IN THE UNITED STATES DISTRICT COURT
FOR THE DISTRICT OF COLUMBIA

DR. JAMES L. SHERLEY, et al., Plaintiffs,	)	
v.	)	Civil Action No. 09-CV-01575-RCL
KATHLEEN SEBELIUS, et al., Defendants.	)	

JOINT APPENDIX

Samuel B. Casey, Cal. Bar No. 76022
ADVOCATES INTERNATIONAL
9691 Main Street, Suite D
Fairfax, VA 22031
(703) 894-1076

Thomas G. Hungar, D.C. Bar No. 447783
Bradley J. Lingo, D.C. Bar No. 490131
Thomas M. Johnson, Jr., D.C. Bar No. 976185
GIBSON, DUNN & CRUTCHER LLP
1050 Connecticut Avenue, N.W.
Washington, D.C. 20036
(202) 955-8500

Steven H. Aden, D.C. Bar No. 466777
ALLIANCE DEFENSE FUND
801 G. Street N.W., Suite 509
Washington, D.C. 20001
(202) 393-8690

Blaine H. Evanson, Cal. Bar No. 254338
Andrew G. Pappas, Cal. Bar No. 266409
GIBSON, DUNN & CRUTCHER LLP
333 S. Grand Avenue
Los Angeles, CA 90071
(213) 229-7000

TABLE OF CONTENTS

and personal information concerning individuals associated with the grant applications, the disclosure of which would constitute a clearly unwarranted invasion of personal privacy.

Name of Committee: National Institute on Alcohol Abuse and Alcoholism. Special Emphasis Panel Alcohol Pharmacotherapy and the Treatment and Prevention of HIV/AIDS. (RFA AA 09 007/008) and Other AIDS Related Research.

Date: August 6, 2009.

Time: 8 a.m. to 11 a.m.

Agenda: To review and evaluate grant applications.

Place: National Institutes of Health, 5635 Fishers Lane, Bethesda, MD 20892. (Telephone Conference Call).

Contact Person: Katrina L Foster, PhD, Scientific Review Officer, National Inst on Alcohol Abuse & Alcoholism, National Institutes of Health, 5635 Fishers Lane, Rm. 2019, Rockville, MD 20852. 301–443–4032. *katrina@mail.nih.gov*.

(Catalogue of Federal Domestic Assistance Program Nos. 93.271, Alcohol Research Career Development Awards for Scientists and Clinicians; 93.272, Alcohol National Research Service Awards for Research Training; 93.273, Alcohol Research Programs; 93.891, Alcohol Research Center Grants; 93.701, ARRA Related Biomedical Research and Research Support Awards, National Institutes of Health, HHS)

Dated: June 29, 2009.

Jennifer Spaeth,

Director, Office of Federal Advisory Committee Policy.

[FR Doc. E9–15847 Filed 7–6–09; 8:45 am]

BILLING CODE 4140–01–M

DEPARTMENT OF HEALTH AND HUMAN SERVICES

National Institutes of Health

National Institute of General Medical Sciences; Notice of Closed Meetings

Pursuant to section 10(d) of the Federal Advisory Committee Act, as amended (5 U.S.C. App.), notice is hereby given of the following meetings.

The meetings will be closed to the public in accordance with the provisions set forth in sections 552b(c)(4) and 552b(c)(6), Title 5 U.S.C., as amended. The grant applications and the discussions could disclose confidential trade secrets or commercial property such as patentable material, and personal information concerning individuals associated with the grant applications, the disclosure of which would constitute a clearly unwarranted invasion of personal privacy.

Name of Committee: National Institute of General Medical Sciences. Special Emphasis Panel Minority Biomedical Research Support.

Date: July 19–20, 2009.

Time: 7 p.m. to 5 p.m.

Agenda: To review and evaluate grant applications.

Place: Hyatt Regency Bethesda, One Bethesda Metro Center, Bethesda, MD 20814.

Contact Person: Margaret J. Weidman, PhD, Scientific Review Officer, Office of Scientific Review, National Institute of General Medical Sciences, National Institutes of Health, 45 Center Drive, Room 3AN18B, Bethesda, MD 20892. 301–594–3663. *weidmanma@nigms.nih.gov.*

Name of Committee: National Institute of General Medical Sciences. Special Emphasis Panel MBRS Score.

Date: July 20–21, 2009.

Time: 8 a.m. to 5 p.m.

Agenda: To review and evaluate grant applications.

Place: Hyatt Regency Bethesda, One Bethesda Metro Center, Bethesda, MD 20814.

Contact Person: Lisa Dunbar, PhD, Scientific Review Officer, Office of Scientific Review, National Institute of General Medical Sciences, National Institutes of Health, 45 Center Drive, Room 3AN12, Bethesda, MD 20892. 301–594–2849. *dunbarl@mail.nih.gov.*

Name of Committee: National Institute of General Medical Sciences. Special Emphasis Panel New Innovator Awards.

Date: July 21, 2009.

Time: 1 p.m. to 5 p.m.

Agenda: To review and evaluate grant applications.

Place: National Institutes of Health, Natcher Building, 45 Center Drive, Bethesda, MD 20892. (Telephone Conference Call).

Contact Person: Richard T. Okita, PhD, Program Director, Pharmacological and Physiological Sciences Branch, National Institute of General Medical Sciences, National Institutes of Health, Natcher Building, Room 2A5–49, Bethesda, MD 20892. 301–594–4469. *okitar@nigms.nih.gov*.

(Catalogue of Federal Domestic Assistance Program Nos. 93.375, Minority Biomedical Research Support; 93.821, Cell Biology and Biophysics Research; 93.859, Pharmacology, Physiology, and Biological Chemistry Research; 93.862, Genetics and Developmental Biology Research; 93.88, Minority Access to Research Careers; 93.96, Special Minority Initiatives, National Institutes of Health, HHS)

Dated: June 29, 2009.

Jennifer Spaeth,

Director, Office of Federal Advisory Committee Policy.

[FR Doc. E9–15846 Filed 7–6–09; 8:45 am]

BILLING CODE 4140–01–M

DEPARTMENT OF HEALTH AND HUMAN SERVICES

National Institutes of Health

National Institutes of Health Guidelines for Human Stem Cell Research

SUMMARY: The National Institutes of Health (NIH) is hereby publishing final ''National Institutes of Health Guidelines for Human Stem Cell Research'' (Guidelines).

On March 9, 2009, President Barack H. Obama issued Executive Order 13505: *Removing Barriers to Responsible Scientific Research Involving Human Stem Cells.* The Executive Order states that the Secretary of Health and Human Services, through the Director of NIH, may support and conduct responsible, scientifically worthy human stem cell research, including human embryonic stem cell (hESC) research, to the extent permitted by law.

These Guidelines implement Executive Order 13505, as it pertains to extramural NIH-funded stem cell research, establish policy and procedures under which the NIH will fund such research, and helps ensure that NIH-funded research in this area is ethically responsible, scientifically worthy, and conducted in accordance with applicable law. Internal NIH policies and procedures, consistent with Executive Order 13505 and these Guidelines, will govern the conduct of intramural NIH stem cell research.

DATES: *Effective Date:* These Guidelines are effective on July 7, 2009.

Summary of Public Comments on Draft Guidelines: On April 23, 2009 the NIH published draft Guidelines for research involving hESCs in the **Federal Register** for public comment, 74 FR 18578 (April 23, 2009). The comment period ended on May 26, 2009.

The NIH received approximately 49,000 comments from patient advocacy groups, scientists and scientific societies, academic institutions, medical organizations, religious organizations, and private citizens. The NIH also received comments from members of Congress. This Notice presents the final Guidelines together with the NIH response to public comments that addressed provisions of the Guidelines.

Title of the Guidelines, Terminology, and Background

Respondents felt the title of the NIH draft guidelines was misleading, in that it is entitled ''National Institutes of Health Guidelines for Human Stem Cell Research,'' yet addresses only one type of human stem cell. The NIH notes that although the Guidelines pertain primarily to the donation of embryos for the derivation of hESCs, one Section also applies to certain uses of both hESCs and human induced pluripotent stem cells. Also, the Guidelines discuss applicable regulatory standards when research involving human adult stem cells or induced pluripotent stem cells constitutes human subject research.

Therefore, the title of the Guidelines was not changed.

Respondents also disagreed with the definition of human embryonic stem cells in the draft Guidelines, and asked that the NIH define them as originating from the inner cell mass of the blastocyst. The NIH modified the definition to say that human embryonic stem cells ''are cells that are derived from the inner cell mass of blastocyst stage human embryos, are capable of dividing without differentiating for a prolonged period in culture, and are known to develop into cells and tissues of the three primary germ layers.''

Financial Gain

Respondents expressed concern that derivers of stem cells might profit from the development of hESCs. Others noted that because the stem cells eligible for use in research using NIH funding under the draft Guidelines are those cells that are subject to existing patents, there will be insufficient competition in the licensing of such rights. These respondents suggested that this could inhibit research, as well as increase the cost of any future clinical benefits. The Guidelines do not address the distribution of stem cell research material. It is, however, the NIH's expectation that stem cell research materials developed with NIH funds, as well as associated intellectual property and data, will be distributed in accordance with the NIH's existing policies and guidance, including ''Sharing Biomedical Research Resources, Principles and Guidelines for Recipients of NIH Grants and Contracts'' and ''Best Practices for the Licensing of Genomic Inventions.'' *http://ott.od.nih.gov/policy/Reports.html* Even where such policies are not directly applicable, the NIH encourages others to refrain from imposing on the transfer of research tools, such as stem cells, any conditions that hinder further biomedical research. In addition, the Guidelines were revised to state that there should be documentation that ''no payments, cash or in kind, were offered for the donated embryos.''

Respondents were concerned that donor(s) be clearly ''apprised up front by any researchers that financial gain may come from the donation and that the donor(s) should know up front if he/she will share in the financial gain.'' The Guidelines address this concern by asking that donor(s) was/were informed during the consent process that the donation was made without any restriction or direction regarding the individual(s) who may receive medical benefit from the use of the stem cells, such as who may be the recipients of cell transplants. The Guidelines also require that the donor(s) receive(s) information that the research was not intended to provide direct medical benefit to the donor(s); that the results of research using the hESCs may have commercial potential, and that the donor(s) would not receive financial or any other benefits from any such commercial development.

IRB Review Under the Common Rule

Respondents suggested that the current regulatory structure of IRB review under the Common Rule (45 CFR Part 46, Subpart A) addresses the core ethical principles needed for appropriate oversight of hESC derivation. They noted that IRB review includes a full review of the informed consent process, as well as a determination of whether individuals were coerced to participate in the research and whether any undue inducements were offered to secure their participation. These respondents urged the NIH to replace the specific standards to assure voluntary and informed consent in the draft Guidelines with a requirement that hESC research be reviewed and approved by an IRB, in conformance with 45 CFR Part 46, Subpart A, as a prerequisite to NIH funding. Respondents also requested that the NIH create a registry of eligible hESC lines to avoid burdensome and repetitive assurances from multiple funding applicants. The NIH agrees that the IRB system of review under the Common Rule provides a comprehensive framework for the review of the donation of identifiable human biological materials for research. However, in the last several years, guidelines on hESC research have been issued by a number of different organizations and governments, and different practices have arisen around the country and worldwide, resulting in a patchwork of standards. The NIH concluded that employing the IRB review system for the donation of embryos would not ameliorate stated concerns about variations in standards for hESC research and would preclude the establishment of an NIH registry of hESCs eligible for NIH funding, because there would be no NIH approval of particular hESCs. To this end and in response to comments, these Guidelines articulate policies and procedures that will allow the NIH to create a Registry. These Guidelines also provide scientists who apply for NIH funding with a specific set of standards reflecting currently recognized ethical principles and practices specific to embryo donation that took place on or after the issuance of the Guidelines, while also establishing procedures for the review of donations that took place before the effective date of the Guidelines.

Federal Funding Eligibility of Human Pluripotent Cells From Other Sources

Respondents suggested that the allowable sources of hESCs potentially available for Federal funding be expanded to include hESC lines from embryos created expressly for research purposes, and lines created, or pluripotent cells derived, following parthenogenesis or somatic cell nuclear transfer (SCNT). The Guidelines allow for funding of research using hESCs derived from embryos created using in vitro fertilization (IVF) for reproductive purposes and no longer needed for these purposes, assuming the research has scientific merit and the embryos were donated after proper informed consent was obtained from the donor(s). The Guidelines reflect the broad public support for Federal funding of research using hESCs created from such embryos based on wide and diverse debate on the topic in Congress and elsewhere. The use of additional sources of human pluripotent stem cells proposed by the respondents involve complex ethical and scientific issues on which a similar consensus has not emerged. For example, the embryo-like entities created by parthenogenesis and SCNT require women to donate oocytes, a procedure that has health and ethical implications, including the health risk to the donor from the course of hormonal treatments needed to induce oocyte production.

Respondents noted that many embryos undergo Pre-implantation Genetic Diagnosis (PGD). This may result in the identification of chromosomal abnormalities that would make the embryos medically unsuitable for clinical use. In addition, the IVF process may also produce embryos that are not transferred into the uterus of a woman because they are determined to be not appropriate for clinical use. Respondents suggested that hESCs derived from such embryos may be extremely valuable for scientific study, and should be considered embryos that were created for reproductive purposes and were no longer needed for this purpose. The NIH agrees with these comments. As in the draft, the final Guidelines allow for the donation of embryos that have undergone PGD.

Donation and Informed Consent

Respondents commented in numerous ways that the draft Guidelines are too procedurally proscriptive in articulating the elements of appropriate informed consent documentation. This over-

reliance on the specific details and format of the informed consent document, respondents argued, coupled with the retroactive application of the Guidelines to embryos already donated for research, would result in a framework that fails to appreciate the full range of factors contributing to the complexity of the informed consent process. For example, respondents pointed to several factors that were precluded from consideration by the proposed Guidelines, such as contextual evidence of the consent process, other established governmental frameworks (representing local and community influences), and the changing standards for informed consent in this area of research over time. Respondents argued that the Guidelines should be revised to allow for a fuller array of factors to be considered in determining whether the underlying ethical principle of voluntary informed consent had been met. In addition to these general issues, many respondents made the specific recommendation that all hESCs derived before the final Guidelines were issued be automatically eligible for Federal funding without further review, especially those eligible under prior Presidential policy, i.e., ''grandfathered.'' The final Guidelines seek to implement the Executive Order by issuing clear guidance to assist this field of science to advance and reach its full potential while ensuring adherence to strict ethical standards. To this end, the NIH is establishing a set of conditions that will maximize ethical oversight, while ensuring that the greatest number of ethically derived hESCs are eligible for Federal funding. Specifically, for embryos donated in the U.S. on or after the effective date of the Guidelines, the only way to establish eligibility will be to either use hESCs listed on the NIH Registry, or demonstrate compliance with the specific procedural requirements of the Guidelines by submitting an assurance with supporting information for administrative review by the NIH. Thus, for future embryo donations in the United States, the Guidelines articulate one set of procedural requirements. This responds to concerns regarding the patchwork of requirements and guidelines that currently exist.

However, the NIH is also cognizant that in the more than a decade between the discovery of hESCs and today, many lines were derived consistent with ethical standards and/or guidelines developed by various states, countries, and other entities such as the International Society for Stem Cell Research (ISSCR) and the National Academy of Sciences (NAS). These various policies have many common features, rely on a consistent ethical base, and require an informed consent process, but they differ in details of implementation. For example, some require specific wording in a written informed consent document, while others do not. It is important to recognize that the principles of ethical research, *e.g.*, voluntary informed consent to participation, have not varied in this time period, but the requirements for implementation and procedural safeguards employed to demonstrate compliance have evolved. In response to these concerns, the Guidelines state that applicant institutions wishing to use hESCs derived from embryos donated prior to the effective date of the Guidelines may either comply with Section II (A) of the Guidelines or undergo review by a Working Group of the Advisory Committee to the Director (ACD). The ACD, which is a chartered Federal Advisory Committee Act (FACA) committee, will advise NIH on whether the core ethical principles and procedures used in the process for obtaining informed consent for the donation of the embryo were such that the cell line should be eligible for NIH funding. This Working Group will not undertake a *de novo* evaluation of ethical standards, but will consider the materials submitted in light of the principles and points to consider in the Guidelines, as well as 45 CFR Part 46 Subpart A. Rather than ''grandfathering,'' ACD Working Group review will enable pre-existing hESCs derived in a responsible manner to be eligible for use in NIH funded research.

In addition, for embryos donated outside the United States prior to the effective date of these Guidelines, applicants may comply with either Section II (A) or (B). For embryos donated outside of the United States on or after the effective date of the Guidelines, applicants seeking to determine eligibility for NIH research funding may submit an assurance that the hESCs fully comply with Section II (A) or submit an assurance along with supporting information, that the alternative procedural standards of the foreign country where the embryo was donated provide protections at least equivalent to those provided by Section II (A) of these Guidelines. These materials will be reviewed by the NIH ACD Working Group, which will recommend to the ACD whether such equivalence exists. Final decisions will be made by the NIH Director. This special consideration for embryos donated outside the United States is needed because donation of embryos in foreign countries is governed by the laws and policies of the respective governments of those nations. Although such donations may be responsibly conducted, such governments may not or cannot change their national donation requirements to precisely comply with the NIH Guidelines. The NIH believes it is reasonable to provide a means for reviewing such hESCs because ethically derived foreign hESCs constitute an important scientific asset for the U.S.

Respondents expressed concern that it might be difficult in some cases to provide assurance that there was a ''clear separation'' between the prospective donor(s)' decision to create embryos for reproductive purposes and the donor(s)' decision to donate the embryos for research purposes. These respondents noted that policies vary at IVF clinics, especially with respect to the degree to which connections with researchers exist. Respondents noted that a particular clinic's role may be limited to the provision of contact information for researchers. A clinic that does not have any particular connection with research would not necessarily have in place a written policy articulating the separation contemplated by the Guidelines. Other respondents noted that embryos that are determined not to be suitable for medical purposes, either because of genetic defects or other concerns, may be donated prior to being frozen. In these cases, it is possible that the informed consent process for the donation might be concurrent with the consent process for IVF treatment. Respondents also noted that the initial consent for IVF may contain a general authorization for donating embryos in excess of clinical need, even though a more detailed consent is provided at the actual time of donation. The NIH notes that the Guidelines specifically state that consent should have been obtained at the time of donation, even if the potential donor(s) had given prior indication of a general intent to donate embryos in excess of clinical need for the purposes of research. Accordingly, a general authorization for research donation when consenting for reproductive treatment would comply with the Guidelines, so long as specific consent for the donation is obtained at the time of donation. In response to comments regarding documentation necessary to establish a separation between clinical and research decisions, the NIH has changed the language of the Guidelines to permit applicant institutions to submit consent forms,

written policies or other documentation to demonstrate compliance with the provisions of the Guidelines. This change should provide the flexibility to accommodate a range of practices, while adhering to the ethical principles intended.

Some respondents want to require that the IVF physician and the hESC researcher should be different individuals, to prevent conflict of interest. Others say they should be the same person, because people in both roles need to have detailed knowledge of both areas (IVF treatment and hESC research). There is also a concern that the IVF doctor will create extra embryos if he/she is also the researcher. As a general matter, the NIH believes that the doctor and the researcher seeking donation should be different individuals. However, this is not always possible, nor is it required, in the NIH's view, for ethical donation.

Some respondents want explicit language (in the Guidelines and/or in the consent) stating that the embryo will be destroyed when the inner cell mass is removed. In the process of developing guidelines, the NIH reviewed a variety of consent forms that have been used in responsible derivations. Several had extensive descriptions of the process and the research to be done, going well beyond the minimum expected, yet they did not use these exact words. Given the wide variety and diversity of forms, as well as the various policy, statutory and regulatory obligations individual institutions face, the NIH declines to provide exact wording for consent forms, and instead endorses a robust informed consent process where all necessary details are explained and understood in an ongoing, trusting relationship between the clinic and the donor(s).

Respondents asked for clarification regarding the people who must give informed consent for the donation of embryos for research. Some commenters suggested that NIH should require consent from the gamete donors, in cases where those individuals may be different than the individuals seeking reproductive treatment. The NIH requests consent from ''the individual(s) who sought reproductive treatment'' because this/these individual(s) is/are responsible for the creation of the embryo(s) and, therefore, its/their disposition. With regard to gamete donation, the risks are associated with privacy and, as such, are governed by requirements of the Common Rule, where applicable.

Respondents also requested clarification on the statement in the draft Guidelines noting that ''although human embryonic stem cells are derived from embryos, such stem cells are not themselves human embryos.'' For the purpose of NIH funding, an embryo is defined by Section 509, Omnibus Appropriations Act, 2009, Public Law 111–8, 3/11/09, otherwise known as the Dickey Amendment, as any organism not protected as a human subject under 45 CFR Part 46 that is derived by fertilization, parthenogenesis, cloning or any other means from one or more human gametes or human diploid cells. Since 1999, the Department of Health and Human Services (HHS) has consistently interpreted this provision as not applicable to research using hESCs, because hESCs are not embryos as defined by Section 509. This long-standing interpretation has been left unchanged by Congress, which has annually reenacted the Dickey Amendment with full knowledge that HHS has been funding hESC research since 2001. These guidelines therefore recognize the distinction, accepted by Congress, between the derivation of stem cells from an embryo that results in the embryo's destruction, for which Federal funding is prohibited, and research involving hESCs that does not involve an embryo nor result in an embryo's destruction, for which Federal funding is permitted.

Some respondents wanted to ensure that potential donor(s) are either required to put their ''extra'' embryos up for adoption before donating them for research, or are at least offered this option. The Guidelines require that all the options available in the health care facility where treatment was sought pertaining to the use of embryos no longer needed for reproductive purposes were explained to the potential donor(s). Since not all IVF clinics offer the same services, the healthcare facility is only required to explain the options available to the donor(s) at that particular facility.

Commenters asked that donor(s) be made aware of the point at which their donation decision becomes irrevocable. This is necessary because if the embryo is de-identified, it may be impossible to stop its use beyond a certain point. The NIH agrees with these comments and revised the Guidelines to require that donor(s) should have been informed that they retained the right to withdraw consent for the donation of the embryo until the embryos were actually used to derive embryonic stem cells or until information which could link the identity of the donor(s) with the embryo was no longer retained, if applicable.

Medical Benefits of Donation

Regarding medical benefit, respondents were concerned that the language of the Guidelines should not somehow eliminate a donor's chances of benefitting from results of stem cell research. Respondents noted that although hESCs are not currently being used clinically, it is possible that in the future such cells might be used for the medical benefit of the person donating them. The Guidelines are meant to preclude individuals from donating embryos strictly for use in treating themselves only or from donating but identifying individuals or groups they do or do not want to potentially benefit from medical intervention using their donated cells. While treatment with hESCs is one of the goals of this research, in practice, years of experimental work must still be done before such treatment might become routinely available. The Guidelines are designed to make it clear that immediate medical benefit from a donation is highly unlikely at this time. Importantly, it is critical to note that the Guidelines in no way disqualify a donor from benefitting from the medical outcomes of stem cell research and treatments that may be developed in the future.

Monitoring and Enforcement Actions

Respondents have expressed concern about the monitoring of funded research and the invocation of possible penalties for researchers who do not follow the Guidelines. A grantee's failure to comply with the terms and conditions of award, including confirmed instances of research misconduct, may cause the NIH to take one or more enforcement actions, depending on the severity and duration of the non-compliance. For example, the following actions may be taken by the NIH when there is a failure to comply with the terms and conditions of any award: (1) Under 45 CFR 74.14, the NIH can impose special conditions on an award, including but not limited to increased oversight/monitoring/reporting requirements for an institution, project, or investigator; and (2) under 45 CFR 74.62 the NIH may impose enforcement actions, including but not limited to withholding funds pending correction of the problem, disallowing all or part of the costs of the activity that was not in compliance, withholding further awards for the project, or suspending or terminating all or part of the funding for the project. Individuals and institutions may be debarred from eligibility for all Federal financial assistance and contracts under 2 CFR part 376 and 48

CFR subpart 9.4, respectively. The NIH will undertake all enforcement actions in accordance with applicable statutes, regulations, and policies.

National Institutes of Health Guidelines for Research Using Human Stem Cells

I. Scope of the Guidelines

These Guidelines apply to the expenditure of National Institutes of Health (NIH) funds for research using human embryonic stem cells (hESCs) and certain uses of induced pluripotent stem cells (See Section IV). The Guidelines implement Executive Order 13505.

Long-standing HHS regulations for Protection of Human Subjects, 45 CFR part 46, subpart A establish safeguards for individuals who are the sources of many human tissues used in research, including non-embryonic human adult stem cells and human induced pluripotent stem cells. When research involving human adult stem cells or induced pluripotent stem cells constitutes human subject research, Institutional Review Board review may be required and informed consent may need to be obtained per the requirements detailed in 45 CFR part 46, subpart A. Applicants should consult *http://www.hhs.gov/ohrp/humansubjects/guidance/45cfr46.htm*.

It is also important to note that the HHS regulation, *Protection of Human Subjects*, 45 CFR part 46, subpart A, may apply to certain research using hESCs. This regulation applies, among other things, to research involving individually identifiable private information about a living individual, 45 CFR 46.102(f). The HHS Office for Human Research Protections (OHRP) considers biological material, such as cells derived from human embryos, to be individually identifiable when they can be linked to specific living individuals by the investigators either directly or indirectly through coding systems. Thus, in certain circumstances, IRB review may be required, in addition to compliance with these Guidelines. Applicant institutions are urged to consult OHRP guidances at *http://www.hhs.gov/ohrp/policy/index.html#topics*.

To ensure that the greatest number of responsibly derived hESCs are eligible for research using NIH funding, these Guidelines are divided into several sections, which apply specifically to embryos donated in the U.S. and foreign countries, both before and on or after the effective date of these Guidelines. Section II (A) and (B) describe the conditions and review processes for determining hESC eligibility for NIH funds. Further information on these review processes may be found at *http://www.NIH.gov*. Sections IV and V describe research that is not eligible for NIH funding.

These guidelines are based on the following principles:

1. Responsible research with hESCs has the potential to improve our understanding of human health and illness and discover new ways to prevent and/or treat illness.

2. Individuals donating embryos for research purposes should do so freely, with voluntary and informed consent.

As directed by Executive Order 13505, the NIH shall review and update these Guidelines periodically, as appropriate.

II. Eligibility of Human Embryonic Stem Cells for Research With NIH Funding

For the purpose of these Guidelines, ''human embryonic stem cells (hESCs)'' are cells that are derived from the inner cell mass of blastocyst stage human embryos, are capable of dividing without differentiating for a prolonged period in culture, and are known to develop into cells and tissues of the three primary germ layers. Although hESCs are derived from embryos, such stem cells are not themselves human embryos. All of the processes and procedures for review of the eligibility of hESCs will be centralized at the NIH as follows:

A. Applicant institutions proposing research using hESCs derived from embryos donated in the U.S. on or after the effective date of these Guidelines may use hESCs that are posted on the new NIH Registry or they may establish eligibility for NIH funding by submitting an assurance of compliance with Section II (A) of the Guidelines, along with supporting information demonstrating compliance for administrative review by the NIH. For the purposes of this Section II (A), hESCs should have been derived from human embryos:

1. That were created using in vitro fertilization for reproductive purposes and were no longer needed for this purpose;

2. That were donated by individuals who sought reproductive treatment (hereafter referred to as ''donor(s)'') and who gave voluntary written consent for the human embryos to be used for research purposes; and

3. For which all of the following can be assured and documentation provided, such as consent forms, written policies, or other documentation, provided:

a. All options available in the health care facility where treatment was sought pertaining to the embryos no longer needed for reproductive purposes were explained to the individual(s) who sought reproductive treatment.

b. No payments, cash or in kind, were offered for the donated embryos.

c. Policies and/or procedures were in place at the health care facility where the embryos were donated that neither consenting nor refusing to donate embryos for research would affect the quality of care provided to potential donor(s).

d. There was a clear separation between the prospective donor(s)'s decision to create human embryos for reproductive purposes and the prospective donor(s)'s decision to donate human embryos for research purposes. Specifically:

i. Decisions related to the creation of human embryos for reproductive purposes should have been made free from the influence of researchers proposing to derive or utilize hESCs in research. The attending physician responsible for reproductive clinical care and the researcher deriving and/or proposing to utilize hESCs should not have been the same person unless separation was not practicable.

ii. At the time of donation, consent for that donation should have been obtained from the individual(s) who had sought reproductive treatment. That is, even if potential donor(s) had given prior indication of their intent to donate to research any embryos that remained after reproductive treatment, consent for the donation for research purposes should have been given at the time of the donation.

iii. Donor(s) should have been informed that they retained the right to withdraw consent for the donation of the embryo until the embryos were actually used to derive embryonic stem cells or until information which could link the identity of the donor(s) with the embryo was no longer retained, if applicable.

e. During the consent process, the donor(s) were informed of the following:

i. That the embryos would be used to derive hESCs for research;

ii. What would happen to the embryos in the derivation of hESCs for research;

iii. That hESCs derived from the embryos might be kept for many years;

iv. That the donation was made without any restriction or direction regarding the individual(s) who may receive medical benefit from the use of the hESCs, such as who may be the recipients of cell transplants;

v. That the research was not intended to provide direct medical benefit to the donor(s);

vi. That the results of research using the hESCs may have commercial potential, and that the donor(s) would not receive financial or any other benefits from any such commercial development;

vii. Whether information that could identify the donor(s) would be available to researchers.

B. Applicant institutions proposing research using hESCs derived from embryos donated in the U.S. before the effective date of these Guidelines may use hESCs that are posted on the new NIH Registry or they may establish eligibility for NIH funding in one of two ways:

1. By complying with Section II (A) of the Guidelines; or

2. By submitting materials to a Working Group of the Advisory Committee to the Director (ACD), which will make recommendations regarding eligibility for NIH funding to its parent group, the ACD. The ACD will make recommendations to the NIH Director, who will make final decisions about eligibility for NIH funding.

The materials submitted must demonstrate that the hESCs were derived from human embryos: (1) That were created using in vitro fertilization for reproductive purposes and were no longer needed for this purpose; and (2) that were donated by donor(s) who gave voluntary written consent for the human embryos to be used for research purposes.

The Working Group will review submitted materials, *e.g.*, consent forms, written policies or other documentation, taking into account the principles articulated in Section II (A), 45 CFR part 46, subpart A, and the following additional points to consider. That is, during the informed consent process, including written or oral communications, whether the donor(s) were: (1) Informed of other available options pertaining to the use of the embryos; (2) offered any inducements for the donation of the embryos; and (3) informed about what would happen to the embryos after the donation for research.

C. For embryos donated outside the United States before the effective date of these Guidelines, applicants may comply with either Section II (A) or (B). For embryos donated outside of the United States on or after the effective date of the Guidelines, applicants seeking to determine eligibility for NIH research funding may submit an assurance that the hESCs fully comply with Section II (A) or submit an assurance along with supporting information, that the alternative procedural standards of the foreign country where the embryo was donated provide protections at least equivalent to those provided by Section II (A) of these Guidelines. These materials will be reviewed by the NIH ACD Working Group, which will recommend to the ACD whether such equivalence exists. Final decisions will be made by the NIH Director.

D. NIH will establish a new Registry listing hESCs eligible for use in NIH funded research. All hESCs that have been reviewed and deemed eligible by the NIH in accordance with these Guidelines will be posted on the new NIH Registry.

III. Use of NIH Funds

Prior to the use of NIH funds, funding recipients should provide assurances, when endorsing applications and progress reports submitted to NIH for projects using hESCs, that the hESCs are listed on the NIH registry.

IV. Research Using hESCs and/or Human Induced Pluripotent Stem Cells That, Although the Cells May Come From Eligible Sources, Is Nevertheless Ineligible for NIH Funding

This section governs research using hESCs and human induced pluripotent stem cells, *i.e.*, human cells that are capable of dividing without differentiating for a prolonged period in culture, and are known to develop into cells and tissues of the three primary germ layers. Although the cells may come from eligible sources, the following uses of these cells are nevertheless ineligible for NIH funding, as follows:

A. Research in which hESCs (even if derived from embryos donated in accordance with these Guidelines) or human induced pluripotent stem cells are introduced into non-human primate blastocysts.

B. Research involving the breeding of animals where the introduction of hESCs (even if derived from embryos donated in accordance with these Guidelines) or human induced pluripotent stem cells may contribute to the germ line.

V. Other Research Not Eligible for NIH Funding

A. NIH funding of the derivation of stem cells from human embryos is prohibited by the annual appropriations ban on funding of human embryo research (Section 509, Omnibus Appropriations Act, 2009, Pub. L. 111–8, 3/11/09), otherwise known as the Dickey Amendment.

B. Research using hESCs derived from other sources, including somatic cell nuclear transfer, parthenogenesis, and/or IVF embryos created for research purposes, is not eligible for NIH funding.

Dated: June 30, 2009.

Raynard S. Kington,

Acting Director, NIH.

[FR Doc. E9–15954 Filed 7–6–09; 8:45 am]

BILLING CODE 4140–01–P

DEPARTMENT OF HOMELAND SECURITY

U.S. Customs and Border Protection

Agency Information Collection Activities: Importer's ID Input Record

AGENCY: U.S. Customs and Border Protection, Department of Homeland Security.

ACTION: 30-Day notice and request for comments; Extension of an existing information collection: 1651–0064.

SUMMARY: U.S. Customs and Border Protection (CBP) of the Department of Homeland Security has submitted the following information collection request to the Office of Management and Budget (OMB) for review and approval in accordance with the Paperwork Reduction Act: Importer's ID Input Record (Form 5106). This is a proposed extension of an information collection that was previously approved. CBP is proposing that this information collection be extended with no change to the burden hours. This document is published to obtain comments from the public and affected agencies. This proposed information collection was previously published in the **Federal Register** (74 FR 16226) on April 9, 2009, allowing for a 60-day comment period. This notice allows for an additional 30 days for public comments. This process is conducted in accordance with 5 CFR 1320.10.

DATES: Written comments should be received on or before August 6, 2009.

ADDRESSES: Interested persons are invited to submit written comments on the proposed information collection to the Office of Information and Regulatory Affairs, Office of Management and Budget. Comments should be addressed to the OMB Desk Officer for Customs and Border Protection, Department of Homeland Security, and sent via electronic mail to *oira_submission@omb.eop.gov* or faxed to (202) 395–5806.

SUPPLEMENTARY INFORMATION: U.S. Customs and Border Protection (CBP) encourages the general public and affected Federal agencies to submit written comments and suggestions on

Matthew Jennings, BSC Coordinator, CDC, Coordinating Office for Terrorism Preparedness and Emergency Response, 1600 Clifton Road, NE., Mailstop D–44, Atlanta, Georgia 30333, Telephone (404) 639–7357.

The Director, Management Analysis and Services Office, has been delegated the authority to sign **Federal Register** notices pertaining to announcements of meetings and other committee management activities for both CDC and the Agency for Toxic Substances and Disease Registry.

Dated: April 17, 2009.

Elaine L. Baker,

Director, Management Analysis and Service Office, Centers for Disease Control and Prevention.

[FR Doc. E9–9331 Filed 4–22–09; 8:45 am]

BILLING CODE 4163–18–P

DEPARTMENT OF HEALTH AND HUMAN SERVICES

National Institutes of Health

Draft National Institutes of Health Guidelines for Human Stem Cell Research Notice

SUMMARY: The National Institutes of Health (NIH) is requesting public comment on draft guidelines entitled ''National Institutes of Health Guidelines for Human Stem Cell Research'' (Guidelines).

The purpose of these draft Guidelines is to implement Executive Order 13505, issued on March 9, 2009, as it pertains to extramural NIH-funded research, to establish policy and procedures under which NIH will fund research in this area, and to help ensure that NIH-funded research in this area is ethically responsible, scientifically worthy, and conducted in accordance with applicable law. Internal NIH procedures, consistent with Executive Order 13505 and these Guidelines, will govern the conduct of intramural NIH research involving human stem cells.

These draft Guidelines would allow funding for research using human embryonic stem cells that were derived from embryos created by *in vitro* fertilization (IVF) for reproductive purposes and were no longer needed for that purpose. Funding will continue to be allowed for human stem cell research using adult stem cells and induced pluripotent stem cells. Specifically, these Guidelines describe the conditions and informed consent procedures that would have been required during the derivation of human embryonic stem cells for research using these cells to be funded by the NIH. NIH funding for research using human embryonic stem cells derived from other sources, including somatic cell nuclear transfer, parthenogenesis, and/or IVF embryos created for research purposes, is not allowed under these Guidelines.

NIH funding of the derivation of stem cells from human embryos is prohibited by the annual appropriations ban on funding of human embryo research (Consolidated Appropriations Act, 2009, Pub. L. 110–161, 3/11/09), otherwise known as the Dickey-Wicker Amendment.

According to these Guidelines, there are some uses of human embryonic stem cells and human induced pluripotent stem cells that, although those cells may come from allowable sources, are nevertheless ineligible for NIH funding.

For questions regarding ongoing NIH-funded research involving human embryonic stem cells, as well as pending applications and those submitted prior to the issuance of Final Guidelines, see the NIH Guide *http://grants.nih.gov/grants/guide/notice-files/NOT-OD-09-085.html.*

DATES: Written comments must be received by NIH on or before May 26, 2009.

ADDRESSES: The NIH welcomes public comment on the draft Guidelines set forth below. Comments may be entered at: *http://nihoerextra.nih.gov/stem_cells/add.htm.* Comments may also be mailed to: NIH Stem Cell Guidelines, MSC 7997, 9000 Rockville Pike, Bethesda, Maryland 20892–7997. Comments will be made publicly available, including any personally identifiable or confidential business information they contain.

SUPPLEMENTARY INFORMATION: On March 9, 2009, President Barack H. Obama issued Executive Order 13505: *Removing Barriers to Responsible Scientific Research Involving Human Stem Cells.* The Executive Order states that the Secretary of Health and Human Services, through the Director of NIH, may support and conduct responsible, scientifically worthy human stem cell research, including human embryonic stem cell research, to the extent permitted by law.

The purpose of these draft Guidelines is to implement Executive Order 13505, issued on March 9, 2009, as it pertains to extramural NIH-funded research, to establish policy and procedures under which NIH will fund research in this area, and to help ensure that NIH-funded research in this area is ethically responsible, scientifically worthy, and conducted in accordance with applicable law. Internal NIH procedures, consistent with Executive Order 13505 and these Guidelines, will govern the conduct of intramural NIH research involving human stem cells.

Long-standing Department of Health and Human Services regulations for Protection of Human Subjects, 45 CFR part 46, establish safeguards for individuals who are the sources of many human tissues used in research, including non-embryonic human adult stem cells and human induced pluripotent stem cells. When research involving human adult stem cells or induced pluripotent stem cells constitutes human subject research, Institutional Review Board review may be required and informed consent may need to be obtained per the requirements detailed in 45 CFR part 46. Applicants should consult *http://www.hhs.gov/ohrp/humansubjects/guidance/45cfr46.htm.*

As described in these draft Guidelines, human embryonic stem cells are cells that are derived from human embryos, are capable of dividing without differentiating for a prolonged period in culture, and are known to develop into cells and tissues of the three primary germ layers. Although human embryonic stem cells are derived from embryos, such stem cells are not themselves human embryos.

Studies of human embryonic stem cells may yield information about the complex events that occur during human development. Some of the most serious medical conditions, such as cancer and birth defects, are due to abnormal cell division and differentiation. A better understanding of the genetic and molecular controls of these processes could provide information about how such diseases arise and suggest new strategies for therapy. Human embryonic stem cells may also be used to test new drugs. For example, new medications could be tested for safety on differentiated somatic cells generated from human embryonic stem cells.

Perhaps the most important potential use of human embryonic stem cells is the generation of cells and tissues that could be used for cell-based therapies. Today, donated tissues and organs are often used to replace ailing or destroyed tissue, but the need for transplantable tissues and organs far outweighs the available supply. Stem cells, directed to differentiate into specific cell types, offer the possibility of a renewable source of replacement cells and tissues to treat diseases and conditions, including Parkinson's disease, amyotrophic lateral sclerosis, spinal cord injury, burns, heart disease, diabetes, and arthritis.

NIH currently funds ongoing research involving human embryonic stem cells as detailed under prior Presidential policy. Under that policy, Federal funds have been used for research on human embryonic stem cells where the derivation process was initiated prior to 9 p.m. EDT August 9, 2001, the embryo was created for reproductive purposes, the embryo was no longer needed for these purposes, informed consent was obtained for the donation of the embryo, and no financial inducements were provided for donation of the embryo.

These draft Guidelines would allow funding for research using only those human embryonic stem cells that were derived from embryos created by *in vitro* fertilization (IVF) for reproductive purposes and were no longer needed for that purpose. Funding will continue to be allowed for human stem cell research using adult stem cells and induced pluripotent stem cells. Specifically, these Guidelines describe the conditions and informed consent procedures that would have been required during the derivation of human embryonic stem cells for research using these cells to be funded by the NIH. NIH funding for research using human embryonic stem cells derived from other sources, including somatic cell nuclear transfer, parthenogenesis, and/or IVF embryos created for research purposes, is not allowed under these Guidelines.

Please note that, for NIH funded research using the permitted human embryonic stem cells, the requirements of the Department's protection of human subjects regulations, 45 CFR part 46, may or may not apply, depending on the nature of the research. For further information, see *Human Embryonic Stem Cells, Germ Cells and Cell Derived Test Articles:* OHRP Guidance for Investigators and Institutional Review Boards.

NIH funding of the derivation of stem cells from human embryos is prohibited by the annual appropriations ban on funding of human embryo research (Consolidated Appropriations Act, 2009, Pub. L. 110–161, 3/11/09), otherwise known as the Dickey-Wicker Amendment.

According to these Guidelines, there are some uses of human embryonic stem cells that, although those cells may come from allowable sources, are nevertheless ineligible for NIH funding.

In developing these draft Guidelines, the NIH consulted its Guidelines issued in 2000, as well as the thoughtful guidelines developed by other national and international committees of scientists, bioethicists, patient advocates, physicians and other stakeholders, including the U.S. National Academies, the International Society for Stem Cell Research, and others.

As directed by Executive Order 13505, the NIH shall review and update these Guidelines periodically, as appropriate.

The Draft Guidelines Follow:

National Institutes of Health Guidelines for Human Stem Cell Research

I. Scope of Guidelines

These Guidelines describe the circumstances under which human embryonic stem cells are eligible for use in extramural NIH-funded research, and they also include a section on uses of human embryonic stem cells or human induced pluripotent stem cells that are ineligible for NIH funding.

For the purpose of these Guidelines, ''human embryonic stem cells'' are cells that are derived from human embryos, are capable of dividing without differentiating for a prolonged period in culture, and are known to develop into cells and tissues of the three primary germ layers. Although human embryonic stem cells are derived from embryos, such stem cells are not themselves human embryos.

II. Guidelines for Eligibility of Human Embryonic Stem Cells for Use in Research

A. The Executive Order: Executive Order 13505, *Removing Barriers to Responsible Scientific Research Involving Human Stem Cells,* states that the Secretary of the Department of Health and Human Services (DHHS), through the Director of the NIH, may support and conduct responsible, scientifically worthy human stem cell research, including human embryonic stem cell research, to the extent permitted by law.

B. Eligibility of Human Embryonic Stem Cells Derived from Human Embryos: Human embryonic stem cells may be used in research using NIH funds, if the cells were derived from human embryos that were created for reproductive purposes, were no longer needed for this purpose, were donated for research purposes, and for which documentation for all of the following can be assured:

1. All options pertaining to use of embryos no longer needed for reproductive purposes were explained to the potential donor(s).

2. No inducements were offered for the donation.

3. A policy was in place at the health care facility where the embryos were donated that neither consenting nor refusing to donate embryos for research would affect the quality of care provided to potential donor(s).

4. There was a clear separation between the prospective donor(s)'s decision to create human embryos for reproductive purposes and the prospective donor(s)'s decision to donate human embryos for research purposes.

5. At the time of donation, consent for that donation was obtained from the individual(s) who had sought reproductive services. That is, even if potential donor(s) had given prior indication of their intent to donate to research any embryos that remained after reproductive treatment, consent for the donation should have been given at the time of the donation. Donor(s) were informed that they retained the right to withdraw consent until the embryos were actually used for research.

6. Decisions related to the creation of human embryos for reproductive purposes were made free from the influence of researchers proposing to derive or utilize human embryonic stem cells in research. Whenever it was practicable, the attending physician responsible for reproductive clinical care and the researcher deriving and/or proposing to utilize human embryonic stem cells should not have been the same person.

7. Written informed consent was obtained from individual(s) who sought reproductive services and who elected to donate human embryos for research purposes. The following information, which is pertinent to making the decision of whether or not to donate human embryos for research purposes, was in the written consent form for donation and discussed with potential donor(s) in the informed consent process:

a. A statement that donation of the embryos for research was voluntary;

b. A statement that donor(s) understood alternative options pertaining to use of the embryos;

c. A statement that the embryos would be used to derive human embryonic stem cells for research;

d. Information about what would happen to the embryos in the derivation of human embryonic stem cells for research;

e. A statement that human embryonic stem cells derived from the embryos might be maintained for many years;

f. A statement that the donation was made without any restriction or direction regarding the individual(s) who may receive medical benefit from the use of the stem cells;

g. A statement that the research was not intended to provide direct medical benefit to the donor(s);

h. A statement as to whether or not information that could identify the donor(s) would be retained prior to the derivation or the use of the human embryonic stem cells (relevant guidance from the DHHS Office for Human Research Protections (OHRP) should be followed, as applicable; see OHRP's *Guidance for Investigators and Institutional Review Boards Regarding Research Involving Human Embryonic Stem Cells, Germ Cells, and Stem Cell-Derived Test Articles* and *Guidance on Research Involving Coded Private Information or Biological Specimens,* or successor guidances); and

i. A statement that the results of research using the human embryonic stem cells may have commercial potential, and a statement that the donor(s) would not receive financial or any other benefits from any such commercial development.

C. Prior to the use of NIH funds: Funding recipients must ensure that: (1) The human embryonic stem cells were derived consistent with sections II.A and B of these Guidelines; and (2) the grantee institution maintains appropriate documentation demonstrating such consistency in accordance with 45 CFR 74.53, which also details rights of access by NIH. The responsible grantee institutional official must provide assurances with respect to (1) and (2) when endorsing applications and progress reports submitted to NIH for projects that utilize these cells.

III. Research Using Human Embryonic Stem Cells and/or Human Induced Pluripotent Stem Cells That, Although the Cells May Come From Allowable Sources, Is Nevertheless Ineligible for NIH Funding

This section governs research using human embryonic stem cells and human induced pluripotent stem cells, *i.e.,* human cells that are capable of dividing without differentiating for a prolonged period in culture, and are known to develop into cells and tissues of the three primary germ layers. There are some uses of these cells that, although they may come from allowable sources, are nevertheless ineligible for NIH funding, as follows:

A. Research in which human embryonic stem cells (even if derived according to these Guidelines) or human induced pluripotent stem cells are introduced into non-human primate blastocysts.

B. Research involving the breeding of animals where the introduction of human embryonic stem cells (even if derived according to these Guidelines) or human induced pluripotent stem cells may have contributed to the germ line.

IV. Other Non-Allowable Research

A. NIH funding of the derivation of stem cells from human embryos is prohibited by the annual appropriations ban on funding of human embryo research (Consolidated Appropriations Act, 2009, Pub. L. 110–161, 3/11/09), otherwise known as the Dickey-Wicker Amendment.

B. NIH funding for research using human embryonic stem cells derived from other sources, including somatic cell nuclear transfer, parthenogenesis, and/or IVF embryos created for research purposes, is not allowed under these Guidelines.

Dated: April 17, 2009.

Raynard S. Kington,

Acting Director, NIH.

[FR Doc. E9–9313 Filed 4–22–09; 8:45 am]

BILLING CODE 4140–01–P

DEPARTMENT OF HEALTH AND HUMAN SERVICES

National Institutes of Health

Government-Owned Inventions; Availability for Licensing

AGENCY: National Institutes of Health, Public Health Service, HHS.

ACTION: Notice.

SUMMARY: The inventions listed below are owned by an agency of the U.S. Government and are available for licensing in the U.S. in accordance with 35 U.S.C. 207 to achieve expeditious commercialization of results of Federally-funded research and development. Foreign patent applications are filed on selected inventions to extend market coverage for companies and may also be available for licensing.

ADDRESSES: Licensing information and copies of the U.S. patent applications listed below may be obtained by writing to the indicated licensing contact at the Office of Technology Transfer, National Institutes of Health, 6011 Executive Boulevard, Suite 325, Rockville, Maryland 20852–3804; telephone: 301/496–7057; fax: 301/402–0220. A signed Confidential Disclosure Agreement will be required to receive copies of the patent applications.

On-Demand In Vitro Assembly of Protein Microarrays

Description of Technology: Protein microarrays are becoming an indispensable biomedical tool to facilitate rapid high-throughput detection of protein-protein, protein-drug and protein-DNA interactions for large groups of proteins. The novel Protein Microarray of this invention is essentially a DNA microarray that becomes a protein microarray on demand and provides an efficient systematic approach to the study of protein interactions and drug target identification and validation, thereby speeding up the discovery process. The technology allows a large number of proteins to be synthesized and immobilized at their individual site of expression on an ordered array without the need for protein purification. As a result, proteins are ready for subsequent use in binding studies and other analysis.

The Protein Microarray is based on high affinity and high specificity of the protein-nucleic acid interaction of the Tus protein and the Ter site of E. coli. The DNA templates are arrayed on the microarray to perform dual function: (1) Synthesizing the protein in situ (cell-free protein synthesis) in the array and (2) at the same time capturing the protein it synthesizes by DNA-protein interaction. This method utilizes an expression vector containing a DNA sequence which serves a dual purpose: (a) Encoding proteins of interest fused to the Tus protein for in vitro synthesis of the protein and (b) encoding the Ter sequence, which captures the fusion protein through the high affinity interaction with the Tus protein.

Applications:

- Simultaneous analysis of interactions of many proteins with other proteins, antibodies, nucleic acids, lipids, drugs, etc, in a single experiment.
- Efficient discovery of novel drugs and drug targets.

Development Status: The technology is in early stages of development.

Inventors: Deb K. Chatterjee, Kalavathy Sitaraman, James L. Hartley, David J. Munroe, Cassio Baptista (NCI).

Patent Status:

U.S. Patent Application No. 11/252,735 filed 19 Oct 2005 (HHS Reference No. E–244–2005/0–US–01).

U.S. Patent Application No. 12/105,636 filed 18 Apr 2008 (HHS Reference No. E–244–2005/1–US–02).

Licensing Status: Available for licensing.

Licensing Contact: Jeffrey A. James, Ph.D.; 301–435–5474; *jeffreyja@mail.nih.gov.*

Collaborative Research Opportunity: The National Cancer Institute Protein Expression Laboratory is seeking statements of capability or interest from parties interested in collaborative research to further develop, evaluate, or

Federal Register

Wednesday,
March 11, 2009

Part IV

The President

Federal Register
Vol. 74, No. 46
Wednesday, March 11, 2009

Presidential Documents

Title 3—

The President

Executive Order 13505 of March 9, 2009

Removing Barriers to Responsible Scientific Research Involving Human Stem Cells

By the authority vested in me as President by the Constitution and the laws of the United States of America, it is hereby ordered as follows:

Section 1. *Policy.* Research involving human embryonic stem cells and human non-embryonic stem cells has the potential to lead to better understanding and treatment of many disabling diseases and conditions. Advances over the past decade in this promising scientific field have been encouraging, leading to broad agreement in the scientific community that the research should be supported by Federal funds.

For the past 8 years, the authority of the Department of Health and Human Services, including the National Institutes of Health (NIH), to fund and conduct human embryonic stem cell research has been limited by Presidential actions. The purpose of this order is to remove these limitations on scientific inquiry, to expand NIH support for the exploration of human stem cell research, and in so doing to enhance the contribution of America's scientists to important new discoveries and new therapies for the benefit of humankind.

Sec. 2. *Research.* The Secretary of Health and Human Services (Secretary), through the Director of NIH, may support and conduct responsible, scientifically worthy human stem cell research, including human embryonic stem cell research, to the extent permitted by law.

Sec. 3. *Guidance.* Within 120 days from the date of this order, the Secretary, through the Director of NIH, shall review existing NIH guidance and other widely recognized guidelines on human stem cell research, including provisions establishing appropriate safeguards, and issue new NIH guidance on such research that is consistent with this order. The Secretary, through NIH, shall review and update such guidance periodically, as appropriate.

Sec. 4. *General Provisions.* (a) This order shall be implemented consistent with applicable law and subject to the availability of appropriations.

(b) Nothing in this order shall be construed to impair or otherwise affect:

(i) authority granted by law to an executive department, agency, or the head thereof; or

(ii) functions of the Director of the Office of Management and Budget relating to budgetary, administrative, or legislative proposals.

(c) This order is not intended to, and does not, create any right or benefit, substantive or procedural, enforceable at law or in equity, by any party against the United States, its departments, agencies, or entities, its officers, employees, or agents, or any other person.

Sec. 5. *Revocations.* (a) The Presidential statement of August 9, 2001, limiting Federal funding for research involving human embryonic stem cells, shall have no further effect as a statement of governmental policy.

(b) Executive Order 13435 of June 20, 2007, which supplements the August 9, 2001, statement on human embryonic stem cell research, is revoked.

THE WHITE HOUSE,
March 9, 2009.

[FR Doc. E9–5441
Filed 3–10–09; 11:15 am]
Billing code 3195–W9–P

Federal Register

Friday,
June 22, 2007

Part III

The President

Executive Order 13435—Expanding Approved Stem Cell Lines in Ethically Responsible Ways

Federal Register
Vol. 72, No. 120
Friday, June 22, 2007

Presidential Documents

Title 3—

The President

Executive Order 13435 of June 20, 2007

Expanding Approved Stem Cell Lines in Ethically Responsible Ways

By the authority vested in me as President by the Constitution and the laws of the United States of America, and to provide leadership with respect to research on pluripotent stem cells derived by ethically responsible techniques so that the potential of pluripotent stem cells can be explored without violating human dignity or demeaning human life, it is hereby ordered as follows:

Section 1. *Research on Alternative Sources of Pluripotent Stem Cells.* (a) The Secretary of Health and Human Services (Secretary) shall conduct and support research on the isolation, derivation, production, and testing of stem cells that are capable of producing all or almost all of the cell types of the developing body and may result in improved understanding of or treatments for diseases and other adverse health conditions, but are derived without creating a human embryo for research purposes or destroying, discarding, or subjecting to harm a human embryo or fetus.

(b) Within 90 days of this order, the Secretary, after such consultation with the Director of the National Institutes of Health (Director), shall issue a plan, including such mechanisms as requests for proposals, requests for applications, program announcements and other appropriate means, to implement subsection (a) of this section, that:

(i) specifies and reflects a determination of the extent to which specific techniques may require additional basic or animal research to ensure that any research involving human cells using these techniques is clearly consistent with the standards established under this order and applicable law;

(ii) prioritizes research with the greatest potential for clinical benefit;

(iii) takes into account techniques outlined by the President's Council on Bioethics, and any other appropriate techniques and research, provided they clearly meet the standard set forth in subsection (a) of this section;

(iv) renames the ''Human Embryonic Stem Cell Registry'' the ''Human Pluripotent Stem Cell Registry;'' and

(v) adds to the registry new human pluripotent stem cell lines that clearly meet the standard set forth in subsection (a) of this section.

(c) Not later than December 31 of each year, the Secretary shall report to the President on the activities carried out under this order during the past fiscal year, including a description of the research carried out or supported by the Department of Health and Human Services, including the National Institutes of Health, and other developments in the science of pluripotent stem cells not derived from human embryos.

Sec. 2. *Policy.* The activities undertaken and supported by and under the direction of the Secretary shall be clearly consistent with the following policies and principles:

(a) the purposes of this order are (i) to direct the Department of Health and Human Services, including the National Institutes of Health, to intensify peer reviewed research that may result in improved understanding of or treatments for diseases and other adverse health conditions, and (ii) to promote the derivation of human pluripotent stem cell lines from a variety

of alternative sources while clearly meeting the standard set forth in section 1(a) of this order;

(b) it is critical to establish moral and ethical boundaries to allow the Nation to move forward vigorously with medical research, while also maintaining the highest ethical standards and respecting human life and human dignity;

(c) the destruction of nascent life for research violates the principle that no life should be used as a mere means for achieving the medical benefit of another;

(d) human embryos and fetuses, as living members of the human species, are not raw materials to be exploited or commodities to be bought and sold; and

(e) the Federal Government has a duty to exercise responsible stewardship of taxpayer funds, both supporting important medical research and respecting ethical and moral boundaries.

Sec. 3. *Interpretation of this Order.* (a) For purposes of this order, the term ''human embryo'' shall mean any organism, not protected as a human subject under 45 CFR 46 as of the date of this order, that is derived by fertilization, parthenogenesis, cloning, or any other means from one or more human gametes or human diploid cells.

(b) For purposes of this order, the term ''subjecting to harm a human embryo'' shall mean subjecting such an embryo to risk of injury or death greater than that allowed for research on fetuses in utero under 45 CFR 46.204(b) and section 498(b) of the Public Health Service Act (42 U.S.C. 289g(b)) as of the date of this order.

(c) Nothing in this order shall be construed to affect any policy, guideline, or regulation regarding embryonic stem cell research, human cloning by somatic cell nuclear transfer, or any other research not specifically authorized by this order, or to forbid the use of existing stem cell lines deemed eligible for other federally funded research in accordance with the presidential policy decision of August 9, 2001, for research specifically authorized by this order.

Sec. 4. *General Provisions.* (a) This order shall be implemented consistent with applicable law and subject to the availability of appropriations.

(b) This order is not intended to, and does not, create any right, benefit, or privilege, substantive or procedural, enforceable at law or in equity, by any party against the United States, its departments, agencies, or entities, its officers, employees, or agents, or any other person.

THE WHITE HOUSE,
June 20, 2007.

[FR Doc. 07–3112
Filed 6–21–07; 11:09 am]
Billing code 3195–01–P

a path to return to peace negotiations based on United Nations Security Council Resolutions 242, 338, and the Madrid Conference. To get to Mitchell, the parties need to resume effective security cooperation and work together to stop terrorism and violence.

I call upon the leaders of the Palestinian Authority and Israel to demonstrate foresight and responsibility by choosing the path toward a better future for their people.

Address to the Nation on Stem Cell Research From Crawford, Texas

August 9, 2001

Good evening. I appreciate you giving me a few minutes of your time tonight so I can discuss with you a complex and difficult issue, an issue that is one of the most profound of our time.

The issue of research involving stem cells derived from human embryos is increasingly the subject of a national debate and dinner table discussions. The issue is confronted every day in laboratories as scientists ponder the ethical ramifications of their work. It is agonized over by parents and many couples as they try to have children or to save children already born. The issue is debated within the church, with people of different faiths, even many of the same faith, coming to different conclusions. Many people are finding that the more they know about stem cell research, the less certain they are about the right ethical and moral conclusions.

My administration must decide whether to allow Federal funds, your tax dollars, to be used for scientific research on stem cells derived from human embryos. A large number of these embryos already exist. They are the product of a process called in vitro fertilization, which helps so many couples conceive children. When doctors match sperm and egg to create life outside the womb, they usually produce more embryos than are implanted in the mother. Once a couple successfully has children, or if they are unsuccessful, the additional embryos remain frozen in laboratories. Some will not survive during long storage; others are destroyed. A number have been donated to science and used to create privately funded stem cell lines. And a few have been implanted in an adoptive mother and born and are today healthy children.

Based on preliminary work that has been privately funded, scientists believe further research using stem cells offers great promise that could help improve the lives of those who suffer from many terrible diseases, from juvenile diabetes to Alzheimer's, from Parkinson's to spinal cord injuries. And while scientists admit they are not yet certain, they believe stem cells derived from embryos have unique potential.

You should also know that stem cells can be derived from sources other than embryos, from adult cells, from umbilical cords that are discarded after babies are born, from human placentas. And many scientists feel research on these types of stem cells is also promising. Many patients suffering from a range of diseases are already being helped with treatments developed from adult stem cells. However, most scientists, at least today, believe that research on embryonic stem cells offer the most promise because these cells have the potential to develop in all of the tissues in the body.

Scientists further believe that rapid progress in this research will come only with Federal funds. Federal dollars help attract the best and brightest scientists. They ensure new discoveries are widely shared at the largest number of research facilities and that the research is directed toward the greatest public good.

The United States has a long and proud record of leading the world toward advances in science and medicine that improve human life. And the United States has a long and proud record of upholding the highest standards of ethics as we expand the limits of science and knowledge. Research on embryonic stem cells raises profound ethical questions, because extracting the stem cell destroys the embryo and thus destroys its potential for life. Like a snowflake, each of these embryos is unique, with the unique genetic potential of an individual human being.

As I thought through this issue, I kept returning to two fundamental questions: First, are these frozen embryos human life and, therefore, something precious to be protected? And second, if they're going to be

destroyed anyway, shouldn't they be used for a greater good, for research that has the potential to save and improve other lives?

I've asked those questions and others of scientists, scholars, bioethicists, religious leaders, doctors, researchers, Members of Congress, my Cabinet, and my friends. I have read heartfelt letters from many Americans. I have given this issue a great deal of thought, prayer, and considerable reflection. And I have found widespread disagreement.

On the first issue, are these embryos human life? Well, one researcher told me he believes this 5-day-old cluster of cells is not an embryo, not yet an individual, but a preembryo. He argued that it has the potential for life, but it is not a life because it cannot develop on its own. An ethicist dismissed that as a callous attempt at rationalization. "Make no mistake," he told me, "that cluster of cells is the same way you and I, and all the rest of us, started our lives. One goes with a heavy heart if we use these," he said, "because we are dealing with the seeds of the next generation."

And to the other crucial question, if these are going to be destroyed anyway, why not use them for good purpose, I also found different answers. Many argue these embryos are byproducts of a process that helps create life, and we should allow couples to donate them to science so they can be used for good purpose instead of wasting their potential. Others will argue there's no such thing as excess life and the fact that a living being is going to die does not justify experimenting on it or exploiting it as a natural resource.

At its core, this issue forces us to confront fundamental questions about the beginnings of life and the ends of science. It lies at a difficult moral intersection, juxtaposing the need to protect life in all its phases with the prospect of saving and improving life in all its stages.

As the discoveries of modern science create tremendous hope, they also lay vast ethical minefields. As the genius of science extends the horizons of what we can do, we increasingly confront complex questions about what we should do. We have arrived at that brave new world that seemed so distant in 1932, when Aldous Huxley wrote about human beings created in test tubes in what he called a "hatchery." In recent weeks, we learned that scientists have created human embryos in test tubes solely to experiment on them. This is deeply troubling and a warning sign that should prompt all of us to think through these issues very carefully.

Embryonic stem cell research is at the leading edge of a series of moral hazards. The initial stem cell researcher was at first reluctant to begin his research, fearing it might be used for human cloning. Scientists have already cloned a sheep. Researchers are telling us the next step could be to clone human beings to create individual designer stem cells, essentially to grow another you, to be available in case you need another heart or lung or liver.

I strongly oppose human cloning, as do most Americans. We recoil at the idea of growing human beings for spare body parts, or creating life for our convenience. And while we must devote enormous energy to conquering disease, it is equally important that we pay attention to the moral concerns raised by the new frontier of human embryo stem cell research. Even the most noble ends do not justify any means.

My position on these issues is shaped by deeply held beliefs. I'm a strong supporter of science and technology and believe they have the potential for incredible good, to improve lives, to save life, to conquer disease. Research offers hope that millions of our loved ones may be cured of a disease and rid of their suffering. I have friends whose children suffer from juvenile diabetes. Nancy Reagan has written me about President Reagan's struggle with Alzheimer's. My own family has confronted the tragedy of childhood leukemia. And like all Americans, I have great hope for cures.

I also believe human life is a sacred gift from our Creator. I worry about a culture that devalues life and believe as your President I have an important obligation to foster and encourage respect for life in America and throughout the world. And while we're all hopeful about the potential of this research, no one can be certain that the science will live up to the hope it has generated.

Eight years ago, scientists believed fetal tissue research offered great hope for cures and treatments, yet the progress to date has

not lived up to its initial expectations. Embryonic stem cell research offers both great promise and great peril. So I have decided we must proceed with great care.

As a result of private research, more than 60 genetically diverse stem cell lines already exist. They were created from embryos that have already been destroyed, and they have the ability to regenerate themselves indefinitely, creating ongoing opportunities for research. I have concluded that we should allow Federal funds to be used for research on these existing stem cell lines, where the life and death decision has already been made.

Leading scientists tell me research on these 60 lines has great promise that could lead to breakthrough therapies and cures. This allows us to explore the promise and potential of stem cell research without crossing a fundamental moral line by providing taxpayer funding that would sanction or encourage further destruction of human embryos that have at least the potential for life.

I also believe that great scientific progress can be made through aggressive Federal funding of research on umbilical cord, placenta, adult, and animal stem cells which do not involve the same moral dilemma. This year, your Government will spend $250 million on this important research.

I will also name a President's council to monitor stem cell research, to recommend appropriate guidelines and regulations, and to consider all of the medical and ethical ramifications of biomedical innovation. This council will consist of leading scientists, doctors, ethicists, lawyers, theologians, and others and will be chaired by Dr. Leon Kass, a leading biomedical ethicist from the University of Chicago. This council will keep us apprised of new developments and give our Nation a forum to continue to discuss and evaluate these important issues.

As we go forward, I hope we will always be guided by both intellect and heart, by both our capabilities and our conscience. I have made this decision with great care, and I pray it is the right one.

Thank you for listening. Good night, and God bless America.

NOTE: The President spoke at 8:01 p.m. at the Bush Ranch.

Digest of Other White House Announcements

The following list includes the President's public schedule and other items of general interest announced by the Office of the Press Secretary and not included elsewhere in this issue.

August 4

In the morning, the President traveled to Bethesda, MD, where he had his annual physical examination at Bethesda Naval Hospital. In the afternoon, he traveled to the Bush Ranch in Crawford, TX.

August 7

In the morning, the President traveled to Waco, TX, and later returned to Crawford.

The White House announced that the President will send U.S. Trade Representative Robert Zoellick to Indonesia on August 10–11 to meet with President Megawati.

August 8

In the morning, the President traveled to Waco, TX, and later returned to Crawford.

August 9

In the morning, the President had a telephone conversation with Secretary of Health and Human Services Tommy G. Thompson concerning the President's decision on stem cell research. In the afternoon, he had a telephone conversation with Dr. Leon Kass of the University of Chicago, also concerning the President's decision on stem cell research.

August 10

The President announced his intention to nominate Ralph Leo Boyce to be Ambassador to Indonesia.

The President announced his intention to nominate John D. Ong to be Ambassador to Norway.

DEPARTMENT OF HEALTH AND HUMAN SERVICES

Centers for Disease Control and Prevention

Disease, Disability, and Injury Prevention and Control Special Emphasis Panel (SEP): Sexually Transmitted Disease (STD) Faculty Expansion Program, Program Announcement #02005

In accordance with section 10(a)(2) of the Federal Advisory Committee Act (Pub. L. 92–463), the Centers for Disease Control and Prevention (CDC) announces the following meeting.

Name: Disease, Disability, and Injury Prevention and Control Special Emphasis Panel (SEP): Sexually Transmitted Disease (STD) Faculty Expansion Program, Program Announcement #02005.

Times and Date: 9 a.m.–9:30 a.m., November 29, 2001 (Open). 9:30 a.m.–4:30 p.m., November 29, 2001 (Closed).

Place: Centers for Disease Control and Prevention, National Center for HIV, STD, and TB Prevention, 10 Corporate Square Blvd, Conference Room 1304, Atlanta, Georgia 30329.

Status: Portions of the meeting will be closed to the public in accordance with provisions set forth in section 552b(c)(4) and (6), Title 5 U.S.C., and the Determination of the Deputy Director for Program Management, CDC, pursuant to Pub. L. 92–463.

Matters to be Discussed: The meeting will include the review, discussion, and evaluation of applications received in response to Program Announcement 02005.

CONTACT PERSON FOR MORE INFORMATION: Elizabeth A. Wolfe, Prevention Support Office, National Center for HIV, STD, and TB Prevention, CDC, Corporate Square Office Park, 8 Corporate Square Boulevard, M/S E07, Atlanta, Georgia 30329, telephone 404/639–8025.

The Director, Management Analysis and Services office has been delegated the authority to sign Federal Register notices pertaining to announcements of meetings and other committee management activities, for both the Centers for Disease Control and Prevention and the Agency for Toxic Substances and Disease Registry.

Dated: November 2, 2001.

John C. Burckhardt,

Acting Director, Management Analysis and Services Office, Centers for Disease Control and Prevention CDC.

[FR Doc. 01–28436 Filed 11–13–01; 8:45 am]

BILLING CODE 4163–18–P

DEPARTMENT OF HEALTH AND HUMAN SERVICES

National Institutes of Health

National Institutes of Health Guidelines for Research Using Human Pluripotent Stem Cells

ACTION: Notice; withdrawal of NIH Guidelines for Research Using Pluripotent Stem Cells Derived from Human Embryos (published August 25, 2000, 65 FR 51976, correctedNovember 21, 2000, 65 FR 69951).

SUMMARY: The National Institutes of Health (NIH) announces the withdrawal of those sections of the NIH Guidelines for Research Using Human Pluripotent Stem Cells, *http://www.nih.gov/news/stemcell/stemcellguidelines.htm.* (NIH Guidelines), that pertain to research involving human pluripotent stem cells derived from human embryos that are the result of *in vitro* fertilization, are in excess of clinical need, and have not reached the stage at which the mesoderm is formed.

The President has determined the criteria that allow Federal funding for research using existing embryonic stem cell lines, *http://www.whitehouse.gov/news/releases/2001/08/print/20010809-1.html.* Thus, the NIH Guidelines as they relate to human pluripotent stem cells derived from human embryos are no longer needed.

FOR FURTHER INFORMATION CONTACT: NIH Office of Extramural Research, NIH, 1 Center Drive, MSC 0152, Building 1, Room 146, Bethesda, MD 20892, or e-mail *DDER@nih.gov.*

Dated: November 2, 2001.

Ruth L. Kirschstein,

Acting Director, National Institutes of Health.

[FR Doc. 01–28426 Filed 11–13–01; 8:45 am]

BILLING CODE 4140–01–P

DEPARTMENT OF THE INTERIOR

Office of the Secretary

Invasive Species Advisory Committee; Notice

AGENCY: Office of the Secretary, Interior.

ACTION: Request for nominations for the Invasive Species Advisory Committee—Extension of Deadline for Nomination Submissions.

SUMMARY: This is an extension of the deadline for nomination submissions due to ongoing delays in surface mail processing in the Washington, DC Metropolitan Area.

DATES: Extended Deadline—Tuesday, November 27, 2001 (6 p.m. EST).

ADDRESSES: Nominations should be sent to Lori Williams, Executive Director, National Invasive Species Council, 1951 Constitution Ave., NW., Room 320, Washington, DC 20240.

FOR FURTHER INFORMATION CONTACT: Kelsey Passé, Program Analyst, at (202) 208–6336, fax: (202) 208–1526, or by e-mail at *Kelsey_Passe@ios.doi.gov.*

SUPPLEMENTARY INFORMATION:

Advisory Committee Scope and Objectives

The purpose and role of the ISAC are to provide advice to the Invasive Species Council (Council), as authorized by Executive Order 13112, on a broad array of issues including preventing the introduction of invasive species, providing for their control, and minimizing the economic, ecological, and human health impacts that invasive species cause. The Council is Co-chaired by the Secretaries of the Interior, Agriculture, and Commerce. The duty of the Council is to provide national leadership regarding invasive species issues. Pursuant to the Executive Order, the Council developed a National Invasive Species Management Plan. The Plan is available on the web at *www.invasivespecies.gov.* The Council is responsible for effective implementation of the Plan. The Council coordinates Federal agency activities concerning invasive species; prepares, revises and issues the National Invasive Species Management Plan; encourages planning and action at local, tribal, State, regional and ecosystem-based levels; develops recommendations for international cooperation in addressing invasive species; facilitates the development of a coordinated network to document, evaluate, and monitor impacts from invasive species; and facilitates establishment of an information-sharing system on invasive species that utilizes, to the greatest extent practicable, the Internet.

The role of ISAC is to maintain an intensive and regular dialogue regarding the aforementioned issues. ISAC provides advice in cooperation with stakeholders and existing organizations addressing invasive species. The ISAC meets up to four (4) times per year.

Terms for current members of the ISAC expire at the end of 2001. Current members of the ISAC are eligible for reappointment. The Secretary of the Interior will appoint members to ISAC in consultation with the Secretaries of Agriculture and Commerce. The Secretary of Interior actively solicits

pharmacokinetic imaging in a non-invasive manner after non-toxic infusion of the spin probe.

However, the disadvantage of EPRI is the lack of proper orientation of the physiological image with respect to anatomy. On the contrary, Magnetic Resonance Imaging (MRI) methods are excellent for providing images with fine anatomical detail, but are often not possible methods that provide physiological information co-registered with anatomy with clinically relevant resolution.

The current invention complements a MRI with EPRI methods to solve each method's problem described above. A low-field MRI(5–30 mT) module is integrated into an EPRI(5—20 mT) system to provide an MRI scout image to properly orient the EPRI physiological information with respect to anatomy (A common magnet/gradient coil assembly is used for both MRI and EPRI scans).

Therefore, the EPR images contain spectral information regarding the local physiological conditions such as oxygen status. This data, when overlaid with anatomical images of MRI (Magnetic Resonance Imaging), co-register anatomical MR images and EPR physiological images.

Dated: November 13, 2000.

Jack Spiegel,

Director, Division of Technology Development and Transfer, Office of Technology Transfer, National Institutes of Health.

[FR Doc. 00–29717 Filed 11–20–00; 8:45 am]

BILLING CODE 4140–01–P

DEPARTMENT OF HEALTH AND HUMAN SERVICES

National Institutes of Health

National Institutes of Health Guidelines for Research Using Human Pluripotent Stem Cells; Correction

ACTION: Notice; correction.

SUMMARY: The National Institutes of Health published in the **Federal Register** on August 25, 2000, the final National Institutes of Health Guidelines for Research Using Human Pluripotent Stem Cells (65 FR 51976). The final Guidelines contained incorrect citations and other errors. The final Guidelines, with the corrections made in this notice, are available on the NIH stem cell information web site at:(*http://www.nih.gov/news/stemcell/index.htm*). For additional information on human pluripotent stem cells, refer to this web site.

FOR FURTHER INFORMATION CONTACT: NIH Office of Science Policy, Attention: HPSCRG, Building 1, Room 218, MSC 0166, 9000 Rockville Pike, Bethesda, MD 20892, (301) 594–7741 or e-mail *stemcell@mail.nih.gov.*

Corrections

1. In Section II.A.2.d of the Guidelines (65 FR 51980, first column), change ''human pluripotent stem cells,'' at the end of the section, to ''embryo.''

2. In Section II.B.1.a. of the Guidelines (65 FR 51980, second column), change ''Section II.A.2'' to ''Section II.B.2.''

3. In Section II.B.2.a. of the Guidelines (65 FR 51980, third column), add the following at the end of the section: ''and with 42 U.S.C. § 289g–2(b).''

4. In Section IV.B. of the Guidelines (65 FR 51981, first column), change ''applications shall'' in the first sentence to ''documentation of compliance with the Guidelines will'' and insert after ''by HPSCRG and'' the words, ''all applications will be reviewed''.

Dated: November 15, 2000.

Ruth L. Kirschstein,

Principal Deputy Director, NIH.

[FR Doc. 00–29791 Filed 11–20–00; 8:45 am]

BILLING CODE 4140–01–M

DEPARTMENT OF HOUSING AND URBAN DEVELOPMENT

[Docket No. FR–4565–N–31]

Notice of Proposed Information Collection: Comment Request; Section 203(k) Rehabilitation Mortgage Insurance Program

AGENCY: Office of the Assistant Secretary for Housing, HUD.

ACTION: Notice.

SUMMARY: The proposed information collection requirement described below will be submitted to the Office of Management and Budget (OMB) for review, as required by the Paperwork Reduction Act. The Department is soliciting public comments on the subject proposal.

DATES: *Comments Due Date:* January 22, 2000.

ADDRESSES: Interested persons are invited to submit comments regarding this proposal. Comments should refer to the proposal by name and/or OMB Control Number and should be sent to: Wayne Eddins, Reports Management Officer, Department of Housing and Urban Development, 451 7th Street, SW, L'Enfant Plaza Building, Room 8001, Washington, DC 20410.

FOR FURTHER INFORMATION CONTACT: Vance T. Morris, Director, Office of Single Family Program Development, Department of Housing and Urban Development, 451 7th Street SW, Washington, DC 20410, telephone (202) 708–2121 (this is not a toll free number) for copies of the proposed forms and other available information.

SUPPLEMENTARY INFORMATION: The Department is submitting the proposed information collection to OMB for review, as required by the Paperwork Reduction Act of 1995 (44 U.S.C. Chapter 35, as amended).

This notice is soliciting comments from members of the public and affected agencies concerning the proposed collection of information to: (1) Evaluate whether the proposed collection is necessary for the proper performance of the functions of the agency, including whether the information will have practical utility; (2) Evaluate the accuracy of the agency's estimate of the burden of the proposed collection of information; (3) Enhance the quality, utility, and clarity of the information to be collected; and (4) Minimize the burden of the collection of information on those who are to respond; including the use of appropriate automated collection techniques or other forms of information technology, e.g., permitting electronic submission of responses.

This Notice also lists the following information:

Title of Proposal: 203(k) Rehabilitation Mortgage Insurance.

OMB Control Number, if applicable: 2502–0527.

Description of the need for the information and proposed use: This request for OMB review involves a reinstatement of a previously approved information collection for 203(k) Rehabilitation Mortgage insurance (OMB control number 2502–0527) that expired on October 31, 2000. The information collection implements recommendations to mitigate program abuses that were cited in an Audit Report of HUD's Office of Inspector General. The information collection focuses on the loan origination process and requires (1) certifications and disclosures concerning identity–of–interest borrowers and program participants, and (2) proficiency testing of home inspectors/consultants. Periodic reporting of the collected information is not required.

Agency form numbers, if applicable: HUD–92700 & HUD–9746–A.

Estimation of the total numbers of hours needed to prepare the information collection including number of respondents, frequency of response, and

Federal Register

Friday,
August 25, 2000

Part IV

Department of Health and Human Services

National Institutes of Health

National Institutes of Health Guidelines for Research Using Human Pluripotent Stem Cells and Notification of Request for Emergency Clearance; Modification of OMB No. 0925–0001/Exp. 2/01, "PHS 398 Research and Research Training Grant Applications and Related Forms"; Notices

DEPARTMENT OF HEALTH AND HUMAN SERVICES

National Institutes of Health

National Institutes of Health Guidelines for Research Using Human Pluripotent Stem Cells

SUMMARY: The National Institutes of Health (NIH) is hereby publishing final ''National Institutes of Health Guidelines for Research Using Human Pluripotent Stem Cells.'' The Guidelines establish procedures to help ensure that NIH-funded research in this area is conducted in an ethical and legal manner.

EFFECTIVE DATE: These Guidelines are effective on August 25, 2000. The moratorium on research using human pluripotent stem cells derived from human embryos and fetal tissue put in place by the Director, NIH, in January 1999, will be lifted on August 25, 2000.

SUMMARY OF PUBLIC COMMENTS ON DRAFT GUIDELINES: On December 2, 1999 (64 FR 67576), the NIH published Draft Guidelines for research involving human pluripotent stem cells (hPSCs) in the **Federal Register** for public comment. The comment period ended on February 22, 2000.

The NIH received approximately 50,000 comments from members of Congress, patient advocacy groups, scientific societies, religious organizations, and private citizens. This Notice presents the final Guidelines together with NIH's response to the substantive public comments that addressed provisions of the Guidelines.

Scope of Guidelines and General Issues

Respondents asked for clarification of terminology used in the Guidelines and some commented that the language was not appropriate or was too technical, particularly the informed consent sections. The NIH agrees that these Guidelines should be clear and understandable. Changes, including some reorganization of the sections, were made to this end. The Guidelines are written primarily for the purpose of informing investigators of the conditions that must be met in order to receive NIH funding for research using hPSCs and, therefore, some technical language is required. The Guidelines do not define the precise language that should appear in informed consent documents because these should be developed by the investigator/clinician specifically for a particular study protocol or procedure for which the consent is being sought. Existing regulatory provisions require (45 CFR 46.116) that the language in informed consent documents be understandable to prospective participants in the study.

Respondents suggested that NIH funding for research using hPSCs would be in violation of the DHHS appropriations law and that derivation of hPSCs cannot be distinguished from their use. For this reason, a number of respondents asked that the NIH withdraw the draft Guidelines. The NIH sought the opinion of the DHHS General Counsel, who determined that ''federally funded research that utilizes hPSCs would not be prohibited by the HHS appropriations law prohibiting human embryo research, because such cells are not human embryos.'' Comments questioning this conclusion did not present information or arguments that justify reconsideration of the conclusion.

Respondents commented that the Guidelines are too restrictive or that there is no need for Federal Guidelines for this arena of research. Comments asserted that federally funded research using hPSCs should go forward without formal requirements, in the same manner as in the private sector. In order to help ensure that the NIH-funded research using hPSCs is conducted in an ethical and legal manner, the NIH felt it was advisable to develop and implement guidelines. To this end, the NIH Director convened a Working Group of the Advisory Committee to the Director, NIH (ACD), to advise the ACD on the development of guidelines and an oversight process for research involving hPSCs. The NIH Director charged the Working Group with developing appropriate guidelines to govern research involving the derivation and use of hPSCs from fetal tissue and research involving the use of hPSCs derived from human embryos that are in excess of clinical need.

Respondents commented regarding the sources of stem cells. Some respondents stated that research on hPSCs was unnecessary because stem cells from adults, umbilical cords, and placentas could be used instead. Other respondents asked the NIH to restrict Federal funding for hPSC research to those cells derived from fetal and adult tissue but not embryos. Other respondents asked that the Guidelines encompass research using stem cells from adult tissues.

As stated under Section I. *Scope of Guidelines*, the Guidelines apply to the use of NIH funds for research using hPSCs derived from human embryos or human fetal tissue. The Guidelines do not impose requirements on Federal funding of research involving stem cells from human adults, umbilical cords, or placentas.

Given the enormous potential of stem cells to the development of new therapies for the most devastating diseases, it is important to simultaneously pursue all lines of promising research. It is possible that no single source of stem cells is best or even suitable/usable for all therapies. Different types or sources of stem cells may be optimal for treatment of specific conditions. In order to determine the very best source of many of the specialized cells and tissues of the body for new treatments and even cures, it is vitally important to study the potential of adult stem cells for comparison to that of hPSCs derived from embryos and fetuses. Unless all stem cell types are studied, the differences between adult stem cells and embryo and fetal-derived hPSCs will not be known.

Moreover, there is evidence that adult stem cells may have more limited potential than hPSCs. First, stem cells for all cell and tissue types have not yet been found in the adult human. Significantly, cardiac stem cells or pancreatic islet stem cells have not been identified in adult humans.

Second, stem cells in adults are often present in only minute quantities, are difficult to isolate and purify, and their numbers may decrease with age. For example, brain cells from adults that may be neural stem cells have been obtained only by removing a portion of the brain of an adult with epilepsy, a complex and invasive procedure that carries the added risk of further neurological damage. Any attempt to use stem cells from a patient's own body for treatment would require that stem cells would first have to be isolated from the patient and then grown in culture in sufficient numbers to obtain adequate quantities for treatment. This would mean that for some rapidly progressing disorders, there may not be sufficient time to grow enough cells to use for treatment.

Third, in disorders that are caused by a genetic defect, the genetic error likely would be present in the patient's stem cells, making cells from such a patient inappropriate for transplantation. In addition, adult stem cells may contain more DNA abnormalities caused by exposure to daily living, including sunlight, toxins, and errors made during DNA replication than will be found in fetal or embryonic hPSCs.

Fourth, there is evidence that stem cells from adults may not have the same capacity to multiply as do younger cells. These potential weaknesses may limit the usefulness of adult stem cells.

Respondents were concerned that these are guidelines and not requirements or regulations. Although these are guidelines and not regulations, they prescribe the documentation and assurances that must accompany requests for NIH funding for research utilizing hPSCs. If the funding requests do not contain the prescribed information, funding for hPSC research will not be provided. Compliance with the Guidelines will be imposed as a condition of grant award.

Respondents commented that there had not been enough widespread public disclosure/discussion of this research or the Guidelines. Prior to the development of draft Guidelines, there were two Congressional hearings on hPSCs. In a further effort to ensure substantial discussion and comment, the NIH convened a Working Group of the Advisory Committee to the Director, NIH (ACD), to advise the ACD on the development of these Guidelines. The Working Group was composed of scientists, patients and patient advocates, ethicists, clinicians, and lawyers. The Working Group met in public session on April 8, 1999, and heard from members of the public, as well as professional associations and Congress. In developing the draft Guidelines, the NIH also considered advice from the National Bioethics Advisory Commission (NBAC). Draft Guidelines were published for public comment in the **Federal Register** on December 2, 1999, for 60 days, and, in response to public interest, the comment period was extended an additional 28 days. Approximately 50,000 comments were received. NIH issued a national press release announcing the **Federal Register** notice and many of the Nation's newspapers carried articles on this area of research and on the Guidelines. Patient groups, scientific societies, and religious organizations convened meetings and discussion groups and disseminated materials about this area of research and about the Guidelines.

Comment was received about whether the Guidelines apply to hPSC lines developed outside of the United States. The Guidelines make no distinction based upon the country in which an hPSC line is developed. All lines to be used in hPSC cell research funded by NIH must meet the same requirements.

Derivation and Use of hPSCs From Fetal Tissue

Respondents made the point that the NIH has specified certain requirements for the use of human fetal tissue to derive hPSCs in addition to those imposed on other areas of human fetal tissue research. These respondents suggested that the section of the Guidelines pertaining to fetal tissue sources be omitted. In order to ensure uniformity in NIH's oversight of research using hPSCs, the Guidelines were extended to govern hPSCs derived from both human embryos and fetal tissue.

Use of hPSCs Derived From Human Embryos

Respondents suggested that the Guidelines refer to ''fertility treatment'' rather than to ''infertility treatment'' in order to clarify that they allow the use of human embryos from treatments that employ assisted reproductive technologies to facilitate reproduction in fertile, as well as in infertile, individuals. The Guidelines have been changed accordingly.

Respondents suggested dropping the word ''early'' throughout the document or more clearly defining ''early.'' The word ''early'' in reference to human embryos has been deleted; the Guidelines make it clear that NIH funding of research using hPSCs derived in the private sector from human embryos can involve only embryos that have not reached the stage at which the mesoderm is formed.

Some respondents were concerned that embryos might be created for research purposes. Other respondents stated there should be no distinction between embryos created for research purposes and those created for fertility treatment. Investigators seeking NIH funds for research using hPSCs are required to provide documentation, prior to the award of any NIH funds, that embryos were created for the purposes of fertility treatment. President Clinton, many members of Congress, the NIH Human Embryo Research Panel, and the NBAC have all embraced the distinction between embryos created for research purposes and those created for reproductive purposes.

Respondents were concerned about the creation of a ''black market'' for human embryos, and expressed concerns that individuals will be coerced into donating embryos. The Guidelines state that there can be no incentives for donation and that a decision to donate must be made free of coercion. In addition, the Guidelines set forth conditions that will help ensure all donations are voluntary. For example, with regard to hPSCs derived from embryos, research using Federal funds may only be conducted if the cells were derived from frozen embryos that were created for the purpose of fertility treatment and that were in excess of clinical need.

Respondents commented on the requirement that human embryos be frozen in order to qualify for derivation of hPSCs to be used in NIH-funded research. Respondents suggested that the freezing requirement would preclude the use of hPSCs derived from embryos that are genetically and chromosomally abnormal, since such embryos are usually not frozen for reproductive purposes. While the NIH acknowledges that research on hPSCs derived from such embryos could yield important scientific information, limiting research to hPSCs derived from frozen human embryos will help ensure that the decision to donate the embryo for hPSC research is distinct and separate from the fertility treatment.

Financial Issues

Respondents expressed concern regarding the sale of fetal tissue for profit and whether hPSC research would encourage such activity. Respondents also were concerned about whether clinics or doctors would profit from the derivation of hPSCs and/or their sale. Section 498B of the Public Health Service Act prohibits any individual from knowingly acquiring or selling human fetal tissue for ''valuable consideration.'' In addition, the Guidelines prohibit any inducement for the donation of human embryos for research purposes. The Guidelines also call for an assurance that the hPSCs to be used in NIH-funded research were obtained through a donation or through a payment that does not exceed the reasonable costs associated with the transportation, processing, preservation, quality control and storage of the hPSCs. All grantees must sign an assurance that they are in compliance with all applicable Federal, State, and local laws. Each funded research institution is responsible for monitoring compliance by individual investigators with any such applicable laws.

Respondents questioned the prohibition against embryo donors benefitting financially from their donation. This clause was retained in the final Guidelines to help ensure that the donating individuals are offered no inducements to donate and that all donations are voluntary.

Respondents suggested that the Guidelines be strengthened to include a waiver of intellectual property rights. This proposed change would be inconsistent with 45 CFR 46.116 of the regulation for the protection of human subjects of research, which provides that no informed consent may include

language through which the subject waives or appears to waive any of the subject's legal rights.

Respondents questioned the reference in the requirements for informed consent related to the commercial potential of donated material. The paragraphs providing for disclosure in the informed consent of the possibility that the donated material could have commercial potential were modified. The reference in these paragraphs to ''donated material'' did not accurately reflect the intent of the provision. The Guidelines now make clear that the ''results of research on the human pluripotent stem cells may have commercial potential.''

Ineligible Research

Respondents objected to the areas of research that the NIH has deemed ineligible, particularly research that is not restricted by statute or regulation, such as research utilizing hPSCs that were derived using somatic cell nuclear transfer, i.e., the transfer of a human somatic cell nucleus into a human egg. The NIH determined that, at this time, research using hPSCs derived from such sources has not received adequate discussion and consideration by the public and is, therefore, ineligible for NIH funding.

Separation of Fertility Treatment and Abortion From Research

Respondents were concerned that hPSC research would encourage abortion. The law and the Guidelines guard against encouraging abortion by requiring that the decision to have an abortion be made apart from and prior to the decision to donate tissue.

Respondents objected to the condition in the Guidelines that the fertility physician could not be the same person as the researcher deriving stem cells. Some respondents stated that the Institutional Review Board (IRB) or an independent physician would be able to guard against this conflict of interest. The restriction was designed so that the person treating the individuals seeking fertility treatment, who is involved in decisions such as how many embryos to produce, is not the person seeking to derive hPSCs. This separation will help ensure that embryos will not be created in numbers greater than necessary for fertility treatment.

Respondents suggested that the clauses regarding donation of fetal tissue or human embryos for derivation of stem cells for eventual use in transplantation be changed explicitly to prevent directed donation. This change has been made.

Identifiers

Respondents were concerned about removing identifiers. There was concern that the investigator would not be able to document compliance with the Guidelines requirements without identifiers, or that the removal of identifiers would make it impossible to conduct certain genetic studies or develop therapeutic materials. The Guidelines have been modified to clarify that the term ''identifier'' refers to any information from which the donor(s) can be identified, directly or through identifiers linked to the donors. However, since information identifying the donor(s) may be necessary if the tissue or cells are to be used in transplantation, the Guidelines have also been modified to state that the informed consent should notify donor(s) whether or not identifiers will be retained.

Respondents commented that DNA is an identifier and that all donors of human embryos or fetal tissue should be told that identifiers such as DNA will be retained with the samples. Although DNA can be used to determine the individual from whom a tissue sample was taken, this can be done only when one has a sample from both the tissue in question and the putative donor; it cannot be used to identify an individual out of a population. Moreover, it is difficult to identify a donor using tissue derived from a fetus or embryo, since the tissue is not genetically identical to the donor.

Informed Consent and IRB Review

Respondents asked why investigators were expected to provide documentation of IRB review of derivation from human embryos, but not for derivation from fetal tissue. Respondents suggested that the requirements be changed so that protocols for both sources of hPSCs must be approved by an IRB. The Guidelines have been changed to make clear that the IRB review requirements regarding the derivation of cells from fetal tissue and human embryos are the same.

Comment was received expressing concern that the informed consent explicitly state that the donor will have no dispositional authority over derived pluripotent stem cells. The Guidelines state that donation of human embryos should have been made without any restriction regarding the individual(s) who may be the recipient of the cells derived from the hPSCs for transplantation. Such a statement is consistent with the statutory provision applicable to the donor informed consent for the use of fetal tissue for transplantation. The Guidelines now provide for the inclusion of a statement to this effect in the informed consent.

Respondents urged that the Guidelines be revised to remove the prohibition on potential donors receiving information regarding subsequent testing of donated tissue in the situation when physicians deem disclosure to be in the donors' best interest. This change has been made.

Respondents requested clarification regarding the persons from whom consent for donation of embryos for research must be obtained. The Guidelines call for informed consent from individual(s) who have sought fertility treatment. Only the individual(s) who were part of the decision to create the embryo for reproductive purposes should have been part of the decision to donate for the derivation of hPSCs.

Respondents urged that fertility clinics should be able to discuss with patients the option of donating embryos for research at the beginning of the IVF process. The Guidelines do not delineate the timeframe during which the general option of donating embryos for research can be discussed. However, according to the Guidelines, obtaining consent for donation of embryos for the purpose of deriving hPSCs should not occur until after the embryos are determined to be in ''excess of clinical need.''

Oversight

Respondents stated that the NIH's oversight in this area of research was very important to the legal and ethical conduct of this research, and asked for more information regarding the oversight process. Information about the operations of the Human Pluripotent Stem Cell Review Group (HPSCRG) can be found in the final Guidelines and on the NIH Web page.

Respondents were concerned about whether and how NIH would monitor research after a researcher receives NIH funds. Compliance with the Guidelines will be largely determined prior to the award of funds. Follow-up to ensure continued compliance with the Guidelines will be conducted in the same manner as for all other conditions of all other NIH grant awards. It is the responsibility of the investigator to file progress reports, and it is the responsibility of the funded institution to ensure compliance with the NIH Guidelines. NIH staff will also monitor the progress of these investigators as part of their regular duties.

Respondents asked about penalties for not following the Guidelines. The following actions may be taken by the NIH when there is a failure to comply with the terms and conditions of any award: (1) Under 45 CFR 74.14, the NIH can impose special conditions on an award, including increased oversight/monitoring/reporting requirements for an institution, project or investigator; and (2) under 45 CFR 74.62, if a grantee materially fails to comply with the terms and conditions of the award, the NIH may withhold funds pending correction of the problem or, pending more severe enforcement action, disallow all or part of the costs of the activity that was not in compliance, withhold further awards for the project, or suspend or terminate all or part of the funding for the project. Individuals and institutions may be debarred from eligibility for all Federal financial assistance and contracts under 45 CFR Part 76 and 48 CFR Subpart 9.4, respectively. Because these sanctions pertain to all conditions of grant award, the NIH did not reiterate them in the Guidelines.

Respondents suggested that the HPSCRG hold periodic Stem Cell Policy Conferences (similar to the Gene Therapy Policy Conferences conducted by the Recombinant DNA Advisory Committee (''RAC'')) in order to solicit and consider public comment from interested parties on the scientific, medical, legal, and ethical issues arising from stem cell research. Members of the HPSCRG will serve as a resource for recommending to the NIH any need for Human Pluripotent Stem Cell Policy Conferences.

Other Changes

Because compliance materials may be made public prior to funding decisions, we have added a sentence requiring the principal investigator's written consent to the disclosure of such material necessary to carry out public review and other oversight procedures.

The draft Guidelines required HPSCRG review of proposals from investigators planning to derive hPSCs from fetal tissue. Because the Guidelines address proposals for NIH funding for the use of hPSCs, this requirement has been removed from the Guidelines.

The text of the final Guidelines follows.

National Institutes of Health Guidelines for Research Using Human Pluripotent Stem Cells

I. Scope of Guidelines

These Guidelines apply to the expenditure of National Institutes of Health (NIH) funds for research using human pluripotent stem cells derived from human embryos (technically known as human embryonic stem cells) or human fetal tissue (technically known as human embryonic germ cells). For purposes of these Guidelines, ''human pluripotent stem cells'' are cells that are self-replicating, are derived from human embryos or human fetal tissue, and are known to develop into cells and tissues of the three primary germ layers. Although human pluripotent stem cells may be derived from embryos or fetal tissue, such stem cells are not themselves embryos. NIH research funded under these Guidelines will involve human pluripotent stem cells derived: (1) From human fetal tissue; or (2) from human embryos that are the result of *in vitro* fertilization, are in excess of clinical need, and have not reached the stage at which the mesoderm is formed.

In accordance with 42 Code of Federal Regulations (CFR) 52.4, these Guidelines prescribe the documentation and assurances that must accompany requests for NIH funding for research using human pluripotent stem cells from: (1) Awardees who want to use existing funds; (2) awardees requesting an administrative or competing supplement; and (3) applicants or intramural researchers submitting applications or proposals. NIH funds may be used to derive human pluripotent stem cells from fetal tissue. NIH funds may not be used to derive human pluripotent stem cells from human embryos. These Guidelines also designate certain areas of human pluripotent stem cell research as ineligible for NIH funding.

II. Guidelines for Research Using Human Pluripotent Stem Cells That Is Eligible for NIH Funding

A. Utilization of Human Pluripotent Stem Cells Derived From Human Embryos

1. Submission to NIH

Intramural or extramural investigators who are intending to use existing funds, are requesting an administrative supplement, or are applying for new NIH funding for research using human pluripotent stem cells derived from human embryos must submit to NIH the following:

a. An assurance signed by the responsible institutional official that the pluripotent stem cells were derived from human embryos in accordance with the conditions set forth in section II.A.2 of these Guidelines and that the institution will maintain documentation in support of the assurance;

b. A sample informed consent document (with patient identifier information removed) and a description of the informed consent process that meet the criteria for informed consent set forth in section II.A.2.e of these Guidelines;

c. An abstract of the scientific protocol used to derive human pluripotent stem cells from an embryo;

d. Documentation of Institutional Review Board (IRB) approval of the derivation protocol;

e. An assurance that the stem cells to be used in the research were or will be obtained through a donation or through a payment that does not exceed the reasonable costs associated with the transportation, processing, preservation, quality control and storage of the stem cells;

f. The title of the research proposal or specific subproject that proposes the use of human pluripotent stem cells;

g. An assurance that the proposed research using human pluripotent stem cells is not a class of research that is ineligible for NIH funding as set forth in section III of these Guidelines; and

h. The Principal Investigator's written consent to the disclosure of all material submitted under Paragraph A.1 of this section, as necessary to carry out the public review and other oversight procedures set forth in section IV of these Guidelines.

2. Conditions for the Utilization of Human Pluripotent Stem Cells Derived From Human Embryos

Studies utilizing pluripotent stem cells derived from human embryos may be conducted using NIH funds only if the cells were derived (without Federal funds) from human embryos that were created for the purposes of fertility treatment and were in excess of the clinical need of the individuals seeking such treatment.

a. To ensure that the donation of human embryos in excess of the clinical need is voluntary, no inducements, monetary or otherwise, should have been offered for the donation of human embryos for research purposes. Fertility clinics and/or their affiliated laboratories should have implemented specific written policies and practices to ensure that no such inducements are made available.

b. There should have been a clear separation between the decision to create embryos for fertility treatment and the decision to donate human embryos in excess of clinical need for research purposes to derive pluripotent stem cells. Decisions related to the creation of embryos for fertility treatment should have been made free

from the influence of researchers or investigators proposing to derive or utilize human pluripotent stem cells in research. To this end, the attending physician responsible for the fertility treatment and the researcher or investigator deriving and/or proposing to utilize human pluripotent stem cells should not have been one and the same person.

c. To ensure that human embryos donated for research were in excess of the clinical need of the individuals seeking fertility treatment and to allow potential donors time between the creation of the embryos for fertility treatment and the decision to donate for research purposes, only frozen human embryos should have been used to derive human pluripotent stem cells. In addition, individuals undergoing fertility treatment should have been approached about consent for donation of human embryos to derive pluripotent stem cells only at the time of deciding the disposition of embryos in excess of the clinical need.

d. Donation of human embryos should have been made without any restriction or direction regarding the individual(s) who may be the recipients of transplantation of the cells derived from the human pluripotent stem cells.

e. Informed Consent

Informed consent should have been obtained from individuals who have sought fertility treatment and who elect to donate human embryos in excess of clinical need for human pluripotent stem cell research purposes. The informed consent process should have included discussion of the following information with potential donors, pertinent to making the decision whether or not to donate their embryos for research purposes.

Informed consent should have included:

(i) A statement that the embryos will be used to derive human pluripotent stem cells for research that may include human transplantation research;

(ii) A statement that the donation is made without any restriction or direction regarding the individual(s) who may be the recipient(s) of transplantation of the cells derived from the embryo;

(iii) A statement as to whether or not information that could identify the donors of the embryos, directly or through identifiers linked to the donors, will be removed prior to the derivation or the use of human pluripotent stem cells;

(iv) A statement that derived cells and/or cell lines may be kept for many years;

(v) Disclosure of the possibility that the results of research on the human pluripotent stem cells may have commercial potential, and a statement that the donor will not receive financial or any other benefits from any such future commercial development;

(vi) A statement that the research is not intended to provide direct medical benefit to the donor; and

(vii) A statement that embryos donated will not be transferred to a woman's uterus and will not survive the human pluripotent stem cell derivation process.

f. Derivation protocols should have been approved by an IRB established in accord with 45 CFR 46.107 and 46.108 or FDA regulations at 21 CFR 56.107 and 56.108.

B. Utilization of Human Pluripotent Stem Cells Derived From Human Fetal Tissue

1. Submission to NIH

Intramural or extramural investigators who are intending to use existing funds, are requesting an administrative supplement, or are applying for new NIH funding for research using human pluripotent stem cells derived from fetal tissue must submit to NIH the following:

a. An assurance signed by the responsible institutional official that the pluripotent stem cells were derived from human fetal tissue in accordance with the conditions set forth in section II.A.2 of these Guidelines and that the institution will maintain documentation in support of the assurance;

b. A sample informed consent document (with patient identifier information removed) and a description of the informed consent process that meet the criteria for informed consent set forth in section II.B.2.b of these Guidelines;

c. An abstract of the scientific protocol used to derive human pluripotent stem cells from fetal tissue;

d. Documentation of IRB approval of the derivation protocol;

e. An assurance that the stem cells to be used in the research were or will be obtained through a donation or through a payment that does not exceed the reasonable costs associated with the transportation, processing, preservation, quality control and storage of the stem cells;

f. The title of the research proposal or specific subproject that proposes the use of human pluripotent stem cells;

g. An assurance that the proposed research using human pluripotent stem cells is not a class of research that is ineligible for NIH funding as set forth in section III of these Guidelines; and

h. The Principal Investigator's written consent to the disclosure of all material submitted under Paragraph B.1 of this section, as necessary to carry out the public review and other oversight procedures set forth in section IV of these Guidelines.

2. Conditions for the Utilization of Human Pluripotent Stem Cells Derived From Fetal Tissue.

a. Unlike pluripotent stem cells derived from human embryos, DHHS funds may be used to support research to derive pluripotent stem cells from fetal tissue, as well as for research utilizing such cells. Such research is governed by Federal statutory restrictions regarding fetal tissue research at 42 U.S.C. 289g–2(a) and the Federal regulations at 45 CFR 46.210. In addition, because cells derived from fetal tissue at the early stages of investigation may, at a later date, be used in human fetal tissue transplantation research, it is the policy of NIH to require that all NIH-funded research involving the derivation or utilization of pluripotent stem cells from human fetal tissue also comply with the fetal tissue transplantation research statute at 42 U.S.C. 289g–1.

b. Informed Consent

As a policy matter, NIH-funded research deriving or utilizing human pluripotent stem cells from fetal tissue should comply with the informed consent law applicable to fetal tissue transplantation research (42 U.S.C. 289g–1) and the following conditions. The informed consent process should have included discussion of the following information with potential donors, pertinent to making the decision whether to donate fetal tissue for research purposes.

Informed consent should have included:

(i) A statement that fetal tissue will be used to derive human pluripotent stem cells for research that may include human transplantation research;

(ii) A statement that the donation is made without any restriction or direction regarding the individual(s) who may be the recipient(s) of transplantation of the cells derived from the fetal tissue;

(iii) A statement as to whether or not information that could identify the donors of the fetal tissue, directly or through identifiers linked to the donors, will be removed prior to the derivation or the use of human pluripotent stem cells;

(iv) A statement that derived cells and/or cell lines may be kept for many years;

(v) Disclosure of the possibility that the results of research on the human pluripotent stem cells may have commercial potential, and a statement that the donor will not receive financial or any other benefits from any such future commercial development; and

(vi) A statement that the research is not intended to provide direct medical benefit to the donor.

c. Derivation protocols should have been approved by an IRB established in accord with 45 CFR 46.107 and 46.108 or FDA regulations at 21 CFR 56.107 and 56.108.

III. Areas of Research Involving Human Pluripotent Stem Cells That Are Ineligible for NIH Funding

Areas of research ineligible for NIH funding include:

A. The derivation of pluripotent stem cells from human embryos;

B. Research in which human pluripotent stem cells are utilized to create or contribute to a human embryo;

C. Research utilizing pluripotent stem cells that were derived from human embryos created for research purposes, rather than for fertility treatment;

D. Research in which human pluripotent stem cells are derived using somatic cell nuclear transfer, *i.e.,* the transfer of a human somatic cell nucleus into a human or animal egg;

E. Research utilizing human pluripotent stem cells that were derived using somatic cell nuclear transfer, *i.e.,* the transfer of a human somatic cell nucleus into a human or animal egg;

F. Research in which human pluripotent stem cells are combined with an animal embryo; and

G. Research in which human pluripotent stem cells are used in combination with somatic cell nuclear transfer for the purposes of reproductive cloning of a human.

IV. Oversight

A. The NIH Human Pluripotent Stem Cell Review Group (HPSCRG) will review documentation of compliance with the Guidelines for funding requests that propose the use of human pluripotent stem cells. This working group will hold public meetings when a funding request proposes the use of a line of human pluripotent stem cells that has not been previously reviewed and approved by the HPSCRG.

B. In the case of new or competing continuation (renewal) or competing supplement applications, all applications shall be reviewed by HPSCRG and for scientific merit by a Scientific Review Group. In the case of requests to use existing funds or applications for an administrative supplement or in the case of intramural proposals, Institute or Center staff should forward material to the HPSCRG for review and determination of compliance with the Guidelines prior to allowing the research to proceed.

C. The NIH will compile a yearly report that will include the number of applications and proposals reviewed and the titles of all awarded applications, supplements or administrative approvals for the use of existing funds, and intramural projects.

D. Members of the HPSCRG will also serve as a resource for recommendations to the NIH with regard to any revisions to the NIH Guidelines for Research Using Human Pluripotent Stem Cells and any need for human pluripotent stem cell policy conferences.

Dated: August 17, 2000.

Ruth L. Kirschstein,

Principal Deputy Director, NIH.

[FR Doc. 00–21760 Filed 8–23–00; 8:45 am]

BILLING CODE 4140–01–P

DEPARTMENT OF HEALTH AND HUMAN SERVICES

National Institutes of Health

Notification of Request for Emergency Clearance; Modification of OMB No. 0925–0001/Exp. 2/01, ''PHS 398 Research and Research Training Grant Applications and Related Forms''

SUMMARY: In accordance with section 3507(j) of the Paperwork Reduction Act of 1995, the National Institutes of Health (NIH) hereby publishes notification of a request for Emergency Clearance for modification of the information collection related to the National Institutes of Health Guidelines for Research Using Human Pluripotent Stem Cells, published elsewhere in today's **Federal Register**. The currently approved information collection OMB No. 0925–0001 permits the NIH to request from applicant institutions information related to application, award, and continued compliance with the terms of Federal assistance for research and research-related training. The approval also covers the information collection authorized in accordance with 42 CFR 52, specifically the obtaining of ''[o]ther pertinent information the Secretary may require to evaluate the proposed project.'' (42 CFR 52.4(f))

The final National Institutes of Health Guidelines for Research Using Pluripotent Stem Cells requires submission of additional documentation in the form of additional institutional records from a limited number of institutions to enable an independent panel of non-Government experts to ascertain institutional compliance with the Guidelines. Compliance with the requirements of existing law and regulations is authorized under OMB No. 0925–0418, Exp. 1/01, ''Protection of Human Subjects: Assurance Identification/Certification/ Declaration.''

The present modification relates to the added reporting requirement of submission of documentation to permit the agency to exercise the oversight responsibility established under the Guidelines.

This modification is essential to the mission of NIH (42 USC 241 and 282(b)) and is of the highest scientific priority as determined by both internal review and external review by a panel of scientific and other experts in the field of stem cell research. After extensive consultation with the public and a public meeting, the NIH published proposed National Institutes of Health Guidelines for Research Using Human Pluripotent Stem Cells in the **Federal Register** on December 2 , 1999 (**Federal Register**, Vol. 64, No. 231, pages 67576–67579). The comment period was extended to February 22, 2000. (**Federal Register**, February 3, 2000, Vol. 65, No. 23, page 539). Following the period of comment, NIH has proceeded to finalize the Guidelines, which are published elsewhere in this issue of the **Federal Register**.

These Guidelines are essential to ensure that NIH-funded research in this area is conducted in an ethical and legal manner. The NIH has determined that the oversight process stipulated in the Guidelines will achieve this objective. The Guidelines will require that institutions requesting or using NIH funds for research using human pluripotent stem cells submit additional documentation to the NIH in the form of institutional records that will permit NIH oversight in accordance with the Guidelines.

NIH has taken all practicable steps to consult with the scientific community and the public, through the process described above and through the careful consideration of all comments received from the public.

In view of the extensive period of comment and the thorough consideration of all views, both prior to the publication of the proposed Guidelines in December 1999 and subsequently, NIH is herewith requesting that OMB approve the modification of the collection of information simultaneously with the publication of the **Federal Register**

DEPARTMENT OF HEALTH & HUMAN SERVICES

Office of the Secretary

The General Counsel
Washington, D.C. 20201

January 11, 2002

MEMORANDUM

TO: Dr. Ruth Kirchstein
Acting Director, National Institutes of Health

FROM: Alex M. Azar II
General Counsel

SUBJECT: Compliance of the President's Embryonic Stem Cell Decision with the Dickey Amendment for Fiscal Year 2002

The National Institutes of Health plan soon to initiate federal funding of research on existing human embryonic stems cells in accordance with the policy announced by the President on August 9, 2001. Prior to the initiation of such funding, you have asked the Office of the General Counsel to provide advice on the legality of the President's policy under the Dickey Amendment to Public Law Number 107-116 (signed Jan. 10, 2002), the appropriations act funding the Department of Health & Human Services (the "Department") for fiscal year 2002.

It is our conclusion that the President's policy comports with the plain language of the Dickey Amendment. This reading is further buttressed by Congress's recent reenactment of the Dickey Amendment and, hence, ratification of the President's policy and by the legislative history accompanying the most recent reenactment of the Dickey Amendment.

The President's Policy

On August 9, 2001 at 9:00 p.m. EDT, President George W. Bush announced his decision to allow federal funds to be used for research on existing human embryonic stem cell lines as long as, prior to his announcement, (1) the derivation process (which commences with the removal of the inner cell mass from the blastocyst) had already been initiated, and (2) the embryo from which the stem cell line was derived no longer had the possibility of development as a human being.

As the President noted, "the life and death decision ha[d] already been made" with respect to those "existing human embryonic stem cell lines." This decision, as the President stated, "allows

188563

us to explore the promise and potential of stem cell research without crossing a fundamental moral line, by providing taxpayer funding that would sanction or encourage further destruction of human embryos that have at least the potential for life." Remarks by the President on Stem Cell Research, Aug. 9, 2001, http://www.whitehouse.gov/news/releases/2001/08/print/20010809-2.html.

The President established the following additional criteria that had to be met for embryonic stem cell research to receive federal funding: (1) the stem cells must have been derived from an embryo that was created for reproductive purposes; (2) the embryo was no longer needed for such purposes; (3) informed consent must have been obtained for the donation of the embryo; and (4) no financial inducements were provided for donation of the embryo. Notice of Criteria for Federal Funding of Research on Existing Human Embryonic Stem Cells and Establishment of NIH Human Embryonic Stem Cell Registry, Nov. 7, 2001, NOT-OD-02-005, Office of the Director, NIH, http://grants.nih.gov/grants/guide/notice-files/NOT-OD-02-005.html; NIH Human Embryonic Stem Cell Registry, http://escr.nih.gov. Pursuant to the President's policy, federal funds will not be used for (1) the derivation or use of stem cell lines derived from newly destroyed embryos; (2) the creation of any human embryos for research purposes; or (3) the cloning of human embryos for any purpose. Fact Sheet, Embryonic Stem Cell Research, Aug. 9, 2001, http://www.whitehouse.gov/news/release/2001/08/print/20010809-1.html.

Pursuant to the President's policy, on August 27, 2001, Secretary Thompson announced the creation of a registry of the embryonic stem cell lines meeting the President's eligibility criteria, such that research on stem cell lines listed on the Registry would be eligible for federal funding. He stated that:

> [t]he NIH wants to expedite this work and is aggressively pursuing several initiatives to facilitate research on all forms of stem cells. The NIH is creating a registry of the embryonic stem cell lines that meet the eligibility criteria so that researchers can contact the owners and gain access to them. The registry will contain basic information about the cells, a unique identifier, the name of the company or laboratory that derived the cells, and contact information about that company or lab. The registry will list these 10 laboratories as well as any other owners of stem cell lines meeting the eligibility criteria who come forward in the future.

Statement by Tommy G. Thompson, Secretary of Health & Human Services, Aug. 27, 2001, http://www.hhs.gov/new/press/2001pres/20010827a.html; *see also* Tommy G. Thompson, Secretary of Health & Human Services, Testimony before the Senate Committee on Health, Education, Labor & Pensions, Sept. 5, 2001, at 4 (discussing NIH's development of "a stem cell registry" and the intent to "mak[e] it available so scientists know exactly what lines are eligible and who they can approach for access" and to post the registry on the NIH website),

http://www.hhs.gov/news/speech/2001/010905.html.

In an NIH Update, the NIH noted that the laboratories or companies that derived the cells listed on the registry that it was creating would provide "a signed assurance that the derivation process was initiated prior to 9:00 p.m. EDT on August 9, 2001, informed consent was obtained for the donation of the embryo, the cells were derived from an excess embryo that was created for reproductive purposes, and there were no financial inducements for the donation of the embryo for research." NIH Update on Existing Human Embryonic Stem Cells, Aug. 27, 2001, at 2-3, http://www.nih.gov/news/stemcell/082701list.html. Shortly thereafter, the NIH entered into a memorandum of understanding with one of the entities that possesses such embryonic stem cell lines, to permit access to those lines by NIH scientists to conduct research and to permit scientists pursuing research funded by the NIH to negotiate access to those lines under the same terms and conditions. *See* NIH Press Release, National Institutes of Health and WiCell Research Institute, Inc. Sign Stem Cell Research Agreement, Sept. 5, 2001, http://www.nih.gov/news/pr/sep2001/od-05.html; Memorandum of Understanding between WiCell Research Institute, Inc. and Public Health Service, US Department of Health & Human Services, effective as of Sept. 5, 2001, http://www.nih.gov/news/stemcell/WicellMOU.pdf; *see also* Tommy G. Thompson, Secretary of Health & Human Services, Testimony before the Senate Committee on Health, Education, Labor & Pensions, Sept. 5, 2001, at 4 (announcing negotiation of the memorandum of understanding permitting research use of WiCell's "five existing stem cell lines that meet the eligibility criteria"), http://www.hhs.gov/news/speech/2001/010905.html.

On November 7, 2001, the NIH posted the Registry of embryonic stem cell lines that comply with the President's policy as announced on August 9, 2001. *See* NIH Human Embryonic Stem Cell Registry, http://escr.nih.gov; Notice of Criteria for Federal Funding of Research on Existing Human Embryonic Stem Cells and Establishment of NIH Human Embryonic Stem Cell Registry, Nov. 7, 2001, NOT-OD-02-005, Office of the Director, NIH, http://grants.nih.gov/grants/guide/notice-files/NOT-OD-02-005.html.

The Dickey Amendment

In construing the meaning of a statute, the starting point of the analysis is the language of the statute. *See, e.g., Central Bank of Denver NA v. First Interstate Bank of Denver NA*, 511 U.S. 164, 173 (1994) (the statutory language is "'the starting point in every case involving construction of a statute'"); *Good Samaritan Hosp. v. Shalala*, 508 U.S. 402, 409 (1993) ("The starting point in interpreting a statute is its language, for '[i]f the intent of Congress is clear, that is the end of the matter.'"); *Ernst & Ernst v. Hochfelder*, 425 U.S. 185, 197 (1976) ("'The starting point in every case involving construction of a statute is the language itself.'"); *Kaiser Aluminum & Chem. Corp. v. Bonjorno*, 494 U.S. 827, 834-44 (1990) (same); *Meredith v. Federal Mine Safety & Health Review Comm'n*, 177 F.3d 1042, 1053 (D.C. Cir. 1999) ("As always, the

starting point of analysis is the text of the statute.").

Since 1995, the Dickey Amendment has been enacted in each of the annual appropriations acts for the Department. For fiscal year 2002, the Amendment provides:

> (a) None of the funds made available in this Act may be used for–
>
> (1) the creation of a human embryo or embryos for research purposes; or
>
> (2) research in which a human embryo or embryos are destroyed, discarded, or knowingly subjected to risk of injury or death greater than that allowed for research on fetuses in utero under 45 CFR 46.208(a)(2) and section 498(b) of the Public Health Service Act (42 U.S.C. 289g(b)).
>
> (b) For purposes of this section, the term 'human embryo or embryos' includes any organism, not protected as a human subject under 45 CFR 46 as of the date of the enactment of this Act, that is derived by fertilization, parthenogenesis, cloning, or any other means from one or more human gametes or human diploid cells.

Pub. L. No. 107-116, § 510. This language is unchanged from the fiscal year 2001 Dickey Amendment.

The President's policy is consistent with the plain language of the Dickey Amendment. The Dickey Amendment contains two basic restrictions. The first prohibits the use of federal funds for "the creation of a human embryo or embryos for research purposes." *See* Pub. L. No. 107-116, § 510(a)(1). It is clear that, under the President's policy, no federal funds will be used for the creation of human embryos for research purposes. *See* Fact Sheet, Embryonic Stem Cell Research, Aug. 9, 2001, http://www.whitehouse.gov/news/release/2001/08/print/20010809-1.html (federal funds will not be used for "creation of any human embryos for research purposes"). Thus, the President's policy comports with the first restriction contained in the Dickey Amendment.

The second restriction of the Dickey Amendment prohibits the use of federal funds for *"research in which* a human embryo or embryos are destroyed, discarded, or knowingly subjected to risk of injury or death greater than that allowed for research on fetuses in utero . . . ." H.R. 3061, § 510(a)(2) (emphasis added). The term "research in which" is not defined in the statute, and our research has not located any cases in which such a term is defined. As such, it is appropriate to look to ordinary and common usage when interpreting those terms. *See FDIC v. Meyer*, 510 U.S. 471, 476 (1994) ("In the absence of such a definition [in the act], we construe a statutory term in accordance with its ordinary or natural meaning."). The word "which," when "[u]sed as a relative pronoun preceded by *that* or a preposition in a clause that defines or restricts the antecedent" means "[t]he thing, animal, group of people, or event previously designated or implied, specifically." *See* The American Heritage Dictionary, New College Edition 1459

(1976). Dictionaries define "in" as meaning "within the confines of; inside"; "within the area covered by"; "during the course of or before the expiration of"; "during or part of the act or process of"; "within the category or class of." *See id.* at 663; *see also* Black's Law Dictionary 683 (5th ed. 1979) (a preposition "expressing relation of presence, existence, situation, inclusion, action, etc.; inclosed or surrounded by limits . . .; also meaning for, in and about, on, within etc.; and is synonymous with expressions 'in regard to', 'respecting', 'with respect to', and 'as is'"). Under the President's policy, federal funding for human embryonic stem cell research is limited to a discrete set of stem cell lines with respect to which the life and death decision had been made prior to the announcement of his policy. The President's policy provides no incentives for the destruction of additional embryos. Moreover, these derivation processes were not funded with federal dollars. So limited, the President's policy does not provide federal funding for "research in which [during the course of, during or part of the act or process of, or within the category or class of] embryos are destroyed, discarded, or knowingly subjected to risk of injury or death greater than that allowed for research on fetuses in utero" within the ordinary, common usage of those terms. The policy is, thus, consistent with the second restriction of the Dickey Amendment.

Congressional Ratification of the Legality of the President's Policy

This plain meaning reading of the Dickey Amendment is bolstered by Congress's reenactment of the Dickey Amendment in identical form after the President's announcement on August 9, 2001. As discussed below, Congress was fully aware of the President's policy decision and the Secretary's steps in implementing that decision. With that knowledge, Congress reenacted the Dickey Amendment in identical form, clearly evidencing its concurrence that the President's policy is consistent with the Dickey Amendment. *See Lorillard v. Pons*, 434 U.S. 575, 580-81 (1978) ("Congress is presumed to be aware of an administrative or judicial interpretation of a statute and to adopt that interpretation when it re-enacts a statute without change."); *Central Bank of Denver*, 511 U.S. at 185-86 ("When Congress reenacts statutory language that has been given a consistent judicial construction, we often adhere to that construction in interpreting the reenacted statutory language."); *Pierce v. Underwood*, 487 U.S. 552, 567 (1988) (same); *City of Pleasant Grove v. United States*, 479 U.S. 462, 468 (1987) ("Congress was aware of the Attorney General's view . . . and implicitly approved it, when it reenacted the Voting Rights Act . . . ."); *San Huan New Materials High Tech, Inc. v. International Trade Comm'n*, 161 F.3d 1347, 1355 (Fed. Cir. 1998) ("The legislative history shows that Congress was fully aware of the agency regulations and practices [regarding consent decrees] at the time of legislating in their area, and absent some special circumstances the failure to change or refer to existing practices is reasonably viewed as ratification thereof.") .

Legislative History of the Dickey Amendment Contained in Pub. L. No. 107-116

The legislative history of the current reenactment of the Dickey Amendment in the appropriations act providing funding for Department for fiscal year 2002 further confirms that Congress understood the contours of the President's policy and believed that the policy complies with the requirements of the Dickey Amendment.

The Committee Report on H.R. 3061, the House version of the Act, published exactly two months after the President's announcement states:

> *Human Stem Cell Research*- The Committee received testimony from NIH institute and center directors, representatives of scientific and medical societies, and members of voluntary health organizations about the potential of both adult and embryonic stem cells for improving the lives of those who suffer with a host of disorders, including diabetes, Alzheimer's, Parkinson's, and cardiovascular disease. The Committee understands that a great deal of basic research is required to determine whether this potential can be realized.
>
> *It is the Committee's intent, that the NIH move ahead expeditiously to implement the President's policy concerning support of scientifically meritorious research involving both adult and human embryonic stem cells.* The Committee commends the NIH for moving quickly to negotiate material transfer agreements *with holders of existing embryonoc [sic] cell lines*. The Director is requested to keep the Committee apprised of program initiatives as well as research progress concerning both adult and embryonic stem cells.

H.R. Rep. 107-229, at 98 (Oct. 9, 2001) (emphases added). In addition, the Committee noted in connection with section 510, the Dickey Amendment, the following:

> Sec. 510. The Committee continues a provision to prohibit the use of funds in the Act concerning research involving human embryos. However, *this language should not be construed to limit federal support for research involving human embryonic stem cells listed on an NIH registry and carried out in accordance with policy outlined by the President.*

H.R. Rep. 107-229, at 180 (Oct. 9, 2001) (emphasis added). The Joint Explanatory Statement of the Committee of Conference directed that "in implementing this agreement [on appropriations], the Departments and agencies should comply with the language and instructions set forth in House Report 107-229 and Senate Report 107-84." *See* Joint Explanatory Statement of the Committee of Conference, H.R. Rep. 107-342, Conference Report on H.R. 3061, at 55 (Dec. 19, 2001). Thus, it would be appropriate to accord to H.R. Rep. 107-229 the weight customarily

given to conference committee explanatory statements. *See Northern Colorado Water Conservancy Dist. v. Federal Energy Regulatory Comm'n*, 730 F.2d 1509, 1518-19 (D.C. Cir. 1984) ("Statements in a conference report, because commended to the entire Congress, carry greater weight than comments from floor debates by individual legislators."); *Vitrano v. Marshall*, 504 F. Supp. 1381, 1383 (D.D.C. 1981) ("Perhaps the most useful document illuminating Congressional purpose is a Conference Report which bears on the final draft that is used by the conferees in explaining to the entire Congress why the bill should pass.").

As a whole, this legislative history expresses the Congress's support for the President's policy and unambiguously confirms that the President's decision is consistent with the Dickey Amendment. *See Thunder Basin Coal Co. v. Reich*, 510 U.S. 200, 209 (1994) ("The legislative history of the Mine Act confirms this interpretation."); *see also San Huan New Materials*, 161 F.3d at 1355 ("The legislative history leaves no doubt that Congress was aware of, and approved of, the Commission's consent order procedure as it existed at the time of the 1988 amendments.").

In sum, whatever legal challenges might be brought, the President's policy is consistent with the Dickey Amendment as evidenced by the plain language of the statute, Congress's reenactment ratification of the President's policy, and the legislative history reflecting Congress's full understanding of the precise contours of the President's policy and that policy's compliance with the Dickey Amendment.

As we move forward with implementation of the President's decision, it should be noted that federal funding of research in the following areas remains barred: (1) the derivation of new stem cells from human embryos; (2) research in which human embryonic stem cells are used to create or contribute to a human embryo; (3) research in which human embryonic stem cells are derived using somatic cell nuclear transfer, i.e., the transfer of a human somatic cell nucleus into a human or animal egg; (4) research using human embryonic stem cells that were derived using somatic cell nuclear transfer, i.e., the transfer of a human somatic cell nucleus into a human or animal egg; (5) research in which human embryonic stem cells are combined with an animal embryo; and (6) research in which human embryonic stem cells are used in combination with somatic cell nuclear transfer for the purposes of reproductive cloning of a human. *See* National Institutes of Health Guidelines for Research Using Human Pluripotent Stem Cells, Part III ("Areas of Research Involving Human Pluripotent Stem Cells that are Ineligible for NIH Funding", listing the above categories of research), 65 FR 51976 (effective Aug. 25, 2000), corrected, 65 FR 69951 (Nov. 21, 2000), www.nih.gov/news/stemcell/stemcellguidelines.html, withdrawn as to those sections pertaining to research involving human pluripotent stem cells derived from human embryos that are the result of in vitro fertilization, are in excess of clinical need, and have not reached the stage at which the mesoderm is formed, Notice of Withdrawal of NIH Guidelines for Research Using Pluripotent Stem Cells, Nov. 7, 2001, NOT-OD-02-007, Office of the Director,

DEPARTMENT OF HEALTH & HUMAN SERVICES

Office of the Secretary

The General Counsel
Washington, D.C. 20201

January 15, 1999

TO: Harold Varmus, M.D.
Director, NIH

FROM: Harriet S. Rabb

SUBJECT: Federal Funding for Research Involving Human Pluripotent Stem Cells

The Office of the General Counsel of the U.S. Department of Health and Human Services (HHS) has prepared the following in response to your request for a legal opinion on whether federal funds may be used for research conducted with human pluripotent stem cells derived from embryos created by *in vitro* fertilization or from primordial germ cells isolated from the tissue of non-living fetuses. This inquiry arises from the recently reported research of: (1) Dr. James A. Thomson of the University of Wisconsin-Madison, who isolated pluripotent stem cells from embryos donated for research by persons undergoing fertility treatment[1]; and (2) Dr. Michael Shamblott of the Johns Hopkins University School of Medicine, who derived pluripotent stem cells from primordial germ cells from non-living fetuses.[2] The research described in these two published reports was not funded by HHS.

Summary Answer

The statutory prohibition on the use of funds appropriated to HHS for human embryo research would not apply to research utilizing human pluripotent stem cells because such cells are not a human embryo within the statutory definition. To the extent human pluripotent stem cells are considered human fetal tissue by law, they are subject to the statutory prohibition on sale for valuable consideration, the restrictions on fetal tissue transplantation research that is conducted or funded by HHS, as well as to the federal criminal prohibition on the directed donation of fetal

[1] James A. Thomson et al., Embryonic Stem Cell Lines Derived from Human Blastocysts, Science, vol. 282, November 6, 1998, pp. 1145-1147.

[2] Michael J. Shamblott et al., Derivation of Pluripotent Stem Cells from Cultured Human Primordial Germ Cells, 95 Proc. Nat'l. Acad. Sci. USA 13726 (Nov. 1998).

tissue. Research involving human pluripotent stem cells excised from a non-living fetus may be conducted only in accordance with any applicable state or local law. Finally, the Presidential Directive banning federal funding of human cloning would apply to pluripotent stem cells, only if they were to be used for that purpose.

Analysis

I. Prohibition on Federal Funding for Human Embryo Research

In the appropriations provision for the Departments of Labor, Health and Human Services, and Education, and Related Agencies in the Omnibus Consolidated and Emergency Supplemental Appropriations Act, Fiscal Year 1999, Public Law 105-277, section 511 provides that none of the funds made available in that appropriation may be used for:

> (1) the creation of a human embryo or embryos for research purposes; or
> (2) research in which a human embryo or embryos are destroyed, discarded or knowingly subjected to risk of injury or death greater than that allowed for research on fetuses in utero under 45 CFR 46.208(a)(2) and section 498(b) of the Public Health Service Act (42 U.S.C. 289g (b)).

The term "human embryo or embryos" is defined in the statute to include "any organism, not protected as a human subject under 45 CFR 46 as of the date of the enactment of this Act, that is derived by fertilization, parthenogenesis, cloning, or any other means from one or more human gametes or human diploid cells."

Pluripotent stem cells are not a human "organism" as that term is used in the definition of human embryo provided by statute. The term "organism" is not itself defined by law, and the question of what is an organism calls for a science-based answer. According to the McGraw-Hill Dictionary of Scientific and Technical Terms (hereinafter McGraw-Hill), an organism is "[a]n individual constituted to carry out all life functions."[3] Pluripotent stem cells are not organisms

---

[3] McGraw-Hill Dictionary of Scientific and Technical Terms 1408 (5th edition 1994). See also N. Campbell, Biology, (4th edition 1996) pp. 8-9, which defines organism as follows:

> While cells are the units of organisms, it is organisms that are the units of life. It's an important distinction. Except for unicellular life, 'cell' does not equal 'organism.' A single-celled organism such as an amoeba is analogous not to one of your cells, but to your whole body. What the amoeba accomplishes with a single cell -- the uptake and processing of nutrients, excretion of wastes, response to environmental stimuli, reproduction, and other functions -- a human or other multicellular organism accomplishes with a division of labor among specialized tissues, organs, and organ systems. Unlike the amoeba, none of your cells could live for long on its own. The organism we recognize as an animal or plant is not a

and do not have the capacity to develop into an organism that could perform all the life functions of a human being -- in this sense they are not even precursors to human organisms.[4] They are, rather, human cells that have the potential to evolve into different types of cells such as blood cells or insulin producing cells.

Moreover, a human embryo, as that term is virtually universally understood, has the potential to develop in the normal course of events into a living human being. The scientific definition of embryo, as described in McGraw-Hill, is "[t]he product of conception up to the third month of human pregnancy."[5] Pluripotent stem cells do not have the capacity to develop into a human being, even if transferred to a uterus.[6] Therefore, in addition to falling outside of the legal definition provided by statute, pluripotent stem cells cannot be considered human embryos consistent with the commonly accepted or scientific understanding of that term. Thus, based on

---

collection of unicells, but a multicellular cooperative with the emergent properties of 'whole organism.'

[4] At a December 2, 1998, stem cell research hearing before the Subcommittee on Labor, Health and Human Services, Education and Related Agencies of the Senate Appropriations Committee, Senator Tom Harkin asked five scientists, two bioethicists, and a theologian testifying before the committee if, in their view, stem cells were organisms. All of the experts who responded concluded that human pluripotent stem cells are not organisms. Use of Fetal Tissue in Brain Stem Cell Research: Hearing Before the Subcomm. on Labor, Health and Human Services, and Education of the Senate Appropriations Comm., 105th Cong. (December 2, 1998) available in LEGI-SLATE, Transcript No. 983360015 [hereinafter Stem Cell Hearing] (statement of Dr. Harold Varmus, Director, National Institutes of Health; Dr. John Gearhart, Johns Hopkins University School of Medicine; Dr. James Thomson, Wisconsin Primate Research Center, University of Wisconsin; Dr. Michael West, Advanced Cell Technology; Dr. Thomas Okarma, Geron Corporation; Dr. Arthur Caplan, Center for Bioethics, University of Pennsylvania Health System; and Mr. Richard Doerflinger, Associate Director for Policy Development, Secretariat of Pro-Life Activities, National Conference of Catholic Bishops). One expert, Dr. Eric Meslin, Executive Director of the National Bioethics Advisory Commission, stated that he could not speak on behalf of the Commission because it had not considered the question. Stem Cell Hearing, supra, (statement of Dr. Eric Meslin).

[5] McGraw-Hill Dictionary, supra note 3, at 673.

[6] See Letter from the Chair of the National Bioethics Advisory Commission, to the President of the United States, response to question no. 2, November 20, 1998; National Institutes of Health, Report of the Human Embryo Research Panel, Sept. 1994, p. 26. See also Stem Cell Hearing, supra note 4, (statements of Dr. Michael West, Advanced Cell Technology; Dr. Thomas Okarma, Geron Corporation; and Dr. Arthur Caplan, Center for Bioethics, University of Pennsylvania Health System).

an analysis of the relevant law and scientific facts, federally funded research that utilizes human pluripotent stem cells would not be prohibited by the HHS appropriations law prohibiting human embryo research, because such stem cells are not human embryos.

II. Restrictions on the Use of Human Fetal Tissue

There are a number of potential sources of human pluripotent stem cells; some of these stem cells may fall within the legal definition of human fetal tissue and would, therefore, be subject to federal regulations. Section 498A of the Public Health Service Act specifies that fetal tissue "means tissue or cells obtained from a dead human embryo or fetus after a spontaneous or induced abortion, or after a stillbirth." 42 U.S.C. 289g-1(g). Some stem cells, for example those derived from the primordial germ cells of non-living fetuses, would be considered human fetal tissue for purposes of Section 498A.

The Public Health Service Act (hereinafter "The Act") contains three relevant provisions governing the use and transfer of human fetal tissue: (1) a criminal prohibition against the sale of human fetal tissue for valuable consideration; (2) restrictions on fetal tissue transplantation research supported by federal funds; and (3) a prohibition on the directed donation of fetal tissue for transplantation. We explore each of these restrictions in turn.

Section 498B(a) of the Act states that it is unlawful for any person to knowingly acquire, receive, or otherwise transfer any human fetal tissue for valuable consideration,[7] if the transfer affects interstate commerce.[8] 42 U.S.C. 289g-2(a). It is common practice for scientists throughout the United States to share research materials through transactions that result in such materials crossing state boundaries. Such exchanges, as well as transactions within the District of Columbia, or exchanges within a state that "affect interstate commerce" would meet the statutory criterion of affecting interstate commerce, but would not fall within the scope of the criminal

---

[7] The term "valuable consideration" encompasses both monetary and non-monetary payments. Section 498B (d)(3) provides that the term does not include "reasonable payments associated with the transportation, implantation, processing, preservation, quality control, or storage of human fetal tissue."

[8] The statute adopts the definition of interstate commerce in section 201(b) of the Federal Food, Drug, and Cosmetic Act, 21 U.S.C. 321(b): ". . . commerce between any State or Territory and any place outside thereof, and . . . commerce within the District of Columbia or within any other Territory not organized with a legislative body." The statute does not define what "affects" interstate commerce, but, in interpreting similar language in another criminal statute the Supreme Court found that "affecting interstate commerce" is an expression of Congress' intent to broadly exercise its Commerce Clause power under the Constitution. Scarborough v. United States, 431 U.S. 563, 571-72 (1977).

prohibition unless the scientist providing the materials sought payment in excess of the expenses included in the statutory definition of "valuable consideration."

In addition, the law places some restrictions on federal support for research on the transplantation of fetal tissue. Section 498A of the Act provides that the Secretary may conduct or support research on the "transplantation of fetal tissue for therapeutic purposes," only if certain statutory requirements are met. 42 U.S.C. 289g-1. These requirements include obtaining: (1) the informed consent of the woman donating the tissue; (2) a statement by the attending physician regarding the woman's consent and the method of obtaining the tissue; (3) a statement by the researcher regarding his or her understanding of the source of the tissue, that such information has been conveyed to the donee, and that the researcher has not participated in any decision regarding termination of the pregnancy.

Finally, section 498B(b) of the Act provides that it shall be unlawful for any person to solicit or knowingly acquire, receive, or accept a donation of human fetal tissue for the purpose of transplantation into another person if the tissue will be or is obtained pursuant to an induced abortion, and there is a promise to the donor: (1) to transplant the tissue into a person specified by the donor; (2) the tissue will be transplanted into a relative of the donor; or (3) the donee of the tissue has provided valuable consideration for the costs associated with the abortion. 42 U.S.C. 289g-2(b). The Act provides criminal penalties for violation of the prohibition on directed donations.

III. Federal Restrictions on Fetal Research

Federal regulation provides that activities involving cells, tissues, or organs excised from a non-living fetus shall be conducted only in accordance with any applicable state or local law. 45 CFR 46.210, Subpart B. This regulation would apply to certain human pluripotent stem cells, including those derived from the primordial germ cells of non-living fetuses.

IV. Prohibition on Federal Funding for Cloning of Human Beings

In a March 4, 1997, memorandum to the heads of executive departments and agencies, the President directed that no federal funds will be used for the cloning of human beings and that federal funds shall not be allocated for that purpose.[9] There are myriad uses for human pluripotent stem cells that are completely unrelated to cloning. However, to the extent such stem cells were to be used for human cloning, the prohibition on the use of federal funds for that purpose would apply.

---

[9] Memorandum from the President of the United States to Heads of Executive Departments and Agencies (March 4, 1997).

Office for Human Research Protections (OHRP)
Department of Health and Human Services

Guidance on Engagement of Institutions in Human Subjects Research

NOTE: This guidance document replaces two previous OHRP guidance documents: (1) "Engagement of Institutions in Research" (January 26, 1999); and (2) "Engagement of Pharmaceutical Companies in HHS-Supported Research" (December 23, 1999).

This guidance represents OHRP's current thinking on this topic and should be viewed as recommendations unless specific regulatory requirements are cited. The use of the word *must* in OHRP guidance means that something is required under HHS regulations at 45 CFR part 46. The use of the word *should* in OHRP guidance means that something is recommended or suggested, but not required. An institution may use an alternative approach if the approach satisfies the requirements of the HHS regulations at 45 CFR part 46. OHRP is available to discuss alternative approaches at 240-453-6900 or 866-447-4777.

Date: October 16, 2008

Scope: This guidance document applies to research involving human subjects that is conducted or supported by the Department of Health and Human Services (HHS). When an institution is *engaged* in non-exempt human subjects research that is conducted or supported by HHS, it must satisfy HHS regulatory requirements related to holding an assurance of compliance and certifying institutional review board (IRB) review and approval. This guidance document describes**:**

(1) scenarios that, in general, would result in an institution being considered *engaged* in a human subjects research project;
(2) scenarios that would result in an institution being considered *not engaged* in a human subjects research project; and
(3) IRB review considerations for cooperative research in which multiple institutions are engaged in the same non-exempt human subjects research project.

The scenarios below of situations where an institution is generally considered to be *engaged* or *not engaged* in human subjects research conducted or supported by HHS apply to all types of institutions, including academic or other non-profit organizations, institutions operating commercial repositories, and pharmaceutical or medical device companies.

Target Audience: IRBs, research administrators and other relevant institutional officials, investigators, and funding agencies that may be responsible for review or oversight of human

subjects research conducted or supported by HHS.

I. Background

Before engaging in HHS-conducted or -supported human subjects research that is not exempt under HHS regulations at 45 CFR 46.101(b), an institution must:

> (1) hold or obtain an OHRP-approved Federalwide Assurance (FWA) [45 CFR 46.103(a)]; and,
>
> (2) certify to the HHS agency conducting or supporting the research that the research has been reviewed and approved by an IRB designated in the FWA and will be subject to continuing review by an IRB [45 CFR 46.103(b)].

Note that the IRBs designated under an FWA may include IRBs of other institutions or independent IRBs. For more information on FWAs and how to designate an IRB of another institution on an FWA, see the following:

- OHRP Assurances Webpage (http://www.hhs.gov/ohrp/assurances/assurances_index.html)
- OHRP FWA Frequently Asked Questions (http://www.hhs.gov/ohrp/FWAfaq.html),
- OHRP Guidance on Extension of an FWA to Cover Collaborating Individual Investigators and Introduction of the Individual Investigator Agreement (http://www.hhs.gov/ohrp/humansubjects/assurance/guidanceonalternativetofwa.htm) , and
- OHRP IRB Registration Frequently Asked Questions (http://www.hhs.gov/ohrp/IRBfaq.html).

The following definitions are relevant for determining whether an institution's activities are covered by the HHS protection of human subjects regulations (45 CFR part 46), and whether the institution is engaged in human subjects research.

Research is defined in 45 CFR 46.102(d) as follows:

> *Research* means a systematic investigation, including research development, testing and evaluation, designed to develop or contribute to generalizable knowledge. Activities which meet this definition constitute research for purposes of this policy, whether or not they are conducted or supported under a program which is considered research for other purposes. For example, some demonstration and service programs may include research activities.

Human subject is defined in 45 CFR 46.102(f) as follows:

> *Human subject* means a living individual about whom an investigator (whether professional or student) conducting research obtains

(1) data through intervention or interaction with the individual, or
(2) identifiable private information.

Intervention includes both physical procedures by which data are gathered (for example, venipuncture) and manipulations of the subject or the subject's environment that are performed for research purposes. Interaction includes communication or interpersonal contact between investigator and subject. *Private information* includes information about behavior that occurs in a context in which an individual can reasonably expect that no observation or recording is taking place, and information which has been provided for specific purposes by an individual and which the individual can reasonably expect will not be made public (for example, a medical record). Private information must be individually identifiable (i.e., the identity of the subject is or may readily be ascertained by the investigator or associated with the information) in order for obtaining the information to constitute research involving human subjects.

Institution is defined in 45 CFR 46.102(b) as any public or private entity or agency (including federal, state, and other agencies).

For purposes of this document, an institution's *employees or agents* refers to individuals who: (1) act on behalf of the institution; (2) exercise institutional authority or responsibility; or (3) perform institutionally designated activities. "Employees and agents" can include staff, students, contractors, and volunteers, among others, regardless of whether the individual is receiving compensation.

II. When to Use This Guidance

This guidance should only be applied to activities that have been determined to be research involving human subjects that are not exempt under HHS regulations at 45 CFR 46.101(b). The following guidance documents available on the OHRP website may be helpful in determining whether research involves human subjects and also whether it is exempt: OHRP Human Subject Regulations Decision Charts (see http://www.dhhs.gov/ohrp/humansubjects/guidance/decisioncharts.htm) and OHRP Guidance on Research Involving Coded Private Information or Biological Specimens (see http://www.hhs.gov/ohrp/humansubjects/guidance/cdebiol.pdf).

Once an activity is determined to involve non-exempt human subjects research, this guidance should be used to determine whether an *institution* involved in some aspect of the research is *engaged* in that human subjects research, because if it is, certain regulatory requirements apply. Specifically, institutions that are engaged in non-exempt human subjects research are required by 45 CFR part 46 to:

(1) hold or obtain an applicable OHRP-approved FWA [45 CFR 46.103(a)]; and
(2) certify to the HHS agency conducting or supporting the research that the research has been reviewed and approved by an IRB designated in the FWA, and will be subject to

continuing review by an IRB [45 CFR 46.103(b)].

OHRP recognizes that many institutions and individuals (e.g., the principal investigator, statistical centers, community physicians, educators, data repositories) may work together on various aspects of a human subjects research project. However, not all participating institutions and individuals need to be covered by an FWA or certify IRB review and approval of the research to the HHS agency conducting or supporting the research. This guidance aims to assist institutions in determining whether they must meet those requirements, that is, whether they are *engaged* in activities covered by the regulations.

III. Interpretation of Engagement of Institutions in Human Subjects Research

In general, an institution is considered *engaged* in a particular non-exempt human subjects research project when its employees or agents for the purposes of the research project obtain: (1) data about the subjects of the research through intervention or interaction with them; (2) identifiable private information about the subjects of the research; or (3) the informed consent of human subjects for the research. The following two sections apply these concepts.

The scenarios in Section A describe the types of institutional involvement that generally would result in an institution being engaged in human subjects research. The scenarios in Section B include the types of institutional involvement that would result in an institution being **not** engaged in human subjects research, but these scenarios are not intended to be all-inclusive. There may be additional scenarios in which an institution would be **not** engaged in human subjects research. The determination of engagement depends on the specific facts of a research study and may be complex.

In applying this guidance, it is important to note that at least one institution must be determined to be engaged in any non-exempt human subjects research project that is conducted or supported by HHS (45 CFR 46.101(a)).

In the scenarios below, employees and agents are individuals acting on behalf of the institution, exercising institutional authority or responsibility, or performing institutionally designated activities.

A. Institutions Engaged in Human Subjects Research

In general, institutions are considered *engaged* in an HHS-conducted or -supported non-exempt human subjects research project (and, therefore, would need to hold or obtain OHRP-approved FWAs and certify IRB review and approval to HHS) when the involvement of their employees or agents in that project includes any of the following:

(1) Institutions that receive an award through a grant, contract, or cooperative agreement directly from HHS for the non-exempt human subjects research (i.e. awardee

institutions), even where all activities involving human subjects are carried out by employees or agents of another institution.

(2) Institutions whose employees or agents intervene for research purposes with any human subjects of the research by performing invasive or noninvasive procedures.

Examples of invasive or noninvasive procedures include drawing blood; collecting buccal mucosa cells using a cotton swab; administering individual or group counseling or psychotherapy; administering drugs or other treatments; surgically implanting medical devices; utilizing physical sensors; and utilizing other measurement procedures.

[See scenarios B.(1), B.(2), and B.(3) below for limited exceptions.]

(3) Institutions whose employees or agents intervene for research purposes with any human subject of the research by manipulating the environment.

Examples of manipulating the environment include controlling environmental light, sound, or temperature; presenting sensory stimuli; and orchestrating environmental events or social interactions.

[See scenarios B.(1) and B.(3) below for limited exceptions.]

(4) Institutions whose employees or agents interact for research purposes with any human subject of the research.

Examples of interacting include engaging in protocol-dictated communication or interpersonal contact; asking someone to provide a specimen by voiding or spitting into a specimen container; and conducting research interviews or administering questionnaires.

[See scenarios B.(1), B.(2), B.(3), and B.(4) below for limited exceptions.]

(5) Institutions whose employees or agents obtain the informed consent of human subjects for the research.

(6) Institutions whose employees or agents **obtain** for research purposes identifiable private information or identifiable biological specimens **from any source** for the research. It is important to note that, in general, institutions whose employees or agents obtain identifiable private information or identifiable specimens for non-exempt human subjects research are considered engaged in the research, even if the institution's employees or agents do not directly interact or intervene with human subjects. In general, obtaining identifiable private information or identifiable specimens includes, but is not limited to:

(a) observing or recording private behavior;
(b) using, studying, or analyzing for research purposes identifiable private information or identifiable specimens provided by another institution; and
(c) using, studying, or analyzing for research purposes identifiable private information or identifiable specimens already in the possession of the investigators.

In general, OHRP considers private information or specimens to be individually identifiable as defined in 45 CFR 46.102(f) when they can be linked to specific individuals by the investigator(s) either directly or indirectly through coding systems.

[See scenarios B.(1), B.(2), B.(3), B.(7), B.(8), B.(9), and B.(10) below for limited exceptions.]

B. Institutions Not Engaged in Human Subjects Research

Institutions would be considered **not** engaged in an HHS-conducted or -supported non-exempt human subjects research project (and, therefore, would not need to hold an OHRP-approved FWA or certify IRB review and approval to HHS) if the involvement of their employees or agents in that project is **limited to one or more** of the following. The following are scenarios describing the types of institutional involvement that would make an institution **not** engaged in human subjects research; there may be additional such scenarios:

(1) Institutions whose employees or agents perform commercial or other services for investigators provided that **all** of the following conditions also are met:

(a) the services performed do not merit professional recognition or publication privileges;
(b) the services performed are typically performed by those institutions for non-research purposes; and
(c) the institution's employees or agents do not administer any study intervention being tested or evaluated under the protocol.

The following are some examples, assuming the services described would not merit professional recognition or publication privileges:

- an appropriately qualified laboratory whose employees perform routine serum chemistry analyses of blood samples for investigators as a commercial service.
- a transcription company whose employees transcribes research study interviews as a commercial service.
- a hospital whose employees obtain blood through a blood draw or collect urine and provide such specimens to investigators as a service.

- a radiology clinic whose employees perform chest x-rays and send the results to investigators as a service.

(2) Institutions (including private practices) not selected as a research site whose employees or agents provide clinical trial-related medical services that are dictated by the protocol and would typically be performed as part of routine clinical monitoring and/or follow-up of subjects enrolled at a study site by clinical trial investigators (e.g., medical history, physical examination, assessment of adverse events, blood test, chest X-ray, or CT scan) provided that **all** of the following conditions also are met:

(a) the institution's employees or agents **do not** administer the study interventions being tested or evaluated under the protocol;
(b) the clinical trial-related medical services are typically provided by the institution for clinical purposes;
(c) the institution's employees or agents do not enroll subjects or obtain the informed consent of any subject for participation in the research; and
(d) when appropriate, investigators from an institution engaged in the research retain responsibility for:
(i) overseeing protocol-related activities; and
(ii) ensuring appropriate arrangements are made for reporting protocol-related data to investigators at an engaged institution, including the reporting of safety monitoring data and adverse events as required under the IRB-approved protocol.

Note that institutions (including private practices) not initially selected as research sites whose employees or agents administer the interventions being tested or evaluated in the study—such as administering either of two chemotherapy regimens as part of an oncology clinical trial evaluating the safety and effectiveness of the two regimens—generally would be engaged in human subjects research (see scenario B.(3) below for a limited exception). If such an institution does not have an FWA, its employees or agents may be covered by the FWA of another institution that is engaged in the research through an Individual Investigator Agreement. See http://www.hhs.gov/ohrp/humansubjects/assurance/guidanceonalternativetofwa.pdf.

(3) Institutions (including private practices) not initially selected as a research site whose employees or agents administer the study interventions being tested or evaluated under the protocol limited to a one-time or short-term basis (e.g., an oncologist at the institution administers chemotherapy to a research subject as part of a clinical trial because the subject unexpectedly goes out of town, or is unexpectedly hospitalized), provided that **all** of the following conditions also are met:
(a) an investigator from an institution engaged in the research determines that it would be in the subject's best interest to receive the study interventions being tested or evaluated under the protocol;
(b) the institution's employees or agents do not enroll subjects or obtain the

informed consent of any subject for participation in the research;

(c) investigators from the institution engaged in the research retain responsibility for:

(i) overseeing protocol-related activities;

(ii) ensuring the study interventions are administered in accordance with the IRB-approved protocol; and

(iii) ensuring appropriate arrangements are made for reporting protocol-related data to investigators at the engaged institution, including the reporting of safety monitoring data and adverse events as required under the IRB-approved protocol; **and**

(d) an IRB designated on the engaged institution's FWA is informed that study interventions being tested or evaluated under the protocol have been administered at an institution **not** selected as a research site.

(4) Institutions whose employees or agents:

(a) inform prospective subjects about the availability of the research;

(b) provide prospective subjects with information about the research (which may include a copy of the relevant informed consent document and other IRB-approved materials) but do not obtain subjects' consent for the research or act as representatives of the investigators;

(c) provide prospective subjects with information about contacting investigators for information or enrollment; and/or

(d) seek or obtain the prospective subjects' permission for investigators to contact them.

An example of this would be a clinician who provides patients with literature about a research study at another institution, including a copy of the informed consent document, and obtains permission from the patient to provide the patient's name and telephone number to investigators.

(5) Institutions (e.g., schools, nursing homes, businesses) that permit use of their facilities for intervention or interaction with subjects by investigators from another institution.

Examples would be a school that permits investigators from another institution to conduct or distribute a research survey in the classroom; or a business that permits investigators from another institution to recruit research subjects or to draw a blood sample at the work site for research purposes.

(6) Institutions whose employees or agents **release** to investigators at another institution identifiable private information or identifiable biological specimens pertaining to the subjects of the research.

Note that in some cases the institution releasing identifiable private information or

identifiable biological specimens may have institutional requirements that would need to be satisfied before the information or specimens may be released, and/or may need to comply with other applicable regulations or laws. In addition, if the identifiable private information or identifiable biological specimens to be released were collected for another research study covered by 45 CFR part 46, then the institution releasing such information or specimens should:

(a) ensure that the release would not violate the informed consent provided by the subjects to whom the information or biological specimens pertain (under 45 CFR 46.116), or
(b) if informed consent was waived by the IRB, ensure that the release would be consistent with the IRB's determinations that permitted a waiver of informed consent under 45 CFR 46.116 (c) or (d).

Examples of institutions that might release identifiable private information or identifiable biological specimens to investigators at another institution include:

(a) schools that release identifiable student test scores;
(b) an HHS agency that releases identifiable records about its beneficiaries; and
(c) medical centers that release identifiable human biological specimens.

Note that, in general, the institutions whose employees or agents **obtain** the identifiable private information or identifiable biological specimens from the releasing institution would be engaged in human subjects research. [See scenario A.(6) above.]

(7) Institutions whose employees or agents:

(a) obtain coded private information or human biological specimens from another institution involved in the research that retains a link to individually identifying information (such as name or social security number); and
(b) are **unable** to readily ascertain the identity of the subjects to whom the coded information or specimens pertain because, for example:

- the institution's employees or agents and the holder of the key enter into an agreement prohibiting the release of the key to the those employees or agents under any circumstances;
- the releasing institution has IRB-approved written policies and operating procedures applicable to the research project that prohibit the release of the key to the institution's employees or agents under any circumstances; or
- there are other legal requirements prohibiting the release of the key to the institution's employees or agents.

For purposes of this document, *coded* means that:

(a) identifying information (such as name or social security number) that would enable the investigator to readily ascertain the identity of the individual to whom the private information or specimens pertain has been replaced with a number, letter, symbol, and/or combination thereof (i.e., the code); and
(b) a key to decipher the code exists, enabling linkage of the identifying information to the private information or specimens.

Although this scenario resembles some of the language in OHRP's Guidance on Research Involving Coded Private Information or Biological Specimens, it is important to note that OHRP's Guidance on Research Involving Coded Private Information or Biological Specimens addresses when research involving coded private information or specimens is or is not research involving *human subjects*, as defined in 45 CFR 46.102(f) (see http://www.dhhs.gov/ohrp/humansubjects/guidance/cdebiol.pdf). As stated above in Section II., this Guidance on Engagement of Institutions in Human Subjects Research should only be applied to research projects that have been determined to involve human subjects and that are not exempt under HHS regulations at 45 CFR 46.101(b).

(8) Institutions whose employees or agents access or utilize individually identifiable private information **only** while visiting an institution that is engaged in the research, provided their research activities are overseen by the IRB of the institution that is engaged in the research.

(9) Institutions whose employees or agents access or review identifiable private information for purposes of study auditing (e.g. a government agency or private company will have access to individually identifiable study data for auditing purposes).

(10) Institutions whose employees or agents receive identifiable private information for purposes of satisfying U.S. Food and Drug Administration reporting requirements.

(11) Institutions whose employees or agents author a paper, journal article, or presentation describing a human subjects research study.

IV. IRB Review Considerations for Cooperative Research

OHRP notes that multiple institutions may be engaged in the same non-exempt human subjects research project. For such cooperative research projects, institutions may enter into joint review arrangements, rely upon the review of another qualified IRB, or make similar arrangements to avoid duplication of effort, in accordance with HHS regulations at 45 CFR 46.114.

When an institution is engaged in only part of a cooperative research project along the lines of scenarios A.(2), A.(3), A.(4), A.(5), or A.(6), the institution must ensure that the IRB(s) designated under its FWA reviews and approves the part(s) of the research in which the institution is engaged. For example, an institution operating the statistical center for a multicenter trial that receives identifiable private information from multiple other institutions must ensure that an IRB designated under its FWA reviews and approves the research activities related to the receipt and processing of the identifiable private information by the statistical center. In such a case, the IRB should ensure that the statistical center has sufficient mechanisms in place to adequately protect the privacy of subjects and maintain the confidentiality of the data. When an institution is engaged in only part of a cooperative research project, the reviewing IRB may decide to review the entire research study, even if information about the entire study is not necessary to approve the institution's part of the research under 45 CFR 46.111.

If you have specific questions about how to apply this guidance, please contact OHRP by phone at (866) 447-4777 (toll-free within the U.S.) or (240) 453-6900, or by e-mail at ohrp@hhs.gov.

Code of Federal Regulations

TITLE 45
PUBLIC WELFARE

Department of Health and Human Services

PART 46
PROTECTION OF HUMAN SUBJECTS

* * *

Revised January 15, 2009
Effective July 14, 2009

SUBPART A—
Basic HHS Policy for Protection of Human Research Subjects

Sec.

SUBPART B—
Additional Protections for Pregnant Women, Human Fetuses and Neonates Involved in Research

Sec.

SUBPART C— Additional Protections Pertaining to Biomedical and Behavioral Research Involving Prisoners as Subjects

Sec.

SUBPART D— Additional Protections for Children Involved as Subjects in Research

Sec.

Authority: 5 U.S.C. 301; 42 U.S.C. 289 (a).

.

SUBPART E — Registration of Institutional Review Boards

Sec.

Editorial Note: The Department of Health and Human Services issued a notice of waiver regarding the requirements set forth in part 46, relating to protection of human subjects, as they pertain to demonstration projects, approved under section 1115 of the Social Security Act, which test the use of cost-sharing, such as deductibles, copayment and coinsurance, in the Medicaid program. For further information see 47 FR 9208, Mar. 4, 1982.

SUBPART A
Basic HHS Policy for Protection of Human Research Subjects

Authority: 5 U.S.C. 301; 42 U.S.C. 289; 42 U.S.C. 300v-1(b).

Source: 56 FR 28012, 28022, June 18, 1991, unless otherwise noted.

§46.101 To what does this policy apply?

(a) Except as provided in paragraph (b) of this section, this policy applies to all research involving human subjects conducted, supported or otherwise subject to regulation by any federal department or agency which takes appropriate administrative action to make the policy applicable to such research. This includes research conducted by federal civilian employees or military personnel, except that each department or agency head may adopt such procedural modifications as may be appropriate from an administrative standpoint. It also includes research conducted, supported, or otherwise subject to regulation by the federal government outside the United States.

(1) Research that is conducted or supported by a federal department or agency, whether or not it is regulated as defined in §46.102(e), must comply with all sections of this policy.

(2) Research that is neither conducted nor supported by a federal department or agency but is subject to regulation as defined in §46.102(e) must be reviewed and approved, in compliance with §46.101, §46.102, and §46.107 through §46.117 of this policy, by an institutional review board (IRB) that operates in accordance with the pertinent requirements of this policy.

(b) Unless otherwise required by department or agency heads, research activities in which the only involvement of human subjects will be in one or more of the following categories are exempt from this policy:

(1) Research conducted in established or commonly accepted educational settings, involving normal educational practices, such as (i) research on regular and special education instructional strategies, or (ii) research on the effectiveness of or the comparison among instructional techniques, curricula, or classroom management methods.

(2) Research involving the use of educational tests (cognitive, diagnostic, aptitude, achievement), survey procedures, interview procedures or observation of public behavior, unless: (i) information obtained is recorded in such manner that human subjects can be identified, directly or through identifiers linked to the subjects; and (ii) any disclosure of the human subjects' responses outside the research could reasonably place the subjects at risk of criminal or civil liability or be damaging to the subjects' financial standing, employability, or reputation.

(3) Research involving the use of educational tests (cognitive, diagnostic, aptitude, achievement), survey procedures, interview procedures, or observation of public behavior that is not exempt under paragraph (b)(2) of this section, if:

(i) the human subjects are elected or appointed public officials or candidates for public office; or (ii) federal statute(s) require(s) without exception that the confidentiality of the personally identifiable information will be maintained throughout the research and thereafter.

(4) Research involving the collection or study of existing data, documents, records, pathological specimens, or diagnostic specimens, if these sources are publicly available or if the information is recorded by the investigator in such a manner that subjects cannot be identified, directly or through identifiers linked to the subjects.

(5) Research and demonstration projects which are conducted by or subject to the approval of department or agency heads, and which are designed to study, evaluate, or otherwise examine:(i) Public benefit or service programs; (ii) procedures for obtaining benefits or services under those programs; (iii) possible changes in or alternatives to those programs or procedures; or (iv) possible changes in methods or levels of payment for benefits or services under those programs.

(6) Taste and food quality evaluation and consumer acceptance studies, (i) if wholesome foods without additives are consumed or (ii) if a food is consumed that contains a food ingredient at or below the level and for a use found to be safe, or agricultural chemical or environmental contaminant at or below the level found to be safe, by the Food and Drug Administration or approved by the Environmental Protection Agency or the Food Safety and Inspection Service of the U.S. Department of Agriculture.

(c) Department or agency heads retain final judgment as to whether a particular activity is covered by this policy.

(d) Department or agency heads may require that specific research activities or classes of research activities conducted, supported, or otherwise subject to regulation by the department or agency but not otherwise covered by this policy, comply with some or all of the requirements of this policy.

(e) Compliance with this policy requires compliance with pertinent federal laws or regulations which provide additional protections for human subjects.

(f) This policy does not affect any state or local laws or regulations which may otherwise be applicable and which provide additional protections for human subjects.

(g) This policy does not affect any foreign laws or regulations which may otherwise be applicable and which provide additional protections to human subjects of research.

h) When research covered by this policy takes place in foreign countries, procedures normally followed in the foreign countries to protect human subjects may differ from those set forth in this policy. [An example is a foreign institution which complies with guidelines consistent with the World Medical Assembly Declaration (Declaration of Helsinki amended 1989) issued either by sovereign states or by an organization whose function for the protection of human research subjects is internationally recognized.] In these circumstances, if a department or agency head determines that the procedures prescribed by the institution afford protections that are at least equivalent to those provided in this policy, the department or agency head may approve the substitution of the foreign procedures in lieu of the procedural requirements provided in this policy. Except when otherwise required by statute, Executive Order, or the department or agency head, notices of these actions as they occur will be published in the FEDERAL REGISTER or will be otherwise published as provided in department or agency procedures.

(i) Unless otherwise required by law, department or agency heads may waive the applicability of some or all of the provisions of this policy to specific research activities or classes of research activities otherwise covered by this policy. Except when otherwise required by statute or Executive Order, the department or agency head shall forward advance notices of these actions to the Office for Human Research Protections, Department of Health and Human Services (HHS), or any successor office, and shall also publish them in the FEDERAL REGISTER or in such other manner as provided in department or agency procedures.[1]

[56 FR 28012, 28022, June 18, 1991; 56 FR 29756, June 28, 1991, as amended at 70 FR 36328, June 23, 2005]

§46.102 Definitions.

(a) *Department or agency head* means the head of any federal department or agency and any other officer or employee of any department or agency to whom authority has been delegated.

(b) *Institution* means any public or private entity or agency (including federal, state, and other agencies).

(c) *Legally authorized representative* means an individual or judicial or other body authorized under applicable law to consent on behalf of a prospective subject to the subject's participation in the procedure(s) involved in the research.

(d) *Research* means a systematic investigation, including research development, testing and evaluation, designed to develop or contribute to generalizable knowledge. Activities which meet this definition constitute research for purposes of this policy, whether or not they are conducted or supported under a program which is considered research for other purposes. For example, some demonstration and service programs may include research activities.

(e) *Research subject to regulation*, and similar terms are intended to encompass those research activities for which a federal department or agency has specific responsibility for regulating as a research activity (for example, Investigational New Drug requirements administered by the Food and Drug Administration). It does not include research activities which are incidentally regulated by a federal department or agency solely as part of the department's or agency's broader responsibility to regulate certain types of activities whether research or non-research in nature (for example, Wage and Hour requirements administered by the Department of Labor).

(f) *Human subject* means a living individual about whom an investigator (whether professional or student) conducting research obtains

(1) Data through intervention or interaction with the individual, or

(2) Identifiable private information.

Intervention includes both physical procedures by which data are gathered (for example, venipuncture) and manipulations of the subject or the subject's environment that are performed for research purposes. Interaction includes communication or interpersonal contact between investigator and subject. Private information includes information about behavior that occurs in a context

in which an individual can reasonably expect that no observation or recording is taking place, and information which has been provided for specific purposes by an individual and which the individual can reasonably expect will not be made public (for example, a medical record).

Private information must be individually identifiable (i.e., the identity of the subject is or may readily be ascertained by the investigator or associated with the information) in order for obtaining the information to constitute research involving human subjects.

(g) *IRB* means an institutional review board established in accord with and for the purposes expressed in this policy.

(h) *IRB approval* means the determination of the IRB that the research has been reviewed and may be conducted at an institution within the constraints set forth by the IRB and by other institutional and federal requirements.

(i) *Minimal risk* means that the probability and magnitude of harm or discomfort anticipated in the research are not greater in and of themselves than those ordinarily encountered in daily life or during the performance of routine physical or psychological examinations or tests.

h) When research covered by this policy takes place in foreign countries, procedures normally followed in the foreign countries to protect human subjects may differ from those set forth in this policy. [An example is a foreign institution which complies with guidelines consistent with the World Medical Assembly Declaration (Declaration of Helsinki amended 1989) issued either by sovereign states or by an organization whose function for the protection of human research subjects is internationally recognized.] In these circumstances, if a department or agency head determines that the procedures prescribed by the institution afford protections that are at least equivalent to those provided in this policy, the department or agency head may approve the substitution of the foreign procedures in lieu of the procedural requirements provided in this policy. Except when otherwise required by statute, Executive Order, or the department or agency head, notices of these actions as they occur will be published in the FEDERAL REGISTER or will be otherwise published as provided in department or agency procedures.

[1]Institutions with HHS-approved assurances on file will abide by provisions of Title 45 CFR part 46 subparts A-D. Some of the other departments and agencies have incorporated all provisions of Title 45 CFR part 46 into their policies and procedures as well. However, the exemptions at 45 CFR 46.101(b) do not apply to research involving prisoners, subpart C. The exemption at 45 CFR 46.101(b)(2), for research involving survey or interview procedures or observation of public behavior, does not apply to research with children, subpart D, except for research involving observations of public behavior when the investigator(s) do not participate in the activities being observed.

§46.103 Assuring compliance with this policy -- research conducted or supported by any Federal Department or Agency.

(a) Each institution engaged in research which is covered by this policy and which is conducted or supported by a federal department or agency shall provide written assurance satisfactory to the department or agency head that it will comply with the requirements set forth in this policy. In lieu of requiring submission of an assurance, individual department or agency heads shall accept the existence of a current assurance, appropriate for the research in question, on file with the Office for Human Research Protections, HHS, or any successor office, and approved for federalwide use by that office. When the existence of an HHS-approved assurance is accepted in lieu of requiring submission of an assurance, reports (except certification) required by this policy to be made to department and agency heads shall also be made to the Office for Human Research Protections, HHS, or any successor office.

(b) Departments and agencies will conduct or support research covered by this policy only if the institution has an assurance approved as provided in this section, and only if the institution has certified to the department or agency head that the research has been reviewed and approved by an IRB provided for in the assurance, and will be subject to continuing review by the IRB. Assurances applicable to federally supported or conducted research shall at a minimum include:

(1)A statement of principles governing the institution in the discharge of its responsibilities for protecting the rights and welfare of human subjects of research conducted at or sponsored by the institution, regardless of whether the research is subject to Federal regulation. This may include an appropriate existing code, declaration, or statement of ethical principles, or a statement formulated by the institution itself. This requirement does not preempt provisions of this policy applicable to department- or agency-supported or regulated research and need not be applicable to any research exempted or waived under §46.101(b) or (i).

(2)Designation of one or more IRBs established in accordance with the requirements of this policy, and for which provisions are made for meeting space and sufficient staff to support the IRB's review and recordkeeping duties.

(3)A list of IRB members identified by name; earned degrees; representative capacity; indications of experience such as board certifications, licenses, etc., sufficient to describe each member's chief anticipated contributions to IRB deliberations; and any employment or other relationship between each member and the institution; for example: full-time employee, part-time employee, member of governing panel or board, stockholder, paid or unpaid consultant. Changes in IRB membership shall be reported to the department or agency head, unless in accord with §46.103(a) of this policy, the existence of an HHS-approved assurance is accepted. In this case, change in IRB membership shall be reported to the Office for Human Research Protections, HHS, or any successor office.

(4)Written procedures which the IRB will follow (i) for conducting its initial and continuing review of research and for reporting its findings and actions to the investigator and the institution; (ii) for determining which projects require review more often than annually and which projects need verification from sources other than the investigators that no material changes have occurred since previous IRB review; and (iii) for ensuring prompt reporting to the IRB of proposed changes in a research activity, and for ensuring that such changes in approved research, during the period for which IRB approval has already been given, may not be initiated without IRB review and approval except when necessary to eliminate apparent immediate hazards to the subject.

(5)Written procedures for ensuring prompt reporting to the IRB, appropriate institutional officials, and the department or agency head of (i) any unanticipated problems involving risks to subjects or others or any serious or continuing noncompliance with this policy or the requirements or determinations of the IRB; and (ii) any suspension or termination of IRB approval.

(c) The assurance shall be executed by an individual authorized to act for the institution and to assume on behalf of the institution the obligations imposed by this policy and shall be filed in such form and manner as the department or agency head prescribes.

(d) The department or agency head will evaluate all assurances submitted in accordance with this policy through such officers and employees of the department or agency and such experts or consultants engaged for this purpose as the department or agency head determines to be appropriate. The department or agency head's evaluation will take into consideration the adequacy of the proposed IRB in light of the anticipated scope of the institution's research activities and the types of subject populations likely to be involved, the appropriateness of the proposed initial and continuing review procedures in light of the probable risks, and the size and complexity of the institution.

(e) On the basis of this evaluation, the department or agency head may approve or disapprove the assurance, or enter into negotiations to develop an approvable one. The department or agency head may limit the period during which any particular approved assurance or class of approved assurances shall remain effective or otherwise condition or restrict approval.

(f) Certification is required when the research is supported by a federal department or agency and not otherwise exempted or waived under §46.101(b) or (i). An institution with an approved assurance shall certify that each application or proposal for research covered by the assurance and by §46.103 of this Policy has been reviewed and approved by the IRB. Such certification must be submitted with the application or proposal or by such later date as may be prescribed by the department or agency to which the application or proposal is submitted. Under no condition shall research covered by §46.103 of the Policy be supported prior to receipt of the certification that the research has been reviewed and approved by the IRB. Institutions without an approved assurance covering the research shall certify within 30 days after receipt of a request for such a certification from the department or agency, that the application or proposal has been approved by the IRB. If the certification is not submitted within these time limits, the application or proposal may be returned to the institution.

(Approved by the Office of Management and Budget under Control Number 0990-0260.)

[56 FR 28012, 28022, June 18, 1991; 56 FR 29756, June 28, 1991, as amended at 70 FR 36328, June 23, 2005]

§§46.104--46.106 [Reserved]

§46.107 IRB membership.

(a) Each IRB shall have at least five members, with varying backgrounds to promote complete and adequate review of research activities commonly conducted by the institution. The IRB shall be sufficiently qualified through the experience and expertise of its members, and the diversity of the members, including consideration of race, gender, and cultural backgrounds and sensitivity to such issues as community attitudes, to promote respect for its advice and counsel in safeguarding the rights and welfare of human subjects. In addition to possessing the professional competence necessary to review specific research activities, the IRB shall be able to ascertain the acceptability of proposed research in terms of institutional commitments and regulations, applicable law, and standards of professional conduct and practice. The IRB shall therefore include persons knowledgeable in these areas. If an IRB regularly reviews research that involves a vulnerable category of subjects, such as children, prisoners, pregnant women, or handicapped or mentally disabled persons, consideration shall be given to the inclusion of one or more individuals who are knowledgeable about and experienced in working with these subjects.

(b) Every nondiscriminatory effort will be made to ensure that no IRB consists entirely of men or entirely of women, including the institution's consideration of qualified persons of both sexes, so long as no selection is made to the IRB on the basis of gender. No IRB may consist entirely of members of one profession.

(c) Each IRB shall include at least one member whose primary concerns are in scientific areas and at least one member whose primary concerns are in nonscientific areas.

(d) Each IRB shall include at least one member who is not otherwise affiliated with the institution and who is not part of the immediate family of a person who is affiliated with the institution.

(e) No IRB may have a member participate in the IRB's initial or continuing review of any project in which the member has a conflicting interest, except to provide information requested by the IRB.

(f) An IRB may, in its discretion, invite individuals with competence in special areas to assist in the review of issues which require expertise beyond or in addition to that available on the IRB. These individuals may not vote with the IRB

§46.108 IRB functions and operations.

In order to fulfill the requirements of this policy each IRB shall:

(a) Follow written procedures in the same detail as described in §46.103(b)(4) and, to the extent required by, §46.103(b)(5).

(b) Except when an expedited review procedure is used (see §46.110), review proposed research at convened meetings at which a majority of the members of the IRB are present, including at least one member whose primary concerns are in nonscientific areas. In order for the research to be approved, it shall receive the approval of a majority of those members present at the meeting.

§46.109 IRB review of research.

(a) An IRB shall review and have authority to approve, require modifications in (to secure approval), or disapprove all research activities covered by this policy.

(b) An IRB shall require that information given to subjects as part of informed consent is in accordance with §46.116. The IRB may require that information, in addition to that specifically mentioned in §46.116, be given to the subjects when in the IRB's judgment the information would meaningfully add to the protection of the rights and welfare of subjects.

(c) An IRB shall require documentation of informed consent or may waive documentation in accordance with §46.117.

(d) An IRB shall notify investigators and the institution in writing of its decision to approve or disapprove the proposed research activity, or of modifications required to secure IRB approval of the research activity. If the IRB decides to disapprove a research activity, it shall include in its written notification a statement of the reasons for its decision and give the investigator an opportunity to respond in person or in writing.

(e) An IRB shall conduct continuing review of research covered by this policy at intervals appropriate to the degree of risk, but not less than once per year, and shall have authority to observe or have a third party observe the consent process and the research.

(Approved by the Office of Management and Budget under Control Number 0990-0260.)

[56 FR 28012, 28022, June 18, 1991, as amended at 70 FR 36328, June 23, 2005]

§46.110 Expedited review procedures for certain kinds of research involving no more than minimal risk, and for minor changes in approved research.

(a) The Secretary, HHS, has established, and published as a Notice in the FEDERAL REGISTER, a list of categories of research that may be reviewed by the IRB through an expedited review procedure. The list will be amended, as appropriate, after consultation with other departments and agencies, through periodic republication by the Secretary, HHS, in the FEDERAL REGISTER. A copy of the list is available from the Office for Human Research Protections, HHS, or any successor office.

(b) An IRB may use the expedited review procedure to review either or both of the following:

(1) some or all of the research appearing on the list and found by the reviewer(s) to involve no more than minimal risk,

(2) minor changes in previously approved research during the period (of one year or less) for which approval is authorized.

Under an expedited review procedure, the review may be carried out by the IRB chairperson or by one or more experienced reviewers designated by the chairperson from among members of the IRB. In reviewing the research, the reviewers may exercise all of the authorities of the IRB except that the reviewers may not disapprove the research. A research activity may be disapproved only after review in accordance with the nonexpedited procedure set forth in §46.108(b).

(c) Each IRB which uses an expedited review procedure shall adopt a method for keeping all members advised of research proposals which have been approved under the procedure.

(d) The department or agency head may restrict, suspend, terminate, or choose not to authorize an institution's or IRB's use of the expedited review procedure.

[56 FR 28012, 28022, June 18, 1991, as amended at 70 FR 36328, June 23, 2005]

§46.111 Criteria for IRB approval of research.

(a) In order to approve research covered by this policy the IRB shall determine that all of the following requirements are satisfied:

(1) Risks to subjects are minimized: (i) By using procedures which are consistent with sound research design and which do not unnecessarily expose subjects to risk, and (ii) whenever appropriate, by using procedures already being performed on the subjects for diagnostic or treatment purposes.

(2) Risks to subjects are reasonable in relation to anticipated benefits, if any, to subjects, and the importance of the knowledge that may reasonably be expected to result. In evaluating risks and benefits, the IRB should consider only those risks and benefits that may result from the research (as distinguished from risks and benefits of therapies subjects would receive even if not participating in the research). The IRB should not consider possible long-range effects of applying knowledge gained in the research (for example, the possible effects of the research on public policy) as among those research risks that fall within the purview of its responsibility.

(3) Selection of subjects is equitable. In making this assessment the IRB should take into account the purposes of the research and the setting in which the research will be conducted and should be particularly cognizant of the special problems of research involving vulnerable populations, such as children, prisoners, pregnant women, mentally disabled persons, or economically or educationally disadvantaged persons.

(4) Informed consent will be sought from each prospective subject or the subject's legally authorized representative, in accordance with, and to the extent required by §46.116.

(5) Informed consent will be appropriately documented, in accordance with, and to the extent required by §46.117.

(6) When appropriate, the research plan makes adequate provision for monitoring the data collected to ensure the safety of subjects.

(7) When appropriate, there are adequate provisions to protect the privacy of subjects and to maintain the confidentiality of data.

(b) When some or all of the subjects are likely to be vulnerable to coercion or undue influence, such as children, prisoners, pregnant women, mentally disabled persons, or economically or educationally disadvantaged persons, additional safeguards have been included in the study to protect the rights and welfare of these subjects.

§46.112 Review by institution.

Research covered by this policy that has been approved by an IRB may be subject to further appropriate review and approval or disapproval by officials of the institution. However, those officials may not approve the research if it has not been approved by an IRB.

§46.113 Suspension or termination of IRB approval of research.

An IRB shall have authority to suspend or terminate approval of research that is not being conducted in accordance with the IRB's requirements or that has been associated with unexpected serious harm to subjects. Any suspension or termination of approval shall include a statement of the reasons for the IRB's action and shall be reported promptly to the investigator, appropriate institutional officials, and the department or agency head.

(Approved by the Office of Management and Budget under Control Number 0990-0260.)

[56 FR 28012, 28022, June 18, 1991, as amended at 70 FR 36328, June 23, 2005]

§46.114 Cooperative research.

Cooperative research projects are those projects covered by this policy which involve more than one institution. In the conduct of cooperative research projects, each institution is responsible for safeguarding the rights and welfare of human subjects and for complying with this policy. With the approval of the department or agency head, an institution participating in a cooperative project may enter into a joint review arrangement, rely upon the review of another qualified IRB, or make similar arrangements for avoiding duplication of effort.

§46.115 IRB records.

(a) An institution, or when appropriate an IRB, shall prepare and maintain adequate documentation of IRB activities, including the following:

(1) Copies of all research proposals reviewed, scientific evaluations, if any, that accompany the proposals, approved sample consent documents, progress reports submitted by investigators, and reports of injuries to subjects.

(2) Minutes of IRB meetings which shall be in sufficient detail to show attendance at the meetings; actions taken by the IRB; the vote on these actions including the number of members voting for, against, and abstaining; the basis for requiring changes in or disapproving research; and a written summary of the discussion of controverted issues and their resolution.

(3) Records of continuing review activities.

(4) Copies of all correspondence between the IRB and the investigators.

(5) A list of IRB members in the same detail as described in §46.103(b)(3).

(6) Written procedures for the IRB in the same detail as described in §46.103(b)(4) and §46.103(b)(5).

(7) Statements of significant new findings provided to subjects, as required by §46.116(b)(5).

(b) The records required by this policy shall be retained for at least 3 years, and records relating to research which is conducted shall be retained for at least 3 years after completion of the research. All records shall be accessible for inspection and copying by authorized representatives of the department or agency at reasonable times and in a reasonable manner.

(Approved by the Office of Management and Budget under Control Number 0990-0260.)

[56 FR 28012, 28022, June 18, 1991, as amended at 70 FR 36328, June 23, 2005]

§46.116 General requirements for informed consent.

Except as provided elsewhere in this policy, no investigator may involve a human being as a subject in research covered by this policy unless the investigator has obtained the legally effective informed consent of the subject or the subject's legally authorized representative. An investigator shall seek such consent only under circumstances that provide the prospective subject or the representative sufficient opportunity to consider whether or not to participate and that minimize the possibility of coercion or undue influence. The information that is given to the subject or the representative shall be in language understandable to the subject or the representative. No informed consent, whether oral or written, may include any exculpatory language through which the subject or the representative is made to waive or appear to waive any of the subject's legal rights, or releases or appears to release the investigator, the sponsor, the institution or its agents from liability for negligence.

(a) Basic elements of informed consent. Except as provided in paragraph (c) or (d) of this section, in seeking informed consent the following information shall be provided to each subject:

(1) A statement that the study involves research, an explanation of the purposes of the research and the expected duration of the subject's participation, a description of the procedures to be followed, and identification of any procedures which are experimental;

(2) A description of any reasonably foreseeable risks or discomforts to the subject;

(3) A description of any benefits to the subject or to others which may reasonably be expected from the research;

(4) A disclosure of appropriate alternative procedures or courses of treatment, if any, that might be advantageous to the subject;

(5) A statement describing the extent, if any, to which confidentiality of records identifying the subject will be maintained;

(6) For research involving more than minimal risk, an explanation as to whether any compensation and an explanation as to whether any medical treatments are available if injury occurs and, if so, what they consist of, or where further information may be obtained;

(7) An explanation of whom to contact for answers to pertinent questions about the research and research subjects' rights, and whom to contact in the event of a research-related injury to the subject; and

(8) A statement that participation is voluntary, refusal to participate will involve no penalty or loss of benefits to which the subject is otherwise entitled, and the subject may discontinue participation at any time without penalty or loss of benefits to which the subject is otherwise entitled.

(b) Additional elements of informed consent. When appropriate, one or more of the following elements of information shall also be provided to each subject:

(1) A statement that the particular treatment or procedure may involve risks to the subject (or to the embryo or fetus, if the subject is or may become pregnant) which are currently unforeseeable;

(2) Anticipated circumstances under which the subject's participation may be terminated by the investigator without regard to the subject's consent;

(3) Any additional costs to the subject that may result from participation in the research;

(4) The consequences of a subject's decision to withdraw from the research and procedures for orderly termination of participation by the subject;

(5) A statement that significant new findings developed during the course of the research which may relate to the subject's willingness to continue participation will be provided to the subject; and

(6) The approximate number of subjects involved in the study.

(c) An IRB may approve a consent procedure which does not include, or which alters, some or all of the elements of informed consent set forth above, or waive the requirement to obtain informed consent provided the IRB finds and documents that:

(1) The research or demonstration project is to be conducted by or subject to the approval of state or local government officials and is designed to study, evaluate, or otherwise examine: (i) public benefit or service programs; (ii) procedures for obtaining benefits or services under those programs; (iii) possible changes in or alternatives to those programs or procedures; or (iv) possible changes in methods or levels of payment for benefits or services under those programs; and

(2) The research could not practicably be carried out without the waiver or alteration.

(d) An IRB may approve a consent procedure which does not include, or which alters, some or all of the elements of informed consent set forth in this section, or waive the requirements to obtain informed consent provided the IRB finds and documents that:

1) The research involves no more than minimal risk to the subjects;

(2) The waiver or alteration will not adversely affect the rights and welfare of the subjects;

(3) The research could not practicably be carried out without the waiver or alteration; and

(4) Whenever appropriate, the subjects will be provided with additional pertinent information after participation.

(e) The informed consent requirements in this policy are not intended to preempt any applicable federal, state, or local laws which require additional information to be disclosed in order for informed consent to be legally effective.

(f) Nothing in this policy is intended to limit the authority of a physician to provide emergency medical care, to the extent the physician is permitted to do so under applicable federal, state, or local law.

(Approved by the Office of Management and Budget under Control Number 0990-0260.)

[56 FR 28012, 28022, June 18, 1991, as amended at 70 FR 36328, June 23, 2005]

§46.117 Documentation of informed consent.

(a) Except as provided in paragraph (c) of this section, informed consent shall be documented by the use of a written consent form approved by the IRB and signed by the subject or the subject's legally authorized representative. A copy shall be given to the person signing the form.

(b) Except as provided in paragraph (c) of this section, the consent form may be either of the following:

(1) A written consent document that embodies the elements of informed consent required by §46.116. This form may be read to the subject or the subject's legally authorized representative, but in any event, the investigator shall give either the subject or the representative adequate opportunity to read it before it is signed; or

(2) A short form written consent document stating that the elements of informed consent required by §46.116 have been presented orally to the subject or the subject's legally authorized representative. When this method is used, there shall be a witness to the oral presentation. Also, the IRB shall approve a written summary of what is to be said to the subject or the representative. Only the short form itself is to be signed by the subject or the representative. However, the witness shall sign both the short form and a copy of the summary, and the person actually obtaining consent shall sign a copy of the summary. A copy of the summary shall be given to the subject or the representative, in addition to a copy of the short form.

(c) An IRB may waive the requirement for the investigator to obtain a signed consent form for some or all subjects if it finds either:

(1) That the only record linking the subject and the research would be the consent document and the principal risk would be potential harm resulting from a breach of confidentiality. Each subject will be asked whether the subject wants documentation linking the subject with the research, and the subject's wishes will govern; or

(2) That the research presents no more than minimal risk of harm to subjects and involves no procedures for which written consent is normally required outside of the research context.

In cases in which the documentation requirement is waived, the IRB may require the investigator to provide subjects with a written statement regarding the research.

(Approved by the Office of Management and Budget under Control Number 0990-0260.)

[56 FR 28012, 28022, June 18, 1991, as amended at 70 FR 36328, June 23, 2005]

§46.118 Applications and proposals lacking definite plans for involvement of human subjects.

Certain types of applications for grants, cooperative agreements, or contracts are submitted to departments or agencies with the knowledge that subjects may be involved within the period of support, but definite plans would not normally be set forth in the application or proposal. These include activities such as institutional type grants when selection of specific projects is the institution's responsibility; research training grants in which the activities involving subjects remain to be selected; and projects in which human subjects' involvement will depend upon completion of instruments, prior animal studies, or purification of compounds. These applications need not be reviewed by an IRB before an award may be made. However, except for research exempted or waived under §46.101(b) or (i), no human subjects may be involved in any project supported by these awards until the project has been reviewed and approved by the IRB, as provided in this policy, and certification submitted, by the institution, to the department or agency.

§46.119 Research undertaken without the intention of involving human subjects.

In the event research is undertaken without the intention of involving human subjects, but it is later proposed to involve human subjects in the research, the research shall first be reviewed and approved by an IRB, as provided in this policy, a certification submitted, by the institution, to the department or agency, and final approval given to the proposed change by the department or agency.

§46.120 Evaluation and disposition of applications and proposals for research to be conducted or supported by a Federal Department or Agency.

(a) The department or agency head will evaluate all applications and proposals involving human subjects submitted to the department or agency through such officers and employees of the department or agency and such experts and consultants as the department or agency head determines to be appropriate. This evaluation will take into consideration the risks to the subjects, the adequacy of protection against these risks, the potential benefits of the research to the subjects and others, and the importance of the knowledge gained or to be gained.

(b) On the basis of this evaluation, the department or agency head may approve or disapprove the application or proposal, or enter into negotiations to develop an approvable one.

§46.121 [Reserved]

§46.122 Use of Federal funds.

Federal funds administered by a department or agency may not be expended for research involving human subjects unless the requirements of this policy have been satisfied.

§46.123 Early termination of research support: Evaluation of applications and proposals.

(a) The department or agency head may require that department or agency support for any project be terminated or suspended in the manner prescribed in applicable program requirements, when the department or agency head finds an institution has materially failed to comply with the terms of this policy.

(b) In making decisions about supporting or approving applications or proposals covered by this policy the department or agency head may take into account, in addition to all other eligibility requirements and program criteria, factors such as whether the applicant has been subject to a termination or suspension under paragraph (a) of this section and whether the applicant or the person or persons who would direct or has/have directed the scientific and technical aspects of an activity has/have, in the judgment of the department or agency head, materially failed to discharge responsibility for the protection of the rights and welfare of human subjects (whether or not the research was subject to federal regulation).

§46.124 Conditions.

With respect to any research project or any class of research projects the department or agency head may impose additional conditions prior to or at the time of approval when in the judgment of the department or agency head additional conditions are necessary for the protection of human subjects.

Subpart B

Additional Protections for Pregnant Women, Human Fetuses and Neonates Involved in Research

Source: 66 FR 56778, Nov. 13, 2001, unless otherwise noted.

§46.201 To what do these regulations apply?

(a) Except as provided in paragraph (b) of this section, this subpart applies to all research involving pregnant women, human fetuses, neonates of uncertain viability, or nonviable neonates conducted or supported by the Department of Health and Human Services (DHHS). This includes all research conducted in DHHS facilities by any person and all research conducted in any facility by DHHS employees.

(b) The exemptions at §46.101(b)(1) through (6) are applicable to this subpart.

(c) The provisions of §46.101(c) through (i) are applicable to this subpart. Reference to State or local laws in this subpart and in §46.101(f) is intended to include the laws of federally recognized American Indian and Alaska Native Tribal Governments.

(d) The requirements of this subpart are in addition to those imposed under the other subparts of this part.

§46.202 Definitions.

The definitions in §46.102 shall be applicable to this subpart as well. In addition, as used in this subpart:

(a) Dead fetus means a fetus that exhibits neither heartbeat, spontaneous respiratory activity, spontaneous movement of voluntary muscles, nor pulsation of the umbilical cord.

(b) Delivery means complete separation of the fetus from the woman by expulsion or extraction or any other means.

(c) Fetus means the product of conception from implantation until delivery.

(d) Neonate means a newborn.

(e) Nonviable neonate means a neonate after delivery that, although living, is not viable.

(f) Pregnancy encompasses the period of time from implantation until delivery. A woman shall be assumed to be pregnant if she exhibits any of the pertinent presumptive signs of pregnancy, such as missed menses, until the results of a pregnancy test are negative or until delivery.

(g) Secretary means the Secretary of Health and Human Services and any other officer or employee of the Department of Health and Human Services to whom authority has been delegated.

(h) Viable, as it pertains to the neonate, means being able, after delivery, to survive (given the benefit of available medical therapy) to the point of independently maintaining heartbeat and respiration. The Secretary may from time to time, taking into account medical advances, publish in the FEDERAL REGISTER guidelines to assist in determining whether a neonate is viable for purposes of this subpart. If a neonate is viable then it may be included in research only to the extent permitted and in accordance with the requirements of subparts A and D of this part.

§46.203 Duties of IRBs in connection with research involving pregnant women, fetuses, and neonates.

In addition to other responsibilities assigned to IRBs under this part, each IRB shall review research covered by this subpart and approve only research which satisfies the conditions of all applicable sections of this subpart and the other subparts of this part.

§46.204 Research involving pregnant women or fetuses.

Pregnant women or fetuses may be involved in research if all of the following conditions are met:

(a) Where scientifically appropriate, preclinical studies, including studies on pregnant animals, and clinical studies, including studies on nonpregnant women, have been conducted and provide data for assessing potential risks to pregnant women and fetuses;

(b) The risk to the fetus is caused solely by interventions or procedures that hold out the prospect of direct benefit for the woman or the fetus; or, if there is no such prospect of benefit, the risk to the fetus is not greater than minimal and the purpose of the research is the development of important biomedical knowledge which cannot be obtained by any other means;

(c) Any risk is the least possible for achieving the objectives of the research;

(d) If the research holds out the prospect of direct benefit to the pregnant woman, the prospect of a direct benefit both to the pregnant woman and the fetus, or no prospect of benefit for the woman nor the fetus when risk to the fetus is not greater than minimal and the purpose of the research is the development of important biomedical knowledge that cannot be obtained by any other means, her consent is obtained in accord with the informed consent provisions of subpart A of this part;

(e) If the research holds out the prospect of direct benefit solely to the fetus then the consent of the pregnant woman and the father is obtained in accord with the informed consent provisions of subpart A of this part, except that the father's consent need not be obtained if he is unable to consent because of unavailability, incompetence, or temporary incapacity or the pregnancy resulted from rape or incest.

(f) Each individual providing consent under paragraph (d) or (e) of this section is fully informed regarding the reasonably foreseeable impact of the research on the fetus or neonate;

(g) For children as defined in §46.402(a) who are pregnant, assent and permission are obtained in accord with the provisions of subpart D of this part;

(h) No inducements, monetary or otherwise, will be offered to terminate a pregnancy;

(i) Individuals engaged in the research will have no part in any decisions as to the timing, method, or procedures used to terminate a pregnancy; and

(j) Individuals engaged in the research will have no part in determining the viability of a neonate.

§46.205 Research involving neonates.

(a) Neonates of uncertain viability and nonviable neonates may be involved in research if all of the following conditions are met:

(1) Where scientifically appropriate, preclinical and clinical studies have been conducted and provide data for assessing potential risks to neonates.

(2) Each individual providing consent under paragraph (b)(2) or (c)(5) of this section is fully informed regarding the reasonably foreseeable impact of the research on the neonate.

(3) Individuals engaged in the research will have no part in determining the viability of a neonate.

(4) The requirements of paragraph (b) or (c) of this section have been met as applicable.

(b) Neonates of uncertain viability. Until it has been ascertained whether or not a neonate is viable, a neonate may not be involved in research covered by this subpart unless the following additional conditions have been met:

(1) The IRB determines that:

(i) The research holds out the prospect of enhancing the probability of survival of the neonate to the point of viability, and any risk is the least possible for achieving that objective, or

(ii) The purpose of the research is the development of important biomedical knowledge which cannot be obtained by other means and there will be no added risk to the neonate resulting from the research; and

(2) The legally effective informed consent of either parent of the neonate or, if neither parent is able to consent because of unavailability, incompetence, or temporary incapacity, the legally effective informed consent of either parent's legally authorized representative is obtained in accord with subpart A of this part, except that the consent of the father or his legally authorized representative need not be obtained if the pregnancy resulted from rape or incest.

(c) Nonviable neonates. After delivery nonviable neonate may not be involved in research covered by this subpart unless all of the following additional conditions are met:

(1) Vital functions of the neonate will not be artificially maintained;

(2) The research will not terminate the heartbeat or respiration of the neonate;

(3) There will be no added risk to the neonate resulting from the research;

(4) The purpose of the research is the development of important biomedical knowledge that cannot be obtained by other means; and

(5) The legally effective informed consent of both parents of the neonate is obtained in accord with subpart A of this part, except that the waiver and alteration provisions of §46.116(c) and (d) do not apply. However, if either parent is unable to consent because of unavailability, incompetence, or temporary incapacity, the informed consent of one parent of a nonviable neonate will suffice to meet the requirements of this paragraph (c)(5), except that the consent of the father need not be obtained if the pregnancy resulted from rape or incest. The consent of a legally authorized representative of either or both of the parents of a nonviable neonate will not suffice to meet the requirements of this paragraph (c)(5).

(d) Viable neonates. A neonate, after delivery, that has been determined to be viable may be included in research only to the extent permitted by and in accord with the requirements of subparts A and D of this part.

§46.206 Research involving, after delivery, the placenta, the dead fetus or fetal material.

(a) Research involving, after delivery, the placenta; the dead fetus; macerated fetal material; or cells, tissue, or organs excised from a dead fetus, shall be conducted only in accord with any applicable federal, state, or local laws and regulations regarding such activities.

(b) If information associated with material described in paragraph (a) of this section is recorded for research purposes in a manner that living individuals can be identified, directly or through identifiers linked to those individuals, those individuals are research subjects and all pertinent subparts of this part are applicable.

§46.207 Research not otherwise approvable which presents an opportunity to understand, prevent, or alleviate a serious problem affecting the health or welfare of pregnant women, fetuses, or neonates.

The Secretary will conduct or fund research that the IRB does not believe meets the requirements of §46.204 or §46.205 only if:

(a) The IRB finds that the research presents

a reasonable opportunity to further the understanding, prevention, or alleviation of a serious problem affecting the health or welfare of pregnant women, fetuses or neonates; and

(b) The Secretary, after consultation with a panel of experts in pertinent disciplines (for example: science, medicine, ethics, law) and following opportunity for public review and comment, including a public meeting announced in the FEDERAL REGISTER, has determined either:

(1) That the research in fact satisfies the conditions of §46.204, as applicable; or

(2) The following:

(i) The research presents a reasonable opportunity to further the understanding, prevention, or alleviation of a serious problem affecting the health or welfare of pregnant women, fetuses or neonates;

(ii) The research will be conducted in accord with sound ethical principles; and

(iii) Informed consent will be obtained in accord with the informed consent provisions of subpart A and other applicable subparts of this part.

Subpart C

Additional Protections Pertaining to Biomedical and Behavioral Research Involving Prisoners as Subjects

Source: 43 FR 53655, Nov. 16, 1978, unless otherwise noted.

§46.301 Applicability.

(a) The regulations in this subpart are applicable to all biomedical and behavioral research conducted or supported by the Department of Health and Human Services involving prisoners as subjects.

(b) Nothing in this subpart shall be construed as indicating that compliance with the procedures set forth herein will authorize research involving prisoners as subjects, to the extent such research is limited or barred by applicable State or local law.

(c) The requirements of this subpart are in addition to those imposed under the other subparts of this part.

§46.302 Purpose.

Inasmuch as prisoners may be under constraints because of their incarceration which could affect their ability to make a truly voluntary and uncoerced decision whether or not to participate as subjects in research, it is the purpose of this subpart to provide additional safeguards for the protection of prisoners involved in activities to which this subpart is applicable.

§46.303 Definitions.

As used in this subpart:

(a) *Secretary* means the Secretary of Health and Human Services and any other officer or employee of the Department of Health and Human Services to whom authority has been delegated.

(b) *DHHS* means the Department of Health and Human Services.

(c) *Prisoner* means any individual involuntarily confined or detained in a penal institution. The term is intended to encompass individuals sentenced to such an institution under a criminal or civil statute, individuals detained in other facilities by virtue of statutes or commitment procedures which provide alternatives to criminal prosecution or incarceration in a penal institution, and individuals detained pending arraignment, trial, or sentencing.

(d) *Minimal risk* is the probability and magnitude of physical or psychological harm that is normally encountered in the daily lives, or in the routine medical, dental, or psychological examination of healthy persons.

§46.304 Composition of Institutional Review Boards where prisoners are involved.

In addition to satisfying the requirements in §46.107 of this part, an Institutional Review Board, carrying out responsibilities under this part with respect to research covered by this subpart, shall also meet the following specific requirements:

(a) A majority of the Board (exclusive of prisoner members) shall have no association with the prison(s) involved, apart from their membership on the Board.

(b) At least one member of the Board shall be a prisoner, or a prisoner representative with appropriate background and experience to serve in that capacity, except that where a particular research project is reviewed by more than one Board only one Board need satisfy this requirement.

[43 FR 53655, Nov. 16, 1978, as amended at 46 FR 8366, Jan. 26, 1981]

§46.305 Additional duties of the Institutional Review Boards where prisoners are involved.

(a) In addition to all other responsibilities prescribed for Institutional Review Boards under this part, the Board shall review research covered by this subpart and approve such research only if it finds that:

(1) The research under review represents one of the categories of research permissible under §46.306(a)(2);

(2) Any possible advantages accruing to the prisoner through his or her participation in the research, when compared to the general living conditions, medical care, quality of food, amenities and opportunity for earnings in the prison, are not of such a magnitude that his or her ability to weigh the risks of the research against the value of such advantages in the limited choice environment of the prison is impaired;

(3) The risks involved in the research are commensurate with risks that would be accepted by nonprisoner volunteers;

(4) Procedures for the selection of subjects within the prison are fair to all prisoners and immune from arbitrary intervention by prison authorities or prisoners. Unless the principal investigator provides to the Board justification in writing for following some other procedures, control subjects must be selected randomly from the group of available prisoners who meet the characteristics needed for that particular research project;

(5) The information is presented in language which is understandable to the subject population;

(6) Adequate assurance exists that parole boards will not take into account a prisoner's participation in the research in making decisions regarding parole, and each prisoner is clearly informed in advance that participation in the research will have no effect on his or her parole; and

(7) Where the Board finds there may be a need for follow-up examination or care of participants after the end of their participation, adequate provision has been made for such examination or care, taking into account the varying lengths of individual prisoners' sentences, and for informing participants of this fact.

(b) The Board shall carry out such other duties as may be assigned by the Secretary.

(c) The institution shall certify to the Secre-

tary, in such form and manner as the Secretary may require, that the duties of the Board under this section have been fulfilled.

§46.306 Permitted research involving prisoners.

(a) Biomedical or behavioral research conducted or supported by DHHS may involve prisoners as subjects only if:

(1) The institution responsible for the conduct of the research has certified to the Secretary that the Institutional Review Board has approved the research under §46.305 of this subpart; and

(2) In the judgment of the Secretary the proposed research involves solely the following:

(i) Study of the possible causes, effects, and processes of incarceration, and of criminal behavior, provided that the study presents no more than minimal risk and no more than inconvenience to the subjects;

(ii) Study of prisons as institutional structures or of prisoners as incarcerated persons, provided that the study presents no more than minimal risk and no more than inconvenience to the subjects;

(iii) Research on conditions particularly affecting prisoners as a class (for example, vaccine trials and other research on hepatitis which is much more prevalent in prisons than elsewhere; and research on social and psychological problems such as alcoholism, drug addiction, and sexual assaults) provided that the study may proceed only after the Secretary has consulted with appropriate experts including experts in penology, medicine, and ethics, and published notice, in the FEDERAL REGISTER, of his intent to approve such research; or

(iv) Research on practices, both innovative and accepted, which have the intent and reasonable probability of improving the health or well-being of the subject. In cases in which those studies require the assignment of prisoners in a manner consistent with protocols approved by the IRB to control groups which may not benefit from the research, the study may proceed only after the Secretary has consulted with appropriate experts, including experts in penology, medicine, and ethics, and published notice, in the FEDERAL REGISTER, of the intent to approve such research.

(b) Except as provided in paragraph (a) of this section, biomedical or behavioral research conducted or supported by DHHS shall not involve prisoners as subjects.

Subpart D

Additional Protections for Children Involved as Subjects in Research

Source: 48 FR 9818, March 8, 1983, unless otherwise noted.

§46.401 To what do these regulations apply?

(a) This subpart applies to all research involving children as subjects, conducted or supported by the Department of Health and Human Services.

(1) This includes research conducted by Department employees, except that each head of an Operating Division of the Department may adopt such nonsubstantive, procedural modifications as may be appropriate from an administrative standpoint.

(2) It also includes research conducted or supported by the Department of Health and Human Services outside the United States, but in appropriate circumstances, the Secretary may, under paragraph (i) of §46.101 of subpart A, waive the applicability of some or all of the requirements of these regulations for research of this type.

(b) Exemptions at §46.101(b)(1) and (b)(3) through (b)(6) are applicable to this subpart. The exemption at §46.101(b)(2) regarding educational tests is also applicable to this subpart. However, the exemption at §46.101 (b)(2) for research involving survey or interview procedures or observations of public behavior does not apply to research covered by this subpart, except for research involving observation of public behavior when the investigator(s) do not participate in the activities being observed.

(c) The exceptions, additions, and provisions for waiver as they appear in paragraphs (c) through (i) of §46.101 of subpart A are applicable to this subpart.

[48 FR 9818, Mar.8, 1983; 56 FR 28032, June 18, 1991; 56 FR 29757, June 28, 1991.]

§46.402 Definitions.

The definitions in §46.102 of subpart A shall be applicable to this subpart as well. In addition, as used in this subpart:

(a) *Children* are persons who have not attained the legal age for consent to treatments or procedures involved in the research, under the applicable law of the jurisdiction in which the research will be conducted.

(b) *Assent* means a child's affirmative agreement to participate in research. Mere failure to object should not, absent affirmative agreement, be construed as assent.

(c) *Permission* means the agreement of parent (s) or guardian to the participation of their child or ward in research.

(d) *Parent* means a child's biological or adoptive parent.

(e) *Guardian* means an individual who is authorized under applicable State or local law to consent on behalf of a child to general medical care.

§46.403 IRB duties.

In addition to other responsibilities assigned to IRBs under this part, each IRB shall review research covered by this subpart and approve only research which satisfies the conditions of all applicable sections of this subpart.

§46.404 Research not involving greater than minimal risk.

HHS will conduct or fund research in which the IRB finds that no greater than minimal risk to children is presented, only if the IRB finds that adequate provisions are made for soliciting the assent of the children and the permission of their parents or guardians, as set forth in §46.408.

§46.405 Research involving greater than minimal risk but presenting the prospect of direct benefit to the individual subjects.

HHS will conduct or fund research in which the IRB finds that more than minimal risk to children is presented by an intervention or procedure that holds out the prospect of direct benefit for the individual subject, or by a monitoring procedure that is likely to contribute to the subject's well-being, only if the IRB finds that:

(a) The risk is justified by the anticipated benefit to the subjects;

(b) The relation of the anticipated benefit to the risk is at least as favorable to the subjects as that presented by available alternative approaches; and

(c) Adequate provisions are made for soliciting the assent of the children and permission of their parents or guardians, as set forth in §46.408.

§46.406 Research involving greater than minimal risk and no prospect of direct benefit to individual subjects, but likely to yield generalizable knowledge about the subject's disorder or condition.

HHS will conduct or fund research in which the IRB finds that more than minimal risk to children is presented by an intervention or procedure that does not hold out the prospect of direct benefit for the individual subject, or by a monitoring procedure which is not likely to contribute to the well-being of the subject, only if the IRB finds that:

(a) The risk represents a minor increase over minimal risk;

(b) The intervention or procedure presents experiences to subjects that are reasonably commensurate with those inherent in their actual or expected medical, dental, psychological, social, or educational situations;

(c) The intervention or procedure is likely to yield generalizable knowledge about the subjects' disorder or condition which is of vital importance for the understanding or amelioration of the subjects' disorder or condition; and

(d) Adequate provisions are made for soliciting assent of the children and permission of their parents or guardians, as set forth in §46.408.

§46.407 Research not otherwise approvable which presents an opportunity to understand, prevent, or alleviate a serious problem affecting the health or welfare of children.

HHS will conduct or fund research that the IRB does not believe meets the requirements of §46.404, §46.405, or §46.406 only if:

(a) the IRB finds that the research presents a reasonable opportunity to further the understanding, prevention, or alleviation of a serious problem affecting the health or welfare of children; and

(b) the Secretary, after consultation with a panel of experts in pertinent disciplines (for example: science, medicine, education, ethics, law) and following opportunity for public review and comment, has determined either:

(1) that the research in fact satisfies the conditions of §46.404, §46.405, or §46.406, as applicable, or (2) the following:

(i) the research presents a reasonable opportunity to further the understanding, prevention, or alleviation of a serious problem affecting the health or welfare of children;

(ii) the research will be conducted in accordance with sound ethical principles;

(iii) adequate provisions are made for soliciting the assent of children and the permission of their parents or guardians, as set forth in §46.408.

§46.408 Requirements for permission by parents or guardians and for assent by children.

(a) In addition to the determinations required under other applicable sections of this subpart, the IRB shall determine that adequate provisions are made for soliciting the assent of the children, when in the judgment of the IRB the children are capable of providing assent. In determining whether children are capable of assenting, the IRB shall take into account the ages, maturity, and psychological state of the children involved. This judgment may be made for all children to be involved in research under a particular protocol, or for each child, as the IRB deems appropriate. If the IRB determines that the capability of some or all of the children is so limited that they cannot reasonably be consulted or that the intervention or procedure involved in the research holds out a prospect of direct benefit that is important to the health or well-being of the children and is available only in the context of the research, the assent of the children is not a necessary condition for proceeding with the research. Even where the IRB determines that the subjects are capable of assenting, the IRB may still waive the assent requirement under circumstances in which consent may be waived in accord with §46.116 of Subpart A.

(b) In addition to the determinations required under other applicable sections of this subpart, the IRB shall determine, in accordance with and to the extent that consent is required by §46.116 of Subpart A, that adequate provisions are made for soliciting the permission of each child's parents or guardian. Where parental permission is to be obtained, the IRB may find that the permission of one parent is sufficient for research to be conducted under §46.404 or §46.405. Where research is covered by §§46.406 and 46.407 and permission is to be obtained from parents, both parents must give their permission unless one parent is deceased, unknown, incompetent, or not reasonably available, or when only one parent has legal responsibility for the care and custody of the child.

(c) In addition to the provisions for waiver contained in §46.116 of subpart A, if the IRB determines that a research protocol is designed for conditions or for a subject population for which parental or guardian permission is not a reasonable requirement to protect the subjects (for example, neglected or abused children), it may waive the consent requirements in Subpart A of this part and paragraph (b) of this section, provided an appropriate mechanism for protecting the children who will participate as subjects in the research is substituted, and provided further that the waiver is not inconsistent with federal, state, or local law. The choice of an appropriate mechanism would depend upon the nature and purpose of the activities described in the protocol, the risk and anticipated benefit to the research subjects, and their age, maturity, status, and condition.

(d) Permission by parents or guardians shall be documented in accordance with and to the extent required by §46.117 of subpart A.

(e) When the IRB determines that assent is required, it shall also determine whether and how assent must be documented.

§46.409 Wards.

(a) Children who are wards of the state or any other agency, institution, or entity can be included in research approved under §46.406 or §46.407 only if such research is:

(1) Related to their status as wards; or

(2) Conducted in schools, camps, hospitals, institutions, or similar settings in which the majority of children involved as subjects are not wards.

(b) If the research is approved under paragraph (a) of this section, the IRB shall require appointment of an advocate for each child who is a ward, in addition to any other individual acting on behalf of the child as guardian or in loco parentis. One individual may serve as advocate for more than one child. The advocate shall be an individual who has the background and experience to act in, and agrees to act in, the best interests of the child for the duration of the child's participation in the research and who is not associated in any way (except in the role as advocate or member of the IRB) with the research, the investigator(s), or the guardian organization.

Subpart E

Registration of Institutional Review Boards

Source: 74 FR 2399, January 15, 2009, unless otherwise noted.

§46.501 What IRBs must be registered?

Each IRB that is designated by an institution under an assurance of compliance approved for federalwide use by the Office for Human Research Protections (OHRP) under §46.103(a) and that reviews research involving human subjects conducted or supported by the Department of Health and Human Services (HHS) must be registered with HHS. An individual authorized to act on behalf of the institution or organization operating the IRB must submit the registration information.

§46.502 What information must be provided when registering an IRB?

The following information must be provided to HHS when registering an IRB:

(a) The name, mailing address, and street address (if different from the mailing address) of the institution or organization operating the IRB(s); and the name, mailing address, phone number, facsimile number, and electronic mail address of the senior officer or head official of that institution or organization who is responsible for overseeing activities performed by the IRB.

(b) The name, mailing address, phone number, facsimile number, and electronic mail address of the contact person providing the registration information.

(c) The name, if any, assigned to the IRB by the institution or organization, and the IRB's mailing address, street address (if different from the mailing address), phone number, facsimile number, and electronic mail address.

(d) The name, phone number, and electronic mail address of the IRB chairperson.

(e)(1) The approximate numbers of:

(i) All active protocols; and

(ii) Active protocols conducted or supported by HHS.

(2) For purpose of this regulation, an ``active protocol'' is any protocol for which the IRB conducted an initial review or a continuing review at a convened meeting or under an expedited review procedure during the preceding twelve months.

(f) The approximate number of full-time equivalent positions devoted to the IRB's administrative activities.

§46.503 When must an IRB be registered?

An IRB must be registered before it can be designated under an assurance approved for federalwide use by OHRP under §46.103(a).

IRB registration becomes effective when reviewed and accepted by OHRP.

The registration will be effective for 3 years.

§46.504 How must an IRB be registered?

Each IRB must be registered electronically through http://ohrp.cit.nih.gov/efile unless an institution or organization lacks the ability to register its IRB(s) electronically. If an institution or organization lacks the ability to register an IRB electronically, it must send its IRB registration information in writing to OHRP.

§46.505 When must IRB registration information be renewed or updated?

(a) Each IRB must renew its registration every 3 years.

(b) The registration information for an IRB must be updated within 90 days after changes occur regarding the contact person who provided the IRB registration information or the IRB chairperson. The updated registration information must be submitted in accordance with §46.504.

(c) Any renewal or update that is submitted to, and accepted by, OHRP begins a new 3-year effective period.

(d) An institution's or organization's decision to disband a registered IRB which it is operating also must be reported to OHRP in writing within 30 days after permanent cessation of the IRB's review of HHS-conducted or -supported research.

National Bioethics Advisory Commission

Ethical Issues in Human Stem Cell Research

VOLUME I

Report and Recommendations of the National Bioethics Advisory Commission

Rockville, Maryland
September 1999

JA069

Ethical Issues in Human Stem Cell Research—Volume I

The National Bioethics Advisory Commission (NBAC) was established by Executive Order 12975, signed by President Clinton on October 3, 1995. NBAC's functions are defined as follows:

a) NBAC shall provide advice and make recommendations to the National Science and Technology Council and to other appropriate government entities regarding the following matters:

   1) the appropriateness of departmental, agency, or other governmental programs, policies, assignments, missions, guidelines, and regulations as they relate to bioethical issues arising from research on human biology and behavior; and

   2) applications, including the clinical applications, of that research.

b) NBAC shall identify broad principles to govern the ethical conduct of research, citing specific projects only as illustrations for such principles.

c) NBAC shall not be responsible for the review and approval of specific projects.

d) In addition to responding to requests for advice and recommendations from the National Science and Technology Council, NBAC also may accept suggestions of issues for consideration from both the Congress and the public. NBAC also may identify other bioethical issues for the purpose of providing advice and recommendations, subject to the approval of the National Science and Technology Council.

National Bioethics Advisory Commission
6100 Executive Boulevard, Suite 5B01, Rockville, Maryland 20892-7508
Telephone: 301-402-4242 • Fax: 301-480-6900 • Website: *www.bioethics.gov*

Ethical Issues in Human Stem Cell Research

VOLUME I
Report and Recommendations of the National Bioethics Advisory Commission

Rockville, Maryland
September 1999

JA071

Table of Contents

NATIONAL BIOETHICS ADVISORY COMMISSION

6100 Executive Blvd
Suite 5B01
Rockville, MD
20892-7508

Telephone
301-402-4242
Facsimile
301-480-6900
Website
www.bioethics.gov

The President
The White House
Washington, DC 20500

September 7, 1999

Dear Mr. President:

On November 14, 1998, you wrote to the National Bioethics Advisory Commission requesting that we "conduct a thorough review of the issues associated with...human stem cell research, balancing all medical and ethical issues." Your request came in response to reports of the successful isolation and culture of these specialized cells, which have simultaneously offered hope of new cures to debilitating and even fatal illness while renewing an important national debate about the ethics of research involving human embryos and cadaveric fetal material. After nine months of careful study, I am pleased to inform you that we have completed our deliberations and now provide you with the Commission's report, *Ethical Issues in Human Stem Cell Research.*

The Commission considered a broad set of scientific, medical, and legal issues, but focused particular attention on the ethical questions relevant to federal sponsorship of research involving human embryonic stem (ES) cells and embryonic germ (EG) cells. In our deliberations, we benefited greatly from the testimony of scientists, religious scholars, ethicists, lawyers, and the public, which testimony fully reflected the wide diversity of moral perspectives on these issues that characterizes our nation. Although wide agreement exists that human embryos deserve respect as a form of human life, there is disagreement both on the form such respect should take and on the level of protection owed at different stages of embryonic development. Moreover, it was clear from the outset that no public policy or set of recommendations could fully bridge these disagreements and satisfy all the thoughtful moral perspectives that are held by members of the American public.

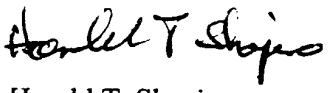
The Commission proposes 13 recommendations in several areas. Perhaps the most important recommendations reflect the Commission's view that federal sponsorship of research that involves the derivation and use of human embryonic stem (ES) cells and human embryonic germ (EG) cells should be limited in two ways. First, such research should be limited to using only two of the current sources of such cells; namely, cadaveric fetal material and embryos remaining after infertility treatments. Second, that such sponsorship be contingent on an appropriate and open system of national oversight and review.

Other recommendations address the requirements for consent from women or from couples who donate cadaveric fetal tissue or embryos remaining following infertility treatments; restrictions on the sale of fetal tissue or embryos and limits on the designation of those who may benefit from their use; the role of federal agencies in the review of research; and the encouragement of the private sector to comply with the same requirements recommended for federally funded researchers.

Taken together, we believe that these recommendations offer a policy framework that will provide the public with the assurance that important, potentially life-saving research can be conducted with federal sponsorship within a publicly accountable and rigorous system of oversight and review.

I would like to thank my fellow Commissioners, whose spirit of public service enabled them to work tirelessly to ensure that our report and its recommendations fully respected the moral worth of a wide variety of thoughtful viewpoints on the issues before us. We appreciate the opportunity to submit this report to you.

Sincerely,



Harold T. Shapiro
Chair

National Bioethics Advisory Commission

*To avoid the appearance of a conflict of interest, Commissioner Charo recused herself from all Commission deliberations as of February 1, 1999. She neither dissents from nor endorses this report and its recommendations.

**To avoid the appearance of a conflict of interest, Commissioner Greider recused herself from Commission deliberations as of July 19, 1999.

National Bioethics Advisory Commission Staff and Consultants

Executive Director
Eric M. Meslin, Ph.D.

Research Staff

Kathi E. Hanna, M.S., Ph.D., *Research Director*
Emily C. Feinstein, *Research Analyst**
Melissa Goldstein, J.D., *Research Analyst**
E. Randolph Hull, Jr., *Research Analyst**
J. Kyle Kinner, J.D., M.P.A., *Presidential Management Intern*
Kerry Jo Lee, *Intern*
Debra McCurry, M.S., *Information Specialist*
Daniel J. Powell, *Intern*
Andrew Siegel, Ph.D., J.D., *Staff Philosopher***
Sean A. Simon, *Research Analyst**
Robert Tanner, J.D., *Research Analyst*

Consultants

Burness Communications, *Communications Consultant*
Sara Davidson, M.A., *Editor*
Elisa Eiseman, Ph.D., *Science Consultant*
Jeffrey P. Kahn, Ph.D., M.P.H., *Bioethics Consultant*
Tamara Lee, *Graphic Designer*
LeRoy Walters, Ph.D., *Bioethics Consultant*

Administrative Staff

Jody Crank, *Secretary to the Executive Director***
Evadne Hammett, *Administrative Officer*
Patricia Norris, *Public Affairs Officer*
Lisa Price, *Secretary***
Margaret C. Quinlan, *Office Manager*
Sherrie Senior, *Secretary*

*Until May 1999
**Until July 1999

Executive Summary

Introduction

In November 1998, President Clinton charged the National Bioethics Advisory Commission with the task of conducting a thorough review of the issues associated with human stem cell research, balancing all ethical and medical considerations. The President's request was made in response to three separate reports that brought to the fore the exciting scientific and clinical prospects of stem cell research while also raising a series of ethical controversies regarding federal sponsorship of scientific inquiry in this area. Scientific reports of the successful isolation and culture of these specialized cells have offered hope of new cures for debilitating and even fatal illness and at the same time have renewed an important national debate about the ethics of research involving human embryos and cadaveric fetal material.

Scientific and Medical Considerations

The stem cell is a unique and essential cell type found in animals. Many kinds of stem cells are found in the body, with some more differentiated, or committed, to a particular function than others. In other words, when stem cells divide, some of the progeny mature into cells of a specific type (e.g., heart, muscle, blood, or brain cells), while others remain stem cells, ready to repair some of the everyday wear and tear undergone by our bodies. These stem cells are capable of continually reproducing themselves and serve to renew tissue throughout an individual's life. For example, they constantly regenerate the lining of the gut, revitalize skin, and produce a whole range of blood cells. Although the term *stem cell* commonly is used to refer to the cells within the adult organism that renew tissue (e.g., hematopoietic stem cells, a type of cell found in the blood), the most fundamental and extraordinary of the stem cells are found in the early stage embryo. These *embryonic stem (ES) cells*, unlike the more differentiated adult stem cells or other cell types, retain the special ability to develop into nearly any cell type. *Embryonic germ (EG) cells*, which originate from the primordial reproductive cells of the developing fetus, have properties similar to ES cells.

It is the potentially unique versatility of the ES and EG cells derived, respectively, from the early stage embryo and cadaveric fetal tissue that presents such unusual scientific and therapeutic promise. Indeed, scientists have long recognized the possibility of using such cells to generate more specialized cells or tissue, which could allow the generation of new cells to be used to treat injuries or diseases, such as Alzheimer's disease, Parkinson's disease, heart disease, and kidney failure. Likewise, scientists regard these cells as an important—perhaps essential—means for understanding the earliest stages of human development and as an important tool in the development of life-saving drugs and cell-replacement therapies to treat disorders caused by early cell death or impairment.

The techniques for deriving these cells have not been fully developed as standardized and readily available research tools, and the development of any therapeutic application remains some years away. Thus, ES and EG cells are still primarily a matter of intense research interest.

At this time, human stem cells can be derived from the following sources:

- human fetal tissue following elective abortion (EG cells),

- human embryos that are created by *in vitro* fertilization (IVF) and that are no longer needed by couples being treated for infertility (ES cells),
- human embryos that are created by IVF with gametes donated for the sole purpose of providing research material (ES cells), and
- potentially, human (or hybrid) embryos generated asexually by somatic cell nuclear transfer or similar cloning techniques in which the nucleus of an adult human cell is introduced into an enucleated human or animal ovum (ES cells).

In addition, although much promising research currently is being conducted with stem cells obtained from adult organisms, studies in animals suggest that this approach will be scientifically and technically limited, and in some cases the anatomic source of the cells might preclude easy or safe access. However, because there are no legal restrictions or new ethical considerations regarding research on adult stem cells (other than the usual concerns about consent and risks), important research can and should go forward in this area. Moreover, because important biological differences exist between embryonic and adult stem cells, this source of stem cells should not be considered an alternative to ES and EG cell research.

Ethical and Policy Considerations

The scientific reports of the successful isolation and culture of ES and EG cells have renewed a longstanding controversy about the ethics of research involving human embryos and cadaveric fetal material. This controversy arises from sharply differing moral views regarding elective abortion or the use of embryos for research. Indeed, an earnest national and international debate continues over the ethical, legal, and medical issues that arise in this arena. This debate represents both a challenge and an opportunity: a challenge because it concerns important and morally contested questions regarding the beginning of life, and an opportunity because it provides another occasion for serious public discussion about important ethical issues. We are hopeful that this dialogue will foster public understanding about the relationships between the opportunities that biomedical science offers to improve human welfare and the limits set by important ethical obligations.

Although we believe most would agree that human embryos deserve respect as a form of human life, disagreements arise regarding both what form such respect should take and what level of protection is required at different stages of embryonic development. Therefore, embryo research that is not therapeutic to the embryo is bound to raise serious concerns and to heighten the tensions between two important ethical commitments: to cure disease and to protect human life. For those who believe that the embryo has the moral status of a person from the moment of conception, research (or any other activity) that would destroy the embryo is considered wrong and should not take place. For those who believe otherwise, arriving at an ethically acceptable policy in this arena involves a complex balancing of a number of important ethical concerns. Although many of the issues remain contested on moral grounds, they co-exist within a broad area of consensus upon which public policy can, at least in part, be constructed.

For most observers, the resolution of these ethical and scientific issues depends to some degree on the source of the stem cells. The use of cadaveric fetal tissue to derive EG cell lines—like other uses of tissues or organs from dead bodies—is generally the most accepted, provided that the research complies with the system of public safeguards and oversight already in place for such scientific inquiry. With respect to embryos and the ES cells from which they can be derived, some draw an ethical distinction between two types of embryos. One is referred to as the *research embryo*, an embryo created through IVF with gametes provided solely for research purposes. Many people, including the President, have expressed the view that the federal government should not fund research that involves creating such embryos. The second type of embryo is that which was created for infertility treatment, but is now intended to be discarded because it is unsuitable or no longer needed for such treatment. The use of these embryos raises fewer ethical questions because it does not alter their final disposition. Finally, the recent demonstration of cloning techniques (somatic cell nuclear transfer) in nonhuman animals suggests that transfer of a human somatic cell nucleus into an oocyte

might create an embryo that could be used as a source of ES cells. The creation of a human organism using this technique raises questions similar to those raised by the creation of research embryos through IVF, and at this time federal funds may not be used for such research. In addition, if the enucleated oocyte that was to be combined with a human somatic cell nucleus came from an animal other than a human being, other issues would arise about the nature of the embryo produced. Thus, each source of material raises ethical questions as well as scientific, medical, and legal ones.

Conscientious individuals have come to different conclusions regarding both public policy and private actions in the area of stem cell research. Their differing perspectives by their very nature cannot easily be bridged by any single public policy. But the development of public policy in a morally contested area is not a novel challenge for a pluralistic democracy such as that which exists in the United States. We are profoundly aware of the diverse and strongly held views on the subject of this report and have wrestled with the implications of these different views at each of our meetings devoted to this topic. Our aim throughout these deliberations has been to formulate a set of recommendations that fully reflects widely shared views and that, in our view, would serve the best interests of society.

Most states place no legal restrictions on any of the means of creating ES and EG cells that are described in this report. In addition, current Food and Drug Administration regulations do not apply to this type of early stage research. Therefore, because the public controversy surrounding such activities in the United States has revolved around whether it is appropriate for the federal government to sponsor such research, this report focuses on the question of whether the scientific merit and the substantial clinical promise of this research justify federal support, and, if so, with what restrictions and safeguards.

Conclusions and Recommendations

This report presents the conclusions that the Commission has reached and the recommendations that the Commission has made in the following areas: the ethical acceptability of federal funding for research that either derives or uses ES or EG cells; the means of ensuring appropriate consent of women or couples who donate cadaveric fetal tissue or embryos remaining after infertility treatments; the need for restrictions on the sale of these materials and the designation of those who may benefit from their use; the need for ethical oversight and review of such research at the national and institutional level; and the appropriateness of voluntary compliance by the private sector with some of these recommendations.

The Ethical Acceptability of Federal Funding of ES and EG Cell Research by the Source of the Material

A principal ethical justification for public sponsorship of research with human ES or EG cells is that this research has the potential to produce health benefits for individuals who are suffering from serious and often fatal diseases. We recognize that it is possible that the various sources of human ES or EG cells eventually could be important to research and clinical application because of, for example, their differing proliferation potential, differing availability and accessibility, and differing ability to be manipulated, as well as possibly significant differences in their cell biology. **At this time, therefore, the Commission believes that federal funding for the use and derivation of ES and EG cells should be limited to two sources of such material: cadaveric fetal tissue and embryos remaining after infertility treatments.** Specific recommendations and their justifications are provided below.

Recommendation 1: EG Cells from Fetal Tissue

Research involving the derivation and use of human EG cells from cadaveric fetal tissue should continue to be eligible for federal funding. Relevant statutes and regulations should be amended to make clear that the ethical safeguards that exist for fetal tissue transplantation also apply to the derivation and use of human EG cells for research purposes.

Considerable agreement exists, both in the United States and throughout the world, that the use of fetal tissue in therapy for people with serious disorders, such

as Parkinson's disease, is acceptable. Research that uses tissue from aborted fetuses is analogous to the use of fetal tissue in transplantation. The rationales for conducting EG research are equally strong, and the arguments against it are not persuasive. The removal of fetal germ cells does not occasion the destruction of a live fetus, nor is fetal tissue intentionally or purposefully created for human stem cell research. Although abortion itself doubtless will remain a contentious issue in our society, the procedures that have been developed to prevent fetal tissue donation for therapeutic transplantation from influencing the abortion decision offer a model for creating such separation in research to derive human EG cells. Because the existing statutes are written in terms of tissue transplantation, which is not a current feature of EG cell research, changes are needed to make it explicit that the relevant safeguards will apply to research to derive EG cells from aborted fetuses. At present, no legal prohibitions exist that would inhibit the use of such tissue for EG cell research.

Recommendation 2: ES Cells from Embryos Remaining After Infertility Treatments

Research involving the derivation and use of human ES cells from embryos remaining after infertility treatments should be eligible for federal funding. An exception should be made to the present statutory ban on federal funding of embryo research to permit federal agencies to fund research involving the derivation of human ES cells from this source under appropriate regulations that include public oversight and review. (See Recommendations 5 through 9.)

The current ban on embryo research is in the form of a rider to the appropriations bill for the Department of Health and Human Services (DHHS), of which the National Institutes of Health (NIH) is a part. The rider prohibits use of the appropriated funds to support any research "in which a human embryo [is] destroyed, discarded, or knowingly subjected to risk of injury greater than that allowed for research on fetuses *in utero*" (Pub. L. No. 105-78, 513(a)). The term "human embryo" in the statute is defined as "any organism . . . that is derived by fertilization, parthenogenesis, cloning, or any other means from one or more human gametes or human diploid cells." The ban is revisited each year when the language of the NIH appropriations bill is considered.

The ban, which concerns only federally sponsored research, reflects a moral point of view either that embryos deserve the full protection of society because of their moral status as persons or that there is sufficient public controversy to preclude the use of federal funds for this type of research. At the same time, however, some effects of the embryo research ban raise serious moral and public policy concerns for those who hold differing views regarding the ethics of embryo research. In our view, the ban conflicts with several of the ethical goals of medicine and related health disciplines, especially healing, prevention, and research. These goals are rightly characterized by the principles of beneficence and nonmaleficence, which jointly encourage pursuing social benefits and avoiding or ameliorating potential harm.

Although some may view the derivation and use of ES cells as ethically distinct activities, we do not believe that these differences are significant from the point of view of eligibility for federal funding. That is, we believe that it is ethically acceptable for the federal government to finance research that both derives cell lines from embryos remaining after infertility treatments and that uses those cell lines. Although one might argue that some important research could proceed in the absence of federal funding for research that derives stem cells from embryos remaining after infertility treatments (i.e., federally funded scientists merely using cells derived with private funds), we believe that it is important that federal funding be made available for protocols that also derive such cells. Relying on cell lines that might be derived exclusively by a subset of privately funded researchers who are interested in this area could severely limit scientific and clinical progress.

Trying to separate research in which human ES cells are used from the process of deriving those cells presents an ethical problem, because doing so diminishes the scientific value of the activities receiving federal support. This separation—under which neither biomedical researchers at NIH nor scientists at universities and other research institutions that rely on federal support could participate in some aspects of this research—rests on the

mistaken notion that the two areas of research are so distinct that participating in one need not mean participating in the other. We believe that this is a misrepresentation of the new field of human stem cell research, and this misrepresentation could adversely affect scientific progress for several reasons.

First, researchers using human ES cell lines will derive substantial scientific benefits from a detailed understanding of the process of ES cell derivation, because the properties of ES cells and the methods for sustaining the cell lines may differ depending on the conditions and methods that were used to derive them. Thus, scientists who conduct basic research and are interested in fundamental cellular processes are likely to make elemental discoveries about the nature of ES cells as they derive them in the laboratory. Second, significant basic research needs to be conducted regarding the process of ES cell derivation before cell-based therapies can be realized, and this work must be pursued in a wide variety of settings, including those exclusively devoted to basic academic research. Third, ES cells are not indefinitely stable in culture. As these cells are grown, irreversible changes occur in their genetic makeup. Thus, especially in the first few years of human ES cell research, it is important to be able to repeatedly derive ES cells in order to ensure that the properties of the cells that are being studied have not changed.

Thus, anyone who believes that federal support of this important new field of research should maximize its scientific and clinical value within a system of appropriate ethical oversight should be dissatisfied with a position that allows federal agencies to fund research using human ES cells but not research through which the cells are derived from embryos. Instead, recognizing the close connection in practical and ethical terms between derivation and use of the cells, it would be preferable to enact provisions applicable to funding by all federal agencies, provisions that would carve out a narrow exception for funding of research to use or to derive human ES cells from embryos that are being discarded by infertility treatment programs.

Recommendation 3: ES Cells from Embryos Made Solely for Research Purposes Using IVF

Federal agencies should not fund research involving the derivation or use of human ES cells from embryos made solely for research purposes using IVF.

ES cells can be obtained from human research embryos created from donor gametes through IVF for the sole purpose of deriving such cells for research. The primary objection to creating embryos specifically for research is that there is a morally relevant difference between generating an embryo for the sole purpose of creating a child and producing an embryo with no such goal. Those who object to creating embryos for research often appeal to arguments about respecting human dignity by avoiding instrumental use of human embryos (i.e., using embryos merely as a means to some other goal does not treat them with appropriate respect or concern as a form of human life).

In 1994, the NIH Human Embryo Research Panel argued in support of federal funding of the creation of embryos for research purposes in exceptional cases, such as the need to create banks of cell lines with different genetic make-ups that encoded various transplantation antigens—the better to respond, for example, to the transplant needs of groups with different genetic profiles. This would require the recruitment of embryos from genetically diverse donors.

In determining how to deal with this issue, a number of points are worth considering. First, it is possible that the creation of research embryos will provide the only way in which to conduct certain kinds of research, such as research into the process of human fertilization. Second, as IVF techniques improve, it is possible that the supply of embryos for research from this source will dwindle. Nevertheless, we have concluded that, either from a scientific or a clinical perspective, there is no compelling reason at this time to provide federal funds for the creation of embryos for research. At the current time, cadaveric fetal tissue and embryos remaining after infertility treatment provide an adequate supply of research resources for federal research projects.

Recommendation 4: ES Cells from Embryos Made Using Somatic Cell Nuclear Transfer into Oocytes

Federal agencies should not fund research involving the derivation or use of human ES cells from embryos made using somatic cell nuclear transfer into oocytes.

Somatic cell nuclear transfer of the nucleus of an adult somatic cell into an enucleated human egg likely

has the potential of creating a human embryo. To date, although little is known about these embryos as potential sources of human ES cells, there is significant reason to believe that their use may have therapeutic potential. For example, the potential use of matched tissue for autologous cell replacement therapy from ES cells may require the use of somatic cell nuclear transfer. The use of this technique to create an embryo arguably is different from all the other cases we considered—due to the asexual origin of the source of the ES cells—although oocyte donation is necessarily involved. The Commission concludes that, at this time, federal funding should not be provided to derive ES cells from this source. Nevertheless, scientific progress and the medical utility of this line of research should be monitored closely.

Requirements for the Donation of Cadaveric Fetal Tissue and Embryos for Research

Potential donors of embryos for ES cell research must be able to make voluntary and informed choices about whether and how to dispose of their embryos. Because of concerns about coercion and exploitation of potential donors, as well as societal controversy about the moral status of embryos, it is important, whenever possible, to separate donors' decisions to dispose of their embryos from their decisions to donate them for research. Potential donors should be asked to provide embryos for research only if they have decided to have those embryos discarded instead of donating them to another couple or storing them. If the decision to discard the embryos precedes the decision to donate them for research purposes, then the research determines only how their destruction occurs, not whether it occurs.

Recommendation 5: Requirements for Donation to Stem Cell Research of Embryos That Would Otherwise Be Discarded After Infertility Treatment

Prospective donors of embryos remaining after infertility treatments should receive timely, relevant, and appropriate information to make informed and voluntary choices regarding disposition of the embryos. Prior to considering the potential research use of the embryos, a prospective donor should have been presented with the option of storing the embryos, donating them to another woman, or discarding them. If a prospective donor chooses to discard embryos remaining after infertility treatment, the option of donating to research may then be presented. (At any point, the prospective donors' questions—including inquiries about possible research use of any embryos remaining after infertility treatment—should be answered truthfully, with all information that is relevant to the questions presented.)

During the presentation about potential research use of embryos that would otherwise be discarded, the person seeking the donation should

- **a) disclose that the ES cell research is not intended to provide medical benefit to embryo donors,**
- **b) make clear that consenting or refusing to donate embryos to research will not affect the quality of any future care provided to prospective donors,**
- **c) describe the general area of the research to be carried out with the embryos and the specific research protocol, if known,**
- **d) disclose the source of funding and expected commercial benefits of the research with the embryos, if known,**
- **e) make clear that embryos used in research will not be transferred to any woman's uterus, and**
- **f) make clear that the research will involve the destruction of the embryos.**

To assure that inappropriate incentives do not enter into a woman's decision to have an abortion, we recommend that directed donation of cadaveric fetal tissue for EG cell derivation be prohibited. Although the ethical considerations supporting a prohibition of the directed donation of human fetal tissue are less acute for EG cell research than for transplantation, certain concerns remain. Potential donors of cadaveric fetal tissue for EG cell derivation would not receive a direct therapeutic incentive to create or abort tissue for research purposes in the same way that such personal interest might arise in a transplant context. However, we agree that the prohibition remains a prudent and appropriate way of assuring that inappropriate incentives, regardless of how remote they may be, are not introduced into a woman's decision to have an abortion. Any suggestion of personal benefit to the donor or to an individual known to the donor would be untenable and possibly coercive.

Recommendation 6: No Promises to Embryo Donors That Stem Cells Will Be Provided to Particular Patient-Subjects

In federally funded research involving embryos remaining after infertility treatments, researchers may not promise donors that ES cells derived from their embryos will be used to treat patient-subjects specified by the donors.

Existing rules prohibit the practice of designated donation, the provision of monetary inducements to women undergoing abortion, and the purchase or sale of fetal tissue. We concur in these restrictions and in the earlier recommendation of the 1988 Human Fetal Tissue Transplantation Research Panel that the sale of fetal tissue for research purposes should not be permitted under any circumstances. The potential for coercive pressure is greatest when financial incentives are present, and the treatment of the developing human embryo or fetus as an entity deserving of respect may be greatly undermined by the introduction of any commercial motive into the donation or solicitation of fetal or embryonic tissue for research purposes.

Recommendation 7: Commerce in Embryos and Cadaveric Fetal Tissue

Embryos and cadaveric fetal tissue should not be bought or sold.

If and when sufficient scientific evidence and societal agreement exist that the creation of embryos specifically for research or therapeutic purposes is justified (specifically through somatic cell nuclear transfer), prohibitions on directed donation should be revisited. For obvious reasons, the use of somatic cell nuclear transfer to develop ES cells for autologous transplantation might require that the recipient be specified.

The Need for National Oversight and Review

The need for national as well as local oversight and review of human stem cell research is crucial. No such system currently exists in the United States. A national mechanism to review protocols for *deriving* human ES and EG cells and to monitor research using such cells would ensure strict adherence to guidelines and standards across the country. Thus, federal oversight can provide the public with the assurance that research involving stem cells is being undertaken appropriately. Given the ethical issues involved in human stem cell research—an area in which heightened sensitivity about the very research itself led the President to request that the Commission study the issue—the public and the Congress must be assured that oversight can be accomplished efficiently, constructively, and in a timely fashion, with sufficient attention to the relevant ethical considerations.

Recommendation 8: Creation and Duties of an Oversight and Review Panel

DHHS should establish a National Stem Cell Oversight and Review Panel to ensure that all federally funded research involving the derivation and/or use of human ES or EG cells is conducted in conformance with the ethical principles and recommendations contained in this report. The panel should have a broad, multidisciplinary membership, including members of the general public, and should

a) review protocols for the derivation of ES and EG cells and approve those that meet the requirements described in this report,

b) certify ES and EG cells lines that result from approved protocols,

c) maintain a public registry of approved protocols and certified ES and EG cell lines,

d) establish a database—linked to the public registry—consisting of information submitted by federal research sponsors (and, on a voluntary basis, by private sponsors, whose proprietary information shall be appropriately protected) that includes all protocols that derive or use ES or EG cells (including any available data on research outcomes, including published papers),

e) use the database and other appropriate sources to track the history and ultimate use of certified cell lines as an aid to policy assessment and formulation,

f) establish requirements for and provide guidance to sponsoring agencies on the social and ethical issues that should be considered in the review of research protocols that derive or use ES or EG cells, and

g) report at least annually to the DHHS Secretary with an assessment of the current state of the science for both the derivation and use of human ES and EG cells, a review of recent developments in the broad category of stem cell research, a summary of any emerging ethical or social concerns associated with this research, and an analysis of the adequacy and continued appropriateness of the recommendations contained in this report.

The Need for Local Review of Derivation Protocols

For more than two decades, prospective review by an Institutional Review Board (IRB) has been the principal method for assuring that federally sponsored research involving human subjects will be conducted in compliance with guidelines, policies, and regulations designed to protect human beings from harm. This system of local review has been subject to criticism, and, indeed, in previous analyses we have identified a number of concerns regarding this system. In the course of preparing this report, we considered a number of proposals that would allow for the local review of research protocols involving human stem cell research, bearing in mind that a decision by the Commission to recommend a role for IRBs might be incorrectly interpreted as endorsing the view that human ES or EG cells or human embryos are human subjects and therefore would be under the purview of the Common Rule.

We adopted the principle, reflected in these recommendations, that for research to derive human ES and EG cells, a system of national oversight and review supplemented by local review would be necessary to ensure that important research could proceed—but only under specific conditions. We recognized that for research proposals involving the derivation of human ES or EG cells, many of the ethical issues associated with these protocols could be considered at the local level, that is, at the institutions at which the research would be taking place. For protocols using but not deriving ES cells (i.e., generating the cells elsewhere), a separate set of ethical deliberations would have occurred. In general, the IRB is an appropriate body to review protocols that aim to derive ES or EG cells. Although few review bodies (including IRBs) have extensive experience in reviewing protocols of this kind, they remain the most visible and expert entities available. It is for this reason, for example, that we make a number of recommendations (8, 9, 10, 11, and 12) that discuss the importance of developing additional guidance for the review of such protocols.

For proposals involving the derivation of human ES or EG cells, particular sensitivities require attention through a national review process. This process should, however, begin at the local level, because institutions that intend to conduct research involving the derivation of human ES cells or EG cells should continue to take responsibility for assuring the ethical conduct of that research. More importantly, however, IRBs can play an important role, particularly by reviewing consent documents and by assuring that collaborative research undertaken by investigators at foreign institutions has satisfied any regulatory requirements for sharing research materials.

Recommendation 9: Institutional Review of Protocols to Derive Stem Cells

Protocols involving the *derivation* of human ES and EG cells should be reviewed and approved by an IRB or by another appropriately constituted and convened institutional review body prior to consideration by the National Stem Cell Oversight and Review Panel. (See Recommendation 8.) This review should ensure compliance with any requirements established by the panel, including confirming that individuals or organizations (in the United States or abroad) that supply embryos or cadaveric fetal tissue have obtained them in accordance with the requirements established by the panel.

Responsibilities of Federal Research Agencies

Federal research agencies have in place a comprehensive system for the submission, review, and approval of research proposals. This system includes the use of a peer review group—sometimes called a study section or initial review group—that is established to assess the scientific merit of the proposals. In addition, in some agencies, such as NIH, staff members review protocols prior to their transmittal to a national advisory council for final approval. These levels of review provide an opportunity to consider ethical issues that arise in the proposals.

When research proposals involve human subjects, federal agencies rely on local IRBs to review and approve the research in order to assure that it is ethically acceptable. (See Recommendation 9.) A grant application should not be funded until ethical issues that are associated with research involving human subjects have been resolved fully. Therefore, at every point in this continuum—from the first discussions that a prospective applicant may have with program staff within a particular institution to the final decision by the relevant national advisory council—ethical and scientific issues can be addressed by the sponsoring agency.

Recommendation 10: Sponsoring Agency Review of Research Use of Stem Cells

All federal agencies should ensure that their review processes for protocols using human ES or EG cells comply with any requirements established by the National Stem Cell Oversight and Review Panel (see Recommendation 8), paying particular attention to the adequacy of the justification for using such cell lines.

Research involving human ES and EG cells raises critical ethical issues, particularly when the proposals involve the derivation of ES cells from embryos remaining after infertility treatments. We recognize that these research proposals may not follow the paradigm usually associated with human subjects research. Nevertheless, research proposals being considered for funding by federal agencies must, in our view, meet the highest standards of scientific merit and ethical acceptability. To that end, the recommendations made in this report, including a proposed set of *Points to Consider in Evaluating Basic Research Involving Human ES Cells and EG Cells*, constitute a set of ethical and policy considerations that should be reflected in the respective policies of federal agencies conducting or sponsoring human ES or EG cell research.

Attention to Issues for the Private Sector

Although this report primarily addresses the ethical issues associated with the use of federal funds for research to derive and use ES and EG cells, we recognize that considerable work in both of these areas will be conducted under private sponsorship. Thus, our recommendations may have implications for those working in the private sector. First, for cell lines to be eligible for use in federally funded research, they must be certified by the National Stem Cell Oversight and Review Panel described in Recommendation 8. Therefore, if a private company aims to make its cell lines available to publicly funded researchers, it must submit its derivation protocol(s) to the same oversight and review process recommended for the public sector, i.e., local review (see Recommendation 9) and for certification that the cells have been derived from embryos remaining after infertility treatments or from cadaveric fetal tissue.

Second, we hope that nonproprietary aspects of protocols developed under private sponsorship will be made available in the public registry, as described in Recommendation 8. The greater the participation of the private sector in providing information on stem cell research, the more comprehensive the development of the science and related public policies in this area.

Third, and perhaps most relevant, in an ethically sensitive area of emerging biomedical research it is important that all members of the research community, whether in the public or private sectors, conduct the research in a manner that is open to appropriate public scrutiny. The last two decades have witnessed an unprecedented level of cooperation between the public and private sectors in biomedical research, which has resulted in the international leadership position of the United States in this arena. Public bodies and other authorities, such as the Recombinant DNA Advisory Committee, have played a crucial role in enabling important medical advances in fields such as gene therapy by providing oversight of both publicly and privately funded research efforts. We believe that voluntary participation by the private sector in the review and certification procedures of the proposed national panel, as well as in its deliberations, can contribute equally to the socially responsible development of ES and EG cell technologies and accelerate their translation into biomedically important therapies that will benefit patients.

Recommendation 11: Voluntary Actions by Private Sponsors of Research That Would Be Eligible for Federal Funding

For privately funded research projects that involve ES or EG cells that would be eligible for federal funding, private sponsors and researchers are

encouraged to adopt voluntarily the applicable recommendations of this report. This includes submitting protocols for the derivation of ES or EG cells to the National Stem Cell Oversight and Review Panel for review and cell line certification. (See Recommendations 8 and 9.)

In this report, we recommend that federally funded research to derive ES cells be limited to those efforts that use embryos remaining after infertility treatment. Some of the recommendations made in this context—such as the requirement for separating the decision by a woman to cease such treatment when embryos still remain and her decision to donate those embryos to research—simply do not apply to efforts to derive ES cells from embryos created (whether by IVF or somatic cell nuclear transfer) solely for research purposes, activities that might be pursued in the private sector. Nevertheless, other ethical standards and safeguards embodied in the recommendations, such as provisions to prevent the coercion of women and the commodification of human reproduction, remain vitally important, even when embryos are created solely for research purposes.

Recommendation 12: Voluntary Actions by Private Sponsors of Research That Would Not Be Eligible for Federal Funding

For privately funded research projects that involve deriving ES cells from embryos created solely for research purposes and that are therefore not eligible for federal funding (see Recommendations 3 and 4)

a) professional societies and trade associations should develop and promulgate ethical safeguards and standards consistent with the principles underlying this report, and

b) private sponsors and researchers involved in such research should voluntarily comply with these safeguards and standards.

Professional societies and trade associations dedicated to reproductive medicine and technology play a central role in establishing policy and standards for clinical care, research, and education. We believe that these organizations can and should play a salutary role in ensuring that all stem cell and embryo research conducted in the United States, including that which is privately funded, conforms to the ethical principles underlying this report. Many of these organizations already have developed policy statements, ethics guidelines, or other directives addressing issues in this report, and the Commission has benefited from a careful review of these materials. These organizations are encouraged to review their professional standards to ensure not only that they keep pace with the evolving science of human ES and EG cell research, but also that their members are knowledgeable about and in compliance with them. For those organizations that conduct research in this area but that lack statements or guidelines addressing the topics of this report, we recommend strongly that they develop such statements or guidelines. No single institution or organization, whether in the public or the private sector, can provide all the necessary protections and safeguards.

The Need for Ongoing Review and Assessment

No system of federal oversight and review of such a sensitive and important area of investigation should be established without simultaneously providing an evaluation of its effectiveness, value, and ongoing need. The pace of scientific development in human ES and EG cell research likely will increase. Although one cannot predict the direction of the science of human stem cell research, in order for the American public to realize the promise of this research and to be assured that it is being conducted responsibly, close attention to and monitoring of all the mechanisms established for oversight and review are required.

Recommendation 13: Sunset Provision for National Panel

The National Stem Cell Oversight and Review Panel described in Recommendation 8 should be chartered for a fixed period of time, not to exceed five years. Prior to the expiration of this period, DHHS should commission an independent evaluation of the panel's activities to determine whether it has adequately fulfilled its functions and whether it should be continued.

There are several reasons for allowing the national panel to function for a fixed period of time and for evaluating its activities before continuing. First, some of the hoped-for results will be available from research projects

that are using the two sources we consider to be ethically acceptable for federal funding. Five years is a reasonable period of time to allow some of this information to amass, offering the panel, researchers, members of Congress, and the public sufficient time to determine whether any of the knowledge or potential health benefits are being realized. The growing body of information in the public registry and database described above (particularly if privately funded researchers and sponsors voluntarily participate) will aid these considerations.

Second, within this period the panel may be able to determine whether additional sources of ES cells are necessary in order for important research to continue. Two arguments are evident for supporting research using embryos created specifically for research purposes: one is the concern that not enough embryos remain for this purpose from infertility treatments, and the other is the recognition that some research requires embryos that are generated particularly for research and/or medical purposes. The panel should assess whether additional sources of ES cells that we have judged to be ineligible for federal funding at this time (i.e., embryos created solely for research purposes) are needed.

Third, an opportunity to assess the relationship between local review of protocols using human ES and EG cells and the panel's review of protocols for the derivation of ES cells will be offered. It will, of course, take time for this national oversight and review mechanism to develop experience with the processes of review, certification, and approval described in this report. Fourth, we hope that the panel will contribute to the national dialogue on the ethical issues regarding research involving human embryos. A recurring theme of our deliberations, and in the testimony we heard, was the importance of encouraging this ongoing national conversation.

The criteria for determining whether the panel has adequately fulfilled its functions should be set forth by an independent body established by DHHS. However, it would be reasonable to expect that the evaluation would rely generally on the seven functions described above in Recommendation 8 and that this evaluation would be conducted by a group with expertise in these areas. In addition, some of the following questions might be considered when conducting this evaluation: Is there reason to believe that the private sector is voluntarily submitting descriptions of protocols involving the derivation of human ES cells to the panel for review? Is the panel reviewing projects in a timely manner? Do researchers find that the review process is substantively helpful? Is the public being provided with the assurance that social and ethical issues are being considered?

Summary

Recent developments in human stem cell research have raised hopes that new therapies will become available that will serve to relieve human suffering. These developments also have served to remind society of the deep moral concerns that are related to research involving human embryos and cadaveric fetal tissue. Serious ethical discussion will (and should) continue on these issues. However, in light of public testimony, expert advice, and published writings, we have found substantial agreement among individuals with diverse perspectives that although the human embryo and fetus deserve respect as forms of human life, the scientific and clinical benefits of stem cell research should not be foregone. We were persuaded that carrying out human stem cell research under federal sponsorship is important, but only if it is conducted in an ethically responsible manner. And after extensive deliberation, the Commission believes that acceptable public policy can be forged, in part, on widely shared views. Through this report, we not only offer recommendations regarding federal funding and oversight of stem cell research, but also hope to further stimulate the important public debate about the profound ethical issues regarding this potentially beneficial research.

Chapter One

Introduction

Introduction

Late in 1998, three separate reports brought to the fore the debate over the scientific and clinical prospects as well as the ethical implications of research using human stem cells—those cells from which the different types of cells in a developing organism grow and that generate new cells throughout an organism's life (Van Blerkom 1994). The initial two reports were published by two independent teams of scientists that had accomplished the isolation and culture of human *embryonic stem cells* (hereafter referred to as *ES cells*) and *embryonic germ cells* (hereafter referred to as *EG cells*). The first report described the successful isolation of EG cells in the laboratory of John Gearhart and his colleagues at The Johns Hopkins University. This team derived stem cells from primordial gonadal tissue obtained from cadaveric fetal tissue (Shamblott et al. 1998). The second described the work of James Thomson and his coworkers at the University of Wisconsin, who derived ES cells from the blastocyst (~100 cells) of an early human embryo donated by a couple who had received infertility treatments (Thomson et al. 1998). Finally, an article in the November 12, 1998, edition of the *New York Times* described work funded by Advanced Cell Technology of Worcester, Massachusetts. Although this work has not yet been verified fully or published in a scientific journal, the company claims that its scientists have caused human somatic cells to revert to the primordial state by fusing them with cow eggs to create a hybrid embryo. From this hybrid embryo, a small clump of cells resembling human ES cells appears to have been isolated (Wade 1998).

Human Stem Cells: An Overview

Although many kinds of stem cells exist within the human body, scientists recognize a hierarchy of types. Some stem cells are more committed—or differentiated—than others. At the earliest stage of embryonic development, the cells of the blastomere are identical to each other and are relatively undifferentiated. Each one is individually capable of generating a whole organism, a quality referred to as *totipotency*. In the next stage, ES cells, although they no longer are capable of producing a complete organism, remain undifferentiated and retain the ability to develop into nearly any cell type found in the human body, representing a type of biological plasticity referred to as *pluripotency*. (The terms *totipotency* and *pluripotency* will be discussed again later in this chapter.) At this point, the ES cells branch out into many types; from each differentiated line, all the specialized cells (e.g., heart, muscle, nerve, skin, or blood) that constitute the tissues and organs of the body will develop (Weiss et al. 1996).

The potential versatility of ES and EG cells derived from the early stage embryo or from cadaveric fetal tissue offers unusual scientific and therapeutic promise. Because these cells have the ability to proliferate and renew themselves over the lifetime of the organism, scientists have long recognized the possibility of using such cells to generate a certain number of specialized cells or tissues, which could permit the generation of new cells or tissue as a treatment for injury or for damage done by diseases such as Alzheimer's disease, Parkinson's disease, heart disease, and kidney failure. Furthermore, scientists regard these cells as an important, perhaps essential, medium for understanding the details of

human development and thus for developing life-saving drugs and other therapies. At the same time, the current source of these cells (the early stage embryo or cadaveric fetal tissue) makes them the subject of significant ethical considerations. Thus, the scientific reports of the successful isolation of these versatile cells simultaneously have raised the prospect of the development of new treatments and perhaps cures for debilitating and even fatal illnesses, while also renewing the debate regarding the ethics of research involving human embryos and cadaveric fetal material.

Ethical Issues

Within days of the publication of these reports and the *New York Times* article, President Clinton wrote to the National Bioethics Advisory Commission with two requests: that the Commission consider the implications of the purported cow-human fusion experiment and report back to him and that it "undertake a thorough review of the issues associated with human stem cell research balancing all ethical and medical considerations." On November 20, 1998, we responded to the President's first request by stating that "any attempt to create a child through the fusion of a human cell and a nonhuman egg would raise profound ethical concerns and should not be permitted." (See Appendix C, which includes these letters of request and response.) Our response was based upon the same principles we relied on when preparing our report to the President entitled *Cloning Human Beings* (1997). We noted, however, that insufficient scientific evidence is available at this time to determine whether the fusing of a human cell with the egg of a nonhuman animal would result in a human embryo. In addition, if the resulting hybrid embryo were to be used as a source of ES cells, it is not clear that those cells would be the same in all respects to those obtained from a nonhybrid human embryo.

The reports of the successful isolation and culture of ES and EG cells have added a new dimension to the ongoing controversy regarding the ethics of research involving human embryos and cadaveric fetal material. This controversy arises from sharply differing moral views regarding elective abortion or the use of embryos for research, and it has fueled the national and international debate over the ethical, legal, and medical issues that arise in this arena. This debate represents both a challenge and an opportunity: a challenge because it concerns important and morally contested questions regarding the beginning of life, and an opportunity because it provides another occasion for serious public discussion about important ethical issues. We are hopeful that this report will contribute to a dialogue that will foster increased public understanding of the ethical issues underlying research on ES and EG cells and an appreciation of the complexity of making responsible public policy in the face of moral disagreement and in light of a realistic appraisal of the scientific and clinical promise of that research.

We believe that most Americans agree that human embryos should be respected as a form of human life, but that disagreement exists both about the form that such respect should take and about what level of protection is owed at different stages of embryonic development. Therefore, embryo research, the purpose of which is not therapeutic to the embryo itself, is bound to raise serious concerns for some about how to resolve the tensions between the ethical imperative to cure diseases and the moral obligation to protect human life. For those who believe that the embryo has the moral status of a person from the moment of conception, research (or any other activity) that would destroy it is considered wrong and should not take place. For others, arriving at an ethically acceptable policy involves a complex balancing of a number of important ethical concerns. Although this is a controversial area, we should not lose sight of a broad area of consensus on which public policy could—in part—be constructed.

In order to respond effectively and responsibly to the President's request to consider issues related to human stem cell research and to "balance all medical and ethical considerations," we determined that it also is necessary to consider certain aspects of the broader issues regarding research using embryonic and/or fetal material. One reason for this approach is that the nature of some of the ethical issues involved depends on the source of the stem cells. For example, ES cells can be derived from early embryos that are destroyed in the process of ES cell

derivation, an act that some people find ethically unacceptable. The use of cadaveric fetal tissue to derive EG cell lines is somewhat less controversial because the fetus is deceased prior to the initiation of the research and because a well-developed system of public oversight for such research is already in place. In addition, the recent demonstration of nuclear transfer techniques (somatic cell nuclear transfer [SCNT]) suggests that transfer of an adult nucleus into an oocyte might under certain conditions create an embryo. However, the use of this technique to combine an animal oocyte with a human diploid nucleus raises additional issues regarding both the nature of the embryo produced and the ethical issues involved. In addition, each source of material bears a unique set of scientific, ethical, and legal distinctions.

We believed that it was especially important to take a broad view of the status of the human embryo and of fetal tissue in relation to biomedical research, because it is likely that science will uncover additional characteristics of the early *ex utero* human embryo or fetal tissues that will raise additional important and unique therapeutic possibilities, separate from those that derive from ES or EG cells. If these developments occur, all of the same ethical considerations that pertain to embryo research and fetal tissue research in general would arise once again.[1] In fact, the 1994 National Institutes of Health Human Embryo Research Panel designated 13 areas in which embryo research could advance scientific knowledge or could lead to important clinical benefits. Among these areas is "the isolation of pluripotential embryonic stem cell lines for eventual differentiation and clinical use in transplantation and tissue repair."[2]

Recent scientific developments require the updating and review of the important work of U.S. bodies that have met previously to address the role of the ethical complexities of human embryo and fetal tissue research, particularly as they relate to the role of federally funded research. In addition, new policy statements from other countries (such as Canada and the United Kingdom) suggest well-thought-out novel approaches that must be considered carefully. In responding to the President's request, therefore, we elected to take a comprehensive approach that built on the work of these reflective efforts, both in this country and abroad.

In our 1997 report, *Cloning Human Beings*, we addressed a specific aspect of cloning, namely where genetic material would be transferred from the nucleus of a somatic cell of an existing human being to an enucleated human egg with the intention of creating a child. At the time that we were preparing this report, the issues surrounding embryo research were not revisited, although we began our discussions recognizing that any effort in humans to transfer a somatic cell nucleus into an enucleated egg likely involves the creation of an embryo, which has the potential to be transferred to a uterus and developed to term. We recognized that ethical concerns surrounding issues of embryo research recently had received extensive analysis and noted that under current law, the use of SCNT to create an embryo solely for research purposes is prohibited in any project involving federal funds. The President's request—together with new developments concerning human ES and EG cell research using embryos remaining after infertility treatments or fetal tissue following elective abortion—requires that we reconsider the appropriateness of using these sources of cells for research purposes.

In this respect it is important to note that research on human embryos, or the creation of human embryos for research purposes, is not only legal in the United States but proceeds without any public oversight as long as 1) federal funds are not involved, 2) Food and Drug Administration regulations do not apply, and 3) the laws of the state in which the research is to be conducted do not forbid such activity. Consequently, most of the public controversy surrounding such activities in the United States has focused on whether it is appropriate for the federal government to sponsor such research when it has significant scientific merit and substantive clinical promise. This question is also the focus of this report.

Framework for This Report

As noted above, President Clinton directed the Commission to conduct a thorough review of the issues associated with human stem cell research balancing all ethical and medical considerations. This approach—balancing or weighing difficult issues—often is used in public policy discussions and has much to recommend

it, particularly when such balancing involves a serious consideration of different moral points of view, the state of scientific and medical developments, and other factors. As discussed more fully in Chapter 4, some of the issues associated with research on human stem cells—the moral status of the human embryo, for example—are especially sensitive and do not lend themselves easily to balancing. We did not, for example, deem the views of those who consider the fetus to have the moral status of a human person from the moment of conception to be of less (or more) moral weight than the views of those who consider the fetus to lack this moral status. Similarly, we did not come to our conclusions simply by balancing potential medical benefits against the potential harms, because the possibility of social benefits, by itself, is not a sufficient reason for federal support of such controversial research, particularly given the interest in stem cell research in the private sector. Nor did we approach this issue based simply upon an interpretation of the existing legal environment. Instead, we combined, as thoughtfully as we could, a number of different perspectives on and approaches to this topic.

Through ongoing discussion and dialogue—informed by scientists, philosophers, legal and religious scholars, members of the public, and others—we developed our moral perspectives on the appropriateness of federal sponsorship of stem cell research involving the derivation and/or use of ES and EG cells, principally focusing on the ethical and scientific issues. We considered the sources of human EG and ES cells and the relevant moral differences that should be evaluated in determining the acceptability of federal funding for the derivation and/or use of cells from each of these sources. In this regard, we were assisted by a number of commissioned papers each of which addressed different aspects of the problem.[3] We also benefited from the input of a group of religious scholars from diverse faith traditions whose views within and across traditions reflected the diversity found within the public as a whole. We then considered some associated ethical issues including voluntary informed consent, the just distribution of potential benefits from stem cell research, and the commodification and sale of the body and its parts. Finally, we considered how and to what extent a mechanism of national oversight and review would provide the necessary assurance that research, conducted responsibly and with accountability, could go forward while protecting and honoring a number of deeply held values. These shared values include

- securing the safety and efficacy of clinical and/or scientific procedures, especially when fundamental ethical and social issues are involved,
- respecting human life at all stages of development, and
- ensuring the responsible pursuit of medical and scientific knowledge.

Although this report primarily addresses the ethical issues associated with the use of federal funds for research to derive and use ES and EG cells, we recognize that considerable work in both of these areas will be conducted under private sponsorship. Thus, our recommendations also may have implications for those working in the private sector.

Definitions Used in This Report

We recognize the need to define clearly the terms that are central to an understanding of this report. Because certain terms, such as *embryo* and *totipotent*, are not always used consistently, it is important to explain how the Commission uses this terminology.

It is most important that the reader understand how the term embryo is used. The Canadian Royal Commission on New Reproductive Technologies elucidated the confusion surrounding the term well in its 1993 report entitled *Proceed with Care: Final Report of the Royal Commission on New Reproductive Technologies*:

> …In the language of biologists, before implantation the fertilized egg is termed a 'zygote' rather than an 'embryo.' The term 'embryo' refers to the developing entity after implantation in the uterus until about eight weeks after fertilisation. At the beginning of the ninth week after fertilisation, it is referred to as a 'fetus,' the term used until time of birth. The terms embryo donation, embryo transfer, and embryo research are therefore inaccurate, since these all occur with zygotes, not embryos. Nevertheless, because the terms are still commonly used in the public debate,

we continue to refer to embryo research, embryo donation, and embryo transfer (607).

For the sake of consistency and accuracy, when referring to the details of the developmental stages of an entity, we use the following terminology: 1) the developing organism is a *zygote* during the first week after fertilization, 2) the organism is an *embryo* during the second through eighth weeks of development, and 3) the organism is a *fetus* from the ninth week of development until the time of birth. However, in other contexts, we will continue to use the broad terms *embryo research, embryo donation,* and *embryo transfer* to refer to zygotes, because this is how the public commonly uses them.

Because there are several sources of human stem cells, we decided that each type of stem cell should be named in a way that clarifies its original source. Therefore, as discussed earlier, cells derived from the inner cell mass of a blastocyst—those cells within the conceptus that form the embryo proper—are called *ES cells*, and cells that are derived from primordial germ cells of embryos and fetuses are called *EG cells*. In addition, cells derived from teratocarcinomas—malignant embryonic tumors—are called *embryonal carcinoma cells*, and stem cells found in the adult organism are called *adult stem (AS) cells*.

Two other terms that require explanation—because the scientific community disagrees about their meaning—are *totipotent* and *pluripotent*. Some differentiate between the two terms by defining totipotency as the ability to develop into a complete organism and pluripotency as the ability to develop into all of the various cell types of an organism without the capability of developing into an entire organism. Others define a totipotent cell as any cell that has the potential to differentiate into all cells of a developing organism, but that does not necessarily have the ability to direct the complete development of an entire organism. These scientists would then define a pluripotent cell as any cell that has the ability to differentiate into multiple (more than two) cell types. Rather than engage in this debate, for the sake of clarity, we decided to avoid using this terminology in this report, unless it refers directly to specific work or to the statements of others in which these words were included. Instead, this report uses descriptions of the stage of development and the differentiation potential of cells to make clear to the reader which types of cells are being discussed.

Organization of This Report

This report comes at a time when the Commission has completed deliberations regarding the use of human biological materials in research (1999). In that report, we recognized that in research involving such materials as DNA, hair, and skin biopsies, a number of significant ethical issues must be addressed by Institutional Review Boards, researchers, and others; these include issues of privacy and confidentiality, potential discrimination, and stigmatization. As important as these issues are—and they must be handled satisfactorily in order for research to proceed with appropriate protections for human subjects—research on human stem cells, whether they are obtained from fetal tissue following elective abortions or from tissue obtained from embryos remaining after infertility treatments, requires additional and perhaps even deeper ethical reflection.

The Commission's primary goal for this report was the development of a set of recommendations that would provide guidance on the appropriateness of permitting the federal government to fund human ES and EG cell research and on what sorts of constraints, if any, should be placed on such support. This report first presents a summary of some of the key scientific issues involved in stem cell research (Chapter 2). To place our analysis in context and to understand the implications of any new recommendations regarding the oversight and regulation of research using fetal tissue and embryos, Chapter 3 describes the historical and current status of law and regulation governing the research use of these materials. Chapter 4 explores the various ethical issues surrounding the moral status of the embryo and cadaveric fetal tissue and ethical concerns governing the acceptable use of these materials in research. Finally, Chapter 5 offers our conclusions and recommendations regarding federal sponsorship of research and appropriate oversight activities in these ethically controversial areas.

Notes

1 For example, it has been generally recommended by most governmental and professional bodies that have previously examined this issue that research on the *ex utero* pre-implantation embryo should not be conducted beyond the 14th day following fertilization. At 14 days, the first stages of organized development begin, leading over the next few days to the first appearance of differentiated tissues of the body. The Commission concurs with this time limit on research involving the *ex utero* human embryo.

2 The 1994 National Institutes of Health Human Embryo Research Panel was asked to consider various areas of research involving the *ex utero* pre-implantation human embryo and to provide areas that 1) are acceptable for federal funding, 2) warrant additional review, and 3) are unacceptable for federal support. The panel did not consider research involving *in utero* human embryos, or fetuses, because guidelines for such research already exist in the form of regulations.

3 See Appendix H for a list of the papers that were prepared for the Commission. These papers are available in Volume II of this report.

References

Canadian Royal Commission on New Reproductive Technologies. 1993. *Proceed with Care: Final Report of the Royal Commission on New Reproductive Technologies*. 2 vols. Ottawa: Minister of Government Services.

National Bioethics Advisory Commission (NBAC). 1997. *Cloning Human Beings*. 2 vols. Rockville, MD: U.S. Government Printing Office.

———. 1999. *Research Involving Human Biological Materials: Ethical Issues and Policy Guidance*. 2 vols. Rockville, MD: U.S. Government Printing Office.

National Institutes of Health (NIH). Human Embryo Research Panel. 1994. *Report of the Human Embryo Research Panel*. 2 vols. Bethesda, MD: NIH.

Shamblott, M.J., J. Axelman, S. Wang, E.M. Bugg, J.W. Littlefield, P.J. Donovan, P.D. Blumenthal, G.R. Huggins, and J.D. Gearhart. 1998. "Derivation of Pluripotent Stem Cells from Cultured Human Primordial Germ Cells." *Proceedings of the National Academy of Sciences USA* 95:13726–13731.

Thomson, J.A., J. Itskovitz-Eldor, S.S. Shapiro, M.A. Waknitz, J.J. Swiergiel, V.S. Marshall, and J.M. Jones. 1998. "Embryonic Stem Cell Lines Derived from Human Blastocysts." *Science* 282:1145–1147.

Van Blerkom, J. 1994. "The History, Current Status, and Future Direction of Research Involving Human Embryos." In NIH Human Embryo Research Panel, *Report of the Human Embryo Research Panel, Vol. II: Papers Commissioned for the Human Embryo Research Panel*, 1–25. Bethesda, MD: NIH.

Wade, N. 1998. "Researchers Claim Embryonic Cell Mix of Human and Cow." *New York Times* 12 November, A-1.

Weiss, S., B.A. Reynolds, A.L. Vescovi, C. Morshead, C.G. Craig, and D. van der Kooy. 1996. "Is There a Neural Stem Cell in the Mammalian Forebrain?" *Trends in Neurosciences* 19(9):387–393.

Chapter 2

Human Stem Cell Research and the Potential for Clinical Application

Introduction

The stem cell is a unique and essential cell type found in animals. Many kinds of stem cells are found in the human body, with some more differentiated—or committed—to a particular function than others. In other words, when stem cells divide, some of the progeny mature into cells of a specific type (e.g., heart, muscle, blood, or brain cells), while others remain stem cells, ready to repair some of the everyday wear and tear undergone by our bodies. These stem cells are capable of continually reproducing themselves and serve to renew tissue throughout an individual's life. For example, they continually regenerate the lining of the gut, revitalize skin, and produce a whole range of blood cells. Although the term *stem cell* commonly is used to refer to the cells within the adult organism that renew tissue (e.g., hematopoietic stem cells, a type of cell found in the blood), the most fundamental and extraordinary of the stem cells are found in the early stage embryo (Van Blerkom 1994). These *embryonic stem (ES) cells*, unlike the more differentiated *adult stem (AS) cells* or other cell types, retain the special ability to develop into nearly any cell type. *Embryonic germ (EG) cells*, which originate from the primordial reproductive cells of the developing fetus, have properties similar to ES cells.

Because stem cells are able to proliferate and renew themselves over the lifetime of the organism—while at the same time retaining all of their multilineage potential—scientists have long recognized that such cells could be used to generate a large number of specialized cells or tissue through amplification, a possibility that could allow the generation of new cells that would treat injury or disease.[1] In fact, if it were possible to control the differentiation of human ES cells in culture, the resulting cells could be used to repair damage caused by such conditions as heart failure, diabetes, and certain neurodegenerative diseases.

In late 1998, three separate reports brought to the fore not only these scientific and clinical prospects but also the controversies inherent in human stem cell research. The first two reports, published by two independent teams of scientists supported by private funds from Geron Corporation, a biotechnology company located in Menlo Park, California, describe the first successful isolation and culture in the laboratory of human ES and EG cells. One team, led by John Gearhart of The Johns Hopkins University School of Medicine in Baltimore, Maryland, derived human EG cells from primordial gonadal tissue, which was obtained from fetal tissue following elective abortion (Shamblott et al. 1998). The second team, led by James Thomson of the University of Wisconsin, derived human ES cells from the blastocyst stage of early embryos donated by couples who had undergone infertility treatment (Thomson et al. 1998). The ES and EG cells derived by each of these means appear to be similar in structure, function, and potential, although additional research is needed in order to verify this claim (Varmus 1998). Finally, an article in the November 12, 1998, edition of the *New York Times* described work funded by Advanced Cell Technology of Worcester, Massachusetts. Although this work has not yet been verified fully or published in a scientific journal, the company claims that its scientists have caused human somatic cells to revert to the primordial state by fusing them with cow eggs to create a hybrid embryo. From this hybrid embryo, a small clump of cells resembling human ES cells appears to have been isolated (Wade 1998).

The methodologies used by these investigators for deriving human ES and EG cells are based on techniques that have been used in mice since the early 1980s and, more recently, from nonhuman primates and other animals. The isolation and culturing of these cells, however, for the first time open certain avenues of important research and future clinical possibilities. At the most basic level, the isolation of these cells allows scientists to focus on how human ES and EG cells differentiate into specific types of cells, with the goal of identifying the genetic and environmental signals that direct their specialization into specific cell types. Such studies using mouse stem cells are ongoing, but comparable studies with human cells will be required in order to determine whether the signals are the same. This research might, for example, lead to the discovery of new ways to treat a variety of conditions, including degenerative diseases, birth defects, and cancer and would build on investigations conducted over the last decade, in which laboratory animals have been used to determine whether ES cells can be used to re-establish tissue in an adult organism (Corn et al. 1991; Diukman and Golbus 1992; Hall and Watt 1989; Hollands 1991). Through processes scientists are only beginning to understand, these primitive stem cells can be stimulated to specialize so that they become precursors to different cell types, which then may be used to replace tissues such as muscle, skin, nerves, or liver. For example, in mid-1999, scientists used mouse ES cells to successfully generate glial (myelin-producing) cells that when transplanted into a rat model of human myelin disease were able to efficiently myelinate axons in the rat's brain and spinal cord (Brustle et al. 1999).

Stem Cell Types

Scientists often distinguish between different kinds of stem cells depending upon their origin and their potential to differentiate. Cells derived from malignant embryonic tumors, or teratocarcinomas, are called *embryonal carcinoma (EC) cells*; cells derived from the inner cell mass of a blastocyst-stage embryo are ES cells, and cells that are derived from precursors of germ cells from a fetus are EG cells. In addition, stem cells can be found in the adult organism, for example, in bone marrow; they may possibly also be found in skin and intestine. These AS cells serve to replenish tissues in which cells often have limited life spans, such as the skin, intestine, and blood. Although interesting new data suggest that stem cells found in the adult organism are not restricted to producing cells from the tissue in which they reside (Bjornson et al. 1999), it is unlikely that these cells are capable of differentiating into all cell types. In contrast, because human ES and EG cells are believed to be capable of differentiating into all cell types, they are likely to be of clinical use in treating a variety of diseases, especially those for which organ-specific stem cells are difficult to isolate and/or use.

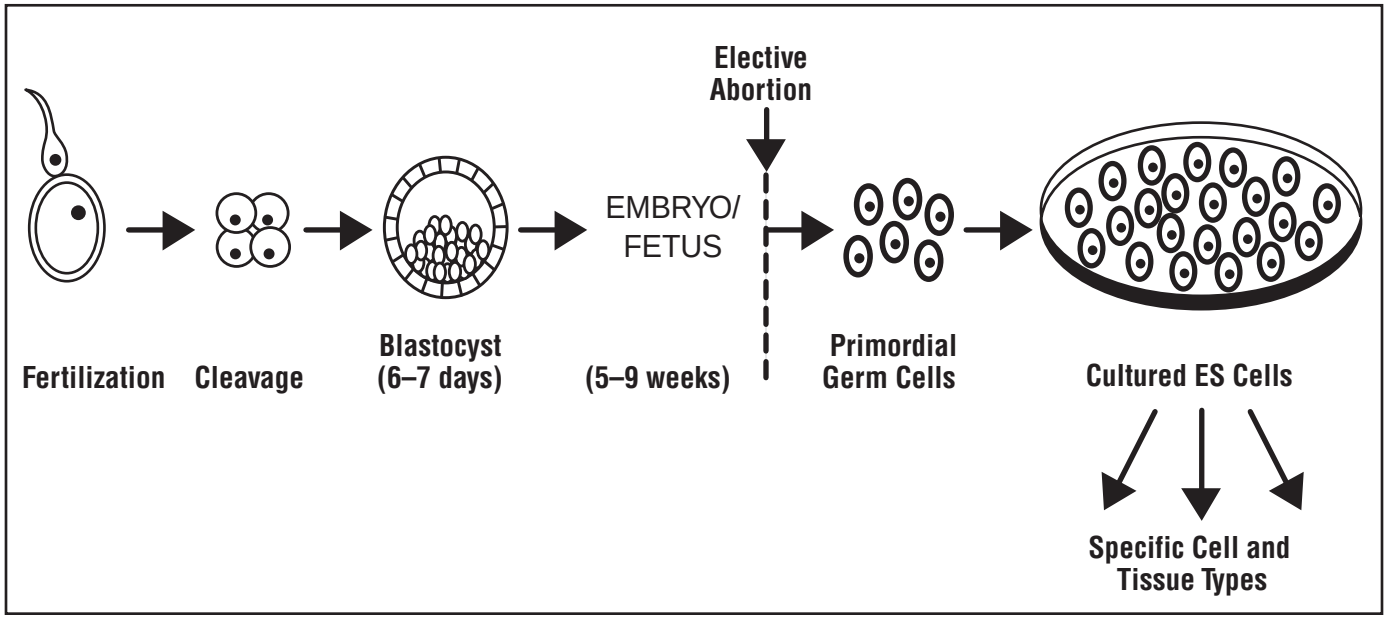
EG Cells

Primordial germ cells are the embryonic precursors of the sperm and ova of the adult animal (Donovan 1998). The establishment of the germline in the embryo involves the separation of primordial germ cells from the somatic cells, the proliferation of primordial germ cells, the migration of these cells to the gonads, and finally their differentiation into gametes (Donovan 1994). Primordial germ cells are the only cells in the body that can give rise to successive generations, while the somatic cells that form the body of the animal lack this capability as soon as they start to differentiate (Matsui 1998).

In culture, primordial germ cells can give rise to EG cells that are capable of differentiating into cells of multiple lineages (Donovan 1998). (See Figure 2-1.) Primordial germ cells normally give rise to gametes, but sometimes if the developmental process goes awry, they become EC cells, the stem cells of benign teratomas and malignant teratocarcinomas, which are tumors containing derivatives of the three primary germ layers (Donovan 1998).

EG cells form embryoid bodies in culture, give rise to teratomas when introduced into histocompatible animals, and form germline chimeras when introduced into a host blastocyst (Donovan 1998). The derivation of EG cells directly from primordial germ cells provides a mechanism to study some aspects of primordial germ cell development, such as imprinting and differentiation (Donovan 1994). At the same time, it may be difficult to obtain an adequate supply of appropriate fetal tissue to

Figure 2-1. Isolation and Culture of Human ES Cells from Embryonic/Fetal Tissue



provide the relevant cell lines needed for both research and clinical uses.

ES Cells

In mammalian embryonic development, cell division gives rise to differentiated daughter cells that eventually comprise the mature animal. As cells become committed to a particular lineage or cell type, a progressive decrease in developmental potential presumably occurs. Early in embryonic development (until about 16 cells), each cell of the early cleavage-stage embryo has the developmental potential to contribute to any embryonic or extra embryonic cell type (Winkel and Pedersen 1988). However, by the blastocyst stage, the cells of the trophectoderm are irreversibly committed to forming the placenta and other trophectoderm lineages (Winkel and Pedersen 1988). By six to seven days postfertilization, the inner cell mass has divided to form two layers, which then give rise to the embryo proper and to extra embryonic tissues (Gardner 1982). (See Exhibit 2-A and Figure 2-2 for a description of early human embryonic development.)

Although the cells of the inner cell mass are precursors to all adult tissues, they can proliferate and replace themselves in the intact embryo only for a limited time before they become committed to specific lineages (Thomson and Marshall 1998). ES cells are derived from cells of the inner cell mass. Once they are placed in the appropriate culture conditions, these cells seem to be capable of extensive, undifferentiated proliferation *in vitro* and maintain the potential to contribute to all adult cell types (Evans and Kaufman 1981; Martin 1981). (See Figure 2-3.)

Even though these embryonic cells are stem cells, they differ substantially from the stem cells found within the fully developed, or adult, organism (see below). Most important, ES cells are highly proliferative, both in the embryo as well as in culture, while some stem cells of the adult can be nearly quiescent and may be more difficult to maintain and expand in culture (Van Blerkom 1994). Therefore, it appears that if stem cells were someday to be used for the treatment of disease, it might be advantageous to use ES cells to treat certain disorders.

Sources of Human ES Cells

We have distinguished between three sources of ES cells, which are derived from early embryos in culture: 1) embryos created by *in vitro* fertilization (IVF) for infertility treatments that were not implanted because they were no longer needed, 2) embryos created by IVF

Exhibit 2-A: Early Development of the Human Embryo

In humans, fertilization (the union of an oocyte [egg] and sperm) occurs in the fallopian tubes and results in the formation of the zygote. In the three to four days it takes for the zygote to travel down the fallopian tube to the uterus, several cell divisions (cleavages) occur.

The first division occurs approximately 36 hours after fertilization, when the zygote begins to cleave into two cells called blastomeres. At about 60 hours following fertilization, the two blastomeres divide again to form four blastomeres. At three days postfertilization, the four blastomeres divide to form eight cells. Each blastomere becomes smaller with each subsequent division. In this early stage of development, all of the blastomeres are of equal size. These cells are unspecialized and have the capacity to differentiate into any of the cell types of the embryo as well as into the essential membranes and tissue that will support the development of the embryo. Therefore, one or more of the blastomeres can be removed without affecting the ability of the other blastomeres to develop into a fetus. In fact, if an embryo separates in half during this early stage of development, identical twins—two genetically identical individuals—will develop.

When the cell division reaches approximately 16 cells, the zygote is called a morula. The morula leaves the fallopian tube and enters the uterine cavity three to four days following fertilization. After reaching the uterus, the developing zygote usually remains in the uterine cavity an additional four to five days before it implants in the endometrium (uterine wall), which means that implantation ordinarily occurs on the seventh or eighth day following fertilization.

Cell division continues, creating a cavity known as a blastocele in the center of the morula. With the appearance of the cavity in the center, the entire structure is now called a blastocyst. This first specialization event occurs just before the zygote attaches to the uterus, approximately six to seven days after fertilization, when approximately 100 cells have developed. This specialization involves the formation of an outer layer of trophoblast cells, which will give rise to part of the placenta, surrounding a group of about 20 to 30 inner cells (the inner cell mass) that remain undifferentiated. At this stage, these cells no longer can give rise to all of the cells necessary to form an entire organism and therefore are incapable of developing into an entire human being. In general, as cells further differentiate, they lose the capacity to enter developmental pathways that were previously open to them.

As the blastocyst attaches to the uterus, the outer layer of cells secretes an enzyme, which erodes the epithelial uterine lining and creates an implantation site for the blastocyst. Once implantation has taken place, the zygote becomes an embryo. The trophoblast and underlying cells proliferate rapidly to form the placenta and the various membranes that surround and nourish the developing embryonic cells.

In the week following implantation, the inner cells of the blastocyst divide rapidly to form the embryonic disc, which will give rise to the three germ layers—the ectoderm, the mesoderm, and the endoderm. These three layers will eventually develop into the embryo. By 14 days, the embryonic disc is approximately 0.5 mm in diameter and consists of approximately 2,000 cells. It is at this time that the first stage of organized development, known as gastrulation, is initiated, leading over the next few days to the first appearance of differentiated tissues of the body, including primitive neural cells. Gastrulation is the process by which the bilaminar (two-layered) embryonic disc is converted into a trilaminar (three-layered) embryonic disc, and its onset at day 14 *in vivo* is marked by the appearance of the primitive streak, a region in which cells move from one layer to another in an organized way.

During the third week, the embryo grows to 2.3 mm long, and the precursors of most of the major organ systems begin to form. At the beginning of the third month, the embryo becomes a fetus. During the third to ninth months, the organ systems and tissues of the fetus continue to develop, until birth.

expressly for research purposes, and 3) embryos resulting from somatic cell nuclear transfer (SCNT) or other cloning techniques. SCNT technology has, in fact, opened the door to a possible alternative approach to creating ES cells. (See Figure 2-4.) If the nucleus is removed from an immature egg (oocyte) and a mature diploid nucleus is inserted, the resulting cell will divide and develop with many characteristics of an embryo. In animal experiments in which a SCNT-derived embryo is transferred to a surrogate mother, a successful pregnancy may be established. (This was the technique used to generate the now-famous cloned sheep Dolly.) If, instead of being transferred to a surrogate, the SCNT-derived embryo is kept in culture, is allowed to divide, and is then dissociated, ES cells can

Figure 2-2. Stages of Development of the Human Embryo and Fetus

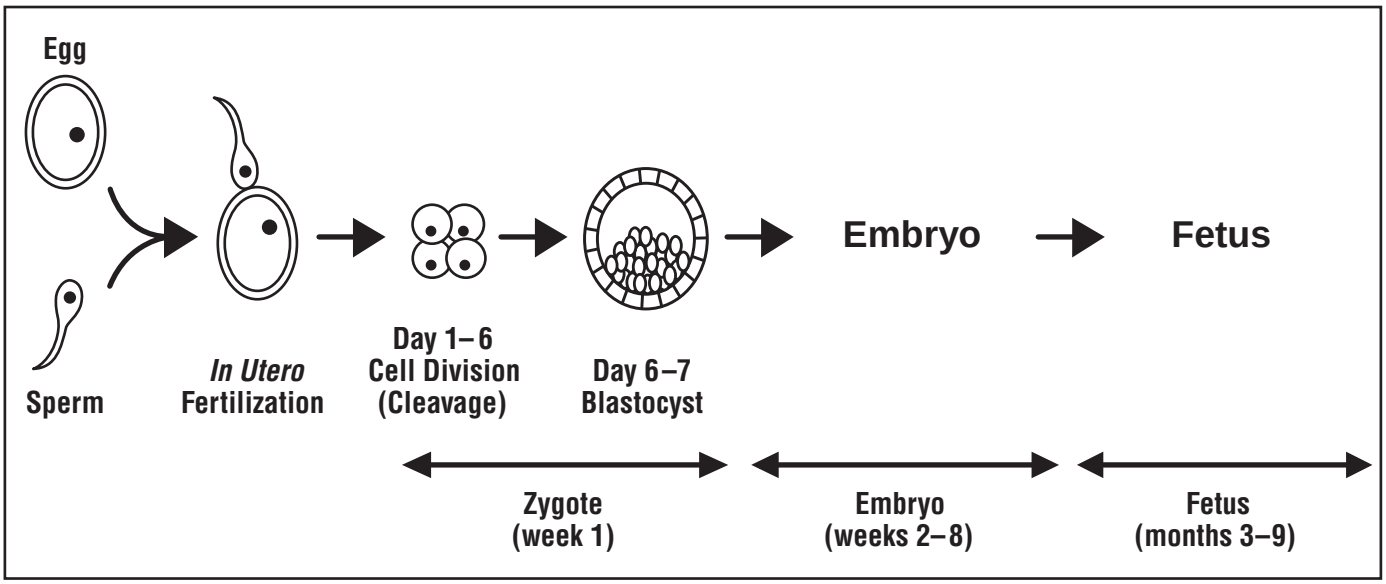
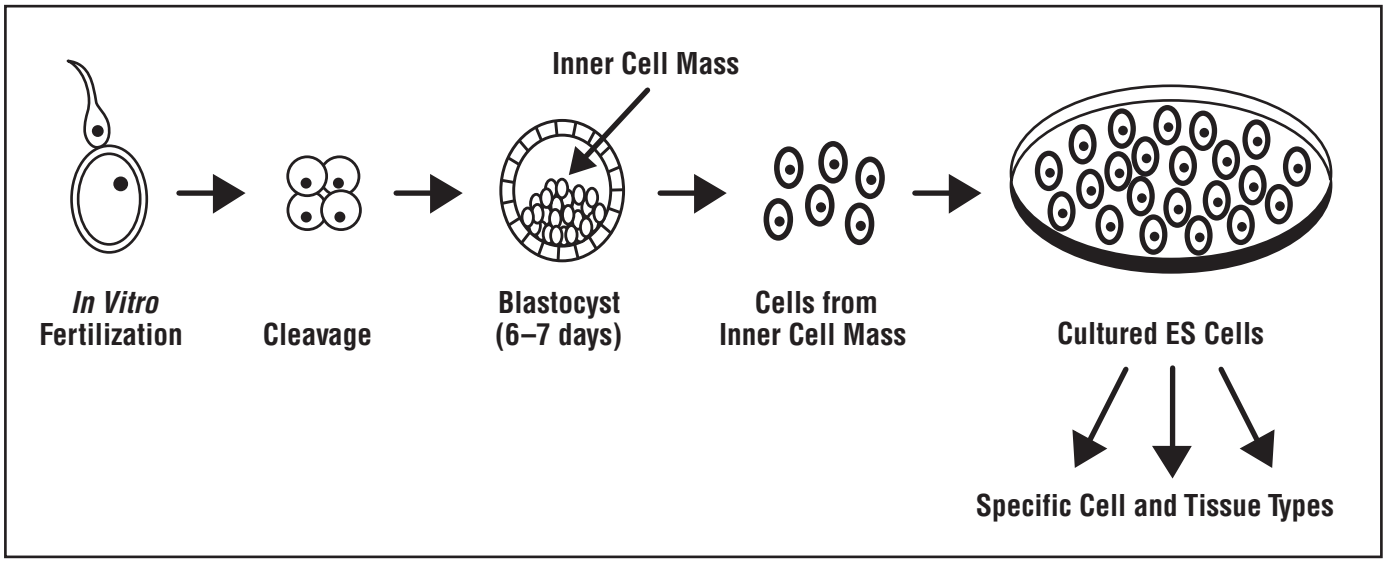


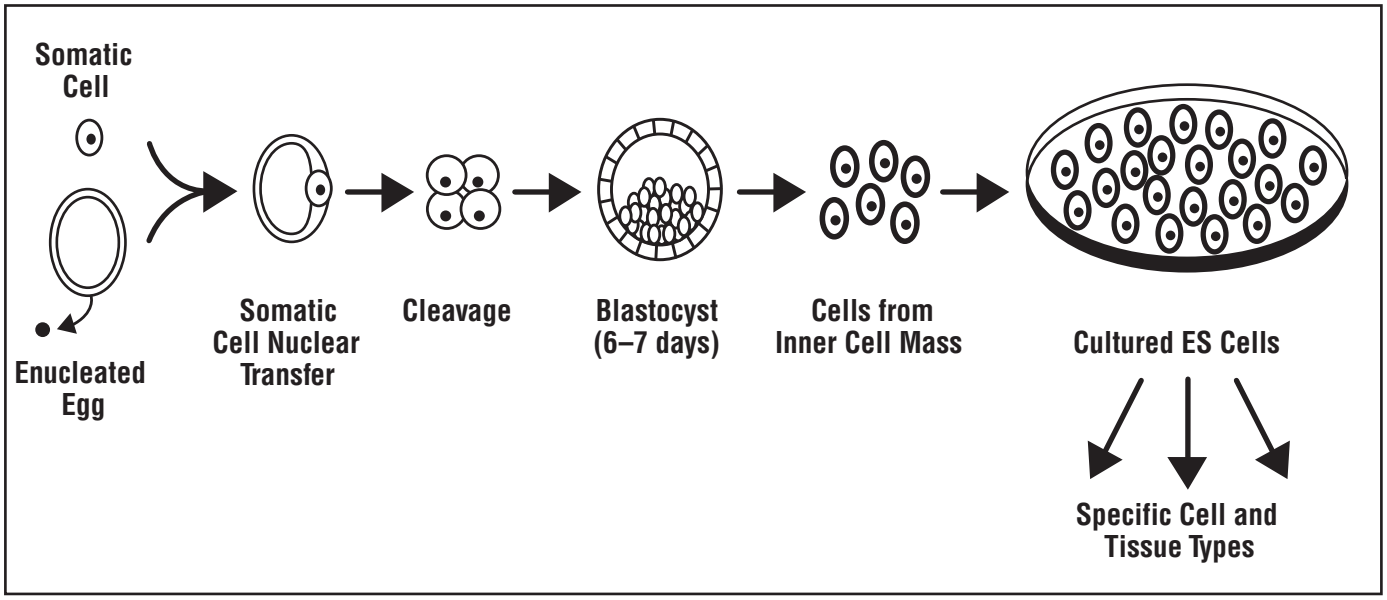
Figure 2-3. Isolation and Culture of Human ES Cells from Blastocysts



be derived. The potential advantage of using SCNT technology to create ES cells is that a somatic cell from an individual can be used to create ES cells that are completely compatible with that individual's tissue type. If cells or tissues are generated from these ES cells for transplant into a person, this tissue type compatibility may avoid many of the problems associated with tissue graft rejection that are currently encountered in the treatment of a variety of diseases.

The use of SCNT into an oocyte has been criticized as an asexual or "unnatural" way of creating a human embryo. However, it is important to distinguish the technique of SCNT from the type of cell that is created; in other words, SCNT techniques also might be used with recipient cells other than oocytes. For example, ES cells with matched tissue types for transplant might be generated by SCNT into an enucleated ES cell.[2] This possibility has not yet been explored, but it may be less morally

Figure 2-4. Isolation and Culture of Human ES Cells from SCNT



problematic to many citizens, because the cell created would not be an embryo with the potential to continue developing.

Stem Cells Found in the Postnatal and Adult Organism

In the adult mammal, cell division occurs in order to maintain a constant number of terminally differentiated cells in tissues in which cells have been lost due to injury, disease, or natural cell death. Cells with a high turnover rate are replaced through a highly regulated process of proliferation, differentiation, and apoptosis (programmed cell death) from relatively undifferentiated stem cells, or precursor cells (Thomson and Marshall 1998). The best known example of an AS cell is the hematopoietic stem cell, which is found in bone marrow and which is responsible for the production of all types of blood cells (Iscove 1990). Other examples of stem cells include the skin epithelium and the epithelium of the small intestine (Hall and Watt 1989). In the human small intestine, for example, approximately 100 billion cells are shed and must be replaced daily (Potten and Loeffler 1990). These tissues contain subpopulations of dividing stem cells that generate replacements for the relatively short-lived, terminally differentiated cells. Much of the debate in the stem cell field revolves around determining the breadth of the potential of these cells: Can they generate only the cells of that organ or are they capable of differentiating into several types of cells when given the proper stimuli?

The successful cloning of Dolly demonstrated that even somatic cells are capable of forming every cell of an organism after nuclear transfer into an oocyte (Wilmut et al. 1997). Preliminary studies of stem cells obtained from various systems of the adult organism suggest that in some cases the reactivation of dormant genetic programs may not require nuclear transfer or experimental modification of the genome. Although research in this area is preliminary, this particular class of stem cells (i.e., AS cells) might be able to differentiate along several cell lineages in response to an appropriate pattern of stimulation.

Neural Stem Cells

For a number of years, scientists have recognized that transplantation of fresh fetal neural tissue into the diseased adult brain may be a promising therapy for neurodegenerative disorders. This type of transplantation recently has been shown to be effective in younger patients with Parkinson's disease (Freed et al. 1999). This technique has several disadvantages, however, such as the need to time the surgery according to the availability of large amounts of fresh fetal tissue, the need to quickly

screen for infectious diseases, and the limited amount of donor fetal tissue available (Bjorklund 1993; Cattaneo and McKay 1991). By developing techniques to culture and expand primary fetal neural cells before transplantation, some of the problems of using fresh tissue may be eliminated. In addition, it might be possible to direct cultured cells to develop along certain lineages or to express specific genes before they are transplanted, so that, for example, dopamine-producing cells could be selectively grown to treat Parkinson's disease (Cattaneo and McKay 1991; Snyder 1994).

Indeed, it has already been demonstrated that neural stem cells are capable of gene (Snyder, Taylor, and Wolfe 1995) and cellular (Rosario et al. 1997; Snyder et al. 1997; Yandava, Billinghurst, and Snyder 1999) replacement in models of neural disease. In many of these experiments, one stable clone of mouse neural stem cells could be used from individual to individual, strain to strain, and disease to disease, regardless of recipient age within the species, without immunorejection or the need for immunosuppression. This suggests that unique immune qualities may exist within stem cells that might allow them to be universal donors. Moreover, the possibility exists that many of the instructive cues for differentiation actually might originate from interaction with damaged central nervous system tissue itself.

The embryonic nervous system arises from the ectoderm. The first cell type to differentiate from the uncommitted precursor cells is the neuron, followed by the oligodendrocyte, and then the astrocyte (Frederiksen and McKay 1988). Recently, Angelo Vescovi, a neurobiologist at the National Neurological Institute Carlo Besta in Milan, Italy, and his colleagues reported that neural stem cells, which give rise to the three main types of brain cells, also can become blood cells when transplanted into mice in which the blood-forming tissue—the bone marrow—has been mostly destroyed (Bjornson et al. 1999). Although the study did not explain what caused the neural cells to turn into blood cells, the investigators speculate that the neural cells might be responding to the same signals that normally stimulate the few remaining blood stem cells to reproduce and mature after irradiation destroys most of the bone marrow (Strauss 1999). Although this research is preliminary and has not yet been conducted using human cells, it raises the possibility of using neural stem cell transplants to treat human blood cell disorders such as aplastic anemia and severe combined immunodeficiency. This is an appealing prospect, because bone marrow stem cells do not replenish themselves well in laboratory cultures. The problem of access to such cells in humans remains, as they must be obtained from the brain—an invasive and risky procedure. This research also opens up the possibility that other apparently restricted AS cells may retain the ability to differentiate into several different types of cells if exposed to a conducive external environment. It is clear that further research is required in this area.

Mesenchymal Stem Cells

Human mesenchymal stem cells, which are present in adult bone marrow, can replicate as undifferentiated cells and have the potential to differentiate into lineages of mesenchymal tissues, including bone, cartilage, fat, tendon, muscle, and marrow stroma (Pittenger et al. 1999). In a recent experiment, cells that have the characteristics of human mesenchymal stem cells were isolated from marrow aspirates of volunteer donors. Individual stem cells were identified that, when expanded to colonies, retained their multilineage potential. These results demonstrate that isolated expanded human mesenchymal stem cells in culture will differentiate, in a controlled manner, to multiple but limited lineages. One might speculate that these particular AS cells could be induced to differentiate exclusively into the adipocytic, chondrocytic, or osteocytic lineages, which then might be used to treat various bone diseases.

The specific environmental cues needed to initiate the proliferation and differentiation of these cells are not understood (Pittenger et al. 1999). The ability to isolate, expand, and direct the differentiation of such cells in culture to particular lineages, however, offers the opportunity to study events associated with cell commitment and differentiation. The human mesenchymal stem cells isolated by Pittenger and colleagues appear to have the ability to proliferate extensively and to maintain the ability to differentiate into certain cell types in culture. Their cultivation and selective differentiation should provide further information about this important progenitor of

multiple tissue types and the potential of new therapeutic approaches for the restoration of damaged or diseased tissue (Pittenger et al. 1999).

Animal Models

ES cells were first derived from mouse embryos, and the mouse has become the principal model for the study of these cells (Evans and Kaufman 1981; Martin 1981). If mouse ES cells are injected into the developing blastocyst, they have the ability to contribute to all three germ layers of the mouse, including the germline, to form a chimeric animal. This is one of the unique properties of the mouse ES cell. More recently, cells with some properties of ES cells have been derived from cows, pigs, rats, sheep, hamsters, rabbits, and primates (Pedersen 1994). (See Table 2-1.) However, only in cows, pigs, and rats did these ES cells contribute to a chimeric animal, and in none of these cases was there contribution to the germline by ES cells, one of the most stringent criteria for defining ES cells.

Mouse ES Cells

ES cells were first isolated from mouse blastocysts in 1981 (Evans and Kaufman 1981; Martin 1981). These blastocysts were placed in culture and allowed to attach to the culture dish so that trophoblast cells spread out, while the undifferentiated inner cells (the inner cell mass) continued to grow as a tight but disorganized cluster. Before the inner cell mass developed into the equivalent of the embryonic disc, it was drawn up into a fine pipette, dissociated into single cells, and dispersed into another dish with a rich culture medium. Under these circumstances, the dissociated cells continued to grow rapidly for an extended period.

Mouse ES cells cannot become organized into an embryo by themselves or implant into the uterus if placed there. However, if the cells are injected back into a new blastocyst, they can intermingle with the host inner cell mass to make a chimera and participate in normal development, eventually contributing to all of the tissues of the adult mouse, including nerve, blood, skin, bone, and germ cells (Robertson and Bradley 1986). This

Table 2-1. Stem Cells Isolated from Mammals

Species	References
Mouse	Evans and Kaufman 1981 Martin 1981
Rat	Iannaccone et al. 1994
Hamster	Doetschman, Williams, and Maeda 1988
Mink	Sukoyan et al. 1992 Sukoyan et al. 1993
Rabbit	Moreadith and Graves 1992 Giles et al. 1993 Graves and Moreadith 1993
Sheep	Handyside et al. 1987 Piedrahita, Anderson, and Bondurant 1990 Notarianni et al. 1991
Pig	Piedrahita et al. 1988 Evans et al. 1990 Notarianni et al. 1990 Piedrahita et al. 1990 Hochereau-de Reiviers and Perreau 1993 Talbot et al. 1993 Wheeler 1994 Shim et al. 1997
Cow	Evans et al. 1990 Saito, Strelchenko, and Niemann 1992 Strelchenko and Stice 1994 Cibelli et al. 1998
Common Marmoset	Thomson et al. 1996
Rhesus Monkey	Thomson et al. 1995
Human	Bongso et al. 1994 Shamblott et al. 1998 Thomson et al. 1998

indicates that mouse ES cells have not lost the capacity to give rise to specialized tissues, but they will not do so unless placed in a conducive environment.

The ability of mouse ES cells to enter the germline in chimeras allows the introduction of specific genetic changes into the mouse genome and offers a direct approach to understanding gene function in the intact animal (Rossant, Bernelot-Moens, and Nagy 1993). Using the technique of homologous recombination in which a gene is either modified or disabled ("knocked out"), mouse ES cells that contain specific gene alterations may be derived. These genetically altered cells can then be used to form chimeras with normal embryos, subsequently generating a mouse lacking one specific gene or containing an extra or altered gene.

Mouse ES cells also have been extremely useful as models of the early differentiation events that occur during the development of mammalian embryos (Pedersen 1994), as shown in the following examples:

- When mouse ES cells were allowed to differentiate in culture, beating heart cells formed spontaneously, providing a model for cardiac-specific gene expression and the development of cardiac muscle and blood vessels (Chen and Kosco 1993; Doetschman et al. 1993; Miller-Hance et al. 1993; Muthuchamy et al. 1993; Robbins et al. 1990; Wobus, Wallukat, and Heschler 1991).
- Blood formation will occur spontaneously in ES cell-derived embryoid bodies and can be augmented by modifying the culture conditions (Snodgrass, Schmitt, and Bruyns 1992). Therefore, hematopoietic stem cells have been studied extensively in an effort to determine the conditions for differentiation, survival, and proliferation of blood cells.
- Several studies have highlighted the importance of growth and differentiation factors in the regulation of mammalian development. For example, the maintenance of mouse ES cells in an undifferentiated state was found to require the presence of leukemia inhibitory factor, a differentiation-inhibiting factor (Fry 1992). Other studies have found several growth and differentiation factors to be important in ES cell development and differentiation, including activins, colony-stimulating factor, erythropoietin, basic fibroblast growth factor, insulin-like growth factor 2, interleukins, parathryoid hormone-related peptide, platelet-derived growth factor, steel factor, and transforming growth factor ß (Pedersen 1994).
- In midgestation embryos and the adult mouse, only one parental allele of imprinted genes is expressed. However, studies have suggested that there is limited relaxation of imprinting in ES cells so that both maternal and paternal alleles are expressed (Pedersen 1994).

By understanding the mechanisms responsible for growth and differentiation in embryonic development, it may then be possible to attempt to regulate the differentiation of ES cells along specific pathways. The knowledge gained from these types of studies could someday lead to the effective treatment of certain important human diseases.

Historically, because of its well-defined genetics, short gestational time, ease of cultivation, and large litters, the mouse has been one of the primary models for the study of mammalian embryonic development. However, there are several differences between early mouse development and early human development, including

- the timing of embryonic genome expression (Braude, Bolton, and Moore 1988),
- the formation, structure, and function of the fetal membranes and placenta (Benirschke and Kaufmann 1990; Luckett 1975, 1978), and
- the formation of an egg cylinder (mouse) as opposed to an embryonic disc (human) (Kaufmann 1992; O'Rahilly 1987).

Thus, other animal models as well as new models that would allow the direct study of human embryonic development are crucial in order to comprehend early human development and to understand the growth requirements of human stem cells of specific lineages.

Bovine ES Cells

The first bovine ES-like cells were reported by Saito, Strelchenko, and Niemann in 1992. More recently, transgenic bovine ES-like cells were derived by using nuclear transfer of fetal fibroblasts to enucleated bovine oocytes (Cibelli et al. 1998). This technique involved introducing a marker gene into bovine fibroblasts from a 55-day-old fetus and then fusing the transgenic fibroblasts to enucleated oocytes to produce blastocyst-stage nuclear

transplant embryos (Cibelli et al. 1998). ES-like cells then were derived from these embryos and were used to create chimeric embryos. When reintroduced into pre-implantation embryos, these transgenic ES-like cells differentiated into derivatives from the three EG layers—ectoderm, mesoderm, and endoderm (Cibelli et al. 1998). Bovine ES cells would be useful in agricultural production of transgenic cows and also may have the potential for generating tissues and organs for use in cross-species transplantation (xenotransplantation) in order to treat human diseases.

Primate ES Cells

Primate ES-like cells have been derived from both the rhesus monkey (Thomson et al. 1995) and the common marmoset (Thomson et al. 1996). When allowed to grow, both marmoset and rhesus ES cells spontaneously differentiate into more complex structures, including cardiac muscle, neurons, endoderm, trophoblast, and numerous unidentified cell types (Thomson and Marshall 1998).

Essential characteristics of these primate ES-like cells include 1) derivation from the pre-implantation or peri-implantation embryo, 2) prolonged undifferentiated proliferation, and 3) stable developmental potential to form derivatives of all three EG layers even after prolonged maintenance in culture (Thomson and Marshall 1998). In addition, although mouse ES cells rarely contribute to trophoblast in chimeras (Beddington and Robertson 1989), primate ES cells differentiate into all three germ layers and trophoblast-like cells (Thomson and Marshall 1998). Furthermore, some primate ES cell lines have maintained a normal karyotype through undifferentiated culture for at least two years, sustained a stable developmental potential throughout this culture period, and maintained the potential to form trophoblast *in vitro* (Thomson et al. 1995, 1996).

Although there is some variation between species, nonhuman primate ES cell lines appear to provide a useful *in vitro* model for understanding the differentiation of human tissues (Thomson and Marshall 1998), and primate ES cells provide a powerful model for understanding human development and disease. Furthermore, because of the similarities between human and primate ES cells, primate ES cells provide a model for developing strategies to prevent immune rejection of transplanted cells and for demonstrating the safety and efficacy of ES cell-based therapies (Thomson et al. 1995).

Human Models

Human ES Cell Lines Derived from Blastocysts

The first successful isolation of cells from the human inner cell mass of blastocysts and their culture *in vitro* for at least two series of cell divisions was reported by Bongso and colleagues in 1994. Starting with 21 spare embryos donated by nine patients in an IVF program,[3] this group isolated cells with typical stem cell characteristics from 17 five-day-old blastocysts (approximately 100 cells) (Bongso et al. 1994). These cells were like ES cells. They were small and round with high nuclear to cytoplasmic ratios, they stained positively for alkaline phosphatase (a biochemical marker for stem cells), and they maintained a normal diploid karyotype. However, after the second subculture, the cells differentiated into fibroblasts or died (Bongso et al. 1994).

In later work, Thomson and his colleagues were able to isolate human ES-like cell lines and grow them continuously in culture for at least five to six months. Although these cells have not passed the most stringent test—as have mouse ES cells—to determine whether they can contribute to the germline, we will continue to use the term *ES cell* throughout this report because both scientists and nonscientists alike have widely applied this term to refer to these cells. This renewable tissue culture source of human cells—capable of differentiating into a wide variety of cell types—is believed to have broad applications in basic research and transplantation therapies (Gearhart 1998).

In Thomson's work, human ES cells were isolated from embryos that were originally produced by IVF for clinical reproductive purposes. (See Exhibit 2-B.) Individuals donated the embryos, following an informed consent process. The consent forms and the entire research protocol were reviewed and approved by an appropriately constituted Institutional Review Board (IRB) (Thomson et al. 1998). Thirty-six embryos were cultured for approximately five days. The inner cell mass was isolated from 14 of the 20 blastocysts that developed,

Exhibit 2-B: *In Vitro* Fertilization (IVF)

The procedure of IVF today is widely available in many countries throughout the world, including the United States. Originally developed for the treatment of infertility due to blocked fallopian tubes, IVF has been extended to assist patients with premature depletion of oocytes, recurrent failure of embryos to implant, and low production of functional sperm. More recently, the technique has been used in conjunction with pre-implantation genetic diagnosis to enable fertile couples at risk for transmitting severe or fatal inherited diseases to have healthy children.

Although details of the IVF procedures vary from center to center, the basic approach is to treat oocyte donors over several days with a regimen of hormones designed to stimulate the final maturation of several follicles within the ovary. This is known as hyperstimulation, a procedure that carries the risk of an adverse reaction of less than 1 percent. Following completion of the hormone treatment, mature follicles are detected by sonography and an average of ten are collected by transvaginal aspiration while the patient is sedated. The oocytes are then fertilized by sperm collected from a male donor and cultured in sterile fluid for about two days. When the zygote has reached the four- to eight-cell stage, between three and six zygotes are transferred to the uterus, and the untransferred embryos, if they are developing normally, are usually frozen. Nonviable embryos are discarded. (See also Figure 2-5.) More recently, IVF specialists have begun culturing embryos to the blastocyst stage before transfer to the uterus.

The efficiency of the IVF procedure is relatively low, with approximately 20 percent of fertilized eggs resulting in successful pregnancies, depending on factors such as age of the recipient and the reason for infertility. In comparison, approximately 30 percent of normally conceived human embryos result in successful pregnancies. Embryos that are not transferred can be cryopreserved and stored indefinitely.

Sources:

National Institutes of Health (NIH). Human Embryo Research Panel. 1994. *Report of the Human Embryo Research Panel*. 2 vols. Bethesda, MD: NIH.

New York State Task Force on Life and the Law. 1998. *Assisted Reproductive Technologies: Analysis and Recommendations for Public Policy*. New York: New York State Task Force on Life and the Law.

and five ES cell lines, originating from five separate embryos, were derived (Thomson et al. 1998). The technique used to derive these human ES cells is essentially the same as that used to isolate nonhuman primate ES cell lines (Thomson et al. 1995).

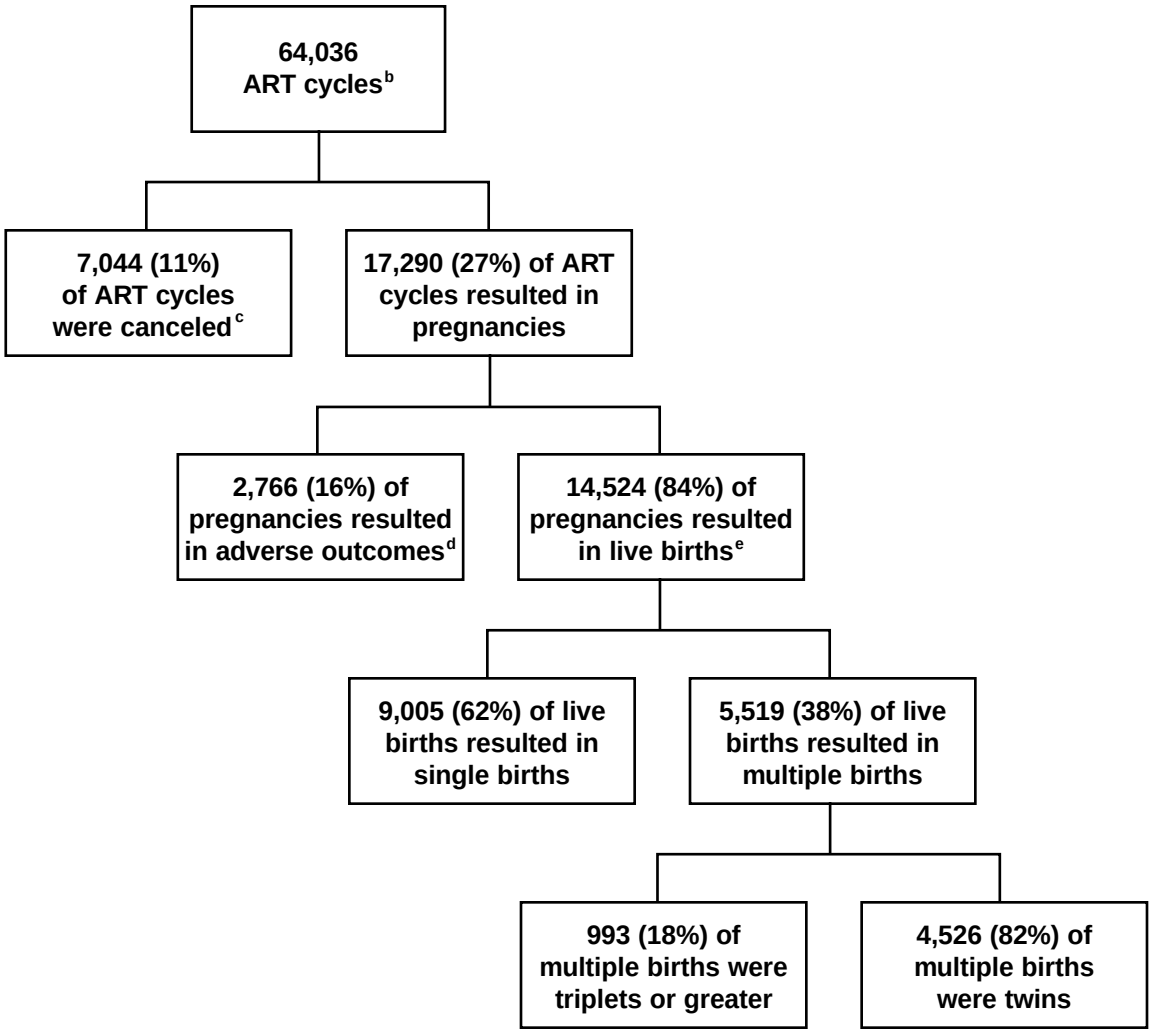
The resulting human ES cell lines had normal karyotypes (two male and three female) and were grown in culture continuously for at least five to six months (Thomson et al. 1998). In addition, the cell lines expressed cell surface markers that also are found on nonhuman primate ES cells (Thomson et al. 1998). Most important, the cell lines maintained the potential to form derivatives of all three EG layers—endoderm, mesoderm, and ectoderm (Thomson et al. 1998).

Many believe that research using human ES cells might offer insights into developmental events that cannot be studied directly in the intact human embryo but that have important consequences in clinical areas such as birth defects, infertility, and miscarriage. Some speculate that the origins of many human diseases (e.g., juvenile-onset diabetes) are due to events that occur early in embryonic development. Such cells also will be particularly valuable for the study of the development and function of tissues that differ between mice and humans. These cells allow for studies that focus on the differentiation of cells into specific tissues and the factors that bring about differentiation, so that cells can be manipulated to generate specific cell types for therapeutic transplantation. Moreover, it may be possible to identify gene targets for new drugs, to manipulate genes that could be used for tissue regeneration therapies, and to understand the teratogenic or toxic effects of certain compounds (Thomson et al. 1998).

Human EG Cells from Fetal Primordial Germ Cells

Primordial germ cells also can give rise to cells with characteristics of ES cells, and, as discussed previously,

Figure 2-5. 1996 Assisted Reproductive Technology (ART) Success Rates[a]



[a]Source: Centers for Disease Control and Prevention (CDC), American Society for Reproductive Medicine, Society for Assisted Reproductive Technology, and RESOLVE. 1998. *1996 Assisted Reproductive Technology Success Rates, National Summary and Fertility Clinic Reports*. Atlanta, GA: CDC.

[b]Data are from 300 U.S. fertility clinics that provided and verified information about the outcomes of all ART cycles started in their clinics in 1996.

[c]Fresh, nondonor cycles were canceled, most commonly because too few (egg) follicles developed. Illness unrelated to the ART procedure also may lead to cancelation. In general, cycles are canceled when chances of success are poor or risks are unacceptably high.

[d]Adverse outcomes included spontaneous abortion (83%), induced abortion (10%), stillbirth (4%), and ectopic pregnancy (3%).

[e]A total of 20,659 babies were born as a result of the 64,036 ART cycles carried out in 1996.

have been designated as EG cells in order to distinguish their tissue of origin (Gearhart 1998). A 1998 report from John D. Gearhart and his colleagues describes the establishment of human EG cell lines from human primordial germ cells (Shamblott et al. 1998). Using an IRB-approved protocol, the human EG cells were isolated from the developing gonads of five- to nine-week-old embryos and fetuses that were obtained following elective abortion (Shamblott et al. 1998). These human EG cell lines have morphological, immunohistochemical, and karyotypic features consistent with those of previously described ES cells and have a demonstrated ability to differentiate *in vitro* into derivatives of the three germ layers (Shamblott et al. 1998).

Fusion of Human Somatic Cells with Cow Eggs to Create Hybrid Embryonic Cells

Advanced Cell Technology of Worcester, Massachusetts, announced in November 1998 that its scientists had made human somatic cells revert to the primordial state by fusing them with cow eggs to create a hybrid embryo (Wade 1998). This work with human cells was performed in 1996 by Jose Cibelli. Using 52 of his own cells—some of them white blood cells and others scraped from the inside of his cheek—Cibelli used a pulse of electricity to fuse each cell with a cow egg from which the nucleus containing the DNA had been removed.[4] Out of these 52 attempts, only one embryo, derived from a cheek epithelial cell, developed into a blastocyst. Approximately 12 days after the fusion of cheek cell and cow egg, sufficient cells existed to allow harvesting of the inner cell mass to produce cells resembling human ES cells. The researchers observed that the hybrid cell quickly became more human-like as the human nucleus took control and displaced bovine proteins with human proteins. However, it is difficult to judge the validity of this work and the nature of the "embryo-like" material produced because the work is extremely preliminary and has not been submitted for peer review or for publication in a scientific journal.

The stated purpose of these experiments was to create an embryo solely for the purpose of establishing an ES cell line that might be used to treat any disease caused by the loss or malfunction of cells, such as Parkinson's disease, diabetes, and heart disease. The researchers emphasized that they had no intention of transferring the resulting hybrid embryos to a uterus, as they considered this to be both unethical and unsafe (Wade 1998).

Growth and Derivation of ES Cells

Human ES cells are different from many adult cells because they have the ability to divide extensively in culture. Although this property has been interpreted by nonscientists as an indication that investigators simply can use existing human ES and EG cell lines (which can be extensively reproduced for a limited time) to study their properties, this is not the case and is a reflection of a misunderstanding of the science that is involved. Evidence from mouse ES cell research suggests that it is essential to derive new ES cell lines repeatedly in order to further our understanding of how to differentiate these cells and grow them extensively in culture.

There are several reasons why it is necessary to repeatedly derive new ES cell lines. First, the properties of ES cells differ depending on the methods used to derive them.[5] Cells derived under some conditions may be limited in their potential to differentiate into a particular tissue type. Second, ES cells are not stable cell types that can simply be mass produced and supplied to an unlimited number of researchers. As these cells grow in culture they accumulate irreversible changes, and the conditions used to grow them can influence the speed at which these changes accumulate. Typically, researchers look only at the ability of ES cells to contribute to some tissues. In one study, however, the ability of ES cells to generate *all* tissue in a mouse was tested (Nagy et al. 1993). This research has shown dramatically that existing cell lines commonly in use by many researchers have lost the ability to generate all mouse tissues and thus to completely generate live mice. When new ES cells were derived and grown for only a short time in culture, they did allow all tissues to be generated. However, after about 14 doublings in culture, even these cells lost their ability to contribute to all tissues. The researchers conclude… "[P]rolonged passage in culture reduces the potential of the ES cell population as a whole. The proportion of cells that retain full potential diminishes with extended

passage" (Nagy et al. 1993). Exactly what changes occur during culture are not yet clear. The chromosome complement remains normal, indicating that this criterion, although frequently used to characterize ES cells, is not a very stringent assay. It could be an accumulation of mutations, changes in gene expression, or epigenetic changes (Nagy et al. 1993). Thus, if one scientist were to obtain cells from a colleague's laboratory, the properties of the cells would depend greatly on the history of how those cells were grown. For this reason, many people who work with mouse ES cells re-derive the cells periodically to be sure the cells have the potential to differentiate into or contribute to many different tissues.

Finally, perhaps the most important reason for deriving new ES cell lines rather than simply working with existing cell lines is that a tremendous amount remains to be learned during the process of derivation itself. It took many laboratories more than ten years to ascertain appropriate conditions for the derivation and growth of mouse ES cells. Research on the growth and derivation of ES cells from other mammalian species is only in its early stages. In fact, only mouse ES cells have the property of contributing to the germline cell lineage—the most stringent criterion for ES cells. Thus, cells from other species are referred to as ES-like cells (Pedersen 1994). Further basic research into the proper conditions to maintain ES cells from many species is ongoing in an attempt to understand the factors necessary to generate stable ES cells. Given that only two successes have been reported on the derivation of human ES and EG cells, it is likely that significant basic research into the appropriate conditions to generate stable stem cells will be needed.

Potential Medical Applications of Human ES Cell and EG Cell Research

Although research into the use of ES and EG cells is still at an early stage, researchers hope to make a contribution to disease treatment in a variety of areas. The ability to elucidate the mechanisms that control cell differentiation is, at the most elemental level, the promise of human ES and EG cell research. This knowledge will facilitate the efficient, directed differentiation of stem cells to specific cell types. The standardized production of large, purified populations of human cells such as cardiomyocytes and neurons, for example, could provide a substantial source of cells for drug discovery and transplantation therapies (Thomson et al. 1998). Many diseases, such as Parkinson's disease and juvenile-onset diabetes mellitus, result from the death or dysfunction of just one or a few cell types, and the replacement of those cells could offer effective treatment and even cures.

Substantial advances in basic cell and developmental biology are required before it will be possible to direct human ES cells to lineages of human clinical importance. However, progress has already been made in the differentiation of mouse ES cells to neurons, hematopoietic cells, and cardiac muscle (Brustle et al. 1997; Deacon et al. 1998; Shamblott et al. 1998). Human ES and EG cells could be put to use in targeting neurodegenerative disorders, diabetes, spinal cord injury, and hematopoietic repopulation, the current treatments for which are either incomplete or create additional complications for those who suffer from them.

Use of Human ES Cells and EG Cells in Transplantation

One of the major causes of organ transplantation and graft failure is immune rejection, and a likely application of human ES and EG cell research is in the area of transplantation. Although much research remains to be done, ES cells derived through SCNT offer the possibility that therapies could be developed from a patient's own cells. In other words, a patient's somatic cells could be fused with an enucleated oocyte and developed to the blastocyst stage, at which point ES cells could be derived for the development of cell-based therapy. This essentially is an autologous transfer. Thus, issues of tissue rejection due to the recognition of foreign proteins by the immune system are avoided entirely. In addition, research to establish xenotransplantation (i.e., interspecies transplantation) as a safe and effective alternative to human organ transplantation is still in its infancy. Alternately, other techniques that would be immunologically compatible for transplantation purposes could be used to generate stem cells, such as

1) banking of multiple cell lines representing a spectrum of major histocompatibility complex (MHC) alleles to serve as a source for MHC matching, and/or

2) creating universal donor lines, in which the MHC genes could be genetically altered so rejection would not occur, an approach that has been tried in the mouse with moderate success (NIAID 1999).

Autologous transplants would obviate the need for immunosuppressive agents in transplantation as it would decrease a central danger to transplant patients—susceptibility to other diseases. Autologous transplants might address problems ranging from the supply of donor organs to the difficulty of finding matches between donors and recipients. Research on ES cells could lead to cures for diseases that require treatment through transplantation, including autoimmune diseases such as multiple sclerosis, rheumatoid arthritis, and systemic lupus erythematosus. These cells also might hold promise for treating type-I diabetes (Melton 1999; Varmus 1998), which would involve the transplantation of pancreatic islet cells or beta cells produced from autologous ES cells. These cells would enter the pancreas and provide normal insulin production by replacing the failing resident islet cells.

Studies of Human Reproduction and Developmental Biology

Research using human ES and EG cells could offer insights into developmental events that cannot be studied directly in the intact human embryo but that have important consequences in clinical areas, including birth defects, infertility, and pregnancy loss (Thomson et al. 1998). ES and EG cells provide large quantities of homogeneous material that can be used for biochemical analysis of the patterns of gene expression and the molecular mechanisms of embryonic differentiation.

Cancer Therapy

Human ES and EG cells may be used to reduce the tissue toxicity brought on by cancer therapy (NCI 1999). Already, bone marrow stem cells, representing a more committed stem cell, are used to treat patients after high-dose chemotherapy. However, the recovered blood cells appear limited in their ability to recognize abnormal cells, such as cancer cells. It is possible that injections of ES and EG cells would revive the complete immune response to patients undergoing bone marrow transplantation. Current approaches aimed at manipulating the immune system after high-dose chemotherapy so that it recognizes cancer cells specifically have not yet been successful.

Diseases of the Nervous System

Some believe that in no other area of medicine are the potential benefits of ES and EG cell research greater than in diseases of the nervous system (Gearhart 1998; Varmus 1998). The most obvious reason is that so many of these diseases result from the loss of nerve cells, and mature nerve cells cannot divide to replace those that are lost. For example, in Parkinson's disease, nerve cells that make the chemical dopamine die; in Alzheimer's disease, it is the cells that make acetylcholine that die; in Huntington's disease the cells that make gamma aminobutyric acid die; in multiple sclerosis, cells that make myelin die; and in amyotrophic lateral sclerosis, the motor nerve cells that activate muscles die. In stroke, brain trauma, spinal cord injury, and cerebral palsy and mental retardation, numerous types of cells are lost with no built-in mechanism for replacing them.

Preliminary results from fetal tissue transplantation trials for Parkinson's disease suggest that supplying new cells to a structure as intricate as the brain can slow or stop disease progression (Freed et al. 1999). Yet the difficulty of obtaining enough cells of the right type—that is, dopamine-producing nerve cells—limits the application of this therapy. In 1999, scientists developed methods in animal models to isolate dopamine precursor cells from the dopamine-producing region of the brain and coax them to proliferate for several generations in cell culture. When these cells were implanted into the brains of rodents with experimental Parkinson's disease, the animals showed improvements in their movement control (NINDS 1999). Scientists also have learned to instruct a stem cell from even a nondopamine region to make dopamine (Wagner et al. 1999). A large supply of "dopamine-competent" stem cells, such as ES cell lines, could remove the barrier of limited amounts of tissue. (See Exhibit 2-C.)

Another recent development eventually may provide treatments for multiple sclerosis and other diseases that attack the myelin coating of nerves. Scientists have successfully generated glial cells that produce myelin from

Exhibit 2-C: Potential Treatment for Parkinson's Disease

Parkinson's disease is a degenerative brain disease that affects 2 percent of the population over age 70. Symptoms include slow and stiff movements, problems with balance and walking, and tremor. In more advanced cases, the patient has a fixed, staring expression, walks with a stooped posture and short, shuffling pace, and has difficulty initiating voluntary movements. Falls, difficulty swallowing, incontinence, and dementia may occur in the late stages. Patients often lose the ability to care for themselves and may become bedridden.

The cause of this illness is a deficiency of the neurotransmitter dopamine in specific areas of the brain. Treatment with drugs such as levodopa often is effective in relieving the symptoms. However, as the disease progresses, treatment often becomes more problematic, with irregular responses, difficulty adjusting doses, and the development of side effects such as involuntary writhing movements. Brain surgery with transplantation of human fetal tissue has shown promise as therapy.

Stem cell transplantation also may be a promising therapy for Parkinson's disease. The injection of stem cells that can differentiate into brain cells may offer a means of replenishing neurons that are capable of synthesizing the deficient neurotransmitter. It is possible that stem cell transplantation may be simpler and more readily available than fetal tissue transplantation.

mouse ES cells (Brustle et al. 1999). When these ES cell-derived glial cells were transplanted in a rat model of human myelin disease, they were able to interact with host neurons and efficiently myelinate axons in the rat's brain and spinal cord (Brustle et al. 1999).

Other diseases that might benefit from similar types of approaches include spinal cord injury, epilepsy, stroke, Tay-Sachs disease, and pediatric causes of cerebral palsy and mental retardation. In mice, neural stem cells already have been shown to be effective in replacing cells throughout the brain and in some cases are capable of correcting neurological defects (Lacorazza et al. 1996; Rosario et al. 1997; Snyder et al. 1997; Snyder, Taylor, and Woolfe 1995; Yandava, Billinghurst, and Snyder 1999). Human neural stem cells also have recently been isolated and have been shown to be responsive to developmental signals and to be willing to replace neurons when transplanted into mice (Flax et al. 1998). These recent discoveries of ways to generate specific types of neural cells from ES cells hold much promise for the treatment of severe neurological disorders that today have no known cure.

Diseases of the Bone and Cartilage

Because ES and EG cells constitute a relatively self-renewing population of cells, they can be cultured to generate greater numbers of bone or cartilage cells than could be obtained from a tissue sample. If a self-renewing, but controlled, population of stem cells can be established in a transplant recipient, it could effect long-term correction of many diseases and degenerative conditions in which bone or cartilage cells are deficient in numbers or defective in function. This could be done either by transplanting ES and EG cells to a recipient or by genetically modifying a person's own stem cells and returning them to the marrow. Such approaches hold promise for the treatment of genetic disorders of bone and cartilage, such as osteogenesis imperfecta and the various chondrodysplasias. In a somewhat different potential application, stem cells perhaps could be stimulated in culture to develop into either bone- or cartilage-producing cells. These cells could then be introduced into the damaged areas of joint cartilage in cases of osteoarthritis or into large gaps in bone that can arise from fractures or surgery. This sort of repair would have a number of advantages over the current practice of tissue grafting (NIAMS 1999).

Blood Disorders

The globin proteins are essential for transport of oxygen in the blood, with different globins expressed at different developmental stages. The epsilon globin gene is expressed only in embryonic red blood cells. When this gene—which is not normally expressed in the adult—is artificially turned on in sickle cell patients, it blocks the sickling of the cells that contain sickle cell hemoglobin. Research involving ES cells could help answer questions about how to turn on the epsilon globin gene in adult blood cells and thereby halt the disease process. Stem cell

research also may help produce transplantable cells that would not contain the sickle cell mutation.

Toxicity and Drug Testing

Human stem cell research offers promise for use in testing the beneficial and toxic effects of biologicals, chemicals, and drugs in the most relevant species for clinical validity—humans. Such studies could lead to fewer, less costly, and better designed human clinical trials yielding more specific diagnostic procedures and more effective systemic therapies. Beyond the drug development screening of pharmacological agents for toxicity and/or efficacy, human stem cell research could define new research approaches for clarifying the complex association of environmental agents with human disease processes (NIEHS 1999). It also makes possible a new means of conducting detailed investigations of the underlying mechanisms of the effects of environmental toxins or mixtures of toxins, including their subtle effects on the developing embryonic and fetal development tissue systems.

Transplantable Organs

Several researchers are investigating ways to isolate AS cells and create transplantable organs that may be used to treat a multitude of diseases that do not rely upon the use of embryonic or fetal tissue. Moreover, if it is found to be possible to differentiate ES cells into specific cell types, such stem cells could be an important source of cells for organ growth. For example, recent developments in animals have shown that it may be possible to create entire transplantable organs from a tissue base in a manner that would overcome such problems as the limited supply of organs and tissue rejection. Such a development—producing this tissue base by directing the growth of human embryonic cells—could be a major breakthrough in the field of whole organ transplantation.

For example, using tissue engineering methods, researchers have successfully grown bladders in the laboratory, implanted them into dogs, and shown them to be functional (Oberpenning et al. 1999). To create the bladders, small biopsies of tissue were taken from dog bladders. The biopsied tissue was then teased apart to isolate the urothelial tissue and muscle tissue, which were then grown separately in culture (Tanne 1999). The tissue was then applied to a mold of biodegradable material with the urothelial tissue on the inside and the muscle tissue on the outside. The new organs were transplanted within five weeks (Tanne 1999).

Dogs that received the tissue-engineered organs regained 95 percent of their original bladder capacity, were continent, and voided normally. When the new organs were examined 11 months later, they were completely covered with urothelial and muscle tissue and had both nerve and blood vessel growth. Dogs that did not undergo reconstructive procedures or only received implants of the biodegradable molds did not regain normal bladder function (Oberpenning et al. 1999). This accomplishment marks the first time a mammalian organ has been grown in a laboratory. The ability to create new organs by seeding molds with cells of specific tissue types would be extremely useful in treating children with congenital malformations of organs and people who have lost organs due to trauma or disease (Tanne 1999).

Summary

Currently, human ES cells can be derived from the inner cell mass of a blastocyst (those cells within the conceptus that form the embryo proper), and EG cells can be derived from the primordial germ cells of fetuses. These cells, present in the earliest stages of embryo and fetal development, can generate all of the human cell types and are capable, at least for some time, of self-renewal. A relatively renewable tissue culture source of human cells that can be used to generate a wide variety of cell types would have broad applications in basic research, transplantation, and other important therapies, and a major step in realizing this goal was taken in 1998 with the demonstration that human ES and EG cells can be grown in culture. The clinical potential for these stem cells is vast—they will be important for *in vitro* studies of normal human embryogenesis, human gene discovery, and drug and teratogen testing and as a renewable source of cells for tissue transplantation, cell replacement, and gene therapies.

Notes

1 For a summary of scientific progress in this field see Eiseman, E., "Human Stem Cell Research," RAND DRU-2171-NBAC, September 1999, a background paper prepared for the National Bioethics Advisory Commission.

2 Thomson, J.A., Testimony before NBAC. January 19, 1999. Washington, DC.

3 Consent to carry out this study was approved by the hospital ethical committee based on the guidelines on Assisted Reproductive Technology of the Ministry of Health, Singapore, that experimentation of human embryos up to day 14 of embryonic growth may be allowed (Bongso et al. 1994).

4 The details of this process are described in a European patent application (PCT/U397/12919 1997) and in testimony before the Commission by ACT President Michael West. November 17, 1998. Miami, FL.

5 Hogan, B., Testimony before NBAC. February 3, 1999. Princeton, NJ.

References

Beddingston, R.S., and E.J. Robertson. 1989. "An Assessment of the Developmental Potential of Embryonic Stem Cells in the Midgestation Mouse Embryo." *Development* 105:733–737.

Benirschke, K., and P. Kaufmann. 1990. *Pathology of the Human Placenta*. New York: Springer-Verlag.

Bjorklund, A. 1993. "Neurobiology: Better Cells for Brain Repair." *Nature* 362:414–415.

Bjornson C.R., R.L. Rietze, B.A. Reynolds, M.C. Magli, and A.L. Vescovi. 1999. "Turning Brain into Blood: A Hematopoietic Fate Adopted by Adult Neural Stem Cells *In Vivo*." *Science* 283:534–537.

Bongso, A., C.Y. Fong, S.C. Ng, and S. Ratnam. 1994. "Isolation and Culture of Inner Cell Mass Cells from Human Blastocysts." *Human Reproduction* 9(11):2110–2117.

Braude, P., V. Bolton, and S. Moore. 1988. "Human Gene Expression First Occurs Between the Four- and Eight-Cell Stages of Pre-Implantation Development." *Nature* 332:459–461.

Brustle, O., A.C. Spiro, K. Karram, K. Choudhary, S. Okabe, and R.D. McKay. 1997. "*In Vitro*-Generated Neural Precursors Participate in Mammalian Brain Development." *Proceedings of the National Academy of Sciences USA* 94:14809–14814.

Brustle, O., K.N. Jones, R.D. Learish, K. Karram, K. Choudhary, O.D. Weistler, I.D. Duncan, and R.D. McKay. 1999. "Embryonic Stem Cell-Derived Glial Precursors: A Source of Myelinating Transplants." *Science* 285:754–756.

Cattaneo, E., and R. McKay. 1991. "Identifying and Manipulating Neuronal Stem Cells." *Trends in Neurosciences* 14(8):338–340.

Chen, U., and M. Kosco. 1993. "Differentiation of Mouse Embryonic Stem Cells *In Vitro*: III. Morphological Evaluation of Tissues Developed After Implantation of Differentiated Mouse Embryoid Bodies." *Developmental Dynamics* 197:217–226.

Cibelli, J.B., S.L. Stice, P.J. Golueke, J.J. Kane, J. Jerry, C. Blackwell, F.A. Ponce de Leon, and J.M. Robl. 1998. "Transgenic Bovine Chimeric Offspring Produced from Somatic Cell-Derived Stem-Like Cells." *Nature Biotechnology* 16:642–646.

Corn, B., M. Reed, S. Dishong, L. Yunsheng, and T. Wagner. 1991. "Culture and Successful Transplantation of Embryonic Yolk-Sac Cells." *Clinical Biotechnology* 3:15.

Deacon, T., J. Dinsmore, L.C. Costantini, J. Ratliff, and O. Isacson. 1998. "Blastula-Stage Stem Cells Can Differentiate into Dopaminergic and Serotenergic Neurons After Transplantation." *Experimental Neurology* 149:28–41.

Diukman, R., and M.S. Golbus. 1992. "*In Utero* Stem Cell Therapy." *Journal of Reproductive Medicine* 37:515–520.

Doetschman, T., P. Williams, and N. Maeda. 1988. "Establishment of Hamster Blastocyst-Derived Embryonic Stem (ES) Cells." *Developmental Biology* 127:224–227.

Doetschman, T., M. Shull, A. Kier, and J.D. Coffin. 1993. "Embryonic Stem Cell Model Systems for Vascular Morphogenesis and Cardiac Disorders." *Hypertension* 22:618–629.

Donovan, P.J. 1994. "Growth Factor Regulation of Mouse Primordial Germ Cell Development." *Current Topics in Developmental Biology* 29:189–225.

———. 1998. "The Germ Cell: The Mother of All Stem Cells." *International Journal of Developmental Biology* 42:1043–1050.

Evans, M.J., and M.H. Kaufman. 1981. "Establishment in Culture of Pluripotential Cells from Mouse Embryos." *Nature* 292:154–156.

Evans, M.J., E. Notarianni, S. Laurie, and R.M. Moor. 1990. "Derivation and Preliminary Characterization of Pluripotent Cell Lines from Porcine and Bovine Blastocysts." *Theriogenology* 33:125–128.

Flax, J.D., S. Aurora, C. Yang, C. Simonin, A.M. Wills, L.L. Billinghurst, M. Jendoubi, R.L. Sidman, J.H. Wolfe, S.U. Kim, and E.Y. Snyder. 1998. "Engraftable Human Neural Stem Cells Respond to Developmental Cues, Replace Neurons and Express Foreign Genes." *Nature Biotechnology* 16:1033–1039.

Frederiksen, K., and R.D. McKay. 1988. "Proliferation and Differentiation of Rat Neuroepithelial Precursor Cells *In Vivo*." *Journal of Neuroscience* 8:1144–1151.

Freed, C.R., R.E. Breeze, C.O. Denver, P.E. Greene, W. Tsai, D. Eidelberg, J.Q. Trojanowski, J.M. Rosenstein, and S. Fahn. 1999. *Double-Blind Controlled Trial of Human Embryonic Dopamine Cell Transplants in Advanced Parkinson's Disease: Study Design, Surgical Strategy, Patient Demographics and Pathological Outcome*. Presentation to the American Academy of Neurology, 21 April.

Fry, R. 1992. "The Effect of Leukemia Inhibitory Factor (LIF) on Embryogenesis." *Reproduction, Fertility, and Development* 4:449–458.

Gardner, R.L. 1982. "Investigation of Cell Lineage and Differentiation in the Extra Embryonic Endoderm of the Mouse Embryo." *Journal of Embryological Experimental Morphology* 68:175–198.

Gearhart, J.D. 1998. "New Potential for Human Embryonic Stem Cells." *Science* 282:1061–1062.

Giles, J.R., X. Yang, W. Mark, and R.H. Foote. 1993. "Pluripotency of Cultured Rabbit Inner Cell Mass Cells Detected by Isozyme Analysis and Eye Pigmentation of Fetuses Following Injection into Blastocysts or Morulae." *Molecular Reproduction and Development* 36:130–138.

Graves, K.H., and R.W. Moreadith. 1993. "Derivation and Characterization of Putative Pluripotential Embryonic Stem Cells from Pre-implantation Rabbit Embryos." *Molecular Reproduction and Development* 36:424–433.

Hall, P. A., and F.M. Watt. 1989. "Stem Cells: The Generation and Maintenance of Cellular Diversity." *Development* 106:619–633.

Handyside, A., M.L. Hooper, M.H. Kaufman, and I. Wilmut. 1987. "Towards the Isolation of Embryonal Stem Cell Lines from the Sheep." *Roux's Archives of Developmental Biology* 196:185–190.

Hochereau-de Reviers, M.T., and C. Perreau. 1993. "*In Vitro* Culture of Embryonic Disc Cells from Porcine Blastocysts." *Reproduction, Nutrition, Development* 33:475–483.

Hollands, P. 1991. "Embryonic Stem Cell Grafting: The Therapy of the Future?" *Human Reproduction* 6:79–84.

Iannaccone, P.M., G.U. Taborn, R.L. Garton, M.D. Caplice, and D.R. Brenin. 1994. "Pluripotent Embryonic Stem Cells from the Rat are Capable of Producing Chimeras." *Developmental Biology* 163:288–292.

Iscove, N. 1990. "Haematopoiesis. Searching for Stem Cells." *Nature* 347:126–127.

Kaufmann, M.H. 1992. *The Atlas of Mouse Development*. London: Academic Press.

Lacorazza, H.D., J.D. Flax, E.Y. Snyder, and M. Jendoubi. 1996. "Expression of Human b-hexosaminidase a-subunit Gene (the Gene Defect of Tay-Sachs Disease) in Mouse Brains upon Engraftment of Transduced Progenitor Cells." *Nature Medicine* 2:424–429.

Luckett, W.P. 1975. "The Development of Primordial and Definitive Amniotic Cavities in Early Rhesus Monkey and Human Embryos." *American Journal of Anatomy* 144:149–167.

———. 1978. "Origin and Differentiation of the Yolk Sac Extra Embryonic Mesoderm in Presomite Human and Rhesus Monkey Embryos." *American Journal of Anatomy* 152:59–97.

Martin, G.R. 1981. "Isolation of a Pluripotent Cell Line from Early Mouse Embryos Cultured in Medium Conditioned by Teratocarcinoma Stem Cells." *Proceedings of the National Academy of Sciences USA* 78:7634–7638.

Matsui, Y. 1998. "Developmental Fates of Mouse Germ Cell Line." *International Journal of Developmental Biology* 42:1037–1042.

Melton, D. 1999. Testimony of the Juvenile Diabetes Foundation International Before the Senate Appropriations Subcommittee on Labor, Health and Human Services, and Education. January 12.

Miller-Hance, W.C., M. LaCorbiere, S.J. Fuller, S.M. Evans, G. Lyons, C. Schmidt, J. Robbins, and K.R. Chien. 1993. "*In Vitro* Chamber Specification During Embryonic Stem Cell Cardiogenesis. Expression of the Ventricular Myosin Light Chain-2 Gene Is Independent of Heart Tube Formation." *Journal of Biological Chemistry* 268:25244–25252.

Moreadith, R.W., and K.H. Graves. 1992. "Derivation of Pluripotential Embryonic Stem Cells from the Rabbit." *Transactions of the Association of American Physicians* 105:197–203.

Muthuchamy, M., L. Pajak, P. Howles, T. Doetschman, and D.F. Wieczorek. 1993. "Developmental Analysis of Tropomyosin Gene Expression in Embryonic Stem Cells and Mouse Embryos." *Molecular Cell Biology* 13:3311–3323.

Nagy, A., J. Rossant, R. Nagy, W. Abramow-Newerly, and J.C. Roder. 1993. "Derivation of Completely Cell Culture-Derived Mice from Early-Passage Embryonic Stem Cells." *Proceedings of the National Academy of Sciences USA* 90:8424–8428.

National Cancer Institute (NCI), NIH. 1999. Response to Senator Specter's Inquiry "What Would You Hope to Achieve from Stem Cell Research?" March 25.

National Institute of Allergy and Infectious Diseases (NIAID), NIH. 1999. Response to Senator Specter's Inquiry "What Would You Hope to Achieve from Stem Cell Research?" March 25.

National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS), NIH. 1999. Response to Senator Specter's Inquiry "What Would You Hope to Achieve from Stem Cell Research?" March 25.

National Institute of Environmental Health Sciences (NIEHS), NIH. 1999. Response to Senator Specter's Inquiry "What Would You Hope to Achieve from Stem Cell Research?" March 25.

National Institute of Neurological Disorders and Stroke (NINDS), NIH. 1999. Response to Senator Specter's Inquiry "What Would You Hope to Achieve from Stem Cell Research?" March 25.

Notarianni, E., S. Laurie, R.M. Moor, and M.J. Evans. 1990. "Maintenance and Differentiation in Culture of Pluripotential Embryonic Cell Lines from Pig Blastocysts." *Journal of Reproduction and Fertility* (Supplement) 41:51–56.

Notarianni, E., L.C. Galli, S. Laurie, R.M. Moor, and M.J. Evans. 1991. "Derivation of Pluripotent, Embryonic Cell Lines from Pig and Sheep." *Journal of Reproduction and Fertility* (Supplement) 43:255–260.

Oberpenning, F., J. Meng, J.J. Yoo, and A. Atala. 1999. "De Novo Reconstruction of a Functional Mammalian Urinary Bladder by Tissue Engineering." *Nature Biotechnology* 17:149–155.

O'Rahilly, R. 1987. *Developmental Stages in Human Embryos*. Washington, DC: Carnegie Institution of Washington.

PCT/U397/12919. 1997. "Embryonic or Stem-Like Cell Lines Produced by Cross Species Nuclear Transplantation." *International Application Published Under the Patent Cooperation Treaty*. WO 98/07/841.

Pedersen, R.A. 1994. "Studies of *In Vitro* Differentiation With Embryonic Stem Cells." *Reproduction, Fertility, and Development* 6:543–552.

Piedrahita, J.A., G.B. Anderson, G.R. Martin, R.H. Bondurant, and R.L. Pashen. 1988. "Isolation of Embryonic Stem Cell-Like Colonies from Porcine Embryos." *Theriogenology* 29:286.

Piedrahita, J.A., G.B. Anderson, and R.H. Bondurant. 1990. "On the Isolation of Embryonic Stem Cells: Comparative Behavior of Murine, Porcine and Ovine Embryos." *Theriogenology* 34:879–891.

Pittenger, M.F., A.M. Mackay, S.C. Beck, R.K. Jaiswal, R.K. Douglas, J.D. Mosca, M.A. Moorman, D.W. Simonetti, S. Craig, and D.R. Marshak. 1999. "Multilineage Potential of Adult Human Mesenchymal Stem Cells." *Science* 284:143–147.

Potten, C.S., and M. Loeffler. 1990. "Stem Cells: Attributes, Cycles, Spirals, Pitfalls and Uncertainties: Lessons for and from the Crypt." *Development* 110:1001–1020.

Robbins, J., J. Gulick, A. Sanchez, P. Howles, and T. Doetschman. 1990. "Mouse Embryonic Stem Cells Express the Cardiac Myosin Heavy Chain Genes During Development *In Vitro*." *Journal of Biological Chemistry* 265:11905–11909.

Robertson, E., and A. Bradley. 1986. "Production of Permanent Cell Lines from Early Embryos and Their Use in Studying Developmental Problems." In *Experimental Approaches to Mammalian Embryonic Development*, eds. J. Rossant and R.A. Pedersen, 475–508. New York: Cambridge University Press.

Rosario, C.M., B.D. Yandava, B. Kosaras, D. Zurakowski, R.L. Sidman, and E.Y. Snyder. 1997. "Differentiation of Engrafted Multipotent Neural Progenitors Towards Replacement of Missing Granule Neurons in Meander Tail Cerebellum May Help Determine the Locus of Mutant Gene Action." *Development* 124:4213–4224.

Rossant J., C. Bernelot-Moens, and A. Nagy. 1993. "Genome Manipulation in Embryonic Stem Cells." *Philosophical Transactions of The Royal Society of London. Series B: Biological Science* 339(1288):207–215.

Saito, S., N.S. Strelchenko, and H. Niemann. 1992. "Bovine Embryonic Stem Cell-Like Cell Lines Cultured over Several Passages." *Roux's Archives of Developmental Biology* 201:134–141.

Shamblott, M.J., J. Axelman, S. Wang, E.M. Bugg, J.W. Littlefield, P.J. Donovan, P.D. Blumenthal, G.R. Higgins, and J.D. Gearhart. 1998. "Derivation of Pluripotent Stem Cells from Cultured Human Primordial Germ Cells." *Proceedings of the National Academy of Sciences USA* 95:13726–13731.

Shim, H., A. Gutierrez-Adan, L.R. Chen, R.H. BonDurant, E. Behboodi, and G.B. Anderson. 1997. "Isolation of Pluripotent Stem Cells from Cultured Porcine Primordial Germ Cells." *Biology of Reproduction* 57:1089–1095.

Snodgrass, H.R., R.M. Schmitt, and E. Bruyns. 1992. "Embryonic Stem Cells and *In Vitro* Hematopoiesis." *Journal of Cellular Biochemistry* 49:225–230.

Snyder, E.Y. 1994. "Grafting Immortalized Neurons to the CNS." *Current Opinion in Neurobiology* 4:742–751.

Snyder, E.Y., R.M. Taylor, and J.H. Wolfe. 1995. "Neural Progenitor Cell Engraftment Corrects Lysosomal Storage Throughout the MPS VII Mouse Brain." *Nature* 374:367–370.

Snyder, E.Y., C. Yoon, J.D. Flax, and J.D. Macklis. 1997. "Multipotent Neural Precursors Can Differentiate Toward Replacement of Neurons Undergoing Targeted Apoptotic Degeneration in Adult Mouse Neocortex." *Proceedings of the National Academy of Sciences USA* 94:11663–11668.

Strauss, E. 1999. "Brain Stem Cells Show Their Potential." *Science* 283:471.

Strelchenko, N., and S. Stice. 1994. "Bovine Embryonic Pluripotent Cell Lines Derived from Morula Stage Embryos." *Theriogenology* 41:304.

Sukoyan, M.A., A.N. Golubitsa, A.I. Zhelezova, A.G. Shilov, S.Y. Vatolin, L.P. Maximovsky, L.E. Andreeva, J. McWhir, S.D. Pack, S.I. Bayborodin, A.Y. Kerkis, H.I. Kizilova, and O.L. Serov. 1992. "Isolation and Cultivation of Blastocyst-Derived Stem Cell Lines from American Mink (Mustela vison)." *Molecular Reproduction and Development* 33:418–431.

Sukoyan, M.A., S.Y. Vatolin, A.N. Golubitsa, A.I. Zhelezova, L.A. Semenova, and O.L. Serov. 1993. "Embryonic Stem Cells Derived from Morulae, Inner Cell Mass, and Blastocysts of Mink: Comparisons of Their Pluripotencies." *Molecular Reproduction and Development* 36:148–158.

Talbot, N.C., C.E. Rexroad Jr., V.G. Pursel, and A.M. Powell. 1993. "Alkaline Phosphatase Staining of Pig and Sheep Epiblast Cells in Culture." *Molecular Reproduction and Development* 36:139–147.

Tanne, J.H. 1999. "Researchers Implant Tissue Engineered Bladders." *British Medical Journal* 318:350B.

Thomson, J.A., J. Itskovitz-Eldor, S.S. Shapiro, M.A. Waknitz, J.J. Swiergiel, V.S. Marshall, and J.M. Jones. 1998. "Embryonic Stem Cell Lines Derived from Human Blastocysts." *Science* 282:1145–1147.

Thomson, J.A., J. Kalishman, T.G. Golos, M. Durning, C.P. Harris, R.A. Becker, and J.P. Hearn. 1995. "Isolation of a Primate Embryonic Stem Cell Line." *Proceedings of the National Academy of Sciences USA* 92:7844–7848.

Thomson, J.A., J. Kalishman, T.G. Golos, M. Durning, C.P. Harris, and J.P. Hearn. 1996. "Pluripotent Cell Lines Derived from Common Marmoset (*Callithrix jacchus*) Blastocysts." *Biology and Reproduction* 55:254–259.

Thomson, J.A., and V.S. Marshall. 1998. "Primate Embryonic Stem Cells." *Current Topics in Developmental Biology* 38:133–165.

Van Blerkom, J. 1994. "The History, Current Status and Future Direction of Research Involving Human Embryos." In National Institutes of Health (NIH) Human Embryo Research Panel, *Report of the Human Embryo Research Panel, Vol. II: Papers Commissioned for the Human Embryo Research Panel*, 1–25. Bethesda, MD: NIH.

Varmus, H. 1998. Testimony Before the Senate Appropriations Subcommittee on Labor, Health and Human Services, and Education. December 2.

Wade, N. 1998. "Researchers Claim Embryonic Cell Mix of Human and Cow." *New York Times* 12 November, A-1.

Wagner, J., P. Åkerud, D.S. Castro, P.C. Holm, J.M. Canals, E.Y. Snyder, T. Perlmann, and E. Arenas. 1999. "Induction of a Midbrain Dopaminergic Phenotype in *Nurr1*-Overexpressing Neural Stem Cells by Type 1 Astrocytes." *Nature Biotechnology* 17:653–659.

Wheeler, M.B. 1994. "Development and Validation of Swine Embryonic Stem Cells: A Review." *Reproduction, Fertility, and Development* 6:563–568.

Wilmut I., A.E. Schnieke, J. McWhir, A.J. Kind, and K.H. Campbell. 1997. "Viable Offspring Derived from Fetal and Adult Mammalian Cells." *Nature* 385:810–813.

Winkel, G.K., and R.A. Pedersen. 1988. "Fate of the Inner Cell Mass in Mouse Embryos as Studied by Microinjection of Lineage Tracers." *Developmental Biology* 127(1):143–156.

Wobus, A.M., G. Wallukat, and J. Hescheler. 1991. "Pluripotent Mouse Embryonic Stem Cells are Able to Differentiate into Cardiomyocytes Expressing Chronotropic Responses to Adrenergic and Cholinergic Agents and Ca2+ Channel Blockers." *Differentiation* 48:173–182.

Yandava, B.D., L.L. Billinghurst, and E.Y. Snyder. 1999. "'Global' Cell Replacement is Feasible via Neural Stem Cell Transplantation: Evidence from the Dysmyelinated *Shiverer* Mouse Brain." *Proceedings of the National Academy of Sciences USA* 96:7029–7034.

Chapter 3

The Legal Framework for Federal Support of Research to Obtain and Use Human Stem Cells

Introduction

In the course of attempting to realize the promise of human *embryonic stem (ES) cell* and *embryonic germ (EG) cell* research to advance basic and applied science as well as to develop new, life-saving therapies, biomedical researchers encounter uncertainties in the law as well as explicit restrictions (including bans on federal research funding) that were created in response to earlier developments in biomedical science and public policy. At the same time, provisions also exist in state and federal law designed to facilitate this field of research and to establish—or offer models for establishing—appropriate safeguards to ensure that all efforts to obtain or use stem cells are carried out in an ethically acceptable way. To date, three sources of ES or EG cells—cadaveric fetal tissue, embryos remaining after infertility treatments, and embryos created solely for research purposes using either *in vitro* fertilization (IVF) or, potentially, somatic cell nuclear transfer (SCNT) techniques—have been identified. The goal of this chapter is to examine separately the legal issues raised by research involving each source of EG or ES cells, noting as appropriate when common issues arise.

The Law Relating to Aborted Fetuses as Sources of EG Cells

Federal law permits funding of some research with cells and tissues from the products of elective as well as spontaneous abortions, and state law facilitates the donation and use of fetal tissue for research. Both state and federal law set forth several requirements for the process of retrieving and using material from this source, although amendments may be needed to federal law in order to make existing safeguards applicable to stem cell research.

Federal Law Regarding Research Using Cells and Tissues from Aborted Fetuses

Since as early as the 1930s, American biomedical research has utilized *ex utero* fetal tissue both as a medium and, increasingly, as an object for experimentation (Gelfand and Levin 1993; Zion 1996). "For many years, the production and testing of vaccines, the study of viral reagents, the propagation of human viruses, and the testing of biological products have been dependent on the unique growth properties of fetal tissue" (Duke 1988, D112, D114). For example, the 1954 Nobel Prize for Medicine was awarded to American immunologists who used cell lines obtained from human fetal kidney cells to grow polio virus in cell cultures, a key advance in the development of polio vaccines (Driscoll 1985; Gelfand and Levin 1993).

In 1972, allegations (some of them quite shocking) about experiments with fetuses both *in* and *ex utero* created an air of controversy (fueled by the greater societal debate about elective abortion) over the use of fetal tissue in research.[1] When Congress established the National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research in 1974, it placed the topic of research using the human fetus at the top of the commission's agenda. Within four months of assuming office, the commissioners were mandated to report on the subject, with the proviso that the presentation of their report to the Secretary of the Department of Health, Education, and Welfare (DHEW)—now the Department of Health and Human Services (DHHS)—would lift the moratorium that Congress had imposed on federal

funding of research using live fetuses.[2] On July 25, 1975, the National Commission submitted its conclusions and recommendations, which formed the basis for regulations that the Department issued later that year on research involving fetuses, pregnant women, and human IVF (1975).

General Regulation of Research with Human Beings Including Fetuses

The 1975 provisions remain as elements of the current federal regulations that aim to protect human subjects participating in research conducted with federal funds—rules that also are followed on a voluntary basis by many institutions in the case of research performed without federal support. The core regulations are set forth in the *Federal Policy for the Protection of Human Subjects*, known as the Common Rule, because the same regulatory provisions have been adopted by most federal agencies and departments that conduct or sponsor research in which human subjects are used. The DHHS regulations appear in Volume 45, Part 46 of the *Code of Federal Regulations*—45 CFR 46. The Common Rule makes up Subpart A of the DHHS regulations, and additional protections for special populations of research subjects appear in three further subparts of 45 CFR 46.

The special provisions applicable to fetal material appear in Subpart B, which covers research on "1) the fetus, 2) pregnant women, and 3) human *in vitro* fertilization" and applies to all DHHS "grants and contracts supporting research, development, and related activities" involving those subjects.[3] The regulations primarily address research that could affect living fetuses adversely. They provide for stringent Institutional Review Board (IRB) consideration, which is based upon the results of preliminary studies on animals and nonpregnant women and on assurances that living fetuses will be exposed only to minimal risk except when the research is intended to meet the health needs of the fetus or its mother.[4] Specific restrictions also are imposed on the inclusion of pregnant women in research activities.

Section 46.210 of Subpart B states that the sole explicit requirement for research involving "cells, tissues, or organs excised from a dead fetus" is that such research "shall be conducted only in accordance with any applicable State or local laws regarding such activities."[5] Some analysts have argued that this is the *only* component of Subpart B applicable to research in which cells or tissues from dead abortuses are used in research (Areen 1988). It appears, however, that even prior to the adoption in 1993 of legislation establishing special rules for using fetal tissue for transplantation, National Institutes of Health (NIH) officials had regarded other, general requirements of Subpart B as applicable to research with tissue from dead fetuses.[6] Specifically, these other provisions exclude researchers from any involvement in the decision to terminate a pregnancy or in an assessment of fetal viability and forbid the payment of any inducements to terminate a pregnancy.[7] This dispute over the scope of Subpart B produces one of the points of uncertainty that may need to be resolved either through legislation or official commentary from NIH's Office for Protection from Research Risks (OPRR), if investigators using cadaveric fetal tissue to generate human EG cells are to proceed with confidence and in an ethical fashion.

The Conditions for Federal Support of Fetal Tissue Transplantation

In the 1980s, medical scientists began experimenting with implanting brain tissue from aborted fetuses into patients with Parkinson's disease as well as patients with other neurological disorders. NIH investigators were among those working in this field, and their protocol to use fetal tissue for transplantation was approved by an internal NIH review body. Although the research complied with Subpart B, then-NIH Director James B. Wyngaarden decided to seek approval from Assistant Secretary for Health Robert E. Windom before proceeding.[8] In March 1988, Windom responded by declaring a temporary moratorium on federally funded transplantation research involving fetal tissue from induced abortions. He also asked NIH to establish an advisory body to consider whether such research should be conducted and under what conditions (Windom 1988). The Human Fetal Tissue Transplantation Research Panel—composed of biomedical investigators, lawyers, ethicists, clergy, and politicians—deliberated until the fall of 1988. Panel members then voted 19-2 to recommend continued funding for fetal tissue transplantation research under guidelines designed to ensure the ethical integrity of any experimental procedures (Adams 1988; Duguay 1992;

Silva-Ruiz 1998). In November 1989, after the transition had been made from the Reagan to the Bush administration, DHHS Secretary Louis Sullivan extended the moratorium indefinitely, based upon the position taken by the minority-voting panel members that fetal tissue transplantation research would increase the incidence of elective abortion (Goddard 1996; Robertson 1993).[9] Attempts by Congress to override the Secretary's decision were not enacted or were vetoed by President Bush.[10]

On January 22, 1993, immediately after President Clinton took office, he instructed the incoming Secretary of DHHS to lift the ban on federal funding for human fetal tissue transplantation research.[11] On February 5, 1993, DHHS Secretary Donna Shalala officially rescinded the moratorium, and, in March 1993, NIH published interim guidelines for research involving human fetal tissue transplantation (OPRR 1994). Provisions to legislate these safeguards were promptly proposed in Congress and included in the NIH Revitalization Act of 1993, which President Clinton signed into law on June 10, 1993.[12]

The 1993 act mirrors most prior statutory and regulatory provisions on research involving tissue from dead fetuses.[13] In general, the Revitalization Act states that any tissue from any type or category of abortion may be used for research on transplantation, but only for "therapeutic purposes." Most agree that this means that research on transplantation that has as its goal the treatment of disease is covered by the act, but that basic laboratory research—which only tangentially can be described as having a therapeutic purpose—would not be covered. Under all conditions, the investigator's research scope is not, however, unfettered. First, research activities in this area must be conducted in accordance with applicable state and local law. The investigator also must obtain a written statement from the donor verifying that a) she is donating fetal tissue for therapeutic purposes, b) no restrictions have been placed on who the recipient will be, and c) the donor has not been informed of the identity of the recipient. Further, the attending physician must sign a statement affirming five additional conditions of the abortion, aimed at insulating a woman's decision to abort from her decision to provide tissue for fetal research. Finally, the person principally responsible for the experiment must also affirm his or her own knowledge of the sources of tissue, that others involved in the research are aware of the tissue status, and that the researcher had no part in the abortion decision or its timing.

The statute provides significant criminal penalties for violation of four prohibited acts: 1) purchase or sale of fetal tissue "for valuable consideration" beyond "reasonable payments [for] transportation, implantation, processing, preservation, quality control, or storage…," 2) soliciting or acquiring fetal tissue through the promise that a donor can designate a recipient, 3) soliciting or acquiring fetal tissue through the promise that the recipient will be a relative of the donor, or 4) soliciting or acquiring fetal tissue after providing "valuable consideration" for the costs associated with the abortion itself.[14]

Research of the type conducted by Gearhart and his colleagues at The Johns Hopkins University, in which primordial germ cells were obtained from the gonadal ridge of human fetuses that had been aborted five to nine weeks after fertilization, arguably is not covered by the fetal tissue transplantation provisions of the 1993 NIH Revitalization Act, because these fetal cells are intended to be cultured and used in laboratory experiments, not transplanted. Nevertheless, if such research were federally supported, it could be subject to the requirements of Subpart B of 45 CFR 46—both the general limitations of § 46.206 (separating the investigators from the abortion process and forbidding payments for pregnancy termination) and the special requirements of § 46.210 for activities involving cells and tissues from dead fetuses. Someday, with the advancement of knowledge about cell differentiation and the like, EG cells derived from dead fetuses may be linked more directly or indirectly with transplantation, at which point the 1993 Act would arguably become applicable.[15] In anticipation of that day, and in order to achieve simplicity in the meantime by applying the same rules to all federally supported research with fetal remains, whether or not for transplantation, it would appear desirable to amend the law to clarify that the safeguards of the 1993 Act apply to research in which EG cells are obtained from dead fetuses after a spontaneous or elective abortion.

State Law Regarding Using Aborted Fetuses as Sources of Stem Cells

As recognized by federal statutes and regulations, state law governs the manner in which cells and tissues from dead fetuses become available for research, principally by statutes, regulations, and case law on organ transplantation. The most basic legal provisions lie in the Uniform Anatomical Gift Act (UAGA), which was first proposed in 1968 and rapidly became the most widely adopted uniform statute. While the UAGA is largely consistent with relevant federal statutes and regulations and should facilitate researchers obtaining cadaveric fetal tissue, a number of states have adopted other statutes that limit or prohibit certain types of research with fetal remains.

Laws Facilitating Donation of Fetal Material for EG Cell Research: The UAGA

The UAGA is relevant not only because federal statutes and regulations explicitly condition funding for research with fetal tissue on compliance with state and local laws, but also because the act applies when EG cell research using fetal tissue does not receive federal funding. The original version of the UAGA was approved by all 50 states and the District of Columbia; a 1987 revision has been enacted by 22 states (Zion 1996).[16] The act establishes a system of voluntary donation of "anatomical gifts" for transplantation, education, and research. It was intended to make it easier for people to authorize gifts of their own body (or parts thereof) through a simple "donor card" executed before the occasion arose, as well as to allow donations to be made with the permission of the next-of-kin, following an order established by the statute. The revised UAGA includes "a stillborn infant or fetus" in the definition of "decedents,"[17] for whom parental consent is determinative.[18] The UAGA also provides that "neither the physician or surgeon who attends the donor at death nor the physician or surgeon who determines the time of death" may be involved in the team that will use the organs removed from the decedent.[19] This section, although it may be waived, seems comparable to the separation that the 1993 NIH Revitalization Act and Subpart B of the DHHS regulations required between the research team and any physicians involved in terminating a pregnancy, determining fetal viability, or assisting in the clinical procedure during which fetal tissue is derived for research purposes.[20]

However, federal law restricts the procedures authorized by the UAGA in one area.[21] The UAGA permits donors to designate recipients—including individual patients—of anatomical gifts. The stricter provisions of the NIH Revitalization Act (which prohibits a donor from having knowledge of an individual transplant recipient) could override this state law in the case of federally supported fetal tissue transplantation, but the issue might not arise regarding stem cell research for two reasons. First, such research does not involve transplantation (and hence at this time is not relevant to the NIH Revitalization Act). Second, according to the Revitalization Act, the only recipient who may be designated by the parents of a dead fetus would be a stem cell researcher or research institution.

Laws Restricting Use of Donated Fetal Material for EG Cell Research

At present, 24 states do not have on their books any statutes "specifically addressing research on embryos or fetuses,"[22] and the restrictions in most of the remaining states principally involve embryos remaining after infertility treatments and limitations aimed at discouraging therapeutic abortions. For example, in 12 states, the law applies only to research with fetuses prior or subsequent to an elective abortion.[23] Six states ban research that involves aborted fetuses or their organs, tissues, or remains,[24] which could cause difficulties for researchers using stem cell lines derived from aborted fetuses "if cell lines are considered 'tissue.'"[25] Six other states permit fetal research when the fetus is deceased, but mandate that the donor must provide consent,[26] although none "specifically address[es] the type of information that must be provided to the progenitors before they are asked for consent."[27] In Pennsylvania, investigators using fetal tissue and recipients of the tissue are required to be informed if the tissue was procured as a result of stillbirth, miscarriage, ectopic pregnancy, abortion, or some other means.[28]

In order to diminish the impact that the potential use of a fetus in research might have on the decision to abort, states have enacted many restrictions on payment for

fetal remains. The broadest prohibitions appear as part of state statutes regulating or prohibiting fetal research. Bans on sale vary in their terminology—an "aborted product of conception,"[29] an "aborted unborn child or the remains thereof,"[30] an "aborted fetus or any tissue or organ thereof,"[31] or an "unborn child"[32] —and exist both in states that permit research on a dead fetus with the mother's consent[33] and in those where it is illegal to conduct research upon any aborted product of conception.[34]

The bans on commercialization have a number of interesting twists. Rhode Island outlaws the selling of an embryo or fetus for purposes that violate the statute (such as research on living embryos or fetuses), but apparently allows payment to the mother for allowing a dead fetus to be used in research, because such research is permissible.[35] Minnesota prohibits the sale of living fetuses or nonrenewable organs but explicitly permits "the buying and selling of a cell culture line or lines taken from a [dead fetus]."[36]

The most widely adopted prohibitions on commercialization of fetal remains are those in Sections 10(a) and (b) of the 1987 revision of the UAGA, which prohibit the sale or purchase of any human body parts for any consideration beyond that necessary to pay for expenses incurred in the removal, processing, and transportation of the tissue.[37] On the federal level, what is in essence the same proscription is included both in the 1993 NIH Revitalization Act, which bars the acquisition or transfer of fetal tissue for "valuable consideration" with the same exceptions,[38] and in the National Organ Transplant Act of 1984 (NOTA), which prohibits the sale of any human organ for "valuable consideration for use in human transplantation"[39] if the sale involves interstate commerce.[40] (In 1988, Congress amended NOTA to include fetal organs within the definition of "human organ," in order to foreclose the sale of fetal tissue as well.[41]) Yet both federal statutes could be interpreted to apply only to sales for transplant or therapeutic purposes, not laboratory research. Moreover, the definition of reasonable processing fees in the federal law (and by extension, the UAGA)[42] is arguably too vague, "leav[ing]...room for unscrupulous tissue processors to abuse the law" (Goddard 1996, 394). If special provisions are adopted to govern federal support of research with fetal material to create human EG cell lines, it would seem advisable to ensure that the provisions lay out more clearly what payments may be made to whom and on what basis for fetal cells and tissues.

The state statutes regulating fetal research have been challenged in several court cases. Generally, limitations have been approved as they relate to live fetuses or to the disposal of aborted fetuses.[43] A few cases have dealt with restrictions on research with dead fetuses or fetal remains. In 1978, Louisiana adopted a statute forbidding virtually all experimentation involving a living fetus ("a live child or unborn child") that was not "therapeutic" to that child, a ban it expanded in 1981 to encompass research with aborted fetal tissue as well.[44] Plaintiffs who argued that the prohibition on research burdened their right of privacy challenged the law.[45] Agreeing, the federal district court concluded that the ban on research did not further the state's compelling interest in protecting the health of the woman, nor did the state's interest in the potential life of the unborn continue past the death of the fetus.[46] Finally, the district court addressed the statute's vagueness, noting that it was not possible, *ex utero*, to distinguish between fetal and maternal tissue and the products of spontaneous and induced abortions.[47] On appeal, the Fifth Circuit ignored the district court's analysis entirely, finding instead that the term "experiment" as used in the statute's prohibition against fetal experimentation was unconstitutionally vague.[48]

The Law Relating to Embryos as Sources of ES Cells

Turning to the second source of human ES cells—embryos created through IVF—one finds that in contrast to the regulatory complexity of the federal and state laws governing research using fetal tissue, the legal framework for research using human embryos is relatively straightforward. With the exception of a few state statutes, no viable regulatory system exists to guide or control the practice of human embryo research in the United States.[49] Regarding federally supported scientists, law prohibits such experimentation, while research conducted in the private sector takes place without any federal medical or bioethical oversight specific to the human embryo.[50] The central issue raised by existing law is whether the recent

scientific developments are important enough to justify modifying, in part, the current blanket ban on federal support by creating a limited exception for certain types of human stem cell research.

Federal Law Regarding Research Using Cells and Tissues from Human Embryos

Federal law regarding research using human embryos by investigators employed or funded by the federal government may best be understood by reviewing Subpart B of the DHHS policy on the protection of human subjects and the rider that has been attached for several years to the DHHS appropriation, most recently in the Omnibus Consolidated and Emergency Supplemental Appropriations Act for Fiscal Year 1999 (OCESAA).[51]

The former, which continues to provide a basic framework for research, even though reasons exist to question its applicability, originated in concerns about research on the human fetus, but it also applies to "grants and contracts supporting research, development, and related activities involving...human *in vitro* fertilization."[52] At the time these provisions were first promulgated, IVF was still an experimental technique: The birth in England of Louise Brown, the first so-called test tube baby, did not occur until 1978. Recognizing that NIH scientists and others would wish to pursue research on IVF and the earliest stages of human development, the regulations provided that "no application or proposal involving human *in vitro* fertilization may be funded by the Department [until it] has been reviewed by the Ethical Advisory Board and the Board has rendered advice as to its acceptability from an ethical standpoint."[53] In 1977, NIH received an application from an academic researcher for support of a study involving IVF. After the application had undergone scientific review within NIH, it was forwarded to the Ethics Advisory Board (EAB) appointed by Joseph Califano, then Secretary of DHEW. At its May 1978 meeting, the EAB agreed to review the research proposal. With the increased public interest that followed the birth of Louise Brown that summer, Secretary Califano asked the EAB to study the broader social, legal, and ethical issues raised by human IVF. On May 4, 1979, in its report to the Secretary, the EAB concluded that federal support for IVF research was "acceptable from an ethical standpoint" provided that certain conditions were met, such as informed consent for the use of gametes, an important scientific goal "not reasonably attainable by other means," and not maintaining an embryo "*in vitro* beyond the stage normally associated with the completion of implantation (14 days after fertilization)" (DHEW EAB 1979, 106, 107). No action was ever taken by the Secretary with respect to the board's report; for other reasons, the Department dissolved the EAB in 1980. Because it failed to appoint another EAB to consider additional research proposals, DHEW effectively forestalled any attempts to support IVF, and no experimentation involving human embryos was ever funded pursuant to the conditions set forth in the May 1979 report or through any further EAB review.

Because the Revitalization Act of 1993 effectively ended the de facto moratorium on IVF and other types of research involving human embryos[54] by nullifying the regulatory provision that mandated EAB review,[55] NIH Director Harold Varmus convened the Human Embryo Research Panel to set forth standards for determining which projects could be funded ethically and which should be considered "unacceptable for federal funding."[56] The panel identified several areas of potential research activity that it considered ethically appropriate for federal support, including studies involving the development of ES cells, though only with embryos resulting from IVF or clinical research that have been donated with the consent of the progenitors. The most controversial aspect of the report was its conclusion that it might be ethical to allow researchers to create human embryos for certain research purposes.[57]

In September 1994, the panel submitted its report to the Advisory Committee to the Director (ACD) of NIH, which formally approved the recommendations and transmitted them to Varmus on December 1, 1994. The following day, pre-empting NIH's response, the President declared that federal funds should not be used to support the creation of human embryos for research purposes and directed that NIH not allocate any resources for such requests.[58] Thereafter, Varmus decided to implement the panel's recommendations not proscribed by the President's directive, concluding that NIH could begin to fund research activities involving "surplus" embryos

(Feiler 1998). Before any funding decisions could be made, however, Congress attached a rider to that year's DHHS appropriations bill that stipulated that none of the funds appropriated could be used to support any activity involving "1) the creation of a human embryo or embryos for research purposes; or 2) research in which a human embryo or embryos are destroyed, discarded, or knowingly subjected to risk of injury or death greater than that allowed for research on fetuses *in utero* under 45 CFR 46.208(a)(2) and section 498(b) of the Public Health Service Act (42 USC 289g(b))."[59]

When the question arose of whether to provide federal funding for human ES cell research using IVF embryos remaining from infertility treatments, Varmus sought the opinion of Harriet Rabb, DHHS General Counsel, regarding the effect of the prohibition in the current appropriations rider. Rabb reported to Varmus that the OCESAA does not prevent NIH from supporting research that uses ES cells derived from this source because the cells themselves do not meet the statutory, medical, or biological definition of a human embryo (NIH OD 1999).[60]

Having concluded that NIH may fund internal and external research that utilizes but does not create human ES cells, NIH has delayed actual funding until an Ad Hoc Working Group of the ACD develops guidelines for the ethical research in this area.[61] The working group began its deliberations in early 1999 and completed draft guidelines on April 8, 1999, which are still undergoing internal review and public comment.[62]

In addition to these guidelines, ES cell research that was supported by federal funds and directly involved human embryos might arguably be subject to the requirements of both Subpart A (the Common Rule) and Subpart B of 45 CFR 46—that is, the research would be required to meet general and specific substantive requirements, would have to be approved by the IRB of the investigator's institution, and might have to undergo further review at the national level. We use the word "arguably" because OPRR has provided no definitive guidance regarding such an interpretation. Indeed, in response to the Commission's inquiry of May 18, 1999, OPRR acknowledged that "although Subpart B does not apply to research involving a human embryo, per se, it does apply to research that involves the process of *in vitro* fertilization. An embryo formed by a means that does not involve *in vitro* fertilization would not be subject to Subpart B."[63] Because no other guidance is provided, we are left to interpret whether embryos (which are not defined in regulation) are human subjects and therefore protected by Subpart B.

Subpart A, which contains the basic requirements for IRB review, informed consent, privacy protection, and the like, aims to protect a "human subject," defined as "a living individual about whom an investigator...obtains (1) data through intervention or interaction...."[64] This definition creates uncertainties about whether the Common Rule applies to embryo research, the derivation of ES cells, and research involving successor stem cells from embryonic sources that require resolution. This is another point upon which a clearer, more accessible interpretation is needed from OPRR if investigators and IRBs are to proceed with confidence regarding a range of stem cell research activities involving human embryos. Assuming that the DHHS regulations apply, the special requirements of Subpart B also would be applicable, because (as previously described) NIH has long taken the position that human IVF research, which is clearly encompassed in Subpart B, encompasses *any* DHHS-funded research involving human embryos not *in utero*. This would mean not only that another EAB could be impaneled by the Secretary pursuant to 45 CFR § 46.204, but also that special responsibility would fall on investigators and IRBs under 45 CFR § 46.205.[65] In addition, special standards would have to be met under 45 CFR § 46.206, including mandates for prior studies involving animals and ensuring the least possible risk. The newly revised 45 CFR 46, Subpart B (not yet finalized) makes no substantive changes that would affect these requirements.[66]

State Law Regarding Research Using Cells and Tissues from Human Embryos

State legislatures have apparently been more concerned about regulating and restricting research using human fetuses or their remains instead of addressing research involving laboratory manipulation of human gametes and early stage embryos. Nonetheless, although

the statutes usually ignore issues (other than commercialization) specific to IVF (Robertson 1990), some could be construed broadly enough to encompass a range of experimental activities involving IVF, including cryopreservation, pre-implantation screening, gene therapy, twinning, cell line development, and basic research (Coleman 1996). The latter two are of obvious relevance to creating stem cell cultures from embryonic sources.

States that regulate cell line development from human embryos either prohibit the practice entirely or restrict it substantially (Coleman 1996). "All ten states that prohibit embryological research have vaguely worded statutes which could encompass cell line development if the statutes were interpreted broadly...[although] some [activity] could be characterized as non-experimental, thus removing it from the scope of experimentation bans" (Coleman 1996, 1358). Issues inherent in cell line development will include the potential for restrictions on downstream commercialization and uncertainty over the extent to which gamete donors must be informed about the nature of and potential commercial uses of the biological materials they donate (Coleman 1996).

Basic research typically involves precommercial scientific activity designed to explore biological processes or to understand genetic and cellular control mechanisms. As noted previously, 24 states and the District of Columbia do not restrict research involving fetuses or embryos.[67] Of the remaining 26 states that regulate embryo or fetal research in one form or another, basic embryological research is prohibited or restricted in 10 (Feiler 1998). Although the degree of regulation of experimental use of embryos under the New Hampshire statute is unlikely to impair ES cell research in that state,[68] the remaining nine states have legislated more broadly, effectively banning all research involving *in vitro* embryos, with penalties mandated in some states, including civil fines and imprisonment.[69]

The subject of commercialization is a potentially important one, affecting both researchers who must acquire embryos from for-profit IVF clinics or other sources and downstream users who may develop derivative, commercial applications from basic embryological and stem cell research. Currently, five states prohibit payment for IVF embryos for research purposes.[70] Eight additional states prohibit payment for human embryos for any purpose.[71] Five states apply ambiguous restrictions that may or may not prohibit sale of embryos, depending upon interpretation or, in some cases, action by state officials.[72] More troubling, some statutes could be interpreted to prevent payment for ES cell lines derived from human embryos (Coleman 1996), although "it is possible that because a cell line is new tissue produced from the genetic material of, but not originally a part of, the embryo, laws proscribing the sale of embryonic tissue may not apply."[73] In line with NOTA and the 1987 revisions of the UAGA, state statutes on organ transplantation now typically prohibit sale of human organs or parts, but none include language likely to impede research involving human embryos.

The Law Relating to Deriving Stem Cells from Organisms Created Through Cloning

The third potential source of human ES cells would involve the use of cloning—that is, SCNT. One possible use of SCNT would be to derive ES cells themselves, thus avoiding the need for embryos. If such a transfer directly into an enucleated stem cell were to be successful, the therapeutic potential of creating cells and tissues for autologous transplantation might be realized without any of the ethical and regulatory problems associated with the creation of embryos.

At present, however, the method for creating human ES cells through SCNT, which has been announced by one scientific team (although not yet published in a scientific journal), involves inserting a somatic cell nucleus into an enucleated oocyte, which, if it then developed, would become a blastocyst from which ES cells would be derived. This approach creates two problems. First, if the blastocyst were characterized as a human embryo (albeit one created asexually rather than by uniting egg and sperm *in vitro*), then the prohibition on federal funding (as well as the restrictions on embryo research in several states) would come into play. Second, the process of carrying out SCNT using human cells has been outlawed by at least two states and may or may not be eligible for federal funding. On March 4, 1997, shortly after the

initial announcement that the Roslin Institute had succeeded in creating Dolly, the cloned sheep, the Office of the White House Press Secretary released a "Memorandum for the Heads of Executive Departments and Agencies," in which the President stated that

> Federal funds should not be used for cloning of human beings. The current restrictions on the use of Federal funds for research involving human embryos do not fully assure this result. In December 1994, I directed the National Institutes of Health not to fund the creation of human embryos for research purposes. The Congress extended this prohibition in FY 1996 and FY 1997 appropriations bills, barring the Department of Health and Human Services from supporting certain human embryo research. However, these restrictions do not explicitly cover human embryos created for implantation and do not cover all Federal agencies. I want to make it absolutely clear that no Federal funds will be used for human cloning. Therefore, I hereby direct that no Federal funds shall be allocated for cloning of human beings.[74]

On June 9, 1997, the President received NBAC's report entitled *Cloning Human Beings* and announced his acceptance of its recommendations, which included a moratorium on publicly or privately funded research to create a child through SCNT but not on laboratory research using the technique. A number of bills have been introduced in Congress to achieve this result—as have other bills that would enact a broader prohibition—but no federal legislation has been adopted. On February 9, 1998, responding to one of those bills (S. 1601, The Human Cloning Prohibition Act), the Executive Office of the President released a Statement of Administration Policy, which provides in part that

> the Administration supports amendments to S. 1601 that would...permit somatic cell nuclear transfer using human cells for the purpose of developing stem cell (unspecified cells capable of giving rise to specific cells and tissues) technology to prevent and treat serious and life-threatening diseases and other medical conditions, including the treatment of cancer, diabetes, genetic disorders, and spinal cord injuries and for basic research that could lead to such treatments.[75]

This statement does not, however, have the force or effect of a Presidential Directive or Executive Order and does not modify the March 1997 Presidential Directive prohibiting funding for human cloning by federal agencies. The resulting uncertainty must be resolved, taking into account the ethical analysis presented in the next chapter.

Summary

As described in Chapter 2, the development of human ES and EG cell lines represents an important advance in biomedicine that promises not only to expand basic scientific understanding but also to improve health and extend life for millions of patients. Even the greatest supporters of this new field recognize, however, that current methods of deriving EG and ES cells from cadaveric fetal tissue and embryos remaining after infertility treatments raise significant ethical issues. Further ethical analysis, which appears in the next chapter of this report, is needed before conclusions can be reached about the goals and principles that should guide policymaking in this field.

Federal law permits the funding of some research that uses tissue from dead fetuses following spontaneous or elective abortion, provided the researchers follow safeguards that aim to separate the decision to abort from the decision to donate material for research, to ensure appropriate consent, and to avoid commercialization of fetal material. The UAGA, which in every state facilitates the process of donating bodies and organs for research as well as transplantation, treats fetuses like other cadavers; the latest version of the statute imposes special conditions on the donation of fetal remains and reinforces the prohibition in federal law against paying for organ donation. The legal framework identified by these statutes is thus favorable to research in which EG cells would be derived from fetal tissue. Some questions remain, however, about the applicability of some of the statutes—for example, the principal set of federal safeguards appears in a statute dealing with fetal tissue transplantation, and EG cell research does not now, and may never, involve directly the transplantation of tissue or cells from a fetus to a patient. Therefore, to overcome the uncertainties and ensure that ethical safeguards are

understood to be applicable to fetal stem cell research, statutory modification and regulatory clarification are desirable.

Confusion also is caused by restrictions and bans in several states on research use of the products of induced abortions; although these statutes seem aimed principally at research with living fetuses, some have—or may be read to have—broader reach. The common theme of these statutes—as in the law on federally funded research—is to erect a significant barrier between a woman's decision to abort a fetus and the separate question of whether fetal remains will be donated for research. To support that barrier, many states employ consent requirements and prohibit payment for fetal remains, so that such material does not become commercialized and thus inappropriately influence the abortion decision.

The picture is clearer but less favorable to research in the area of embryos remaining after infertility treatments. In addition to restrictions and even outright prohibitions in the law of a number of states, riders to DHHS appropriation statutes in recent years rule out the use of these funds in any process in which human embryos are created for research or are destroyed or subject to a risk of injury. Once it has developed special guidelines to ensure that investigators will safeguard the ethics of the process, NIH will fund suitable research projects using human ES cells derived from IVF embryos, although it will not fund the derivation process itself. This position has been denounced by many members of Congress who supported the ban on federal funding of research with embryos and who believe that however the statutory language may be read, its intent clearly is to prohibit research that depends upon the prohibited acts.

The questions raised by this disagreement go beyond interpretation of the language and intent of the DHHS appropriations rider. First, is the justification for research using human ES cells compelling enough to permit an exception to the ban on federal funding for embryo research? Second, can an exception be crafted in a way that continues to give appropriate weight to the values that underlie the ban in the first place? And third, is the justification for using ES cells strong enough to permit funding of the process of deriving these cells from IVF embryos remaining after infertility treatments? Answers to these questions will require evaluation of the scientific and medical aspects of human ES cell research that are described in Chapter 2 in the context of the ethical considerations that are discussed in Chapter 4.

Notes

1 Proposed guidelines for fetal tissue research were released by NIH and DHEW in 38 *Fed. Reg.* 31,738 (1973) (Gelfand and Levin 1993).

2 See National Research Act, Public Law 93-348, Section 201(a), 88 Stat. 348 (1974).

3 45 CFR § 46.201(a) (1997). "The purpose of this subpart [is] to...assure that [applicable research] conform[s] to appropriate ethical standards and relate[s] to important societal needs" (Ibid. at § 46.202).

4 The portions of Subpart B dealing with research on living fetuses were re-enforced by the Human Research Extension Act of 1985. The act directs that no federally supported research may be conducted on a nonviable living human fetus *ex utero* or on a living human fetus *ex utero* for whom viability has not been determined, unless a) the research or experimentation may enhance the health, well-being, or probability of survival of the fetus itself; or b) will pose no added risk of suffering, injury, or death to the fetus where the research or experimentation is for "the development of important biomedical knowledge which cannot be obtained by other means." In either instance, the degree of risk must be the same for fetuses carried to term as for those intended to be aborted (42 USC § 289g 1998).

5 On May 20, 1998, DHHS released for public comment proposed revisions of Subpart B, most of which relate to research with living fetuses. In these revisions, § 46.210 would become § 46.206, which would retain the requirement that research with material from a dead fetus would have to conform to state law. The revised regulation would add that any living individual who becomes personally identified as a result of research on dead fetal or placental material must be treated as a research subject and accorded the protections of the federal Common Rule.

6 During the period of 1987–92, the NIH Office of Science Policy repeatedly stated that NIH applies Subpart B broadly to a range of fetal research activities. For example, in a 1988 memorandum, NIH Director James B. Wyngaarden informed Assistant Secretary for Health Robert E. Windom that "[a]s you know, the NIH conducts all human fetal tissue research in accordance with Federal Guidelines (45 CFR 46)," and provided a 1987 summary of fetal tissue research at NIH that stated that "NIH-supported human fetal tissue research is conducted in compliance with all Federal… regulations regarding the use of human fetal tissue. These regulations include restrictions on tissue procurement [Subpart B] that are intended to prevent possible ethical abuses" (NIH 1987; Memorandum from James B. Wyngaarden to Robert E. Windom, February 2, 1988).

7 45 CFR §§ 46.206 (a)(3) and 46.206(b)(1997).

8 "Although such approval was not required, the Assistant Secretary was consulted because of the scientific and ethical implications of the study" (Ryan 1991, 687).

9 Letter from Louis Sullivan to William Raub, November 2, 1999.

10 See H.R. 2507, 102d Cong., 1st Sess. (1991) (amending Part G of Title IV of the Public Health Service Act). See also H.R. 5495, 102d Cong., 2nd Sess. (1992) (amending Part G of Title IV of the Public Health Service Act and incorporating the establishment of a federally operated national tissue bank as provided by Exec. Order No. 12,806 [1992]). During this period, in an apparent attempt to find an alternative to fetal tissue derived from elective abortion, the administration established (without success) a tissue bank to collect fetal tissue for research from ectopic pregnancies and miscarriages. Exec. Order No. 12,806, 57 *Fed. Reg.* 21,589 (1992). Because spontaneously aborted tissue may contain viral infections or pathological defects, the use of ectopic and miscarried abortuses is disfavored for transplantation and most other research. In October 1992, a consortium of disease advocacy organizations filed suit against DHHS Secretary Sullivan, alleging that the Hyde Amendment, which bars federal funding for abortions, Departments of Labor, Health, Education, and Welfare Appropriations Act of 1977, Public Law 94-439, did not apply to research on and transplantation of fetal tissue. The plaintiffs argued, moreover, that the fetal tissue transplantation research ban was beyond the Department's statutory authority under the law (Bell 1994).

11 See 58 *Fed. Reg.* 7457 (1993).

12 The administration's policies on fetal tissue transplantation did not entirely quell public controversy or congressional interest (GAO 1997).

13 The policy initiated by President Clinton in 1993 and formalized in the 1993 NIH Revitalization Act is in line with the position taken in many other countries that the use of fetal tissue from elective abortions in therapy for people with conditions such as Parkinson's disease is acceptable. As with U.S. laws and regulations, international guidelines emphasize the need to separate the decision to terminate pregnancy from the decision to donate fetal tissue and the need for informed consent for the donation. See Knowles, L.P., 1999, "International Perspectives on Human Embryo and Fetal Tissue Research." This background paper was prepared for NBAC and is available in Volume II of this report.

14 42 USC § 289g-2(a)-(c) (1997). But see Goddard (1996).

15 DHHS General Counsel Harriet Rabb apparently believes that research of the type conducted by Gearhart is already sufficiently connected to transplantation to be subject to the NIH Revitalization Act, though she does not explain how she reached that conclusion. In a January 15, 1999, memorandum to NIH Director Varmus, Rabb concluded that "[t]o the extent human pluripotent stem cells are considered human fetal tissue by law, they are subject to…the restrictions on fetal tissue transplantation research that is conducted or funded by DHHS, as well as to the federal criminal prohibition on the directed donation of fetal tissue." Rabb examined the definition of "fetal tissue" at 42 USC 289g-1(g) which defines it as "tissue or cells obtained from a dead human embryo or fetus after a spontaneous or induced abortion, or after a stillbirth" and observed that "some stem cells, for example those derived from the primordial germ cells of non-living fetuses, would be considered human fetal tissue for purposes of [federal law]." Having concluded that primordial germ cells extracted from nonliving fetuses are a type of fetal tissue, the General Counsel went on, without further explanation, to apply the prohibition on sale of fetal tissue, the firewall restrictions, and the donative limitations stipulated in the NIH Revitalization Act, as well as the requirements of 45 CFR § 46.210.

16 National Conference of Commissioners on Uniform State Laws (NCCUSL), *A Few Facts About the Revised Uniform Anatomical Gift Act*, 1987.

17 Uniform Anatomical Gift Act (UAGA) § 1(3). But see Zion (1996): "UAGA…does not differentiate between a fetus donated from a miscarriage or one given through an elective abortion. Presumably, either type of donation is included, but a certain determination is difficult" (1293).

18 Under § 3 of the UAGA, the first two categories of individuals who may consent to donate are a spouse or adult child of the decedent, which would be irrelevant in the case of a fetus, thus giving priority to the next class, the parents. Usually, permission from any member of a class is adequate, unless a majority of the class objects, though as revised, the "UAGA makes the mother's consent determinative unless the father objects, and...does not provide for notice to the father" (Gelfand and Levin 1993, 679). Gelfand and Levin contrast this UAGA provision with 45 CFR § 46.209(d), which requires the father's consent unless his identity or whereabouts "cannot reasonably be ascertained" or he is "unavailable" to consent; however, these provisions apply only "until it has been ascertained whether or not a fetus *ex utero* is viable," and do not apply to donation of a dead fetus or fetal remains.

19 UAGA § 8(b).

20 See, for example, 45 CFR § 46.206(a)(3) ("Individuals engaged in the activity [of research] will have no part in: (i) Any decisions as to the timing, method, and procedures used to terminate the pregnancy, and (ii) determining the viability of the fetus at the termination of the pregnancy"); see also Zion (1996): "These provisions create a 'Chinese Wall' between the individuals effecting the abortion and those conducting fetal tissue research and transplantation.…While this language standing alone would likely preclude most undue influence, the UAGA also provides for the waiver of the 'Chinese Wall'....[R]evision may be necessary" (1294).

21 There are also state laws whose restrictions regarding choosing tissue recipients are broader, and may have implications for stem cell research. In Pennsylvania, for example, "No person who consents to the procurement or use of any fetal tissue or organ may designate the recipient of that tissue or organ, nor shall any other person or organization act to fulfill that designation" (18 Pa. Cons. Stat. Ann. § 3216(b)(5)). This law unintentionally would create the

situation where an IVF patient could donate her excess embryo for stem cell research, but she could specify that it be used by a particular medical center. She would have to blindly turn it over, and risk it going to a researcher or entity (such as a for-profit company) that she might not approve of. See Andrews, L.B., 1999, "State Regulation of Embryo Stem Cell Research." This background paper was prepared for NBAC and is available in Volume II of this report.

22 Andrews 1999.

23 See Ariz. Rev. Stat. Ann. § 36-2302(A) (subsequent); Ark. Stat. Ann. § 20-17-802 (subsequent); Cal. Health and Safety Code § 123440 (subsequent); Fla. Stat. Ann. § 390.0111(6) (prior or subsequent); Ind. Code Ann. § 16-34-2-6 (subsequent); Ky. Rev. Stat. § 436.026 (subsequent); Mo. Ann. Stat. § 188.037 (prior or subsequent); Neb. Rev. Stat. § 28-346 (subsequent); Ohio Rev. Code Ann. § 2919.14(A) (subsequent); Okla. Stat. Ann. tit. 63, § 1-735(A) (prior or subsequent); Tenn. Code Ann. § 39-15-208 (subsequent); Wyo. Stat. Ann. § 35-6-115 (subsequent).

24 Ariz. Rev. Stat. Ann. § 36-2302, -2303; Ind. Code Ann. § 1 6.34-2-6; N.D. Cent. Code § 14-02.2-01 to -02; Ohio Rev. Code Ann. § 2919.14; Okla. Stat. Ann. tit. 63, § 1-735; S.D. Codified Laws Ann. § 34-23A-17.

25 Andrews 1999. Similarly, Arizona's statute provides that a "person shall not knowingly use any human fetus or embryo, living or dead, or any parts, organs or fluids of any such fetus or embryo resulting from an induced abortion in any manner" (Ariz. Rev. Stat. § 36-2302(A)).

26 Ark. Stat. Ann. § 20-17-802(2); Mass. Ann. Laws ch. 112 § 12J(a)(II); Mich. Comp. Laws Ann. § 333.2687 (must also comply with state's version of the UAGA, Mich. Comp. Laws Ann. § 333.10101 et seq.); 18 Pa. Cons. Stat. Ann. § 3216(b)(1) (mother's consent valid only after decision to abort has been made; no compensation allowed); R.I. Gen. Laws § 11-54-1(d); Tenn. Code Ann. § 39-15-208(a).

27 Even in the context of research on live fetuses, only New Mexico's statute describes the information that must be provided before consent to research involving a fetus is valid. Under the New Mexico law, a woman who is asked to participate in research must be "fully informed regarding possible impact on the fetus" (Andrews 1999, citing N.M. Stat. Ann. § 24-9A-2(b)).

28 18 Pa. Cons. Stat. Ann. § 3216(b)(4).

29 Ohio Rev. Code Ann. § 2919.14.

30 Okla. Stat. Ann. § 1-735.

31 N.D. Cent. Code § 14-02.2-01(2); Mo. Stat. Ann. § 188.036(5).

32 Tenn. Code Ann. § 39-15-208 (also prohibits sale of an aborted fetus); Utah Code Ann. § 76-7-311.

33 Ark. Stat. Ann. § 20-17-802(c); also a crime to possess such material, § 20-17-802(d).

34 See, for example, Ind. Stat. § 35-46-5-1 (applies both to aborted and stillborn fetuses); Ohio Rev. Code Ann. § 2919.14(A); Okla. Stat. Ann. § 1-735(A).

35 R.I. Geb. Laws § 11-54-1(f).

36 Minn. Stat. Ann. § 145.422(3).

37 Of the 23 states in which organ transplant laws forbid payment, two appear inapplicable to using fetal remains in stem cell research: Arizona's statute defines a decedent to include a stillborn infant but not a fetus (Ariz. Rev. Stat. § 36-849(1)), and Kentucky excludes "fetal parts or…any products of the birth or conception" from its definition of "transplantable organs" that may not be sold (Ky. Rev. Stat. Ann. § 311.165(5)(b)).

38 42 USC § 289g-2(a) (1997).

39 National Organ Transplant Act (NOTA) 42 USC § 274e(a) (1997). "Valuable consideration" is defined at 42 USC § 274e(c)(2) (1997) negatively: "'valuable consideration' does not include the reasonable payments associated with the removal, transportation, implantation, processing, preservation, quality control, and storage of a human organ or the expenses of travel, housing, and lost wages incurred by the donor of a human organ in connection with the donation of the organ." A similar definition (excluding donor costs) is provided in the NIH Revitalization Act at 42 USC § 289g-2(d)(3) (1997).

40 Because the definition of "interstate commerce" in NOTA is based upon the Federal Food, Drug and Cosmetic Act, which defines it as "commerce between any State or Territory and any place outside thereof," 21 USC § 321(b), NOTA's prohibitions extend to purchasing organs abroad for importation into the United States. Most countries explicitly prohibit the commercialization of human fetal tissue. The Canadian Royal Commission on New Reproductive Technologies stated that the noncommercialization of reproduction should be considered a guiding principle. The commission recommended that no for-profit trade be permitted in fetal tissue and that the "prohibition on commercial exchange of fetuses and fetal tissue extend to tissues imported from other countries" (1993). This prohibition was intended to prevent the exploitation of poor women, especially in developing countries, who might be persuaded to begin and end pregnancies for compensation.

41 Organ Transplants Amendment Act of 1988, 42 USC § 274(e)(c)(1) (1997). The amendment was specifically intended to prevent the "sale or exchange for any valuable consideration" of fetal organs and tissue. 134 *Cong. Rec.* S10, 131 (27 July 1988).

42 As enacted in six states, the statutes prohibit the sale of human organs but fail to include a definition of "valuable consideration" that stipulates an exemption for miscellaneous overhead expenses; sixteen states provide such an exemption (Andrews 1999).

43 See for example, *Doe v. Rampton*, 366 F. Supp. 189, 194 (D. Utah 1973) (suggesting in dicta that statute provision prohibiting research on live fetus may not be otherwise unconstitutional), vacated and remanded, 410 U.S. 950 (1973) (directing further consideration in light of *Roe*); *Wolfe v. Schroering*, 388 F. Supp. 631, 638 (W.D. Ky. 1974), aff'd in part, rev'd in part on other grounds, 541 F.2d 523 (6th Cir. 1976) (upholding prohibition on experimentation on a viable fetus due to state's interest in the fetus after viability); *Planned Parenthood Association v. Fitzpatrick*, 401 F. Supp.

554 (E.D. Penn. 1975), aff'd without opin sub nom.; *Franklin v. Fitzpatrick*, 428 U.S. 901 (1976) (affirming legitimate state interest in disposal of fetal remains); *Wynn v. Scott*, 449 F. Supp. 1302, 1322 (N.D. Ill. 1978) (medical researchers have no fundamental rights under the Constitution to perform fetal experiments), aff'd on other grounds sub nom.; *Wynn v. Carey*, 599 F.2d 193 (7th Cir. 1979) (upholding state's rational interest in regulating medicine as to viable fetus); *Leigh v. Olson*, 497 F. Supp. 1340 (D.N.D. 1980) (striking fetal disposal statute as vague where it left "humane disposal" undefined and required mother to determine method of disposal); *Akron v. Akron Center for Reproductive Health, Inc.*, 462 U.S. 416 (1983) (struck down local ordinance that, inter alia, mandated humane and sanitary disposal of fetal remains, finding the provision impermissibly vague because it was unclear whether it mandated a decent burial of the embryo at the earliest stages of formation); *Planned Parenthood Association v. City of Cincinnati*, 822 F.2d 1390, 1391 (6th Cir. 1987) (struck down on other grounds, the court noted in dicta that the wording used by the municipal code regulating disposal of aborted fetal tissue might be precise enough to survive scrutiny); *Planned Parenthood of Minnesota v. Minnesota*, 910 F.2d 479 (8th Cir. 1990) (upholding Minnesota's fetal disposal statute against challenge of vagueness and infringement of privacy).

44 La. Rev. Stat. Ann. § 40:1299.35.13. See Clapp (1988): "The Louisiana statute effectively prohibits any research, experimentation, or even observational study on any embryo, fetus, or aborted fetal tissue. The ban encompasses a range of activities, including studies of the safety of ultrasound and pathological study of fetal tissues removed from a woman for the purpose of monitoring her health. Research on IVF is likewise barred. Since the aborted previable fetus is not living or cannot survive for long, no procedure performed upon it could be considered 'therapeutic,' and therefore use of this tissue is likewise prohibited. If performed on tissues from a miscarriage, such experimentation would be acceptable under the statutory scheme" [footnote omitted] (1076–1077).

45 *Margaret S. v. Treen*, 597 F. Supp. 636 (E.D. La. 1984), aff'd sub nom.; *Margaret S. v. Edwards*, 794 F.2d 994 (5th Cir. 1986). See Clapp (1988): The court "specifically note[d] that reproductive choice was 'not limited to abortion decisions...but extends to both childbirth and contraception.' Prohibiting experimentation on fetal tissues could deny a woman knowledge that would influence her own future pregnancies, as well as prohibit procedures of immediate medical benefit such as pathological examination of tissues. The court also found that the prohibition curtailed the development and use of prediagnostic techniques, including amniocentesis. This result constituted a 'denial of health care' and a 'significant burden' on choice made during the first trimester" [footnote omitted] (1078–1079).

46 *Margaret S. v. Treen*, 597 F. Supp. 636, 674-75 (E.D. La. 1984). See Clapp (1988): "The court further suggested the statute would fail even a rational relation test because it failed to serve its own stated purpose of treating the fetus like a human being, since it treated fetal tissue differently from other human tissue" (1079).

47 *Margaret S. v. Treen*, 597 F. Supp. 636, 675-76 (E.D. La. 1984).

48 *Margaret S. v. Edwards*, 794 F.2d 994, 999 (5th Cir. 1986). "The whole distinction between experimentation and testing, or between research and practice, is…almost meaningless, [such that] 'experiment' is not adequately distinguishable from 'test'...every medical test that is now 'standard' began as an 'experiment.'" But see Clapp (1988): "[T]he court hypothesized that the statute was intended 'to remove some of the incentives for research-minded physicians…to promote abortion' and was therefore 'rationally related to an important state interest.' This language suggests that if the statute had not been vague, the court would have applied less than strict scrutiny to a ban on fetal research. The court also implied, in dicta, that the rationale was based on the 'peculiar nature of abortion and the state's legitimate interest in discouraging' it, relying on *H.L. v. Matheson*, 450 U.S. 398, 411–413 (1981)" (1080). A concurring opinion "criticized the majority for avoiding the real constitutional issue raised—that any statutory ban on experimentation would inevitably limit the kinds of tests available to women and their physicians and thus could not help but infringe on fundamental rights" Ibid. at 999–1002 (Williams, J., concurring) (Clapp 1988, 1080). See also *Jane L. v. Bangerter*, 61 F.3d 1493 (10th Cir. 1995) (striking down as vague Utah's criminal prohibition on fetal research which permitted experimentation aimed at acquiring genetic information about the embryo or fetus).

49 Members of Congress who have opposed stem cell funding maintain that "current law...also specifically covers cells and tissue obtained from embryos," citing as applicable 42 USC § 289g-1(b)(2)(ii) ("no alternation of the timing, method, or procedures used to terminate the pregnancy...made solely for the purposes of obtaining the [fetal] tissue") (Members of the House of Representatives 1999). The apparent basis for this assertion is the definition of "human fetal tissue" at 42 USC § 289g-1(g) ("for purposes of this section, the term 'human fetal tissue' means tissue or cells obtained from a dead human embryo or fetus after a spontaneous or induced abortion"). Two elements render the congressional arguments unpersuasive: 1) neither 42 USC § 289g-1 nor 289g-2 is directed at embryo or IVF research; rather, both sections are exclusively centered in a conventional understanding of aborted fetal tissue and the issues arising from fetal tissue research; and 2) biological embryology, IVF, and ES cell research typically include only "live" embryos that are maintained in a living state for research purposes until they are either implanted, disaggregated for living unicellular components, or terminated upon the experiment's completion. A "dead human embryo" would, by definition, comprise a multicellular tissue mass in which all cellular functions associated with life activity had previously ceased (clinical cell death), and would be more in the nature of a stored pathology specimen. The draft guidelines of the NIH Ad Hoc Working Group of the Advisory Committee to the Director support this interpretation (NIH Ad Hoc Working Group 1999, 5).

50 Some private sector biotechnology companies have voluntarily undertaken to self-regulate their research activities using IVF embryos through the use of advisory boards and ethical protocols (Geron Ethics Advisory Board 1998).

51 Public Law No. 105-277, 112 Stat. 2681 (1998).

52 45 CFR § 46.201(a).

53 45 CFR § 46.204(d), nullified by section 121(c) of the NIH Revitalization Act of 1993, Public Law 103-43, June 10, 1993; see 59 *Fed. Reg.* 28276 (June 1, 1994).

54 DHHS has considered human embryo research only under the category of IVF research, as defined in Subpart B ("any fertilization of human ova which occurs outside the body of a female, either through admixture of donor human sperm and ova or by any other means," 45 CFR § 46.203(g)) and hence it had been subject to the requirement of EAB review prior to funding.

55 The 1993 Act deleted the requirement that IVF research be reviewed by an EAB before it could be funded, but it did not remove the remaining subsections of 45 CFR § 46.204, which prescribe the basic structure and functions of the "one or more Ethical Advisory Boards" that "shall be established by the Secretary" to provide advice as needed on individual applications or "general policies, guidelines, and procedures" covered by Subpart B, including the setting of "class of applications or proposals which: (1) must be submitted to the Board, or (2) need not be submitted to the Board" 45 CFR § 46.204 (a)-(c).

56 59 *Fed. Reg.* 28874, 28875 (June 3, 1994) (notice of meeting); (NIH 1994, vol. 1, ix).

57 "[It] would not be wise to prohibit altogether the fertilization and study of oocytes for research purposes....[H]owever, the embryo merits respect as a developing form of human life and should be used in research only for the most serious and compelling reasons....The Panel believes that the use of oocytes fertilized expressly for research should be allowed only under two conditions. The first condition is when the research by its very nature cannot otherwise be validly conducted. The second condition...is when a compelling case can be made that this is necessary for the validity of a study that is potentially of outstanding scientific and therapeutic value" (NIH 1994, vol. 1, xi–xii).

58 "The Director of the National Institutes of Health has received a recommendation regarding federal funding of research on human embryos. The subject raises profound ethical and moral questions as well as issues concerning the appropriate allocation of federal funds. I appreciate the work of the committees that have considered this complex issue and I understand that advances in *in vitro* fertilization research and other areas could derive from such work. However, I do not believe that federal funds should be used to support the creation of human embryos for research purposes, and I have directed that NIH not allocate any resources for such research. In order to ensure that advice on complex bioethical issues that affect our society can continue to be developed, we are planning to move forward with the establishment of a National Bioethics Advisory Commission over the next year" (Office of the White House Press Secretary, Statement by the President, December 2, 1994). Although technically superseded in its effect by the congressional appropriations rider governing DHHS, the Directive remains effective throughout other Executive agencies. This has not been formally inscribed as an Executive Order.

59 Public Law No. 104-99, Title I, § 128, 110 Stat. 26, 34 (1996). The rider defines "human embryo" as "any organism, not protected as a human subject under 45 CFR 46 as of the date of the enactment of this Act, that is derived by fertilization, parthenogenesis, cloning, or any other means from one or more human gametes or human diploid cells." NIH has described the effect of the ban as prohibiting "*in vitro* fertilization of a human egg for research purposes where there is no direct therapeutic intent...as well as...research with embryos resulting from clinical treatment and research on parthenogenesis." The rider has been attached to the subsequent DHHS appropriations, through the current Fiscal Year. See Public Law No. 104-208, Div. A, § 101(e), Title V, § 512, 110 Stat. 3009, 3009-270 (1996); Public Law No. 105-78, Title V, § 513, 111 Stat. 1467, 1517 (1997); Public Law No. 105-277, 112 Stat. 2461 (1998).

60 Memorandum from Harriet Rabb to Harold Varmus, January 15, 1999.

61 "NIH funds (including equipment, facilities, and supplies purchased on currently funded grants) should not be used to conduct research using human pluripotent stem cells derived from human fetal tissue or human embryos until further notice....While the NIH proposes to support research utilizing these human pluripotent stem cells, it will not do so until public consultation has occurred, guidelines are issued, and an oversight committee has ensured that each project is in accord with these guidelines. Research on human stem cells derived from sources other than human embryos or fetal tissue will not be subject to these guidelines and oversight: this research will continue to be funded under existing policies and procedures" (NIH 1999). The NIH Director's caution has not avoided public controversy, however (Members of the House of Representatives 1999; Lanza, Arrow, Axelrod, et al. 1999).

62 "Opening Statement of Co-Chair Ezra C. Davidson, Jr., M.D.," Meeting of the NIH Ad Hoc Working Group of the Advisory Committee to the Director, April 8, 1999 (NIH Ad Hoc Working Group 1999).

63 Letter from Gary B. Ellis, Director of the Office for Protection from Research Risks, to Eric M. Meslin, Executive Director of the National Bioethics Advisory Commission (NBAC), June 3, 1999.

64 45 CFR § 46.102(f).

65 In addition to their other duties, IRBs reviewing research subject to Subpart B must "1) Determine that all aspects of the activity meet the requirements of this subpart; 2) Determine that adequate consideration has been given to the manner in which potential subjects will be selected, and adequate provision has been made by the applicant or offeror for monitoring the actual informed consent process (e.g., through such mechanisms, when appropriate, as participation by the Institutional Review Board or subject advocates in: i) Overseeing the actual process by which individual consents required by this subpart are secured either by approving induction of each individual into the activity or verifying, perhaps through sampling, that approved procedures for induction of individuals into the activity are being followed, and ii) monitoring the progress of the activity and intervening as necessary through such steps as

visits to the activity site and continuing evaluation to determine if any unanticipated risks have arisen); 3) Carry out such other responsibilities as may be assigned by the Secretary" (45 CFR § 46.205(a) (1997)). See also 45 CFR § 46.205(c) (1997) ("Applicants or offerors seeking support for activities covered by this subpart must provide for the designation of an Institutional Review Board, subject to approval by the Secretary, where no such Board has been established under Subpart A of this part.").

66 See 45 CFR §§ 46.201-210, Subpart B, "Additional DHHS Protections for Pregnant Women, Human Fetuses, and Newborns Involved as Subjects in Research, and Pertaining to Human *In Vitro* Fertilization," *Fed. Reg.* 27794–27804 (May 20, 1998).

67 "In those states...embryo stem cell research is not banned," but see D.C. Code § 6-2601 (1998) prohibiting sale of any part of human body (even cells), a restriction that may extend to human embryos (Andrews 1999).

68 N.H. Rev. Stat. Ann. § 168-B:15 (limiting the maintenance of *ex utero* pre-implantation embryo in a noncryopreserved state to under 15 days and prohibiting the transfer of research embryo to the uterine cavity).

69 Louisiana broadly prohibits research involving IVF embryos. La. Rev. Stat. Ann. §§ 9:121–122 (West 1991). Eight other states restrict embryo research indirectly, banning all research on "live" embryos or fetuses. Fla. Stat. Ann. § 390.0111(6); Me. Rev. Stat. Ann. tit. 22, § 1593 (West 1992); Mass. Ann. Laws ch. 112, § 12j(a)(I) (Law. Co-op. 1996); Mich. Comp. Laws Ann. §§ 333.2685, 333.2686, 333.2692 (West 1992); Minn. Stat. Ann. § 145.422 Subd. 1,2 (West 1989); N.D. Cent. Code §§ 14-02.2-01, 14-02.2-02 (1991); 18 Pa. Cons. Stat. Ann. § 3216(a) (Supp. 1995); R.I. Gen. Laws § 11-54-1(a)-(c) (1994) (Andrews 1999).

70 Me. Rev. Stat. Ann. tit. 22 § 1593; Mass. Ann. Laws ch. 112 § 12(j)(A)(Iv); Mich. Comp. Laws § 333.2609; N.D. Cent. Code § 14-02.2-02(4); and R.I. Gen. Laws § 11-54-1(f).

71 Fla. Stat. Ann. § 873.05; Georgia Code Ann. § 16-12-160 (A) (Except for Health Services Education); Ill. Stat. Ann. Ch 110½ Para. 308.1; La. Rev. Stat. Ann. § 9:122; Minn. Stat. Ann. § 145.422(3) (Live); 18 Pa. Cons. Stat. Ann. § 3216(b)(3) (forbids payment for the procurement of fetal tissue or organs); Texas Penal Code § 48.02; Utah Code Ann. § 76-7-311. But see Feiler (1998): "Although some state laws prohibit the sale of fertilized embryos, they do nothing to prevent the sale of gametes (sperm and eggs), which can easily be converted into research embryos through deliberate fertilization. Payment for sperm and eggs is widespread among American infertility clinics" [citations omitted] (2455).

72 nn. 66; 75; 76; 80. Tenn. Code Ann. § 39-15-208 (199_) and Utah Code Ann. § 76-7-311 (199_) prohibit sale of an "unborn child"; D.C. Code § 6-2601 (199_) and Va. Code § 32.1-289.1 (199_) prohibit sale of all or a portion of the "human body" (D.C.) or a "natural body part" (Va.); two state statutes prohibit sale of specified organs (not including embryos), but permit state health officials to expand the list under prescribed conditions. N.Y. Public Health Law § 4307 (199_); W. Va. Code § 68.50.610(2) (199_) (Andrews 1999).

73 At least one state "prohibits the sale of living [embryos] or non-renewable organs but does allow 'the buying and selling of a cell culture line or lines taken from a non-living human [embryo],'" ibid., citing Minn. Stat. Ann. § 145.422(3) (Andrews 1999, citing Minn. Stat. Ann. § 145.422(3)).

74 Office of the White House Press Secretary, "Memorandum for the Heads of Executive Departments and Agencies," March 4, 1997.

75 Executive Office of the President of the United States, 1998, *Statement of Administration Policy [on] S.1601 (Human Cloning Prohibition Act)* (Washington, DC: Executive Office of the President).

References

Adams, A.M. 1988. "Background Leading to Meeting of the Human Fetal Tissue Transplantation Research Panel Consultants." *Report of the Human Fetal Tissue Transplantation Research Panel*. Vol. 2, A3–A5. Bethesda, MD: National Institutes of Health (NIH).

Areen, J.C. 1988. "Statement on Legal Regulation of Fetal Tissue Transplantation for the Human Fetal Tissue Transplantation Research Panel." *Report of the Human Fetal Tissue Transplantation Research Panel*. Vol. 2, D21–D26. Bethesda, MD: NIH.

Bell, N.M.C. 1994. "Regulating Transfer and Use of Fetal Tissue in Transplantation Procedures: The Ethical Dimensions." *American Journal of Law and Medicine* 20:277–294.

Canadian Royal Commission on New Reproductive Technologies. 1993. *Proceed with Care: Final Report of the Royal Commission on New Reproductive Technologies*. 2 vols. Ottawa: Minister of Government Services.

Clapp, M. 1988. "State Prohibition of Fetal Experimentation and the Fundamental Right of Privacy." *Columbia Law Review* 88:1073–1097.

Coleman, J. 1996. "Playing God or Playing Scientist: A Constitutional Analysis of State Laws Banning Embryological Procedures." *Pacific Law Journal* 27:1331–1399.

Department of Health, Education, and Welfare (DHEW). Ethics Advisory Board (EAB). 1979. *Report and Conclusions: HEW Support of Research Involving Human* In Vitro *Fertilization and Embryo Transfer*. Washington, DC: U.S. Government Printing Office.

Driscoll, Jr., E. 1985. "Nobel Recipient John Enders, 88; Virus Work Led to Polio Vaccine." Obituary. *Boston Globe*, 10 September, 85.

Duguay, K.F. 1992. "Fetal Tissue Transplantation: Ethical and Legal Considerations." *CIRCLES: Buffalo Women's Journal of Law and Social Policy* 1:36.

Duke, R.C. 1988. "Statement of the Population Crisis Committee." In *Report of the Human Fetal Tissue Transplantation Research Panel*. Vol. 2. D112–D121. Bethesda, MD: NIH.

Feiler, C.L. 1998. "Human Embryo Experimentation: Regulation and Relative Rights." *Fordham Law Review* 66:2435–2469.

General Accounting Office (GAO). 1997. *Report to the Chairmen and Ranking Minority Members, Committee on Labor and Human Resources, U.S. Senate, and Committee on Commerce, House of Representatives: National Institutes of Health-Funded Research: Therapeutic Human Fetal Tissue Transplantation Projects Meet Federal Requirements*. Washington, DC: U.S. Government Printing Office.

Gelfand, G., and T.R. Levin. 1993. "Fetal Tissue Research: Legal Regulation of Human Fetal Tissue Transplantation." *Washington and Lee Law Review* 50:647–694.

Geron Ethics Advisory Board. Geron Corporation. 1998. *A Statement on Human Embryonic Stem Cells*.

Goddard, J.E. 1996. "The National Institutes of Health Revitalization Act of 1993 Washed Away Many Legal Problems with Fetal Tissue Transplantation Research But a Stain Remains." *Southern Methodist University Law Review* 49:375–399.

Lanza, R.P., K.J. Arrow, J. Axelrod, et al. 1999. "Science over Politics." *Science* 284:1849–1850.

Members of the House of Representatives. 1999. *Statement of the Members of the House of Representatives to the Working Group of the Advisory Committee to the Director of the National Institutes of Health*. April 8.

National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research. 1975. *Research on the Fetus: Report and Recommendations*. Washington, DC: U.S. Government Printing Office.

NIH. 1987. "Human Fetal Tissue Research Supported by the National Institutes of Health." *Summary Highlights of FY 1987*. Bethesda, MD: NIH.

———. 1999. "National Institutes of Health Position on Human Pluripotent Stem Cell Research." Bethesda, MD: NIH. April 19, www.nih.gov/grants/policy/stemcells.htm.

NIH. Ad Hoc Working Group of the Advisory Committee to the Director. 1999. "Draft Guidelines for Research Involving Pluripotent Stem Cell Research." Bethesda, MD: NIH.

NIH. Human Embryo Research Panel. 1994. *Report of the Human Embryo Research Panel*. 2 vols. Bethesda, MD: NIH.

NIH. Office of the Director (OD). 1999. "Fact Sheet on Stem Cell Research." Bethesda, MD: NIH, www.nih.gov/news/pr/apr99/od-21.htm.

Office for Protection from Research Risks (OPRR). 1994. *Human Subjects Protections: Fetal Tissue Transplantation—Ban on Research Replaced by New Statutory Requirements*. 29 April.

Robertson, J.A. 1990. "Reproductive Technology and Reproductive Rights. In the Beginning: The Legal Status of Early Embryos." *Virginia Law Review* 76:437–517.

———. 1993. "Abortion to Obtain Fetal Tissue for Transplant." *Suffolk University Law Review* 27:1359–1389.

Ryan, K.J. 1991. "Tissue Transplantation from Aborted Fetuses, Organ Transplantation from Anencephalic Infants and Keeping Brain-Dead Pregnant Women Alive Until Fetal Viability." *Southern California Law Review* 65:683–696.

Silva-Ruiz, P.F. 1998. "Section II: The Protection of Persons in Medical Research and Cloning of Human Beings." *American Journal of Comparative Law* 46:151–163.

Windom, R.E. 1988. "Memorandum to James B. Wyngaarden, March 22, 1988." *Report of the Human Fetal Tissue Transplantation Research Panel*. Vol. 2, A3. Bethesda, MD: NIH.

Zion, C. 1996. "The Legal and Ethical Issues of Fetal Tissue Research and Transplantation." *Oregon Law Review* 75:1281–1296.

Chapter Four

Ethical Issues in Human Stem Cell Research

Ethical Issues Relating to the Sources of Human Embryonic Stem or Embryonic Germ Cells

Research involving human embryonic stem (ES) cells and embryonic germ (EG) cells raises several important ethical issues, principally related to the current sources and/or methods of deriving these cells. If, for example, ES and EG cells could be derived from sources other than human embryos or cadaveric fetal material, fewer ethical concerns would be involved in determining a policy for their use for scientific research or clinical therapies. At present, however, the only methods available to isolate and culture human ES and EG cells involve the use of human embryos or cadaveric fetal tissue. Therefore, careful consideration of the ethical issues involved in the use of these sources is an unavoidable component of the advancement of this type of research.

This chapter first considers the ethical issues arising from research involving the derivation and/or use of ES or EG cells from three potential sources: cadaveric fetal tissue, embryos resulting from and remaining after infertility treatments, and embryos created solely for research purposes either by *in vitro* fertilization (IVF) or somatic cell nuclear transfer (SCNT) techniques. The chapter then reviews separately the specific arguments for and against federal funding of this research. Finally, the chapter discusses relevant ethical issues in federal oversight and review of research involving the derivation and/or use of ES or EG cells.[1]

Research with EG Cells Derived from Cadaveric Fetal Tissue

Many of the ethical questions regarding research involving the use of cadaveric fetal tissue were analyzed in depth by the 1988 National Institutes of Health (NIH) Human Fetal Tissue Transplantation Research Panel. What is new in the present context is that, in the near term at least, the materials derived from this tissue would not be transplanted; rather, gonadal tissue (both male and female) would be used as a source for human EG cells. Initially, these cell lines would be used in basic research to determine their nature, to understand their relationship to human development, and to identify differentiation factors that enable such cells to develop into particular tissue types. Later, such cell lines also might be used for the development of transplantation for particular tissue types. The value of cadaveric fetal tissue already has been demonstrated; a broad variety of research materials and reagents derived from cadaveric fetal tissue currently are used in federally funded research.[2]

The ethical acceptability of deriving EG cells from the tissue of aborted fetuses is, for some, closely connected to the ethical acceptability of abortion. Those who believe that elective abortions are morally acceptable are less likely to identify insurmountable ethical barriers to research that involves the derivation and use of EG cells derived from cadaveric fetal tissue. This group might agree that it is necessary to restrict such research by requiring that the decision to donate fetal tissue be separate from the decision to terminate the pregnancy. The purpose of such a requirement would be to protect the pregnant woman against coercion and exploitation rather than to protect the fetus. In addition, even those who find it acceptable to use cadaveric fetal tissue in research might hold that certain uses of such tissue—for example, uses that treat it as nothing more than any other bodily tissue—should be ruled out as disrespectful.

Those who view elective abortions as morally unjustified often—but not always—oppose the research use of tissue derived from aborted fetuses. They usually have no moral difficulty with the use of tissue from spontaneously aborted fetuses or—if they recognize exceptions to the moral prohibition on abortion—from fetuses in cases that they believe are morally justifiable abortions (e.g., to save the pregnant woman's life). However, in general they do not believe that it is possible to derive and use tissue from what they believe are unjustifiably aborted fetuses without inevitable and unacceptable association with those abortions. This association, they believe, usually taints the actions of all those involved in using these materials or in financing research protocols that rely on such tissue. Nevertheless, some opponents of elective abortions believe that it is still possible to support such research as long as effective safeguards are in place to separate abortion decisions from the procurement and use of fetal tissue in research. For them, when appropriate safeguards are in place, using cadaveric fetal tissue from elective abortions for research is relevantly similar to using nonfetal cadavers donated for scientific and medical purposes.

Association with Abortion

Opponents of the research use of fetal materials obtained from elective abortions dispute the claim that it is possible to separate the moral issues surrounding the abortion from those involved in obtaining and using fetal material. They argue that those who obtain and use fetal material from elective abortion inevitably become associated, in ethically unacceptable ways, with the abortions that are the source of the material.[3] They identify two major types of unacceptable association or cooperation with abortion: 1) causal responsibility for abortions and 2) symbolic association with abortions.

1. Causal Responsibility

Some believe that those who provide cadaveric fetal tissue in research are indirectly, if not directly, responsible for the choice of some women to have an abortion. Direct causal responsibility exists where, in this case, someone's actions directly lead a pregnant woman to have an abortion—for example, the researcher offers financial compensation for cadaveric fetal tissue and this compensation leads the pregnant woman to have an abortion she would not otherwise have had. In part because of concerns about direct causal responsibility, the Human Fetal Tissue Transplantation Research Panel (1988) recommended the following safeguards to separate the pregnant woman's decision to abort from her decision to donate fetal tissue:

- The consent of women for abortions must be obtained prior to requesting or obtaining consent for the donation of fetal tissue.
- Those who seek a woman's consent to donate should not discuss fetal tissue donation prior to her decision to abort, unless she specifically requests such information.
- Women should not be paid for providing fetal tissue.
- A separation must be maintained between abortion clinic personnel and those involved in using fetal tissue.
- There should be a prohibition against any alteration of the timing of or procedures used in an abortion solely for the purpose of obtaining tissue.
- Donors of cadaveric fetal tissue should not be allowed to designate a specific recipient of transplanted tissue.

As noted in Chapter 3, several of these safeguards were later adopted in federal legislation regarding the use of aborted fetal tissue in transplantation research, and they appear to be sufficient to avoid direct causal responsibility for abortions in human EG research as well as in transplantation research.

Those involved in research uses of EG cells derived from fetal tissue could be indirectly responsible for abortions if the perceived potential benefits of the research contributed to an increase in the number of abortions. Opponents of fetal tissue research argue that it is unrealistic to suppose that a woman's decision to abort can be kept separate from considerations of donating fetal tissue, as many women facing the abortion decision are likely to have gained knowledge about fetal tissue research through the media or other sources. The knowledge that having an elective abortion might have benefits for future patients through the donation of fetal tissue for research may tip the balance in favor of going through with an abortion for some women who are ambivalent about it. Some argue that the benefits achieved through the routine use of fetal tissue will further legitimize abortion and result in more permissive societal attitudes and policies concerning elective abortion.

It is impossible to eliminate the possibility completely, however slight it may be, that knowledge of the promise of research on EG cells derived from fetal tissue will play a role in some elective abortion decisions, even if only rarely. However, it is not clear how much moral weight ultimately attaches to this possibility. One might be justified in some instances in asserting that if it were not for the research use of fetal tissue following an abortion, a woman might not have chosen to terminate her pregnancy.

But one could assign this kind of causal responsibility to a number of factors that figure into abortion decisions without making ascriptions of indirect causal responsibility, or what is sometimes called moral complicity. For example, a woman might choose to have an abortion principally because she does not want to slow the advancement of her education and career. She might not have had an abortion in the absence of expectations that encourage women to develop their careers. Yet, we would not think it appropriate to charge those who promote such expectations and/or policies as complicit in her abortion. In both this case and that of research, the opportunity to choose abortion is a consequence of a legitimate social policy. The burden on those seeking to end such policies is to show that the risks of harm—both the probability and the magnitude of harm—resulting from the policies outweigh the expected benefits (Childress 1991). This criterion minimally requires evidence of a high probability of a large number of elective abortions that would not have occurred in the absence of those policies. There is, however, no such evidence at present. If compelling evidence did emerge that elective abortions did, or probably would, increase as a result of the research use of cadaveric fetal tissue, this would require a re-examination of the balance of benefits and harms as well as the safeguards that had been put into place to eliminate the potential for direct causal responsibility and reduce the likelihood of indirect causal responsibility for abortions.

2. Symbolic Association

People can become inappropriately associated with what they believe are wrongful acts for which they are not causally responsible. Particularly problematic for many is an association that appears to symbolize approval of the wrongdoing. For example, James Burtchaell maintains that those involved in research on fetal tissue enter a symbolic alliance with the practice of abortion in producing or deriving benefits from it (1988).

A common response is that persons can benefit from what they might consider immoral acts without tacitly approving of those acts. For example, transplant surgeons and transplant recipients may benefit (the latter more directly than the former) from donated organs from victims of murder or drunken driving but nevertheless condemn those wrongful acts (Robertson 1988; Vawter et al. 1991). A researcher who uses cadaveric fetal material in studies to answer important research questions or to study its potential therapeutic effects or the patient who receives the donated tissue need not sanction the act of abortion any more than the transplant surgeon who uses the organs of a murder victim approves of the homicidal act.

Some opponents of fetal tissue research maintain that it implicates those involved in a kind of wrongdoing that cannot be attributed to the transplant surgeon in the example above. Unlike drunken driving and murder, abortion is an institutionalized practice in which certain categories of human life (the members of which are considered by some to have the same moral status as human adults) are allowed to be killed. In this respect, some opponents of abortion go so far as to suggest that fetal tissue research is more analogous to research that benefits from experiments conducted by Nazi doctors during World War II (Bopp 1994).

But whatever one thinks of comparisons between the victims of Nazi crimes and aborted fetuses—and many are outraged by these comparisons—it is possible to concede the comparisons without concluding that human stem cell research involving cadaveric fetal tissue is morally problematic. Of course, some believe that those who use data derived from Nazi experiments are morally complicit with those crimes. For example, William Seidelman writes:

> By giving value to (Nazi) research we are, by implication, supporting Himmler's philosophy that the subjects' lives were 'useless.' This is to argue that, by accepting data derived from their misery we are, post mortem, deriving utility from otherwise 'useless' life. Science could thus stand accused of giving greater value to knowledge than to human life itself (1988, 232).

But one need not adopt this stance. Instead, one can reasonably believe that a scientist's actions must be understood and judged not by their consequences or uses but rather by several other factors, including the scientist's intentions, the social practices of which his or her actions are a part, and the social context in which those practices are embedded. As philosopher Benjamin Freedman wrote:

> A moral universe such as our own must, I think, rely on the authors of their own actions to be primarily responsible for attaching symbolic significance to those actions...[I]n using the Nazi data, physicians and scientists are acting pursuant to their own moral commitment to aid patients and to advance science in the interest of humankind. The use of data is predicated upon that duty, and it is in seeking to fulfill that duty that the symbolic significance of the action must be found (1992, 151).

It is likewise reasonable to maintain that the symbolic significance of support for research using EG cells derived from aborted fetal tissue lies in the commitment and desire to gain knowledge, promote health, and save lives. This research is allied with a worthy cause, and any taint that might attach from the source of the cells appears to be outweighed by the potential good that the research may yield.

Consent and Donation

In previous debates about the use of fetal tissue in research, questions have been raised about who has the moral authority to donate the material. Some assert that, from an ethical standpoint, a woman who chooses abortion forfeits her rights to determine the disposition of the dead fetus. Burtchaell, for instance, argues that "the decision to abort, made by the mother, is an act of such violent abandonment of the maternal trusteeship that no further exercise of such responsibility is admissible" (1988, 9). By contrast, John Robertson argues that this position mistakenly assumes that the persons disposing of cadaveric remains act only as the guardians or proxies of the deceased. Instead, "a more accurate account of their role is to guard their own feelings and interests in assuring that the remains of kin are treated respectfully" (1988, 6).

In our view, obtaining consent to donate fetal tissue is an ethical prerequisite for using such material to derive EG cells, even though the woman or couple are not research subjects per se, and even though the cadaveric fetus is not a human subject. This view is consistent with the conclusion of the Human Fetal Tissue Transplantation Research Panel, which held that "[e]xpress donation by the pregnant woman after the abortion decision is the most appropriate mode of transfer of fetal tissues because it is the most congruent with our society's traditions, laws, policies, and practices, including the Uniform Anatomical Gift Act and current Federal research regulations" (1988, 6). According to this panel, a woman's choice of a legal abortion does not disqualify her legally and should not disqualify her morally from serving "as the primary decisionmaker about the disposition of fetal remains, including the donation of fetal tissue for research." She "has a special connection with the fetus and she has a legitimate interest in its disposition and use." In addition, her decision to donate fetal tissue would not violate the dead fetus's interests. The panel concluded that "in the final analysis, any mode of transfer other than maternal donation appears to raise more serious ethical problems" (6). Fetal tissue should not be used without the woman's consent. Not only should her consent be necessary, it should also be sufficient to donate the tissue, except where the father's objection is known.

We concur with the Human Fetal Tissue Transplantation Research Panel that a woman undergoing an elective abortion should be authorized to donate fetal tissue, unless the father is known to object. We further agree with the panel and with subsequent federal legislation that it is important to establish safeguards to separate the pregnant woman's decision to abort from the decision to donate cadaveric fetal tissue. The guidelines already in place for fetal tissue transplantation research generally are appropriate and appear to be sufficient if they also apply to research involving human EG cells.

As already noted, some opponents of elective abortion can support fetal tissue research as long as there are safeguards to avoid direct causal responsibility and to reduce the likelihood of indirect causal responsibility. Many who view elective abortion as morally problematic, even if not always morally unjustified, also may endorse

these safeguards as a way to avoid certain forms of association with morally problematic actions and at the same time as a way to prevent the exploitation and coercion of pregnant women. Even those who do not find elective abortions morally problematic may accept these safeguards in order to protect pregnant women from exploitation and coercion as well as to sustain social practices that reflect important social and cultural values and to respect the moral concerns of opponents of elective abortion. We believe, therefore, that there can be wide agreement on appropriate safeguards for the process of donating cadaveric fetal tissue.

At a minimum, these safeguards should separate the decision to have an abortion from the decision to donate by ensuring, as much as possible, that the former occurs before the latter by not providing before the abortion decision is made information about the possibility of using fetal materials in research and by prohibiting the provision of financial compensation for the fetal tissue to the woman (or to the couple) having the abortion. If these and other requirements that already have been adopted in regulations governing federally funded human fetal tissue transplantation research do not clearly extend to research to generate EG cells from cadaveric fetal tissue, the regulations should be modified to do so.

Research with ES Cells Derived from Embryos Remaining After Infertility Treatments

Ethical issues arising from research involving the use of human embryos have generated a sustained public policy discussion and a valuable body of literature that spans at least 20 years. Some of these issues were considered in depth by the Department of Health, Education and Welfare (DHEW) Ethics Advisory Board (EAB) in 1978 and in 1979 (DHEW Ethics Advisory Board 1979). The ethical debate was continued here and abroad by other national advisory bodies, including the British Warnock Committee (Committee of Inquiry 1984) and the Canadian Royal Commission on New Reproductive Technologies (1993). In 1994, the NIH Human Embryo Research Panel considered multiple types of present and future human embryo research and discussed both ethical and public policy issues (NIH 1994). In contrast, for example, SCNT has been seriously debated in the United States and elsewhere only for about two years, and the research use of SCNT has been debated for an even shorter period.

One source of embryos for ES cells is those remaining after infertility treatments. Couples who provide such embryos have decided that they no longer need them to achieve their reproductive goals. If the couple prefers to discontinue storing the remaining embryos and does not wish to donate them to other couples, the only alternatives are to direct that the embryos be discarded (that is, to destroy them through the thawing process) or to donate them for research. When only these latter two alternatives remain, the situation is somewhat similar to that in which a woman is deciding whether to donate fetal tissue for research following elective abortion and the situation in which families are deciding whether to donate the organs or tissues of a loved one who has recently died. However, whether this similarity is decisive depends upon one's perception of the moral status of embryos. Derivation of ES cells involves destroying the embryos, whereas abortion precedes the donation of the fetal tissue and death precedes the donation of whole organs for transplantation.

The Moral Status of Embryos

To say that an entity has "moral status" is to say something both about how one should act towards that thing or person and about whether that thing or person can expect certain treatment from others. The debate about the moral status of embryos traditionally has revolved around the question of whether the embryo has the same moral status as children and adult humans do—with a right to life that may not be sacrificed by others for the benefit of society. At one end of the spectrum of attitudes is the view that the embryo is a mere cluster of cells that has no more moral status than any other collection of human cells. From this perspective, one might conclude that there are few, if any, ethical limitations on the research uses of embryos.

At the other end of the spectrum is the view that embryos should be considered in the same moral category as children or adults. According to this view, research involving the destruction of embryos is absolutely prohibited. Edmund D. Pellegrino, a professor of bioethics at Georgetown University, described this perspective in testimony given before the Commission:

> The Roman Catholic perspective…rejects the idea that full moral status is conferred by degrees or at some arbitrary point in development. Such arbitrariness is liable to definition more in accord with experimental need than ontological or biological reality.[4]

In contrast, scholars representing other religious traditions testified that moral status varies according to the stage of development.[5] For example, Margaret Farley, a professor of Christian ethics at Yale University, pointed out that

> There are clear disagreements among Catholics—whether moral theologians, church leaders, ordinary members of the Catholic community—on particular issues of fetal and embryo research….A growing number of Catholic moral theologians, for example, do not consider the human embryo in its earliest stages…to constitute an individualized human entity.[6]

Other scholars from Protestant, Jewish, and Islamic traditions noted that major strands of those traditions support a view of fetal development that does not assign full moral status to the early embryo.[7] For example, Jewish scholars testified that the issue of the moral status of extra-corporeal embryos is not central to an assessment of the ethical acceptability of research involving ES cells. Rabbi Elliot Dorff noted that

> Genetic materials outside the uterus have no legal status in Jewish law, for they are not even a part of a human being until implanted in a woman's womb and even then, during the first 40 days of gestation, their status is 'as if they were water.' As a result, frozen embryos may be discarded or used for reasonable purposes, and so may stem cells be procured from them.[8]

As a result, for some Jewish thinkers, the derivation and use of ES cells from embryos remaining after infertility treatments may be less problematic than the use of aborted fetal tissue, at least following morally unjustified abortions.

On this issue, the Commission adopted what some have described as an intermediate position, one with which many likely would agree: that the embryo merits respect as a form of human life, but not the same level of respect accorded persons. We recognize that, on such a morally contested issue, there will be strong differences of opinion. Moreover, it is unlikely that, by sheer force of argument, those with particularly strong beliefs on either side will be persuaded to change their opinions (Murray 1996). However, there is, in our judgment, considerable value in describing some of these positions, not only to reveal some of the difficulties of resolving the issue, but to seek an appropriate set of recommendations that can reflect the many values we share as well as the moral views of those with diverse ethical commitments.

A standard approach taken by those who deny that embryos are persons with the same moral status as children and adults is to identify one or more psychological or cognitive capacities that are considered essential to personhood (and a concomitant right to life) but that embryos lack. Most commonly cited are consciousness, self-consciousness, and the ability to reason (Feinberg 1986; Tooley 1983; Warren 1973). The problem with such accounts is that they appear to be either under- or over-inclusive, depending on which capacities are invoked. For example, if one requires self-consciousness or the ability to reason as an essential condition for personhood, most very young infants will not be able to satisfy this condition. On the other hand, if sentience is regarded as the touchstone of the right to life, then nonhuman animals also possess this right.

Those who deny that embryos have the same moral status as persons might maintain that the embryo is simply too nascent a form of human life to merit the kind of respect accorded more developed humans. However, some would argue that, in the absence of an event that decisively (i.e., to everyone's satisfaction) identifies the first stage of human development—a stage at which destroying human life is morally wrong—it is not permissible to destroy embryos.

The fundamental argument of those who oppose the destruction of human embryos is that these embryos are human beings and, as such, have a right to life. The very humanness of the embryo is thus thought to confer the moral status of a person. The problem is that, for some, the premise that all human lives at any stage of their development are persons in the moral sense is not self-evident. Indeed, some believe that the premise conflates two categories of human beings: namely, beings that belong to the species *homo sapiens*, and beings that

belong to a particular moral community (Warren 1973). According to this view, the fact that an individual is a member of the species *homo sapiens* is not sufficient to confer upon it membership in the moral community of persons. Although it is not clear that those who advance this view are able to establish the point at which, if ever, embryos first acquire the moral status of persons, those who oppose the destruction of embryos likewise fail to establish, in a convincing manner, why society should ascribe the status of persons to human embryos.

It is not surprising that these different views on the moral status of the embryo appear difficult to resolve, given their relationship to the issues surrounding the abortion debate, a debate the philosopher Alastair MacIntyre describes as interminable: "I do not mean by this just that such debates go on and on and on—although they do—but also that they can apparently find no terminus. There seems to be no rational way of securing moral agreement in our culture" (1984, 6). This difficulty has led most concerned observers to search for a position that respects the moral integrity of different perspectives, but to the extent possible, focuses public policy on ethical values that may be broadly shared.

The Importance of Shared Views

Once again, we are aware that the issue of the moral status of the embryo has occupied the thoughtful attention of previous bodies deliberating about fetal tissue and embryo research.[9] Further, as already noted, we do not presume to be in a position to settle this debate, but instead have aimed to develop public policy recommendations regarding research involving the derivation and use of ES cells that are formulated in terms that people who hold differing views on the status of the embryo can accept. As Thomas Nagel argues, "In a democracy, the aim of procedures of decision should be to secure results that can be acknowledged as legitimate by as wide a portion of the citizenry as possible" (1995, 212). In this vein, Amy Gutmann and Dennis Thompson argue that the construction of public policy on morally controversial matters should involve a "search for significant points of convergence between one's own understandings and those of citizens whose positions, taken in their more comprehensive forms, one must reject" (1996, 85).

R. Alta Charo suggests an approach for informing policy in this area that seeks to accommodate the interests of individuals who hold conflicting views on the status of the embryo. Charo argues that the issue of moral status can be avoided altogether by addressing the proper limits of embryo research in terms of political philosophy rather than moral philosophy:

> The political analysis entails a change in focus, away from the embryo and the research and toward an ethical balance between the interests of those who oppose destroying embryos in research and those who stand to benefit from the research findings. Thus, the deeper the degree of offense to opponents and the weaker the opportunity for resorting to the political system to impose their vision, the more compelling the benefits must be to justify the funding (1995, 20).

In Charo's view, once one recognizes that the substantive conflict among fundamental values surrounding embryo research cannot be resolved in a manner that will satisfy all sides, the most promising approach is to seek to balance all the relevant considerations in determining whether to proceed with the research. Thus, although it is clear that embryo research would offend some people deeply, she would argue that the potential health benefits for this and future generations outweigh the pain experienced by opponents of the research.

It is, however, questionable whether Charo's analysis successfully avoids the issue of moral status. It might be argued, for example, that placing the lives of embryos in this kind of utilitarian calculus will seem appropriate only to those who presuppose that embryos do not have the status of persons. Those who believe—or who genuinely allow for the possibility—that embryos have the status of persons will regard such consequentialist grounds for destroying embryos as extremely problematic.

In our view, an appropriate approach to public policy in this arena is to develop policies that demonstrate respect for all reasonable alternative points of view and that focus, when possible, on the shared fundamental values that these divergent opinions, in their own ways, seek to affirm. This particular perspective was recommended by Patricia King in her testimony before the Commission and elsewhere (1997).[10] As long as the

disagreement is cast strictly as one between those who think the embryo is a person with a right to life and those who think it has little or no moral status, the quest for convergence will be an elusive one. But there are grounds for supposing that this may be a misleading depiction of the conflict. Indeed, there may be a sufficiently broad consensus regarding the respect to be accorded to embryos to justify, under certain conditions, not only the research use of stem cells but also the use of embryos remaining after infertility treatments to generate ES cells.

The abortion debate offers an illustration of the complex middle ground that might be found in ethically and politically contentious areas of public policy. Philosopher Ronald Dworkin maintains that, despite their rhetoric, many who oppose abortion do not actually believe that the fetus is a person with a right to life. This is revealed, he claims, through a consideration of the exceptions that they often permit to their proposed prohibitions on abortion.

For example, some hold that abortion is morally permissible when a pregnancy is the result of rape or incest. Yet, as Dworkin comments, "[i]t would be contradictory to insist that a fetus has a right to live that is strong enough to justify prohibiting abortion even when childbirth would ruin a mother's or a family's life, but that ceases to exist when the pregnancy is the result of a sexual crime of which the fetus is, of course, wholly innocent" (1994, 32).

The importance of reflecting on the meaning of such exceptions in the context of the research uses of embryos is that they suggest that even in an area of great moral controversy it may be possible to identify some common ground. If it is possible to find common ground in the case of elective abortions, we might be able to identify when it would be permissible in the case of destroying embryos. For example, conservatives allow such exceptions implicitly hold with liberals that very early forms of human life may sometimes be sacrificed to promote the interests of other humans.[11] Although liberals and conservatives disagree about the range of ends for which embryonic or fetal life may ethically be sacrificed, they may be able to reach some consensus. Conservatives who accept that destroying a fetus is permissible when necessary to save a pregnant woman or spare a rape victim additional trauma might agree with liberals that it also is permissible to destroy embryos when it is necessary to save lives or prevent extreme suffering. We recognize, of course, that these cases are different, as the existence of the fetus may directly conflict with the pregnant woman's interests, while a particular *ex utero* embryo does not threaten anyone's interests. But this distinction obscures the fact that these two cases share an implicit attribution of greater value to the interests of children and adults.

We believe that the following would seem to be a reasonable statement of the kind of agreement that could be possible on this issue:

> Research that involves the destruction of embryos remaining after infertility treatments is permissible when there is good reason to believe that this destruction is necessary to develop cures for life-threatening or severely debilitating diseases and when appropriate protections and oversight are in place in order to prevent abuse.

Given the great promise of ES cell research for saving lives and alleviating suffering, such a statement would appear to be sufficient to permit, at least in certain cases, not only the use of ES cells in research, but also the use of certain embryos to generate ES cells. Some might object, however, that the benefits of the research are too uncertain to justify a comparison with the conditions under which one might make an exception to permit abortion. But the lower probability of benefits from research uses of embryos is balanced by a much higher ratio of potential lives saved relative to embryonic lives lost and by two other characteristics of the embryos used to derive ES cells: first, that they are at a much earlier stage of development than is usually true of aborted fetuses, and second, that they are about to be discarded after infertility treatment and thus have no prospect for survival even if they are not used in deriving ES cells. In our view, the potential benefits of the research outweigh the harms to embryos that are destroyed in the research process.

Another objection is that the availability of alternative means of obtaining (and sources of) stem cells makes it unnecessary to use embryos to obtain ES cells for research. Richard Doerflinger of the National Conference

of Catholic Bishops testified before the Commission that "it is now clearer than ever that new research involving adult stem cells…offers the promise that embryonic stem cells may simply be irrelevant to future medical progress."[12] In our judgment, the derivation of stem cells from embryos remaining following infertility treatments is justifiable only if no less morally problematic alternatives are available for advancing the research. But as we have noted, ES cells from embryos appear to be different in scientifically important ways from AS cells and also appear to offer greater promise of therapeutic breakthroughs. The claim that there are alternatives to using stem cells derived from embryos is not, at the present time, supported scientifically. We recognize, however, that this is a matter that must be revisited continually as science advances.

Nevertheless, if research is to proceed with the derivation and use of ES cells from embryos that remain following infertility treatments, we must consider what kinds of conditions and constraints should apply to this work. Many of these conditions, discussed below, also are reflected in our recommendations that are provided in the next chapter.

First, ideally, those who have the authority to decide about the disposition of the remaining embryos should make the decision about whether to donate them to another couple, to continue to store them, or to destroy them before they are asked about donating them for research. This will reduce the likelihood that a desire to benefit research will lead to a choice to destroy the embryos. If the decision to destroy the embryos precedes the decision to donate them for research purposes, then the research use of such embryos affects only how, not whether, the destruction occurs. Obviously, this separation may not be possible, particularly because the couple may be given several options simultaneously, either at the outset of treatment for infertility or after its completion. Indeed, some infertility programs provide patients with multiple consent forms at the outset of treatment, forms that include options to donate to research, discard, or transfer any embryos that remain. But even then, it may be appropriate to view the options as consisting of donation of the embryos to another couple, their continued storage, or their destruction, with destruction of the embryos taking one of two forms—discarding them through thawing or through the process of research. If embryo destruction is permissible, then it certainly should be permissible to destroy them in a way that would generate stem cells for bona fide research.

Second, the couple's or the individual's decision to donate any remaining embryos for research should be a voluntary one, free from coercion and undue pressure. Third, donors of embryos for research should not be allowed to designate or restrict the recipients of derivative tissues or cell lines for research or therapy. Fourth, even though it is legal to sell sperm and ova, it should remain illegal to sell embryos; the demonstration of respect for embryos requires this prohibition. Fifth, only the minimum number of embryos that are needed to derive sufficient stem cells for important research should be used in this way.

Sixth, it is important to develop and widely disseminate additional professional standards of practice in reproductive medicine that will reduce the likelihood that infertility clinics will increase the numbers of embryos remaining after infertility treatments in order to increase the supply for possible research purposes. These standards could address issues such as the production of embryos, the number of embryos implanted and allowed to develop to term, and the care and handling of gametes and embryos.

Seventh, any research use of embryos or embryonic cell lines imported from outside of the country must satisfy all the regulations for the use of such materials when they are produced in the United States. Eighth, if possible, institutions, researchers, and potential recipients of therapies should be informed in some way about the source of the stem cells—perhaps by tagging the cells—so that all concerned can avoid using any cells that are believed to have been derived unethically. This last condition is intended to enable institutions, researchers, and patients to make their own conscientious choices about the acceptability of using stem cells that have been derived from ethically controversial sources.

Ethical Distinctions and Relationships Between the Derivation and Use of ES Cells Derived from Embryos Remaining After Infertility Treatments

There is significant debate regarding whether the *use* of cultures of ES cells should be regarded or treated differently from the *derivation* of such cells, given that the derivation arises from the destruction of an intact embryo. For purposes of this report, three questions will help frame this issue: First, are derivation and use ethically distinct? Second, is the use of ES cells, their derivation, or both ethically justifiable? Third, should use, derivation, or both be eligible for public funding? Here we discuss our views on the first two questions. Later in this chapter, we discuss the third question in more detail.

Even though many individuals would want to avoid the use of ES cells because of their source, the processes of derivation and use are sufficiently different to warrant being regarded as morally distinct from one another. The NIH Human Embryo Research Panel reached this conclusion as well (1994). Moreover, we heard testimony that would support this distinction.[13] However, there is vigorous debate regarding whether this distinction, even if morally relevant, is morally decisive or determinative for judgments about particular actions and public policies.

As previously discussed, most moral concerns about the derivation of ES cells from embryos that remain after infertility treatments focus on the fact that derivation involves destruction. If embryos could be destroyed by allowing them to thaw—the standard approach to discarding them—and researchers could then derive ES cells, the moral issues would be parallel to those that arise in the derivation of germ cells from aborted fetuses. Destruction and derivation could be separated in principle as well as through various practical measures. However, in practice, destruction and derivation cannot be separated; therefore, this option is not available. The question, then, is whether the use of ES cells derived in a process that destroys the embryos can be morally separated from that of derivation.

There are several possible responses. One position holds that such use is morally unacceptable because it necessarily involves association with the wrongful act of embryo destruction. Another position is that the problem of associating the use of the cells and the destruction of the embryo disappears if the destruction of the embryo is not viewed as problematic, as some traditions hold. There is no association with wrongdoing if the initial act is not on balance wrong. A third position holds that even if embryo destruction is viewed as morally wrong, there still may be ways to separate at least some uses from derivation. John Robertson suggests that there may be some circumstances in which researchers using ES cells would not be considered complicit with the destruction of embryos. He indicates, for example, that there would be no meaningful association where an investigator's "research plans or actions had no effect on whether the original immoral derivation occurred" (1999, 113).

Some commentators hold that it would be ethically justifiable, though regrettable, to use existing cell lines that were derived through unethical embryo destruction. A version of this position was suggested by Father Demetrios Demopulos, who explained his views from the perspective of Eastern Orthodoxy in testimony before the Commission:

> …I cannot condone any procedure that threatens viability, dignity, and sanctity of that life. In my view the establishment of embryonic stem cell lines…was done at the cost of human lives. Even though not yet a human person, an embryo should not be used for or sacrificed in experimentation, no matter how noble the goal may seem.[14]

Yet, in response to a Commissioner's inquiry about whether it might still be permissible to use existing ES cell lines, Demopulos stated:

> In my opinion, yes, since the lines exist and they have some benefit. I wish they had not been derived in the way that they were but since they are there….I do not think it would be a good thing to not take advantage of [their availability].[15]

In our reflections on both the distinction and relationship between derivation and use, especially for purposes of determining ethically acceptable public policy, we were influenced by testimony that stressed how important it is for public policy to be clear and to be justified in terms that are widely understood. Individuals representing widely differing views about the moral

status of the embryo and the moral justifiability of embryo destruction offered similar testimony. For example, Gilbert Meilaender called for the Commission to avoid misleading and even deceptive language in its statement and justification of public policies, whatever those policies turned out to be, on the grounds that misleading language would be a disservice to public discourse.[16] While affirming a different view regarding the moral status of embryos and embryo destruction, Dena Davis made a similar point by stressing that public policy and its rationale should pass the "straight-face test," a test failed, in her judgment, by an interpretation of federal law that permits federal funding of research using stem cells while denying federal funding of research that involves deriving the cells themselves. According to Davis, "it is disrespectful to suggest that those who believe that human embryos are persons look the other way when embryos are destroyed to obtain stem cells as long as public funding only kicks in once the stem cells are derived." Moreover, she argued that it is "more respectful, both of individuals opposed to the research and the public discourse generally, to be explicit about what is going on here and to acknowledge the ethical if not legal linkage between embryo destruction and the deriving of stem cells."[17]

The legal opinion rendered by the Department of Health and Human Services distinguishes the current legality of providing federal funds for the downstream use of ES cells from the legality of providing funds for the derivation of these cells. Indeed, as noted in Chapter 3, our own independent legal analysis reached a similar conclusion.[18] However, because our report focuses on the ethical issues involved in human ES and EG cell research, we find that there is no inconsistency between accepting this legal analysis and, at the same time, concluding that research involving both the derivation and use of these cells can under certain circumstances be justified ethically and that federal funds should be provided for both. We examine the ethical arguments for and against funding both derivation and use after we consider another possible source of stem cells–embryos that are created solely for research.

Research with ES Cells Derived from Embryos Created Solely for Research

Ever since the NIH Human Embryo Research Panel recommended that under certain conditions embryos could be created solely for research purposes (1994), there has been an ongoing discussion about the ethical and scientific merit of such a practice. Following is a discussion of this issue as it relates to two sources of ES cells derived from embryos that are created solely for research purposes.

Embryos Created Using IVF Procedures

There are two significant arguments in favor of creating human embryos using IVF technologies solely for stem cell research: The first is that there may be an inadequate supply of embryos remaining after infertility treatments. The second is that important research that could be of great medical benefit cannot be undertaken except with well-defined embryos that are created specifically for research and/or medical purposes. However, recommending federal funding for research using or deriving ES cells from embryos expressly created for research purposes presents two ethical problems. First, unlike in the case of embryos that remain following infertility treatments, there does not appear to be sufficient societal agreement on the moral acceptability of this practice at this time. Second, it is unclear whether an adequate supply of ES cells from embryos is available to meet scientific need or whether specialized cells are needed. We do not, at this time, support the federal sponsorship of research involving the creation of embryos solely for research purposes. However, we recognize that, in the future, scientific evidence and public support for this type of stem cell research may be sufficient in order to proceed. Therefore, to promote ongoing dialogue on this topic, we offer the following discussion.[19]

The "Discarded-Created" Distinction: On the Importance of Intentions

Various parties have discussed whether there is a moral difference between conducting research on embryos created with the intention of using them for reproduction and conducting research on embryos created with the intention of using them for research (Annas,

Caplan, and Elias 1996; Capron 1999; Davis 1995; Edwards 1990). Embryos created with the intention of using them for reproduction become available for research only when it is known that they are no longer intended to be used for infertility treatments; only then are they considered discarded, and only then do they become potentially available for research. The second group of embryos—research embryos—are those that are created without the intention that they will be used for procreative purposes. Rather, they are developed solely for research purposes or to generate research and medical materials such as stem cells or other cell lines, clones, DNA sequences, or proteins.

For some observers, it is difficult to defend an ethical distinction between what one can do with an embryo that has been created solely for research purposes and what one can do with an embryo remaining from infertility treatments (Davis 1995). For others, conducting research on embryos that were originally created for reproduction but which were then discarded is far easier to justify than is research conducted on embryos that were originally created for research (Harris 1998).

An ethical intuition that seems to motivate the "discarded-created" distinction is that the act of creating an embryo for reproduction is respectful in a way that is commensurate with the moral status of embryos, while the act of creating an embryo for research is not. Embryos that are discarded following the completion of IVF treatments were presumably created by individuals who had the primary intention of implanting them for reproductive purposes. These individuals did not consider the destruction of these embryos until it was determined that they were no longer needed. By contrast, research embryos are created for use in research and, in the case of stem cell research, their destruction in the process of research. Hence, one motivation that encourages serious consideration of the "discarded-created" distinction is a concern about instrumentalization—treating the embryo as a mere object—a practice that may increasingly lead us to think of embryos generally as means to our ends rather than as ends in themselves.

The Use of SCNT to Produce ES Cells

Somatic cell nuclear transfer of a diploid nucleus into an oocyte also has been suggested as a method to generate embryos from which ES cells could be derived. If successful, tissues derived from such cells could be useful in avoiding graft rejection if the donor nucleus were taken from the eventual transplant recipient. Although fertilization of an egg with sperm *in vitro* clearly results in a human zygote that will divide to become an embryo and has the potential to develop into a human if implanted, it is less clear whether the embryo created through SCNT has that potential. Nevertheless, the fact that this technique can produce living animals such as sheep and cows strongly suggests that it is likely that the cell that results from insertion of an adult nucleus into an oocyte is a zygote and can become an embryo.

Some have argued, however, that it is not clear that a zygote produced in this manner is similar to an embryo created by fertilization, because there are significant differences in the ability to generate different animals using these techniques, and we do not understand the potential of the human cell in this context. Because it is unclear whether SCNT works equally well in all species, we do not yet know whether this technique works in humans. Currently, therefore, we are uncertain whether cells created using SCNT have the full potential to become human. Because of previous work showing the potential of SCNT to create an animal in some situations, many would argue that similar concerns about the creation of embryos for research purposes apply to embryos created by SCNT. Thus, because of moral concerns outlined above regarding the creation of life only for research purposes, this category of research is disturbing to some. In the future, however, research may define conditions under which SCNT can be carried out while culturing the cells in such a manner that the resulting cell is directed to immediately differentiate into a specific tissue, precluding further development into an embryo. Perhaps in the future, then, it will be possible to use SCNT without the creation of an embryo.

One major distinction between IVF and SCNT embryos is that while creation of embryos by IVF would only generate more embryos, generation of embryos by SCNT would generate a specific kind of cell that might be useful in treating disease by allowing autologous transplant of a specific tissue type. Thus, in balancing the moral concern over the creation of an embryo and the value to society of the SCNT embryo, the potential therapeutic uses of the resulting ES cells from SCNT embryos must be evaluated carefully. At the present time, insufficient scientific evidence exists to evaluate this potential; however, within the next several years, such information should become more abundant. We recognize that if our

recommendations are accepted, the most likely way that this information will accumulate is through research carried out in the private sector.

We are aware, however, that if the use of SCNT to create embryos for research purposes were deemed to be both scientifically and medically necessary, other ethical issues still would need to be addressed. For example, we would need to revisit the current prohibition on designating a recipient of fetal or embryonic tissue, in light of the likelihood that this would be an important motivator for producing such embryos.

The Arguments Relating to Federal Funding of Research Involving the Derivation and/or Use of ES and EG Cells

This chapter has described several issues that arise when considering the ethical acceptability of stem cell research, depending on the source of the ES or EG cells. These issues are not unique to the source of funding, however, as they could apply equally to stem cell research conducted in either the private or public sector. Because our main interest is in providing advice and guidance regarding the federal government's role in funding research that involves the derivation and/or use of ES and EG cells, we now turn to an examination of arguments both for and against such funding.

Arguments Against Federal Funding of Certain Types of Human Stem Cell Research

In our deliberations, we considered three major arguments against federal funding of certain types of stem cell research: its association with abortion and embryo destruction, objections by some citizens to having federal funds used for research they consider to be objectionable, and the possibility that federal funds could be used for research using AS cells rather than ES or EG cells. Each argument is briefly considered below.

Association with Abortion and Embryo Destruction

As discussed earlier, research in this area is controversial in part because of the belief, held by some, that there is a direct or indirect association with abortion. For those who hold this belief, federal funding of research that derives EG cells from cadaveric fetal tissue after elective abortion also would involve moral association with the act of abortion.[20] Similarly, federal funding for the use of embryos remaining after infertility treatments to obtain ES cells would involve the federal government in deliberately destroying biologically human entities.

Federal Funding for ES and/or EG Cell Research Violates the Deeply Held Moral Beliefs of Some Citizens

By funding research of this kind, opponents argue that the federal government is violating the beliefs of some citizens, including the belief that they should not be required to subsidize a practice they consider to be morally objectionable. If it is possible to achieve essentially the same legitimate public goals with a policy that does not offend some citizens' sincere moral sensibilities, it would be better to do so. Sometimes, the federal government decides not to support an activity because it would be offensive to many people and because the benefits lost from this support are minimal, either because the activity is of only marginal value or because other sponsors will ensure that a worthwhile activity receives the support it needs. Not infrequently, however, activities that produce valuable results and that are legitimate objects of government funding receive such support despite the objections raised by some taxpayers. Providing such support does not violate democratic principles or infringe on the rights of dissent of those in the minority. Of course, the existence of such strongly held dissenting views makes more necessary a careful assessment of the arguments in favor of government support of the activity.

Funding Alternative Sources of Stem Cell Research Is Morally Preferable

The Commission has considered the argument that a targeted and vigorous program that aims to develop alternative sources of human stem cells could discover ways to achieve the same therapeutic goals with the use of ethically less controversial means. As noted above and in Chapter 2, research on AS cells is still developing and should be encouraged, but on scientific grounds there is good reason to believe that ES cells will provide a more reliable source of cells that can differentiate into a variety of tissues. It also should be noted that the harvesting of AS cells is technically difficult and risky to human beings. For some types of adult cells, such as bone marrow cells,

a certain amount of pain and discomfort is involved. For other types of stem cells, such as neuronal cells from the brain, there are significant risks to the donor from the brain biopsy procedure.

Although these objections to federal funding are important, they are not decisive. Regarding the objection based on association with wrongdoing, this report joins others in supporting various safeguards in the context of abortion in order to avoid any direct causal responsibility, to reduce the likelihood of any indirect causal responsibility, and to blunt symbolic association. Our report also proposes safeguards to prevent inappropriate and unnecessary use of embryos that remain following IVF. Regarding the second objection—avoiding offense to those who are morally opposed to using embryos for this purpose—we believe that public policy should avoid such offense in cases where the costs are not great, and we propose ways in which to reduce such offense. However, in this area of moral controversy, we believe that the arguments in favor of federal funding outweigh the offense that federal funding would create for some. Finally, we agree that alternative sources of stem cells should be sought when possible and that federal funds should be allocated to finding those sources. However, at the same time, we believe that on balance the ethical and scientific arguments support pursuing important research with EG cells obtained from cadaveric fetal tissue, with ES cells from embryos remaining after infertility treatments, and with other promising alternative sources. We now turn to additional arguments that lead us to support federal funding for certain types of ES and EG cell research.

Arguments in Favor of Federal Funding for Certain Types of Stem Cell Research

One of the principal ethical justifications for public sponsorship of research with human ES and EG cells is the same as for all biomedical and behavioral research in this country: Such research has the potential to produce health benefits for individuals suffering from disease. Many of the potential benefits of research using human ES or EG cells are discussed in Chapter 2.

The appeal to the potential benefits of stem cell research provides strong moral grounds for federal support of such research, but these potential benefits are not necessarily sufficient to justify this support. The pursuit of social benefit is always subject to moral constraint. Concerns for justice and respect for the rights of individuals can trump the morally laudable pursuit of potential benefits. Such concerns also may justify additional constraints on public funding of research.

The Enhancement of Scientific Progress Through Federal Support of the Derivation of ES Cells

Although ES cell lines already exist from studies conducted in the past year, relying upon these lines or upon the few other cell lines that might be derived by private companies for basic research on human stem cells could severely limit progress in this area of science. As discussed in Chapter 2, the potential to realize the possible medical benefits of ES cells depends on additional research into the nature and properties of ES cells. There are three main scientific reasons why it is beneficial for a broader segment of the scientific community to conduct research that involves both the derivation and use of ES cells.

First, there is great scientific value in understanding the process of ES cell derivation. Basic scientists who are interested in fundamental cellular processes are likely to make important discoveries about the nature of ES cells as they derive them in the laboratory. Moreover, by funding both derivation and use, under appropriate circumstances, federally funded researchers will be able to take advantage of the knowledge that arises from a detailed understanding of the source of the materials and the methods of derivation. Experience with animal studies indicates that research that involves both the derivation and use of particular cell lines has the greatest probability of generating promising new results.

Second, the properties of ES cells differ depending upon the conditions that were used to derive them. Moreover, the conditions for derivation of human ES cells that will differentiate into all tissue types are not yet fully understood by researchers. It is clear that the conditions used for mouse ES cells do not translate directly when using cells from other mammals. There is a significant amount of basic research that needs to be done regarding the process of ES cell derivation before the benefits from cell-based therapies can be realized.

Third, ES cells in culture are not stable indefinitely. As the cells are grown in culture, irreversible changes occur in their genetic makeup. Thus, especially in the first few

years of human ES cell research, it is important to repeatedly derive ES cells to be sure that the properties of the cells that are being studied have not changed.

The Benefits of Encouraging Both Public and Private Support for ES and EG Cell Research

We anticipate that in order for stem cell research to proceed most effectively, it will require an environment in which both public and private funding will be available. Indeed, in his testimony before the Commission, David Blumenthal suggested that "since prohibition of federal funding of stem cell research will result in reliance on private companies to support almost all the investigation utilizing stem cells, the differences between industrially funded and publicly funded university investigation are pertinent to your [deliberations]."[21] Increasingly, research is being supported and conducted by industry. Support for biomedical research and development from private sector pharmaceutical and biotechnology companies now outstrips the funding from all federal sources for this research, and it is likely that the field will continue to develop even if no federal funding is forthcoming. The drug industry recently estimated that $24 billion will be spent on drug research and development in 1999, up from $2 billion in 1980 (PhRMA 1998). In light of this, some might question whether federal funding for the derivation and use of ES cells from embryos remaining from infertility treatments is necessary for future progress in this field.

We believe that a combination of federal and private sector funding is more likely to produce rapid progress in this field than would private sector funding alone. An entire cadre of researchers is likely to be drawn into this field of research through the establishment of a federal funding program. Perhaps an analogy with the field of higher education is useful. It would be possible for all college and university education in the United States to be offered solely by privately funded colleges or universities. However, the combination of publicly and privately funded schools allows the higher education system as a whole to capitalize on the unique strengths of each type of institution. Competing, yet often working together, the two types of institutions may be able to achieve levels of excellence that neither type could achieve by itself.

Synergy from a Combined Federal Effort for Research Involving Use and Derivation

Federal funding provides the opportunity for collaboration and coordination among a much larger group of researchers. Moreover, the availability of federal funding would likely increase greatly the number of scientists carrying out ES and EG cell research and thus increase the chance of important findings. Federal support for research will encourage basic research on the biology of stem cells, in addition to the product-oriented research typically supported by biotechnology firms that are focused on developing marketable products. However, in the long run, advances in the basic biology of stem cells—for example, increased understanding of the conditions and signals that lead stem cells to differentiate or of the detailed mechanisms of differentiation—are essential for therapeutic advances. Such basic research will require long-term efforts, which traditionally have been supported by NIH.*

*Commissioner Capron makes the following observations: "As described in Chapter 3 and mentioned earlier in this chapter, NIH, relying on the opinion of the General Counsel of DHHS, has concluded that the present rider to the Department's appropriation allows the funding of research using but not deriving ES cells from embryos because the latter would involve destroying embryos for research purposes. The alternative policy urged in this report would, in addition to its scientific benefits, also enable the federal government to play a stronger role in ensuring that ethically acceptable processes are used in deriving the ES cells that federally supported scientists use in their research. Specifically, adopting a limited exception to the funding ban solely to allow support of ES cell line derivation from embryos donated from fertility programs provides a stronger platform for the federal government to enforce the distinction between research using this group of embryos and that which would use embryos created solely for research purposes.

Of course, even if NIH funds only 'use' research, it could still try to require that the ES cells used not be derived from embryos created for research purposes. But its moral leverage is undermined by its own rationale: By insisting that federal funding of research using human ES cells does not implicate federal sponsors in the process by which the ES cells have been derived, it limits its ability to mandate that one process rather than another be used. Plainly, federal law could restrict federal support to activities that do not, for example, cause unlawful pollution; by extension, the limitation could extend to activities that do not purchase materials that were produced in processes that pollute. In the present case, however, the appropriations rider bans federal support for research that creates or destroys human embryos, which means that a federal agency cannot claim to be implementing federal policy were it to limit funding to research that uses only those ES cells that were derived from discarded embryos but not from embryos created for the purpose of deriving ES cells. Thus, NIH may be hard pressed to justify differentiation based on the type of embryos from which ES cells are derived, thereby losing an opportunity to oversee the derivation process directly and to enforce an important ethical distinction.

Adopting a limited exception to the embryo research ban solely for research to derive ES cells from embryos remaining from fertility programs would also avoid relying on the theoretical line between derivation and use research that underlies the NIH policy. Such a line is difficult to defend in practical terms when the question is not whether an activity is inherently licit or illicit but whether it ought to be paid for with federal research dollars. Any such line is merely theoretical because the funding provided for research using ES cells would of course flow directly to researchers deriving those cells, perhaps even in an adjacent laboratory. The only difference would be that the federal funds would not go directly as salary and laboratory expenses for the derivation process but indirectly in the form of funds to purchase the ES cells (which funds would then pay salaries, laboratory expenses, and so forth)."

Requiring That Recipients Conduct Their Research in Accordance with the Federal Regulations

As with all federally sponsored research, conditions attached to funding provide the federal government with the authority to require compliance with relevant regulations, policies, and guidelines. Among these regulations are those pertaining to human subjects research, tissue donation and transplantation, oversight, and review. In addition, federal funding agencies can stipulate that recipients of federal funding for human stem cell research must share both research results and research materials (including cell lines) with other recipients of federal funds or with all other researchers. Thus, federal funding may lead to more widespread dissemination of findings and sharing of materials, which ultimately may enhance scientific discoveries.

In contrast, many privately funded studies require that the scientists not distribute their findings until after a review by the company and that materials can be shared only after the institution receiving the materials has signed a material transfer agreement. Some of these agreements make it difficult for scientists to share or secure the reagents necessary for their research, even if they wish to do so. As the Institute of Medicine noted in its report, *Resource Sharing in Biomedical Research*, "The perception that scientific data and research materials (e.g., animals, reagents, etc.) have potential commercial value frequently causes universities to be even more reluctant than individual scientists with respect to sharing" (1996, 81).

Sustaining U.S. Leadership in Science and Technology

In supporting federal funding for certain types of stem cell research, we are not opposing research in the private sector. On the contrary, we recognize the value for the nation's investment in science and technology for research sponsored and conducted by both the public and the private sectors and the quality of private sector research. Indeed, stem cell research is receiving, and probably will continue to receive, increasing support from industry. There are, however, certain specific advantages that arise from the federal investment in science that should be acknowledged. An observation made by the Office of Technology Assessment, in its 1986 report, *Research Funding as an Investment*, is relevant in this context.

> The goal of federally funded research is not profitability, but a means of achieving social objectives, whether they are health, national security, or the enhancement of knowledge and education. The Federal research infrastructure is designed to provide a stable environment for these goals, despite a changing political environment….In addition, Federal research programs must be responsive to many more groups than industrial research efforts, and this affects the manner in which the research agenda is shaped. (1986, 61)

Federal funding is probably required in order for the United States to sustain a leadership position in this increasingly important area of research. By funding research, the federal government conveys the clear message that, under particular conditions and constraints, certain types of human stem cell research can be morally legitimate research that is worthy of public support.

Just Distribution of Potential Benefits from Stem Cell Research

Much of the testimony we heard indicated that the just distribution of the benefits of stem cell research, including both the knowledge gained and any potential therapeutic benefits, should be taken into account in any recommendation that would permit the federal government to support ES and EG cell research. For example, there was widespread agreement among the religious scholars who testified before us that in order for this research to be morally acceptable, several "background factors" must be in place, including equitable access to the benefits of the research and appropriate prioritization of this research relative to other social needs, both of which involve procedural and substantive justice. (See Appendix E.)

Issues of procedural and substantive justice are not unique to stem cell research but rather arise in various societal decisions about the use of funds for research, medical care, and other goods. Although we can note these issues here, we cannot resolve them. In addition, federal funding of stem cell research does not guarantee

that greater numbers of the American public will have access to the fruits of basic or applied research or that this will occur more quickly than it would if federal funding were not available. However, by recommending federal funding for certain types of human stem cell research, we acknowledge that there is a basis for an argument for broader access to any therapies developed from that research.

Ethical Issues in Adopting Federal Oversight and Review Policies for ES and EG Cell Research

Concerns have been expressed regarding the likelihood of accountability depending on whether ES and EG cell research is sponsored and/or conducted by the public or private sector. Arthur Caplan, a bioethicist at the University of Pennsylvania, in testimony before the Senate Subcommittee on Labor, Health and Human Services, Education and Related Agencies, said that

> …it is better to do things in this area that are accountable and public, than it is to ask them to become private and commercial. And if we continue the policies we have, we're not going to be able to bring the nuanced supervision and oversight that this area of stem cell research requires from us….That's why we need public funding, public accountability, to make the right tradeoffs.[22]

One of the principal benefits of federal funding of biomedical and behavioral research is that it is relatively easy to put in place an effective system of public oversight and review. By oversight, we are referring to the mechanism of monitoring categories of research or other activities to determine compliance with policies, procedures, rules, guidelines, and regulations and to prevent abuses. It is a policy strategy designed to provide the appropriate checks and balances and ensure ethically acceptable research protocols. The existing federal system of oversight has its origins both in the legislative and executive branches of the federal government: Congress, through its appropriations authority, may (and often does) direct that certain research be undertaken or avoided. Seen in this way, federal oversight can provide the public with two assurances: first, that stem cell research will receive national attention and scrutiny through the appropriations process undertaken by Congress; and second, that stem cell research would be conducted in accordance with relevant federal regulations. These oversight components are necessary but not sufficient for providing the public with confidence that research, especially research involving human subjects, is being undertaken appropriately. There also are mechanisms maintained by individual agencies such as the Food and Drug Administration.

In contrast, review usually refers to the evaluation of individual research protocols involving human subjects to assess their scientific merit and ethical acceptability—the activity usually carried out by Institutional Review Boards. As noted above, however, some research involving human stem cells may not be considered research involving human subjects, as defined by the Common Rule. In our view, the considerable sensitivity and public concern regarding stem cell research merits both national and local approaches to oversight and review, the details of which are described in the following chapter. We are persuaded that federal oversight and review of some types of stem cell research is required in order to make federal funding available to support such research. The types of questions about ES and EG cell research that we consider important for such an oversight and review body to ask are enumerated in Appendix F.

Summary

We were asked by the President to thoroughly review the issues associated with stem cell research, "balancing all ethical and medical considerations." In this chapter, we have endeavored to do just that. Specifically, we recognized that there are many different views on the ethical appropriateness of this type of research and also on the appropriateness of providing federal funding for such research. We believe that the ethical arguments that support the use of federal funds for stem cell research using cadaveric fetal tissue and for both deriving and using ES cells from embryos remaining after infertility treatments have considerable merit. However, such research should be conducted only within the context of a framework of national oversight and review. At the same time, we were

not persuaded that we should recommend that federal funds be available at this time for the creation of embryos solely for research purposes. We arrived at these conclusions with full awareness of the strongly held views (from both religious and secular ethical perspectives) on all sides of the main issues regarding the morality of stem cell research.

Notes

1 The arguments presented here were helpfully informed by two papers prepared for the National Bioethics Advisory Commission (NBAC) by Fletcher, J.C., 1999, "Deliberating Incrementally on Human Pluripotential Stem Cell Research," and Siegel, A.W., 1999, "Locating Convergence: Ethics, Public Policy and Human Stem Cell Research." Both of these papers are available in Volume II of this report.

2 Eiseman, E., 1999, "Quick Response: Use of Human Fetal Tissue in Federally Funded Research." This paper was prepared for NBAC and is available in Volume II of this report.

3 Several terms have been used to refer to inappropriate connections between one agent's actions and another agent's wrongdoing. We have mainly used the term *association*, but other terms include *cooperation, collaboration,* and *complicity*. See, for example, Childress (1990). For a discussion of cooperation and complicity with evil in Roman Catholic moral theology, see Maguire (1986).

4 Pellegrino, E.D., Testimony before NBAC. May 7, 1999. Washington, DC. Meeting transcript, 10.

5 For a summary of these positions, see Appendix E.

6 Farley, M., Testimony before NBAC. May 7, 1999. Washington, DC. Meeting transcript, 18.

7 Dorff, E., M. Tendler, L. Zoloth, A. Sachedina. Testimony before NBAC. May 7, 1999. Washington, DC.

8 Dorff, E., Testimony before NBAC. May 7, 1999. Washington, DC. Meeting transcript, 48.

9 For a discussion of these issues see the paper prepared for NBAC by Knowles, L.P., 1999, "International Perspectives on Human Embryo and Fetal Tissue Research," available in Volume II of this report.

10 King, P.A., Testimony before NBAC. January 19, 1999. Washington, DC.

11 The terms *liberal* and *conservative* used here are used in the context intended by Dworkin (1994), *Life's Dominion*.

12 Doerflinger, R., Written testimony before NBAC. April 16, 1999. Charlottesville, VA. Meeting transcript, 1.

13 Pellegrino, E.D., Testimony before NBAC. May 7, 1999. Washington, DC.

14 Demopulos, D., Testimony before NBAC. May 7, 1999. Washington, DC. Meeting transcript, 89.

15 Ibid.

16 Meilaender, G., Testimony before NBAC. May 7, 1999, Washington, DC.

17 Davis, D., Testimony before NBAC. May 7, 1999. Washington, DC. Meeting transcript, 164.

18 This opinion was provided by Flannery, E., 1999, in "Analysis of Federal Laws Pertaining to Funding of Human Pluripotent Stem Cell Research," available in Volume II of this report.

19 For a discussion of these issues see the paper prepared for NBAC by Parens, E., 1999, "What Has the President Asked of NBAC? On the Ethics and Politics of Embryonic Stem Cell Research," available in Volume II of this report.

20 It is important to note, however, that the abortion exceptions, which serve as the basis for the type of shared views identified above, are exceptions to the law banning federal funding for abortions (Title V, Labor, HHS, and Education Appropriations, 112 Stat. 3681-385, Sec. 509 (a) (1)&(2)). Thus, federal funding for research use of cadaveric fetal tissue, within appropriate limits, might be viewed as consistent with current federal funding practices in the abortion context.

21 Blumenthal, D., Written testimony before NBAC. February 2, 1999. Princeton, NJ. Meeting transcript, 1.

22 Caplan, A.L., Testimony before the Senate Appropriations Subcommittee on Labor, Health, and Human Services, Education and Related Agencies. December 2, 1998.

References

Annas, G.J., A.L. Caplan, and S. Elias. 1996. "The Politics of Human-Embryo Research: Avoiding Ethical Gridlock." *New England Journal of Medicine* 334:1329–1332.

Bopp, J., 1994. "Fetal Tissue Transplantation and Moral Complicity with Induced Abortion." In *The Fetal Tissue Issue: Medical and Ethical Aspects*, eds. P.J. Cataldo and A. Moracszewski, 61–79. Braintree, MA: Pope John Center.

Burtchaell, J.T. 1988. "University Policy on Experimental Use of Aborted Fetal Tissue." *IRB* 10(4):7–11.

Canadian Royal Commission on New Reproductive Technologies. 1993. *Proceed with Care: Final Report of the Royal Commission on New Reproductive Technologies*. 2 vols. Ottawa: Minister of Government Services.

Capron, A.M. 1999. "Good Intentions." *Hastings Center Report* 29(2):26–27.

Charo, R.A. 1995. "The Hunting of the Snark: The Moral Status of Embryos, Right-to-Lifers, and Third World Women." *Stanford Law and Policy Review* 6:11–27.

Childress, J.F. 1990. "Disassociation from Evil: The Case of Human Fetal Tissue Transplantation Research." *Social Responsibility: Business, Journalism, Law, Medicine* 16:32–49.

———. 1991. "Ethics, Public Policy, and Human Fetal Tissue Transplantation Research." *Kennedy Institute of Ethics Journal* 1(2):93–121.

Committee of Inquiry into Human Fertilisation and Embryology. 1984. *Report of the Committee of Inquiry into Human Fertilisation and Embryology*. London: Her Majesty's Stationary Office.

Davis, D. 1995. "Embryos Created for Research Purposes." *Kennedy Institute of Ethics Journal* 5(4):343–354.

Department of Health, Education, and Welfare (DHEW). Ethics Advisory Board. 1979. *Report and Conclusions, HEW Support of Research Involving Human* In Vitro *Fertilization and Embryo Transfer*. Washington, DC: U.S. Government Printing Office.

Dworkin, R. 1994. *Life's Dominion: An Argument About Abortion, Euthanasia, and Individual Freedom*. New York: Vintage.

Edwards, R. 1990. "Ethics and Embryology: The Case for Experimentation." In *Experiments on Embryos*, eds. A. Dyson and J. Harris, 42–54. New York: Routledge.

Feinberg, J. 1986. "Abortion." In *Matters of Life and Death*, ed. T. Regan, 256–293. New York: Random House.

Freedman, B. 1992. "Moral Analysis and the Use of Nazi Experimental Results." In *When Medicine Went Mad: Bioethics and the Holocaust*, ed. A.L. Caplan, 141–154. Totowa, NJ: Humana Press.

Gutmann, A., and D. Thompson. 1996. *Democracy and Disagreement*. Cambridge, MA: Belknap Press.

Harris, J. 1998. *Clones, Genes and Immortality: Ethics and the Genetic Revolution*. New York: Oxford University Press.

Institute of Medicine (IOM). Committee on Resource Sharing in Biomedical Research. 1996. *Resource Sharing in Biomedical Research*. Washington, DC: National Academy Press.

King, P.A. 1997. "Embryo Research: The Challenge for Public Policy." *Journal of Medicine and Philosophy* 22(5):441–455.

MacIntyre, A. *After Virtue*. 1984. Notre Dame: University of Notre Dame Press.

Maguire, D.C. 1986. "Cooperation with Evil." In *Westminster Dictionary of Christian Ethics*, 2d ed., eds. J.F. Childress and J. Maquarrie. Philadelphia: Westminster Press.

Murray, T.H. 1996. *The Worth of a Child*. Berkeley: University of California Press.

Nagel, T. 1995. "Moral Epistemology." In *Society's Choices*, eds. R.E. Bulger, E.M. Bobby, and H.V. Fineberg, 201–214. Washington, DC: National Academy Press.

National Institutes of Health (NIH). 1988. *Report of the Human Fetal Tissue Transplantation Research Panel*. 2 vols. Bethesda, MD: NIH.

NIH. Human Embryo Research Panel. 1994. *Report of the Human Embryo Research Panel*. 2 vols. Bethesda, MD: NIH.

Office of Technology Assessment (OTA). 1986. *Research Funding as an Investment: Can We Measure the Returns? A Technical Memorandum*. Washington, DC: U.S. Government Printing Office.

Pharmaceutical Manufacturers and Research Association. (PhRMA). 1998. "Drug Companies Add 39 New Treatments to Nation's Medicine Chest, Increase R & D Investment to $24 Billion," www.phrma.org/charts/nda_i98.html.

Robertson, J.A. 1988. "Fetal Tissue Transplant Research Is Ethical: A Response to Burtchaell: II." *IRB* 10(6):5–8.

———. 1999. "Ethics and Policy in Embryonic Stem Cell Research." *Kennedy Institute of Ethics Journal* 9(2):109–136.

Seidelman, W.E. 1988. "Mengele Medicus: Medicine's Nazi Heritage." *The Milbank Quarterly* 66:221–239.

Tooley, M. 1983. *Abortion and Infanticide*. New York: Oxford University Press.

Vawter, D.E., W. Kearney, K.G. Gervais, A.L. Caplan, D. Garry, and C. Tauer. 1991. "The Use of Human Fetal Tissue: Scientific, Ethical and Policy Concerns." *International Journal of Bioethics* 2(3):189–196.

Warren, M.A. 1973. "On the Moral and Legal Status of Abortion." *The Monist* 57:43–61.

Chapter Five

Conclusions and Recommendations

Introduction

In November 1998, President Clinton charged the National Bioethics Advisory Commission with the task of conducting a thorough review of the issues associated with human stem cell research, balancing all ethical and medical considerations. The President's request was made in response to three separate reports that brought to the fore the exciting scientific and clinical prospects of stem cell research while also raising a series of ethical controversies regarding federal sponsorship of scientific inquiry in this area. Such research raises ethical issues because it involves the derivation of human *embryonic germ (EG) cells* from aborted fetuses or the derivation of human *embryonic stem (ES) cells* from early stage embryos remaining after infertility treatments. A number of these important ethical concerns previously have been identified in public debate, both here and abroad. The Commission reviewed these concerns in light of both the medical and scientific promise in this significant new field and the existing statutes and regulations that affect research in this area. Our task, however, was neither to engage in moral analysis for its own sake nor to address all the regulatory issues that might be raised, but rather to offer advice on how the balance of ethical, scientific, and medical considerations should shape policies on the use of federal funds to support research that involves deriving or using human ES or EG cells.

Scientific and Medical Considerations

The stem cell is a unique and essential cell type found in animals. Many kinds of stem cells are found in the human body, with some more differentiated, or committed, to a particular function than others. In other words, when stem cells divide, some of the progeny mature into cells of a specific type (heart, muscle, blood, or brain cells), while others remain stem cells, ready to repair some of the everyday wear and tear undergone by our bodies. These stem cells are capable of continually reproducing themselves and serve to renew tissue throughout an individual's life. For example, they continually regenerate the lining of the gut, revitalize skin, and produce a whole range of blood cells. Although the term *stem cell* commonly is used to refer to those cells within the adult organism that renew tissue (e.g., hematopoietic stem cells, a type of cell found in the blood), the most fundamental and extraordinary of the stem cells are found in the early stage embryo. These ES cells, unlike the more differentiated adult stem (AS) cells or other cell types, retain the special ability to develop into nearly any cell type. EG cells, which originate from the primordial reproductive cells of the developing fetus, have properties similar to ES cells.

It is the potentially unique versatility of the ES and EG cells derived, respectively, from the early stage embryo and cadaveric fetal tissue that presents such unusual scientific and therapeutic promise. Indeed, scientists have long recognized the possibility of using such cells to generate more specialized cells or tissue, which could allow the newly generated cells to be used to treat injuries or diseases such as Alzheimer's disease, Parkinson's disease, heart disease, and kidney failure. In addition, scientists regard these cells as important—perhaps essential—in understanding the earliest stages of human development and in developing life-saving drugs and cell-replacement therapies to treat disorders caused by early cell death or

impairment. At the same time, the techniques for deriving these cells have not been fully developed as standardized and readily available research tools, and the development of any therapeutic application remains some years away.

Research also is under way to determine whether human stem cells could be obtained from the differentiated stem cells of fully developed organisms. Thus far, however, studies in animals indicate that this approach faces substantial scientific and technical limitations; indeed, the anatomic source of certain cells might preclude easy or safe access in human beings. In addition, important biological differences apparently exist between ES cells, EG cells, and AS cells. Furthermore, differences among species mean that for full scientific and clinical benefits to be realized, some research will need to be conducted with human ES and EG cells, even as the emphasis remains on laboratory and animal research. In summary, research using stem cells from animals or from human adults is not a substitute for human ES and EG cell research, and it is toward the latter that we direct our ethical and policy analyses.

Ethical and Policy Considerations

The longstanding controversy about the ethics of research involving human embryos and cadaveric fetal material arises from fundamental and sharply differing moral views regarding elective abortion or the use of embryos for research. Indeed, an earnest national and international debate continues over the ethical, legal, and medical issues that arise in this arena. This debate represents both a challenge and an opportunity: a challenge because it concerns important and morally contested questions regarding the beginning of life, and an opportunity because it provides another occasion for serious public discussion about important ethical issues. We are hopeful that this dialogue will foster public understanding about the relationships between the opportunities that biomedical science offers to improve human welfare and the limits set by important ethical obligations.

Although we believe most would agree that human embryos deserve respect as a form of human life, disagreements arise regarding both what form such respect should take and what level of protection is required at different stages of embryonic development. Therefore, embryo research that is not therapeutic to the embryo is bound to raise serious concerns for some about how to resolve the tensions between two important ethical commitments: to cure disease and to protect human life. For those who believe that from the moment of conception the embryo has the moral status of a person, research (or any other activity) that would destroy the embryo is considered wrong and should be prohibited. For those who believe otherwise, arriving at an ethically acceptable policy in this arena involves a complex balancing of many important ethical concerns. Although many of the issues remain contested on moral grounds, they can exist within a broad area of consensus upon which public policy can, at least in part, be constructed.

For most observers, the resolution of these ethical and scientific issues depends to some degree upon the source of the stem cells. The use of cadaveric fetal tissue to derive EG cell lines—like other uses of tissues or organs from dead bodies—is generally the most acceptable of these sources, provided that the research complies with the system of public safeguards and oversight already in place for such scientific inquiry. With respect to embryos and the ES cells from which they can be derived, some draw an ethical distinction among three potential types of embryos. One is referred to as the *research embryo*, an embryo created through *in vitro* fertilization (IVF), with gametes provided solely for research purposes. Many people, including the President, have expressed the view that the federal government should not fund research that involves creating such embryos. The second type of embryo is that which was created for treatment of infertility, but is now intended to be discarded because it is unsuitable or no longer needed for such treatment. The use of these embryos raises fewer ethical questions because it does not alter their final disposition. Finally, the recent demonstration of cloning techniques (somatic cell nuclear transfer [SCNT]) in nonhuman animals suggests that the transfer of a human somatic cell nucleus into an oocyte might create an embryo that could be used as a source of ES cells. The creation of a human organism using this technique raises questions similar to those raised by the creation of research embryos through IVF, and at this time federal funds may not be used for such

research. In addition, if the enucleated oocyte that was to be combined with a human somatic cell nucleus came from a nonhuman animal, other issues would arise about the nature of the embryo produced. Thus, each source of material raises distinct ethical questions as well as scientific, medical, and legal ones.

Conscientious individuals have reached different conclusions regarding both public policy and private actions in the area of stem cell research. Their differing perspectives by their very nature cannot easily be bridged by any single public policy. But the development of such policy in a morally contested area is not a novel challenge for a pluralistic democracy such as that which exists in the United States. We are profoundly aware of the diverse and strongly held views on the subject of this report and has wrestled with the implications of these different views at each of our meetings devoted to this topic. Our aim throughout these deliberations has been to formulate a set of recommendations that fully reflects widely shared views and that, in our view, would serve the best interests of society.

Most states place no legal restrictions on any of the means of creating ES and EG cells that are described in this report. In addition, current Food and Drug Administration (FDA) regulations do not apply to this type of early stage research. (See Appendix D.) Therefore, because the public controversy surrounding such activities in the United States has revolved around whether it is appropriate for the federal government to sponsor such research, this report focuses on the question of whether the scientific merit and the substantive clinical promise of this research justify federal support, and, if so, with what restrictions and safeguards.

Views about the status of embryos and fetuses vary widely. Some believe that what matters is the potential for a new human life that arises at the moment of conception, while others identify the relevant concept as personhood, which they say begins only at some postembryonic stage. We heard from many members of the public, including those who are eager for this area of research to move forward as rapidly as possible, as well as those who oppose the research if it is built upon any activity that is connected to abortion or to the destruction of fertilized human eggs. In addition, our deliberations have been informed by testimony from scientists and physicians, lawyers and other experts in governmental regulation, philosophers, and Catholic, Protestant, Jewish, Islamic, and Eastern Orthodox theologians. As a result of these discussions, it has become clear that the question of whether federal policy should sponsor human ES or EG cell research is characterized by a tension between the desire to realize the great therapeutic benefits that may be derived from such work and the need to recognize that the materials involved must be treated with respect. We concluded that sufficient safeguards can be put in place in order to prevent abuse and to ensure that any use of embryos that remain after infertility treatments—like any use of fetal remains following elective abortion—is based upon and embodies the kind of respect for the embryos that most Americans would expect and demand of any activity that is carried out with the support of the federal government. Beyond the regulatory effects of the rules adopted to govern federal support for research in this area—with which we hope private sponsors of research involving ES and EG cells will comply voluntarily—the states also can influence research in this field through statutes and regulations on abortion, embryo research, and the donation of human body parts, embryos, and gametes.

Conclusions and Recommendations

The conclusions and recommendations presented in this chapter are grouped into several categories:

- the ethical acceptability of federal funding for research that either derives or uses ES or EG cells,
- the requirements for the donation of cadaveric fetal tissue and embryos for research,
- restrictions on the sale of these materials and designation of those who may benefit from their use,
- the need—and the means—for national oversight and institutional review,
- the need for local review of derivation protocols,
- the responsibilities of federal research agencies,
- the issues that must be considered regarding the private sector, and
- the need for ongoing review and assessment.

The Ethical Acceptability of Federal Funding of ES Cell and EG Cell Research

Despite the enormous scientific and clinical potential offered by research use of ES or EG cells derived from various sources, many find that certain sources are more ethically problematic than others. Our recommendations reflect respect for these diverse views, which varied even among the Commissioners, regarding the ethical acceptability of the derivation and use of ES and EG cells from various sources.

As described in Chapter 2, human ES and EG cells can be derived from the following sources:

- human fetal tissue following elective abortion (EG cells),
- human embryos that are created by IVF and that are no longer needed by couples being treated for infertility (ES cells),
- human embryos that are created by IVF with gametes donated for the sole purpose of providing research material (ES cells), and
- potentially, human (or hybrid) embryos generated asexually by SCNT or similar cloning techniques in which the nucleus of an adult human cell is introduced into an enucleated human or animal ovum (ES cells).

A principal ethical justification for public sponsorship of research with human ES or EG cells is that this research has the potential to produce health benefits for those who are suffering from serious and often fatal diseases. We recognize that it is possible that all the various sources of human ES or EG cells eventually could be important to research and clinical application because of, for example, their differing proliferation potential, differing availability and accessibility, and differing ability to be manipulated, as well as possibly significant differences in their cell biology.

Although each source of stem cells poses its own scientific, ethical, and legal challenges and opportunities, much of the ethical analysis leading to public policy recommendations depends upon the scientific and/or clinical necessity of using a specific source of the cells. In our judgment, the immediate scientific uses of ES or EG cells can be satisfied by the derivation and use of cell lines derived from fetal tissues (i.e., EG cells) and from embryos (i.e., ES cells) remaining after infertility treatments have ended.

The potential use of matched tissue for autologous cell-replacement therapy from ES cells may in the future require the use of cell lines developed by SCNT techniques. In addition, embryos created through IVF specifically as a source of ES cells might be essential for creating banks of multiple cell lines representing a spectrum of alleles for the major histocompatibility complex. This goal might require that ova and sperm of persons with specific genotypes be selected to make embryos from which to derive particular classes of ES cells.

Finally, although much promising research currently is being conducted with stem cells obtained from adult organisms, studies in animals suggest that this approach will be scientifically and technically limited, and, in some cases, the anatomic source of the cells might preclude easy or safe access. Important research can and should go forward in this area, although important biological differences exist between ES and AS cells, and the use of AS cells should not be considered an alternative to ES and EG cell research.

Much research into the generation of specific tissue types from stem cells can be conducted using EG cells derived from fetal material and ES cells derived from embryos remaining after infertility treatments. In the future, adequate scientific evidence and increased prospect for medical benefits may be available to generate public support for using human ES cells derived from embryos produced through IVF for research purposes or by SCNT for autologous transplant. We note, however, that a responsible federal science policy does not necessarily require public funding for access to all sources of ES or EG cells at once. **At this time, therefore, the Commission believes that federal funding for the use and derivation of ES and EG cells should be limited to two sources of such material: cadaveric fetal tissue and embryos remaining after infertility treatments**. Specific recommendations and their justifications are provided below.

Recommendation 1:

Research involving the derivation and use of human EG cells from cadaveric fetal tissue should continue to be eligible for federal funding. Relevant statutes and regulations should be amended to make clear that the ethical safeguards that exist for fetal tissue transplantation also

apply to the derivation and use of human EG cells for research purposes.

Considerable agreement exists, both in the United States and throughout the world, that the use of fetal tissue in therapy for those with serious disorders, such as Parkinson's disease, is acceptable.[1] Research that uses cadaveric tissue from aborted fetuses is analogous to the use of fetal tissue in transplantation. The rationales for conducting EG research are equally strong, and the arguments against it are not persuasive. The removal of fetal germ cells does not occasion the destruction of a live fetus, nor is fetal tissue intentionally or purposefully created for human stem cell research. Although abortion itself doubtless will remain a contentious issue in our society, the procedures that have been developed to prevent fetal tissue donation for therapeutic transplantation from influencing the abortion decision offer a model for creating such separation in research to derive human EG cells. Because the existing statutes are written in terms of tissue transplantation, which is not a current feature of EG cell research, changes are needed to make explicit that the relevant safeguards will apply to research to derive EG cells from aborted fetuses.

Due to the contentious and polarizing nature of the abortion debate in the United States, restrictions were enacted over a decade ago to block the use of federal funding of fetal tissue transplantation therapy research. Until 1993, the only permissible source of tissue for such research was tissue from spontaneously aborted fetuses or ectopic pregnancies—sources that were largely unsuitable for research. In 1993, President Clinton lifted the ban on the use of fetal tissue from elective abortions for fetal tissue transplantation research.

Previous moral opposition to fetal tissue transplant research, because of its association with abortion, helped shape a system of safeguards to prevent the encouragement of the practice. These rules require that the consent process for women making abortion decisions must precede separately from the consent process for donation of fetal tissue for transplant research. Although some disagree, sufficient consensus exists that society should respect the autonomous choices of women who have chosen to have legal abortions to donate fetal tissue for research. If women have a right to choose to have an abortion, it follows that the self-determination or autonomy expressed in that right extends to the choice to donate fetal tissue for research purposes.

Research using fetal tissue obtained after legal elective abortions will greatly benefit biomedical science and also will provide enormous therapeutic benefits to those suffering from various diseases and other conditions. In our view, there is no overriding reason for society to discourage or prohibit this research and thus forgo an important opportunity to benefit science and those who are suffering from illness and disease—especially in light of the legality of elective abortions that provide access to fetal tissue and of the risks involved in losing these valuable opportunities. Indeed, the consequences of forgoing the benefits of the use of fetal tissue may well be harmful. Moreover, if not used in research, this tissue will be discarded.

The Acceptability of Federal Support for Research Using Embryos Remaining After Infertility Treatments to Derive ES Cells

The current congressional ban on embryo research prohibits federal support of any research "in which a human embryo…[is] destroyed, discarded, or knowingly subjected to risk of injury greater than that allowed for research on fetuses *in utero*."[2] The term *human embryo* in the statute is defined as "any organism, that is derived by fertilization, parthenogenesis, cloning, or any other means from one or more human gametes or human diploid cells."

The ban, which concerns only federally sponsored research, reflects a moral stance either that embryos deserve some measure of protection from society because of their moral status as persons, or that sufficient public controversy exists such that federal funds should not be used for this type of research. However, some effects of the embryo research ban raise serious moral and public policy concerns for those who hold differing views of the ethics of embryo research. In our view, the ban conflicts with several of the ethical goals of medicine, especially healing, prevention, and research—goals that are rightly characterized by the principles of beneficence and nonmaleficence, jointly encouraging the pursuit of each social benefit and avoiding or ameliorating potential harm.

In the United States, moral disputes—especially those concerning certain practices in the area of human reproduction—are sometimes resolved by denying federal

funding for those practices (e.g., elective abortion), while not interfering with the practice in the private sector. In this case, investigative embryo research guided only by self-regulation is a widespread practice in the private sector, and the ban on embryo research has served to discourage the development of a coherent public policy, not only regarding embryo research but also regarding health research more generally. The ban also may have more profound effects on other areas of federally supported research that are dedicated to the relief of human suffering, raising concerns about the distribution and allocation of federal research resources. For example, by limiting the federal government's ability to fund promising areas of basic research, a complete ban on embryo research could prevent promising, collaborative studies in other areas, such as cancer and genetics. We recognize that many factors affect how federal research priorities are set in this country. However, in our view, the intentional withholding of federal funds for research that may lead to promising treatments may be considered unjust or unfair.

Although no consensus has been reached regarding the moral status of the embryo, there is agreement that if embryo research is permissible, some limitations and/or regulations are necessary and appropriate. Such regulation reflects an appreciation of the disparate views regarding the acceptability and unacceptability of this area of scientific investigation and serves as a way of providing accountability, allaying public anxiety, promoting beneficial research, and demonstrating respect for human embryos.

Recommendation 2:

Research involving the derivation and use of human ES cells from embryos remaining after infertility treatments should be eligible for federal funding. An exception should be made to the present statutory ban on federal funding of embryo research to permit federal agencies to fund research involving the derivation of human ES cells from this source under appropriate regulations that include public oversight and review. (See Recommendations 5 through 9.)

Based on advice from the Department of Health and Human Services (DHHS) General Counsel, the Director of the National Institutes of Health (NIH) announced in January 1999 that NIH will apply the ban only to research involving the derivation of ES cells from human embryos but not to research involving only the use of ES cells. NIH has indicated that research proposals that involve the use of ES cells will be considered for funding once NIH has established a set of special guidelines that are currently under development. The DHHS General Counsel concluded that ES cells are not, in themselves, organisms and hence cannot be embryos as defined by the statute. Thus, it could be surmised from this interpretation that the only activity that could amount to "research in which a human embryo or embryos are destroyed" would be an attempt to derive ES cells from living embryos. This, in fact, is the interpretation adopted by DHHS and NIH. More than 70 members of Congress have protested this interpretation, claiming that whatever the language of the statute, Congress clearly intended to prohibit not just research in which human embryos are destroyed but also research that depends on the prior destruction of a human embryo. Yet the plain meaning of the statutory wording differs from this interpretation, and nothing in its legislative history indicates that either proponents or opponents of the rider anticipated a situation in which research that destroyed the embryo would be conducted separately from research that used the cells derived from the embryo. Thus, in legal terms, the General Counsel's interpretation appears to be reasonable, even though it does not address any of the ethical concerns involved.

Although some may view the derivation and use of ES cells as ethically distinct activities, we believe that it is ethically acceptable for the federal government to finance research that both derives cell lines from embryos remaining after infertility treatments and that uses those cell lines. Although one might argue that some important research could proceed in the absence of federal funding for research that derives stem cells from embryos remaining after infertility treatments (i.e., federally funded scientists merely using cells derived with private funds), we believe that it is important that federal funding be made available for protocols that also derive such cells. Relying on cell lines that might be derived exclusively by a subset of privately funded researchers who are

interested in this area could severely limit scientific and clinical progress.

An ethical problem is presented in trying to separate research in which human ES cells are used from the process of deriving those cells, because doing so diminishes the scientific value of the activities receiving federal support. This division—under which neither biomedical researchers at NIH nor scientists at universities and other research institutions who rely on federal support could participate in some aspects of this research—rests on the mistaken notion that derivation and use can be neatly separated without affecting the expansion of scientific knowledge. We believe that this misrepresentation of the new field of human stem cell research has several implications.

First, researchers using human ES cell lines will derive substantial scientific benefits from a detailed understanding of the process of ES cell derivation, because the properties of ES cells and the methods for sustaining the cell lines may differ depending upon the conditions and methods used to derive them. Thus, scientists who conduct basic research and who are interested in fundamental cellular processes are likely to make elemental discoveries about the nature of ES cells as they derive them in the laboratory. Second, significant basic research must be conducted regarding the process of ES cell derivation before cell-based therapies can be fully realized, and this work must be pursued in a wide variety of settings, including those exclusively devoted to basic academic research. Third, ES cells are not indefinitely stable in culture. As these cells are grown, irreversible changes occur in their genetic makeup. Thus, especially in the first few years of human ES cell research, it is important to be able to repeatedly derive ES cells in order to ensure that the properties of the cells that are being studied have not changed.

Thus, anyone who believes that federal support of this important new field of research should maximize its scientific and clinical value within a system of appropriate ethical oversight should be dissatisfied with a position that allows federal agencies to fund research using human ES cells but not research through which the cells are derived from embryos. Instead, recognizing the close connection in practical terms between the derivation and the use of these cells, it would be preferable to enact provisions that apply to funding by all federal agencies, provisions that would carve out a narrow exception for funding of research to use or to derive human ES cells from embryos that would otherwise be discarded by infertility treatment programs.

The Ethical Acceptability of Creating Embryos Through IVF Specifically as a Source of ES Cells

ES cells can be obtained from human research embryos created from donor gametes through IVF for the sole purpose of deriving such cells for research. The primary objection to creating embryos specifically for research is that many believe that there is a morally relevant difference between producing an embryo for the sole purpose of creating a child and producing an embryo with no such goal. Those who object to creating embryos for research often appeal to arguments that speak to respecting human dignity by avoiding the instrumental use of human embryos (i.e., using embryos merely as a means to some other goal does not treat them with appropriate respect or concern as a form of human life). Currently, we believe that cadaveric fetal tissue and embryos remaining after infertility treatments provide an adequate supply of research resources for federal research projects involving human embryos. Therefore, embryos created specifically for research purposes are not needed at the current time in order to conduct important research in this area.

Recommendation 3:

Federal agencies should not fund research involving the derivation or use of human ES cells from embryos made solely for research purposes using IVF.

In 1994, the NIH Human Embryo Research Panel argued in support of federal funding of the creation of embryos for research purposes in exceptional cases, such as the need to create banks of cell lines with different genetic make-ups that encoded various transplantation antigens—the better to respond, for example, to the transplant needs of groups with different genetic profiles. Such a project would require the recruitment of embryos from genetically diverse donors.

A number of points are worth considering in determining how to deal with this issue. First, it is possible that the creation of research embryos will provide the only opportunity to conduct certain kinds of research—such as research into the process of human fertilization. Second, as IVF techniques improve, it is possible that the supply of embryos for research from this source will dwindle. Nevertheless, we have concluded that, either from a scientific or a clinical perspective, there is no compelling reason to provide federal funds for the creation of embryos for research at this time.

The Use of SCNT to Obtain ES Cells

The use of SCNT to transfer the nucleus of an adult somatic cell into an enucleated human egg likely has the potential of creating a human embryo. To date, although little is known about these embryos as potential sources of human ES cells, there is significant reason to believe that their use may have therapeutic potential. For example, the possible use of matched tissue for autologous cell-replacement therapy from ES cells may require the use of SCNT. Arguably, the use of this technique to create an embryo is different from the other cases we have considered—because of the asexual origin of the source of the ES cells—although oocyte donation is necessarily involved. We conclude that at this time, because other sources are likely to provide the cells needed for the preliminary stages of research, federal funding should not be provided to derive ES cells from SCNT. Nevertheless, the medical utility and scientific progress of this line of research should be monitored closely.

Recommendation 4:

Federal agencies should not fund research involving the derivation or use of human ES cells from embryos made using SCNT into oocytes.

Requirements for the Donation of Cadaveric Fetal Tissue and Embryos for Research

Potential donors of embryos for ES cell research must be able to make voluntary and informed choices about whether and how to dispose of their embryos. Because of concerns about coercion and exploitation of potential donors, as well as controversy regarding the moral status of embryos, it is important, whenever possible, to separate donors' decisions to dispose of their embryos from their decisions to donate them for research. Potential donors should be asked to provide embryos for research only if they have decided to have those embryos discarded instead of donating them to another couple or storing them. If the decision to discard the embryos precedes the decision to donate them for research purposes, then the research determines only how their destruction occurs, not whether it occurs.

Recommendation 5:

Prospective donors of embryos remaining after infertility treatments should receive timely, relevant, and appropriate information to make informed and voluntary choices regarding disposition of the embryos. Prior to considering the potential research use of the embryos, a prospective donor should have been presented with the option of storing the embryos, donating them to another woman, or discarding them. If a prospective donor chooses to discard embryos remaining after infertility treatment, the option of donating to research may then be presented. (At any point, the prospective donors' questions—including inquiries about possible research use of any embryos remaining after infertility treatment—should be answered truthfully, with all information that is relevant to the questions presented.)

During the presentation about potential research use of embryos that would otherwise be discarded, the person seeking the donation should

- **a) disclose that the ES cell research is not intended to provide medical benefit to embryo donors,**
- **b) make clear that consenting or refusing to donate embryos to research will not affect the quality of any future care provided to prospective donors,**
- **c) describe the general area of the research to be carried out with the embryos and the specific research protocol, if known,**
- **d) disclose the source of funding and expected commercial benefits of the research with the embryos, if known,**
- **e) make clear that embryos used in research will not be transferred to any woman's uterus, and**
- **f) make clear that the research will involve the destruction of the embryos.**

This proposal also stresses the separation that existing laws and policies seek between the pregnant

woman's decision to abort and her decision to donate cadaveric fetal tissue for transplantation research. Recommendation 1 proposes to extend that separation to the donation of cadaveric fetal tissue for stem cell research. It may be difficult to achieve this separation in making decisions about embryos that remain after infertility treatments, in part because potential donors at the outset of treatment may have chosen to donate them to research. But, however difficult it may be to achieve, this separation will reduce the chance that potential donors could be pressured or coerced into donating their embryos for stem cell research.

The parts of this recommendation that deal with providing information to donors are designed to ensure that potential donors understand the range of available options and that their decisions are not influenced by anticipated personal medical benefits or by concerns about the quality of subsequent care; that they understand that the research will involve the destruction of the embryos; and that they understand the nature of the proposed research, its source of funding, and its anticipated commercial benefits, if known. Several additional suggested information items are proposed in a document entitled "Points to Consider in Evaluating Basic Research Involving Human Embryonic Stem Cells and Embryonic Germ Cells," presented in Appendix F.

Although the ethical considerations that support the prohibition of the designated donation of human fetal tissue are less acute for EG cell research than they are for transplantation, cause for concern remains. Potential donors of cadaveric fetal tissue for EG cell derivation would not have a direct therapeutic incentive to create or abort tissue for research purposes, as might occur in a transplant context. However, we agree that the prohibition remains a prudent and appropriate way to assure that no incentive—however remote—is introduced into a woman's decision to have an abortion. Any suggestion of personal benefit to the donor or to an individual known to the donor would be untenable and potentially coercive. Thus, the potential donor should be informed both before and after the decision to donate that there is no obligation to make such a gift, that no personal benefit will accrue as a result of the decision to donate, and that no penalty or sanction will result from a decision to refuse to donate.

Recommendation 6:

In federally funded research involving embryos remaining after infertility treatments, researchers may not promise donors that ES cells derived from their embryos will be used to treat patient-subjects specified by the donors.

Current provisions regulating fetal tissue research (42 USC § 289g-1 and g-2) have been narrowly interpreted by NIH and DHHS to apply only where fetal cellular material is intended for transfer into a living human recipient for therapeutic or clinical purposes. No comparable rules exist for human embryos. We believe that this statute should be applied more broadly to include *any* research involving human fetal or embryonic tissue, regardless of its immediate or eventual therapeutic benefit or intended method of intervention. Advances in EG cell research have demonstrated that bioethical concerns are not limited to fetal tissue transplantation.

As noted in Chapter 3, the Uniform Anatomical Gift Act (UAGA), currently enacted in some form in all 50 states and the District of Columbia, also may require clarification. Current versions of the UAGA explicitly permit donors to make an anatomical gift of the human body or body parts. Because a fetus is included within the UAGA's definition of *decedent*, either directly or by implication depending upon the statutory language enacted, the statute's anatomical gift provision undermines any federal prohibition of designated donation of human fetal tissue. What would otherwise qualify for federal statutory preemption is clouded by provisions of the NIH Revitalization Act of 1993 and the federal Common Rule, which direct that fetal tissue transplant researchers must abide by local and state laws, including (by implication) the UAGA.

Finally, if and when sufficient scientific evidence becomes available, clinical benefits are clearly anticipated, and agreement is reached among the various elements in society that the creation of embryos specifically for research or therapeutic purposes is justified (specifically through the use of SCNT), prohibitions on directed donation should be revisited. For obvious reasons, the use of SCNT to develop ES cells for autologous transplantation might require that the recipient be specified.

Prohibitions Against the Sale of Embryonic and Fetal Material

Existing rules prohibit the practice of designated donation, the provision of monetary inducements to women undergoing abortion, and the purchase or sale of fetal tissue. We concur in these restrictions and in the recommendation of the 1988 Human Fetal Tissue Transplantation Research Panel that the sale of fetal tissue for research purposes should not be permitted under any circumstances. The potential for coercion is greatest when financial incentives are present, and the treatment of the developing human embryo or fetus as an entity deserving of respect may be greatly undermined by the introduction of any commercial motive into the donation or solicitation of fetal or embryonic tissue for research purposes.

Recommendation 7:

Embryos and cadaveric fetal tissue should not be bought or sold.

Policies already in place state that no for-profit trade in fetal tissue should be permitted, and some recommend that the "prohibition on commercial exchange of fetuses and fetal tissue extend to tissues imported from other countries" (Canadian Royal Commission 1993). This prohibition is intended to prevent the exploitation of poor women—especially those in developing countries—who might be persuaded to begin and end pregnancies for money. An important distinction must be made between the possible exploitation of persons that occurs when they are coerced or inappropriately induced to sell parts of their bodies and the exchanges that occur when companies, research institutions, or other groups provide reasonable compensation. This is a familiar issue in discussions about remuneration for participation in research and about which federal regulations defer to Institutional Review Boards (IRBs) for their judgment.

Current regulations (42 USC §§ 289g-2(a), 289g-2(b)(3), 274e, and 42 CFR § 46.206(b)) attempt to codify this recommendation. Further, depending upon whether a state has enacted the most recent revision of the UAGA (and not all states have enacted the UAGA restriction) and has included the fetus within its definition of *decedent*, the sale of fetal remains may or may not be prohibited by individual state statutes. Many states appear to rely on federal statutes and regulations to prohibit fetal tissue sale, and none address human embryos, except by implication.

We strongly encourage those who will draft modified legislation to frame their language in clear terms that are specifically defined. In particular, terms such as *valuable consideration, processing*, and *reasonable payments* require precise definitions.

We believe that, with respect to these regulations, different categories of research intermediaries should be treated differently. One approach would be to establish three intermediary categories: 1) entities responsible for tissue harvest or embryo collection, 2) entities responsible for stem cell derivation or other preresearch preparation *and* postderivation investigators; and 3) *de minimis* intermediaries (including courier or supply services, off-site specimen evaluation, pathological or chemical analysis for research suitability, and other insignificant non- or preresearch patient or specimen interactions). We believe that the first category warrants the greatest degree of regulation. An abortion provider, IVF clinic, or other third party responsible for obtaining consent and/or collecting biological materials should not be able to commercially solicit, pay for, or be paid for the fetal or embryonic material it obtains (permitting only a specifically defined, cost-based reimbursement exception for entities in that category). By placing such prohibitions against paying those who obtain the embryos, it is our intention to discourage the creation of excess embryos during routine infertility procedures, which would later be used for research purposes.

The National Organ Transplant Act (NOTA) prohibition on tissue sale (42 USC § 274e(a)) has been criticized for a statutory construction that focuses exclusively on the sale of human organs. Although we agree that fetal organ sale (as well as the sale of embryonic material) should be prohibited, we believe that NOTA's terms are unacceptably narrow and that pre-organ tissues characteristic of early fetal and embryonic development should be included in the tissue sale prohibition.

The Need for National Oversight and Review

The need for national oversight and review of ES and EG cell research is crucial. At present, no such system

exists in the United States. A national mechanism to review protocols for deriving human ES and EG cells and to monitor research using such cells would ensure strict adherence to guidelines and standards across the country. Thus, federal oversight can provide the public with the assurance that research involving stem cells is being undertaken appropriately. Given the ethical issues involved in ES and EG cell research—an area in which heightened sensitivity about the very research itself led the President to request that the Commission study the issue—the public and the Congress must be assured that oversight can be accomplished efficiently, constructively, in a timely fashion, and with sufficient attention to the relevant ethical considerations.

Several countries, such as the United Kingdom, have recommended the establishment of regulatory boards or national commissions to license and regulate assisted reproductive treatments and embryo research. The use of a national oversight mechanism of this kind has certain advantages, particularly because the use of law to regulate (rather than to set limits) in this area would be burdensome, given the rapid development of biomedical science and technology. On the other hand, some kind of national commission or authority could provide the necessary flexibility and adaptability, and, in addition, such an entity could ensure more consistent ongoing application of safeguards as well as greater public accountability.[3]

In 1994, the NIH Human Embryo Research Panel considered and then explicitly rejected reconstituting the Ethics Advisory Board (EAB) for the purpose of reviewing proposals involving embryos or fertilized eggs:

> Although revisiting the EAB experience offers the potential for public consensus development and a consistent application of the new guidelines, it nonetheless has significant disadvantages. These include the creation of an additional standing government board, the likelihood of significant delay before embryo research could be funded in order to meet legal requirements for new rulemaking prior to the official creation of the government body, and possible further delay if all proposals for embryo research were required to be considered individually by an EAB-type board, despite appearing to be consistent with a developed consensus at NIH about acceptability for funding (1994, 72).

Instead, the NIH panel recommended that

> national review of all protocols by a diverse group of experts is warranted for a time. It is the hope of the Panel that this ad hoc group will develop additional guidance gained from experience with actual protocols that can be communicated to IRBs through existing mechanisms at NIH (1994, 73).

These recommendations envisioned a time when, following sufficient experience by the ad hoc panel, guidelines for embryo research review could be decentralized to the local IRBs. (It was recommended that the ad hoc panel function for at least three years.) We used similar reasoning in a previous report when recommending that the Secretary of Health and Human Services convene a Special Standing Panel to review certain categories of research involving persons with mental disorders (NBAC 1998). Like the NIH panel, we did not specify when such guidelines could be decentralized; but unlike the NIH panel, we did recommend that the panel be a standing rather than an ad hoc body.

The NIH panel's recommendations must be viewed in the context of its reporting relationship: the panel was charged with advising the NIH Director about research that could be sponsored or conducted by that agency. We note that NIH is not the only federal agency that might be interested in sponsoring or conducting research involving human stem cells; thus, some accommodation must be made for the review of proposals that are not funded by NIH.

Other elements of the NIH panel's recommendation also require additional assessment. For example, the panel recommended that "all such research proposals continue to be specially monitored by the councils and the NIH Office for Protection from Research Risks [OPRR]" (1994, 74). We are less sanguine than the NIH panel about the ability of OPRR to provide the needed oversight and monitoring for ES and EG cell research at this time, particularly given the recent decision to move this office from NIH to DHHS. Although OPRR's role in the oversight of human subjects research, like that of the FDA, remains central to the structure of human subjects protections in this country, we believe that at this time, an additional mechanism is needed for the review and oversight of federally sponsored research involving human ES and EG cells.

We do, however, share the concern of the 1994 NIH panel, investigators, and IRBs that the process of protocol review should not be viewed as simply a bureaucratic hurdle that researchers must successfully leap solely to satisfy a procedural or regulatory requirement. Done well, protocol review often improves the quality of studies by identifying concerns in the areas of study design, selection of subjects, recruitment, informed consent, and dissemination of results.

Recommendation 8:

DHHS should establish a National Stem Cell Oversight and Review Panel to ensure that all federally funded research involving the derivation and/or use of human ES or EG cells is conducted in conformance with the ethical principles and recommendations contained in this report. The panel should have a broad, multidisciplinary membership, including members of the general public, and should

a) review protocols for the derivation of ES and EG cells and approve those that meet the requirements described in this report,

b) certify ES and EG cells lines that result from approved protocols,

c) maintain a public registry of approved protocols and certified ES and EG cell lines,

d) establish a database—linked to the public registry—consisting of information submitted by federal research sponsors (and, on a voluntary basis, by private sponsors, whose proprietary information shall be appropriately protected) that includes all protocols that derive or use ES or EG cells (including any available data on research outcomes, including published papers),

e) use the database and other appropriate sources to track the history and ultimate use of certified cell lines as an aid to policy assessment and formulation,

f) establish requirements for and provide guidance to sponsoring agencies on the social and ethical issues that should be considered in the review of research protocols that derive or use ES or EG cells, and

g) report at least annually to the DHHS Secretary with an assessment of the current state of the science for both the derivation and use of human ES and EG cells, a review of recent developments in the broad category of stem cell research, a summary of any emerging ethical or social concerns associated with this research, and an analysis of the adequacy and continued appropriateness of the recommendations contained in this report.

We recommend several functions that the panel should carry out. In order to accomplish its purposes, the panel should maintain a public registry of federally funded protocols that employ or derive human ES and EG cells and, to the degree possible, a comprehensive listing of privately funded protocols. The purpose of the registry is to make it possible to track not only the protocols themselves and their adherence to the principles described above, but also their outcomes and the outcomes of all research based on their results. The panel should be able to describe the history and trajectory of research that uses these cells and to guard against the promiscuous use of the cells. As they are submitted, new federally funded protocols involving the derivation of ES cells must include a statement that only certified cell lines will be used.

Knowledge about the history and ultimate outcome and use of research using human ES and EG cells should be open to the public. Thus, the information accumulated by the panel through the registry should be used not only for ethical review, but also for public education. This is an important educational and informational function that may encourage the active participation of the private sector in the registry—even in the absence of any federal regulatory requirement to do so. In addition, within five years, the panel and the registry should be independently reviewed. This review, which should include an evaluation of the processes of the oversight and review mechanisms, will help to determine whether the level of limitations on this area of research is appropriate as well as to determine whether case-by-case review of derivation protocols is still warranted.

There are several benefits to a national review process for all federally funded research on ES and EG cells. These include preventing ethically problematic research,

assuring the public that the research is scientifically meritorious and ethically acceptable; providing information by which to evaluate issues of social justice in the use of the knowledge or other products of the research; providing public oversight of controversial research practices; assuring consistency in the review of protocols; evaluating this type of research; and educating the public.

Although we are aware that NIH will likely conduct and/or fund the majority of federally sponsored stem cell research in the country and will be developing its own set of guidelines for the conduct of ES and EG cell research, we are persuaded that it is important to distance to some degree the review and oversight of stem cell research from what is the principal source of funding in this country. The proximity of NIH within DHHS (our recommended location for the panel) makes it possible for a number of beneficial arrangements to develop. These include developing requirements for data sharing as a condition for receiving grants; developing guidelines for sharing cell lines; and providing a common review mechanism for other federal agencies that are conducting/funding research involving ES and EG cells (e.g., through a Memorandum of Understanding).

The Need for Local Review of Derivation Protocols

For more than two decades, prospective review by an IRB has been the principal method for assuring that federally sponsored research involving human subjects will be conducted in compliance with guidelines, policies, and regulations designed to protect human beings from harm. This system of local review has been subject to criticism, and, indeed, in previous analyses we have identified a number of concerns regarding this system of review. In preparing this report, we considered a number of proposals that would allow for the local review of research protocols involving human ES and EG cell research, bearing in mind that a decision by the Commission to recommend a role for IRBs might be incorrectly interpreted as endorsing the view that human ES or EG cells or human embryos are human subjects.

We adopted the principle, reflected in these recommendations, that for research involving the derivation of ES and EG cells, a system of national oversight and review would be needed to ensure that important federal sponsorship of stem cell research could proceed—but only under specific conditions. We recognized that for such research proposals, many of the ethical issues could be considered at the local level—that is, at the institutions where the research would be conducted. In general, the IRB is an appropriate body for reviewing protocols that aim to derive ES or EG cells. Although few review bodies (including IRBs) have extensive experience in the review of such protocols, IRBs remain the most visible and expert entities available. It is for this reason, for example, that a number of recommendations presented in this report (8, 9, 10, 11, and 12) discuss the importance of developing additional guidance for the review of protocols that involve human stem cell research.

Recommendation 9:

Protocols involving the *derivation* of human ES and EG cells should be reviewed and approved by an IRB or by another appropriately constituted and convened institutional review body prior to consideration by the National Stem Cell Oversight and Review Panel. (See Recommendation 8.) This review should ensure compliance with any requirements established by the panel, including confirming that individuals or organizations (in the United States or abroad) that supply embryos or cadaveric fetal tissue have obtained them in accordance with the requirements established by the panel.

As noted earlier, for research proposals that involve the derivation of human ES or EG cells, particular ethical issues require attention through a national review process. However, this process should begin at the local level, because institutions that intend to conduct research involving the derivation of human ES cells or EG cells should continue to take responsibility for ensuring the ethical conduct of that research. More important, however, IRBs can play an important role—particularly by reviewing consent documents and by assuring that collaborative research undertaken by investigators at foreign institutions has satisfied any regulatory requirements for the sharing of research materials.

We noted in Chapter 3 that currently there is no definitive answer to the question of whether the

Common Rule, 45 CFR 46, and/or Subpart B apply to research involving fetal tissue transplantation, to human embryo research, and by extension to EG and ES cell research. If the regulations do apply, then IRBs would be expected to review protocols, consistent with the regulatory requirements. We have indicated, however, that even if these regulations do not apply, we believe that IRBs should be expected to review derivation protocols to assess their ethical acceptability without having to commit to a position that the activities are human subjects research as defined by the regulations. If, as a matter of public policy, ES and EG cell research were found to be human subjects research, certain clarifying changes in the regulations might be needed. For example, Subpart B would need to provide clearly that *any* living donor of human biological material constitutes a human subject for purposes of research protection, and IRB review and informed consent under all subparts of the DHHS version of the Common Rule would need to apply. Similarly, we have made clear that the authorization a woman may give to donate fetal tissue following an elective abortion may better be understood as consent to donate—analogous to donating organs—rather than as providing informed consent for research participation. Even if these models differ, the principle we adopt remains the same: opportunity for consent should rest exclusively with the individual or individuals legally empowered to assume a donative role.[4]

Responsibilities of Federal Research Agencies

We have recommended that protocols involving the *derivation* of ES or EG cells should be reviewed by both a local review group and the national panel described in Recommendation 8. For protocols that involve only the use but not the derivation of ES or EG cells, oversight and review are still necessary, but these protocols do not require reliance on such a system. In our judgment, these protocols can be appropriately reviewed using the existing system for the submission, review, and approval of research proposals that is in place at federal research agencies, which includes the use of a peer review group—sometimes called a study section or initial review group—that is established to assess the scientific merit of the proposals. In addition, in some agencies, such as NIH, staff members review protocols before they are transmitted to a national advisory council for final approval. These levels of review all provide an opportunity to consider ethical issues that arise in the proposals. When research proposals involve human subjects, in order to assure that it is ethically acceptable, federal agencies rely on local IRBs for review and approval. (See Recommendation 9.) At every point in this continuum—from the first discussions that a prospective applicant may have with program staff within a particular institute to the final decision by the relevant national advisory council—ethical and scientific issues can be addressed by the sponsoring agency. But even if—based on a particular interpretation of the federal regulation—these research proposals do not involve human subjects, we believe the system of oversight and review can adequately address the relevant ethical issues.

Recommendation 10:

All federal agencies should ensure that their review processes for protocols using human ES or EG cells comply with any requirements established by the National Stem Cell Oversight and Review Panel (see Recommendation 8), paying particular attention to the adequacy of the justification for using such cell lines.

Research involving human ES and EG cells raises critical ethical issues, particularly when the proposals involve the derivation of ES cells from embryos that remain after infertility treatments. We recognize that these research proposals may not follow the paradigm that is usually associated with human subjects research. Nevertheless, research proposals that are being considered for funding by federal agencies must, in our view, meet the highest standards of scientific merit and ethical acceptability. To that end, the recommendations made in this report, including a proposed set of points to consider in evaluating basic research involving human ES cells and EG cells (see Appendix F), constitute a set of ethical and policy considerations that should be reflected in the respective policies of federal agencies conducting or sponsoring human ES or EG cell research.

Attention to Issues for the Private Sector

Although this report primarily addresses the ethical issues associated with the use of federal funds for research involving the derivation and/or use of ES and

EG cells, we recognize that considerable work in both of these areas will be conducted under private sponsorship. Thus, our recommendations may have implications for those working in the private sector. First, for cell lines to be eligible for use in federally funded research, they must be certified by the National Stem Cell Oversight and Review Panel described in Recommendation 8. Therefore, if a private company aims to make its cell lines available to publicly funded researchers, it must submit its derivation protocol(s) to the same oversight and review process recommended for the public sector, (i.e., local review; see Recommendation 9) and for certification by the proposed national panel that the cells have been derived from embryos remaining after infertility treatments or from cadaveric fetal tissue.

Second, we hope that nonproprietary aspects of protocols developed under private sponsorship will be made available in the public registry, as described in Recommendation 8. The greater the participation of the private sector in providing information on human ES and EG cell research, the more comprehensive the development of the science and related public policies in this area.

Third, and perhaps most relevant in an ethically sensitive area of emerging biomedical research, it is important that all members of the research community, whether in the public or private sector, conduct the research in a manner that is open to appropriate public scrutiny. During the last two decades, we have witnessed an unprecedented level of cooperation between the public and private sectors in biomedical research, which has resulted in the international leadership position of the United States in this area. Public bodies and other authorities, such as the Recombinant DNA Advisory Committee, have played a crucial role in enabling important medical advances in fields such as gene therapy by providing oversight of both publicly and privately funded research efforts. We believe that voluntary participation by the private sector in the review and certification procedures of the proposed national panel, as well as in its deliberations, can contribute equally to the socially responsible development of ES and EG cell technologies and accelerate their translation into biomedically important therapies that will benefit patients.

Recommendation 11:

For privately funded research projects that involve ES or EG cells that would be eligible for federal funding, private sponsors and researchers are encouraged to adopt voluntarily the applicable recommendations of this report. This includes submitting protocols for the derivation of ES or EG cells to the National Stem Cell Oversight and Review Panel for review and cell line certification. (See Recommendations 8 and 9.)

In this report, we recommend that federally funded research that involves the derivation of ES cells should be limited to those efforts that use embryos that remain after infertility treatments. Some of the recommendations made in this context—such as the requirement for separating the decision by a woman to cease infertility treatment when embryos still remain from her decision to donate those embryos to research—simply do not apply to efforts to derive ES cells from embryos created (whether by IVF or by SCNT) solely for research purposes—activities that might be pursued in the private sector. Nevertheless, other ethical standards and safeguards embodied in the recommendations, such as provisions to prevent the coercion of women and the promotion of commerce in human reproduction, remain vitally important, even when embryos are created solely for research purposes.

Recommendation 12:

For privately funded research projects that involve deriving ES cells from embryos created solely for research purposes and that are therefore not eligible for federal funding (see Recommendations 3 and 4)

a) professional societies and trade associations should develop and promulgate ethical safeguards and standards consistent with the principles underlying this report, and

b) private sponsors and researchers involved in such research should voluntarily comply with these safeguards and standards.

Professional societies and trade associations dedicated to reproductive medicine and technology play a central role in establishing policy and standards for clinical care, research, and education. We believe that these organizations

can and should play a salutary role in ensuring that all embryo research conducted in the United States, including that which is privately funded, conforms to the ethical principles underlying this report. Many of these organizations already have developed policy statements, ethics guidelines, or other directives addressing issues in this report, and we have benefited from a careful review of these materials. These organizations are encouraged to review their professional standards to ensure not only that they keep pace with the evolving science of human ES and EG cell research, but also that their members are knowledgeable about and in compliance with them. For those organizations that conduct research in this area but that lack statements or guidelines addressing the topics of this report, we recommend strongly that they develop such statements or guidelines. No single institution or organization, whether in the public or the private sector, can provide all the necessary protections and safeguards.

The Need for Ongoing Review and Assessment

No system of federal oversight and review of such a sensitive and important area of investigation should be established without at the same time providing an evaluation of its effectiveness, value, and ongoing need. The pace of scientific development in human ES and EG cell research likely will increase. Although one cannot predict the direction of the science of human stem cell research, in order for the American public to realize the promise of this research and to be assured that it is being conducted responsibly, close attention to and monitoring of all the mechanisms established for oversight and review are required.

Recommendation 13:

The National Stem Cell Oversight and Review Panel described in Recommendation 8 should be chartered for a fixed period of time, not to exceed five years. Prior to the expiration of this period, DHHS should commission an independent evaluation of the panel's activities to determine whether it has adequately fulfilled its functions and whether it should be continued.

There are several reasons for allowing the national panel to function for a fixed period of time and for evaluating its activities before it continues its work. First, some of the hoped-for results will be available from research projects that are using the two sources we consider to be ethically acceptable for federal funding. Five years is a reasonable period in which to allow some of this information to amass, offering the panel, researchers, members of Congress, and the public sufficient time to determine whether any of the knowledge or potential health benefits are being realized. The growing body of information in the public registry and database described above (particularly if privately funded researchers and sponsors voluntarily participate) will aid these considerations.

Second, within this period the panel may be able to determine whether additional sources of ES cells are necessary in order for important research to continue. Two arguments have been offered for supporting research using embryos created specifically for research purposes: One is the concern that not enough embryos remain for this purpose from infertility treatments, and the other is the recognition that some research requires embryos that are generated for specific research and/or medical purposes. The panel should assess whether additional sources of ES cells that we have judged to be ineligible for federal funding at this time (i.e., embryos created solely for research purposes) are legitimately needed.

Third, an opportunity to assess the relationship between local review of protocols using human ES and EG cells and the panel's review of protocols for the derivation of ES cells will be offered. It will, of course, take time for this national oversight and review mechanism to develop experience with the processes of review, certification, and approval described in this report.

Fourth, we hope that the panel will contribute to the broad and ongoing national dialogue on the ethical issues regarding research involving human embryos. A recurring theme of our deliberations, and in the testimony we heard, was the importance of encouraging this national conversation.

The criteria for determining whether the panel has adequately fulfilled its functions should be set forth by an independent body established by DHHS. However, it would be reasonable to expect that the evaluation would rely generally on the seven functions described above in Recommendation 8 and that this evaluation would be conducted by a group with the requisite expertise. In

addition, some of the following questions might be considered when conducting this evaluation: Is there reason to believe that the private sector is voluntarily submitting descriptions of protocols involving the derivation of human ES cells to the panel for review? Is the panel reviewing projects in a timely manner? Do researchers find that the review process is substantively helpful? Is the public being provided with the assurance that social and ethical issues are being considered?

Summary

Recent developments in human ES and EG cell research have raised the prospect that new therapies will become available that will serve to relieve human suffering. These developments also have served to remind society of the deep moral concerns that are related to research involving human embryos and cadaveric fetal tissue. Serious ethical discussion will (and should) continue on these issues. However, in light of public testimony, expert advice, and published writings, we have found substantial agreement among individuals with diverse perspectives that although the human embryo and fetus deserve respect as forms of human life, the scientific and clinical benefits of stem cell research should not be foregone. We were persuaded that carrying out human ES cell research under federal sponsorship is important, but only if it is conducted in an ethically responsible manner. After extensive deliberation, the Commission believes that acceptable public policy can be forged, in part, based upon these widely shared views. Through this report, we not only offer recommendations regarding federal funding and oversight of stem cell research, but also hope to further stimulate the important public debate about the profound ethical issues regarding this potentially beneficial research.

Notes

1 Use of fetal tissue in research is also permitted in Canada, the United Kingdom, Australia, and in most countries in the European Union. Germany, for example, does not permit embryo research but does permit the use of fetal tissue for the derivation of EG cells. The German statement concerning human ES cells upholds the ban on destructive embryo research, effectively banning the derivation of ES cells, because the option of deriving EG cells exists in that country. See the German statement concerning the question of human ES cells, March 1999, 8–10 (DFG 1999).

2 Public Law No. 105-78, 513(a) (1997).

3 EGE *Opinion* (1998) at Art. 2.11. See also the Australian NHMRC *Guidelines* (1996) advocating that complementary national assisted reproductive technology standards or legislation be adopted in the Australian States.

4 See *Fed. Reg.* 27804, proposed rule 45 CFR § 46.204(d)-(e) and Table 1, "Current and Proposed 45 CFR 46, Subpart B," 27798, explanatory text ("consent of the father is not required"; rather, "consent of the mother *or* her legally authorized representative is required" [after she is]..."informed of the reasonably foreseeable impact of the research on the fetus").

References

Canadian Royal Commission on New Reproductive Technologies. 1993. *Proceed with Care: Final Report of the Royal Commission on New Reproductive Technologies*. 2 vols. Ottawa: Minister of Government Services.

Deutsche Forschungsgemeinschaf (DFG). 1999. "Statement Concerning the Question of Human Embryonic Stem Cells," www.dfg-bonn.de/english/press/eszell_e.html.

European Commission. European Group on Ethics (EGE). 1998. "Opinion of the European Group on Ethics in Science and New Technologies."

National Bioethics Advisory Commission (NBAC). 1998. *Research Involving Persons with Mental Disorders That May Affect Decisiomaking Capacity*. 2 vols. Rockville, MD: U.S. Government Printing Office.

National Health and Medical Research Council (NHMRC). 1996. *Draft Statement on Ethical Guidelines on Assisted Reproductive Technology*. Canberra, Australia: Australian Government Publishing Service.

National Institutes of Health (NIH). Human Embryo Research Panel. 1994. *Report of the Human Embryo Research Panel*. 2 vols. Bethesda, MD: NIH.

Appendix A

Acknowledgments

This report benefited from the input of many individuals and groups. Several organizations responded to a February 1999 request from the National Bioethics Advisory Commission for input on the scientific, medical, and ethical issues involved in human stem cell research, and nearly 40 scientific, medical, professional, religious, and health organizations were asked to provide their perspectives on these complex issues. The Commission gratefully acknowledges the thoughtful comments provided by the following groups:

- American Bioethics Advisory Commission (Stafford, Virginia)
- The American College of Obstetricians and Gynecologists (Washington, DC)
- The American Society for Cell Biology (Bethesda, Maryland)
- American Society for Reproductive Medicine (Birmingham, Alabama)
- Association of American Medical Colleges (Washington, DC)
- Biotechnology Industry Association (Washington, DC)
- College of American Pathologists (Northfield, Illinois)
- Pharmaceutical Research and Manufacturers of America (Washington, DC)
- RESOLVE (Somerville, Massachusetts)

In addition, the Commission asked the following individuals to review portions of the draft report for scientific, legal, and ethical accuracy. The comments provided by these individuals improved the quality and outcome of the report and are greatly appreciated:

- Brigid L.M. Hogan (Vanderbilt University School of Medicine; Nashville, Tennessee)
- Anna Mastroianni (University of Washington; Seattle, Washington)
- John A. Robertson (The University of Texas; Austin, Texas)
- Janet Rossant (Samuel Lunenfeld Research Institute, Mt. Sinai Hospital; Toronto, Ontario)
- Lee Silver (Princeton University; Princeton, New Jersey)
- Evan Y. Snyder (Harvard Medical School; Cambridge, Massachusetts)
- James A. Thomson (University of Wisconsin; Madison, Wisconsin)

We are also grateful to Michelle Myer, a graduate student at the University of Virginia, for preparing a summary of the presentations that were provided to the Commission on May 7, 1999, on religious perspectives relating to research involving human stem cells. The summary appears as Appendix E of this report.

Appendix B

Glossary

adult stem (AS) cells – stem cells found in the adult organism (e.g., in bone marrow, skin, and intestine) that replenish tissues in which cells often have limited life spans. They are more differentiated than embryonic stem (ES) cells or embryonic germ (EG) cells.

ART (assisted reproductive technology) – all treatments or procedures that involve the handling of human eggs and sperm for the purpose of helping a woman become pregnant. Types of ART include *in vitro* fertilization, gamete intrafallopian transfer, zygote intrafallopian transfer, embryo cryopreservation, egg or embryo donation, and surrogate birth.

blastocyst – a mammalian embryo in the stage of development that follows the morula. It consists of an outer layer of trophoblast to which is attached an inner cell mass.

blastomere – one of the cells into which the egg divides after its fertilization; one of the cells resulting from the division of a fertilized ovum.

chimera – an organism composed of two genetically distinct types of cells.

cloning – the production of a precise genetic copy of a molecule (including DNA), cell, tissue, plant, or animal.

differentiation – the specialization of characteristics or functions of cell types.

diploid cell – the cell containing two complete sets of genes derived from the father and the mother respectively; the normal chromosome complement of somatic cells (in humans, 46 chromosomes).

ectoderm – the outer layer of cells in the embryo; the origin of skin, the pituitary gland, mammary glands, and all parts of the nervous system.

embryo – 1) the beginning of any organism in the early stages of development, 2) a stage (between the ovum and the fetus) in the prenatal development of a mammal, 3) in humans, the stage of development between the second and eighth weeks following fertilization, inclusive.

embryonic stem (ES) cells – cells that are derived from the inner cell mass of a blastocyst embryo.

embryonic germ (EG) cells – cells that are derived from precursors of germ cells from a fetus.

endoderm – the innermost of the three primary layers of the embryo; the origin of the digestive tract, the liver, the pancreas, and the lining of the lungs.

ex utero – outside of the uterus.

fibroblast – a cell present in connective tissue, capable of forming collagen fibers.

gamete – 1) any germ cell, whether ovum or spermatozoon, 2) a mature male or female reproductive cell.

gastrulation – the process of transformation of the blastula into the gastrula, at which point the embryonic germ layers or structures begin to be laid out.

germ cells – gametes (ova and sperm) or the cells that give rise directly to gametes.

haploid cell – a cell with half the number of chromosomes as the somatic diploid cell, such as the ova or sperm. In humans, the haploid cell contains 23 chromosomes.

in vivo – in the natural environment (i.e., within the body).

in vitro – in an artificial environment, such as a test tube or culture medium.

***in vitro* fertilization (IVF)** – a process by which a woman's eggs are extracted and fertilized in the laboratory and then transferred after they reach the embryonic stage into the woman's uterus through the cervix. Roughly 70 percent of assisted reproduction attempts involve IVF, using fresh embryos developed from a woman's own eggs.

karyotype – the chromosome characteristics of an individual cell or of a cell line, usually presented as a systematic array of metaphase chromosomes from a photograph of a single cell nucleus arranged in pairs in descending order of size.

mesoderm – the middle of the three primary germ layers of the embryo; the origin of all connective tissues, all body musculature, blood, cardiovascular and lymphatic systems, most of the urogenital system, and the lining of the pericardial, pleural, and peritoneal cavities.

morula – 1) the mass of blastomeres resulting from the early cleavage divisions of the zygote, 2) solid mass of cells resembling a mulberry, resulting from the cleavage of an ovum.

oocyte – 1) a diploid cell that will undergo meiosis (a type of cell division of germ cells) to form an egg, 2) an immature ovum.

ovum – female reproductive or germ cell.

pluripotent cells – cells, present in the early stages of embryo development, that can generate all of the cell types in a fetus and in the adult and that are capable of self-renewal. Pluripotent cells are not capable of developing into an entire organism.

pre-implantation embryo – 1) the embryo before it has implanted in the uterus, 2) commonly used to refer to *in vitro* fertilized embryos before they are transferred to a woman's uterus.

somatic cells – [from *soma* - the body] 1) cells of the body which in mammals and flowering plants normally are made up of two sets of chromosomes, one derived from each parent, 2) all cells of an organism with the exception of germ cells.

stem cells – cells that have the ability to divide indefinitely and to give rise to specialized cells as well as to new stem cells with identical potential.

totipotent – having unlimited capacity. Totipotent cells have the capacity to differentiate into the embryo and into extra-embryonic membranes and tissues. Totipotent cells contribute to every cell type of the adult organism.

trophoblast – the outermost layer of the developing blastocyst of a mammal. It differentiates into two layers, the cytotrophoblast and syntrophoblast, the latter coming into intimate relationship with the uterine endometrium with which it establishes nutrient relationships.

zygote – 1) the cell resulting from the fusion of two gametes in sexual reproduction, 2) a fertilized egg (ovum), 3) the diploid cell resulting from the union of a sperm and an ovum, 4) the developing organism during the first week after fertilization.

Appendix C

Letters of Request and Response

THE WHITE HOUSE

WASHINGTON

November 14, 1998

Dr. Harold Shapiro
Chair
National Bioethics Advisory Commission
Suite 3C01
6100 Executive Boulevard
Bethesda, Maryland 20892-7508

Dear Dr. Shapiro:

This week's report of the creation of an embryonic stem cell that is part human and part cow raises the most serious of ethical, medical, and legal concerns. I am deeply troubled by this news of experiments involving the mingling of human and non-human species. I am therefore requesting that the National Bioethics Advisory Commission consider the implications of such research at your meeting next week, and to report back to me as soon as possible.

I recognize, however, that other kinds of stem cell research raise different ethical issues, while promising significant medical benefits. Four years ago, I issued a ban on the use of federal funds to create human embryos solely for research purposes; the ban was later broadened by Congress to prohibit any embryo research in the public sector. At that time, the benefits of human stem cell research were hypothetical, while the ethical concerns were immediate. Although the ethical issues have not diminished, it now appears that this research may have real potential for treating such devastating illnesses as cancer, heart disease, diabetes, and Parkinson's disease. With this in mind, I am also requesting that the Commission undertake a thorough review of the issues associated with such human stem cell research, balancing all ethical and medical considerations.

I look forward to receiving your reports on these important issues.

Sincerely,

Bill Clinton

NATIONAL BIOETHICS ADVISORY COMMISSION

6100 Executive Blvd
Suite 5B01
Rockville, MD
20892-7508

Telephone
301-402-4242
Facsimile
301-480-6900
Website
www.bioethics.gov

November 20, 1998

The President
The White House
Washington, DC 20500

Dear Mr. President:

I am responding to your letter of November 14, 1998 requesting that the National Bioethics Advisory Commission discuss at its meeting in Miami this week the ethical, medical, and legal concerns arising from the fusion of a human cell with a cow egg.

The Commission shares your view that this development raises important ethical and potentially controversial issues that need to be considered, including concerns about crossing species boundaries and exercising excessive control over nature, which need further careful discussion. This is especially the case if the product resulting from the fusion of a human cell and the egg from a non-human animal is transferred into a woman's uterus and, in a different manner, if the fusion products are embryos even if no attempt is made to bring them to term. In particular, we believe that any attempt to create a child through the fusion of a human cell and a non-human egg would raise profound ethical concerns and should not be permitted.

We devoted time at our meeting to discussing various aspects of this issue, benefiting not only from the expertise of the Commissioners, but from our consultation (via telephone) with Dr. Ralph Brinster, a recognized expert in the field of embryology, from the University of Pennsylvania. Also in attendance at our meeting was Dr. Michael West, of Advanced Cell Technology, who was given an opportunity to answer questions from Commission members. As you know, however, the design and results of this experiment are not yet publicly available, and as a consequence the Commission was unable to evaluate fully its implications.

As a framework for our initial discussion, we found it helpful to consider three questions:

1. ***Can the product of fusing a human cell with the egg of a non-human animal, if transferred into a woman's uterus, develop into a child?***

At this time, there is insufficient scientific evidence to answer this question. What little evidence exists, based on other fusions of non-human eggs with non-human cells from a different species, suggests that a pregnancy cannot be maintained. If it were possible, however, for a child to develop from these fused cells, then profound ethical issues would be raised. An attempt to develop a child from these fused cells should not be permitted.

This objection is consistent with our views expressed in *Cloning Human Beings*, in which we concluded that:

> "...at this time it is morally unacceptable for anyone in the public or private sector, whether in a research or clinical setting, to attempt to create a child using somatic cell nuclear transfer cloning."

2. ***Does the fusion of a human cell and an egg from a non-human animal result in a human embryo?***

The common understanding of a human embryo includes, at least, the concept of an organism at its earliest stage of development, which has the potential, if transferred to a uterus, to develop in the normal course of events into a living human being. At this time, however, there is insufficient scientific evidence to be able to say whether the combining of a human cell and the egg of a non-human animal results in an embryo in this sense. In our opinion, if this combination does result in an embryo, important ethical concerns arise, as is the case with all research involving human embryos. These concerns will be made more complex and controversial by the fact that these hybrid cells will contain both human and non-human biological material.

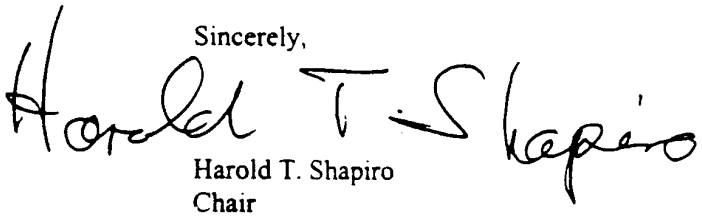
It is worth noting that these hybrid cells should not be confused with human embryonic stem cells. Human embryonic stem cells, while derived from embryos, are not themselves capable of developing into children. The use of human embryonic stem cells, for example to generate cells for transplantation, does not directly raise the same type of moral concerns.

3. ***If the fusion of a human cell and the egg of a non-human animal does not result in an embryo with the potential to develop into a child, what ethical issues remain?***

If this line of research does not give rise to human embryos, we do not believe that totally new ethical issues arise. We note that scientists routinely conduct non-controversial and highly beneficial research that involves combining material from human and other species. This research has led to such useful therapies as: blood clotting factor for hemophilia, insulin for diabetes, erythropoietin for anemia, and heart valves for transplants. Combining human cells with non-human eggs might possibly lead some day to methods to overcome transplant rejections without the need to create human embryos, or to subject women to invasive, risky medical procedures to obtain human eggs.

We recognize that some of the issues raised by this type of research may also be pertinent to stem cell research in general. We intend to address these and other issues in the report that you requested regarding human stem cell research.

Sincerely,



Harold T. Shapiro
Chair

Appendix D

The Food and Drug Administration's Statutory and Regulatory Authority to Regulate Human Stem Cells[1]

An Overview of Food and Drug Administration Regulations Pertinent to Human Cellular Materials and Tissues

The Food and Drug Administration (FDA) has had in place a regulatory framework for cellular and tissue materials that has evolved over time as the development and use of such biological materials for therapeutic purposes has increased. The Public Health Service Act (PHS Act), 42 USC 262 and 264, the Federal Food, Drug, and Cosmetic Act (FD&C Act), 21 USC 201 et seq., and implementing regulations of the FDA provide the agency with broad authority to regulate both the research into and the use of human stem cells that are *intended to be used as* biological products, drugs, or medical devices in order to prevent, treat, cure, or diagnose a disease or condition.[2] Scientific research not intended for use in the development of any FDA-regulated product is not under the oversight and control of the FDA.

In order for the FDA to assert its regulatory authority over stem cell-related research and products, such research *and* products must fall within one of the product categories over which the FDA exercises jurisdiction and must move in interstate commerce. To the extent that the FDA determines that a particular product falls within the definition of a biological product, a drug, or a medical device, it will assert its jurisdiction. Whether a particular product falls within the definition of any of the FDA-regulated product categories will depend, in part, upon the intended use of the product.

The manufacturer's objective intent—as evidenced by labeling, promotional, and other relevant materials for the product—has long been regarded as the primary source for establishing a product's intended use and thus its status for purposes of FDA regulation.[3] Although this approach would seem to grant manufacturers unlimited control over the regulatory status of their products, courts in fact have recognized the FDA's right to look beyond the express claims of manufacturers in order to consider more subjective indicia of intent—such as the foreseeable and actual use of a product—to prove that its intended use subjects it to agency jurisdiction.[4]

Regardless of whether the FDA or the manufacturer is characterizing the intended use of a product for purposes of evaluating FDA jurisdiction, it is clear that FDA regulatory authority will not extend automatically to all scientific research on stem cells. Indeed, to the extent that such nonhuman research is preliminary in nature and/or is undertaken without intent to develop a therapeutic product, stem cell research is not subject to FDA jurisdiction. Thus, for example, basic research to develop stem cell models to evaluate the safety and efficacy of therapeutic products would not be regulated directly. Instead, the FDA would review any scientific data generated from such a model and submitted as part of a marketing application. It is only at the juncture when the science of stem cell research has progressed to the point that development of a particular therapeutic product and its use in humans is envisioned that FDA regulatory authority will apply, and further research then must be conducted in compliance with FDA requirements.

Even if a product falls within one of the defined categories over which the FDA asserts its jurisdiction, no

statutory authority over the product exists unless it moves in interstate commerce. The FDA takes an expansive view of what constitutes interstate commerce; in regard to biological products, the FDA has been particularly aggressive. For example, in its 1993 policy statement regarding somatic cell therapy products, the FDA concluded that

> [t]he interstate commerce nexus needed to require premarket approval under the statutory provisions governing biological products and drugs may be created in various ways in addition to shipment of the finished product by the manufacturer. For example, even if a biological drug product is manufactured entirely with materials that have not crossed State lines, transport of the product into another State by an individual patient creates the interstate commerce nexus. If a component used in the manufacture of the product moves interstate, the interstate commerce prerequisite for the prohibition against drug misbranding is also satisfied even when the finished product stays within the State. Products that do not carry labeling approved in a PLA (or NDA) are misbranded under section 502(f)(1) of the [FD&C] Act....Moreover, falsely labeling a biological product is prohibited under section 351(b) of the PHS Act without regard to any interstate commerce nexus (42 U.S.C. 262(b)) (58 *Fed. Reg.* at 53250).

It can be expected that the FDA would apply the same logic to all cellular and tissue materials that are used in the prevention, treatment, cure, or diagnosis of a disease or condition.

Application to Stem Cells

In recent congressional testimony, National Institutes of Health Director Harold Varmus described three potential applications of research using human "pluripotent stem cells" that illustrate the inconsistencies of FDA regulation. He noted that the FDA does not regulate two of the examples, but will regulate one. First, stem cell research could include basic research such as "the identification of the factors involved in the cellular decision-making process that determines cell specialization."[5] Second, "[h]uman pluripotent stem cell research could also dramatically change the way we develop drugs and test them for safety and efficacy. Rather than evaluating safety and efficacy of a candidate drug in an animal model of a human disease, these drugs could be tested against a human cell line that had been developed to mimic the disease process."[6] It is unlikely that the FDA would regulate either of these potential applications directly. Varmus also made the following comments:

> Perhaps the most far-reaching potential application of human pluripotent stem cells is the generation of cells and tissue that could be used for transplantation, so-called cell therapies. Pluripotent stem cells stimulated to develop into specialized cells offer the possibility of a renewable source of replacement cells and tissue to treat a myriad of diseases, conditions and disabilities including Parkinson's and Alzheimer's disease, spinal cord injury, stroke, burn, heart disease, diabetes, osteoarthritis and rheumatoid arthritis.[7]

These stem cell products, based on their *intended use*, would be subject to FDA regulation.

Case-by-Case Regulation

The FDA has been cautious in exercising its regulatory discretion regarding cellular and tissue materials and in fact never has overseen a single regulatory program for human cellular and tissue-based products. Instead, the FDA has regulated these products on a case-by-case basis, responding as it deemed appropriate to the particular characteristics of and concerns raised by each type of product.[8]

One example has been the FDA's approach to regulating bone marrow. Although for years the FDA has licensed blood and blood components pursuant to section 351 of the PHS Act (42 USC 262), it voluntarily has refrained from regulating minimally manipulated bone marrow, the earliest source of stem cells used for transplantation, despite its status as a blood component. Indeed, not until the early 1990s did the FDA announce that to the extent that bone marrow was subject to extensive manipulation prior to transplantation, it would be treated the same as somatic cell therapy and gene therapy products subject to the investigational new drug (IND) regulations and would require PHS Act licensure (58 *Fed. Reg.* 53248, 53249 (Oct. 14, 1993)).

Also in 1993, in response to concerns regarding the transmission of the human immunodeficiency virus (HIV) and other infectious diseases, the FDA published an

emergency final rule that mandated certain processing, testing, and recordkeeping procedures for specific types of tissue products.[9] This rule, however, did *not* mandate premarket approval or notification for all tissues, but rather provided, among other things, for donor screening, documentation of testing, and FDA inspection of tissue facilities.[10]

Another example of the FDA's case-by-case approach is the publication in 1996 of a guidance that stated that manipulated autologous structural cells (autologous cells manipulated and then returned to the body for structural repair or reconstruction) would be subject to PHS licensure.[11] In addition, until recently, the FDA carefully chose not to regulate reproductive tissues. Then, in 1997, it proposed that, in the future, certain reproductive tissues (i.e., semen, ova, and embryos) should be regulated in some form.

Traditional tissue products (including but not limited to bone, skin, corneas, and tendons) also have been subject to the FDA's piecemeal regulatory approach. Historically, the FDA regulated these products on an ad hoc basis as medical devices under section 201 of the FD&C Act. However, with the advent of HIV and the potential for its transmission, the FDA concluded in the early 1990s that a more comprehensive program for regulating the use of traditional tissues was necessary. In 1991, the FDA concluded that human heart valves were medical devices subject to premarket approval requirements.[12] Following litigation, the FDA decided that while these products were indeed medical devices, they would not be subject to premarket approval requirements.[13] In defining tissue subject to this rule, the FDA exempted a number of products, including vascularized organs, *dura mater*, allografts, and umbilical cord vein grafts.

A New Approach to Regulating Human Cellular and Tissue-Based Products

In February 1997 the FDA proposed a new approach to the regulation of human cellular and tissue-based products. This framework is intended to "protect the public health without imposing unnecessary government oversight" ("Reinventing the Regulation of Human Tissue," *National Performance Review*, February 1997). Although it is still considered a proposed approach, the 1997 document utilizes FDA's existing statutory authority under the PHS and FD&C Acts to regulate a broad array of cellular and tissue materials. The framework proposed is a tiered approach to regulation (FDA, "A Proposed Approach to the Regulation of Cellular and Tissue-Based Products," February 28, 1997). Products that pose increased risks to health or safety would be subject to increased levels of regulation (i.e., either licensure under the PHS Act or premarket approval under the FD&C Act), while products that pose little or no risk of transmitting infectious disease would be subject to minimal regulation (e.g., facility registration and product listing). However, products that are 1) highly processed (more-than-minimally manipulated); 2) are used for other than their usual purpose; 3) are combined with nontissue components (e.g., devices or other therapeutic products); or 4) are used for metabolic purposes (e.g., systemic, therapeutic purposes) will be subject to clinical investigation as INDs, must be documented with investigational device exemption applications (IDEs), and will be subject to premarket approval as biological products, medical devices, or new drugs.

This proposed approach addresses the FDA's regulation of stem cell products. In the case of a minimally manipulated product for autologous use and allogeneic use of cord blood stem cells by a close blood relative, the FDA has proposed requiring compliance with standards consistent with section 361 of the PHS Act, rather than an IND and licensure pursuant to section 351 of the act. However, minimally manipulated products that will be used by an unrelated party will be regulated under section 351 of the Act. The FDA also intends to develop standards—including disease screening requirements, establishment controls, processing controls, and product standards: "If sufficient data are not available to develop processing and product standards after a specified period of time, the stem cell products would be subject to IND and marketing application requirements."[14] Stem cell products that are more-than-minimally manipulated will require INDs and licensing under section 351 of the PHS Act. For example, stem cell products that are to be used for a nonhomologous function or are more-than-minimally manipulated will be required to be licensed under

section 351. The FDA also has articulated "increased safety and effectiveness concerns for cellular and tissue-based products that are used for nonhomologous function, because there is less basis on which to predict the product's behavior."[15]

Implementation of the Proposed Approach

The FDA has begun to implement the proposed approach with the publication on January 20, 1998, of a *Request for Proposed Standards for Unrelated Allogeneic Peripheral and Placental/Umbilical Cord Blood Hematopoietic Stem/Progenitor Cell Products*" (63 *Fed. Reg.* 2985), utilizing its standards-setting authority under section 361 of the PHS Act.[16] In this notice, the FDA requests product standards to ensure the safety and effectiveness of stem cell products, which should be supported by clinical and nonclinical laboratory data. The FDA also announced its intention to phase in over a three-year period implementation of IND application and license application requirements for minimally manipulated unrelated allogeneic hematopoietic stem/progenitor cell products. The notice states that "[i]f adequate information can be developed, the agency intends to issue guidance for establishment controls, processing controls, and product standards....FDA intends to propose that, in lieu of individual applications containing clinical data, licensure may be granted for products certified as meeting issued standards." If, however, the FDA determines that adequate standards cannot be developed, the agency has expressed its intention to enforce IND and licensing requirements at the end of three years. Proposals are due on or before January 20, 2000.

On May 14, 1998, the FDA proposed *Establishment Registration and Listing for Manufacturers of Human Cellular and Tissue-Based Products* (63 *Fed. Reg.* 26744). The agency describes the proposed registration and listing requirements as a first step towards accomplishing its goal of putting into place a comprehensive new system of regulation for human cellular and tissue-based products. Registration and listing is intended to allow the FDA to assess the state of the cell and tissue industry, "to accrue basic knowledge about the industry that is necessary for its effective regulation," and to facilitate communication between the agency and industry (Ibid. at 26746). As proposed, the registration and listing requirements would apply to human cellular and tissue-based products that the FDA will regulate under section 361 of the PHS Act.[17] Among the products designated for regulation under that section and consequently subject to registration and listing are bone, tendons, skin, corneas, as well as peripheral and cord blood stem cells under certain conditions, and sperm, oocytes, and embryos for reproductive use (Ibid. at 26746).

FDA Discretion Entitled to Great Deference

Today there is a vast array of biological products that have been approved by the FDA and many others that are awaiting FDA action.[18] These products are scientifically complex and rarely lend themselves to categorization. As a result, the FDA invariably is required to determine on a case-by-case basis whether its existing statutory authority applies to a new product, which particular authority to apply, and, if so, what evidence will adequately demonstrate proof of safety, purity, and potency (efficacy). The decision of whether and how to regulate a product is made based upon the FDA's expert determination and upon the particular facts and circumstances, the historical application of the law to similar products, the applicable statutory and regulatory criteria, and the state of the FDA's scientific understanding at the time of the approval.

The FDA's exercise of the significant discretion provided to the agency by Congress is entitled to great deference by the courts.[19] In a recent challenge to the FDA's approval of a biological product under the PHS Act, the District Court for the District of Columbia held that "FDA's policies and its interpretation of its own regulations will be paid special deference *because of the breadth of the Congress' delegation of authority to FDA and because of FDA's scientific expertise*."[20]

Moreover, even if the FDA has not asserted jurisdiction previously with regard to reproductive tissue, for example, it is within the agency's statutory authority that its policies are evolutionary. The Supreme Court has recognized that expert administrative agency interpretations are not "carved in stone. On the contrary, the

agency...must consider varying interpretations and the wisdom of its policy *on a continuing basis*" (emphasis added).[21] Furthermore, the Court has acknowledged that "regulatory agencies do not establish rules of conduct to last forever....[A]n agency must be given ample latitude to 'adapt their rules and policies to the demands of changing circumstances.'"[22]

Conclusion

The FDA has developed a comprehensive approach to the regulation of cellular and tissue-based therapeutic products under its jurisdiction, including human stem cells. Nonclinical and clinical stem cell research undertaken to develop a therapeutic product intended to treat human disease will continue to be regulated by the FDA, while basic scientific research and other nonhuman research will remain outside of the agency's purview.

Notes

1 The content of this appendix is based upon a paper commissioned by the National Bioethics Advisory Commission and prepared by Brady, R.P., M.S. Newberry, and V.W. Girard, "The Food and Drug Administration's Statutory and Regulatory Authority to Regulate Human Pluripotent Stem Cells," available in Volume II of this report.

2 The scope of this appendix is limited to human stem cells. The FDA has a similar regulatory structure to regulate animal stem cell products used as animal drugs (21 USC 360b). The U.S. Department of Agriculture has the authority to regulate animal stem cell products used in animal vaccines (21 USC 151).

3 See *United States v. An Article…Sudden Change*, 409 F.2d 734, 739 (2d Cir. 1969).

4 See *National Nutritional Foods Ass'n. v. Mathews*, 557 F.2d. 325, 334 (2d Cir. 1977); *Action on Smoking and Health v. Harris*, 655 F.2d 236, 240–41 (D.C. Cir. 1980).

5 Statement of Harold Varmus, M.D., Director, National Institutes of Health, before the Senate Appropriations Subcommittee on Labor, Health and Human Services, Education and Related Agencies. December 2, 1998. Meeting transcript, 3.

6 Ibid.

7 Ibid. 3–4.

8 63 *Fed. Reg.* 26744 (May 14, 1998) (FDA Proposed Rule "Establishment and Listing for Manufacturers of Human Cellular and Tissue-Based Products").

9 "Human Tissue Intended for Transplantation" 58 *Fed. Reg.* 65514 (Dec. 14, 1993).

10 In 1997, FDA finalized its 1993 emergency rule establishing processing, testing, and recordkeeping requirements for all tissue products. "Human Tissue Intended for Transplantation" 62 *Fed. Reg.* 40429 (July 29, 1997).

11 CBER, Guidance on Applications for Products Comprised of Living Autologous Cells Manipulated *Ex Vivo* and Intended for Structural Repair or Reconstruction (May 1996).

12 "Cardiovascular Devices; Effective Date of Requirement for Premarket Approval; Replacement Heart Valve Allograft" 56 *Fed. Reg.* 29177 (June 26, 1991).

13 FDA Rescission Notice, 59 *Fed. Reg.* 52078 (October 14, 1994).

14 Proposed Approach, 25.

15 Proposed Approach, 16.

16 While FDA may choose to implement this policy through regulation, FDA also may implement it on a case-by-case basis. See infra, Section VI.

17 Consistent with the discussion supra, Section III. A., the preamble to the proposed rule states that "use of human cellular or tissue-based products solely for nonclinical scientific or educational purposes does not trigger the registration or listing requirements. Any use for implantation, transplantation, infusion, or transfer into humans is considered clinical use and would be subject to part 1271 [the registration and listing requirements]" Ibid. 26748.

18 Today, biological products are available or under development to treat, diagnose, or prevent virtually every serious or life-threatening disease. Available products include, but are not limited to, vaccines (manufactured both in traditional ways and through the use of biotechnology); human blood and blood-derived products; monoclonal or polyclonal immunoglobulin products; human cellular (i.e., gene therapy) products; protein, peptide, and carbohydrate products; protein products produced in animal body fluids by genetic alteration of the animal (i.e., transgenic animals); animal venoms; and allergenic products.

19 *U.S. v. Rutherford*, 442 U.S. 544, 553 (1979); *Bristol-Myers Squibb Co. v. Shalala*, 923 F. Supp. 212, 216 (D.D.C. 1996).

20 *Berlex Laboratories, Inc. v. FDA* et al., 942 F. Supp. 19 (D.D.C. 1996) (emphasis added). See also *Lyng v. Payne*, 476 U.S. 926 (1986).

21 *Chevron, U.S.A., Inc. v. Natural Resources Defense Council, Inc.*, 467 U.S. 837, 863–64 (1984).

22 *Motor Vehicle Mfrs. Ass'n. of the U.S. v. State Farm Mut. Auto. Ins. Co.*, 463 U.S. 29, 42 (1983) (citations omitted).

Appendix E

Summary of Presentations on Religious Perspectives Relating to Research Involving Human Stem Cells, May 7, 1999

Introduction

As part of the National Bioethics Advisory Commission's deliberations for this report, a meeting was convened on May 7, 1999, at Georgetown University in order for the Commission to hear testimony from prominent scholars of religious ethics on their traditions' views of human stem cell research. Although it would be inappropriate for religious views to determine public policy in our country, such views are the products of long traditions of ethical reflection, and they often overlap with secular views. Thus, the Commission believed that testimony from scholars of religious ethics was crucial to its goal of informing itself about the range, content, and rationale of various ethical positions regarding research in this area.

The Commission heard testimony from scholars who work within the Roman Catholic, Protestant, Eastern Orthodox, Jewish, and Islamic faiths. Although the presenters were able to reach consensus on several significant issues related to embryonic stem (ES) and embryonic germ (EG) cell research, disagreement emerged among the religious traditions represented and often within each tradition itself, particularly between restrictive and permissive positions on several issues.

Roman Catholic Perspectives

The restrictive, "official" position within Roman Catholicism opposes EG and ES cell research, primarily because obtaining stem cells from either aborted fetal tissue or embryos that remain following clinical *in vitro* fertilization (IVF) procedures involves the intentional destruction of a genetically unique, living member of the human species. According to this view, it is impermissible to obtain stem cells from *in vitro* fertilized blastocysts, because doing so results in the destruction of the blastocyst—a human life worthy of full moral protection from the moment of conception. No amount of benefit to others can justify the destruction of the blastocyst, an act that would be equivalent to murder.

Similarly, from this perspective, it is impermissible to obtain EG cells from the gonadal tissue of aborted fetuses, because although such harvesting is not directly responsible for the death of the fetus, it nevertheless involves complicity with the evil of abortion. Moreover, to make use of any therapy derived from research on either human embryonic or fetal tissue and to contribute to the development or application of such research through general taxation would involve complicity in the destruction of human life. Federal funding, which in a sense would make all citizens complicit in this research, thus would greatly impose upon the consciences of Catholics.

However, even the restrictive position of the Roman Catholic Church does not oppose stem cell research per se. The central moral impediment to such research concerns the sources from which stem cells are derived. The act of harvesting stem cells from other sources—miscarried fetuses, placental blood, or adult tissues—would not be intrinsically immoral. In fact, this perspective, recognizing the potential benefits to human health of stem cell research, encourages investigation into the feasibility of

such alternative sources. In practice, however, stem cell research, even with alternative stem cell sources, would remain morally problematic for two reasons. First, some are concerned that any safeguards will be ineffective because, in the face of potentially promising and lucrative research, the temptation to transgress such safeguards might be irresistible. Second, many fear that the benefits of this research might not be distributed equitably and are concerned that stem cell research perhaps may not be the best use of national resources, given the preponderance of so many other unmet human needs.

Although all Roman Catholics share a variety of important basic convictions, individual Catholics often differ in how to interpret them in practice. According to a less restrictive Catholic perspective, this disagreement is due, at least in part, to a commitment to the theory of natural law—a commitment that, while a fundamental part of the Catholic tradition, also involves reliance upon an "imperfect science." A commitment to natural law involves belief in a moral order that can be "seen" by all human beings in the reality of creation itself. But because the act of "looking" entails "a complex process of discernment and deliberation, and a structuring of insights, a determination of meaning, from the fullest vantage point available, given a particular history—one that includes the illumination of Scripture and the accumulated wisdom of the tradition"—what any two human beings see will not always be the same.[1]

With respect to stem cell research, the major areas of disagreement among Catholics are also those upon which the restrictive voice within Catholicism most strongly bases its opposition: the moral status of the embryo and the moral permissibility of using aborted fetuses as sources of stem cells. In contrast to this restrictive view of the embryo, another Catholic might, with the aid of science, look to the reality of the early human embryo and see that which is not yet an "individualized human entity with the settled inherent potential to become a human person."[2] Because the early embryo, according to this less restrictive view, is not a person, it is sometimes permissible to use it in research, though as human life it must always be accorded some respect. Similarly, one might decide that adequate barriers—such as a prohibition against the directed donation of cadaveric fetal tissue, and the distinction between somatic cell nuclear transfer (SCNT) for research or therapy and SCNT for reproduction—can be erected between the use of aborted fetal tissue in research and the act of abortion itself so that engaging in the former does not amount to complicity in the latter. From this perspective, then, a Catholic may be able to support ES cell research without sacrificing a commitment to the fundamental principles that define Catholicism, including the duties to protect human life, honor the sacred, and promote distributive justice in health care. Finally, because of the diversity within and among ethical traditions, this perspective is congruent with the restrictive Catholic view that individuals who oppose this research should not be forced to contribute to it but, contrary to the restrictive view, favors an approach that would allow federal funding, but with accommodations made to permit conscientious objection.

To summarize the testimony of the Roman Catholic panel, all agree that in light of certain agreed-upon principles, major Catholic concerns with regard to both embryonic and nonembryonic stem cell research include the following issues: 1) the moral status of the early embryo, 2) complicity with abortion in using fetal tissue as a source of stem cells, 3) the need for safeguards, distributive justice, and just allocation of national resources, and 4) the difficulty in federally funding research to which many are opposed on moral and religious grounds. The major disagreements arise from conflicting interpretations of the broad principles, which in turn lead to different responses to these four major concerns.

Jewish Perspectives

The two main sources of Jewish ethics—theology and law—yield several principles relevant to a Jewish ethical analysis of stem cell research. First, human beings are merely the stewards of their bodies, which belong to God. Moreover, God has placed conditions on the use of the human body, including the command that health and life must be preserved. Second, human beings are God's partners in healing, and in order to fulfill God's command, they have a duty to use any means available to heal themselves, whether these means are natural or artificial. Third, because all human beings, regardless of

ability, are created in the image of God, they are valuable. Fourth, human beings, unlike God, lack perfect knowledge of the consequences of their actions and in the process of trying to improve themselves or the world must, therefore, be careful to avoid causing harm to them.

Four potential moral impediments to EG and ES cell research arise from these Jewish principles: 1) the moral status of the fetus and of the act of abortion, 2) potential complicity with evil, 3) the commandments to respect the dead, and 4) the moral status of the embryo.

According to Conservative Judaism, the fetus until the 40th day after conception is "like water." Although the fetus becomes a potential and partial person after the 40th day, and is thus entitled to a certain amount of respect and protection, it remains primarily a part of the pregnant woman's body, and does not become an independent person with full moral rights until the greater part of its body emerges from the womb during birth. Because of the command to preserve human health and life, if either the health or the life of the woman is clearly threatened by the fetus, abortion is not only permissible but obligatory, as she is a full person while the fetus remains only a part of her and a potential person. When the woman's health is at some increased risk but is not clearly compromised by the pregnancy, abortion is permissible but not obligatory. More recently, some Jewish authorities also permit abortion in cases in which the fetus has a terminal disease or serious malformations.

According to Orthodox Judaism, on the other hand, after 40 days of gestation, the fetus becomes a person with full moral rights and may not be aborted except to protect the pregnant woman's health. Yet, even though abortion after 40 days is viewed by the Orthodox Jews as homicide, it does not follow from this perspective that life-saving use of stem cells procured from illegitimately aborted fetuses is impermissible (although the question of who can legitimately give consent to such procurement is problematic from this perspective). Although this perspective recognizes the possibility that therapeutic use of aborted fetuses may make abortion appear less heinous, the strength of the commandment to preserve life, for which all other laws must be suspended except those prohibiting murder, idolatry, and sexual transgressions, overrides this concern. Thus, despite the disagreement within Judaism regarding the moral status of the fetus and the permissibility of abortion after 40 days, all agree that neither source of stem cells is illegitimate. One caveat to this consensus is that some within Conservative Judaism who accept the permissibility of abortion to preserve the life or health of the woman nevertheless require that stem cells be procured only from fetuses that have been legitimately aborted; Orthodox Judaism, by contrast, appears to hold that although abortion after 40 days postconception is generally impermissible, there is no complicity involved in using these aborted fetuses as sources of stem cells.

Jewish thinkers agree that commandments to respect the dead, which require that corpses not be mutilated or left unburied longer than necessary, can be suspended in order to save lives. Because of the strong commandment to preserve life and health, for example, Jewish law permits both autopsies and organ procurement when they will benefit the living. Reasoning by analogy, if tissue procurement from the cadavers of full persons in order to benefit human health and life is permitted, then tissue procurement from dead fetuses—which according to some Jewish perspectives are less than full persons—must also be permitted for the same purpose provided that (for some interpreters) the abortion itself was permissible according to Jewish law.

There is also wide consensus within Judaism that no serious moral impediments exist to using IVF embryos as sources of stem cells because extra-corporeal embryos have no status under Jewish law. These entities lack status because all embryos prior to 40 days postconception are "like water" and because as extra-corporeal entities, they lack the status of potential and partial person that is accorded to fetuses, which develop from embryos implanted in a uterus. Although extra-corporeal embryos merit a certain respect as human life, they are closest in moral status to gametes and thus may be discarded, frozen, or used as life-saving sources of stem cells. In fact, so long as they are never implanted, there is no clear legal prohibition against creating embryos for research purposes, although extra-legal norms may raise ethical questions about this practice.

Because stem cells can be permissibly procured either from extra-corporeal embryos or from legitimately aborted fetuses, stem cell research is not considered intrinsically immoral. Rather, stem cell research becomes morally problematic when applied in a variety of contexts. First, Judaism views the provision of health care as a communal duty. Thus, a context in which the benefits of stem cell research are not accessible to all persons who are in need would be problematic. Similarly, it may be problematic to focus national resources in this area of research rather than in other areas of need. In addition, although obtaining consent to procure stem cells is necessary, it may be challenging. Finally, there is widespread agreement that stem cell research should not be used to enhance human beings, although some disagreement exists over whether it may be used to improve health or whether it must be reserved only for life-saving purposes.

Eastern Orthodox Perspectives

According to Eastern Orthodoxy, all human beings are created in the image of God and grow continuously toward the likeness of God. Although the embryo, fetus, and adult are each at different stages of this process, all share the same potential for attaining authentic personhood, and each, with God's grace, will attain such personhood. According to this belief, God has given us medicine in order to heal, and any misuse of this gift that results in the destruction of potentially authentic persons is considered illegitimate. Thus, although miscarried fetuses may be used as sources of EG cells, neither electively aborted fetuses nor blastocysts may be so used. However, despite the impermissibility of procuring ES cells from blastocysts, because cell lines from this source already exist and have the potential to save lives, it is considered wasteful to discard these lines, and it is in fact permissible to use them. No complicity is thought to arise from such use. On the other hand, it is not permissible to procure EG cells from aborted fetuses, as such procurement *would* involve complicity.

Even assuming that stem cells could be permissibly procured, Eastern Orthodoxy shares with other religious traditions a variety of concerns about the context in which stem cell research might be applied, including addressing the problems of equitable access to the benefits of the research and other problems that can occur when market forces control the research; using the research for eugenic or cosmetic purposes, rather than for healing; and obtaining the informed, voluntary consent of the woman or couple.

Islamic Perspectives

Islam consists of two major schools of thought—Sunni and Shi'i—both of which refer to the same historical sources. Although these two schools differ somewhat in their views of abortion, in general, Islam regards the life of the fetus as developing over several stages, and personhood is considered a process. Although from the moment of conception the embryo is a human life meriting some protection, it is not commonly thought to attain personhood until it is ensouled, some time around the fourth month of gestation. Thus, because of the enormous potential to improve human health through this type of research, the vast majority of followers of Islam would agree that it is permissible to use early human embryonic life for this purpose. Moreover, it is permissible to use the tissue from illegitimately aborted fetuses to save lives, just as it is permissible to use cadaveric organs to save lives, even when the cadaveric organ source has been wrongfully killed. Finally, with caution, it can be deduced that creating embryos for research purposes is also permissible from an Islamic perspective, as long as those embryos are not implanted.

Protestant Perspectives

Protestant positions range dramatically from the highly restrictive to the nonrestrictive in this area. For example, according to restrictive Protestant view, a person is not defined by his or her capacities; rather, a person is a human being with a personal history, regardless of whether he or she is aware of that history. From this perspective, embryos are simply the weakest and least advantaged people among us. Because procuring stem cells from embryos requires the destruction of the embryo, such procurement thus raises serious moral issues, despite the ease with which it might be used to

attain undeniably positive consequences for others, and rather than accepting the use of illicit means to achieve a good end, we should search for alternative, permissible means. Similarly, using aborted fetuses as sources of EG cells amounts to complicity with evil, and procurement of EG cells even from permissibly aborted fetuses (however that category is defined) would involve using a human life twice for another's benefit—first, to benefit the woman who aborted and then to benefit society through EG cell research. Therefore, from this perspective, it is impermissible to derive stem cells from embryos, whether spare or created for this purpose, and from aborted fetuses, whether permissibly aborted or not. The use of alternative sources of stem cells—for example, from bone marrow or umbilical cord blood—would, however, be permissible.

For Protestants whose views are less restrictive on this issue, the moral status of the embryo is more ambiguous. Although even nascent human life—which retains the potential for full human life—deserves respect and protection from callous disregard, the early embryo and the late fetus are viewed in moral terms as significantly different. Because the potential benefits of ES and EG cell research are so substantial, the moral difference between the early embryo and the developed fetus becomes compelling in this case, and it is thus permissible to use human life at the blastocyst stage to benefit other lives. No embryos should be created solely for this purpose, however, unless no other sources are available, and attempts should be made to locate alternative sources of stem cells that do not involve the destruction of embryos. It is permissible to procure EG cells from aborted fetuses, as long as safeguards are erected to prevent the therapeutic use of aborted fetal tissue from either increasing the frequency of abortion or encouraging a callous view of early human life. Moreover, although less restrictive Protestant views permit the procurement of stem cells from both proposed sources, this procurement must occur within a context of respect for nascent human life, only when significant benefit can be derived from it, and only after broad public discussion and acceptance of such research. If the general public is excluded from a discussion of this research, then public support of this and future beneficial research may be compromised. Furthermore, the requirement that all members of society have the opportunity to participate in open, sustained dialogue about these decisions is critical from this perspective, and if federal funds are to be allocated toward this research, conscientious objectors should be accommodated. Finally, most Protestants share previously articulated contextual concerns regarding 1) ensuring global access to the benefits of this research, 2) avoiding the negative consequences that might come with market-controlled research, and 3) assessing the priority of these research efforts relative to other current and pending health-related research projects.

Summary of Broad Areas of Agreement and Disagreement

Not surprisingly, the panelists did not reach unanimity on all aspects of human ES and EG cell research. Although some differences exist among the various religious traditions, these mostly concern the appropriate sources and methods of religious-ethical reasoning. On substantive issues, less restrictive individuals across most religious traditions appear to have more in common with each other than with restrictive members of their own faiths. (The same is true for commonalities among restrictive members of all faiths.) The substantive issues relevant to stem cell research on which there is internal disagreement include the following:

1) *The moral status of the embryo*. The perceived status of the embryo ranges from full moral personhood with correlative inviolable rights to life to an early, extracorporeal biological entity lacking any significant moral status. Between these poles, although the embryo tends to be viewed as valuable because of its current status as a form of human life and its potential status as a person, it is ultimately, if tragically, subordinate to the health needs of actual persons.
2) *Whether the use of EG cells derived from aborted fetuses involves complicity with the perceived evil of abortion*. On one end of the spectrum is the view that many abortions are permissible. Thus, complicity with evil is either never or rarely a consideration. On the other end of the spectrum is the view that all deliberate abortions are immoral, and that any use of EG cells derived from aborted fetuses involves complicity.

Those who take more moderate positions argue that even when abortion is wrong, it is not wrong to use tissue that would otherwise be discarded, or that complicity can be avoided by erecting barriers between abortion and stem cell procurement, such as a prohibition of directed donation.

3) *Whether stem cell research should, ideally, be federally funded*. Some, based on their belief in the duty to heal, hold that stem cell research should proceed as quickly as possible (given certain conditions; see below), while others hold that any federal funding that enables immoral research is itself immoral and would involve conscientious citizens in complicity against their will. The moderate view holds that in the absence of agreement on such issues as the moral status of the embryo, conscientious objectors should be allowed to opt out of federal support for the research and that without any federal support, privatized human ES and EG cell research will make contextual goals such as distributive justice even more difficult to realize.

Despite these areas of disagreement, widespread consensus was reached both within and among the various religious traditions on several important issues in ES and EG cell research:

1) Stem cell research is not inherently immoral, and in fact has the potential to contribute important knowledge that can lead to therapies for certain diseases, provided that morally legitimate sources of cells are used (although this is defined differently), and provided that important contextual factors of justice and regulation are addressed. (See #3 below.)
2) If society chooses to embark upon federally funded ES and EG cell research, it must do so under conditions of respect for the humanity of the embryo. It would be preferable if there existed alternative sources of stem cells that did not involve the direct or indirect destruction of human life, and efforts should be made to identify such sources.
3) In order for the research to be morally permissible, several "background factors" must be in place, including
   - assurance of equitable access to the benefits of the research,
   - appropriate prioritization of this research relative to other social needs,
   - assurance that the research will be used to treat disease, not enhance humans,
   - public education, discussion, and acceptance of human stem cell research, and
   - public scrutiny, oversight, and regulation of the research.
4) Assuming that privately funded research will continue in this area, it is preferable that a public body—even one that is funded with tax dollars—be required by law to review all private sector research and to make this review part of the public record, despite the possibility that the connection between the government and ES and EG cell research may be perceived as legitimating research that some citizens will continue to consider immoral.

Meeting Participants

Catholicism
Kevin W. Wildes, S.J., Ph.D., Georgetown University
Edmund D. Pellegrino, M.D., Georgetown University
Margaret Farley, Ph.D., Yale University

Judaism
Rabbi Elliot N. Dorff, Ph.D., University of Judaism
Rabbi Moshe Tendler, Ph.D., Yeshiva University
Laurie Zoloth, Ph.D., San Francisco State University

Eastern Orthodoxy
Father Demetrios Demopulos, Ph.D., Holy Trinity Greek Orthodox Church

Islam
Abdulaziz Sachedina, Ph.D., University of Virginia

Protestantism
Gilbert C. Meilander, Jr., Ph.D., Valparaiso University
Nancy J. Duff, Ph.D., Princeton University Theological Seminary
Ronald Cole-Turner, M.Div., Ph.D., Pittsburgh Theological Seminary

Notes

1 Farley, M., "Roman Catholic Views on Research Involving Human Embryonic Stem Cells." Testimony before NBAC. May 7, 1999. Washington DC. Meeting transcript, 3.

2 Ibid. 5.

Appendix F

Points to Consider in Evaluating Basic Research Involving Human Embryonic Stem Cells and Embryonic Germ Cells

This document describes some of the ethical, scientific, and legal issues that could be considered when designing and/or reviewing studies that involve access to and use of human stem cells. These *Points to Consider* are relevant only for designing and evaluating studies in which the role of the individual(s) who provide gametes, cadaveric fetal tissue, or embryos is limited to providing these materials for research intended to develop generalizable new knowledge. This document results from the recommendations described in this report and therefore is intended for use by those who design, conduct, and review research involving human embryonic stem (ES) and embryonic germ (EG) cells using federal funds. Private researchers and sponsors also may find this document to be of use. These *Points to Consider* do not apply to situations in which an individual would be the recipient of a stem cell-based therapy, nor do they apply to studies involving human/animal hybrids.

I. Scientific and Research Design Considerations

The ethical acceptability of any research protocol depends, in part, on its scientific merit, the qualifications of investigators, the protocol's overall design characteristics, and the precise nature of the materials and operations employed. In these respects, several issues arise when designing research involving human ES and EG cells, consideration of which would help ensure not only that the research is well designed, important, feasible, and timely, but also that a number of important ethical matters are considered. These issues are of particular significance given the nature of the materials to be used in research.

A. What are the sources from which human ES and EG cells will be obtained?
   1. From existing cell lines
   2. From cadaveric fetal tissue (following elective abortion or surgical termination of ectopic pregnancy)
   3. From embryos remaining after infertility treatments
   4. From embryos created solely for research purposes[1]

B. Has previous and requisite research been conducted using nonhuman animal models?

C. Are there valid alternatives to using human ES and EG cells in the proposed research?

D. What are the future plans for conservation of gametes, cadaveric fetal tissue, and embryos?
   1. Will ES or EG cells be produced and stored for later use?
   2. If a particular protocol is being proposed that uses embryos remaining after infertility treatments, does it propose to use only the minimum number of embryos necessary?
   3. What plans exist in the event that additional ES or EG stem cells are needed?

E. In what setting will the research be conducted?
   1. Are the investigators scientifically qualified to carry out the proposed research?
   2. Is the research environment (including facilities) appropriate for the conduct of research involving stem cells?

II. Identification of Providers and Donors and Recruitment Practices and Compensation

Several issues should be considered when identifying individuals (or couples) who may be asked to consider providing gametes, fetal tissue, or embryos for research; consideration of these issues could help to ensure that no inappropriate burden, inducement, or exploitation would occur.

A. Identification and recruitment practices
   1. Are potential donors or providers identified through advertisements to the general public? Are they identified through direct solicitation? Do they self-select?
   2. Is the selection of such individuals equitable and fair?
   3. Are these individuals vulnerable to undue influence, coercion, or exploitation? Does the recruitment method raise concerns about undue influence or coercion of the prospective donors?
   4. Are the potential donors capable of consenting?
   5. In which circumstances is it appropriate to identify and recruit an individual as well as his or her partner?

B. Compensation and reimbursement
   1. Will any financial compensation be paid to individuals (or couples) who donate materials; and if so, will the details of this compensation be disclosed?
   2. Does the compensation reimburse the individual (or couple) solely for the additional expenses that relate to this particular project?
   3. When is the offer of compensation made relative to an individual's (or couple's) decision to make available the materials from which stem cells will be derived?

III. Consent to Donate

Several issues arise in the process of providing information to individuals and couples who may be donating cadaveric fetal tissue or embryos remaining after infertility treatments. Considering these issues would help to ensure that prospective donors or providers of source materials would receive timely, relevant, and appropriate information to make informed and voluntary choices. In some cases, these issues are unique to the provision of gametes, embryos, or fetal tissue; in other cases. the items are important in other situations as well.

A. General considerations for individuals (or couples) who donate cadaveric fetal tissue or embryos remaining after infertility treatments
   1. Who will seek the consent? Will a clinician and/or researcher be available to answer questions?
   2. Is it appropriate for others to participate in the consent process (e.g., partner or family member)?
   3. Will psychological support mechanisms be in place if needed?
   4. Are the purposes of ES or EG cell research (in general) described fully?
   5. Will the consent form clearly disclose that stem cell research is not intended to benefit the donor directly?
   6. Is it clear that decisions to consent to or refuse the procedures to obtain stem cells will not affect the quality of care the patient will receive?
   7. Will individuals be informed that no medical or genetic information about the fetal tissue, embryos, or stem cells derived from these sources will be available to any outside individual or entity?
   8. What measures will be taken to protect the privacy and confidentiality of individuals who provide cadaveric fetal tissue or embryos?
   9. Is the source of funding for the research (public, private, public/private, philanthropic) disclosed?
   10. What known commercial benefits, if any, are expected to arise for the investigators seeking to obtain human ES or EG cells?

B. Additional considerations specific to consent to donate cadaveric fetal tissue
   1. Is there a description of what usually is done with fetal tissue at the institution at which a pregnancy will be terminated? Is this information available in written form and provided to individuals?
   2. Is permission to conduct research immediately available?

C. Additional considerations specific to consent to donate embryos remaining after infertility treatments
   1. Are the methods of disposal of embryos remaining after infertility treatments described? Is this information available in written form and provided to patients?
   2. Will information be made available about whether the embryos were viable and normal or not?
   3. Is there a description of the options available (e.g., permit material to be used in research, cryopreserve, discard, or donate to another couple for infertility treatment)?
   4. Is it clear that the embryos used in research will not, under any circumstances, be transferred to any woman's uterus?
   5. Is it clear that the research will result in the destruction of the embryo? Is the method described?

IV. Review Issues

Because of the special nature of human ES and EG cells, several issues arise in the review and oversight of research involving their use. The Commission has recommended a system of national oversight and review, combined with local monitoring. Careful and thoughtful consideration of these issues will provide assurance that, regardless of the source of funding, appropriate compliance with applicable regulations, guidelines, and other standards will occur. These considerations would supplement, not replace, applicable federal and state regulations.

A. Applicability of relevant regulations
   1. What current guidelines, regulations, rules, or policies apply to the conduct of this research? If ambiguity exists, how will it be resolved?
   2. What mechanisms are in place to assure compliance with these regulations?
   3. What regulations apply for collaborating with international researchers (e.g., importing fetal tissue or embryos from other countries)?

B. Applicability of professional practice standards

C. Submission of research findings for publication

D. Other responsibilities of investigators and collaborating clinicians

Note

1 The National Bioethics Advisory Commission has recommended that federal agencies should not fund research involving the derivation or use of human ES cells from embryos created solely for research purposes. (See Recommendations 3 and 4.)

Appendix G

Public and Expert Testimony

January 19, 1999 (Washington, DC)

Public:
E.J. Suh, Collegians Activated to Liberate Life
Kneale Ewing, Collegians Activated to Liberate Life
Olga Fairfax
Will Goodman

Expert:
Harold Varmus, National Institutes of Health
John Gearhart, The Johns Hopkins University
James Thomson, University of Wisconsin
Austin Smith, University of Edinburgh
Daniel Perry, Alliance for Aging Research
Patricia King, Georgetown University School of Law
John Robertson, University of Texas School of Law
Erik Parens, The Hastings Center
Françoise Baylis, Dalhousie University
Ted Peters, Center for Theology and the Natural Sciences
Karen Lebacqz, Pacific School of Religion

February 2–3, 1999 (Princeton, New Jersey)

Expert:
David Blumenthal, Massachusetts General Hospital
Brigid Hogan, Vanderbilt University
Barbara Mishkin, Hogan & Hartson L.L.P.
Robert Brady, Hogan & Hartson L.L.P.

March 2–3, 1999 (Vienna, Virginia)

Expert:
John Fletcher, University of Virginia
Lori Knowles, The Hastings Center
LeRoy Walters, Georgetown University

April 16, 1999 (Charlottesville, Virginia)

Public:
Richard Doerflinger, National Conference of Catholic Bishops
Edward Furton, National Catholic Bioethics Center
Karen Poehailos
Sidney Gunst, Jr.
Ida Chow, American Society of Cell Biology
Ethics and Religious Liberty Commission of the Southern Baptist Convention (submitted written testimony)

May 7, 1999 (Washington, DC)

Public:
Dena Davis, Cleveland-Marshall College of Law
Richard Doerflinger, National Conference of Catholic Bishops

Expert:
Kevin Wildes, Georgetown University
Edmund Pellegrino, Georgetown University
Margaret Farley, Yale University
Demetrios Demopulos, Holy Trinity Greek Orthodox Church
Elliot Dorff, University of Judaism
Moshe Tendler, Yeshiva University
Laurie Zoloth, San Francisco State University
Abdulaziz Sachedina, University of Virginia
Gilbert Meilander, Jr., Valparaiso University
Nancy Duff, Princeton University Theological Seminary

May 11–12, 1999 (Northbrook, Illinois)

Public:
Daniel McConchie, Center of Bioethics and Human Dignity

Expert:
Lori Andrews, Chicago–Kent College of Law
Sander Shapiro, University of Wisconsin–Madison

June 28, 1999 (Washington, DC)

Public:
Phil Noguchi, Food and Drug Administration

Appendix H

Commissioned Papers

The following papers, prepared for the National Bioethics Advisory Commission, are available in Volume II of this report:

State Regulation of Embryo Stem Cell Research
Lori B. Andrews
Chicago-Kent College of Law

The Food and Drug Administration's Statutory and Regulatory Authority to Regulate Human Pluripotent Stem Cells
Robert P. Brady, Molly S. Newberry, and Vicki W. Girard
Hogan & Hartson L.L.P.

Quick Response: Use of Human Fetal Tissue in Federally Funded Research
Elisa Eiseman
RAND Science and Technology Policy Institute

Analysis of Federal Laws Pertaining to Funding of Human Pluripotent Stem Cell Research
Ellen J. Flannery and Gail H. Javitt
Covington & Burling

Deliberating Incrementally on Human Pluripotential Stem Cell Research
John C. Fletcher
University of Virginia

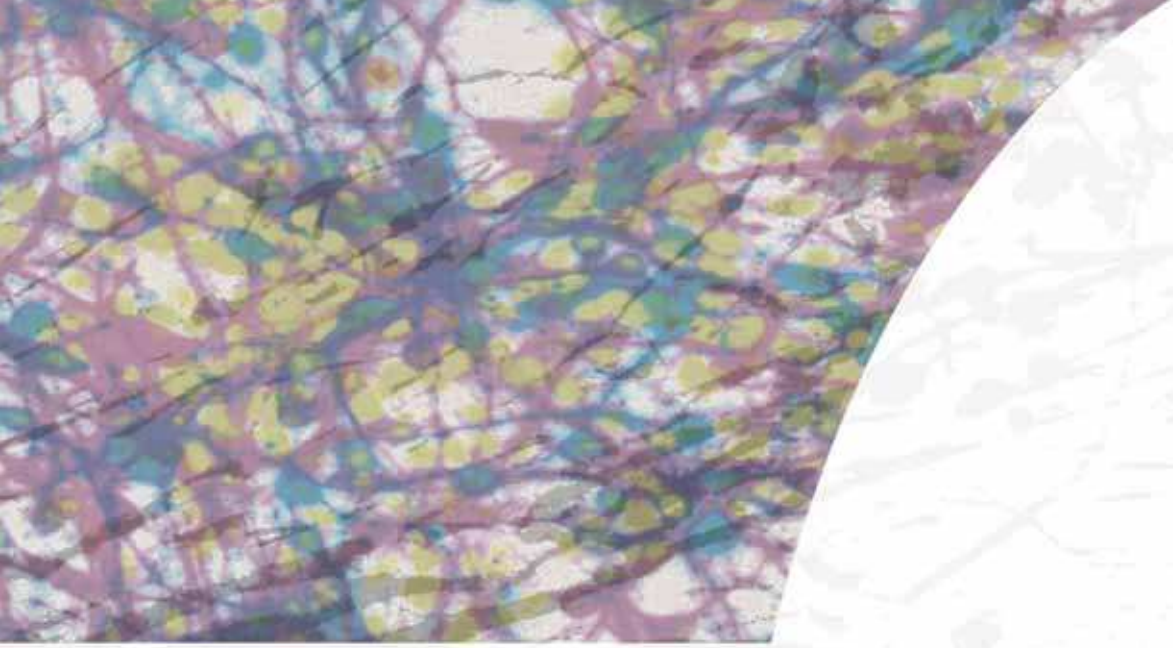
Bioethical Regulation of Human Fetal Tissue and Embryonic Germ Cellular Material: Legal Survey and Analysis
J. Kyle Kinner, Presidential Management Intern
National Bioethics Advisory Commission

Regulating Embryonic Stem Cell Research: Biomedical Investigation of Human Embryos
J. Kyle Kinner, Presidential Management Intern
National Bioethics Advisory Commission

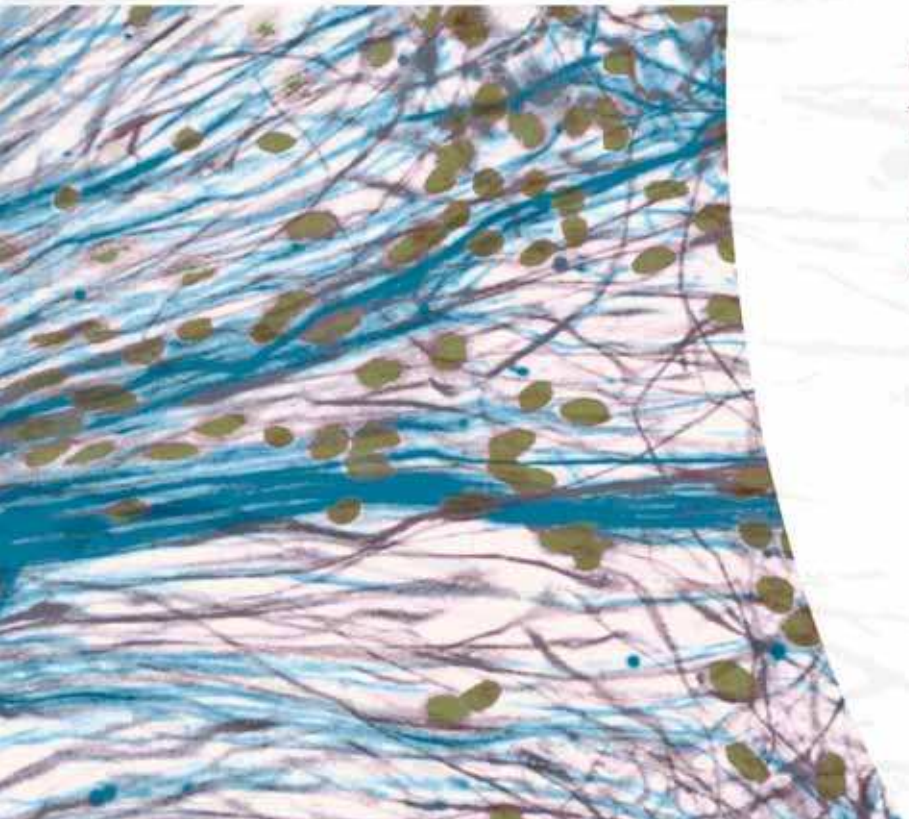
International Perspectives on Human Embryo and Fetal Tissue Research
Lori P. Knowles
The Hastings Center

What Has the President Asked of NBAC? On the Ethics and Politics of Embryonic Stem Cell Research
Erik Parens
The Hastings Center

Locating Convergence: Ethics, Public Policy, and Human Stem Cell Research
Andrew W. Siegel
The Johns Hopkins University



UNDERSTANDING STEM CELLS



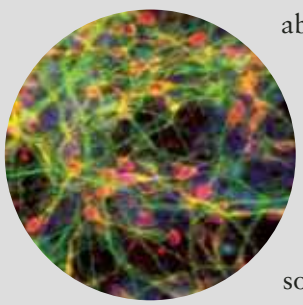
AN OVERVIEW OF THE SCIENCE AND ISSUES FROM THE NATIONAL ACADEMIES

National Academy of Sciences
National Academy of Engineering
Institute of Medicine
National Research Council

THE NATIONAL ACADEMIES
Advisers to the Nation on Science, Engineering, and Medicine

IN THIS BOOKLET. . .

For centuries, scientists have known that certain animals can regenerate missing parts of their bodies. Humans actually share this ability with animals like the starfish and the newt. Although we can't replace a missing leg or a finger, our bodies are constantly regenerating blood, skin, and other tissues. The identity of the powerful cells that allow us to regenerate some tissues was first revealed when experiments with bone marrow in the 1950s established the existence of *stem cells* in our bodies and led to the development of bone marrow transplantation, a therapy now widely used in medicine. This discovery raised hope in the medical potential of regeneration. For the first time in history, it became possible for physicians to regenerate a damaged tissue with a new supply of healthy cells by drawing on the unique ability of stem cells to create many of the body's specialized cell types.

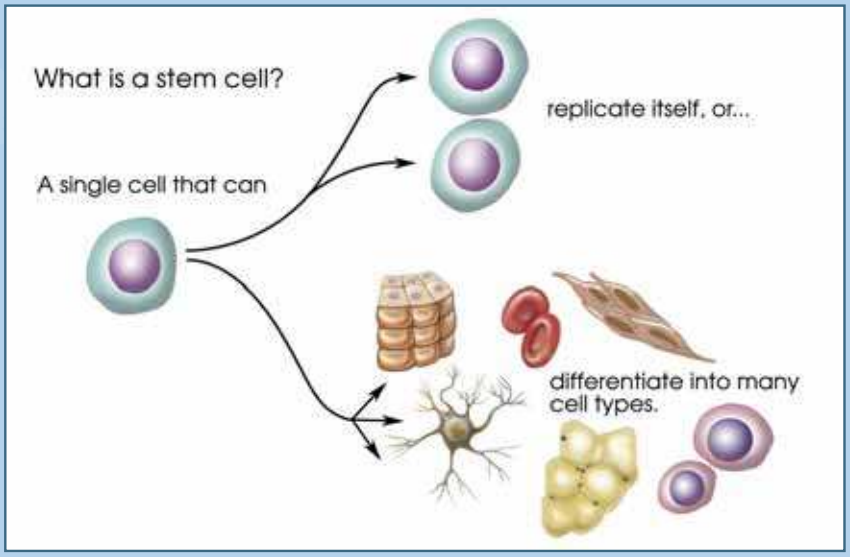


Once they had recognized the medical potential of regeneration through the success of bone marrow transplants, scientists sought to identify similar cells within the embryo. Early studies of human development had demonstrated that the cells of the embryo were capable of producing every cell type in the human body. Scientists were able to extract embryonic stem cells from mice in the 1980s, but it wasn't until 1998 that a team of scientists from the University of Wisconsin–Madison became the first group to isolate human embryonic stem cells and keep them alive in the laboratory. The team knew that they had in fact isolated stem cells because the cells could remain unspecialized for long periods of time, yet maintained the ability to transform into a variety of specialized cell types, including nerve, gut, muscle, bone, and cartilage cells.

Stem cell research is being pursued in the hope of achieving major medical breakthroughs. Scientists are striving to create therapies that rebuild or replace damaged cells with tissues grown from stem cells and offer hope to people suffering from cancer, diabetes, cardiovascular disease, spinal-cord injuries, and many other disorders. Both adult and embryonic stem cells may also provide a route for scientists to develop valuable new methods of drug discovery and testing. They are also powerful tools for doing the research that leads to a better understanding of the basic biology of the human body. By drawing on expert scientists, doctors, bioethicists, and others, the National Academies have examined the potential of stem cell technologies for medicine and provided a forum for discussing the ethical implications and moral dilemmas of stem cell research.

WHAT IS A STEM CELL?

Ultimately, every cell in the human body can be traced back to a fertilized egg that came into existence from the union of egg and sperm. But the body is made up of over 200 different types of cells, not just one. All of these cell types come from a pool of *stem cells* in the early embryo. During early development, as well as later in life, various types of stem cells give rise to the *specialized* or *differentiated* cells that carry out the specific functions of the body, such as skin, blood, muscle, and nerve cells.



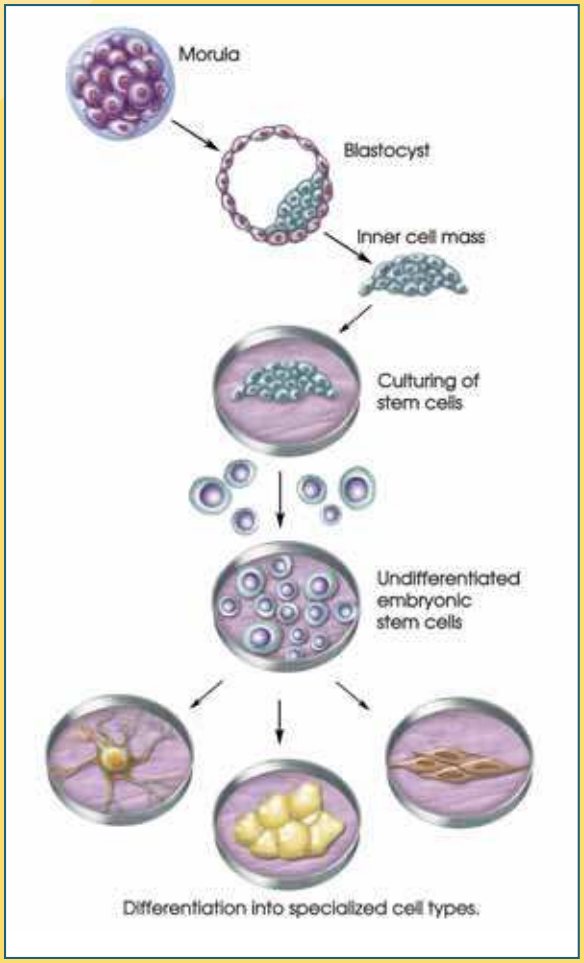
Over the past two decades, scientists have been gradually deciphering the processes by which unspecialized stem cells become the many specialized cell types in the body. Stem cells can regenerate themselves or produce specialized cell types. This property makes stem cells appealing for scientists seeking to create medical treatments that replace lost or damaged cells.

TYPES OF STEM CELLS

Stem cells are found in all of us, from the early stages of human development to the end of life. All stem cells may prove useful for medical research, but each of the different types has both promise and limitations. *Embryonic stem cells*, which can be derived from a very early stage in human development, have the potential to produce all of the body's cell types. *Adult stem cells*, which are found in certain tissues in fully developed humans, from babies to adults, may be limited to producing only certain types of specialized cells. Recently, scientists have also identified stem cells in umbilical cord blood and the placenta that can give rise to the various types of blood cells.

Embryonic Stem Cells

A *blastocyst* (BLAST-oh-sist), is a pre-implantation embryo that develops 5 days after the fertilization of an egg by a sperm. It contains all the material necessary for the development of a complete human being. The blastocyst is a mostly hollow sphere of cells that is smaller than the period at the end of this sentence. In its interior is the inner cell mass, which is composed of 30-34 cells that are referred to by scientists as *pluripotent* because they can differentiate into all of the cell types of the body. In comon usage, "embryo" can refer to all stages of development from fertilization until a somewhat ill-defined stage when it is called a fetus. Scientists use terms such as "morula" and "blastocyst" to refer to precise, specific stages of pre-implantation development. In order to be as precise as possible, this booklet uses the scientific terms when describing scientific concepts but uses the term "embryo" where more precision seemed likely to confuse rather than clarify.



Embryonic stem cells are derived from the inner cell mass of the blastocyst. In culture, they can self-replicate or produce specialized cell types.

In normal development, the blastocyst would implant in the wall of the uterus to become the embryo and continue developing into a mature organism. Its outer cells would begin to form the placenta and the inner cell mass would begin to differentiate into the progressively more specialized cell types of the body.

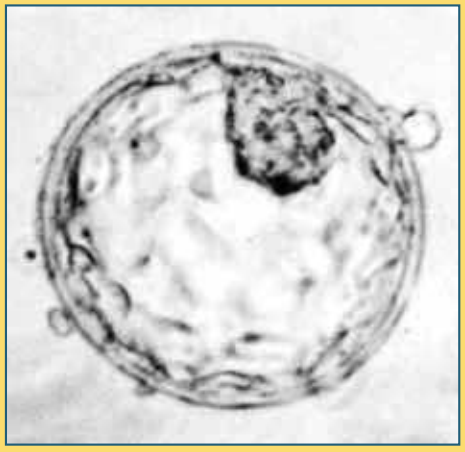
When the blastocyst is used for stem cell research, scientists remove the inner cell mass and place these cells in a culture dish with a nutrient-rich liquid where they give rise to embryonic stem cells. Embryonic stem cells seem to be more flexible than stem cells found in adults, because they have the potential to produce every cell type in the human body. They are also generally easier to collect, purify and maintain in the laboratory than adult stem cells.

Scientists can induce embryonic stem cells to replicate themselves in an *undifferentiated* state for very long periods of time before stimulating them to create specialized cells. This means that just a few embryonic stem cells can build a large bank of stem cells to be used in experiments. However, such undifferentiated stem cells could not be used directly for tissue transplants because they can cause a type of tumor called a teratoma. To be used for therapies, embryonic stem cells would first need to be differentiated into specialized cell types.

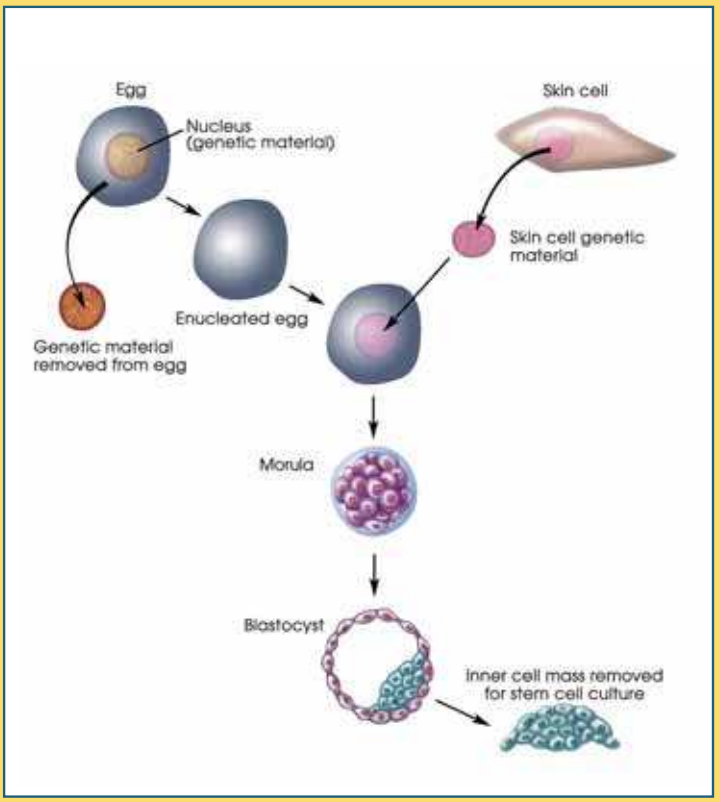
Some find embryonic stem cell research to be morally objectionable, because when scientists remove the inner cell mass, the blastocyst no longer has the potential to become a fully developed human being.

Sources of Embryonic Stem Cells

In Vitro Fertilization: The largest potential source of blastocysts for stem cell research is from in vitro fertilization (IVF) clinics. The process of IVF requires the retrieval of a woman's eggs via a surgical procedure after undergoing an intensive regimen of "fertility drugs," which stimulate her ovaries to produce multiple mature eggs. When IVF is used for reproductive purposes, doctors typically fertilize all of the donated eggs in order to maximize their chance of producing a viable blastocyst that can be implanted in the womb. Because not all the fertilized eggs are implanted, this has resulted in a large bank of "excess" blastocysts that are currently stored in freezers around the country. The blastocysts stored in IVF clinics could prove to be a major source of embryon-



A human blastocyst, which is produced about 5 days after fertilization, is smaller than the period at the end of this sentence. NIH/Mr. J. Conaghan.



Through nuclear transfer, scientists could produce a blastocyst by inserting the nucleus from an adult cell (for example, a skin cell) into an egg without a nucleus. All the stem cells derived from this blastocyst are genetically matched to the adult cell.

ic stem cells for use in medical research. However, because most of these blastocysts were created before the advent of stem cell research, most donors were not asked for their permission to use these left-over blastocysts for research.

The in vitro fertilization (IVF) technique could potentially also be used to produce blastocysts specifically for research purposes. This would facilitate the isolation of stem cells with specific genetic traits necessary for the study of particular diseases. For example, it may be possible to study the origins of an inherited disease like cystic fibrosis using stem cells made from egg and sperm donors who have this disease. The creation of stem cells specifically for research using IVF is, however, ethically problematic for some people because it involves intentionally creating a blastocyst that will never develop into a human being.

Nuclear Transfer: The process called *nuclear transfer* offers another potential way to produce embryonic stem cells. In animals, nuclear transfer has been accomplished by inserting the nucleus of an already differentiated adult cell—for example, a skin cell—into a donated egg that has had its nucleus removed. This egg, which now contains the genetic material of the skin cell, is then stimulated to form a blastocyst from which embryonic stem cells can be derived. The stem cells that are created in this way are therefore copies or "clones" of the original adult cell because their nuclear DNA matches that of the adult cell.

As of the summer of 2006, nuclear transfer has not been successful in the production of human embryonic stem cells,[1] but progress in animal research suggests that scientists may be able to use this technique to develop human stem cells in the future.

[1]Claims by Korean scientists of successful derivation of human embryonic stem cells using nuclear transfer have been found to be invalid and were retracted.

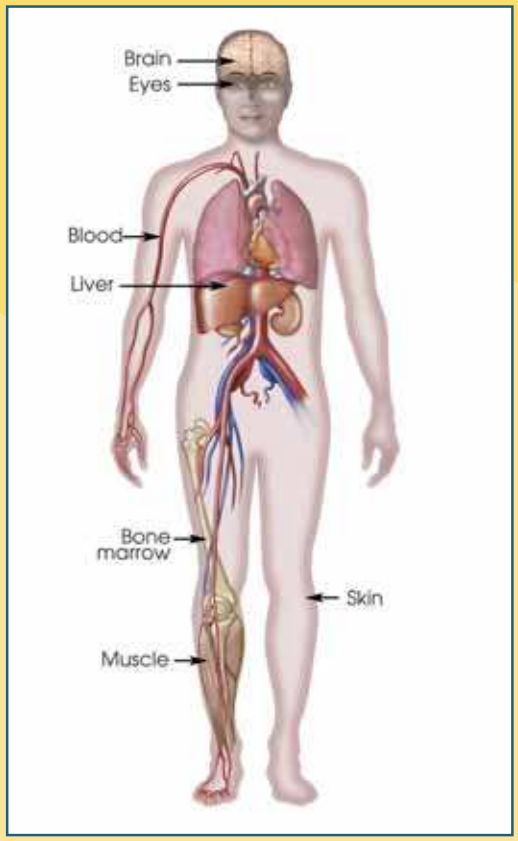
Producing Embryonic Stem Cells Using Nuclear Transfer Is Not the Same as Reproductive Cloning

The use of nuclear transfer to develop disease-specific stem cells can be called *research cloning*, and the use of this technique for personalized tissue transplants is sometimes called *therapeutic cloning*. These terms must be carefully distinguished from *reproductive cloning*, in which the intent is to implant a cloned embryo in a female's womb and allow it to develop fully into an individual. This was the technique by which Dolly the sheep was made and is now widely used for reproductive cloning in animals. In humans, however, reproductive cloning has been actively discouraged by most in the scientific community. The National Academies concluded, "Human reproductive cloning should not now be practiced. It is dangerous and likely to fail" in the 2002 report *Scientific and Medical Aspects of Human Reproductive Cloning*.

Although using nuclear transfer to produce stem cells is not the same as reproductive cloning, some are concerned about the potential misapplication of the technique for reproductive cloning purposes. Other ethical considerations include egg donation, which requires informed consent, and the possible destruction of blastocysts.

Adult Stem Cells

Adult stem cells are hidden deep within organs, surrounded by millions of ordinary cells, and may help replenish some of the body's cells when needed. In fact, some adult stem cells are currently being used in therapies. They have been found in several

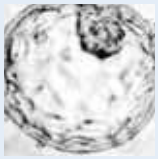


Some of the known sources of adult stem cells.

Scientists believe that if they are able to use nuclear transfer to derive human stem cells, it could allow them to study the development and progression of specific diseases by creating stem cells containing the genes responsible for certain disorders. In the future, scientists may also be able to create "personalized" stem cells that contain only the DNA of a specific patient. The embryonic stem cells created by nuclear transfer would be genetically matched to a person needing a transplant, making it far less likely that the patient's body would reject the new cells than it would be with traditional tissue transplant procedures.

organs that need a constant supply of cells, such as the blood, skin, and lining of the gut, and have also been found in surprising places like the brain, which is not known to readily replenish its cells. Unlike embryonic stem cells, adult stem cells are already somewhat specialized. For example, blood stem cells normally only give rise to the many types of blood cells, and nerve stem cells can only make the various types of brain cells. Recent research however, suggests that some adult stem cells might be more flexible than previously thought, and may be made to produce a wider variety of cell types. For example, some experiments have suggested that blood stem cells isolated from adult mice may also be able to produce liver, muscle, and skin cells, but these results are not yet proven and have not been demonstrated with human cells. Nevertheless, scientists are working on finding a way to stimulate adult stem cells, or even other types of adult cells, to be more versatile. If they succeed, it could provide another source of unspecialized stem cells.

COMPARISON OF THE DIFFERENT SOURCES OF STEM CELLS

	Embryonic Stem Cells		Adult Stem Cells
			
	In Vitro Fertilization	**Nuclear Transfer**	**Adult Tissues**
Attributes	• can produce all cell types • relatively easy to identify, isolate, maintain, and grow in the laboratory • large source of "excess" blastocysts from IVF clinics	• can produce all cell types • relatively easy to identify, isolate, maintain, and grow in the laboratory • stem cells may be genetically matched to patient	• demonstrated success in some treatments • stem cells may be genetically matched to patient
Limitations	• limited number of cell lines available for federally funded research • risk of creating teratomas (tumors) from implanting undifferentiated stem cells	• not yet achieved with human cells • risk of creating teratomas (tumors) from implanting undifferentiated stem cells	• produce limited number of cell types • not found in all tissues • difficult to identify, isolate, maintain, and grow in the laboratory
Ethical Concerns	• destruction of human blastocysts • donation of blastocysts requires informed consent	• destruction of human blastocysts • donation of eggs requires informed consent • concern about misapplication for reproductive cloning	• no major ethical concerns have been raised

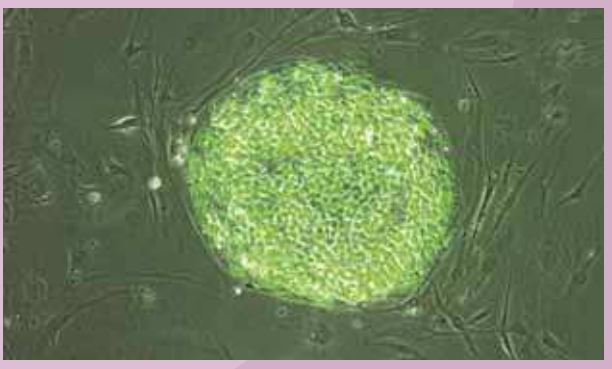
WORKING WITH STEM CELLS

The day-to-day work that goes on in the laboratories across the country studying stem cells begins with developing ways to identify stem cells, culture cell lines, and stimulate stem cells to differentiate. Once these first steps have been achieved, work on animals plays an important role in furthering basic research and developing medical applications. This work is necessary to form the foundation of knowledge that will point the way to medical advances.

Identifying Stem Cells

As early as 1961, scientists knew that adult bone marrow contained cells that could make all of the blood cell types. But it wasn't until 1988 that those stem cells were isolated as pure populations. Why did it take so long? The techniques for identifying stem cells have only recently been developed. Partly, this is because adult stem cells are, by their very nature, inconspicuous in shape, size, and function. They also tend to hide deep in tissues and are present only in very low numbers, making their identification and isolation like finding a needle in a haystack.

How do scientists know when they have found a stem cell? Every cell displays an array of proteins on its surface; different cell types have different proteins. Scientists can use these surface proteins as "markers" that characterize individual cell types—a type of "molecular ID." For example, using molecules that recognize and attach to specific surface proteins and that can fluoresce under certain wavelengths of light, scientists can visually tell the difference between a blood stem cell and a mature white blood cell. Unfortunately, not all stem cells can now be identified in this manner because scientists have not yet identified markers for all stem cell types. Scientists also identify stem cells by observing their behavior in the laboratory: stem cells must be able to remain unspecialized and self-renew for long periods of time.



Fluorescent markers can be used to identify stem cells hidden among ordinary adult cells. Here, human embryonic stem cells are recognized by the marker proteins they express (green). Courtesy of Paul J. Tesar, Laboratory of Molecular Biology, NINDS and the NIH Stem Cell Unit.

Scientists believe that there might be more types of adult stem cells than the handful that have already been identified, but finding them is a difficult process.

Culturing Cell Lines and Stimulating Stem Cells to Differentiate

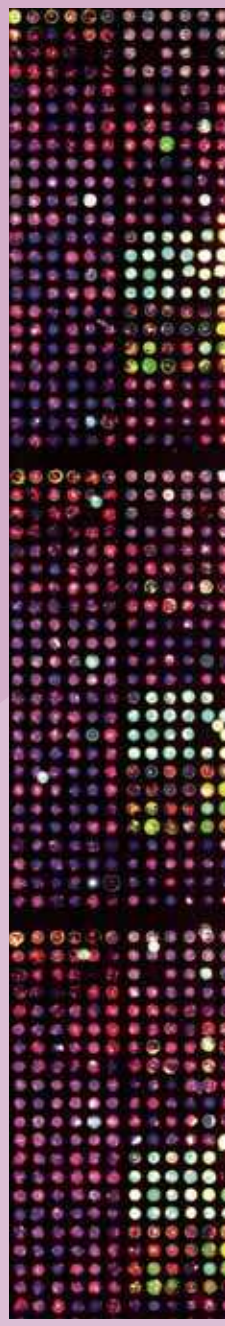
Cell culture is a term that refers to the growth and maintenance of cells in a controlled environment outside of an organism. A successful stem cell culture is one that keeps the cells healthy, dividing, and unspecialized. The culturing of stem cells is the first step in establishing a stem cell line—a propagating collection of genetically identical cells. Cell lines are important because they provide a long-term supply of multiplying cells that can be shared among scientists for research and therapy development. The National Academies report *Stem Cells and the Future of Regenerative Medicine* (2001) described some of the challenges of maintaining cell lines: "Over time, all cell lines…change, typically accumulating harmful genetic mutations. There is no reason to expect stem cell lines to behave differently. While there is much that can be learned using existing stem cell lines…such concerns necessitate continued monitoring of these cells as well as the development of new stem cell lines in the future."

Once they have established a stable stem cell line, scientists start the process of causing the stem cells to differentiate into specialized cell types. The cellular environment in which stem cells naturally reside provides scientists with clues about how to make them differentiate in a culture dish. For example, in the bone marrow, where blood stem cells reside, bone cells send physical and chemical signals that tell the blood stem cells when to differentiate. Scientists are just beginning to understand these signals and have developed ways to mimic the natural processes in cell cultures. Usually, the technology involves adding certain proteins to the cell culture and, in some cases, introducing specific genes into the stem cells.

It will be essential that scientists are sure that stem cells have fully differentiated before they can use them for medical applications. If completely undifferentiated stem cells (such as embryonic stem cells) are implanted directly into an organism, they can cause a type of tumor called a *teratoma*, which scientists have observed in experiments using mice. Semi-specialized adult stem cells and differentiated cells derived from embryonic stem cells are unlikely to cause teratomas.

The Role of Animals in Stem Cell Research

For medical research, as well as for research that explores the basic processes in the development of organisms and diseases, scientists often rely on animals. Implanting human cells into animals



Center Photo: Scientists can test whether they have successfully caused embryonic stem cells to differentiate by labeling for specific *marker proteins* found in specialized cells. Courtesy of Dr. Daniel Anderson, MIT.

such as mice has long been common practice in order to test the safety and effectiveness of new drugs, procedures, and medical devices before clinical testing in human volunteers. For stem cell research, scientists use animals to make sure the stem cells are able to incorporate into the tissue, do not cause any harmful consequences, and function in concert with the rest of the body. For example, before using stem cells to replace the pancreatic cells that are destroyed by type I diabetes in humans, scientists will transplant human stem cells into a mouse to see whether the stem cells yield healthy, insulin-producing cells. If their methods prove successful in mice, scientists may eventually apply the technology to developing treatments for diabetes in humans.

Animal studies can also reveal how human cells differentiate during normal development. For example, scientists may implant human stem cells into a developing mouse to observe the processes involved in building and organizing the different tissue types that make up the human body. Scientists can also trace the development and progression of certain diseases within an animal. By implanting human stem cells that lead to a particular disease into a mouse blastocyst, scientists can observe when and how the afflicted cells begin to show signs of disease and can test drugs that might prevent that process.

Many research mice are *chimeras* because they contain both human and mouse cells. Courtesy of Advanced Cell Technology, Inc., Alameda, CA.

Organisms that contain cells or tissues from another individual of the same or a different species are called *chimeras*. A common example of a chimera is a mouse that has been injected with some human cells so that it can be used for studying a human disease or testing a new drug. A person who has had a blood transfusion or a person who has received a heart valve transplant from a pig is technically a chimera, as well. The making of chimeras for research has unique ethical implications that have been the topic of discussions among scientists, ethicists and the public, especially when the chimeras contain both human and animal cells.

Alternatives to Using Embryos in Stem Cell Research

To address ethical concerns about the destruction of blastocysts, scientists are trying to find new ways of obtaining stem cells that behave like embryonic stem cells but that don't require harming a blastocyst. As the science progresses, ethical issues surrounding these alternatives may also arise. Some possible alternatives include:

Courtesy of Leonard I. Zon

- Cells collected from the *morula* (MOR-yoo-la), the developmental stage prior to the blastocyst. The morula, a solid ball of about 16–30 cells, seems able to sustain the loss of a few cells without developmental damage so that the remaining cells can continue to develop. Cell extraction from the morula is already being used in some clinics to screen for genetic disorders in embryos produced by in vitro fertilization. Researchers have recently shown that cells isolated from a mouse morula can give rise to embryonic stem cells while the remaining morula cells develop into a healthy mouse. However, this process may still be morally objectionable to some because of the chance of harm to the morula, and because the long-term effects of removing cells from a morula are not yet known.

- The creation of embryonic stem cells through a process called *altered nuclear transfer* (ANT). In this variation of the nuclear transfer technique, scientists create a blastocyst whose genetic material has been changed so that further development and implantation into the uterus is not possible. It aims to create embryo-like entities that are not truly embryos but that can be a source of pluripotent stem cells. ANT, so far only tested with mouse blastocysts, could allow the creation of embryonic stem cells without destroying a viable human blastocyst. Some who object to embryonic stem cell research support ANT because the resulting blastocyst could never develop into a full human being and therefore would not have the moral status of a human embryo. However, this procedure is objectionable to some because they believe that it involves the creation of an imperfect blastocyst that is designed to be destroyed.

- Causing an adult cell to act like an embryonic stem cell. During development, as cells become more and more specialized, they gradually lose the ability to turn on the genes that allow embryonic stem cells to be so versatile. The silencing of these genes seems to be responsible for keeping specialized cells specialized and limiting the differentiation capacities of adult stem cells. By "reprogramming" adult stem cells so that they can turn on the genes that allow versatility, scientists hope to cause them to revert to a more flexible state. It is even possible that scientists could one day "reprogram" any cell, not only stem cells. However, research in this area is in the early stages and scientists may be many years away from making an adult cell as versatile as an embryonic stem cell.

WHY STEM CELL RESEARCH IS BEING PURSUED

Right now, only a few diseases are treatable with stem cell therapies because scientists can only regenerate a few types of tissues. However, the success of the most established stem cell-based therapies—blood and skin transplants—gives hope that someday stem cells will allow scientists to develop therapies for a variety of diseases previously thought to be incurable. Many major diseases are caused by the loss of a single type of cell or tissue. For example, type I diabetes (juvenile-onset) is caused by the loss of the insulin-producing cells of the pancreas, and its treatment is limited to merely alleviating the symptoms. Finding a cure for such diseases would be much easier if scientists could simply re-grow the missing or damaged cells and implant them into patients.

Blood Stem Cells

After scraping a knee or donating blood, the body replenishes the blood cells that are lost by drawing on a small number of semi-specialized *hematopoietic* (heem-AT-oh-poh-EH-tik) stem cells contained in the blood and bone marrow. For decades, scientists have been using this type of adult stem cell to treat patients with diseases such as leukemia, sickle cell anemia, bone marrow damage, and some metabolic disorders and immunodeficiencies where the body has lost its ability to replenish its own set of healthy blood cells. Hematopoietic stem cells give rise to all the blood cell types, from infection-fighting white blood cells to blood-clotting platelets. Preliminary results have suggested that they may also be able to produce other cell types not found in blood, but this is not yet proven.

In the past, the only way to use hematopoietic stem cells for therapies was through bone marrow transplants. Extracting bone marrow is an uncomfortable and invasive procedure, and in order for a transplant to work, the donor and recipient must be genetically similar. If they are too genetically different, the blood cells produced from the transplanted marrow may recognize the patient's body as foreign and fight against the patient's own cells and organs. Additionally, the patient's immune system may reject the transplant, causing a dangerous "war" within the patient's body.

More recently, scientists have developed ways to derive hematopoietic stem cells from the blood contained in the umbilical cord and placenta at birth. The stem cells isolated from a person's own umbilical cord blood and placenta, if used for therapies later in life, would be less likely to cause an "internal war" within the recipient's body. They are also more accessible than the stem cells in bone marrow because the extraction of this blood poses no risk to the mother or infant.

The Changed Face of Skin Grafts

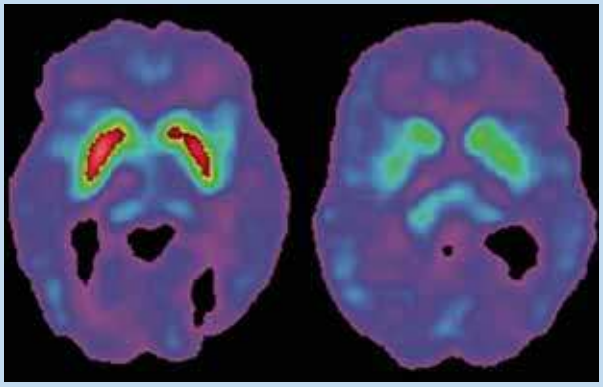
For many years, scientists have been harnessing the regenerative capabilities of human skin to treat victims of severe burns using skin transplants. Skin transplants are possible because of the existence of stem cells located just under the top layer of skin. Every day, thousands of new skin cells are produced to replace those that have been shed. When someone suffers severe burns that destroy the source of these stem cells, their skin can no longer regenerate on its own. Traditionally, doctors treated severe burns by transplanting sections of skin from undamaged areas of the body onto the burned areas, but if doctors could not find enough unharmed skin to cover the burned areas, the patient could die. Now, scientists can grow vast sheets of new skin by culturing the stem cells from small pieces of healthy skin. This practice, which is a type of tissue engineering, has become routine for treating burn victims over the past 20 years. Recently, scientists have identified other types of stem cells in hair follicles and deeper layers of the skin. The inclusion of these new stem cells into engineered skin should help create more natural-looking skin transplants in the future.

Stem Cells Found in Umbilical Cord Blood

In 2005, the National Academies issued a report, *Cord Blood: Establishing a National Hematopoietic Stem Cell Bank Program*, which recommended that a national cord blood "bank" be established to harness the medical potential of this source of stem cells. Such a bank would not only benefit the people from whom the blood was collected but anyone in need of blood transplants. As with blood banks for blood transfusions, scientists could screen the bank to find the best match for each patient, providing a safer, more personalized living-cell therapy.

Possible Future Treatment for Parkinson's Disease?

When most people reach for a pen, their body acts in one smooth and controlled movement. This is because the instant a person thinks of grabbing the pen, a series of nerve cells fire in an orchestrated symphony from the brain to the muscles responsible for that action. For the movement to be precise and smooth, all the nerve cells in the "grabbing-the-pen network" must function properly, including cells that tell unneeded muscles to stay still. In Parkinson's disease, the brain cells responsible for keeping unneeded muscles from moving degenerate and die. This results in progressively more dramatic and uncontrolled movements, tremors, and spasms. To date, there is no cure for Parkinson's disease because no one has figured out a way to bring back the specialized nerve cells that have died.



Parkinson's disease is caused by the loss of a single type of nerve cell. These brain scans show the difference between a normal brain (left) and the brain of a Parkinson's patient (right). Courtesy of Dr. David A. Rottenberg, Professor of Neurology and Radiology, University of Minnesota.

Are the Promises of Stem Cell Therapies Realistic?

The list of medical achievements stem cells could offer seems to be expanding at an incredible pace. The role of stem cells in medicine is already very real, but there is a danger of exaggerating the promise of new medical developments. What tend to be "over-promised" are not only the potential outcomes of both embryonic and adult stem cell research, but also the time scales that are involved. The basic research needed to develop viable therapeutic options is a lengthy process that may extend over many years and decades. Even after science has moved from basic research to developing medical applications, it still takes many years to thoroughly test those applications and demonstrate that they are safe to prescribe for patients. This is true for all medical treatments, including the development of new drugs, procedures, and medical equipment, and is not specific to the living cell therapies made possible by stem cell research.

There are also many legal and social questions that must be addressed before stem cell-based therapies become clinically available. Legal issues that will affect stem cell applications include how to address intellectual property concerns and how to apply and enforce diverse and sometimes conflicting state and national laws. Social issues include concerns about the destruction of embryos, the distribution of the benefits of the research, and the protection of both physical and privacy interests of egg and sperm donors and clinical research subjects.

Because Parkinson's disease results from the loss of one specific type of nerve cell, stem cells offer a very tangible possibility for treatment. Researchers have recently learned how to differentiate embryonic stem cells into the specific type of brain cell that is lost in Parkinson's disease. They have also successfully transplanted adult nerve stem cells into rat brains. When this technique is proven to be effective and safe, transplantation of stem cells into the brains of patients may one day allow doctors to reverse the burden of Parkinson's disease and restore control of movement. Another strategy currently under study is the addition of chemicals or growth factors that aim to induce the patient's own stem cells to repair the damaged nerves without needing to grow and transplant stem cells.

Possible Fix for Diabetes?

In people who suffer from type I diabetes, the beta cells of the pancreas that normally produce insulin are destroyed by the patient's overactive immune system. Without insulin, the cells of the body cannot take up glucose and they starve. Patients with type I diabetes

STEM CELL TIMELINE

1956

First successful bone marrow transplant

1981

Embryonic stem cells are isolated from mouse blastocysts

1988

Hematopoietic (blood) stem cells from adult mice are purified and characterized

1992

Stem cells are identified in the adult human brain

1998

The first human embryonic stem cells are isolated

require insulin injections several times a day for their entire lives. The only current cure is a pancreatic transplant from a recently deceased donor, but the demand for transplants far outweighs the supply. While adult stem cells have not yet been found in the pancreas, scientists have made progress transforming embryonic stem cells into insulin-producing cells. Combining beta-cell transplants with methods to "fix" the patient's immune system—including chemotherapy to destroy malfunctioning immune-system cells and blood transplants to replenish healthy white blood cells—could offer great hope for the many Americans suffering with type I diabetes.

Cancer: Getting to the Root of the Problem

Why are some cancers so hard to eliminate, even after many rounds of chemotherapy? The answer may lie in a few abnormal stem cells. Cancerous stem cells were first identified in 1997 when a research group from the University of Toronto transferred a few blood stem cells from human leukemia patients into mice and watched leukemia develop in the mice. Stem cell-like cells have also recently been found in breast and brain tumors. Like normal stem cells, tumor stem cells exist in very low numbers, but they can replicate and give rise to a multitude of cells. Unlike normal stem cells, however, cancerous stem cells lack the controls that tell them when to stop dividing. Traditional chemotherapy kills off the majority of the tumor cells, but if any of the cancerous stem cells survive the treatment, the cancer may return. Research into the differences in gene expression between normal and tumor stem cells may lead to treatments where the root of the problem—the cancer stem cell—is targeted.

2001

Mouse embryonic stem cells are created by nuclear transfer

2002

Pancreatic cells derived from mouse embryonic stem cells cure diabetes in mice

2004

The type of nerve cell lost in Parkinson's disease is produced from human embryonic stem cells

Stem cell research continues to advance. Preliminary results from recent studies support the promise of stem cells for conducting basic research that may eventually lead to medical achievements. For example, in 2005, human embryonic stem cells were shown to differentiate into active functioning nerve cells when placed in mouse brains. Scientists also made significant progess in deriving pancreatic cells from adult stem cells. In 2006, scientists were able to derive embryonic stem cells from the morula of a mouse, and embryonic stem cells were first grown without animal products in the culture. Results of these and other recent experiments must be replicated and consistently demonstrated by other researchers before they become generally accepted by the scientific community.

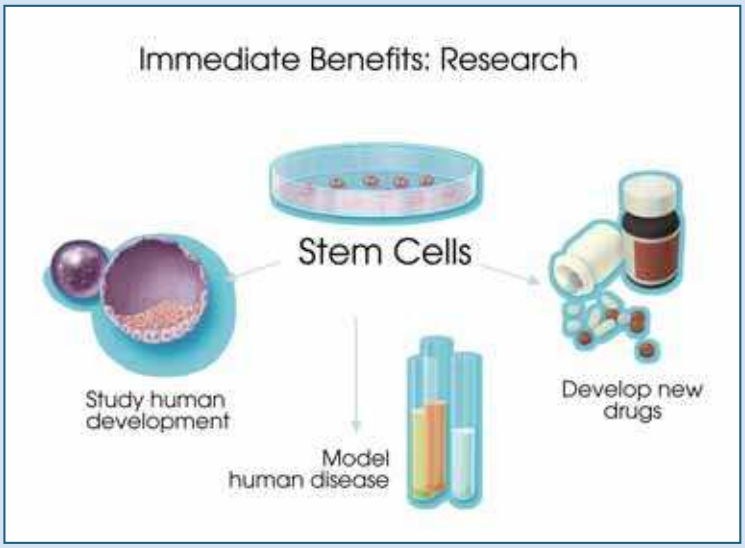
The Role of Stem Cells in Basic Research

Stem cells offer opportunities for scientific advances that go far beyond regenerative medicine. They offer a window for addressing many of biology's most fundamental questions. Watching embryonic stem cells give rise to specialized cells is like peeking into the earliest development of the many tissues and organs of the human body. Stem cell research may help clarify the role genes play in human development and how genetic mutations affect normal processes. They can be used to study how infectious agents invade and attack human cells, to investigate the genetic and environmental factors that are involved in cancer and other diseases, and to decipher what happens during aging.

Stem cells may also revolutionize traditional chemical medicine. Because embryonic stem cells can continue to divide for long periods of time and produce a variety of cell types, they could provide a valuable source of human cells for testing drugs or measuring the effects of toxins on normal tissues without risking the health of a single human volunteer. In the future, thousands of compounds could be quickly tested on a wide assortment of cell types derived from stem cells, making drug discovery more efficient and cost effective.

Using nuclear transfer to produce stem cells could be particularly useful for testing drugs for disorders that are of genetic origin. For example, it is difficult to study the progression of Alzheimer's and Parkinson's diseases in the brains of live patients—but by using the cells of an Alzheimer's patient to create stem cell lines with nuclear transfer, scientists could trace the development of the disease in a culture dish and test drugs that regenerate lost nerve cells with no danger to the patient.

Stem cells may also help scientists calculate the effects of toxic substances in drugs, food, and the environment.



Courtesy of Dr. Leonard I. Zon.

ETHICS, MORAL VALUES, AND U.S. LAW

Scientists and society as a whole must consider the ethical implications of stem cell research. As discussed throughout this booklet, different ethical issues are raised by the wide range of stem cell research activities. In 2005, the National Academies published guidelines for scientists who do research with human embryonic stem cells to encourage responsible and ethically sensitive conduct in their work. Although the guidelines are not expressly legally binding, many researchers have voluntarily adopted them as a guide to what constitutes appropriate conduct in human embryonic stem cell research. Yet for some people, such guidelines are inadequate because they aim to govern a practice that they see as intrinsically unethical.

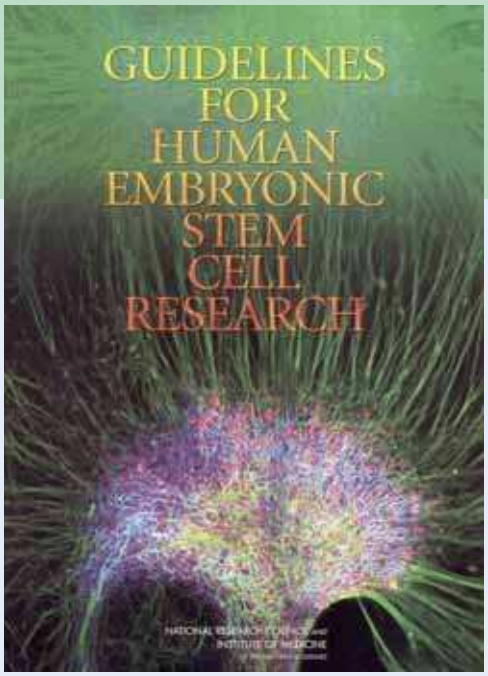
As the science advances, it is essential that scientists; religious, moral, and political leaders; and society as a whole continue to evaluate and communicate about the ethical implications of stem cell research.

The National Academies published the *Guidelines for Human Embryonic Stem Cell Research* in 2005. Here, members of the committee present at a report briefing.

Is an Embryo a Person?

The controversy over embryonic stem cell research touches on some of the same fundamental questions that society has grappled with in the debates over contraception, abortion, and in vitro fertilization. The questions at the center of the controversy concern the nature of early human life and the legal and moral status of the human embryo. Embryonic stem cell research often involves removing the inner cell mass from "excess" blastocysts that are unneeded by couples who have completed their fertility treatment. This prevents those blastocysts from continuing to develop. Although such blastocysts would likely be discarded (and thus destroyed) by the clinics in any case, some believe that this does not make it morally acceptable

to use them for research or therapeutic purposes. They believe that the life of a human being begins at the moment of conception and that society undermines a commitment to human equality and to the protection of vulnerable individuals if blastocysts are used for such purposes. Some cultures and religious traditions oppose the use of human life as a means to some other end, no matter how noble that end might be. Other traditions support embryonic stem cell research because they believe that the embryo gains the moral status of a human being only after a few weeks or months of development. Many traditions emphasize obligations to heal the sick and ease suffering—goals for which embryonic stem cell research holds great potential—and favor embryonic stem cell research for this reason. Several religious groups are currently involved in internal discussions about the status of the human embryo and have not yet established official opinions on the matter. Public opinion polls suggest that the majority of both religious and non-religious



The National Academies' Guidelines for Human Embryonic Stem Cell Research

In order to provide all scientists—those working in universities and private companies and with both public and private funding—with a common set of scientific and ethical guidelines, the National Academies published the *Guidelines for Human Embryonic Stem Cell Research* in 2005. The report outlines the need for institutional oversight mechanisms for monitoring all human embryonic stem cell research and provides specific guidance regarding the derivation of new stem cell lines. Under the guidelines, certain activities, such as experimenting on human embryos by inserting stem cells into them, are not permitted. The guidelines also require that all egg, sperm, and blastocyst donations follow appropriate informed consent and confidentiality procedures. Because the ethical and technical questions associated with human embryonic stem cell research are likely to change as science advances, in 2006, the National Academies established a panel of experts to monitor and review scientific developments and changing ethical, legal, and policy issues and to prepare periodic reports to update the guidelines as needed. For more information on the guidelines, please visit www.nationalacademies.org/stemcells.

Americans support embryonic stem cell research, although public opinion seems divided about the creation or use of human blastocysts solely for research.

The Relationship of Stem Cell Research to Reproductive Cloning

Although cloning and stem cell research are often lumped together in the context of ethical debates, the goals and results of the two are very different. The common factor between current attempts at reproductive cloning and stem cell research is a laboratory technique called *nuclear transfer*. Using nuclear transfer, scientists can create blastocysts containing stem cells that are "clones" of a single adult cell by inserting the genetic material from an adult cell (for example, a skin cell) into an egg whose nucleus has been removed (this process is described in more detail on page 6). Scientists hope that they could derive stem cells from the cells inside such blastocysts and grow replacement tissues that are genetically matched to specific patients, thus offering patients a safer alternative to traditional tissue transplants.

Reproductive cloning, such as the process that was used to create Dolly the sheep, also uses the nuclear transfer technique. However, instead of removing the inner cell mass to derive a stem cell line, the blastocyst is implanted into the uterus and allowed to develop fully. In 2002, the National Academies issued the report *Scientific and Medical Aspects of Human Reproductive Cloning*, which concluded "Human reproductive cloning should not now be practiced. It is dangerous and likely to fail."

"Human reproductive cloning should not now be practiced. It is dangerous and likely to fail."

—*Scientific and Medical Aspects of Human Reproductive Cloning,* National Academies Press, 2005

The Ethics of Human-Animal Chimeras

Chimeras are organisms composed of cells or tissues from more than one individual. Chimeras have been produced for research for many years, but when human and animal cells are mixed in the laboratory, there is a clear need for heightened ethical consideration. Cells from different organisms can be combined either in the early developmental stages (for example, introducing human cells into a mouse blastocyst to observe certain developmental processes) or after an individual is fully developed (for example, implanting

human stem cell-derived pancreatic cells into a mouse to test their ability to function in a living body). Chimeras are considered essential for advancing stem cell research to viable therapies, since no therapy can be tested in humans without research in animals first.

Some people believe that the creation of chimeras involving human cells for medical research is morally acceptable as long as the chimera has no level of human consciousness. Therefore, research in which it is possible for human stem cells to produce part of an animal's brain should be conducted with great care. The National Academies' guidelines prohibit the introduction of human cells into the blastocyst of a non-human primate, or the introduction of any animal or human cells into a human blastocyst. The guidelines also prohibit the breeding of human-animal chimeras in the unlikely event that any human genetic material would be contained in their reproductive cells.

Is it legal?

Currently, all forms of stem cell research in the U.S. are legal at the federal level. That is, it is not illegal to make or work with new embryonic stem cell lines. However, the use of federal funds for human embryonic stem cell research is restricted to the cell lines that were available as of August 9, 2001. Therefore, the derivation of new embryonic stem cell lines can only occur when scientists are working with non-federal funding. Some states and private foundations have been supporting this work. Some requirements of federal law, such as human subjects protections, apply to state- and privately funded stem cell research. For a complete discussion of the mechanisms for oversight of stem cell research, see the National Academies' report *Guidelines for Human Embryonic Stem Cell Research*.

It is legal to conduct research using blastocysts and to derive new cell lines in most states, with some exceptions. Because stem cell legislation is an area of active debate, please visit the National Conference of State Legislatures at http://www.ncsl.org/programs/health/genetics/embfet.htm to learn about the laws in a particular state.

ABOUT THE NATIONAL ACADEMIES

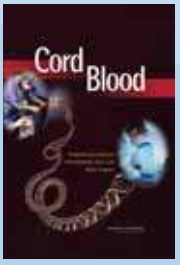
The National Academies are private, nonprofit organizations that were set up to provide independent scientific and technological advice to the U.S. government and nation. The National Academies includes three honorary societies that elect new members to their ranks each year—the National Academy of Sciences, National Academy of Engineering, and Institute of Medicine—and the National Research Council, the operating arm that conducts the bulk of the institution's science policy and technical work. The Academies enlist committees of the nation's top scientists, engineers, and other experts, all of whom volunteer their time to study specific issues and concerns.

RELATED REPORTS FROM THE NATIONAL ACADEMIES

Stem Cells and the Future of Regenerative Medicine (2001).
http://www.nap.edu/catalog/10195.html

Scientific and Medical Aspects of Human Reproductive Cloning (2002).
http://www.nap.edu/catalog/10285.html

Guidelines for Human Embryonic Stem Cell Research (2005).
http://www.nap.edu/catalog/11278.html
(free download is available)



Cord Blood: Establishing a National Hematopoietic Stem Cell Bank Program (2005).
http://www.nap.edu/catalog/11269.html

These and other National Academies reports are available from the National Academies Press, 500 Fifth Street, NW, Washington, DC 20001; 800-624-6242; http://www.nap.edu. Reports are available online in a fully searchable format.

JA231

This booklet and other information about activities related to stem cells at the National Academies are available at www.nationalacademies.org/stemcells.

For more information, contact the Board on Life Sciences at bls@nas.edu or visit www.nationalacademies.org/bls. This brochure was prepared by National Research Council staff Anne Jurkowski, Giovanna Guerrero, Fran Sharples, and Adam Fagen in collaboration with Bruce Altevogt and Andrew Pope of the Institute of Medicine's Health Sciences Policy Board. It was designed by Michele de la Menardiere.

Division on Earth and Life Studies

Subscribe to the Earth and Life Studies Gateway at http://dels.nas.edu to receive notification of report releases, events, and news about stem cells or other specific areas of interest.

Institute of Medicine

Sign up to receive IOM News, a free, bi-monthly e-mail newsletter that announces new IOM publications, upcoming events, and new online content. See www.iom.edu/subscribe. To visit the Health Sciences Policy Board, see http://iom.edu/hsp.

PHOTO CREDITS

Cover images: (top) Nerve cells derived from human embryonic stem cells. PNAS 101(34):12543, Copyright 2004, National Academy of Sciences, U.S.A. (bottom) Nerve cells derived from human embryonic stem cells in the laboratory of Professor Su-Chun Zhang at the University of Wisconsin–Madison. Used with permission from the University of Wisconsin's Board of Regents. (inside front and back cover) Neural progenitor cell isolated from human embryonic stem cells. Nat. Biotech. 18:399 (2000). Image Courtesy of Martin Pera.

Page 2: (left and right) Nerve cells derived from human embryonic stem cells. PNAS 101(34):12543, Copyright 2004, National Academy of Sciences, U.S.A. (middle) Nerve cells derived from human embryonic stem cells in the laboratory of Professor Su-Chun Zhang at the University of Wisconsin–Madison. Used with permission from the University of Wisconsin's Board of Regents.

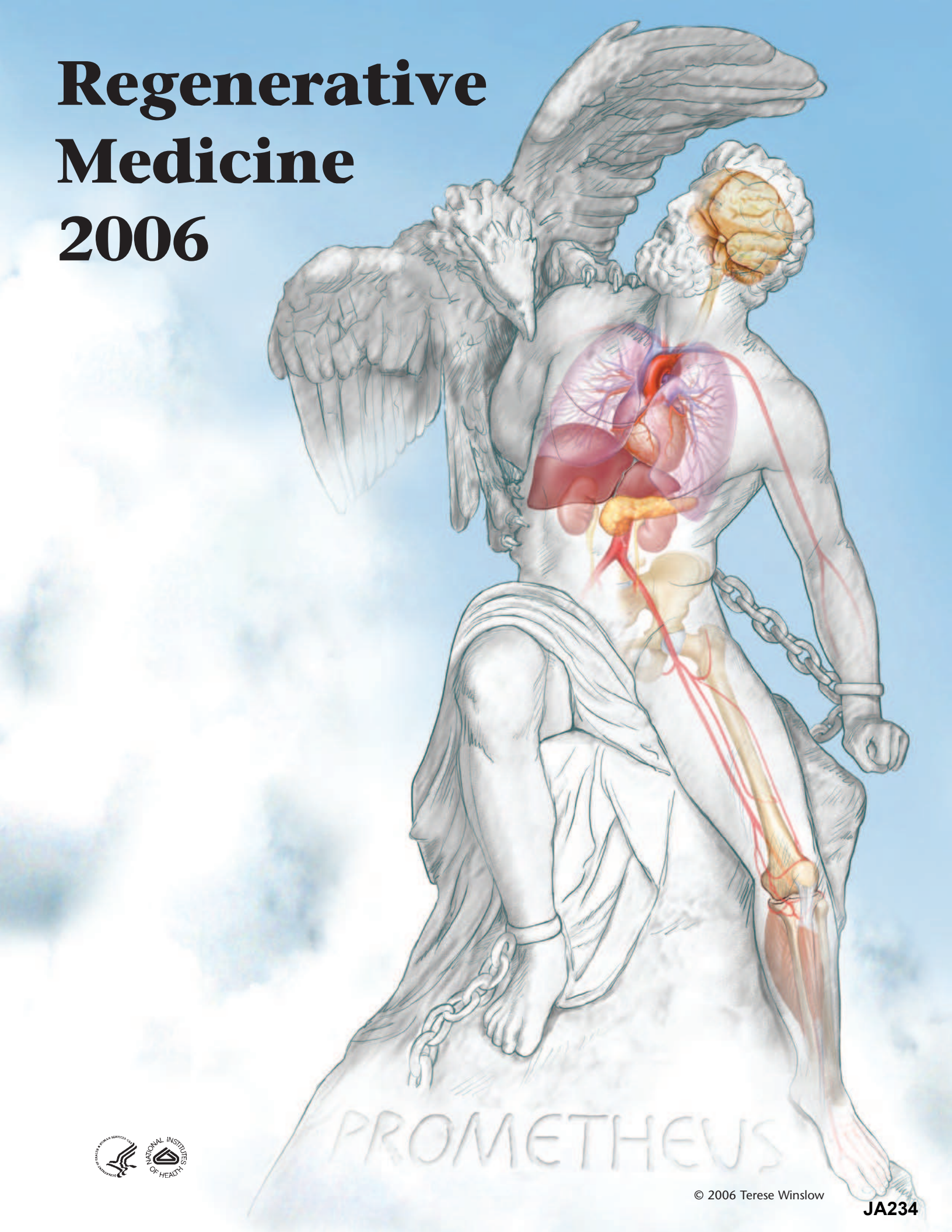
Page 8: (left) NIH/Mr. J. Conaghan. (middle) Kitai Kim, Children's Hospital. (right) Suslov, Oleg N. et al. PNAS 99:14506. Copyright 2002, The National Academy of Sciences, U.S.A.

Over the past decade, stem cells have gained a place in most Americans' vocabularies—discussions of them appear on TV and radio news programs, in newspapers and magazines, and even in political campaigns across the country. As stem cells have come to the forefront of medical research, the ethical controversies over embryonic stem cells have become prominent. This booklet is designed to provide basic knowledge to facilitate thinking about and understanding the scientific and ethical issues surrounding stem cells. It is intended to help readers more easily interpret news about stem cells, as the science advances or new controversies develop.

THE NATIONAL ACADEMIES™
Advisers to the Nation on Science, Engineering, and Medicine

The nation turns to the National Academies—National Academy of Sciences, National Academy of Engineering, Institute of Medicine, and National Research Council—for independent, objective advice on issues that affect people's lives worldwide.
www.national-academies.org

Regenerative Medicine 2006



JA234

TABLE OF CONTENTS

2007 UPDATES

INTRODUCTION

The Greek Titan, Prometheus, is a fitting symbol for regenerative medicine. As punishment for giving fire to Humankind, Zeus ordered Prometheus chained to a rock and sent an eagle to eat his liver each day. However, Prometheus' liver was able to regenerate itself daily, enabling him to survive. The scientific researchers and medical doctors of today hope to make the legendary concept of regeneration into reality by developing therapies to restore lost, damaged, or aging cells and tissues in the human body.

This report features chapters written by experts in several areas of enormous potential for regenerative medicine. Drs. Junying Yu and James A. Thomson explain the basic features of embryonic stem cells, how they are being used in research, and how they may lead to human therapies. Drs. Jos Domen, Amy Wagers, and Irving Weissman describe the historical origins of blood-forming stem cell research, basic features of these adult stem cells, progress on using these cells for human therapies, and future possibilities. Dr. David Panchision explores ways to use cell-based therapies to restore lost function in the human nervous system. Dr. Thomas Zwaka explains how stem cells may be used for gene therapy, and Dr. Mark L. Rohrbaugh explains the current state of intellectual property issues associated with research using human embryonic stem cells.

[This page intentionally left blank]

1. EMBRYONIC STEM CELLS

by Junying Yu and James A. Thomson***

Human embryonic stem (ES) cells capture the imagination because they are immortal and have an almost unlimited developmental potential *(Fig. 1.1: How hESCs are derived).* After many months of growth in culture dishes, these remarkable cells maintain the ability to form cells ranging from muscle to nerve to blood — potentially any cell type that makes up the body. The proliferative and developmental potential of human ES cells promises an essentially unlimited supply of specific cell types for basic research and for transplantation therapies for diseases ranging from heart disease to Parkinson's disease to leukemia. Here we discuss the origin and properties of human ES cells, their implications for basic research and human medicine, and recent research progress since August 2001, when President George W. Bush allowed federal funding of this research for the first time. A previous report discussed progress prior to June 17, 2001 (http://stemcells.nih.gov/info/scireport/.)

WHAT ARE EMBRYONIC STEM CELLS?

Embryonic stem cells are derived from embryos at a developmental stage before the time that implantation would normally occur in the uterus. Fertilization normally occurs in the oviduct, and during the next few days, a series of cleavage divisions occur as the embryo travels down the oviduct and into the uterus. Each of the cells (blastomeres) of these cleavage-stage embryos are undifferentiated, *i.e.* they do not look or act like the specialized cells of the adult, and the blastomeres are not yet committed to becoming any particular type of differentiated cell. Indeed, each of these blastomeres has the potential to give rise to any cell of the body. The first differentiation event in humans occurs at approximately five days of development, when an outer layer of cells committed to becoming part of the placenta (the trophectoderm) separates from the inner cell mass (ICM). The ICM cells have the potential to generate any cell type of the body, but after implantation, they are quickly depleted as they differentiate to other cell types with more

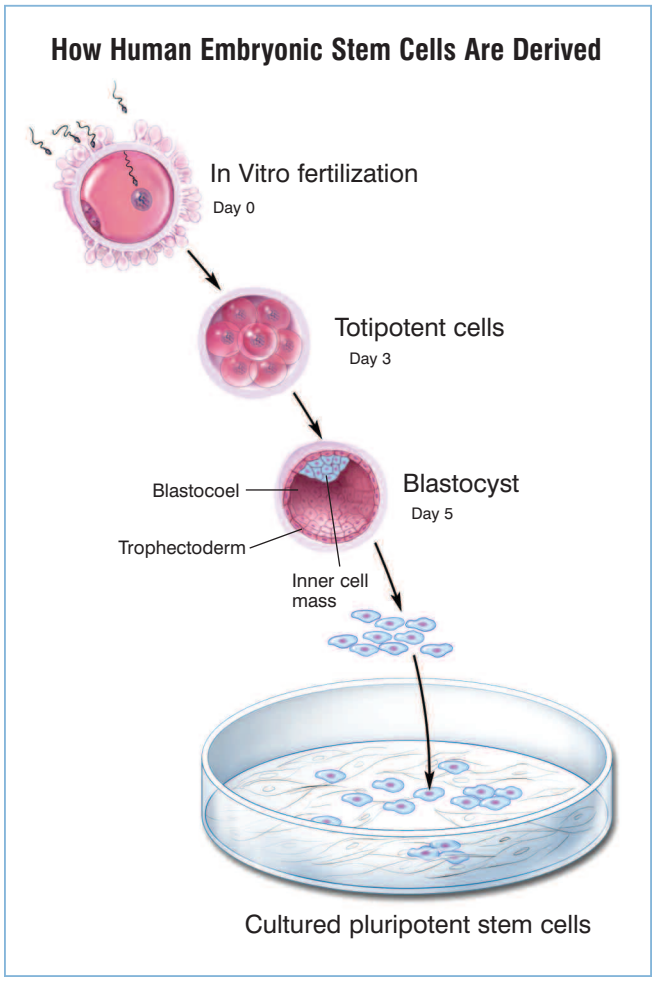


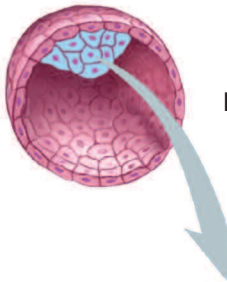
Figure 1.1. How Human Embryonic Stem Cells are Derived

* Genetics and Biotechnology Building, Madison, WI 53706, *Email:* jyu@primate.wisc.edu

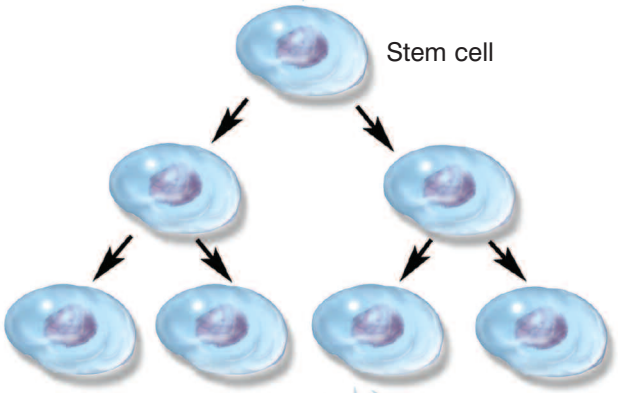
** John D. MacArthur Professor, Department of Anatomy, University of Wisconsin–Madison Medical School, The Genome Center of Wisconsin, and The Wisconsin National Primate Research Center, Madison, WI 53715, *Email:* thomson@primate.wisc.edu

Characteristics of Embryonic Stem Cells

1. Origin:
Derived from pre-implantation or peri-implantation embryo



Blastocyst

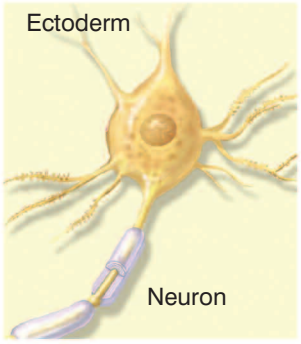


Stem cell

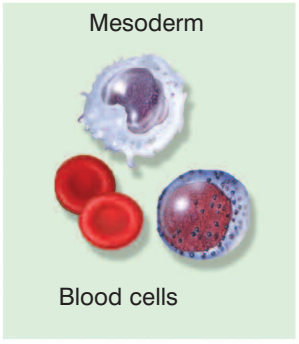
2. Self-Renewal:
The cells can divide to make copies of themselves for a prolonged period of time without differentiating.

3. Pluripotency:
Embryonic stem cells can give rise to cells from all three embryonic germ layers even after being grown in culture for a long time.

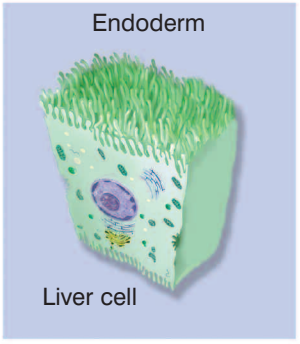
The three germ layers and one example of a cell type derived from each layer:



Ectoderm gives rise to: brain, spinal cord, nerve cells, hair, skin, teeth, sensory cells of eyes, ears nose, and mouth, and pigment cells.



Mesoderm gives rise to: muscles, blood, blood vessels, connective tissues, and the heart.



Endoderm gives rise to: the gut (pancreas, stomach, liver, etc.), lungs, bladder, and germ cells (eggs or sperm)

Figure 1.2.Characteristics of Embryonic Stem Cells.

limited developmental potential. However, if the ICM is removed from its normal embryonic environment and cultured under appropriate conditions, the ICM-derived cells can continue to proliferate and replicate themselves indefinitely and still maintain the developmental potential to form any cell type of the body (*"pluripotency"; see Fig. 1.2: Characteristics of ESCs*). These pluripotent, ICM-derived cells are ES cells.

The derivation of mouse ES cells was first reported in 1981,[1,2] but it was not until 1998 that derivation of human ES cell lines was first reported.[3] Why did it take such a long time to extend the mouse results to humans? Human ES cell lines are derived from embryos produced by *in vitro* fertilization (IVF), a process in which oocytes and sperm are placed together to allow fertilization to take place in a culture dish. Clinics use this method to treat certain types of infertility, and sometimes, during the course of these treatments, IVF embryos are produced that are no longer needed by the couples for producing children. Currently, there are nearly 400,000 IVF-produced embryos in frozen storage in the United States alone,[4] most of which will be used to treat infertility, but some of which (~2.8%) are destined to be discarded. IVF-produced embryos that would otherwise have been discarded were the sources of the human ES cell lines derived prior to President Bush's policy decision of August 2001. These human ES cell lines are now currently eligible for federal funding. Although attempts to derive human ES cells were made as early as the 1980s, culture media for human embryos produced by IVF were suboptimal. Thus, it was difficult to culture single-cell fertilized embryos long enough to obtain healthy blastocysts for the derivation of ES cell lines. Also, species-specific differences between mice and humans meant that experience with mouse ES cells was not completely

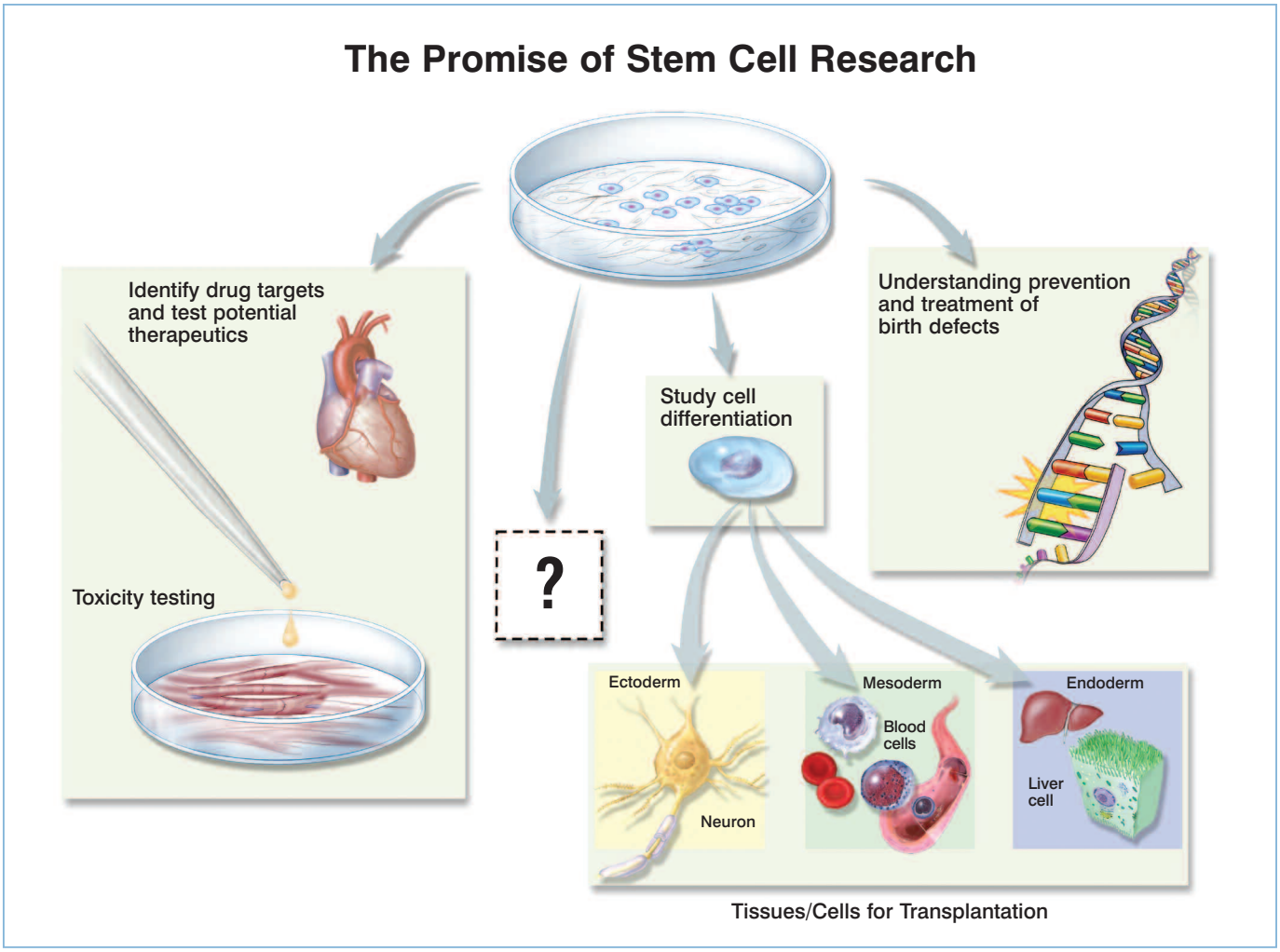


Figure 1.3: The Promise of Stem Cell Research

applicable to the derivation of human ES cells. In the 1990s, ES cell lines from two non-human primates, the rhesus monkey[5] and the common marmoset,[6] were derived, and these offered closer models for the derivation of human ES cells. Experience with non-human primate ES cell lines and improvements in culture medium for human IVF-produced embryos led rapidly to the derivation of human ES cell lines in 1998.[3]

Because ES cells can proliferate without limit and can contribute to any cell type, human ES cells offer an unprecedented access to tissues from the human body. They will support basic research on the differentiation and function of human tissues and provide material for testing that may improve the safety and efficacy of human drugs *(Figure 1.3: Promise of SC Research).*[7,8] For example, new drugs are not generally tested on human heart cells because no human heart cell lines exist. Instead, researchers rely on animal models. Because of important species-specific differences between animal and human hearts, however, drugs that are toxic to the human heart have occasionally entered clinical trials, sometimes resulting in death. Human ES cell-derived heart cells may be extremely valuable in identifying such drugs before they are used in clinical trials, thereby accelerating the drug discovery process and leading to safer and more effective treatments.[9-11] Such testing will not be limited to heart cells, but to any type of human cell that is difficult to obtain by other sources.

Human ES cells also have the potential to provide an unlimited amount of tissue for transplantation therapies to treat a wide range of degenerative diseases. Some important human diseases are caused by the death or dysfunction of one or a few cell types, *e.g.,* insulin-producing cells in diabetes or dopaminergic neurons in Parkinson's disease. The replacement of these cells could offer a lifelong treatment for these disorders. However, there are a number of challenges to develop human ES cell-based transplantation therapies, and many years of basic research will be required before such therapies can be used to treat patients. Indeed, basic research enabled by human ES cells is likely to impact human health in ways unrelated to transplantation medicine. This impact is likely to begin well before the widespread use of ES cells in transplantation and ultimately could have a more profound long-term effect on human medicine. Since August 2001, improvements in culture of human ES cells, coupled with recent insights into the nature of pluripotency, genetic manipulation of human ES cells, and differentiation, have expanded the possibilities for these unique cells.

CULTURE OF ES CELLS

Mouse ES cells and human ES cells were both originally derived and grown on a layer of mouse fibroblasts (called "feeder cells") in the presence of bovine serum. However, the factors that sustain the growth of these two cell types appear to be distinct. The addition of the cytokine, leukemia inhibitory factor (LIF), to serum-containing medium allows mouse ES cells to proliferate in the absence of feeder cells. LIF modulates mouse ES cells through the activation of STAT3 (signal transducers and activators of transcription) protein. In serum-free culture, however, LIF alone is insufficient to prevent mouse ES cells from differentiating into neural cells. Recently, Ying *et al.* reported that the combination of bone morphogenetic proteins (BMPs) and LIF is sufficient to support the self-renewal of mouse ES cells.[12] The effects of BMPs on mouse ES cells involve induction of inhibitor of differentiation (Id) proteins, and inhibition of extracellular receptor kinase (ERK) and p38 mitogen-activated protein kinases (MAPK).[12,13] However, LIF in the presence of serum is not sufficient to promote the self-renewal of human ES cells,[3] and the LIF/STAT3 pathway appears to be inactive in undifferentiated human ES cells.[14,15] Also, the addition of BMPs to human ES cells in conditions that would otherwise support ES cells leads to the rapid differentiation of human ES cells.[16,17]

Several groups have attempted to define growth factors that sustain human ES cells and have attempted to identify culture conditions that reduce the exposure of human ES cells to non human animal products. One important growth factor, bFGF, allows the use of a serum replacement to sustain human ES cells in the presence of fibroblasts, and this medium allowed the clonal growth of human ES cells.[18] A "feeder-free" human ES cell culture system has been developed, in which human ES cells are grown on a protein matrix (mouse Matrigel or Laminin) in a bFGF-containing medium that is previously "conditioned" by co-culture with fibroblasts.[19] Although this culture system eliminates direct contact of human ES cells with the fibroblasts, it does not remove the potential for mouse pathogens being introduced into the culture via the fibroblasts. Several different sources of human feeder

cells have been found to support the culture of human ES cells, thus removing the possibility of pathogen transfer from mice to humans.[20–23] However, the possibility of pathogen transfer from human to human in these culture systems still remains. More work is still needed to develop a culture system that eliminates the use of fibroblasts entirely, which would also decrease much of the variability associated with the current culture of human ES cells. Sato *et al.* reported that activation of the Wnt pathway by 6-bromoindirubin-3'-oxime (BIO) promotes the self-renewal of ES cells in the presence of bFGF, Matrigel, and a proprietary serum replacement product.[24] Amit *et al.* reported that bFGF, TGFβ, and LIF could support some human ES cell lines in the absence of feeders.[25] Although there are some questions about how well these new culture conditions will work for different human ES cell lines, there is now reason to believe that defined culture conditions for human ES cells, which reduce the potential for contamination by pathogens, will soon be achieved*.

Once a set of defined culture conditions is established for the derivation and culture of human ES cells, challenges to improve the medium will still remain. For example, the cloning efficiency of human ES cells — the ability of a single human ES cell to proliferate and become a colony — is very low (typically less than 1%) compared to that of mouse ES cells. Another difficulty is the potential for accumulation of genetic and epigenetic changes over prolonged periods of culture. For example, karyotypic changes have been observed in several human ES cell lines after prolonged culture, and the rate at which these changes dominate a culture may depend on the culture method.[26,27] The status of imprinted (epigenetically modified) genes and the stability of imprinting in various culture conditions remain completely unstudied in human ES cells**. The status of imprinted genes can clearly change with culture conditions in other cell types.[28,29] These changes present potential problems if human ES cells are to be used in cell replacement therapy, and optimizing medium to reduce the rate at which genetic and epigenetic changes accumulate in culture represents a long-term endeavor. The ideal human ES cell medium, then, (a) would be cost-effective and easy to use so that many more investigators can use human ES cells as a research tool; (b) would be composed entirely of defined components not of animal origin; (c) would allow cell growth at clonal densities; and (d) would minimize the rate at which genetic and epigenetic changes accumulate in culture. Such a medium will be a challenge to develop and will most likely be achieved through a series of incremental improvements over a period of years.

Among all the newly derived human ES cell lines, twelve lines have gained the most attention. In March 2004, a South Korean group reported the first derivation of a human ES cell line (SCNT-hES-1) using the technique of somatic cell nuclear transfer (SCNT). Human somatic nuclei were transferred into human oocytes (nuclear transfer), which previously had been stripped of their own genetic material, and the resultant nuclear transfer products were cultured *in vitro* to the blastocyst stage for ES cell derivation.[30]*** Because the ES cells derived through nuclear transfer contain the same genetic material as that of the nuclear donor, the intent of the procedure is that the differentiated derivatives would not be rejected by the donor's immune system if used in transplantation therapy. More recently, the same group reported the derivation of eleven more human SCNT-ES cell lines*** with markedly improved efficiency (16.8 oocytes/line vs. 242 oocytes/line in their previous report).[31]*** However, given the abnormalities frequently observed in cloned animals, and the costs involved, it is not clear how useful this procedure will be in clinical applications. Also, for some autoimmune diseases, such as type I diabetes, merely providing genetically-matched tissue will be insufficient to prevent immune rejection.

Additionally, new human ES cell lines were established from embryos with genetic disorders, which were detected during the practice of preimplantation genetic diagnosis (PGD). These new cell lines may provide an excellent *in vitro* model for studies on the effects that the genetic mutations have on cell proliferation and differentiation.[32]

---

* **Editor's note:** Papers published since this writing report defined culture conditions for human embryonic stem cells. See Ludwig et al., Nat. Biotech 24: 185-187, 2006; and Lu et al., PNAS 103:5688-5693, 2006.08.14.

** **Editor's note:** Papers published since the time this chapter was written address this: see Maitra et al., Nature Genetics 37, 1099-1103, 2005; and Rugg-Gunn et al., Nature Genetics 37:585-587, 2005.

*** **Editor's note:** Both papers referenced in 30 and 31 were later retracted: see Science 20 Jan 2006; Vol. 311. No. 5759, p. 335.

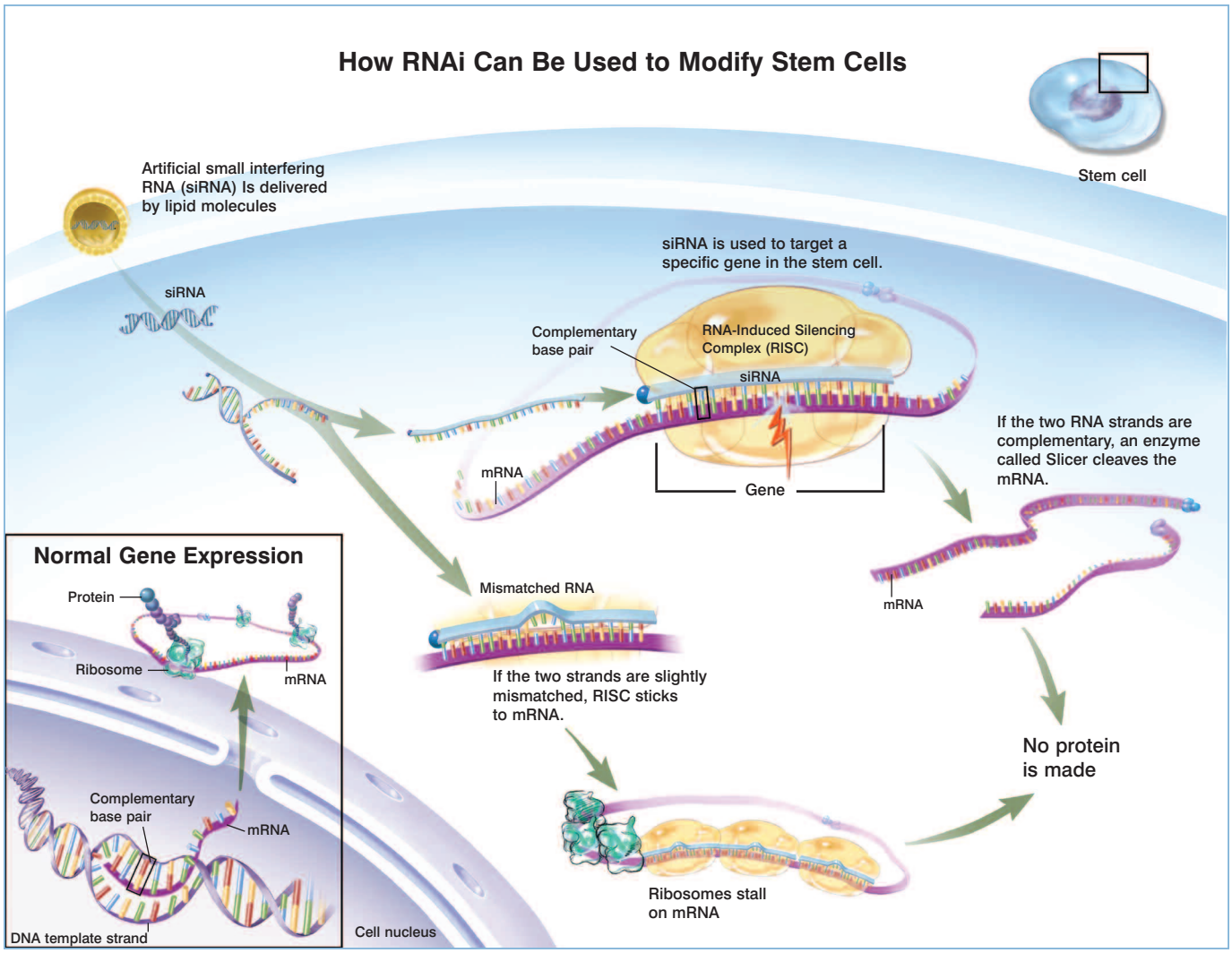


Figure 1.4. How RNAi Can Be Used To Modify Stem Cells

To date, more than 120 human ES cell lines have been established worldwide,[33]* 67 of which are included in the National Institutes of Health (NIH) registry (http://stemcells.nih.gov/research/registry/). As of this writing, 21 cell lines are currently available for distribution, all of which have been exposed to animal products during their derivation. Although it has been eight years since the initial derivation of human ES cells, it is an open question as to the extent that independent human ES cell lines differ from one another. At the very least, the limited number of cell lines cannot represent a reasonable sampling of the genetic diversity of different ethnic groups in the United States, and this has consequences for drug testing, as adverse reactions to drugs often reflect a complex genetic component. Once defined culture conditions are well established for human ES cells, there will be an even more compelling need to derive additional cell lines.

PLURIPOTENCY OF ES CELLS

The ability of ES cells to develop into all cell types of the body has fascinated scientists for years, yet remarkably little is known about factors that make one cell pluripotent and another more restricted in its developmental potential. The transcription factor Oct4 has

* **Editor's note:** One recent report now estimates 414 hESC lines, see Guhr et al., www.StemCells.com early online version for June 15, 2006: "Current State of Human Embryonic Stem Cell Research: An Overview of Cell Lines and their Usage in Experimental Work."

been used as a key marker for ES cells and for the pluripotent cells of the intact embryo, and its expression must be maintained at a critical level for ES cells to remain undifferentiated.[34] The Oct4 protein itself, however, is insufficient to maintain ES cells in the undifferentiated state. Recently, two groups identified another transcription factor, Nanog, that is essential for the maintenance of the undifferentiated state of mouse ES cells.[35,36] The expression of Nanog decreased rapidly as mouse ES cells differentiated, and when its expression level was maintained by a constitutive promoter, mouse ES cells could remain undifferentiated and proliferate in the absence of either LIF or BMP in serum-free medium.[12] Nanog is also expressed in human ES cells, though at a much lower level compared to that of Oct4, and its function in human ES cells has yet to be examined.

By comparing gene expression patterns between different ES cell lines and between ES cells and other cell types such as adult stem cells and differentiated cells, genes that are enriched in the ES cells have been identified. Using this approach, Esg-1, an uncharacterized ES cell-specific gene, was found to be exclusively associated with pluripotency in the mouse.[37] Sperger *et al.* identified 895 genes that are expressed at significantly higher levels in human ES cells and embryonic carcinoma cell lines, the malignant counterparts to ES cells.[38] Sato *et al.* identified a set of 918 genes enriched in undifferentiated human ES cells compared with their differentiated counterparts; many of these genes were shared by mouse ES cells.[39] Another group, however, found 92 genes, including Oct4 and Nanog, enriched in six different human ES cell lines, which showed limited overlap with those in mouse ES cell lines.[40] Care must be taken to interpret these data, and the considerable differences in the results may arise from the cell lines used in the experiments, methods to prepare and maintain the cells, and the specific methods used to profile gene expression.

GENETIC MANIPULATION OF ES CELLS

Since establishing human ES cells in 1998, scientists have developed genetic manipulation techniques to determine the function of particular genes, to direct the differentiation of human ES cells towards specific cell types, or to tag an ES cell derivative with a certain marker gene. Several approaches have been developed to introduce genetic elements randomly into the human ES cell genome, including electroporation, transfection by lipid-based reagents, and lentiviral vectors.[41–44] However, homologous recombination, a method in which a specific gene inside the ES cells is modified with an artificially introduced DNA molecule, is an even more precise method of genetic engineering that can modify a gene in a defined way at a specific locus. While this technology is routinely used in mouse ES cells, it has recently been successfully developed in human ES cells *(See chapter 5: Genetically Modified Stem Cells),* thus opening new doors for using ES cells as vehicles for gene therapy and for creating *in vitro* models of human genetic disorders such as Lesch-Nyhan disease.[45,46] Another method to test the function of a gene is to use RNA interference (RNAi) to decrease the expression of a gene of interest *(see Figure 1.4: RNA interference).* In RNAi, small pieces of double-stranded RNA (siRNA; small interfering RNA) are either chemically synthesized and introduced directly into cells, or expressed from DNA vectors. Once inside the cells, the siRNA can lead to the degradation of the messenger RNA (mRNA), which contains the exact sequence as that of the siRNA. mRNA is the product of DNA transcription and normally can be translated into proteins. RNAi can work efficiently in somatic cells, and there has been some progress in applying this technology to human ES cells.[47–49]

DIFFERENTIATION OF HUMAN ES CELLS

The pluripotency of ES cells suggests possible widespread uses for these cells and their derivatives. The ES cell-derived cells can potentially be used to replace or restore tissues that have been damaged by disease or injury, such as diabetes, heart attacks, Parkinson's disease or spinal cord injury. The recent developments in these particular areas are discussed in detail in other chapters, and Table 1 summarizes recent publications in the differentiation of specific cell lineages.

The differentiation of ES cells also provides model systems to study early events in human development. Because of possible harm to the resulting child, it is not ethically acceptable to experimentally manipulate the postimplantation human embryo. Therefore, most of what is known about the mechanisms of early human embryology and human development, especially in the early postimplantation period, is based on histological sections of a limited number of human embryos and on analogy to the experimental embryology of the

mouse. However, human and mouse embryos differ significantly, particularly in the formation, structure, and function of the fetal membranes and placenta, and the formation of an embryonic disc instead of an egg cylinder.[50–52] For example, the mouse yolk sac is a well-vascularized, robust, extraembryonic organ throughout gestation that provides important nutrient exchange functions. In humans, the yolk sac also serves important early functions, including the initiation of hematopoiesis, but it becomes essentially a vestigial structure at later times or stages in gestation. Similarly, there are dramatic differences between mouse and human placentas, both in structure and function. Thus, mice can serve in a limited capacity as a model system for understanding the developmental events that support the initiation and maintenance of human pregnancy. Human ES cell lines thus provide an important new *in vitro* model that will improve our understanding of the differentiation of human tissues, and thus provide important insights into processes such as infertility, pregnancy loss, and birth defects.

Human ES cells are already contributing to the study of development. For example, it is now possible to direct human ES cells to differentiate efficiently to trophoblast, the outer layer of the placenta that mediates implantation and connects the conceptus to the uterus.[17,53] Another use of human ES cells is for the study of germ cell development. Cells resembling both oocytes and sperm have been successfully derived from mouse ES cells *in vitro*.[54–56] Recently, human ES cells have also been observed to differentiate into cells expressing genes characteristic of germ cells.[57] Thus it may also be possible to derive oocytes and sperm from human ES cells, allowing the detailed study of human gametogenesis for the first time. Moreover, human ES cell studies are not limited to early differentiation, but are increasingly being used to understand the differentiation and functions of many human tissues, including neural, cardiac, vascular, pancreatic, hepatic, and bone *(see Table 1)*. Moreover, transplantation of ES-derived cells has offered promising results in animal models.[58–67]

Although scientists have gained more insights into the biology of human ES cells since 2001, many key questions remain to be addressed before the full potential of these unique cells can be realized. It is surprising, for example, that mouse and human ES cells appear to be so different with respect to the molecules that mediate their self-renewal, and perhaps even in their developmental potentials. BMPs, for example, in combination with LIF, promote the self-renewal of mouse ES cells. But in conditions that would otherwise support undifferentiated proliferation, BMPs cause rapid differentiation of human ES cells. Also, human ES cells differentiate quite readily to trophoblast, whereas mouse ES cells do so poorly, if at all. One would expect that at some level, the basic molecular mechanisms that control pluripotency would be conserved, and indeed, human and mouse ES cells share the expression of many key genes. Yet we remain remarkably ignorant about the molecular mechanisms that control pluripotency, and the nature of this remarkable cellular state has become one of the central questions of developmental biology. Of course, the other great challenge will be to continue to unravel the factors that control the differentiation of human ES cells to specific lineages, so that ES cells can fulfill their tremendous promise in basic human biology, drug screening, and transplantation medicine.

Table 1. Publications on Differentiation of Human Embryonic Stem Cells since 2001

Cell types	Publications	References
Neural	8	61, 66, 68-73
Cardiac	6	9-11, 74-76
Endothelial (Vascular)	2	77, 78
Hematopoietic (Blood)	8	79-86
Pancreatic (Islet-like)	2	87, 88
Hepatic (Liver)	3	89-91
Bone	1	92
Trophoblast	2	17, 53
Multilineages	9	16, 57, 93-99

ACKNOWLEDGEMENT

We thank Lynn Schmidt, Barbara Lewis, Sangyoon Han and Deborah J. Faupel for proofreading this report.

REFERENCES

1. Evans MJ, Kaufman MH. Establishment in culture of pluripotential cells from mouse embryos. *Nature.* 1981;292:154-156.

2. Martin GR. Isolation of a pluripotent cell line from early mouse embryos cultured in medium conditioned by teratocarcinoma stem cells. *Proc Natl Acad Sci USA.* 1981;78:7634-7638.

3. Thomson JA, Itskovitz-Eldor J, Shapiro SS, et al. Embryonic stem cell lines derived from human blastocysts. *Science.* 1998;282:1145-1147.

4. Hoffman DI, Zellman GL, Fair CC, et al. Cryopreserved embryos in the United States and their availability for research. *Fertil Steril.* 2003;79:1063-1069.

5. Thomson JA, Kalishman J, Golos TG, et al. Isolation of a primate embryonic stem cell line. *Proc Natl Acad Sci USA.* 1995;92:7844-7848.

6. Thomson JA, Kalishman J, Golos TG, Durning M, Harris CP, Hearn JP. Pluripotent cell lines derived from common marmoset (Callithrix jacchus) blastocysts. *Biol Reprod.* 1996;55:254-259.

7. Bremer S, Hartung T. The use of embryonic stem cells for regulatory developmental toxicity testing in vitro — the current status of test development. *Curr Pharm Des.* 2004;10:2733-2747.

8. Rolletschek A, Blyszczuk P, Wobus AM. Embryonic stem cell-derived cardiac, neuronal and pancreatic cells as model systems to study toxicological effects. *Toxicol Lett.* 2004;149:361-369.

9. He JQ, Ma Y, Lee Y, Thomson JA, Kamp TJ. Human embryonic stem cells develop into multiple types of cardiac myocytes: action potential characterization. *Circ Res.* 2003;93:32-39.

10. Mummery C, Ward-van Oostwaard D, Doevendans P, et al. Differentiation of human embryonic stem cells to cardiomyocytes: role of coculture with visceral endoderm-like cells. Circulation. 2003;107:2733-2740.

11. Vanderlaan RD, Oudit GY, Backx PH. Electrophysiological profiling of cardiomyocytes in embryonic bodies derived from human embryonic stem cells. Circ Res. 2003;93:1-3.

12. Ying QL, Nichols J, Chambers I, Smith A. BMP induction of Id proteins suppresses differentiation and sustains embryonic stem cell self-renewal in collaboration with STAT3. *Cell.* 2003;115:281-292.

13. Qi X, Li TG, Hao J, et al. BMP4 supports self-renewal of embryonic stem cells by inhibiting mitogen-activated protein kinase pathways. *Proc Natl Acad Sci USA.* 2004;101:6027-6032.

14. Daheron L, Optiz SL, Zaehres H, et al. LIF/STAT3 signaling fails to maintain self-renewal of human embryonic stem cells. *Stem Cells.* 2004;22:770-778.

15. Humphrey RK, Beattie GM, Lopez AD, et al. Maintenance of pluripotency in human embryonic stem cells is STAT3 independent. *Stem Cells.* 2004;22:522-530.

16. Pera MF, Andrade J, Houssami S, et al. Regulation of human embryonic stem cell differentiation by BMP-2 and its antagonist noggin. *J Cell Sci.* 2004;117:1269-1280.

17. Xu RH, Chen X, Li DS, et al. BMP4 initiates human embryonic stem cell differentiation to trophoblast. *Nat Biotechnol.* 2002;20:1261-1264.

18. Amit M, Carpenter MK, Inokuma MS, et al. Clonally derived human embryonic stem cell lines maintain pluripotency and proliferative potential for prolonged periods of culture. *Dev Biol.* 2000;227:271-278.

19. Xu C, Inokuma MS, Denham J, et al. Feeder-free growth of undifferentiated human embryonic stem cells. *Nat Biotechnol.* 2001;19:971-974.

20. Amit M, Margulets V, Segev H, et al. Human feeder layers for human embryonic stem cells. *Biol Reprod.* 2003;68:2150-2156.

21. Lee JB, Lee JE, Park JH, et al. Establishment and maintenance of human embryonic stem cell lines on human feeder cells derived from uterine endometrium under serum-free condition. *Biol Reprod.* 2004.

22. Richards M, Fong CY, Chan WK, Wong PC, Bongso A. Human feeders support prolonged undifferentiated growth of human inner cell masses and embryonic stem cells. *Nat Biotechnol.* 2002;20:933-936.

23. Richards M, Tan S, Fong CY, Biswas A, Chan WK, Bongso A. Comparative evaluation of various human feeders for prolonged undifferentiated growth of human embryonic stem cells. *Stem Cells.* 2003;21:546-556.

24. Sato N, Meijer L, Skaltsounis L, Greengard P, Brivanlou AH. Maintenance of pluripotency in human and mouse embryonic stem cells through activation of Wnt signaling by a pharmacological GSK-3-specific inhibitor. *Nat Med.* 2004;10:55-63.

25. Amit M, Shariki K, Margulets V, Itskovitz-Eldor J. Feeder layer- and serum-free culture of human embryonic stem cells. *Biol Reprod.* 2004;70:837-845.

26. Draper JS, Smith K, Gokhale P, et al. Recurrent gain of chromosomes 17q and 12 in cultured human embryonic stem cells. *Nat Biotechnol.* 2004;22:53-54.

27. Inzunza J, Sahlen S, Holmberg K, et al. Comparative genomic hybridization and karyotyping of human embryonic stem cells reveals the occurrence of an isodicentric X chromosome after long-term cultivation. *Mol Hum Reprod.* 2004;10:461-466.

28. Doherty AS, Mann MR, Tremblay KD, Bartolomei MS, Schultz RM. Differential effects of culture on imprinted H19 expression in the preimplantation mouse embryo. *Biol Reprod.* 2000;62:1526-1535.

29. Mann MR, Lee SS, Doherty AS, et al. Selective loss of imprinting in the placenta following preimplantation development in culture. *Development.* 2004;131:3727-3735.

30. Hwang WS, Ryu YJ, Park JH, et al. Evidence of a pluripotent human embryonic stem cell line derived from a cloned blastocyst. *Science.* 2004;303:1669-1674.

31. Hwang WS, Roh SI, Lee BC, et al. Patient-specific embryonic stem cells derived from human SCNT blastocysts. *Science.* Jun 17 2005;308(5729):1777-1783.

32. Verlinsky Y, Strelchenko N, Kukharenko V, et al. Human embryonic stem cell lines with genetic disorders. *Reprod Biomed Online.* 2005;10:105-110.

33. Stojkovic M, Lako M, Strachan T, Murdoch A. Derivation, growth, and applications of human embryonic stem cells. *Reproduction.* 2004;128:259-267.

34. Niwa H, Miyazaki J, Smith AG. Quantitative expression of Oct-3/4 defines differentiation, dedifferentiation or self-renewal of ES cells. *Nat Genet.* 2000;24:372-376.

35. Chambers I, Colby D, Robertson M, et al. Functional expression cloning of Nanog, a pluripotency sustaining factor in embryonic stem cells. *Cell.* 2003;113:643-655.

36. Mitsui K, Tokuzawa Y, Itoh H, et al. The homeoprotein Nanog is required for maintenance of pluripotency in mouse epiblast and ES cells. Cell. 2003;113:631-642.

37. Tanaka TS, Kunath T, Kimber WL, et al. Gene expression profiling of embryo-derived stem cells reveals genes associated with pluripotency and lineage specificity. *Genome Res.* 2002;12:1921-1928.

38. Sperger JM, Chen X, Draper JS, et al. Gene expression patterns in human embryonic stem cells and human pluripotent germ cell tumors. *Proc Natl Acad Sci USA.* 2003;100:13350-13355.

39. Sato N, Sanjuan IM, Heke M, Uchida M, Naef F, Brivanlou AH. Molecular signature of human embryonic stem cells and its comparison with the mouse. *Dev Biol.* 2003;260:404-413.

40. Bhattacharya B, Miura T, Brandenberger R, et al. Gene expression in human embryonic stem cell lines: unique molecular signature. Blood. 2004;103:2956-2964.

41. Eiges R, Schuldiner M, Drukker M, Yanuka O, Itskovitz-Eldor J, Benvenisty N. Establishment of human embryonic stem cell-transfected clones carrying a marker for undifferentiated cells. *Curr Biol.* 2001;11:514-518.

42. Gropp M, Itsykson P, Singer O, et al. Stable genetic modification of human embryonic stem cells by lentiviral vectors. *Mol Ther.* 2003;7:281-287.

43. Lakshmipathy U, Pelacho B, Sudo K, et al. Efficient transfection of embryonic and adult stem cells. *Stem Cells.* 2004;22:531-543.

44. Ma Y, Ramezani A, Lewis R, Hawley RG, Thomson JA. High-level sustained transgene expression in human embryonic stem cells using lentiviral vectors. *Stem Cells.* 2003;21:111-117.

45. Urbach A, Schuldiner M, Benvenisty N. Modeling for Lesch-Nyhan disease by gene targeting in human embryonic stem cells. *Stem Cells.* 2004;22:635-641.

46. Zwaka TP, Thomson JA. Homologous recombination in human embryonic stem cells. *Nat Biotechnol.* 2003;21:319-321.

47. Matin MM, Walsh JR, Gokhale PJ, et al. Specific knockdown of Oct4 and beta2-microglobulin expression by RNA interference in human embryonic stem cells and embryonic carcinoma cells. *Stem Cells.* 2004;22:659-668.

48. Vallier L, Rugg-Gunn PJ, Bouhon IA, Andersson FK, Sadler AJ, Pedersen RA. Enhancing and diminishing gene function in human embryonic stem cells. Stem Cells. 2004;22:2-11.

49. Velkey JM, O'Shea KS. Oct4 RNA interference induces trophectoderm differentiation in mouse embryonic stem cells. *Genesis.* 2003;37:18-24.

50. Castellucci M, Scheper M, Scheffen I, Celona A, Kaufmann P. The development of the human placental villous tree. *Anat Embryol (Berl).* 1990;181:117-128.

51. Luckett WP. The development of primordial and definitive amniotic cavities in early Rhesus monkey and human embryos. *Am J Anat.* 1975;144:149-167.

52. Luckett WP. Origin and differentiation of the yolk sac and extraembryonic mesoderm in presomite human and rhesus monkey embryos. *Am J Anat.* 1978;152:59-97.

53. Gerami-Naini B, Dovzhenko OV, Durning M, Wegner FH, Thomson JA, Golos TG. Trophoblast differentiation in embryoid bodies derived from human embryonic stem cells. *Endocrinology.* 2004;145:1517-1524.

54. Geijsen N, Horoschak M, Kim K, Gribnau J, Eggan K, Daley GQ. Derivation of embryonic germ cells and male gametes from embryonic stem cells. *Nature.* 2004;427:148-154.

55. Hubner K, Fuhrmann G, Christenson LK, et al. Derivation of oocytes from mouse embryonic stem cells. *Science.* 2003;300:1251-1256.

56. Toyooka Y, Tsunekawa N, Akasu R, Noce T. Embryonic stem cells can form germ cells in vitro. *Proc Natl Acad Sci USA.* 2003;100:11457-11462.

57. Clark AT, Bodnar MS, Fox M, et al. Spontaneous differentiation of germ cells from human embryonic stem cells in vitro. *Hum Mol Genet.* 2004;13:727-739.

58. Bjorklund LM, Sanchez-Pernaute R, Chung S, et al. Embryonic stem cells develop into functional dopaminergic neurons after transplantation in a Parkinsonian rat model. *Proc Natl Acad Sci USA.* 2002;99:2344-2349.

59. Chiba S, Ikeda R, Kurokawa MS, et al. Anatomical and functional recovery by embryonic stem cell-derived neural tissue of a mouse model of brain damage. *J Neurol Sci.* 2004;219:107-117.

60. Kim D, Gu Y, Ishii M, et al. In vivo functioning and transplantable mature pancreatic islet-like cell clusters differentiated from embryonic stem cells. *Pancreas.* 2003;27:e34-e41.

61. Lee DH, Park S, Kim EY, et al. Enhancement of re-closure capacity by the intra-amniotic injection of human embryonic stem cells in surgically induced spinal open neural tube defects in chick embryos. *Neurosci Lett.* 2004;364:98-100.

62. Marchetti S, Gimond C, Iljin K, et al. Endothelial cells genetically selected from differentiating mouse embryonic stem cells incorporate at sites of neovascularization in vivo. *J Cell Sci.* 2002;115:2075-2085.

63. Min JY, Yang Y, Converso KL, et al. Transplantation of embryonic stem cells improves cardiac function in postinfarcted rats. *J Appl Physiol.* 2002;92:288-296.

64. Miyagi T, Takeno M, Nagafuchi H, Takahashi M, Suzuki M. Flk1+ cells derived from mouse embryonic stem cells reconstitute hematopoiesis in vivo in SCID mice. *Exp Hematol.* 2002;30:1444-1453.

65. Nishimura F, Yoshikawa M, Kanda S, et al. Potential use of embryonic stem cells for the treatment of mouse parkinsonian models: improved behavior by transplantation of in vitro differentiated dopaminergic neurons from embryonic stem cells. *Stem Cells.* 2003;21:171-180.

66. Park S, Kim EY, Ghil GS, et al. Genetically modified human embryonic stem cells relieve symptomatic motor behavior in a rat model of Parkinson's disease. *Neurosci Lett.* 2003;353:91-94.

67. von Unge M, Dirckx JJ, Olivius NP. Embryonic stem cells enhance the healing of tympanic membrane perforations. *Int J Pediatr Otorhinolaryngol.* 2003;67:215-219.

68. Carpenter MK, Inokuma MS, Denham J, Mujtaba T, Chiu CP, Rao MS. Enrichment of neurons and neural precursors from human embryonic stem cells. *Exp Neurol.* 2001;172:383-397.

69. Park S, Lee KS, Lee YJ, et al. Generation of dopaminergic neurons in vitro from human embryonic stem cells treated with neurotrophic factors. *Neurosci Lett.* 2004;359:99-103.

70. Reubinoff BE, Itsykson P, Turetsky T, et al. Neural progenitors from human embryonic stem cells. *Nat Biotechnol.* 2001;19:1134-1140.

71. Schuldiner M, Eiges R, Eden A, et al. Induced neuronal differentiation of human embryonic stem cells. *Brain Res.* 2001;913:201-205.

72. Schulz TC, Palmarini GM, Noggle SA, Weiler DA, Mitalipova MM, Condie BG. Directed neuronal differentiation of human embryonic stem cells. *BMC Neurosci.* 2003;4:27.

73. Zhang SC, Wernig M, Duncan ID, Brustle O, Thomson JA. In vitro differentiation of transplantable neural precursors from human embryonic stem cells. *Nat Biotechnol.* 2001;19:1129-1133.

74. Kehat I, Amit M, Gepstein A, Huber I, Itskovitz-Eldor J, Gepstein L. Development of cardiomyocytes from human ES cells. *Methods Enzymol.* 2003;365:461-473.

75. Satin J, Kehat I, Caspi O, et al. Mechanism of spontaneous excitability in human embryonic stem cell derived cardiomyocytes. *J Physiol.* 2004;559:479-496.

76. Xu C, Police S, Rao N, Carpenter MK. Characterization and enrichment of cardiomyocytes derived from human embryonic stem cells. *Circ Res.* 2002;91:501-508.

77. Gerecht-Nir S, Ziskind A, Cohen S, Itskovitz-Eldor J. Human embryonic stem cells as an in vitro model for human vascular development and the induction of vascular differentiation. *Lab Invest.* 2003;83:1811-1820.

78. Levenberg S, Golub JS, Amit M, Itskovitz-Eldor J, Langer R. Endothelial cells derived from human embryonic stem cells. *Proc Natl Acad Sci USA.* 2002;99:4391-4396.

79. Cerdan C, Rouleau A, Bhatia M. VEGF-A165 augments erythropoietic development from human embryonic stem cells. *Blood.* 2004;103:2504-2512.

80. Chadwick K, Wang L, Li L, et al. Cytokines and BMP-4 promote hematopoietic differentiation of human embryonic stem cells. *Blood.* 2003;102:906-915.

81. Kaufman DS, Hanson ET, Lewis RL, Auerbach R, Thomson JA. Hematopoietic colony-forming cells derived from human embryonic stem cells. *Proc Natl Acad Sci USA.* 2001;98:10716-10721.

82. Lu SJ, Li F, Vida L, Honig GR. CD34+CD38- hematopoietic precursors derived from human embryonic stem cells exhibit an embryonic gene expression pattern. *Blood.* 2004;103:4134-4141.

83. Tian X, Kaufman DS. Hematopoietic development of human embryonic stem cells in culture. *Methods Mol Biol.* 2004;290:149-162.

84. Vodyanik MA, Bork JA, Thomson JA, Slukvin II. Human embryonic stem cell-derived CD34+ cells: efficient production in the co-culture with OP9 stromal cells and analysis of lymphohematopoietic potential. *Blood.* 2004.

85. Wang L, Li L, Shojaei F, et al. Endothelial and hematopoietic cell fate of human embryonic stem cells originates from primitive endothelium with hemangioblastic properties. *Immunity.* 2004;21:31-41.

86. Zhan X, Dravid G, Ye Z, et al. Functional antigen-presenting leucocytes derived from human embryonic stem cells in vitro. *Lancet.* 2004;364:163-171.

87. Assady S, Maor G, Amit M, Itskovitz-Eldor J, Skorecki KL, Tzukerman M. Insulin production by human embryonic stem cells. *Diabetes.* 2001;50:1691-1697.

88. Segev H, Fishman B, Ziskind A, Shulman M, Itskovitz-Eldor J. Differentiation of human embryonic stem cells into insulin-producing clusters. *Stem Cells.* 2004;22:265-274.

89. Lavon N, Yanuka O, Benvenisty N. Differentiation and isolation of hepatic-like cells from human embryonic stem cells. *Differentiation.* 2004;72:230-238.

90. Rambhatla L, Chiu CP, Kundu P, Peng Y, Carpenter MK. Generation of hepatocyte-like cells from human embryonic stem cells. *Cell Transplant.* 2003;12:1-11.

91. Shirahashi H, Wu J, Yamamoto N, et al. Differentiation of human and mouse embryonic stem cells along a hepatocyte lineage. *Cell Transplant.* 2004;13:197-211.

92. Sottile V, Thomson JA, McWhir J. In vitro osteogenic differentiation of human ES cells. *Cloning Stem Cells.* 2003;5:149-155.

93. Calhoun JD, Rao RR, Warrenfeltz S, et al. Transcriptional profiling of initial differentiation events in human embryonic stem cells. *Biochem Biophys Res Commun.* 2004;323:453-464.

94. Conley BJ, Trounson AO, Mollard R. Human embryonic stem cells form embryoid bodies containing visceral endoderm-like derivatives. *Fetal Diagn Ther.* 2004;19:218-223.

95. Dang SM, Gerecht-Nir S, Chen J, Itskovitz-Eldor J, Zandstra PW. Controlled, scalable embryonic stem cell differentiation culture. *Stem Cells.* 2004;22:275-282.

96. Dang SM, Zandstra PW. Scalable production of embryonic stem cell-derived cells. *Methods Mol Biol.* 2004;290:353-364.

97. Gertow K, Wolbank S, Rozell B, et al. Organized development from human embryonic stem cells after injection into immunodeficient mice. *Stem Cells Dev.* 2004;13:421-435.

98. Goldstein RS, Drukker M, Reubinoff BE, Benvenisty N. Integration and differentiation of human embryonic stem cells transplanted to the chick embryo. *Dev Dyn.* 2002;225:80-86.

99. Levenberg S, Huang NF, Lavik E, Rogers AB, Itskovitz-Eldor J, Langer R. Differentiation of human embryonic stem cells on three-dimensional polymer scaffolds. *Proc Natl Acad Sci USA.* 2003;100:12741-12746.

2. BONE MARROW (HEMATOPOIETIC) STEM CELLS

by Jos Domen, Amy Wagers** and Irving L. Weissman****

CONTENTS

* Cellerant Therapeutics, 1531 Industrial Road, San Carlos, CA 94070. Current address: Department of Surgery, Arizona Health Sciences Center, 1501 N. Campbell Avenue, P.O. Box 245071, Tucson, AZ 85724-5071, *Email:* jdomen@surgery.arizona.edu

** Section on Developmental and Stem Cell Biology, Joslin Diabetes Center, One Joslin Place, Boston, MA 02215, *Email:* amy.wagers@joslin.harvard.edu

*** Director, Institute for Cancer/Stem Cell Biology and Medicine, Professor of Pathology and Developmental Biology, Stanford University School of Medicine, Stanford, CA 94305, *Email:* Irv@Stanford.edu

INTRODUCTION

Blood and the system that forms it, known as the hematopoietic system, consist of many cell types with specialized functions *(see Figure 2.1).* Red blood cells (erythrocytes) carry oxygen to the tissues. Platelets (derived from megakaryocytes) help prevent bleeding. Granulocytes (neutrophils, basophils and eosinophils) and macrophages (collectively known as myeloid cells) fight infections from bacteria, fungi, and other parasites such as nematodes (ubiquitous small worms). Some of these cells are also involved in tissue and bone remodeling and removal of dead cells. B-lymphocytes produce antibodies, while T-lymphocytes can directly kill or isolate by inflammation cells recognized as foreign to the body, including many virus-infected cells and cancer cells. Many blood cells are short-lived and need to be replenished continuously; the average human requires approximately one hundred billion new hematopoietic cells each day. The continued production of these cells depends directly on the presence of Hematopoietic Stem Cells (HSCs), the ultimate, and only, source of all these cells.

HISTORICAL OVERVIEW

The search for stem cells began in the aftermath of the bombings in Hiroshima and Nagasaki in 1945. Those who died over a prolonged period from lower doses of radiation had compromised hematopoietic systems that could not regenerate either sufficient white blood cells to protect against otherwise nonpathogenic infections or enough platelets to clot their blood. Higher doses of radiation also killed the stem cells of the intestinal tract, resulting in more rapid death. Later, it was demonstrated that mice that were given doses of whole body X-irradiation developed the same radiation syndromes; at the minimal lethal dose, the mice died from hematopoietic failure approximately two weeks after radiation exposure.[1] Significantly, however, shielding a single bone or the spleen from radiation prevented this irradiation syndrome. Soon thereafter, using inbred strains of mice, scientists showed that whole-body-irradiated mice could be rescued from otherwise fatal hematopoietic failure by injection of suspensions of cells from blood-forming organs such as the bone marrow.[2] In 1956, three laboratories

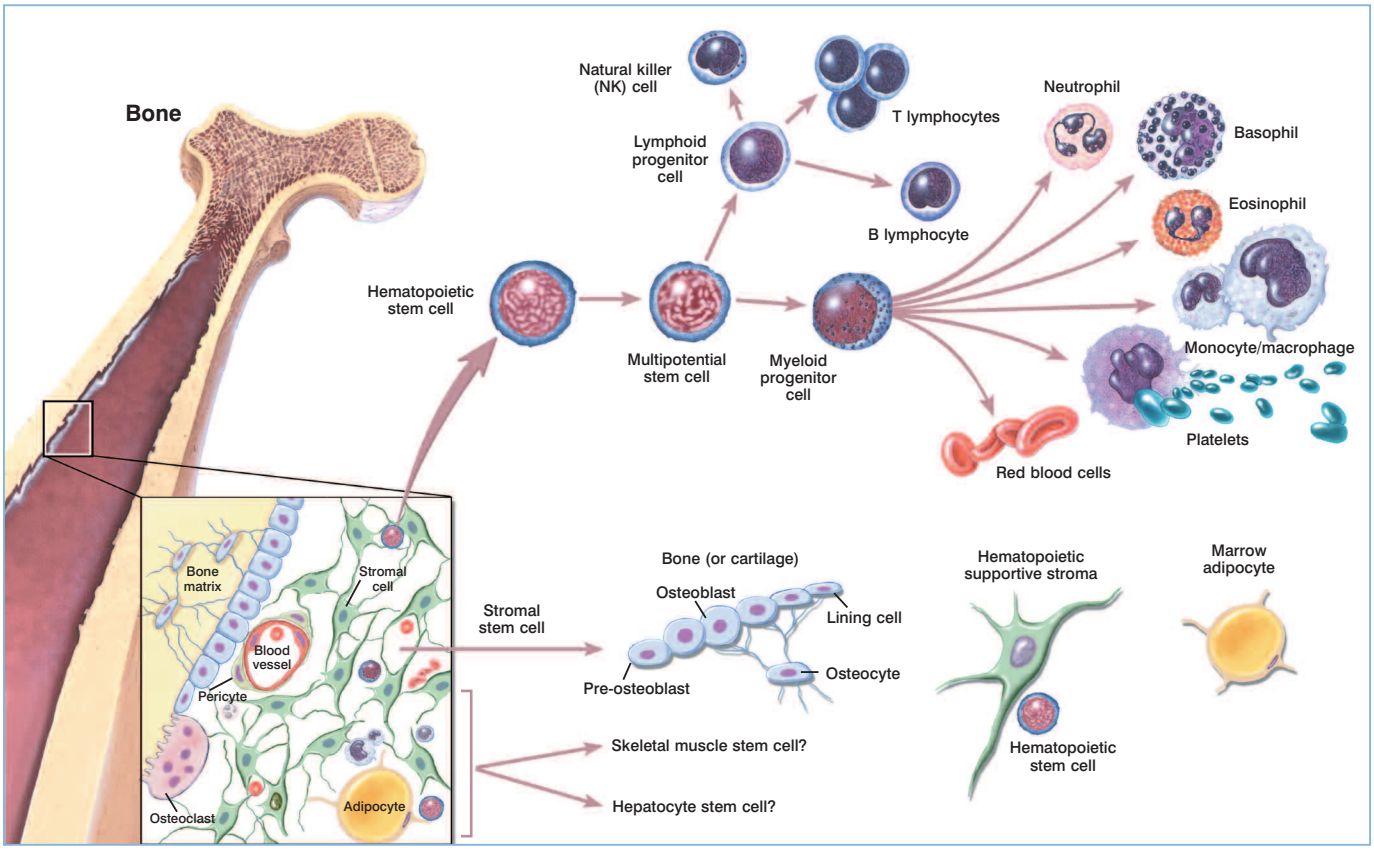


Figure 2.1. Hematopoietic and stromal cell differentiation.

demonstrated that the injected bone marrow cells directly regenerated the blood-forming system, rather than releasing factors that caused the recipients' cells to repair irradiation damage.[3–5] To date, the only known treatment for hematopoietic failure following whole body irradiation is transplantation of bone marrow cells or HSCs to regenerate the blood-forming system in the host organisms.[6,7]

The hematopoietic system is not only destroyed by the lowest doses of lethal X-irradiation (it is the most sensitive of the affected vital organs), but also by chemotherapeutic agents that kill dividing cells. By the 1960s, physicians who sought to treat cancer that had spread (metastasized) beyond the primary cancer site attempted to take advantage of the fact that a large fraction of cancer cells are undergoing cell division at any given point in time. They began using agents (*e.g.,* chemical and X-irradiation) that kill dividing cells to attempt to kill the cancer cells. This required the development of a quantitative assessment of damage to the cancer cells compared that inflicted on normal cells. Till and McCulloch began to assess quantitatively the radiation sensitivity of one normal cell type, the bone marrow cells used in transplantation, as it exists in the body. They found that, at sub-radioprotective doses of bone marrow cells, mice that died 10–15 days after irradiation developed colonies of myeloid and erythroid cells *(see Figure 2.1 for an example)* in their spleens. These colonies correlated directly in number with the number of bone marrow cells originally injected (approximately 1 colony per 7,000 bone marrow cells injected).[8] To test whether these colonies of blood cells derived from single precursor cells, they pre-irradiated the bone marrow donors with low doses of irradiation that would induce unique chromosome breaks in most hematopoietic cells but allow some cells to survive. Surviving cells displayed radiation-induced and repaired chromosomal breaks that marked each clonogenic (colony-initiating) hematopoietic cell.[9] The researchers discovered that all dividing cells within a single spleen colony, which contained different types of blood cells, contained the same unique chromosomal marker. Each colony displayed its own unique chromosomal marker, seen in its dividing cells.[9] Furthermore, when cells from a single spleen colony were re-injected into a second set of lethally-irradiated mice, donor-derived spleen colonies that contained the same unique chromosomal marker were often observed, indicating that these colonies had been regenerated from the same, single cell that had generated the first colony. Rarely, these colonies contained sufficient numbers of regenerative cells both to radioprotect secondary recipients (*e.g.,* to prevent their deaths from radiation-induced blood cell loss) and to give rise to lymphocytes and myeloerythroid cells that bore markers of the donor-injected cells.[10,11] These genetic marking experiments established the fact that cells that can both self-renew and generate most (if not all) of the cell populations in the blood must exist in bone marrow. At the time, such cells were called *pluripotent* HSCs, a term later modified to *multipotent* HSCs.[12,13] However, identifying stem cells in retrospect by analysis of randomly chromosome-marked cells is not the same as being able to isolate pure populations of HSCs for study or clinical use.

Achieving this goal requires markers that uniquely define HSCs. Interestingly, the development of these markers, discussed below, has revealed that most of the early spleen colonies visible 8 to 10 days after injection, as well as many of the later colonies, visible at least 12 days after injection, are actually derived from progenitors rather than from HSCs. Spleen colonies formed by HSCs are relatively rare and tend to be present among the later colonies.[14,15] However, these findings do not detract from Till and McCulloch's seminal experiments to identify HSCs and define these unique cells by their capacities for self-renewal and multilineage differentiation.

THE ISOLATION OF HSCS IN MOUSE AND MAN

While much of the original work was, and continues to be, performed in murine model systems, strides have been made to develop assays to study human HSCs. The development of Fluorescence Activated Cell Sorting (FACS) has been crucial for this field *(see Figure 2.2).* This technique enables the recognition and quantification of small numbers of cells in large mixed populations. More importantly, FACS-based cell sorting allows these rare cells (1 in 2000 to less than 1 in 10,000) to be purified, resulting in preparations of near 100% purity. This capability enables the testing of these cells in various assays.

HSC Assays

Assays have been developed to characterize hematopoietic stem and progenitor cells *in vitro* and *in vivo (Figure 2.3).*[16,17] *In vivo* assays that are used to study

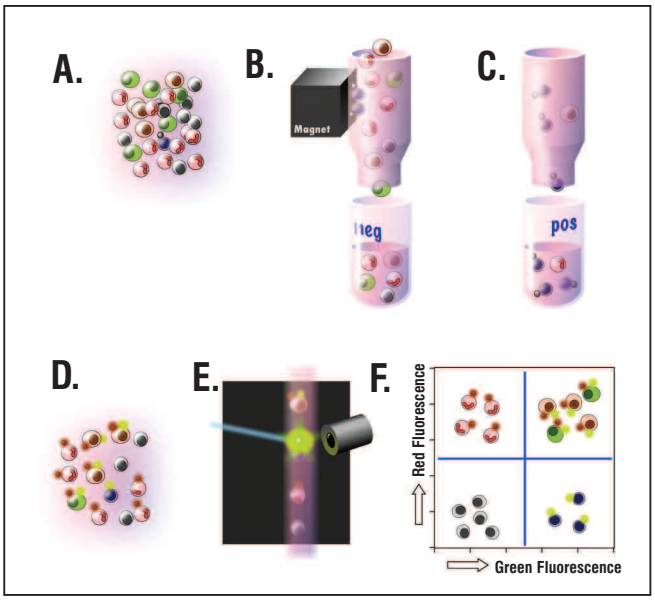


Figure 2.2. Enrichment and purification methods for hematopoietic stem cells. Upper panels illustrate column-based magnetic enrichment. In this method, the cells of interest are labeled with very small iron particles (A). These particles are bound to antibodies that only recognize specific cells. The cell suspension is then passed over a column through a strong magnetic field which retains the cells with the iron particles (B). Other cells flow through and are collected as the depleted negative fraction. The magnet is removed, and the retained cells are collected in a separate tube as the positive or enriched fraction (C). Magnetic enrichment devices exist both as small research instruments and large closed-system clinical instruments.

Lower panels illustrate Fluorescence Activated Cell Sorting (FACS). In this setting, the cell mixture is labeled with fluorescent markers that emit light of different colors after being activated by light from a laser. Each of these fluorescent markers is attached to a different monoclonal antibody that recognizes specific sets of cells (D). The cells are then passed one by one in a very tight stream through a laser beam (blue in the figure) in front of detectors (E) that determine which colors fluoresce in response to the laser. The results can be displayed in a FACS-plot (F). FACS-plots (see figures 3 and 4 for examples) typically show fluorescence levels per cell as dots or probability fields. In the example, four groups can be distinguished: Unstained, red-only, green-only, and red-green double labeling. Each of these groups, *e.g.,* green fluorescence-only, can be sorted to very high purity. The actual sorting happens by breaking the stream shown in (E) into tiny droplets, each containing 1 cell, that then can be sorted using electric charges to move the drops. Modern FACS machines use three different lasers (that can activate different set of fluorochromes), to distinguish up to 8 to 12 different fluorescence colors and sort 4 separate populations, all simultaneously.

Magnetic enrichment can process very large samples (billions of cells) in one run, but the resulting cell preparation is enriched for only one parameter (*e.g.,* CD34) and is not pure. Significant levels of contaminants (such as T-cells or tumor cells) remain present. FACS results in very pure cell populations that can be selected for several parameters simultaneously (*e.g.,* Lin^{neg}, $CD34^{pos}$, $CD90^{pos}$), but it is more time consuming (10,000 to 50,000 cells can be sorted per second) and requires expensive instrumentation.

HSCs include Till and McCulloch' s classical spleen colony forming (CFU-S) assay,[8] which measures the ability of HSC (as well as blood-forming progenitor cells) to form large colonies in the spleens of lethally irradiated mice. Its' main advantage (and limitation) is the short-term nature of the assay (now typically 12 days). However, the assays that truly define HSCs are reconstitution assays.[16,18] Mice that have been "preconditioned" by lethal irradiation to accept new HSCs are injected with purified HSCs or mixed populations containing HSCs, which will repopulate the hematopoietic systems of the host mice for the life of the animal. These assays typically use different types of markers to distinguish host and donor-derived cells.

For example, allelic assays distinguish different versions of a particular gene, either by direct analysis of DNA or of the proteins expressed by these alleles. These proteins may be cell-surface proteins that are recognized by specific monoclonal antibodies that can distinguish between the variants (*e.g.,* CD45 in Figure 2.3) or cellular proteins that may be recognized through methods such as gel-based analysis. Other assays take advantage of the fact that male cells can be detected in a female host by detecting the male-cell-specific Y-chromosome by molecular assays (*e.g.,* polymerase chain reaction, or PCR).

Small numbers of HSCs (as few as one cell in mouse experiments) can be assayed using competitive reconstitutions, in which a small amount of host-type bone marrow cells (enough to radioprotect the host and thus ensure survival) is mixed in with the donor-HSC population. To establish long-term reconstitutions in mouse models, the mice are followed for at least 4 months after receiving the HSCs. Serial reconstitution, in which the bone marrow from a previously-irradiated and reconstituted mouse becomes the HSC source for

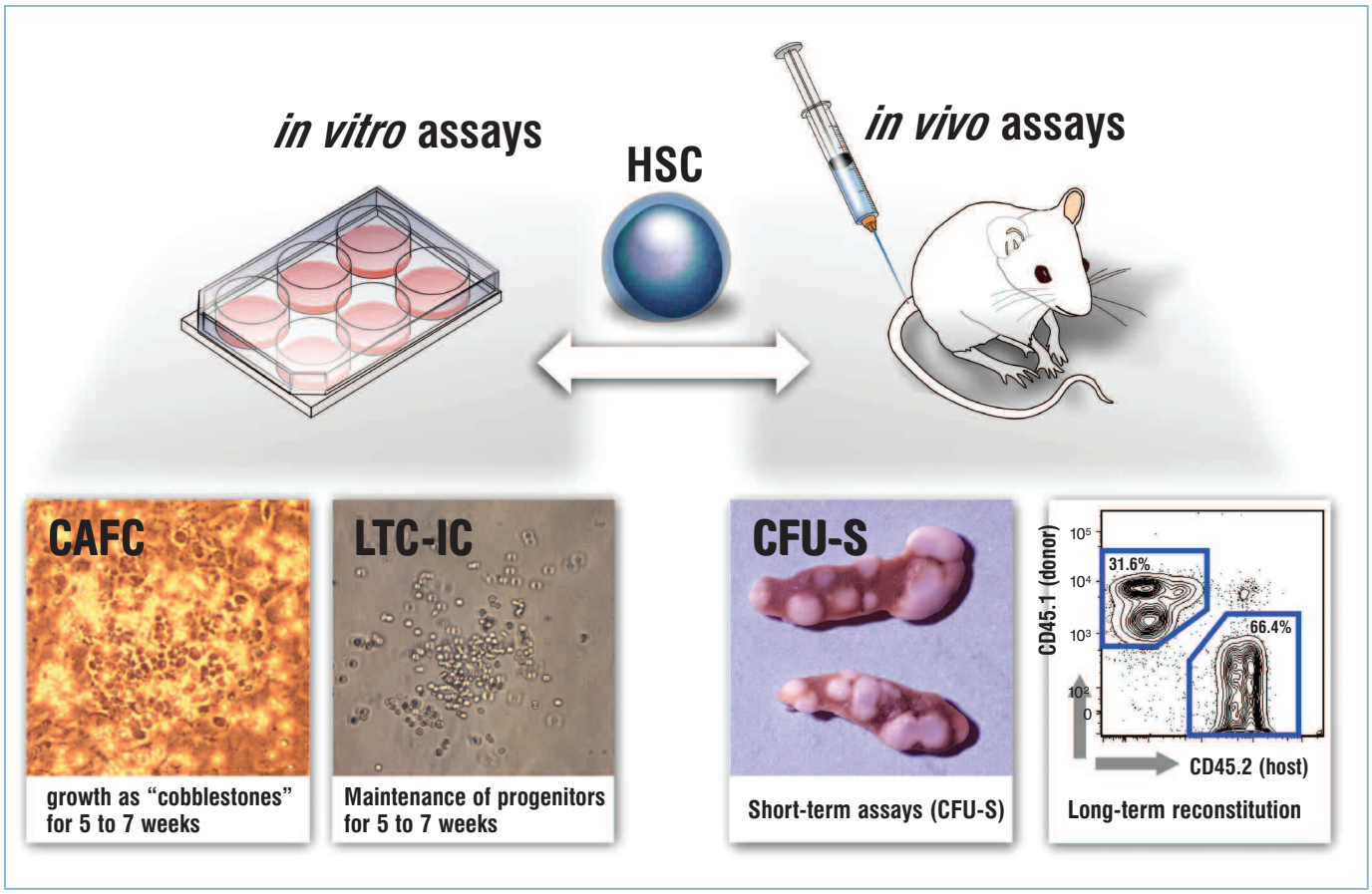


Figure 2.3. Assays used to detect hematopoietic stem cells. The tissue culture assays, which are used frequently to test human cells, include the ability of the cells to be tested to grow as "cobblestones" (the dark cells in the picture) for 5 to 7 weeks in culture. The Long Term Culture-Initiating Cell assay measures whether hematopoietic progenitor cells (capable of forming colonies in secondary assays, as shown in the picture) are still present after 5 to 7 weeks of culture.

In vivo assays in mice include the CFU-S assay, the original stem cell assay discussed in the introduction. The most stringent hematopoietic stem cell assay involves looking for the long-term presence of donor-derived cells in a reconstituted host. The example shows host-donor recognition by antibodies that recognize two different mouse alleles of CD45, a marker present on nearly all blood cells. CD45 is also a good marker for distinguishing human blood cells from mouse blood cells when testing human cells in immunocompromised mice such as NOD/SCID. Other methods such as pcr-markers, chromosomal markers, and enzyme markers can also be used to distinguish host and donor cells.

second irradiated mouse, extends the potential of this assay to test lifespan and expansion limits of HSCs. Unfortunately, the serial transfer assay measures both the lifespan and the transplantability of the stem cells. The transplantability may be altered under various conditions, so this assay is not the *sine qua non* of HSC function. Testing the *in vivo* activity of human cells is obviously more problematic.

Several experimental models have been developed that allow the testing of human cells in mice. These assays employ immunologically-incompetent mice (mutant mice that cannot mount an immune response against foreign cells) such as SCID[19–21] or NOD-SCID mice.[22,23] Reconstitution can be performed in either the presence or absence of human fetal bone or thymus implants to provide a more natural environment in which the human cells can grow in the mice. Recently NOD/SCID/cγ$^{-/-}$ mice have been used as improved recipients for human HSCs, capable of complete reconstitution with human lymphocytes, even in the absence of additional human tissues.[24] Even more promising has been the use of newborn mice with an impaired immune system (Rag-2$^{-/-}$Cγ$^{-/-}$), which results in reproducible production of human B- and T-lymphoid and myeloerythroid cells.[25] These assays

are clearly more stringent, and thus more informative, but also more difficult than the *in vitro* HSC assays discussed below. However, they can only assay a fraction of the lifespan under which the cells would usually have to function. Information on the long-term functioning of cells can only be derived from clinical HSC transplantations.

A number of assays have been developed to recognize HSCs *in vitro* (*e.g.,* in tissue culture). These are especially important when assaying human cells. Since transplantation assays for human cells are limited, cell culture assays often represent the only viable option. *In vitro* assays for HSCs include Long-Term Culture-Initializing Cell (LTC-IC) assays[26–28] and Cobble-stone Area Forming Cell (CAFC) assays.[29] LTC-IC assays are based on the ability of HSCs, but not more mature progenitor cells, to maintain progenitor cells with clonogenic potential over at least a five-week culture period. CAFC assays measure the ability of HSCs to maintain a specific and easily recognizable way of growing under stromal cells for five to seven weeks after the initial plating. Progenitor cells can only grow in culture in this manner for shorter periods of time.

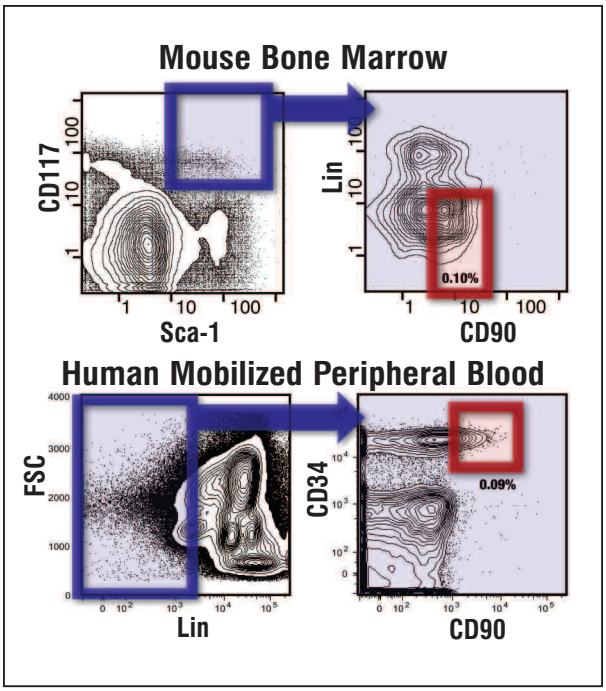


Figure 2.4. Examples of Hematopoietic Stem Cell staining patterns in mouse bone marrow (top) and human mobilized peripheral blood (bottom). The plots on the right show only the cells present in the left blue box. The cells in the right blue box represent HSCs. Stem cells form a rare fraction of the cells present in both cases.

Cell Markers Can Identify HSCs

While initial experiments studied HSC activity in mixed populations, much progress has been made in specifically describing the cells that have HSC activity. A variety of markers have been discovered to help recognize and isolate HSCs. Initial marker efforts focused on cell size, density, and recognition by lectins (carbohydrate-binding proteins derived largely from plants),[30] but more recent efforts have focused mainly on cell surface protein markers, as defined by monoclonal antibodies. For mouse HSCs, these markers include panels of 8 to 14 different monoclonal antibodies that recognize cell surface proteins present on differentiated hematopoietic lineages, such as the red blood cell and macrophage lineages (thus, these markers are collectively referred to as "Lin"),[13,31] as well as the proteins Sca-1,[13,31] CD27,[32] CD34,[33] CD38,[34] CD43,[35] CD90.1 (Thy-1.1),[13,31] CD117 (c-Kit),[36] AA4.1,[37] and MHC class I,[30] and CD150.[38] Human HSCs have been defined with respect to staining for Lin,[39] CD34,[40] CD38,[41] CD43,[35] CD45RO,[42] CD45RA,[42] CD59,[43] CD90,[39] CD109,[44] CD117,[45] CD133,[46,47] CD166,[48] and HLA DR (human).[49,50] In addition, metabolic markers/dyes such as rhodamine123 (which stains mitochondria),[51] Hoechst33342 (which identifies MDR-type drug efflux activity),[52] Pyronin-Y (which stains RNA),[53] and BAAA (indicative of aldehyde dehydrogenase enzyme activity)[54] have been described. While none of these markers recognizes functional stem cell activity, combinations (typically with 3 to 5 different markers, see examples below) allow for the purification of near-homogenous populations of HSCs. The ability to obtain pure preparations of HSCs, albeit in limited numbers, has greatly facilitated the functional and biochemical characterization of these important cells. However, to date there has been limited impact of these discoveries on clinical practice, as highly purified HSCs have only rarely been used to treat patients (discussed below). The undeniable advantages of using purified cells (*e.g.,* the absence of contaminating tumor cells in autologous transplantations) have been offset by practical difficulties and increased purification costs.

Cell Surface Marker Combinations That Define Hematopoietic Stem Cells

HSC assays, when combined with the ability to purify HSCs, have provided increasingly detailed insight into the cells and the early steps involved in the differentiation process. Several marker combinations have been developed that describe murine HSCs, including [CD117^{high}, CD90.1^{low}, Lin$^{neg/low}$, Sca-1^{pos}],[15] [CD90.1^{low}, Lin^{neg}, Sca-1^{pos} Rhodamine123^{low}],[55] [CD34$^{neg/low}$, CD117^{pos}, Sca-1^{pos}, Lin^{neg}],[33] [CD150 ^{pos}, CD48^{neg}, CD244^{neg}],[38] and "side-population" cells using Hoechst-dye.[52] Each of these combinations allows purification of HSCs to near-homogeneity. Figure 2.4 shows an example of an antibody combination that can recognize mouse HSCs. Similar strategies have been developed to purify human HSCs, employing markers such as CD34, CD38, Lin, CD90, CD133 and fluorescent substrates for the enzyme, aldehyde dehydrogenase. The use of highly purified human HSCs has been mainly experimental, and clinical use typically employs enrichment for one marker, usually CD34. CD34 enrichment yields a population of cells enriched for HSC and blood progenitor cells but still contains many other cell types. However, limited trials in which highly FACS-purified CD34^{pos} CD90^{pos} HSCs *(see Figure 2.4)* were used as a source of reconstituting cells have demonstrated that rapid reconstitution of the blood system can reliably be obtained using only HSCs.[56–58]

The purification strategies described above recognize a rare subset of cells. Exact numbers depend on the assay used as well as on the genetic background studied.[16] In mouse bone marrow, 1 in 10,000 cells is a hematopoietic stem cell with the ability to support long-term hematopoiesis following transplantation into a suitable host. When short-term stem cells, which have a limited self-renewal capacity, are included in the estimation, the frequency of stem cells in bone marrow increases to 1 in 1,000 to 1 in 2,000 cells in humans and mice. The numbers present in normal blood are at least ten-fold lower than in marrow.

None of the HSC markers currently used is directly linked to an essential HSC function, and consequently, even within a species, markers can differ depending on genetic alleles,[59] mouse strains,[60] developmental stages,[61] and cell activation stages.[62,63] Despite this, there is a clear correlation in HSC markers between divergent species such as humans and mice. However, unless the ongoing attempts at defining the complete HSC gene expression patterns will yield usable markers that are linked to essential functions for maintaining the "stemness" of the cells,[64,65] functional assays will remain necessary to identify HSCs unequivocally.[16]

Cell Surface Marker Patterns of Hematopoietic Progenitor Cells

More recently, efforts at defining hematopoietic populations by cell surface or other FACS-based markers have been extended to several of the progenitor populations that are derived from HSCs *(see Figure 2.5)*. Progenitors differ from stem cells in that they have a reduced differentiation capacity (they can generate only a subset of the possible lineages) but even more importantly, progenitors lack the ability to self-renew. Thus, they have to be constantly regenerated from the HSC population. However, progenitors do have extensive proliferative potential and can typically generate large numbers of mature cells. Among the progenitors defined in mice and humans are the Common Lymphoid Progenitor (CLP),[66,67] which in adults has the potential to generate all of the lymphoid but not myeloerythroid cells, and a Common Myeloid Progenitor (CMP), which has the potential to generate all of the mature myeloerythroid, but not lymphoid, cells.[68,69] While beyond the scope of this overview, hematopoietic progenitors have clinical potential and will likely see clinical use.[70,71]

HALLMARKS OF HSCS

HSCs have a number of unique properties, the combination of which defines them as such.[16] Among the core properties are the ability to choose between self-renewal (remain a stem cell after cell division) or differentiation (start the path towards becoming a mature hematopoietic cell). In addition, HSCs migrate in regulated fashion and are subject to regulation by apoptosis (programmed cell death). The balance between these activities determines the number of stem cells that are present in the body.

Self-Renewal

One essential feature of HSCs is the ability to self-renew, that is, to make copies with the same or very similar potential. This is an essential property because

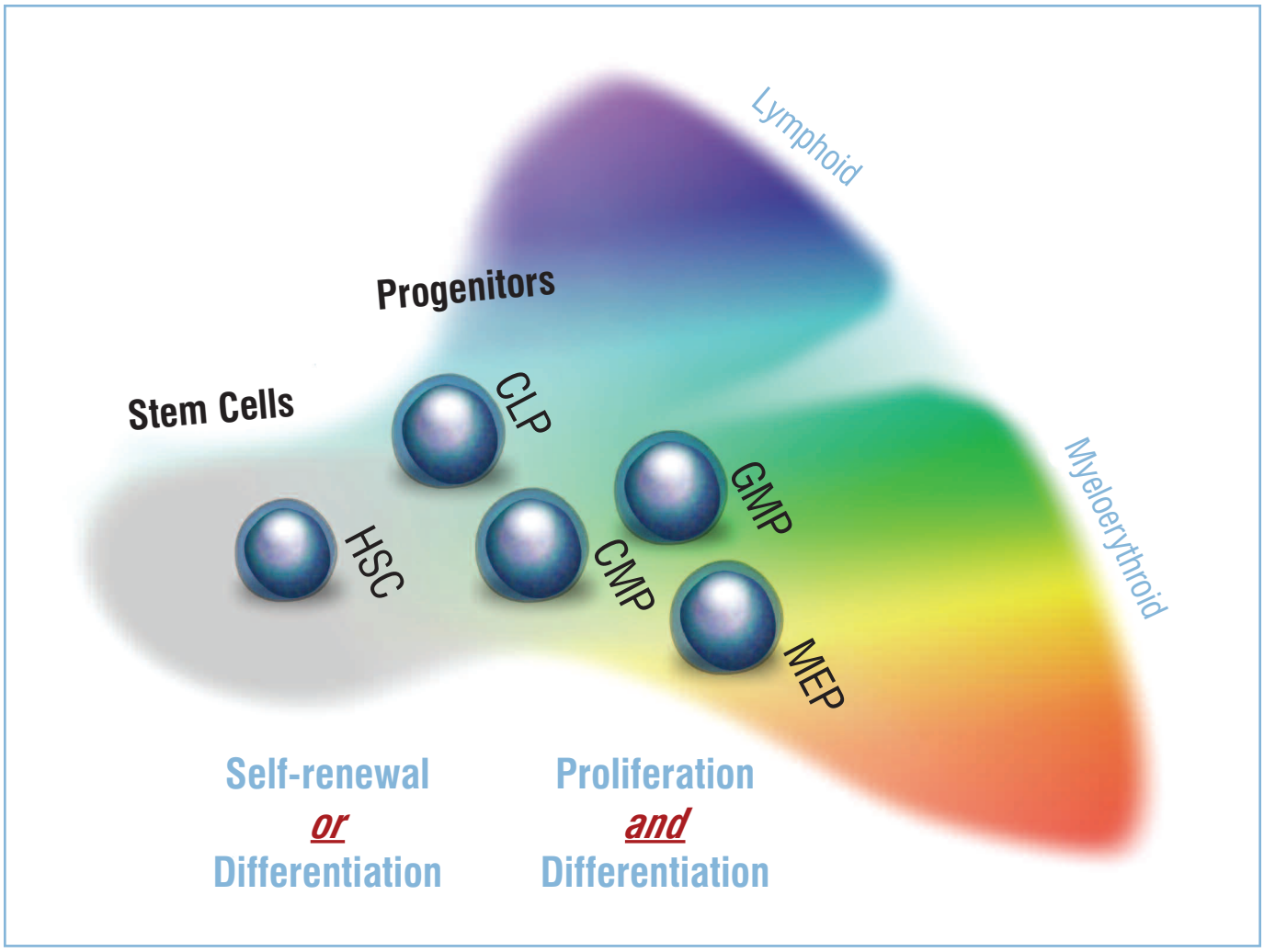


Figure 2.5. Relationship between several of the characterized hematopoietic stem cells and early progenitor cells. Differentiation is indicated by colors; the more intense the color, the more mature the cells. Surface marker distinctions are subtle between these early cell populations, yet they have clearly distinct potentials. Stem cells can choose between self-renewal and differentiation. Progenitors can expand temporarily but always continue to differentiate (other than in certain leukemias). The mature lymphoid (T-cells, B-cells, and Natural Killer cells) and myeloerythroid cells (granulocytes, macrophages, red blood cells, and platelets) that are produced by these stem and progenitor cells are shown in more detail in Figure 2.1.

more differentiated cells, such as hematopoietic progenitors, cannot do this, even though most progenitors can expand significantly during a limited period of time after being generated. However, for continued production of the many (and often short-lived) mature blood cells, the continued presence of stem cells is essential. While it has not been established that adult HSCs can self-renew indefinitely (this would be difficult to prove experimentally), it is clear from serial transplantation experiments that they can produce enough cells to last several (at least four to five) lifetimes in mice. It is still unclear which key signals allow self-renewal. One link that has been noted is telomerase, the enzyme necessary for maintaining telomeres, the DNA regions at the end of chromosomes that protect them from accumulating damage due to DNA replication. Expression of telomerase is associated with self-renewal activity.[72] However, while absence of telomerase reduces the self-renewal capacity of mouse HSCs, forced expression is not sufficient to enable HSCs to be transplanted indefinitely; other barriers must exist.[73,74]

It has proven surprisingly difficult to grow HSCs in culture despite their ability to self-renew. Expansion in culture is routine with many other cells, including

neural stem cells and ES cells. The lack of this capacity for HSCs severely limits their application, because the number of HSCs that can be isolated from mobilized blood, umbilical cord blood, or bone marrow restricts the full application of HSC transplantation in man (whether in the treatment of nuclear radiation exposure or transplantation in the treatment of blood cell cancers or genetic diseases of the blood or blood-forming system). Engraftment periods of 50 days or more were standard when limited numbers of bone marrow or umbilical cord blood cells were used in a transplant setting, reflecting the low level of HSCs found in these native tissues. Attempts to expand HSCs in tissue culture with known stem-cell stimulators, such as the cytokines stem cell factor/steel factor (KitL), thrombopoietin (TPO), interleukins 1, 3, 6, 11, plus or minus the myeloerythroid cytokines GM-CSF, G-CSF, M-CSF, and erythropoietin have never resulted in a significant expansion of HSCs.[16,75] Rather, these compounds induce many HSCs into cell divisions that are always accompanied by cellular differentiation.[76] Yet many experiments demonstrate that the transplantation of a single or a few HSCs into an animal results in a 100,000-fold or greater expansion in the number of HSCs at the steady state while simultaneously generating daughter cells that permitted the regeneration of the full blood-forming system.[77–80] Thus, we do not know the factors necessary to regenerate HSCs by self-renewing cell divisions. By investigating genes transcribed in purified mouse LT-HSCs, investigators have found that these cells contain expressed elements of the Wnt/fzd/beta-catenin signaling pathway, which enables mouse HSCs to undergo self-renewing cell divisions.[81,82] Overexpression of several other proteins, including HoxB4[83-86] and HoxA9[87] has also been reported to achieve this. Other signaling pathways that are under investigation include Notch and Sonic hedgehog.[75] Among the intracellular proteins thought to be essential for maintaining the "stem cell" state are Polycomb group genes, including Bmi-1.[88] Other genes, such as c-*Myc* and JunB have also been shown to play a role in this process.[89,90] Much remains to be discovered, including the identity of the stimuli that govern self-renewal *in vivo,* as well as the composition of the environment (the stem cell "niche") that provides these stimuli.[91] The recent identification of osteoblasts, a cell type known to be involved in bone formation, as a critical component of this environment[92,93] will help to focus this search. For instance, signaling by Angiopoietin-1 on osteoblasts to Tie-2 receptors on HSCs has recently been suggested to regulate stem cell quiescence (the lack of cell division).[94] It is critical to discover which pathways operate in the expansion of human HSCs to take advantage of these pathways to improve hematopoietic transplantation.

Differentiation

Differentiation into progenitors and mature cells that fulfill the functions performed by the hematopoietic system is not a unique HSC property, but, together with the option to self-renew, defines the core function of HSCs. Differentiation is driven and guided by an intricate network of growth factors and cytokines. As discussed earlier, differentiation, rather than self-renewal, seems to be the default outcome for HSCs when stimulated by many of the factors to which they have been shown to respond. It appears that, once they commit to differentiation, HSCs cannot revert to a self-renewing state. Thus, specific signals, provided by specific factors, seem to be needed to maintain HSCs. This strict regulation may reflect the proliferative potential present in HSCs, deregulation of which could easily result in malignant diseases such as leukemia or lymphoma.

Migration

Migration of HSCs occurs at specific times during development (*i.e.,* seeding of fetal liver, spleen and eventually, bone marrow) and under certain conditions (*e.g.,* cytokine-induced mobilization) later in life. The latter has proven clinically useful as a strategy to enhance normal HSC proliferation and migration, and the optimal mobilization regimen for HSCs currently used in the clinic is to treat the stem cell donor with a drug such as cytoxan, which kills most of his or her dividing cells. Normally, only about 8% of LT-HSCs enter the cell cycle per day,[95,96] so HSCs are not significantly affected by a short treatment with cytoxan. However, most of the downstream blood progenitors are actively dividing,[66,68] and their numbers are therefore greatly depleted by this dose, creating a demand for a regenerated blood-forming system. Empirically, cytokines or growth factors such as G-CSF and KitL can increase the number of HSCs in the blood, especially if administered for several days following a cytoxan pulse. The optimized protocol of cytoxan plus G-CSF results in several self-renewing cell divisions for

each resident LT-HSC in mouse bone marrow, expanding the number of HSCs 12- to 15-fold within two to three days.[97] Then, up to one-half of the daughter cells of self-renewing dividing LT-HSCs (estimated to be up to 10^5 per mouse per day[98]) leave the bone marrow, enter the blood, and within minutes engraft other hematopoietic sites, including bone marrow, spleen, and liver.[98] These migrating cells can and do enter empty hematopoietic niches elsewhere in the bone marrow and provide sustained hematopoietic stem cell self-renewal and hematopoiesis.[98,99] It is assumed that this property of mobilization of HSCs is highly conserved in evolution (it has been shown in mouse, dog and humans) and presumably results from contact with natural cell-killing agents in the environment, after which regeneration of hematopoiesis requires restoring empty HSC niches. This means that functional, transplantable HSCs course through every tissue of the body in large numbers every day in normal individuals.

Apoptosis

Apoptosis, or programmed cell death, is a mechanism that results in cells actively self-destructing without causing inflammation. Apoptosis is an essential feature in multicellular organisms, necessary during development and normal maintenance of tissues. Apoptosis can be triggered by specific signals, by cells failing to receive the required signals to avoid apoptosis, and by exposure to infectious agents such as viruses. HSCs are not exempt; apoptosis is one mechanism to regulate their numbers. This was demonstrated in transgenic mouse experiments in which HSC numbers doubled when the apoptosis threshold was increased.[76] This study also showed that HSCs are particularly sensitive and require two signals to avoid undergoing apoptosis.

SOURCES OF HSCS

Bone Marrow and Mobilized Peripheral Blood

The best-known location for HSCs is bone marrow, and bone marrow transplantation has become synonymous with hematopoietic cell transplantation, even though bone marrow itself is increasingly infrequently used as a source due to an invasive harvesting procedure that requires general anesthesia. In adults, under steady-state conditions, the majority of HSCs reside in bone marrow. However, cytokine mobilization can result in the release of large numbers of HSCs into the blood. As a clinical source of HSCs, mobilized peripheral blood (MPB) is now replacing bone marrow, as harvesting peripheral blood is easier for the donors than harvesting bone marrow. As with bone marrow, mobilized peripheral blood contains a mixture of hematopoietic stem and progenitor cells. MPB is normally passed through a device that enriches cells that express CD34, a marker on both stem and progenitor cells. Consequently, the resulting cell preparation that is infused back into patients is not a pure HSC preparation, but a mixture of HSCs, hematopoietic progenitors (the major component), and various contaminants, including T cells and, in the case of autologous grafts from cancer patients, quite possibly tumor cells. It is important to distinguish these kinds of grafts, which are the grafts routinely given, from highly purified HSC preparations, which essentially lack other cell types.

Umbilical Cord Blood

In the late 1980s, umbilical cord blood (UCB) was recognized as an important clinical source of HSCs.[100,101] Blood from the placenta and umbilical cord is a rich source of hematopoietic stem cells, and these cells are typically discarded with the afterbirth. Increasingly, UCB is harvested, frozen, and stored in cord blood banks, as an individual resource (donor-specific source) or as a general resource, directly available when needed. Cord blood has been used successfully to transplant children and (far less frequently) adults. Specific limitations of UCB include the limited number of cells that can be harvested and the delayed immune reconstitution observed following UCB transplant, which leaves patients vulnerable to infections for a longer period of time. Advantages of cord blood include its availability, ease of harvest, and the reduced risk of graft-versus-host-disease (GVHD). In addition, cord blood HSCs have been noted to have a greater proliferative capacity than adult HSCs. Several approaches have been tested to overcome the cell dose issue, including, with some success, pooling of cord blood samples.[101,102] *Ex vivo* expansion in tissue culture, to which cord blood cells are more amenable than adult cells, is another approach under active investigation.[103]

The use of cord blood has opened a controversial treatment strategy — embryo selection to create a related UCB donor.[104] In this procedure, embryos are conceived by *in vitro* fertilization. The embryos are

tested by pre-implantation genetic diagnosis, and embryos with transplantation antigens matching those of the affected sibling are implanted. Cord blood from the resulting newborn is then used to treat this sibling. This approach, successfully pioneered at the University of Minnesota, can in principle be applied to a wide variety of hematopoietic disorders. However, the ethical questions involved argue for clear regulatory guidelines.[105]

Embryonic Stem Cells

Embryonic stem (ES) cells form a potential future source of HSCs. Both mouse and human ES cells have yielded hematopoietic cells in tissue culture, and they do so relatively readily.[106] However, recognizing the actual HSCs in these cultures has proven problematic, which may reflect the variability in HSC markers or the altered reconstitution behavior of these HSCs, which are expected to mimic fetal HSC. This, combined with the potential risks of including undifferentiated cells in an ES-cell-derived graft means that, based on the current science, clinical use of ES cell-derived HSCs remains only a theoretical possibility for now.

HSC PLASTICITY

An ongoing set of investigations has led to claims that HSCs, as well as other stem cells, have the capacity to differentiate into a much wider range of tissues than previously thought possible. It has been claimed that, following reconstitution, bone marrow cells can differentiate not only into blood cells but also muscle cells (both skeletal myocytes and cardiomyocytes),[107–111] brain cells,[112,113] liver cells,[114,115] skin cells, lung cells, kidney cells, intestinal cells,[116] and pancreatic cells.[117] Bone marrow is a complex mixture that contains numerous cell types. In addition to HSCs, at least one other type of stem cell, the mesenchymal stem cell (MSC), is present in bone marrow. MSCs, which have become the subject of increasingly intense investigation, seem to retain a wide range of differentiation capabilities i*n vitro* that is not restricted to mesodermal tissues, but includes tissues normally derived from other embryonic germ layers (*e.g.,* neurons).[118–120] MSCs are discussed in detail in Dr. Catherine Verfaillie's testimony to the President's Council on Bioethics at this website: http://bioethicsprint.bioethics.gov/transcripts/apr02/apr25session2.html and will not be discussed further here. However, similar claims of differentiation into multiple diverse cell types, including muscle,[111] liver,[114] and different types of epithelium[116] have been made in experiments that assayed partially- or fully-purified HSCs. These experiments have spawned the idea that HSCs may not be entirely or irreversibly committed to forming the blood, but under the proper circumstances, HSCs may also function in the regeneration or repair of non-blood tissues. This concept has in turn given rise to the hypothesis that the fate of stem cells is "plastic," or changeable, allowing these cells to adopt alternate fates if needed in response to tissue-derived regenerative signals (a phenomenon sometimes referred to as "trans-differentiation"). This in turn seems to bolster the argument that the full clinical potential of stem cells can be realized by studying only adult stem cells, foregoing research into defining the conditions necessary for the clinical use of the extensive differentiation potential of embryonic stem cells. However, as discussed below, such "transdifferentiation" claims for specialized adult stem cells are controversial, and alternative explanations for these observations remain possible, and, in several cases, have been documented directly.

While a full discussion of this issue is beyond the scope of this overview, several investigators have formulated criteria that must be fulfilled to demonstrate stem cell plasticity.[121,122] These include *(i)* clonal analysis, which requires the transfer and analysis of single, highly-purified cells or individually marked cells and the subsequent demonstration of both "normal" and "plastic" differentiation outcomes, *(ii)* robust levels of "plastic" differentiation outcome, as extremely rare events are difficult to analyze and may be induced by artefact, and *(iii)* demonstration of tissue-specific function of the "transdifferentiated" cell type. Few of the current reports fulfill these criteria, and careful analysis of individually transplanted KTLS HSCs has failed to show significant levels of non-hematopoietic engraftment.[123,124] In addition, several reported trans-differentiation events that employed highly purified HSCs, and in some cases a very strong selection pressure for trans-differentiation, now have been shown to result from fusion of a blood cell with a non-blood cell, rather than from a change in fate of blood stem cells.[125–127] Finally, in the vast majority of cases, reported contributions of adult stem cells to cell types outside their tissue of origin are exceedingly rare, far too rare to be considered therapeutically useful. These findings have raised significant doubts about the

biological importance and immediate clinical utility of adult hematopoietic stem cell plasticity. Instead, these results suggest that normal tissue regeneration relies predominantly on the function of cell type-specific stem or progenitor cells, and that the identification, isolation, and characterization of these cells may be more useful in designing novel approaches to regenerative medicine. Nonetheless, it is possible that a rigorous and concerted effort to identify, purify, and potentially expand the appropriate cell populations responsible for apparent "plasticity" events, characterize the tissue-specific and injury-related signals that recruit, stimulate, or regulate plasticity, and determine the mechanism(s) underlying cell fusion or transdifferentiation, may eventually enhance tissue regeneration via this mechanism to clinically useful levels.

HSC SYSTEMS BIOLOGY

Recent progress in genomic sequencing and genome-wide expression analysis at the RNA and protein levels has greatly increased our ability to study cells such as HSCs as "systems," that is, as combinations of defined components with defined interactions. This goal has yet to be realized fully, as computational biology and system-wide protein biochemistry and proteomics still must catch up with the wealth of data currently generated at the genomic and transcriptional levels. Recent landmark events have included the sequencing of the human and mouse genomes and the development of techniques such as array-based analysis. Several research groups have combined cDNA cloning and sequencing with array-based analysis to begin to define the full transcriptional profile of HSCs from different species and developmental stages and compare these to other stem cells.[64,65,128–131] Many of the data are available in online databases, such as the NIH/NIDDK Stem Cell Genome Anatomy Projects (http://www.scgap.org). While transcriptional profiling is clearly a work in progress, comparisons among various types of stem cells may eventually identify sets of genes that are involved in defining the general "stemness" of a cell, as well as sets of genes that define their exit from the stem cell pool (*e.g.,* the beginning of their path toward becoming mature differentiated cells, also referred to as *commitment*). In addition, these datasets will reveal sets of genes that are associated with specific stem cell populations, such as HSCs and MSCs, and thus define their unique properties. Assembly of these datasets into pathways will greatly help to understand and to predict the responses of HSCs (and other stem cells) to various stimuli.

CLINICAL USE OF HSCS

The clinical use of stem cells holds great promise, although the application of most classes of adult stem cells is either currently untested or is in the earliest phases of clinical testing.[132,133] The only exception is HSCs, which have been used clinically since 1959 and are used increasingly routinely for transplantations, albeit almost exclusively in a non-pure form. By 1995, more than 40,000 transplants were performed annually world-wide.[134,135] Currently the main indications for bone marrow transplantation are either hematopoietic cancers (leukemias and lymphomas), or the use of high-dose chemotherapy for non-hematopoietic malignancies (cancers in other organs). Other indications include diseases that involve genetic or acquired bone marrow failure, such as aplastic anemia, thalassemia sickle cell anemia, and increasingly, autoimmune diseases.

Autologous versus Allogeneic Grafts

Transplantation of bone marrow and HSCs are carried out in two rather different settings, autologous and allogeneic. Autologous transplantations employ a patient's own bone marrow tissue and thus present no tissue incompatibility between the donor and the host. Allogeneic transplantations occur between two individuals who are not genetically identical (with the rare exceptions of transplantations between identical twins, often referred to as syngeneic transplantations). Non-identical individuals differ in their human leukocyte antigens (HLAs), proteins that are expressed by their white blood cells. The immune system uses these HLAs to distinguish between "self" and "non-self." For successful transplantation, allogeneic grafts must match most, if not all, of the six to ten major HLA antigens between host and donor. Even if they do, however, enough differences remain in mostly uncharacterized minor antigens to enable immune cells from the donor and the host to recognize the other as "non-self." This is an important issue, as virtually all HSC transplants are carried out with either non-purified, mixed cell populations (mobilized peripheral blood, cord blood, or bone marrow) or cell populations that have been enriched for HSCs (*e.g.,* by column selection

for CD34+ cells) but have not been fully purified. These mixed population grafts contain sufficient lymphoid cells to mount an immune response against host cells if they are recognized as "non-self." The clinical syndrome that results from this "non-self" response is known as graft-versus-host disease (GVHD).[136]

In contrast, autologous grafts use cells harvested from the patient and offer the advantage of not causing GVHD. The main disadvantage of an autologous graft in the treatment of cancer is the absence of a graft-versus-leukemia (GVL) or graft-versus-tumor (GVT) response, the specific immunological recognition of host tumor cells by donor-immune effector cells present in the transplant. Moreover, the possibility exists for contamination with cancerous or pre-cancerous cells.

Allogeneic grafts also have disadvantages. They are limited by the availability of immunologically-matched donors and the possibility of developing potentially lethal GVHD. The main advantage of allogeneic grafts is the potential for a GVL response, which can be an important contribution to achieving and maintaining complete remission.[137,138]

CD34+-Enriched versus Highly Purified HSC Grafts

Today, most grafts used in the treatment of patients consist of either whole or CD34+-enriched bone marrow or, more likely, mobilized peripheral blood. The use of highly purified hematopoietic stem cells as grafts is rare.[56–58] However, the latter have the advantage of containing no detectable contaminating tumor cells in the case of autologous grafts, therefore not inducing GVHD, or presumably GVL,[139–141] in allogeneic grafts. While they do so less efficiently than lymphocyte-containing cell mixtures, HSCs alone can engraft across full allogeneic barriers (*i.e.,* when transplanted from a donor who is a complete mismatch for both major and minor transplantation antigens).[139–141] The use of donor lymphocyte infusions (DLI) in the context of HSC transplantation allows for the controlled addition of lymphocytes, if necessary, to obtain or maintain high levels of donor cells and/or to induce a potentially curative GVL-response.[142,143] The main problems associated with clinical use of highly purified HSCs are the additional labor and costs[144] involved in obtaining highly purified cells in sufficient quantities.

While the possibilities of GVL and other immune responses to malignancies remain the focus of intense interest, it is also clear that in many cases, less-directed approaches such as chemotherapy or irradiation offer promise. However, while high-dose chemotherapy combined with autologous bone marrow transplantation has been reported to improve outcome (usually measured as the increase in time to progression, or increase in survival time),[145–154] this has not been observed by other researchers and remains controversial.[155–161] The tumor cells present in autologous grafts may be an important limitation in achieving long-term disease-free survival. Only further purification/purging of the grafts, with rigorous separation of HSCs from cancer cells, can overcome this limitation. Initial small scale trials with HSCs purified by flow cytometry suggest that this is both possible and beneficial to the clinical outcome.[56] In summary, purification of HSCs from cancer/lymphoma/leukemia patients offers the only possibility of using these cells post-chemotherapy to regenerate the host with cancer-free grafts. Purification of HSCs in allotransplantation allows transplantation with cells that regenerate the blood-forming system but cannot induce GVHD.

Non-Myeloablative Conditioning

An important recent advance in the clinical use of HSCs is the development of non-myeloablative preconditioning regimens, sometimes referred to as "mini transplants."[162–164] Traditionally, bone marrow or stem cell transplantation has been preceded by a preconditioning regimen consisting of chemotherapeutic agents, often combined with irradiation, that completely destroys host blood and bone marrow tissues (a process called *myeloablation*). This creates "space" for the incoming cells by freeing stem cell niches and prevents an undesired immune response of the host cells against the graft cells, which could result in graft failure. However, myeloablation immunocompromises the patient severely and necessitates a prolonged hospital stay under sterile conditions. Many protocols have been developed that use a more limited and targeted approach to preconditioning. These non-myeloablative preconditioning protocols, which combine excellent engraftment results with the ability to perform hematopoietic cell transplantation on an outpatient basis, have greatly changed the clinical practice of bone marrow transplantation.

Additional Indications

FACS purification of HSCs in mouse and man completely eliminates contaminating T cells, and thus GVHD (which is caused by T-lymphocytes) in allogeneic transplants. Many HSC transplants have been carried out in different combinations of mouse strains. Some of these were matched at the major transplantation antigens but otherwise different (Matched Unrelated Donors or MUD); in others, no match at the major or minor transplantation antigens was expected. To achieve rapid and sustained engraftment, higher doses of HSCs were required in these mismatched allogeneic transplants than in syngeneic transplants.[139–141,165–167] In these experiments, hosts whose immune and blood-forming systems were generated from genetically distinct donors were permanently capable of accepting organ transplants (such as the heart) from either donor or host, but not from mice unrelated to the donor or host. This phenomenon is known as transplant-induced tolerance and was observed whether the organ transplants were given the same day as the HSCs or up to one year later.[139,166] Hematopoietic cell transplant-related complications have limited the clinical application of such tolerance induction for solid organ grafts, but the use of non-myeloablative regimens to prepare the host, as discussed above, should significantly reduce the risk associated with combined HSC and organ transplants. Translation of these findings to human patients should enable a switch from chronic immunosuppression to prevent rejection to protocols wherein a single conditioning dose allows permanent engraftment of both the transplanted blood system and solid organ(s) or other tissue stem cells from the same donor. This should eliminate both GVHD and chronic host transplant immunosuppression, which lead to many complications, including life-threatening opportunistic infections and the development of malignant neoplasms.

We now know that several autoimmune diseases — diseases in which immune cells attack normal body tissues — involve the inheritance of high risk-factor genes.[168] Many of these genes are expressed only in blood cells. Researchers have recently tested whether HSCs could be used in mice with autoimmune disease (*e.g.,* type 1 diabetes) to replace an autoimmune blood system with one that lacks the autoimmune risk genes. The HSC transplants cured mice that were in the process of disease development when non-myeloablative conditioning was used for transplant.[169] It has been observed that transplant-induced tolerance allows co-transplantation of pancreatic islet cells to replace destroyed islets.[170] If these results using non-myeloablative conditioning can be translated to humans, type 1 diabetes and several other auto-immune diseases may be treatable with pure HSC grafts. However, the reader should be cautioned that the translation of treatments from mice to humans is often complicated and time-consuming.

Hematopoietic Stem Cell Banking

Banking is currently a routine procedure for UCB samples. If expansion of fully functional HSCs in tissue culture becomes a reality, HSC transplants may be possible by starting with small collections of HSCs rather than massive numbers acquired through mobilization and apheresis. With such a capability, collections of HSCs from volunteer donors or umbilical cords could be theoretically converted into storable, expandable stem cell banks useful on demand for clinical transplantation and/or for protection against radiation accidents. In mice, successful HSC transplants that regenerate fully normal immune and blood-forming systems can be accomplished when there is only a partial transplantation antigen match. Thus, the establishment of useful human HSC banks may require a match between as few as three out of six transplantation antigens (HLA). This might be accomplished with stem cell banks of as few as 4,000–10,000 independent samples.

LEUKEMIA (AND CANCER) STEM CELLS

Leukemias are proliferative diseases of the hematopoietic system that fail to obey normal regulatory signals. They derive from stem cells or progenitors of the hematopoietic system and almost certainly include several stages of progression. During this progression, genetic and/or epigenetic changes occur, either in the DNA sequence itself (genetic) or other heritable modifications that affect the genome (epigenetic). These (epi)genetic changes alter cells from the normal hematopoietic system into cells capable of robust leukemic growth. There are a variety of leukemias, usually classified by the predominant pathologic cell types and/or the clinical course of the disease. It has been proposed that these are diseases in which self-

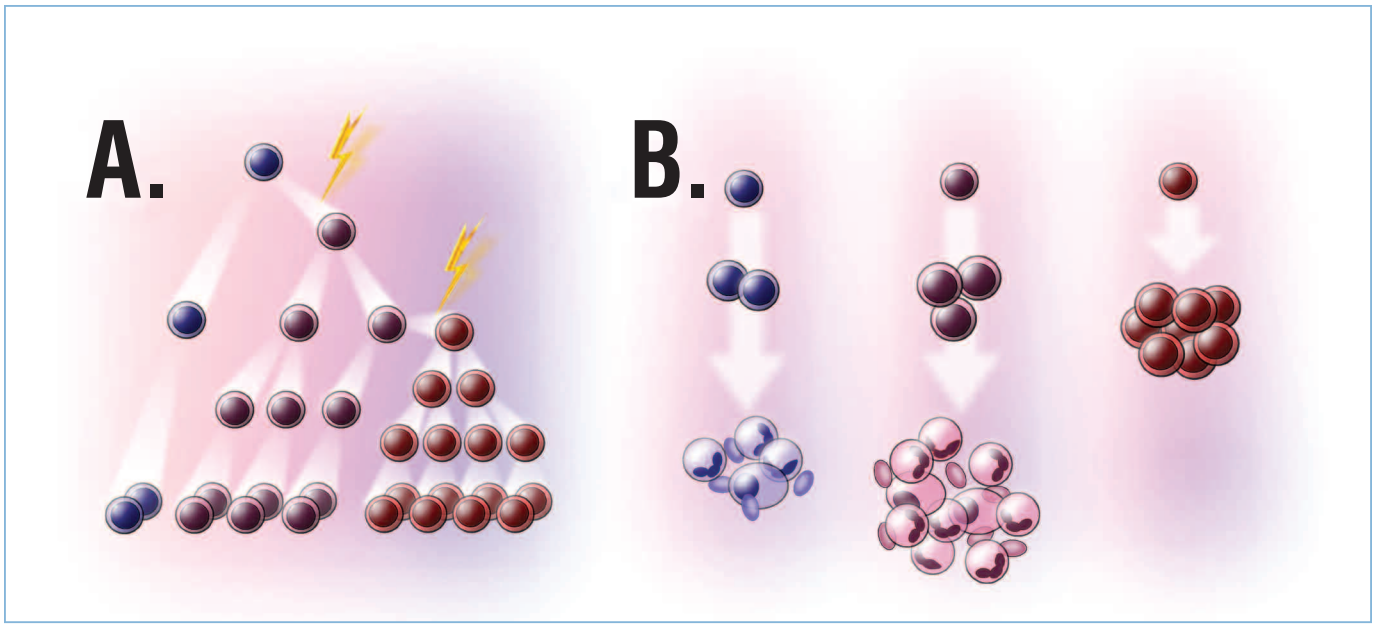


Figure 2.6. Leukemic progression at the hematopoietic stem cell level. Self-renewing HSCs are the cells present long enough to accumulate the many activating events necessary for full transformation into tumorigenic cells. Under normal conditions, half of the offspring of HSC cell divisions would be expected to undergo differentiation, leaving the HSC pool stable in size. (A) (Pre) leukemic progression results in cohorts of HSCs with increasing malignant potential. The cells with the additional event (two events are illustrated, although more would be expected to occur) can outcompete less-transformed cells in the HSC pool if they divide faster (as suggested in the figure) or are more resistant to differentiation or apoptosis (cell death), two major exit routes from the HSC pool. (B) Normal HSCs differentiate into progenitors and mature cells; this is linked with limited proliferation (left). Partially transformed HSCs can still differentiate into progenitors and mature cells, but more cells are produced. Also, the types of mature cells that are produced may be skewed from the normal ratio. Fully transformed cells may be completely blocked in terminal differentiation, and large numbers of primitive blast cells, representing either HSCs or self-renewing, transformed progenitor cells, can be produced. While this sequence of events is true for some leukemias (*e.g.,* AML), not all of the events occur in every leukemia. As with non-transformed cells, most leukemia cells (other than the leukemia stem cells) can retain the potential for (limited) differentiation.

renewing but poorly regulated cells, so-called "leukemia stem cells" (LSCs), are the populations that harbor all the genetic and epigenetic changes that allow leukemic progression.[171–176] While their progeny may be the characteristic cells observed with the leukemia, these progeny cells are not the self-renewing "malignant" cells of the disease. In this view, the events contributing to tumorigenic transformation, such as interrupted or decreased expression of "tumor suppressor" genes, loss of programmed death pathways, evasion of immune cells and macrophage surveillance mechanisms, retention of telomeres, and activation or amplification of self-renewal pathways, occur as single, rare events in the clonal progression to blast-crisis leukemia. As LT HSCs are the only self-renewing cells in the myeloid pathway, it has been proposed that most, if not all, progression events occur at this level of differentiation, creating clonal cohorts of HSCs with increasing malignancy *(see Figure 2.6).* In this disease model, the final event, explosive self-renewal, could occur at the level of HSC or at any of the known progenitors *(see Figures 2.5 and 2.6).* Activation of the β-catenin/lef-tcf signal transduction and transcription pathway has been implicated in leukemic stem cell self-renewal in mouse AML and human CML.[177] In both cases, the granulocyte-macrophage progenitors, not the HSCs or progeny blast cells, are the malignant self-renewing entities. In other models, such as the JunB-deficient tumors in mice and in chronic-phase CML in humans, the leukemic stem cell is the HSC itself.[90,177] However, these HSCs still respond to regulatory signals, thus representing steps in the clonal progression toward blast crisis *(see Figure 2.6).*

Many methods have revealed contributing proto-oncogenes and lost tumor suppressors in myeloid leukemias. Now that LSCs can be isolated, researchers should eventually be able to assess the full sequence of events in HSC clones undergoing leukemic

transformation. For example, early events, such as the AML/ETO translocation in AML or the BCR/ABL translocation in CML can remain present in normal HSCs in patients who are in remission (*e.g.,* without detectable cancer).[177,178] The isolation of LSCs should enable a much more focused attack on these cells, drawing on their known gene expression patterns, the mutant genes they possess, and the proteomic analysis of the pathways altered by the proto-oncogenic events.[173,176,179] Thus, immune therapies for leukemia would become more realistic, and approaches to classify and isolate LSCs in blood could be applied to search for cancer stem cells in other tissues.[180]

SUMMARY

After more than 50 years of research and clinical use, hematopoietic stem cells have become the best-studied stem cells and, more importantly, hematopoietic stem cells have seen widespread clinical use. Yet the study of HSCs remains active and continues to advance very rapidly. Fueled by new basic research and clinical discoveries, HSCs hold promise for such indications as treating autoimmunity, generating tolerance for solid organ transplants, and directing cancer therapy. However, many challenges remain. The availability of (matched) HSCs for all of the potential applications continues to be a major hurdle. Efficient expansion of HSCs in culture remains one of the major research goals. Future developments in genomics and proteomics, as well as in gene therapy, have the potential to widen the horizon for clinical application of hematopoietic stem cells even further.

REFERENCES

1. Jacobsen LO, Marks EK, Gaston EO, Zirkle RE. Effect of spleen protection on mortality following X-irradiation. *J Lab Clin Med.* Vol 34; 1949:1538-1543.
2. Lorenz E, Uphoff ED, Reid TR, Shelton E. Modification of acute irradiation injury in mice and guinea pigs by bone marrow injection. *Radiology.* Vol 58; 1951:863-877.
3. Gengozian N, Urso IS, Congdon CC, Conger AD, Makinodan T. Thymus specificity in lethally irradiated mice treated with rat bone marrow. *Proc Soc Exp Biol Med.* Vol 96; 1957:714-720.
4. Ford CE, Hamerton JL, Barnes DWH, Loutit JF. Cytological identification of radiation-chimaeras. *Nature.* Vol 177; 1956:452-454.
5. Nowell PC, Cole LJ, Habermyer JG, Roan PL. Growth and continued function of rat marrow cells in x-irradiated mice. *Cancer Res.* Vol 16; 1956:258-261.
6. Weissman IL. Translating stem and progenitor cell biology to the clinic: barriers and opportunities. *Science.* Vol 287; 2000:1442-1446.
7. Manz MG, Akashi K, Weissman IL. Biology of Hematopoietic Stem and Progenitor Cells. In: Appelbaum FR, ed. *Thomas' Hematopoietic Cell Transplantation.* Third ed. Malden, MA: Blackwell Publishing; 2004.
8. Till J, McCulloch E. A direct measurement of the radiation sensitivity of normal mouse bone marrow cells. *Radiat Res.* Vol 14; 1961:213-224.
9. Becker A, McCulloch E, Till J. Cytological demonstration of the clonal nature of spleen colonies derived from transplanted mouse marrow cells. *Nature.* Vol 197; 1963:452-454.
10. Siminovitch L, McCulloch E, Till J. The distribution of colony-forming cells among spleen colonies. *J Cell Comp Physiol.* Vol 62; 1963:327-336.
11. Wu AM, Till JE, Siminovitch L, McCulloch EA. Cytological evidence for a relationship between normal hematopoietic colony-forming cells and cell of the lymphoid system. *J Exp Med.* Vol 127; 1963:455-467.
12. Weissman IL. Stem cells: units of development, units of regeneration, and units in evolution. *Cell.* Vol 100; 2000:157-168.
13. Spangrude GJ, Heimfeld S, Weissman IL. Purification and characterization of mouse hematopoietic stem cells. *Science.* Vol 241; 1988:58-62.
14. Na Nakorn T, Traver D, Weissman IL, Akashi K. Myeloerythroid-restricted progenitors are sufficient to confer radioprotection and provide the majority of day 8 CFU-S. *J Clin Invest.* 2002;109:1579-1585.
15. Morrison SJ, Weissman IL. The long-term repopulating subset of hematopoietic stem cells is deterministic and isolatable by phenotype. *Immunity.* 1994;1:661-673.
16. Domen J, Weissman IL. Self-renewal, differentiation or death: regulation and manipulation of hematopoietic stem cell fate. *Mol Med Today.* Vol 5; 1999:201-208.
17. Ploemacher RE. Stem cells: characterization and measurement. *Baillieres Clin Haematol.* Vol 10; 1997:429-444.
18. Morrison SJ, Uchida N, Weissman IL. The biology of hematopoietic stem cells. *Annu Rev Cell Dev Biol.* Vol 11; 1995:35-71.
19. McCune JM, Namikawa H, Kaneshima H, Schultz LD, al e. The SCID-hu mouse: murine model for the analysis of human hematolymphoid differentiation and function. *Science.* Vol 241; 1988:1632-1639.
20. Kamel-Reid S, Dick JE. Engraftment of immune deficient mice with human hematopoietic stem cells. *Science.* Vol 242; 1988:1706-1709.
21. Peault B, Weissman IL, Baum C, McCune JM, Tsukamoto A. Lymphoid reconstitution of the human fetal thymus in SCID mice with CD34+ precursor cells. *J Exp Med.* Vol 174; 1991:1283-1286.

22. Larochelle A, Vormoor J, Hanenberg H, et al. Identification of primitive human hematopoietic cells capable of repopulating NOD/SCID mouse bone marrow: implications for gene therapy. *Nat Med.* Vol 2; 1996:1329-1337.

23. Bhatia M, Wang JC, Kapp U, Bonnet D, Dick JE. Purification of primitive human hematopoietic cells capable of repopulating immune-deficient mice. *Proc Natl Acad Sci USA.* Vol 94; 1997:5320-5325.

24. Hiramatsu H, Nishikomori R, Heike T, et al. Complete reconstitution of human lymphocytes from cord blood CD34+ cells using the NOD/SCID/gammacnull mice model. *Blood.* Vol 102; 2003:873-880.

25. Traggiai E, Chicha L, Mazzucchelli L, et al. Development of a human adaptive immune system in cord blood cell-transplanted mice. *Science.* Vol 304; 2004:104-107.

26. Dexter TM, Allen TD, Lajtha LG. Conditions controlling the proliferation of haemopoietic stem cells in vitro. *J Cell Physiol.* Vol 91; 1977:335-344.

27. Whitlock CA, Witte ON. Long-term culture of B lymphocytes and their precursors from murine bone marrow. *Proc Natl Acad Sci USA.* Vol 79; 1982:3608-3612.

28. Sutherland HJ, Lansdorp PM, Henkelman DH, Eaves AC, Eaves CJ. Functional characterization of individual human hematopoietic stem cells cultured at limiting dilution on supportive marrow stromal layers. *Proc Natl Acad Sci USA.* Vol 87; 1990:3584-3588.

29. Ploemacher R, van der Sluijs J, Voerman JSA, Bron NHC. An in vitro limiting-dilution assay of long-term repopulating hematopoietic stem cell in the mouse. *Blood.* Vol 74; 1989:2755-2763.

30. Bauman JG, de Vries P, Pronk B, Visser JW. Purification of murine hemopoietic stem cells and committed progenitors by fluorescence activated cell sorting using wheat germ agglutinin and monoclonal antibodies. *Acta Histochem Suppl.* Vol 36; 1988:241-253.

31. Uchida N, Weissman IL. Searching for hematopoietic stem cells: evidence that Thy-1.1lo Lin- Sca-1+ cells are the only stem cells in C57BL/Ka-Thy-1.1 bone marrow. *J Exp Med.* Vol 175; 1992:175-184.

32. Wiesmann A, Phillips RL, Mojica M, et al. Expression of CD27 on murine hematopoietic stem and progenitor cells. *Immunity.* Vol 12; 2000:193-199.

33. Osawa M, Hanada K, Hamada H, Nakauchi H. Long-term lymphohematopoietic reconstitution by a single CD34-low/negative hematopoietic stem cell. Science. Vol 273; 1996:242-245.

34. Randall TD, Lund FE, Howard MC, Weissman IL. Expression of murine CD38 defines a population of long-term reconstituting hematopoietic stem cells. *Blood.* Vol 87; 1996:4057-4067.

35. Moore T, Huang S, Terstappen LW, Bennett M, Kumar V. Expression of CD43 on murine and human pluripotent hematopoietic stem cells. *J Immunol.* Vol 153; 1994:4978-4987.

36. Ikuta K, Weissman IL. Evidence that hematopoietic stem cells express mouse c-kit but do not depend on steel factor for their generation. *Proc Natl Acad Sci USA.* Vol 89; 1992:1502-1506.

37. Jordan CT, McKearn JP, Lemischka IR. Cellular and developmental properties of fetal hematopoietic stem cells. *Cell.* Vol 61; 1990:953-963.

38. Kiel MJ, Yilmaz OH, Iwashita T, Terhorst C, Morrison SJ. SLAM family receptors distinguish hematopioetic stem and progenitor cells and reveal enothelial niches for stem cells. *Cell.* 2005;121:1109-1121.

39. Baum CM, Weissman IL, Tsukamoto AS, Buckle AM, Peault B. Isolation of a candidate human hematopoietic stem-cell population. *Proc Natl Acad Sci USA.* Vol 89; 1992:2804-2808.

40. Civin CI, Strauss LC, Brovall C, Fackler MJ, Schwartz JF, Shaper JH. Antigenic analysis of hematopoiesis. III. A hematopoietic progenitor cell surface antigen defined by a monoclonal antibody raised against KG- 1a cells. *J Immunol.* Vol 133; 1984:157-165.

41. Terstappen LW, Huang S, Safford M, Lansdorp PM, Loken MR. Sequential generations of hematopoietic colonies derived from single nonlineage-committed CD34+CD38– progenitor cells. *Blood.* Vol 77; 1991:1218-1227.

42. Lansdorp PM, Sutherland HJ, Eaves CJ. Selective expression of CD45 isoforms on functional subpopulations of CD34+ hemopoietic cells from human bone marrow. *J Exp Med.* Vol 172; 1990:363-366.

43. Hill B, Rozler E, Travis M, et al. High-level expression of a novel epitope of CD59 identifies a subset of CD34+ bone marrow cells highly enriched for pluripotent stem cells. *Exp Hematol.* Vol 24; 1996:936-943.

44. Sutherland DR, Yeo EL, Stewart AK, et al. Identification of CD34+ subsets after glycoprotease selection: engraftment of CD34+Thy-1+Lin– stem cells in fetal sheep. *Exp Hematol.* Vol 24; 1996:795-806.

45. Gunji Y, Nakamura M, Osawa H, et al. Human primitive hematopoietic progenitor cells are more enriched in KITlow cells than in KIThigh cells. *Blood.* Vol 82; 1993:3283-3289.

46. Miraglia S, Godfrey W, Yin AH, et al. A novel five-transmembrane hematopoietic stem cell antigen: isolation, characterization, and molecular cloning. *Blood.* Vol 90; 1997:5013-5021.

47. Yin AH, Miraglia S, Zanjani ED, et al. AC133, a novel marker for human hematopoietic stem and progenitor cells. *Blood.* Vol 90; 1997:5002-5012.

48. Uchida N, Yang Z, Combs J, et al. The characterization, molecular cloning, and expression of a novel hematopoietic cell antigen from CD34+ human bone marrow cells. *Blood.* Vol 89; 1997:2706-2716.

49. Srour EF, Brandt JE, Leemhuis T, Ballas CB, Hoffman R. Relationship between cytokine-dependent cell cycle progression and MHC class II antigen expression by human CD34+ HLA-DR- bone marrow cells. *J Immunol.* Vol 148; 1992:815-820.

50. Tsukamoto A, Weissman I, Chen B, et al. Phenotypic and functional analysis of hematopoietic stem cells in mouse and man. In: Mertelsman La, ed. *Hematopoietic Stem Cells, Biology and Therapeutic Applications:* Phenotypic and functional analysis of hematopoietic stem cells in mouse and man.; 1995.

51. Spangrude GJ, Johnson GR. Resting and activated subsets of mouse multipotent hematopoietic stem cells. *Proc Natl Acad Sci USA.* Vol 87; 1990:7433-7437.

52. Goodell MA, Brose K, Paradis G, Conner AS, Mulligan RC. Isolation and functional properties of murine hematopoietic stem cells that are replicating in vivo. *J Exp Med.* Vol 183; 1996:1797-1806.

53. Gothot A, Pyatt R, McMahel J, Rice S, Srour EF. Functional heterogeneity of human CD34(+) cells isolated in subcompartments of the G0 /G1 phase of the cell cycle. *Blood.* Vol 90; 1997:4384-4393.

54. Storms RW, Trujillo AP, Springer JB, et al. Isolation of primitive human hematopoietic progenitors on the basis of aldehyde dehydrogenase activity. *Proc Natl Acad Sci USA.* Vol 96; 1999:9118-9123.

55. Kim M, Cooper DD, Hayes SF, Spangrude GJ. Rhodamine-123 staining in hematopoietic stem cells of young mice indicates mitochondrial activation rather than dye efflux. *Blood.* Vol 91; 1998:4106-4117.

56. Negrin RS, Atkinson K, Leemhuis T, et al. Transplantation of highly purified CD34+Thy-1+ hematopoietic stem cells in patients with metastatic breast cancer. *Biol Blood Marrow Transplant.* Vol 6; 2000:262-271.

57. Michallet M, Philip T, Philip I, et al. Transplantation with selected autologous peripheral blood CD34+Thy-1+ hematopoietic stem cells (HSCs) in multiple myeloma: impact of HSC dose on engraftment, safety, and immune reconstitution. *Exp Hematol.* Vol 28; 2000:858-870.

58. Vose JM, Bierman PJ, Lynch JC, et al. Transplantation of highly purified CD34+Thy-1+ hematopoietic stem cells in patients with recurrent indolent non-Hodgkin's lymphoma. *Biol Blood Marrow Transplant.* Vol 7; 2001:680-687.

59. Spangrude GJ, Brooks DM. Phenotypic analysis of mouse hematopoietic stem cells shows a Thy-1- negative subset. *Blood.* Vol 80; 1992:1957-1964.

60. Spangrude GJ, Brooks DM. Mouse strain variability in the expression of the hematopoietic stem cell antigen Ly-6A/E by bone marrow cells. *Blood.* Vol 82; 1993:3327-3332.

61. Morrison SJ, Hemmati HD, Wandycz AM, Weissman IL. The purification and characterization of fetal liver hematopoietic stem cells. *Proc Natl Acad Sci USA.* Vol 92; 1995:10302-10306.

62. Randall TD, Weissman IL. Phenotypic and functional changes induced at the clonal level in hematopoietic stem cells after 5-fluorouracil treatment. *Blood.* Vol 89; 1997:3596-3606.

63. Sato T, Laver JH, Ogawa M. Reversible Expression of CD34 by Murine Hematopoietic Stem Cells. *Blood.* Vol 94; 1999:2548-2554.

64. Ramalho-Santos M, Yoon S, Matsuzaki Y, Mulligan RC, Melton DA. "Stemness": transcriptional profiling of embryonic and adult stem cells. *Science.* Vol 298; 2002:597-600.

65. Ivanova NB, Dimos JT, Schaniel C, Hackney JA, Moore KA, Lemischka IR. A stem cell molecular signature. *Science.* Vol 298; 2002:601-604.

66. Kondo M, Weissman IL, Akashi K. Identification of clonogenic common lymphoid progenitors in mouse bone marrow. *Cell.* Vol 91; 1997:661-672.

67. Galy A, Travis M, Cen D, Chen B. Human T, B, natural killer, and dendritic cells arise from a common bone marrow progenitor cell subset. *Immunity.* Vol 3; 1995:459-473.

68. Akashi K, Traver D, Miyamoto T, Weissman IL. A clonogenic common myeloid progenitor that gives rise to all myeloid lineages. *Nature.* Vol 404; 2000:193-197.

69. Manz MG, Miyamoto T, Akashi K, Weissman IL. Prospective isolation of human clonogenic common myeloid progenitors. *Proc Natl Acad Sci USA.* Vol 99; 2002:11872-11877.

70. Arber C, BitMansour A, Sparer TE, et al. Common lymphoid progenitors rapidly engraft and protect against lethal murine cytomegalovirus infection after hematopoietic stem cell transplantation. *Blood.* Vol 102; 2003:421-428.

71. BitMansour A, Burns SM, Traver D, et al. Myeloid progenitors protect against invasive aspergillosis and Pseudomonas aeruginosa infection following hematopoietic stem cell transplantation. *Blood.* Vol 100; 2002:4660-4667.

72. Greenwood MJ, Lansdorp PM. Telomeres, telomerase, and hematopoietic stem cell biology. *Arch Med Res.* Vol 34; 2003:489-495.

73. Allsopp RC, Morin GB, Horner JW, DePinho R, Harley CB, Weissman IL. Effect of TERT over-expression on the long-term transplantation capacity of hematopoietic stem cells. *Nat Med.* Vol 9; 2003:369-371.

74. Allsopp RC, Morin GB, DePinho R, Harley CB, Weissman IL. Telomerase is required to slow telomere shortening and extend replicative lifespan of HSCs during serial transplantation. *Blood.* Vol 102; 2003:517-520.

75. Reya T. Regulation of hematopoietic stem cell self-renewal. *Recent Prog Horm Res.* Vol 58; 2003:283-295.

76. Domen J, Weissman IL. Hematopoietic stem cells need two signals to prevent apoptosis; BCL-2 can provide one of these, Kitl/c-Kit signaling the other. *J Exp Med.* Vol 192; 2000:1707-1718.

77. Smith LG, Weissman IL, Heimfeld S. Clonal analysis of hematopoietic stem-cell differentiation in vivo. *Proc Natl Acad Sci USA.* Vol 88; 1991:2788-2792.

78. Wagers AJ, Sherwood RI, Christensen JL, Weissman IL. Little evidence for developmental plasticity of adult hematopoietic stem cells. *Science.* Vol 297; 2002:2256-2259.

79. Morrison SJ, Wandycz AM, Hemmati HD, Wright DE, Weissman IL. Identification of a lineage of multipotent hematopoietic progenitors. *Development.* Vol 124; 1997:1929-1939.

80. Iscove NN, Nawa K. Hematopoietic stem cells expand during serial transplantation in vivo without apparent exhaustion. *Curr Biol.* Vol 7; 1997:805-808.

81. Reya T, Duncan AW, Ailles L, et al. A role for Wnt signalling in self-renewal of haematopoietic stem cells. *Nature.* Vol 423; 2003:409-414.

82. Willert K, Brown JD, Danenberg E, et al. Wnt proteins are lipid-modified and can act as stem cell growth factors. *Nature.* Vol 423; 2003:448-452.

83. Sauvageau G, Thorsteinsdottir U, Eaves CJ, et al. Overexpression of HOXB4 in hematopoietic cells causes the selective expansion of more primitive populations in vitro and in vivo. *Genes Dev.* Vol 9; 1995:1753-1765.

84. Antonchuk J, Sauvageau G, Humphries RK. HOXB4-induced expansion of adult hematopoietic stem cells ex vivo. *Cell.* Vol 109; 2002:39-45.

85. Krosl J, Austin P, Beslu N, Kroon E, Humphries RK, Sauvageau G. In vitro expansion of hematopoietic stem cells by recombinant TAT-HOXB4 protein. *Nat Med.* Vol 9; 2003:1428-1432.

86. Amsellem S, Pflumio F, Bardinet D, et al. Ex vivo expansion of human hematopoietic stem cells by direct delivery of the HOXB4 homeoprotein. *Nat Med.* Vol 9; 2003:1423-1427.

87. Thorsteinsdottir U, Mamo A, Kroon E, et al. Overexpression of the myeloid leukemia-associated Hoxa9 gene in bone marrow cells induces stem cell expansion. *Blood.* Vol 99; 2002:121-129.

88. Valk-Lingbeek ME, Bruggeman SW, van Lohuizen M. Stem cells and cancer; the polycomb connection. *Cell.* Vol 118; 2004:409-418.

89. Wilson A, Murphy MJ, Oskarsson T, et al. c-Myc controls the balance between hematopoietic stem cell self-renewal and differentiation. *Genes Dev.* Vol 18; 2004:2747-2763.

90. Passegue E, Wagner EF, Weissman IL. JunB deficiency leads to a myeloproliferative disorder arising from hematopoietic stem cells. *Cell.* Vol 119; 2004:431-443.

91. Watt FM, Hogan BL. Out of Eden: stem cells and their niches. *Science.* Vol 287; 2000:1427-1430.

92. Calvi LM, Adams GB, Weibrecht KW, et al. Osteoblastic cells regulate the haematopoietic stem cell niche. *Nature.* Vol 425; 2003:841-846.

93. Zhang J, Niu C, Ye L, et al. Identification of the haematopoietic stem cell niche and control of the niche size. *Nature.* Vol 425; 2003:836-841.

94. Arai F, Hirao A, Ohmura M, et al. Tie2/angiopoietin-1 signaling regulates hematopoietic stem cell quiescence in the bone marrow niche. *Cell.* Vol 118; 2004:149-161.

95. Cheshier SH, Morrison SJ, Liao X, Weissman IL. In vivo proliferation and cell cycle kinetics of long-term self- renewing hematopoietic stem cells. *Proc Natl Acad Sci USA.* Vol 96; 1999:3120-3125.

96. Bradford GB, Williams B, Rossi R, Bertoncello I. Quiescence, cycling, and turnover in the primitive hematopoietic stem cell compartment. *Exp Hematol.* Vol 25; 1997:445-453.

97. Morrison SJ, Wright DE, Weissman IL. Cyclophosphamide/granulocyte colony-stimulating factor induces hematopoietic stem cells to proliferate prior to mobilization. *Proc Natl Acad Sci USA.* Vol 94; 1997:1908-1913.

98. Wright DE, Wagers AJ, Gulati AP, Johnson FL, Weissman IL. Physiological migration of hematopoietic stem and progenitor cells. *Science.* Vol 294; 2001:1933-1936.

99. Abkowitz JL, Robinson AE, Kale S, Long MW, Chen J. Mobilization of hematopoietic stem cells during homeostasis and after cytokine exposure. *Blood.* Vol 102; 2003:1249-1253.

100. Barker JN, Wagner JE. Umbilical cord blood transplantation: current practice and future innovations. *Crit Rev Oncol Hematol.* Vol 48; 2003:35-43.

101. Koh LP, Chao NJ. Umbilical cord blood transplantation in adults using myeloablative and nonmyeloablative preparative regimens. *Biol Blood Marrow Transplant.* Vol 10; 2004:1-22.

102. Wagner Je J, Barker J. Umbilical cord blood transplantation: Novel approaches toward improving engraftment. *Biol Blood Marrow Transplant.* Vol 10; 2004:733.

103. Jaroscak J, Goltry K, Smith A, et al. Augmentation of umbilical cord blood (UCB) transplantation with ex vivo-expanded UCB cells: results of a phase 1 trial using the AastromReplicell System. *Blood.* Vol 101; 2003:5061-5067.

104. Grewal SS, Kahn JP, MacMillan ML, Ramsay NK, Wagner JE. Successful hematopoietic stem cell transplantation for Fanconi anemia from an unaffected HLA-genotype-identical sibling selected using preimplantation genetic diagnosis. *Blood.* Vol 103; 2004:1147-1151.

105. Wolf SM, Kahn JP, Wagner JE. Using preimplantation genetic diagnosis to create a stem cell donor: issues, guidelines & limits. *J Law Med Ethics.* Vol 31; 2003:327-339.

106. Vodyanik MA, Bork JA, Thomson JA, Slukvin, II. Human embryonic stem cell-derived CD34+ cells: efficient production in the co-culture with OP9 stromal cells and analysis of lymphohematopoietic potential. *Blood;* 2004.

107. Ferrari G, Cusella-De Angelis G, Coletta M, et al. Muscle regeneration by bone marrow-derived myogenic progenitors. *Science.* Vol 279; 1998:1528-1530.

108. Gussoni E, Soneoka Y, Strickland CD, et al. Dystrophin expression in the mdx mouse restored by stem cell transplantation. *Nature.* Vol 401; 1999:390-394.

109. Orlic D, Kajstura J, Chimenti S, et al. Bone marrow cells regenerate infarcted myocardium. *Nature.* Vol 410; 2001:701-705.

110. Orlic D, Kajstura J, Chimenti S, et al. Mobilized bone marrow cells repair the infarcted heart, improving function and survival. *Proc Natl Acad Sci USA.* Vol 98; 2001:10344-10349.

111. Jackson KA, Majka SM, Wang H, et al. Regeneration of ischemic cardiac muscle and vascular endothelium by adult stem cells. *J Clin Invest.* Vol 107; 2001:1395-1402.

112. Brazelton TR, Rossi FM, Keshet GI, Blau HM. From marrow to brain: expression of neuronal phenotypes in adult mice. *Science.* Vol 290; 2000:1775-1779.

113. Mezey E, Chandross KJ, Harta G, Maki RA, McKercher SR. Turning blood into brain: cells bearing neuronal antigens generated in vivo from bone marrow. *Science.* Vol 290; 2000:1779-1782.

114. Lagasse E, Connors H, Al-Dhalimy M, et al. Purified hematopoietic stem cells can differentiate into hepatocytes in vivo. *Nat Med.* Vol 6; 2000:1229-1234.

115. Petersen BE, Bowen WC, Patrene KD, et al. Bone marrow as a potential source of hepatic oval cells. *Science.* Vol 284; 1999:1168-1170.

116. Krause DS, Theise ND, Collector MI, et al. Multi-organ, multi-lineage engraftment by a single bone marrow-derived stem cell. *Cell.* Vol 105; 2001:369-377.

117. Ianus A, Holz GG, Theise ND, Hussain MA. In vivo derivation of glucose-competent pancreatic endocrine cells from bone marrow without evidence of cell fusion. *J Clin Invest.* Vol 111; 2003:843-850.

118. Prockop DJ. Marrow stromal cells as stem cells for nonhematopoietic tissues. *Science.* Vol 276; 1997:71-74.

119. Pittenger MF, Mackay AM, Beck SC, et al. Multilineage potential of adult human mesenchymal stem cells. *Science.* Vol 284; 1999:143-147.

120. Verfaillie CM, Schwartz R, Reyes M, Jiang Y. Unexpected potential of adult stem cells. *Ann NY Acad Sci.* Vol 996; 2003:231-234.

121. Lemischka I. A few thoughts about the plasticity of stem cells. *Exp Hematol.* Vol 30; 2002:848-852.

122. Verfaillie CM. Adult stem cells: assessing the case for pluripotency. *Trends Cell Biol.* Vol 12; 2002:502-508.

123. Wagers AJ, Weissman IL. Plasticity of adult stem cells. *Cell.* Vol 116; 2004:639-648.

124. Sherwood RI, Christensen JL, Conboy IM, et al. Isolation of Adult Mouse Myogenic Progenitors; Functional Heterogeneity of Cells within and Engrafting Skeletal Muscle. *Cell.* Vol 119; 2004:543-554.

125. Wang X, Willenbring H, Akkari Y, et al. Cell fusion is the principal source of bone-marrow-derived hepatocytes. *Nature.* Apr 24 2003;422(6934):897-901.

126. Alvarez-Dolado M, Pardal R, Garcia-Verdugo JM, et al. Fusion of bone-marrow-derived cells with Purkinje neurons, cardiomyocytes and hepatocytes. *Nature.* Vol 425; 2003:968-973.

127. Camargo FD, Finegold M, Goodell MA. Hematopoietic myelomonocytic cells are the major source of hepatocyte fusion partners. *J Clin Invest.* Vol 113; 2004:1266-1270.

128. Phillips RL, Ernst RE, Brunk B, et al. The genetic program of hematopoietic stem cells. *Science.* Vol 288; 2000:1635-1640.

129. Terskikh AV, Easterday MC, Li L, et al. From hematopoiesis to neuropoiesis: evidence of overlapping genetic programs. *Proc Natl Acad Sci USA.* Vol 98; 2001:7934-7939.

130. Park IK, He Y, Lin F, et al. Differential gene expression profiling of adult murine hematopoietic stem cells. *Blood.* Vol 99; 2002:488-498.

131. Terskikh AV, Miyamoto T, Chang C, Diatchenko L, Weissman IL. Gene expression analysis of purified hematopoietic stem cells and committed progenitors. *Blood.* Vol 102; 2003:94-101.

132. Koc ON, Gerson SL, Cooper BW, et al. Rapid hematopoietic recovery after coinfusion of autologous-blood stem cells and culture-expanded marrow mesenchymal stem cells in advanced breast cancer patients receiving high-dose chemotherapy. *J Clin Oncol.* Vol 18; 2000:307-316.

133. Wakitani S, Imoto K, Yamamoto T, Saito M, Murata N, Yoneda M. Human autologous culture expanded bone marrow mesenchymal cell transplantation for repair of cartilage defects in osteoarthritic knees. *Osteoarthritis Cartilage.* Vol 10; 2002:199-206.

134. Santos GW. Historical background to hematopoietic stem cell transplantation. In: Atkinson K, ed. *Clinical bone marrow and blood stem cell transplantation.* Cambridge, UK: Cambridge University Press; 2000:1-12.

135. Horowitz MM. Uses and growth of hematopoietic cell transplantationj. In: Forman SJ, ed. *Hematopoietic cell transplantation.* Second ed. Malden, MA: Blackwell Science Inc; 1999:12-18.

136. Ferrara JL, Levy R, Chao NJ. Pathophysiologic mechanisms of acute graft-vs.-host disease. *Biol Blood Marrow Transplant.* Vol 5; 1999:347-356.

137. Thomas ED, Blume KG, Forman SJ. Hematopoietic cell transplantation. 2nd ed. Malden, MA: Blackwell Science, Inc.; 1999.

138. Atkinson K. Clinical bone marrow and blood stem cell transplantation. Cambridge, UK: Cambridge University Press; 2000.

139. Gandy KL, Weissman IL. Tolerance of allogeneic heart grafts in mice simultaneously reconstituted with purified allogeneic hematopoietic stem cells. *Transplantation.* Vol 65; 1998:295-304.

140. Shizuru JA, Jerabek L, Edwards CT, Weissman IL. Transplantation of purified hematopoietic stem cells: requirements for overcoming the barriers of allogeneic engraftment. *Biol Blood Marrow Transplant.* Vol 2; 1996:3-14.

141. Wang B, El-Badri NS, Cherry, Good RA. Purified hematopoietic stem cells without facilitating cells can repopulate fully allogeneic recipients across entire major histocompatibility complex transplantation barrier in mice. *Proc Natl Acad Sci USA.* Vol 94; 1997:14632-14636.

142. Slavin S, Morecki S, Weiss L, Or R. Donor lymphocyte infusion: the use of alloreactive and tumor-reactive lymphocytes for immunotherapy of malignant and non-malignant diseases in conjunction with allogeneic stem cell transplantation. *J Hematother Stem Cell Res.* Vol 11; 2002:265-276.

143. Feinstein L, Sandmaier B, Maloney D, et al. Nonmyeloablative hematopoietic cell transplantation. Replacing high-dose cytotoxic therapy by the graft-versus-tumor effect. *Ann NY Acad Sci.* Vol 938; 2001:328-337; discussion 337-329.

144. Goldman JM. Autologous hematopoietic stem cell transplantation for chronic myeloid leukemia. In: Atkinson K, ed. *Clinical bone marrow and blood stem cell transplantation.* Cambridge, UK: Cambridge University Press; 2000:279-286.

145. Attal M, Harousseau JL, Stoppa AM, et al. A prospective, randomized trial of autologous bone marrow transplantation and chemotherapy in multiple myeloma. Intergroupe Francais du Myelome. *N Engl J Med.* Vol 335; 1996:91-97.

146. Haioun C, Lepage E, Gisselbrecht C, et al. Benefit of autologous bone marrow transplantation over sequential chemotherapy in poor-risk aggressive non-Hodgkin's lymphoma: updated results of the prospective study LNH87-2. Groupe d'Etude des Lymphomes de l'Adulte. *J Clin Oncol.* Vol 15; 1997:1131-1137.

147. Gianni AM, Bregni M, Siena S, et al. High-dose chemotherapy and autologous bone marrow transplantation compared with MACOP-B in aggressive B-cell lymphoma. *N Engl J Med.* Vol 336; 1997:1290-1297.

148. Matthay KK, Villablanca JG, Seeger RC, et al. Treatment of high-risk neuroblastoma with intensive chemotherapy, radiotherapy, autologous bone marrow transplantation, and 13-cis- retinoic acid. Children's Cancer Group. *N Engl J Med.* Vol 341; 1999:1165-1173.

149. Damon LE, Hu WW, Stockerl-Goldstein KE, et al. High-dose chemotherapy and hematopoietic stem cell rescue for breast cancer: experience in California. *Biol Blood Marrow Transplant.* Vol 6; 2000:496-505.

150. Nieto Y, Champlin RE, Wingard JR, et al. Status of high-dose chemotherapy for breast cancer: a review. *Biol Blood Marrow Transplant.* Vol 6; 2000:476-495.

151. Somlo G, Chow W, Hamasaki V, et al. Tandem-cycle high-dose melphalan and cisplatin with peripheral blood progenitor cell support in patients with breast cancer and other malignancies. *Biol Blood Marrow Transplant.* Vol 7; 2001:284-293.

152. Child JA, Morgan GJ, Davies FE, et al. High-dose chemotherapy with hematopoietic stem-cell rescue for multiple myeloma. *N Engl J Med.* Vol 348; 2003:1875-1883.

153. Rodenhuis S, Bontenbal M, Beex LV, et al. High-dose chemotherapy with hematopoietic stem-cell rescue for high-risk breast cancer. *N Engl J Med.* Vol 349; 2003:7-16.

154. Monitoring treatment and survival in chronic myeloid leukemia. Italian Cooperative Study Group on Chronic Myeloid Leukemia and Italian Group for Bone Marrow Transplantation. *J Clin Oncol.* Vol 17; 1999:1858-1868.

155. Harousseau JL, Cahn JY, Pignon B, et al. Comparison of autologous bone marrow transplantation and intensive chemotherapy as postremission therapy in adult acute myeloid leukemia. The Groupe Ouest Est Leucemies Aigues Myeloblastiques (GOELAM). *Blood.* Vol 90; 1997:2978-2986.

156. Cassileth PA, Harrington DP, Appelbaum FR, et al. Chemotherapy compared with autologous or allogeneic bone marrow transplantation in the management of acute myeloid leukemia in first remission. *N Engl J Med.* Vol 339; 1998:1649-1656.

157. Stevens RF, Hann IM, Wheatley K, Gray RG. Marked improvements in outcome with chemotherapy alone in paediatric acute myeloid leukemia: results of the United Kingdom Medical Research Council's 10th AML trial. MRC Childhood Leukaemia Working Party. *Br J Haematol.* Vol 101; 1998:130-140.

158. Hortobagyi GN, Buzdar AU, Theriault RL, et al. Randomized trial of high-dose chemotherapy and blood cell autografts for high-risk primary breast carcinoma. *J Natl Cancer Inst.* Vol 92; 2000:225-233.

159. Bashey A, Corringham S, Garrett J, et al. A phase II study of two cycles of high-dose chemotherapy with autologous stem cell support in patients with metastatic breast cancer who meet eligibility criteria for a single cycle. *Bone Marrow Transplant.* Vol 25; 2000:519-524.

160. Stadtmauer EA, O'Neill A, Goldstein LJ, et al. Conventional-dose chemotherapy compared with high-dose chemotherapy plus autologous hematopoietic stem-cell transplantation for metastatic breast cancer. Philadelphia Bone Marrow Transplant Group. *N Engl J Med.* Vol 342; 2000:1069-1076.

161. Tallman MS, Gray R, Robert NJ, et al. Conventional adjuvant chemotherapy with or without high-dose chemotherapy and autologous stem-cell transplantation in high-risk breast cancer. *N Engl J Med.* Vol 349; 2003:17-26.

162. Slavin S. Non-myeloablative stem cell transplantation for induction of host-versus-graft tolerance for adoptive immunotherapy of malignant and nonmalignant diseases and towards transplantation of organ allografts. *Transplant Proc.* Vol 34; 2002:3371-3373.

163. Spitzer TR. The expanding applications of non-myeloablative stem cell transplantation. *Pediatr Transplant.* Vol 7 Suppl 3; 2003:95-100.

164. Maris M, Sandmaier BM, Maloney DG, et al. Non-myeloablative hematopoietic stem cell transplantation. *Transfus Clin Biol.* Vol 8; 2001:231-234.

165. Uchida N, Tsukamoto A, He D, Friera AM, Scollay R, Weissman IL. High doses of purified stem cells cause early hematopoietic recovery in syngeneic and allogeneic hosts. *J Clin Invest.* Vol 101; 1998:961-966.

166. Shizuru JA, Weissman IL, Kernoff R, Masek M, Scheffold YC. Purified hematopoietic stem cell grafts induce tolerance to alloantigens and can mediate positive and negative T cell selection. *Proc Natl Acad Sci USA.* Vol 97; 2000:9555-9560.

167. Gandy KL, Domen J, Aguila H, Weissman IL. *CD8+ TCR+ and CD8+ TCR+ cells in whole bone marrow facilitate the engraftment of hematopoietic stem cells across allogeneic barriers:* Immunity; 1999.

168. McDevitt HO. The role of MHC class II molecules in susceptibility and resistance to autoimmunity. *Curr Opin Immunol.* 1998;10:677-681.

169. Beilhack GF, Landa RR, Masek MA, Shizuru JA. Prevention of type 1 diabetes with major histocompatibility complex-compatible and nonmarrow ablative hematopoietic stem cell transplants. *Diabetes.* 2005;54:1770-1779.

170. Beilhack GF, Scheffold YC, Weissman IL, et al. Purified allogeneic hematopoietic stem cell transplantation blocks diabetes pathogenesis in NOD mice. *Diabetes.* 2003;52:59-68.

171. Reya T, Morrison SJ, Clarke MF, Weissman IL. Stem cells, cancer, and cancer stem cells. *Nature.* Vol 414; 2001:105-111.

172. Hope KJ, Jin L, Dick JE. Human acute myeloid leukemia stem cells. *Arch Med Res.* Vol 34; 2003:507-514.

173. Guzman ML, Jordan CT. Considerations for targeting malignant stem cells in leukemia. *Cancer Control.* Vol 11; 2004:97-104.

174. Scadden DT. Cancer stem cells refined. *Nat Immunol.* Vol 5; 2004:701-703.

175. Passegue E, Jamieson CH, Ailles LE, Weissman IL. Normal and leukemic hematopoiesis: are leukemias a stem cell disorder or a reacquisition of stem cell characteristics? *Proc Natl Acad Sci USA.* Vol 100 Suppl 1; 2003:11842-11849.

176. Al-Hajj M, Becker MW, Wicha M, Weissman I, Clarke MF. Therapeutic implications of cancer stem cells. *Curr Opin Genet Dev.* Vol 14; 2004:43-47.

177. Jamieson CH, Ailles LE, Dylla SJ, et al. Granulocyte-macrophage progenitors as candidate leukemic stem cells in blast-crisis CML. *N Engl J Med.* Vol 351; 2004:657-667.

178. Miyamoto T, Weissman IL, Akashi K. AML1/ETO-expressing nonleukemic stem cells in acute myelogenous leukemia with 8;21 chromosomal translocation. *Proc Natl Acad Sci USA.* Vol 97; 2000:7521-7526.

179. Jones RJ, Matsui WH, Smith BD. Cancer stem cells: are we missing the target? *J Natl Cancer Inst.* Vol 96; 2004:583-585.

180. Al-Hajj M, Clarke MF. Self-renewal and solid tumor stem cells. *Oncogene.* Vol 23; 2004:7274-7282.

3. REPAIRING THE NERVOUS SYSTEM WITH STEM CELLS

*by David M. Panchision**

Diseases of the nervous system, including congenital disorders, cancers, and degenerative diseases, affect millions of people of all ages. Congenital disorders occur when the brain or spinal cord does not form correctly during development. Cancers of the nervous system result from the uncontrolled spread of aberrant cells. Degenerative diseases occur when the nervous system loses functioning of nerve cells. Most of the advances in stem cell research have been directed at treating degenerative diseases. While many treatments aim to limit the damage of these diseases, in some cases scientists believe that damage can be reversed by replacing lost cells with new ones derived from cells that can mature into nerve cells, called neural stem cells. Research that uses stem cells to treat nervous system disorders remains an area of great promise and challenge to demonstrate that cell-replacement therapy can restore lost function.

STRATEGIES TO REPAIR THE NERVOUS SYSTEM

The nervous system is a complex organ made up of nerve cells (also called neurons) and glial cells, which surround and support neurons *(see Figure 3.1)*. Neurons send signals that affect numerous functions including thought processes and movement. One type of glial cell, the oligodendrocyte, acts to speed up the signals of neurons that extend over long distances, such as in the spinal cord. The loss of any of these cell types may have catastrophic results on brain function.

Although reports dating back as early as the 1960s pointed towards the possibility that new nerve cells are formed in adult mammalian brains, this knowledge was not applied in the context of curing devastating brain diseases until the 1990s. While earlier medical research focused on limiting damage once it had occurred, in recent years researchers have been working hard to find out if the cells that can give rise to new neurons can be coaxed to restore brain function. New neurons in the adult brain arise from slowly-dividing cells that appear to be the remnants of stem cells that existed during fetal brain development. Since some of these adult cells still retain the ability to generate both neurons and glia, they are referred to as adult neural stem cells.

These findings are exciting because they suggest that the brain may contain a built-in mechanism to repair itself. Unfortunately, these new neurons are only generated in a few sites in the brain and turn into only a few specialized types of nerve cells. Although there are many different neuronal cell types in the brain, we now know that these new neurons can "plug in" correctly to assist brain function.[1] The discovery of these cells has spurred further research into the characteristics of neural stem cells from the fetus and the adult, mostly using rodents and primates as model species. The hope is that these cells may be able to replenish those that are functionally lost in human degenerative diseases such as Parkinson's Disease, Huntington's Disease, and amyotrophic lateral sclerosis (ALS, also known as Lou Gehrig's disease), as well as from brain and spinal cord injuries that result from stroke or trauma.

Scientists are applying these new stem cell discoveries in two ways in their experiments. First, they are using current knowledge of normal brain development to modulate stem cells that are harvested and grown in culture. Researchers can then transplant these cultured cells into the brain of an animal model and allow the brain's own signals to differentiate the stem cells into neurons or glia. Alternatively, the stem cells

* Investigator, Center for Neuroscience Research and Assistant Professor, Dept. of Pediatrics and Pharmacology, George Washington University School of Medicine, Children's Research Institute, Children's National Medical Center, Washington, DC 20010-2970, *Email:* dpanchision@cnmcresearch.org

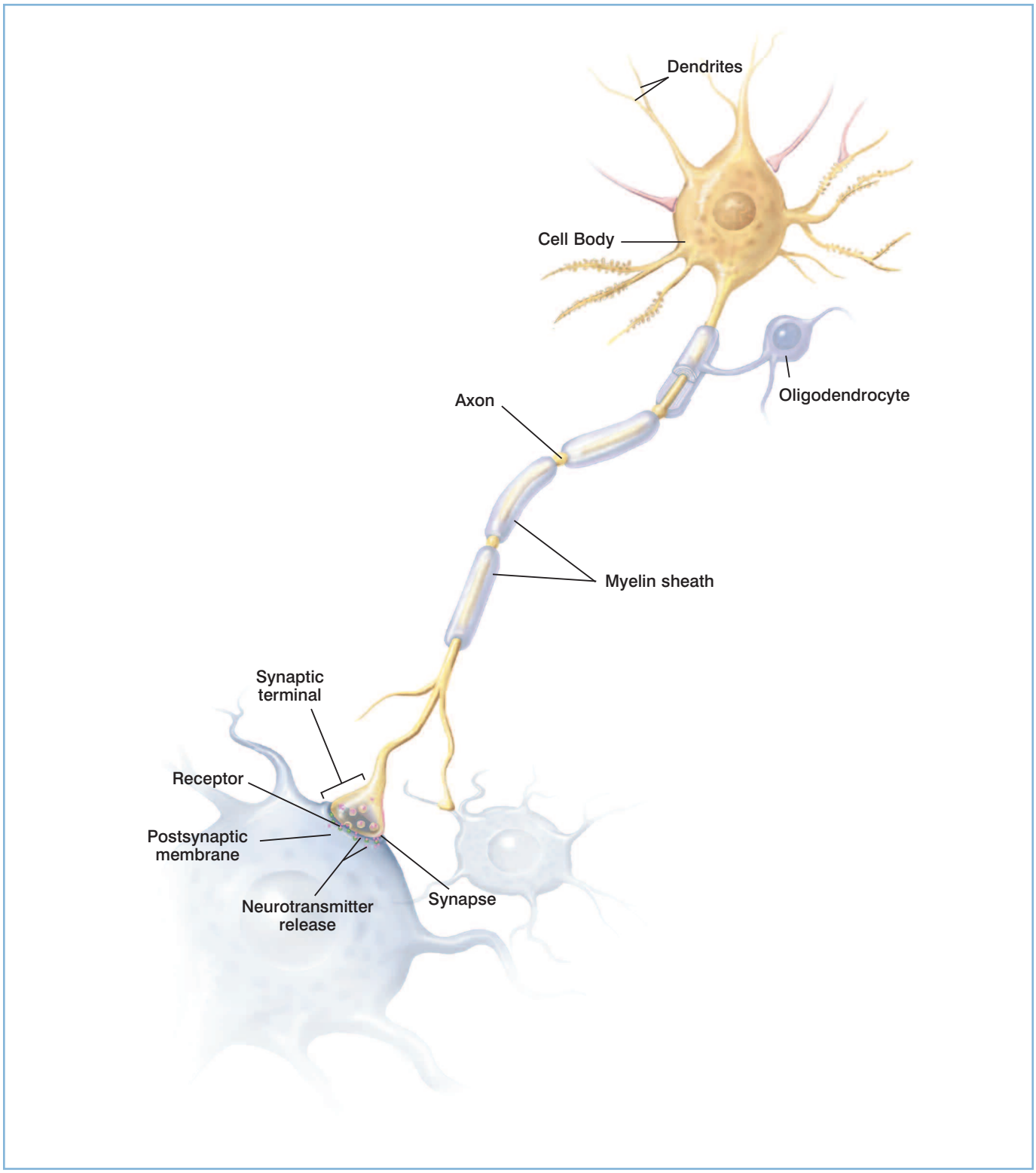


Figure 3.1. The Neuron.
When sufficient neurotransmitters cross synapses and bind receptors on the neuronal cell body and dendrites, the neuron sends an electrical signal down its axon to synaptic terminals, which in turn release neurotransmitters into the synapse that affects the following neuron. The brain neurons that die in Parkinson's Disease release the transmitter dopamine. Oligodendrocytes supply the axon with an insulating myelin sheath.

can be induced to differentiate into neurons and glia while in the culture dish, before being transplanted into the brain. Much progress has been made the last several years with human embryonic stem (ES) cells that can differentiate into all cell types in the body. While ES cells can be maintained in culture for relatively long periods of time without differentiating, they usually must be coaxed through many more steps of differentiation to produce the desired cell types. Recent studies, however, suggest that ES cells may differentiate into neurons in a more straightforward manner than may other cell types.

Second, scientists are identifying growth (trophic) factors that are normally produced and used by the developing and adult brain. They are using these factors to minimize damage to the brain and to activate the patient's own stem cells to repair damage that has occurred. Each of these strategies is being aggressively pursued to identify the most effective treatments for degenerative diseases. Most of these studies have been carried out initially with animal stem cells and recipients to determine their likelihood of success. Still, much more research is necessary to develop stem cell therapies that will be useful for treating brain and spinal cord disease in the same way that hematopoietic stem cell therapies are routinely used for immune system replacement (see Chapter 2).

The majority of stem cell studies of neurological disease have used rats and mice, since these models are convenient to use and are well-characterized biologically. If preliminary studies with rodent stem cells are successful, scientists will attempt to transplant human stem cells into rodents. Studies may then be carried out in primates (*e.g.,* monkeys) to offer insight into how humans might respond to neurological treatment. Human studies are rarely undertaken until these other experiments have shown promising results. While human transplant studies have been carried out for decades in the case of Parkinson's disease, animal research continues to provide improved strategies to generate an abundant supply of transplantable cells.

PARKINSON'S DISEASE — A MAJOR TARGET FOR STEM CELL RESEARCH

The intensive research aiming at curing Parkinson's disease with stem cells is a good example for the various strategies, successful results, and remaining challenges of stem cell-based brain repair. Parkinson's disease is a progressive disorder of motor control that affects roughly 2% of persons 65 years and older. Triggered by the death of neurons in a brain region called the substantia nigra, Parkinson's disease begins with minor tremors that progress to limb and bodily rigidity and difficulty initiating movement. These neurons connect via long axons to another region called the striatum, composed of subregions called the caudate nucleus and the putamen. These neurons that reach from the substantia nigra to the striatum release the chemical transmitter dopamine onto their target neurons in the striatum. One of dopamine's major roles is to regulate the nerves that control body movement. As these cells die, less dopamine is produced, leading to the movement difficulties characteristic of Parkinson's disease. Currently, the causes of death of these neurons are not well understood.

For many years, doctors have treated Parkinson's disease patients with the drug levodopa (L-dopa), which the brain converts into dopamine. Although the drug works well initially, levodopa eventually loses its effectiveness, and side-effects increase. Ultimately, many doctors and patients find themselves fighting a losing battle. For this reason, a huge effort is underway to develop new treatments, including growth factors that help the remaining dopamine neurons survive and transplantation procedures to replace those that have died.

RESEARCH ON FETAL TISSUE TRANSPLANTS IN PARKINSON'S DISEASE

The strategy to use new cells to replace lost ones is not new. Surgeons first attempted to transplant dopamine-releasing cells from a patient's own adrenal glands in the 1980s.[2,3] Although one of these studies reported a dramatic improvement in the patients' conditions, U.S. surgeons were only able to achieve modest and temporary improvement, insufficient to outweigh the risks of such a procedure. As a result, these human studies were not pursued further.

Another strategy was attempted in the 1970s, in which cells derived from fetal tissue from the mouse substantia nigra was transplanted into the adult rat eye and found to develop into mature dopamine neurons.[4] In the 1980s, several groups showed that transplantation of this type of tissue could reverse Parkinson's-like symptoms in rats and monkeys when placed in the damaged areas.The success of the animal studies led to

several human trials beginning in the mid-1980s.[5,6] In some cases, patients showed a lessening of their symptoms. Also, researchers could measure an increase in dopamine neuron function in the striatum of these patients by using a brain-imaging method called positron emission tomography (PET) *(see Figure 3.2).*[7]

The NIH has funded two large and well-controlled clinical trials in the past 15 years in which researchers transplanted tissue from aborted fetuses into the striatum of patients with Parkinson's disease.[7,8] These studies, performed in Colorado and New York, included controls where patients received "sham" surgery (no tissue was implanted), and neither the patients nor the scientists who evaluated their progress knew which patients received the implants. The patients' progress was followed for up to eight years. Unfortunately, both studies showed that the transplants offered little benefit to the patients as a group. While some patients showed improvement, others began to suffer from dyskinesias, jerky involuntary movements that are often side effects of long-term L-dopa treatment. This effect occurred in 15% of the patients in the Colorado study.[7] and more than half of the patients in the New York study.[8] Additionally, the New York study showed evidence that some patients' immune systems were attacking the grafts.

However, promising findings emerged from these studies as well. Younger and milder Parkinson's patients responded relatively well to the grafts, and PET scans of patients showed that some of the transplanted dopamine neurons survived and matured. Additionally, autopsies on three patients who died of unrelated causes, years after the surgeries, indicated the presence of dopamine neurons from the graft. These cells appeared to have matured in the same way as normal dopamine neurons, which suggested that they were acting normally in the brain.

Researchers in Sweden followed the severity of dyskinesia in patients for eleven years after neural transplantation and found that the severity was

Dopamine-Neuron Transplantation

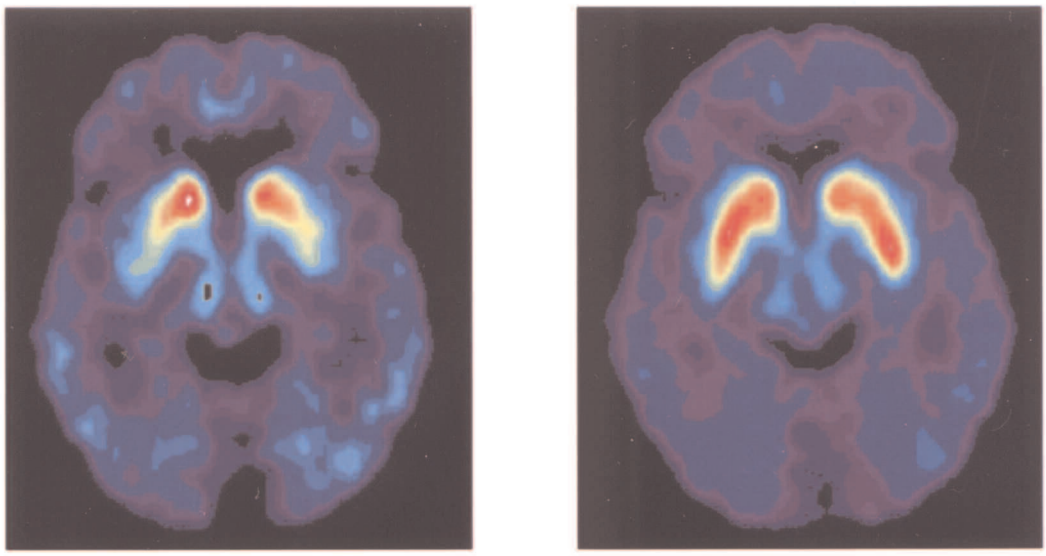


Figure 3.2. Positron Emission Tomography (PET) images from a Parkinson's patient before and after fetal tissue transplantation. The image taken before surgery (left) shows uptake of a radioactive form of dopamine (red) only in the caudate nucleus, indicating that dopamine neurons have degenerated. Twelve months after surgery, an image from the same patient (right) reveals increased dopamine function, especially in the putamen. (Reprinted with permission from N Eng J Med 2001;344 (10) p. 710.)

typically mild or moderate. These results suggested that dyskinesias were due to effects that were distinct from the beneficial effects of the grafts.[9] Dyskinesias may therefore be related to the ways that transplantation disturbs other cells in the brain and so may be minimized by future improvements in therapy. Another study that involved the grafting of cells both into the striatum (the target of dopamine neurons) and the substantia nigra (where dopamine neurons normally reside) of three patients showed no adverse effects and some modest improvement in patient movement.[10] To determine the full extent of therapeutic benefits from such a procedure and confirm the reliability of these results, this study will need to be repeated with a larger patient population that includes the appropriate controls.

The limited success of these studies may reflect variations in the fetal tissue used for transplantation, which is of limited quantity and can not be standardized or well-characterized. The full complement of cells in these fetal tissue samples is not known at present. As a result, the tissue remains the greatest source of uncertainty in patient outcome following transplantation.

STEM CELLS AS A SOURCE OF NEURONS FOR TRANSPLANTATION IN PARKINSON'S DISEASE

The major goal for Parkinson's investigators is to generate a source of cells that can be grown in large supply, maintained indefinitely in the laboratory, and differentiated efficiently into dopamine neurons that work when transplanted into the brain of a Parkinson's patient. Scientists have investigated the behavior of stem cells in culture and the mechanisms that govern dopamine neuron production during development in their attempts to identify optimal culture conditions that allow stem cells to turn into dopamine-producing neurons.

Preliminary studies have been carried out using immature stem cell-like precursors from the rodent ventral midbrain, the region that normally gives rise to these dopamine neurons. In one study these precursors were turned into functional dopamine neurons, which were then grafted into rats previously treated with 6-hydroxy-dopamine (6-OHDA) to kill the dopamine neurons in their substantia nigra and induce Parkinson's-like symptoms. Even though the percentage of surviving dopamine neurons was low following transplantation, it was sufficient to relieve the Parkinson's-like symptoms.[11] Unfortunately, these fetal cells cannot be maintained in culture for very long before they lose the ability to differentiate into dopamine neurons.

Cells with features of neural stem cells have been derived from ES-cells, fetal brain tissue, brain tissue from neurosurgery, and brain tissue that was obtained after a person's death. There is controversy about whether other organ stem cell populations, such as hematopoietic stem cells, either contain or give rise to neural stem cells

Many researchers believe that the more primitive ES cells may be an excellent source of dopamine neurons because ES-cells can be grown indefinitely in a laboratory dish and can differentiate into any cell type, even after long periods in culture. Mouse ES cells injected directly into 6-OHDA-treated rat brains led to relief of Parkinson-like symptoms. Further investigation showed that these ES cells had differentiated into both dopamine and serotonin neurons.[12] This latter type of neuron is generated in an adjacent region of the brain and may complicate the response to transplantation. Since ES cells can generate all cell types in the body, unwanted cell types such as muscle or bone could theoretically also be introduced into the brain. As a result, a great deal of effort is being currently put into finding the right "recipe" for turning ES cells into dopamine neurons — and only this cell type — to treat Parkinson's disease. Researchers strive to learn more about normal brain development to help emulate the natural progression of ES cells toward dopamine neurons in the culture dish.

The recent availability of human ES cells has led to further studies to examine their potential for differentiation into dopamine neurons. Recently, dopamine neurons from human embryonic stem cells have been generated.[13] One research group used a special type of companion cell, along with specific growth factors, to promote the differentiation of the ES cells through several stages into dopamine neurons. These neurons showed many of the characteristic properties of normal dopamine neurons.[13] Furthermore, recent evidence of more direct neuronal differentiation methods from mouse ES cells fuels hope that scientists can refine and streamline the production of transplantable human dopamine neurons.

One method with great therapeutic potential is nuclear transfer. This method fuses the genetic material from one individual donor with a recipient egg cell that has had its nucleus removed. The early embryo that develops from this fusion is a genetic match for the donor. This process is sometimes called "therapeutic cloning" and is regarded by some to be ethically questionable. However, mouse ES cells have been differentiated successfully in this way into dopamine neurons that corrected Parkinsonian symptoms when transplanted into 6-OHDA-treated rats.[14] Similar results have been obtained using parthenogenetic primate stem cells, which are cells that are genetic matches from a female donor with no contribution from a male donor.[15] These approaches may offer the possibility of treating patients with genetically-matched cells, thereby eliminating the possibility of graft rejection.

ACTIVATING THE BRAIN'S OWN STEM CELLS TO REPAIR PARKINSON'S DISEASE

Scientists are also studying the possibility that the brain may be able to repair itself with therapeutic support. This avenue of study is in its early stages but may involve administering drugs that stimulate the birth of new neurons from the brain's own stem cells. The concept is based on research showing that new nerve cells are born in the adult brains of humans. The phenomenon occurs in a brain region called the dentate gyrus of the hippocampus. While it is not yet clear how these new neurons contribute to normal brain function, their presence suggests that stem cells in the adult brain may have the potential to re-wire dysfunctional neuronal circuitry.

The adult brain's capacity for self-repair has been studied by investigating how the adult rat brain responds to transforming growth factor alpha (TGFα), a protein important for early brain development that is expressed in limited quantities in adults.[16] Injection of TGFα into a healthy rat brain causes stem cells to divide for several days before ceasing division. In 6-OHDA-treated (Parkinsonian) rats, however, the cells proliferated and migrated to the damaged areas. Surprisingly, the TGFα-treated rats showed few of the behavioral problems associated with untreated Parkinsonian rats.[16] Additionally, in 2002 and 2003, two research groups isolated small numbers of dividing cells in the substantia nigra of adult rodents.[17,18] These findings suggest that the brain can repair itself, as long as the repair process is triggered sufficiently. It is not clear, though, whether stem cells are responsible for this repair or if the TGFα activates a different repair mechanism.

POSSIBILITIES FOR STEM CELLS IN THE TREATMENT OF OTHER NERVOUS SYSTEM DISORDERS

Many other diseases that affect the nervous system hold the potential for being treated with stem cells. Experimental therapies for chronic diseases of the nervous system, such as Alzheimer's disease, Lou Gehrig's disease, or Huntington's disease, and for acute injuries, such as spinal cord and brain trauma or stoke, are being currently developed and tested. These diverse disorders must be investigated within the contexts of their unique disease processes and treated accordingly with highly adapted cell-based approaches.

Although severe spinal cord injury is an area of intense research, the therapeutic targets are not as clear-cut as in Parkinson's disease. Spinal cord trauma destroys numerous cell types, including the neurons that carry messages between the brain and the rest of the body. In many spinal injuries, the cord is not actually severed, and at least some of the signal-carrying neuronal axons remain intact. However, the surviving axons no longer carry messages because oligodendrocytes, which make the axons' insulating myelin sheath, are lost. Researchers have recently made progress to replenish these lost myelin-producing cells. In one study, scientists cultured human ES cells through several steps to make mixed cultures that contained oligodendrocytes. When they injected these cells into the spinal cords of chemically-demyelinated rats, the treated rats regained limited use of their hind limbs compared with un-grafted rats.[19] Researchers are not certain, however, whether the limited increase in function observed in rats is actually due to the remyelination or to an unidentified trophic effect of the treatment.

Getting neurons to grow new axons through the injury site to reconnect with their targets is even more challenging. While myelin promotes normal neuronal function, it also inhibits the growth of new axons following spinal injury. In a recent study to attempt post-trauma axonal growth, Harper and colleagues

treated ES cells with a combination of factors that are known to promote motor neuron differentiation.[20] The researchers then transplanted these cells into adult rats that had received spinal cord injuries. While many of these cells survived and differentiated into neurons, they did not send out axons unless the researchers also added drugs that interfered with the inhibitory effects of myelin. The growth effect was modest, and the researchers have not yet seen evidence of functional neuron connections. However, their results raise the possibility that signals can be turned on and off in the correct order to allow neurons to reconnect and function properly. Spinal injury researchers emphasize that additional basic and preclinical research must be completed before attempting human trials using stem cell therapies to repair the trauma-damaged nervous system.

Since myelin loss is at the heart of many other degenerative diseases, oligodendrocytes made from ES cells may be useful to treat these conditions as well. For example, scientists recently cultured human ES cells with a combination of growth factors to generate a highly enriched population of myelinating oligodendrocyte precursors.[21,22] The researchers then tested these cells in a genetically-mutated mouse that does not produce myelin properly. When the growth factor-cultured ES cells were transplanted into affected mice, the cells migrated and differentiated into mature oligodendrocytes that made myelin sheaths around neighboring axons. These researchers subsequently showed that these cells matured and improved movement when grafted in rats with spinal cord injury.[23] Improved movement only occurred when grafting was completed soon after injury, suggesting that some post-injury responses may interfere with the grafted cells. However, these results are sufficiently encouraging to plan clinical trials to test whether replacement of myelinating glia can treat spinal cord injury.

Amyotrophic lateral sclerosis (ALS), also known as Lou Gehrig's disease, is characterized by a progressive destruction of motor neurons in the spinal cord. Patients with ALS develop increasing muscle weakness over time, which ultimately leads to paralysis and death. The cause of ALS is largely unknown, and there are no effective treatments. Researchers recently have used different sources of stem cells to test in rat models of ALS to test for possible nerve cell-restoring properties. In one study, researchers injected cell clusters made from embryonic germ (EG) cells into the spinal cord fluid of the partially-paralyzed rats.[24] Three months after the injections, many of the treated rats were able to move their hind limbs and walk with difficulty, while the rats that did not receive cell injections remained paralyzed. Moreover, the transplanted cells had migrated throughout the spinal fluid and developed into cells that displayed molecular characteristics of mature motor neurons. However, too few cells matured in this way to account for the recovery, and there was no evidence that the transplanted cells formed functional connections with muscles. The researchers suggest that the transplanted cells may be promoting recovery in some other way, such as by producing trophic factors.

This possibility was addressed in a second study in which scientists grew human fetal CNS stem cells in culture and genetically modified them to produce a trophic factor that promotes the survival of cells that are lost in ALS. When grafted into the spinal cords of the ALS-like rats, these cells secreted the desired growth factor and promoted the survival of the neurons that are normally lost in the ALS-like rats.[25] While promising, these results highlight the need for additional basic research into functional recovery in ALS disease models.

Stroke affects about 750,000 patients per year in the U.S. and is the most common cause of disability in adults. A stroke occurs when blood flow to the brain is disrupted. As a consequence, cells in affected brain regions die from insufficient amounts of oxygen. The treatment of stroke with anti-clotting drugs has dramatically improved the odds of patient recovery. However, in many patients the damage cannot be prevented, and the patient may permanently lose the functions of affected areas of the brain. For these patients, researchers are now considering stem cells as a way to repair the damaged brain regions. This problem is made more challenging because the damage in stroke may be widespread and may affect many cell types and connections.

However, researchers from Sweden recently observed that strokes in rats cause the brain's own stem cells to divide and give rise to new neurons.[26] However, these neurons, which survived only a couple of weeks, are few in number compared to the extent of damage caused. A group from the University of Tokyo added a growth factor, bFGF, into the brains of rats after stroke and showed that the hippocampus was able to generate

large numbers of new neurons.[27] The researchers found evidence that these new neurons were actually making connections with other neurons. These and other results suggest that future stroke treatments may be able to coax the brain's own stem cells to make replacement neurons.

Taking an alternative approach, another group attempted transplantation as a means to treat the loss of brain mass after a severe stroke. By adding stem cells onto a polymer scaffold that they implanted into the stroke-damaged brains of mice, the researchers demonstrated that the seeded stem cells differentiated into neurons and that the polymer scaffold reduced scarring.[28] Two groups transplanted human fetal stem cells in independent studies into the brains of stroke-affected rodents; these stem cells not only survived but migrated to the damaged areas of the brain.[29,30] These studies increase our knowledge of how stem cells are attracted to diseased areas of the brain.

There is also increasing evidence from numerous animal disease models that stem cells are actively drawn to brain damage. Once they reach these damaged areas, they have been shown to exert beneficial effects such as reducing brain inflammation or supporting nerve cells. It is hoped that, once these mechanisms are better understood, this stem cell recruitment can potentially be exploited to mobilize a patient's own stem cells.

Similar lines of research are being considered with other disorders such as Huntington's Disease and certain congenital defects. While much attention has been called to the treatment of Alzheimer's Disease, it is still not clear if stem cells hold the key to its treatment. But despite the fact that much basic work remains and many fundamental questions are yet to be answered, researchers are hopeful that repair for once-incurable nervous system disorders may be amenable to stem cell based therapies.

Considerable progress has been made the last few years in our understanding of stem cell biology and devising sources of cells for transplantation. New methods are also being developed for cell delivery and targeting to affected areas of the body. These advances have fueled optimism that new treatments will come for millions of persons who suffer from neurological disorders. But it is the current task of scientists to bring these methods from the laboratory bench to the clinic in a scientifically sound and ethically acceptable fashion.

REFERENCES

1. van Praag H, Schinder AF, Christie BR, Toni N, Palmer TD, Gage FH. Functional neurogenesis in the adult hippocampus. *Nature.* 2/28/2002 2002;415(6875):1030-1034.
2. Backlund EO, Granberg PO, Hamberger P, et al. Transplantation of adrenal medullary tissue to striatum in parkinsonism. First clinical trials. *J Neurosurg.* 1985;62:169-173.
3. Madrazo I, Drucker-Colin R, Diaz V, Martinez-Mata J, Torres C, Becerril JJ. Open microsurgical autograft of adrenal medulla to the right caudate nucleus in two patients with intractable Parkinson's disease. *N Engl J Med.* 1987;316:831-834.
4. Olson L, Malmfors T. Growth characteristics of adrenergic nerves in the adult rat. Fluorescence histochemical and 3H-noradrenaline uptake studies using tissue transplantations to the anterior chamber of the eye. *Acta Physiol Scand Suppl.* 1970;348:1-112.
5. Madrazo I, Leon V, Torres C, et al. Transplantation of fetal substantia nigra and adrenal medulla to the caudate nucleus in two patients with Parkinson's disease. *N Engl J Med.* 1988;318:51.
6. Lindvall O, Rehncrona S, Brundin P, et al. Human fetal dopamine neurons grafted into the striatum in two patients with severe Parkinson's disease. A detailed account of methodology and a 6-month follow-up. *Arch Neurol.* 1989;46:615-631.
7. Freed CR, Greene PE, Breeze RE, et al. Transplantation of embryonic dopamine neurons for severe Parkinson's disease. *N Engl J Med.* 2001;344:710-719.
8. Olanow CW, Goetz CG, Kordower JH, et al. A double-blind controlled trial of bilateral fetal nigral transplantation in Parkinson's disease. *Ann Neurol.* 2003;54:403-414.
9. Hagell P, Piccini P, Bjorklund A, et al. Dyskinesias following neural transplantation in Parkinson's disease. *Nat Neurosci.* 2002;5:627-628.
10. Mendez I, Dagher A, Hong M, et al. Simultaneous intrastriatal and intranigral fetal dopaminergic grafts in patients with Parkinson disease: a pilot study. Report of three cases. *J Neurosurg.* 2002;96:589-596.
11. Studer L, Tabar V, McKay RD. Transplantation of expanded mesencephalic precursors leads to recovery in parkinsonian rats. *Nat Neurosci.* 1998;1:290-295.

12. Bjorklund LM, Sanchez-Pernaute R, Chung S, et al. Embryonic stem cells develop into functional dopaminergic neurons after transplantation in a Parkinsonian rat model. *Proc Natl Acad Sci USA.* 2002;99:2344-2349.

13. Perrier AL, Tabar V, Barberi T, et al. Derivation of midbrain dopamine neurons from human embryonic stem cells. *Proc Natl Acad Sci USA.* 2004;101:12543-12548.

14. Barberi T, Klivenyi P, Calingasan NY, et al. Neural subtype specification of fertilization and nuclear transfer embryonic stem cells and application in parkinsonian mice. *Nat Biotechnol.* 2003;21:1200-1207.

15. Vrana KE, Hipp JD, Goss AM, et al. Nonhuman primate parthenogenetic stem cells. *Proc Natl Acad Sci USA.* 2003;100 Suppl 1:11911-11916.

16. Fallon J, Reid S, Kinyamu R, et al. In vivo induction of massive proliferation, directed migration, and differentiation of neural cells in the adult mammalian brain. *Proc Natl Acad Sci USA.* 2000;97:14686-14691.

17. Lie DC, Dziewczapolski G, Willhoite AR, Kaspar BK, Shults CW, Gage FH. The adult substantia nigra contains progenitor cells with neurogenic potential. *J Neurosci.* 2002;22:6639-6649.

18. Zhao M, Momma S, Delfani K, et al. Evidence for neurogenesis in the adult mammalian substantia nigra. *Proc Natl Acad Sci USA.* 2003;100:7925-7930.

19. Liu S, Qu Y, Stewart TJ, et al. Embryonic stem cells differentiate into oligodendrocytes and myelinate in culture and after spinal cord transplantation. *Proc Natl Acad Sci USA.* 2000;97:6126-6131.

20. Harper JM, Krishnan C, Darman JS, et al. Axonal growth of embryonic stem cell-derived motoneurons in vitro and in motoneuron-injured adult rats. *Proc Natl Acad Sci USA.* 2004;101:7123-7128.

21. Nistor GI, Totoiu MO, Haque N, Carpenter MK, Keirstead HS. Human embryonic stem cells differentiate into oligodendrocytes in high purity and myelinate after spinal cord transplantation. *Glia.* Feb 2005;49(3):385-396.

22. Brustle O, Jones KN, Learish RD, et al. Embryonic stem cell-derived glial precursors: a source of myelinating transplants. *Science.* 1999;285:754-756.

23. Keirstead HS, Nistor G, Bernal G, et al. Human embryonic stem cell-derived oligodendrocyte progenitor cell transplants remyelinate and restore locomotion after spinal cord injury. *J Neurosci.* May 11 2005;25(19):4694-4705.

24. Kerr DA, Llado J, Shamblott MJ, et al. Human embryonic germ cell derivatives facilitate motor recovery of rats with diffuse motor neuron injury. *J Neurosci.* 2003;23:5131-5140.

25. Klein SM, Behrstock S, McHugh J, et al. GDNF delivery using human neural progenitor cells in a rat model of ALS. *Hum Gene Ther.* Apr 2005;16(4):509-521.

26. Arvidsson A, Collin T, Kirik D, Kokaia Z, Lindvall O. Neuronal replacement from endogenous precursors in the adult brain after stroke. *Nat Med.* 2002;8:963-970.

27. Nakatomi H, Kuriu T, Okabe S, et al. Regeneration of hippocampal pyramidal neurons after ischemic brain injury by recruitment of endogenous neural progenitors. *Cell.* 2002;110:429-441.

28. Park KI, Teng YD, Synder EY. The injured brain interacts reciprocally with neural stem cells supported by scaffolds to reconstitute lost tissue. *Nat Biotechnol.* 2002;20:1111-1117.

29. Kelly S, Bliss TM, Shah AK, et al. Transplanted human fetal neural stem cells survive, migrate, and differentiate in ischemic rat cerebral cortex. *Proc Natl Acad Sci USA.* 2004;101:11839-11844.

30. Imitola J, Raddassi K, Park KI, et al. Directed migration of neural stem cells to sites of CNS injury by the stromal cell-derived factor 1alpha/CXC chemokine receptor 4 pathway. *Proc Natl Acad Sci USA.* Dec 28 2004;101(52):18117-18122.

[This page intentionally left blank]

4. USE OF GENETICALLY MODIFIED STEM CELLS IN EXPERIMENTAL GENE THERAPIES

*by Thomas P. Zwaka**

INTRODUCTION

Gene therapy is a novel therapeutic branch of modern medicine. Its emergence is a direct consequence of the revolution heralded by the introduction of recombinant DNA methodology in the 1970s. Gene therapy is still highly experimental, but has the potential to become an important treatment regimen. In principle, it allows the transfer of genetic information into patient tissues and organs. Consequently, diseased genes can be eliminated or their normal functions rescued. Furthermore, the procedure allows the addition of new functions to cells, such as the production of immune system mediator proteins that help to combat cancer and other diseases.

Originally, monogenic inherited diseases (those caused by inherited single gene defects), such as cystic fibrosis, were considered primary targets for gene therapy. For instance, in pioneering studies on the correction of adenosine deaminase deficiency, a lymphocyte-associated severe combined immunodeficiency (SCID), was attempted.[1] Although no modulation of immune function was observed, data from this study, together with other early clinical trials, demonstrated the potential feasibility of gene transfer approaches as effective therapeutic strategies. The first successful clinical trials using gene therapy to treat a monogenic disorder involved a different type of SCID, caused by mutation of an X chromosome-linked lymphocyte growth factor receptor.[2]

While the positive therapeutic outcome was celebrated as a breakthrough for gene therapy, a serious drawback subsequently became evident. By February 2005, three children out of seventeen who had been successfully treated for X-linked SCID developed leukemia because the vector inserted near an oncogene (a cancer-causing gene), inadvertently causing it to be inappropriately expressed in the genetically-engineered lymphocyte target cell.[3] On a more positive note, a small number of patients with adenosine deaminase-deficient SCID have been successfully treated by gene therapy without any adverse side effects.[4]

A small number of more recent gene therapy clinical trials, however, are concerned with monogenic disorders. Out of the approximately 1000 recorded clinical trials (January 2005), fewer than 10% target these diseases *(see Figure 4.1)*. The majority of current clinical trials (66% of all trials) focus on polygenic diseases, particularly cancer.

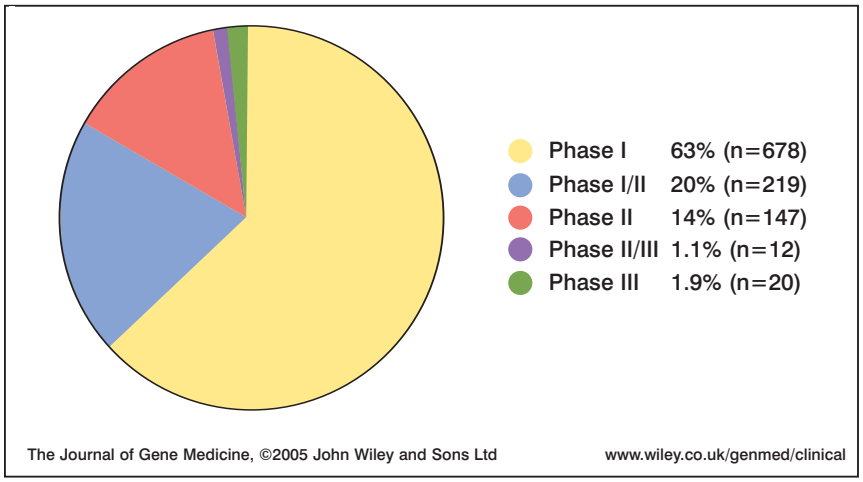


Figure 4.1. Indications Addressed by Gene Therapy Clinical Trials.

Gene therapy relies on similar principles as traditional pharmacologic therapy; specifically, regional specificity for the targeted tissue, specificity of the introduced gene function in relation to disease, and stability and controllability of expression of the introduced gene. To integrate all these aspects into a successful therapy is an exceedingly complex process that requires expertise from many disciplines, including molecular and cell biology, genetics and virology, in addition to bioprocess manufacturing capability and clinical laboratory infrastructure.

* Center for Cell and Gene Therapy & Department of Molecular and Cellular Biology, Baylor College of Medicine, Houston, Texas 77030, Email: tpzwaka@bcm.tmc.edu

THE TWO PATHS TO GENE THERAPY

Gene therapy can be performed either by direct transfer of genes into the patient or by using living cells as vehicles to transport the genes of interest. Both modes have certain advantages and disadvantages.

Direct gene transfer is particularly attractive because of its relative simplicity. In this scenario, genes are delivered directly into a patient's tissues or bloodstream by packaging into liposomes (spherical vessels composed of the molecules that form the membranes of cells) or other biological microparticles. Alternately, the genes are packaged into genetically-engineered viruses, such as retroviruses or adenoviruses. Because of biosafety concerns, the viruses are typically altered so that they are not toxic or infectious (that is, they are replication incompetent). These basic tools of gene therapists have been extensively optimized over the past 10 years.

However, their biggest strength — simplicity — is simultaneously their biggest weakness. In many cases, direct gene transfer does not allow very sophisticated control over the therapeutic gene. This is because the transferred gene either randomly integrates into the patient's chromosomes or persists unintegrated for a relatively short period of time in the targeted tissue. Additionally, the targeted organ or tissue is not always easily accessible for direct application of the therapeutic gene.

On the other hand, therapeutic genes can be delivered using living cells. This procedure is relatively complex in comparison to direct gene transfer, and can be divided into three major steps. In the first step, cells from the patient or other sources are isolated and propagated in the laboratory. Second, the therapeutic gene is introduced into these cells, applying methods similar to those used in direct gene transfer. Finally, the genetically-modified cells are returned to the patient. The use of cells as gene transfer vehicles has certain advantages. In the laboratory dish *(in vitro),* cells can be manipulated much more precisely than in the body *(in vivo).* Some of the cell types that continue to divide under laboratory conditions may be expanded significantly before reintroduction into the patient. Moreover, some cell types are able to localize to particular regions of the human body, such as hematopoietic (blood-forming) stem cells, which return to the bone marrow. This "homing" phenomenon may be useful for applying the therapeutic gene with regional specificity.

A major disadvantage, however, is the additional biological complexity brought into systems by living cells. Isolation of a specific cell type requires not only extensive knowledge of biological markers, but also insight into the requirements for that cell type to stay alive *in vitro* and continue to divide. Unfortunately, specific biological markers are not known for many cell types, and the majority of normal human cells cannot be maintained for long periods of time *in vitro* without acquiring deleterious mutations.

STEM CELLS AS VEHICLES FOR GENE THERAPY

Stem cells can be classified as embryonic or adult, depending on their tissue of origin. The role of adult stem cells is to sustain an established repertoire of mature cell types in essentially steady-state numbers over the lifetime of the organism. Although adult tissues with a high turnover rate, such as blood, skin, and intestinal epithelium, are maintained by tissue-specific stem cells, the stem cells themselves rarely divide. However, in certain situations, such as during tissue repair after injury or following transplantation, stem cell divisions may become more frequent. The prototypic example of adult stem cells, the hematopoietic stem cell, has already been demonstrated to be of utility in gene therapy.[4,5] Although they are relatively rare in the human body, these cells can be readily isolated from bone marrow or after mobilization into peripheral blood. Specific surface markers allow the identification and enrichment of hematopoietic stem cells from a mixed population of bone marrow or peripheral blood cells.

After *in vitro* manipulation, these cells may be re-transplanted into patients by injection into the bloodstream, where they travel automatically to the place in the bone marrow in which they are functionally active. Hematopoietic stem cells that have been explanted, *in vitro* manipulated, and re-transplanted into the same patient (autologous transplantation) or a different patient (allogeneic transplantation) retain the ability to contribute to all mature blood cell types of the recipient for an extended period of time (when patients' cells are temporarily grown "outside the body" before being returned to them, the *in vitro* process is typically referred to as an *"ex vivo"* approach).

Another adult bone marrow-derived stem cell type with potential use as a vehicle for gene transfer is the mesenchymal stem cell, which has the ability to form cartilage, bone, adipose (fat) tissue, and marrow stroma (the bone marrow microenvironment).[6] Recently, a related stem cell type, the multipotent adult progenitor cell, has been isolated from bone marrow that can differentiate into multiple lineages, including neurons, hepatocytes (liver cells), endothelial cells (such as the cells that form the lining of blood vessels), and other cell types.[7] Other adult stem cells have been identified, such as those in the central nervous system and heart, but these are less well characterized and not as easily accessible.[8]

The traditional method to introduce a therapeutic gene into hematopoietic stem cells from bone marrow or peripheral blood involves the use of a vector derived from a certain class of virus, called a retrovirus. One type of retroviral vector was initially employed to show proof-of-principle that a foreign gene (in that instance the gene was not therapeutic, but was used as a molecular tag to genetically mark the cells) introduced into bone marrow cells may be stably maintained for several months.[9] However, these particular retroviral vectors were only capable of transferring the therapeutic gene into actively dividing cells. Since most adult stem cells divide at a relatively slow rate, efficiency was rather low. Vectors derived from other types of retroviruses (lentiviruses) and adenoviruses have the potential to overcome this limitation, since they also target non-dividing cells.

The major drawback of these methods is that the therapeutic gene frequently integrates more or less randomly into the chromosomes of the target cell. In principle, this is dangerous, because the gene therapy vector can potentially modify the activity of neighboring genes (positively or negatively) in close proximity to the insertion site or even inactivate host genes by integrating into them. These phenomena are referred to as "insertional mutagenesis." In extreme cases, such as in the X-linked SCID gene therapy trials, these mutations contribute to the malignant transformation of the targeted cells, ultimately resulting in cancer.

Another major limitation of using adult stem cells is that it is relatively difficult to maintain the stem cell state during *ex vivo* manipulations. Under current suboptimal conditions, adult stem cells tend to lose their stem cell properties and become more specialized, giving rise to mature cell types through a process termed "differentiation." Recent advances in supportive culture conditions for mouse hematopoietic stem cells may ultimately facilitate more effective use of human hematopoietic stem cells in gene therapy applications.[10,11]

EMBRYONIC STEM CELL: "THE ULTIMATE STEM CELL"

Embryonic stem cells are capable of unlimited self-renewal while maintaining the potential to differentiate into derivatives of all three germ layers. Even after months and years of growth in the laboratory, they retain the ability to form any cell type in the body. These properties reflect their origin from cells of the early embryo at a stage during which the cellular machinery is geared toward the rapid expansion and diversification of cell types.

Murine (mouse) embryonic stem cells were isolated over 20 years ago,[12,13] and paved the way for the isolation of nonhuman primate, and finally human embryonic stem cells.[14] Much of the anticipated potential surrounding human embryonic stem cells is an extrapolation from pioneering experiments in the mouse system. Experiments performed with human embryonic stem cells in the last couple of years indicate that these cells have the potential to make an important impact on medical science, at least in certain fields. In particular, this impact includes: a) differentiation of human embryonic stem cells into various cell types, such as neurons, cardiac, vascular, hematopoietic, pancreatic, hepatic, and placental cells, b) the derivation of new cell lines under alternative conditions, c) and the establishment of protocols that allow the genetic modification of these cells.

THE POTENTIAL OF HUMAN EMBRYONIC STEM CELLS FOR GENE THERAPY

Following derivation, human embryonic stem cells are easily accessible for controlled and specific genetic manipulation. When this facility is combined with their rapid growth, remarkable stability, and ability to mature *in vitro* into multiple cell types of the body, human embryonic stem cells are attractive potential tools for gene therapy. Two possible scenarios whereby human embryonic stem cells may benefit the gene therapy field are discussed below.

First, human embryonic stem cells could be genetically manipulated to introduce the therapeutic gene. This

gene may either be active or awaiting later activation, once the modified embryonic stem cell has differentiated into the desired cell type. Recently published reports establish the feasibility of such an approach.[15] Skin cells from an immunodeficient mouse were used to generate cellular therapy that partially restored immune function in the mouse. In these experiments, embryonic stem cells were generated from an immunodeficient mouse by nuclear transfer technology. The nucleus of an egg cell was replaced with that from a skin cell of an adult mouse with the genetic immunodeficiency. The egg was developed to the blastula stage at which embryonic stem cells were derived. The genetic defect was corrected by a genetic modification strategy designated "gene targeting." These "cured" embryonic stem cells were differentiated into hematopoietic "stem" cells and transplanted into immunodeficient mice. Interestingly, the immune function in these animals was partially restored. In principle, this approach may be employed for treating human patients with immunodeficiency or other diseases that may be corrected by cell transplantation.

However, significant advances must first be made. The levels of immune system reconstitution observed in the mice were quite modest (<1% of normal), while the methodology employed to achieve hematopoietic engraftment is not clinically feasible. This methodology involved using a more severely immunodeficient mouse as a recipient (which also had the murine equivalent of the human X-linked SCID mutation) and genetically engineering the hematopoietic engrafting cells with a potential oncogene prior to transplantation.

Embryonic stem cells may additionally be indirectly beneficial for cellular gene therapy. Since these cells can be differentiated *in vitro* into many cell types, including presumably tissue-specific stem cells, they may provide a constant *in vitro* source of cellular material. Such "adult" stem cells derived from embryonic stem cells may thus be utilized to optimize protocols for propagation and genetic manipulation techniques.[16] To acquire optimal cellular material from clinical samples in larger quantities for experimental and optimization purposes is usually rather difficult since access to these samples is limited.

GENETIC MANIPULATION OF STEM CELLS

The therapeutic gene needs to be introduced into the cell type used for therapy. Genes may be introduced into cells by transfection or transduction. Transfection utilizes chemical or physical methods to introduce new genes into cells. Usually, small molecules, such as liposomes, as well as other cationic-lipid based particles are employed to facilitate the entry of DNA encoding the gene of interest into the cells. Brief electric shocks are additionally used to facilitate DNA entry into living cells. All of these techniques have been applied to various stem cells, including human embryonic stem cells. However, the destiny of the introduced DNA is relatively poorly controlled using these procedures. In most cells, the DNA disappears after days or weeks, and in rare cases, integrates randomly into host chromosomal DNA. *In vitro* drug selection strategies allow the isolation and expansion of cells that are stably transfected, as long as they significantly express the newly introduced gene.

Transduction utilizes viral vectors for DNA transfer. Viruses, by nature, introduce DNA or RNA into cells very efficiently. Engineered viruses can be used to introduce almost any genetic information into cells. However, there are usually limitations in the size of the introduced gene. Additionally, some viruses (particularly retroviruses) only infect dividing cells effectively, whereas others (lentiviruses) do not require actively dividing cells. In most cases, the genetic information carried by the viral vector is stably integrated into the host cell genome (the total complement of chromosomes in the cell).

An important parameter that must be carefully monitored is the random integration into the host genome, since this process can induce mutations that lead to malignant transformation or serious gene dysfunction. However, several copies of the therapeutic gene may also be integrated into the genome, helping to bypass positional effects and gene silencing. Positional effects are caused by certain areas within the genome and directly influence the activity of the introduced gene. Gene silencing refers to the phenomenon whereby over time, most artificially introduced active genes are turned off by the host cell, a mechanism that is not currently well understood. In these cases, integration of several copies may help to achieve stable gene expression, since a subset of the introduced genes may integrate into favorable sites. In the past, gene silencing and positional effects were a particular problem in mouse hematopoietic stem cells.[17] These problems led to the optimization of retroviral and lentiviral vector systems by the addition of

genetic control elements (referred to as chromatin domain insulators and scaffold/matrix attachment regions) into the vectors, resulting in more robust expression in differentiating cell systems, including human embryonic stem cells.[18]

In some gene transfer systems, the foreign transgene does not integrate at a high rate and remains separate from the host genomic DNA, a status denoted "episomal". Specific proteins stabilizing these episomal DNA molecules have been identified as well as viruses (adenovirus) that persist stably for some time in an episomal condition. Recently, episomal systems have been applied to embryonic stem cells.[19]

An elegant way to circumvent positional effects and gene silencing is to introduce the gene of interest specifically into a defined region of the genome by the gene targeting technique referred to previously.[20] The gene targeting technique takes advantage of a cellular DNA repair process known as homologous recombination.[21] Homologous recombination provides a precise mechanism for defined modifications of genomes in living cells, and has been used extensively with mouse embryonic stem cells to investigate gene function and create mouse models of human diseases. Recombinant DNA is altered in vitro, and the therapeutic gene is introduced into a copy of the genomic DNA that is targeted during this process. Next, recombinant DNA is introduced by transfection into the cell, where it recombines with the homologous part of the cell genome. This in turn results in the replacement of normal genomic DNA with recombinant DNA containing genetic modifications.

Homologous recombination is a very rare event in cells, and thus a powerful selection strategy is necessary to identify the cells in which it occurs. Usually, the introduced construct has an additional gene coding for antibiotic resistance (referred to as a selectable marker), allowing cells that have incorporated the recombinant DNA to be positively selected in culture. However, antibiotic resistance only reveals that the cells have taken up recombinant DNA and incorporated it somewhere in the genome. To select for cells in which homologous recombination has occurred, the end of the recombination construct often includes the thymidine kinase gene from the herpes simplex virus. Cells that randomly incorporate recombinant DNA usually retain the entire DNA construct, including the herpes virus thymidine kinase gene. In cells that display homologous recombination between the recombinant construct and cellular DNA, an exchange of homologous DNA sequences is involved, and the non-homologous thymidine kinase gene at the end of the construct is eliminated. Cells expressing the thymidine kinase gene are killed by the antiviral drug ganciclovir in a process known as negative selection. Therefore, those cells undergoing homologous recombination are unique in that they are resistant to both the antibiotic and ganciclovir, allowing effective selection with these drugs *(see Figure 4.2).*

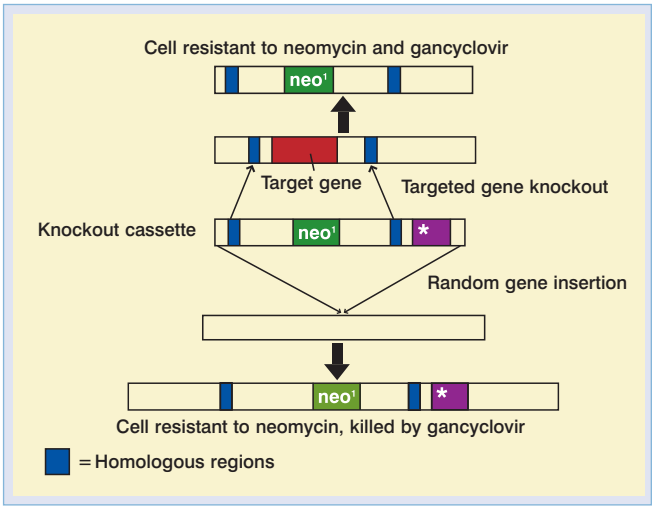


Figure 4.2. Gene targeting by homologous recombination.

Gene targeting by homologous recombination has recently been applied to human embryonic stem cells.[22] This is important for studying gene functions *in vitro* for lineage selection and marking. For therapeutic applications in transplantation medicine, the controlled modification of specific genes should be useful for purifying specific embryonic stem cell-derived, differentiated cell types from a mixed population, altering the antigenicity of embryonic stem cell derivatives, and adding defined markers that allow the identification of transplanted cells. Additionally, since the therapeutic gene can now be introduced into defined regions of the human genome, better controlled expression of the therapeutic gene should be possible. This also significantly reduces the risk of insertional mutagenesis.

FUTURE CHALLENGES FOR STEM CELL-BASED GENE THERAPY

Despite promising scientific results with genetically modified stem cells, some major problems remain to be overcome. The more specific and extensive the

genetic modification, the longer the stem cells have to remain *in vitro*. Although human embryonic stem cells in the culture dish remain remarkably stable, the cells may accumulate genetic and epigenetic changes that might harm the patient (epigenetic changes regulate gene activity without altering the genetic blueprint of the cell). Indeed, sporadic chromosomal abnormalities in human embryonic stem cell culture have been reported, and these may occur more frequently when the cells are passaged as bulk populations. This observation reinforces the necessity to optimize culture conditions further, to explore new human embryonic stem cell lines, and to monitor the existing cell lines.[23,24] Additionally undifferentiated embryonic stem cells have the potential to form a type of cancer called a teratocarcinoma. Safety precautions are therefore necessary, and currently, protocols are being developed to allow the complete depletion of any remaining undifferentiated embryonic stem cells.[25] This may be achieved by rigorous purification of embryonic stem cell derivatives or introducing suicide genes that can be externally controlled.

Another issue is the patient's immune system response. Transgenic genes, as well as vectors introducing these genes (such as those derived from viruses), potentially trigger immune system responses. If stem cells are not autologous, they eventually cause immuno-rejection of the transplanted cell type. Strategies to circumvent these problems, such as the expression of immune system-modulating genes by stem cells, creation of chimeric, immunotolerable bone marrow or suppression

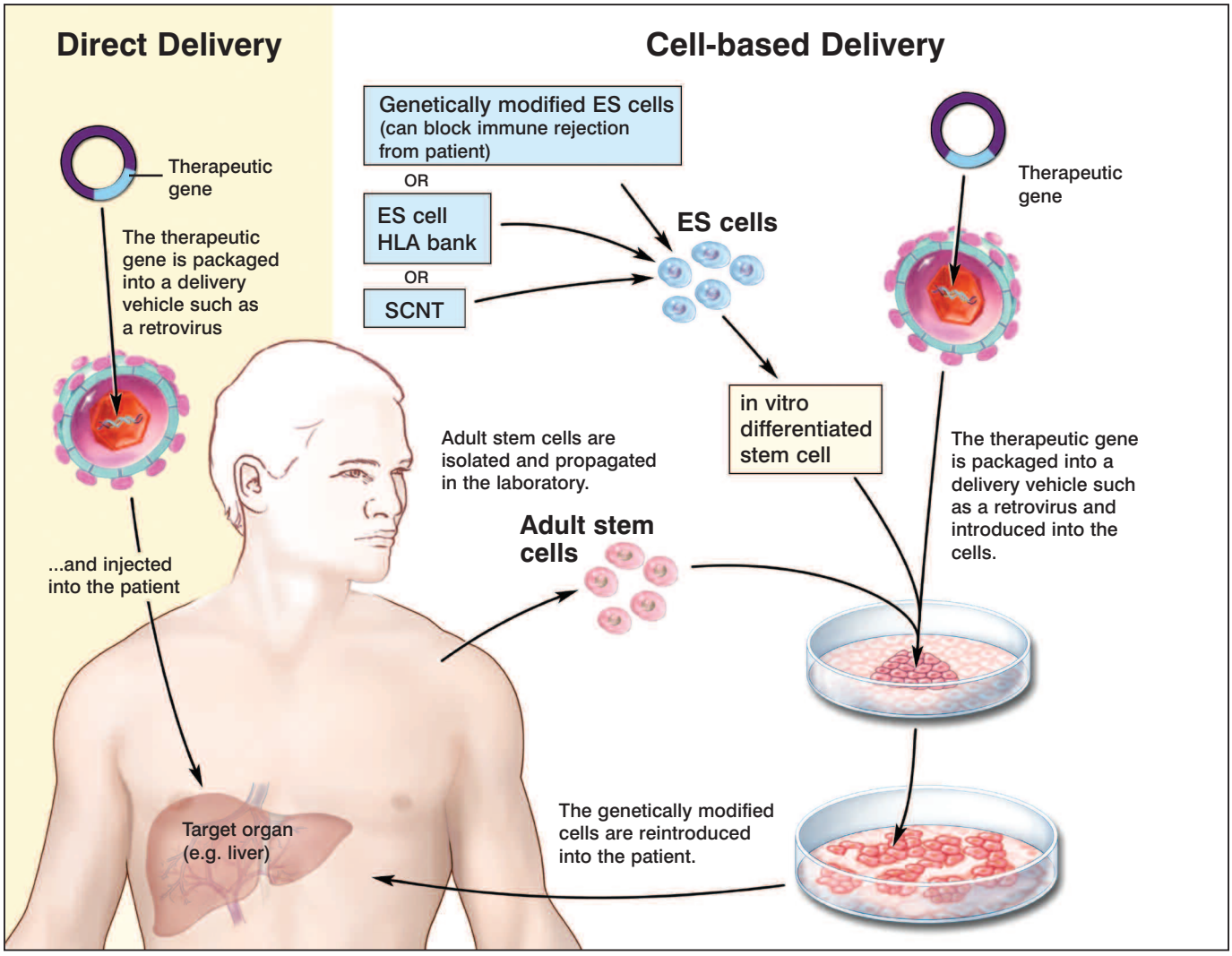


Figure 4.3. Strategies for Delivering Therapeutic Transgenes into Patients.

of HLA genes have been suggested.[25] In this context, nuclear transfer technology has been recently extended to human embryonic stem cells.[26]* Notably, immune-matched human embryonic stem cells have now been established from patients, including an individual with an immunodeficiency disease, congenital hypogammaglobulinemia.[27]* Strategies that combine gene targeting with embryonic stem cell-based therapy are thus potential novel therapeutic options.

The addition of human embryonic stem cells to the experimental gene therapy arsenal offers great promise in overcoming many of the existing problems of cellular based gene therapy that have been encountered in clinic trials *(see Figure 4.3)*. Further research is essential to determine the full potential of both adult and embryonic stem cells in this exciting new field.

REFERENCES

1. Mullen CA, Snitzer K, Culver KW, Morgan RA, Anderson WF, Blaese RM. Molecular analysis of T lymphocyte-directed gene therapy for adenosine deaminase deficiency: long-term expression in vivo of genes introduced with a retroviral vector. *Hum Gene Ther.* 1996;7:1123-1129.
2. Hacein-Bey-Abina S, Le Deist F, Carlier F, et al. Sustained correction of X-linked severe combined immunodeficiency by ex vivo gene therapy. *N Engl J Med.* Apr 18 2002;346(16):1185-1193.
3. Hacein-Bey-Abina S, von Kalle C, Schmidt M, et al. A serious adverse event after successful gene therapy for X-linked severe combined immunodeficiency. *N Engl J Med.* Jan 16 2003;348(3):255-256.
4. Aiuti A, Slavin S, Aker M, et al. Correction of ADA-SCID by stem cell gene therapy combined with nonmyeloablative conditioning. *Science.* 2002;296:2410-2413.
5. Hacein-Bey-Abina S, Le Diest F, Carlier F, et al. Sustained correction of X-linked severe combined immunodeficiency by ex vivo gene therapy. *N Engl J Med.* 2002;346:1185-1193.
6. Gregory CA, Prockop DJ, Spees JL. Non-hematopoietic bone marrow stem cells: molecular control of expansion and differentiation. *Exp Cell Res.* 2005;306:330-335.
7. Jiang Y, Jahagirdar BN, Reinhardt RL, et al. Pluripotency of mesenchymal stem cells derived from adult marrow. *Nature.* Jul 4 2002;418(6893):41-49.
8. Stocum DL. Stem cells in CNS and cardiac regeneration. *Adv Biochem Eng Biotechnol.* 2005;93:135-159.
9. Brenner MK, Rill DR, Holladay MS, et al. Gene marking to determine whether autologous marrow infusion restores long-term haemopoiesis in cancer patients. *Lancet.* Nov 6 1993;342(8880):1134-1137.
10. Reya T, Duncan AW, Ailles L, et al. A role for Wnt signalling in self-renewal of haematopoietic stem cells. *Nature.* May 22 2003;423(6938):409-414.
11. Willert K, Brown JD, Danenberg E, et al. Wnt proteins are lipid-modified and can act as stem cell growth factors. *Nature.* May 22 2003;423(6938):448-452.
12. Evans MJ, Kaufman MH. Establishment in culture of pluripotential cells from mouse embryos. *Nature.* Jul 9 1981;292(5819):154-156.
13. Martin GR. Isolation of a pluripotent cell line from early mouse embryos cultured in medium conditioned by teratocarcinoma stem cells. *Proc Natl Acad Sci USA.* Dec 1981;78(12):7634-7638.
14. Thomson JA, Itskovitz-Eldor J, Shapiro SS, et al. Embryonic stem cell lines derived from human blastocysts. *Science.* Nov 6 1998;282(5391):1145-1147.
15. Rideout WM, 3rd, Hochedlinger K, Kyba M, Daley GQ, Jaenisch R. Correction of a genetic defect by nuclear transplantation and combined cell and gene therapy. *Cell.* Apr 5 2002;109(1):17-27.
16. Barberi T, Willis LM, Socci ND, Studer L. Derivation of multipotent mesenchymal precursors from human embryonic stem cells. *PLoS Med.* 2005;2:e161.
17. Challita PM, Kohn DB. Lack of expression from a retroviral vector after transduction of murine hematopoietic stem cells is associated with methylation in vivo. *Proc Natl Acad Sci USA.* Mar 29 1994;91(7):2567-2571.
18. Ma Y, Ramezani A, Lewis R, Hawley RG, Thomson JA. High-level sustained transgene expression in human embryonic stem cells using lentiviral vectors. *Stem Cells.* 2003;21(1):111-117.
19. Aubert J, Dunstan H, Chambers I, Smith A. Functional gene screening in embryonic stem cells implicates Wnt antagonism in neural differentiation. *Nat Biotechnol.* Dec 2002;20(12):1240-1245.
20. Kyba M, Perlingeiro RC, Daley GQ. HoxB4 confers definitive lymphoid-myeloid engraftment potential on embryonic stem cell and yolk sac hematopoietic progenitors. *Cell.* Apr 5 2002;109(1):29-37.
21. Smithies O. Forty years with homologous recombination. *Nat Med.* Oct 2001;7(10):1083-1086.
22. Zwaka TP, Thomson JA. Homologous recombination in human embryonic stem cells. *Nat Biotechnol.* Mar 2003;21(3):319-321.

* **Editor's note:** Both papers referenced in 26 and 27 were later retracted. See Science 20 January 2006: Vol. 311. no. 5759, p. 335.

23. Draper JS, Smith K, Gokhale P, et al. Recurrent gain of chromosomes 17q and 12 in cultured human embryonic stem cells. *Nat Biotechnol.* Jan 2004;22(1):53-54.

24. Cowan CA, Klimanskaya I, McMahon J, et al. Derivation of embryonic stem-cell lines from human blastocysts. *N Engl J Med.* Mar 25 2004;350(13):1353-1356.

25. Gerecht-Nir S, Itskovitz-Eldor J. Cell therapy using human embryonic stem cells. *Transpl Immunol.* Apr 2004;12(3-4):203-209.

26. Hwang WS, Ryu YJ, Park JH, et al. Evidence of a pluripotent human embryonic stem cell line derived from a cloned blastocyst. *Science.* Mar 12 2004;303(5664):1669-1674.

27. Hwang WS, Roh SI, Lee BC, et al. Patient-specific embryonic stem cells derived from human SCNT blastocysts. *Science.* 2005;308:1777-1783.

5. INTELLECTUAL PROPERTY OF HUMAN PLURIPOTENT STEM CELLS

*by Mark L. Rohrbaugh**

This report will provide an update in the area of intellectual property issues related to human pluripotent stem cells, and specifically, to human embryonic stem cells (hESCs). As anticipated, the patent landscape with respect to stem cells continues to become more complex in the United States, with new patents issued in various areas involving differentiated or modified cells and methods to differentiate cells. In Europe, some patent claims that involve unmodified hESCs currently stand rejected, although their ultimate outcomes are undetermined, as several parties have appealed the rejections they have received.

THE UNITED STATES PATENT LANDSCAPE

Since Thomson and colleagues were issued a patent on March 13, 2001 that specifically claimed hESCs,[1] a number of patents have issued in the U.S. involving claims to methods of using, maintaining, or inducing differentiation of hESCs or to the modified or differentiated cells themselves. According to data provided by the United States Patent and Trademark Office (USPTO) on October 22, 2004, nearly 300 patents had been issued with claims to embryonic stem (ES) cells or processes, of which approximately 38 encompass human products or processes. Approximately 700 pending patent applications had been published with claims to ES cells or processes, of which approximately 200 encompass human products or processes. Approximately 150 published patient applications encompass "totipotent" ES cells or processes. These patents claim various cell types that would be used in regenerative medicine (as described below) or auxiliary technologies, such as conditioned medium for cell growth, that support the use of hESCs.[2]

Among the patents issued more recently, one stands out in particular — a patent issued to Geron with broad claims to cells grown feeder-free.[3] One broad claim from this patent states, "A cellular composition comprising undifferentiated primate primordial stem (pPS) cells proliferating on an extracellular matrix, wherein the composition is free of feeder cells." Another recites, "A cell population consisting essentially of primate embryonic stem (ES) cells proliferating in culture on an extracellular matrix in a manner such that at least 50% of the proliferating ES cells are undifferentiated." The term "primordial" as used in the application refers to pluripotent or totipotent cells such as embryonic germ cells and ES cells. The claims cover cells that have been weaned from feeder cells as well as those that were derived *de novo* in feeder-free cultures. This patented technology, along with the original Thomson hESC technology, will likely be necessary in the use of many anticipated therapeutic applications of hESCs.

Other patents have issued to methods of inducing differentiation and to partially or fully differentiated cells. Such patents include the University of Utah's patent claiming neuroepithelial stem cells and Geron's patent claiming "directed differentiation of human pluripotent stem cells to cells of the hepatocyte lineage."[4] The Thomson patent will dominate such technologies to the extent that they utilize hESCs as starting or intermediate materials. However, technologies exist that do not require the use of the Thomson patent claims because they rely on lineage-specific stem cells obtained from sources other than hESCs. One such technology patented by Snyder *et al.* is a "pluripotent and self-renewing neural stem cell of human origin" isolated from embryonic neural tissue.[5] Another patent claim is directed to a method of obtaining a "substantially homogeneous population of pluripotent brain stem cells" from brain tissue rather than from hESCs.[6]

* Director, National Institutes of Health Office of Technology Transfer, Bethesda, MD 20892, *Email:* rohrbaugh@nih.gov

Scientists and physicians envision therapeutic uses of stem cells that are genetically modified in some manner to enhance their utility. For example, a pluripotent stem cell could be modified with a gene construct that enhances the ability to remove trace undifferentiated hESCs from an otherwise differentiated population of cells. This construct might include a gene encoding an enzyme that converts a pro-drug to a toxic drug linked to a promoter that is active only in undifferentiated hESCs. After isolating a differentiated population of cells modified in this manner, the pro-drug could be added to the culture, where it would be converted to a toxin in any residual undifferentiated cells.[7] The depletion of undifferentiated cells from a population of differentiated cells prior to implantation into patients reduces the risk that "contaminating" undifferentiated cells would form tumors.

THE EUROPEAN PATENT LANDSCAPE

In Europe, the first patents claiming unmodified stem cells have been denied based on a European Patent Convention (EPC) rule that excludes inventions involving the use of human embryos for industrial or commercial purposes. These denials include that of James Thomson of the Wisconsin Alumni Research Foundation (WARF).[8–10] While it does not appear that unmodified human embryonic stem cell patents will issue in Europe, the door has not yet been closed, as these decisions are currently being appealed.[11]

In arriving at the decision to deny the WARF application, the Examining Division maintained that the EPC rule against patenting embryos did not apply to downstream products from embryos as long as those products did not necessitate the use of a human embryo. Because the WARF technology necessitates use of a human embryo, it could not be patented. Commentators opposed to this decision view the rule more narrowly, arguing that the limits of ethical acceptability as defined by the rule should not be so broad as to include claims that involve starting materials that are already embryonic cells or cell mixtures. Such reasoning would limit the exclusion to claims that include a preliminary step of producing freshly disaggregated cells by destroying a human embryo, but not necessarily to isolated human embryonic stem cells *per se,* which are available through legal importation in many European countries.[10]

FACILITATING ACCESS TO STEM CELLS

Several new model agreements have been approved by NIH for use in distributing hESCs under Infrastructure Grants. These include model material transfer agreements (MTAs) from MizMedi Hospital, Seoul, Korea; Technion-Israel Institute of Technology, Haifa, Israel; and Cellartis, AB, Göteborg, Sweden (for details, see http://stemcells.nih.gov/research/registry/eligibilityCriteria.asp). The terms are similar to the previous model agreements that the NIH has entered into or approved for use with NIH-funded hESC distribution.

CONCLUSIONS

To date, two patents, one from WARF and one from Geron, dominate most of the anticipated commercial uses of hESCs in the U.S. Europe has taken a different course by not currently permitting the patenting of unmodified hESCs. In both North America and Europe, it is likely that more patents will continue to issue on other types of pluripotent stem cells, tissue-specific stem cells, methods that use these cells, and materials and methods associated with their propagation. More stem cells are now available for broad distribution with U.S. Federal funding under terms that permit reasonably unrestricted use in non-profit research.

While many scientists have received hESCs for non-profit research, fewer have been able to reach agreements with providers for collaborative research that directly benefits the commercial sector. In these instances, the research is high-risk and often does not result in new intellectual property, yet the industrial collaborator seeks an agreement in advance that includes the right to license new inventions, particularly new uses of the materials, should they occur. The industrial collaborator usually must negotiate an agreement and pay a fee in advance to patent holders and owners of the cell lines. This can be a high hurdle for small companies that have limited funds and for large companies that do not have a strong interest in the field but want to protect their investment in proprietary materials while providing them to non-profit researchers. Finally, WiCell, recipient of the NIH contract for the National Stem Cell Bank, must reach agreements with owners of patents and proprietary cell lines to facilitate the distribution of the cells through the Bank while protecting the interests of all parties.

The NIH experience with agreements to transfer proprietary materials from companies to government researchers suggests that only a small fraction of these collaborations lead to new inventions, yet they result in important scientific publications that advance biomedical research. Hopefully, patent owners, cell providers, and researchers will work together to facilitate these public-private partnerships.

REFERENCES

1. Thomson JA, inventor; Wisconsin Alumni Research Foundation, assignee. Primate embryonic stem cells. US patent 6,200,806, 2001.
2. Xu C, Gold JD, inventors; Geron Corporation, assignee. Conditioned media for propagating human pluripotent stem cells, 2003.
3. Bodnar AG, Chiu C-P, Gold JD, Inokuma M, Murai JT, West MD, inventors; Geron Corporation, assignee. Methods and materials for the growth of primate-derived primordial stem cells in feeder-free culture. US patent 6,800,480, 2004.
4. Rambhatla L, Carpenter MK, inventors; Geron Corporation, assignee. Hepatocyte lineage cells derived from pluripotent stem cells. US patent 6,458,589, 2002.
5. Snyder EY, Wolfe JH, Kim SU, inventors; The Children's Medical Center Corporation, University of British Columbia, University of Pennsylvania,, assignee. Engraftable human neural stem cells. US patent 6,680,198, 2004.
6. Steindler DA, Laywell ED, Kukekou VG, Thomas LB, inventors; University of Tennessee Research Foundation, assignee. Isolated mammalian neural stem cells. US patent 6,638,763, 2003.
7. Gold JD, Lebkowski JS, inventors; Geron Corporation, assignee. Methods for providing differentiated stem cells. US patent 6,576,464, 2003.
8. Decision to refuse European Patent application #96 903 521.1-2401, Primate embryonic stem cells, applicant: Wisconsin Alumni Research Foundation. *Examining Division;* 2004.
9. Webber P. Embryonic cell patenting. *Biosci Law Rev.* 2004;3:87-98.
10. Germinario C. The value of life. *Patent World.* 2004(June):87-98.
11. Gallagher K. Battles loom over basic patent on stem cells. *Milwaukee J Sentinel.* October 13, 2004.

[This page intentionally left blank]

6. MENDING A BROKEN HEART: STEM CELLS AND CARDIAC REPAIR

Charles A. Goldthwaite, Jr., Ph.D.

HEART FAILURE: THE DISEASE AND ITS CAUSES

Cardiovascular disease (CVD), which includes hypertension, coronary heart disease (CHD), stroke, and congestive heart failure (CHF), has ranked as the number one cause of death in the United States every year since 1900 except 1918, when the nation struggled with an influenza epidemic.[1] In 2002, CVD claimed roughly as many lives as cancer, chronic lower respiratory diseases, accidents, diabetes mellitus, influenza, and pneumonia combined. According to data from the 1999-2002 National Health and Nutrition Examination Survey (NHANES), CVD caused approximately 1.4 million deaths (38.0 percent of all deaths) in the U.S. in 2002. Nearly 2600 Americans die of CVD each day, roughly one death every 34 seconds. Moreover, within a year of diagnosis, one in five patients with CHF will die. CVD also creates a growing economic burden; the total health care cost of CVD in 2005 was estimated at $393.5 billion dollars.

Given the aging of the U.S. population and the relatively dramatic recent increases in the prevalence of cardiovascular risk factors such as obesity and type 2 diabetes,[2,3] CVD will continue to be a significant health concern well into the 21st century. However, improvements in the acute treatment of heart attacks and an increasing arsenal of drugs have facilitated survival. In the U.S. alone, an estimated 7.1 million people have survived a heart attack, while 4.9 million live with CHF.[1] These trends suggest an unmet need for therapies to regenerate or repair damaged cardiac tissue.

Ischemic heart failure occurs when cardiac tissue is deprived of oxygen. When the ischemic insult is severe enough to cause the loss of critical amounts of cardiac muscle cells (cardiomyocytes), this loss initiates a cascade of detrimental events, including formation of a non-contractile scar, ventricular wall thinning, an overload of blood flow and pressure, ventricular remodeling (the overstretching of viable cardiac cells to sustain cardiac output), heart failure, and eventual death.[4] Restoring damaged heart muscle tissue, through repair or regeneration, therefore represents a fundamental mechanistic strategy to treat heart failure. However, endogenous repair mechanisms, including the proliferation of cardiomyocytes under conditions of severe blood vessel stress or vessel formation and tissue generation via the migration of bone-marrow-derived stem cells to the site of damage, are in themselves insufficient to restore lost heart muscle tissue (myocardium) or cardiac function.[5] Current pharmacologic interventions for heart disease, including beta-blockers, diuretics, and angiotensin-converting enzyme (ACE) inhibitors, and surgical treatment options, such as changing the shape of the left ventricle and implanting assistive devices such as pacemakers or defibrillators, do not restore function to damaged tissue. Moreover, while implantation of mechanical ventricular assist devices can provide long-term improvement in heart function, complications such as infection and blood clots remain problematic.[6] Although heart transplantation offers a viable option to replace damaged myocardium in selected individuals, organ availability and transplant rejection complications limit the widespread practical use of this approach.

The difficulty in regenerating damaged myocardial tissue has led researchers to explore the application of embryonic and adult-derived stem cells for cardiac repair. A number of stem cell types, including embryonic stem (ES) cells, cardiac stem cells that naturally reside within the heart, myoblasts (muscle stem cells), adult bone marrow-derived cells, mesenchymal cells (bone marrow-derived cells that give rise to tissues such as muscle, bone, tendons, ligaments, and adipose tissue), endothelial progenitor cells (cells that give rise to the endothelium, the interior lining of blood vessels), and umbilical cord blood cells, have been investigated to varying extents as possible sources for regenerating

damaged myocardium. All have been tested in mouse or rat models, and some have been tested in large animal models such as pigs. Preliminary clinical data for many of these cell types have also been gathered in selected patient populations.

However, clinical trials to date using stem cells to repair damaged cardiac tissue vary in terms of the condition being treated, the method of cell delivery, and the primary outcome measured by the study, thus hampering direct comparisons between trials.[7] Some patients who have received stem cells for myocardial repair have reduced cardiac blood flow (myocardial ischemia), while others have more pronounced congestive heart failure and still others are recovering from heart attacks. In some cases, the patient's underlying condition influences the way that the stem cells are delivered to his/her heart (see the section, "Methods of Cell Delivery" for details). Even among patients undergoing comparable procedures, the clinical study design can affect the reporting of results. Some studies have focused on safety issues and adverse effects of the transplantation procedures; others have assessed improvements in ventricular function or the delivery of arterial blood. Furthermore, no published trial has directly compared two or more stem cell types, and the transplanted cells may be autologous (i.e., derived from the person on whom they are used) or allogeneic (i.e., originating from another person) in origin. Finally, most of these trials use unlabeled cells, making it difficult for investigators to follow the cells' course through the body after transplantation (see the section "Considerations for Using These Stem Cells in the Clinical Setting" at the end of this article for more details).

Despite the relative infancy of this field, initial results from the application of stem cells to restore cardiac function have been promising. This article will review the research supporting each of the aforementioned cell types as potential source materials for myocardial regeneration and will conclude with a discussion of general issues that relate to their clinical application.

MECHANISMS OF ACTION

In 2001, Menasche, *et.al.* described the successful implantation of autologous skeletal myoblasts (cells that divide to repair and/or increase the size of voluntary muscles) into the post-infarction scar of a patient with severe ischemic heart failure who was undergoing coronary artery bypass surgery.[8] Following the procedure, the researchers used imaging techniques to observe the heart's muscular wall and to assess its ability to beat. When they examined patients 5 months after treatment, they concluded that treated hearts pumped blood more efficiently and seemed to demonstrate improved tissue health. This case study suggested that stem cells may represent a viable resource for treating ischemic heart failure, spawning several dozen clinical studies of stem cell therapy for cardiac repair (see Boyle, *et.al.*[7] for a complete list) and inspiring the development of Phase I and Phase II clinical trials. These trials have revealed the complexity of using stem cells for cardiac repair, and considerations for using stem cells in the clinical setting are discussed in a subsequent section of this report.

The mechanism by which stem cells promote cardiac repair remains controversial, and it is likely that the cells regenerate myocardium through several pathways. Initially, scientists believed that transplanted cells differentiated into cardiac cells, blood vessels, or other cells damaged by CVD.[9-11] However, this model has been recently supplanted by the idea that transplanted stem cells release growth factors and other molecules that promote blood vessel formation (angiogenesis) or stimulate "resident" cardiac stem cells to repair damage.[12-14] Additional mechanisms for stem-cell mediated heart repair, including strengthening of the post-infarct scar[15] and the fusion of donor cells with host cardiomyocytes,[16] have also been proposed.

METHODS OF CELL DELIVERY

Regardless of which mechanism(s) will ultimately prove to be the most significant in stem-cell mediated cardiac repair, cells must be successfully delivered to the site of injury to maximize the restored function. In preliminary clinical studies, researchers have used several approaches to deliver stem cells. Common approaches include intravenous injection and direct infusion into the coronary arteries. These methods can be used in patients whose blood flow has been restored to their hearts after a heart attack, provided that they do not have additional cardiac dysfunction that results in total occlusion or poor arterial flow.[12, 17] Of these two methods, intracoronary infusion offers the advantage of directed local delivery, thereby increasing the number of cells that reach the target tissue relative to the number that will home to the heart once they

have been placed in the circulation. However, these strategies may be of limited benefit to those who have poor circulation, and stem cells are often injected directly into the ventricular wall of these patients. This endomyocardial injection may be carried out either via a catheter or during open-heart surgery.[18]

To determine the ideal site to inject stem cells, doctors use mapping or direct visualization to identify the locations of scars and viable cardiac tissue. Despite improvements in delivery efficiency, however, the success of these methods remains limited by the death of the transplanted cells; as many as 90% of transplanted cells die shortly after implantation as a result of physical stress, myocardial inflammation, and myocardial hypoxia.[4] Timing of delivery may slow the rate of deterioration of tissue function, although this issue remains a hurdle for therapeutic approaches.

TYPES OF STEM CELLS INVESTIGATED TO REGENERATE DAMAGED MYOCARDIAL TISSUE

Embryonic and adult stem cells have been investigated to regenerate damaged myocardial tissue in animal models and in a limited number of clinical studies. A brief review of work to date and specific considerations for the application of various cell types will be discussed in the following sections.

Embryonic Stem (ES) Cells

Because ES cells are pluripotent, they can potentially give rise to the variety of cell types that are instrumental in regenerating damaged myocardium, including cardiomyocytes, endothelial cells, and smooth muscle cells. To this end, mouse and human ES cells have been shown to differentiate spontaneously to form endothelial and smooth muscle cells *in vitro*[19] and *in vivo,*[20,21] and human ES cells differentiate into myocytes with the structural and functional properties of cardiomyocytes.[22-24] Moreover, ES cells that were transplanted into ischemically-injured myocardium in rats differentiated into normal myocardial cells that remained viable for up to four months,[25] suggesting that these cells may be candidates for regenerative therapy in humans.

However, several key hurdles must be overcome before human ES cells can be used for clinical applications. Foremost, ethical issues related to embryo access currently limit the avenues of investigation. In addition, human ES cells must go through rigorous testing and purification procedures before the cells can be used as sources to regenerate tissue. First, researchers must verify that their putative ES cells are pluripotent. To prove that they have established a human ES cell line, researchers inject the cells into immunocompromised mice; i.e., mice that have a dysfunctional immune system. Because the injected cells cannot be destroyed by the mouse's immune system, they survive and proliferate. Under these conditions, pluripotent cells will form a teratoma, a multi-layered, benign tumor that contains cells derived from all three embryonic germ layers. Teratoma formation indicates that the stem cells have the capacity to give rise to all cell types in the body.

The pluripotency of ES cells can complicate their clinical application. While undifferentiated ES cells may possibly serve as sources of specific cell populations used in myocardial repair, it is essential that tight quality control be maintained with respect to the differentiated cells. Any differentiated cells that would be used to regenerate heart tissue must be purified before transplantation can be considered. If injected regenerative cells are accidentally contaminated with undifferentiated ES cells, a tumor could possibly form as a result of the cell transplant.[4] However, purification methodologies continue to improve; one recent report describes a method to identify and select cardiomyocytes during human ES cell differentiation that may make these cells a viable option in the future.[26]

This concern illustrates the scientific challenges that accompany the use of all human stem cells, whether derived from embryonic or adult tissues. Predictable control of cell proliferation and differentiation requires additional basic research on the molecular and genetic signals that regulate cell division and specialization. Furthermore, long-term cell stability must be well understood before human ES-derived cells can be used in regenerative medicine. The propensity for genetic mutation in the human ES cells must be determined, and the survival of differentiated, ES-derived cells following transplantation must be assessed. Furthermore, once cells have been transplanted, undesirable interactions between the host tissue and the injected cells must be minimized. Cells or tissues derived from ES cells that are currently available for use in humans are not tissue-matched to patients and thus would require immunosuppression to limit immune rejection.[18]

Skeletal Myoblasts

While skeletal myoblasts (SMs) are committed progenitors of skeletal muscle cells, their autologous origin, high proliferative potential, commitment to a myogenic lineage, and resistance to ischemia promoted their use as the first stem cell type to be explored extensively for cardiac application. Studies in rats and humans have demonstrated that these cells can repopulate scar tissue and improve left ventricular function following transplantation.[27] However, SM-derived cardiomyocytes do not function in complete concert with native myocardium. The expression of two key proteins involved in electromechanical cell integration, N-cadherin and connexin 43, are downregulated *in vivo,*[28] and the engrafted cells develop a contractile activity phenotype that appears to be unaffected by neighboring cardiomyocytes.[29]

To date, the safety and feasibility of transplanting SM cells have been explored in a series of small studies enrolling a collective total of nearly 100 patients. Most of these procedures were carried out during open-heart surgery, although a couple of studies have investigated direct myocardial injection and transcoronary administration. Sustained ventricular tachycardia, a life-threatening arrhythmia and unexpected side-effect, occurred in early implantation studies, possibly resulting from the lack of electrical coupling between SM-derived cardiomyocytes and native tissue.[30,31] Changes in pre-implantation protocols have minimized the occurrence of arrhythmias in conjunction with the use of SM cells, and Phase II studies of skeletal myoblast therapy are presently underway.

Human Adult Bone-Marrow Derived Cells

In 2001, Jackson, *et.al.* demonstrated that cardiomyocytes and endothelial cells could be regenerated in a mouse heart attack model through the introduction of adult mouse bone marrow-derived stem cells.[9] That same year, Orlic and colleagues showed that direct injection of mouse bone marrow-derived cells into the damaged ventricular wall following an induced heart attack led to the formation of new cardiomyocytes, vascular endothelium, and smooth muscle cells.[11] Nine days after transplanting the stem cells, the newly-formed myocardium occupied nearly 70 percent of the damaged portion of the ventricle, and survival rates were greater in mice that received these cells than in those that did not. While several subsequent studies have questioned whether these cells actually differentiate into cardiomyocytes,[32,33] the evidence to support their ability to prevent remodeling has been demonstrated in many laboratories.[7]

Based on these findings, researchers have investigated the potential of human adult bone marrow as a source of stem cells for cardiac repair. Adult bone marrow contains several stem cell populations, including hematopoietic stem cells (which differentiate into all of the cellular components of blood), endothelial progenitor cells, and mesenchymal stem cells; successful application of these cells usually necessitates isolating a particular cell type on the basis of its' unique cell-surface receptors. In the past three years, the transplantation of bone marrow mononuclear cells (BMMNCs), a mixed population of blood and cells that includes stem and progenitor cells, has been explored in more patients and clinical studies of cardiac repair than any other type of stem cell.7

The results from clinical studies of BMMNC transplantation have been promising but mixed. However, it should be noted that these studies have been conducted under a variety of conditions, thereby hampering direct comparison. The cells have been delivered via open-heart surgery and endomyocardial and intracoronary catheterization. Several studies, including the Bone Marrow Transfer to Enhance ST-Elevation Infarct Regeneration (BOOST) and the Transplantation of Progenitor Cells and Regeneration Enhancement in Acute Myocardial Infarction (TOPCARE-AMI) trials, have shown that intracoronary infusion of BMMNCs following a heart attack significantly improves the left ventricular (LV) ejection fraction, or the volume of blood pumped out of the left ventricle with each heartbeat.[34-36] However, other studies have indicated either no improvement in LV ejection fraction upon treatment[37] or an increased LV ejection fraction in the control group.[38] An early study that used endomyocardial injection to enhance targeted delivery indicated a significant improvement in overall LV function.[39] Discrepancies such as these may reflect differences in cell preparation protocols or baseline patient statistics. As larger trials are developed, these issues can be explored more systematically.

Mesenchymal (Bone Marrow Stromal) Cells

Mesenchymal stem cells (MSCs) are precursors of non-hematopoietic tissues (e.g., muscle, bone, tendons, ligaments, adipose tissue, and fibroblasts) that are obtained relatively easily from autologous bone marrow. They remain multipotent following expan-

sion *in vitro,* exhibit relatively low immunogenicity, and can be frozen easily. While these properties make the cells amenable to preparation and delivery protocols, scientists can also culture them under special conditions to differentiate them into cells that resemble cardiac myocytes. This property enables their application to cardiac regeneration. MSCs differentiate into endothelial cells when cultured with vascular endothelial growth factor[40] and cardiomyogenic (CMG) cells when treated with the DNA-demethylating agent, 5-azacytidine.41 More important, however, is the observation that MSCs can differentiate into cardiomyocytes and endothelial cells *in vivo* when transplanted to the heart following myocardial infarct (MI) or non-injury in pig, mouse, or rat models.[42-45] Additionally, the ability of MSCs to restore functionality may be enhanced by the simultaneous transplantation of other stem cell types.[43]

Several animal model studies have shown that treatment with MSCs significantly increases myocardial function and capillary formation.[5,41] One advantage of using these cells in human studies is their low immunogenicity; allogeneic MSCs injected into infarcted myocardium in a pig model regenerated myocardium and reduced infarct size without evidence of rejection.[46] A randomized clinical trial implanting MSCs after MI has demonstrated significant improvement in global and regional LV function,[47] and clinical trials are currently underway to investigate the application of allogeneic and autologous MSCs for acute MI and myocardial ischemia, respectively.

Resident Cardiac Stem Cells

Recent evidence suggests that the heart contains a small population of endogenous stem cells that most likely facilitate minor repair and turnover-mediated cell replacement.[7] These cells have been isolated and characterized in mouse, rat, and human tissues.[48,49] The cells can be harvested in limited quantity from human endomyocardial biopsy specimens[50] and can be injected into the site of infarction to promote cardiomyocyte formation and improvements in systolic function.[49] Separation and expansion *ex vivo* over a period of weeks are necessary to obtain sufficient quantities of these cells for experimental purposes. However, their potential as a convenient resource for autologous stem cell therapy has led the National Heart, Lung, and Blood Institute to fund forthcoming clinical trials that will explore the use of cardiac stem cells for myocardial regeneration.

Endothelial Progenitor Cells

The endothelium is a layer of specialized cells that lines the interior surface of all blood vessels (including the heart). This layer provides an interface between circulating blood and the vessel wall. Endothelial progenitor cells (EPCs) are bone marrow-derived stem cells that are recruited into the peripheral blood in response to tissue ischemia.[4] EPCs are precursor cells that express some cell-surface markers characteristic of mature endothelium and some of hematopoietic cells.[19,51-53] EPCs home in on ischemic areas, where they differentiate into new blood vessels; following a heart attack, intravenously injected EPCs home to the damaged region within 48 hours.[12] The new vascularization induced by these cells prevents cardiomyocyte apoptosis (programmed cell death) and LV remodeling, thereby preserving ventricular function.[13] However, no change has been observed in non-infarcted regions upon EPC administration. Clinical trials are currently underway to assess EPC therapy for growing new blood vessels and regenerating myocardium.

Other Cells: Umbilical Cord Blood Stem Cells, Fibroblasts, and Peripheral Blood CD34$^+$ Cells

Several other cell populations, including umbilical cord blood (UCB) stem cells, fibroblasts (cells that synthesize the extracellular matrix of connective tissues), and peripheral blood CD34$^+$ cells, have potential therapeutic uses for regenerating cardiac tissue. Although these cell types have not been investigated in clinical trials of heart disease, preliminary studies in animal models indicate several potential applications in humans.

Umbilical cord blood contains enriched populations of hematopoietic stem cells and mesencyhmal precursor cells relative to the quantities present in adult blood or bone marrow.[54,55] When injected intravenously into the tail vein in a mouse model of MI, human mononuclear UCB cells formed new blood vessels in the infarcted heart.[56] A human DNA assay was used to determine the migration pattern of the cells after injection; although they homed only to injured areas within the heart, they were also detected in the marrow, spleen, and liver. When injected directly into the infarcted area in a rat model of MI, human mononuclear UCB cells improved ventricular function.[57] Staining for CD34 and other markers found on the cell surface of hematopoietic stem cells indicated that some of the cells survived in the myocardium. Results similar to these have been

observed following the injection of human unrestricted somatic stem cells from UCB into a pig MI model.[58]

Adult peripheral blood $CD34^+$ cells offer the advantage of being obtained relatively easily from autologous sources.[59] Although some studies using a mouse model of MI claim that these cells can transdifferentiate into cardiomyocytes, endothelial cells, and smooth muscle cells at the site of tissue injury,[60] this conclusion is highly contested. Recent studies that involve the direct injection of blood-borne or bone marrow-derived hematopoietic stem cells into the infarcted region of a mouse model of MI found no evidence of myocardial regeneration following injection of either cell type.[33] Instead, these hematopoietic stem cells followed traditional differentiation patterns into blood cells within the microenvironment of the injured heart. Whether these cells will ultimately find application in myocardial regeneration remains to be determined.

Autologous fibroblasts offer a different strategy to combat myocardial damage by replacing scar tissue with a more elastic, muscle-like tissue and inhibiting host matrix degradation.[4] The cells may be manipulated to express muscle-specific transcription factors that promote their differentiation into myotubes such as those derived from skeletal myoblasts.[61] One month after these cells were implanted into the post-infarction scar in a rat model of MI, they occupied a large portion of the scar but were not functionally integrated.[61] Although the effects on ventricular function were not evaluated in this study, authors noted that modified autologous fibroblasts may ultimately prove useful in elderly patients who have a limited population of autologous skeletal myoblasts or bone marrow stem cells.

CONSIDERATIONS FOR USING THESE STEM CELLS IN THE CLINICAL SETTING

As these examples indicate, many types of stem cells have been applied to regenerate damaged myocardium. In select applications, stem cells have demonstrated sufficient promise to warrant further exploration in large-scale, controlled clinical trials. However, the current breadth of application of these cells has made it difficult to compare and contextualize the results generated by the various trials. Most studies published to date have enrolled fewer than 25 patients, and the studies vary in terms of cell types and preparations used, methods of delivery, patient populations, and trial outcomes. However, the mixed results that have been observed in these studies do not necessarily argue against using stem cells for cardiac repair. Rather, preliminary results illuminate the many gaps in understanding of the mechanisms by which these cells regenerate myocardial tissue and argue for improved characterization of cell preparations and delivery methods to support clinical applications.

Future clinical trials that use stem cells for myocardial repair must address two concerns that accompany the delivery of these cells: 1) safety and 2) tracking the cells to their ultimate destination(s). Although stem cells appear to be relatively safe in the majority of recipients to date, an increased frequency of non-sustained ventricular tachycardia, an arrhythmia, has been reported in conjunction with the use of skeletal myoblasts.[30,62-64] While this proarrhythmic effect occurs relatively early after cell delivery and does not appear to be permanent, its presence highlights the need for careful safety monitoring when these cells are used. Additionally, animal models have demonstrated that stem cells rapidly diffuse from the heart to other organs (e.g., lungs, kidneys, liver, spleen) within a few hours of transplantation,[65,66] an effect observed regardless of whether the cells are injected locally into the myocardium. This migration may or may not cause side-effects in patients; however, it remains a concern related to the delivery of stem cells in humans. (*Note:* Techniques to label stem cells for tracking purposes and to assess their safety are discussed in more detail in other articles in this publication).

In addition to safety and tracking, several logistical issues must also be addressed before stem cells can be used routinely in the clinic. While cell tracking methodologies allow researchers to determine migration patterns, the stem cells must target their desired destination(s) and be retained there for a sufficient amount of time to achieve benefit. To facilitate targeting and enable clinical use, stem cells must be delivered easily and efficiently to their sites of application. Finally, the ease by which the cells can be obtained and the cost of cell preparation will also influence their transition to the clinic.

CONCLUSIONS

The evidence to date suggests that stem cells hold promise as a therapy to regenerate damaged myocardium. Given the worldwide prevalence of

cardiac dysfunction and the limited availability of tissue for cardiac transplantation, stem cells could ultimately fulfill a large-scale unmet clinical need and improve the quality of life for millions of people with CVD. However, the use of these cells in this setting is currently in its infancy — much remains to be learned about the mechanisms by which stem cells repair and regenerate myocardium, the optimal cell types and modes of their delivery, and the safety issues that will accompany their use. As the results of large-scale clinical trials become available, researchers will begin to identify ways to standardize and optimize the use of these cells, thereby providing clinicians with powerful tools to mend a broken heart.

REFERENCES

1. American Heart Association. *Heart Disease and Stroke Statistics–2005.* Dallas: American Heart Association; 2005.
2. Mokdad AH, Ford ES, Bowman BA, et al. Prevalence of obesity, diabetes, and obesity-related health-risk factors, 2001. *JAMA.* 2003;289:76-79.
3. Flegal KM, Carroll MD, Ogden CL, Johnson CL. Prevalence and trends in obesity among US adults, 1999-2000. *JAMA.* 2002;288:1723-1727.
4. Rosenstrauch D, Poglajen G, Zidar N, Gregoric ID. Stem cell therapy for ischemic heart failure. *Tex Heart Ist J.* 2005;32:339-347.
5. Mangi AA, Noiseux N, Kong D, et al. Mesenchymal stem cells modified with Akt prevent remodeling and restore performance of infarcted hearts. *Nat Med.* 2003;9:1195-1201.
6. Lietz K, Long JW, Kfoury AG, et al. Outcomes of left ventricular assist device implantation as destination therapy in the post-REMATCH era: implications for patient selection. *Circulation.* 2007;116:497-505.
7. Boyle AJ, Schulman SP, Hare JM. Is stem cell therapy ready for patients? Stem cell therapy for cardiac repair. *Circulation.* 2006;114:339-352.
8. Menasche P, Hagege AA, Scorsin M, et al. Myoblast transplantation for heart failure. *Lancet.* 2001;357:279-280.
9. Jackson KA, Majka SM, Wang H, et al. Regeneration of ischemic cardiac muscle and vascular endothelium by adult stem cells. *J Clin Invest.* 2001;107:1395-1402.
10. Condorelli G, Borello U, De Angelis L, et al. Cardiomyocytes induce endothelial cells to transdifferentiate into cardiac muscle: implications for myocardium regeneration. *Proc Natl Acad Sci USA.* 2001;98:10733-10738.
11. Orlic D, Kajstura J, Chimenti S, et al. Bone marrow cells regenerate infarcted myocardium. *Nature.* 2001; 410:701-705.
12. Kocher AA, Schuster MD, Szaboles MJ, et al. Neovascularization of ischemic myocardium by human bone-marrow derived angioblasts prevents cardiomyocyte apoptosis, reduces remodelling and improves cardiac function. *Nat Med.* 2001;7:430-436.
13. Schuster MD, Kocher AA, Seki T, et al. Myocardial neovascularization by bone marrow angioblasts results in cardiomyocyte regeneration. Am J Physiol Heart Circ Physiol. 2004;287:H525-H532.
14. Gnecchi M, He H, Liang OD, et al. Paracrine action accounts for marked protection of ischemic heart by Akt-modified mesenchymal stem cells. *Nat Med.* 2005;11:367-368.
15. Fujii T, Yau TM, Weisel RD, et al. Cell transplantation to prevent heart failure: a comparison of cell types. *Ann Thorac Surg.* 2003;76:2062-2070.
16. Nygren JM, Jovinge S, Breitbach M, et al. Bone marrow-derived hematopoietic cells generate cardiomyocytes at low frequency through cell fusion, but not transdifferentiation. *Nat Med.* 2004;10:494-501.
17. Strauer BE, Brehm M, Zeus T, et al. Repair of infarcted myocardium by autologous intracoronary mononuclear bone marrow cell transplantation in humans. *Circulation.* 2002;106:1913-1918.
18. Oettgen P. Cardiac stem cell therapy: need for optimization of efficacy and safety monitoring. *Circulation.* 2006;114:353-358.
19. Vittet D, Prandidni MH, Berthier R, et al. Embryonic stem cells differentiate in vitro to endothelial cells through successive maturation steps. *Blood.* 1996;88:3424-3431.
20. Marchetti S, Gimond C, Iljin K, et al. Endothelial cells genetically selected from differentiating mouse embryonic stem cells incorporate at sites of neovascularization in vivo. *J Cell Sci.* 2002;115:2075-2085.
21. Yamashita J, Itoh H, Hirashima M, et al. Flk1-positive cells derived from embryonic stem cells serve as vascular progenitors. *Nature.* 2000;408:92-96.
22. Kehat I, Kenyagin-Karsenti D, Snir M, et al. Human embryonic stem cells can differentiate into myocytes with structural and functional properties of cardiomyocytes. *J Clin Invest.* 2001;108:407-414.
23. Kehat I, Gepstein A, Spira A, Itskovitz-Eldor J, Gepstein L. High-resolution electrophysiological assessment of human embryonic stem cell-derived cardiomyocytes: a novel in vitro model for the study of conduction. *Circ Res.* 2002;91:659-661.
24. Westfall MV, Pasyk KA, Yule DI, Samuelson LC, Metzger JM. Ultrastructure and cell-cell coupling of cardiac myocytes differentiating in embryonic stem cell cultures. *Cell Motil Cytoskel.* 1998;36:43-54.
25. Min JY, Yang Y, Converso KL, et al. Transplantation of embryonic stem cells improves cardiac function in postinfarcted rats. *J Appl Physiol.* 2002;92:288-296.

26. Huber I, Itzhaki I, Caspi O, et al. Identification and selection of cardiomyocytes during human embryonic stem cell differentiation. *FASEB J.* 2007;21:2551-2563.

27. Dowell JD, Rubart M, Pasumarthi KB, Soonpaa MH, Field LJ. Myocyte and myogenic stem cell transplantation in the heart. *Cardiovasc Res.* 2003;58:336-350.

28. Reinecke H, MacDonald GH, Hauschka SD, Murry CE. Electromechanical coupling between skeletal and cardiac muscle. Implications for infarct repair. *J Cell Biol.* 2000;149:731-740.

29. Leobon B, Garcin I, Menasche P, Vilquin JT, Audinat E, Charpak S. Myoblasts transplanted into rat infarcted myocardium are functionally isolated from their host. *Proc Natl Acad Sci USA.* 2003;100:7808-7811.

30. Menasche P, Hagege AA, Vilquin J-T, et al. Autologous skeletal myoblast transplantation for severe postinfarction left ventricular dysfunction. *J Am Coll Cardiol.* 2003;41:1078-1083.

31. Siminiak T, Kalawski R, Fiszer D, et al. Autologous skeletal myoblast transplantation for the treatment of post-infarction myocardial injury: phase I clinical study with 12 months of follow-up. *Am Heart J.* 2004;148:531-537.

32. Murry CE, Soonpaa MH, Reinecke H, et al. Haematopoietic stem cells do not transdifferentiate into cardiac myocytes in myocardial infarcts. *Nature.* 2004;428:664-668.

33. Balsam LB, Wagers AJ, Christensen JL, Kofidis T, Weissman IL, Robbins RC. Haematopoietic stem cells adopt mature haematopoietic fates in ischaemic myocardium. *Nature.* 2004;428:668-673.

34. Assmus B, Schachinger V, Teupe C, et al. Transplantation of Progenitor Cells and Regeneration Enhancement in Acute Myocardial Infarction (TOPCARE-AMI). *Circulation.* 2002;106:3009-3017.

35. Schachinger V, Assmus B, Britten MB, et al. Transplantation of progenitor cells and regeneration enhancement in acute myocardial infarction: final one-year results of the TOPCARE-AMI Trial. *J Am Coll Cardiol.* 2004;44:1690.

36. Wollert KC, Meyer GP, Lotz J, et al. Intra-coronary autologous bone-marrow cell transfer after myocardial infarction: the BOOST randomised controlled clinical trial. *Lancet.* 2004;364:141-148.

37. Janssens S, Dubois C, Bogaert J, et al. Autologous bone marrow-derived stem-cell transfer in patients with ST-segment elevation myocardial infarction: double-blind, randomised, controlled trial. *Lancet.* 2005;367:113-121.

38. Cleland JG, Freemantle N, Coletta AP, Clark AL. Clinical trials update from the American Heart Association: REPAIR-AMI, ASTAMI, JELIS, MEGA, REVIVE-II, SURVIVE, and PROACTIVE. *Eur J Heart Fail.* 2006;8:105-110.

39. Perin EC, Dohmann HFR, Borojevic R, et al. Transendocardial, autologous bone marrow cell transplantation for severe, chronic ischemic heart failure. *Circulation.* 2003;107:2294-2302.

40. MacKenzie TC, Flake AW. Human mesenchymal stem cells: insights from a surrogate in vivo assay system. *Cells Tissues Organs.* 2002;171:90-95.

41. Davani S, Marandin A, Mersin N, et al. Mesenchymal progenitor cells differentiate into an endothelial phenotype, enhance vascular density, and improve heart function in a rat cellular cardiomyoplasty model. *Circulation.* 2003;108(suppl II):II-253-II-258.

42. Makino S, Fukuda K, Miyoshi S, et al. Cardiomyocytes can be generated from marrow stromal cells in vitro. *J Clin Invest.* 1999;103:697-705.

43. Min J-Y, Sullivan MF, Yang Y, et al. Significant improvement of heart function by cotransplantation of human mesenchymal stem cells and fetal cardiomyocytes in postinfarcted pigs. *Ann Thorac Surg.* 2002;74:1568-1575.

44. Shake JG, Gruber PJ, Baumgartner WA, et al. Mesenchymal stem cell implantation in a swine myocardial infarct model: engraftment and functional effects. *Ann Thorac Surg.* 2002;73:1919-1926.

45. Toma C, Pittenger MF, Cahill KS, Byrne BJ, Kessler PD. Human mesenchymal stem cells differentiate to a cardiomyocyte phenotype in the adult murine heart. *Circulation.* 2002;105:93-98.

46. Amado LC, Saliaris AP, Schuleri KH, et al. Cardiac repair with intramyocardial injection of allogeneic mesenchymal stem cells after myocardial infarction. *Proc Natl Acad Sci USA.* 2005;102:11474-11479.

47. Chen S-I, Fang W-W, Ye F, et al. Effect on left ventricular function of intracoronary transplantation of autologous bone marrow mesenchymal stem cells in patients with acute myocardial infarction. *Am J Cardiol.* 2004;94:92-95.

48. Beltrami AP, Barlucchi L, Torella D, et al. Adult cardiac stem cells are multipotent and support myocardial regeneration. *Cell.* 2003;114:763-776.

49. Messina E, De Angelis L, Frati G, et al. Isolation and expansion of adult cardiac stem cells from human and murine heart. *Circ Res.* 2004;95:911-921.

50. Smith RR, Barile L, Cho HC, et al. Unique phenotype of cardiospheres derived from human endomyocardial biopsies. *Circulation.* 2005;112(suppl II):II-334.

51. Eichmann A, Corbel C, Nataf V, Vaigot P, Breant C, Le Dourain NM. Ligand-dependent development of the endothelial and hematopoietic lineages from embryonic mesodermal cells expressing vascular endothelial growth factor receptor 2. *Proc Natl Acad Sci USA.* 1997; 94:5141-5146.

52. Sato TN, Quin Y, Kozak CA, Audus KL. Tie-1 and Tie-2 define another class of putative receptor tyrosine kinase genes expressed in early embryonic vascular system. *Proc Natl Acad Sci USA.* 1993;90:9355-9358.

53. Suri C, Jones PF, Patan S, et al. Requisite role of angiopoietin-1, a ligand for the TIE2 receptor, during embryonic angiogenesis. *Cell.* 1996;87:1171-1180.

54. Erices A, Conget P, Minguell JJ. Mesenchymal progenitor cells in human umbilical cord blood. *Br J Haematol.* 2000;109:235-242.

55. Mayani H, Lansdorp PM. Biology of human umbilical cord blood-derived hematopoietic stem/progenitor cells. *Stem Cells.* 1998;16:153-165.

56. Ma N, Stamm C, Kaminski A, et al. Human cord blood cells induce angiogenesis following myocardial infarction in NOD/scid-mice. *Cardiovasc Res.* 2005;66:45-54.

57. Hirata Y, Sata M, Motomura N, et al. Human umbilical cord blood cells improve cardiac function after myocardial infarction. *Biochem Biophys Res Commun.* 2005; 327:609-614.

58. Kim B-O, Tian H, Prasongsukarn K, et al. Cell transplantation improves ventricular function after a myocardial infarction: a preclinical study of human unrestricted somatic stem cells in a porcine model. *Circulation.* 2005; 112 (suppl I):I-96-I-104.

59. Korbling M, Katz RL, Khanna A, et al. Hepatocytes and epithelial cells of donor origin in recipients of peripheral-blood stem cells. *N Engl J Med.* 2002;346:738-746.

60. Yeh ET, Zhang S, Wu HD, Korbling M, Willerson JT, Estrov Z. Transdifferentiation of human peripheral blood $CD34^+$-enriched cell population into cardiomyocytes, endothelial cells, and smooth muscle cells in vivo. *Circulation.* 2003;108:2070-2073.

61. Etzion S, Barbash IM, Feinberg MS, et al. Cellular cardiomyoplasty of cardiac fibroblasts by adenoviral delivery of MyoD ex vivo: an unlimited source of cells for myocardial repair. *Circulation.* 2002;106(12 Suppl 1):I125-I130.

62. Smits PC, van Genus RJ, Poldermans D, et al. Catheter-based intramyocardial injection of autologous skeletal myoblasts as a primary treatment of ischemic heart failure: clinical experience with six-month follow-up. *J Am Coll Cardiol.* 2003;42:2063-2069.

63. Dib N, McCarthy P, Campbell A, et al. Feasibility and safety of autologous myoblast transplantation in patients with ischemic cardiomyopathy. *Cell Transplant.* 2005;14:11-19.

64. Pagani FD, DerSimonian H, Zawadzka A, et al. Autologous skeletal myoblasts transplanted to ischemia-damaged myocardium in humans: histological analysis of cell survival and differentiation. *J Am Coll Cardiol.* 2003;41:879-888.

65. Dow J, Simkhovich BZ, Kedes L, Kloner RA. Washout of transplanted cells from the heart: a potential new hurdle for cell transplantation therapy. *Cardiovasc Res.* 2005;67:301-307.

66. Muller-Ehmsen J, Whittaker P, Kloner RA, et al. Survival and development of neonatal rat cardiomyocytes transplanted into adult myocardium. *J Mol Cell Cardiol.* 2002;34:107-116.

7. ARE STEM CELLS THE NEXT FRONTIER FOR DIABETES TREATMENT?

Charles A. Goldthwaite, Jr., Ph.D.

Diabetes is a devastating disease that affects millions of people worldwide. The major forms of the disease are type 1 and type 2 diabetes. In type 1 diabetes, the body's immune system aberrantly destroys the insulin-producing beta cells (β-cells) of the pancreas. Type 2 diabetes, the more common form, is characterized both by insulin resistance, a condition in which various tissues in the body no longer respond properly to insulin action, and by subsequent progressive decline in β-cell function to the point that the cells can no longer produce enough additional insulin to overcome the insulin resistance. Researchers are actively exploring cell replacement therapy as a potential strategy to treat type 1 diabetes, because patients with this disease have lost all or nearly all β-cell function. However, if a safe and cost-effective means for replenishing β-cells were developed, such a treatment strategy could also be useful for the larger population with type 2 diabetes. One of the major challenges of cell replacement therapy is the current insufficient supply of β-cells from human organ donors. This article focuses on stem cells as potential sources for deriving new β-cells.

DIABETES: A CRITICAL HEALTH ISSUE FOR THE 21ST CENTURY

According to the International Diabetes Federation, diabetes currently affects 7% of the world's population — nearly 250 million individuals worldwide.[1] This total is expected to rise to 380 million by 2025 as a result of aging populations, changing lifestyles, and a recent worldwide increase in obesity. Although projections for increases in diabetes prevalence suggest that the greatest percentage gains will occur in Asia and South America,[2,3] all nations will experience a rising disease burden.

According to the National Diabetes Fact Sheet, which was compiled using information from the Centers for Disease Control and Prevention and other Federal and non-Federal organizations, 20.8 million U.S. children and adults have diabetes (6.2 million of whom are currently undiagnosed).[4] An estimated 54 million Americans have "pre-diabetes", a condition defined by blood glucose levels that are above normal but not sufficiently high to be diagnosed as diabetes. In 2005, 1.5 million new cases of diabetes were diagnosed in Americans aged 20 years or older.[4] If present trends continue, 1 in 3 Americans (1 in 2 minorities) born in 2000 will develop diabetes in their lifetimes.[5]

Diabetes is currently the sixth leading cause of death in the U.S.[4] It is associated with numerous health complications, including increased risk for heart disease, stroke, kidney disease, blindness, and amputations. In 2007, the total annual economic cost of diabetes was estimated to be $174 billion dollars.[6] Direct medical expenditures account for the vast majority of this total ($116 billion), although lost productivity and other indirect costs approached nearly $58 billion. The American Diabetes Association estimates that one out of every 10 health care dollars currently spent in the U.S. is used for diabetes and its complications.[6]

While diabetes can be managed, at present it cannot be cured. As a result, it is a lifelong and often disabling disease that can severely impact the quality of life of those who are afflicted. Based on several recent discoveries, however, researchers have begun to ask if a new treatment approach is on the horizon — can stem cells that are derived from adult or embryonic tissues generate new pancreatic β-cells to replace those that have failed or been destroyed? Cell replacement therapy is one of many research avenues being pursued as a potential treatment strategy for type 1 diabetes. The strategy may also have implications for ameliorating type 2 diabetes. One of the key obstacles to advancing such therapy is the current inadequate supply of cadaveric donor pancreata as a source of cells for transplantation. Additionally, it is not currently possible

to induce a patient's own cells to regenerate new β-cells within the body. Thus, researchers are actively investigating potential sources of new beta cells, including different types of stem cells. This article will focus on the various types of stem cells that are candidates for use in pancreatic regeneration and will discuss the challenges of using such cells as therapy for diabetes.

DEFINING DIABETES

Diabetes results from the body's inability to regulate the concentration of sugar (glucose) in the blood. Blood glucose concentration is modulated by insulin, a hormone produced by pancreatic β-cells and released into the bloodstream to maintain homeostasis. In healthy individuals, β-cells counteract sharp increases in blood glucose, such as those caused by a meal, by releasing an initial "spike" of insulin within a few minutes of the glucose challenge. This acute release is then followed by a more sustained release that may last for several hours, depending on the persistence of the elevated blood glucose concentration. The insulin release gradually tapers as the body's steady-state glucose concentration is reestablished. While postprandial insulin release is stimulated by factors other than blood glucose, the blood sugar concentration is the major driver. When the β-cells fail to produce enough insulin to meet regulatory needs, however, the blood glucose concentration rises. This elevated concentration imposes a metabolic burden on numerous body systems, dramatically increasing the risk of premature cardiovascular disease, stroke, and kidney failure. Moreover, the risk for certain diabetes-related complications increases even at blood glucose concentrations below the threshold for diagnosing diabetes.

At present, there is no cure for diabetes. β-cell failure is progressive[7]; once the condition is manifest, full function usually cannot be restored. Those with type 1 diabetes require daily insulin administration to survive. Persons with type 2 diabetes must control their elevated blood glucose levels through various means, including diet and exercise, oral antihyperglycemic (blood glucose-lowering) drugs, and/or daily insulin shots. Most people who live with type 2 diabetes for a period of time will eventually require insulin to survive.

As noted earlier, there are different forms of diabetes. Type 1 diabetes results when a person's immune system mistakenly attacks and destroys the β-cells. This type of diabetes was once referred to as "juvenile-onset diabetes," because it usually begins in childhood. Type 1 diabetes accounts for 5–10% of diabetes cases, and people with type 1 diabetes depend on daily insulin administration to survive.

By contrast, type 2 diabetes is a metabolic disorder that results from a decline in β-cell function combined with insulin resistance, or the inability to use insulin effectively in peripheral tissues such as the liver, muscles, and fat.[8] Onset is associated with genetic factors and with obesity, and type 2 diabetes disproportionately affects certain minority groups.[3] Unlike type 1 diabetes, type 2 is largely preventable. Numerous studies have suggested that the environmental and behavioral factors that promote obesity (e.g., a sedentary lifestyle, a high-calorie diet) have profoundly influenced the recent rise in the prevalence of type 2 diabetes.[9] This trend suggests that type 2 diabetes will continue to be a major health care issue.

THE CASE FOR STEM CELLS

There is great interest in developing strategies to expand the population of functional β-cells. Possible ways to achieve this include physically replacing the β-cell mass via transplantation, increasing β-cell replication, decreasing β-cell death, and deriving new β-cells from appropriate progenitor cells.[10] In 1990, physicians at the Washington University Medical Center in St. Louis reported the first successful transplant of donor-supplied pancreatic islet tissue (which includes β-cells; *see below*) in humans with type 1 diabetes.[11] By the end of the decade, many other transplants had been reported using various protocols, including the widely-known "Edmonton protocol" (named for the islet transplantation researchers at the University of Alberta in Edmonton).[12-14] This protocol involves isolating islets from the cadaveric pancreatic tissue of multiple donors and infusing them into the recipient's portal vein. However, the lack of available appropriate donor tissue and the strenuous regimen of immunosuppressive drugs necessary to keep the body from rejecting the transplanted tissue limit the widespread use of this approach. Moreover, the isolation process for islets damages the transplantable tissue; as such, 2–3 donors are required to obtain the minimal β-cell mass sufficient for transplantation into a single recipient.[13] While these strategies continue to be improved, islet

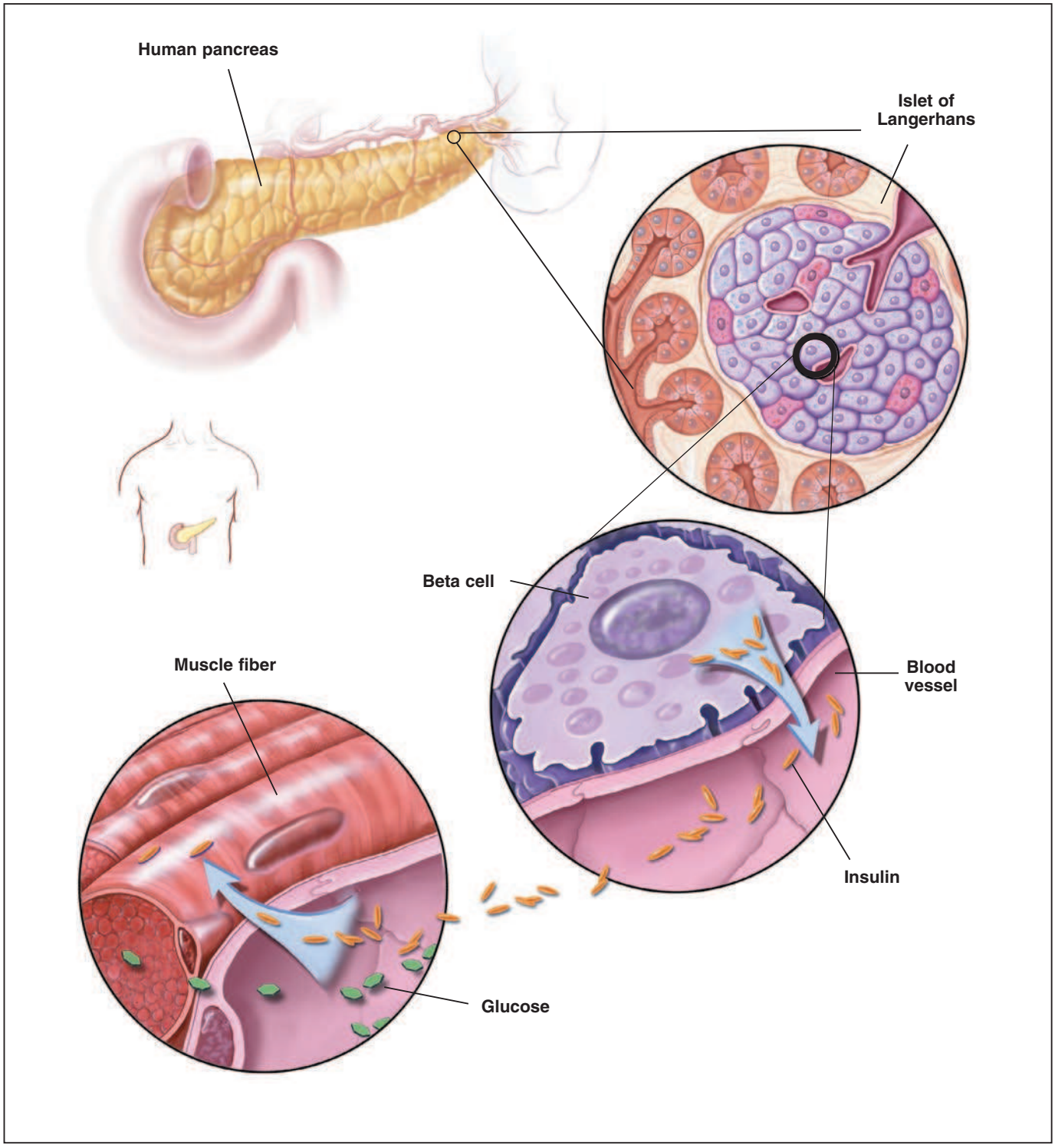


Figure 7.1. Insulin Production in the Human Pancreas.
The pancreas is located in the abdomen, adjacent to the duodenum (the first portion of the small intestine). A cross-section of the pancreas shows the islet of Langerhans which is the functional unit of the endocrine pancreas. Encircled is the beta cell that synthesizes and secretes insulin. Beta cells are located adjacent to blood vessels and can easily respond to changes in blood glucose concentration by adjusting insulin production. Insulin facilitates uptake of glucose, the main fuel source, into cells of tissues such as muscle.

function declines relatively rapidly post-transplant. For example, a long-term follow-up study of Edmonton transplant patients indicated that less than 10% of recipients remained insulin-independent five years after transplant.[15]

These challenges have led researchers to explore the use of stem cells a possible therapeutic option. Type 1 diabetes is an appropriate candidate disease for stem cell therapy, as the causative damage is localized to a particular cell type. In theory, stem cells that can differentiate into β-cells in response to molecular signals in the local pancreatic environment could be introduced into the body, where they would migrate to the damaged tissue and differentiate as necessary to maintain the appropriate β-cell mass. Alternately, methods could be developed to coax stem cells grown in the laboratory to differentiate into insulin-producing β-cells. Once isolated from other cells, these differentiated cells could be transplanted into a patient. As such, stem cell therapy would directly benefit persons with type 1 diabetes by replenishing β-cells that are destroyed by autoimmune processes, although it would still be necessary to mitigate the autoimmune destruction of β-cells. The strategy would also benefit those with type 2 diabetes to a lesser extent by replacing failing β-cells, although the insulin resistance in peripheral tissues would remain present. As discussed in the following sections, however, debate continues about potential source(s) of pancreatic stem cells.

SEARCHING FOR THE "PANCREATIC STEM CELL"

The pancreas is a complex organ made up of many cell types. The majority of its mass is comprised of exocrine tissue, which contains acinar cells that secrete pancreatic enzymes into the intestine to aid in food digestion. Dispersed throughout this tissue are thousands of islets of Langerhans, clusters of endocrine cells that produce and secrete hormones into the blood to maintain homeostasis. The insulin-producing β-cell is one type of endocrine cell in the islet; other types include alpha cells (α-cells), which produce glucagon, gamma cells (γ-cells), which produce pancreatic polypeptide, and delta cells (δ-cells), which produce somatostatin.

Each of these cell types arises from a precursor cell type during the process of development. Therefore, the key step for using stem cells to treat diabetes is to identify the precursor cell(s) that ultimately give rise to the β-cell. However, generating these cells is more complex than simply isolating a hypothetical "pancreatic stem cell." Experiments have indicated that embryonic and adult stem cells can serve as sources of insulin-secreting cells,[16] leading researchers to explore several avenues through which stem cells could feasibly be used to regenerate β-cells. However, many challenges must be addressed before a particular cell type will become established for this approach.

The human body has inherent mechanisms to repair damaged tissue, and these mechanisms remain active throughout life. Thus, there is reason to speculate that the adult pancreas may be aided by some type of regenerative system that replaces worn-out cells and repairs damaged tissue in response to injury. Such a system could theoretically be supported by precursor or stem cells, located in the endocrine pancreas or elsewhere, which could be coaxed to differentiate in response to select molecular or chemical stimuli. But do these cells exist? If so, how can they be recognized, isolated, and cultured for therapeutic use? How quickly could they produce sufficient numbers of β-cells to offset damage caused by diabetes processes? Alternately, what if cells that have the capability to regenerate β-cells exist in the body but are committed to differentiate into some other cell type? Could embryonic stem (ES) cell lines, which have the potential to develop into cells from all lineages, then be derived *in vitro* and be directed to differentiate into β-cells? These questions will be explored in the following sections, which review the types of candidate stem cells for diabetes.

ARE ADULT PANCREATIC STEM CELLS PRESENT IN THE PANCREAS?

Whether β-cell progenitors are present in the adult pancreas is a controversial topic in diabetes research. Several recent studies in rodents have indicated that the adult pancreas contains some type of endocrine progenitor cells that can differentiate toward β-cells.[16] However, researchers have not reached consensus about the origin of the bona fide pancreatic stem cell (if it exists) or the mechanism(s) by which β-cells are regenerated.[17] For example, a pivotal study by Dor and colleagues used genetic lineage tracing in adult mice to determine how stem cells contribute to the development of β-cells.[18] Their analysis indicated that new β-cells arise from pre-existing ones, rather

than from pluripotent stem cells, in adult mice. As such, the authors noted that β-cells can proliferate *in vivo,* thereby "cast[ing] doubt on the idea that adult stem cells have a significant role in beta-cell replenishment." Soon after this report was published, Seaberg and coworkers reported the identification of multipotent precursor cells from the adult mouse pancreas.[19] These novel cells proliferated in vitro to form colonies that could differentiate into pancreatic α-, β-, and δ-cells as well as exocrine cells, neurons, and glial cells. Moreover, the beta-like cells demonstrated glucose-dependent insulin release, suggesting possible therapeutic application to diabetes. Several subsequent studies have also reported the existence of pancreatic stem/precursor cells *in vitro* or *in vivo.*[20-22] One recent report suggests that such cells exist in the pancreatic ductal lining and can be activated autonomously in response to injury, increasing the β-cell mass through differentiation and proliferation.[23]

The study of pancreatic regeneration continues to evolve, and many claims have been made regarding cells believed to be involved in the process. In the last decade, reports have described various putative pancreatic stem cells embedded in the pancreatic islets,[24,25] pancreatic ducts,[23,26] among the exocrine acinar cells,[20,21] and in unspecified pancreatic locales[19,27] in rodent models, as well as from human adult pancreatic cell lines,[28] islet tissue,[29] and non-islet tissues discarded after islets have been removed for transplantation.[30-32] These cells are identified by the presence of one or more cell-surface proteins, or markers, known to be associated with a particular stem cell lineage. However, these studies illustrate several challenges shared by all researchers who seek to identify the "pancreatic stem cell". First, all potential stem cell candidates identified to date are relatively rare; for instance, the precursor cells identified by Seaberg are present at the rate of 1 cell per 3,000–9,000 pancreatic cells.[19] Because there are so few of these putative stem cells, they can be difficult to identify. Additionally, the choice of marker can select for certain stem cell populations while possibly excluding others. Interestingly, the progenitor cells identified in the Seaberg study lacked some known β-cell markers such as HNF3β, yet they were able to generate β-cells. Thus, a hypothetical experiment that used only HNF3β as a marker for β-cell differentiation would likely not identify this stem cell population. Moreover, techniques used to study the pancreatic tissue, such as the genetic lineage technique of Dor, *et.al.* could possibly interfere with the generation of new β-cells from stem or precursor cells.[33]

As such, the possibility remains that β-cells could be regenerated by differentiation of endogenous stem cells, by proliferation of existing β-cells, or a combination of the two mechanisms.

Further research to elucidate conditions under which β-cells can proliferate may help to develop new therapeutic approaches. For example, several advances have recently been made from studies of pregnancy and pregnancy-related diabetes (gestational diabetes) in mice. During pregnancy, pancreatic islet cells normally expand in number to meet increased metabolic demands. Researchers have found that the protein HNF4-alpha helps increase β-cell mass, and that pregnancy-related decreases in levels of another protein, menin, also enable β-cell proliferation.[34,35] Insights may also arise from research on another organ, the liver. Unlike the pancreas, the liver has an inherently high capacity for regeneration. New strategies for inducing pancreatic islet cell growth may emerge from knowledge of how liver cells develop from progenitor cells during early development such that the resulting adult organ retains substantial regenerative capacity.[36] In another research avenue, scientists are exploring whether it may be possible to redirect adult pancreatic cells in the body to change from their original cell type into β-cells.

OTHER POTENTIAL SOURCES OF STEM CELLS DERIVED FROM ADULT CELLS

Furthermore, various reports have also described putative stem cells in the liver, spleen, central nervous system, and bone marrow that can differentiate into insulin-producing cells.[17] While it is possible that such pathways may exist, these results are currently under debate within the research community. In another research avenue, scientists recently reported that differentiated cells, including adult human skin cells, can be genetically "reprogrammed" to revert to a pluripotent state, resembling that of embryonic stem (ES) cells.[37] The researchers refer to these cells as induced pluripotent stem (iPS) cells. Their method involved introducing a defined set of genes into the differentiated cells. This approach may facilitate the establishment of human iPS cell lines from patients with specific diseases that could be used as research tools. This technique, or variations of it, may also one day allow patient-specific stem cells to be generated

for use in stem cell-based therapies. However, the genes used for reprogramming were introduced into the cells using a virus-based method, which could have adverse clinical effects. If, however, safe alternate methods based on this research can be developed for reprogramming cells, then iPS cells may lead to novel, personalized therapies.

CAN EMBRYONIC STEM CELLS BE USED?

The challenges associated with identifying and isolating adult "pancreatic stem cells" has led some researchers to explore the use of ES cells as a source of insulin-producing cells. Several factors make ES cells attractive for this application.[33] First, given the complexity of pancreatic tissue, identified β-cell precursors would likely be difficult to isolate from the adult pancreas. If isolated, the cells would then need to be replicated *ex vivo* while keeping them directed toward a β-cell lineage. Second, protocols to grow and expand mature β-cells in culture have met with technical challenges. ES cells, which are pluripotent cell lines (they can give rise to all cell types of the embryo) that can be induced to develop into various lineages based on culture conditions, may therefore represent a future option for β-cell regeneration.

To date, several human ES cells lines have been successfully derived.[38-40] While these cell lines serve as resources for exploring the mechanisms of development, their potential use in a clinical setting is limited by several factors, most notably ethical concerns and the risk of teratoma development. (For a more detailed discussion of the scientific challenges associated with clinical application of ES cells, see Chapter 6, "Mending a Broken Heart: Stem Cells and Cardiac Repair," p.59). In addition, researchers are only beginning to unlock the myriad factors that come into play as a once-pluripotent cell differentiates into a unipotent cell, one that can contribute to only one mature cell type.[41] For example, several recent reports indicate that mouse[42] and human[43] ES cells can be successfully differentiated into endodermal cells, the precursors of pancreatic cells. In addition, insulin-producing cells have been derived from mouse[44,45] and human[46] ES cells.

However, it should be noted that directed differentiation of ES cells toward the β-cell has not been reported. Beta cells appear relatively late during embryonic development, suggesting that their presence involves the temporal control of a considerable number of genes. Moreover, the creation of patient-specific, stem cell-derived β-cells for transplantation requires genetic matching to lessen the immune response. Generating immune-matched tissues requires the therapeutic cloning of human ES cells, which has not been accomplished to date. A fraudulent claim to the contrary in 2005 by South Korean researcher Woo Suk Hwang[47] ignited international controversy within the scientific community[48] and illustrated the scientific and ethical challenges of using ES cells as a source of transplant tissue. Despite current gaps in knowledge, researchers recognize the potential of ES cells as sources of specialized cells such as the β-cell, and the study of ES cells provides insight into the processes that govern differentiation and specialization.

CLINICAL CHALLENGES

Clearly, using stem cells to treat diabetes will require additional knowledge, both in the laboratory and in the clinic. This section will suggest several envisioned approaches for stem-cell derived diabetes therapies and discuss key considerations that must be addressed for their successful application.

Contingent upon the development of appropriate protocols, stem cells could theoretically be used to treat diabetes through two approaches.[49] Both strategies would require the isolation and *in vitro* expansion of a homogenous population of β-cell precursor cells from appropriate donor tissue. Once a population of these cells has been generated, they could either 1) be induced to differentiate into insulin-producing cells *in vitro* and then be transplanted into the diabetic patient's liver, or 2) be injected into the circulation along with stem cell stimulators, with the hope that the cells will "home in" to the injured islets and differentiate into a permanent self-renewing β-cell population.

Because type 1 diabetes is an autoimmune disease, controlling the autoimmune response is critical to the success of any potential stem cell-based therapy. Type 1 diabetes is characterized by the action of β-cell-specific, autoreactive T-cells. Even if the regenerative properties of the pancreas remain functional, the continued presence of these T-cells effectively counteracts any endogenous repair and would likely decimate populations of newly-regenerated or transplanted insulin-producing cells. However, the autoimmune response has been successfully averted in non-obese diabetic mice either by using anti-T-cell antibodies to

eliminate the majority of the autoreactive cells[50] or by transplanting bone marrow from a diabetes-resistant donor (with a sublethal dose of irradiation) into the diabetic animal.[51-53] Both strategies appear to enable the replenishment of insulin-secreting cells and the eventual restoration of normal blood glucose levels, although the process requires weeks to months and may necessitate additional therapy. Other strategies being explored include altering the immune tolerance through the use of monoclonal antibodies,[54] proteins,[55] and oligonucleotides.[56]

Other clinical challenges, including safety, tracking of the stem cells, delivery of the cells to the targeted tissue within a clinically relevant time frame (for transplanted cells), identification of ways to promote long-term survival and functioning of regenerated β-cells, ease of obtaining the cells, and cost, parallel those encountered with all applications of stem cell-based regenerative therapy. These issues must be addressed once the "pancreatic stem cell" population has been identified conclusively. Given current debate on this issue, the routine clinical application of stem-cell based regenerative therapy for the treatment of diabetes remains a future goal, albeit one with great potential.

As an additional source of information, an extensive discussion of research challenges and strategies for achieving the goal of cell replacement therapy for Type 1 diabetes is presented in *Advances and Emerging Opportunities in Type 1 Diabetes Research: A Strategic Plan,* available on the NIH web site at http://www2.niddk.nih.gov/AboutNIDDK/ResearchAndPlanning/Type1Diabetes/.

CONCLUSIONS

The results discussed in this article demonstrate the many challenges that must be addressed before stem cells can be used to regenerate islet tissue in persons with diabetes. Debate continues on the identification of the "pancreatic stem cell," and at present it is difficult to ascertain which cell type has the greatest potential for diabetes therapy. Moreover, modulating the autoimmune response in type 1 diabetes remains a significant challenge regardless of the type of cell that is transplanted, and it will also be important to address the insulin resistance in type 2 diabetes, as well as factors that contribute to obesity. However, diabetes is a disease with a major deficiency in the functioning of one type of cell, and there is potential of stem cells to treat type 1 diabetes and to improve the quality of life for those with type 2 diabetes. As researchers learn more about the mechanisms that govern stem cell programming, differentiation, and renewal, their ability to identify, isolate, and culture candidate stem cells will continue to improve. While stem cells can be currently considered a frontier for diabetes therapy, they may one day become its basis.

REFERENCES

1. International Diabetes Federation. Facts & figures: diabetes prevalence. http://www.idf.org/home/index.cfm?node=264. Accessed September 15, 2007.
2. King H, Aubert RE, Herman WH. Global burden of diabetes, 1995-2025: prevalence, numerical estimates, and projections. *Diabetes Care.* 1998;21:1414-1431.
3. Zimmet P, Alberti KG, Shaw J. Global and societal implications of the diabetes epidemic. *Nature.* 2001;414:782-787.
4. Centers for Disease Control and Prevention. National diabetes fact sheet: general information and national estimates on diabetes in the United States, 2005. http://www.cdc.gov/diabetes/pubs/pdf/ndfs_2005.pdf. Accessed April 24, 2008.
5. Narayan KM, Boyle JP, Thompson TJ, Sorensen SW, Williamson DF. Lifetime risk for diabetes mellitus in the United States. *JAMA.* 2003;290:1884-1890.
6. American Diabetes Association. Economic costs of diabetes in the U.S. In 2007. *Diabetes Care.* 2008;31:596-615.
7. UK Prospective Diabetes Study Group. Overview of 6 years' therapy of type II diabetes: a progressive disease (UKPDS 16). *Diabetes.* 1995;44:1249-1258.
8. Ferranninni E. Insulin resistance versus insulin deficiency in non-insulin-dependent diabetes mellitus: problems and prospects. *Endocr Rev.* 1998;19:477-490.
9. Hill JO, Peters JC. Environmental contributions to the obesity epidemic. *Science.* 1998;280:1371-1374.
10. Lipsett M, Finegood DT. Beta-cell neogenesis during prolonged hyperglycemia in rats. *Diabetes.* 2001;51:1834-1841.
11. Scharp DW, Lacy PE, Santiago JV, et al. Insulin independence after islet transplantation into type I diabetic patient. *Diabetes.* 1990(39):515-518.
12. Ryan EA, Lakey JR, Paty BW, et al. Successful islet transplantation: continued insulin reserve provides long-term glycemic control. *Diabetes.* 2002;51:2148-2157.
13. Ryan EA, Lakey JR, Rajotte RV, et al. Clinical outcomes and insulin secretion after islet transplantation with the Edmonton protocol. *Diabetes.* 2001;50:710-719.

14. Shapiro AM, Lakey JR, Ryan EA, et al. Islet transplantation in seven patients with type 1 diabetes mellitus using a glucocorticoid-free immunosuppressive regimen. *N Engl J Med.* 2000;343:230-238.

15. Ryan EA, Paty BW, Senior PA, et al. Five-year follow-up after clinical islet transplantation. *Diabetes.* 2005;54:2060-2069.

16. Soria B, Bedoya FJ, Martin F. Gastrointestinal stem cells: I. Pancreatic stem cells. *Am J Physiol Gastrointest Liver Physiol.* 2005;289:G177-G180.

17. Zulewski H. Stem cells with potential to generate insulin-producing cells in man. *Swiss Med Wkly.* 2006;136:647-654.

18. Dor Y, Brown J, Martinez OI, Melton DA. Adult pancreatic beta-cells are formed by self-duplication rather than stem-cell differentiation. *Nature.* 2004;429:41-46.

19. Seaberg RM, Smukler SR, Kieffer TJ, et al. Clonal identification of multipotent precursors from adult mouse pancreas that generate neural and pancreatic lineages. *Nat Biotechnol.* 2004;22:1115-1124.

20. Baeyens L, De Breuck S, Lardon J, Mfopou JK, Rooman I, Bouwens L. In vitro generation of insulin-producing beta cells from adult exocrine pancreatic cells. *Diabetologia.* 2005;48:49-57.

21. Minami K, Okuno M, Miyawaki K, et al. Lineage tracing and characterization of insulin-secreting cells generated from adult pancreatic acinar cells. *Proc Natl Acad Sci USA.* 2005;102:15116-15121.

22. Lee CS, De Leon DD, Kaestner KH, Stoffers DA. Regeneration of pancreatic islets after partial pancreatectomy in mice does not involve the reactivation of neurogenin-3. *Diabetes.* 2006;55:269-272.

23. Xu X, D'Hoker J, Stangé G, et al. Beta cells can be generated from endogenous progenitors in injured adult mouse pancreas. *Cell.* 2008;132:197-207.

24. Guz Y, Nasir I, Teitelman G. Regeneration of pancreatic beta cells from intra-islet precursor cells in an experimental model of diabetes. *Endocrinology.* 2001;142:4956-4968.

25. Zulewski H, Abraham EJ, Gerlach MJ, et al. Multipotential nestin-positive stem cells isolated from adult pancreatic islets differentiate ex vivo into pancreatic endocrine, exocrine, and hepatic phenotypes. *Diabetes.* 2001;50:521-533.

26. Oshima Y, Suzuki A, Kawashimo K, Ishikawa M, Ohkohchi N, Taniguchi H. Isolation of mouse pancreatic ductal progenitor cells expressing CD133 and c-Met by flow cytometric cell sorting. *Gastroenterology.* 2007;132:720-732.

27. Raimya VK, Maraist M, Arfors KE, Schatz DA, Peck AB, Cornelius JG. Reversal of insulin-dependent diabetes using islets generated in vitro from pancreatic stem cells. *Nat Med.* 2000;6:278-282.

28. Tsang WG, Zheng T, Wang Y, et al. Generation of functional islet-like clusters after monolayer culture and intracapsular aggregation of adult human pancreatic islet tissue. *Transplantation.* 2007;83:685-693.

29. Abraham EJ, Leech CA, Lin JC, Zulewski H, Habener JF. Insulinotropic hormone glucagon-like peptide-1 differentiation of human pancreatic islet-derived progenitor cells into insulin-producing cells. *Endocrinology.* 2002;143:3152-3161.

30. Todorov I, Nair I, Ferreri K, et al. Multipotent progenitor cells isolated from human adult pancreatic tissue. *Transplant Proc.* 2005;37:3420-3421.

31. Todorov I, Omori K, Pascual M, et al. Generation of human islets through expansion and differentiation of non-islet pancreatic cells discarded (pancreatic discard) after islet isolation. *Pancreas.* 2006;32:130-138.

32. Bonner-Weir S, Taneja M, Weir GC, et al. In vitro cultivation of human islets from expanded ductal tissue. *Proc Natl Acad Sci USA.* 2000;97:7999-8004.

33. Trucco M. Regeneration of the pancreatic beta cell. *J Clin Invest.* 2005;115:5-12.

34. Gupta RK, Gao N, Gorski RK, et al. Expansion of adult beta-cell mass in response to increased metabolic demand is dependent on HNF-4alpha. *Genes Dev.* 2007;21:756-769.

35. Karnik SK, Chen H, McLean GW, et al. Menin controls growth of pancreatic beta-cells in pregnant mice and promotes gestational diabetes mellitus. *Science.* 2007;318:806-809.

36. Zaret KS. Genetic programming of liver and pancreas progenitors: lessons for stem-cell differentiation. *Nat Rev Genet.* 2008;9:329-340.

37. Park IH, Zhao R, West JA, et al. Reprogramming of human somatic cells to pluripotency with defined factors. *Nature.* 2008;451:141-146.

38. Thomson JA, Itskovitz-Eldor J, Shapiro SS, et al. Embryonic stem cell lines derived from human blastocysts. *Science.* 1998;282:1145-1147.

39. Shamblott MJ, Axelman J, Wang S, et al. Derivation of pluripotent stem cells from cultured human primordial germ cells. *Proc Natl Acad Sci USA.* 1998;95:13726-13731.

40. Cowan CA, Klimanskaya I, McMahon J, et al. Derivation of embryonic stem-cell lines from human blastocysts. *N Engl J Med.* 2004;350:1353-1356.

41. Wagers AJ, Weissman IL. Plasticity of adult stem cells. *Cell.* 2004;116:639-648.

42. Yasunaga M, Tada S, Torikai-Nishikawa S, et al. Induction and monitoring of definitive and visceral endoderm differentiation of mouse ES cells. *Nat Biotechnol.* 2005;23:1542-1550.

43. D'Amour KA, Agulnick AD, Eliazer S, Kelly OG, Kroon E, Baetge EE. Efficient differentiation of human embryonic stem cells to definitive endoderm. *Nat Biotechnol.* 2005;23:1534-1541.

44. Lumelsky N, Blondel O, Laeng P, Velasco I, Ravin R, McKay R. Differentiation of embryonic stem cells to insulin-secreting structures similar to pancreatic islets. *Science.* 2001;292:1389-1394.

45. Soria B, Roche E, Berna G, Leon-Quinto T, Reig JA, Martin F. Insulin-secreting cells from embryonic stem cells normalize glycemia in streptozotocin-induced diabetic mice. *Diabetes.* 2000;49:157-162.

46. Seguev H, Fishman B, Ziskind A, Shulman M, Itskovitz-Eldor J. Differentiation of human stem cells into insulin-producing cell clusters. *Stem Cells.* 2004;22:265-274.

47. Hwang WS, Roh SI, Lee BC, et al. Patient-specific embryonic stem cells derived from human SCNT blastocysts. *Science.* 2005;308:1777-1783.

48. Chong AS, Normile D. Stem cells: how young Korean researchers helped unearth a scandal… *Science.* 2006;311:22-25.

49. Lechner A, Habener JF. Stem/progenitor cells derived from adult tissues: potential for the treatment of diabetes mellitus. *Am J Physiol Endocrinol Metab.* 2003;284:E259-E266.

50. Ogawa N, List JF, Habener JF, Maki T. Cure of overt diabetes in NOD mice by transient treatment with anti-lymphocyte serum and exendin-4. *Diabetes.* 2004;53:1700-1705.

51. Ikehara S, Ohtsuki H, Good RA, et al. Prevention of type 1 diabetes in nonobese diabetic mice by allogenic bone marrow transplantation. *Proc Natl Acad Sci USA.* 1985;82:7743-7747.

52. Li H, Kaufman CL, Boggs SS, Johnson PC, Patrene KD, Ildstad ST. Mixed allogeneic chimerism induced by a sublethal approach prevents autoimmune diabetes and reverses insulitis in nonobese diabetic (NOD) mice. *J Immunol.* 1996;156:380-388.

53. Zorina TD, Subbotin VM, Bertera S, et al. Distinct characteristics and features of allogeneic chimerism in the NOD mouse model of autoimmune diabetes. *Cell Transplant.* 2002;11:113-123.

54. Chatenoud L, Primo J, Bach J-F. CD3 antibody-induced dominant self tolerance in overtly diabetic NOD mice. *J Immunol.* 1997;158:2947-2954.

55. Zheng XX, Sanchez-Fueyo A, Sho M, Domenig C, Sayegh MH, Strom TB. Favorably tipping the balance between cytopathic and regulatory T cells to create transplantation tolerance. *Immunity.* 2003;19:503-514.

56. Machen J, Harnaha J, Lakomy R, Styche A, Trucco M, Giannoukakis N. Antisense oligonucleotides down-regulating costimulation confer diabetes-preventive properties to nonobese diabetic mouse dendritic cells. *J Immunol.* 2004;173:4331-4341.

8. ALTERNATE METHODS FOR PREPARING PLURIPOTENT STEM CELLS

James F. Battey, Jr., MD, PhD; Laura K. Cole, PhD; and Charles A. Goldthwaite, Jr., PhD

THE CLINICAL APPLICATION OF PLURIPOTENT CELLS: THE PROMISE AND THE CHALLENGES

Stem cells are distinguished from other cells by two characteristics: (1) they can divide to produce copies of themselves (self-renewal) under appropriate conditions and (2) they are pluripotent, or able to differentiate into any of the three germ layers: the endoderm (which forms the lungs, gastrointestinal tract, and interior lining of the stomach), mesoderm (which forms the bones, muscles, blood, and urogenital tract), and ectoderm (which forms the epidermal tissues and nervous system). Pluripotent cells, which can differentiate into any mature cell type, are distinct from multipotent cells (such as hematopoietic, or blood-forming, cells) that can differ into a limited number of mature cell types. Because of their pluripotency and capacity for self-renewal, stem cells hold great potential to renew tissues that have been damaged by conditions such as type 1 diabetes, Parkinson's disease, heart attacks, and spinal cord injury. Although techniques to transplant multipotent or pluripotent cells are being developed for many specific applications, some procedures are sufficiently mature to be established options for care. For example, human hematopoietic cells from the umbilical cord and bone marrow are currently being used to treat patients with disorders that require replacement of cells made by the bone marrow, including Fanconi's anemia and chemotherapy-induced bone marrow failure after cancer treatment.

However, differentiation is influenced by numerous factors, and investigators are just beginning to understand the fundamental properties of human pluripotent cells. Researchers are gradually learning how to direct these cells to differentiate into specialized cell types and to use them for research, drug discovery, and transplantation therapy (see Figure 8.1). However, before stem cell derivatives are suitable for clinical application, scientists require a more complete understanding of the molecular mechanisms that drive pluripotent cells into differentiated cells. Scientists will need to pilot experimental transplantation therapies in animal model systems to assess the safety and long-term stable functioning of transplanted cells. In particular, they must be certain that any transplanted cells do not continue to self-renew in an unregulated fashion after transplantation, which may result in a teratoma, or stem

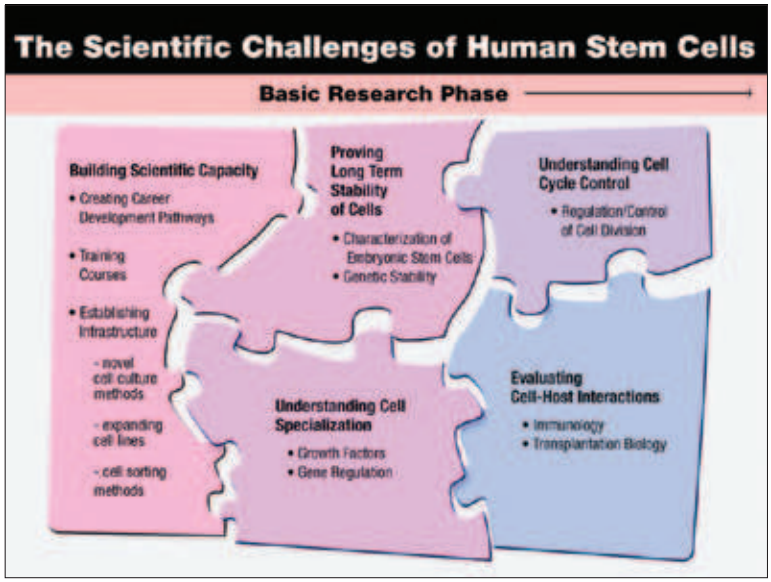


Figure 8.1. The Scientific Challenge of Human Stem Cells
The state of the science currently lies in the development of fundamental knowledge of the properties of human pluripotent cells. The scientific capacity needs to be built, an understanding of the molecular mechanisms that drive cell specialization needs to be advanced, the nature and regulation of interaction between host and transplanted cells needs to be explored and understood, cell division needs to be understood and regulated, and the long-term stability of the function in transplanted cells needs to be established.

cell tumor. In addition, scientists must ascertain that cells transplanted into a patient are not recognized as foreign by the patient's immune system and rejected.

Stem cells derived from an early-stage human blastocyst (an embryo fertilized in vitro and grown approximately five days in culture) have the capacity to renew indefinitely, and can theoretically provide an unlimited supply of cells. It is also possible to derive stem cells from non-embryonic tissues, including amniotic fluid, placenta, umbilical cord, brain, gut, bone marrow, and liver. These stem cells are sometimes called "adult" stem cells, and they are typically rare in the tissue of origin. For example, blood-forming (hematopoietic) stem cell experts estimate that only 1 in 2000 to fewer than 1 in 10,000 cells found in the bone marrow is actually a stem cell.[1] Because so-called "adult" stem cells include cells from the placenta and other early stages of development, they are more correctly termed "non-embryonic stem cells." Non-embryonic stem cells are more limited in their capacity to self renew in the laboratory, making it more difficult to generate a large number of stem cells for a specific experimental or therapeutic application. Under normal conditions, non-embryonic stem cells serve as a repair pool for the body, so they typically differentiate only into the cell types found in the organ of origin. Moreover, there is little compelling evidence for trans-differentiation, whereby a stem cell from one organ differentiates into a mature cell type of a different organ. New discoveries may overcome these limitations of stem cells derived from non-embryonic sources, and research directed toward this goal is currently underway in a number of laboratories.

THE ROLE OF CULTURED CELLS IN UNDERSTANDING THE DIFFERENTIATION PROCESS

Cultures of human pluripotent, self-renewing cells enable researchers to understand the molecular mechanisms that regulate differentiation (see Figure 8.2), including epigenetic changes (traits that may be inherited that do not arise from changes in the DNA sequence) in the chromatin structure, developmental changes in gene expression, exposure to growth factors, and interactions between adjacent cells. Understanding these basic mechanisms may enable future scientists to mobilize and differentiate endogenous populations of pluripotent cells to replace a cell type ravaged by injury or disease. Alternatively, scientists may some day be able to coax human pluripotent cells grown in the laboratory to become a specific type of specialized cell, which physicians could subsequently transplant into a patient to replace cells damaged by these same disease processes.

Scientists are gradually learning to direct the differentiation of pluripotent cell cultures into a specific type of cell, which can then be used as cellular models of human disease for drug discovery or toxicity studies. While it is not possible to predict the myriad ways that a basic understanding of stem cell differentiation may lead to new approaches for treating patients with cellular degenerative diseases, some avenues can be theorized. For example, in the case of Huntington's disease, a fatal neurodegenerative disorder, one could imagine that pluripotent cells derived from an embryo that carries Huntington's disease and differentiated into neurons in culture could be used to test drugs to delay or prevent degeneration.

Despite the incredible growth in knowledge that has occurred in stem cell research within the last couple of decades, investigators are just beginning to unravel the process of differentiation. Human pluripotent cell lines are an essential tool to understand this process and to facilitate the ultimate use of these cells in the clinic. To provide background on this fundamental topic, this article reviews the various potential sources and approaches that have been used to generate human pluripotent and multipotent cell lines, both of embryonic and non-embryonic origin.

ESTABLISHING HUMAN PLURIPOTENT STEM CELL LINES FROM EMBRYONIC OR FETAL TISSUES

Currently, at least six embryonic sources have been used to establish human pluripotent stem cell lines. All approaches involve isolation of viable cells during an early phase of development, followed by growth of these cells in appropriate culture medium. The various sources of these initial cell populations are discussed in brief below. It should be noted that the manipulation and use of embryonic tissues has raised a number of ethical issues.[2,3] This article focuses on the scientific and technical issues associated with creating pluripotent cells, with the understanding that some of these techniques are currently subject to debates that extend beyond discussions of their scientific merits.

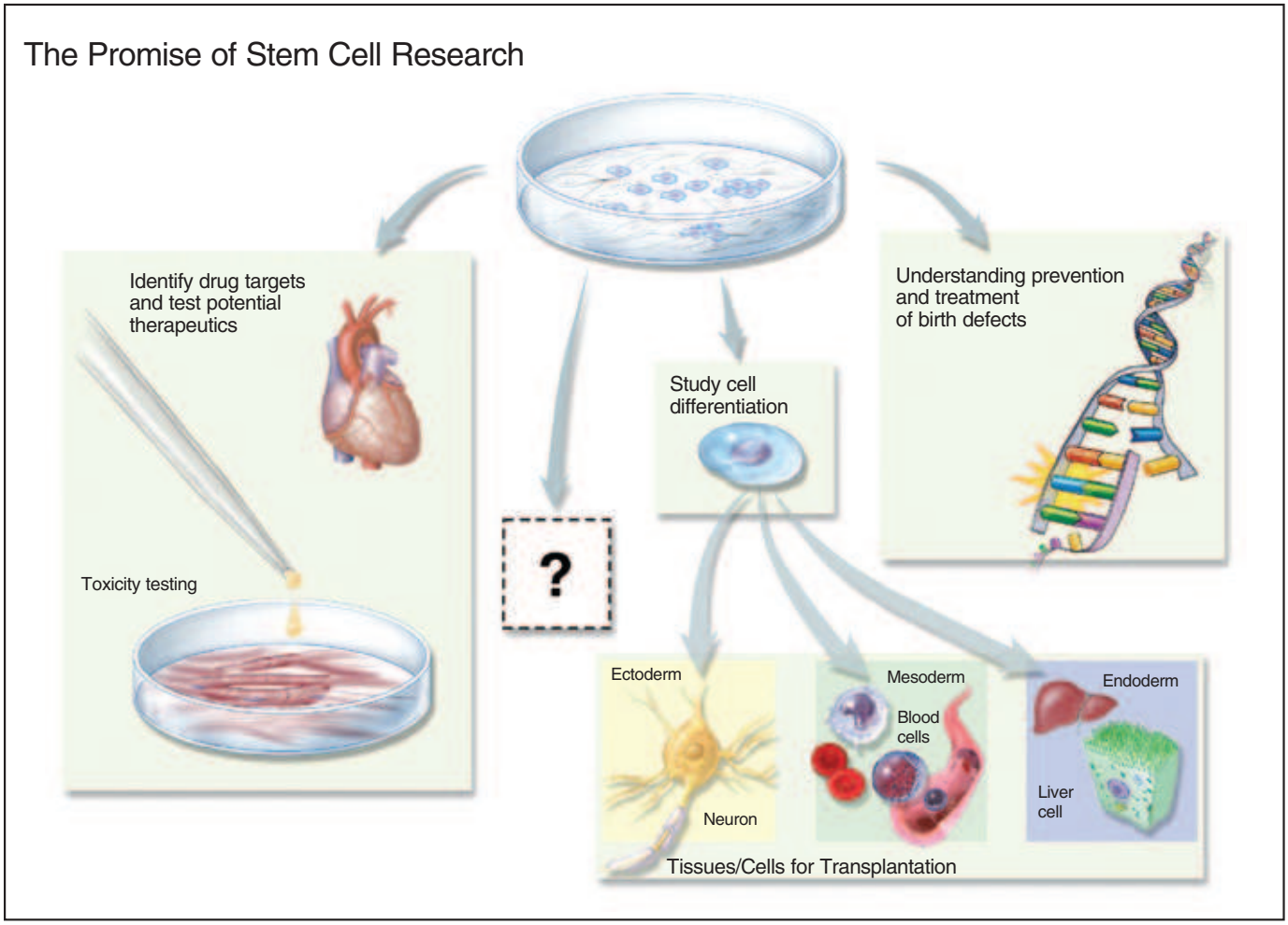


Figure 8.2. The Promise of Stem Cell Research
Stem cell research provides a useful tool for unraveling the molecular mechanisms that determine the differentiation fate of a pluripotent cell and for understanding the gene expression properties and epigenetic modifications essential to maintain the pluripotent state. In the future, this knowledge may be used to generate cells for transplantation therapies, whereby a specific cell population compromised by disease is replaced with new, functional cells. Differentiated derivatives of human pluripotent cells may also prove to be useful as models for understanding the biology of disease and developing new drugs, particularly when there is no animal model for the disease being studied. The greatest promise of stem cell research may lie in an area not yet imagined.

Traditional Human Embryonic Stem Cell (hESC) Line Generation

Drawing upon twenty years of communal expertise with mouse ES cells[4] and on human inner cell mass culture conditions developed by Ariff Bongso and colleagues[5], James Thomson and colleagues at the University of Wisconsin generated the first hESC lines in 1998 using tissue from embryos fertilized *in vitro*.[6] This method uses embryos generated for *in vitro* fertilization (IVF) that are no longer needed for reproductive purposes. During IVF, medical professionals usually produce more embryos than a couple attempting to start a family may need. Spare embryos are typically stored in a freezer to support possible future attempts for additional children if desired. It is estimated that there are approximately 400,000 such spare embryos worldwide.[7] If these embryos are never used by the couple, they either remain in storage or are discarded as medical waste. Alternatively, these embryos can potentially be used to generate a hESC line.

To generate a hESC line, scientists begin with a donated blastocyst-stage embryo, at approximately five days after IVF (see Figure 8.3a). The blastocyst consists of approximately 150–200 cells that form a hollow sphere of cells, the outer layer of which is called the trophectoderm. During normal development, the trophoblast becomes the placenta and umbilical cord. At one pole of this hollow sphere, 30–50 cells form a

cluster that is called the inner cell mass (ICM), which would give rise to the developing fetus. ICM cells are pluripotent, possessing the capacity to become any of the several hundred specialized cell types found in a developed human, with the exception of the placenta and umbilical cord.

Scientists remove the ICM from the donated blastocyst and place these cells into a specialized culture medium. In approximately one in five attempts, a hESC line begins to grow. Stem cells grown in such a manner can then be directed to differentiate into various lineages, including neural precursor cells,[8] cardiomyocytes,[9] and hematopoietic (blood forming) precursor cells.[10]

However, hESC lines are extremely difficult to grow in culture; the cells require highly specialized growth media that contain essential ingredients that are difficult to standardize. Yet the culture conditions are critical to maintain the cells' self-renewing and pluripotent properties. Culture requires the support of mouse or human cells, either directly as a "feeder" cell layer[6,11,12] or indirectly as a source of conditioned medium in feeder-free culture systems.[13] The feeder cells secrete important nutrients and otherwise support stem cell growth, but are treated so they cannot divide. Although the complete role of these feeder cells is not known, they promote stem cell growth, including detoxifying the culture medium and secreting proteins that participate in cell growth.[14] hESC lines used to produce human cells for transplantation therapies may need to be propagated on a human feeder cell layer to reduce the risk of contamination by murine viruses or other proteins that may cause rejection. Thus, hESC lines often grow only under highly specific culture conditions, and the identification of ideal growth conditions presents a challenge regardless of the source of the hESCs.

Furthermore, human ES cell cultures must be expanded using an exacting protocol to avoid cell death and to control spontaneous differentiation. Since a limited number of laboratories in the United States are growing these cells, there is a shortage of people well-versed in the art and science of successful hESC culture. In the short term, challenges of working with these cells include developing robust culture conditions and protocols, understanding the molecular mechanisms that direct differentiation into specific cell types, and developing the infrastructure to advance this scientific opportunity. Once these challenges have been met, scientists will need to conduct transplantation studies in animal models (rodent and non-human primates) to demonstrate safety, effectiveness, and long-term benefit before stem cell therapies may enter clinical trials.

hESC Lines from Human Primordial Germ Cells

A second method for generating human pluripotent stem cell lines was published in 1998 by John Gearhart and coworkers at The Johns Hopkins Medical School.[15] These researchers isolated specialized cells known as primordial germ cells (PGCs) from a 5–7-week-old embryo and placed these cells into culture (see Figure 8.3b). PGCs are destined to become either oocytes or sperm cells, depending on the sex of the developing embryo. The resulting cell lines are called embryonic germ cell lines, and they share many properties with ES cells. As with ES cells, however, PGCs present challenges with sustained growth in culture.[16,17] Spontaneous differentiation, which hinders the isolation of pure clonal lines, is a particular issue. Therefore, the clinical application of these cells requires a more complete understanding of their derivation and maintenance *in vitro.*

hESC Lines from Dead Embryos

Embryos that stop dividing after being fertilized *in vitro* are not preferentially selected for implantation in a woman undergoing fertility treatment. These embryos are typically either frozen for future use or discarded as medical waste. In 2006, scientists at the University of Newcastle, United Kingdom, generated hESC lines from IVF embryos that had stopped dividing.[18] These scientists used similar methods as described under "Traditional hESC Line Generation" except that their source material was so-called "dead" IVF embryos (see Figure 8.3c). The human stem cells created using this technique behaved like pluripotent stem cells, including producing proteins critical for "stemness" and being able to produce cells from all three germ layers. It has been proposed that an IVF embryo can be considered dead when it ceases to divide.[19] If one accepts this definition, such an embryo that "dies" from natural causes presumably cannot develop into a human being, thereby providing a source to derive human ES cells without destroying a living embryo.

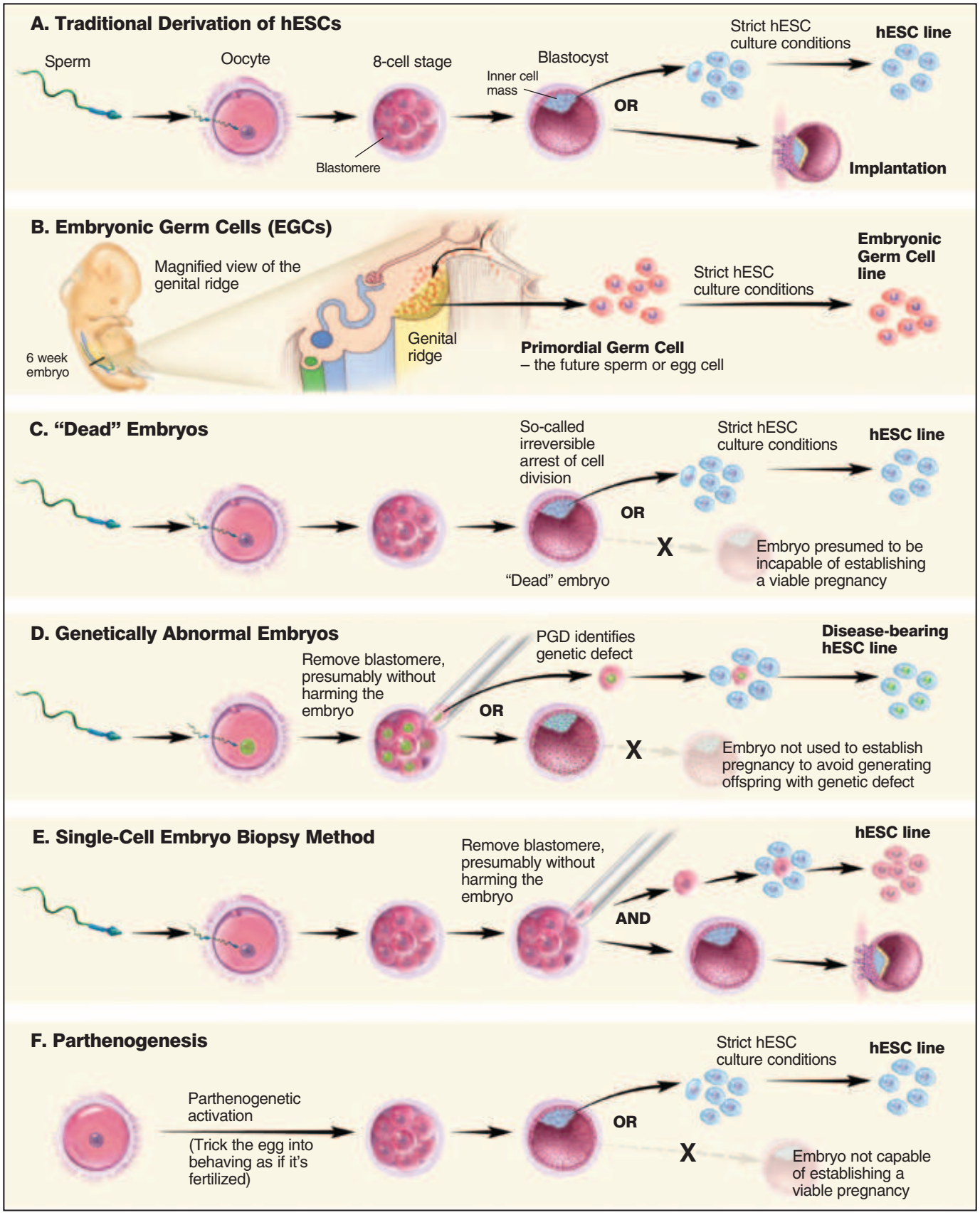


Figure 8.3. Alternative Methods for Preparing Pluripotent Stem Cells

hESC Lines from Genetically Abnormal Embryos

Couples who have learned that they carry a genetic disorder sometimes use pre-implantation genetic diagnosis (PGD) and IVF to have a child that does not carry the disorder. PGD requires scientists to remove one cell from a very early IVF human embryo and test it for diseases known to be carried by the hopeful couple. Normally, embryos identified with genetic disorders are discarded as medical waste. However, Dr.Yuri Verlinsky

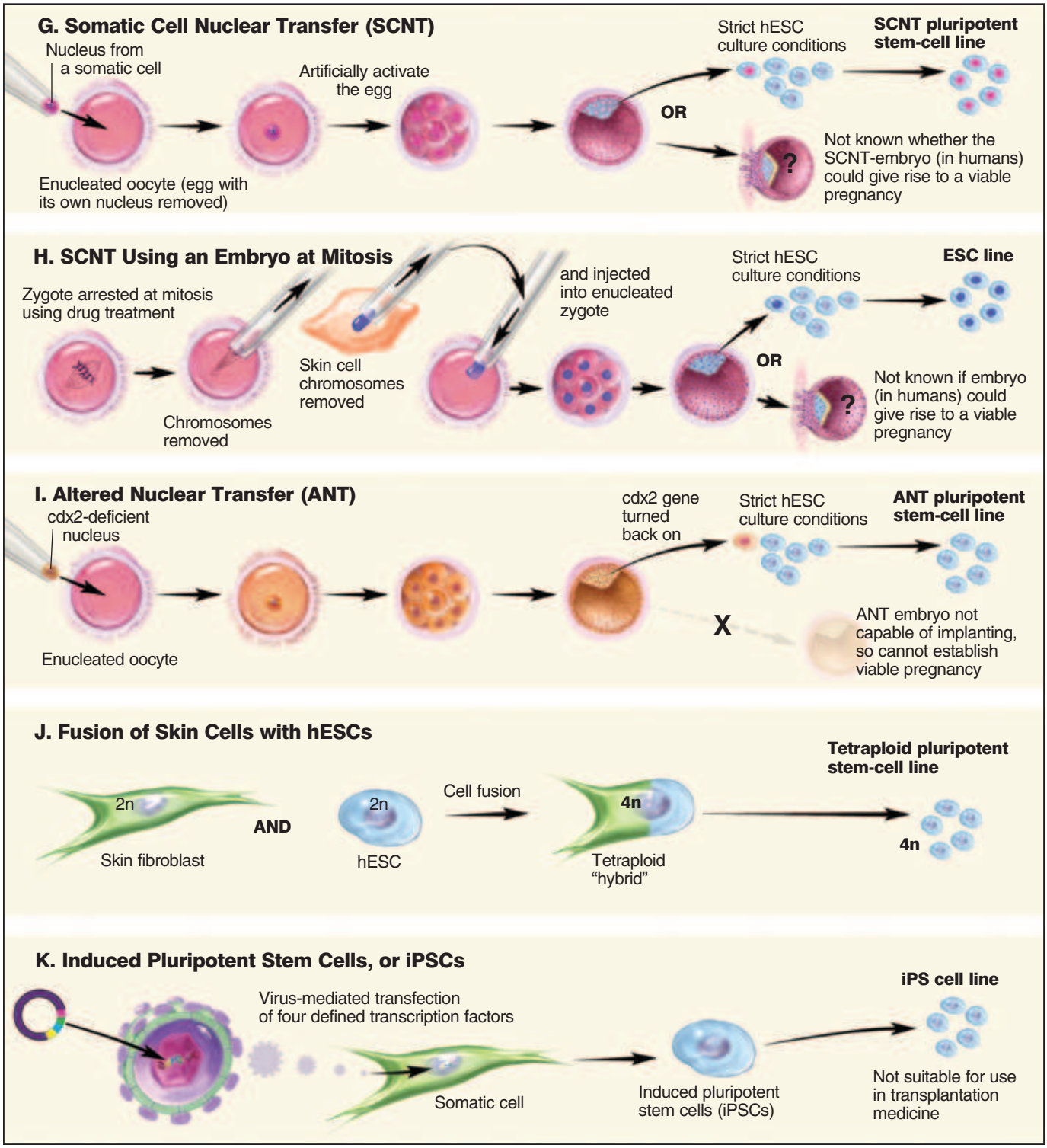


Figure 8.3. Alternative Methods for Preparing Pluripotent Stem Cells

and colleagues have capitalized on these embryos as a way to further our understanding of the diseases they carry (see Figure 8.3d) by deriving hESC lines from them.[20] These stem cell lines can then be used to help scientists understand genetically-based disorders such as muscular dystrophy, Huntington's disease, thalessemia, Fanconi's anemia, Marfan syndrome, adrenoleukodystrophy, and neurofibromatosis.

hESC Lines from Single Cell Embryo Biopsy

In 2006, Dr. Robert Lanza and colleagues demonstrated that it is possible to remove a single cell from a pre-implantation mouse embryo and generate a mouse ES cell line.[21] This work was based upon their experience with cleavage-stage mouse embryos. Later that same year, Dr. Lanza's laboratory reported that it had successfully established hESC lines (see Figure 8.3e) from single cells taken from pre-implantation human embryos.[22] The human stem cells created using this technique behaved like pluripotent stem cells, including making proteins critical for "stemness" and producing cells from all three germ layers. Proponents of this technique suggest that since it requires only one embryonic cell, the remaining cells may yet be implanted in the womb and develop into a human being. Therefore, scientists could potentially derive human embryonic stem cells without having to destroy an embryo. However, ethical considerations make it uncertain whether scientists will ever test if the cells remaining after removal of a single cell can develop into a human being, at least in embryos that are not at risk for carrying a genetic disorder. Moreover, it is unclear whether the single cell used to generate a pluripotent stem cell line has the capacity to become a human being.

hESC Lines Created via Parthenogenesis

Parthenogenesis is the creation of an embryo without fertilizing the egg with a sperm, thus omitting the sperm's genetic contributions. To achieve this feat, scientists "trick" the egg into believing it is fertilized, so that it will begin to divide and form a blastocyst (see Figure 8.3f). In 2007, Dr. E.S. Revazova and colleagues reported that they successfully used parthenogenesis to derive hESCs.[23] These stem cell lines, derived and grown using a human feeder cell layer, retained the genetic information of the egg donor and demonstrated characteristics of pluripotency. This technique may lead to the ability to generate tissue-matched cells for transplantation to treat women who are willing to provide their own egg cells.[24] It also offers an alternate method for deriving tissue-matched hESCs that does not require destruction of a fertilized embryo.

HUMAN STEM CELL LINES WHOSE POTENCY IS CURRENTLY BEING DETERMINED: AMNIOTIC FLUID STEM CELLS

Amniotic fluid surrounding the developing fetus contains cells shed by the fetus and is regularly collected from pregnant women during amniocentesis. In 2003, researchers identified a subset of cells in amniotic fluid that express Oct-4, a marker for pluripotent human stem cells that is expressed in ES cells and embryonic germ cells.[25] Since then, investigators have shown that amniotic fluid stem cells can differentiate into cells of all three embryonic germ layers and that these cells do not form tumors *in vivo*.[26,27]

For example, Anthony Atala and colleagues at the Wake Forest University have recently generated non-embryonic stem cell lines from cells found in human and rat amniotic fluid.[27] They named these cells amniotic fluid-derived stem cells (AFS). Experiments demonstrate that AFS can produce cells that originate from each of the three embryonic germ layers, and the self-renewing cells maintained the normal number of chromosomes after a prolonged period in culture. However, undifferentiated AFS did not produce all of the proteins expected of pluripotent cells, and they were not capable of forming a teratoma. The scientists developed *in vitro* conditions that enabled AFS to produce nerve cells, liver cells, and bone-forming cells. AFS-derived human nerve cells could make proteins typical of specialized nerve cells and were able to integrate into a mouse brain and survive for at least two months. Cultured AFS-derived human liver cells secreted urea and made proteins characteristic of normal human liver cells. Cultured AFS-derived human bone cells made proteins expected of human bone cells and formed bone in mice when seeded onto scaffolds and implanted under the mouse's skin. Although scientists do not yet know how many different cell types AFS can generate, AFS may one day allow researchers to establish a bank of cells for transplantation into humans.

STRATEGIES TO "REPROGRAM" NON-PLURIPOTENT CELLS TO BECOME PLURIPOTENT CELLS

An alternative to searching for an existing population of stem cells is to create a new one from a population of non-pluripotent cells. This strategy, which may or may not involve the creation of an embryo, is known as "reprogramming." This section will summarize reprogramming approaches, including several recent breakthroughs in the field.

Reprogramming through Somatic Cell Nuclear Transfer (SCNT)

In SCNT (see Figure 8.3g), human oocytes (eggs) are collected from a volunteer donor who has taken drugs that stimulate the production of more than one oocyte during the menstrual cycle. Scientists then remove the nucleus from the donated oocyte and replace it with the nucleus from a somatic cell, a differentiated adult cell from elsewhere in the body. The oocyte with the newly-transferred nucleus is then stimulated to develop. The oocyte may develop only if the transplanted nucleus is returned to the pluripotent state by factors present in the oocyte cytoplasm. This alteration in the state of the mature nucleus is called nuclear reprogramming. When development progresses to the blastocyst stage, the ICM is removed and placed into culture in an attempt to establish a pluripotent stem cell line. To date, the technique has been successfully demonstrated in two primates: macaque monkeys[28] and humans.[29]

However, successful SCNT creates an embryo-like entity, thereby raising the ethical issues that confront the use of spare IVF embryos. However, pluripotent cell lines created by embryos generated by SCNT offer several advantages over ES cells. First, the nuclear genes of such a pluripotent cell line will be identical to the genes in the donor nucleus. If the nucleus comes from a cell that carries a mutation underlying a human genetic disease such as Huntington's disease, then all cells derived from the pluripotent cell line will carry this mutation. In this case, the SCNT procedure would enable the development of cellular models of human genetic disease that can inform our understanding of the biology of disease and facilitate development of drugs to slow or halt disease progression. Alternatively, if the cell providing the donor nucleus comes from a specific patient, all cells derived from the resulting pluripotent cell line will be genetically matched to the patient with respect to the nuclear genome. If these cells were used in transplantation therapy, the likelihood that the patient's immune system would recognize the transplanted cells as foreign and initiate tissue rejection would be reduced. However, because mitochondria also contain DNA, the donor oocyte will be the source of the mitochondrial genome, which is likely to carry mitochondrial gene differences from the patient which may still lead to tissue rejection.

A technique reported in 2007 by Dr. Kevin Eggan and colleagues at Harvard University may expand scientists' options when trying to "reprogram" an adult cell's DNA[30]. Previously, successful SCNT relied upon the use of an unfertilized egg. Now, the Harvard scientists have demonstrated that by using a drug to stop cell division in a fertilized mouse egg (zygote) during mitosis, they can successfully reprogram an adult mouse skin cell by taking advantage of the "reprogramming factors" that are active in the zygote at mitosis. They removed the chromosomes from the single-celled zygote's nucleus and replaced them with the adult donor cell's chromosomes (see Figure 8.3h). The active reprogramming factors present in the zygote turned genes on and off in the adult donor chromosomes, to make them behave like the chromosomes of a normally fertilized zygote. After the zygote was stimulated to divide, the cloned mouse embryo developed to the blastocyst stage, and the scientists were able to harvest embryonic stem cells from the resulting blastocyst. When the scientists applied their new method to abnormal mouse zygotes, they succeeded at reprogramming adult mouse skin cells and harvesting stem cells. If this technique can be repeated with abnormal human zygotes created in excess after IVF procedures, scientists could use them for research instead of discarding them as medical waste.

Reprogramming Through Altered Nuclear Transfer (ANT)

Altered nuclear transfer is a variation on standard SCNT that proposes to create patient-specific stem cells without destroying an embryo. In ANT, scientists turn off a gene needed for implantation in the uterus *(Cdx2)* in the patient cell nucleus before it is transferred into the donor egg (see Figure 8.3i). In 2006, Dr. Rudolph

Jaenisch and colleagues at MIT demonstrated that ANT can be carried out in mice.[31] Mouse ANT entities whose *Cdx2* gene is switched off are unable to implant in the uterus and do not survive to birth. Although ANT has been used to create viable stem cell lines capable of producing almost all cell types, the authors point out that this technique must still be tested with monkey and human embryos. Moreover, the manipulation needed to control *Cdx2* expression introduces another logistical hurdle that may complicate the use of ANT to derive embryonic stem cells. Proponents of ANT, such as William Hurlbut of the Stanford University Medical Center, suggest that the entity created by ANT is not a true embryo because it cannot implant in the uterus.[32,33] However, the technique is highly controversial, and its ethical implications remain a source of current debate.[3,32]

Reprogramming Through Cell Fusion

In 2005, Kevin Eggan and colleagues at Harvard University reported that they had fused cultured adult human skin cells with hESCs (see Figure 8.3j).[36] The resulting "hybrid" cells featured many characteristics of hESCs, including a similar manner of growth and division and the manufacture of proteins typically produced by hESCs. Some factor(s) within the hESCs enabled them to "reprogram" the adult skin cells to behave as hESCs. However, these cells raised a significant technical barrier to clinical use. Because fused cells are tetraploid (they contain four copies of the cellular DNA rather than the normal two copies), scientists would need to develop a method to remove the extra DNA without eliminating their hESC-like properties. The fusion method serves as a useful model system for studying how stem cells "reprogram" adult cells to have properties of pluripotent cells. However, if the reprogramming technique could be carried out without the fusion strategy, a powerful avenue for creating patient-specific stem cells without using human eggs could be developed.

Induced Pluripotent Stem Cells (iPSCs): Reprogramming Adult Somatic Cells to Become Pluripotent Stem Cells

In 2007, two independent research groups published manuscripts that described successful genetic reprogramming of human adult somatic cells into pluripotent human stem cells.[34,35] Although some technical limitations remain, this strategy suggests a promising new avenue for generating pluripotent cell lines that can inform drug development, models of disease, and ultimately, transplantation medicine. These experiments, which are discussed below, were breakthroughs because they used adult somatic cells to create pluripotent stem cells that featured hallmarks of ES cells.

In 2006, Shinya Yamanaka and colleagues at Kyoto University reported that they could use a retroviral expression vector to introduce four important stem cell factors into adult mouse cells and reprogram them to behave like ES cells (see Figure 8.3k).[37] They called the reprogrammed cells "iPSCs," for induced pluripotent stem cells. However, iPSCs produced using the original technique failed to produce sperm and egg cells when injected into an early mouse blastocyst and did not make certain critical DNA changes. These researchers then modified the technique to select for iPSCs that can produce sperm and eggs,[38] results that have since been reproduced by Rudolph Jaenisch and colleagues at the Massachusetts Institute of Technology (MIT).[39] In addition, the MIT scientists determined that iPSCs DNA is modified in a manner similar to ES cells, and important stem cell genes are expressed at similar levels. They also demonstrated that iPSCs injected into an early mouse blastocyst can produce all cell types within the developing embryo, and such embryos can complete gestation and are born alive.

Once these research advances were made in mice, they suggested that similar techniques might be used to reprogram adult human cells. In 2007, Yamanaka and coworkers reported that introducing the same four genetic factors that reprogrammed the mouse cells into adult human dermal fibroblasts reprogrammed the cells into human iPSCs.[35] These iPSCs were similar to human ES cells in numerous ways, including morphology, proliferative capacity, expression of cell surface antigens, and gene expression. Moreover, the cells could differentiate into cell types from the three embryonic germ layers both *in vitro* and in teratoma assays. Concurrent with the Yamanaka report, James Thomson and coworkers at the University of Wisconsin published a separate manuscript that detailed the creation of human iPSCs through somatic cell reprogramming using four genetic factors (two of which were in common with the Yamanaka report).[34] The cells generated by the Thomson group met all

defining criteria for ES cells, with the exception that they were not derived from embryos.

These breakthroughs have spurred interest in the field of iPSCs research. In early 2008, investigators at the Massachusetts General Hospital[40] and the University of California, Los Angeles[41] reported generating reprogrammed cells. As scientists explore the mechanisms that govern reprogramming, it is anticipated that more reports will be forthcoming in this emerging area. Although these reprogramming methods require the use of a virus, non-viral strategies may also be possible in the future. In any case, these approaches have created powerful new tools to enable the "dedifferentation" of cells that scientists had previously believed to be terminally differentiated.[42,43]

Although further study is warranted to determine if iPS and ES cells differ in clinically significant ways, these breakthrough reports suggest that reprogramming is a promising strategy for future clinical applications. Induced pluripotent cells offer the obvious advantage that they are not derived from embryonic tissues, thereby circumventing the ethical issues that surround use of these materials. Successful reprogramming of adult somatic cells could also lead to the development of stem cell lines from patients who suffer from genetically-based diseases, such as Huntington's Disease, spinal muscular atrophy, muscular dystrophy, and thalessemia. These lines would be invaluable research tools to understand the mechanisms of these diseases and to test potential drug treatments. Additionally, reprogrammed cells could potentially be used to repair damaged tissues; patient-specific cell lines could greatly reduce the concerns of immune rejection that are prevalent with many transplantation strategies.

However, several technical hurdles must be overcome before iPSCs can be used in humans. For example, in preliminary experiments with mice, the virus used to introduce the stem cell factors sometimes caused cancers.[37] The viral vectors used in these experiments will have to be selected carefully and tested fully to verify that they do not integrate into the genome, thereby harboring the potential to introduce genetic mutations at their site of insertion. This represents a significant concern that must be addressed before the technique can lead to useful treatments for humans. However, this strategy identifies a method for creating pluripotent stem cells that, together with studies of other types of pluripotent stem cells, will help researchers learn how to reprogram cells to repair damaged tissues in the human body.

OTHER SOURCES OF PLURIPOTENT AND/OR MULTIPOTENT CELLS

Stem cell research is a rapidly evolving field, and researchers continue to isolate new pluripotent cells and create additional cell lines. This section briefly reviews other sources of pluripotent cells and the implications that their discovery may have on future research.

Epiblast Cells. While rodent and human ES cells are pluripotent, they maintain their respective pluripotencies through different molecular signaling pathways. It is not known why these differences exist. Recently, several research groups have reported the generation of stable, pluripotent cell lines from mouse and rat epiblast, a tissue of the post-implantation embryo that ultimately generates the embryo proper.[44,45] These cells are distinct from mouse ES cells in terms of the signals that control their differentiation. However, the cells share patterns of gene expression and signaling responses with human ES cells. The establishment of epiblast cell lines can therefore provide insight into the distinctions between pluripotent cells from different species and illuminate ways that pluripotent cells pursue distinct fates during early development.

Existing Adult Stem Cells. As has been discussed in other chapters, numerous types of precursor cells have been isolated in adult tissues.[46] Although these cells tend to be relatively rare and are dispersed throughout the tissues, they hold great potential for clinical application and tissue engineering. For example, tissues created using stem cells harvested from an adult patient could theoretically be used clinically in that patient without engendering an immune response. Moreover, the use of adult stem cells avoids the ethical concerns associated with the use of ES cells. In addition, adult-derived stem cells do not spontaneously differentiate as do ES cells, thus eliminating the formation of teratomas often seen with implantation of ES cells. The potential of adult stem cells for regenerative medicine is great; it is likely that these various cells will find clinical application in the upcoming decades.

CONCLUSION: PLURIPOTENT CELL LINES ARE TOOLS FOR FUTURE RESEARCH

Although the recent advances in reprogramming of adult somatic cells has generated a wave of interest in the scientific community, these cell lines will not likely replace hESC lines as tools for research and discovery. Rather, both categories of cells will find unique uses in the study of stem cell biology and the development and evaluation of therapeutic strategies. Pluripotent cells offer a number of potential clinical applications, especially for diseases with a genetic basis. However, researchers are just beginning to unlock the many factors that govern the cells' growth and differentiation. As scientists make strides toward understanding how these cells can be manipulated, additional applications, approaches, and techniques will likely emerge. As such, pluripotent cells will play a pivotal role in future research into the biology of development and the treatment of disease.

REFERENCES

1. Domen J, Wagers A, Weissman IL. Bone marrow (hematopoietic) stem cells. *Regenerative Medicine* [http://stemcells.nih.gov/info/scireport/2006report. Accessed February 22, 2008.
2. Rao M, Condic ML. Alternative sources of pluripotent stem cells: scientific solutions to an ethical dilemma. *Stem Cells Dev.* 2008;17:1-10.
3. The President's Council on Bioethics. White paper: alternative sources of human pluripotent stem cells. http://www.bioethics.gov/reports/white_paper/index.html Accessed February 26, 2008.
4. Downing GJ, Battey JF Jr. Technical assessment of the first 20 years of research using mouse embryonic stem cell lines. *Stem Cells.* 2004;22:1168-1180.
5. Bongso A, Chui-Yee F, Soon-Chye N, Ratnam S. Isolation and culture of inner cell mass cells from human blastocysts. Hum Reprod 1994; 9: 2110-2117.
6. Thomson JA, Itskovitz-Eldor J, Shapiro SS, et al. Embryonic stem cell lines derived from human blastocysts. *Science.* 1998;282:1145-1147.
7. Hoffman DI, Zellman GL, Fair CC, et al. Cryopreserved embryos in the United States and their availability for research. *Fertil Steril.* 2003;79:1063-1069.
8. Zhang SC, Wernig M, Duncan ID, Brustle O, Thomson JA. In vitro differentiation of transplantable neural precursors from human embryonic stem cells. *Nat Biotechnol.* 2001;19:1129-1133.
9. He JQ, Ma Y, Lee Y, Thomson JA, Kamp TJ. Human embryonic stem cells develop into multiple types of cardiac myocytes: action potential characterization. *Circ Res.* 2003;93:32-39.
10. Wang L, Li L, Menendez P, Cerdan C, Bhatia M. Human embryonic stem cells maintained in the absence of mouse embryonic fibroblasts or conditioned media are capable of hematopoietic development. *Blood.* 2005;105:4598-4603.
11. Richards M, Fong CY, Chan WK, Wong PC, Bongso A. Human feeders support prolonged undifferentiated growth of human inner cell masses and embryonic stem cells. *Nat Biotechnol.* 2002;20:933-936.
12. Richards M, Tan S, Fong CY, Biswas A, Chan WK, Bongso A. Comparative evaluation of various human feeders for prolonged undifferentiated growth of human embryonic stem cells. *Stem Cells.* 2003;21:546-556.
13. Xu C, Inokuma MS, Denham J, et al. Feeder-free growth of undifferentiated human embryonic stem cells. *Nat Biotechnol.* 2001;19:971-974.
14. Lim JWE, Bodnar A. Proteome analysis of conditioned medium from mouse embryonic fibroblast feeder layers which support the growth of human embryonic stem cells. *Proteomics.* 2002;2:1187-1203.
15. Shamblott MJ, Axelman J, Wang S, et al. Derivation of pluripotent stem cells from cultured human primordial germ cells. *Proc Natl Acad Sci USA.* 1998;95:13726-13731.
16. Turnpenny L, Brickwood S, Spalluto CM, et al. Derivation of human embryonic germ cells: an alternative source of pluripotent stem cells. *Stem Cells.* 2003;21:598-609.
17. Aflatoonian B, Moore H. Human primordial germ cells and embryonic germ cells, and their use in cell therapy. *Curr Opin Biotechnol.* 2005;16:530-535.
18. Zhang X, Stojkovic P, Przyborski S, et al. Derivation of human embryonic stem cells from developing and arrested embryos. *Stem Cells.* 2006;24:2669-2676.
19. Landry DW, Zucker HA. Embryonic death and the creation of human embryonic stem cells. *J Clin Invest.* 2004;114:1184-1186.
20. Verlinsky Y, Strelchenko N, Kukharenko V, et al. Human embryonic stem cell lines with genetic disorders. *Reprod Biomed Online.* 2005;10:105-110.
21. Chung Y, Klimanskaya I, Becker S, et al. Embryonic and extraembryonic stem cell lines derived from single mouse blastomeres. *Nature.* 2006;439:216-219.
22. Klimanskaya I, Chung Y, Becker S, Lu SJ, Lanza R. Human embryonic stem cell lines derived from single blastomeres. *Nature.* 2006;444:481-485.
23. Revazova ES, Turovets NA, Kochetkova OD, et al. Patient-specific stem cell lines derived from human parthenogenetic blastocysts. *Cloning Stem Cells.* 2007;9:432-449.

24. Revazova ES, Turovets NA, Kochetkova OD, et al. HLA homozygous stem cell lines derived from human parthenogenetic blastocysts. *Cloning Stem Cells.* 2007;Dec 19:epub ahead of print.

25. Prusa AR, Marton E, Rosner M, Bernaschek G, M H. Oct-4-expressing cells in human amniotic fluid: a new source for stem cell research? *Hum Reprod.* 2003;18:1489-1493.

26. Siegel N, Rosner M, Hanneder M, Valli A, Hengstschlager M. Stem cells in amniotic fluid as new tools to study human genetic diseases. *Stem Cell Rev.* 2007;3:256-264.

27. De Coppi P, Bartsch G Jr, Siddiqui MM, et al. Isolation of amniotic stem cell lines with potential for therapy. *Nat Biotechnol.* 2007;25:100-106.

28. Byrne JA, Pedersen DA, Clepper LL, et al. Producing primate embryonic stem cells by somatic cell nuclear transfer. *Nature.* 2007;450:497-502.

29. French A, Adams C, Anderson L, Kitchen J, Hughes M, Wood S. Development of human cloned blastocysts following somatic cell nuclear transfer (SCNT) with adult fibroblasts. *Stem Cells.* 2008;Epub ahead of print.

30. Egli, D, Rosains, J, Birkhoff, G, Eggan, K. Developmental reprogramming after chromosome transfer into mitotic mouse zygotes. *Nature.* 2007; 447: 679-685.

31. Meissner A, Jaenisch R. Generation of nuclear transfer-derived pluripotent ES cells from cloned *Cdx2*-deficient blastocysts. *Nature.* 2006;439:212-215.

32. Hurlbut WB. Ethics and embryonic stem cell research: altered nuclear transfer as a way forward. *BioDrugs.* 2007;21:79-83.

33. Hurlbut WB. Altered nuclear transfer: a way forward for embryonic stem cell research. *Stem Cell Rev.* 2005;1:293-300.

34. Yu J, Vodyanik MA, Smuga-Otto K, et al. Induced pluripotent stem cell lines derived from human somatic cells. *Science.* 2007;318:1917-1920.

35. Takahashi K, Tanabe K, Ohnuki M, et al. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell.* 2007;131:1-12.

36. Cowan CA, Atienza J, Melton DA, Eggan K. Nuclear reprogramming of somatic cells after fusion with human embryonic stem cells. *Science.* 2005;309:1369-1373.

37. Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell.* 2006;126:663-676.

38. Okita K, Ichisaka T, Yamanaka S. Generation of germline-competent induced pluripotent stem cells. *Nature.* 2007;448:313-317.

38. Wernig M, Meissner A, Foreman R, et al. In vitro reprogramming of fibroblasts into a pluripotent ES-cell-like state. *Nature.* 2007;448:318-324.

40. Park IH, Zhao R, West JA, et al. Reprogramming of human somatic cells to pluripotency with defined factors. *Nature.* 2008;451:141-146.

41. Lowry WE, Richter L, Yachechko R, et al. Generation of human induced pluripotent stem cells from dermal fibroblasts. *Proc Natl Acad Sci USA.* 2008; Epub ahead of print.

42. Jaenisch R, Young R. Stem cells, the molecular circuitry of pluripotency and nuclear reprogramming. *Cell.* 2008;132:567-582.

43. Collas P. Dedifferentiation of cells: new approaches. *Cytotherapy.* 2007;9:236-244.

44. Tesar PJ, Chenoweth JG, Brook FA, et al. New cell lines from mouse epiblast share defining features with human embryonic stem cells. *Nature.* 2007;448:196-199.

45. Brons IGM, Smither LE, Trotter MWB, et al. Derivation of pluripotent epiblast stem cells from mammalian embryos. *Nature.* 2007;448:191-195.

46. Young HE, Duplaa C, Katz R, et al. Adult-derived stem cells and their potential for use in tissue repair and molecular medicine. *J Cell Mol Med.* 2005;9:753-769.

Stem Cell Information

The National Institutes of Health resource for stem cell research

Frequently Asked Questions (FAQs)

Basic Questions

1. **What are human embryonic stem cells?**
   Stem cells are cells that have the remarkable potential to develop into many different cell types in the body. Serving as a sort of repair system for the body, they can theoretically divide without limit to replenish other cells for as long as the person or animal is still alive. When a stem cell divides, each "daughter" cell has the potential to either remain a stem cell or become another type of cell with a more specialized function, such as a muscle cell, a red blood cell, or a brain cell.

   A more detailed primer on stem cells can be found at Stem Cell Basics.

2. **What classes of stem cells are there?**
   There are three classes of stem cells: totipotent, multipotent, and pluripotent.

   - A fertilized egg is considered totipotent, meaning that its potential is total; it gives rise to all the different types of cells in the body.
   - Pluripotent stem cells can give rise to any type of cell in the body except those needed to develop a fetus.
   - Stem cells that can give rise to a small number of different cell types are generally called multipotent.

3. **Where do stem cells come from?**
   There are several sources of stem cells. Pluripotent stem cells can be isolated from human embryos that are a few days old. Cells from these embryos can be used to create pluripotent stem cell "lines" —cell cultures that can be grown indefinitely in the laboratory. Pluripotent stem cell lines have also been developed from fetal tissue (older than 8 weeks of development).

   In late 2007, scientists identified conditions that would allow some specialized adult human cells to be "reprogrammed genetically to assume a stem cell-like state. This new type of stem cells is called induced pluripotent stem cells (iPSCs). IPSCs are adult cells that have been genetically reprogrammed to an embryonic stem cell–like state by being forced to express genes and factors important for maintaining the defining properties of embryonic stem cells. Although these cells meet the defining criteria for pluripotent stem cells, it is not known if iPSCs and embryonic stem cells differ in clinically significant ways. Mouse iPSCs were first reported in 2006, and human iPSCs were first reported in late 2007. Mouse iPSCs demonstrate important characteristics of pluripotent stem cells, including expressing stem cell markers, forming tumors containing cells from all three germ layers, and being able to contribute to many different tissues when injected into mouse embryos at a very early stage in development. Human iPSCs also express stem cell markers and are capable of generating cells characteristic of all three germ layers.

   Although additional research is needed, iPSCs are already useful tools for drug development and modeling of diseases, and scientists

hope to use them in transplantation medicine. Viruses are currently used to introduce the reprogramming factors into adult cells, and this process must be carefully controlled and tested before the technique can lead to useful treatments for humans. In animal studies, the virus used to introduce the stem cell factors sometimes causes cancers. Researchers are currently investigating non-viral delivery strategies.

Non-embryonic, or "adult" stem cells have been identified in many organs and tissues. Typically there is a very small number of stem cells in each tissue, and these cells have a limited capacity for proliferation, thus making it difficult to generate large quantities of these cells in the laboratory. Stem cells are thought to reside in a specific area of each tissue (called a "stem cell niche") where they may remain quiescent (non-dividing) for many years until they are activated by a normal need for more cells, or by disease or tissue injury. Among adult tissues reported to contain stem cells are brain, bone marrow, peripheral blood, blood vessels, skeletal muscle, skin, intestine, teeth, heart, teeth, gut, liver, ovarian epithelium, and testis.

4. **Why do scientists want to use stem cell lines?**
Once a stem cell line is established from a cell in the body, it is essentially immortal, no matter how it was derived. That is, the researcher using the line will not have to go through the rigorous procedure necessary to isolate stem cells again. Once established, a cell line can be grown in the laboratory indefinitely and cells may be frozen for storage or distribution to other researchers.

   Stem cell lines grown in the lab provide scientists with the opportunity to "engineer" them for use in transplantation or treatment of diseases. For example, before scientists can use any type of tissue, organ, or cell for transplantation, they must overcome attempts by a patient's immune system to reject the transplant. In the future, scientists may be able to modify human stem cell lines in the laboratory by using gene therapy or other techniques to overcome this immune rejection. Scientists might also be able to replace damaged genes or add new genes to stem cells in order to give them characteristics that can ultimately treat diseases.

Up to Top

Healthcare Questions

1. **Why are doctors and scientists so excited about human embryonic stem cells?**
Stem cells have potential in many different areas of health and medical research. To start with, studying stem cells will help us to understand how they transform into the dazzling array of specialized cells that make us what we are. Some of the most serious medical conditions, such as cancer and birth defects, are due to problems that occur somewhere in this process. A better understanding of normal cell development will allow us to understand and perhaps correct the errors that cause these medical conditions.

   Another potential application of stem cells is making cells and tissues for medical therapies. Today, donated organs and tissues are often used to replace those that are diseased or destroyed. Unfortunately, the number of people needing a transplant far exceeds the number of organs available for transplantation. Pluripotent stem cells offer the possibility of a renewable source of replacement cells and tissues to treat a myriad of diseases, conditions, and disabilities including Parkinson's disease, amyotrophic lateral sclerosis, spinal cord injury, burns, heart disease, diabetes, and arthritis.

2. **Have human embryonic stem cells been used successfully to treat any human diseases yet?**
Scientists have only been able to do experiments with human embryonic stem cells (hESC) since 1998, when a group led by Dr. James Thomson at the University of Wisconsin developed a technique to isolate and grow the cells. Moreover, federal funds to support hESC research have only been available since August 9, 2001, when President Bush announced his decision on federal funding for hESC research. Because many academic researchers rely on federal funds to support their laboratories, they are just beginning to learn how to grow and use the cells. Thus, although hESC are thought to offer potential cures and therapies for many devastating diseases, research using them is still in its early stages.

   In late January 2009, the California-based company Geron received FDA clearance to begin the first human clinical trial of cells derived from human embryonic stem cells.

   - Read the Geron press release

   Adult stem cells such as blood-forming stem cells in bone marrow (called hematopoietic stem cells, or HSCs) are currently the only type of stem cell commonly used to treat human diseases. Doctors have been transferring HSCs in bone marrow transplants for over 40 years. More advanced techniques of collecting, or "harvesting", HSCs are now used in order to treat leukemia, lymphoma and several inherited blood disorders.

   The clinical potential of adult stem cells has also been demonstrated in the treatment of other human diseases that include diabetes and advanced kidney cancer. However, these newer uses have involved studies with a very limited number of patients.

3. **What will be the best type of stem cell to use for therapy?**
Pluripotent stem cells, while having great therapeutic potential, face formidable technical challenges. First, scientists must learn how to control their development into all the different types of cells in the body. Second, the cells now available for research are likely to be rejected by a patient's immune system. Another serious consideration is that the idea of using stem cells from human embryos or human fetal tissue troubles many people on ethical grounds.

   Until recently, there was little evidence that multipotent adult stem cells could change course and provide the flexibility that researchers need in order to address all the medical diseases and disorders they would like to. New findings in animals, however, suggest that even after a stem cell has begun to specialize, it may be more flexible than previously thought.

   There are currently several limitations to using adult stem cells. Although many different kinds of multipotent stem cells have been identified, adult stem cells that could give rise to all cell and tissue types have not yet been found. Adult stem cells are often present in only minute quantities and can therefore be difficult to isolate and purify. There is also evidence that they may not have the same capacity to multiply as embryonic stem cells do. Finally, adult stem cells may contain more DNA abnormalities—caused by sunlight, toxins, and errors in making more DNA copies during the course of a lifetime. These potential weaknesses might limit the usefulness of adult stem cells.

4. **I have Parkinson's Disease. Is there a clinical trial that I can participate in that uses stem cells as therapy?**
The public may search a database of NIH-sponsored clinical trials at www.clinicaltrials.gov. Enter the search terms of interest (in this case, *Parkinson's Disease and stem cells*) to search for applicable clinical trials.

5. **Where can I donate umbilical cord stem cells?**

NIH cannot accept donated umbilical cord stem cells from the general public. The National Marrow Donor Program maintains a Web page on donating cord blood at http://www.marrow.org/HELP/Donate_Cord_Blood_Share_Life/index.html, and the International Cord Blood Society has one at http://www.cordblood.org/index.php?rm=common_page&id=10.

Up to Top

Research and Policy Questions

1. **Which research is best to pursue?**
   The development of stem cell lines that can produce many tissues of the human body is an important scientific breakthrough. This research has the potential to revolutionize the practice of medicine and improve the quality and length of life. Given the enormous promise of stem cells therapies for so many devastating diseases, NIH believes that it is important to simultaneously pursue all lines of research and search for the very best sources of these cells.

2. **Why not use adult stem cells instead of using human embryonic stem cells in research?**
   Human embryonic stem cells are thought to have much greater developmental potential than adult stem cells. This means that embryonic stem cells may be pluripotent—that is, able to give rise to cells found in all tissues of the embryo except for germ cells rather than being merely multipotent—restricted to specific subpopulations of cell types, as adult stem cells are thought to be.

3. **What are the NIH Guidelines on the utilization of stem cells derived from human fetal tissue (embryonic germ cells)?**
   The Federal Register Announcement National Institutes of Health Guidelines for Research Using Human Pluripotent Stem Cells (230k PDF; get Adobe Reader), published August 25, 2000, was "superceded as it pertains to embryonic stem cell research" on November 14, 2001). However, Section II. B, titled "Utilization of Human Pluripotent Stem Cells Derived from Human Fetal Tissue," still governs human embryonic germ cell research. In addition, Section III, titled "Areas of Research Involving Human Pluripotent Stem Cells That Are Ineligible for NIH Funding," governs both human embryonic stem cell and human embryonic germ cell research.

4. **May individual states pass laws to permit human embryonic stem cell research?**
   Individual states have the authority to pass laws to permit human embryonic stem cell research using state funds. Unless Congress passes a law that bans it, states may pay for research using human embryonic stem cell lines that are not eligible for federal funding.

5. **Where can I find information about patents obtained for stem cells?**
   The U.S. Patent and Trademark Office offers a full-text search of issued patents and published applications. Try searching for "stem cell" or "stem cells."

Up to Top

Cell Line Availability and the Registry

1. **I am a scientist funded by the NIH. How many cell lines are available to me, and how do I get them?**
   As of March 2007, there are 21 independent, fully developed stem cell lines available for widespread distribution to researchers. Some of these cell lines are available through the National Stem Cell Bank at reduced cost. The remaining lines may be purchased by contacting the cell line providers directly. Information on the lines and how to contact the National Stem Cell Bank and the individual providers can be found on the NIH Stem Cell Registry.

   In addition, this site provides researchers with a unique NIH identifier code to apply for federal funds to do research using human embryonic stem cells.

2. **I'm interested in purchasing more than one cell line from the NIH Stem Cell Registry. What is known about the status of the cell lines and their availability?**
   Many of the cell lines have been characterized as embryonic stem cells by detecting expression of surface antigen markers specific to embryonic stem cells, determining if the cells are pluripotent, and demonstrating that the cells are undifferentiated. A number of scientific publications have described the characterization of human embryonic stem cells. Although the characterization approaches may differ across laboratories, an example of the strategies used can be found in Thomson et al. (1998), *Science,* 282,1145–1147.

   The National Stem Cell Bank and the individual providers of the federally eligible cells are working to make them available to researchers. This includes developing quality control measures to grow and reproduce the cell lines in sufficient numbers, having the administrative structure to receive and process requests, and establishing material transfer agreements with research purchasers. The National Stem Cell Bank and the individual providers of federally eligible cell lines have the most up-to-date information on availability. A list of these sources and contact information is available on the NIH Stem Cell Registry.

3. **Who owns the cells?**
   The stem cell lines remain the property of the individual stem cell providers, as listed on the NIH Stem Cell Registry. Researchers may negotiate a material transfer agreement (MTA) with either the National Stem Cell Bank on behalf of the individual providers, or directly with the cell providers in order to specify their rights and responsibilities concerning resulting data, publications, and potential patents. Examples of MTAs negotiated between the Department of Health and Human Services/NIH and various stem cell line providers are listed by provider on the NIH Stem Cell Registry.

4. **When does NIH anticipate that more stem cell lines will become available?**
   As of March 2007, there are 21 independent, fully developed stem cell lines available for widespread distribution to researchers. Providers of these 21 cell lines all received an NIH Infrastructure award. This number compares to 17 in 2004 and 1 in April 2002. The increased availability of the lines is a direct consequence of NIH's funding of Infrastructure awards to support cell providers to develop their eligible lines into distribution-quality, well-characterized cell lines. Up-to-date information on available lines can be found on the NIH Stem Cell Registry. The remaining 31 independent derivations are all at institutions that do not have NIH Infrastructure awards to develop their cell lines. The NIH does not know when these 31 derivations might become available for distribution.

5. **What policies govern use of stem cell lines from WiCell Research Institute?**
   WiCell has published FAQs About WiCell's Policies on the Use of Its hESC Lines (136k PDF file; get Adobe Reader) to address this question.

Up to Top

Page citation: FAQs . In *Stem Cell Information* [World Wide Web site]. Bethesda, MD: National Institutes of Health, U.S. Department of Health and Human Services, 2010 [cited Friday, September 17, 2010] Available at <http://stemcells.nih.gov/info/faqs>

National Institutes of Health (NIH)
9000 Rockville Pike
Bethesda, Maryland 20892

Department of Health
and Human Services (DHHS)

USA.gov
The U.S. government's official web portal

Stem Cell Information

The National Institutes of Health resource for stem cell research

Stem Cell Basics

I. Introduction: What are stem cells, and why are they important?

Stem cells have the remarkable potential to develop into many different cell types in the body during early life and growth. In addition, in many tissues they serve as a sort of internal repair system, dividing essentially without limit to replenish other cells as long as the person or animal is still alive. When a stem cell divides, each new cell has the potential either to remain a stem cell or become another type of cell with a more specialized function, such as a muscle cell, a red blood cell, or a brain cell.

Stem cells are distinguished from other cell types by two important characteristics. First, they are unspecialized cells capable of renewing themselves through cell division, sometimes after long periods of inactivity. Second, under certain physiologic or experimental conditions, they can be induced to become tissue- or organ-specific cells with special functions. In some organs, such as the gut and bone marrow, stem cells regularly divide to repair and replace worn out or damaged tissues. In other organs, however, such as the pancreas and the heart, stem cells only divide under special conditions.

Until recently, scientists primarily worked with two kinds of stem cells from animals and humans: **embryonic stem cells** and non-embryonic "**somatic**" or **"adult" stem cells**. The functions and characteristics of these cells will be explained in this document. Scientists discovered ways to derive embryonic stem cells from early mouse embryos nearly 30 years ago, in 1981. The detailed study of the biology of mouse stem cells led to the discovery, in 1998, of a method to derive stem cells from human embryos and grow the cells in the laboratory. These cells are called **human embryonic stem cells**. The embryos used in these studies were created for reproductive purposes through ***in vitro* fertilization** procedures. When they were no longer needed for that purpose, they were donated for research with the informed consent of the donor. In 2006, researchers made another breakthrough by identifying conditions that would allow some specialized adult cells to be "reprogrammed" genetically to assume a stem cell-like state. This new type of stem cell, called **induced pluripotent stem cells (iPSCs)**, will be discussed in a later section of this document.

Stem cells are important for living organisms for many reasons. In the 3- to 5-day-old embryo, called a **blastocyst**, the inner cells give rise to the entire body of the organism, including all of the many specialized cell types and organs such as the heart, lung, skin, sperm, eggs and other tissues. In some adult tissues, such as bone marrow, muscle, and brain, discrete populations of adult stem cells generate replacements for cells that are lost through normal wear and tear, injury, or disease.

Given their unique regenerative abilities, stem cells offer new potentials for treating diseases such as Parkinson's disease, diabetes, and heart disease. However, much work remains to be done in the laboratory and the clinic to understand how to use these cells for **cell-based therapies** to treat disease, which is also referred to as **regenerative or reparative medicine**.

Laboratory studies of stem cells enable scientists to learn about the cells' essential properties and what makes them different from specialized cell types. Scientists are already using stem cells in the laboratory to screen new drugs and to develop model systems to study normal growth and identify the causes of birth defects.

Research on **stem cells** continues to advance knowledge about how an organism develops from a single cell and how healthy cells replace damaged cells in adult organisms. Stem cell research is one of the most fascinating areas of contemporary biology, but, as with many expanding fields of scientific inquiry, research on stem cells raises scientific questions as rapidly as it generates new discoveries.

I. Introduction | Next

Page citation: Stem Cell Basics: Introduction . In *Stem Cell Information* [World Wide Web site]. Bethesda, MD: National Institutes of Health, U.S. Department of Health and Human Services, 2009 [cited Friday, September 17, 2010] Available at <http://stemcells.nih.gov/info/basics/basics1>

National Institutes of Health (NIH)
9000 Rockville Pike
Bethesda, Maryland 20892

Department of Health

and Human Services (DHHS)

USA.gov
The U.S. government's official web portal

Stem Cell Information

The National Institutes of Health resource for stem cell research

Stem Cell Basics

II. What are the unique properties of all stem cells?

Stem cells differ from other kinds of cells in the body. All stem cells—regardless of their source—have three general properties: they are capable of dividing and renewing themselves for long periods; they are unspecialized; and they can give rise to specialized cell types.

Stem cells are capable of dividing and renewing themselves for long periods. Unlike muscle cells, blood cells, or nerve cells—which do not normally replicate themselves—stem cells may replicate many times, or **proliferate**. A starting population of stem cells that proliferates for many months in the laboratory can yield millions of cells. If the resulting cells continue to be unspecialized, like the parent stem cells, the cells are said to be capable of **long-term self-renewal**.

Scientists are trying to understand two fundamental properties of stem cells that relate to their **long-term self-renewal**:

1. why can **embryonic stem cells** proliferate for a year or more in the laboratory without differentiating, but most **non-embryonic stem cells** cannot; and
2. what are the factors in living organisms that normally regulate stem cell **proliferation** and self-renewal?

Discovering the answers to these questions may make it possible to understand how cell proliferation is regulated during normal embryonic development or during the abnormal **cell division** that leads to cancer. Such information would also enable scientists to grow embryonic and non-embryonic stem cells more efficiently in the laboratory.

The specific factors and conditions that allow stem cells to remain unspecialized are of great interest to scientists. It has taken scientists many years of trial and error to learn to derive and maintain stem cells in the laboratory without them spontaneously differentiating into specific cell types. For example, it took two decades to learn how to grow **human embryonic stem cells** in the laboratory following the development of conditions for growing mouse stem cells. Therefore, understanding the signals in a mature organism that cause a stem cell population to proliferate and remain unspecialized until the cells are needed. Such information is critical for scientists to be able to grow large numbers of unspecialized stem cells in the laboratory for further experimentation.

Stem cells are unspecialized. One of the fundamental properties of a stem cell is that it does not have any tissue-specific structures that allow it to perform specialized functions. For example, a stem cell cannot work with its neighbors to pump blood through the body (like a heart muscle cell), and it cannot carry oxygen molecules through the bloodstream (like a red blood cell). However, unspecialized stem cells can give rise to specialized cells, including heart muscle cells, blood cells, or nerve cells.

Stem cells can give rise to specialized cells. When unspecialized stem cells give rise to specialized cells, the process is called **differentiation**. While differentiating, the cell usually goes through several stages, becoming more specialized at each step. Scientists are just beginning to understand the signals inside and outside cells that trigger each stem of the differentiation process. The internal **signals** are controlled by a cell's **genes**, which are interspersed across long strands of DNA, and carry coded instructions for all cellular structures and functions. The external signals for cell differentiation include chemicals secreted by other cells, physical contact with neighboring cells, and certain molecules in the **microenvironment**. The interaction of signals during differentiation causes the cell's DNA to acquire **epigenetic** marks that restrict DNA expression in the cell and can be passed on through cell division.

Many questions about stem cell differentiation remain. For example, are the internal and external signals for cell differentiation similar for all kinds of stem cells? Can specific sets of signals be identified that promote differentiation into specific cell types? Addressing these questions may lead scientists to find new ways to control stem cell differentiation in the laboratory, thereby growing cells or tissues that can be used for specific purposes such as **cell-based therapies** or drug screening.

Adult stem cells typically generate the cell types of the tissue in which they reside. For example, a blood-forming adult stem cell in the bone marrow normally gives rise to the many types of blood cells such as red blood cells, white blood cells, and platelets. It is generally accepted that a blood-forming cell in the bone marrow—which is called a **hematopoietic stem cell**—cannot give rise to the cells of a very different tissue, such as nerve cells in the brain. Experiments over the last several years have purported to show that stem cells from one tissue may give rise to cell types of a completely different tissue. This remains an area of great debate within the research community. This controversy demonstrates the challenges of studying adult stem cells and suggests that additional research using adult stem cells is necessary to understand their full potential as future therapies.

Previous | II. What are the unique properties of all stem cells? | Next

Page citation: Stem Cell Basics: What are the unique properties of all stem cells? . In *Stem Cell Information* [World Wide Web site]. Bethesda, MD: National Institutes of Health, U.S. Department of Health and Human Services, 2009 [cited Friday, September 17, 2010] Available at <http://stemcells.nih.gov/info/basics/basics2>

National Institutes of Health (NIH)
9000 Rockville Pike
Bethesda, Maryland 20892

Department of Health
and Human Services (DHHS)

USA.gov
The U.S. government's official web portal

Stem Cell Information

The National Institutes of Health resource for stem cell research

Stem Cell Basics

III. What are embryonic stem cells?

A. What stages of early embryonic development are important for generating embryonic stem cells?

Embryonic stem cells, as their name suggests, are derived from embryos. Most embryonic stem cells are derived from embryos that develop from eggs that have been fertilized ***in vitro***—in an ***in vitro* fertilization** clinic—and then donated for research purposes with informed consent of the donors. They are *not* derived from eggs fertilized in a woman's body. The **embryos** from which **human embryonic stem cells** are derived are typically four or five days old and are a hollow microscopic ball of cells called the **blastocyst**. The blastocyst includes three structures: the **trophoblast**, which is the layer of cells that surrounds the **blastocoel**, hollow cavity inside the blastocyst; and the **inner cell mass**, which is a group of cells at one end of the blastocoel that develop into the embryo proper.

B. How are embryonic stem cells grown in the laboratory?

Growing cells in the laboratory is known as **cell culture**. Human embryonic stem cells are isolated by transferring the **inner cell mass** into a plastic laboratory culture dish that contains a nutrient broth known as **culture medium**. The cells divide and spread over the surface of the dish. The inner surface of the culture dish is typically coated with mouse embryonic skin cells that have been treated so they will not divide. This coating layer of cells is called a **feeder layer**. The mouse cells in the bottom of the culture dish provide the inner cell mass cells a sticky surface to which they can attach. Also, the feeder cells release nutrients into the culture medium. Researchers have devised ways to grow embryonic stem cells without mouse feeder cells. This is a significant scientific advance because of the risk that viruses or other macromolecules in the mouse cells may be transmitted to the human cells.

The process of generating an embryonic stem cell line is somewhat inefficient, so lines are not produced each time an inner cell mass is placed into a culture dish. However, if the plated inner cell mass cells survive, divide and multiply enough to crowd the dish, they are removed gently and plated into several fresh culture dishes. The process of re-plating or subculturing the cells is repeated many times and for many months. Each cycle of **subculturing** the cells is referred to as a **passage**. When the cell line is established, the original cells yield millions of embryonic stem cells. Embryonic stem cells that have proliferated in cell culture for six or more months without differentiating, are **pluripotent**, and appear genetically normal are referred to as an **embryonic stem cell line** at any stage in the process.

Batches of cells can be frozen and shipped to other laboratories for further culture and experimentation.

C. What laboratory tests are used to identify embryonic stem cells?

At various points during the process of generating embryonic stem cell lines, scientists test the cells to see whether they exhibit the fundamental properties that make them embryonic stem cells. This process is called characterization.

Scientists who study human embryonic stem cells have not yet agreed on a standard battery of tests that measure the cells' fundamental properties. However, laboratories that grow human embryonic stem cell lines use several kinds of tests, including:

Growing and subculturing the stem cells for many months. This ensures that the cells are capable of long-term growth and self-renewal. Scientists inspect the cultures through a microscope to see that the cells look healthy and remain **undifferentiated**

Using specific techniques to determine the presence of transcription factors that are typically produced by undifferentiated cells. Two or more important transcription factors are Nanog and Oct4. Transcription factors help turn **genes** on and off at the right time, which is an important part of the processes of cell **differentiation** and embryonic development. In this case, both Oct 4 and Nanog are associated with maintaining the stem cells in an undifferentiated state, capable of self-renewal.

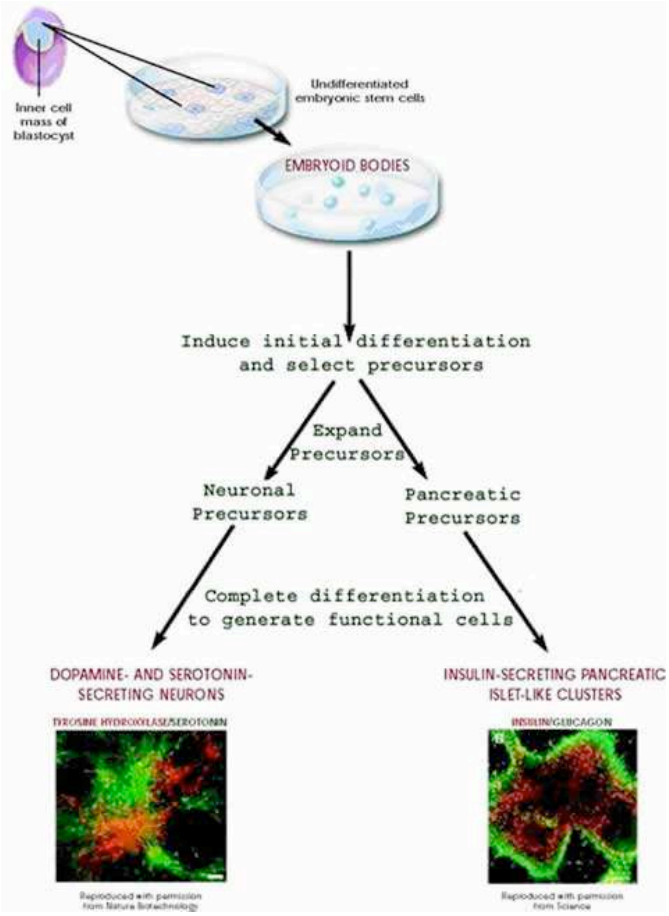
Using specific techniques to determine the presence of paricular cell surface markers that are typically produced by undifferentiated cells.

Examining the chromosomes under a microscope. This is a method to assess whether the chromosomes are damaged or if the number of chromosomes has changed. It does not detect genetic mutations in the cells.

Determining whether the cells can be re-grown, or subcultured, after freezing, thawing, and re-plating.

Testing whether the human embryonic stem cells are pluripotent by 1) allowing the cells to differentiate spontaneously in cell culture; 2) manipulating the cells so they will differentiate to form cells characteristic of the three germ layers; or 3) injecting the cells into a mouse with a suppressed immune system to test for the formation of a benign tumor called a **teratoma**. Since the mouse's immune system is suppressed, the injected human stem cells are not rejected by the mouse immune system and scientists can observe growth and differentiation of the human stem cells. Teratomas typically contain a mixture of many differentiated or partly differentiated cell types—an indication that the embryonic stem cells are capable of differentiating into multiple cell types.

D. How are embryonic stem cells stimulated to differentiate?



As long as the embryonic stem cells in culture are grown under appropriate conditions, they can remain undifferentiated (unspecialized). But if cells are allowed to clump together to form **embryoid bodies**, they begin to differentiate spontaneously. They can form muscle cells, nerve cells, and many other cell types. Although spontaneous differentiation is a good indication that a culture of embryonic stem cells is healthy, it is not an efficient way to produce cultures of specific cell types.

So, to generate cultures of specific types of differentiated cells—heart muscle cells, blood cells, or nerve cells, for example—scientists try to control the differentiation of embryonic stem cells. They change the chemical composition of the culture medium, alter the surface of the culture dish, or modify the cells by inserting specific genes. Through years of experimentation, scientists have established some basic protocols or "recipes" for the **directed differentiation** of embryonic stem cells into some specific cell types (Figure 1). (For additional examples of directed differentiation of embryonic stem cells, refer to the NIH stem cell reports available at http://stemcells.nih.gov/info/2006report/ and http://stemcells.nih.gov/info/2001report/2001report.htm.)

If scientists can reliably direct the differentiation of embryonic stem cells into specific cell types, they may be able to use the resulting, differentiated cells to treat certain diseases in the future. Diseases that might be treated by transplanting cells generated from human embryonic stem cells include Parkinson's disease, diabetes, traumatic spinal cord injury, Duchenne's muscular dystrophy, heart disease, and vision and hearing loss.

Previous | III. What are embryonic stem cells? | Next

Page citation: Stem Cell Basics: What are embryonic stem cells? . In *Stem Cell Information* [World Wide Web site]. Bethesda, MD: National Institutes of Health, U.S. Department of Health and Human Services, 2010 [cited Friday, September 17, 2010] Available at <http://stemcells.nih.gov/info/basics/basics3>

National Institutes of Health (NIH)
9000 Rockville Pike
Bethesda, Maryland 20892

Department of Health

and Human Services (DHHS)

USA.gov
The U.S. government's official web portal

Stem Cell Information

The National Institutes of Health resource for stem cell research

Stem Cell Basics

IV. What are adult stem cells?

An adult stem cell is an **undifferentiated** cell found among differentiated cells in a tissue or organ that can renew itself and can differentiate to yield some or all of the major specialized cell types of the tissue or organ. The primary roles of **adult stem cells** in a living organism are to maintain and repair the tissue in which they are found. Scientists also use the term **somatic stem cell** instead of adult stem cell, where somatic refers to cells of the body. Unlike **embryonic stem cells**, which are defined by their origin (the **inner cell mass** of the **blastocyst**), the origin of adult stem cells in some mature tissues is still under investigation.

Research on adult stem cells has recently generated a great deal of excitement. Scientists have found adult stem cells in many more tissues than they once thought possible. This finding has led researchers and clinicians to ask whether adult stem cells could be used for transplants. In fact, adult hematopoietic, or blood-forming, stem cells from bone marrow have been used in transplants for 40 years. Scientists now have evidence that stem cells exist in the brain and the heart. If the differentiation of adult stem cells can be controlled in the laboratory, these cells may become the basis of transplantation-based therapies.

The history of research on adult stem cells began about 50 years ago. In the 1950s, researchers discovered that the bone marrow contains at least two kinds of stem cells. One population, called **hematopoietic stem cells**, forms all the types of blood cells in the body. A second population, called **mesenchymal stem cells**, was discovered a few years later. Mesenchymal stem cells make up a small proportion of the **stromal cells** in the bone marrow, and can generate bone, cartilage, fat, and fibrous connective tissue.

In the 1960s, scientists who were studying rats discovered two regions of the brain that contained dividing cells that ultimately become nerve cells. Despite these reports, most scientists believed that the adult brain could not generate new nerve cells. It was not until the 1990s that scientists agreed that the adult brain does contain stem cells that are able to generate the brain's three major cell types—**astrocytes** and **oligodendrocytes**, which are non-neuronal cells, and **neurons**, or nerve cells.

A. Where are adult stem cells found, and what do they normally do?

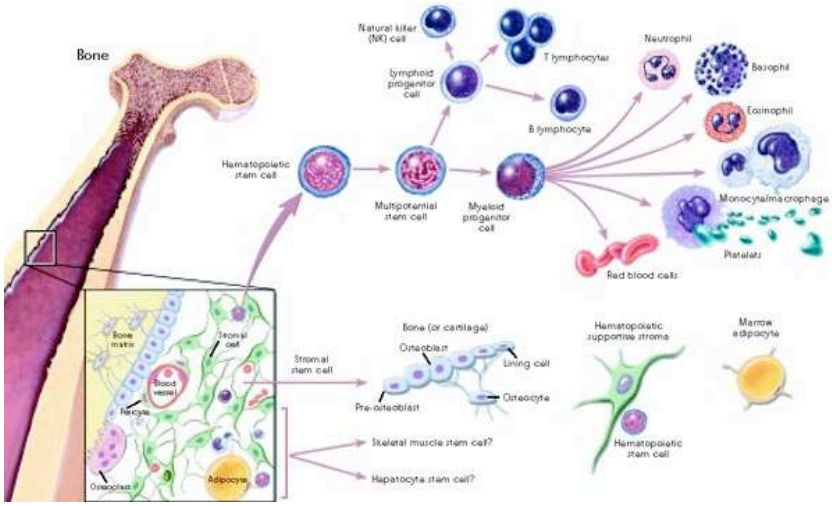
Adult stem cells have been identified in many organs and tissues. Typically there is a very small number of stem cells in each tissue, and these cells have a limited capacity for proliferation, thus making it difficult to generate large quantities of these cells in the laboratory. Stem cells are thought to reside in a specific area of each tissue (called a "stem cell niche") where they may remain quiescent (non-dividing) for many years until they are activated by a normal need for more cells, or by disease or tissue injury. Among adult tissues reported to contain stem cells are brain, bone marrow, peripheral blood, blood vessels, skeletal muscle, skin, teeth, heart, gut, liver, ovarian epithelium, and testis.

Scientists in many laboratories are trying to find ways to grow large quantities of adult stem cells in **cell culture** and manipulate them to generate specific cell types so they can be used to treat injury or disease. Some examples of potential treatments include developing insulin-producing cells for type I diabetes, and repairing damaged heart muscle following a heart attack with cardiac muscle cells.

B. What tests are used for identifying adult stem cells?

Scientists often use one or more of the following methods to identify adult stem cells: (1) label the cells in a living tissue with molecular markers and then determine the specialized cell types they generate; (2) remove the cells from a living animal, label them in cell culture, and transplant them back into another animal to determine whether the cells replace (or "repopulate") their tissue of origin; and (3) isolate the cells, grow them in cell culture, and manipulate them, often by adding growth factors or introducing new **genes**, to determine what differentiated cell types they can become.

C. What is known about adult stem cell differentiation?



As indicated above, scientists have reported that adult stem cells occur in many tissues and that they enter normal **differentiation** pathways to form the specialized cell types of the tissue in which they reside.

Normal differentiation pathways of adult stem cells. In a living animal, adult stem cells can divide for a long period and can give rise to mature cell types that have characteristic shapes and specialized structures and functions of a particular tissue. The following are examples of differentiation pathways of adult stem cells (Figure 2) that have been demonstrated *in vitro* or *in vivo*.

- Hematopoietic stem cells give rise to all the types of blood cells: red blood cells, B lymphocytes, T lymphocytes, natural killer cells, neutrophils, basophils, eosinophils, monocytes and macrophages.
- **Mesenchymal stem cells** give rise to a variety of cell types: bone cells (osteocytes), cartilage cells (chondrocytes), fat cells (adipocytes), and other kinds of connective tissue cells such as those in tendons.
- **Neural stem cells** in the brain give rise to its three major cell types: nerve cells (neurons) and two categories of non-neuronal cells—astrocytes and **oligodendrocytes**.
- Epithelial stem cells in the lining of the digestive tract occur in deep crypts and give rise to several cell types: absorptive cells, goblet cells, paneth cells, and enteroendocrine cells.
- Skin stem cells occur in the basal layer of the epidermis and at the base of hair follicles. The epidermal stem cells give rise to keratinocytes, which migrate to the surface of the skin and form a protective layer. The follicular stem cells can give rise to both the hair follicle and to the epidermis.

Transdifferentiation. A number of experiments have reported that certain adult stem cell types can differentiate into cell types seen in organs or tissues other than those expected from the cells' predicted lineage (i.e., brain stem cells that differentiate into blood cells or blood-forming cells that differentiate into cardiac muscle cells, and so forth). This reported phenomenon is called **transdifferentiation**. Although isolated instances of transdifferentiation have been observed in some vertebrate species, whether this phenomenon actually occurs in humans is under debate by the scientific community. It should be noted that in instances in which transdifferentiation has been detected, only a very small percentage of cells undergoes the process.

In a variation of transdifferentiation experiments, scientists have recently demonstrated that certain adult cell types can be "reprogrammed" into other cell types *in vivo* using a well-controlled process of genetic modification (see Section VI for a discussion of the principles of reprogramming). This strategy may offer a way to reprogram available cells into other cell types that have been lost or damaged due to disease. For example, one recent experiment shows how pancreatic beta cells, the insulin-producing cells that are lost or damaged in diabetes, could possibly be created by reprogramming other pancreatic cells. By "re-starting" expression of three critical beta-cell genes in differentiated adult pancreatic exocrine cells, researchers were able to create beta cell-like cells that can secrete insulin. The reprogrammed cells were similar to beta cells in appearance, size, and shape; expressed genes characteristic of beta cells; and were able to partially restore blood sugar regulation in mice whose own beta cells had been chemically destroyed. While not transdifferentiation by definition, this method for reprogramming adult cells may be used as a model for directly reprogramming other adult cell types.

D. What are the key questions about adult stem cells?

Many important questions about adult stem cells remain to be answered. They include:

- How many kinds of adult stem cells exist, and in which tissues do they exist?
- What are the sources of adult stem cells in the body? Are they "leftover" embryonic stem cells, or do they arise in some other way? Why do they remain in an undifferentiated state when all the cells around them have differentiated?
- Do adult stem cells have the capacity to transdifferentiate, and it is possible to control this process to improve its reliability and efficiency?
- What are the factors that control adult stem cell proliferation and differentiation?
- What are the factors that stimulate stem cells to relocate to sites of injury or damage?

Previous | IV. What are adult stem cells? | Next

Page citation: Stem Cell Basics: What are adult stem cells? . In *Stem Cell Information* [World Wide Web site]. Bethesda, MD: National Institutes of Health, U.S.

Department of Health and Human Services, 2010 [cited Friday, September 17, 2010] Available at <http://stemcells.nih.gov/info/basics/basics4>

National Institutes of Health (NIH)
9000 Rockville Pike
Bethesda, Maryland 20892

Department of Health
and Human Services (DHHS)

USA.gov
The U.S. government's official web portal

Stem Cell Information

The National Institutes of Health resource for stem cell research

Stem Cell Basics

V. What are the similarities and differences between embryonic and adult stem cells?

Human embryonic and **adult stem cells** each have advantages and disadvantages regarding potential use for **cell-based regenerative therapies**. One major difference between adult and embryonic stem cells is their different abilities in the number and type of differentiated cell types they can become. **Embryonic stem cells** can become all cell types of the body because they are **pluripotent**. Adult stem cells are thought to be limited to differentiating into different cell types of their tissue of origin.

Embryonic stem cells can be grown relatively easily in culture. Adult stem cells are rare in mature tissues, so isolating these cells from an adult tissue is challenging, and methods to expand their numbers in **cell culture** have not yet been worked out. This is an important distinction, as large numbers of cells are needed for stem cell replacement therapies.

Scientists believe that tissues derived from embryonic and adult stem cells may differ in the likelihood of being rejected after transplantation. We don't yet know whether tissues derived from embryonic stem cells would cause transplant rejection, since the first phase 1 clinical trial testing the safety of cells derived from hESCS has only recently been approved by the United States Food and Drug Administration (FDA).

Adult stem cells, and tissues derived from them, are currently believed less likely to initiate rejection after transplantation. This is because a patient's own cells could be expanded in culture, coaxed into assuming a specific cell type (**differentiation**), and then reintroduced into the patient. The use of adult stem cells and tissues derived from the patient's own adult stem cells would mean that the cells are less likely to be rejected by the immune system. This represents a significant advantage, as immune rejection can be circumvented only by continuous administration of immunosuppressive drugs, and the drugs themselves may cause deleterious side effects

Previous | V. What are the similarities and differences between embryonic and adult stem cells? | Next

Page citation: Stem Cell Basics: What are the similarities and differences between embryonic and adult stem cells? . In *Stem Cell Information* [World Wide Web site]. Bethesda, MD: National Institutes of Health, U.S. Department of Health and Human Services, 2009 [cited Friday, September 17, 2010] Available at <http://stemcells.nih.gov/info/basics/basics5>

National Institutes of Health (NIH)
9000 Rockville Pike
Bethesda, Maryland 20892

Department of Health
and Human Services (DHHS)

USA.gov
The U.S. government's official web portal

Stem Cell Information

The National Institutes of Health resource for stem cell research

Stem Cell Basics

VI. What are induced pluripotent stem cells?

Induced pluripotent stem cells (iPSCs) are adult cells that have been genetically reprogrammed to an embryonic stem cell–like state by being forced to express genes and factors important for maintaining the defining properties of embryonic stem cells. Although these cells meet the defining criteria for pluripotent stem cells, it is not known if iPSCs and embryonic stem cells differ in clinically significant ways. Mouse iPSCs were first reported in 2006, and human iPSCs were first reported in late 2007. Mouse iPSCs demonstrate important characteristics of pluripotent stem cells, including expressing stem cell markers, forming tumors containing cells from all three germ layers, and being able to contribute to many different tissues when injected into mouse embryos at a very early stage in development. Human iPSCs also express stem cell markers and are capable of generating cells characteristic of all three **germ layers**.

Although additional research is needed, iPSCs are already useful tools for drug development and modeling of diseases, and scientists hope to use them in transplantation medicine. Viruses are currently used to introduce the reprogramming factors into adult cells, and this process must be carefully controlled and tested before the technique can lead to useful treatments for humans. In animal studies, the virus used to introduce the stem cell factors sometimes causes cancers. Researchers are currently investigating non-viral delivery strategies. In any case, this breakthrough discovery has created a powerful new way to "de-differentiate" cells whose developmental fates had been previously assumed to be determined. In addition, tissues derived from iPSCs will be a nearly identical match to the cell donor and thus probably avoid rejection by the immune system. The iPSC strategy creates pluripotent stem cells that, together with studies of other types of pluripotent stem cells, will help researchers learn how to reprogram cells to repair damaged tissues in the human body.

Previous | VI. What are induced pluripotent stem cells? | Next

Page citation: . In *Stem Cell Information* [World Wide Web site]. Bethesda, MD: National Institutes of Health, U.S. Department of Health and Human Services, 2009 [cited Friday, September 17, 2010] Available at <http://stemcells.nih.gov/info/basics/basics10>

National Institutes of Health (NIH)
9000 Rockville Pike
Bethesda, Maryland 20892

Department of Health
and Human Services (DHHS)

USA.gov
The U.S. government's official web portal

Stem Cell Information

The National Institutes of Health resource for stem cell research

Stem Cell Basics

VII. What are the potential uses of human stem cells and the obstacles that must be overcome before these potential uses will be realized?

There are many ways in which human stem cells can be used in research and the clinic. Studies of **human embryonic stem cells** will yield information about the complex events that occur during human development. A primary goal of this work is to identify how **undifferentiated** stem cells become the differentiated cells that form the tissues and organs. Scientists know that turning **genes** on and off is central to this process. Some of the most serious medical conditions, such as cancer and birth defects, are due to abnormal **cell division** and **differentiation**. A more complete understanding of the genetic and molecular controls of these processes may yield information about how such diseases arise and suggest new strategies for therapy. Predictably controlling cell proliferation and differentiation requires additional basic research on the molecular and genetic signals that regulate cell division and specialization. While recent developments with iPS cells suggest some of the specific factors that may be involved, techniques must be devised to introduce these factors safely into the cells and control the processes that are induced by these factors.

Human stem cells could also be used to test new drugs. For example, new medications could be tested for safety on differentiated cells generated from human **pluripotent** cell lines. Other kinds of cell lines are already used in this way. Cancer cell lines, for example, are used to screen potential anti-tumor drugs. The availability of pluripotent stem cells would allow drug testing in a wider range of cell types. However, to screen drugs effectively, the conditions must be identical when comparing different drugs. Therefore, scientists will have to be able to precisely control the differentiation of stem cells into the specific cell type on which drugs will be tested. Current knowledge of the signals controlling differentiation falls short of being able to mimic these conditions precisely to generate pure populations of differentiated cells for each drug being tested.

Perhaps the most important potential application of human stem cells is the generation of cells and tissues that could be used for **cell-based therapies**. Today, donated organs and tissues are often used to replace ailing or destroyed tissue, but the need for transplantable tissues and organs far outweighs the available supply. Stem cells, directed to differentiate into specific cell types, offer the possibility of a renewable source of replacement cells and tissues to treat diseases including Parkinson's and Alzheimer's diseases, spinal cord injury, stroke, burns, heart disease, diabetes, osteoarthritis, and rheumatoid arthritis.

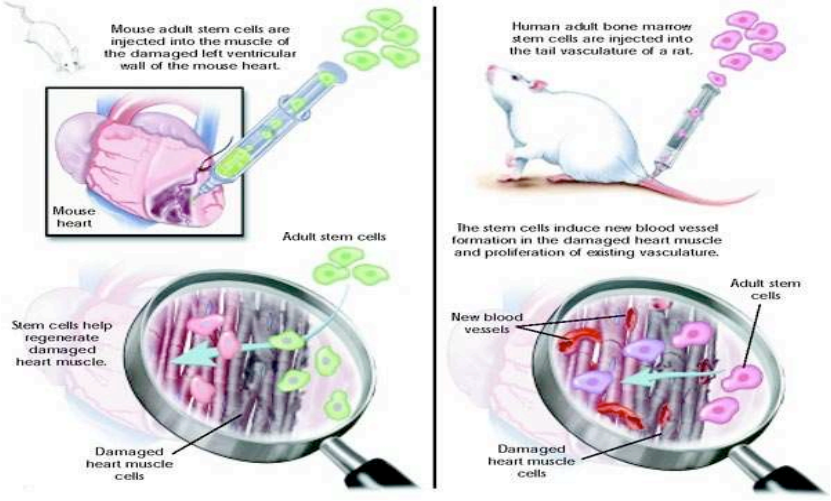


Figure 3. Strategies to repair heart muscle with adult stem cells. Click here for larger image.
© 2001 Terese Winslow

For example, it may become possible to generate healthy heart muscle cells in the laboratory and then transplant those cells into patients with chronic heart disease. Preliminary research in mice and other animals indicates that bone marrow stromal cells, transplanted into a damaged heart, can have beneficial effects. Whether these cells can generate heart muscle cells or stimulate the growth of new blood vessels that repopulate the heart tissue, or help via some other mechanism is actively under investigation. For example, injected cells may accomplish repair by secreting growth factors, rather than actually incorporating into the heart. Promising results from animal studies have served as the basis for a small number of exploratory studies in humans (for discussion, see call-out box, "Can Stem Cells Mend a Broken

Heart?"). Other recent studies in **cell culture** systems indicate that it may be possible to direct the **differentiation** of embryonic stem cells or adult bone marrow cells into heart muscle cells (Figure 3).

Can Stem Cells Mend a Broken Heart?: Stem Cells for the Future Treatment of Heart Disease

Cardiovascular disease (CVD), which includes hypertension, coronary heart disease, stroke, and congestive heart failure, has ranked as the number one cause of death in the United States every year since 1900 except 1918, when the nation struggled with an influenza epidemic. Nearly 2600 Americans die of CVD each day, roughly one person every 34 seconds. Given the aging of the population and the relatively dramatic recent increases in the prevalence of cardiovascular risk factors such as obesity and type 2 diabetes, CVD will be a significant health concern well into the 21st century.

Cardiovascular disease can deprive heart tissue of oxygen, thereby killing cardiac muscle cells (cardiomyocytes). This loss triggers a cascade of detrimental events, including formation of scar tissue, an overload of blood flow and pressure capacity, the overstretching of viable cardiac cells attempting to sustain cardiac output, leading to heart failure, and eventual death. Restoring damaged heart muscle tissue, through repair or regeneration, is therefore a potentially new strategy to treat heart failure.

The use of embryonic and adult-derived stem cells for cardiac repair is an active area of research. A number of stem cell types, including embryonic stem (ES) cells, cardiac stem cells that naturally reside within the heart, myoblasts (muscle stem cells), adult bone marrow-derived cells including mesenchymal cells (bone marrow-derived cells that give rise to tissues such as muscle, bone, tendons, ligaments, and adipose tissue), endothelial progenitor cells (cells that give rise to the endothelium, the interior lining of blood vessels), and umbilical cord blood cells, have been investigated as possible sources for regenerating damaged heart tissue. All have been explored in mouse or rat models, and some have been tested in larger animal models, such as pigs.

A few small studies have also been carried out in humans, usually in patients who are undergoing open-heart surgery. Several of these have demonstrated that stem cells that are injected into the circulation or directly into the injured heart tissue appear to improve cardiac function and/or induce the formation of new capillaries. The mechanism for this repair remains controversial, and the stem cells likely regenerate heart tissue through several pathways. However, the stem cell populations that have been tested in these experiments vary widely, as do the conditions of their purification and application. Although much more research is needed to assess the safety and improve the efficacy of this approach, these preliminary clinical experiments show how stem cells may one day be used to repair damaged heart tissue, thereby reducing the burden of cardiovascular disease.

In people who suffer from type I diabetes, the cells of the pancreas that normally produce insulin are destroyed by the patient's own immune system. New studies indicate that it may be possible to direct the differentiation of human embryonic stem cells in cell culture to form insulin-producing cells that eventually could be used in transplantation therapy for persons with diabetes.

To realize the promise of novel cell-based therapies for such pervasive and debilitating diseases, scientists must be able to manipulate stem cells so that they possess the necessary characteristics for successful differentiation, transplantation, and engraftment. The following is a list of steps in successful cell-based treatments that scientists will have to learn to control to bring such treatments to the clinic. To be useful for transplant purposes, stem cells must be reproducibly made to:

- Proliferate extensively and generate sufficient quantities of tissue.
- Differentiate into the desired cell type(s).
- Survive in the recipient after transplant.
- Integrate into the surrounding tissue after transplant.
- Function appropriately for the duration of the recipient's life.
- Avoid harming the recipient in any way.

Also, to avoid the problem of immune rejection, scientists are experimenting with different research strategies to generate tissues that will not be rejected.

To summarize, stem cells offer exciting promise for future therapies, but significant technical hurdles remain that will only be overcome through years of intensive research.

Previous | VII. What are the potential uses of human stem cells and the obstacles that must be overcome before these potential uses will be realized? | Next

Page citation: Stem Cell Basics: What are the potential uses of human stem cells and the obstacles that must be overcome before these potential uses will be realized? . In *Stem Cell Information* [World Wide Web site]. Bethesda, MD: National Institutes of Health, U.S. Department of Health and Human Services, 2009 [cited Friday, September 17, 2010] Available at <http://stemcells.nih.gov/info/basics/basics6>

National Institutes of Health (NIH)
9000 Rockville Pike
Bethesda, Maryland 20892

Department of Health

and Human Services (DHHS)

USA.gov
The U.S. government's official web portal

Stem Cell Information

The National Institutes of Health resource for stem cell research

Stem Cell Basics

VIII. Where can I get more information?

For a more detailed discussion of stem cells, see the NIH's Stem Cell Reports page. Check the Frequently Asked Questions page for quick answers to specific queries. The navigation table at right can connect you to the information you need.

The following websites, which are not part of the NIH Stem Cell site, also contain information about stem cells. The NIH is not responsible for the content of these sites.

- http://www.isscr.org/public/index.htm
  Stem cell information for the public from the International Society for Stem Cell Research (ISSCR).
- http://www.nlm.nih.gov/medlineplus/stemcells.html
  Medline Plus is a consumer health database that includes news, health resources, clinical trials, and more
- http://www.explorestemcells.co.uk
  A United Kingdom-based resource for the general public that discusses the use of stem cells in medical treatments and therapies.
- http://www.stemcellresearchnews.com
  A commercial, online newsletter that features stories about stem cells of all types.

Previous | VIII. Where can I get more information?

Page citation: Stem Cell Basics: Where can I get more information? . In *Stem Cell Information* [World Wide Web site]. Bethesda, MD: National Institutes of Health, U.S. Department of Health and Human Services, 2009 [cited Friday, September 17, 2010] Available at <http://stemcells.nih.gov/info/basics/basics7>

National Institutes of Health (NIH)
9000 Rockville Pike
Bethesda, Maryland 20892

Department of Health
and Human Services (DHHS)

USA.gov
The U.S. government's official web portal

Stem Cell Information

The National Institutes of Health resource for stem cell research

Highlights of Stem Cell Research

Due to copyright restrictions, the full text of articles linked below is available only to the NIH community. Those outside the NIH community can access citations and abstracts.

Other Years

2002 Articles ‖ 2003 Articles ‖ 2004 Articles ‖ 2005 Articles ‖ 2006 Articles ‖ 2007 Articles ‖ 2008 Articles ‖

2009 Articles

- **Induced Pluripotent Stem Cells Able to Produce Live Mice**
  In the field of animal stem cell research, a stem cell's ability to produce a live mouse in a tetraploid complementation assay is the gold standard test for pluripotency. Until recently, only mouse embryonic stem cells had demonstrated this ability. Now, two groups of scientists in China report that they have reprogrammed adult mouse cells using the four pluripotency genes reported in the first mouse iPSC publication **Scientists Reprogram Adult Mouse Skin Cells by Adding Defined Factors** and generated iPSC-derived embryos that survived gestation and were born alive after tetraploid complementation. One laboratory also demonstrated that an iPSC-generated male mouse was capable of impregnating a female and passing on its iPSC-derived characteristics. This research is an important demonstration that iPSCs are truly pluripotent. *Nature Advance Online Publication doi 10.1038*, laboratory of Q. Zhou; *Cell Stem Cell Advance Online Publication doi: 10.1016*, laboratory of S. Gao.

- **Cancer-destroying Cells Generated from Human Embryonic Stem Cells**
  Natural Killer, or NK cells, are a specialized type of white blood cells that continuously patrol the body, eliminating cells that have become abnormal, such as cancer cells. In healthy individuals, NK cells eliminate cancerous cells before they can cause problems. In some individuals, however, their native NK cells are unable to eliminate all cancerous cells. NK cells generated from umbilical cord blood (UCB-NK cells) are already being used to treat individuals with cancer, but they are neither consistent nor efficient in destroying cancer cells. NIH-supported scientists at the University of Minnesota hope to generate a more potent version of NK cells for use in cancer therapies, and developed a way to coax human embryonic stem cells (hESCs) to differentiate into NK cells. The scientists studied mice with human cancers to compare the cancer-destroying ability of hESC-derived NKs to the abilities of UCB-NK cells. They found that hESC-derived NKs were better than UCB-NKs at destroying both leukemia (blood cancer) and solid tumors, such as breast and prostate cancer. The hESC-derived NK cells not only destroyed human cancers in mice, but also protected them from recurrence and metastasis. Further studies will address whether other hESC lines are capable of generating such potent NK cells. This research marks an important advance for scientists working to understand how NK cells work and how they may be used to attack and destroy human cancers. *Blood*, laboratory of D.S. Kaufman. 2009 Jun 11.

- **Human Corneal Stem Cells Repair Defective Corneas in Mice**
  The cornea helps to protect the eye from environmental irritants and serves as the eye's outermost lens, contributing between 65—75 percent of the eye's total focusing power. In most cases, scratches on the cornea caused by irritants or trauma can be repaired by the cornea's own stem cells. However, deeper scratches can cause corneal scarring, resulting in a haze on the cornea that can greatly impair vision. In this case, the cornea is unable to repair itself, and a corneal transplant may be needed. As with most transplant organs, corneas are in low supply. NIH-supported scientists transplanted stem cells from the adult human corneal stroma (cells that make up the transparent cornea) into the eyes of mice that exhibit corneal cloudiness. These mice's eyes lack the ability to produce a protein called lumican, which organizes the cornea's collagen in order to make it transparent. After injection of the human cornea stromal stem cells, the transparency of the treated corneas was comparable to those in mice with normal corneas. Treated mice did not reject the transplanted human cells. The scientists will now try to reproduce this result in animals with cornea scarring. If successful, the scientists may be able to develop a potential stem cell therapy for cornea scarring in humans. *Stem Cells* epub 2009, laboratory of J. Funderburgh.

- **Another Safety Improvement for Generating Induced Pluripotent Stem Cells (iPSCs)**
  Scientists funded by the Juvenile Diabetes Research Foundation, the United Kingdom, and Canada reprogrammed mouse and human fibroblasts without using potentially dangerous viruses. For both types of fibroblasts, the reprogramming genes and an inducible transcription factor (can be used to turn expression on and off) were carried into the cells by naked DNA sequences. The naked DNA carriers also contained marking sequences that are targeted and "cut out" by specific enzymes. Using these special carriers, the scientists were able to insert reprogramming genes, turn them on for a specific period of time, and then remove the reprogramming genes and the transcription factor by adding the specific enzyme that zeroes in on and cuts out its targets. This method has several benefits: temporary expression of the reprogramming genes, the ability to remove inserted DNA after reprogramming is accomplished, use of a single carrier for all four reprogramming genes, and carriers' seeming increased resistantance to "silencing," or being inactivated (which could explain the higher efficiency as compared to other non-viral carriers).

  This method has some potential drawbacks. Insertion of the reprogramming factors is random and could still temporarily interfere with an important gene. Part of the carrier DNA is often left behind even after removal. The DNA cuts made at the DNA removal site are not always repaired correctly. The PiggyBac method used for some of the experiments employs a transposon, or "jumping gene." Jumping genes are known to cause human diseases such as muscular dystrophy or hemophilia, as well as increase susceptibility to cancer. The bottom line: These methods are another step toward improving our ability to reprogram cells and increasing our understanding of reprogramming. However, these methods could still pose a danger to human health if derivatives of these cells are used to treat humans. The cells generated by this method are a valuable research tool and provide useful means to screen drugs and establish human disease models in culture. *Nature* advance online publication, laboratory of A. Nagy; *Nature* advance online publication, laboratory of K. Woltjen. 2009 Mar 6.

- **Induced Pluripotent Stem Cell–Derived Working Heart Muscle Cells**
  Heart transplants are done as a life-saving measure for end-stage heart failure when medical treatment and less drastic surgery have failed. Fortunately, most heart transplant recipients (about 90 percent) can come close to resuming their normal daily activities; however,

donor hearts are in short supply. NIH-supported scientists have been able to grow heart muscle cells (cardiomyocytes) from induced pluripotent stem cells (iPSCs). They compared cardiomyocytes derived from iPSCs with cardiomyocytes derived from human embryonic stem cells (hESCs). All cardiomyocytes in the study were derived using an embryoid body (EB) method. Both iPSC- and hESC-derived cardiomyocytes showed a reduction in gene expression for OCT4 and NANOG (known to regulate pluripotency) as they differentiated. However, pluripotency gene expression was more variable in iPSC-derived cardiomyocytes. Both types of cardiomyocytes demonstrated heart muscle–specific characteristics, such as organized bands of contraction proteins, and electrical activity that causes them to spontaneously contract. Overall, the iPSC-derived cardiomyocytes are very similar to hESC-derived cardiomyocytes. Due to the short supply of donor hearts for transplantation, these iPSC-derived cardiomyocytes may one day provide an important treatment for the substantial number of people with heart disease. By reprogramming their own skin cells into cardiomyocytes for repairing their heart muscle, patients can avoid the immune-suppressing drugs that accompany traditional heart transplant. Scientists also hope that the derived cardiomyocytes will be useful for testing potential drugs and for understanding the underlying cause of heart disease. *Circulation Research* advance online publication, laboratory of T. Kamp. 2009 Feb 12.

Page citation: Highlights of Stem Cell Research . In *Stem Cell Information* [World Wide Web site]. Bethesda, MD: National Institutes of Health, U.S. Department of Health and Human Services, 2010 [cited Thursday, September 23, 2010] Available at <http://stemcells.nih.gov/research/scilit/highlights/defaultpage>

Frequently Asked Questions
What are stem cells?
Can they cure diseases?
Are there ethical issues?
What is the U.S. policy?
More FAQs
Links to related resources
Stem Cell Research
NIH Stem Cell Unit
Current Research
Upcoming Events
Funding for Research
Training Programs
Scientific Literature
Tools
Site Map
Glossary
Downloads
Text size: smaller | BIGGER

Home › Research Topics › Scientific Literature › Highlights of Stem Cell Research › 2008 Articles

2008 Articles

Due to copyright restrictions, the full text of articles linked below is available only to the NIH community. Those outside the NIH community can access citations and abstracts.

Other Years

2002 Articles ‖ 2003 Articles ‖ 2004 Articles ‖ 2005 Articles ‖ 2006 Articles
2007 Articles ‖ 2009 Articles ‖ 2009 Articles ‖ 2010 Articles ‖

Induced Pluripotent Stem Cell-Derived Motor Neurons Show Symptoms of Disease

Scientists are deriving induced pluripotent stem cells (iPSCs) from individuals with hereditary diseases, in hopes of generating cells and tissues from the iPSCs to learn more about the cause of the disease and in some cases, to develop new treatments. However, scientists don't know whether the iPSC-derived cells and tissues will exhibit disease characteristics. NIH-supported scientists generated an iPSC line from a child with spinal muscular atrophy (SMA) and from the child's mother, who does not have SMA. SMA is a group of hereditary diseases that cause weakness and wasting of the voluntary muscles of infants' and children's arms and legs. The diseases are caused by an abnormal or missing gene known as the survival motor neuron gene (SMN1), which is responsible for the production of a protein essential to motor neurons. The scientists found that motor neurons derived from the SMA-affected (SMA-iPSC-derived) child began to die after a month in culture, while motor neurons derived from the child's mother's iPSCs survived. The death of SMA-iPSC-derived motor neurons mirrors what happens in an affected child and indicates that the cells exhibit at least one important characteristic of SMA. The SMA-iPSC-derived motor neurons also responded to treatment with drugs known to increase the production of the missing motor neuron protein. These SMA-iPSC-derived motor neurons provide an important new in *vitro* model of SMA, and scientists can use them to test new drugs for SMA and to study how and when SMA develops. *Nature* advance online publication, laboratory of C. Svendsen. 2008 Dec 21.

Safer Reprogramming of Human Cells

In 2007, scientists generated induced pluripotent stem cells, or iPSCs, from adult skin fibroblasts (see Human Skin Cells Reprogrammed). Two features of the original technique make it unlikely that these cells will be used to derive cells for human transplantation. First, the reprogramming factors include cancer-promoting genes. Second, the factors are carried into the adult cells using inactivated viruses that integrate at random into the host DNA, introducing the possibility that the virus will interrupt or otherwise damage a critical gene. Scientists have recently developed several new alternative iPSC production methods. The iPSCs produced in each new method appear to be very similar to iPSCs produced in the traditional method. Each new method has its own advantages and disadvantages as compared to the original method, and each provides insight into how scientists may be able to develop iPSCs that are safe for use in clinical trials.

Privately funded investigators at Harvard University added a potent chemical, valproic acid, to newborn human skin (fibroblast) cells in culture. This treatment unravels the DNA to permit access to genes, and the scientists then needed to add only two reprogramming factors to the cells, rather than the usual four factors needed in traditional iPS cell reprogramming. The two eliminated factors are the potent cancer-promoting genes *c-Myc* and *Klf4*. This technique uses newborn rather than adult cells, and still uses potentially harmful viruses to reprogram. However, by successfully eliminating the 2 cancer-promoting factors, scientists hope they may

one day be able to use chemicals rather than viruses to reprogram human cells. *Nature* advance online publication, laboratory of D. Melton. 2008 Oct 12.

NIH-supported scientists at Harvard University developed a method that uses a different virus—an adenovirus—as a means of carrying the reprogramming factors into newborn mouse skin and adult mouse liver cells. The adenovirus has the advantage of not usually integrating into the DNA, and thus avoids the potential for interrupting or otherwise damaging a critical gene. The virus needs to be present for only a short time (several days) in order to accomplish reprogramming. However, the technique is very inefficient when compared to generating iPSCs with retroviruses, still uses cancer-promoting genes, and the adenovirus may still integrate into the host DNA at low frequencies. The scientists are now working to use adenoviruses to reprogram human cells. *Science* advance online publication, laboratory of K. Hochedlinger. 2008 Sep 25.

A third group reported success at generating iPSCs without using any viruses. Japanese researchers successfully reprogrammed mouse cells by using only "naked" DNA of the reprogramming factors—in a circular, or plasmid form. The scientists introduced the plasmids into mouse embryonic skin cells via transfection, and were able to generate iPSCs. This method has the advantage of avoiding any use of viruses, but still uses cancer-promoting genes to accomplish reprogramming. It is also much less efficient than the original reprogramming method, and begins with embryonic skin cells, which may be more amenable to reprogramming than adult skin cells. *Science* advance online publication, laboratory of S. Yamanaka. 2008 Oct 9.

- **Pluripotent Stem Cells from Adult Human Testis**
  In 2006, German scientists succeeded in coaxing adult mouse stem cells that normally produce sperm (spermatogonial stem cells, or SSCs) to instead behave in a manner similar to embryonic stem cells (ESCs; see Pluripotent Stem Cells Found in Adult Mouse Testicles). Now, another team of German scientists has succeeded in generating pluripotent stem cells from tissue biopsied from human testicles. They call the cells human adult GSCs, for germline stem cells. Human adult GSCs demonstrate many characteristics of pluripotent cells, including the ability to form teratomas and generate cells characteristic of all three germ layers. Scientists hope to learn more about development and differentiation by comparing these human adult GSCs with both human embryonic stem cells (hESCs)and induced pluripotent stem cells (iPSCs). *Nature* advance online publication, lab of T. Skutella. 2008 Oct. 8.
- **Adult Human Skin Cells Reprogrammed into Insulin-Secreting Cells**
  Scientists are developing a variety of options that may one day enable replacement of the insulin-producing beta islet cells of the pancreas that are lost in individuals with type 1 diabetes. Previously, NIH-funded scientists reprogrammed differentiated pancreatic exocrine cells in adult mice into cells that closely resemble beta cells. (See Adult Pancreas Cells Directly Reprogrammed to Insulin-Secreting Beta Cells.) In that study, mouse cells were not taken all the way back to a primitive, or embryonic-like state, but were converted to another fate, in a process the scientists termed "direct reprogramming". Now, privately funded scientists report reprogramming human foreskin fibroblasts into induced pluripotent stem cells, or iPSCs. The iPSCs were then differentiated into islet-like clusters (ILCs) that secreted insulin when glucose was added to the cell cultures in the laboratory. The researchers are now working to develop iPSCs from individuals with diabetes, in the hope of one day producing patient-specific insulin producing cells. Although transplantation can restore insulin production, it does not address the autoimmune destruction of the individual's own beta cells that initially results in type 1 diabetes and may recur, thereby destroying the newly transplanted cells. Scientists also hope that cells generated from individuals with type 1 diabetes will be useful for testing potential diabetes drugs and for understanding the underlying cause of the disease. *Journal of Biological Chemistry*, laboratory of Y. Zhang. 2008 Sep 9.
- **Adult Pancreas Cells Directly Reprogrammed to Insulin-Secreting Beta Cells**
  The success of iPS cell reprogramming (see Scientists Reprogram Adult Mouse Skin Cells by Adding Defined Factors) led scientists to wonder if adult cells could be directly reprogrammed from one type to another, without the need to take them all

the way back to a pluripotent stem cell. NIH-funded scientists now report successful direct reprogramming of adult exocrine cells from the pancreas into cells that resemble beta cells. Based on their knowledge of normal beta cell development, they were able to "re-start" expression of three critical beta cell genes in the differentiated adult exocrine pancreas cells. The reprogrammed cells are similar to beta cells in appearance, size, and shape; express genes characteristic of beta cells; and are able to partially restore blood sugar regulation in mice whose own beta cells have been chemically destroyed. This method for reprogramming adult cells may now be used as a model for directly reprogramming other adult cell types. *Nature* advance online publication, laboratory of D. Melton. 2008 Aug 27.

- **Adult Stem Cell Lines Created for 10 Additional Human Diseases**
  One week after the report of induced pluripotent stem cells (iPS cells) generated from an Amyotrophic Lateral Sclerosis (ALS) patient, NIH-funded scientists reported generating iPS cell lines carrying 10 additional human diseases. The new iPS cell lines were generated from individuals with Duchenne muscular dystrophy, Becker muscular dystrophy, juvenile-onset (type 1) diabetes, Parkinson's disease, Huntington's disease, Down syndrome, ADA severe combined immunodeficiency, Shwachman-Bodian-Diamond syndrome, Gaucher disease, and a carrier of Lesch-Nyhan Syndrome. As before, scientists must still determine whether the relevant cell types derived from these lines demonstrate symptoms of the diseases. For example—do muscle cells from the Duchenne MD iPS line behave as they do in individuals with the disease? The cell lines are capable of long term self-renewal in culture, thus providing a potentially endless supply of material for study of disease processes and testing of potential drugs on human cells. Scientists may now be able to generate and compare iPS lines from individuals with the same disease but different symptoms—a potential insight into what is due to inheritance and what is due to environment. *Cell* advance online publication, laboratories of C. Cowan, K. Hochedlinger, G. Daley. 2008 August 6.
- **Mouse Embryonic Stem Cells Used to Predict Human Breast Cancer Risk**
  Scientists at the National Cancer Institute (NCI) at the NIH report a research breakthrough in predicting human breast cancer risk. *Nature Medicine* 14(8), laboratory of S. Sharan. 2008 August 1.
- **Scientists Generate Stem Cell Line from Patient with Lou Gehrig's Disease**
  Privately funded scientists report successfully generating stem cells from a patient with an inherited form of Lou Gehrig's disease, or amyotrophic lateral sclerosis (ALS). Starting with skin cells from the patient, the scientists used viruses to insert factors to reprogram the adult skin cells into induced pluripotent stem cells (iPSC) (see Human Skin Cells Reprogrammed). Once they had generated an ALS-iPSC line, the scientists coaxed the cells into becoming the type of motor neurons that are destroyed in ALS. These iPSC-derived motor neurons carry genes responsible for ALS and hold great potential for investigating the ALS disease process in human cells. Scientists are still uncertain whether the iPSC-derived motor neurons will degenerate in the same way as the patient's naturally occurring motor neurons. Ongoing experiments are comparing healthy motor neurons to the ALS-iPSC–derived motor neurons. If the iPSC-derived motor neurons show signs of ALS-like degeneration, they will be invaluable for observing events in the course of the ALS disease process and for testing potential ALS drugs on human cells in the laboratory before the drugs are used in humans. *Science* advance online publication, laboratory of K. Eggan. 2008 July 31.
- **Adult Mouse Neural Stem Cells Reprogrammed Using Fewer Factors**
  The current techniques for reprogramming adult cells require the use of viruses to insert several pluripotency factors into each cell's DNA (see Human Skin Cells Reprogrammed). Both viruses and DNA insertions could cause negative health consequences, so elimination of one or both is desirable if reprogrammed cells are to be used in clinical applications. German scientists selected a starting cell type (adult neural stem cells) that already expresses high levels of two factors known to be important for reprogramming. Using these cells, the scientists generated reprogrammed adult cells by inserting fewer factors. If this technique works in other types of adult cells with high levels of endogenous reprogramming factor expression, it will bring the field one step closer to enabling the use of stem cells to treat humans. *Nature* 454:646–50, laboratory of H. Scholer. 2008 July 31.

- **Transplanted Adult Stem Cells Improve Muscle Function in Mouse Model of Muscular Dystrophy**
  In January, privately funded scientists reported improvement in a mouse model of muscular dystrophy (MD) treated with muscle cells derived from mouse embryonic stem cells. Now, NIH-funded scientists have developed a method to isolate a specific type of adult mouse muscle stem cells that improves muscle function when transplanted into mice suffering from muscular dystrophy. The transplanted muscle stem cells were also able to establish a pool of non-diseased cells for continued repair and replacement of damaged muscle. This method uses mouse muscle stem cells, rather than beginning with undifferentiated embryonic stem cells and driving them to become muscle. Scientists hope to identify a similar human adult muscle stem cell population in order to learn more about what enables the cells to self-renew and possibly to learn to boost their regenerative potential. This research may one day lead to treatments for individuals with MD. *Cell* 134(1):37–47, laboratory of A. Wagers. 2008 July 11.

- **Human Embryonic Stem Cells Generate Heart Progenitor Cells**
  Heart disease, or coronary artery disease, is a leading cause of death in the United States. Although treatments for heart disease are constantly improving, scientists would also like to be able to replace damaged heart tissue. One possible source is human heart cells derived from human embryonic stem cells (hESCs). Previously, scientists were able to drive mouse embryonic stem cells to become mouse cardiovascular progenitor cells, which have the capacity to become any of the three distinct cell types that compose the adult heart. Now the same scientists have induced hESCs to become human heart progenitor cells. As with the mouse heart progenitor cells, the human heart progenitor cells can produce the three main heart cell types—cardiomyocytes (contractile heart muscle cells), endothelial cells (cells that line the blood vessels), and vascular smooth muscle cells (cells that provide elasticity to blood vessels). The scientists identified key characteristics that enabled them to sort the heart progenitor cells from other cells in culture. Finally, they verified that cardiomyocytes derived via this process are functional by examining their expression of cardiac genes, their ability to conduct electrical current, and their ability to repair the pumping ability of mouse hearts damaged by induced heart attacks. This work provides a first step for developing human heart cells to be used for human heart tissue transplantation, or to test prospective heart drugs. *Nature* 453(7194):524–8, laboratory of G. Keller. 2008 May 22.

- **Neurons from Reprogrammed Adult Mouse Skin Cells Improve Symptoms in Rat Model of Parkinson's Disease**
  Scientists hope one day to replace the dopamine-producing nerve cells (neurons) lost in Parkinson's Disease with neurons derived from stem cells. Previously, scientists coaxed human embryonic stem cells (hESCs) into becoming dopamine-producing neurons. Another team of scientists now report generating dopamine-producing neurons from mouse-induced pluripotent stem cells (iPSCs). They tested the function of their derived dopamine-producing neurons by injecting them into the brains of rats used as a model for Parkinson's disease. Treated rats showed improvement in their Parkinsonian symptoms. These results demonstrate that animal iPSCs are capable of replacing lost cells and improving disease in animal models. They also offer hope that human iPSCs may one day enable scientists to develop patient-specific cells for replacing those lost or damaged by disease. *Proceedings of the National Academy of Sciences of the USA* 105(15):5856–5861, laboratory of R. Jaenisch. 2008 April 15.

- **What Molecular Changes Enable Reprogramming?**
  Scientists have successfully reprogrammed adult mouse and human cells to behave like embryonic stem cells (ESCs). These reprogrammed adult cells are known as induced pluripotent stem cells, or iPS cells. Although iPS cells share many characteristics of ESCs, scientists have not yet identified what molecular changes enable reprogramming. To address these questions, NIH-funded scientists developed a special virus that allowed them to start and stop the expression of genes used in reprogramming (Oct4, Sox2, c-Myc, and Klf4) at will. Using their new "on/off switch," they determined the minimum amount of time that an adult cell must be exposed to these gene products in order to be reprogrammed. They also identified specific events, such as changes in level of gene expression or gene activation

versus inactivation, that are indicative of cells at different stages of the reprogramming process. Scientists can now use this information to sort cells that are reprogrammed from those that are not. They will also be able to use what they know about the stages of reprogramming and exposure time as they develop new reprogramming techniques that eliminate the potential cancer risks of the viruses and genes used in the current methods. *Cell Stem Cell* 2(3):230–240, laboratory of K. Hochedlinger. 2008 March 6.

- **Human Embryonic Stem Cell-Derived Neurons Treat Stroke in Rats**
  Scientists hope to use embryonic stem cells to generate neurons to replace those lost to disease, including the loss of nerve cells (neurons) in the brain that happens after a stroke. Scientists can already drive human embryonic stem cells (hESCs) into becoming neurons. However, transplants of these cells into animal models of human diseases sometimes "overgrow" and form tumors, suggesting that the transplants contain both desirable neurons and undesirable undifferentiated cells. NIH-funded scientists now report developing a cell culturing method that selects only human neural stem cells (hNSCs), and then drives them to become mature neurons, with no undifferentiated cells remaining. Transplants of these cells into rats did not produce any tumors, at least within the 2 month period of observation. In addition, rats that had suffered a stroke and subsequently stopped using one front paw began using that paw again after receiving transplanted human neurons. Post-mortem tissue sections of the treated rats' brains showed transplanted human neurons grew towards the site of neuron loss and did not appear to generate any tumors. The scientists now hope to study these hESC-derived neurons to learn how they differentiate and how they are different from human neurons derived from other culturing methods or tissue sources. Scientists also hope to adapt this technique to treat human stroke patients. *PLOS One* 3(2): e1644, laboratory of G.K. Steinberg. 2008 February 20.

- **Muscular Dystrophy in Mice Treated with Muscle from Mouse Embryonic Stem Cells**
  Muscular dystrophy (MD) is an inherited disease characterized by progressive weakness and degeneration of the skeletal muscles that control movement. Current treatments for MD aim to slow the disease's progression but can't cure it or completely halt its progression. One possible hope lies in replacing diseased muscles with new muscle cells, generated from embryonic stem cells (ESCs). However, scientists have had difficulty generating skeletal muscle from ESCs, due in part to a lack of useful ways to identify developing skeletal muscle amidst other cell types. Privately funded scientists have now developed such a method in mice, as well as another method to sort muscle cells from undifferentiated stem cells that could divide uncontrollably and produce tumors after transplantation. The scientists injected the mouse ESC–derived skeletal muscle cells into mice with an MD-like muscle-wasting condition. Tests showed that treated mice's muscles had an improved ability to contract, and treated mice fared better than untreated diseased mice on standard tests for muscle function. In the future, scientists hope to test the ability of human embryonic stem cell (hESC)–derived muscle cells to treat human MD. *Nature* advance online publication, laboratory of R.C.R. Perlingeiro. 2008 Jan 20.

- **Single Cell Biopsy Successfully Generates Human Embryonic Stem Cell Line; Biopsied Embryos Develop to Blastocyst Stage**
  In 2006, privately funded scientists successfully established human embryonic stem cell (hESC) lines using cells taken from pre-implantation human embryos (see Scientists Generate Human Embryonic Stem Cell Lines from Single Cells). However, the previous method involved dissociation of the embryos (i.e., the embryos were destroyed) and co-culture with existing hESCs. In this latest publication from the same laboratory, the scientists removed only one or two cells from each embryo via a biopsy procedure. These one or two cells were used to generate hESC lines. At the same time, the scientists cultured the biopsied embryos to the blastocyst stage and then froze them. One of the cell lines developed was cultured with a protein called laminin instead of being cultured with existing hESCs. However, ethical considerations make it uncertain whether scientists will ever test if the cells remaining after removal of a single cell can develop into a human being, at least in embryos that are not at risk for carrying a genetic disorder. *Cell Stem Cell*

doi:10.1016/j.stem.2007.12.013, laboratory of R. Lanza. 2008 Jan 10.

Other Years

2002 Articles ‖ 2003 Articles ‖ 2004 Articles ‖ 2005 Articles ‖ 2006 Articles
2007 Articles ‖ 2009 Articles ‖

2007 Articles

Due to copyright restrictions, the full text of articles linked below is available only to the NIH community. Those outside the NIH community can access citations and abstracts.

Other Years

2002 Articles ‖ 2003 Articles ‖ 2004 Articles ‖ 2005 Articles ‖ 2006 Articles
2008 Articles ‖ 2009 Articles ‖ 2009 Articles ‖ 2010 Articles ‖

Reprogrammed Mouse Skin Cells Cure Mouse Sickle Cell Anemia

Individuals with sickle cell anemia inherit a defective hemoglobin gene that causes their red blood cells (RBCs) to assume a sickle shape. The sickled RBCs clump together and block blood flow, causing pain and organ damage. NIH-funded scientists used a published method (see Human Skin Cells Reprogrammed) to reprogram mouse skin cells derived from a mouse carrying the defective hemoglobin gene, producing induced pluripotent stem cells (iPS cells). They then used homologous recombination to repair the defective hemoglobin gene and directed the iPS cells to become blood-forming (hematopoietic) stem cells. Hematopoietic stem cells are found in bone marrow and produce all the blood cells in the body. Finally, the scientists transplanted the repaired hematopoietic stem cells into the bone marrow of sickle cell mice whose own marrow was destroyed in order to eliminate the defective hematopoietic cells. The transplanted cells were able to regenerate the mice's blood systems, including production of normal rather than sickled red blood cells. This research advance demonstrates that reprogrammed adult mouse cells (iPS cells) are capable of producing cells that can treat disease in mice. However, the methods used in this study include use of a cancer-promoting gene and inactivated viruses, and are not likely to be used to treat humans. If scientists can develop safer methods to reprogram adult cells, iPS cells could one day generate cells and tissues to treat human diseases. *Science* 318:1920–23, laboratory of R. Jaenisch. 2007 Dec 6.

Human Skin Cells Reprogrammed

In 2006, Japanese scientists were able to reprogram adult mouse skin cells to behave like mouse embryonic stem cells, although the reprogrammed cells could not produce eggs or sperm (gametes). The scientists named the cells iPS cells, for induced pluripotent stem cells. In 2007, the Japanese researchers successfully generated gametes from iPS cells, and their results were verified and extended by another independent laboratory. Now, simultaneous publications from the Japanese scientists and a team of NIH-supported scientists report that they have each succeeded at reprogramming adult human skin cells to behave like human embryonic stem cells (hESCs). The Japanese team forced adult skin cells to express *Oct3/4, Sox2, Klf4,* and *c-Myc,* while the NIH-supported team forced adult skin cells to express *OCT4, SOX2, NANOG,* and *LIN28.* The genes were all chosen for their known importance in maintaining the so-called "stemness" properties of stem cells. In both reports, the adult skin cells are thus reprogrammed into human iPS cells that demonstrate important characteristics of pluripotency, including the ability to differentiate into cells characteristic of each embryonic germ layer. The techniques reported by these research teams will enable scientists to generate patient-specific and disease-specific human stem cell lines for laboratory study, and to test potential drugs on human cells in culture. However, these human iPS cells are not yet suitable for use in transplantation medicine. The current techniques use viruses that could generate tumors or other undesirable mutations in cells derived from iPS cells. Scientists are now working to accomplish reprogramming in adult human cells

without using potentially dangerous viruses. *Cell* 131:861–72, laboratory of S. Yamanaka, 2007 Nov 30; *Science* 318:1917–1920, laboratory of J. Thomson, 2007 Dec 21.

- **Monkey Embryonic Stem Cells Produced following SCNT**
  One possible way to produce patient-specific tissues for therapies is to generate them from stem cells produced by somatic cell nuclear transfer, or SCNT. However, the standard protocol for SCNT in other species has not been successful in primates, such as monkeys and humans. Now scientists using a modified SCNT protocol have successfully generated stem cell lines from rhesus macaque embryos (see the NIH Research Matters article Embryonic Stem Cell Milestone Achieved in Primates). The scientists eliminated the use of a stain (Hoechst) to visualize the egg's chromosomes, opting instead to use a specialized imaging system that enables visualization of the chromosomes without use of a stain. The scientists also removed calcium and magnesium from the medium bathing the eggs, in an attempt to keep the eggs from spontaneously "activating," or releasing internal stores of calcium ions. The monkey embryonic stem cells thus produced demonstrated key characteristics of pluripotency, including the ability to form tissues from all of the embryonic germ layers. Since monkeys are close to humans in evolution, scientists may be able to study these monkey stem cells and learn how to generate similar cells in humans. *Nature* 450:497–502, laboratory of S.M. Mitalipov. 2007 Nov 22.
- **Heart Cells Derived from Human Embryonic Stem Cells Help Restore Rat Heart Function**
  Heart disease impairs the heart's ability to pump blood and sustain the body's organs and tissues. Scientists hope to one day repair or replace damaged heart muscle cells with stem cells, but they face many critical challenges. These include generating enough new heart cells, making sure transplanted heart cells are not contaminated with immature or other cell types, and ensuring the heart cells' survival after transplantation. NIH-funded investigators developed a new technique to generate large numbers of pure cardiomyocytes (heart muscle cells) from human embryonic stem cells (hESCs). They also formulated a "prosurvival" cocktail (PSC) of factors designed to overcome several known causes of transplanted cell death. The scientists then induced heart attacks in rats and injected the rat hearts with either hESC-derived human cardiomyocytes plus PSC (treatment group) or one of several control preparations. Four weeks later, the scientists identified human cardiomyocytes being supported by rat blood vessels in treated rat hearts. The treated rat hearts also demonstrated an improved ability to pump blood. The scientists did not identify any surviving human cells in the control animals, and they saw no improvement in heart function. This work demonstrates that hESC-derived cardiomyocytes can survive and improve function in damaged rat hearts. Scientists now hope to learn how the human cells improved the rat hearts, and eventually to test this method to treat human heart disease. *Nature Biotechnology* 25(9):1015–1024, laboratory of CE Murry. 2007 Sept.
- **Researchers Isolate Adult Stem Cells for First Time in Tendon**
  This research advance was featured in a press release from the National Institute of Dental and Craniofacial Research (NIDCR). *Nature Medicine* (10):1219–27, laboratory of M. Young. 2007 Oct.
- **Scientists Uncover the Origin of the Korean Stem Cell Line SCNT-hES1**
  In 2004, scientists led by Woo-Suk Hwang at the Seoul National University in South Korea reported that they had succeeded in using somatic cell nuclear transfer (SCNT) to establish a human embryonic stem cell (hESC) line (see original report here). They claimed to have combined the DNA of a woman's mature cell with her donated egg (nucleus removed) and stimulated the newly combined cell to divide. They named their new hESC line SCNT-hES1. In January 2006, the editors of the journal *Science* retracted this and a subsequent paper from the Hwang research laboratory, citing Seoul National University's investigative report (PDF; get Adobe Reader), which determined that "a significant amount of the data presented in both papers is fabricated." A multinational group of NIH-funded investigators developed extensive experience identifying the origin of stem cell lines based upon their patterns of genetic recombination. Recently, this group examined the genetic recombination patterns of SCNT-hES1 and determined that it was likely derived via parthenogenesis instead of SCNT. The authors speculate that the Hwang lab's cell

line was the result of unsuccessful enucleation (removal of the nucleus), or that it fused with its own polar body after enucleation. *Cell Stem Cell* doi:10,1016/j.stem.2007.07.001 (PDF; get Adobe Reader), laboratory of G.Q. Daley. 2007 Sep.

- **Human Embryonic Stem Cells (hESC) Prefer to Become Different Types of Neurons**
  Possible successful treatment for individuals with neurodegenerative diseases may be achieved by adequately replacing their damaged or missing nerve cells with new nerve cells created from human embryonic stem cells (hESCs). Scientists have shown that hESCs can differentiate into nerve, heart, and other cells that can be implanted to restored damaged tissue. However, it has been difficult to determine the correct conditions to grow the hESCs to produce a specific cell type. Now, NIH- and privately supported scientists have compared mature neurons grown from two hESCs on the NIH Stem Cell Registry. They developed procedures to differentiate the two stem cell lines first into neural progenitor cells, and then into mature neurons. The scientists studied the neurons in a new culture technique to observe the biology, genetics, and development of synapses, which are the critical junctions between neurons where much of the signaling and communication occurs. They also compared the genetic microRNAs, small snippets of genetic material that are believed to be significant regulators of stem cell differentiation, produced by the two types of neurons. This study also showed that the two different hESC lines had the tendency to produce different types of neurons. Determining why different hESC lines grow and differentiate differently will help scientists start with any hESC line to produce particular cell types that can be used to help repair or regenerate damaged tissues. *Proceedings of the National Academy of Sciences of the USA* 104(34):13821–13826, laboratory of Y. Sun. 2007 Aug 21.

- **International Stem Cell Initiative Compares Embryonic Stem Cells Throughout the World**
  The International Stem Cell Initiative (ISCI) was established to compare a large and diverse set of human embryonic stem cell (hESC) lines derived and maintained in different research laboratories throughout the world. The ISCI has published its comparison of 59 hESC lines from 17 individual laboratories. Overall, the lines were remarkably similar. However, the ISCI identified differences in expression of imprinted genes and in X-chromosome inactivation. *Nature Biotechnology* 25(7):803–16. 2007 Jul.

- **Tissue-Matched Human Stem Cells Created without Cloning**
  Scientists have proposed the use of somatic cell nuclear transfer, or SCNT, to create stem cells that are tissue-matched to an individual. This process is also known as therapeutic cloning. However, due to exchange of genetic information between pairs of like chromosomes (homologous recombination) during the egg's meiosis, the stem cells created using this method may still not be a precise match for the nucleus donor. Previously, scientists derived stem cells from a mouse embryo that was created using a process known as parthenogenesis (see Tissue-Matched Stem cells Created in Mice without Cloning). Parthenogenesis describes an embryo created without fertilization of the egg by a sperm, thus omitting the sperm's genetic contributions. Now, privately funded scientists have used parthenogenesis to derive human embryonic stem cell lines (hESCs). These identified stem cell lines retained the identical "self" (genetic information of the egg donor) and were shown to be pluripotent. These hESC lines were also derived and grown on a human feeder layer. This technique may lead to the ability to generate tissue-matched cells for transplantation to treat women who are willing to provide their own egg cells. This technique could also offer an alternative method for deriving tissue-matched hESCs that do not require destruction of a fertilized embryo. *Cloning Stem Cells* advance online publication, laboratory of J.D. Janus. 2007 Dec 19.

- **Counterparts: Rodent Embryonic Stem Cell and Human Embryonic Stem Cell**
  Typically, embryonic stem cell (ESC) lines have been derived from the inner cell mass of a blastocyst-stage pre-implanted embryo. However, scientists have now reported that ESC lines can be derived from the epiblast, a derivative of the inner cell mass in an embryo at a later stage of development. It has also been known that rodent ESCs are similar to human ESCs (hESC), but they differ in how they maintain pluripotency, the ability to develop into virtually any cell type in the body. Now, two

independent teams of British, U.S., and Swedish scientists supported by the NIH, the British government, and other UK sources have reported these mouse and rat epiblast-derived stem cell (EpiSC) lines are even more similar to hESCs. Unlike mouse ESCs, which require culture conditions different from hESCs to grow, EpiSCs grow better in culture conditions similar to those for hESCs. The EpiSCs also share other molecular characteristics and cell surface markers with hESCs. Because of the similarities between EpiSCs and hESCs, these studies suggest an additional method for creating pluripotent stem cells that may offer a new animal model for understanding how human stem cells grow and differentiate. *Nature* advance online publication and *Nature* advance online publication, laboratories of R. McKay and R. Pedersen. 2007 June 27.

- **New Therapeutic Cloning Technique Does Not Require Unfertilized Eggs**
  A new technique developed by NIH-funded scientists at Harvard University may expand scientists' options when trying to "reprogram" an adult cell's DNA. Previously, successful somatic cell nuclear transfers (SCNT, or cloning) relied upon the use of an unfertilized egg. Now, the Harvard scientists have demonstrated that by using a drug to stop cell division in a fertilized mouse egg (zygote) at mitosis, they can successfully reprogram an adult mouse skin cell by taking advantage of the "reprogramming factors" that are active in the zygote at mitosis. They removed the chromosomes from the single-celled zygote's nucleus and replaced them with the adult donor cell's chromosomes. The active reprogramming factors turned genes on and off in the adult donor chromosomes, to make them behave like the chromosomes of a normally fertilized zygote. After the zygote was stimulated to divide, the cloned mouse embryo developed to the blastocyst stage, and the scientists were able to harvest embryonic stem cells from it. When the scientists applied their new method to abnormal mouse zygotes, they succeeded at reprogramming adult mouse skin cells and harvesting stem cells. If this technique can be repeated with abnormal human zygotes created in excess after in vitro fertilization (IVF) procedures, scientists could use them for research instead of discarding them as medical waste. Human embryonic stem cells generated in this way would be a genetic match for the chromosome donor (see therapeutic cloning), helping to avoid the problem of transplant rejection. In addition, use of excess IVF zygotes for SCNT would eliminate the need for human egg donations. This technique may overcome some ethical objections to deriving stem cells from 5-day-old human embryos, since the abnormal zygotes that would be used for this technique are not believed capable of surviving until birth. *Nature* 447:679–686, laboratory of K. Eggan. 2007 Jun 7.

- **New Advances in Reprogramming Adult Mouse Cells**
  In 2006, Japanese scientists reported that they could use a virus to introduce four important stem cell factors into adult mouse cells and reprogram them to behave like embryonic stem (ES) cells (see Scientists Reprogram Adult Mouse Skin Cells by Adding Defined Factors). They called the reprogrammed cells iPS, for induced pluripotent stem cells. However, iPS produced using the original technique cannot do everything that ES cells can do. Notably, the original iPS cells do not make sperm and egg cells when injected into an early mouse blastocyst, and they do not make some changes to their DNA that help silence genes. Now the same scientists have modified their original technique, and they report that they can select for iPS that can make sperm and eggs. Their report is accompanied by another from an NIH-funded laboratory, which successfully reproduced the Japanese group's results. In addition, the NIH-funded scientists determined that iPS DNA is modified in a manner similar to ES cells, and important stem cell genes are expressed at similar levels. They also demonstrated that iPS injected into an early mouse blastocyst can produce all cell types within the developing embryo, and such embryos can complete gestation and are born alive. These research advances were made in mice, and scientists must still determine if the same techniques can reprogram cells of adult humans. If this can be accomplished, scientists should be able to develop stem cell lines from patients who suffer from genetic diseases, such as Huntington's Disease, spinal muscular atrophy, muscular dystrophy, and thalessemia. Such lines would be invaluable research tools for understanding specific diseases and testing potential drugs to treat them. A second use of reprogrammed cells would be to repair damaged tissues in the human body. The Japanese scientists noted that the virus

used to introduce the stem cell factors sometimes caused cancers in the mice. This represents a significant obstacle that must be overcome before the technique can lead to useful treatments for humans. This work suggests an additional method for creating pluripotent stem cells that, together with studies of other types of pluripotent stem cells, will help scientists learn how to reprogram cells to repair damaged tissues in the human body. *Nature* advance online publications, 6 June 2007. Laboratories of R. Jaenisch and S. Yamanaka.

- **Scientists Identify Olfactory Stem Cells in Mammals**
  The odor-detecting tissue lining the nose (olfactory epithelium, or OE) is exposed to a wide variety of environmental insults—dirt, chemicals, other pollutants, viruses, and bacteria. These insults frequently kill cells in the OE—yet most humans can still detect odors. This is possible because the OE can regenerate itself. Although scientists presumed that the regenerative capability was due to division of resident stem cells, there were two possible candidates for the stem cell: globose basal cells (GBCs) or horizontal basal cells (HBCs). Scientists supported by the NIH's National Institute on Deafness and Other Communication Disorders (NIDCD) used a genetic tag to label early mouse HBCs and all of their cellular offspring, or daughter cells. Under normal circumstances the HBCs divided only rarely, and GBCs replaced any lost cells. Yet after severe damage that destroyed even the GBCs, the HBCs divided to produce GBCs—which subsequently produced all cell types in the OE (except HBCs). Scientists can now study how damaged OE stimulates division of its HBC stem cell population. This type of investigation may also help scientists figure out how to "jump start" stem cell division to help repair other organs, such as damaged nerves or insulin-producing cells. *Nature Neuroscience* 10(6):720–6, laboratory of R.R. Reed. 2007 Jun.

- **Mice Regenerate Hair Follicles**
  This research article was featured in NIH Research Matters, a review of NIH research from the Office of Communications and Public Liaison, Office of the Director, National Institutes of Health. *Nature* 447(17):316–320, laboratory of G. Cotsarelis. 2007 May.

- **Human Embryonic Stem Cells Give Rise to Lung Tissue**
  If scientists treating disease or injury by transplanting cells that were derived from human embryonic stem cells (hESCs) accidentally transplanted some undifferentiated cells, the undifferentiated cells might keep dividing, resulting in a tumor. Thus, before using hESCs to treat humans, scientists must first be able to generate a pure population of a specific cell type. NIH-funded scientists have developed a method to coax hESCs into becoming cells that resemble lung epithelial cells. The scientists engineered a virus (modified to eliminate its disease-transmitting function) to infect cells with two genes simultaneously, one that drives them into becoming a specialized type of lung cell and another that enables them to resist being killed by a drug (neomycin). Only those cells that express the two genes survived when the scientists treated the culture dish with neomycin. In this way, they were able to generate a pure population of lung-like cells, with no contaminating cells. The surviving cells had the appearance and shape of lung-lining cells called alveolar type 2 cells. These cells help maximize air exchange, remove fluid from the lungs, serve as a pool of repair cells, and fight airborne diseases. The hESC-derived alveolar type 2–like cells also made proteins characteristic of that cell type. This research represents an important step toward developing hESCs for use in treating humans. In addition to its usefulness for creating lung cells, this technique may also be used to generate pure populations of other types of desired human cells. *Proceedings of the National Academy of Sciences of the USA* 104(11):4449–4454, laboratory of R.A. Wetsel. 2007 March.

- **Adult Stem Cells Derived from Blood Vessels Can Regenerate into Skeletal Muscle**
  Blood vessels in skeletal muscle are composed of two cell types, endothelial and perivascular (also known as pericytes, vascular smooth muscle cells, or mural cells). Recently, scientists funded by the Muscular Dystrophy Association (MDA), Italian government, and other sources have discovered that "pericyte-derived" stem cells are located around small blood vessels in muscle tissue and have the potential to regenerate skeletal muscle in individuals with muscular dystrophy. The scientists injected the pericyte-derived cells taken from healthy human muscle tissue into

immune-deficient mice missing the dystrophin protein (the cause of human Duchenne muscular dystrophy). The mice showed functional improvement in walking and holding onto a moving rod. Unlike satellite cells in the muscle that can also regenerate skeletal muscle but need to be injected directly into the affected muscle, the new pericyte-derived cells could repair the muscle and reconstitute the muscle cell population by crossing the blood vessel wall into the muscle. Therefore, if these new pericyte-derived stem cells taken from a individual's own muscle could be easily injected into the bloodstream, this would be an ideal treatment for muscular dystrophy. *Nature Cell Biology* 9(3):255–267, laboratories of G. Cossu and P. Bianco. 2007 March.

- **Stem Cells Improve Symptoms of Neurodegenerative Disease**
  In humans, Sandhoff Disease kills nerve cells (neurons) throughout the body because faulty enzymes cause a toxic buildup of debris inside the neurons. Individuals with the disease usually die by age 3, and there is currently no effective treatment. NIH-funded scientists tested whether stem cells from different sources could improve disease symptoms in a mouse model of human Sandhoff Disease. They transplanted either adult mouse neural stem cells, fetal human neural stem cells, or neural stem cells derived from human embryonic stem cells into brains of mice with the disease. All types of transplanted neural stem cells prolonged the lifespan and delayed loss of motor function in treated mice. However, the number of transplanted cells that replaced dead neurons was not sufficient to account for all aspects of the mice's improvement. Examination of treated mouse brains showed the scientists that the majority of transplanted cells did not replace dead neurons. Instead, most remained as neural stem cells or became supporting cells. These cells stayed near damaged neurons and supplied them with a non-faulty version of the enzyme, which corrects the deficiency that causes debris buildup and cell death. Rescuing dying neurons, in turn, helped reduce inflammation and further loss of neurons. This research demonstrates that transplanted neural stem cells can improve disease symptoms not only by replacing lost or damaged cells, but also by rescuing defective nerve cells and helping reverse disease symptoms such as inflammation. Scientists can take advantage of all of these therapeutic benefits of transplanted cells to develop treatments for Sandhoff Disease and other neurodegenerative diseases that are currently untreatable, including Alzheimer's Disease and Lou Gehrig's Disease (Amyotrophic Lateral Sclerosis, or ALS). *Nature Medicine* doi: 10.1038/nm1548, laboratory of E.Y. Snyder. 2007 Mar.
- **Further Evidence that Mice Can Be Cloned from Adult Stem Cell Types**
  Scientists have proposed the use of somatic cell nuclear transfer (SCNT) to examine how adult cell nuclei could be reprogrammed and then potentially used to create embryonic stem cells. A group of NIH-supported scientists used SCNT to clone mice by using the nuclei from the sensory neurons found in the nose. These adult stem cells are known for their ability to regenerate themselves. In addition, this finding shows that it is possible to reprogram an adult nucleus by using SCNT. Now, another group of NIH-supported scientists have used the same technique to clone robust and healthy mice using the nucleus from keratinocyte adult stem cells found in a part of the hair follicle called the bulge. These stem cells are involved in hair growth and in repairing skin wounds. In addition, because they reside in the skin, the cells are easily accessible. The oldest of these cloned mice is now nearly two years, which is old age for a mouse. If scientists are able to determine how the adult nucleus is reprogrammed in the egg during SCNT, then they could learn how to reprogram adult stem cells without using SCNT (the egg) to produce pluripotent stem cells that could be used to repair or regenerate certain tissues in the body without the destruction of an embryo. *Proceedings of the National Academy of Sciences of the USA* 104:2738–2743, laboratory of E. Fuchs. 2007 Feb 20.
- **Nonembryonic Human Stem Cells Survive and Mature in Rat Spinal Cord**
  Scientists are actively pursuing the use of many types of stem cells to treat spinal cord injuries. In 2006, NIH-supported scientists used mouse embryonic stem cells to restore some movement abilities to paralyzed rats. Now, another group of NIH-supported scientists reports that cultured human fetal spinal cord cells survive and mature when transplanted into normal or injured rat spinal cords. The human cells' mature fate depended on their location: those located near the center of the spinal cord became neurons, while those located near a protective membrane called the

pia mater stayed immature or matured into a specific type of supporting cell called an astrocyte. Although the cells survived and differentiated, more research will need to determine if the cells actually function and help treated rats recover mobility. However, these results suggest that, at least in rats, the damaged spinal cord does not prevent stem cells from surviving and differentiating. This study provides more hope that scientists may one day be able to use stem cells to treat spinal cord injury and neurodegenerative diseases. *PLoS Medicine* 4(2):e39, laboratory of V.E. Koliatsos. 2007 Feb 13.

- **Found: Stem Cells Responsible for Pancreatic Cancer**

  Scientific data has shown that the ability of a tumor to grow and spread is dependent on a small group of rogue cells within the tumor, called cancer stem cells. Finding these stem cells is particularly critical for individuals with pancreatic cancer, which has the worst survival rate of any major cancer type. Fortunately, for the first time, privately supported scientists have identified a small population of human pancreatic cancer stem cells. The scientists examined tissue samples from 10 separate pancreatic cancer tumors. The samples then were implanted into mice and aggressively drove tumor formation. When the tumors were examined, the scientists were able to isolate cells that express the characteristics and cellular markers found in stem cells. These pancreatic cancer stem cells composed 1 percent of the total cell population in the tumors grown in the mice. This discovery will help scientists to develop therapeutic approaches to treat pancreatic cancer. *Cancer Research* 67(3):1030–7, laboratory of D. Simeone. 2007 Feb 1.

- **Mother's Stem Cells Passed to Baby—Suggests Possible Way to Treat Diabetes**

  In type 1 diabetes, an individual's immune system attacks and destroys their own insulin-producing beta cells in the pancreas. Insulin is necessary to efficiently metabolize sugars in foods, and without it, individuals with diabetes must inject themselves with insulin to survive. Scientists are trying to determine why the body attacks its own beta cells, with the hope of developing treatments to halt or reverse the disease process. Umbilical cord blood specimens from male infants contain female cells, believed to cross the placenta from the mother to the child during pregnancy. NIH-funded scientists designed a study to test the hypothesis that in type 1 diabetes, too many maternal cells cross the placenta, contribute to organs in the developing fetus, and stimulate the child's immune system to attack those organs after the child is born. The scientists developed a method for identifying non-child (maternal) DNA in cells and tissues and used it to examine blood samples from individuals with type 1 diabetes, from their siblings who do not have diabetes, and from unrelated healthy individuals. Blood samples from individuals with type 1 diabetes contained more maternal cells than blood from their siblings without diabetes, and significantly higher numbers of maternal cells than in blood from unrelated healthy individuals. The scientists next examined male pancreatic autopsy specimens of children or infants for evidence of maternal cells. Although they found more maternal cells in one specimen from a child with diabetes, the cells did not seem to be under autoimmune attack. Instead, the evidence suggested that the mother's cells had become functional beta cells, helping the child produce insulin after the loss of his own beta cells. The scientists concluded that rather than initiating an immune system attack in individuals with type 1 diabetes, the maternal stem cells may instead increase in number and migrate to the pancreas to replace lost beta cells. They theorize that the child's body tolerates the maternal cells because the immune system is still developing at the time of maternal cell entry into the child's body. They are now investigating this process, and hope to one day use maternal stem cells to treat children with type 1 diabetes. *Proceedings of the National Academy of Sciences of the USA* 104(5):1637–42, laboratory of E.A.M. Gale. 2007 Jan 30.

- **Stem Cell Lines Generated from Amniotic Fluid**

  Amniotic fluid surrounding the developing fetus contains cells shed by the fetus and is regularly collected from pregnant women during amniocentesis. Scientists have previously reported that some of these cells can differentiate into fat, muscle, bone, and nerve cells. Now, privately funded scientists have generated non-embryonic stem cell lines from cells found in both human and rat amniotic fluid. They named the cells amniotic fluid-derived stem cells (AFS). Tests demonstrate that AFS can

produce cells that originate from each of the three embryonic germ layers. The cells are self-renewing and maintain the normal number of chromosomes after a long time in culture. However, undifferentiated AFS did not make all of the proteins expected in pluripotent cells, and they were not capable of forming a teratoma. The scientists developed in vitro conditions that enabled them to produce nerve cells, liver cells, and bone-forming cells from AFS. AFS-derived human nerve cells could make proteins typical of specialized nerve cells and were able to integrate into a mouse brain and survive for at least two months. Cultured AFS-derived human liver cells secreted urea and made proteins characteristic of normal human liver cells. Cultured AFS-derived human bone cells made proteins expected of human bone cells and formed bone in mice when seeded onto 3-D scaffolds and implanted under the mouse's skin. Although scientists do not yet know how many different cell types AFS are capable of generating, AFS may one day allow scientists to establish a bank of cells for transplantation into human beings. *Nature Biotechnology* 25(1):100–6, laboratory of A. Atala. 2007 Jan.

- **Tissue-Matched Stem Cells Created in Mice without Cloning**
  Scientists have proposed the use of somatic cell nuclear transfer (SCNT) to create stem cells that are tissue-matched to an individual. This process is also known as therapeutic cloning. However, due to exchange of genetic information between pairs of like chromosomes (homologous recombination) during the egg's meiosis, the stem cells created using this method may still not be a precise match for the nucleus donor. In an attempt to improve the degree of tissue-matching, scientists recently derived stem cells from a mouse embryo created using a process known as parthenogenesis. Parthenogenesis describes an embryo created without fertilization of the egg by a sperm, thus omitting the sperm's genetic contributions. The scientists identified stem cell lines retaining the identical "self" genetic information of the egg donor and used them to generate tissues for transplantation into the egg donor. These transplanted tissues were not rejected by the egg donor mouse's immune system. If scientists can repeat this technique using human eggs, they may be able to generate tissue-matched cells for transplantation to treat women who are willing to provide their own egg cells for this purpose. This technique could also offer an alternative method for deriving tissue-matched human embryonic stem cells that does not require destruction of a fertilized embryo. *Science* 315:482–6, laboratory of G.Q. Daley. 2007 Jan 26.

- **Multipotent Adult Progenitor Cells (MAPCs) Regenerate Blood in Mice**
  In 2001, scientists isolated a special type of non-blood stem cells from human bone marrow. They named these cells *multipotent adult progenitor cells,* or MAPCs. MAPCs are able to generate cells of all three embryonic germ layers. Initially, MAPCs were notoriously difficult to isolate and grow in culture. In 2006, scientists reported improved MAPC isolation and culture conditions. Now a collaborative group of NIH-supported scientists successfully used mouse MAPCs to regenerate the blood-forming system in mice. The scientists speculate that MAPCs may arise earlier in development than blood-forming stem cells, because transplanted MAPCs generated both long-term blood-forming stem cells and all types of early blood cells. Although MAPC-derived cells that did not make blood-specific proteins (i.e., not blood cells) were identified in tissues outside of the blood, they also did not make proteins characteristic of the tissue in which they were found. The scientists have not yet determined the identity of these cells. Transplanted MAPC-derived cells did not appear to form tumors in recipient mice. MAPCs' ability to grow and divide in culture and to regenerate the blood-forming system in mice provides hope that scientists may be able to use human MAPCs to treat diseases of the blood. Doctors may also be able to induce transplant tolerance in human beings by using MAPCs to generate both immune cells and tissues for repair or replacement. *The Journal of Experimental Medicine* 204(1):129–39, laboratory of C. Verfaillie. 2007 Jan 22.

Other Years

2002 Articles ‖ 2003 Articles ‖ 2004 Articles ‖ 2005 Articles ‖ 2006 Articles
2008 Articles ‖ 2009 Articles ‖

ID	Status	Date_Stamp	Comments
2726	Redacted	5/1/2009 10:18:53 AM	Presbyterians Pro-Life upholds the value of human life from fertilization until natural death. Our faith in God leads us to believe that all life belongs to him and as human beings we are prohibited from taking the life of another innocent human being. The first responsibility of any local, state, or national government is to protect the lives of those under its care. That responsibility applies to every human life, no matter its size, ability, or stage of development. These guidelines are thoughtful in the restrictions they apply to protect those couples donating embryos, but no consideration is written herein to protect the lives of the embryos themselves. No where in this document is it stated that the research in question ALWAYS results in the death of the embryo. This is a serious omission and the primary fact that causes Presbyterians Pro-Life to urge NIH to withdraw this document. The only way to "ensure that NIH-funded research in this area is ethically responsible, scientifically worthy," is to restrict all stem cell research funding to the use of adult stem cells and those derived through IPS. The guidelines state that "Although human embryonic stem cells are derived from embryos, such stem cells are not themselves human embryos." You omit the pertinent truth that a human embryo is destroyed in order to obtain "embryonic stem cells." This statement misleads potential donors. The guidelines require that the donor receive "Information about what would happen to the embryos in the derivation of human embryonic stem cells for research", but the language is not specific in requiring fertility clinics to state that the embryo WILL DIE in the process. Still, these problems with the guidelines are not the reason we urge you to withdraw these guidelines, but because the destruction of human embryos for research is morally wrong and taxpayers should not be forced to fund the death of innocent human beings at any stage of development. These lives, as soon as they are fertilized, are not "owned" by the parents or by the government, but by our Creator. Our Constitution states plainly that we are endowed by our Creator with certain inalienable rights and the first of these is the right to life. I urge you to retain the current Stem Cell Guidlines and to continue to deny government funding for any research that results in the death of human embryos. Sincerely, ***** Presbyterians Pro-Life Research, Education, & Care, Inc. Attached: Position Paper on Stem Cells

Position Statement on Stem Cells
Presbyterians Pro-Life

The world will never starve for want of wonders, but only for want of wonder.
--G. K. Chesterton

"We are the first generation to contemplate killing our very young children and grandchildren to use their body parts for our benefit."

As the frontiers of medical research advance over time, the Church in each age is called to accurately evaluate the moral questions of its era and to live faithfully in the midst of new discoveries and possibilities.

The whole counsel of God, concerning all things necessary for his own glory, man's salvation, faith, and life, is either expressly set down in Scripture, or by good and necessary consequence may be deduced from Scripture 1

With those words, the Westminster Confession affirms the confidence of the Church that in every aspect of life, Christians can receive sufficient direction from Scripture to discern God's will and to respond obediently to all moral challenges, including those which have not been faced by previous generations. We believe that our limited and fallen understanding and reasoning must always be subject to the authority of Scripture.

Scripture commands us to love our neighbors and to demonstrate compassion for all who suffer. The Bible also teaches that we are forbidden to take innocent human lives and that there is a continuity between life before and after birth. Those key biblical principles provide the guidance we need to live faithfully when confronting new challenges and opportunities in the areas of life, death and biotechnology.

"Stem cell research" is a prominent contemporary topic. The popular understanding (though false) is that although "embryonic stem cells" could provide cures for those now suffering from Alzheimer's disease, Parkinson's disease, multiple sclerosis, diabetes and numerous other devastating illnesses, such research is being unreasonably opposed by uncaring people, who are unmoved by the suffering of others. It is a controversy in which emotions sometimes run high, but the scientific and moral dimensions of the discussion are seldom well elucidated.

What are stem cells?

Human "stem cells" are cells present throughout the biological stages of human life, from human embryo, to fetus, to baby, to child, to adult. Stem cells have the amazing potential to make identical copies of themselves and to "differentiate" into the more than 200 types of cells with specialized functions that are needed to support a human life -- from a neuron in the brain, to a muscle cell of the heart, to a lymphocyte circulating in the bloodstream producing antibodies to fight infection.

The process that leads a fertilized egg, over time, to produce all the cells, organs, and complex structure that characterize an adult human being is an incredible one, which science has barely begun to understand. The beginnings of that process provide the background and terminology used in discussions of stem cells. At the time of fertilization—the union of a sperm and an egg—a new, genetically unique individual is formed. In the earliest stages of development, the fertilized egg ("zygote") divides and forms a ball of cells. That ball of cells develops a cavity (blastocyst stage) and comes to consist of two portions, the "trophoblast" (which will develop into the placenta and umbilical cord) and the "inner cell mass" (which will become the fetus). The "inner cell mass" will produce the three primary "germ layers" of cells that will later give rise to all the cell types of the body.2 The "ectoderm" (external layer) is the source of cells which include skin cells and neurons of the brain. The "mesoderm" (middle layer) produces cells including muscle cells and blood cells. The "endoderm" (internal layer) yields cells such as pancreatic cells and alveolar cells of the lung.3

Scientists use the term "totipotent" to describe a cell having the potential to generate all the cells that make up the embryo plus its supporting structures (placenta and umbilical cord). The term "pluripotent" is used to describe stem cells which can give rise to all the cells of the human body (cells from all three germ layers). The term "unipotent" describes more limited stem cells which can produce cells of only one of the three lines.3 The zygote is described as totipotent. Scientists have found embryonic stem cells to be pluripotent. It was initially believed that adult stem cells were unipotent.
However, "studies have shown that blood stem cells (derived from mesoderm) may be able to generate both skeletal muscle (also derived from mesoderm) and neurons (derived from ectoderm). That realization has been triggered by a flurry of papers reporting that stem cells derived from one adult tissue can change their appearance and assume characteristics that resemble those of differentiated cells from other tissues. The term plasticity . . . means that a stem cell from one adult tissue can generate the differentiated cell types of another tissue." 4 Adult stem cells are present in relatively low numbers and are mixed with differentiated cells in the tissues, therefore it is more time-consuming to isolate them, but adult stem cells have been isolated which developed from all three germ layers and adult stem cells have demonstrated the capability to differentiate into tissues other than the ones from which they originated.

What is the moral issue?

When stem cells for use in research or for treatment of disease are obtained in a manner that does not harm the donor, there is no ethical dilemma. The situation is analogous to a healthy person donating a unit of blood to benefit others, or to a person with two healthy kidneys donating one to help another. No one is harmed and there is great potential to save or significantly improve the life of someone else. "Adult stem cells" pose no moral problems because they can be obtained without harm to the donor. The list of adult tissues reported to contain stem cells is growing and includes bone marrow, peripheral blood, brain, spinal cord, dental pulp, blood vessels, skeletal muscle, epithelium of the skin and digestive system, cornea, retina, liver, and pancreas.4 Bone marrow transplants, in which stem cells capable of producing all the types of blood cells are transfused into a person who needs them, have been performed successfully for a number of years. Umbilical cord blood from newborn babies5 is a readily-available source of stem cells. Recovering stem cells from cord blood poses no moral problems and may have some advantages since the cells are younger and have not undergone the deleterious effects that aging may have on stem cells recovered from adults.

The only types of human stem cells which raise moral concern are human "embryonic stem cells" or fetal stem cells which require the killing of the donor to obtain the stem cells. The "embryonic stem cells" causing current controversy are obtained by allowing an embryo to develop in the laboratory to the "blastocyst" stage (a stage that occurs just before the embryo would implant in the uterine wall in a normal pregnancy) and then, in a process that ends the development of that individual, the embryo is destroyed and cells from the "inner cell mass" (which would have developed into the fetus) are separated from the others. Those cells are then propagated in the laboratory as embryonic stem cell lines for various uses, but they will not develop into a baby because the baby's life was ended when its stem cells were removed.

The embryo is very small and is only about a week old when it is destroyed to obtain embryonic stem cells. In most discussions of abortion, the prenatal life being ended is one to which we can easily relate. Even very early in a pregnancy, say from eight to twelve weeks, the fetus already has easily-recognized features and a beating heart which can be seen on ultrasound. In the destruction of human embryos to create embryonic stem cell lines, the life that is being destroyed may appear, to our examination, to be just a collection of cells. But it is no ordinary group of cells. At the time of fertilization, when the 23 chromosomes of the sperm merge with the 23 chromosomes of the egg, a new human life comes into existence as a single, 46-chromosome cell called a "zygote." The zygote is just one cell, but already the genetic characteristics of that future human adult -- gender, blood type, hair and eye color, and all other genetic characteristics -- have been determined.

Even more remarkable, contained in that zygote are all of the instructions for how and when that cell will divide, which genes will be turned on and off at what times, and what types of specialized cells will be created in what locations in order to produce the more than 200 types of cells that are needed. The cells are not randomly produced and distributed, but rather are organized into the appropriate organs. For example, astrocytes, oligodendrocytes, and neurons are located in the brain while the insulin-producing cells reside in the pancreas. The various organs and tissues assemble into a complex structure, the human body, with head and trunk, arms and legs, right and left, front and back all in proper position. The cells in the brain capable of sight extend forward in the face forming eyes, a beating four-chambered heart connects to a network of blood vessels, propelling blood, delivering nutrients and oxygen to every cell of the body and removing toxic cellular waste products. The nervous system, digestive system, reproductive system are all intricately formed to provide for life. As in post-natal life, programmed cell death is part of the process of life. In utero, this means that instead of webbed fingers and toes, certain cells destroy themselves so that fingers and toes develop as separate structures.2

The zygote and early embryo may not be impressive to the human eye, but given the opportunity to implant in the uterine wall, in nine months that group of cells -- that embryo -- will be a baby, capable of independent life.

How should we treat an early human embryo?

Does the early embryo qualify as a human life which we are required to protect rather than to destroy? Scripture clearly teaches that God places a higher value on humans than on the rest of creation, that the meaning and purpose of God for each human life begins before birth, that God forbids us to kill innocent human life, and that we are to protect and care for innocent life. 6 The biblical theme of continuity of life before and after birth is particularly relevant.

The biblical writers did not use different words to label prenatal and postnatal life. The same Hebrew and Greek terms are often used to refer both to the born and the unborn. For example, Geber is a Hebrew noun usually translated man, male, or husband. In Job 3:3, Job curses the night in which it was said, "a man-child [geber] is conceived." Yeled is a term in Hebrew commonly translated child or boy. Yet Genesis 25:22 refers to yeladim (children) struggling inside the womb of Rebekah. Moses recites a law in which a Yeled (child, boy) comes forth from a woman (born prematurely).

In Greek, brephos is often used of infants and the newly born (Luke 18:15; 1 Peter 2;2; Acts 7:19). But in Luke 1:41 and 44, brephos is used of John the Baptist leaping in the womb of Elizabeth. Huios in the Greek means son and is used in Luke 1:36 of John being conceived by Elizabeth: "'And behold, even your relative Elizabeth has also conceived a son in her old age; and she who was called barren is now in her sixth month.'" 7

Although it might seem convenient if the facts were otherwise, neither Scripture nor biology gives us a basis to treat the zygote and embryo as anything other than the unique human lives that they are. By using the same words to describe prenatal and postnatal life, Scripture shows continuity between life before and after birth. The biological process of human development from zygote, to embryo, to fetus, to baby, to child, to mature adult is a continuous biological process. The only beginning point is fertilization, when a new individual is created. There is no basis for drawing any other conclusion.

If embryos are going to be destroyed anyway, isn't it better to use them to obtain stem cells? Some have suggested that it is morally acceptable for "leftover" human embryos from in vitro fertilization clinics to be donated by their parents to be used as a source of stem cells since these frozen embryos will never be implanted and therefore will never develop into children. Two professors at the University of Minnesota effectively addressed the assertion that since no relative harm is done, such a practice would be moral:

The argument that research is justified as long as no relative harm is done

to the subject and there is potential gain for others appears powerful at first inspection, and indeed it has proven powerful in the past. Gilbert Meilaender -- the Richard and Phyllis Duesenberg professor of Christian Ethics at Valparaiso University and a member of the President's Council on Bioethics -- cited two previous applications of the argument in a lecture at the University of Minnesota this past November.

The Tuskegee syphilis trials allowed black men with syphilis to go untreated to determine the effects of the disease. Access to "comfort" care for those men actually was improved by their participation in the trial, since their usual access to care was so poor. The fate of these men had been determined (by others) prior to the study. If no relative harm was done to them by participating in the study, and there was the promise of some gain for others, why not proceed?

Meilaender's second example was Nazi medical experimentation on prisoners at Auschwitz. Upon arrival at Auschwitz, prisoners were graded according to their "life prospects," and some were condemned to death (by others). If no relative harm was done to these prisoners -- already condemned to death -- and there was the promise of some gain for others, why not proceed? 8

Such illustrations sharpen the focus on the moral issue involved in using "unwanted" embryos to obtain stem cells: What is wrong is wrong, regardless of the potential good that might result for others. "Shall we do evil that good may come of it?" (Romans 3:8) We are the first generation to contemplate killing our very young children and grandchildren to use their body parts for our benefit.

Embryonic vs. adult stem cells

Although all stem cells are believed to have wide potential, early research indicates that embryonic stem cells behave differently than stem cells from other sources. At this time, in fact, embryonic stem cells have not been shown to be helpful in alleviating any medical problems whereas work with adult stem cells, which poses no moral problem, has resulted in a number of successes. Doctor Nigel M. de S. Cameron, Ph.D., chair of the Advisory Board for The Center for Bioethics and Human Dignity and founding editor of the international journal Ethics & Medicine, summarized the status of stem cell research this way in July 2004:

Even the more honest advocates of embryo stem cell research have admitted that cures are a long, long way off. This is patently clear to those who have followed the animal experiments, which have so far yielded very little evidence of cures and many problems . . .

I gave a presentation at the Experimental Biology conference in Washington, D.C. a few weeks ago, where I was surveying the ethical pros and cons of stem cell research. Alongside me were other speakers who are experts in embryo and stem cell research. The embryo research expert talked about basic research. The adult stem cell expert, on the other hand, talked about patients with what had been thought to be incurable diseases going home from the hospital cured. (If you want to read some of the latest research go to www.stemcellresearch.org . . .) 9

Even if human embryonic stem cells were to be effective and even if they were the only means of obtaining effective treatments, the principle that it is wrong to take an innocent human life still applies. Doctor Cameron articulately summarized the moral challenge:

For the question we face is distinctly ethical in character. At the heart of our conception of civilization lies the principle of restraint: that there are things we shall not do, shall never do, even though they may bring us benefit; some things we shall never do, though the heavens fall.

As we stand on the threshold of the biotech century, we could hardly confront a decision that is more onerous, since the promised benefits from this technology may be great . . . If there are things that we should not do, it is easy for us to refuse to do them when they offer no benefit. When the benefit they offer is modest, the choice is still not hard. The challenge to morals and to public policy lies precisely here, where the benefits seem great. Yet it is here also that our intuitive respect for the early embryo requires us to pay a price. 10

We are hopeful that adult stem cells will one day provide new avenues for treatment of diseases which are currently untreatable and will alleviate the suffering of many. Research done thus far suggests that work with adult stem cells has great potential and promise. But even if it were true that adult stem cells do not accomplish the cures that embryonic stem cells might achieve, we must limit our work to that which can be done in a way that is morally right and does not kill one human life in the hopes of helping another, even if the human who must be killed is small -- even a very tiny human embryo, which each of us once was.

Endnotes

1. Westminster Confession, Book of Confessions, 6.006.
2. Stem Cells: Scientific Progress and Future Research Directions. Appendix A: Early Development. Department of Health and Human Services. June 2001. http://stemcells.nih.gov/info/scireport
3. Ibid., Chapter 1: The Stem Cell.
4. Ibid., Chapter 4: The Adult Stem Cell.
5. During the process of development in the womb, the placenta and umbilical cord are tissues produced by the baby to support its growth. The blood that circulates from the baby, through the umbilical cord, to the placenta, and back to the baby is the baby's blood, produced by the developing child, and is different from the mother's blood. Because the umbilical cord is discarded at birth, stem cells can be obtained from the blood remaining in the umbilical cord after birth without harming the baby.
6. Position Statement on Abortion, Presbyterians Pro-Life Research, Education and Care, Inc., Allison Park, PA, adopted June 1988, rev 9/93.
7. Fowler, Paul B., Abortion: Toward an Evangelical Consensus, 1987, Multnomah Press, Portland, Oregon, pp 144, 145.
8. Dowd, Bryan and Chris Macosko, "Key question for research on human embryos is whether it is moral," Commentary, Minneapolis Star Tribune, March 12, 2004.
9. Cameron, Nigel, "The stem cell debate gets hotter," Biotech Commentary, Council for Biotechnology Policy, July 19, 2004. http://www.pfm.org/BiotechTemplate.cfm?Section=Biotech_Home&Template=/ContentManagement/ContentDisplay.cfm&ContentID=13134
10. Testimony of Nigel Cameron, Ph.D., given August 1, 2001 before the United States Senate Committee on Appropriations, Subcommittee on Labor, Health and Human Services, Education, and Related Agencies Hearing on Embryonic Stem Cell Research http://www.thecbc.org/redesigned/research_display.php?id=61

ID	Status	Date_Stamp	Comments
4618		5/1/2009 4:40:50 PM	I am opposed to Federal Funding (my dollars) being used for Embryonic Stem Cell Research. If this were a fruitful endeavor commercial firms would have long since embraced this practice. There is adequate evidence that adult stem cell research is an effective alternative and does not require my tax dollars for funding.
4619		5/1/2009 4:41:14 PM	I am opposed to federal funding of stem cell research!
4620		5/1/2009 4:41:22 PM	It doesn't seem logical that something so simple escapes and eludes the most intelligent minds in our society. It's really simple Embryonic Stem Cell-NO! Adult Stem Cell-YES!!!!! Embryonic Stem Cells create tumors....period!!! They have not now, or will they ever become the miracle component of curing diseases.....period. Adult Stem Cells have eliminated or assisted in curing 70+ diseases, aiding the healing process of burn victims, and has no side effects that could set back its progress. You don't have to be an Einstein genius to acknowledge that Adult Stem Cells are the way to go, but you do have to have an open mind; the realization that embryos harvasted from abortions could be looked upon as murder, because the goal is certainly greed, corruption, power and egos. Sadly enough, what goes around comes around. Fate, Karma, etc. never miss you when it's got you in its sights. I shall pray for your blackened soul....you should pray too!
4621	Redacted	5/1/2009 4:41:30 PM	National Institute of Health: We are writing to oppose the current draft of allowing Stem Cell research using embryonic stem cells. Our opposition comes from two perspectives. The first is the devaluing of human life that comes from the killing of embryos. As a country, we have a long history of valuing life and have resisted the temptation many times to place potential unproven research gains in precedence over the value of preserving life. Our second concern is based upon the current scientific research that overwhelmingly points to the dramatic differences in results of adult stem cell progress in research, to very little hope of progress and no results at the embryonic level. The current body of scientific evidence would urge us to continue promoting and funding adult stem cell research as the private sector has chosen to do, and refrain from unproductive research that has not been proven, and is morally misguided. As an agency that works in the community to promote the active integration of good research, and strong moral guidelines, we urge you to not remove the current ban on embryonic stem cell research, and fund continuing research on adult stem cells. ***** Christian Family & Children's Center
4622		5/1/2009 4:41:34 PM	I am vehemently opposed to the human embryonic stem cell research (ESCR)and to the public funding of such research. I would respectfully request that President Obama's policy on this be rescinded.Thank you.
4623	Redacted	5/1/2009 4:41:39 PM	It is time this country moved into the 21st century after years of being in the dark ages in regard to scientific investigation. I strongly support stem cell research and hope that it advances the needed cures for diseases yet to be cured.
4624		5/1/2009 4:41:42 PM	Most all of the success with stem cells has been Adult Stem Cells. What you are doing is morally wrong.

ID	Status	Date_Stamp	Comments
26154		5/18/2009 1:25:59 PM	I am a graduate student at Arizona State, working on the history of embryological research. I support the draft guidelines as written, under current law. However, I do wish to stress the importance of the repeal of the Dickey-Wicker amendment in the appropriations bill. I am pleased to see that the NIH guidelines include strict measures of informed consent for those who donate their IVF embryos. These informed consent measures are robust and precautionary, as they require that consent be gotten both at the time of creation *and* at the time of donation, which enables donors to change their minds. Is it clear that the embryos become the property of research institutions after donation, and no longer the property of the donors? I am also pleased to see that no inducements to donate can be offered. While I'm still concerned about the unequal access to IVF because of cost, I would not want to see women who can not afford treatment induced to donate embryos because it would give her a discount. Good job, NIH!
26155		5/18/2009 1:26:01 PM	I wholeheartedly support embryonic stem cell research, and am glad restrictions are being loosened.
26156		5/18/2009 1:26:14 PM	I support embryonic stem cell research, and am glad some of the restrictions are being loosened. Also, I would hope that whoever is tallying these comments takes into account the deliberate efforts by pro-life and far-right religious groups to flood these comments with anti-stem cell positions to create the illusion of public support for their side. And regardless, the science should take precedent in the formation of these guidelines, not the opinions of the uneducated masses who will just regurgitate what their pastors and Fox News tells them. Thank you.
26157		5/18/2009 1:26:27 PM	I support all forms of stem cell research and applaud the President's quick work to allow researchers to do their jobs.
26158	redacted	5/18/2009 1:26:33 PM	From a medical or scientific point of view, there is no legitimacy to any proposition that human life begins at any moment other than the fertilization of a human ovum by a human sperm. This conclusion is based on microscopic observation, the life span (days) of human gametes vs. the life span (120+ years) of the result of their union, the haploid nature of human gametes contrasted to the diploid nature of the zygote and his/her unique DNA makeup throughout development in utero, birth, post-natal development and maturation. Based on the fact of that beginning, and being an organization of physicians, nurses, dentists, therapists, scientists and other health professionals dedicated to the Hippocratic principal of a respect for all human life, we oppose the killing of human embryos. The proposed regulations will force taxpayers to fund research that is unethical because it requires the destruction of human embryonic life. Furthermore, expanding funding to new human embryonic stem cell (ESC)lines will divert federal funds away from promising work aimed at treating people now with adult stem cell therapies, and will divert funds from other sources of induced multipotent stem cells (ISPs) that can be generated without the use of any human embryos. The proposed regulations create a financial incentive for the creation of more embryonic humans that would be destined for destruction in order to obtain their embryonic stem cells. The guidelines do not require any separation between an IVF physician and an ESC researcher. The guidelines say they "should" be separate, but only when practical. The guidelines allow any IVF physician to create more embryos than are needed for fertility purposes in order to generate more so-called "leftover" embryos for embryonic stem cell research (ESCR)using taxpayer funds. Thus, instead of preventing any future expansion of funding for ESCR on unethical experiments involving human clones and human-animal hybrids, these regulations open the door for such funding whenever NIH wants to do so in the future. Moreover, the guidelines do not require full informed consent from the parents of the human embryos as to their options for their embryonic offspring to be adopted by other infertile couples. Thank you for your attention.

ID	Status	Date_Stamp	Comments
38059	Redacted	5/22/2009 9:02:53 AM	COMMENTS ON NATIONAL INSTITUTES OF HEALTH (NIH)PROPOSED GUIDELINES FOR HUMAN STEM CELL RESEARCH BY THE CHRISTIAN MEDICAL ASSOCIATION Submitted by:***** ***** ***** ***** The draft guidelines as published by NIH in the FEDERAL REGISTER on April 23, 2009 (74 Fed. Reg. 18578) (the "Guidelines") present specific problems regarding enforcement, current law and the prospect of future rulemaking involving even more controversial areas. The guidelines also engender serious general concerns regarding ethical principles and practical considerations. The guidelines underscore a fundamental departure from historic American standards regarding our respect for nascent human life. We request that this letter and its attachments be made part of the public record of the proceedings and that NIH consider this letter and attachments as relevant matter to be taken into account in any statement of the basis and purpose of this rulemaking action under 5 U.S.C. § 553. Specific concerns regarding enforcement, current law and the prospect of future rulemaking 1. Some of the provisions intended to enforce "ethical standards" may be unenforceable. In section II, Part B, number 6, th Guidelines state that "Whenever it was practicable, the attending physician responsible for reproductive clinical care and the researcher deriving and/or proposing to utilize human embryonic stem cells should not have been the same person." This raises concerns about potential conflict of interest if the researcher and attending physician advising the family are one and the same. The researcher may have an incentive to encourage certain patients to have excess embryos created to benefit his/her research. 2. The prohibition in the Dickey-Wicker Amendment cited in the Guidelines is in re ality much broader than that noted by NIH. Dickey-Wicker prohibits federal funding of creating human embryos by any method, explicitly including human cloning, or any "research in which" human embryos are harmed in any way. As the Dickey-Wicker statute states, "SEC. 509. (a) None of the funds made available in this Act may be used for (1) the creation of a human embryo or embryos for research purposes; OR (2) research in which a human embryo or embryos are destroyed, discarded, or knowingly subjected to risk of injury or death greater than that allowed for research on fetuses in utero under 45 CFR 46.204(b) and section 498(b) of the Public Health Service Act (42 U.S.C. 289g(b)). (b) For purposes of this section, the term "human embryo or embryos" includes any organism, not protected as a human subject under 45 CFR 46 as of the date of the enactment of this Act, that is derived by fertilization, parthenogenesis, cloning, or any other means from one or more human gametes or human diploid cells. By eliminating the existing requirement that the stem cell lines pre-exist the promulgation of the policy, the Guidelines patently and knowingly subject all human embryos that are currently living in in-vitro fertilization laboratories and are no longer wanted for reproductive purposes by their biological parents to "the risk of …death" prescribed by Dickey-Wicker due to the incentives in the form of federal funding and the related academic or scientific prestige such funding entails for doing research on the stem cells of these human embryos as the Guidelines now propose. 3. Even when finalized, these draft guidelines will not exclude further rule making to fund other types of embryo-destructive research. President Obama's executive order broadly ordered NIH to conduct research "to the extent permitted by law," and specifically states that "the NIH shall review and update these Guidelines periodically, as appropriate." Further actions may be taken to fund research on stem cell lines obtained by human cloning. 4. The guidelines do not include any criteria to promote the non-embryo- destructive research that offers the greatest potential for patient benefit in the near term. This oversight may deemphasize research that is already showing usefulness

ID	Status	Date_Stamp	Comments
38059		5/22/2009 9:02:53 AM	in treating patients. General concerns regarding ethical principles and practical considerations The proposed NIH guidelines provide an incentive for scientists to destroy human e mbryos for embryonic stem cell research and are ethically irresponsible, scientifically unworthy and medically unnecessary. It is wrong to kill one person, even to save the life of another. The fact that an embryo is not wanted by its biological parents does not change the essence of what the embryo is. A street child abandoned by its parents still has incalculable moral worth and is worthy of protection just as an abandoned embryo does in an IVF lab. Just because a frozen embryo will ultimately die does not change its moral worth. How long a human being will live has no bearing on the value of that individual human being. The NIH has created an artificial dichotomy through utilitarian reasoning. It a sserts that since these embryos are going to die anyway and the parents don't want them, then why not get some good out of them by using them for research? Justifying such flawed utilitarian reasoning by saying that the parents have given an informed consent for such fatal research on their human embryos, is like saying that a medical doctor can kill a comatose but living child by extracting his much-needed organs for research just because his parents don't want to take care of their child anymore or think that such research is more valuable than their child is. We should never start down this slippery slope, whether in organ donation or in stem cell research, where the human embryo is more than a human organ. It is a unique, living human being. It is lamentable that such research is not banned, and it should never be carried on at the expense of taxpayers, many of whom object to such unethical human experimentation and could not in good conscience use any therapy essentially derived from the killing of other human beings. But there is another ethically acceptable way to deal with parental aba ndonment of embryos. Rather than authorize such abandonment as proposed by the Guidelines, parents should be encouraged and even required to transfer their unwanted embryonic children to infertile couples who desperately want to have children. Over two million American couples suffer from infertility. In the same way that we protect older children, the government should mandate that if embryos are not used after a specified number of years, they must be put emancipated and put up for adoption. Indeed, it is telling and inconsistent with the existing federal policy encouraging human embryo adoption that the Guidelines do not include the option of human embryo adoption in "written informed consent" information required in Part II.B.7 of the Guidelines The only way the NIH can justify the destruction of embryonic human bei ngs is to consider them to be property owned by their parents, rather than the nascent human beings they in fact are. Given this perspective, the Guidelines serve to corrupt the parent-child relationship and parental responsibilities to their genetic offspring. Science also becomes corrupted in this process. The American Medical A ssociation ethical statement on research states, "Adequate safeguards must be provided for the welfare, safety and comfort of the subject. It is fundamental social policy that the advancement of scientific knowledge must always be secondary to primary concern for the individual." The Nuremburg Code, adopted after WWII atrocities involving elite German physicians and medical institutions, states, "No experiment should be conducted where there is a priori reason to believe that death or disabling injury will occur." The greatest atrocities in modern science have occurred when scientists have depersonalized their research subjects and adopted an end justifies the means mentality, particularly when the subjects can't speak for themselves and are represented by others whose interests conflict with their own. Not only are the proposed guidelines ethically irresponsible; they are also are scientifically unworthy. After almost ten years of embryonic stem cell (embryonic stem cell) research scientists have found that these cells are difficult to culture, difficult to control their differentiation, are likely to form tumors, have genomic instability and often the cells derived are functionally abnormal. Even if all these hurdles were somehow surmounted, each patient would have to be cloned to create his or her identical twin to kill for its stem cells to differentiate into tissue. The lack of human trials in human beings using embryonic stem cells is not du e to a lack of money. California alone is putting as much money into embryonic stem cell research as was spent on the entire Human Genome Project.

ID	Status	Date_Stamp	Comments
38059		5/22/2009 9:02:53 AM	Even if technical problems were solved, this highly customized thera py would cost over $200,000 dollars per patient, putting it out of the reach of all but the wealthiest. In addition, a significant percentage of the population would object to this therapy out of moral considerations. The government should not be putting tax payer's dollars into a line of research that many American's would refuse to use. The embryonic stem cell therapy that NIH proposes to fund is unnecessary. No n-embryonic stem cell therapies are already available for over 75 diseases. The NIH reports over 1,200 human trials underway in this arena. The ability to develop induced pluripotent stem cells using somatic cells makes the sacrifice of human embryos on the altar of science unnecessary. This technology provides an unlimited and cheap source of embryonic stem like cells for experimentation. They can be used to develop disease specific cultures and well as ones that are histocompatible with individual patients. Research dollars should be focused on real cures for real people in the sho rtest amount of time. If there are two paths to th cures everyone wants, but one is expensive, technologically difficult, will take an inordinate amount of time and requires killing human beings, while the other path is cheaper, quick and morally acceptable, we should go down the latter path. It is clear that the path to breakthrough treatments for patients in the near future is not the path of embryonic stem cell research. The government, which has as its primary purpose to protect human life, should not be through funding inducing scientists to destroy embryos. For these reasons and the additional reasons set forth in comments submitted b y DO NO HARM et. al., in which CMA has also joined, the 15,000 members of the Christian Medical Association urge the withdrawal of the proposed Guidelines and the continuance of NIH's existing stem cell research guidelines permitting federal funding for human stem cell research using adult stem cells and induced pluripotent stem cells" that do not require or depend upon the destruction of human embryos.
38060		5/22/2009 9:03:04 AM	OPPOSE DESTRUCTIVE EMBRYONIC STEM CELL RESEARCH National Institutes of Health (NIH) is proposing guidelines to destroy human embryos derived from in vitro fertilization - a "create to kill" policy. Our tax dollars will pay for this research! Please complete the following form, cut it out and send to the NIH: I oppose the destruction of embryonic stem cells for body parts and cloning.
38061		5/22/2009 9:03:42 AM	I am opposed to your draft guidelines for embryonic stem cell research, which force me as a taxpayer to subsidize research requiring the destruction of innocent human life. Support should be directed to stem cell research and treatments that harm no one and are already producing good results. In no case should government support be extended to human cloning or the human embryos for research purposes.
38062		5/22/2009 9:03:46 AM	I am opposed to your draft guidelines for embryonic stem cell research. These guidelines force me as a taxpayer to subsidize research requiring the destruction of innocent human life. Support should be directed to stem cell research and treatments that harm no one and are already producing good results. In no case should government support be extended to human cloning or the human embryos for research purposes.

ID	Status	Date_Stamp	Comments
48190		5/26/2009 10:22:03 PM	I represent Citizens for Science and Ethics, a group of scientific, medical, business, and private individuals who are opposed to the NIH draft guidelines for embryonic stem cell research which, for the first time, will encourage the destruction of human life subsidized by taxpayers when more sound science prevails. These guidelines promote a biased and rushed consent process by allowing use of embryos that were never frozen, thus, pressuring women for informed consent at a time when they are wrestling with the problem of infertility. Furthermore, the proposed policy goes far beyond the proposal to use frozen embryos that may be discarded by allowing the option upfront for parents to donate their embryos for destructive research. Finally, as even embryonic stem cell (ESC) proponents admit, the most likely use for ESC will not be in developing new treatments and therapies, but rather in drug safety testing and disease modeling. This is clearly not the ESC "promise" that was made by President Obama to the American public. In addition to promoting an unethical approach to procuring ESC, the draft guidelines are also naïve, since it is unquestionably true that the proven technologies of adult and induced pluripotent stem cells (iPSC) are vastly superior to the "promise" of ESCs. Ian Wilmut, former outspoken cheerleader for ESC research, has recently commented that the "availability and capacities of iPSC are unquestionable", and any remaining technical challenges will require very little time to overcome. He acknowledges that the availability of iPSC not only abrogates the cloning requirement necessitated by conventional ESC, but that iPSC are also "more useful than embryonic cells" in providing for disease modeling and drug screening, because one can study the inherited disease of a patient without having to introduce the genetic error. President Obama's Executive Order ignores the 70+ diseases already being clinically treated with ethically-procured adult stem cells, and sends us back in time by spending federal monies on archaic technology. Science has generated and improved the method to produce more useful embryonic-like stem cells in iPSC without destroying embryos. World-renowned investigators and institutes are studying iPSC and reporting their advantages over ESC. The Scripps Institute, the University of Peking, and the University of California, San Diego joint publication in January, for example, describes that their developed human iPSC, unlike human ESCs, are a counterpart to the conventional and most useful mouse ESCs that have already generated a large volume of data, therefore offering an advantage in biomedical research by allowing knowledge gained from iPSC to be more directly translatable to human cells. Furthermore, Britain, the home of Dr. Wilmut and a hotbed of ESC funding, is experiencing a "brain drain" of some of its most notable scientists because of the imbalanced focus and preferential funding of ESC over non-ESC stem cell research, which has resulted in not a single patient being treated by ESCs for any disease or disorder. This is a powerful rebuke of the President's prediction that our best scientists will leave for other countries if ESC research is not funded. NIH has the responsibility for funding research in accordance with its published criteria and goals: "scoring that reflects the overall impact that the project could have on the advancement of science", and to "advance our understanding of biological systems, to improve the control of disease, and to enhance health". Funding influenced by well-established investigators and research consortiums who are politically pressing for use of ESCs in the light of both their limitations and the availability of more advanced tools is scientifically and fiscally irresponsible. If the NIH and the President stand behind the President's promise to "make scientific decisions based on fact" they should be funding only iPS and adult stem cell research rather than wasting taxpayers' money by squandering the limited financial resources of the NIH on clearly inferior, ethically challenged science. CSE recently initiated circulation of a petition opposing use of tax dollars for unethical and clinically dubious experiments that require the destruction of human embryos and requesting the President to keep cures on the fast track while maintaining the highest ethical standards. The very early response has yielded 1,500 signatures that supported this petition. This is merely a reflection of the larger mass of people who have come to share this view as they become educated on the real progress that has been made in this research field and want their tax money used to further promising and ethical science.

ID	Status	Date_Stamp	Comments
47063	Redacted	5/26/2009 2:57:15 PM	May 26, 2009 NIH Stem Cell Guidelines, MSC 7997 9000 Rockville Pike Bethesda, Maryland 20892-7997 Subject: Draft Guidelines on Human Embryonic Stem Cell Research To Whom It May Concern: The New Jersey Catholic Conference (NJCC) offers the following comments on draft guidelines proposed by the National Institutes of Health (NIH) to authorize federally funded human embryonic stem cell research, published at 74 Fed. Reg. 18578-80 (April 23, 2009) (Guidelines). Founded in 1949, NJCC represents the Catholic Bishops of New Jersey on matters of public policy. There are more than 600 parishes and more than 3.5 million Catholics registered in seven dioceses throughout New Jersey. Monsignor David J. Malloy, S.T.D., General Secretary of the United States Conference of Catholic Bishops (USCCB) provided comprehensive comments on May 22, 2009. The NJCC supports and endorses Monsignor Malloy's comments without reservation or qualification. We add our voice to that of the USCCB to emphasize that the dignity and inviolability of human life at every stage of development is a foundational principle of a civilized society. Subsequent to President Obama's March 9, 2009 executive order, the NIH has proposed guidelines for federally funded embryonic stem cell research. If these Guidelines are approved, it would be the first time ever that federal regulation would allow the use of taxpayer funds to encourage the killing of embryonic human beings to obtain their stem cells. Through the support of embryonic stem cell research, we treat innocent human beings as mere sources of body parts, as commodities for our use. Rather than destroying human embryos, alternative methods of stem cell research are available and have been shown to be beneficial and effective. Adult stem cell and cord blood research are now showing great promise to treat ailments and disabling conditions without harming human life. Peer-reviewed studies have shown that reprogramming ordinary adult cells into "induced pluripotent stem cells" has provided significant advances in treating cancer, juvenile diabetes, Parkinson's disease, spinal cord injury, and heart disease. We would hope that the NIH will recognize that science is moving away from embryonic stem cell research. Let us all be thankful that the ability to reprogram ordinary adult cells into "induced pluripotent stem cells" (iPS cells) is rapidly replacing embryonic stem cells in research among some of the world's most distinguished researchers. Let us say yes to induced pluripotent stem cell research and no to embryonic stem cell research.

ID	Status	Date_Stamp	Comments
47063		5/26/2009 2:57:15 PM	Thank you for your consideration. Sincerely, ***** ***** c. The Catholic Bishops of New Jersey
47064		5/26/2009 2:57:34 PM	I support federal funding for stem cell research. I believe the guidelines should be expanded to provide funding for all existing stem cell lines, including those that have been developed since 1998, since they permit the most comprehensive study of disease. I also support innovative technologies such as somatic cell nuclear transfer. Federal funding should support stem cell research in a broad and flexible way, not in a narrow, restrictive way. Thank you.
47065	Redacted	5/26/2009 2:57:37 PM	Very supportive of the policy and think it is the most sensible way to go forward.
47066		5/26/2009 2:57:47 PM	I write this on behalf of my mother, who has Parkinson's. Embryonic stem cell research offers hope for the millions with Parkinson's and similar diseases for a real cure. Embryonic stem cell research holds great promise for millions of Americans suffering from many diseases and disorders. I am not a scientist, but I am a member of the Parkinson's community and have been following progress in this field with great interest. Significant strides have been made over the past decade, and the final guidelines issued by NIH must build on this progress so that cures and new therapies can get to patients as quickly as possible. The final guidelines should not create new bureaucratic hurdles that will slow the pace of progress. I am pleased that these draft guidelines -- in Section II B -- would appear to permit federal funding of stem cell lines previously not eligible for federal funding and for new lines created in the future from surplus embryos at fertility clinics. However, as drafted, Section II B does not ensure that any current stem cell line will meet the criteria outlined and thus be eligible for federal funding. It will be important for the final guidelines to allow federal funds for research using all stem cell lines created by following ethical practices at the time they were derived. This will ensure that the final guidelines build on progress that has already been made. I also believe that the final guidelines should permit federal funding for stem cell lines derived from sources other than excess IVF embryos, such as somatic cell nuclear transfer (SCNT). Sections II B and IV of the draft guidelines do not permit such federal funding and I recommend that the final guidelines provide federal funding using stem cell lines derived in other ways. If not, it is essential that the NIH continue to monitor developments in this exciting research area and to update these guidelines as the research progresses.

ID	Status	Date_Stamp	Comments
47160	Redacted	5/26/2009 3:28:18 PM	

May 26, 2009

NIH Stem Cell Guidelines, MSC 7997
9000 Rockville Pike
Bethesda, Maryland 20892-7997

Re: Draft Guidelines on Human Embryonic Stem Cell Research

Dear Sir or Madam:

This letter is in response to the Administration's invitation to submit comments on the draft National Institutes of Health guidelines to expand the circumstances under which federal funding of embryonic stem cell research will be available. The Thomas More Society strongly opposes the proposed guidelines. This opposition is based upon the well-recognized medical and scientific fact that human life begins at conception, understood as fertilization, and the principle that no innocent human life should be intentionally destroyed for research or any other asserted reason. The Thomas More Society also notes, in comments that are developed in greater detail in other submissions, that, unlike adult stem cells, embryonic stem cells have never been used successfully to cure or ameliorate any known disease or condition.

The Thomas More Society is a public interest law firm based in Chicago, Illinois. It was founded in 1997 to meet the burgeoning needs of the pro-life movement. Incorporated as a § 501(c)(3) not-for-profit corporation under the laws of the State of Illinois, the Thomas More Society provides legal advice and assistance to those who face harassment, employment discrimination, unjust treatment, civil litigation or criminal prosecution as a result of their pro-life views or their peaceful protest activities. In recent years, the Thomas More Society has provided legal services and assistance in a wide range of cases, including several in the United States Supreme Court, where it has represented parties and amici curiae. In addition to the legal representation it provides, the Thomas More Society also recognizes (and supports before legislative, executive and judicial bodies) appropriate public policy measures that are intended to protect innocent prenatal human life. Consistent with its core mission and the respect for human life that mission implies, the Thomas More Society respectfully opposes the Department's proposal to expand the circumstances under which federal funding is available for embryonic stem cell research.

What is the Applicable Principle?

It is a well accepted moral and legal principle that forbids the intentional destruction of innocent human life. Application of this principle runs the gamut from prohibiting the terror-bombing of civilian populations in war time to restricting the necessity defense in criminal law to the infliction of non-lethal injuries. Although, by virtue of the Supreme Court's decision in Roe v. Wade, 410 U.S. 113 (1973), this principle cannot presently be applied to ban abortion, nothing in Roe requires either the federal government or the States to subsidize or otherwise pay for the costs of elective or therapeutic abortions. See Harris v. McRae, 448 U.S. 297 (1980); Williams v. Zbaraz, 448 U.S. 358 (1980). Accordingly, regardless of whether the intentional destruction of human embryos ex utero for purported research needs falls inside or outside the scope of the "abortion liberty" recognized in Roe, there is clearly no constitutional obligation to fund such research (indeed, for the reasons set forth in the submission by the United States Conference of Catholic Bishops, such funding is prohibited by the Dickey-Wicker Amendment). Thus, the principle that forbids the intentional destruction of innocent human life should bar public funding of embryonic stem cell research if such research would result in the intentional

ID	Status	Date_Stamp	Comments
47160		5/26/2009 3:28:18 PM	destruction of "human life." It is undisputed that embryos used for research purposes will not be implanted and allowed to mature naturally in utero. In other words, they will die. Does their death mark the end of a "human life"? The answer to that question turns upon the answer to another question, when does human life begin? Does the Principle Apply to the Intentional Destruction of Human Embryos? The morality of destroying human embryos for purposes of research does not involve the termination of a "pregnancy," as such, but in answering the question, "When does human life begin?," it is instructive to consider whether pregnancy itself is understood as commencing with conception, understood as fertilization, or implantation. Although the American College of Obstetricians & Gynecologists (ACOG) has taken the position that a "pregnancy" does not begin until implantation of the embryo in the uterine wall (sometimes referred to as an "established pregnancy"), ACOG's position is not one widely shared in the medical and scientific communities. The American Medical Association defines "pregnancy" as "[t]he process of carrying a developing embryo or fetus in the uterus from conception on." AMA Complete Medical Encyclopedia 1011 (2003). "Conception," in turn, is defined as "[t]he fertilization of an egg by a sperm that initiates pregnancy." Id. at 392.[n. 1] The AMA's terminology is supported by a wealth of medical and scientific sources, including standard embryology texts,[n. 2] obstetrics texts,[n. 3] and medical dictionaries.[n. 4] Although two medical dictionaries define conception solely in terms of implantation,[n. 5] the majority of medical dictionaries and medical encyclopedias now in use agree with the AMA in defining conception as "[t]he fertilization of an egg by a sperm that initiates pregnancy." In addition to the definitions from Melloni's, Mosby's, Dye and Barron's, quoted above, the following dictionary and encyclopedia definitions may be cited: Black's Medical Dictionary 156 (41st ed. 2006): "Conception signifies the complex set of changes which occur in the OVUM and in the body of the mother at the beginning of pregnancy. The precise moment of conception is that at which the male element, or spermatozoon, and the female element, or ovum, fuse together." Dorland's defines an "embryo" (in humans) as "the developing organism from fertilization to the end of the eighth week [of pregnancy]." Dorland's Illustrated Medical Dictionary 614 (31st ed. 2007). And "pregnancy" is defined as "the condition of having a developing embryo or fetus in the body, after union of an oocyte and spermatozoon." Id. at 1531. Stedman's defines an embryo (in humans) as "the developing organism from conception until approximately the end of the second month [of pregnancy]." Stedman's Medical Dictionary 627 (28th ed. 2006). And "conception" is defined as "[f]ertilization of [an] oocyte by a sperm." Id. at 425. See also Bantam Medical Dictionary 146 (5th ed. 2004)(same).[n. 6] One medical encyclopedia defines "pregnancy" as "[t]he period from conception to birth," Gale Encyclopedia of Medicine, Vol. 4, p. 3005 (3rd ed. 2006), "conception" being understood as fertilization. Further, pregnancy is described as "a state in which a woman carries a fertilized egg inside of her body." Id., vol. 4, p. 3006. The understanding of "conception" as "fertilization" is also reflected in standard English language dictionaries.[n. 7] Given the weight of medical and scientific opinion that pregnancy begins with conception, understood as fertilization, not implantation, the intentional destruction of a fertilized embryo may be said to end a human life. Apart from determining when pregnancy begins, the fact that human life, in biological terms, begins at conception (understood as fertilization) is supported by a wealth of scientific and medical evidence. After reviewing many authorities and hearing testimony from world-renowned geneticists, biologists and physicians, the Subcommittee on Separation of Powers of the Senate Judiciary Committee stated: "[C]ontemporary scientific evidence points to a clear conclusion: the life of a human being begins at conception, the time when the process of fertilization is complete." Report of the Subcommittee on Separation of Powers, Senate Judiciary Committee, on S. 158, the Human Life Bill, 97th Congress, 1st Sess, at 7 (1991). "Physicians, biologists, and other scientists agree that conception marks the beginning of the life of a

ID	Status	Date_Stamp	Comments
47160		5/26/2009 3:28:18 PM	human being–of a being that is alive and a member of the human species." Id. And that scientific consensus continues to the present day. In addition to the authorities cited above, especially Moore and Persaud, The Developing Human at 15, see M.J.T. Fitzgerald and M. Fitzgerald, Human Embryology 1 (1994) ("[t]he prenatal period of life commences at the moment of fertilization, and terminates at birth"); R. O'Rahilly and F. Muller, Human Embryology & Teratology 8 (3rd ed. 1996) ("[a]lthough life is a continuous process, fertilization . . . is a critical landmark because, under ordinary circumstances, a new, genetically distinct human organism is formed when the chromosomes of the male and female pronuclei blend in the oocyte"); F.J. Dye, Human Life Before Birth 53 (2000) ("[t]wo cells on the verge of death are the participant in fertilization, one of the most though-provoking events in biology. If these two cells undergo fertilization, a new individual may result"); Wm. Larsen, Human Embryology 1 (3rd ed. 2001) ("we begin our description of the developing human with the formation and differentiation of the male and female sex cells or gametes, which will unite at fertilization to initiate the embryonic development of a new individual").[n. 8] Both legislatures and courts have recognized this scientific and medical reality. After a review of the current medical and scientific evidence on human development, a special task force created by the South Dakota Legislature found that "the new recombinant DNA technologies indisputably prove that the unborn child is a whole human being from the moment of fertilization . . . ." Report of the South Dakota Task Force to Study Abortion 31 (December 2005). More recently, the Eighth Circuit Court of Appeals considered the constitutionality of a South Dakota informed consent statute that requires a physician to advise a woman seeking an abortion that the procedure "will terminate the life of a whole, separate, unique, living human being." S.D. Codified Laws § 34-23A-10.1(1)(b). "Human being," in turn, is defined as "an individual living member of the species of Homo sapiens, including the unborn human being during the entire embryonic and fetal ages from fertilization to full gestation." Id. § 34-23A-1(4). The court of appeals held that, taking into account the definition of "human being" set forth in § 34-23A-1(4), the disclosure required by § 34-23A-10.1(1)(b) is neither "untruthful [n]or misleading." Planned Parenthood Minnesota, North Dakota, South Dakota vs. Rounds, 530 F.3d 724, 737 (8th Cir. 2008) (en banc). Rather, the statute simply requires the physician "to disclose truthful and non-misleading information as part of obtaining informed consent to a procedure." Id. There is a scientific and medical consensus that human life, in biological terms, begins with conception, understood as fertilization. By definition, embryos are fertilized ova. Accordingly, their intentional destruction, for research or other purposes, violates the principle that forbids the intentional destruction of innocent human life. Accordingly, federal funding of such research should not be expanded. Conclusion We oppose the draft guidelines for expanding federal funding for embryonic stem cell research and respectfully request that they be withdrawn in accordance with the principles set forth in this submission. Very truly yours, ***** ***** Thomas More Society Thomas More Society Notes

ID	Status	Date_Stamp	Comments
47160		5/26/2009 3:28:18 PM	1. This usage continues in the AMA's Concise Medical Encyclopedia 1, 184, 565 (2006). 2. See, e.g., Keith L. Moore and T.V.N. Persaud, The Developing Human, Clinically Oriented Embryology 2 (8th ed. 2008) ("Human development is a continuous process that begins when an oocyte (ovum) from a female is fertilized by a sperm (spermatazoon) from a male"), id. at 15 ("[h]uman development begins at fertilization when a male gamete or sperm unites with a female gamete or oocyte to form a single cell, a zygote. This highly specialized, totipotent cell marks the beginning of each of us as a unique individual"); R. Jones and K. Lopez, Human Reproductive Biology 253 (3rd ed. 2006) (stating that "pregnancy begins at conception"), id. at 231 (defining "conception" in terms of "fertilization"); G. Thibodeau and K. Patton, Anatomy and Physiology 1167 (6th ed. 2007). 3. See Scott, DiSaia, Hammond and Spellacy, Danforth's Obstetrics and Gynecology 29 (8th ed. 1999); Cunningham, Gant, Leveno, Gilstrap, Hauth and Wenstrom, Williams Obstetrics 86-87 & Figure 2-1 (21st ed. 2001) (defining conception in terms of fertilization and distinguishing conception from implantation); see also Cunningham, Leveno, Bloom, Hauth, Gilstrap and Wenstrom, Williams Obstetrics 92 & Figure 4-1 (22nd ed. 2005) (equating conception with fertilization). 4. See Melloni's Illustrated Medical Dictionary 526 (4th ed. 2002) (defining "pregnancy" as the "[c]ondition of the female from conception to delivery of the fetus or embryo," id. at 138 (defining "conception" in terms of fertilization); Mosby's Dictionary of Medicine, Nursing & Health Professions 1512 (7th ed. 2006) (defining "pregnancy" as "the gestational process, comprising the growth and development within a woman of a new individual from conception through the embryonic and fetal periods to birth"), id. at 436 (defining "conception" as "the beginning of pregnancy, usually taken to be the instant that a spermatozoon enters an ovum and forms a viable zygote," or, alternatively, "[f]ertilization of [an] oocyte by a sperm"); F.J. Dye, Dictionary of Developmental Biology and Embryology 124 (2002) (defining "pregnancy" as "[t]he condition of a woman who is carrying a conceptus (the product of conception or fertilization"), id. at 31 (defining "conceptus" as "[t]hat which results from conception (fertilization), i.e., the embryo or fetus and its associated membranes"); Mikel A. Rothenberg and Charles E. Chapman, Barron's Dictionary of Medical Terms 471 (5th ed. 2006) (defining "pregnancy" as "the period during which a woman carries a developing fetus in the uterus, from the time of conception to the birth of the child"), id. ("[p]regnancy lasts 266 days from the day of fertilization"), id. at 137 (defining "conception" as the "fertilization of the female egg cell (ovum) by a male spermatozoon, the beginning of pregnancy"). 5. See Joseph C. Segan, Concise Dictionary of Modern Medicine 159 (2006), and Taber's Cyclopedic Medical Dictionary 464 (20th ed. 2005). 6. Two other medical dictionaries define "conception" as either fertilization or implantation See Merriam-Webster's Medical Dictionary 163 (rev. ed. 2005) (defining "conception" as "the process of becoming pregnant involving fertilization or implantation or both"); Miller-Keane, Encyclopedia and Dictionary of Medicine, Nursing and Allied Health 406 (7th ed. 2003) (defining "conception" as "the onset of pregnancy, marked by implantation of the BLASTOCYST; the formation of a viable ZYGOTE"), id. at 662-63 (fertilization occurs when the head of the sperm unites with the oocyte to form the zygote). 7. See Webster's Third New International Dictionary (unabridged) 469 (2002) (defining "conception" as the "act of becoming pregnant; formation of a viable zygote"); Funk and Wagnalls New International Dictionary of the English Language 270 (2003) (defining "conception," in biological terms, as "[t]he impregnation of an ovum"); Random House Webster's Unabridged Dictionary 422 (2nd ed. 1998) (defining "conception" as "fertilization; inception of pregnancy").

ID	Status	Date_Stamp	Comments
47160		5/26/2009 3:28:18 PM	8. Additional authorities may be found in R. George and C. Tollefsen, Embryo: A Defense of Human Life (2008).
47161		5/26/2009 3:28:22 PM	Embryonic stem cell research holds great promise for millions of Americans suffering from many diseases and disorders. I am entering the medical feild and, having a mother with a neurodegenerative disease who I see wax and wane in her health, I strongly beliee that stem cell therapy may become her only option. Significant strides have been made over the past decade, and the final guidelines issued by NIH must build on this progress so that cures and new therapies can get to patients as quickly as possible. The final guidelines should not create new bureaucratic hurdles that will slow the pace of progress.
47162		5/26/2009 3:28:29 PM	Stem cell research holds much promise in the search for a cure and better treatments for the nearly 24 million American adults and children with diabetes, as well as those with many other serious medical conditions. This research will allow scientists an opportunity to better explore how to control and direct stem cells so they can grow insulin-producing beta cells found in the pancreas. Creating new beta cells could mean a cure for type 1 diabetes and could provide a powerful tool for controlling type 2 diabetes. I strongly support the draft guidelines on embryonic stem cell research. They demonstrate the ability of NIH to create a research framework that will allow for the potential of embryonic stem cell research while maintaining the highest safety and ethical standards. As this process moves forward, however, I hope that NIH will consider adapting the guidelines to ensure they include funding not only new stem cell lines, but current stem cell lines that have been developed using prevailing ethical practices. Research on these current stem cell lines should be eligible for federal funding as part of the final rule. Given the enormous promise of stem cells for diseases such as diabetes, it is important to allow federal funding for all forms of stem cell research, including research on embryonic stem cells, and that NIH continue to adapt as our scientists learn more about the promise of stem cell research. I commend NIH for taking this important action to support research that provides the potential for new treatments, and ultimately a cure, for diabetes.
47163	Redacted	5/26/2009 3:28:42 PM	As a senior postdoctoral researcher of the University of Michigan Center for Stem Cell Biology, I would like to comment on the draft NIH Guidelines for Human Stem Cell Research, which appeared in the Federal Register on April 23, 2009. The guidelines were drafted in response to Executive Order 13505, Removing Barriers to responsible Scientific Research Involving Human Stem Cells. To be brief I would only like to say that I support NIH's efforts to loosen restrictions on embryonic stem cell research, and that I fully support comments on the guidelines that have been submitted by ISSCR or ASCB. Sincerely,

ID	Status	Date_Stamp	Comments
47348		5/26/2009 4:39:19 PM	May 26, 2009 Dr. Raynard Kington Acting NIH Director NIH Stem Cell Guidelines, MSC 7997 9000 Rockville Pike Bethesda, Maryland, 20892-7997 Comment on Stem Cell Guidelines for Human Stem Cell Research Dear Dr. Kington: As Members of Congress who support human dignity, the advancement of science, including ethical stem cell research, and the alleviation of disease, we submit the following comment in response to the draft Guidelines for Human Stem Cell Research published by the National Institutes of Health in the Federal Register on April 23, 2009. We appreciate the stated purpose of the guidelines, which read in part, "The purpose of these draft Guidelines is…to help ensure that NIH-funded research in this area is ethically responsible, scientifically worthy, and conducted in accordance with applicable law." However, we believe that the kind of research permitted by these guidelines fails on all three counts, allowing research that is not ethically responsible, scientifically justified, or in keeping with current law. With regard to ethical responsibility, it is not possible to permit, let alone promote, research that incentivizes the intentional destruction of human life. Yet the current draft guidelines, if implemented, will create a federally-subsidized incentive for the creation and destruction of additional human embryos. In fact, the new policy is even more expansive than previous proposals. For example, the current guidelines ask parents to decide about the destruction of their embryo without even the benefit of any kind of waiting period as suggested in the Clinton guidelines of 2000 or having made a prior decision to "discard" them as in legislation passed by the Congress. Incentivizing the death of any member of the human species is not ethically responsible. Scientifically, embryo-destructive research has failed to show clinical benefit to patients, yet there are ethical stem cell therapies that are currently being used to treat thousands of patients for various diseases and conditions. We strongly encourage you to focus NIH funding on clinical research using stem cells derived from ethical sources, like adult stem cells, that have been showing increasing treatment possibilities and have helped many patients already. For basic research, we further urge the NIH to focus funding on induced Pluripotent Stem (iPS) cells. These cells are highly desired, easy to create and use, and thought by many scientists to likely replace both embryonic and cloning research as a superior kind of stem cell research. If we are to truly follow the science, it would be prudent to devote our limited federal resources toward stem cell research that makes sense both scientifically and ethically. Finally, we have concerns about the current interpretation of the Dickey-Wicker amendment. As you know, this provision of law prohibits federal funding for "(1) the creation of a human embryo or embryos for research purposes; or (2) research in which a human embryo or embryos are destroyed, discarded, or knowingly subjected to risk of injury or death...." In establishing this law, the intent of Congress was to prohibit federal funding for any kind of research that directly or indirectly created, destroyed, or harmed a human embryo. We strenuously object to federal funding for embryo-destructive stem cell research and its expansion. The NIH should take stronger measures to prohibit the creation of human embryos for destruction. The guidelines, as currently drafted, have left the door open for revisions as well as the possibility of separate

ID	Status	Date_Stamp	Comments
47348		5/26/2009 4:39:19 PM	guidelines permitting research involving human cloning (SCNT) or creating animal-human hybrids. We urge the Administration and the NIH to take an unequivocal stance against these clearly unethical and de-humanizing practices. In conclusion, we strongly encourage you to take a closer look at the moral status of and respect due to the human embryo. Specifically, we ask you to consider the conclusion of President Clinton's National Bioethics Advisory Commission which said, "In our judgment, the derivation of stem cells from embryos remaining following infertility treatments is justifiable only if no less morally problematic alternatives are available for advancing the research." (Sept. 1999) Given the reality of adult stem cells and the positive outcomes of that research as well as the very exciting possibilities of induced pluripotent stem cell research, there are less ethically problematic alternatives that show embryonic stem cell research to be unnecessary as well as immoral. We urge you to redraft the guidance for human stem cell research in a way that reflects the moral complexity of dealing with the human embryo and treats each embryo with the dignity required for any member of the human species. Sincerely, Senator Roger Wicker (R-MS) Rep. Bart Stupak (D-MI) Rep. Christopher H. Smith (R-NJ) Rep. John Boehner (R-OH) Rep. Eric Cantor (R-VA) Rep. Dan Lipinski (D-IL) Senator Sam Brownback (R-KS) Rep. Joe Pitts (R-PA) Rep. Mark Souder (R-IN) Rep. John Boozman (R-AR) Rep. Dan Lungren (R-CA) Senator Tom Coburn (R-OK) Rep. Rodney Alexander (R-LA) Rep. John Campbell (R-CA) Rep. Michele Bachmann (R-MN) Rep. Todd Tiahrt (R-KS) Senator Jim Bunning (R-KY) Rep. Jim Jordan (R-OH) Senator John Thune (R-SD) Rep. Lincoln Davis (D-TN) Rep. Paul Ryan (R-WI) Senator David Vitter (R-LA) Rep. Jean Schmidt (R-OH) Rep. Doug Lamborn (R-CO) Rep. Robert Aderholt (R-AL) Rep. Ron Paul (R-TX) Rep. Jeb Hensarling (R-TX) Rep. Virginia Foxx (R-NC) Rep. Randy Forbes (R-VA)

ID	Status	Date_Stamp	Comments
47348		5/26/2009 4:39:19 PM	Rep. Patrick McHenry (R-NC) Senator Jim Inhofe (R-OK) Rep. Sam Johnson (R-TX) Rep. Donald Manzullo (R-IL) Rep. Henry Brown (R-SC) Rep. Jerry Moran (R-KS) Rep. Patrick Tiberi (R-OH) Rep. Pete Olson (R-TX) Senator James Risch (R-ID) Senator Mel Martinez (R-FL) Rep. Robert Latta (R-OH) Rep. John Linder (R-GA) Rep. Jeff Fortenberry (R-NE) Rep. Joe Wilson (R-SC) Rep. Trent Franks (R-AZ) Rep. Steve King (R-IA) Rep. John Kline (R-MN) Rep. Tom Cole (R-OK) Rep. Louie Gohmert (R-TX) Rep. John Fleming, M.D. (R-LA) Rep. Mike Conaway (R-TX) Rep. Wally Herger (R-CA) Rep. Roscoe Bartlett (R-MD) Rep. Paul Broun, M.D. (R-GA) Rep. Glenn Thompson (R-PA) Senator Mike Crapo (R-ID) Rep. Mary Fallin (R-OK) Rep. Michael McCaul (R-TX) Senator Mike Johanns (R-NE) Rep. Pete Hoekstra (R-MI) Rep. Spencer Bachus (R-AL) Senator John Ensign (R-NV) Rep. Scott Garrett (R-NJ) Rep. Jerry Costello (D-IL) Rep. Tim Murphy (R-PA) Rep. Gregg Harper (R-MS) Senator Mike Enzi (R-WY) Senator Chuck Grassley (R-IA) Senator George Voinovich (R-OH)

ID	Status	Date_Stamp	Comments
47992	Redacted	5/26/2009 8:58:33 PM	NIH Stem Cell Guidelines, MSC 7997 9000 Rockville Pike Bethesda, Maryland 20892-7997 May 26, 2009 Dear Sir/Madam: The National Institutes of Health (NIH) is requesting public comment on draft guidelines titled "National Institutes of Health Guidelines for Human Stem Cell Research" (Guidelines). The Catholic Medical Association (CMA) is a nonprofit corporation organized under the laws of the Commonwealth of Virginia and the largest association of Catholic physicians in the United States. This issue is of profound consequence for CMA members in clinical practice and research, and for their thousands of patients. CMA submits the following observations and suggestions: In his March 9, 2009, statement accompanying Executive Order 13505, President Obama decried "the false choice between sound science and moral values." Unfortunately, his prescribed solution to this apparent dilemma ignores both sound ethical values and the most up-to-date findings of scientific research. To the extent that the draft Guidelines are based upon the terms of this prescribed solution, they are fundamentally flawed in their nature and require substantial revision. 1. Human Embryonic Stem Cell Research Is Unethical. Research on human embryonic stem cells (hESC), derived from destroying a human embryo at 4-5 days of gestation, is unethical. Each human being possesses inherent dignity as a unique, unrepeatable person created in the image and likeness of God. Principled respect for human life has characterized the medical profession in Western civilization since the founding of the Hippocratic School. In the 20th century, in response to evidence of profound violations of human rights and dignity – violations sanctioned both by government and by members of the medical profession, respect for human life was explicitly recognized by the Nuremburg Code and by the Universal Declaration on Human Rights. In recent U.S. law, respect for human life and well-being, particularly in research, has been protected by 45 C.F.R. Part 46 (in particular by 45 CFR 46.208(a)(2) and Section 498(b) of the Public Health Service Act [1](42 U.S.C. 289g(b)) (Title 42, Section 289g(b) and by the Dickey-Wicker Amendment, which helps to implement these legal protections. 2. Human Embryonic Stem Cell Research Is Unnecessary. Both President Obama and the NIH in its request for comment cite the need to find cures for serious diseases as a primary reason for providing federal funding for hESC research. However, the (perhaps once understandable) perception that hESC were indispensable for curing serious diseases has been effectively rebutted. Demonstrated success in treating scores of diseases with adult stem cell (ASC)-based therapies shows that ethical, accessible alternatives to destroying human embryos in the name of science already exist. Moreover, recent research into induced pluripotent stem cells (iPSC) shows that the pluripotency once thought to be available only through hESC is in fact available without having to resort to violating both ethical principles and human dignity. And, iPSCs, at least in principle, avoid a difficulty intrinsic to any hESC-based therapies—the challenge of immune rejection. 3. NIH Should Publish a Clear Statement of Ethical Limits and Justification Therefor for Any Research Deliberately Destructive of Human Life. CMA opposes any federal funding of research deliberately destructive of human life. However, given that President Obama and NIH seem determined to proceed with such funding and research, we think that NIH owes the scientific community and the public a clear statement of what worth and dignity nascent human lives possess, and where and how clear lines of principle and procedure will be drawn to protect this dignity.

ID	Status	Date_Stamp	Comments
47992		5/26/2009 8:58:33 PM	President Obama's comments accompanying Executive Order 13505 were unduly expansive—affirming unprecedented federal funding and support for research on early human life—excluding only human reproductive cloning. Yet, the draft Guidelines contain certain limits (e.g., federal funding for research using human embryonic stem cells derived from certain sources, including somatic cell nuclear transfer, parthenogenesis, and/or IVF embryos created for research purposes, is disallowed under these Guidelines), but without explanation of why these lines have been drawn, and whether these protections will hold in the future. NIH should bear in mind that even the Clinton National Bioethics Advisory Commission, in its report "Ethical Issues in Human Stem Cell Research" (1999), acknowledged the need to show respect for human embryonic life, stating that: "In our judgment, the derivation of stem cells from embryos remaining following infertility treatments is justifiable only if no less morally problematic alternatives are available for advancing the research." Of course, less morally problematic and more effective alternatives are now available! Still, NIH ought to be more transparent, and provide the scientific community and the public with a clear explanation of the principles that will guide respect for human life in research. Only in this way can people register assent or dissent to public policy. And, NIH should issue a clear statement making permanent the limit it has expressed in these draft Guidelines—namely, that federal funding will never be provided to fund the creation and destruction of human embryos for research purposes. 4. NIH Should Strengthen the Existing Draft Guidelines. While anything short of full respect for the dignity of every human life represents a serious departure from sound science and ethics, CMA holds that NIH should establish guidelines that are as protective of human life as possible (rather than as broad as politically and financially expedient). In this regard, we suggest the following improvements: • The Guidelines currently call for a "clear separation between the prospective donor(s)'s decision to create human embryos for reproductive purposes and . . . to donate human embryos for research purposes." The Guidelines do not sufficiently define the nature of this separation. Given that the decision to pursue assisted reproductive technology (A.R.T.) is fraught with emotional complexity (not to mention technological and financial complexity), it makes sense to establish a clear separation here, including specific waiting periods, to avoid the worst abuses of the informed consent process. • The Guidelines note that: "Whenever it was practicable, the attending physician responsible for reproductive clinical care and the researcher deriving and/or proposing to utilize human embryonic stem cells should not have been the same person." This is clearly an inadequate standard to avoid abuse. NIH standards should demand no less than a clear and complete separation of persons, similar, for example, to that required in the decision to donate organs. • The Guidelines state that some uses of hESC, even when such cells are derived from "allowable sources, are nevertheless ineligible for NIH funding," e.g., (A) "Research in which human embryonic stem cells . . . or human induced pluripotent stem cells are introduced into non-human primate blastocysts"; and (B) "Research involving the breeding of animals where the introduction of human embryonic stem cells . . . or human induced pluripotent stem cells may have contributed to the germ line." Not only is the rationale for this limitation not explained, but it still provides an unacceptable range of scenarios in which human embryonic stem cells could be introduced into animals. The Guidelines should be amended to read: "Human embryonic stem cells . . . or human induced pluripotent stem cells never may be introduced into animal embryos, or used in any respect to create human/animal hybrids." 5. Concluding Thoughts. On April 23, 2009, in his comments on Holocaust Remembrance Day, President Obama aptly noted: "It is the grimmest of ironies that one of the most savage, barbaric acts of evil in history began in one of the most modernized societies of its time, where so many markers of human progress became tools of human depravity: science that

ID	Status	Date_Stamp	Comments
47992		5/26/2009 8:58:33 PM	can heal, used to kill; education that can enlighten, used to rationalize away basic moral impulses; the bureaucracy that sustains modern life, used as the machinery of mass death, a ruthless, chillingly efficient system where many were responsible for the killing, but few got actual blood on their hands." With regard to this unprecedented change in federal policy in funding research intrinsically linked to destroying human life, America stands at an important threshold. We acknowledge that many involved in this debate have high hopes, noble intentions and, ostensibly, the technology and financial means to pursue their goals. However, it can still be asked whether the ethical principles necessary to prevent this initiative from devolving into a serious, systemic abuse of human rights and dignity have been honestly discussed and acknowledged. This is no small matter—for the soul of science and of American society. We ask NIH to step back and reconsider these weighty questions before committing the Institute and federal funding to a course that is bound to end in ethical disaster, few, if any, cures and many dashed hopes. We ask, finally, for NIH to consider well the dialogue from the end of the well-known movie "Judgment at Nuremburg": Ernst Janning (Burt Lancaster): [T]hose millions of people... I never knew it would come to that. YOU must believe it, YOU MUST believe it. Judge Dan Haywood (Spencer Tracy): Herr Janning, it came to that the first time you sentenced a man to death you knew to be innocent. Thank you for your attention to this most serious matter. Sincerely, *****, M.D. ***** *****, Ph.D. *****
47993		5/26/2009 8:58:37 PM	The National Institutes of Health should rescind its guidelines proposing to use federal funds for stem cell research that requires destroying live human embryos. It is especially troubling that some supporters of this research are urging the NIH to endorse an even broader policy, encouraging the deliberate use of in-vitro fertilization or cloning to produce human embryos for stem cell research. Such creation of new life solely to destroy it would mark the final reduction of human beings to mere objects or commodities. My tax dollars should not be used to promote destructive embryonic stem cell research or any form of human cloning. Instead support should be directed to adult stem cell research, which is ethically sound, harms no one, and is already helping suffering patients with dozens of conditions.

ID	Status	Date_Stamp	Comments
48143		5/26/2009 10:04:43 PM	May 26, 2009 VIA ELECTRONIC SUBMISSION AND E-MAIL NIH Stem Cell Guidelines MSC 7997 9000 Rockville Pike Bethesda, MD 20892-7997 Re: Draft NIH Guidelines for Human Stem Cell Research, 74 Federal Register 18578-18580 (April 23, 2009); Comment Period Ending: May 26, 2009 To Dr. Raynard S. Kington: Bioethics Defense Fund, on behalf of neurobiologist and stem cell researcher Dr. Maureen L. Condic and the Westchester Institute for Ethics and the Human Person (collectively referred to as "Commentators"), whose interests are more fully described in Appendix A, respectfully submit the following comments on the above-referenced "Draft NIH Guidelines for Human Stem Cell Research" ("Guidelines"). We request that this comment be made part of the public record of the proceedings and that NIH consider this letter as relevant matter to be taken into account in any statement of the basis and purpose of this rulemaking action under 5 U.S.C. § 553. COMMENTS For the reasons set forth below, Commentators respectfully request that the NIH reject the proposed Guidelines and cease any effort to use federal tax dollars to fund research involving newly created human embryonic stem cells (hESC) lines. Federal funding of research on the basis of the proposed Guidelines is both ethically irresponsible and scientifically unworthy, especially in light of the continuing breakthroughs in induced pluripotent stem cells (iPSCs). The 2007 breakthrough in induced pluripotent stem cells, or iPS cells, provides patient-specific stem cells that are the functional equivalent of embryonic stem cells. iPSC research meets every mark of good science and has the following ethical advantages: It does not destroy human embryos; it does not use human oocytes (eggs) harvested from women; and it does not alienate a large part of the country's citizens by engaging in research that they find deeply immoral. Commentators present the following ten myths and facts for consideration by the NIH: 1. Myth: Despite the 2007 and ongoing breakthroughs in iPSC research, disease resea rch should also include the use of human embryonic stem cells. Fact: Direct reprogramming to create iPS cells from patients' skin cells provides a scientifically feasible and promising alternative to human embryonic stem cell research. The Obama administration should therefore adopt the policy of President Clinton's bioethics commission, which concluded that human embryo destruction posed a moral problem and was "justifiable" only if there were no alternatives: "In our judgment, the derivation of stem cells from embryos remaining following infertility treatments is justifiable only if

ID	Status	Date_Stamp	Comments
48143		5/26/2009 10:04:43 PM	no less morally problematic alternatives are available for advancing the research. But as we have noted, stem cells from embryos appear to be different in scientifically important ways from AS cells and also appear to offer greater promise of therapeutic breakthroughs. The claim that there are alternatives to using stem cells derived from embryos is not, at the present time, supported scientifically. We recognize, however, that this is a matter that must be revisited continually as science advances." National Bioethics Advisory Commission, Ethical Issues in Human Stem Cell Research (Sept. 1999), Volume I, p. 53. 2. Myth: Human embryonic stem cell research involves only embryos t hat will be discarded by fertility clinics. Fact: Human embryo cloning is the endgame. Unlike iPS cells, hES cells from "surplus embryos" are not genetically identical to patients, and would be rejected by the immune system. For hESCs to be patient-specific like iPS cells, "surplus embryos" from fertility clinics will not be sufficient. Instead, cloned embryos will have to be intentionally produced in the laboratory, and then destroyed to obtain stem-cell lines. NIH funding of so-called "surplus" human embryos in fertility clinics will serve only to coarsen the conscience of the nation regarding the use of human life as raw material for science experiments. The proposed Guidelines, if adopted, will pave the way for current congressional proposals to repeal the Dickey-Wicker amendment so that federal funding can be used to clone, fertilize and destroy human embryos solely for the purpose of experimentation. 3. Myth: We don't know whether iPSCs or hESCs will be better for research . Fact: There are at least three significant reasons why iPS cells are better for research: First, patient-specific iPSCs are available "here and now," compared to the merely theoretical prospects of stem cells from human-embryo cloning. Direct reprogramming is the ONLY way to derive pluripotent cells from specific adult patients (i.e. patient-specific stem cells) for research on human genetic diseases at this time. In the last year, multiple disease-specific human iPS cell lines have already been produced. Second, direct reprogramming makes multiple iPSC lines from an individual patient's skin cells without any additional cost or effort—an enormous scientific advantage. Obtaining iPSCs does not require access to a fertility clinic, simplifying the requirements for research,. iPS cells are easier to produce than hESCs, so more scientists will work with them and research will advance much more quickly. In the last year, over 800 new laboratories have begun conducting research on iPS cells. Third, because iPS cells do not involve human embryos or human eggs, they will be subject to significantly simpler regulatory requirements. IPS cells are fully eligible now for funding by the NIH without the need for the newly proposed Guidelines, and in fact the initial iPSC study by Dr. Thomson was partly funded by the NIH. 4. Myth: We don't know whether iPSCs or hESCs will be better for therapies. Fact: Currently, clinical trials for both hESCs or iPSCs are problematic because of concerns regarding safety (cancer risk)

ID	Status	Date_Stamp	Comments
48143		5/26/2009 10:04:43 PM	and efficacy (ability to differentiate into useful cell types). However, if these obstacles can be overcome, there are at least two significant reasons why iPSCs will be better for human therapies: First, iPS cells are patient-specific, a huge advantage for therapeutic use, compared to hES cells from "surplus" fertility clinic embryos that are not patient-specific and would require immune suppression. Second, iPS cells do not use human eggs, making it possible to develop therapies without imposing significant medical risks on women who are induced with thousands of dollars to be given high doses of hormones to produce numerous eggs per cycle for egg production and surgical extraction. 5. Myth: Embryonic stem cells are better because they are natural cells, not laboratory -produced like iPSCs. Fact: Just because embryonic stem cells, known as ES cells, are isolated from embryos does not mean that they are unchanged by the isolation process. Multiple studies have shown that ES cells are not identical to natural cells of the embryo; rather they are a laboratory-produced cell type, just as iPS cells are. This is precisely why ES cells can be patented as "inventions." 6. Myth: Scientists still need to compare iPSCs to the "gold standard" of hESCs. Fact: Yes, but this does not require the on-going destruction of human embryos to make more hESC lines. Existing hESC lines are more than sufficient for this comparison. The currently eligible and available cell lines are listed here: http://stemcells.nih.gov/research/registry/eligibilityCriteria.asp. Furthermore, the primate system permits the best in-depth platform for comparative studies. From Rhesus macaque monkeys, primate pluripotent stem cells are available from all conceivable sources: IVF embryos, naturally conceived embryos (removed from the fallopian tube after fertilization), somatic cell nuclear transfer-cloned embryos, parthenotes, and, recently, primate iPS cells as well. 7. Myth: We don't really know if iPSCs and hESCs are equivalent. Fact: Dr. James Thomson, the first scientist ever to isolate, culture, and characterize human embryonic stem cells in 1998, and author of one of the 2007 initial human iPSC studies, found that iPS cells "meet the defining criteria" for embryonic stem cells "with the significant exception that the iPS cells are not derived from embryos." Yu J, Vodyanik MA, Smuga-Otto K, Antosiewicz-Bourget J, Frane JL, Tian S, Nie J, Jonsdottir GA, Ruotti V, Stewart R, Slukvin II, Thomson JA, Induced pluripotent stem cell lines derived from human somatic cells, SCIENCE, 2007 Dec 21;318(5858):1917-20. Epub 2007 Nov 20. Mouse iPSCs have passed the strictest possible scientific tests for being functional equivalents of mouse ESCs. Tests for human cells are more limited, but human iPSCs have met all the available criteria for being the functional equivalent of hESCs. This can be established with greater certainty through comparisons with the existing hESC lines available through the Bush registry, which have been used in the vast majority of human ESC studies throughout the world.

ID	Status	Date_Stamp	Comments
48143		5/26/2009 10:04:43 PM	It is important to note that although iPS cells are the functional equivalent of hES cells harvested from embryos, neither type of stem cells can turn into a human embryo. To be an embryo a cell must be able to do two important things; make all the cell types found in the body and, more importantly, organize those cells into a coherent, functional body. Many kinds of tumors and some types of stem cells (including iPS cells) can make all the cell types found in the body. However, human iPS cells are only weakly able to make the cell types found in the placenta, and could not produce enough placental cells to allow for implantation into the uterus or to support the needs of a developing baby. More importantly, iPS cells are not able to organize the cells they produce into a functional body. Like human embryonic stem cells, iPS cells produce disorganized tumors containing all the cell types found in the body in a chaotic mass. They do not produce babies. Only human zygotes (one-cell embryos) and possibly individual cells from embryos up to the 4-cell stage are truly "totipotent" – able to both make all cell types and organize them into a functioning human body. Furthermore, the ability of a blastocyst stage embryo to twin does not mean that hES cells or iPS cells are able to become a human embryo. The ability of a split embryo to produce two identical twins reflects a property of the embryo as a whole organism, not a property of the cells extracted from the embryo. At this early stage, the embryo has only two major cell types, trophectoderm (TE) which becomes the placenta, and inner cell mass (ICM) which organizes to form the fetal body. So long as each half of a split embryo contains some of each cell type, it is able to repair this injury, and continue on as a whole organism—with TE cells replacing the missing parts of the TE, and ICM cells replacing the missing parts of the ICM. In contrast, embryonic stem cells are produced from only a part of the embryo, the ICM, and cannot replace the missing TE. Just as an isolated heart would not be able to "regenerate" the whole person it was taken from, an isolated ICM "part" cannot not replace the whole embryo it was taken from. 8.□ Myth: We shouldn't limit research to iPSCs because these cells can make tumors and convert to cancer cells. Fact: Multiple scientific studies show that all pluripotent cells, including human embryonic stem cells, form tumors (teratomas) and can convert to cancer cells. The risk of tumor formation from iPS cells was initially greater than that of embryo-derived stem cells because the genes used for reprogramming remained inserted in the cell. However, over the last year, the iPS technique has been significantly improved. Current approaches have eliminated any added risk of tumor formation, and iPS cells are now no more likely to produce tumors or cause cancer than are hESCs. The risk of tumor formation that is NOT due to the reprogramming procedure but common to all pluripotent stem cells can theoretically be addressed by converting pluripotent stem cells into mature cells that do not form tumors and can be transplanted safely to patients. It is important to understand that the efficient conversion of pluripotent stem cells to transplantable cells useful in the clinic is not yet possible for any human cell type, although much progress has been made. Thus, no immediate therapies should be expected from human pluripotent stem cells, either embryo-derived or iPSC. 9. Myth: Human embryos are just a ball of cells, but patients are human being s who are suffering. Fact: All human beings began life as a one-cell embryo. The argument that small size and immaturity are sufficient reasons to destroy one human individual, in the hope of benefiting someone of larger size or greater maturity is clearly an unethical line of reasoning. The critical question is whether human embryos at early stages are mere collections of human cells or developing human beings. This question has been thoroughly addressed by the scientific evidence: Embryos are developing human beings, not tumors or disorganized collections of human cells. They are small and immature, as all human beings once were, but they are human individuals. As Dr. Leon Kass, former chairman of the President's Council on

ID	Status	Date_Stamp	Comments
48143		5/26/2009 10:04:43 PM	Bioethics, has stated in the Washington Post: "The moral issue does not disappear just because the embryos are very small or because they are no longer wanted for reproductive purposes: Because they are living human embryos, destroying them is not a morally neutral act. Just as no society can afford to be callous to the needs of suffering humanity, none can afford to be cavalier about how it treats nascent human life." Leon R. Kass, Playing Politics With the Sick, WASH. POST, Oct. 8, 2004, at A35. 10. Myth: Opponents of human embryonic stem cell research are illegitimately imposing their 'religious' views. Fact: The objection to using human life as raw material for science experiments is based on ethics and morals that recognize the dignity of every member of the human family, and not any spiritual religious belief. Religious objections are those that appeal to specific religious traditions, invoking religious authorities or teachings, such as those found in the Quran, the Torah, or the Bible. An objection to eating during daylight hours in the month of Ramadan, to eating pork at any time, or to eating meat on Fridays during the season of Lent would be examples of "religious" objections stemming from the Islamic, Jewish, and Catholic traditions, respectively. Such objections should indeed be confined to members of the religion itself. By contrast, objections to human embryo destructive research are not religious; they are ethical and moral objections. They are based on religiously neutral reasoning that takes into consideration both the scientific evidence establishing that human embryos are human individuals and the current U.S. law that prohibits harming human beings (including prenatal human beings) in scientific experiments. The protection of human beings who participate in scientific research is an important ethical consideration. The Nazi experiments on Jews, the Tuskegee syphilis experiments on black men, and the Japanese hypothermia experiments on prisoners of war were unethical, and were not justified simply because they led to new and exciting discoveries that benefited patients. Science, like all human endeavors, must operate within an ethical framework. This is not a religious objection; it is a basic tenet of universal human rights. For the above reasons, Commentators urge the NIH to reject the proposed Guidelines that provide for federal funding of the unethical, immoral and scientifically unworthy research using stem cells derived from newly destroyed human embryos. Sincerely, Nikolas T. Nikas Dorinda C. Bordlee BIOETHICS DEFENSE FUND 6811 E. Voltaire Avenue Scottsdale, AZ 85254

ID	Status	Date_Stamp	Comments
48143		5/26/2009 10:04:43 PM	On behalf of Dr. Maureen L. Condic and the Westchester Institute for Ethics and the Human Person APPENDIX A Statements of Interest: Bioethics Defense Fund Nikolas T. Nikas, President and General Counsel Dorinda C. Bordlee, Senior Counsel 6811 E. Voltaire Avenue Scottsdale, AZ 85254 (480) 483-3597 E-Mail: info@bdfund.org Bioethics Defense Fund ("BDF") is a national public interest law and policy organization that advocates for the human right to life through litigation, legislation and public education in the public policy arenas of abortion, human cloning/human embryonic stem cell research and end-of-life issues. In courts, legislative bodies, law schools, medical conferences and in the media, Bioethics Defense Fund educates policy shapers and citizens about the objective facts surrounding human reproduction and embryology and its role in bioethics law and policy. Bioethics Defense Fund lawyers served as lead counsel in a Missouri case challenging deceptive wording in ballot initiative that purported to ban human cloning, Missourians Against Human Cloning v. Carnahan (Circuit Court of Cole County, 2006); and filed an amicus brief on behalf of Princeton professor and member of the President's Council on Bioethics, Robert P. George, D.Phil., J.D. and Dr. Maureen L. Condic in Cures Without Cloning, et al. v. Carnahan, Case No. WD 69376 (W.D. Mo. 2008). Maureen L. Condic, Ph.D. 207 5th Avenue Salt Lake City, UT 84103-2501 Email: mlcondic@neuro.utah.edu Dr. Condic is an Associate Professor of Neurobiology and Anatomy at the University of Utah School of Medicine, with an adjunct appointment in the department of Pediatrics. She received her undergraduate degree from the University of Chicago, her doctorate from the University of California at Berkeley and postdoctoral training at the University of Minnesota. Since her appointment at the University of Utah in 1997, Dr. Condic's primary research focus has been the development and regeneration of the nervous system. In 1999, she was awarded the Basil O'Connor Young Investigator Award for her studies of peripheral nervous system development. In 2002, she was named a McKnight Neuroscience of Brain Disorders Investigator, in recognition of her research in the field of adult spinal cord regeneration. In addition to her scientific research, Dr. Condic teaches both graduate and medical students. Her teaching focuses primarily on embryonic development, and she directed the University of Utah School of Medicine's course in Human

ID	Status	Date_Stamp	Comments
48143		5/26/2009 10:04:43 PM	Embryology. Westchester Institute for Ethics and the Human Person Fr. Thomas Berg, Ph.D., Executive Director P.O. Box 10 Hopewell Junction, NY 12533 Email: tberg@westchesterinstitute.net The Westchester Institute for Ethics & the Human Person is a research institute conducting interdisciplinary, natural law analysis of complex, contemporary moral issues yet unresolved among Judeo-Christian scholars. Anchored in the classic perennial and Catholic view of the human person, our moral inquires are first and foremost of a scholarly nature. However, we pursue answers to these disputed questions with an eye toward enriching the quality of contemporary moral discourse, and fostering sound prudential judgment in cultural and political matters. We are currently dedicated to the following issues: • The genesis of human life & the moral status of the human embryo • The search for scientifically and morally feasible alternatives to embryo-based biomedical research • The use of emergency contraception in rape protocols • The determination of human death, and end-of-life issues • The relationship between religion, science, and reason as sources of moral insight for modern society. The Westchester Institute and its scholars have become a distinguished resource and point of reference for think-tanks, centers for applied research, and institutes of public policy analysis. Together, we seek to make a significant contribution to the common good and to contemporary culture. ###
48144		5/26/2009 10:04:52 PM	I object to any tax dollars being used to fund embryonic stem cell research which is a result of the destruction of human life. Research in the area of adult stem cells, which is not controversial, has already shown medical benefits which embryonic stem cells do not. No tax dollars should be used for embryonic stem cell research! The proposed regulations do not prevent future funding for embryonic stem cell research that could lead to the creation of clones and human-animal hybrids. This loophole must be closed immediately.
48145	Redacted	5/26/2009 10:04:53 PM	Dear NIH, I am opposed to your draft guidelines for embryonic stem cell research, which force me as a taxpayer to subsidize research requiring the destruction of innocent human life. Support should be directed to stem cell research and treatments that harm no one and are already producing good results. In no case should government support be extended to human cloning or other morally reprehensible creation of human embryos for research purposes.

ID	Status	Date_Stamp	Comments
47067	Redacted	5/26/2009 2:57:53 PM	Please see .txt attachment and .pdf version sent via e-mail. Do No Harm: The Coalition of Americans for Research Ethics, molecular biologists and stem cell researchers Dr. Theresa Deisher and Dr. James L. Sherley, the Family Research Council, Concerned Women for America, the Christian Medical Association, Advocates International, and the Alliance Defense Fund (collectively "Do No Harm et al." or "Commentators"), whose interests are more fully described in Appendix A, hereby respectfully submit the following comments (including the accompanying attachments) on the above-referenced "Draft NIH Guidelines for Human Stem Cell Research" ("Guidelines"). We request that this letter and each of its appendices be made part of the public record of the proceedings and that NIH consider this letter and its appendices as relevant matter to be taken into account in any statement of the basis and purpose of this rulemaking action under 5 U.S.C. § 553.
47068		5/26/2009 2:58:30 PM	Viable stem cell lines have been created by private funding and state governments in order to work around the limitations set by the Bush Administration. Please do not make our scientist start over. Instead, allow them to use these cells and lines created under other policies. Make sure that the new NIH guidelines allow these cells to be implemented into continued research using federal funds.
47069		5/26/2009 2:58:36 PM	I oppose killing human embryos. The proposed regulations will force taxpayers like me to fund research I believe is unethical because it requires the killing of human embryos. Expanding funding to new human embryonic stem cell lines will divert federal funds away from promising research that is treating people now with non-embryonic stem cells and will also divert funds away from other sources of embryonic-like stem cells that have been generated without the use of human embryos. The proposed regulations create a financial incentive for the creation of more human embryos to be destroyed to obtain their embryonic stem cells. The guidelines do not require any separation between an IVF doctor and an ESCR researcher. The guidelines say they "should" be separate, but only when practicable. The guidelines allow any IVF doctor to create more embryos than are needed for fertility purposes in order to generate more so-called "leftover" embryos for ESCR research using taxpayer funds. Instead of preventing any future expansion of funding for ESCR on unethical experiments involving human clones and human-animal hybrids, these regulations open the door for such funding upon the order of NIH. The guidelines do not require full informed consent for the parents of the human embryos so that they understand that their options include permission for infertile couples to adopt them.
47070		5/26/2009 2:58:45 PM	The National Institutes of Health should rescind its guidelines proposing to use federal funds for stem cell research that requires destroying live human embryos. It is especially troubling that some supporters of this research are urging the NIH to endorse an even broader policy, encouraging the deliberate use of in vitro fertilization or cloning to produce human embryos for stem cell research. Such creation of new life solely to destroy it would mark the final reduction of human beings to mere objects or commodities. My tax dollars should not be used to promote destructive embryonic stem cell research or any form of human cloning. Instead support should be directed to adult stem cell research, which is ethically sound, harms no one, and is already helping suffering patients with dozens of conditions.
47071		5/26/2009 2:59:04 PM	I am opposed to embryonic stem cell research. Life begins at conception. This is conducting research on a living being. I am opposed to it in all respects.

47067_Do_No_Harm_et_al._Comments_re_Proposed_NIH_Stem_Cell_Guidelines

May 26, 2009

VIA ELECTRONIC SUBMISSION AND E-MAIL

NIH Stem Cell Guidelines

MSC 7997

9000 Rockville Pike

Bethesda, MD 20892-7997

Re: Draft NIH Guidelines for Human Stem Cell Research,
74 Federal Register 18578-18580 (April 23, 2009);
Comment Period Ending: May 26, 2009

To Dr. Raynard S. Kington:

Do No Harm: The Coalition of Americans for Research Ethics, molecular biologists and stem cell researchers Dr. Theresa Deisher and Dr. James L. Sherley, the Family Research Council, Concerned Women for America, the Christian Medical Association, Advocates International, and the Alliance Defense Fund (collectively "Do No Harm et al." or "Commentators"), whose interests are more fully described in Appendix A, hereby respectfully submit the following comments (including the accompanying attachments) on the above-referenced "Draft NIH Guidelines for Human Stem Cell Research" ("Guidelines"). We request that this letter and each of its appendices be made part of the public record of the proceedings and that NIH consider this letter and its appendices as relevant matter to be taken into account in any statement of the basis and purpose of this rulemaking action under 5 U.S.C. § 553.

GENERAL COMMENTS

The Guidelines were purportedly drafted "to help ensure that NIH-funded research in [the area of human embryonic stem cells] is ethically responsible, scientifically worthy, and conducted in accordance with applicable law." Guidelines, Summary, 74 Fed. Reg. 18578. As proposed, however, the Guidelines fail to achieve even one of these goals. For the scientific, legal, and ethical reasons set forth below, we respectfully request that the NIH decide not to issue the proposed Guidelines and take no further steps to fund research invlving "human embryonic stem cells," other than the ongoing research on the stem cell lines in the NIH's Human Embryonic Stem Cell Registry permitted under the NIH's current guidelines, as set forth in NIH Notice Number NOT-OD-09-085. Any federal funding of research on the basis of the proposed Guidelines at this time:

(1) Is illegal.

(a) Federal funding of human embryonic stem cell research violates the plain language and clear intent of applicable federal law.1

(b) This federal funding also promotes the destruction of human embryos in a manner that may violate applicable state law.2

(2) Is unnecessary and inappropriate due to several advances in scientific research and medical understanding that promise to achieve each of the stated purposes for the proposed Guidelines without violating the legal and ethical boundaries implicated by the use of human embryonic stem cells:

(a) Scientific developments achieved utilizing adult stem cells provide or promise to provide actual cell-based therapies that will lead to beneficial results for patients suffering from the diseases and conditions amenable to such therapies noted in the proposed Guidelines.

(b) Recent scientific developments provide the ability to create induced human pluripotent stem cells already approved for funding by NIH, which offer an ethical, viable alternative to embryonic stem cell research. Such cells are fully capable of achieving the Guidelines' stated desires "to test new drugs" and obtain "a better understanding of the genetic and molecular controls" involved in serious medical conditions such as cancer and birth defects, which arise due to abnormal cell division and differentiation. Guidelines, Supplementary Information.

(c) The body of scientific evidence indicates that human embryonic stem cells are abnormal, tumor-producing cells that cannot achieve the very purposes for embryonic stem cell research that are offered in the proposed Guidelines.

(3) Lacks necessary and sufficient "conflicts of interest" safeguards because:

(a) The proposed Guidelines do not prohibit contractual, agency or corporate relationships between the IVF clinic that creates and then cryogenically stores the human embryo, the researchers (a/k/a the "derivers") who kill that human embryo to harvest its stem cells, and the researchers (a/k/a the "users") who will be funded by NIH to continue the research process with respect to these human embryo stem

cells. Indeed, it appears that the Guidelines do not even prohibit the deriver and the user from being the very same person. See Guidelines, Part II.B.6.

(b) The proposed Guidelines erroneously presume that the parents of the human embryo have the legal right under applicable state law, as well as the moral and ethical authority, to substitute their judgment for the judgment of the legally incompetent human embryo to withhold essential life-supporting medical care from the human embryo, thereby assuring that their embryonic child will surely die. Not only is state law on this point far from settled, but the parents' moral and ethical authority to do so is far from accepted. At a bare minimum, as a prerequisite for funding, the Guidelines should require a judicial proceeding, in which the human life interests of the human embryo(s) in question are represented by a court-appointed attorney pro vita (for life), and court approval before such a parental "donation" would be deemed lawful under state law and free of the obvious conflicts of interest presented when the parents of an offspring initially conceived to be their child are now proposing to terminate its life solely for medical research purposes supported by federal tax dollars, particularly when there has been no showing that: (1) other more life-preserving options have been explored for the embryonic child and reasonably excluded; and (2) the federally financed researcher has established that a compelling governmental interest exists to perform the research, which interest cannot otherwise be satisfied without destroying the lives of these human embryos.3

(4) Lacks necessary and sufficient "informed consent" safeguards because the proposed Guidelines do not even require the parents of the human embryos (a/k/a "the potential donors") to be informed that:

(a) Scientifically speaking, each of their human embryos is a living human being;

(b) Legally speaking, many states hold that human life begins at conception. In these states, the "donation" of human embryos for research may be deemed to be the taking of human life.4 In multiple states, research involving human embryos is effectively banned.5

(c) Insofar as each of these human beings is "no longer needed," Guidelines, Supplementary Information at 18579, it is now possible for the parents to place each embryo up for adoption as an alternative to having the human embryo killed for research purposes.6

SPECIFIC COMMENTS

1. It is axiomatic that regulations of a federal agency cannot violate an applicable federal statute.7 The proposed Guidelines violate an applicable federal statute. Current federal law prohibits federal funding of any "research in which a human embryo or embryos are destroyed, discarded, or knowingly subjected to risk of injury or death greater than that allowed for research on fetuses in utero under 45 CFR 46.204(b) and section 498(b) of the Public Health Service Act (42 U.S.C. 289g(b))." Federal Funding Ban, subsection (a)(2). The proposed Guidelines (including in particular Part II.B, authorizing federal funding for embryonic stem cell research) would infringe this and other current laws and regulations protecting the human embryo from harmful experiments at federal expense, because the research to be funded by the Guidelines necessarily involves and entails the destruction of human embryos. Indeed, human embryonic stem cell research cannot be conducted without destroying the human embryos involved, and thus the clear and inevitable purpose and effect of the Guidelines is to necessitate and encourage the destruction of human embryos for research in direct violation of the Federal Funding Ban. Such a purpose and effect is contrary to Congress' clear intent to maintain the status quo by re-enacting the current ban on federal funding for destructive human embryonic research. As one legal commentator has explained that legislative history:

The history behind federal funding of human embryo research evinces uneasy disapproval of this type of experimentation. Since 1980, the federal government has withheld funding for human embryo research by de facto moratorium. Until 1993, [45 C.F.R. § 46.204(d)] authorized federal funding of embryo research subject to approval of such projects by a Department of Health and Human Services Ethical Advisory Board ("EAB"). The first-and only-EAB appointed to evaluate embryo research concluded that it was ethical as a theoretical matter for the purpose of developing IVF techniques. Despite this approval, the NIH neither took action on a specific project nor appointed additional EAB's, and funding was never allocated for

projects involving embryo research.
The National Institutes of Health Revitalization Act of 1993 eliminated the EAB approval requirements of 45 C.F.R. § 46.204(d). . . .
Before allocating any funds, however, the NIH convened the Human Embryo Research Panel. The Panel gathered nineteen participants with expertise in clinical research, ethics, law, social science, public health, and public policy to consider the moral and ethical implications of human embryo research, and to develop funding Guidelines for that research.
After listening to testimony from more than forty witnesses and reviewing correspondence from 30,000 individuals, the Panel [affirmed the NIH's prior recommendation] that embryo research should be funded by the federal government. The members found that human embryo experimentation would generate significant advances in scientific research-particularly in the areas of infertility, genetic defects, and disease therapy. The Panel struggled, however, with the ethical implications of research conducted with deliberately fertilized embryos. While they did not define the precise moral or legal status of the embryo, they attempted to design their recommendations with "respect" for the embryo as a symbol of human life. The Panel believed that their Guidelines and corresponding public funding would also stimulate ethical and scientific review of privately funded embryo research. . . .
The Advisory Committee to the Director of the NIH ("ACD") approved all of the Panel's recommendations-including the one permitting deliberate creation of research embryos-and passed the recommendations on to the NIH Director, Harold Varmus, for the ultimate funding decision. Within hours of that vote, however, President Clinton stated: "I do not believe that federal funds should be used to support the creation of human embryos for research purposes, and I have directed that the NIH not allocate any resources for such research." William Galston, deputy director of Clinton's Domestic Policy Council, later confirmed that the Clinton administration had decided even before the ACD's meeting that deliberate creation of human embryos for experimentation exceeded the public's tolerance for "exotic" research.
The President's announcement did not prevent Varmus from implementing the NIH Panel's other recommendations--such as . . . funding for experimentation on "surplus" embryos. Congress, however, has since passed broader restrictions. Under Public Law 105-78 [continued under Pub. L. No. 110-329], federal funds are presently unavailable not only for the creation of research embryos, but also for any type of research in which human embryos are destroyed, discarded, or knowingly subjected to risk of injury or death. In effect, the moratorium on federally-funded embryo research continues. No federal legislation, however, exists to regulate embryo research conducted in the private sector.8

The foregoing legislative history makes clear that the purpose of the Federal Funding Ban was to prevent NIH from implementing the very strategy that the Guidelines are now being proposed to implement-federal funding for experimentation on "surplus" human embryos. Given the nature of the living human embryo, any human stem cell research is, in the words of the Federal Funding Ban, a type of "research in which a human embryo or embryos are destroyed, discarded, or knowingly subjected to risk of injury or death. . . ." It is patently impossible for NIH, in good conscience, to promulgate these Guidelines and begin funding human embryo research without knowing that such funding means that human embryos are thereby being "subjected to risk of injury or death" in patent violation of the Federal Funding Ban-a risk that would not exist were it not for the incentives to destroy embryos created by the availability of NIH funding.9 There will be no way for NIH to wash its hands of its complicity in the destruction of human embryos involved in the research projects it funds; the funding proposed in the Guidelines can serve only to create the very "risk of injury or death" prohibited by the Federal Funding Ban. Indeed, the Guidelines confirm this understanding, because they directly regulate the manner in which consent for embryo destruction is obtained from the parents and determine the categories of embryos that should be destroyed for federally funded research projects. See Guidelines, Part II.B. Moreover, such funding plainly contradicts NIH's prior pronouncement that the early human embryo "warrants serious moral consideration as developing form of human life." NIH, Final Report of the Human Embryo Research Panel, Page 2 (1994). Killing a defenseless human being, then asking every taxpayer to pay for research on that human being's cells, is the exact opposite of "moral consideration"-it is callous inhumanity.

2. This Federal Funding Ban has been in place since 1996, and its meaning has not changed. There is no justification for ignoring the plain language of the Federal Funding Ban. Any research involving cells derived through the destruction of human embryos is necessarily research "in which a human embryo" is destroyed. Indeed, HHS itself has acknowledged that, in order for any guidelines to comply with the Federal Funding Ban, it is critical that "human embryonic stem cell research [be] limited to a discrete set of stem cell lines with respect to which the life and death decision has been made prior to the announcement of the policy . . . [which would] provide[] no incentives for the destruction of additional embryos."10

3. By necessarily entailing, promoting, and encouraging the destruction of human embryos, the federally funded research envisioned by the Guidelines also potentially violates various state laws and policies, without even considering, let alone justifying, these intrusions on state law and policy.11 For example, 21 states have fetal homicide statutes that apply without regard to gestational age. See Appendix B, Part I. Eight states have wrongful death statutes that apply regardless of gestational age. Id. at Part II.

Still other states explicitly proclaim that life begins at conception.12 The overwhelming majority of medical authorities equate the terms "conception" and "fertilization." For example, a medical text commonly used near the time these definitions were adopted stated:

The term conception refers to the union of the male and female pronuclear elements of procreation from which a new living being develops. It is synonymous with the terms fecundation, impregnation, and fertilization.

J. Greenhill and E. Friedman, Biological Principles and Modern Practice of Obstetrics, 17 (1974) (emphasis in original).13 This usage continues to the present day, as the majority of medical dictionaries now in use follow the American Medical Association in defining conception as "[t]he fertilization of an egg by a sperm that initiates pregnancy." AMA Complete Medical Encyclopedia 392 (2003).14 In those states that acknowledge and protect life from the moment of fertilization or conception, "donation" of human embryos for the purpose of destruction is properly viewed as a state criminal violation. The Guidelines fail even to consider this possibility, and improperly seek to encourage and fund potentially illegal activity.

4. As further discussed below, adult stem cell research has already provided a wide array of vastly important real-world medical benefits and promises future advances of similar quality. It is, therefore, a worthy scientific priority meriting federal funding so long as it is pursued in a lawful, ethical and scientifically appropriate fashion on the basis of broad public consensus as to what is socially acceptable for American taxpayers to fund. The public generally supports adult stem cell research that does no harm to anyone, but many millions of American taxpayers oppose research like human embryo stem cell research that relies on destroying one human life in the speculative (and illusory) hope of perhaps making another human being's life better somehow, some day. Under these circumstances it would be arbitrary and capricious for the NIH to force every American taxpayer to pay for research that is scientifically unnecessary and that many Americans believe to be unethical, particularly where alternative research avenues exist for pursuing the same goals in a more uncontroversial, lawful, and ethical fashion.

Adult stem cells have verifiably treated countless individuals suffering from a wide variety of diseases including, but not limited to, ovarian cancer, retinoblastoma, brain tumors, testicular cancer, chronic and acute leukemias, breast cancer, renal cell carcinoma, anemias, Crohn's disease, rheumatoid arthritis, and juvenile (Type I) diabetes.15 Adult stem cells also present the following benefits that embryonic stem cells ("ESCs") cannot:16

* Adult stem cells provide a readily available and flexible source of stem cells for the treatment of disease.
* Adult stem cells can be harvested from various tissue sources, including virtually all body tissues, as well as tissues normally discarded after birth (umbilical cord blood, placenta).
* Adult stem cells can be harvested as well as grown in numbers sufficient for patient treatments.
* Adult stem cells can provide matched tissue transplants, especially in the majority of cases where the patient's own cells are used, and also in donor

transplants.
* Adult stem cells do not present a risk of tumor formation, making these cells a safe therapeutic strategy.
* Adult stem cells show some ability to home to sites of tissue damage, and the homing ability can be further enhanced to increase efficacy and delivery.
* Adult stem cells have shown efficacy at repairing damaged and diseased tissue in numerous animal models of disease and injury.
* Adult stem cells have already demonstrated their efficacy in improving the health and saving the lives of thousands of patients.

5. Not only has adult stem cell research progressed in recent years, so has human induced pluripotent stem cell ("iPSC") research. This research provides an ethical alternative to human embryonic stem cell research. Accordingly, even if NIH had reason to believe that research involving human embryonic stem cells would be as valuable from a scientific and medical standpoint as research involving human adult stem cells (which it does not), it would be arbitrary and capricious for NIH to fund embryonic stem cell research when it could achieve the same scientific and medical goals through research involving human induced pluripotent stem cells that does not pose the same moral and ethical problems. Below is a summary of the advantages of iPSCs:17

* Induced pluripotent stem cells ("iPSCs") are a substitute for embryonic stem cells (ESCs), and have additional advantages over ESCs.
* iPSCs are indistinguishable from ESCs in their morphology and cellular behavior.
* iPSCs can be created through reprogramming of virtually any somatic cell type.
* iPSC lines can be created more easily and less expensively than ESC lines.
* iPSC creation does not require use of embryos, eggs, or nuclear transfer (organismal) cloning, thus bypassing ethical concerns associated with use of embryos, eggs, and cloning in stem cell research.
* iPSC lines can be created from a specific individual, allowing creation of patient-specific cell lines. Several such lines have already been created from individuals with specific diseases so that disease mechanisms and potential drug-based therapies can be studied in the laboratory. There is also the potential that such lines would provide cells that would not be rejected if transplanted into the same individual from whom they were derived.

6. In addition to the facts that adult stem cell research has made significant strides, provides a wide array of life-saving treatments, and offers the prospect of many further medical and scientific advances, recent scientific research suggests that human embryonic stem cells ("hESCs") can never be transplanted into children or adults as a safe and effective therapeutic. In fact, research suggests that hESCs will not lead to safe and effective human therapeutics-thus obviating the need for human embryonic stem cell research at all, since, as the Guidelines recognize, the very purpose of this research is to develop cures or treatments for various diseases. Guidelines, Supplementary Information, 74 Fed. Reg. 18578.

Human embryonic stem cells ("hESCs") will not lead to safe human therapeutics and are therefore inappropriate federal funding targets for the following reasons:18

A. ESCs are not normal cells.

1. While the cells of the inner cell mass give rise to the organism during normal embryonic development, the derivation of embryonic stem cells ("ESCs") from the inner cell mass generates cells that exhibit epigenetic changes and that form tumors in vivo, even after in vivo tetraploid fetus derivation. The formation of tumors by hESCs is an essential characteristic used to identify a cell as a pluripotent hESC, and is a quality control test used by commercial suppliers of hESCs. Additionally, the formation of tumors after in vivo injection of ESCs is a uniform event in animal models of sufficient duration when adequate quantities of ESCs have been injected to achieve long term ESC survival.

2. Clonal analysis of ESC-generated tumors reveals that the tumor is not clonal, demonstrating that tumor formation is not the product of a single aberrant

ESC. Rather, tumor formation is an inherent property of all ESC injections, which explains the development of polyclonal tumors.

3. This quality of ESCs cannot be dismissed as a normal characteristic of a pluripotent cell removed from its endogenous environment. The use of hESCs for medical therapy does not imagine the re-introduction of hESCs into a normal embryonic environment, but rather the injection of hESCs into a non-embryonic recipient, and science teaches us that the clinical result will be tumor formation.

B. ESCs do not differentiate into desired adult phenotype cells, but to fetal, immature phenotype cells.

1. Both in vitro and after in vivo injection, ESCs differentiate into fetal or immature cell phenotypes, rather than into fully functioning adult phenotype cells needed for therapeutic treatments. When attempts are made to differentiate ESCs in vitro prior to in vivo injection, in order to reduce tumor formation, the ESCs do not then differentiate into adult cell phenotypes in vivo, and actually do not survive long term in the in vivo environment.

2. Experience has taught us that in vivo use of fetal tissue or cells leads to dangerous, uncontrolled cell growth and tumor formation.

3. Fetal cells are not adequate cell replacements for lost adult cells. Fetal insulin-producing cells do not produce therapeutically effective levels of insulin.

C. ESCs are neither useful nor required for research using other pluripotent cells such as spermatogonial stem cells (SSCs) or induced pluripotent stem cells (iPSCs).

1. The quality assurance test used by commercial suppliers of ESCs as well as by research laboratories to demonstrate cellular pluripotency is tumor formation. This test to determine whether other cell types are pluripotent does not require ESCs at any step.

2. If pluripotent cells will have any therapeutic utility they must be differentiated into adult, functional phenotype cells. This requires the use of the desired adult cell type as an in vitro and in vivo comparator. Because the only possible comparator is adult stem cells, ESCs are neither required nor useful at any step of these comparative tests.

D. hESCs will not cure the targeted diseases listed in the Draft National Institutes of Health Guidelines for Human Stem Cell Research Notice.

1. Complex, polygenic, autoimmune diseases such as Parkinson's Disease, amyotrophic lateral sclerosis, diabetes and arthritis are not amenable to stem cell therapy because hESCs will not address the pathology underlying these diseases.

2. Effective treatment of these types of diseases requires medical intervention to significantly dampen if not eradicate the autoimmune attack prior to any attempt to regenerate tissue.

3. Stem cell therapy in the environment of autoimmune activity will not lead to long term functional recovery, as any tissue replacement will eventually suffer the same autoimmune attack and destruction.

4. Clinical studies using this approach have demonstrated that autoimmune blockade or eradication can be sufficient to allow endogenous tissue regeneration to occur leading to profound clinical benefits in patients suffering from rheumatoid arthritis, type I diabetes, multiple sclerosis and osteoporosis.

Because the Guidelines fail to make any showing that human embryonic stem cell research is currently necessary and sufficient to accomplish vitally important research that cannot otherwise be accomplished through the use of adult stem cells and/or iPSCs, NIH should not promulgate the Guidelines and should instead withdraw

the notice of proposed rulemaking.

Until the publication of these Guidelines, it has been NIH's position that human embryos are to be used for research only if "the research goals cannot otherwise be accomplished" by other means. NIH, Final Report of the Human Embryo Research Panel (1994), at page 3. More recently, the National Bioethics Advisory Commission said that the derivation of stem cells from embryos is justifiable "only if no less morally problematic alternatives are available for advancing the research." NBAC, Ethical Issues in Human Stem Cell Research (Rockville, MD: September 1999, Volume I, at page 53.) Given what we know about the progress of adult stem cell and iPSC research, NIH should not promulgate the Guidelines and should instead continue to fund only the latter types of research as the "less morally problematic" alternatives to achieve the same medical and scientific goals.

7. The Guidelines are also problematic to the extent they do not prevent conflicts of interest between the reproductive facility and the research facility. By virtue of these Guidelines, NIH directs how embryos are obtained for destruction, regulates the process for obtaining consent from the parents, and determines which categories of embryos may be destroyed for the federally funded research project. The Guidelines clearly establish that the process of destroying the embryo for its stem cells is an integral and federally regulated part of the research project receiving federal funds. But under the Guidelines, the person or organization destroying the embryos can even be the same person who then uses the stem cells thus obtained-simply using different funds for the two activities. Indeed, so long as the "attending physician responsible for reproductive clinical care and the researcher deriving and/or proposing to utilize human embryonic stem cells [is not] one and the same person" "where practicable," nothing prevents an IVF facility from being both the human embryo killer and the human embryo stem cell researcher. See Guidelines, Part II.B.6. Thus, the overly vague Guidelines allow the unacceptable "conflicts of interest" that the proponents of these Guidelines state they were trying to avoid.

8. These Guidelines only set the stage for further abuses by limiting federal funding to cell lines that are derived from embryos that are "no longer needed" for reproductive purposes. 74 Fed. Reg. 18579. But the distinction between "spare" embryos that are "no longer needed" and those specially created for research is easy to evade: Infertility clinics can simply create more embryos at the outset, ostensibly for fertility treatment, so they will have more "spares" left for research. Ironically, the funding separation attempted by these Guidelines, requiring the NIH to accept at face value the assurances provided by researchers regarding their use of private funds to obtain and destroy embryos, makes it even less likely that such abuses will be detected or stopped. Thus, the Guidelines tend to encourage, not avoid, the very sort of abuses that will degrade public trust in the entire enterprise.19

9. The Guidelines purport to implement "ethically responsible" research procedures, but it is not ethically responsible to ignore the humanity of the human embryo. By restricting human embryonic stem cell research to cell lines derived from human embryos that were "donated" for research, and providing that the living human embryo's parents are to think of themselves only as "donors," the Guidelines completely disregard the unique status, worth, and life of human embryos. Guidelines, Part II.B. It is unseemly for NIH, at the taxpayers' expense, to state in federal regulations that living human beings can and ought to be "donated" by their legal guardians "for research" in this country. Human embryos are not mere tissue, nor are they personal property. Under the terms of the Federal Funding Ban, they are living human beings deserving of the same respect due to other protected human subjects under 45 C.F.R. 46. See Federal Funding Ban. Since 1975, embryos in the womb at this same stage of development (about a week old) have been seen by the federal government as "human subjects" to be protected from harmful research (see 45 CFR § 46.201 et seq.). Yet the NIH now proposes to fund research that necessarily entails the destruction and exploitation of identical human embryos in vitro. Even NIH's own Human Embryo Research Panel in 1994, and President Clinton's National Bioethics Advisory Commission in 1999, admitted that a human embryo is a developing form of human life that deserves considerably more respect than would be accorded the human embryo in the Guidelines.

10. The Guidelines ought to afford living human embryos more respect than the

requirement "that researchers may not create embryos solely for research purposes." Samuel B. Casey and Nathan A. Adams, Specially Respecting the Living Human Embryo by Adhering to Standard Human Subject Experimentation Rules, 2 Yale J. Health Pol'y, L. & Ethics 111, 119 (2001). Under the proposed Guidelines, embryos are deemed eligible for destructive research if they were originally created for reproductive purposes but are now "no longer needed for this purpose" (i.e., are unwanted by the parents). But current federal law on embryo research was clearly designed to extend the same protection to these embryos that is now provided for the unborn child in the womb.20 That law prohibits any effort to select an unborn child for risky or lethal research because he or she is "unwanted" and slated for a future abortion. Because that principle is ignored here, the Guidelines should be deemed to be contrary to the plain meaning and intent of the Federal Funding Ban.

11. The Guidelines create further conflicts of interest by erroneously presupposing, without explanation, that due to the obvious incompetence of the human embryo to speak for itself, the biological parents of the human embryos are legally and morally empowered to substitute their judgment for that of the human embryo in consenting to the destruction of the human embryo. Guidelines, Part II.B. In recent years, courts have encountered, with increasing frequency, requests for permission to withhold life-supporting medical treatment from incompetent individuals. Some courts have employed the so-called doctrine of substituted judgment to decide cases where the surrogate decision-maker's motives are not self-interested and can be further shown to reflect the true intentions of the incompetent patient, particularly where the imminent terminal outcome for the patient can be shown or safely presumed regardless of the medical care provided. However, any application of that doctrine in the instant situation to be regulated by the Guidelines suffers from theoretical incoherence and practical un-workability where a terminal outcome for the human embryo can be readily avoided by cryopreservation and implantation in adoptive mothers,21 and the surrogate decision-makers must be presumed to have an exclusively self-interested motive to always destroy the human embryo because the Guidelines presume the parents will only be asked to "donate" human embryos "no longer needed" for their reproductive purposes. Guidelines, Part II.B.

Given the lack of legal and moral support for NIH's unjustified assumption that the parents of a human embryo ex utero can or ought to be so authorized to speak for the human embryo under these circumstances, the Guidelines should, at the very least, be revised to provide that such authority will be legally recognized only when the human embryo's interests are represented by a court-appointed guardian, rather than merely by his or her parents. Surely, if the interests of science are as great as the Guidelines suggest, the cost of requiring these judicial proceedings would be a small price to pay for the certainty that the decision to kill the human embryos was made by a neutral third party in conformance with applicable state law, untainted by conflicts of interest, and in a fully informed fashion.

Moreover, the Guidelines should not presume that the parents of the human embryo ex utero can legally and morally substitute their judgment for that of their incompetent human embryos who find themselves in the unfortunate position of being "no longer needed." As one commentator has suggested under these circumstances:

[P]erhaps the best way to preclude the exploitation of the relative defenselessness of incompetents would be to set up a standard whereby incompetents would be treated as if they were competent individuals desiring life. Such a standard would not require the application of useless treatments, since these are irrelevant to a human embryo's desire and ability to live. Nor would this standard mandate a life-at-all-costs approach: if a given treatment option would not be open to a competent individual (whether because of expense or impracticability, or because the requisite resources are already occupied elsewhere), it would also not be available to the incompetent. But this approach would require that an incompetent not be denied beneficial treatments solely on the basis of his incompetence to choose them. This approach, which might be denominated the presumptions approach, seeks to strain out improper decisional bases. The notion that some classes of humans have less value before the law, for example, might otherwise serve as an implicit or explicit basis for decision-making.

The presumptions approach also serves to check motivations originating in third party selfishness. The strong presumption against the wishes of parental "donors"

provides a stronger safeguard for the incompetent than does a standard which too readily accedes to the [parents'] requests. Those who seek the court's aid often want desperately to believe that they are acting for the incompetent's good, and not just their own convenience. Presumptions to the contrary test this belief and force its reexamination by both the court and, ideally, the parties seeking relief.22

12. The Guidelines fail to require that parents receive sufficient information to be able to give truly informed consent. The Guidelines require only that "[i]nformation about what would happen to the embryos in the derivation of human embryonic stem cells for research" be provided to the parents, Guidelines, Part II.B(d), but that information, standing alone, fails to inform the donor(s) clearly and explicitly that the embryo will be destroyed. The National Bioethics Advisory Commission has recommended that such information "make clear that the research will involve the destruction of the embryos." NBAC, Ethical Issues in Human Stem Cell Research (Rockville, MD: September 1999, Volume I, at page 72.)

13. The Guidelines are also inadequate in terms of "informed consent" because they fail to require that the embryo's parents be informed that the "donation" will "terminate the life of a whole, separate, unique, living human being." Planned Parenthood v. Rounds, 530 F.3d 724, 726 (8th Cir. 2008). Without the vital information that the human embryo is a living, unique human individual, the embryo's parents have not been fully informed before consenting to the destruction of the human embryos.

14. Attached as Appendix J is The Founding Statement of Do No Harm: The Coalition of Americans for Research Ethics (July 1, 1999). The Statement, which has been signed by a growing group of several hundred doctors, medical researchers, nurses, bio-ethicists, law professors, attorneys, and theologians, makes the following points, all of which support our request to withdraw the notice of proposed rulemaking and not issue the Guidelines:23

Recent scientific advances in human stem cell research have brought into fresh focus the dignity and status of the human embryo. . . . [H]uman stem cell research requiring the destruction of human embryos is objectionable on legal, ethical, and scientific grounds. Moreover, destruction of human embryonic life is unnecessary for medical progress, as alternative methods of obtaining human stem cells and of repairing and regenerating human tissue exist and continue to be developed.

Do No Harm's Statement makes the following points, which the Guidelines fail to adequately consider or address:

A. Human embryonic stem cell research violates existing law and policy:

States: Homicide laws in all 50 states protect human life and the dignity of every human being-especially the vulnerable. In addition, a number of states already specifically protect vulnerable embryonic human beings outside the womb, while others prohibit destructive human embryo and human fetal research.

National: The present Congressional ban on federally funded human embryo research explicitly excludes "research in which a human embryo or embryos are destroyed, discarded, or knowingly subjected to risk of injury or death"; existing laws requiring separation between the death of an unborn child in abortion and research objectives using the unborn child's tissues preclude the destruction of human embryos as a means of achieving research objectives. "Obviously, Congress' intent here was not merely to prohibit the use of federal funds for embryo destruction, but to prohibit the use of such funds for research dependent in any way upon such destruction. Therefore, the opinion of HHS that human embryonic stem cell research may receive federal funding clearly violates both the language of and intention behind the existing law. Congress and the courts should ensure that the law is properly interpreted and enforced to ban federal funding for research which harms, destroys, or is dependent upon the destruction of human embryos."

International: Documents such as the Nuremberg Code, the World Medical Association's Declaration of Helsinki, and the United Nations Declaration of Human Rights reject the use of human beings in experimental research without their informed consent, and permit research on incompetent subjects only if there is a legal surrogate, minimal risk, and therapeutic benefit for the human subject.

B. Human embryonic stem cell research is unethical:

* Recent history provides tragic examples of attempts to justify gross violations of the rights of human beings in medical research on the utilitarian basis of "social and medical benefit": the Tuskegee experiments on African Americans, U.S. government-sponsored radiation research; the Nazi medical war crimes, etc.

* Good ends (e.g., health) do not justify the use of unethical means (e.g., killing human beings).
* Scientifically, the international consensus of embryologists is that human beings begin at fertilization (or cloning)-i.e., when their genetic code is complete and operative; even before implantation they are far more than a "bunch of cells" or merely "potential human beings."

C. Human embryonic stem cell research is scientifically unnecessary:

* Other research methods which use stem cells from adults to develop treatments for many diseases have recently been shown to be quite promising. See Appendix G.
* The use of a patient's own stem cells is even preferable to using embryonic stem cells because it avoids the problem of the body rejecting cells other than its own.
* Other new methods such as somatic cell gene therapy are increasingly successful in tissue regeneration and otherwise treating disease.

15. The NIH has provided the public with insufficient time to meaningfully comment on the Draft Guidelines. A mere 34-day comment period does not afford interested parties an adequate opportunity to comprehensively review and comment on the Guidelines-especially given the scientific complexity and ethical ramifications of the Guidelines. See Fla. Power & Light Co. v. United States, 846 F.2d 765, 771 (D.C. Cir. 1988) (explaining that a notice of proposed rulemaking must provide "adequate time for comments," and noting that interested parties should be able "to comment meaningfully"); In re Estate of Smith v. Bowen, 656 F. Supp. 1093, 1097-99 (D. Colo. 1987) (holding that a 60-day period was inadequate because, inter alia, the issue involved "such great numbers of interested persons and organizations . . . [that would need] to go through their own bureaucratic processes to arrive at their comments"). Moreover, the inadequate comment period precludes the NIH from having sufficient information to engage in informed rulemaking.

Making matters worse, the NIH has failed even to create an appearance that it will thoroughly consider, with an open mind, the comments submitted within the 34-day window. Indeed, a full week prior to publishing the Draft Guidelines, the NIH had already announced that it was accepting applications for human embryonic stem cell research, reflecting an obvious decision to authorize such research regardless of the comments received. See Implementation of Executive Order on Removing Barriers to Responsible Scientific Research Involving Human Stem Cells, NOT-OD-09-085 (Apr. 17, 2009), available at http://grants.nih.gov/grants/guide/notice-files/NOT-OD-09-085.html ("NIH will accept applications for research proposing to use human embryonic stem cells during the period of Guidelines development . . . .").

16. Acting Director Raynard Kington should be excluded from crafting and approving the final guidelines because he has made clear that his mind is made up about the merits of the policy and the formulation of the guidelines. In Association of National Advertisers, Inc. v. FTC, the D.C. Circuit held that an agency member should be excluded from a rulemaking proceeding when there is a "clear and convincing showing that the agency member has an unalterably closed mind on matters critical to the disposition of the proceeding." 627 F.2d 1151, 1170 (D.C. Cir. 1979). The member need not be excluded because of a "mere discussion of policy or advocacy on a legal question." Id. at 1171. But, when a decision maker enters a rulemaking proceeding with an unalterably closed mind about the merits of the proposed rule, the effect is to entirely deprive interested parties of the required opportunity to comment. See Nehemiah Corp. of America v. Jackson, 546 F. Supp. 2d 830, 847 (E.D. Ca. 2008) (holding that HUD Secretary Jackson should have been excluded after stating that HUD "intend[ed] to approve the new rule by the end of the year even if the agency receive[d] critical comments").

Like Secretary Jackson, Acting Director Kington has made clear his views on the funding of embryonic stem cell research. Indeed, Kington reported to the press that NIH "will expand greatly the number of cell lines eligible for funding." Guatam Naik, NIH Offers Rules for Embryonic Stem Cell Research, Wall Street Journal, Apr. 17, 2009, http://online.wsj.com/article/SB123999343505429693.html. Furthermore, Kington and the NIH have demonstrated their judgment of the merits of the proposed Guidelines by allowing applications for funding of hESC research to be submitted even before final promulgation of the Guidelines. See Implementation of Executive Order on Removing Barriers to Responsible Scientific Research Involving Human Stem Cells, http://grants.nih.gov/grants/guide/notice-files/NOT-OD-09-085.html

(last visited May 22, 2009). Kington's statement and this action goes beyond a "mere discussion of policy" and demonstrates that Kington (and potentially other NIH officials as well) has an "unalterably closed mind" in regards to the merits of the NIH Guidelines. Thus, because Acting Director Kington has demonstrated clearly by both his words and his actions that he has prejudged the merits of the Guidelines proposed by the NIH, he should be excluded from the decision-making process, together with any other NIH officials who share his unalterably close-minded approach to this issue.

Thank you for your consideration of these comments.

Very truly yours,

Thomas G. Hungar
of GIBSON, DUNN & CRUTCHER LLP

Of Counsel:
Samuel B. Casey
General Counsel
ADVOCATES INTERNATIONAL

APPENDIX A

Statement of Interests

DO NO HARM: The Coalition of Americans for Research Ethics
Gene Tarne, Communications Director
1100 H Street NW, Suite 700
Washington, DC 20005
(202) 347-6840
E-Mail: gtarne@comcast.net

Do No Harm: The Coalition of Americans for Research Ethics (www.stemcellresearch.org) is composed of a growing coalition of more than 350 scientists, researchers, bioethicists, medical academic and other professionals, patient advocates and concerned individuals who advocate the ethical pursuit of stem cell research and regenerative medicine in general.

Do No Harm has reviewed the Draft National Institutes of Health (NIH) Guidelines for Human Stem Cell Research (the "Guidelines") as published on April 23, 2009 in the Federal Register (74 Fed Reg. 18578), as well Executive Order 13505 issued by President Obama on March 9, 2009, directing the Director of NIH to "support and conduct responsible, scientifically worthy human stem cell research, including human embryonic stem cell research, to the extent permitted by law." Do No Harm joins in the accompanying joint comments because, for the reasons set forth in the accompanying comments, Do No Harm believes the federal funding of human embryonic stem cell research as proposed in the Guidelines is neither responsible, scientifically worthy or even permitted by existing federal law.

Since issuing its founding statement in 1999, Do No Harm has opposed stem cell research that relies on the destruction of human life and on human cloning and supports such alternatives as adult and cord blood stem cell research and the more recent advances involving induced pluripotent stem cells (iPSCs). The coalition is non-sectarian and not affiliated with any religious denomination or church.

Do No Harm's opposition to human embryonic stem cell research arises from the serious ethical concerns about the commodification of human life represented by such research. This type of research destroys a human life by turning it into raw research material. This concern is even more urgent because proponents of embryonic stem cell research admit that creating new human embryos by cloning is the only way that this type of research can advance. Thus human life becomes a mere commodity to be created, manipulated and destroyed as a means to another's end. This destruction-and now creation-of new human life is at the heart of the controversy

over embryonic stem cell research. The coalition maintains that human life must never be reduced to a mere commodity, to be created and destroyed at will, in the name of scientific advancement. The coalition maintains that science does not need to kill in order to cure. This is a position that a person of any faith or no faith at all can share.

From a practical point of view, to date, no human patient has been successfully treated with embryonic stem cells for any disease or condition, and their success in animal models has been very limited. One thing embryonic stem cells have been shown to do well is to produce tumors; in fact in several animal studies, the animals being treated with embryonic stem cells died from tumors produced by them . . .

Nor was human embryonic stem cell research in any way instrumental in leading to the iPSC breakthrough of 2007. Japan's Shinya Yamanaka is the scientist credited with the original iPSC breakthrough in animal models, and one of two scientists to develop human iPSC (the other being James Thomson of the University of Wisconsin, who was also the first to isolate human embryonic stem cells). Both scientists worked indepentently and published their results in November, 2007. According to Yamanaka, human embryonic stem cells (hESCs) were not crucial to his work. Yamanaka's initial work in reprogramming utilized mice, not human, embryonic stem cells and he used the same method for human iPSC production. According to him, "[n]either eggs nor embryos are necessary. I've never worked with either." (Nature, June 7 2007, p. 618). In fact, it was precisely Yamanaka's ethical concerns to avoid lethal experiments with human embryos that led to his breakthrough. Recalling looking at a human embryo through a microscope several years earlier, Yamanaka said: "When I saw the embryo, I suddenly realized there was such a small difference between it and my daughters. . . . I thought, we can't keep destroying embryos for our research. There must be another way." ("Risk Taking in His Genes," The New York Times, 12/11/07.)

James Thomson, the stem cell pioneer from the University of Wisconsin who was the first to grow human embryonic stem cells in 1998, is also an independent co-discoverer of human induced pluripotent (iPS) stem cells along with Japanese scientists. Already these reprogrammed cells have eclipsed the value of those harvested from embryos, Dr. Thomson has said, because of significantly lower cost, ease of production, and genetic identity with the patient. They also bring unique application to medical and pharmaceutical research, because cells cultivated from patients with certain diseases readily become laboratory models for developing and testing therapy. That iPS cells overcome ethical concerns about creating and sacrificing embryos is an added plus.

Finally, for the many reasons set forth in Appendix I of the accompanying comments, Do No Harm submits that human embryonic cells will not, in all likelihood, lead to safe human therapies, including therapies for the various diseases identified by NIH in the proposed Guidelines as a purpose for such hESC research.

Given this poor record for human embryonic stem cell research, Do No Harm maintains that resources are far better used to support those areas of stem cell research, such as adult and cord blood, that have actually demonstrated benefits for human patients.

Thus, along with opposition to destructive human embryonic stem cell research, Do No Harm actively advocates for increased public awareness and support for ethically non-contentious avenues of stem cell research such as research using adult and cord blood stem cells (well documented in the accompanying Appendix G), as well as the more recent advances in the creation of iPCs (well documented in the accompanying Appendix H). In contrast to human embryonic stem cells, adult and cord blood stems have and are continuing to provide therapeutic benefits to human patients for at least 73 diseases and conditions including diabetes, multiple sclerosis, heart disease, spinal cord injury, Parkinson's, lupus and others (see: http://www.stemcellresearch.org/facts/treatments.htm). Adult stem cells have been used for corneal regeneration (one Japanese group used stem cells from lining of

mouth to make corneal cells and transplant onto patients' eyes); liver repair; wound and bone repair; growing new bladders; and in a fairly recent development, growing a new windpipe from bone marrow. These are not yet "cures," but according to the peer-reviewed literature, as Do No Harm noted in two letters published in Science, they are applications that have provided "observable and measurable benefit to patients, a necessary step toward formal FDA approval and what is expected of new, cutting-edge medical applications." (SCIENCE 19 January 2007: Vol. 315. no. 5810, p. 328; see also, SCIENCE 8 June 2007: Vol. 316. no. 5830, pp. 1422 - 1423.)

This is a proven track record for adult and cord blood stem cells, and in the interests of putting patients first, any guidelines involving federal funding for stem cell research should give priority to research that is actually improving their lives today.

Do No Harm maintains that the proposed Guidelines are little more than an attempt to put an ethical gloss on an inherently unethical avenue of research. By its very nature, human embryonic stem cell research requires the destruction of human life. By its very nature, human embryonic stem cell research commodifies human life, declares some life more valuable than others, and reduces some human life to a mere means to another's ends. This violates all international standards for the conduct of medical research involving human subjects and also U.S. law, which under the Federal Funding Ban, prohibits federal funding of research in which embryos are even "subjected to risk of injury or death." It also violates America's foundational commitment to the worth and human dignity of every human being.

As then-candidate, now-President Obama famously said during the recent presidential campaign, "you can put lipstick on a pig-but it's still a pig." The NIH may attempt to propose guidelines to make destructive embryonic stem cell research appear ethical, but as Do No Harm has been saying for years, it still remains an inherently unethical enterprise that actually violates President Obama's Order because, in the very words of that Order, quoted in the proposed Guidelines, hESC research is neither "responsible," "scientifically worthy" of taxpayer support, nor permitted by the "existing" federal "laws" and the "laws" of many states barring such research.

Dr. James L. Sherley, M.D., Ph.D.
64 Grove Street,
Watertown, Massachusetts 02472
E-Mail: sherleyj@bbri.org

Dr. James L. Sherley, M.D., Ph.D., is a senior scientist currently working at the Boston Biomedical Research Institute where he and his research team are pursuing the study of normal molecular and biochemical processes in adult stem cells that are involved in cancer initiation and that contribute to aging. Adult stem cells are rare tissue cells that continuously replace expired tissue cells. Investigations of their specialized properties will yield new therapies for injured, diseased, and aging tissue cells. Dr. Sherley employs an integrated approach, incorporating both basic and applied research strategies, to elucidate novel mechanisms of adult stem cell-specific functions and apply the knowledge to improve methods for identifying adult stem cells and producing them in large number for therapeutic development.

Dr. Sherley has been the recipient of many awards and recognitions in his field of molecular biological research, including the NIH Director's Pioneer Award and the honor of testifying before the Australian Parliament in 2006 on the current state of stem cell science.

Prior to his current position on the faculty of BBRI, Dr. Sherley served as an Associate Professor in the Department of Biological Engineering at the Massachusetts Institute of Technology. Prior to that appointment, Dr. Sherley was an associate member of the staff working in the Department of Molecular Oncology, Division of Medical Science at the Fox Chase Cancer Center in Philadelphia. Dr. Sherley has his B.A. in Biology from Harvard; his M.D. and Ph.D. in molecular biology from John Hopkins University School of Medicine's Department of Molecular Biology and Genetics, and he did his post-doctoral research work at Princeton University's Department of Molecular Biology.

47067_Do_No_Harm_et_al._Comments_re_Proposed_NIH_Stem_Cell_Guidelines

Dr. Sherley has reviewed the Draft National Institutes of Health (NIH) Guidelines for Human Stem Cell Research (the "Guidelines") as published on April 23, 2009 in the Federal Register (74 Fed Reg. 18578), as well Executive Order 13505 issued by President Obama on March 9, 2009, directing the Director of NIH to "support and conduct responsible, scientifically worthy human stem cell research, including human embryonic stem cell research, to the extent permitted by law." Dr. Sherley joins in the accompanying comments submitted by DO NO HARM: The Coalition of Americans for Research Ethics et al. because, for the reasons set forth in the accompanying comments, he believes the federal funding of human embryonic stem cell research as proposed in the Guidelines is neither responsible, scientifically worthy nor even permitted by existing federal law.

Dr. Sherley is most centrally concerned that the proposed Guidelines fail to acknowledge that the scientific fact that human embryos are living human beings. The President's Executive Order 13435 that purportedly seeks to "remove barriers to responsible scientific research involving human stem cells" fails to acknowledge this scientific truth, and previous NIH documents, including the proposed Guidelines, omit it as well; and the two most quoted leaderships of scientific organizations (NAS and ISSCR) omit it too. Therefore, Dr. Sherley is concerned that the proposed Guidelines for human embryonic stem cell (hESC) research are publicly deceptive in the same manner as the recently issued respective recommendations from the U.S. National Academy of Sciences (NAS), headquartered in Washington, D.C., and the International Society for Stem Cell Research (ISSCR), headquartered in Boston.

Like the NAS and ISSCR documents, the proposed Guidelines consider ethical treatment only from the perspective of so-called "donors" of human embryos for research. In fact, the "human research subjects," who are due ethical protection under the NIH's existing regulations for human research studies, are the human embryos, not their biological parents who are donating these human subjects for research.

Existing regulations for research studies with human subjects require a clear statement of the eligibility criteria for participants, including their state of well-being. Like the NAS and ISSCR documents, the NIH guidelines do not acknowledge the scientific fact that embryos are living human beings and, as such, are due the same protections. Along with this omission, they falsely represent embryos as "human materials" obtained from protected donors. The language of the Guidelines falsely equates living human embryos with tissues obtained from donors for induced pluripotent stem cell (iPSC) research. The NIH does this with full expert knowledge that, whereas tissue material harvested for iPSC research has the same human genome as its donor, because it is the donor's own tissue, embryos have a different and unique genome. Embryos are not tissues harvested from consenting donors. They are non-consenting, distinct human individuals, and they deserve the same protections for human subjects research as any other human subject. Using embryos for hESC research is equivalent to injurious research with children, which is not permitted.

Although the NIH guidelines acknowledge the priority of the Federal Funding ban, which prohibits federal funding of research in which human embryos are injured, more is needed for adequate protection of human embryos. The NIH's recommendation of research with existing hESC lines will motivate private funding of the federally prohibited research. Surely, NIH scientists must recognize that promoting the use of existing hESC lines, while at the same time prohibiting the production of new ones, is an ethically conflicted policy.

Dr. Sherley insists that NIH revise its existing guidelines to meet its own regulations for ethical treatment of human research subjects. For any chance of validity, the Guidelines must state that "human embryos are living human beings" that cannot properly give consent; and NIH must adopt an ethically consistent policy that disallows both the unethical production of hESCs going forward and the use of existing cell lines that were produced in the past in violation of NIH regulations for ethical treatment of human research subjects.

Dr. Theresa Deisher, Ph.D.
Managing Member and Research and Development Director
AVM Biotechnology
City Centre Building,
1420 Fifth Avenue, Suite 2650

Seattle, WA 98101
(202) 906-0022
E-Mail: tdeisher@avmbiotech.com

Dr. Deisher, an internationally renowned expert in the field of adult stem cell therapies and regenerative medicine, brings 17 years of experience in scientific and corporate leadership positions involving research, discovery, production and commercialization of human therapeutics. Dr. Deisher's penchant for groundbreaking scientific discovery and her distinguished scientific research has resulted in 23 patents issued. She has published numerous scientific manuscripts and is a frequent invited lecturer and guest speaker in the area of stem cell technology and regenerative medicine.

Throughout her career, Dr. Deisher has been recruited by some of the country's top biotechnology companies, including Genentech, Repligen, ZymoGenetics, Immunex and Amgen. She has managed and mentored undergraduate honors students, post-doctoral fellows, scientific executives and over 20 research assistants/scientists at all levels of responsibility.

Dr. Deisher graduated with honors and distinction from Stanford University, and obtained her Ph.D. in Molecular and Cellular Physiology from Stanford University.

Subsequent to obtaining her Ph.D. from Stanford, Dr. Deisher was recruited by Repligen Corporation (Cambridge, MA) and accepted a position as Research Scientist where she managed a staff of associates and scientists and directed the development of research and clinical assays in support of Phase I and Phase II clinical trials for various Repligen developmental efforts. Additionally, Dr. Deisher was selected by Senior Management to participate in strategic alliance initiatives, including serving on the Repligen / Eli Lilly joint development committee.

Dr. Deisher has reviewed the Draft National Institutes of Health (NIH) Guidelines for Human Stem Cell Research (the "Guidelines") as published on April 23, 2009 in the Federal Register (74 Fed. Reg. 18578), as well as Executive Order 13505 issued by President Obama on March 9, 2009, directing the Director of NIH to "support and conduct responsible, scientifically worthy human stem cell research, including human embryonic stem cell research, to the extent permitted by law." Dr. Deisher joins in the accompanying comments submitted by DO NO HARM: The Coalition of Americans for Research Ethics et al. because, for the reasons set forth in the accompanying comments, she believes the federal funding of human embryonic stem cell research as proposed in the Guidelines is neither responsible, scientifically worthy nor even permitted by existing federal law.

For the ample reasons based upon the published data set forth in the accompanying Appendix I she has prepared, Dr. Deisher is most concerned that the human embryonic stem cell research being proposed in the Guidelines cannot possibly be useful for any of the potential purposes cited for funding such research in the proposed Guidelines. Dr. Deisher believes that the human stem cell research using adult stem cells and induced pluripotent stem cells already permitted under federal law and fundable under existing NIH Guidelines is more than sufficient to accomplish all the necessary scientific investigation and work on the development of the various therapies mentioned in the proposed Guidelines.

Christian Medical Association
Dr. David Stevens, M.D.
Chief Executive Officer
2604 Hwy. 421
Bristol, TN 37621
(423) 844-1000
E-Mail: ceo@cmda.org

The 15,000 members of the Christian Medical Association (CMA) include thousands of physicians committed to the Hippocratic tradition of medicine that

requires physicians to "first, do no harm." In accordance with this tradition and a commitment to biblical principles, CMA members have officially adopted the following ETHICAL STATEMENT ON HUMAN STEM CELL RESEARCH AND USE:

"The field of stem cell research offers great promise for the advancement of medical science. Adult stem cells are presently being used to treat a variety of illnesses. However, the isolation of human embryonic stem cells in 1998 and resultant research have raised moral concerns because current methods of procuring embryonic stem cells require the destruction of human life.

"CMA recognizes the potential value of stem cell technology:

- We endorse the goals of stem cell research to treat human illness and relieve human suffering.
- We endorse retrieval and use of adult stem cells from a variety of sources - umbilical cord blood, placenta, amniotic fluid, adult organs, etc.
- We endorse human adult stem cell research and use if it is safe for human subjects.
- We endorse animal stem cell research provided it is not cruel to experimental animals.

"CMA has moral concerns regarding embryonic human stem cell research and use. We recognize the sacred dignity and worth of human life from fertilization to death.

- The destruction of nascent individual human life even for the benefit of others is immoral.
- We condemn specious arguments that "excess" embryos may be used as a source for embryonic stem cells, "because they would have been destroyed anyway and that good may come." There is a moral difference between intentionally taking a human being's life and the embryo dying a natural death.
- We are concerned that stem cell research will involve exploitation of women (especially poor women) by using them to produce the eggs necessary for stem cell research, thereby subjecting them to the risk of attendant procedures and potential complications.
- We are concerned that the instrumental production, use, commodification or destruction of any human being will coarsen our society's attitude toward human life itself.

"CMA advances the following moral guidelines to direct stem cell research and therapy:

- No human life should be produced by any means for primarily utilitarian purposes - no matter how noble the ends or widespread the benefit.
- Technology and research must not involve the abuse or destruction of human life.
- We encourage the careful and ethical development of alternative methods for procuring stem cells that do not involve the destruction of human life.

"CMA encourages life-honoring stem cell research for the advancement of medical science and the benefit of all patients.

In this pursuit, CMA advocates the protection of all human life, for humans are made in the Image of God."

Besides following the principles expressed in the above ethics statement, CMA physicians do not want to advance a path of research that is unlikely to produce useable therapies for patients in the near future or at all. CMA physicians believe that stem cell research should focus on the ethical path that has most clearly and substantially contributed to therapies for real patients, and that path is non-destructive adult stem cell research. For these reasons and the additional reasons set forth in separate comments submitted by CMA, the 15,000 members of the Christian Medical Association urge the withdrawal of the proposed Guidelines and the continuance of NIH's existing stem cell research guidelines permitting federal

funding for human stem cell research using adult stem cells and induced pluripotent stem cells that do not require or depend upon the destruction of human embryos.

Family Research Council
Dr. David A. Prentice, Ph.D.
Senior Fellow for Life Sciences,
Center for Human Life and Bioethics
801 G Street, NW
Washington, D.C. 20001
(202) 393-2100
E-Mail: dap@frc.org

Since 2004, Dr. David Prentice has served as Senior Fellow for Life Sciences at Family Research Council (FRC). While advances in science, medicine, and technology may hold promises of improved health and well-being, FRC believes such advances may also devalue human life and human dignity. Stem cells, cloning, genetic engineering, and other new technologies need to be evaluated carefully within both a scientific and an ethical framework. FRC opposes research that destroys, harms, or manipulates an embryonic human being. However, FRC vigorously supports research and therapies using "adult" stem cells (such as from bone marrow and umbilical cord blood), which is not ethically problematic and has already resulted in useful therapies in human patients. FRC opposes all forms of human cloning, whether "reproductive" to bring an infant to term, or "therapeutic," to destroy the cloned embryo for experiments. FRC believes that good science is also ethical science, and supports biotechnologies that advance scientific knowledge and medical treatments, while valuing all human life and maintaining human dignity.

Prior to joining FRC in July 2004, Dr. Prentice spent almost 20 years as Professor of Life Sciences, Indiana State University, and Adjunct Professor of Medical and Molecular Genetics, Indiana University School of Medicine. He received his Ph.D. in Biochemistry from the University of Kansas, and was at Los Alamos National Laboratory and the University of Texas Medical School-Houston before joining Indiana State University, where he served as Acting Associate Dean of Arts and Sciences, Assistant Chair of Life Sciences, and was recognized with the University's Distinguished Teaching Award and Distinguished Service Award.

Dr. Prentice is a Founding Member of Do No Harm: The Coalition of Americans for Research Ethics, a Fellow of the Wilberforce Forum Council for Biotechnology Policy, a Fellow of the Institute on Biotechnology and the Human Future, and an Advisory Board Member for the Center for Bioethics and Human Dignity. He received the 2007 Walter C. Randall Award in Biomedical Ethics from the American Physiological Society, given for promoting the honor and integrity of biomedical science through example and mentoring in the classroom and laboratory.

Dr. Prentice's research interests encompass aspects of cell growth; one major focus is adult stem cells. Dr. Prentice is an internationally-recognized expert on stem cells and cloning, and has testified before the U.S. Congress, numerous state legislatures, the U.S. National Academy of Sciences, the President's Council on Bioethics, European Parliament, British Parliament, Canadian Parliament, Australian Parliament, German Bundestag, French Senate, Swedish Parliament, the Vatican, and the United Nations. Dr. Prentice was selected by the U.S. President's Council on Bioethics to write their comprehensive review of adult stem cell research. His defense of Adult Stem Cell Treatments with extensive literature documentation was published by Science in January 2007.

Dr. Prentice has reviewed the Draft National Institutes of Health (NIH) Guidelines for Human Stem Cell Research (the "Guidelines") as published on April 23, 2009 in the Federal Register (74 Fed Reg. 18578), as well as Executive Order 13505 issued by President Obama on March 9, 2009, directing the Director of NIH to "support and conduct responsible, scientifically worthy human stem cell research, including human embryonic stem cell research, to the extent permitted by law." On behalf of FRC, Dr. Prentice joins in the accompanying comments submitted by Do No Harm: The Coalition of Americans for Research Ethics et al. because, for the reasons

set forth in the accompanying comments, he believes the federal funding of human embryonic stem cell research as proposed in the Guidelines is neither responsible nor scientifically worthy, and is questionable under existing federal law.

As demonstrated by the extensive documented evidence set forth in accompanying Appendices G and H, Dr. Prentice is particularly concerned that NIH acknowledge and the American public realize that taxpayer-funded human stem cell research using adult stem cells and induced pluripotent stem cells, as currently authorized under existing federal law and eligible for funding under existing NIH guidance, is more than sufficient to satisfy all the purposes offered by NIH in its proposed Guidelines to needlessly extend such funding to embryonic stem cells.

Concerned Women for America
Wendy Wright, President
1015 15th Street, N.W., Suite 1100
Washington, DC 20005
E-Mail: wwright@cwfa.org

The vision of CWA is for women and like-minded men, from all walks of life, to come together and restore the family to its traditional purpose and thereby allow each member of the family to realize their God-given potential and be more responsible citizens. CWA supports the protection of all innocent human life from conception until natural death. While CWA believes in seeking medical cures for debilitating diseases with which we or our loved ones might suffer, America, particularly at the expense of millions of taxpayers who object to unethical research, must not seek such cures at the much dearer expense of innocent human life, including all human embryos in vivo or in vitro.

Stem cell science is not controversial. Killing living human embryos is. Only research that requires the destruction of a human embryo is objectionable to CWA. What the media and proponents of embryonic stem cell research ignore is that embryonic stem cells have not cured any diseases or successfully treated a single patient. In fact, embryonic stem cell research has yielded only unstable, deadly tumors and patient immune rejection.

CWA believes that the good news is that there are ethical alternatives to embryonic stem cell research that are working, treating and curing without the destruction of the tiniest human life. Skin cells that are reprogrammed to act like embryos-induced pluripotent stem cells-hold the same research potential as stem cells from embryos. The induced pluripotent stem cells can be created from the body cells of anyone, so the ensuing stem cell lines are plentiful and avoid the high risk of tissue rejection. They have already been used to make heart muscle, brain neurons, motor neurons, blood and insulin-secreting cells.

One of the researchers who discovered the induced pluripotent stem cell alternative and the researcher to first to identify embryonic stem cells confirms CWA's concerns. Dr. James Thomson, a University of Wisconsin stem cell scientist, states: "If embryonic stem cell research does not make you at least a little bit uncomfortable, you have not thought about it enough." CWA is further confirmed by Dr. Mehmet Oz, a cardiovascular surgeon at Columbia University, who recently appeared on the Oprah Winfrey Show, and in the presence of Oprah and Michael J. Fox declared that the "stem cell debate is dead" because of the successes using adult stem cells and induced pluripotent stem cells.

CWA's views are further confirmed by an article in the March 4 issue of U.S. News and World Report titled "Why embryonic stem cells are obsolete," wherein Dr. Bernadine Healy, the former head of the NIH, wrote that "adult stem cell research successes have 'diminished' the prospect that embryonic stem cell research is the future of regenerative medicine."

CWA acknowledges that all leading science textbooks on the subject clearly state that human life begins at conception when the human egg is fertilized. Life at that moment receives its entire DNA, all its genetic makeup, its gender, hair

color, etc. This is a point from which we all began. To devalue life at this point is comparable to saying that the life of a toddler is of less worth than that of a young adult simply because of her size or because she is not as developed. Should we relegate a toddler to research material to benefit the young adult?

Human embryos are not simply tissue to be researched. The underlying utilitarian belief that some humans need to be sacrificed for the betterment of others is morally and ethically wrong. Experimentation on human embryos contradicts existing federal law and all applicable medical codes of ethics involving experimentation on human subjects, including the Nuremberg Code, ethical guidelines established after World War II, which prohibits such experimentation that knowingly causes injury or death to humans.

CWA has reviewed the Draft National Institutes of Health (NIH) Guidelines for Human Stem Cell Research (the "Guidelines") as published on April 23, 2009 in the Federal Register (74 Fed. Reg. 18578), as well as Executive Order 13505 issued by President Obama on March 9, 2009, directing the Director of NIH to "support and conduct responsible, scientifically worthy human stem cell research, including human embryonic stem cell research, to the extent permitted by law." CWA joins in the accompanying comments submitted by Do No Harm: The Coalition of Americans for Research Ethics et al. because, for the reasons set forth in the accompanying comments, CWA believes the federal funding of human embryonic stem cell research as proposed in the Guidelines is neither responsible, scientifically worthy nor even permitted by existing federal law.

CWA is particularly concerned that the informed consent provisions in Part II.B.7 of the proposed Guidelines are wholly inadequate to properly inform the donors what they are doing and what they are giving up. Moreover, no donor has the right to sacrifice the live of another living human being for any purpose, much less unnecessary human experimentation that provides no benefits to the human being so sacrificed.

Advocates International
Samuel B. Casey, General Counsel
800 Braddock Road, Suite 300
Springfield, VA 222151
(703) 894-1076
E-Mail: sbcasey@advocatesinternational.org

Advocates International ("AI") is an international organization of attorneys and other public policy advocates in over 150 nations that seeks to do justice with compassion including, through its Global Task Force on Life, protecting or defending in all available legal fora the inalienable right to life and dignity of every human being from his or her biological conception in vitro or vivo to natural death. AI does not object to all human pluripotent stem cell research, however. Human pluripotent stem cells can be obtained from three four sources: living human embryos, fetal tissue derived from aborted deceased pre-born children, human adult stem cells, and induced pluripotent stem cells. As is currently permitted under federal law and NIH guidance, AI supports federal funding for stem cell research using human adult stem cells and induced pluripotent stem cells. As yet, there is no reported scientific evidence that types of stem cells, along with the stem cells in the NIH's Human Stem Cell Registry already approved by Congress for research under the Dickey-Wicker Amendment will not be sufficient to accomplish the basic scientific research and achieve all of the medical therapies that is currently being offered as the excuse for ignoring all of the legal and ethical barriers and other scientific reservations involved in human embryo stem cell research.

Alliance Defense Fund
Steven H. Aden, Senior Legal Counsel
Matthew Bowman, Legal Counsel
801 G Street, NW, Suite 509
Washington, D.C. 20001
(202) 637-4610

E-Mail: mbowman@telladf.org

The Alliance Defense Fund ("ADF") is a legal alliance defending the sanctity of human life, religious freedom, marriage and the family. ADF is involved in direct litigation and amicus briefing throughout the United States to defend the right to life of preborn children and the right of the government to protect the unborn and specifically to defend the personhood of human embryos. ADF helped fund the 2002 lawsuit Nightlight Christian Adoptions v. Thompson, 1:01-cv-502-RCL (D.D.C. filed Mar. 8, 2001), which challenged the Clinton-era NIH's proposed policy to fund human embryo research according to a questionable interpretation of the Dickey-Wicker amendment that was subsequently withdrawn by the NIH. ADF has supported several state court cases involving questions of the humanity of preborn children, including an amicus brief in the Texas embryo custody appeal Roman v. Roman, 193 S.W.3d 40 (Tex. Ct. App. 2006). ADF also funded litigation in Missouri to protect the right of voters to propose a ban on cloning without having their proposal deceptively characterized on the ballot by the Secretary of State. ADF submitted written comments to HHS in September 2008 discussing the federal legal status of the pre-implantation human embryo in general and as it relates to regulations that implement federal laws that prohibit fund recipients violating the religious beliefs of pro-life medical providers. ADF also submitted comments in 2007 in the United Kingdom analyzing the Human Tissue and Embryos Draft Bill, which proposed allowing the creation of human-animal hybrid and chimera embryos.

Appendix B

DO NO HARM et al. Comments on Draft NIH Guidelines for Human Stem Cell Research, 74 Federal Register 18578-18580 (April 23, 2009)

Part I: Fetal Homicide Statutes that Apply Without Regard to Gestational Age

Alabama: 2006 Ala. Acts ch. 419 (amending the definition of "person," when referring to the victim of a criminal homicide or assault, to mean "a human being, including an unborn child in utero at any stage of development, regardless of viability").

Arizona: ARIZ. REV. STAT. ANN. §§ 13-1102(A), (B) (negligent homicide), 13-1103(A)(5), -(B) (manslaughter), 13-1104(A), (B) (second degree murder), 13-1105(A)(1), -(C) (first degree murder) (West Supp. 2005).

Idaho: IDAHO CODE § 18-4016 (definition of human embryo and fetus); §§ 18-4001 (definition of murder), 18-4006 (definition of manslaughter) (2004).

Illinois: 720 ILCS §§ 5/9-1.2 (intentional homicide of an unborn child), 5/9-2.1 (voluntary manslaughter of an unborn child), 5/9-3.2 (involuntary manslaughter or reckless homicide of an unborn child) (West 2002).

Indiana: IND. CODE ANN. § 35-42-1-6 (Michie 2004) (feticide).

Kentucky: KY. REV. STAT. § 507A.010 et seq. (Michie Supp. 2005) (fetal homicide).

Louisiana: LA. REV. STAT. ANN. § 14:2(11) (West 1997) (definition of "unborn child"); § 14:32.5 (definition of "feticide"), §§ 14:32.6 (first degree feticide), 14:32.7 (second degree feticide), 14:32.8 (third degree feticide) (West 1997 & Supp. 2006).

Michigan: MICH. COMP. LAWS ANN. § 750.90a et seq. (West 2004).

Minnesota: MINN. STAT. ANN. §§ 609.266 (definition of unborn child), 609.2661 (first degree murder of an unborn child), 609.2662 (second degree murder of an unborn child), 609.2663 (third degree murder of an unborn child), 609.2664 (manslaughter of an unborn child in the first degree), 609.2665 (manslaughter of an unborn child in the second degree), 609.268(1) (felony murder of an unborn child), 609.21 subd. 3 (vehicular homicide of an unborn child) (West 2003 & Supp. 2006).

Mississippi: MISS. CODE ANN. § 97-3-37(1) (2005) (homicide and assault offenses).

Missouri: MO. ANN. STAT. §§ 565.020 subd. 1 (first degree murder), 565.021 subd. 2 (second degree felony murder), and 565.024 (involuntary manslaughter) (West 1999), interpreted in light of § 1.205 (West 2000); see State v. Knapp, 843 S.W.2d 345 (Mo. 1992); State v. Holcomb, 956 S.W.2d 286 (Mo. Ct. App. 1997); State v. Rollen, 133 S.W.3d 57 (Mo. Ct. App. 2003), transfer denied, May 25, 2004 (Missouri Supreme Court).

Nebraska: NEB. REV. STAT. ANN. § 28-388 et seq. (Michie 2003).

North Dakota: N.D. CENT. CODE § 12.1-17.1-01 et seq. (1997).

Ohio: Under Ohio law, "the unlawful termination of another's pregnancy" may be punished as aggravated murder, murder, voluntary manslaughter, involuntary manslaughter, reckless homicide, negligent homicide or aggravated vehicular homicide, vehicular homicide or vehicular manslaughter, see OHIO REV. CODE ANN. §§ 2903.01(A), -(B), 2903.02(A), -(B), 2903.03(A), 2903.04(A), -(B), 2903.041(A), 2903.05(A), 2903.06(A) (Anderson 2003 & Supp. 2005). "Unlawful termination of another's pregnancy" is defined as "causing the death of an unborn member of the species homo sapiens, who is or was carried in the womb of another, as a result of injuries inflicted during the period that begins with fertilization and that continues unless and until live birth occurs." OHIO REV. CODE ANN. § 2903.09(A), -(B) (Anderson 2003).

Oklahoma: OKLA. STAT. ANN. tit. 21, § 713 (West Supp. 2006) (killing an unborn child), interpreted in light of the definition of "unborn child" in tit. 63, § 1-730(2) (West 2004).

Pennsylvania: 18 PA. CONS. STAT. ANN. § 1102 (West 1998), § 2601 et seq. (1998) (homicide).

South Dakota: S.D. CODIFIED LAWS § 22-17-6 (Michie 1998) (intentional killing of human fetus); §§ 22-16-1 (defining homicide), 22-16-1.1 (fetal homicide) (Michie 1998), read in conjunction with § 22-1-2(31) (definition of "person") (Michie Supp. 2003), and § 22-1-2(50A) (Michie Supp. 2003) (definition of unborn child).

Texas: TEX. PENAL CODE § 1.07(a)(26) (West Supp. 2005) (defining the term "individual," as used in the Texas Penal Code, to mean "a human being who is alive, including an unborn child at every stage of gestation from fertilization until birth").

Utah: UTAH CODE ANN. § 76-5-201(1)(a) (2003) (when referring to the victim of a criminal homicide, the term "another human being" includes "an unborn child at any stage of its development").

West Virginia: W. VA. CODE § 61-2-30 (2005) (recognizing an embryo or fetus as a distinct unborn victim of certain crimes against the person, including homicide).

Wisconsin: WIS. STAT. ANN. § 939.75(1) (West 2005) (defining unborn child as "any individual of the human species from fertilization until birth that is gestating inside a woman"); §§ 940.01(1)(b) (first degree intentional homicide), 940.02(1m) (first degree reckless homicide), 940.05(2g) (second degree intentional homicide), 940.06(2) (second degree reckless homicide), 940.08(2) (homicide by negligent handling of a dangerous weapon, explosive or fire), 940.09(1)(c), -(cm), -(d), -(e) (homicide by intoxicated use of a vehicle), 940.09(1g)(c), (1g)(cm), -(d) (homicide by intoxicated use of a firearm), 940.10(2) (homicide by negligent operation of a vehicle) (West 2005); WIS. STAT. ANN. § 940.04(1) (West 2005) (intentional destruction of the life of an unborn child).

Part II: Wrongful Death Statutes That Apply Without Regard to the State of Gestation or Development

Illinois: 740 ILCS § 180/2.2 (West 2002) (right to maintain a wrongful death action is not foreclosed by "[t]he state of gestation or development of a human being").

Louisiana: Danos v. St. Pierre, 402 So.2d 633, 639 (La. 1981) (rejecting any gestational requirement to maintain wrongful death action); LA. CIV. CODE ANN. art. 26 (West 1999) (codifying holding in Danos).

Michigan: MICH. COMP. LAWS ANN. § 600.2922a (West Supp. 2006) (amending statute to provide liability for "a wrongful or negligent act [committed] against a pregnant individual . . . if the act results in a miscarriage or stillbirth by that individual, or physical injury to or the death of the embryo or fetus").

Missouri: Connor v. Monkem, 898 S.W.2d 89 (Mo. 1995) (interpreting statute setting forth rule of construction).

Nebraska: NEB. REV. STAT. § 30-809(1) (Supp. 2005) (amending wrongful death statute to include "an unborn child in utero at any stage of gestation").

South Dakota: S.D. CODE LAWS ANN. § 21-5-1 (1987) (amending wrongful death statute to include "an unborn child").

Texas: TEX. CIV. PRAC. & REM. CODE ANN. § 71.001(4) (West Supp. 2006) (defining "individual" in wrongful death statute to include "an unborn child at every stage of gestation from fertilization until birth").

West Virginia: Farley v. Sartin, 466 S.E.2d 522 (W. Va. 1995) (interpreting wrongful death statute).

Part III: Courts Rejecting Constitutional Challenges to Fetal Homicide Statutes That Apply Without Regard to the Age of the Unborn Child

People v. Campos, 227 Ill. App. 3d 434, 451-52, 592 N.E.2d 85, 97 (1992) (twenty and one-half weeks pregnant), appeal denied, 146 Ill. 2d 635 (1992).

United States. ex rel Campos v. Peters, 827 F. Supp. 1359 (N.D. Ill. 1993) (denying habeas corpus relief to the defendant in the Campos case), affirmed without opinion, 37 F.3d 1501 (7th Cir. 1994), cert. denied, 514 U.S. 1024 (1995).

People v. Ford, 221 Ill. App. 3d 354, 366-73, 581 N.E.2d 1189, 1197-1202 (1991) (five and one half months), appeal denied, 143 Ill. 2d 642 (1992) Ford v. Ahtow, 104 F.3d 926 (7th Cir. 1997) (denying habeas corpus relief in the previously cited cased).

State v. Bauer, 471 N.W.2d 363, 365-66 (Minn. Ct. App. 1991), review denied, July 24, 1991 (Minnesota Supreme Court).

State v. Merrill, 450 N.W.2d 318, 321-24 (Minn. 1990) (twenty-eight days), cert. denied, 496 U.S. 931 (1990).

State v. Rollen, 133 S.W.3d 57, 63 (Mo. Ct. App. 2003) (sixteen weeks pregnant), transfer denied, May 25, 2004 (Missouri Supreme Court).

State v. Holcomb, 956 S.W.2d 286, 289-93 (Mo. Ct. App. 1997) (twenty-six to twenty-eight weeks), transfer denied, Dec. 23, 1997 (Missouri Supreme Court).

State v. Knapp, 843 S.W.2d 345, 349 (Mo. 1992) (six months pregnant).

State v. Alfieri, 724 N.E.2d 477, 481-84 (Ohio Ct. App. 1998) (six months pregnant), appeal denied, 709 N.E.2d 849 (Ohio 1999).

State v. Moore, Ohio Ct. App. (Second District), Oct. 30, 1998, slip op. at 2-5, 1998 WL 754603, 1998 Ohio App. Lexis 5040 (six months pregnant).

State v. Coleman, 705 N.E.2d 419, 420-22 (Ohio Ct. App. 1997) (age of unborn child not indicated), appeal denied, 691 N.E.2d 1058 (Ohio 1998).

Coleman v. DeWitt, 282 F.3d 908 (6th Cir. 2002) (denying habeas corpus relief in the previously cited cased).

Commonwealth v. Bullock, 868 A.2d 516, 521-25 (Pa. Super. Ct. 2005) (22 to 23 weeks pregnant), allocatur allowed, 885 A.2d 40 (Pa. 2005).

State v. MacGuire, 84 P.3d 1171, 1174-78 (Utah 2004) (thirteen to fifteen weeks).

Courts Recognizing that Roe v. Wade Does Not Prevent States from Providing Liability under Wrongful Death Statutes for Prenatal Injuries Resulting in the Death of Unborn Children Prior to Viability

Santana v. Zilog, Inc. 95 F.3d 780, 784-85 n. 4 (9th Cir. 1996) ("In the wrongful death context, Roe's use of viability to denote when the balance of competing interests shifts is simply irrelevant.").

Summerfield v. Superior Court, 698 P.2d 712, 723 (Ariz. 1985) ("Roe v. Wade balances the rights of the fetus against the rights of its mother and concludes that the latter's right to privacy outweighs the former's right to life in the first trimester of pregnancy; it 'neither prohibits nor compels' the inclusion of a fetus as a person for the purposes of other enactments") (citation omitted).

Wiersma v. Maple Leaf Farms, 543 N.W.2d 787, 790 n. 2 (S.D. 1996) ("Nothing in Roe prohibits the Legislature from including a nonviable fetus in its definition of a person under our State's wrongful death act.").

Farley v. Sartin, 466 S.E.2d 522, 534 (W. Va. 1995) ("Our definition of 'person' within the confines of the wrongful death statute neither affects nor interferes with the constitutional protection afforded a woman who chooses to have an abortion, as was set forth originally in Roe v. Wade.") (citation omitted).

APPENDIX C
DO NO HARM et al. Comments on Draft NIH Guidelines for Human Stem Cell Research, 74 Federal Register 18578-18580 (April 23, 2009)
The Legal Consensus on the Beginning of Life
[See generally Elizabeth Spahn and Barbara Andrade, Mis-Conceptions: The Moment of Conception in
Religion, Science and Law, 32 U.S.F.L.Rev. 261 (1998); Paul B. Linton, PLANNED PARENTHOOD v.
CASEY: The Flight From Reason in the Supreme Court 13 St. Louis U. Pub. L. Rev. 15 9 (1993)]
Alabama:
Trent v. State, 73 So. 834, 836 (Ala. Civ. App. 1916) (interpreting state abortion law) ("does not the new being, from the first day of its uterine life, acquire a legal and moral status that entitles it to the same protection as that guaranteed to human beings in extra-uterine life?") (quoting from the 1911 Transactions of the Medical Association of Alabama).
Wolfe v. Isbell, 280 So.2d 758, 761 (Ala. 1973) (rejecting viability requirement in wrongful death action where death occurs after live birth):
[T]he more recent authorities emphasize that there is no valid medical basis for a distinction based on viability, especially where the child has been born alive. These [decisions] proceed on the premise that the fetus is just as much an independent being prior to viability as it is afterwards, and that from the moment of conception, the fetus or embryo is not a part of the mother, but rather has a separate existence within the body of the mother.
Alabama Constitutional Convention Call (S.J. Res. 9, 1980 Ala. Acts 396):
[A]pplies to the Congress . . . to call a convention for the sole and exclusive purpose of proposing an amendment to the Constitution that would protect the lives

of all human beings including unborn children at every stage of their biological development and providing that neither the United States nor any state shall deprive any human being, from the moment of fertilization, of the right to life without due process of law, nor shall any state deny any human being, from the moment of fertilization, the equal protection of the laws, except where pregnancy results from rape or incest; or where abortion is necessary to save the life of the mother; or where testing revealed abnormality or deformity of the fetus.

Arizona:

Nelson v. Planned Parenthood Ctr. of Tucson, 505 P.2d 580, 586 (Ariz. Ct. App. 1973) (construing state abortion law):

One cannot gainsay a legislative determination that an embryonic or fetal organism is "life." Once begun, the inevitable result is a human being, barring prior termination of the pregnancy.

ARIZ. REV. STAT. ANN. § 13-1103(A)(5) (1989) (defining offense of manslaughter to include "[k]nowingly or recklessly causing the death of an unborn child at any stage of its development by any physical injury to the mother of such child which would be murder if death of the mother had occurred").

Arkansas:

ARK. CONST. amend. 68, § 2 ("[t]he policy of Arkansas is to protect the life of every unborn child from conception until birth, . . .")

Arkansas Constitutional Convention Call (Res. of Feb. 17, 1977, H.R.J. Res. 2):

Requests Congress to call a convention to propose a constitutional amendment which would provide that every human being subject to the jurisdiction of the United States or any state shall be deemed from the moment of fertilization to be a person and entitled 'to the right of life; provides that Congress and the states shall have concurrent powers to enforce such an amendment.

California:

CAL. PENAL CODE, § 187(a) (West 1988) ("[m]urder is the unlawful killing of a human being, or a fetus, with malice aforethought").

Scott v. McPheeters, 92 P.2d 678, 681 (Cal. App. 1939) (it is "an established and recognized fact by science and by everyone of understanding" that "an unborn child is a human being separate and distinct from its mother").

Connecticut:

Simon v. Mullin, 380 A.2d 1353, 1357 (Conn. Supp. 1977) (rejecting viability requirement in wrongful death action where death occurs after live birth) ("[t]he development of the principle of law that now permits recovery by or on behalf of a child born alive for prenatal injuries suffered at any time after conception, without regard to the viability of the fetus, is a notable illustration of the viability of our common law").

Delaware:

Scott v. State, 117 A.2d 831, 835-36 (Del. 1955) (characterizing abortion law as one that defines an offense against the lives and persons of individuals).

Delaware Constitutional Convention Call (Res. of May 23, 1978, H.R. Con. Res. 9):

Requests Congress to call a convention to propose a constitutional amendment that would protect the lives of all human beings, including unborn children at every stage of their biological development.

District of Columbia:

Bonbrest v. Kotz, 65 F. Supp. 138, 140 (D.D.C. 1946) (recognizing cause of action for prenatal injuries) ("[f]rom the viewpoint of the civil law and the law of property, a child en ventre sa mere is not only regarded as [a] human being, but as such from the moment of conception--which it is in fact").

Florida:

Day v. Nationwide Mut. Ins. Co., 328 So.2d 560, 561 (Fla. Dist. Ct. App. 2d Dist. 1976) (rejecting viability requirement in case of prenatal injuries) (quoting with approval WILLIAM L. PROSSER, HANDBOOK OF THE LAW OF TORTS §55, at 336 (4th ed. 1971)):

Viability of course does not affect the question of the legal existence of the foetus, and therefore of the defendant's duty; and it is a most unsatisfactory criterion, since it is a relative matter, depending on the health of mother and child and many other matters in addition to the stage of development. Certainly the infant may be no less injured; and all logic is in favor of ignoring the stage at which it occurs.

Georgia:
Hornbuckle v. Plantation Pipe Line Co., 93 S.E.2d 727, 728 (Ga. 1956) (rejecting viability requirement in case of prenatal injuries) ("[i]f a child born after an injury sustained at any period of its prenatal life can prove the effect on it of a tort, it would have a right to recover") (a dissent characterized majority opinion as holding, in effect, "that an infant becomes a 'person' from the moment of conception, with the right to sue for a tortious injury after its birth"); id. at 729.
Morrow v. Scott, 7 Ga. 535, 537 (1849) ("[i]n . . . general, a child is to be, considered as in being, from the time of its conception, where it will be for the benefit of such child to be so considered").

Idaho:
Nash v. Meyer, 31 P.2d 273, 280 (Idaho 1934) (construing state abortion law) (criminal abortion statute intended "to discourage abortions because thereby the life of a human being, the unborn child, is taken").
Blake v. Cruz, 698 P.2d 315, 323 (Idaho 1984) (Bistline, J., concurring in part and dissenting in part) ("[t]his Court recently committed itself to the proposition that an unborn child is a person in being," citing Volk v. Baldazo, 651 P.2d 11 (Idaho 1982) (rejecting live birth requirement in wrongful death action where death occurs after viability)).
Idaho Constitutional Convention Call (S. Con. Res. 132, 45th Legis. 2d Sess., 1980 Idaho Sess. Laws 1005):
[R]equest[s] that the Congress . . . call a constitutional convention for the specific and exclusive purpose of proposing an amendment . . . [to provide that]:
(a) From the moment of conception a person shall be guaranteed all personal rights extended to all individuals under the constitution and laws of the United States of America and the state or states of residence and only under extreme circumstances shall it be otherwise; namely, to save the life of the mother, or other extenuating circumstances where at least two consulting physicians, one not having previously been involved in the case, and after due and thorough consultation with all persons having the legal right to be involved, find it is necessary and just that the life of the unborn shall be terminated.
(b) Provide that the several states shall have the power to enforce such an amendment, and establish priority of life by appropriate legislation.
Illinois:
720 ILL. COMP. STAT. ANN. § 510/1 (Smith-Hurd 1993) (preamble to Illinois Abortion Law of 1975):
[T]he General Assembly of the State of Illinois do solemnly declare and find in reaffirmation of the longstanding policy of this State, that the unborn child is a human being from the time of conception and is, therefore, a legal person for purposes of the unborn child's right to life and is entitled to the right to life from conception under the laws and Constitution of this State.
740 ILL. COMP. STAT. ANN. § 180/2.2 (Smith-Hurd 1993) (amending wrongful death statute to allow wrongful death action to be brought on behalf of an unborn child without regard to the stage of pregnancy when the child is injured or whether there is a live birth).
720 ILL. COMP. STAT. ANN. § 5/9-1.2(b)(1) (Smith-Hurd 1993) (defining "unborn child" as "any individual of the human species from fertilization until birth").
720 ILL. COMP. STAT. ANN. §§ 5/9-1.2, 5/9-2.1, 519-3.2, 5/12- 3.2, 5/12-4.4 (Smith-Hurd 1993) (amending criminal code to define broad range of crimes, including homicide, that can be committed against unborn child, regardless of gestational age).
Indiana:
Cheaney v. State, 285 N.E.2d 265, 268 (1972) cert. denied, 410 U.S. 991 (1973) (construing state abortion law) ("[i]t is now established that some sort of independent life begins at conception," rejecting quickening and viability as outdated and arbitrary distinctions).
Kansas:
City of Wichita v. Tilson, Case No. 91 MC 108 (Sedgwick County Court, July 21, 1991) (accepting necessity defense) (slip op. at 22) ("the medical and scientific communities . . . are of the opinion that life in homo sapiens begins at conception"), appeal sustained without discussion of this point, 855 P.2d 911, 918

(Kan. 1993), cert. denied, Nov. 16, 1993, 62 U.S. L. W. 3348 (Docket 93-467).
State v. Harris, 136 P. 264, 267 (Kan. 1913) (construing state abortion law):
The arbitrary refusal of the common law to regard the foetus as alive . . . until quick[ening] was based on no sound physiological principle . . . . [T]he movement recognized by the mother, and which is supposed to prove that her unborn child is alive, is merely one evidence of life, whereas unless life had existed long before the most disastrous consequences to the mother must have already been suffered . . .
.
For many purposes the law regards the infant as alive from its conception.
Kentucky:
KY. REV. STAT. ANN. § 311.710(5) (Michie/Bobbs-Merrill 1990):
If . . . the United States constitution is amended or relevant judicial decisions are reversed or modified, the declared policy of this Commonwealth to recognize and to protect the lives of all human beings regardless of their degree of biological development shall be fully restored.
KY. REV. STAT. ANN. §§ 311.720(5), (6) (Michie/Bobbs-Merrill 1990) (abortion regulations) (defining "fetus" as "a human being from fertilization until birth" and "human being" as "any member of the species homo sapiens from fertilization until death").
Hollis v. Commonwealth, 652 S.W.2d 61, 66-67 (Ky. 1983) (Wintersheimer, J., dissenting) (noting that "[b]iologically speaking, human life begins at the moment of conception" and that "[m]edical authority has long recognized that the child is in existence from the moment of conception").
Kentucky Constitutional Convention Call (H.R. Res. 7, 1978 Gen. Assembly, Reg. Sess., 1978 Ky. Acts 1401):
[R]equest[s] the Congress . . . to call a convention for the sole purpose of proposing the following article as an amendment to the Constitution . . .:
Section 1. With respect to the right to life, the word person as used in this article and in the Fifth and Fourteenth Articles of Amendment to this Constitution applies to all human beings irrespective of age, health, function, or condition of dependency, including their unborn offspring at every stage of their biological development.
Section 2. No unborn person shall be deprived of life by any person, provided, however, that nothing in this article shall prohibit a law permitting only those medical procedures required to prevent the death of the mother.
Section 3. The Congress and the several states shall have the power to enforce this article by appropriate legislation.
Louisiana:
LA. REV. STAT. ANN. § 14:2(7) (West 1986) (defining "person" for purposes of criminal code to include "a human being from the moment of fertilization and implantation").
LA. REV. STAT. ANN. §§ 14:32.5-32.8 (West 1992 Supp.) (defining fetal homicide offenses).
Danos v. St. Pierre, 383 So. 2d 1019, 1027 (La. Ct. App. 1980), aff'd, 402 So. 2d 633 (La. 1981) (Lottinger, J., concurring):
This definition [LA. REV. STAT. ANN. § 14:2(7) (West 1986)] added to the Criminal Code in 1976, reflects a legislative intent to classify an unborn child as a "person" for purposes of violent criminal conduct like homicide and battery. The definition reveals an express recognition by the legislature that life begins at the moment of conception and that this form of life can indeed be the victim of a harm, i.e., a murder or battery.
1991 La. Acts. § 1, No. 26 (amending state abortion law):
It is declared to be the public policy of the state of Louisiana that it has a legitimate compelling interest in protecting, to the greatest extent possible, the life of the unborn from the time of conception until birth. We also affirm our belief that life begins at conception and that life thereafter is a continuum until the time of death.
Johnson v. New Orleans Light & Traction Co., Docket 9048 (La. App. Orl. Dec. 10, 1923) (rejecting live birth and viability requirements in cause of action for wrongful death) (quoted with approval in Danos v. St. Pierre, 402 So. 2d 633, 639 (La. 1981)):
The argument of the defendant is that the infant before it is born is not a child, not a human being, that it is only a thing, a part of the anatomy of the mother, as

are her organs. We cannot accept that theory. We believe the infant is a child from the moment of conception although life may be in a state of suspended animation, the subject of love, affection and hope and that the injury or killing of it in its mother's womb is covered by the [wrongful death statute] and gives its bereaved parents to a right of action against the guilty parties for their grief and mental anguish.

Danos v. St. Pierre, 383 So. 2d 1019, 1029 (La. Ct. App. 1980), aff'd, 402 So. 2d 633 (La. 1981) (rejecting live birth requirement in action for wrongful death of a viable unborn child) (Lottinger, J., concurring):

Viability has not been the controlling factor in some previous Louisiana cases allowing recovery [for wrongful death of a stillborn child], and there is no need to make it a controlling factor in this decision. Just as live birth is an arbitrary cutoff point for wrongful death purposes, viability is equally arbitrary in deciding whether the fetus is a "person" whose wrongful killing is compensable.

Louisiana Constitutional Convention Call (Res. of July 16, 1976, S. Con Res. 70):

Requests Congress to call a convention to propose a constitutional amendment extending the term "person" in the Fifth and Fourteenth amendments to apply to all human beings "irrespective of age, health, function or condition of dependency, including unborn offspring at every stage of their biological development;" permits states to adopt laws necessary to preserve the woman's life; requests state legislative bodies to apply to Congress to call a convention to propose this constitutional amendment; grants Congress and the states the power to enforce the amendment.

Maryland:

Damasiewicz v. Gorsuch, 79 A.2d 550, 559 (Md. 1951) (recognizing cause of action for prenatal injuries) ("from a medical point of view, a child is alive within the mother before the time arrives when it can live apart from her"), id. at 560 (theory that "an unborn child is a part of the mother" is "an outworn point of view, now rejected by modern medicine").

Group Health Ass'n v. Blumenthal, 453 A.2d 1198, 1207 (Md. 1983) ("a cause of action lies for the wrongful death of a child born alive who dies as a result of injuries sustained while en ventre sa mere") (rejecting viability requirement).

Massachusetts:

Commonwealth v. Cass, 467 N.E.2d 1324, 1325 (Mass. 1984) (viable fetus is a "person" within meaning of vehicular homicide statute):

In keeping with approved usage, and giving terms their ordinary meaning, the word "person" is synonymous with the term "human being." An offspring of human parents cannot reasonably be considered to be other than a human being, and therefore a person, first within, and then in the normal course outside, the womb . . . . By the use of the term[] "person" . . . the Legislature has given no hint of a contemplated distinction between pre-born and born human beings.

Torigian v. Watertown News Co., 225 N.E.2d 926, 927 (Mass. 1967) (rejecting viability requirement in wrongful death action where death follows live birth).

Massachusetts Constitutional Convention Call (Act of June 8, 1977, H.R. 5984):

Requests Congress to call a convention to propose a constitutional amendment extending the term "person" in the Fifth and Fourteenth amendments to apply to all human beings "irrespective of age, health, function or condition of dependency, including unborn offspring at every stage of their biological development;" permits states to adopt laws necessary to preserve the woman's life; grants Congress and the states the power to enforce the amendment.

Michigan:

Womack v. Buchhorn, 187 N.W.2d 218, 222 (Mich. 1971) (recognizing cause of action for prenatal injuries and rejecting viability requirement because "a child has a legal right to begin life with a sound mind and body").

O'Neill v. Morse, 188 N.W.2d 785 (Mich. 1971) (recognizing cause of action for wrongful death of a viable stillborn child).

Larkin v. Cahalan, 208 N.W.2d 176, 179 (Mich. 1973) (construing state abortion law) ("statutes proscribing manslaughter by abortion are designed to protect human life and carry the necessary implication that that life, the destruction of which is punishable as manslaughter, is human life").

Minnesota:

MINN. STAT. ANN. §§ 609.266, 609.2661-609.2665, 609.267, 609.2671, 609.2672, 609.268 (West 1987 & 1992 Supp.) (amending criminal code to include a broad range of crimes,

including homicide, that can be committed against an unborn child, regardless of gestational age).
Verkennes v. Corniea, 38 N.W.2d 838, 840 (Minn. 1949) (rejecting live birth requirement in wrongful death action) (quoting with approval federal district court opinion in Bonbrest v. Kotz, 65 F. Supp. 138, 140 (D.D.C. 1946), where court said "[f]rom the viewpoint of the civil law and the law of property, a child en ventre sa mere is not only regarded as [a] human being, but as such from the moment of conception--which it is in fact").
Missouri:
MO. ANN. STAT. § 1.205.1(1) (Vernon Supp. 1992) (preamble to Missouri Abortion Law) ("[t]he life of each human being begins at conception").
MO. ANN. STAT§ 188.015(8) (Vernon Supp. 1992) (abortion regulations) (defining "unborn child" as, "the offspring of human beings from the moment of conception until birth and at every stage of its biological development, including the human conceptus, zygote, morula, blastocyst, embryo, and fetus").
Rodgers v. Danforth, 486 S.W.2d 258, 259 (Mo. 1972) (construing criminal abortion law) (accepting stipulation that "unborn children have all the qualities and attributes of adult human persons differing only in age or maturity" and that "[m]edically, human life is a continuum from conception to death").
Missouri Constitutional Convention Call (Res. of Apr. 24, 1975, S. Con. Res. 7):
Requests Congress to call a convention to propose a constitutional amendment extending the term "person" in the Fifth and Fourteenth amendments to apply to all human beings "irrespective of age, health, function, or condition of dependency, including unborn offspring at every stage of their biological development;" permits states to adopt laws necessary to preserve the woman's life; grants Congress and the states the power to enforce the amendment.
Montana:
MONT. CODE ANN. § 50-20-102 (1993) (statement of legislative purpose and intent--abortion regulations):
The legislature reaffirms the tradition of the state of Montana to protect every human life, whether unborn or aged, healthy or sick. In keeping with this tradition and in the spirit of our constitution, we reaffirm the intent to extend the protection of the laws of Montana in favor of all human life.
MONT. CODE ANN. § 41-1-103 (1993) ("[a] child conceived but not yet born is to be deemed an existing person, so far as may be necessary for its interests in the event of its subsequent birth").
Nebraska:
Nebraska Constitutional Convention Call (Res. of Apr. 21, 1978, Legis. Res. 152):
"Legislature . . . petition[s] . . . Congress . . . to call a convention for the sole purpose of proposing the following article as an amendment to the Constitution of the United States."
ARTICLE
Section 1. With respect to the right to life, the word person as used in this article and in the Fifth and Fourteenth Articles of Amendment to this Constitution applies to all human beings irrespective of age, health, function, or condition of dependency, including their unborn offspring at every stage of their biological development.
Section 2. No unborn child shall be deprived of life by any person, provided, however, that nothing in this article shall prohibit a law permitting only those medical procedures required to prevent the death of the mother.
Section 3. The Congress and the several states shall have the power to enforce this article by appropriate legislation.
Nevada:
White v. Yup, 458 P.2d 617, 623 (Nev. 1969) (recognizing cause of action for prenatal injuries and for the wrongful death of a viable, stillborn child) (proposition that "[a]n unborn child is a part of its mother until birth and thus has no juridical existence" "has no scientific or medical basis in fact").
Nevada Constitutional Convention Call (S.J. Res. 27, 60th Legis., 1979 Nev. Stat. 2014):
[L]egislature requests . . . Congress . . . to call a convention limited to proposing an amendment to the Constitution . . . to protect human life by restricting abortion [subject to exceptions in cases where the pregnancy results from rape or incest and where continuation of the pregnancy would seriously endanger

the life of the mother].
New Hampshire:
Bennett v. Hymers, 147 A.2d 108, 110 (N.H. 1958) (rejecting viability requirement in cause of action for prenatal injuries) ("[w]e adopt the opinion that the fetus from the time of conception becomes a separate organism and remains so throughout its life").
Wallace v. Wallace, 421 A.2d 134, 136 (N.H. 1980) (wrongful death action) ("[t]o deny a nonviable fetus a [wrongful death] cause of action is not to deny that life begins with conception").
New Jersey:
Smith v. Brennan, 157 A.2d 497, 502 (N.J. 1960) (rejecting viability requirement in cause of action for prenatal injuries) ("[m]edical authorities have long recognized that a child is in existence from the moment of conception, and not merely a part of its mother's body"):
We see no reason for denying recovery for a prenatal injury because it occurred before the infant was capable of separate existence. In the first place, age is not the sole measure of viability, and there is no real way of determining in a borderline case whether or not a fetus was viable at the time of the injury, unless it was immediately born. Therefore, the viability rule is. impossible of practical application . . . . In addition, . . . medical authority recognizes that an unborn child is a distinct biological entity from the time of conception, and many branches of the law afford the unborn child protection throughout the period of gestation. The most important consideration, however, is that the viability distinction has no relevance to the injustice of denying recovery for harm which can be proved to have resulted from the wrongful act of another. Whether viable or not at the time of the injury, the child sustains the same harm after birth, and therefore, should be given the same opportunity for redress.
Id. at 504.
Gleitman v. Cosgrove, 227 A.2d 689, 696 n.3 (1967) (Francis, J., concurring) (rejecting cause of action for wrongful life) ("[i]t was noted 30 years ago that the increase in knowledge of embryology had revealed that the child has separate existence from the moment of conception"), overruled, Bermarr v. Allan, 404 A.2d 8 (N.J. 1979) (reorganizing action).
New Jersey Constitutional Convention Call (Act of Apr. 21, 1977, S. 1271):
Requests Congress to call a convention to propose a constitutional amendment which would provide that every human being subject to the jurisdiction of the United States or any state shall be deemed from the moment of fertilization to be a person and entitled to the right to life; provides that Congress and the states shall have concurrent powers to enforce such an amendment.
New York:
New York City Health & Hosp. Corp., 286 N.E.2d 887, 888 (N.Y. 1972), appeal dismissed, 410 U.S. 949 (1973) (rejecting challenge to pre-Roe abortion law which allowed abortion on demand through the twenty-fourth week of gestation but recognizing that human life begins at conception):
It is not effectively contradicted, if it is contradicted at all, that modern biological disciplines accept that upon conception a fetus has an independent genetic "package" with potential to become a full-fledged human being and that it has an autonomy of development and character although it is for the period of gestation dependent upon the mother. It is human, if only because it may not be characterized as not human, and it is unquestionably alive.
Kelly v. Gregory, 125 N.Y.S.2d 696, 697 (N.Y. App. Div. 1953) (rejecting viability requirement in cause of action for prenatal injuries) ("legal separability should begin where there is biological separability" and "separability begins at conception"):
The mother's biological contribution from conception on is nourishment and protection; but the foetus has become a separate organism and remains so throughout its life. That it may not live if its protection and nourishment are cut off earlier than the viable stage of its development is not to destroy its separability; it is rather to describe conditions under which life will not continue. Succeeding conditions exist, of course, that have that result at every stage of its life, postnatal as well as prenatal.
Id. at 697.
North Carolina:

DiDonato v. Wortman, 358 S.E.2d 489, 496 (N.C. 1987) (recognizing cause of action for wrongful death of a viable unborn child) ("[t]he public policy of this state as expressed by the legislature in our statutes recognizes that an unborn infant is a person") (Martin, J., concurring in part and dissenting in part).
Corkey v. Edwards, 322 F. Supp. 1248, 1252 (W.D.N.C. 1971), vacated and remanded, 410 U.S. 950 (1973) (construing criminal abortion statute):
Apart, the sperm and the unfertilized egg will die; neither has the capacity to grow and develop independently as does the fertilized egg. During fertilization, sperm and egg pool their nucleii and chromosomes. Biologically, a living organism belonging to the species homo sapiens is created out of this organization. Genetically, the adult man was from such a beginning all that the essentially has become in every cell and human attribute.
North Dakota:
N.D. CENT. CODE §§ 12.1-17.1-02 through 12.1-17.1-06 (Supp. 1991) (amending criminal code to define broad range of crimes, including homicide, that can be committed against unborn child, regardless of gestational age).
Statute providing that "[a] child conceived but not born is to be deemed an existing person so far as may be necessary for its interests in the event of its subsequent birth" was intended "to ensure and to protect the interests of a child subsequent to its conception but prior to its birth," Hopkins v. McBane, 359 N.W.2d 862, 864 (N.D. 1984).
Ohio:
Steinberg v. Brown, 321 F. Supp. 741, 746 (N.D. Ohio 1970) (construing criminal abortion law) (holding that human life is entitled to federal constitutional protection from conception) ("a new life comes into being with the union of human egg and sperm cells" and "[s]uch terms as 'quick' or 'viable', which are frequently encountered in legal discussion, are scientifically imprecise and without recognized medical meaning").
Williams v. Marion Rapid Transit, 87 N.E.2d 334, 340 (Ohio 1949) (recognizing cause of action for prenatal injuries):
To hold that the plaintiff in the instant case [a viable unborn child] did not suffer an injury in her person would require this court to announce that as a matter of law the infant is part of the mother until birth and has no existence in law until that time. In our view such a ruling would deprive the infant of the right [to a remedy] conferred by the [Ohio] Constitution upon all persons, by the application of a time worn fiction not founded on fact and within common knowledge untrue and unjustified.
The court also quoted with approval WILLIAM L. PROSSER, HANDBOOK OF THE LAW OF TORTS § 31, 189 (1941). Professor Prosser stated, "So far as duty is concerned, if existence at the time [of injury] is necessary, medical authority has recognized long since that the child is in existence from the moment of conception, and for many purposes its existence is recognized by the law." Id. at 339.
Oklahoma:
OKLA. STAT. ANN. tit. 63, § 1-730(2) (West 1997) (abortion regulations) (defining "unborn child" as "the unborn offspring of human beings from the moment of conception, through pregnancy, and until live birth including the human conceptus, zygote, morula, blastocyst, embryo and fetus . . . .").
Evans v. Olson, 550 P.2d 924, 926 (Okla. 1976) (rejecting viability requirement in cause of action for prenatal injuries and live birth requirement in wrongful death actions) ("there is no medical or scientific basis" for the proposition that "an unborn child has no judicial existence apart from its mother").
Oregon:
State v. Ausplund, 167 P. 1019, 1022-23 (Or. 1917) (construing criminal abortion law):
The statute refers to "any woman pregnant with a child" without reference to the stage of pregnancy. When a virile spermatozoon unites with a fertile ovum in the uterus, conception is accomplished. Pregnancy at once ensues, and under normal circumstances continues until parturition. During all this time the woman is "pregnant with a child" within the meaning of the statute. She cannot be pregnant with anything else than a child. From the moment of conception a new life has begun, and is protected by the enactment. The product of conception during its entire course is imbued with life, and is capable of being destroyed as contemplated by the law. By such destruction the death of a child is produced and often that of

its mother as well.
Mallison v. Pomeroy, 291 P.2d 225, 228 (Or. 1955) (recognizing cause of action for prenatal injuries) (Thin Oregon we have recognized by statute the separate entity of an unborn child by protecting him in his property rights and against criminal conduct . . . .").
Libbee v. Permanente Clinic, 518 P.2d 636 (Or. 1974) (recognizing cause of action for the wrongful death of a viable stillborn child).
Pennsylvania:
28 PA. Code § 29.31 (1995) (abortion regulations) (defining "unborn child" as a human being from fertilization until birth and includes a fetus).
Amadio v. Levin, 501 A.2d 1085, 1087 (Pa. 1985) (rejecting live birth requirement in wrongful death actions) ("a child en ventre sa mere is a separate individual from the moment of conception'').
Sinkler v. Kneale, 164 A.2d 93, 96 (Pa. 1960) (rejecting viability requirement in cause of action for prenatal injuries) (viability has "little to do with the basic right to recover, when the foetus is regarded as having existence as a separate creature from the moment of conception").
Pennsylvania Constitutional Convention Call (H.R. 71, 1978 Gen. Assembly, 1978 Pa. Laws 1431):

> [A]pplication to the Congress . . . to call a convention for drafting and proposing an amendment to the Constitution . . . to guarantee the right to life to the unborn fetus by doing the following:
>
> (a) With respect to the right to life guaranteed in the United States Constitution, provide that every human being subject to the jurisdiction of the United States or any state shall be deemed from the moment of fertilization to be a person and entitled to the right to life.
>
> (b) Provide that Congress and the several states shall have concurrent powers to enforce such an amendment by appropriate legislation.
>
> ***
>
> (d) Nothing in this article shall prohibit a law permitting only those medical procedures required to prevent the death of the mother.

Rhode Island:
Sylvia v. Gobeille, 220 A.2d 222, 223-24 (R.I. 1966) (rejecting viability requirement in cause of action for prenatal injuries) (noting "the medical fact that a fetus becomes a living human being from the moment of conception" and rejecting viability as a "decisive criterion" because "there is no sound reason for drawing a line at the precise moment of the fetal development when the child attains the capability of an independent existence").
Presley v. Newport Hosp., 365 A.2d 748, 751 (R.I. 1976) (rejecting live birth requirement in wrongful death of a viable unborn child) (citing with approval the civil law proposition that "from the moment of conception a separate organism with its own identity comes into existence" and the medical proposition that "an ovum, once it is fertilized, is a separate living entity"):

> [V]iability is a concept bearing no relation to the attempts of the law to provide remedies for civil wrongs. If we profess allegiance to reason, it would be seditious to adopt so arbitrary and uncertain a concept as viability as a dividing line between those persons who shall enjoy the protection of our remedial laws and those who shall become, for most intents and purposes, nonentities. It seems that if live birth is to be characterized, as it so frequently has been, as an arbitrary line of demarcation, then viability, when enlisted to serve that same purpose, is a veritable non sequitur.

Id. at 753-54 (dicta in plurality opinion) (disapproved Miccolis v. Amica Mutual Ins. Co., 587 A.2d 611 (R.I. 1991)).
Rhode Island Constitutional Convention Call (Act. of Apr. 21, 1977, H.R. 5150):

> Requests Congress to call a convention to propose a constitutional amendment which would provide that every human being subject to the jurisdiction of the United States or any state shall be deemed from the moment of fertilization to be a person and entitled to the right to life; provides that Congress and the states shall have concurrent power to enforce such an amendment.

South Dakota:
State v. Munson, 201 N.W.2d 123, 126 (S.D. 1972), vacated and remanded, 410 U.S. 950 (1973) (construing criminal abortion law) (citing with approval holding in Steinberg v. Brown, 321 F. Supp. 741 (N.D. Ohio 1970), that human life is entitled to federal

constitutional protection from conception).
S.D. CODIFIED LAWS ANN. § 21-5-1 (1987) (amending wrongful death statute to include "an unborn child" without regard to gestational age).
S.D. CODIFIED LAWS ANN. § 22-17-6 (1988) ("[a]ny person who intentionally kills a human fetus by causing an injury to its mother . . . is guilty of a Class 4 felony").
S.D. CODIFIED LAWS ANN. § 26-1-2 (1992) ("[a] child conceived, but not born, is to be deemed an existing person so far as may be necessary for its interests in the event of its subsequent birth").
Texas:
Thompson v. State, 493 S.W.2d 913, 918 (Tex. Crim. App. 1971) vacated and remanded, 410 U.S. 950 (1973) (construing criminal abortion law):
The State of Texas is committed to preserving the lives of its citizens so that no citizen "shall be deprived of life, . . . except by the due course of the law of the land." [Citation omitted]. [The Texas abortion law] is designed to protect fetal life . . . and this justifies prohibiting termination of the life of the fetus or embryo except for the purpose of saving the life of the mother.
Leal v. C.C. Pitts Sand & Gravel, Inc., 419 S.W.2d 820, 822 (Tex. 1967) (recognizing cause of action for wrongful death for prenatal injuries where death occurs after live birth), rev'g 413 S.W.2d 825 (Tex. Civ. App. 1967) (denying cause of action) and app'g dissenting opinion of Justice Cadena, 413 S.W.2d at 828 ("medical science . . . consider[s] that life begins at conception"), id. at 829 ("legalistic concept that the unborn child is but a part of its mother" is "contrary to scientific fact and common sense").
Witty v. Am. Gen. Capital Distrib., Inc., 727 S.W.2d 503, 505 (Tex. 1987) (denying cause of action for wrongful death of viable child who was stillborn but recognizing "the fetus as having an existence separate from its mother").
Delgado v. Yandell, 468 S.W.2d 475 (Tex. Civ. App. 1971), writ ref'd n.r.e. 471 S.W.2d 569 (Tex. 1971) (per curiam) (rejecting viability requirement in cause of action for prenatal injuries).
Utah:
UTAH CODE ANN. § 76-7-301.1(2): "The state of Utah has a compelling interest in the protection of the lives of unborn children."
UTAH CODE ANN. § 76-5-201(1) (1992 Supp.) (defining offense of criminal homicide as causing "the death of another human being, including an unborn child at any stage of its development").
Utah Constitutional Convention Call (H.R.J. Res. 28, 42nd Legis., Reg. Sess., 1977 Utah Laws 1317, 1318):
[A]pplies to the Congress . . . to call a convention for the purpose of drafting and submitting for ratification by the states, . . . an amendment to the Constitution that will guarantee to every human life, from the moment of fertilization throughout its natural existence, in every state, territory, and possession of the United States, the full protection of all laws respecting life, excepting an unborn child whose mother's life would otherwise be lost.
Virginia:
Kalafut v. Gruver, 389 S.E.2d 681, 683-84 (Va. 1990) (rejecting viability rule in cause of action for prenatal injuries or for wrongful death following live birth) (noting "developments in medical science, especially in the field of embryology," court held that "an action may be maintained for recovery of damages for any injury occurring after conception, provided the tortious conduct and the proximate cause of the harm can be established").
Wisconsin:
WIS. STAT. ANN. § 940.04(6) (West 1982) (criminal abortion statute defining "unborn child" as "a human being from the time of conception until it is born alive")
Puhl v. Milwaukee Auto. Ins. Co., 99 N.W.2d 163, 170 (Wis. 1959) (rejecting viability requirement in cause of action for prenatal injuries), overruled on other grounds, In re Estate of Stromsted, 299 N.W.2d 226 (Wis. 1980):
The viability theory has been challenged as unrealistic in that it draws an arbitrary line between viability and nonviability, and fails to recognize the biological fact there is a living human being before viability. A child is no more a part of its mother before it becomes viable than it is after viability. It would be more accurate to say that the fetus from conception lives within its mother rather than as a part of her. The claim of a child injured before viability is just

as meritorious as that of a child injured during the viable stage. Kwaterski v. State Farm Mut. Auto. Ins. Co., 148 N.W.2d 107, 111 (Wis. 1967) (rejecting born alive requirement in wrongful death actions) (assertion that "[a] child has no juridical existence apart from its mother" has "no scientific or medical basis in fact").

APPENDIX D
DO NO HARM et al. Comments on Draft NIH Guidelines for Human Stem Cell Research, 74 Federal Register 18578-18580 (April 23, 2009)
Frozen Embryos:
The Adoption Solution
RONALD L. STODDART, ESQ. (c) (November 5, 1999), as updated May 22, 2009
THE BACKGROUND

The increase in the use of "reproductive technology" has resulted in the birth of children through an alphabet soup of conception techniques. For those families who have gone through infertility treatment, terms such as IVF, GIFT, ZIFT, AHA, etc. sometimes obscure the fact that achieving a pregnancy and having a family is the goal. But while pursuing the goal, families find themselves creating new issues as frequently as they resolve existing ones.

For example, one of the by-products of in-vitro fertilization of eggs is the creation of embryos which are not immediately implanted. Where economics and technology clash, the economy of scale has typically prevailed and left the "fertility challenged" parents with "extra" embryos that can be frozen and stored for later implantation. Whether the first implantations are unsuccessful or the parents desire additional children, the availability of stored embryos is an attractive service offered by the fertility physicians.

By some estimates, there are hundreds of thousands of frozen embryos currently in storage in the United States. A recent report indicated that there were over 25,000 frozen embryos being stored in Massachusetts, alone, due to their favorable health insurance coverage requirements for infertility procedures.

Eventually the genetic parents will be confronted with the need to make a decision on the future of their stored embryos when they have completed their own family. The three choices they are given are (1) to donate the embryos for implantation, (2) to donate the embryos for research or (3) to have the embryos destroyed. Physicians, bioethicists, social workers, clergy and other "experts" have weighed in on these choices with arguments reminiscent of the Pro-Life -Pro-Choice debate. Although I am strongly Pro-Life, this issue is largely irrelevant when dealing with the focus of this article, the adoption of frozen embryos.

For the record, however, I would like to state the fundamental argument for "adopting" frozen embryos rather than transferring them through some other contractual means. A frozen embryo is a pre-born child with the potential for development into a viable fetus and ultimately a new born baby. Regardless of the debate surrounding the creation of the embryos that are now frozen and stored, the movement to offer the genetic parents the full rights of birth parents in an adoption proceeding recognizes the deep emotional bonds that exist between genetic parents and their children - regardless of how they come to be born.

THE LAW

As one might imagine, the law has lagged far behind reproductive technology and generally responds to disputes that test the wisdom of Solomon. In California, the Penal Code has brought the transfer of embryos under the common law Statue of Frauds by requiring a written agreement. The further regulation of such transfers, however, are woefully lacking any specifics or protections for either party to the written agreement, other than those provided by the Health & Safety Code sections dealing with tissue transfer and health issues.

California Penal Code Section 367(b) provides the legal basis for the formalities required in an embryo transfer as follows:
"It shall be unlawful for anyone to knowingly implant sperm, ova, or embryos, through the use of assisted reproduction technology, into a recipient who is not the sperm, ova, or embryo provider, without the signed written consent of the sperm, ova, or embryo provider and recipient."
There are certainly clinics and physicians that are transferring embryos with the

most abbreviated consent forms imaginable. To those families who are comfortable with the designation "provider" and "recipient", perhaps such informality is sufficient. But the law has always treated the adoption of human beings with a bit more respect.

Seven states have laws in effect which provide some general guidance for embryo donation or adoption. With the exception of statutes in Louisiana, most statutes are geared toward the respective rights of those donating and receiving embryos, rather than the embryos themselves. With the exception of Louisiana and Florida, four states solely use the term embryo donation as opposed to embryo adoption.

1. CALIFORNIA

a. California civil law provides that each individual undergoing fertility treatment must be informed of all possible options for unused embryos. It also details possible dispositions for embryos belonging to individuals or couples who die, separate, divorce, or fail to pay storage fees. CAL HEALTH & SAFETY CODE §12315 (2007).

b. California criminal law prohibits the use of embryos for anything other than that to which the embryo provider consents. Cal. Penal Code § 367g (2007).

2. FLORIDA

Florida law provides that donors of embryos relinquish all parental rights with respect to the donation of embryos or the resulting children. FLA. STAT. § 742.14 (2007). Additionally, embryo adoption is included in a listing of fertility techniques. FLA. STAT. § 63.213 (2007).

3. LOUISIANA

Louisiana law provides for a wide range of embryo protection, stating that an embryo is a juridical person (not fully human under the law, but deserving of some rights), and has a legal status in which it is recognized as a separate entity apart from the physician or the sperm and egg donors. Embryos may not be intentionally destroyed. Louisiana also allows for embryo adoption if IVF patients renounce parental rights. LA. REV. STAT. ANN. §§ 9:122-130 (2007).

4. OHIO

Ohio law provides that a woman who gives birth to a child as the result of embryo donation will be regarded as the natural mother and establishes that embryo donors have no parental rights or responsibilities. OHIO REV. CODE ANN. §§ 3111.97 (2007).

5. OKLAHOMA

Oklahoma law provides basic guidelines for human embryo transfer and donation and establishes that donors of embryos relinquish all parental rights with respect to the donation or any resulting children. OKLA. STAT. ANN. tit. 10, § 556 (2007).

6. TEXAS

Texas law includes embryo donation in the definition of assisted reproduction technology (ART). TEX. FAM. CODE ANN. § 160.102 (2007).

7. GEORGIA

Georgia law, enacted April 3, 2009, called the Option of Adoption Act, specifically provides procedures for genetic parents to relinquish their rights to embryos before birth and allow the recipient intended parents to be the legal parents of the child that may be born as a result of the embryo transfer. Additionally, the bill changes the definition of "child" to include an in vitro human embryo and offers the same legal rights to adoption as an in utero or already born human being.

ADOPTION LAW

The basic elements of an adoption, even ignoring the considerable evidence supporting the importance of "open adoption", include:

1. Complete and thorough advisement of legal rights to the birth parent(s), generally accompanied by psychological counseling.
2. Complete and thorough screening and education of the adopting parent(s), generally through the home study process.
3. Formal execution of consent documents by both birth parents and adopting parents.
4. Court decree recognizing the sufficiency of the process and-the protection of the best interests of the child.

5. Promulgation of a new birth certificate reflecting the legal status of adopting parents and child.

When dealing with embryo adoptions, the first three elements of an adoption can be satisfied, and should be satisfied for the protection of the child and the adult parties to the adoption. As will be shown below, the need for a new birth certificate is obviated in an embryo adoption and there is no statutory basis for an adoption decree (although some courts may be willing to issue ceremonial decrees).

DEFINING THE ROLES

As with any new area of the law, defining the roles of the participants - and the terminology applied to them - is often the first hurdle to overcome. To ease the understanding of the roles - both emotionally and legally - of the parties we have adopted the following definitions.

Genetic Parents: The genetic parents fill the role most commonly associated with "birth parents" in adoptions. The frozen embryo is the pre-born child of the genetic parents. The genetic parents have the legal right to custody and control of the frozen embryos, which custody has generally been assigned temporarily to a fertility clinic or cryobank laboratory. With some exceptions, the law recognizes this right of custody more as an ownership interest than parental rights and obligations.

For purposes of this article, the genetic parents are assumed to have been the source of the eggs and sperm used to create the embryos. In the case where donor eggs or donor sperm were used, the genetic parents are the individuals with the legal right to determine the future of the frozen embryos.

Pre-born Child: A frozen embryo is a pre-born child, subject to many of the same risks of survival as any pre-born child. Our purpose in emphasizing the personhood of the frozen embryo is not to subject the genetic parents to a moral and religious argument for not destroying the embryo - although certainly that is our unequivocal position. Rather, it is easier to understand and plan for the future emotional needs of the "adopted" embryo by recognizing its identity at the earliest possible time.

Adopting Parents: The adopting parents are the recipients of the frozen embryo and therefore the child's "birth parents" under the law. The frozen embryo would be implanted in the adopting mother after it has been legally "relinquished" or transferred to the adopting parents. No additional legal proceedings would be necessary for the adopting parents/birth parents to secure full legal and physical custody to the child.

Relinquishment: The term relinquishment, rather than donation, legal transfer or gift, is used to describe the procedure for the genetic parents to terminate their legal rights to the frozen embryo. It is important that this be accomplished with the same safeguards as are found in a more traditional adoption in order to best prepare and educate all of the parties involved. It is also important that the relinquishment be accomplished prior to the implantation of the frozen embryo into the adopting mother so that there is no later dispute as to the legal roles of the parties.

Genetic Siblings: One of the little noticed, but important factors in treating the transfer of a frozen embryo to another family as an "adoption" is to safeguard the later needs of the genetic family, including genetic siblings. Unlike other forms of in-vitro fertilization used in infertility cases, the placement of frozen embryos for adoption generally involves genetic parents who have already been successful in giving birth to children using the contemporaneously created embryos.

ADVANTAGES OF EMBRYO ADOPTION

There are a number of advantages to embryo adoption to all of the parties involved. Let's review what some of those advantages might be.

1. Advantages to Genetic Parents

As was discussed earlier, once genetic parents have completed their families and have no further desire to give birth to additional children, the decision as to the future of any remaining frozen embryos must be made. Regardless of the medical status of the embryo, which may be as few as 4 cells, the genetic parents are frequently emotionally invested in the future of "all" of their children, even those that carry the label "potential" children. For those genetic parents who believe that the embryos are more than tissue, and who would like to give each embryo a fair chance at life, adoption is the most satisfying answer.

Mere release of the embryos for implantation in unknown parents is similar to the

old "closed adoption" system that left birth mothers grieving for far too long when simple information as to the child's welfare would have been a healing balm. Like birth mothers in an open adoption, genetic parents can be as involved or uninvolved in the selection of adopting parents as they choose. In addition, they can maintain the security of knowing that the genetic siblings of their own children will always be known in the event of medical emergencies or to later answer imponderable questions.

2. Advantages to Adopting Parents

For infertile couples, it was thought that the closest experience to giving birth was adopting a new born baby and taking the baby home directly from the hospital. Although some women who have experienced labor and delivery may disagree, the opportunity to become pregnant with your adopted child, carry the child to term and then give birth to your adopted child truly maximizes the parenting experience. For those experts who extol the virtues of "pre-natal bonding", frozen embryo adoption is the great equalizer.

MODEL EMBRYO ADOPTION PROGRAM

I. Services to the Genetic Parents

Similar to traditional adoptions, genetic parents should be offered counseling as to all of the options available to them. Adoption of the frozen embryos should be described as a lifelong commitment to the children who may be born from the implantation of the embryos in an adopting mother. As with open adoption, genetic parents should be encouraged to participate in the establishment of criteria for the adopting parents and even in the actual selection of parents.

Genetic parents will provide complete medical information, including recent HIV test results. Such information must be disclosed to the adopting parents and the physician assisting with the embryo implantation.

Post-adoption services, including counseling must also be made available to genetic parents. Every effort should be made to maintain contact through the agency involved with the genetic parents.

II. Services to Adopting Parents

Potential adopting parents should complete a home study as would any other adopting parents. It is important that the family be counseled as to the life long issues of adoption, even though they will be giving birth to their adopted child. To try to ignore the fact that the child is adopted could result in emotional upheaval for the child later in life. Although the way of explaining to a birthed child that the child is adopted may seem bizarre to us now, children will soon find the realities of reproductive technology very common place. Education and support will be as important in frozen embryo adoptions as they are in other more traditional adoptions.

Potential adopting mothers must also show, through recommendations from her physician, that she is capable of carrying a child to term even though she may suffer from other infertility problems. It is also highly desirable that the adopting parents have the willingness to provide continuing information on their child(ren) to the agency and genetic parents. It should be remembered that the tie between genetic parents and adopting parents is particularly strong when the presence of genetic siblings are recognized.

III. The Role of the Adoption Agency

The role of the adoption agency is critical to the future of frozen embryo adoptions. Without the recognition that adoptions of frozen embryos are entitled to the same safeguards and protections as other adoptions, the potential for a "market" in frozen embryos being created is very real. Just as the law regulates who may act as an intermediary in traditional adoptions (either the birth parent(s) directly or a licensed adoption agency), it is equally important to regulate who may act as an intermediary in a frozen embryo adoption. It should also be noted that even when birth parent(s) place a child directly with adopting parents, the law still requires a home study and court approval of the adoption.

In the case of frozen embryo adoption, until the law catches up with the science, the appropriate adoption expertise to apply to frozen embryo adoption will come from licensed agencies. The agency can offer the counseling, screening, education and formal relinquishment services that should be the hallmarks of a frozen embryo adoption. Until the legislature or courts provide for other formalities or protections, the adoption community should encourage, even advocate, for the necessity of such an adoption model.

CONCLUSIONS

Although many physicians and facilitators may point to the success of their myriad varieties of egg, sperm and embryo transfers, at some point the treatment of embryos must conform to that afforded children rather than property. To wait until we have a generation of displaced children, with little knowledge or understanding of their roots, crying for "open records" and their "right to know' their history, would reflect too little appreciation for the 'past errors of adoption practice. The time to develop a progressive and thoughtful approach to dealing with the futures of the hundreds of thousands of stored frozen embryos is now.

There are more than 10 million infertile couples in the U.S. In the last decade, the infertility industry has grown from about 30 to over 300 clinics earning revenues in excess of 1 billion dollars. It is estimated that 11-25% of couples who experience difficulty conceiving or carrying a pregnancy to term consider adoption. The National Adoption Information Clearinghouse reports that about 200,000 couples are actively seeking to adopt each year. It is estimated that in the U.S. in 2007, about 1% of the live births (or more than 42,000 infants) will be born as a result of IVF - about the same number that will be available through traditional unrelated infant adoption. At the same time, more than 400,000 human embryos are now frozen, suspended in liquid nitrogen tanks on the premises of IVF clinics (with more than 19,000 frozen embryos estimated to be added to each year). While many proponents of embryonic stem cell research claim that these 400,000 frozen embryos are "unwanted leftovers" that ought to be used for research, the facts prove otherwise. According to the most definitive 2003 Rand Corporation study, only 2.8% (or about 11,000) of the frozen embryos are "designated for research" by their biological parents, 88.2% are designated by the biological families for their own "family-building," 2.3% (or about 9,200) for donation or "adoption by others," 2.2% are to be "discarded," and 4.5% have experienced "lost contact with biological 'patients,' patient death, abandonment or divorce." Thus, aside from the unethical nature of destructive human embryo research, there are not even enough human embryos designated for research to create the number of genetically diverse stem cell lines demanded by embryonic research proponents

Although the program developed by Christian Adoption & Family Services (called Snowflakes) is certainly a "work in progress", it does recognize the unique nature of each embryo and the real needs of the genetic parents in planning for their future.1

APPENDIX E

Legislative and Administrative History
of the
Federal Funding Ban
on
Destructive Human Embryo Research

May 26, 2009

by
Samuel B. Casey,1 General Counsel
ADVOCATES INTERNATIONAL

The federal funding ban on destructive human embryo research [popularly known as the "Dickey-Wicker Amendment" after its original sponsor, former Cong. Jay. Dickey (R-AK) and current Senator Roger Wicker (R-MS) who was then a member of the House of Representatives)], included in every Health and Human Services ("HHS") appropriations bill since 1995,2 states, "None of the funds made available by this Act may be used for . . . research in which a human embryo or embryos are destroyed, discarded or knowingly subjected to risk of injury or death greater than that allowed for research on fetuses in utero. . . ." Interpreting this language, then-HHS General Counsel Harriet S. Rabb issued a memorandum on January 15, 1999, cleverly claiming that the Dickey-Wicker Amendment bans federal funding of the derivation of embryonic stem cells - a euphemism for the procedure killing the living human embryo - but not research utilizing the derived embryonic stem cells.3

On August 25, 2000, based almost exclusively on the 'derivation' vs. 'use' distinction in the Rabb memorandum, NIH published and made effective its "GUIDELINES FOR RESEARCH USING HUMAN PLURIPOTENT STEM CELLS." 65 Fed. Reg. 51976 (hereafter the "Clinton Guidelines"). Contrary to HHS's decades-long practice of refusing to fund research that threatens or destroys human embryos, and in direct contradiction of Congress's plainly expressed intent, the Clinton Guidelines allowed federal funding of research using embryonic stem cells derived from the destruction of human embryos by others not funded by the federal government. The Clinton Guidelines were never implemented due to the end of the Clinton Administration, litigation in the Nightlight Adoption case staying their enforcement, and their ultimate withdrawal by the National Institutes of Health (NIH) on November 7, 2001, based upon the President's Executive Statement of August 9, 2001.4

In January 2002, following President Bush's August 9, 2001 announcement of his administration's stem cell research policy, the Bush Administration formally withdrew the Clinton Guidelines and issued its own guidance in the following documents (hereafter the Bush Guidelines) that remain the law today, subject to the NIH review ordered by President Obama in his March 11, 2009 Executive Order 13505:

1. HHS General Counsel Memorandum, January 11, 2002, Alex M. Azar II to Dr. Ruth Kirchstein, Acting Director, NIH

2. Notice of Criteria for Federal Funding of Research on Existing Human Embryonic Stem Cells and Establishment of NIH Human Embryonic Stem Cell Registry, November 7, 2001, NOT-OD-02-005, Office of the Director, NIH (http://grants.nih.gov/grants/guide/notice-files/NOT-OD-02-006.html; see generally http://stemcells.nih.gov/).

All research involving human embryonic stem cells necessarily entails extraction of stem cells from living human embryos. The process by which human embryonic stem cells are extracted from human embryos necessarily destroys the human embryos. Accordingly, research using embryonic stem cells necessarily involves the destruction or discarding of embryos and/or places such embryos at more than a minimal risk, without biomedical necessity. The proposed NIH Guidelines nevertheless provide for federal funding of research involving human embryonic stem cells, so long as the funds are not directly used to pay for the act of extracting the stem cells from the human embryos for research.

The proposed NIH Draft Guidelines on Human Embryonic Stem Cell Research, as published by NIH for comment on April 23, 2009 (77 Fed. Reg. 18578-18580)(the "proposed Guidelines) fail to account for, and substantially undermine, the laws of numerous States that protect human life from the moment of conception or otherwise protect human embryos from being destroyed or placed at risk for the purpose of medical experimentation. Similarly, the proposed Guidelines fail to account for longstanding ethical norms that protect human life from medical exploitation and experimentation.

The proposed Guidelines cannot be justified by any attempt to resurrect the thinking originally set forth in a single legal memorandum, dated January 15, 1999, issued by HHS General Counsel Harriett S. Rabb (the "Rabb memo"). See 65 Fed. Reg. 51796. The Rabb memo claimed that despite the federal funding ban, federal funds could still be used to pay for research involving stem cells obtained by deliberately destroying human embryos so long as the federal funds do not pay for the specific procedure by which the stem cells are extracted from the living human embryos.

In attempting to justify the purported legality of federally funding human embryonic stem cell research, the Rabb memo concluded that research on embryonic stem cells "would not be prohibited by the HHS appropriations law prohibiting human embryo research, because such stem cells are not human embryos." In support of this conclusion, the Rabb memo asserted that human embryonic stem cells "are not organisms and do not have the capacity to develop into an organism that could perform all the life functions of a human being - in this sense they are not even precursors to human organisms." Further, the Rabb memo stated that human embryonic

"stem cells do not have the capacity to develop into a human being, even if transferred into a uterus." In support of these assertions, the Rabb memo mischaracterized testimony before a Senate Subcommittee's Hearings, ignored other critical testimony provided during the course of those same hearings, and failed to discuss scientific evidence suggesting that the conclusions stated in the Rabb memo are inaccurate.

In a letter dated February 11, 1999, approximately 75 members of Congress requested that then-Secretary Shalala correct the HHS General Counsel's misinterpretation of the federal funding ban on destructive embryo research.

On February 12, 1999, seven United States Senators signed and delivered a letter to Secretary Shalala expressing "deep[] concern[]" over certain testimony by then-NIH Director Varmus suggesting the NIH's willingness to fund embryonic stem cell research. The Senators expressly disagreed with Director Varmus's "contention . . . that once the stem cells are derived, federal funding of research which directly relies on such destruction is acceptable." The Senators made clear that "Congress never intended for the National Institutes of Health to give incentives for the killing of human embryos for the purpose of stem cell research." The letter also expressed concern over Director Varmus's sworn testimony admitting that he was "unsure" whether so-called "pluripotent stem cells may come together in culture to begin developing as an embryo." The Senators noted that if, as some researchers have found, such development is possible, then even under the reasoning employed in the Rabb memo, embryonic stem cell research would unquestionably violate Congress's ban on any research that destroys, discards, or places human embryos at risk.

Despite these congressional warnings, the NIH, on December 2, 1999, published a Notice of its Draft Guidelines for Research Involving Human Pluripotent Stem Cells in the Federal Register and invited public comment for a period of 60 days. See 64 Fed. Reg. 67576 (Dec. 2, 1999). The NIH subsequently extended the original 60-day comment period for an additional 28 days. The comment period ended on February 22, 2000.

The NIH received approximately 50,000 comments from members of Congress, patient advocacy groups, scientific societies, religious organizations, and private citizens. The vast majority of these comments were opposed to the Guidelines.

The Clinton Guidelines allowed for funding of research involving human embryonic stem cells "only if the cells were derived (without Federal funds) from human embryos that were created for the purposes of fertility treatment and were in excess of the clinical need of the individuals seeking such treatment." 65 Fed. Reg. 51979. The Clinton Guidelines also "prescribe the documentation and assurances that must accompany requests for NIH funding for research using human [embryonic] stem cells from: (1) Awardees who want to use existing funds; (2) awardees requesting an administrative or competing supplement; and (3) applicants or intramural researchers submitting applications or proposals." 65 Fed. Reg. 51979.

The Clinton Guidelines provided no scientific, or any other, support for the primary premise upon which NIH relied and apparently still relies in the proposed Guidelines, namely, its assumption that human embryonic stem cells are not protectable as human embryos. Rather, the Guidelines merely repeated HHS General Counsel Rabb's unscientific and unfounded assertion that "[a]lthough human pluripotent stem cells may be derived from embryos or fetal tissue, such stem cells are not themselves embryos." 65 Fed. Reg. 51979.

In responding to numerous comments objecting to the Clinton Guidelines on the ground that NIH funding for human embryonic stem cell research plainly violates HHS appropriations law, NIH merely cited HHS General Counsel Rabb's unsupported assertion that "'federally funded research that utilizes [human embryonic stem cells] would not be prohibited by the HHS appropriations law prohibiting human embryo research, because such cells are not human embryos.'" 65 Fed. Reg. 51976. NIH asserted without explanation that these comments "did not present information or arguments that justify reconsideration of the [HHS General Counsel's] conclusion."

Id.

NIH defended its decision to fund embryonic stem cell research, rather than relying on adult stem cell research, on the grounds that "[i]t is possible that no single source of stem cells is best or even suitable/usable for all therapies," and that "[d]ifferent types or sources of stem cells may be optimal for treatment of specific conditions." 65 Fed. Reg. 51976 (emphases added). Those speculative and unsubstantiated assertions, however, fell far short of Congress's requirement that embryonic research can be conducted only if, in addition to not posing a non-minimal risk to the human embryo, "the purpose of the activity is the development of important biomedical knowledge which cannot be obtained by other means." 45 C.F.R. § 46.208(a)(2) (emphases added); Pub. L. No. 106-554, Omnibus Consolidated Appropriations Act of 2001, § 510.

On March 8, 2001, the Nightlight plaintiffs sued HHS to prevent implementation of the Clinton Administration's Rabb-influenced Guidelines for Research Involving Pluripotent Stem Cells,6 because this interpretation flatly contradicted legislative history through 2000, and the original purpose for passing the Dickey-Wicker Amendment: to prevent destructive human embryo research.7 Until 1994, a de facto federal ban on human embryo research existed.8 The Clinton Administration took steps to reverse this ban, pursuant to the recommendation of an ad hoc advisory committee, the Human Embryo Research Panel ("HERP"),9 while still prohibiting the creation of embryos for research purposes.10 In testimony before the House Appropriations Committee, NIH Director Varmus stated that he "firmly agree[d]" with several portions of the HERP report, and told the Committee that NIH was currently deciding whether to go forward with funding.11

Before NIH could approve any grants, Congress passed the Dickey-Wicker Amendment for the first time.12 Opponents of the amendment objected to it on the grounds that it would foreclose action on the HERP report and "segregate [human embryo] research into private laboratories, which are not subject to any set scientific or ethical guidelines."13 Sen. Boxer agreed that the Dickey-Wicker Amendment amounted to "a total prohibition of Federal funding for human embryo research."14 That first year, the House Appropriations Committee rejected an alternative rider offered by Rep. John Porter (R-IL), which would have codified President Clinton's directive by prohibiting only the funding of the creation of embryos for research purposes.15

During the 1997 reauthorization cycle, the full House roundly rejected (167-256) an amendment identical to the Porter Amendment offered by Rep. Lowey (D-NY).16 Again, the proponents and opponents of embryo research operated on the same premise; i.e., that the Dickey-Wicker Amendment banned federal funding of all research dependent upon the destruction of an embryo.17 Rep. Porter argued, for example, that repeal of the Dickey-Wicker Amendment was necessary, because federal funding of research "could also lead to breakthroughs in the use of embryonic stem cells."18 No further attempts were made to modify the Dickey-Wicker Amendment until the 2001 reauthorization cycle.

In 2001, the House reauthorized the amendment without change, with a statement in the House report describing its action as consistent with the announced Bush Administration stem cell policy as articulated by President Bush on August 9, 2001.19 Rep. McDermott and Sen. Arlen Specter proposed amendments permitting liberal embryonic stem cell research.20 Both failed, with the Specter bill defeated due to the Bush Administration's public opposition.21 The resulting Amendment is not a vindication of the Rabb memo's derivation-versus-use dichotomy. Nor is it a vindication of the limited protection that President Clinton, Reps. Lowey, Porter, and McDermott, and Sen. Specter offered (i.e., prohibiting the funding merely of the creation of embryos for research purposes).

Rather, the resulting amendment is at most a vindication of the principles permitting research on already dead fetuses. President Bush refused to justify research on living human embryos based on the derivation-versus-use dichotomy; he authorized research only on embryos terminated before August 9, 2001, without creating federal incentives to kill more.

On January 14, 2002, without waiver of the right to re-file the case should circumstances change, the Nighlight case was voluntarily dismissed by the plaintiffs

because the objectives of the injunctive relief action they had filed in federal court the prior March against the Department of Health & Human Services and the National Institutes of Health - enjoining destructive human embryo research - have been achieved by two actions taken by the Bush Administration: (1) the President's action the prior week signing the 2002 Labor/HHS Appropriations Act (H.R. 3061) that continues the complete federal funding ban on destructive human embryo research; and (2) the action of the HHS General Counsel on January 11, 2002 issuing his legal memorandum to NIH confirming that HHS and NIH will now properly interpret the law to completely ban any federal funding for destructive human embryo research.22

Since that time federal law and policy has firmly prohibited the federal funding of destructive human embryonic stem cell research. In President Bush's words when he signed the 2002 Labor/HHS Appropriations Act (H.R. 3061:

"I am pleased that the final version of the [Labor/HHS Appropriations] bill retains the prohibition against research in which human embryos are destroyed, and reinforces my determination on August 9, 2001, to support federally funded stem cell research in an ethical manner."

According to the HHS General Counsel's January 11, 2002 legal opinion supporting the President's action:

"Under the President's policy, federal funding for human embryonic stem cell research is limited to a discrete set of stem cells with respect to which the life and death decision had been made prior to the announcement of his policy. The President's policy provides no incentives for the destruction of additional embryos....So limited, the President's policy does not provide federal funding for research in which (during the course of, during or part of the act or process of, or within the category of class of] embryos are destroyed, discarded, or knowingly subjected to risk of injury or death....within the ordinary, common usage of those terms. The policy is, thus, consistent with the second restriction of the [2002 Labor/HHS Appropriations Act].

Viewed in this perspective, the proposed Guidelines violate the intent of federal funding ban because by their terms they are not "limited to a discrete set of stem cells with respect to which the life and death decision had been made prior to the announcement [of their new] policy and they do "provide incentives for the destruction of additional embryos" in the form of federal research dollars. Indeed, the proposed Guidelines detailed protocol for how to regulate the consent process and obtain embryos for destruction is best described as the initial phase of a larger research project which will receive funds from the federal government and must be viewed as a blatant attempt to violate the existing federal law set forth in the federal funding ban and for the first time illegally authorize the use of federal funds in the precise case prohibited by the federal funding ban, that is "research in which" human embryos are "harmed, destroyed or subjected to risks" not permitted for unborn children in the womb.

Indeed, interpreting paragraph (2) of the federal funding ban to cover only the act of destruction itself would violate two principles of statutory construction applicable to the federal funding ban.

First, a statute must be construed to avoid rendering any of its word superfluous. Walters v. Metropolitan Educational Enterprises, 519 U.S. 202, 209-210 (1997); United States v. Menasche, 348 U.S. 528, 538-539 (1955). While the NIH in the proposed Guidelines acknowledges the existence of federal funding ban, it fails to give any legal basis for the proposed Guidelines other than the Executive Order that merely instructs it to "support and conduct responsible scientifically worth human stem research...to the extent permitted by law." Nonetheless, the unspoken interpretation apparently used by the NIH in the proposed Guidelines would render the words in paragraph (2) of the federal funding ban "research in which" superfluous.

Second, when Congress chooses different language in proximate subsections of the same statute - one narrow, the other broad - the statute must be construed to give effect to those differences. Russello v. United States, 464 U.S. 16, 23 (1983) and cases cites therein. Thus, NIH is correct when it says that the federal funding

ban in the Dickey-Wicker Amendment prohibits "NIH funding of the derivation of stem cells from human embryos." Guidelines, IV.A., 74 Fed. Reg. at 18580. But its proposed Guidelines violate the federal funding ban by failing to acknowledge that it prohibits more than that, and also prescribes "research in which" human embryos are "harmed, destroyed or subjected to risks" not permitted for unborn children in the womb.

APPENDIX F
HHS General Counsel Memorandum, January 11, 2002
Alex M. Azar II to Dr. Ruth Kirchstein, Acting Director, NIH
January 11, 2002
Via Facsimile
Thomas G. Hungar, Esq.
Gibson, Dunn & Crutcher, LLP
1050 Connecticut Avenue, N.W.
Washington, D.C. 20036
Re: Nightlight Christian Adoption, et al., Civil No. 01-0502 (RCL) (DDC)
Dear Mr. Hungar:
On November 7, 2001, Defendants in the above-referenced case gave notice that they had completed their review of the National Institutes of Health ("NIH") Guidelines for Research using Human Pluripotent Stem Cells, 65 Fed. Reg. 51976 (Aug. 25, 2000) ("Guidelines"), which review had resulted in withdrawal of those Guidelines and issuance of a Notice of Criteria for Federal Funding of Research on Existing Human Embryonic Stem Cells and Establishment of NIH Human Embryonic Stem Cell Registry announcing new criteria that must be met to allow Federal funds to be used for research on human embryonic stem cell lines.
As you know, NIH plans soon to initiate federal funding of research on existing human embryonic stem cells in accordance with the policy announced by the President on August 9, 2001. For your information, please find enclosed a Memorandum dated January 11, 2002 from Alex M. Azar II, General Counsel at the Department of Health & Human Services, to Dr. Ruth Kirchstein, Acting Director of NIH, concluding that the President's policy with respect to embryonic stem cell research comports with the so-called Dickey Amendment.
Please do not hesitate to contact me if you have any questions or concerns.
Sincerely,
/s/ Robert D. McCallum, Jr.
Robert D. McCallum, Jr.
Assistant Attorney General

January 11, 2002
MEMORANDUM
TO: Dr. Ruth Kirchstein
Acting Director, National Institutes of Health
FROM Alex M. Azar II
General Counsel
SUBJECT: Compliance of the President's Embryonic Stem Cell Decision with the Dickey Amendment for Fiscal Year 2002
The National Institutes of Health plan soon to initiate federal funding of research on existing human embryonic stems cells in accordance with the policy announced by the President on August 9, 2001. Prior to the initiation of such funding, you have asked the Office of the General Counsel to provide advice on the legality of the President's policy under the Dickey Amendment to Public Law Number 107-116 (signed Jan. 10, 2002), the appropriations act funding the Department of Health & Human Services (the "Department") for fiscal year 2002.
It is our conclusion that the President's policy comports with the plain language of the Dickey Amendment. This reading is further buttressed by Congress's recent reenactment of the Dickey Amendment and, hence, ratification of the President's policy and by the legislative history accompanying the most recent reenactment of the Dickey Amendment.
The President's Policy
On August 9, 2001 at 9:00 p.m. EDT, President George W. Bush announced his decision to allow federal funds to be used for research on existing human embryonic stem cell lines as long as, prior to his announcement, (1) the derivation process (which

commences with the removal of the inner cell mass from the blastocyst) had already been initiated, and (2) the embryo from which the stem cell line was derived no longer had the possibility of development as a human being.

As the President noted, "the life and death decision ha[d] already been made" with respect to those "existing human embryonic stem cell lines." This decision, as the President stated, "allows us to explore the promise and potential of stem cell research without crossing a fundamental moral line, by providing taxpayer funding that would sanction or encourage further destruction of human embryos that have at least the potential for life." Remarks by the President on Stem Cell Research, Aug. 9, 2001, http://www.whitehouse.gov/news/releases/2001/08/print/20010809-2.html.

The President established the following additional criteria that had to be met for embryonic stem cell research to receive federal funding: (1) the stem cells must have been derived from an embryo that was created for reproductive purposes; (2) the embryo was no longer needed for such purposes; (3) informed consent must have been obtained for the donation of the embryo; and (4) no financial inducements were provided for donation of the embryo. Notice of Criteria for Federal Funding of Research on Existing Human Embryonic Stem Cells and Establishment of NIH Human Embryonic Stem Cell Registry, Nov. 7, 2001, NOT-OD-02-005, Office of the Director, NIH, http://grants.nih.gov/grants/guide/notice-files/NOT-OD-02-005.html; NIH Human Embryonic Stem Cell Registry, http://escr.nih.gov. Pursuant to the President's policy, federal funds will not be used for (1) the derivation or use of stem cell lines derived from newly destroyed embryos; (2) the creation of any human embryos for research purposes; or (3) the cloning of human embryos for any purpose. Fact Sheet, Embryonic Stem Cell Research, Aug. 9, 2001, http://www.whitehouse.gov/news/release/2001/08/print/20010809-1.html.

Pursuant to the President's policy, on August 27, 2001, Secretary Thompson announced the creation of a registry of the embryonic stem cell lines meeting the President's eligibility criteria, such that research on stem cell lines listed on the Registry would be eligible for federal funding. He stated that:

[t]he NIH wants to expedite this work and is aggressively pursuing several initiatives to facilitate research on all forms of stem cells. The NIH is creating a registry of the embryonic stem cell lines that meet the eligibility criteria so that researchers can contact the owners and gain access to them. The registry will contain basic information about the cells, a unique identifier, the name of the company or laboratory that derived the cells, and contact information about that company or lab. The registry will list these 10 laboratories as well as any other owners of stem cell lines meeting the eligibility criteria who come forward in the future.

Statement by Tommy G. Thompson, Secretary of Health & Human Services, Aug. 27, 2001, http://www.hhs.gov/new/press/2001pres/20010827a.html; see also Tommy G. Thompson, Secretary of Health & Human Services, Testimony before the Senate Committee on Health, Education, Labor & Pensions, Sept. 5, 2001, at 4 (discussing NIH's development of "a stem cell registry" and the intent to "mak[e] it available so scientists know exactly what lines are eligible and who they can approach for access" and to post the registry on the NIH website), http://www.hhs.gov/news/speech/2001/010905.html.

In an NIH Update, the NIH noted that the laboratories or companies that derived the cells listed on the registry that it was creating would provide "a signed assurance that the derivation process was initiated prior to 9:00 p.m. EDT on August 9, 2001, informed consent was obtained for the donation of the embryo, the cells were derived from an excess embryo that was created for reproductive purposes, and there were no financial inducements for the donation of the embryo for research." NIH Update on Existing Human Embryonic Stem Cells, Aug. 27, 2001, at 2-3, http://www/nih.gov/news/stemcell/082701list.html. Shortly thereafter, the NIH entered into a memorandum of understanding with one of the entities that possesses such embryonic stem cell lines, to permit access to those lines by NIH scientists to conduct research and to permit scientists pursuing research funded by the NIH to negotiate access to those lines under the same terms and conditions. See NIH Press Release, National Institutes of Health and WiCell Research Institute, Inc. Sign Stem Cell Research Agreement, Sept. 5, 2001, http://www.nih.gov/news/pr/sep2001/od-05.html; Memorandum of Understanding between WiCell Research Institute, Inc. and Public Health Service, US Department of Health & Human Services, effective as of Sept. 5, 2001, http://www.nih.gov/news/stemcell/WiCellMOU.pdf; see also Tommy G.

Thompson, Secretary of Health & Human Services, Testimony before the Senate Committee on Health, Education, Labor & Pensions, Sept. 5, 2001, at 4 (announcing negotiation of the memorandum of understanding permitting research use of WiCell's "five existing stem cell lines that meet the eligibility criteria"), http://www.hhs.gov/news/speech/2001/010905.html.

On November 7, 2001, the NIH posted the Registry of embryonic stem cell lines that comply with the President's policy as announced on August 9, 2001. See NIH Human Embryonic Stem Cell Registry, http://escr.nih.gov; Notice of Criteria for Federal Funding of Research on Existing Human Embryonic Stem Cells and Establishment of NIH Human Embryonic Stem Cell Registry, Nov. 7, 2001, NOT-OD-02-005, Office of the Director, NIH, http://grants.nih.gov/grants/guide/ notice-files/NOT-OD-02-005.html.

The Dickey Amendment

In construing the meaning of a statute, the starting point of the analysis is the language of the statute. See, e.g., Central Bank of Denver NA v. First Interstate Bank of Denver NA, 511 U.S. 164, 173 (1994) (the statutory language is "'the starting point in every case involving construction of a statute'"); Good Samaritan Hosp. v. Shalala, 508 U.S. 402, 409 (1993) ("The starting point in interpreting a statute is its language, for '[i]f the intent of Congress is clear, that is the end of the matter.'"); Ernst & Ernst v. Hochfelder, 425 U.S. 185, 197 (1976) ("'The starting point in every case involving construction of a statute is the language itself.'"); Kaiser Aluminum & Chem. Corp. v. Bonjorno, 494 U.S. 827, 834-44 (1990) (same); Meredith v. Federal Mine Safety & Health Review Comm'n, 177 F.3d 1042, 1053 (D.C. Cir. 1999) ("As always, the starting point of analysis is the text of the statute.").

Since 1995, the Dickey Amendment has been enacted in each of the annual appropriations acts for the Department. For fiscal year 2002, the Amendment provides:

(a) None of the funds made available in this Act may be used for-

(1) the creation of a human embryo or embryos for research purposes; or

(2) research in which a human embryo or embryos are destroyed, discarded, or knowingly subjected to risk of injury or death greater than that allowed for research on fetuses in utero under 45 CFR 46.208(a)(2) and section 498(b) of the Public Health Service Act (42 U.S.C. 289g(b)).

(b) For purposes of this section, the term 'human embryo or embryos' includes any organism, not protected as a human subject under 45 CFR 46 as of the date of the enactment of this Act, that is derived by fertilization, parthenogenesis, cloning, or any other means from one or more human gametes or human diploid cells.

Pub. L. No. 107-116 § 510. This language is unchanged from the fiscal year 2001 Dickey Amendment.

The President's policy is consistent with the plain language of the Dickey Amendment. The Dickey Amendment contains two basic restrictions. The first prohibits the use of federal funds for "the creation of a human embryo or embryos for research purposes." See Pub. L. No. 107-116, § 510(a)(1). It is clear that, under the President's policy, no federal funds will be used for the creation of human embryos for research purposes. See Fact Sheet, Embryonic Stem Cell Research, Aug. 9, 2001, http://www.whitehouse.gov/news/release/2001/08/print/20010809-1.html (federal funds will not be used for "creation of any human embryos for research purposes"). Thus, the President's policy comports with the first restriction contained in the Dickey Amendment.

The second restriction of the Dickey Amendment prohibits the use of federal funds for "research in which a human embryo or embryos are destroyed, discarded, or knowingly subjected to risk of injury or death greater than that allowed for research on fetuses in utero . . . ." H.R. 3061, § 510(a)(2) (emphasis added). The term "research in which" is not defined in the statute, and our research has not located any cases in which such a term is defined. As such, it is appropriate to look to ordinary and common usage when interpreting those terms. See FDIC v. Meyer, 510 U.S. 471, 476 (1994) ("In the absence of such a definition [in the act], we construe a statutory term in accordance with its ordinary or natural meaning."). The word "which," when "[u]sed as a relative pronoun preceded by that or a preposition in a clause that defines or restricts the antecedent" means "[t]he thing, animal, group of people, or event previously designated or implied, specifically." See The American Heritage Dictionary, New College Edition 1459 (1976). Dictionaries define "in" as meaning "within the confines of; inside";

"within the area covered by"; "during the course of or before the expiration of"; "during or part of the act or process of"; "within the category or class of." See id. at 663; see also Black's Law Dictionary 683 (5th ed. 1979) (a preposition "expressing relation of presence, existence, situation, inclusion, action, etc.; inclosed or surrounded by limits . . .; also meaning for, in and about, on, within etc.; and is synonymous with expressions 'in regard to', 'respecting', 'with respect to', and 'as is'"). Under the President's policy, federal funding for human embryonic stem cell research is limited to a discrete set of stem cell lines with respect to which the life and death decision had been made prior to the announcement of his policy. The President's policy provides no incentives for the destruction of additional embryos. Moreover, these derivation processes were not funded with federal dollars. So limited, the President's policy does not provide federal funding for "research in which [during the course of, during or part of the act or process of, or within the category or class of] embryos are destroyed, discarded, or knowingly subject to risk of injury or death greater than that allowed for research on fetuses in utero" within the ordinary, common usage of those terms. The policy is, thus, consistent with the second restriction of the Dickey Amendment.

Congressional Ratification of the Legality of the President's Policy

This plain meaning reading of the Dickey Amendment is bolstered by Congress's reenactment of the Dickey Amendment in identical form after the President's announcement on August 9, 2001. As discussed below, Congress was fully aware of the President's policy decision and the Secretary's steps in implementing that decision. With that knowledge, Congress reenacted the Dickey Amendment in identical form, clearly evidencing its concurrence that the President's policy is consistent with the Dickey Amendment. See Lorillard v. Pons, 434 U.S. 575, 580-81 (1978) ("Congress is presumed to be aware of an administrative or judicial interpretation of a statute and to adopt that interpretation when it re-enacts a statute without change."); Central Bank of Denver, 511 U.S. at 185-86 ("When Congress reenacts statutory language that has been given a consistent judicial construction, we often adhere to that construction in interpreting the reenacted statutory language."); Pierce v. Underwood, 487 U.S. 552, 567 (1988) (same); City of Pleasant Grove v. United States, 479 U.S. 462, 468 (1987) ("Congress was aware of the Attorney General's view . . . and implicitly approved it, when it reenacted the Voting Rights Act . . . ."); San Huan New Materials High Tech, Inc. v. International Trade Comm'n, 161 F.3d 1347, 1355 (Fed. Cir. 1998) ("The legislative history shows that Congress was fully aware of the agency regulations and practices [regarding consent decrees] at the time of legislating in their area, and absent some special circumstances the failure to change or refer to existing practices is reasonably viewed as ratification thereof.").

Legislative History of the Dickey Amendment Contained in Pub. L. No. 107-116

The legislative history of the current reenactment of the Dickey Amendment in the appropriations act providing funding for Department for fiscal year 2002 further confirms that Congress understood the contours of the President's policy and believed that the policy complies with the requirements of the Dickey Amendment. The Committee Report on H.R. 3061, the House version of the Act, published exactly two months after the President's announcement states:

Human Stem Cell Research- The Committee received testimony from NIH institute and center directors, representatives of scientific and medical societies, and members of voluntary health organizations about the potential of both adult and embryonic stem cells for improving the lives of those who suffer with a host of disorders, including diabetes, Alzheimer's, Parkinson's, and cardiovascular disease. The Committee understands that a great deal of basic research is required to determine whether this potential can be realized.

It is the Committee's intent, that the NIH move ahead expeditiously to implement the President's policy concerning support of scientifically meritorious research involving both adult and human embryonic stem cells. The Committee commends the NIH for moving quickly to negotiate material transfer agreements with holders of existing embryonoc [sic] cell lines. The Director is requested to keep the Committee apprised of program initiatives as well as research progress concerning both adult and embryonic stem cells.

H.R. Rep. 107-229, at 98 (Oct. 9, 2001) (emphases added). In addition, the Committee noted in connection with section 510, the Dickey Amendment, the following:

Sec. 510. The Committee continues a provision to prohibit the use of funds in the

Act concerning research involving human embryos. However, this language should not be construed to limit federal support for research involving human embryonic stem cells listed on an NIH registry and carried out in accordance with policy outlined by the President.
H.R. Rep. 107-229, at 180 (Oct. 9, 2001) (emphasis added). The Joint Explanatory Statement of the Committee of Conference directed that "in implementing this agreement [on appropriations], the Departments and agencies should comply with the language and instructions set forth in House Report 107-229 and Senate Report 107-84." See Joint Explanatory Statement of the Committee of Conference, H.R. Rep. 107-342, Conference Report on H.R. 3061, at 55 (Dec. 19, 2001). Thus, it would be appropriate to accord to H.R. Rep. 107-229 the weight customarily given to conference committee explanatory statements. See Northern Colorado Water Conservancy Dist. v. Federal Energy Regulatory Comm'n, 730 F.2d 1509, 1518-19 (D.C. Cir. 1984) ("Statements in a conference report, because commended to the entire Congress, carry greater weight than comments from floor debates by individual legislators."); Vitrano v. Marshall, 504 F. Supp. 1381, 1383 (D.D.C. 1981) ("Perhaps the most useful document illuminating Congressional purpose is a Conference Report which bears on the final draft that is used by the conferees in explaining to the entire Congress why the bill should pass.")
As a whole, this legislative history expresses the Congress's support for the President's policy and unambiguously confirms that the President's decision is consistent with the Dickey Amendment. See Thunder Basin Coal Co. v. Reich, 510 U.S. 200, 209 (1994) ("The legislative history of the Mine Act confirms this interpretation."); see also San Huan New Materials, 161 F.3d at 1355 ("The legislative history leaves no doubt that Congress was aware of, and approved of, the Commission's consent order procedure as it existed at the time of the 1988 amendments.").
In sum, whatever legal challenges might be brought, the President's policy is consistent with the Dickey Amendment as evidenced by the plain language of the statute, Congress's reenactment ratification of the President's policy, and the legislative history reflecting Congress's full understanding of the precise contours of the President's policy and that policy's compliance with the Dickey Amendment.
As we move forward with implementation of the President's decision, it should be noted that federal funding of research in the following areas remains barred: (1) the derivation of new stem cells from human embryos; (2) research in which human embryonic stem cells are used to create or contribute to a human embryo; (3) research in which human embryonic stem cells are derived, using somatic cell nuclear transfer, i.e., the transfer of a human somatic cell nucleus into a human or animal egg; (4) research using human embryonic stem cells that were derived using somatic cell nuclear transfer, i.e., the transfer of a human somatic cell nucleus into a human or animal egg; (5) research in which human embryonic stem cells are combined with an animal embryo; and (6) research in which human embryonic stem cells are used in combination with somatic cell nuclear transfer for the purposes of reproductive cloning of a human. See National Institutes of Health Guidelines for Research Using Human Pluripotent Stem Cells, Part III ("Areas of Research Involving Human Pluripotent Stem Cells that are Ineligible for NIH Funding", listing the above categories of research), 65 FR 51976 (effective Aug. 25, 2000), corrected, 65 FR 69951 (Nov. 21, 2000), www.nih.gov/news/stemcell/stemcellguidelines.html, withdrawn as to those sections pertaining to research involving human pluripotent stem cells derived from human embryos that are the result of in vitro fertilization, are in excess of clinical need, and have not reached the stage at which the mesoderm is formed, Notice of Withdrawal of NIH Guidelines for Research Using Pluripotent Stem Cells, Nov. 7, 2001, NOT-OD-02-007, Office of the Director, NIH, http://grants.nih.gov/grants/guide/notice-files/NOT-OD-02-007.html; NIH Office of Extramural Research, Implementation Issues for Human Embryonic Stem Cell Research Frequently Asked Questions, Nov. 16, 2001, http://grants.nih.gov/grants/stem_cell_faqs.html.

APPENDIX G
DO NO HARM et al. Comments on Draft NIH Guidelines for Human Stem Cell Research, 74 Federal Register 18578-18580 (April 23, 2009)

47067_Do_No_Harm_et_al._Comments_re_Proposed_NIH_Stem_Cell_Guidelines

Adult Stem Cell Research

By: David A. Prentice, Ph.D.

Only adult stem cells-not embryonic stem cells-have shown any successes in therapeutic applications. A wealth of published scientific papers document that adult stem cells are a much more promising source of stem cells for regenerative medicine. Some adult stem cells actually do show pluripotent flexibility in generation of tissues, meaning that they can generate most or all of the different tissues of the body. In 2001, researchers found that one adult bone marrow stem cell could form not only marrow and blood, but also form liver, lung, digestive tract, skin, heart, muscle.1 Other researchers have found pluripotent ability of adult stem cells from various sources, including bone marrow,2,3,4 peripheral blood,5 inner ear,6 umbilical cord blood,7,8 nasal mucosa,9 amniotic fluid,10,11 and placental amniotic membrane.12 Indeed, a report from researchers at Wake Forest11 that amniotic fluid and placenta contains stem cells that can be easily harvested, show extended growth in culture, show similar flexibility to form other tissues of the body, and can be transplanted without tumors, emphasizes the range of abilities that adult and tissue stem cells have without the negatives associated with embryonic stem cells. Furthermore, testicular biopsies have shown that animal and human pluripotent stem cells can be obtained from this tissue source.13,14,15

The true test of the usefulness of any stem cell is not its pluripotency, but rather its ability for use in regenerative medicine, repairing damaged and diseased tissue and improving health. Pre-clinical results provide voluminous evidence that adult stem cells are effective in treating animal models of disease, including examples such as diabetes,16 stroke,17 spinal cord injury,18 Parkinson's disease,19 retinal degeneration,20 ALS,21 and cardiac damage.22

More importantly, adult stem cells are already being used clinically to treat many diseases in human patients. While it is true that bone marrow transplants have been used successfully in patients since the 1960's and the first successful cord blood transplant was in 1988,23 the human bone marrow stem cell was not actually isolated until 1992.24 Thus, it is only in recent times that a real focus on adult stem cells as a separate cell type and not an unidentified entity or phenomenon within a tissue has been possible. Given this recent development makes it all the more amazing that clinical applications have moved ahead as rapidly as they have done so. There has also been a bias against adult stem cells as a reparative stem cell with multipotent capabilities. This is exemplified in a statement from the National Institutes of Health in its 2001 review of stem cell science:

It was not until recently that anyone seriously considered the possibility that stem cells in adult tissues could generate the specialized cell types of another type of tissue from which they normally reside-either a tissue derived from the same embryonic germ layer or from a different germ layer.25

A search of clinicaltrials.gov shows well over 2,000 clinical trials currently with adult stem cells, and the number grows weekly. The published successful results with patients continue to pour forth with increasing frequency. Early successes and many of the continuing results use adult stem cells, most often from bone marrow or umbilical cord blood, in conjunction with chemotherapy or radiation, in treatments for various cancers, including ovarian cancer,26 retinoblastoma,27 amyloidosis,28 brain tumors,29 Merkel cell carcinoma,30 mantle cell lymphoma,31 testicular cancer,32 various lymphomas including Hodgkin's lymphoma33 and Non-Hodgkin's lymphoma,34 chronic35 and acute36 leukemias, breast cancer,37 renal cell carcinoma,38 and numerous other cancers (for a representative list of references, please see: http://www.sciencemag.org/cgi/data/315/5810/328b/DC1/1 and http://stemcellresearch.org/facts/asc-refs.pdf). Similar methodology has utilized adult stem cells in treatments for various anemias, including sickle cell anemia39 and Fanconi's anemia40 (for a representative list of references, please see: http://www.sciencemag.org/cgi/data/315/5810/328b/DC1/1 and http://stemcellresearch.org/facts/asc-refs.pdf). In the last decade, this technique has also been used successfully to treat patients with various autoimmune diseases, including multiple sclerosis,41 systemic lupus,42 Crohn's disease,43 rheumatoid arthritis,44 and juvenile (Type I) diabetes45 (for a representative list of references, please see: http://www.sciencemag.org/cgi/data/315/5810/328b/DC1/1 and http://stemcellresearch.org/facts/asc-refs.pdf). Various immunodeficiencies including SCID have been treated successfully as well46 (for a representative list

of references, please see: http://www.sciencemag.org/cgi/data/315/5810/328b/DC1/1 and http://stemcellresearch.org/facts/asc-refs.pdf). Adult stem cells have also shown success in protocols to ameliorate the effects of various genetic metabolic disorders such as Hurler's syndrome,47 Krabbe's leukodystrophy,48 and other genetic disorders (for a representative list of references, please see: http://www.sciencemag.org/cgi/data/315/5810/328b/DC1/1 and http://stemcellresearch.org/facts/asc-refs.pdf). These life-saving treatments continue to improve and to increase with further federally-funded clinical trials. The utility of adult stem cells to save lives and improve health is not, however, limited to use as an adjunct or rescue technique to chemotherapy. Published patient results have also shown their abilities for repair of acute and chronic cardiac damage49 (for a representative list of references, please see: http://www.sciencemag.org/cgi/data/315/5810/328b/DC1/1 and http://stemcellresearch.org/facts/asc-refs.pdf). Adult stem cells have also been used to grow new corneas to restore sight to blind patients50 (for a representative list of references, please see: http://www.sciencemag.org/cgi/data/315/5810/328b/DC1/1 and http://stemcellresearch.org/facts/asc-refs.pdf). Successful results have also been obtained for treatment of limb ischemia and wounds51 (for a representative list of references, please see: http://www.sciencemag.org/cgi/data/315/5810/328b/DC1/1 and http://stemcellresearch.org/facts/asc-refs.pdf). Early, ongoing trials have shown evidence of successful amelioration of the effects of stroke52 (for a representative list of references, please see: http://www.sciencemag.org/cgi/data/315/5810/328b/DC1/1 and http://stemcellresearch.org/facts/asc-refs.pdf). Early results with adult stem cells show effectiveness at treating liver disease53 (for a representative list of references, please see: http://www.sciencemag.org/cgi/data/315/5810/328b/DC1/1 and http://stemcellresearch.org/facts/asc-refs.pdf). An early clinical trial has shown effectiveness of the patient's own adult stem cells at treating Parkinson's disease.54 Several reports now document clinical improvement using adult stem cells for treatment of spinal cord injury.55

Adult stem cells have also already shown their utility in tissue engineering applications to treat patients, including growth of functional bladders56 and a published case of a new windpipe.57

Adult stem cells have distinct advantages over other stem cell types. In most cases the patient's own stem cells can be used for the treatment, circumventing problems of immune rejection. Adult stem cells do not have the problem of tumor formation that is associated with embryonic stem cells. Adult stem cells also show a homing ability to damaged tissue, allowing development of minimally invasive administration techniques.

The citations given above for adult stem cells are only a sampling. Adult stem cells already show ability to deliver therapeutic benefit to countless patients suffering from a wide array of diseases, and the greatest possible resources should be devoted to improving current adult stem cell therapies and developing the full promise of these useful cells.

APPENDIX H

DO NO HARM et al. Comments on Draft NIH Guidelines for Human Stem Cell Research, 74 Federal Register 18578-18580 (April 23, 2009)

Human Induced Pluripotent Stem Cell Research

By: David A. Prentice, Ph.D.

Induced pluripotent stem (iPS) cells provide a relatively easy method for creation of embryonic stem cells (ESC) directly from virtually any tissue source or individual. These cells were first developed in 2006 in mice by the Japanese scientist Shinya Yamanka.1 Several groups have now verified the ability to produce embryonic-like iPS cells from mice.2 In November 2007, Yamanaka's lab and the lab of Thomson in the U.S. showed that this same technique could work for human cells as well, easily producing human iPS cells directly from human tissue.3 The straightforward technique involves "reprogramming" the genetic expression of a cell, similar to reprogramming a computer to run a different program. The technique essentially reverses the developmental clock of the cell, inducing it to behave as if it was an ESC. The original Yamanaka reprogramming technique involved adding four genes directly to a human cell such as a fibroblast (e.g., skin) cell, with the

genes added using a viral vector. While there was initial concern over possible cancers because at least one of the genes used (c-Myc, which is an oncogene) and because the original viral vector (retroviruses) have cancer-causing potential, subsequent work has shown that reprogramming can proceed without the need for c-Myc,4 the number of genes necessary for reprogramming has been reduced, sometimes by combining the genetic signal with chemical compounds,5 safer viral vectors have been developed,6 as well as vectors that do not require viruses.7 Additional work has also demonstrated the ability to completely remove any residual genetic sequences that were added to reprogram the iPS cells.8 Reprogramming of iPS cells has now been accomplished completely without the use of added DNA sequences, by using added protein reprogramming factors.9

Using numerous tests, the characteristics of iPS cells have been shown to be virtually indistinguishable from ESC. For example, the telomeres of iPS cells acquire the same characteristics as those found in ESC.10 Thomson's group in their first paper showing production of human iPS cells noted:

The human iPS cells described here meet the defining criteria we originally proposed for human ES cells, with the significant exception that the iPS cells are not derived from embryos.11

Hearing of the impending announcement of the first human iPS cells, Prof. Ian Wilmut, cloner of Dolly the sheep, publicly forsook cloning technology to work on the new iPS cell technology.12 Wilmut has noted that "the technique of cloning is no longer applicable;" "The de-differentiation of somatic cells didn't require the use of human embryos as, technically speaking, it wasn't necessary. The first iPS cells were produced and identified through studies on mouse embryos;" "The iPS technique to obtain stem cells is now the most efficient technique for researchers, in particular for research on inherited diseases;" and "iPS cells are more useful than embryonic cells."13

The iPS cells from mice have already been used in proof-of-principle experiments to ameliorate disease in mouse models of sickle cell anemia,14 Parkinson's disease,15 and murine hemophilia.16

iPS cells can be created from virtually any cell type. Besides common fibroblast cells, human iPS cells have been generated from plucked human hair17 and from human blood cells.18

The iPS cells have succeeded where cloning had previously failed.19 Discussing this real advance with iPS cells in mice, the researchers noted:

This demonstrates that IPS cells have the same potential for therapy as embryonic stem cells, without the ethical and practical issues raised in creating embryonic stem cells," says Jaenisch.20

Additionally:

Townes says he and Jaenisch initially collaborated on a project that used nuclear transfer to make corrected stem cells, a process called therapeutic cloning. But the experiments failed, he says, because nuclear transfer was too inefficient to produce the needed cells. The iPS cell technique "is amazingly efficient," he says.21

Thus, iPS cells fulfill the desire to create embryonic-type stem cells, with the potential for transplant match, but do so without the use of embryos, eggs, or cloning.

Due to the ease of preparation, numerous human iPS cell lines have already been created. Within one year after announcement of the first human iPS cell lines, at least 315 human iPS cell lines had been generated, and over 500 total human iPS cell lines have been reported. In addition, iPS cell lines from patients suffering from various diseases have been created, covering 13 different diseases. See Table 1 at the end.

In summary, iPS cells provide all of the characteristics of pluripotent ESC, and also distinct advantages in terms of their ethical creation as well as ease and cost of creation, and production directly from patients.

TABLE 1. HUMAN INDUCED PLURIPOTENT STEM (iPS) CELL LINES

Publications-Human iPS Cell Lines
Detailed Lines

Total Lines
Additional Information
1
Takahashi K et al. (Yamanaka), Cell 131, 861-872
published online 20 November 2007
3
32

2
Yu J et al.(Thomson), Science 318, 1917-1920
published online 20 November 2007
8
62

3
Nakagawa M et al., Nature Biotechnology 26, 101-106
published online 30 November 2007
7
7

4
Park I-H et al., Nature 451, 141-147
published online 23 December 2007
15
15

5
Lowry WE et al., Proc. Natl. Acad. Sci. USA 105, 2883-2888
published online 16 February 2008
7
30

6
Liao J et al., Cell Research 18, 600-603
published May 2008
1
1
Paper indicates large number of colonies
7
Mali P et al., Stem Cells 26, 1998-2005
published online 29 May 2008
15
15
possibly more lines
8
Park I-H et al., Nature Protocols 3, 1180-1186
published online 26 June 2008

protocol for lines as developed in #4 above
9
Dimos JT et al., Science 321, 1218-1221
published online 31 July 2008
3
8
ALS disease-specific lines
10
Park I-H et al., Cell 134, 877-886
published online 7 August 2008
22
22
21 disease-specific lines, 10 diseases: ADA-SCID, Gaucher, Duchenne MD, Becker MD, Down's, Parkinson's, Type I Diabetes, Shwachman-Bodian-Diamond, Huntington's,

Lesch-Nyhan
11
Tateishi K et al., J. Biological Chemistry 283, 31601-31607
published online 9 Sept 2008
9
9
made insulin-secreting islet clusters
12
Maherali N et al., Cell Stem Cell 3, 340-345
11 Sept 2008
15
15
possibly more lines
13
Hockemeyer D et al., Cell Stem Cell 3, 346-353
11 Sept 2008
8
8

14
Huangfu D et al., Nature Biotechnology 26, 1269-1275
published online 12 October 2008
9
34

15
Aasen T et al., Nature Biotechnology 26, 1276-1284
published online 17 October 2008
8
31

16
Zhao Y et al., Cell Stem Cell 3, 475-479
6 November 2008
26
26
possibly more lines

>>>--One year, at least 315 lines--<<<

17
Ebert AD et al., Nature 457, 277-280
published online 21 December 2008
3
3
2 lines--Spinal Muscular Atrophy
18
Choi K-D et al., Stem Cells 27, 559-567
published online 8 January 2009
3
3
Hematopoietic and Endothelial Differentiation
19
Li W et al., Cell Stem Cell 4, 16-19
9 January 2009
4
4
possibly more lines
20
Park TS et al., Stem Cells 27, 783-795
published online 22 January 2009

used 2 lines from #5 above,
Derivation of Primordial Germ Cells
21
Zhang J et al., Circulation Research 104, e30-e41
published online 12 February 2009

used lines from #2 above,
Functional Cardiomyocytes
22
Karumbayaram S et al., Stem Cells 27, 806-811
published online 23 February 2009

used lines from #5 above,
Active Motor Neurons
23
Chambers SM et al., Nature Biotechnology 27, 275-280
published online 1 March 2009
2
2
Neural Conversion
24
Kaji K et al., Nature 458, 771-775
published online 1 March 2009
3
3

25
Woltjen K et al., Nature 458, 766-770
published online 1 March 2009
4
4

26
Zhang D et al., Cell Research 19, 429-438
published online 3 March 2009

used lines from #16 above,
pancreatic insulin-producing cells
27
Soldner F et al., Cell 136, 964-977
6 March 2009
25
25
23 Parkinson's lines
28
Loh Y-H et al., Blood xxx doi: 10.1182/blood-2009-02-204800
published online 18 March 2009
2
8

29
Yu J et al., Science 324, 797-801
published online 26 March 2009
2
12
and at least 24 subclones
30
Deng J et al., Nature Biotechnology 27, 353-360

published online 29 March 2009

used lines from #2, #4, and #12 above
31
Ball MP et al., Nature Biotechnology 27, 361-368
published online 29 March 2009
3
3

32
Hotta A et al., Nature Methods 6, 370-376
published online 26 April 2009
6
135
at least 1 Rett syndrome line

213
517

Detailed Lines
Total Lines
13 diseases

APPENDIX I
DO NO HARM et al. Comments on Draft NIH Guidelines for Human Stem Cell Research, 74 Federal Register 18578-18580 (April 23, 2009)
Human Embryonic Stem Cell Research
By: Theresa Deisher, Ph.D.

Human embryonic stem cells (hESCs) will not lead to human therapeutics and are therefore inappropriate federal funding targets for the following reasons: hESCs are not normal cells; hESCs do not differentiate into the desired adult phenotype cells; hESCs are not necessary for pluripotent stem cell research; and hESCs will not provide the over-promised cures for diseases that are per se not amenable to stem cell therapy.

While the cells of the blastocyst's inner cell mass give rise to the organism during normal embryonic development, the derivation of ESC from this inner cell mass generates cells that are not normal. The derived hESC cells exhibit epigenetic instability demonstrated by altered methylation patterns (1)1. Of great concern are studies demonstrating that this epigenetic instability is independent of hESC isolation methods or hESC culture conditions, indicating that this is a universal characteristic of hESCs (2) (3) (4). Additionally, culture of hESCs leads to well-documented genetic and chromosomal instability (5) (6) (7) (8). However, even in hESC lines that do not exhibit gross evidence of chromosomal instability using standard cytogenetics measures, neoplastic changes are readily apparent, which include high proliferative capacity and growth factor independence (7) (9). The hESC lines studied in a 2009 Nature Biotechnology publication had amplifications, deletions and mosaicism demonstrated by array comparative genomic hybridization. Indeed, genomic amplifications at 20q11 have been associated with oncogenic transformation and most likely provide a selection advantage to hESCs in culture

(8).

Scientists who want to continue to derive new hESC lines have argued that this genetic and chromosomal instability is the result of removing the cells from their normal tissue environment, the embryo. However, even when ESCs are re-injected into the natural embryonic development environment, using tetraploid embryo complementation techniques, the resulting fetuses derived solely from the implanted ESC continue to exhibit altered gene methylation and expression patterns and abnormal phenotype (1) (10). There is no evidence to suggest that hESCs will behave otherwise. In fact, merely culturing fertilized embryos in vitro has been shown to lead to epigenetic abnormalities. Epigenetic abnormalities are observed at significantly higher rates in ART (assisted reproductive technology) children than in naturally conceived children (11) (12) (13) (14).

In addition to concerns about the genetic instability of hESCs, we are also faced with the challenges of overcoming another universal characteristic of hESCs: teratoma, or tumor, formation. In fact, teratoma formation is one of the quality control assays used by commercial suppliers of hESCs lines to validate the identity of their cells as hESCs (15) (16). The ability of hESCs to form teratomas when implanted in mice is the sole quality control assay that demonstrates the pluripotency of these cells. Not only do commercial hESC suppliers rely on the teratoma forming assay to characterize their products, but academic and individual scientists routinely and commonly utilize this assay to demonstrate the pluripotency of their hESC cells. The teratoma formation has been shown to be polyclonal, further evidence that this is an innate characteristic of ESCs and not the result of an aberrant contaminating cell within the ESC culture (17) (18). The anti-apoptotic factor surviving appears to contribute to ESC teratoma formation, and is highly expressed in hESCs and teratomas, but not in the embryoid bodies from which the hESCs are derived (19). Additionally, the teratoma formation cannot be ascribed to culture conditions that include animal cells or animal growth factors, as derivation of new hESC lines in conditions lacking animal cell feeder layers or growth factors produces hESC lines that also form teratomas (20).

Science answered the question of whether ESCs would form teratomas in an organism years ago (18) (21) (22) (23), and acknowledges this insurmountable hurdle by having invested substantial resources into developing sensitive imaging techniques to monitor the formation of teratomas in vivo (24) (25) (26) (27) (28) (16) (29) (30) (18) (31) (7), and into developing methods to prevent teratoma formation, without success (32) (19) (33) (34) (35) (36). One of the attempted means to prevent in vivo teratoma formation in response to ESC treatment has been to differentiate the ESCs in vitro towards a somatic phenotype and then to implant these differentiated cells. Careful assessment of differentiated hESCs demonstrates however, that even differentiated hESCs rapidly formed teratomas (25). Published claims that differentiated ESCs show reduced teratoma formation in in vivo models need to be substantiated by documenting the continued presence of engrafted ESCs in high enough numbers and for substantial periods of time, at least 10-12 months, in order for the claim of no teratoma formation to be made with validity (16). Indeed, engraftment of differentiated ESCs has been demonstrated to be efficient and effective only in immune-compromised rodents such as the SCID mouse or athymic nude rats (37), indicating that life-long immunosuppression would be necessary in humans with its associated severe side effects that can include diabetes, hypertension, and osteoporosis.

Additionally, several therapeutic problems have been routinely observed with the approach of using differentiated ESCs for in vivo therapy. First, the ESC-derived differentiated cells exhibit immature or fetal phenotypes that are not therapeutically useful (23) (38) (39) (40) (19) (41) (35). For instance, several reports claim the derivation of insulin and C-peptide producing cells for the treatment of diabetes, but the derived cells have differentiated only to the fetal stage and do not produce therapeutic levels of insulin (42). Unfortunately, the fetal or immature phenotype cells do not further differentiate toward a fully functioning adult phenotype after being introduced into the organism (35) (37). Furthermore, the ESC-derived differentiated cells do not survive in vivo (23) (39)

(43) (37) (34) (44) (35), and have required complex cocktails of gene therapy, in vitro growth factors additives, and in vivo growth factor treatments and immune suppression (44) (36). Of even greater concern is the fact that human experience has already taught us the hard lesson that the clinical use of fetal cells or tissue leads to uncontrolled cellular growth and tumor formation (45) (46) (47) (48).

In vitro evidence of neoplastic qualities of hESC (7) (8) has been substantiated by in vivo demonstration of hESC formation of malignant tumors in SCID mice, not merely teratoma formation (49). Indeed, both teratoma formation and malignant tumor formation may be intrinsic qualities of pluripotent stem cells (50) that cannot be avoided without also losing the sought-after potency of the cells themselves (16). Again, one cannot ascribe the malignant tumor formation to the situation of removing a pluripotent stem cell from its intrinsic environment. Okita and Yamanaka have shown that chimeric mice derived partially from induced pluripotent cells have a malignant tumor incidence of 29% (50).

The discovery and publication of pluripotent stem cells equivalent to hESCs, induced pluripotent stem cells (iPSCs) and spermatogonial or testicular stem cells (SSCs) eliminates any justification to destroy a human embryo in order to derive pluripotent stem cells. However, scientists continue to argue and publish their perceived need for newly derived hESC lines. They claim that they need to continue to derive new hESC lines in order to use these as a comparator for the pluripotent properties and differentiation capacity of iPSCs or SSCs. The arguments are fallacious for the following reasons. The only, and the sufficient, assay to establish the pluripotency of a stem cell, either in the culture dish or in vivo, is the teratoma-forming assay. The test to determine whether a cell is a pluripotent cell involves injecting the cell in question into an animal, and watching for teratoma formation. While ESCs are, in some instances, the "tested" cell, at no step in this test are ESCs needed or required when other potentially pluripotent cells are being tested. The teratomas are well characterized and therefore the assays do not require ESCs at any step in the process. In regards to differentiation capacity, we have already discussed the tendency of ESCs to differentiate into immature or fetal phenotypes, rather than adult, fully functioning phenotypes. The necessary and sufficient comparators for the differentiation capacity of iPSCs or SSCs are the adult phenotype cells that are the replacement target for in vivo stem cell regenerative therapy. Derivation of new hESCs lines cannot be justified by either of these above arguments.

The targeted diseases listed in the Draft National Institutes of Health Guidelines for Human Stem Cell Research Notice include Parkinson's disease, amyotrophic lateral sclerosis, diabetes and arthritis. These are complex, polygenic diseases with an autoimmune component (51) (52) (53) (54) (55) (56) (57) (58) (59) (60) (61) (62) (63) (64) (65) (66) (67). Effective treatment of these types of diseases requires medical intervention to significantly dampen if not eradicate the autoimmune attack prior to any attempt to regenerate tissue. Stem cell therapy in the environment of autoimmune activity will not lead to long term functional recovery, as any tissue replacement will eventually suffer the same autoimmune attack and destruction. It is correct that adult stem cell treatment is being investigated, with exciting results, in the context of treating and/or curing type I diabetes, lupus and multiple sclerosis. However, the stem cell treatments utilized in these clinical trials are for the specific purpose of regenerating the blood/marrow systems following non-myeloablative chemotherapy (68) (69) (70) (71) (72). The autoimmune attack is reduced or eliminated by the ablative destruction of the mature self-reactive immune cells. Unfortunately, ablation of the self-reactive immune cells also damages normal blood and marrow cells, requiring administration of autologous stem cells for marrow rescue to prevent infectious complications and/or death from the ablative therapy. Stem cell therapy will not treat autoimmune disease until the underlying pathological organ or tissue attack is controlled, and therefore, hESCs are improbable, if not absolutely unlikely, candidates for the diseases highlighted in the proposed guidelines.

hESC research proponents also promise cures for the devastating disease of Alzheimer's, again over-promising and over-simplifying a complex, polygenic, poorly

understood disease process that is unlikely to be amenable to stem cell therapy (73). More to the point, early Alzheimer's disease appears to be driven by aberrant reactivation of fetal neural synapse pruning processes (74) (75) (76) (77), as well as being driven by an inflammatory/immune component compromising the blood-brain-barrier integrity (78). Delivery of further levels of embryonic or fetal genes and microRNAs to the brain of an Alzheimer's patient by attempting to treat them with embryonic or fetal stem cells would be the last thing one would want to do to a patient with Alzheimer's.

In conclusion, hESCs are not safe for human therapy due to their intrinsic teratoma and neoplastic properties. Nor are the necessary for research using other pluripotent cell lines. Most importantly, hESCs will not treat the myriad of diseases promised by hESC research proponents. In contrast, less pluripotent stem cells, such as those found in the mononuclear fractions of our bone marrow, are safe, affordable, and effectively treating patients in clinic and clinical trials.

REFERENCES

1. Epigenetic Instability in ES Cells and Cloned Mice. Humphreys D, Eggan K, Akutsu H, Hochedlinger K, Rideout III WM, Biniszkiewicz D, Yanagimachi R, Jaenisch R. 2001, Science, Vol. 293, pp. 95-97.
2. Status of genomic imprinting in human embryonic stem cells as revealed by a large cohort of independently derived and maintained lines. Rugg-Gunn PJ, Fergusin-Smith AC, Pedersen RA. r2, 2007, Human Molecular Genetics, Vol. 16, pp. R243-R251.
3. Characterization of human embryonic stem cell lines by the International Stem Cell Initiative. Adewumi O, Aflatoonian B, Ahrlund-Richter L, Amit M, Andrews PW, Beighton G, Bello PA, Benvenisty N, Berry LS, Bevan S. 2007, Nature Biotechnology, Vol. 25, pp. 803-816.
4. Gene-specific vulnerability to imprinting variability in human embryonic stem cell lines. Kim KP, Thurston A, Mummery C et al. 2007, Genome Res, Vol. 17, pp. 1731-1742.
5. Recurrent gain of chromosomes 17q and 12 in cultured human mebryonic stem cells. Draper JS, Smith K, Gokhale P, Moore HD, Maltby E, Johnson J, Meisner L, Zwaka TP, Thomson JA, Andrews PW 2004, Nature Biotechnology, Vol. 22, pp. 53-54.
6. Correspondence: Karyotype of human ES cells during extended culture. Buzzard JJ, Gough NM, Crook JM, Colman A, Andrews PW Nature Biotechnology.
7. Characterization of human embryonic stem cells with features of neoplastic progression. Werbowetski-Ogilvie TE, Bosse M, Stewart M, Schnerch A, Ramos-Mejia V, Rouleau A, Wynder T, Smith MJ, Dingwall S, Carter T, Williams C, Harris C, Dolling J, Wynder C, Boreham D, Bhatia M 1, Jan 2009, Nature Biotechnology, Vol. 27, pp. 91-97.
8. Human embryonic stem cells reveal recurrent genomic instability at 20q11.21. Lefort N, Feyeux M, Bas C, Feraud O, Bennaceur-Griscelli A, Tachdjian G, Peschanski M, Perrier AL. 12, Dec 2008, Nature Biotechnology, Vol. 26, pp. 1364-1366.
9. Statins inhibit the growth of variant human embryonic stem cells and cancer cells in vitro but not normal human embryonic stem cells. Gauthaman K, manasi N, Bongso A. May 2009, Br J Pharmacology. epub May 8 2009.
10. Altered imprinted gene methylation and expression in completely ES cell-derived mouse fetuses : association with aberrant phenotypes. Dean W, Bowden L, Aitchison A, Klose J, Moore T, Meneses JJ, Reik W, Feil R. 1998, Development, Vol. 125, pp. 2273-2282.
11. Association of in vitro fertilization with Beckwith-Wiedemann syndrome and epigenetic alterations of LIT1 and H19. DeBaun MR, Niemitz EL, Feinberg AP. 2003, Am J Hum Genet, Vol. 72, pp. 156-160.
12. In vitro fertilization may increase the risk of Beckwith-Wiedemann syndrome related to the abnormal imprinting of the KCN1OT gene. Gicquel C, Gaston V, Mandelbaum J, Siffroi JP, Flahault A, LeBouc Y. 2003, Vol. 72, pp. 1338-1341.
13. Beckwith-Wiedemann syndrome and IVF : a case-control study. Halliday J, Oke K, Breheny S, Algar E, Amor DJ. 2004, Am J hum Genet, Vol. 75, pp. 526-528.
14. Epigenetic risks related to assisted reproductive technologies : epigenetics, imprinting, ART and icebergs? Maher ER, Afnan M, Barratt CL. 2003, Hum Reprod, Vol. 18, pp. 2508-2511.
15.

http://www.millipore.com/publications.nsf/a73664f9f981af8c852569b9005b4eee/23abecafc823d01785257306007515d8/$FILE/SCC020.PDF.
http://www.millipore.com/techpublications/tech1/74k554. [Online] [Cited: May 19, 2009.]
http://www.millipore.com/publications.nsf/a73664f9f981af8c852569b9005b4eee/23abecafc823d01785257306007515d8/$FILE/SCC020.PDF.
16. Deconstructing Stem Cell Tumorigenicity : A Roadmap to Safe Regenerative Medicine. PS, Knoepfler. 5, Feb 12, 2009, Stem Cells, Vol. 27, pp. 1050-1056.
17. Clonal Analysis of Human Embryonic Stem Cell Differentiation into Teratomas. Blum B, Benvenisty N. 2007, Stem Cells, Vol. 25, pp. 1924-1930.
18. The tumorigenicity of human embryonic stem cells. Blum B, Benvenisty N. 2008, Adv Cancer Res, Vol. 100, pp. 133-158.
19. The anti-apoptotic gene survivin contributes to teratoma formation by human embryonic stem cells. Blum B, Bar-Nur O, Golan-Lev T, Benvenisty N. 3, 2009, Nature Biotechnology, Vol. 27, pp. 281-287.
20. Characterization of six new human embryonic stem cell lines (HSF7, -8, -9, -10, -12, and -13) derived under minimal-animal component conditions. Chavez SL, Meneses JJ, Nguyen HN, Kim SK, Pera RA. 2, 2008, Stem Cells Dev., Vol. 17, pp. 535-546.
21. Teratoma Formation Leads to Failure of Treatment for Type I Diabetes Using Embryonic Stem Cell-Derived Insulin-Producing Cells. Fujikawa T, Oh SH, Pi L, Hatch HM, Shupe T, Petersen BE. 6, 2005, American Journal of Pathology, Vol. 166, pp. 1781-1791.
22. Regeneration Gaps. Observations on Stem Cells and Cardiac Repair. Murry CE, Reinecke H, Pabon LM. 9, May 2, 2006, J Am Coll Cardiol, Vol. 47, pp. 1777-1785.
23. Differentiation, Survival, and Function of Embryonic Stem Cell Derived Endothelial Cells for Ischemic Heart Disease. Li Z, Wu JC, Sheikh AY, Kraft D, Cao F, Xie X, Patel M, Gambhir SS, Robbins RC, Cooke JP, Wu JC. 2007, Circulation, Vol. 116, pp. I-46 - I-54.
24. Noninvasive de novo imaging of human mebryonic stem cell-derived teratoma formation. Cao F, Li Z, Liu Z, Chen K, Wang H, Cai W, Chen X, Wu JC. 7, 2009, Cancer Res, Vol. 69, pp. 2709-2713.
25. Teratoma formation by human mebryonic stem cells : Evaluation of essential parameters for future safety studies. Hentze H, Soong PL, Wang ST, Phillips BW, Putti TC, Dunn NR. Feb 12, 2009, Stem Cell Res. epub ahead of print.
26. Molecular imaging of human embryonic stem cells. Narsinh KH, Cao F, Wu JC. 2009, Methods Mol Biol, Vol. 515, pp. 13-32. developed to serially image teratomas and monitor in vivo ablation if necessary.
27. Transplantation of undifferentiated murine embryonic stem cells in the heart : teratoma formation and immune response. Nussbaum J, Minami E, Laflamme MA, Virag JAI, Ware CB, Masino A, Muskheli V, Pabon L, Reinecke H, Murry CE. 2007, FASEB, Vol. 21, pp. 1345-1357.
28. Quantitative identification of teratoma tissues formed by human embryonic stem cells with TeratomEye. Oh SK, Chua P, FoonKL, Ng E, Chin A, Srinivasan R. 5, May 2009, Biotechnol Lett, Vol. 31, pp. 653-659.
29. Serial imaging of human embryonic stem-cell engraftment and teratoma formation in live mouse models. Pomper MG, Hammond H, Yu X, Ye Z, Foss CA, Lin DD, Fox JJ, Cheng L. 3, Mar 2009, Cell Res, Vol. 19, pp. 370-379.
30. Teratoma Formation by Human Embryonic Stem Cells is site-dependent and enhanced by the presence of Matrigel. Prokhorova TA, Harkness LM, Frandsen U, Ditzel N, Burns JS, Schroeder HD, Kassem M. Apr 7, 2008, Stem Cells Dev. epub ahead of print.
31. Clinical hurdles for the transplantation of cardiomyocytes derived from human mebryonic stem cells : role of molecular imaging. Swijnenburg RJ, van der Boat KE, Sheikh AY, Cao F, Wu JC. 1, Feb 2007, Curr Opin Biotechnology, Vol. 18, pp. 38-45.
32. The Production and Directed Differentiation of Human Embryonic Stem Cells. A, Trounson. 2, 2006, Endocrine Reviews, Vol. 27, pp. 208-219.
33. Embryonic Stem Cells in cardiac Repair and Regeneration. DK, Singla. Feb 9, 2009, Antioxid Redox Signal. epub - teratoma is still biggest problem
34. Embryonic Stem Cell-Derived Neuronally Committed Precursor Cells with Reduced Teratoma Formation After Transplantation into the Lesioned Adult Mouse Brain. Dihne M, Bernreuther C, Hagel C, Wesche KO, Schachner M. 2006, Stem Cells, Vol. 24, pp. 1458-1466.
35. Differentiation in vivo of Cardiac Committed Human Embryonic Stem Cells in Post-myocardial Infarcted Rats. Tomescot A, Leschik J, Bellamy V, Dubois G, Messas

E, Bruneval P, Desnos M, Hagege AA, Amit M, Itskovitz J, Menasche P, Puceat M. 2007, Stem Cells. epud May 31, 2007.
36. Cardiomyocytes derived from human embryonic stem cells in pro-survival factors enhance function of infarcted rat hearts. Laflamme MA, Chen KY, Naumova AV, Muskheli V, Fugate JA, Dupras SK, Reinecke H, Xu C, Hassanipour M, Police S, O'Sullivan C, Collins L, Chen Y, Minami E, Gill EA, Ueno S, Yuan C, Gold J, Murry CE. 2007, Nature Biotechnology.
37. Survival and maturation of human embryonic stem cell-derived cardiomyocytes in rat hearts. Dai W, Field LJ, Rubart M, Reuter S, Hale SL, Zweigerdt R, Graichen RE, Kay GL, Jyrala AJ, Colman A, Davidson BP, Pera M, Kloner RA. 4, Oct 2007, J Mol Cell Cardiol, Vol. 43, pp. 504-516. Patients would have to immune suppressed permanently.
38. Endothelial cells derived from human mebryonic stem cells. Levenberg S, Golub JS, Amit M, Itskovitz-Eldor J, Langer R. 7, 2002, Proc. Nat'l Acad Sci, Vol. 99, pp. 4391-4396.
39. In vitro and in vivo analyses of human embryonic stem cell-derived dopamine neurons. Park CH, Minn YK, Lee JY, Choi DH, Chang MY, Shim JW, Ko JY, Koh HC, Kang MJ, Kang JS, Rhie DJ, Lee YS, Son H, Moon SY, Kim KS, Lee SH. 2005, J Neurochem, Vol. 92, pp. 1265-1276.
40. Embryonic stem cells differentiate in vitro to endothelial cells through successive maturation steps. Vittet D, Prandini MH, Berthier R, Schweitzer A, Martin-Sisteron H, Uzan G, Dejana E. 1996, Blood, Vol. 88, pp. 3424-3431.
41. Embryonic Stem Cell-Derived Dendritic Cells Expressing Glypican-3, a Recently Identified Oncofetal Antigen, Induce Protective Immunity against Highly Metastatic Mouse Melanoma, B16-F10. Motomura Y, Senju S, Nakatsura T, Matsuyoshi H, Hirata S, Monji M, Komori H, Fukuma D, Baba H, Nishimura Y. 4, 2006, Cancer Res, Vol. 66, pp. 2414-2422. ESC go to fetal DC with onco antigen.
42. Differentiation of human mebryonic stem cells into insulin-producing clusters. Segev H, Fishman B, Ziskind A, Shulman M, Itskovitz-Eldor J. 2004, Stem Cells, Vol. 22, pp. 265-274.
43. Dopaminergic differentiation of human embryonic stem cells. Zeng X, Cai J, Chen J, Luo Y, You ZB, Fotter E, Wang Y, Harvey B, Miura T, Backman C, Chen GJ, Rao MS, Freed WJ. 2004, Stem Cells, Vol. 22, pp. 925-940.
44. Embryonic Stem Cell Immunogenicity Increases Upon Differentiation After Transplantation Into Ischemic Myocardium Swijnenburg R-J, Tanaka M, Vogel H, Baker J, Kofidis T, Gunawan F, Lebl DR, Caffarelli AD, de Bruin JL, Fedoseyeva EV, Robbins RC. 2005, Circ, Vol. 112, pp. 166-172.
45. Factors affecting the clinical outcome after neural transplantation in parkinson's disease. Piccini P, Pavese N, Hagell P, Reimer J, Bjorklund A, Oertel WH, Quinn NP, Brooks DJ, Lindvall O. 2005, Brain, Vol. 128, pp. 2977-2986.
46. Dyskinesia after Fetal Cell Transplantation for Parkinsonism: A PET Study. Ma Y, Feigin A, Dhawan V, Fukuda M, Shi Q, Greene P, Breeze R, Fahn S, Freed C, Eidelberg D. 2002, Ann Neurol, Vol. 52, pp. 628-634.
47. A Double-blind Controlled Trial of Bilateral Fetal Nigral Transplantation in Parkinson's Disease. Olanow CW, Goetz CG, Kordower JH, Stoessl AJ, Sossi V, Brin MF, Shannon KM, Nauert GM, Perl DP, Godbold J, Freeman TB. 2003, Ann Neurol, Vol. 54, pp. 403-414.
48. Transplantation of insulin-secreting tissues. Karl RC, Scharp DW, Ballinger WF, Lacy PE. 1977, Gut, Vol. 18, pp. 1062-1072.
49. Human embryonic stem cells are prone to generate primitive, undifferentiated tumors in engrafted human fetal tissues in severe combined immunodeficiency mice. Shih CC, Forman SJ, Chu P et. al. 2007, Stem Cells Dev, Vol. 16, pp. 893-902. hESC form malignant tumors in SCID.
50. Generation of germline-competent induced pluripotent stem cells. Okita K, Ichisaka T, Yamanaka S. 2007, Nature, Vol. 448, pp. 313-317. iPS form malignant tumors.
51. Multiple genes/multiple autoantigens role in type 1 diabetes. Abiru N, Eisenbarth GS. 1, 2000, Clin Rev Allergy Immunology, Vol. 18, pp. 27-40.
52. The tumorigenicity of human embryonic stem cells. Blum B, Benvensity N. 2008, Adv Cancer Research, Vol. 100, pp. 133-158.
53. wikipedia. http://en.wikipedia.org/wiki/Diabetes_mellitus_type_1. wikipedia. [Online] 2009. http://en.wikipedia.org/wiki/Diabetes_mellitus_type_1.
54. Type I diabetes: polygenic inheritance, multiple autoantigens and 'dual' parameter prediction. Ziegler R, Jackson RA, Ziegler AG, Eisenbarth GS. s1, 1990, J

Autoimmunity, Vol. 3, pp. 69-74.
55. Initiation of autoimmune type 1 diabetes and molecular cloning of a gene encoding for islet cell-specific 37kd autoantigen. Jun HS, Yoon JW. 1994, Adv Exp Med Biology, Vol. 347, pp. 207-220.
56. wikipedia. http://en.wikipedia.org/wiki/Rheumatoid_arthritis. wikipedia. [Online] http://en.wikipedia.org/wiki/Rheumatoid_arthritis.
57. Familial clustering of rheumatoid arthritis with other autoimmune diseases. Lin JP, Cash JM, Doyle SZ, Peden S, Kanik K, Amos CI, Bale SJ, Wilder RL. 4, 1998, Human Genetics, Vol. 103, pp. 475-482.
58. wikipedia. http://en.wikipedia.org/wiki/Osteoarthritis. wikipedia. [Online] http://en.wikipedia.org/wiki/Osteoarthritis.
59. Therapeutic Targtes for Osteoarthritis. S, Edmonds. 2009, Maturitas. epub ahead of print.
60. The contribution of genes to osteoarthritis. Valdes AM, Spector TD. 1, 2009, Med Clin North America, Vol. 93, pp. 45-66.
61. Molecular genetics of Alzheimer's disease and aging. Cacabelos R, Fernandez-Novoa L, Lombardi V, Kubota Y, Takeda M sA, 2005, Methods Find Exp Clin Pharmacology, Vol. 27, pp. 1-573.
62. Micro-RNA speciation in fetal, adult and Alzheimer's disease hippocampus. WJ, Lukiw. 3, 2007, Neuroreport, Vol. 18, pp. 297-300.
63. Synaptic retrogenesis and amyloid-beta in Alzheimer's disease. Wasling P, Daborg J, Riebe I, Andersson M, Portelius E, Blennow K, Hanse E, Zetterberg H. 1, 2009, J Alzheimer's Dis, Vol. 16, pp. 1-14.
64. Activity-dependent synapse modulation and the pathogenesis of Alzheimer disease. PG, Nelson. 5, 2005, Curr Alzheimer Res, Vol. 2, pp. 497-506.
65. Synaptic remodeling during aging and in Alzheimer's disease. Masliah E, Crews L, Hansen L. s3, 2006, J Alzheimers Dis, Vol. 9, pp. 91-99.
66. wikipedia. http://en.wikipedia.org/wiki/Parkinson's_disease . wikipedia. [Online] http://en.wikipedia.org/wiki/Parkinson's_disease .
67. Neuroinflammation and peripheral immune infiltration in Parkinson's disease : an autoimmune hypothesis. Monahan AJ, Warren M, Carvey PM. 4, 2008, Cell Transplant, Vol. 17, pp. 363-372.
68. Blood-brain barrier pathology in Alzheimer's and Parkinson's disease : implications for drug therapy. Desai BS, Monahan AJ, Carvey PM, Hendey B. 3, 2007, Cell Transplant, Vol. 16, pp. 285-299.
69. An inflammatory pathomechanism for Parkinson's disease? Wersinger C, Sidhu A. 5, 2006, Curr Med Chem, Vol. 13, pp. 591-602.
70. Is Parkinson's disease an autoimmune disorder of endogenous vasoactive neuropeptides? DR, Syaines. 6, 2007, Med Hypotheses, Vol. 69, pp. 1208-1211.
71. Are multiple sclerosis and amyotrophic lateral sclerosis autoimmune disorders of endogenous vasoactive neuropeptodes? DR, Staines. 2, 2008, Med Hypotheses, Vol. 70, pp. 413-418.
72. High-Resolution Linkage Mapping for Susceptibility Genses in Human Polygenic Disease : Insulin-dependent Diabetes Mellitus and Chromosome 11q. Hyer RN, Julier C, Buckley JD, Trucco M, Rotter J, Spielman R, Barnett A, Bain S, Boitard C, Deschamps I, Todd JA, Bell JI, Lathrop GM. 1991, Am J Hum Genet, Vol. 48, pp. 243-257.
73. Does the locus on chromosome 11 implicated in susceptibility to HLA-DR4 dependent type I diabetes mellitus also affect susceptibility to rheumatoid arthritis? Pile KD, Wordsworth BP, Bell JI. 1992, Annals of the Rheumatic Diseases, Vol. 51, pp. 1250-1251.
74. Familial Aggregation of Systemic Lupus Erythematosus, Rheumatoid Arthritis, and Other Autoimmune Diseases in 1,177 Lupus Patients from the GLADEL Cohort. Alarcon-Segovia D, Alarcon-Riquelme ME, Cardiel MH, Caeiro F, Massardo L, Villa AR, Pons-Estel BA, GLADEL. 4, 2005, Arthritis & Rheumatism, Vol. 52, pp. 1138-1147.
75. S, Everts. Getting To The Root of Cancer. The Dark Side of stem cells is initiating a revolution in cancer research. Chemical & Engineering News. January 15, 2007, pp. 28-29.
76. Generation of Insulin-producing Islet-like Clusters from Human Embryonic Stem Cells. Jiang J, Au M, Lu K, Eshpeter A, Korbutt G, Fisk G, Majumdar AS. 2007, Stem Cells. epub May 7 2007.
77. G1 to S phase cell cycle transition in somatic and embryonic stem cells. Neganova I, Lako M. 1, 2008, J Anatomy, Vol. 213, pp. 30-44.
78. Differentiation and Enrichment of Expandable Chondrogenic Cells from Human

Embryonic Stem Cells in Vitro. Toh WS, Guo XM, Choo AB, Lu K, Lee EH, Cao T. May 1, 2009, J Cell Mol Med. epub - full chondrocytes and no teratomas.
79. The anti=apoptotic gene survivin contributes to teratoma formation by human embryonic stem cells. Blum B, Bar-Nur O, Golan-Lev T, Benvenisty N. 3, Mar 2009, Nature Biotechnology, Vol. 27, pp. 281-287. teratomas are polyclonal.
80. The MLLgnant consequences of reverting to an embryonic transcriptional program C, Jamieson. 2, Feb 6, 2009, Cell Stem Cell, Vol. 4, pp. 97-98.
81. Whole-blastocyst culture followed by laser drilling technology enhances the efficiency of inner cell mass isolation and embryonic stem cell derivation from good- and poor-quality mouse embryos: new insights for derivation of human embryonic stem cell lines. Cortes JL, Sanchez L, Catalina P, Cobo F, Bueno C, Martinex-Ramirez A, Barroso A, Cabrera C, Ligero G, Montes R, Rubio R, Nieto A, Menendez P. 2, Apr 2008, Stem Cells Dev, Vol. 17, pp. 255-267.
82. Ilic D, Genbacev O, Krtolica A. Derivation of hESC from intact blastocysts. 2007. p. 1A.2.
83. Methods for Derivation of Human Embryonic Stem Cells. Kim HS, Oh SK, Park YB, Ahn HJ, Sung KC, Kang MJ, Lee AL, Suh CS, Kim SH, Kim D-W, Moon SY. 2005, Stem Cells, Vol. 23, pp. 1228-1233.
84. Deconstructing Stem Cell Tumorigenicity: A Roadmap to Safe Regenerative Medicine. PS, Knoepfler. 5, Feb 2009, Stem Cells, Vol. 27, pp. 1050-1056.

APPENDIX J
DO NO HARM et al. Comments on Draft NIH Guidelines for Human Stem Cell Research, 74 Federal Register 18578-18580 (April 23, 2009)
DO NO HARM: THE COALITION OF AMERICANS FOR RESEARCH ETHICS
STATEMENT
ON HUMAN EMBRYOS AND STEM CELL RESEARCH:
AN APPEAL FOR LEGALLY AND ETHICALLY
RESPONSIBLE SCIENCE AND PUBLIC POLICY
Released July 1, 1999

Recent scientific advances in human stem cell research have brought into fresh focus the dignity and status of the human embryo. These advances have prompted a decision by the Department of Health and Human Services (HHS) and the National Institutes of Health (NIH) to fund stem cell research which is dependent upon the destruction of human embryos. Moreover, the National Bioethics Advisory Commission (NBAC) is calling for a modification of the current ban against federally funded human embryo research in order to permit direct federal funding for the destructive harvesting of stem cells from human embryos. These developments require that the legal, ethical, and scientific issues associated with this research be critically addressed and articulated. Our careful consideration of these issues leads to the conclusion that human stem cell research requiring the destruction of human embryos is objectionable on legal, ethical, and scientific grounds. Moreover, destruction of human embryonic life is unnecessary for medical progress, as alternative methods of obtaining human stem cells and of repairing and regenerating human tissue exist and continue to be developed.

Human Embryonic Stem Cell Research Violates Existing Law and Policy

In November 1998, two independent teams of U.S. scientists reported that they had succeeded in isolating and culturing stem cells obtained from human embryos and fetuses. Stem cells are the cells from which all 210 different kinds of tissue in the human body originate. Because many diseases result from the death or dysfunction of a single cell type, scientists believe that the introduction of healthy cells of this type into a patient may restore lost or compromised function. Now that human embryonic stem cells can be isolated and multiplied in the laboratory, some scientists believe that treatments for a variety of diseases-such as diabetes, heart disease, Alzheimer's, and Parkinson's-may be within reach. While we in no way dispute the fact that the ability to treat or heal suffering persons is a great good, we also recognize that not all methods of achieving a desired good are morally or legally justifiable. If this were not so, the medically accepted and legally required practices of informed consent and of seeking to do no harm to the patient could be ignored whenever some "greater good" seems achievable.

One of the great hallmarks of American law has been its solicitous protection of the lives of individuals, especially the vulnerable. Our nation's traditional protection of human life and human rights derives from an affirmation of

the essential dignity of every human being. Likewise, the international structure of human rights law-one of the great achievements of the modern world-is founded on the conviction that when the dignity of one human being is assaulted, all of us are threatened. The duty to protect human life is specifically reflected in the homicide laws of all 50 states. Furthermore, federal law and the laws of many states specifically protect vulnerable human embryos from harmful experimentation. Yet in recently publicized experiments, stem cells have been harvested from human embryos in ways which destroy the embryos.

Despite an existing congressional ban on federally-funded human embryo research, the Department of Health and Human Services (HHS) determined on January 15, 1999 that the government may fund human embryonic stem cell research. The stated rationales behind this decision are that stem cells are not embryos (which itself may be a debatable point) and that research using cells obtained by destroying human embryos can be divorced from the destruction itself. However, even NBAC denies this latter claim, as is evident by the following statement in its May 6, 1999 Draft Report on Stem Cell Research:

Whereas researchers using fetal tissue are not responsible for the death of the fetus, researchers using stem cells derived from embryos will typically be implicated in the destruction of the embryo. This is true whether or not researchers participate in the derivation of embryonic stem cells. As long as embryos are destroyed as part of the research enterprise, researchers using embryonic stem cells (and those who fund them) will be complicit in the death of embryos.

If the flawed rationales of HHS are accepted, federally-funded researchers may soon be able to experiment on stem cells obtained by destroying embryonic human beings, so long as the act of destruction does not itself receive federal funds. However, the very language of the existing ban prohibits the use of federal funds to support "research in which a human embryo or embryos are destroyed, discarded, or knowingly subjected to risk of injury or death...." (Sec. 511(a)(2)). Obviously, Congress' intent here was not merely to prohibit the use of federal funds for embryo destruction, but to prohibit the use of such funds for research dependent in any way upon such destruction. Therefore, the opinion of HHS that human embryonic stem cell research may receive federal funding clearly violates both the language of and intention behind the existing law. Congress and the courts should ensure that the law is properly interpreted and enforced to ban federal funding for research which harms, destroys, or is dependent upon the destruction of human embryos.

It is important to recognize also that research involving human embryos outside the womb-such as embryos produced in the laboratory by in vitro fertilization (IVF) or cloning-has never received federal funding. Initially, this was because a federal regulation of 1975 prevented government funding of IVF experiments unless such experiments were deemed acceptable by an Ethics Advisory Board. Following the failure of the first advisory board to reach a consensus on the matter, no administration chose to appoint a new board. After this regulation was rescinded by Congress in 1993, the Human Embryo Research Panel recommended to the National Institutes of Health (NIH) that certain kinds of harmful nontherapeutic experiments using human embryos receive federal funding. However, these recommendations were rejected in part by President Clinton and then rejected in their entirety by Congress.

Further, it is instructive to note that the existing law which permits researchers to use fetal tissue obtained from elective abortions requires that the abortions are performed for reasons which are entirely unrelated to the research objectives. This law thus prohibits HHS from promoting the destruction of human life in the name of medical progress, yet medical progress is precisely the motivation and justification offered for the destruction of human life that occurs when stem cells are obtained from human embryos.

Current law against funding research in which human embryos are harmed and destroyed reflects well-established national and international legal and ethical norms against the misuse of any human being for research purposes. Since 1975, those norms have been applied to unborn children at every stage of development in the womb, and since 1995 they have been applied to the human embryo outside the womb as well. The existing law on human embryonic research is a reflection of universally accepted principles governing experiments on human subjects-principles reflected in the Nuremberg Code, the World Medical Association's Declaration of

Helsinki, the United Nations Declaration of Human Rights, and many other statements. Accordingly, members of the human species who cannot give informed consent for research should not be the subjects of an experiment unless they personally may benefit from it or the experiment carries no significant risk of harming them. Only by upholding such research principles do we prevent treating people as things-as mere means to obtaining knowledge or benefits for others.

It may strike some as surprising that legal protection of embryonic human beings can co-exist with the U.S. Supreme Court's 1973 legalization of abortion. However, the Supreme Court has never prevented the government from protecting prenatal life outside the abortion context, and public sentiment also seems even more opposed to government funding of embryo experimentation than to the funding of abortion. The laws of a number of states-including Louisiana, Maine, Massachusetts, Michigan, Minnesota, Pennsylvania, Rhode Island, and Utah-specifically protect embryonic human beings outside the womb. Most of these provisions prohibit experiments on embryos outside the womb. We believe that the above legally acknowledged protections against assaults on human dignity must be extended to all human beings-irrespective of gender, race, religion, health, disability, or age. Consequently, the human embryo must not be subject to willful destruction even if the stated motivation is to help others. Therefore, on existing legal grounds alone, research using stem cells derived from the destruction of early human embryos is proscribed.

Human Embryonic Stem Cell Research Is Unethical

The HHS decision and the recommendations of NBAC to federally fund research involving the destruction of human embryos would be profoundly disturbing even if this research could result in great scientific and medical gain. The prospect of government-sponsored experiments to manipulate and destroy human embryos should make us all lie awake at night. That some individuals would be destroyed in the name of medical science constitutes a threat to us all. Recent statements such as "stem cell research is too promising to be slowed, impeded, or stopped" underscore the sort of utopianism and hubris that could blind us to the truth of what we are doing and the harm we could cause to ourselves and others. Human embryos are not mere biological tissues or clusters of cells; they are the tiniest of human beings. Thus, we have a moral responsibility not to deliberately harm them.

An international scientific consensus now recognizes that human embryos are biologically human beings beginning at fertilization, and acknowledges the physical continuity Of human growth and development from the one-cell stage forward. In the 1970s and 1980s, some frog and mouse embryologists referred to the human embryo in its first week or two of development as a "pre embryo," claiming that it deserved less respect than embryos in later stages of development. However, some embryology textbooks now openly refer to the term "pre-embryo" as a scientifically invalid and "inaccurate" term which has been "discarded" and others which once used the term have quietly dropped it from new editions. Both the Human Embryo Research Panel and the National Bioethics Advisory Commission have also rejected the term, describing the human embryo from its earliest stages as a living organism and a "developing form of human life." The claim that an early human embryo becomes a human being only after 14 days or implantation in the womb is therefore a scientific myth. Finally, the historic and well-respected 1995 Ramsey Colloquium statement on embryo research acknowledges that:

The [embryo] is human; it will not articulate itself into some other kind of animal. Any being that is human is a human being. If it is objected that, at five days or fifteen days, the embryo does not look like a human being, it must be pointed out that this is precisely what a human being looks like-and what each of us looked like-at five or fifteen days of development.

Therefore, the term "pre-embryo," and all that it implies, is scientifically invalid.

The last century and a half has been marred by numerous atrocities against vulnerable human beings in the name of progress and medical benefit. In the 19th century, vulnerable human beings were bought and sold in the town square as slaves and bred as though they were animals. In this century, the vulnerable were executed mercilessly and subjected to demeaning experimentation at Dachau and Auschwitz. At mid-century, the vulnerable were subjects of our own government's radiation experiments without their knowledge or consent. Likewise, vulnerable African-Americans in Tuskegee, Alabama were victimized as subjects of a

government-sponsored research project to study the effects of syphilis. Currently, we are witness to the gross abuse of mental patients used as subjects in purely experimental research. These experiments were and are driven by a crass utilitarian ethos which results in the creation of a "sub-class" of human beings, allowing the rights of the few to be sacrificed for the sake of potential benefit to the many. These unspeakably cruel and inherently wrong acts against human beings have resulted in the enactment of laws and policies which require the protection of human rights and liberties, including the right to be protected from the tyranny of the quest for scientific progress. The painful lessons of the past should have taught us that human beings must not be conscripted for research without their permission-no matter what the alleged justification-especially when that research means the forfeiture of their health or lives. Even if an individual's death is believed to be otherwise imminent, we still do not have a license to engage in lethal experimentation-just as we may not experiment on death row prisoners or harvest their organs without their consent..

We are aware that a number of Nobel scientists endorse human embryonic stem cell research on the basis that it may offer a great good to those who are suffering. While we acknowledge that the desire to heal people is certainly a laudable goal and understand that many have invested their lives in realizing this goal, we also recognize that we are simply not free to pursue good ends via unethical means. Of all human beings, embryos are the most defenseless against abuse. A policy promoting the use and destruction of human embryos would repeat the failures of the past. The intentional destruction of some human beings for the alleged good of other human beings is wrong. Therefore, on ethical grounds alone, research using stem cells obtained by destroying human embryos is ethically proscribed.

Human Embryonic Stem Cell Research is Scientifically Questionable

Integral to the decision to use federal funds for research on human embryonic stem cells is the distinction between stem cells and embryos. HHS has stated that federal funds may be used to support human embryonic stem cell research because stem cells are not embryos. A statement issued by the National Institutes of Health (NIH) regarding this decision asserts that "The congressional prohibition on the use of [government] funds for . . . embryciresearch does not apply to research utilizing human pluripotent stem cells because such cells are not an embryo as defined by statute. Moreover, because pluripotent stem cells do not have the capacity to develop into a human being, they cannot be considered human embryos consistent with the commonly accepted or scientific understanding of that term."

It is important to note that the materials used in an experiment, as well as the methods of experimentation, are considered to be part of scientific research. When a scientific study is published, the first part of the article details the methods and materials used to conduct the research. Ethical and scientific evaluation of an experiment takes into account both the methods and materials used in the research process. Therefore, the source of stem cells obtained for research is both a scientifically and ethically relevant consideration.

Research on human embryonic stem cells is objectionable due to the fact that such research necessitates the prior destruction of human embryos; however, the HHS's claim that stem cells are not, and cannot develop into, embryos may itself be subject to dispute. Some evidence suggests that stem cells cultured in the laboratory may have a tendency to recongregate and form an aggregate of cells capable of beginning to develop as an embryo. In 1993, Canadian scientists reported that they successfully produced a live-born mouse from a cluster of mouse stem cells. While it is true that these stem cells had to be wrapped in placenta-like cells in order to implant in a female mouse, it seems that at least some doubt has been cast on the claim that a cluster of stem cells is not embryonic in nature. If embryonic stem cells do indeed possess the ability to form or develop as a human embryo (without any process of activation which affects the transformation of the cell into a human embryo), research on such stem cells could itself involve the creation and/or destruction of human life and would thereby certainly fall under the existing ban on federally-funded embryo research. It would be irresponsible for the HHS to conduct and condone human embryonic stem cell research without first discerning the status of these cells. Their use in any research in which they could be converted into human embryos should likewise be banned.

Methods of Repairing and Regenerating Human Tissue Exist

Which Do Not Require the Destruction of Human Embryos

While proponents of human embryonic stem cell research lobby aggressively for government funding of research requiring the destruction of human embryos, alternative methods for repairing and regenerating human tissue render such an approach unnecessary for medical progress.

For instance, a promising source of more mature stem cells for the treatment of disease is hematopoietic (blood cell-producing) stem cells from bone marrow or even from the placenta or umbilical cord blood in live births. These cells are already widely used in cancer treatment and in research on treating leukemia and other diseases. Recent experiments have indicated that their versatility is even greater than once thought. For example, given the right environment, bone marrow cells can be used to regenerate muscle tissue, opening up a whole new avenue of potential therapies for muscular dystrophies. In April 1999, new advances were announced in isolating mesenchymal cells from bone marrow and directing them to form fat, cartilage, and bone tissue. Experts in stem cell research believe that these cells may allow for tissue replacement in patients suffering from cancer, osteoporosis, dental disease, or injury.

An enormously promising new source of more mature stem cells is fetal bone marrow, is many times more effective than adult bone marrow and umbilical cord blood. It appears that fetal bone marrow cells do not provoke immune reactions to the same degree as adult or even newborn infant cells. This is true whether the unborn child is the donor or the recipient-that is, fetal cells can be used to treat adults, or adult bone marrow cells can be used to treat a child in the womb without the usual risk of harmful immune reactions. Such cells would not need to be derived from fetuses who were intentionally aborted, but could instead be obtained from spontaneously aborted fetuses or stillborn infants.

In 1999, unprecedented advances were also made in isolating and culturing neural stem cells from living human nerve tissue and even from adult cadavers. Such advances render it quite possible that treatment of neural diseases such as Parkinson's and Alzheimer's, as well as spinal cord injuries, will not depend upon destructive embryo research.

Earlier claims that embryonic stem cells are uniquely capable of "self-renewal" and indefinite growth can also now be seen as premature. For example, scientists have isolated an enzyme, telomerase, which may allow human tissues to grow almost indefinitely. Although this enzyme has been linked to the development of cancer, researchers have been able to use it in a controlled way to "immortalize" useful tissue without producing cancerous growths or other harmful side effects. Thus, cultures of non-embryonic stem cells may be induced to grow and develop almost indefinitely for clinical use.

One of the most exciting new advances in stem cell research is the January 1999 announcement that Canadian and Italian researchers succeeded in producing new blood cells from neural stem cells taken from an adult mouse. Until recently, it was believed that adult stem cells were capable of producing only a particular type of cell: for example, a neural stem cell could develop only into cells belonging to the nervous system. Researchers believed that only embryonic stem cells retained the capacity to form all kinds of tissue in the human body. However, if stem cells taken from adult patients can produce cells and tissues capable of functioning within entirely different systems, new brain tissue needed to treat a patient with Parkinson's disease, for example, might be generated from blood stem cells derived from the patient's bone marrow. Conversely, neural stem cells might be used to produce needed blood and bone marrow. Use of a patient's own stem cells would circumvent one of the major obstacles posed by the use of embryonic stem cells-namely, the danger that tissue taken from another individual would be rejected when transplanted into a patient. Thus, in commenting on this finding, the British Medical Journal remarked on January 30, 1999 that the use of embryonic stem cells "may soon be eclipsed by the more readily available and less controversial adult stem cells." Given that the function of the adult stem cells was converted without the cells first having to pass through an embryonic stage, the use of such cells would not be subject to the ethical and legal objections raised by the use of human embryonic stem cells. The Director of the NIH has pointed out that evidence that adult stem cells can take on different functions has emerged only from studies on mice. However, his own claim that human embryonic stem cell research can produce treatments for diabetes and other diseases is also based solely on experimental

success in mice.

One approach to tissue regeneration that does not rely on stem cells at all, but on somatic cell gene therapy, is already in use as an experimental treatment. A gene that controls production of growth factors can be injected directly into a patient's own cells, with the result that new blood vessels will develop. In early trials, this type of therapy saved the legs of patients who would have otherwise undergone amputation. It was reported in January 1999 that the technique has generated new blood vessels in the human heart and improved the condition of 19 out of 20 patients with blocked cardiac blood vessels. Such growth factors are now being explored as a means for growing new organs and tissues of many kinds.

The above recent advances suggest that it is not even necessary to obtain stem cells by destroying human embryos in order to treat disease. A growing number of researchers believe that adult stem cells may soon be used to develop treatments for afflictions such as cancer, immune disorders, orthopedic injuries, congestive heart failure, and degenerative diseases. Such researchers are working to further research on adult, rather than embryonic, stem cells. In light of these promising new scientific advances, we urge Congress to provide federal funding for the development of methods to repair and regenerate human tissue which do not require the destruction of embryonic human life. However, even if such methods do not prove to be as valuable in treating disease as are human embryonic stem cells, use of the latter in the name of medical progress is still neither legally nor ethically justifiable for the reasons stated in this document.

Conclusion

We believe that an examination of the legal, ethical, and scientific issues associated with human embryonic stem cell research leads to the conclusion that the use of federal funds to support any such research that necessitates the destruction of human embryos is, and should remain, prohibited by law. Therefore, we call on Congress to (1) maintain the existing ban against harmful federally-funded human embryo research and make explicit its application to stem cell research requiring the destruction of human embryos and (2) provide federal funding for the development of alternative treatments which do not require the destruction of human embryonic life. If anything is to be gained from the cruel atrocities committed against human beings in the last century and a half, it is the lesson that the utilitarian devaluation of one group of human beings for the alleged benefit of others is a price we simply cannot afford to pay.

For more information visit http://www.stemcellresearch.org

A referenced version is available upon request. If desired, contact The Center for Bioethics and Human Dignity at 847-317-8180.

100646741_6.DOC

1 The current funding ban is found in the Consolidated Security, Disaster Assistance, and Continuing Appropriations Act of 2009, Pub. L. No. 110-329, Division A (2008) (incorporating by reference, and continuing the effectiveness of, Consolidated Appropriations Act of 2008, Pub. L. No. 110-161, § 509, 121 Stat. 1844 (2007)) (hereinafter "Dickey-Wicker" or "Federal Funding Ban"). It provides that "none of the funds made available in this Act may be used for (1) the creation of a human embryo or embryos for research purposes; or (2) research in which a human embryo or embryos are destroyed, discarded, or knowingly subjected to risk of injury or death greater than that allowed for research on fetuses in utero under 45 C.F.R. 46.204(b) and section 498(b) of the Public Health Service Act (42 U.S.C. 289g(b))." Id.

2 Part II.B.7.h of the Guidelines requires that the donor(s) be issued a "statement as to whether or not information that could identify the donor(s) would be retained prior to the derivation or the use of the human embryonic stem cells." The Guidelines cite OHRP's Guidance for Investigators and Institutional Review Boards Regarding Research Involving Human Embryonic Stem Cells, Germ Cells, and Stem Cell-Derived Test Articles. This document provides that HHS-conducted or supported research involving human cell lines where donor(s) may be identified constitutes human subject research that is subject to the consent requirements in 45 C.F.R. 46. Id. at p. 3. As discussed infra, Comments 9-11, it is impossible to follow the

consent procedures when deriving human embryonic stem cells, because that process necessarily results in the destruction of the human embryo, which is a unique human individual. As a result, in order to avoid application of the consent procedures, researchers will undoubtedly strip the embryonic stem cells of all identifiers associated with the embryo. This means that it will be impossible to prove that any particular stem cell line was not derived from the destruction of a human embryo in violation of state law.

3 For a thoughtful analysis of the substituted judgment rule and the inherent conflicts of interests involved in health care decisions terminating life, see Walter Weber, Substituted Judgment Doctrine: A Critical Analysis, 1 Issues L. & Med. 131 (1985).

4 For an analysis of state law protecting human life from conception, see the accompanying Appendix B. Part I of Appendix B lists states' fetal homicide statutes that apply without regard to gestational age. Part II lists wrongful death statutes that apply without regard to the state of gestation or development. Finally, Part III identifies courts that have rejected constitutional challenges to fetal homicide statutes that apply without regard to the age of the unborn child.

5 See Appendix C, The Legal Consensus on the Beginning of Life.

6 For a legal analysis of the adoption alternative that legally and ethically should be part of any informed consent procedure involving frozen embryos in excess of clinical need, see the accompanying Appendix D, The Frozen Embryos: The Adoption Solution.

7 The Administrative Procedure Act declares it "unlawful" for "agency action, findings, and conclusions . . . to be . . . contrary to constitutional right, power, privilege, or immunity [or] in excess of statutory jurisdiction, authority, or limitations, or short of statutory right." 5 U.S.C. § 706; see also Iowa Telecomms. Servs., Inc. v. Iowa Utils. Bd., 545 F. Supp. 2d 869 (S.D. Iowa 2008) (Agencies only possess powers conferred by statute; they do not possess inherent powers); Agro Dutch Indus. Ltd. v. United States., 508 F.3d 1024 (Fed. Cir. 2007) (An agency literally has no power to act unless and until Congress confers power upon it.); Portland Gen. Elec. Co. v. Bonneville Power Admin., 501 F.3d 1009 (9th Cir. 2007) (Regardless of how serious the problem an administrative agency seeks to address, it may not exercise its authority in a manner that is inconsistent with the administrative structure that Congress enacted into law, because an administrative agency's power to regulate in the public interest must always be grounded in a valid grant of authority from Congress.); Elec. Power Supply Ass'n v. F.E.R.C., 391 F.3d 1255 (D.C. Cir. 2004) (When an agency acts in violation of an express congressional mandate, its motives are irrelevant); In re Sealed Case, 237 F.3d 657 (D.C. Cir. 2001) (Agencies are not empowered to carve out exceptions to statutory limits on their authority); Birth Hope Adoption Agency, Inc. v. Arizona Health Care Cost Containment Sys., 218 F.3d 1040 (9th Cir. 2000) (The scope of an agency's power is measured by statute and may not be expanded by agency fiat); United States v. Amdahl Corp., 786 F.2d 387 (Fed. Cir. 1986) (Administrative actions taken in violation of statutory authorization or requirement are of no effect).

8 See Christine L. Feiler, Note: Human Embryo Experimentation: Regulation and Relative Rights, 66 Fordham L. Rev. 2435, 2459-61 (1998) (emphasis added). The current funding ban, found in the Consolidated Security, Disaster Assistance, and Continuing Appropriations Act of 2009, Pub. L. No. 110-329, Division A (2008) (incorporating by reference, and continuing the effectiveness of, Consolidated Appropriations Act of 2008, Pub. L. No. 110-161, § 509, 121 Stat. 1844 (2007)), provides that "none of the funds made available in this Act may be used for (1) the creation of a human embryo or embryos for research purposes; or (2) research in which a human embryo or embryos are destroyed, discarded, or knowingly subjected to risk of injury or death greater than that allowed for research on fetuses in utero under 45 C.F.R. 46.204(b) and section 498(b) of the Public Health Service Act (42 U.S.C. 289g(b))." The standard of risk referenced in 45 C.F.R. § 46.204(b) limits in utero fetal research to that where the "risk to the fetus is caused solely by interventions or procedures that hold out the prospect of direct benefit for the woman or the fetus; or, if there is no such prospect of benefit, the risk to the fetus is not greater than minimal and the purpose of the research is the development of important biomedical knowledge which cannot be obtained by other means." The term "minimal risk" is defined at 45 C.F.R. § 46.102(i) as "mean[ing] that the probability and magnitude of harm or discomfort anticipated in the research are not

greater in and of themselves than those ordinarily encountered in daily life or during the performance of routine physical or psychological examinations or tests." The standard of risk referenced in section 498(b) of the Public Health Service Act (42 U.S.C. § 289g(b)) provides that "[i]n administering the regulations for the protection of human research subjects [at 45 C.F.R. 46] . . . the Secretary [of Health and Human Services] shall require that the risk standard . . . be the same for fetuses which are intended to be aborted and fetuses which are intended to be carried to term." See also Exhibit E, Samuel B. Casey, Legislative & Administrative History of the Federal Funding Ban on Destructive Human Embryo Research (May 26, 2009).

9 The Federal Funding Ban (see supra, fn. 1) defines an embryo as "any organism, not protected as a human subject under 45 CFR 46 as of the date of enactment of this Act, that is derived by fertilization, parthenogenesis, cloning, or any other means. . . ." This provision proceeds to require that this embryo be treated exactly like other protected human subjects, by extending to the embryo in the laboratory the protective standard already in effect for all fetuses in utero.

10 A copy of the HHS Legal Memorandum re Compliance of the President's Embryonic Stem Cell Decision with the Dickey Amendment for Fiscal Year 2002, dated January 11, 2002, is attached as Appendix F.

11 See Appendix C, The Legal Consensus on the Beginning of Life.

12 See id.

13 This use of "conception" clearly refers to fertilization, not implantation. At least seven medical dictionaries published at or near the time the General Assembly defined conception as fertilization. See Butterworth's Medical Dictionary 400 (2d ed. 1978) (conception: "1. The act of becoming pregnant. 2. The fertilization of the ovum by a spermatazoon and the beginning of the growth of the embryo."); Blakiston's Gould Medical Dictionary 305 (4th ed. 1979) (conception: "the fertilization of the ovum by the spermatazoon"); Black's Medical Dictionary 217 (33rd ed. 1981) ("Conception signifies the complex set of changes which occur in the ovum and in the body of the mother at the beginning of pregnancy. The precise moment of conception is that at which the male element, or spermatazoon, and the female element, or ovum, fuse together."); Urdang Dictionary of Current Medical Terms 91 (1981) (conception: "1. (in gynecology) the start of pregnancy when a male germ cell (sperm) fertilizes a female germ cell (ovum) in the fallopian tube."); Mosby's Medical and Nursing Dictionary 258 (1983) (conception: "1. the beginning of pregnancy, usually taken to be the instant that a spermatazoon enters an ovum, 2. the act or process of fertilization"); Taber's Cyclopedic Medical 12 Dictionary 368 (15th ed. 1984) (conception: "2. fertilization"); Melloni's Illustrated Medical Dictionary 108 (2d ed. 1985) (conception: "2. The fertilization of an ovum or the act of becoming pregnant.").

14 See also, R. Jones & K. Lopez, Human Reproductive Biology 23 (3d ed. 2006) ("The process of fertilization, or conception, involves fusion of the nucleus of a male gamete (sperm) and a female gamete (ovum) to form a new individual.") (emphasis in original); id. at 540 ("Conception[:] See Fertilization."); G. Thibodeau & K. Patton, Anatomy & Physiology 1167 (6th ed. 2007) (equating conception with fertilization).

15 See Appendix G-5 through G-7 for a more detailed discussion of adult stem cell success stories, and accompanying references.

16 See Appendix G for a thorough discussion on the benefits of adult stem cell research.

17 See Appendix H for a thorough discussion on iPSC research, with supporting authorities.

18 For a more detailed explanation of the reasons why stem cells can never be transplanted into children or adults as a safe and effective therapeutic, and citations to supporting authority, see Appendix I.

19 The NIH is no stranger to the damage to public confidence such abuses engender. In January 1997, media controversy erupted when NIH-supported geneticist and former HERP panelist Mark Hughes from Georgetown University was found to have violated the restrictions governing the use of human embryos. Hughes had included Federal equipment and personnel in lab experiments on prenatal embryo diagnosis, violating strict segregation rules designed to implement the Federal Funding Ban. NIH Director Harold Varmus severed ties with the scientist and told a

Congressional committee investigating the incident that NIH had taken "several steps to further diminish the risk of subsequent violations." See Rick Weiss, Georgetown Geneticist Admits Disobeying Test Ban on Embryos, WASH. POST, Jan. 15, 1997, at 3; Testimony of Harold E. Varmus, M.D., Director, NIH, Before the Subcommittee on Oversight and Investigations Committee on Commerce, United States House of Representatives, June 19, 1997 (Serial No. 105-26; ISBN 0-16-055330-X). At that time, Dr. Varmus testified that Dr. Hughes' pre-implantation genetic diagnostic research of human embryos using federal equipment and funds violated "[federal] appropriations laws prohibited the use of federal resources for human embryo research." Id. at 3-4; Congressional Statement at 2; and Letter from John J. Callahan, Assistant Secretary for Management and Budget, DHHS, to DHHS Institutional Officials (February 1997) (reinforcing the legal requirements of the Federal Funding Ban). If the Hughes incident, which did not even result in the deaths of any human embryos, violated the Federal Funding Ban, the Guidelines clearly do so as well.

20 See supra Comment 1.

21 See, e.g., Natalie Lester, Embryo Adoption Becoming the Rage, WASH. TIMES, Apr. 19, 2009, available at http://washingtontimes.com/news/2009/apr/19/embryo-adoption-becoming-rage/.

22 Walter Weber, Substituted Judgment Doctrine: A Critical Analysis, 1 Issues L & Med. 131, 154-54 (1985).

23 For the full Statement and other information, see DO NO HARM-THE COALITION OF AMERICANS FOR RESEARCH ETHICS, available at http://www.stemcellresearch.org/statement/statement.htm

1 It is existing federal policy to promote human embryo adoption as currently authorized by Congressional appropriations and implemented by HHS. For updated information on the federally-funded Embryo Adoption Awareness Campaign see www.embryoadoption.org/. Biological and adoptive parents interested in human embryo adoption can also obtain additional information from Nightlight Christian Adoptions (www.nightlight.org/snowflake adoption.htm), the National Embryo Donation Center (www.embryodonation. org), Embryos Alive (www.embryosalive.com) and Miracles Waiting (www.miracleswaiting.org).

1 Mr. Casey was co-counsel for plaintiffs in Nightlight Christian Adoptions et al. v. Thompson (Civil Action No. 1.01CV00502-RCL, U.S. District Court, District of Columbia, hereafter "Nightlight"), the case that ultimately dismissed without prejudice when the Bush Administration agreed to withdraw the HHS regulations issued by the Clinton Administration (65 F.R. 51976 et seq.).

2 The current funding ban is found in the CONSOLIDATED SECURITY, DISASTER ASSISTANCE, AND CONTINUING APPROPRIATIONS ACT OF 2009, Pub. L. No. 110-329, Division A (2008) (incorporating by reference, and continuing the effectiveness of, CONSOLIDATED APPROPRIATIONS ACT OF 2008, Pub. L. No. 110-161, § 509, 121 Stat. 1844 (2007)) (hereinafter "Dickey-Wicker" or "Federal Funding Ban"). For earlier legislation containing the same amendment, see e.g. BALANCED BUDGET DOWNPAYMENT ACT, Pub. L. No. 104-99, 110 Stat. 26, 34, Title I, § 128 (January 26, 1996); Omnibus Bill, Pub. L. No. 104-208 § 512 (Sept. 30, 1996); Labor/HHS/Education Appropriations Act, Pub. L. No. 105-78 § 513 (Nov. 13, 1997); Omnibus Bill, Pub. L. No. 105-277 § 511 (Oct. 21, 1998); Omnibus Bill, Pub. L. No. 106-113 § 510 (Nov. 29, 1999); Omnibus Consolidated Appropriations Act of 2001, Pub L. No. 106-554 § 510 (December 21, 2000); Labor/HHS/Education Appropriations Act; H.R. 3061/S. 1536, 107th Cong. (2001) (conference report approved by both houses on 12-20-01); Omnibus Bill. Pub. L. No. 107-116, § 510 (January 22, 2002).

3 See 65 Fed. Reg. 51796 (2000).

4 This legal history is summarized in the attached legal memorandum to NIH's Acting Director, Dr. Ruth Kirchstein, from Alex M. Azar, II, NIH's General Counsel, dated January 11, 2002, Appendix F.

5 As recently as June 14, 2004, Associated Press reports that the Bush Administration is rejecting calls by former President Reagan's family to change its policy on stem cell research. Press Secretary Scott McClellan reportedly said, "[t]he policy remains the same." He adds: "We are looking at other ways to combat disease." On June 22, 2007, President Bush issued his Executive Order 13435 reaffirming his presidential policy decision of August 9, 2001 and "expanding the approved stem cell lines in ethically responsible ways" to include "alternative sources of pluripotent stem cells" that are "derived without creating a human embryo for research purposes or destroying, discarding or subjecting to harm a human embryo

or fetus." 72 Fed. Reg. 34591. President Bush's Order sought to explore the "potential of pluripotent stem cells...without violating human dignity or demeaning human life." Id. Section 2 of the Order set forth the following ethical principles: (b) it is critical to establish moral and ethical boundaries to allow the Nation to move forward vigorously with medical research, while also maintaining the highest ethical standards and respecting human life and human dignity; (c) the destruction of nascent life for research violates the principle that no life should be used as a mere means for achieving the medical benefit of another; (d) human embryos and fetuses, as living members of the human species, are not raw materials to be exploited or commodities to be bought and sold; and (e) the Federal Government has a duty to exercise responsible stewardship of taxpayer funds, both supporting important medical research and respecting ethical and moral boundaries. On March 11, 2009, President Obama issued his Executive Order 13505 that "revoked" Executive Order 13435. 74 Fed. Reg. 10667.

6 Nightlight Christian Adoptions et al. v. Tommy G. Thompson, Civil Action No. 1.01CV00502-RCL, U.S. District Court, District of Columbia (March 8, 2001).

7 See Appendix A. See also Samuel B. Casey and Nathan A. Adams, IV, Specially Respecting the Living Human Embryo by Adhering to Standard Human Subject Experimentation Rules. YALE J. HEALTH, POL'Y, L. & ETHICS (forthcoming).

8 Although federal funding for IVF research projects was permissible, it required the approval of an Ethical Advisory Board ("EAB"). 45 C.F.R. § 46.204(d), nullified by section 121(c) of the NIH Revitalization Act of 1993, Pub. L. No. 103-43, 107 Stat. 122, June 10, 1993. HHS declined to direct an EAB to perform any funding review of a proposed IVF research project until September 1978. That board concluded that certain funding was theoretically ethical, but NIH declined to take any action on this conclusion. In early-1993, the Clinton Administration proposed, and Congress subsequently passed, legislation intended to eliminate the EAB approval prerequisite, as well as the executive moratorium on fetal tissue research. Pub. L. No. 103-43.

9 NIH, Report of the Human Embryo Research Panel, Vol. I at 49 (1994) ("HERP Report"); see also id. at xvii, 2, 8, 26-27, 47, 49, 50, 76 (recommending federal funding for human embryonic stem cell research using "spare" embryos from IVF clinics).

10 30 Weekly Comp. Pres. Doc. 2459 (December 2, 1994)

11 Department of Labor, Health and Human Services, Education, and Related Agencies Appropriations for 1996: Hearings Before a Subcomm. of the House Comm. on Appropriations, 104th Cong., 1st Sess. 139, 144 (1995); see also NIH, Background Information on the Impact of the Human Embryo Research Amendment at 2 (June 30, 1996) (NIH would have funded six out of nine applications for grants involving embryo-related research "if the NIH had been able to proceed according to the [Human Embryo Research Panel's] recommendations and the President's directive.")

12 H.R. Rep. No. 104-209, at 384 (emphasis added).

13 Id. at 385.

14 142 Cong. Rec. S429, S433 (1996) (emphasis added).

15 H.R. Rep. No. 104-209, at 213-14 (1995).

16 Id. at H7364; 142 Cong. Rec. H7339 (July 11, 1996).

17 Id. at H7339-43.

18 Id. at H7340 (emphasis added).

19 The House report language states: "The committee continues a provision to prohibit the use of funds in the Act concerning research involving human embryos. However, this language should not be construed to limit federal support for research involving human embryonic stem cells listed on an NIH registry and carried out in accordance with policy outlined by the President." H.R. REP. NO. 107-229, § 510 (2001).

20 H.R. 2059, 107th Cong. (2001) (killed in committee); S. 723, 107th Cong. (2001) (killed in committee); S. 1536, 107th Cong. § 510 (2001) (adding to the Dickey-Wicker Amendment part (c) "Federal dollars are permitted, at the discretion of the President, solely for the purpose of stem cell research, on embryos that have been created in excess of clinical need and will be discarded, and donated with the written consent of the progenitors.")

21 Office of Management and Budget, Statement of Administration Policy (October 30, 2001), available on the web at

www.whitehouse.gov/omb/legislative/sap/107-1/S1536-s.html.

22 The lawsuit dismissed in 2002 was originally commenced by a group of plaintiffs, including the Nightlight Christian Adoption Agency that (through its "Snowflakes" program, www.snowflakes.org) successfully arranges for infertile couples to adopt human embryos stored at in vitro fertilization clinics; the Christian Medical Association (www.cmdahome.org), a national association of doctors ethically opposed to the destructive human experimentation on human embryos, several couples who desire to adopt human embryos; and Dr. David Prentice, a researcher specializing in research using stem cells derived from adults without the loss of human life.

1 Krause DS et al.; "Multi-Organ, Multi-Lineage Engraftment by a Single Bone Marrow-Derived Stem Cell"; Cell 105, 369-377; 4 May 2001

2 Jiang Y et al.; "Pluripotency of mesenchymal stem cells derived from adult marrow"; Nature 418, 41-49; 4 July 2002

3 D'Ippolito G et al., "Marrow-isolated adult multilineage inducible (MIAMI) cells, a unique population of postnatal young and old human cells with extensive expansion and differentiation potential", J. Cell Science 117, 2971-2981, 15 July 2004

4 Yoon Y-s et al., "Clonally expanded novel multipotent stem cells from human bone marrow regenerate myocardium after myocardial infarction", Journal of Clinical Investigation 115, 326-338, February 2005

5 Zhao Y et al.; "A human peripheral blood monocyte-derived subset acts as pluripotent stem cells"; Proceedings of the National Academy of Sciences USA 100, 2426-2431; 4 March 2003

6 Li H et al., "Pluripotent stem cells from the adult mouse inner ear", Nature Medicine 9, 1293-1299, October 2003

7 Kögler G et al., "A new human somatic stem cell from placental cord blood with intrinsic pluripotent differentiation potential", J. Experimental Medicine 200, 123-135, 19 July 2004

8 McGuckin CP et al., Production of stem cells with embryonic characteristics from human umbilical cord blood, Cell Proliferation 38, 245-255, August 2005

9 Murrell W et al., "Multipotent stem cells from adult olfactory mucosa", Developmental Dynamics published online 21 March 2005

10 Prusa A-R, Marton E, Rosner M, et al. Oct-4-expressing cells in human amniotic fluid: a new source for stem cell research? Hum Reprod 18, 1489-1493, 2003

11 De Coppi et al., Isolation of amniotic stem cell lines with potential for therapy, Nature Biotechnology published online 7 January 2007; doi:10.1038/nbt1274

12 Miki T et al., Stem cell characteristics of amniotic epithelial cells, Stem Cells published online 4 Aug 2005; doi:10.1634/stemcells.004-0357

13 Guan K et al., Pluripotency of spermatogonial stem cells from adult mouse testis, Nature 440, 1199-1203, 27 April 2006

14 Seandel M et al., Generation of functional multipotent adult stem cells from GPR125+ germline progenitors, Nature 449, 346-350, 20 Sept 2007

15 Conrad S et al., Generation of pluripotent stem cells from adult human testis, Nature 344-349, 20 November 2008

16 Lee RH et al., Multipotent stromal cells from human marrow home to and promote repair of pancreatic islets and renal glomeruli in diabetic NOD/scid mice, PNAS 103, 17438-17443, November 14, 2006; Oh S-H et al., "Adult bone marrow-derived cells

transdifferentiating into insulin-producing cells for the treatment of type I diabetes," Laboratory Investigation published online 22 March 2004; Kodama S et al., "Islet regeneration during the reversal of autoimmune diabetes in NOD mice", Science 302, 1223-1227; 14 Nov 2003; Hess D et al., "Bone marrow-derived stem cells initiate pancreatic regeneration", Nature Biotechnology 21, 763-770; July 2003

17 Ohtaki H et al., Stem/progenitor cells from bone marrow decrease neuronal death in global ischemia by modulation of inflammatory/immune responses, Proc. Natl. Acad. Sci. USA 105-14638-14643, 23 Sept 2008; Kolb B et al., Growth factor-stimulated generation of new cortical tissue and functional recovery after stroke damage to the motor cortex of rats, J Cerebral Blood Flow Metabolism 27, 983-997, May 2007; Shyu W-C et al., "Functional recovery of stroke rats induced by granulocyte colony-stimulating factor-stimulated stem cells", Circulation 110, 1847-1854, 2004; Willing AE et al., "Mobilized peripheral blood stem cells administered intravenously produce functional recovery in stroke", Cell Transplantation 12, 449-454; 2003; Arvidsson A et al.; "Neuronal replacement from endogenous precursors in the adult brain after stroke"; Nature Medicine 8, 963-970; Sept 2002; Riess P et al.; "Transplanted neural stem cells survive, differentiate, and improve neurological motor function after experimental traumatic brain injury"; Neurosurgery 51, 1043-1052; Oct 2002

18 Ziv Y et al., Synergy between immune cells and adult neural stem/progenitor cells promotes functional recovery from spinal cord injury, Proceedings of the National Academy of Sciences USA 103, 13174-13179, August 29, 2006; Zurita M & Vaquero J, Bone marrow stromal cells can achieve cure of chronic paraplegic rats: functional and morphological outcome one year after transplantation, Neuroscience Letters 410, 51-56, July 10, 2006; Karimi-Abdolrazaee S et al., Delayed transplantation of adult neural precursor cells promotes remyelination and functional neurological recovery after spinal cord injury, J Neuroscience 26, 3377-3389, 29 March 2006; Kuh S-U et al., Functional recovery after human umbilical cord blood cells transplantation with brain-derived neurotrophic factor into the spinal cord injured rat, Acta Neurochir (Wien) 147, 985-992, 2005; Ohta M et al., Bone marrow stromal cells infused into the cerebrospinal fluid promote functional recovery of the injured rat spinal cord with reduced cavity formation, Experimental Neurology 187, 266-278, 2004; Hofstetter CP et al., "Marrow stromal cells form guiding strands in the injured spinal cord and promote recovery", Proc Natl Acad Sci USA 99, 2199-2204; 19 February 2002; Sasaki M et al., "Transplantation of an acutely isolated bone marrow fraction repairs demyelinated adult rat spinal cord axons," Glia 35, 26-34; July 2001; Ramón-Cueto A et al., "Functional recovery of paraplegic rats and motor axon regeneration in their spinal cords by olfactory ensheathing glia," Neuron 25, 425-435; February 2000

19 Murrell W et al., Olfactory mucosa is a potential source for autologous stem cell therapy for Parkinson's disease, Stem Cells 26, 2183-2192, June 2008; Weiss ML, et al., Human umbilical cord matrix stem cells: preliminary characterization and effect of transplantation in a rodent model of parkinson's disease, Stem Cells 24, 781-792, March 2006; Mari Dezawa et al., Specific induction of neuronal cells from bone marrow stromal cells and application for autologous transplantation, Journal of Clinical Investigation 113:1701-1710, 2004; Liker MA et al.; "Human neural stem cell transplantation in the MPTP-lesioned mouse"; Brain Research 971, 168-177; May 2003; Åkerud P et al.; "Persephin-overexpressing neural stem cells regulate the function of nigral dopaminergic neurons and prevent their degeneration in a model of Parkinson's disease"; Molecular and Cellular Neuroscience 21, 205-222; Nov 2002; Ourednik J et al.; "Neural stem cells display an inherent mechanism for rescuing dysfunctional neurons"; Nature Biotechnology 20, 1103-1110; Nov 2002

20 Harris JR et al., CD133 Progenitor Cells from the Bone Marrow Contribute to Retinal Pigment Epithelium Repair, Stem Cells 27, 457 -466, Feb 2009; Arnhold S et al., Transplantation of bone marrow-derived mesenchymal stem cells rescue photoreceptor cells in the dystrophic retina of the rhodopsin knockout mouse, Graefes Arch Clin Exp Ophthalmol. 245, 414-422, Mar 2007; Harris JR et al., Bone marrow-derived cells home to and regenerate retinal pigment epithelium after injury, Invest Ophthalmol Vis Sci. 47, 2108-2113, May 2006; Otani A et al., "Rescue of retinal degeneration by intravitreally injected adult bone marrow-derived

lineage-negative hematopoietic stem cells", J. Clinical Investigation 114, 765-774, September 2004; Otani A et al., "Bone marrow derived stem cells target retinal astrocytes and can promotes or inhibit retinal angiogenesis"; Nature Medicine 8, 1004-1010; Sept 2002; Tomita M et al., "Bone marrow derived stem cells can differentiate into retinal cells in injured rat retina"; Stem Cells 20, 279-283; 2002

21 Corti S et al., Wild-type bone marrow cells ameliorate the phenotype of SOD1-G93A ALS mice and contribute to CNS, heart and skeletal muscle tissues, Brain 127, 2518-2532, 2004

22 Dawn B et al., "Cardiac stem cells delivered intravascularly traverse the vessel barrier, regenerate infarcted myocardium, and improve cardiac function", Proceedings of the National Academy of Sciences USA 102, 3766-3771, 8 March 2005; Yoon Y-s et al., "Clonally expanded novel multipotent stem cells from human bone marrow regenerate myocardium after myocardial infarction", Journal of Clinical Investigation 115, 326-338, February 2005; Orlic D et al., "Mobilized bone marrow cells repair the infarcted heart, improving function and survival"; Proceedings of the National Academy of Sciences USA 98, 10344-10349, 28 August 2001

23 Gluckman E et al., Hematopoietic reconstitution in a patient with Fanconi's anemia by means of umbilical-cord blood from an HLA-identical sibling, N. Engl. J. Med. 321, 1174-1178, 1989

24 Baum CM et al., Isolation of a candidate human hematopoietic stem-cell population, Proc. Natl. Acad. Sci. USA 89, 2804-2808, April 1992

25 Stem Cells: Scientific Progress and Future Research Directions, National Institutes of Health, June 2001; Pg. 26
26 Stiff PJ et al.; "High-dose chemotherapy and autologous stem-cell transplantation for ovarian cancer: An autologous blood and marrow transplant registry report"; Ann. Intern. Med. 133, 504-515; Oct. 3, 2000

27 Hertzberg H et al.; "Recurrent disseminated retinoblastoma in a 7-year-old girl treated successfully by high-dose chemotherapy and CD34-selected autologous peripheral blood stem cell transplantation"; Bone Marrow Transplant 27(6), 653-655; March 2001

28 E.g., Seldin D et al. Successful treatment of AL amyloidosis with high-dose melphalan and autologous stem cell transplantation in patients over age 65, Blood 108, 3945-3947, Dec 2006
29 Dunkel, IJ; "High-dose chemotherapy with autologous stem cell rescue for malignant brain tumors"; Cancer Invest. 18, 492-493; 2000

30 Waldmann V et al.; "Transient complete remission of metastasized merkel cell carcinoma by high-dose polychemotherapy and autologous peripheral blood stem cell transplantation"; Br. J. Dermatol. 143, 837-839; Oct 2000

31 Geisler CH et al., Long-term progression-free survival of mantle cell lymphoma after intensive front-line immunochemotherapy with in vivo-purged stem cell rescue, Blood 112, 2687-2693, Oct 2008

32 Bhatia S et al.; "High-dose chemotherapy as initial salvage chemotherapy in patients with relapsed testicular cancer"; J. Clin. Oncol. 18, 3346-3351; Oct. 19, 2000

33 Peggs KS et al., "Clinical evidence of a graft-versus-Hodgkin's-lymphoma effect after reduced-intensity allogeneic transplantion", Lancet 365, 1934-1941, 4 June 2005; Tabata M et al.; "Peripheral blood stem cell transplantation in patients over 65 years old with malignant lymphoma--possibility of early completion of chemotherapy and improvement of performance status"; Intern Med 40, 471-474; June 2001

34 Buadi FK et al., Autologous hematopoietic stem cell transplantation for older patients with relapsed non-Hodgkin's lymphoma, Bone Marrow Transplant 37, 1017-1022, June 2006

35 Elliott MA et al., Allogeneic stem cell transplantation and donor lymphocyte infusions for chronic myelomonocytic leukemia, Bone Marrow Transplantation 37, 1003-1008, 2006

36 Eapen M et al., Outcomes of transplantation of unrelated donor umbilical cord blood and bone marrow in children with acute leukaemia: a comparison study, Lancet 369, 1947-1954, 2007
37 Damon LE et al.; "High-dose chemotherapy and hematopoietic stem cell rescue for breast cancer: experience in California"; Biol. Blood Marrow Transplant 6, 496-505; 2000
38 Barkholt L et al., Allogeneic haematopoietic stem cell transplantation for metastatic renal carcinoma in Europe, Annals of Oncology published online 28 April 2006

39 Krishnamurti L et al., Stable long-term donor engraftment following reduced-intesity hematopoietic cell transplantation for sickle cell disease, Biol. Blood Marrow Transplant 14, 1270-1278, 2008; Bernaudin F et al., Long-term results of related myeloablative stem-cell transplantation to cure sickle cell disease, Blood 110, 2749-2756, 2007
40 Bitan M et al., Fludarabine-based reduced intensity conditioning for stem cell transplantation of fanconi anemia patients from fully matched related and unrelated donors, Biol Blood Marrow Transplant. 12, 712-718, July 2006
41 Burt RK et al., Autologous non-myeloablative haemopoietic stem cell transplantation in relapsing-remitting multiple sclerosis: a phase I/II study, The Lancet Neurology 8, 244-253, March 2009
42 Burt RK et al., Nonmyeloablative hematopoietic stem cell transplantation for systemic lupus erythematosus, Journal of the American Medical Association 295, 527-535, February 1, 2006
43 Kreisel W et al., Complete remission of Crohn's disease after high-dose cyclophosphamide and autologous stem cell transplantation, Bone Marrow Transplantation 32, 337-340, 2003
44 Burt RK et al., "Induction of remission of severe and refractory rheumatoid arthritis by allogeneic mixed chimerism", Arthritis & Rheumatism 50, 2466-2470, August 2004
45 Couri CEB et al., C-peptide levels and insulin independence following autologous nonmyeloablative hematopoietic stem cell transplantation in newly diagnosed type 1 diabetes mellitus, JAMA 301, 1573-1579, 2009; Voltarelli JC et al., Autologous Nonmyeloablative Hematopoietic Stem Cell Transplantation in Newly Diagnosed Type 1 Diabetes Mellitus, Journal of the American Medical Association 297, 1568-1576, 11 April 2007
46 Grunebaum E et al., Bone marrow transplantation for severe combined immune deficiency, Journal of the American Medical Association 295, 508-518, 1 February 2006
47 Cox-Brinkman J et al., Haematopoietic cell transplantation (HCT) in combination with enzyme replacement therapy (ERT) in patients with Hurler syndrome, Bone Marrow Transplantation 38, 17-21, 2006
48 Escolar ML et al., "Transplantation of umbilical cord-blood in babies with infantile Krabbe's disease", New England Journal of Medicine 352, 2069-2081, 19 May 2005
49 Herbots L et al., improved regional function after autologous bone marrow-derived stem cell transfer in patients with acute myocardial infarction: a randomized, double-blind strain rate imaging study, Eur. Heart Journal 30, 662-670, 2009; Burt RK et al., Clinical applications of blood-derived and marrow-derived stem cells for nonmalignant diseases, JAMA 299, 925-936, Feb 2008; Joseph J et al., Safety and effectiveness of granulocyte-colony stimulating factor in mobilizing stem cells and improving cytokine profile in advanced chronic heart failure, American Journal of Cardiology 97, 681-684, 1 March 2006; Strauer BE et al., Regeneration of human infarcted heart muscle by intracoronary autologous bone marrow cell transplantation in chronic coronary artery disease, Journal of the American College of Cardiology

46, 1651-1658, 1 November 2005
50 Inatomi T et al., Midterm results on ocular surface reconstruction using cultivated autologous oral mucosal epithelial transplantation, American Journal of Ophthalmology 141, 267-275, February 2006
51 Tateishi-Yuyama E et al.; "Therapeutic angiogenesis for patients with limb ischaemia by autologous transplantation of bone-marrow cells: a pilot study and a randomised controlled trial"; Lancet 360, 427-435; 10 August 2002; Badiavas EV and Falanga V, "Treatment of chronic wounds with bone marrow-derived cells", Archives of Dermatology 139, 510-516, 2003
52 Shyu W-C et al., Granulocyte colony-stimulating factor for acute ischemic stroke: a randomized controlled trial, Canadian Medical Association Journal 174, 927-933, 28 March 2006
53 Terai S et al., Improved liver function in liver cirrhosis patients after autologous bone marrow cell fusion therapy, Stem Cells 24, 2292-2298, Oct 2006
54 Levesque MF et al., Therapeutic microinjection of autologous adult human neural stem cells and differentiated neurons for Parkinson's disease: five-year post-operative outcome, The Open Stem Cell Journal 1, 20-29, 2009
55 Geffner LF et al., Administration of autologous bone marrow stem cells into spinal cord injury patients via multiple routes is safe and improves their quality of life: Comprehensive case studies, Cell Transplantation 17, 1277-1293, 2008; Mackay-Sim A et al., Autologous olfactory ensheathing cell transplantation in human paraplegia: a 3-year clinical trial, Brain 131, 2376 - 2386, September 2008; Lima C et al., Olfactory mucosa autografts in human spinal cord injury: A pilot clinical study, Journal of Spinal Cord Medicine 29, 191-203, June 2006
56 Atala A et al., Tissue-engineered autologous bladders for patients needing cytoplasty, The Lancet 367, 1241-1246, 15 April 2006
57 Macchiarini P et al., Clinical transplantation of a tissue-engineered airway, The Lancet doi: 10.1016/S0140-6736(08)61598-6, published online 19 November 2008
1 Takahashi K and Yamanaka S, Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors, Cell 126, 663-676, 25 August 2006

2 Okita K et al., Generation of germline-competent induced pluripotent stem cells, Nature 448, 313-317, 19 July 2007; online 6 June 2007; Wernig M et al., In vitro reprogramming of fibroblasts into a pluripotent ES-cell-like state, Nature 448, 318-324, 19 July 2007; published online 6 June 2007; Maherali N et al., Directly reprogrammed fibroblasts show global epigenetic remodeling and widespread tissue contribution, Cell Stem Cell 1, 55-70, July 2007; published online 6 June 2007; Meissner A et al., Direct reprogramming of genetically unmodified fibroblasts into pluripotent stem cells, Nature Biotechnology 25, 1177-1181, October 2007; published online 27 August 2007; Blelloch R et al., Generation of induced pluripotent stem cells in the absence of drug selection, Cell Stem Cell 1, 245-247, Sept 2007

3 Takahashi K et al., Induction of pluripotent stem cells from adult human fibroblasts by defined factors, Cell 131, 861-872, 30 November 2007; published online 20 November 2007; Yu J et al., Induced pluripotent stem cell lines derived from human somatic cells, Science 318, 1917-1920, 21 December 2007, published online 20 November 2007
4 Nakagawa M et al., Generation of induced pluripotent stem cells without Myc from mouse and human fibroblasts, Nature Biotechnology 26, 101-106, January 2008, published online 30 November 2007; Wernig W et al., C-Myc is dispensable for direct reprogramming of mouse fibroblasts, Cell Stem Cell 2, 10-12, 10 January 2008, published online 28 December 2007
5 Kim JB et al., Pluripotent stem cells induced from adult neural stem cells by reprogramming with two factors, Nature 454, 646-650, 31 July 2008; published online 29 June 2008; Eminli S et al., Reprogramming of neural progenitor cells into induced pluripotent stem cells in the absence of exogenous Sox2 expression, Stem Cells 26, 2467-2474, October 2008; published online 17 July 2008; Huangfu D et al., Induction of pluripotent stem cells from primary human fibroblasts with only Oct4 and Sox2, Nature Biotechnology 26, 1269-1275, published online 12 October 2008; Shi Y et al., Induction of pluripotent stem cells from mouse embryonic fibroblasts by Oct4 and Klf4 with small-molecule compounds, Cell Stem Cell 3, 568-574, 6 November 2008
6 Stadtfeld M et al., Induced pluripotent stem cells generated without viral

integration, Science 322, 945-949, 7 November 2008; published online 25 Sept 2008; Sommer CA et al., iPS Cell Generation Using a Single Lentiviral Stem Cell Cassette, Stem Cells 27, 543-549, March 2009; Chang C-W et al., Polycistronic Lentiviral Vector For Hit and Run Reprogramming Of Adult Skin Fibroblasts To Induced Pluripotent Stem Cells, Stem Cells 27, 1042-1049, May 2009, published online 12 February 2009

7 Okita K et al., Generation of mouse induced pluripotent stem cells without viral vectors, Science 322, 949-953, 7 November 2008

8 Kaji K et al., Virus-free induction of pluripotency and subsequent excision of reprogramming factors, Nature 458, 771-775, 9 April 2009, published online 1 March 2009; Woltjen K et al., piggyBac transposition reprograms fibroblasts to induced pluripotent stem cells, Nature 458, 766-770, 9 April 2009, published online 1 March 2009; Yu J et al., Human induced pluripotent stem cells free of vector and transgene sequences, Science 324, 797-801, 8 May 2009, published online 26 March 2009; Yusa K et al., Generation of transgene-free induced pluripotent mouse stem cells by the piggyBac transposon, Nature Methods published online 31 March 2009

9 Zhou H et al., Generation of Induced Pluripotent Stem Cells Using Recombinant Proteins, Cell Stem Cell 4, 381-384, 8 May 2009, published online 23 April 2009

10 Marion RM et al., Telomeres acquire embryonic stem cell characteristics in induced pluripotent stem cells, Cell Stem Cell 4, 141-154, 6 February 2009

11 Yu J et al., Induced pluripotent stem cell lines derived from human somatic cells, Science 318, 1917-1920, 21 December 2007, published online 20 November 2007

12 Roger Highfield, Dolly creator Prof Ian Wilmut shuns cloning, The Telegraph, November 16, 2007

13 "Interview du professeur Ian WILMUT par Gènéthique", accessed at: http://www.genethique.org/tribunes_mensuelles/mai_2009.asp ; for English translation, see: http://ethicalstemcellresearch.blogspot.com/2009/05/read-this-wilmut-king-of-cloning-says.html

14 Hanna J et al., Treatment of sickle cell anemia mouse model with iPS cells generated from autologous skin, Science 318, 1920-1923, 21 December 2007

15 Wernig M et al., Neurons derived from reprogrammed fibroblasts functionally integrate into the fetal brain and improve symptoms of rats with Parkinson's disease, Proc. Natl. Acad. Sci. USA 105, 5856-5861, 15 April 2008

16 Xu D et al., Phenotypic correction of murine hemophilia A using an iPS cell-based therapy, Proc. Natl. Acad. Sci. USA 106, 808-813, 20 January 2009

17 Aasen T et al., Efficient and rapid generation of induced pluripotent stem cells from human keratinocytes, Nature Biotechnology 26, 1276-1284, November 2008; published online 17 October 2008

18 Loh Y-H et al., Generation of induced pluripotent stem cells from human blood, Blood published online 18 March 2009, doi: 10.1182/blood-2009-02-204800

19 Hanna J et al., Treatment of sickle cell anemia mouse model with iPS cells generated from autologous skin, Science 318, 1920-1923, 21 December 2007, online 6 Dec 2007

20 Reprogrammed adult cells treat sickle-cell anemia in mice, published 14:10 EST, December 06, 2007, http://physorg.com/news116172622.html

21 Gretchen Vogel, Reprogrammed Skin Cells Strut Their Stuff, ScienceNOW Daily News, 6 December 2007

1 Citations are to the references attached at the end of this summary.

??

46253_Mkochik_comment_on_NIH_Guidelines_for_Human_Stem_Cell_Research

May 26, 2009

Raynard Kingston
Acting Director
National Institute of Health
NIH Stem Cell Guidelines
MSC 7997
9000 Rockville Pike
Bethesda, MD. 20892-7997

RE: Draft NIH Guidelines for Human Stem Cell Research, 74 F.R. 18578 (April 29, 2009)

Dear Dr. Kingston:

I serve as ***** of the National Catholic Partnership on Disability. NCPD was established by the U.S. Catholic Bishops in 1982 to implement their Pastoral Statement on People with Disabilities. A central aim of NCPD's mission is "to increase the public's sensitivity toward the needs of ... [disabled] people ... and support their rightful demand for justice."

On behalf of NCPD and the 14 million disabled Catholics it represents, I urge you not to promulgate the draft NIH guidelines on human stem cell research. Rather than aiding disabled people, the guidelines will ultimately compromise their lives by advancing the proposition that human beings with disabling conditions are expendable.

The guidelines authorize NIH funded research on stem cells derived from human embryos. As an organism with both a human chromosomal structure and a principle of internal development distinct from its parents, human embryos are human beings from the moment of fertilization. The fact that many embryos never come to term says nothing about their nature but rather about their fragility and is a cause for efforts to further their lives, not an excuse to end them

To derive stem cells for research, the subject embryos must be destroyed. In other words, human beings are killed in order to further research aimed at benefiting others. Yet, reducing humans to the status of mere instruments for the welfare of others is what, from ancient times,1 we have given the name of "slavery."

The main purpose offered for these killings is that the stem cells extracted have the potential for curing disabling conditions and disease. Yet, "[t]here is not one documented successful treatment in humans of a pathology using embryonic stem cells, even though this research has been conducted for decades."2 But the fact that such killings are gratuitous, since "[r]ecent advances have obviated the need for destroying human embryos in order to obtain pluripotent stem cells[,]"3is most telling of all as it exposes the underlying assumption that such embryos are simply expendable.

That the guidelines limit funding to research on stem cells already extracted will not relieve NIH of responsibility for the destruction of embryos that such research requires. NIH would have no reason to issue the guidelines if it did not anticipate embryos destroyed in large numbers to furnish stem cells for research. By encouraging such research through making its funds available, NIH shares responsibility for promoting the destruction of the embryos that make such research possible.

Of particular concern is that the guidelines offer an incentive for destroying embryos with genetic anomalies. Many in vitro fertilization clinics practice pre-implantation genetic testing which now can screen for up to 120 different disorders. Under the guidelines, clinicians can continue counseling parents against implanting embryos with detected anomalies as poor candidates for "reproductive purposes." Between the remaining alternatives-- discarding such embryos, freezing

them indefinitely with its diminishing prospects for revival, or donating them for stem cell research, the choice for most will undoubtedly be what the guidelines expect.

The rationale offered for destroying such embryos is that research on the extracted stem cells may suggest strategies for treating disorders resulting from abnormal cell development, ironically sacrificing some with disabilities in order to treat the disabling conditions of others. In choosing donation, parents will doubtless be seeking to salvage some good from the regrettable circumstance of their offsprings' genetic conditions. Yet, that decision inescapably views the lives they agree to destroy as better off dead, an outlook the guidelines endorse by offering funds for research that requires such deaths. Replying that most parents would choose to discard these embryos anyway ignores the impact government sanction of such killings will have on social attitudes toward the worth of disabled life generally.

It is a monstrous thing for government to back research which fosters the belief that any class of human beings, and particularly those as defenseless as embryos with genetic conditions, are of value only when dead. On behalf of NCPD and the disabled Catholics it represents, I therefore urge NIH not to promulgate these guidelines.

Respectfully submitted,

*****, J.D.
*****
*****
National Catholic Partnership on Disability
415 Michigan Avenue, Suite 95
Washington, DC 20017

1 See, e.g., Aristotle, Politics, 1254a15-1254a17.

2 Letter of *****, *****, National Catholic Bioethics Center, to Raynard Kingston, Acting Director, NIH (Apr. 28, 2009).

3 Ibid.

ID	Status	Date_Stamp	Comments
46402	Redacted	5/26/2009 11:29:52 AM	May 26, 2009 Via Electronic Submission National Institutes of Health ATT'N: NIH Stem Cell Guidelines MSC 7997 9000 Rockville Pike Washington, DC 20892-7997 Re: □ "National Institutes of Health Guidelines for Human Stem Cell Research" (Draft Guidelines) 74 Fed. Reg. 18,578 (April 23, 2009) Dear Director, National Institutes of Health: On behalf of the Family Research Council (FRC), this document responds to the above-captioned public notice in which the National Institutes of Health (NIH) has requested comment on draft guidelines titled "National Institutes of Health Guidelines for Human Stem Cell Research." The draft guidelines were written to implement President Barack Obama's Executive Order 13505, issued on March 9, 2009. SUMMARY OF ISSUE Human embryonic stem (ES) cell research is legal and unrestricted by federal law (though some states have restrictions), so researchers can create and kill as many embryos as they choose for any reason. Family Research Council (FRC) submits comment in response to guidance from the National Institutes of Health (NIH) on federal funding of human ES cell research. The current debate concerns whether taxpayers should pay for research in which embryos are killed for their stem cells. This debate is not about "stem cell research". It is legal to perform research with stem cells that exist throughout various body organs, such as pancreas, liver, bone marrow, nose, and brain, and it is legal to do research on stem cells that are derived from human embryos. The only question is whether the federal government should fund human embryo research. FRC objects to funding human ES cell research for several reasons. First, such research requires the destruction of human embryonic life and is therefore unethical. Second, FRC believes that such funding violates the legal prohibition on funding research in which embryos are created, harmed, or destroyed in research, a law known as the Dickey-Wicker provision (P.L. 110-161, the Consolidated Appropriations Act, 2009), which first became law in 1996. Third, funding such research creates an incentive for researchers to create more human embryos for destruction, and the proposed NIH guidelines are guilty of creating this financial incentive even though they propose funding human ES cells from so-called "leftover" embryos.

ID	Status	Date_Stamp	Comments
46402		5/26/2009 11:29:52 AM	Finally, funding such research diverts limited federal funds away from stem cell therapies that have shown and continue to show real therapeutic benefit for patients suffering from over 70 conditions. The preoccupation with human ES cells is unfortunate given inherent biological barriers to using these cells in patients, such as tumor formation, immune rejection, and chromosomal abnormalities, among others. While such research is currently legal, FRC believes that the American public would be better served by NIH focusing funding on stem cell research showing benefit to patients experiencing a host of diseases. This is not a debate over the legality of the issue, but what is and what should be funded by the federal government. BRIEF HISTORY In 1975, the federal government recognized that human embryos in the womb are to be protected as "human subjects" in federally funded research. It is important to note that in the current debate, human embryos that researchers want to destroy for their stem cells are at the same stage of development as those embryos in the womb that are protected by federal regulations. Since 1996, the Dickey-Wicker appropriations rider has prevented federal funding for any research "in which" embryos are destroyed (P.L. 104-99). The law states that federal funds may not be used for "(1) the creation of a human embryo or embryos for research purposes; or (2) research in which a human embryo or embryos are destroyed, discarded, or knowingly subjected to risk of injury or death greater than that allowed for research on fetuses in utero" (in the womb). Since 1996, federal law has prohibited the use of federal funds to pay for research that would result in the killing of human embryos or placing them at risk, including research in which federal dollars do not pay for the direct destruction of the human embryo. In 2000, the NIH guidelines approved by the Clinton administration allowed federal funding for research on stem cells derived from human embryos, so long as the specific act of destroying the embryos was not carried out with the use of federal funds. These new rules, promulgated by then-NIH Director Harold Varmus, were based on a 1999 HHS General Counsel memo written by Harriet S. Rabb ("Rabb Memo") expressing the opinion that the use of federal tax dollars for research using such stem cells would not violate the Dickey-Wicker ban as long as federal funds did not pay for the act of killing the embryo. Though these rules were issued in 2000, President Bush prevented them from being implemented. On August 9, 2001, President Bush announced, in an address to the nation, his decision to begin federal funding of research on stem cell lines derived from human embryos who were killed prior to his announcement. This was the first time the federal government funded human ES cell research. Some commentators strongly disapproved of this policy, whereas others thought the policy was ethically defensible and that it was a political compromise that prevented implementation of the Clinton-era NIH guidelines. Both the Bush and Clinton administrations seem to have adopted the legal interpretation from the Rabb Memo that the Dickey-Wicker provision would not be violated so long as funds were not used on research that kills the human embryo, and since the ES cells are not human embryos, the ban accordingly did and does not apply. However, Bush's policy differed substantially from the Clinton rules in that, though the Clinton rules would have prevented using funds to directly destroy human embryos, they would have simultaneously created a continuing financial incentive to create and destroy embryos for research. In contrast, Bush's policy not only ensured that no federal funds would be used to directly destroy embryos, but it also restricted funds to stem cells that were derived from embryos in which the life-and-death decision had been previously made. Arguably, the Bush policy avoided generating any financial incentive to create more embryos for destructive research since no funds would be available for

ID	Status	Date_Stamp	Comments
46402		5/26/2009 11:29:52 AM	research on stem cells obtained from newly destroyed embryos after August 9, 2001. FRC believes this legal interpretation is misguided in that Dickey-Wicker prevents any funding for research "in which" human embryos are created, harmed, or destroyed. Given the current science, human embryos are destroyed when the stem cells are obtained from them. Research on human ES cells is research tied to the destruction of the human embryos from whom they came. After the August 9, 2001 Bush announcement, the NIH established a human ES cell registry that listed lines that were eligible to receive federal funding, and NIH is now funding infrastructure grants to make the ES cells available. NIH determined that there were 78 ES cell lines eligible for research funding in accordance with President Bush's policy. Since that time, NIH has worked to attract researchers to apply for grants to perform research on the eligible lines. Of the 78 eligible lines, 21 are currently receiving federal funds. The NIH reported as late as 2007 that over 3,000 additional shipments of human ES cells were available to researchers upon request. The NIH has stated that the approved ES cells reproduce indefinitely. The NIH has also stated they have been able to fulfill requests for basic research. Since President Bush's decision, federal funding has increased to over $90 million per year on human ES cells, totaling almost $480 million since 2002. Despite such funding levels, and in addition to over $1 billion in non-human ES cell research during the same period,1 ES cells have yielded no treatments for any condition. On the contrary, there continue to be breakthroughs with adult stem cell research for a variety of conditions. In fact, researchers have used non-ES cells to treat human patients for over 70 diseases2 and shown novel ways of creating embryonic-like stem cells without harming or destroying human embryos. In addition to breakthroughs in early 2007 involving amniotic stem cells, Japanese and U.S. scientists in November 2007 published studies showing the capacity to reprogram normal human body cells into human embryonic-like stem cells that are identical in character to ES cells.3 However, these pluripotent stem cells do not involve human embryos at all, nor do they involve the extraction and use of women's eggs or the controversial process of human cloning (somatic cell nuclear transfer). Researchers call these cells induced pluripotent stem cells, or iPS cells. They behave identically to human ES cells, can be created directly from patients for disease-specific cell lines, and potentially could genetically match the patients. Thus iPS cells could potentially bypass problems with immune rejection when using human ES cells in the clinical setting. On March 9, 2009 President Barack Obama issued Executive Order 13505, which overturns President Bush's previous policy of funding human ES cells. The new executive order opens federal funding for newly created human ES cell lines utilizing newly created and destroyed human embryos so long as, per the Dickey-Wicker provision, the funds are not used directly to destroy the embryos. FRC objects to the executive order because it opens the floodgates for funding more ES cell research and generates an incentive for researchers to create and destroy more human embryos. Moreover, President Obama's executive order is vastly broader than even most proponents of such research claim is needed. Specifically, President Obama's executive order does several things. First, it opens the door to funding research on stem cells taken from so-called "leftover" embryos created during the in vitro fertilization (IVF) process that were created initially for baby-making. Second, the executive order rescinded the statement of President Bush that allowed funding for research on human ES cells created prior to August 9, 2001. Third, it revoked President Bush's Executive Order 13435 of June 20, 2007, which supplanted the August 9, 2001 statement. Executive Order 13435 expanded funding of research involving alternative methods of producing pluripotent stem cells, including the possible derivation of human ES cells without harming or destroying human embryos. Moreover, this executive order also placed priority on stem cell research with the greatest potential for near-term clinical benefit. By

ID	Status	Date_Stamp	Comments
46402		5/26/2009 11:29:52 AM	revoking President Bush's executive order, President Obama eliminates any such priority for NIH. Fourth, President Obama's executive order established a new policy for federal funding of stem cell research that involves cloned human embryos, human-animal hybrid embryos, and human parthenogenetic embryos. President Obama designated NIH to draft guidelines for distributing funds for stem cell research. On April 23, NIH officially posted draft guidelines to regulate federal funding for human ES cell research. The proposed guidelines would fund research on human ES cells derived from human embryos created by the IVF process and that were created initially for the purpose of childbearing. ETHICAL PROBLEMS WITH PROPOSED GUIDELINES Proponents of federal funding of ES cell research argue that ES cells are the most promising to treat upwards of 100 million patients. Although they claim that it is unethical to create human embryos for the sole purpose of destructive research, they argue it is ethical to fund research on "leftover" human embryos that "would otherwise be discarded". They are referring here to embryos created by IVF but that have not yet been transferred to the womb for gestation to produce children. Proponents have argued that we should fund research on these "excess" IVF embryos. In 2003, Rand published a report4 showing over 400,000 frozen human embryos in storage in the United States. This report generated a renewed call for President Bush to expand his policy to incorporate these new embryos, especially since ES cell research proponents claim "they will be destroyed anyway." However, the current estimated number of 400,000 "leftover" embryos will not satisfy the demands of research, especially if federal funds are promoting ES cell research and human embryo destruction. According to the Rand report, 88% of the 400,000 frozen embryos are destined for later transfer for gestation by the parents. The percent of embryos that are designated for research is 2.8%; that is, about 11,000 frozen embryos potentially available for ES cell research. Even if all of these embryos were made available for research, the best scientific estimate of the number of stem cell lines that would be derived from these embryos would be extremely limited. Rand estimated that at most, only 275 ES cell lines might result from existing available embryos. Dr. Raynard Kington, Acting Director of NIH, has publicly claimed that the draft guidelines could potentially fund research on a total of 700 human ES cell lines. However, it is unclear how Dr. Kington determined that figure. The NIH guidelines should also require the publication of information disclosing the location of the human embryos that were used to obtain the funded ES cell lines. We reject as entirely utilitarian the argument that ES cell research is ethically legitimate given that embryos are supposedly going to be discarded and are of potential use in treating millions of patients. FRC believes that the destruction of innocent human life, including nascent human life, is unethical. FRC believes as a corollary that the federal government should not fund research that involves the destruction of human embryonic life. The NIH draft guidelines would ensure American taxpayers' complicity in what millions reasonably believe is the unethical destruction of human life. While the debate over the utility of ES cell research continues as a scientific question, it clearly continues to be debated as an ethical and public policy matter. There is simply no clear consensus showing that the majority of Americans support funding for the use of any embryos in experiments. Even the NIH guidelines acknowledge an implied concern about the moral status of the human embryo. This is evident in NIH's decision not to fund research on ES cells derived from human embryos specifically created for research, as well as

ID	Status	Date_Stamp	Comments
46402		5/26/2009 11:29:52 AM	ES cells from cloned embryos and parthenogenetic embryos. FRC believes that the NIH should not fund research that the agency itself acknowledges raises ethical concerns. The Obama administration has stated that in its deliberative process it consulted with other bioethics and scientific bodies. It would be well to note that under the Clinton administration, the National Bioethics Advisory Committee (NBAC), which recommended funding of research that destroyed human embryos for stem cells, acknowledged that the government should only fund such research if no other alternatives were available. NBAC concluded: "In our judgment, the derivation of stem cells from embryos remaining following infertility treatments is justifiable only if no less morally problematic alternatives are available for advancing the research… The claim that there are alternatives to using stem cells derived from embryos is not, at the present time, supported scientifically. We recognize, however, that this is a matter that must be revisited continually as science advances."5 Here the science can and should inform the discussion. Since 1999, advances using adult stem cells have shown positive benefit in patients for over 70 diseases and injuries. Moreover, alternative methods of obtaining "pluripotent" stem cells have been discovered. In short, the science has provided a way out of the ethical dilemma by offering "less morally problematic alternatives" that are already treating patients as well as providing ample stem cells for basic research, all without the need for human embryos. The NIH guidelines are in fact scientifically dated as well as morally problematic. TECHNICAL PROBLEMS WITH PROPOSED GUIDELINES The NIH draft guidelines also suffer from other, more specific problems. They state: "These draft Guidelines would allow funding for research using only those human ES cells that were derived from embryos created by IVF for reproductive purposes and were no longer needed for that purpose." Additionally, the NIH guidelines state that they will not fund (at present) research on human ES cells derived from embryos created by cloning, parthenogenesis or IVF embryos specifically created for research. Despite the draft guidelines' statement to that effect, NIH offers no legal basis for not funding such research given the fact that Executive Order 13505 clearly gives NIH the authority to fund such research. The draft guidelines contain no reporting requirements or benchmarks for determining at a later time whether they will proceed to fund such controversial research. The draft guidelines should contain rigorous criteria to be used to justify proceeding to fund any such research. The guidelines offer several criteria for determining which ES cell lines are eligible for funding. First, the human embryos must have been created for reproductive purposes. Second, the human embryos must no longer be "needed" for reproductive purposes. Third, the human embryos must be donated for research purposes. Fourth, additional restrictions on the facilities are outlined. The proposed criteria contain large loopholes that would lead to the creation of additional embryos in order to destroy them for their stem cells. Regarding the first criterion, NIH gives no explanation of how it will determine which embryos were created for which purpose or whether multiple purposes (reproduction and research) are permissible for funding. There is nothing in these guidelines to ensure a researcher cannot claim that human embryos were created as part of the IVF process to generate a child, when they were in fact created in excess to obtain more embryos for stem cell research. Moreover, there is nothing to prevent researchers from applying pressure on parents from the outset to ensure that additional embryos are created so the researcher can obtain "leftover" embryos for stem cell work. Second, the NIH guidelines offer no criteria or explanations for determining whether the embryos in question are "no

ID	Status	Date_Stamp	Comments
46402		5/26/2009 11:29:52 AM	longer needed" for reproduction. That many parents decide later to have more children after storing their embryos for years is not considered here, in terms of any waiting period for the decision to give embryos to research. The guidelines also ignore the options parents have to give their embryos to other infertile couples wanting to adopt their embryos. Thus, the embryos are indeed still "needed" for reproduction. That the NIH guidelines would be silent on a matter that Congress has supported in the Embryo Adoption Information Campaign is very troubling. Third, there is no requirement as to when the embryos are to be donated for research. There is no defined separation between the time a couple chooses to go through the IVF process for the purpose of reproduction and when they decide to donate their embryos for research. Parents making informed decisions about their choices should be given time to consider all the options. Unfortunately, the guidelines do not require any period of separation for such decisions to be made by the parents. Fourth, the guidelines lack requirements for documentation of several additional factors pertinent to sound public policy. The guidelines should require documentation that all the options pertaining to the use of embryos "no longer needed" are explained to the parents. It is not evident who will be required to explain the various options to the parents, and this could even, under the proposed guidelines, include the ES cell researcher. Such a dual role creates a serious conflict of interest, thereby ensuring that the parents will receive biased information. Moreover, the guidelines fail to specify what, if any, options must be offered to the parents. Would parents be told in the documentation that hundreds of infertile couples have now pursued embryo adoption successfully to have children? Additionally, documentation is purportedly required to show that no financial inducements were offered for the donation of embryos to research. The guidelines fail to specify what, if any, compensation would be permissible. Nor do they limit the source of the compensation. The ambiguous requirement could be interpreted to mean that federal funding cannot be used as compensation to the parents, but private funding could. Fifth, one of the most egregious loopholes in the guidelines is that they permit the stem cell researcher and the IVF doctor to be one and the same. These guidelines provide a financial incentive for IVF doctors to apply for federal funds for ES cell research. The guidelines say the IVF doctor and the ES cell researcher "should" be separate, but only when practicable, and do not in fact require any actual separation between the two. The guidelines allow the likely scenario where the IVF doctor creates more embryos than are needed for fertility purposes in order to generate more so-called "leftover" embryos for the doctor's own ES cell research using taxpayer funds. Fundamentally, these guidelines create a conflict-of-interest for IVF doctors who are professionally obligated to be dedicated to the creation and preservation of healthy embryos for the purpose of baby-making for the parents. In allowing the IVF doctor to receive federal funds for human ES cell research, the guidelines encourage an attending physician to bring another set of goals into the doctor-patient relationship. Clearly, if the stem cell researcher is also the IVF parent's doctor, he or she would certainly have a financial incentive to create more embryos than are needed for gestation so that he or she would have more "leftover" embryos for stem cell research using federal funds. The fact that the guidelines do not require separation between the IVF physician and the researcher deriving the ES cells from the embryos is a grave flaw. Sixth, the guidelines do not prevent funding in which human ES cells are used to create human-animal hybrids or human-human chimeras. The guidelines only prohibit research in which human ES cells are "introduced into non-human primate blastocysts," but experiments in which human ES cells are placed into other animal embryos (e.g., mouse, cow, sheep) are not prohibited. Likewise, there are no prohibitions on introduction of human ES cells into human embryos to form a human-human chimera, nor is there any prohibition on using human ES cells to form tetraploid embryos. This is grossly unethical.

ID	Status	Date_Stamp	Comments
46402		5/26/2009 11:29:52 AM	Lastly, the guidelines do not require the donor(s) of human embryos to sign an informed consent agreement while generating embryos for reproductive purposes. They could be offered separate consent forms at the same time (thereby creating the scenario where the embryos are created for both reproduction and research, which would not qualify under the guidelines). Or different consent forms could be offered by the IVF doctor and the ES cell researcher in order to elicit donation. DIVERTING FUNDS AWAY FROM REAL TREATMENTS The NIH Guidelines will divert federal funding away from promising research treating people now with adult stem cells and will divert funds away from more promising sources of embryonic-like stem cells generated without the use of any human embryos. EMBRYONIC STEM CELLS ARE UNSUITED FOR CLINICAL APPLICATIONS The NIH Guidelines define human pluripotent stem cells as "human cells that are capable of dividing without differentiating for a prolonged period in culture, and are known to develop into cells and tissues of the three primary germ layers." Proponents of federal funding for human ES cell research argue that because ES cells are pluripotent, they are the most promising to treat numerous diseases. Yet pluripotent stem cells, and particularly ES cells, are an unrealistic source for actual clinical therapies. The rapid growth of ES cells coupled with the lack of control over specific differentiation often leads to tumors in experimental animals. The bulk of the scientific evidence indicates that human ES cells are tumorigenic cells, unsuitable for the purposes outlined in the proposed guidelines, and therefore inappropriate for federal funding. Animal studies highlight the danger of ES cells in transplants. Sensitive assays show that as few as two ES cells are enough to form a tumor.6 The risk of tumor formation seen for ES cells is increased when using homologous hosts (e.g., mouse ES cells into mice,7 or potentially human ES cells into humans). Moreover, differentiation into specialized, non-growing cell types does not preclude tumor formation; ES cells appear to reverse specialization into a growing, tumor-forming state.8 ES cells tend rapidly to accumulate mutations, increasing the chances of tumor formation.9 A recent study notes that many IVF embryos, the targets of these guidelines, have chromosomal abnormalities,10 increasing the likelihood that the result of implementing these guidelines will be even more abnormal ES cells. Indeed, studies note that ES cells have more in common with cancer cells than with normal cells.11 A recent cautionary report showing tumor formation caused by fetal stem cells in a young boy12 emphasizes the fact that young, pluripotent stem cells are clinically unsuitable. ES cells also face significant hurdles related to transplant rejection. The cells actually seem to increase their immunogenicity upon differentiation, making them more susceptible to transplant rejection and inflammatory responses.13 INDUCED PLURIPOTENT STEM CELLS (iPS CELLS) Induced pluripotent stem (iPS) cells provide a relatively easy method for creation of ES cells directly from virtually any tissue source or individual. These cells were first developed in 2006 in mice by the Japanese scientist Shinya Yamanaka.14 In November 2007, Yamanaka's lab and the lab of Thomson in the U.S. showed that this same technique could work for human cells as well, easily producing human iPS cells directly from human tissue.15 The straightforward technique involves "reprogramming" the genetic expression of a cell, altering the gene expression of a normal body cell by adding several master genes, and inducing the cell to behave as if it were an ES cell. The original Yamanaka reprogramming technique involved adding four genes directly to a human cell such as a skin fibroblast cell, with the genes

ID	Status	Date_Stamp	Comments
46402		5/26/2009 11:29:52 AM	added using a viral vector. The technique has advanced rapidly in less than three years, and reprogramming of iPS cells has now been accomplished completely without the use of added DNA sequences, by using added protein reprogramming factors.16 The behavior of iPS cells appears virtually indistinguishable from ES cells. Thomson's group in their seminal paper producing human iPS cells noted: "The human iPS cells described here meet the defining criteria we originally proposed for human ES cells, with the significant exception that the iPS cells are not derived from embryos."17 Thomson has also pointed out the ethical advantage of iPS cells: "These cells possess the therapeutically desired characteristics of ES cells, namely indefinite self-renewal and pluripotency, without the requirement of human embryo destruction."18 Prof. Ian Wilmut, cloner of Dolly the sheep, has noted that "the technique of cloning is no longer applicable," "The de-differentiation of somatic cells didn't require the use of human embryos as, technically speaking, it wasn't necessary. The first iPS cells were produced and identified through studies on mouse embryos," and "The iPS technique to obtain stem cells is now the most efficient technique for researchers, in particular for research on inherited diseases," and "iPS cells are more useful than embryonic cells."19 Thus, iPS cells fulfill the desire to create ES cells, with the added advantage of easy and cheap creation directly from a patient, and the potential for transplant match, but do all of this without the use of embryos, eggs, or cloning. Within one year after announcement of the first human iPS cells, at least 315 human iPS cell lines had been generated, and over 500 total human iPS cell lines have been reported. In addition, iPS cell lines from patients suffering from various diseases have been created, covering 13 different diseases.20 In summary, iPS cells provide all of the desired characteristics of pluripotent ES cells, and also distinct advantages in terms of their ethical creation as well as ease and cost of creation, and production directly from patients. ADULT STEM CELLS Adult stem cells provide a readily available and flexible source of stem cells for the treatment of disease. Only adult stem cells have shown any real successes in therapeutic applications. A wealth of published scientific papers document that adult stem cells are a much more promising source of stem cells for regenerative medicine. Some adult stem cells actually do show pluripotent flexibility in generation of tissues, meaning that they can generate most or all of the different tissues of the body; such sources include bone marrow,21 peripheral blood,22 umbilical cord blood,23 nasal mucosa,24 amniotic fluid,25 and testicular tissue.26 The real success for adult stem cells, however, is their ability to repair and replace damaged tissue, i.e., actually accomplish regenerative medicine. Pre-clinical results provide voluminous evidence that adult stem cells are effective in treating animal models of disease. More importantly, adult stem cells are already being used clinically to treat dozens of diseases in human patients, relieving suffering and saving lives. Early successes and many of the continuing results use adult stem cells, most often from bone marrow or umbilical cord blood, in conjunction with chemotherapy or radiation, in treatments for various cancers, including ovarian cancer, retinoblastoma, brain tumors, testicular cancer,27 various lymphomas including Hodgkin's lymphoma28 and Non-Hodgkin's lymphoma,29 chronic30 and acute31 leukemias, breast

ID	Status	Date_Stamp	Comments
46402		5/26/2009 11:29:52 AM	cancer,32 renal cell carcinoma,33 and numerous other cancers. Similar methodology has utilized adult stem cells in treatments for various anemias, including sickle cell anemia34 and Fanconi's anemia35. This technique has also been used successfully to treat patients with various autoimmune diseases, including multiple sclerosis,36 systemic lupus,37 Crohn's disease,38 and juvenile (Type I) diabetes.39 Various immunodeficiencies including SCID have been treated successfully as well.40 Adult stem cells have also shown success in ameliorating the effects of various genetic metabolic disorders such as Hurler's syndrome,41 Krabbe's leukodystrophy,42 and others. These life-saving treatments continue to improve and to increase, but need increased support with further federally funded clinical trials. Published patient results have also shown the usefulness of adult stem cells for repair of acute and chronic cardiac damage,43 growing new corneas to restore sight to blind patients,44 treatment of limb ischemia and wounds,45 successful amelioration of the effects of stroke,46 and treating liver disease.47 An early clinical trial has shown effectiveness of the patient's own adult stem cells at treating Parkinson's disease,48 and several reports now document clinical improvement using adult stem cells for treatment of spinal cord injury.49 Adult stem cells have also already shown their utility in tissue-engineering applications to treat patients, including growth of functional bladders50 and a published case of a new windpipe.51 Adult stem cells have distinct advantages over other stem cell types. In most cases the patient's own stem cells can be used for the treatment, circumventing problems of immune rejection. Adult stem cells do not have the problem of tumor formation that is associated with embryonic stem cells. Adult stem cells also show a homing ability to damaged tissue, allowing development of minimally invasive administration techniques. The citations given above for adult stem cells are only a sampling (for a representative list of references, please see: http://www.sciencemag.org/cgi/data/315/5810/328b/DC1/1 and http://stemcellresearch.org/facts/asc-refs.pdf). Adult stem cells already show the ability to deliver therapeutic benefit to patients, and resources should be devoted to improving current adult stem cell therapies and developing the full promise of these useful cells. CONCLUSION 1. ES cell research is legal and unrestricted. However, just as U.S. taxpayers should not have to pay for abortions, they should not have to pay for destructive research on embryos. 2. Furthermore, ES cell research should not be funded when there are ethical alternatives such as adult stem cells and iPS cells. In 1999, even President Clinton's National Bioethics Advisory Commission (NBAC) acknowledged broad agreement in our society that early human embryos "deserve respect as a form of human life" (NBAC, Ethical Issues in Human Stem Cell Research, 1999, p. ii). The Commission actually concluded that research requiring the destruction of these human lives should be seen as a last resort, saying: "In our judgment, the derivation of stem cells from embryos remaining following infertility treatments is justifiable only if no less morally problematic alternatives are available for advancing the research." (Id., p. 53). The Commission recommended funding ES cell research because it thought at that time that no alternatives existed; but it said this factual judgment "must be revisited continually as science advances." 3. There now exist several alternatives to ES cells. The iPS cell reprogramming technique produces cells that are indistinguishable from ES cells without the use of embryos, eggs, or cloning, and with the advantage that this technique is easier and cheaper and produces cells directly from a patient. 4. The successes of adult stem cells in improving health and saving lives are now well documented. Studies over the past decade show that adult stem cells can effectively and ethically deliver therapeutic benefit to patients. If the federal government considers the patients first, stem cell research funding would be directed primarily to adult stem cells. Thank you for your consideration of these comments.

ID	Status	Date_Stamp	Comments
46402		5/26/2009 11:29:52 AM	Respectfully submitted on behalf of the Family Research Council, ***** ***** ***** ***** ***** ***** ***** ***** ***** 1 From NIH "Estimates of Funding for Various Research, Condition, and Disease Categories (RCDC)", Table Updated January 15, 2009 accessed 25 May 2009 at http://report.nih.gov/rcdc/categories/PFSummaryTable.aspx 2 See references at http://www.sciencemag.org/cgi/data/315/5810/328b/DC1/1 and http://stemcellresearch.org/facts/asc-refs.pdf 3 Takahashi K et al., Induction of pluripotent stem cells from adult human fibroblasts by defined factors, Cell 131, 861-872, 30 November 2007; published online 20 November 2007; Yu J et al., Induced pluripotent stem cell lines derived from human somatic cells, Science 318, 1917-1920, 21 Decmber 2007, published online 20 November 2007 4 Hoffman DI et al., Cryopreserved embryos in the United States and their availability for research, Fertility and Sterility 79, 1063-1069, 2003 5 National Bioethics Advisory Commission, Ethical Issues in Human Stem Cell Research, Rockville, MD: September 1999, Volume I, at page 53. 6 Lawrenz B et al., Highly sensitive biosafety model for stem-cell-derived grafts, Cytotherapy 6, 212-222, 2004 7 Erdo F et al., Host-dependent tumorigenesis of embryonic stem cell transplantation in experimental stroke, J Cerebral Blood Flow Metab 23, 780-785, 2003 8 Roy NS et al., Functional engraftment of human ES cell-derived dopaminergic neurons enriched by coculture with telomerase-immortalized midbrain astrocytes, Nature Medicine 12, 1259-1268, November 2006; Sipione S et al., Insulin expressing cells from differentiated embryonic stem cells are not beta cells, Diabetologia 47, 499-508, 2004 9 Maitra A et al., Genomic alterations in cultured human embryonic stem cells, Nature Genetics ; 37, 1099-1103, October 2005; Draper JS et al., "Recurrent gain of chromosomes 17q and 12 in cultured human embryonic stem cells", Nature Biotechnology 22, 53-54; January 2004; Humpherys D et al.; Epigenetic instability in ES cells and cloned mice; Science 293, 95-97; 6 July 2001 10 Vanneste E et al., Chromosome instability is common in human cleavage-stage embryos, Nature Medicine 15, 577-583, May 2009 11 Werbowetski-Ogilvie TE et al. Characterization of human embryonic stem cells with features of neoplastic progression, Nature Biotechnology 27, 91-97, January 2009; Somervaille TCP et al., Hierarchical maintenance of MLL myeloid leukemia stem cells employs a transcriptional program shared with embryonic rather than adult stem cells, Cell Stem Cell 4, 129-140, 6 Feb 2009 12 Amariglio N et al., Donor-Derived Brain Tumor Following Neural Stem Cell Transplantation in an Ataxia Telangiectasia Patient, PLoS Med 6: e1000029. doi:10.1371/journal.pmed.1000029, 17 Feb 2009 13 Swijnenburg R-J et al., Embryonic stem cell immunogenicity increases upon differentiation after transplantation into ischemic myocardium, Circulation 112, I-166-I-172, 30 August 2005; Kofidis T et al., They are not stealthy in the heart: embryonic stem cells trigger cell infiltration, humoral and T-lymphocyte-based host immune response, European Journal

ID	Status	Date_Stamp	Comments
46402		5/26/2009 11:29:52 AM	of Cardio-thoracic Surgery 28, 461–466, 2005 14 Takahashi K and Yamanaka S, Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors, Cell 126, 663-676, 25 August 2006 15 Takahashi K et al., Induction of pluripotent stem cells from adult human fibroblasts by defined factors, Cell 131, 861-872, 30 November 2007; published online 20 November 2007; Yu J et al., Induced pluripotent stem cell lines derived from human somatic cells, Science 318, 1917-1920, 21 Decmber 2007, published online 20 November 2007 16 Zhou H et al., Generation of Induced Pluripotent Stem Cells Using Recombinant Proteins, Cell Stem Cell 4, 381-384, 8 May 2009, published online 23 April 2009 17 Yu J et al., Induced pluripotent stem cell lines derived from human somatic cells, Science 318, 1917-1920, 21 December 2007, published online 20 November 2007 18 Swaney DL et al., Human embryonic stem cell phosphoproteome revealed by electron transfer dissociation tandem mass spectrometry, Proc. Natl. Acad. Sci. USA 106, 995-1000, 27 January 2009 19 “Interview du professeur Ian Wilmut par Gènéthique”, accessed at: http://www.genethique.org/tribunes_mensuelles/mai_2009.asp ; for English translation, see: http://ethicalstemcellresearch.blogspot.com/2009/05/read-this-wilmut-king-of-cloning-says.html 20 For a list of iPS cell publications and current human iPS cell lines, please see the links at: http://www.frcblog.com/2009/05/update-on-ips-cells/ 21 D’Ippolito G et al., Marrow-isolated adult multilineage inducible (MIAMI) cells, a unique population of postnatal young and old human cells with extensive expansion and differentiation potential, J. Cell Science 117, 2971-2981, 15 July 2004 22 Zhao Y et al.; A human peripheral blood monocyte-derived subset acts as pluripotent stem cells; Proceedings of the National Academy of Sciences USA 100, 2426-2431; 4 March 2003 23 McGuckin CP et al., Production of stem cells with embryonic characteristics from human umbilical cord blood, Cell Proliferation 38, 245-255, August 2005 24 Murrell W et al., Multipotent stem cells from adult olfactory mucosa, Developmental Dynamics published online 21 March 2005 25 De Coppi et al., Isolation of amniotic stem cell lines with potential for therapy, Nature Biotechnology published online 7 January 2007; doi:10.1038/nbt1274 26 Conrad S et al., Generation of pluripotent stem cells from adult human testis, Nature 344-349, 20 November 2008 27 Bhatia S et al.; High-dose chemotherapy as initial salvage chemotherapy in patients with relapsed testicular cancer; J. Clin. Oncol. 18, 3346-3351; Oct. 19, 2000 28 Peggs KS et al., Clinical evidence of a graft-versus-Hodgkin’s-lymphoma effect after reduced-intensity allogeneic transplantion, Lancet 365, 1934-1941, 4 June 2005; Tabata M et al.; Peripheral blood stem cell transplantation in patients over 65 years old with malignant lymphoma--possibility of early completion of chemotherapy and improvement of performance status; Intern Med 40, 471-474; June 2001 29 Buadi FK et al., Autologous hematopoietic stem cell transplantation for older patients with relapsed non-Hodgkin's lymphoma, Bone Marrow Transplant 37, 1017-1022, June 2006 30 Elliott MA et al., Allogeneic stem cell transplantation and donor lymphocyte infusions for chronic myelomonocytic leukemia, Bone Marrow Transplantation 37, 1003-1008, 2006 31 Eapen M et al., Outcomes of transplantation of unrelated donor umbilical cord blood and bone marrow in children with acute leukaemia: a comparison study, Lancet 369, 1947-1954, 2007 32 Damon LE et al.; High-dose chemotherapy and hematopoietic stem cell rescue for breast cancer: experience in California; Biol. Blood Marrow Transplant 6, 496-505; 2000 33 Barkholt L et al., Allogeneic haematopoietic stem cell transplantation for metastatic renal carcinoma in Europe, Annals of Oncology published online 28 April 2006

ID	Status	Date_Stamp	Comments
46402		5/26/2009 11:29:52 AM	34 Krishnamurti L et al., Stable long-term donor engraftment following reduced-intesity hematopoietic cell transplantation for sickle cell disease, Biol. Blood Marrow Transplant 14, 1270-1278, 2008; Bernaudin F et al., Long-term results of related myeloablative stem-cell transplantation to cure sickle cell disease, Blood 110, 2749-2756, 2007 35 Bitan M et al., Fludarabine-based reduced intensity conditioning for stem cell transplantation of fanconi anemia patients from fully matched related and unrelated donors, Biol Blood Marrow Transplant. 12, 712-718, July 2006 36 Burt RK et al., Autologous non-myeloablative haemopoietic stem cell transplantation in relapsing-remitting multiple sclerosis: a phase I/II study, The Lancet Neurology 8, 244-253, March 2009 37 Burt RK et al., Nonmyeloablative hematopoietic stem cell transplantation for systemic lupus erythematosus, Journal of the American Medical Association 295, 527-535, February 1, 2006 38 Kreisel W et al., Complete remission of Crohn's disease after high-dose cyclophosphamide and autologous stem cell transplantation, Bone Marrow Transplantation 32, 337-340, 2003 39 Couri CEB et al., C-peptide levels and isulin independence following autologous nonmyeloablative hematopoietic stem cell transplantation in newly diagnosed type 1 diabetes mellitus, Journal of the American Medical Association 301, 1573-1579, 2009; Voltarelli JC et al., Autologous nonmyeloablative hematopoietic stem cell transplantation in newly diagnosed type 1 diabetes mellitus, Journal of the American Medical Association 297, 1568-1576, 11 April 2007 40 Grunebaum E et al., Bone marrow transplantation for severe combined immune deficiency, Journal of the American Medical Association 295, 508-518, 1 February 2006 41 Cox-Brinkman J et al., Haematopoietic cell transplantation (HCT) in combination with enzyme replacement therapy (ERT) in patients with Hurler syndrome, Bone Marrow Transplantation 38, 17-21, 2006 42 Escolar ML et al., Transplantation of umbilical cord-blood in babies with infantile Krabbe's disease, New England Journal of Medicine 352, 2069-2081, 19 May 2005 43 Herbots L et al., Improved regional function after autologous bone marrow-derived stem cell transfer in patients with acute myocardial infarction: a randomized, double-blind strain rate imaging study, Eur. Heart Journal 30, 662-670, 2009; Burt RK et al., Clinical applications of blood-derived and marrow-derived stem cells for nonmalignant diseases, Journal of the American Medical Association 299, 925-936, Feb 2008; Joseph J et al., Safety and effectiveness of granulocyte-colony stimulating factor in mobilizing stem cells and improving cytokine profile in advanced chronic heart failure, American Journal of Cardiology 97, 681-684, 1 March 2006; Strauer BE et al., Regeneration of human infarcted heart muscle by intracoronary autologous bone marrow cell transplantation in chronic coronary artery disease, Journal of the American College of Cardiology 46, 1651-1658, 1 November 2005 44 Inatomi T et al., Midterm results on ocular surface reconstruction using cultivated autologous oral mucosal epithelial transplantation, American Journal of Ophthalmology 141, 267-275, February 2006 45 Tateishi-Yuyama E et al.; Therapeutic angiogenesis for patients with limb ischaemia by autologous transplantation of bone-marrow cells: a pilot study and a randomised controlled trial; Lancet 360, 427-435; 10 August 2002; Badiavas EV and Falanga V, Treatment of chronic wounds with bone marrow-derived cells, Archives of Dermatology 139, 510-516, 2003 46 Shyu W-C et al., Granulocyte colony-stimulating factor for acute ischemic stroke: a randomized controlled trial, Canadian Medical Association Journal 174, 927-933, 28 March 2006 47 Terai S et al., Improved liver function in liver cirrhosis patients after autologous bone marrow cell fusion therapy, Stem Cells 24, 2292-2298, Oct 2006 48 Levesque MF et al., Therapeutic microinjection of autologous adult human neural stem cells and differentiated neurons for Parkinson's disease: five-year post-operative outcome, The Open Stem Cell Journal 1, 20-29, 2009 49 Geffner LF et al., Administration of autologous bone marrow stem cells into spinal cord injury patients via multiple routes is safe and improves their quality of life: Comprehensive case studies, Cell Transplantation 17, 1277-1293, 2008; Mackay-Sim A et al., Autologous olfactory ensheathing cell transplantation in human paraplegia: a 3-year clinical trial, Brain 131, 2376 - 2386, September 2008; Lima C et al., Olfactory mucosa autografts in human spinal cord injury: A pilot

ID	Status	Date_Stamp	Comments
46402		5/26/2009 11:29:52 AM	clinical study, Journal of Spinal Cord Medicine 29, 191-203, June 2006 50 Atala A et al., Tissue-engineered autologous bladders for patients needing cytoplasty, The Lancet 367, 1241-1246, 15 April 2006 51 Macchiarini P et al., Clinical transplantation of a tissue-engineered airway, The Lancet doi: 10.1016/S0140-6736(08)61598-6, published online 19 November 2008
46403		5/26/2009 11:30:41 AM	Dear Sir or Madam: I oppose the proposed guidelines which encourage and further research using embryonic stem cells. This research kills tiny human beings and plays with them as if they were not human. The proposed guidelines force me as a taxpayer to pay for this. Additionally, it diverts money away from useful and ethical research such as adult stem cell research that has already produced cures and treatments to help peoples' lives. Finally, taxpayer money should not be used for human cloning and similar experiments.
46404		5/26/2009 11:31:00 AM	I am against the destruction of these human embroy's. The research shows that any advancement that has occured has NOT been from embryonic stems cells but adult stem cells. These embroys are human beings, please please let us not continue to kill these embroys.
46405		5/26/2009 11:31:05 AM	I am opposed to your draft guidelines for embryonic stem cell research, which force me as a taxpayer to subsidize research requiring the destruction of innocent human life. Support should be directed to stem cell research and treatments that harm no one and are already producing good results. In no case should government support be extended to human cloning or other morally reprehensible creation of human embryos for research purposes.
46406		5/26/2009 11:31:07 AM	I am opposed to your draft guidelines for embryonic stem cell research, which force me as a taxpayer to subsidize research requiring the destruction of innocent human life. Support should be directed to stem cell research and treatments that harm no one and are already producing good results. In no case should government support be extended to human cloning or the human embryos for research purposes.
46407		5/26/2009 11:31:12 AM	I oppose all federal funding for human embryonic stem cell research. It should not matter that the parents of embryos have given their consent to such research or experimentation. The embryo is a human being distinct from the mother and father. Any experimentation upon the embryo, or at its expense, which is not of its nature ordered to the well-being of the distinct human embryo, or which causes direct harm to it, should not be within the domain of the parents to authorize, just as parents are not possessed of the right to allow such experimentation or harm upon their new-born child. It certainly should not be funded by federal funds.
46408		5/26/2009 11:31:16 AM	I am against embryonic stem cell research and totally against you using my money for this research which I do not support. I would much rather you use the money to fund research for existing stem cell research (adult stem cell) which harms no one and is already producing good results.

CERTIFICATE OF SERVICE

I hereby certify that on November 9, 2010, I caused a true and correct copy of the foregoing Joint Appendix to be served on Defendants' counsel electronically by means of the Court's ECF system.

/s/ Thomas M. Johnson, Jr.
Thomas M. Johnson, Jr.