EXHIBIT Q
Part 1 of 2

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UNITED STATES DISTRICT COURT
DISTRICT OF MASSACHUSETTS

AMGEN, INC.,

Plaintiff,

v.

F. HOFFMANN-LA ROCHE, LTD., ROCHE DIAGNOSTICS GMBH, and HOFFMANN-LA ROCHE, INC.

Defendants.

Civil Action No. 05-CV-12237 WGY

EXPERT REPORT OF DR. EDWARD EVERETT HARLOW, JR.

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EXPERT REPORT OF DR. EDWARD EVERETT HARLOW, JR.

I. BACKGROUND

1. I am Professor and Chair of the Department of Biological Chemistry and Molecular Pharmacology at Harvard Medical School. My responsibilities at Harvard Medical School include: (i) running the administration of a large basic science research and teaching department that currently has 31 faculty members, (ii) teaching graduate and medical students in courses around my own research interests, and (iii) running my own research lab where we study the basic differences between normal and cancer cells. The research group for which I am responsible consists of approximately 40 individuals including graduate students, post-doctoral fellows, clinical fellows, technicians, and senior research scientists. Since 1974, when I graduated from college, the focus of my scientific research has been on various aspects of basic cancer research. I am also an Associate Director of the Dana Farber/Harvard Cancer Center, where I am responsible for planning and evaluation for a large multi-institution research consortium of that unites approximately 900 research laboratories within the Harvard community with interests in cancer.

2. I earned a B.S. in Microbiology from the University of Oklahoma in 1974. In 1978, I received an M.S., also from the University of Oklahoma. In 1982, I earned a Ph.D. at Imperial Cancer Research Fund Laboratories, London, England, with my degree from Kings College, University of London. Here, I was part of a team of scientists that successfully cloned the mouse tumor suppressor gene now known as p53. We were the second team to successfully clone this gene. During this time, I became aware of and used many of the methods described in the work for the patents discussed in this report. Later, in 1985, while a staff scientist at Cold Spring Harbor, I became the first to clone and express the human p53 gene.

3. Since graduating from college in 1974, I have held many positions as a researcher, scientist, and teacher. Among these positions, I was Senior Staff Scientist at Cold Spring Harbor Laboratory in Cold Spring Harbor, NY, from 1988 to 1991. I was also Adjunct Assistant Professor in the Department of Microbiology at SUNY Stony Brook and, as stated above, am currently a professor and department chair at Harvard Medical School. I was the Scientific Director of the Massachusetts General Hospital Cancer Center from 1990 to 2002. From July 1996 to October 1998, I was Associate Director for Science Policy at the National Cancer Institute of the National Institutes of Health. I have also held a number of visiting professorships and named lecturer positions, including at Columbia University, Massachusetts Institute of Technology, University of California San Francisco, Baylor University, and Memorial Sloan-Kettering Cancer Center. Since 1992 I have been an American Cancer Society Research Professor.

4. I have been fortunate to receive many honors and awards for scientific achievement. Among these, I was elected to the National Academy of Sciences in 1993. In 1996, I was elected a Fellow of the American Academy of Arts and Sciences. In 1999, I was elected to the Institute of Medicine. I also received the Alfred P. Sloan, Jr. Prize from the General Motors Cancer Research Foundation in 1995, the Dickson Prize in Medicine from the University of Pittsburgh in 1996, and the Bristol Myers-Squib Award for Distinguished Achievement in Cancer Research in 1991.

5. I hold a number of positions on scientific advisory, award, and review committees. For example, I am a member of the Board on Life Sciences for the National Research Council. I currently sit of the External Advisory Board of several cancer centers including the University of California at San Francisco, Stanford University, and New York

University. I am a member of or chair of several award committees including the Jane Coffin Childs Memorial Fund for Medical Research and the Smith Family Medical Foundation. I currently am a member of several scientific advisory boards for biotechnology companies. Currently I chair the Scientific Advisory Board of 3V Biosciences in Zurich, and I am a member of the Scientific Advisory Board of Alnylam Pharmaceuticals in Boston. In the past I have chaired or been a member of several advisory boards for such companies as Onyx Pharmaceuticals and Genomics Collaborative.

6. I am the author or co-author of a number of scientific articles and textbooks. I have had articles published in such peer-reviewed journals as *Nature*, *Science*, *Cell, Genes and Development*, *Journal of Virology*, *Molecular Cell Biology*, *Oncogene*, and the *Proceedings of the National Academy of Science U.S.A.*, among others. I also co-authored *Antibodies: A Laboratory Manual*, first published by Cold Spring Harbor Laboratory Press in 1988. This is a basic laboratory manual on immunology and immunochemical methods. A revised edition of *Antibodies* was published in 1998.

7. A more complete list of my educational and professional experience, honors, and publications is found in my Curriculum Vitae attached as Exhibit 1 to this report. I will discuss various parts of my CV to provide my scientific background.

8. I have listed on my Curriculum Vitae attached to this report the publications I have authored within the last ten years, and before that I intend to go through my CV to explain my background and expertise.

9. I have not testified as an expert at trial or by deposition within the last four years.

10. My fee for consulting to Hoffmann-La Roche's attorneys during this case is $700 per hour.

II. MATERIALS REVIEWED

11. I have read and studied the patents-in-suit, U.S. Patent Nos. 5,547,933 ('933 patent), 5,621,080 ('080 patent), 5,955,422 ('422 patent), 5,618,698 ('698 patent), 5,441,868 ('868 patent), and 5,756,349 ('349 patent). I have also reviewed the prosecution histories of these patents. I have read and studied U.S. Patent No. 4,667,016 ('016 patent). I have also reviewed the prosecution history of this patent. I have read and studied U.S. Patent No. 4,703,008 ('008 patent).

12. I have reviewed the following literature, as well as the references in footnotes:

a. Scahill, et al., "Expression and characterization of the product of a human immune interferon cDNA gene in Chinese hamster ovary cells," PROC. NAT'L ACAD. SCI. 80: 4654-4658 (1983);

b. Oi, et al., "Immunoglobulin gene expression in transformed lymphoid cells," PROC. NAT'L. ACAD. SCI. 80:825-829 (1983);

c. Neuberger, "Expression and regulation of immunoglobulin heavy chain gene transfected into lymphoid cells," EMBO J. 2:1373-1378 (1983);

d. Crowley et al., "Plasmid-Directed Synthesis of Hepatitis B Surface Antigen in Monkey Cells," MOL. & CELL BIOL. 3:44-55 (1983);.

e. Fiers, et al., "The human fibroblast and human immune interferon genes and their expression in homologous and heterologous cells," PHILOS. TRANS R. SOC. LONDON BIOL SCI., 299:29-38 (1982);

f. Gray and Goeddel, "Cloning and Expression of Murine Immune Interferon cDNA," PROC. NAT'L. ACAD. SCI., 80:5842-5846 (1983);

g. Haynes et al, "Constitutive, long-term production of human interferons by hamster cells containing multiple copies of a cloned interferon gene," NUCL. ACIDS RES., 11: 687-706 (1983).

h. Rose and Bergmann, "Expression from Cloned cDNA of Cell-Surface Secreted Forms of the Glycoprotein of Vesicular Stomatitis Virus in Eucaryotic Cells," CELL, 30:753-762 (1982);

i. Wigler et al., "Transformation of mammalian cells with an amplifiable dominant-acting gene," PROC. NATL. ACAD. SCI., 77:3567-3570 (1980);

j. Murray et al., "Construction and Use of a Dominant, Selectable Marker: a Harvey Sarcoma Virus-Dihydrofolate Reductase Chimera," MOL. & CELL. BIOL. 3:32-43 (1983);

k. Kaufman and Sharp, "Amplification and Expression of Sequences Cotransfected with a Modular Dihydrofolate Reductase Complementary DNA Gene," J. MOL. BIOL. 159:601-621 (1982);

l. Lau et al., "Amplification and expression of human α-globin genes in Chinese hamster ovary cells," MOL. & CELL. BIOL. 4: 1469-1475 (1984).

m. Kawakita et al., "Characterization of human megakaryocyte colony-stimulating factor in the urinary extracts from patients with aplastic anemia and idiopathic thrombocytopenic purpura," BLOOD, 61:556-560 (1983); and

n. Goldwasser and Kung, "Purification of Erythropoietin," PROC. NATL. ACAD. SCI., 68:697-698 (1971).

13. In forming my below-stated opinions, I have relied upon my education, training, experience, and documents cited in this report. My knowledge and experience is further informed by numerous articles, books and journals that I have read over the years which have informed my understanding of the field of cell or molecular biology.

14. I reserve the right to rely upon other materials generated in the further discovery proceedings or presented at trial. In connection with my testimony, I may rely upon certain graphic or demonstrative exhibits including those attached hereto as Exhibit 2. I assume copies of these exhibits will be provided to Amgen as required.

III. METHODS FOR EXPRESSING AND RECOVERING RECOMBINANT PROTEINS FROM MAMMALIAN CELL CULTURES WERE WELL KNOWN IN 1983[1]

A. Recombinant techniques call for transfecting or transforming a cell with an exogenous DNA sequence

15. The field of recombinant protein synthesis traces its roots to the discovery of recombinant DNA techniques by Stanley Cohen and Herbert Boyer in 1973. In these methods, DNA segments of interest are combined to form a new combination of DNA molecules not found naturally. The following are some generalized principles to frame the discussion of Amgen's work.

16. The central dogma of biology sets forth – and has set forth since well before 1983 – that the DNA in each cell contains all the information necessary to create a new cell. Although there are many newly discovered intricacies and variations, the basic principles of how information is stored and is used to manage the activities of a cell were well established in 1983. The cell's DNA is organized in a precise code that provides all the instructions to run the cell. The DNA is faithfully copied and replicated each time the cell divides, and thus each daughter cell has an authentic copy of the instructions. The instructions are encoded using a simple alphabet of only four letters, and these letters are organized into various sequences that lead to the DNA code being enacted. For the discussions in this report, we are primarily interested in how a DNA code is and was used to direct the synthesis of a protein. Proteins are essentially flexible strings of amino acids made by attaching one to another in a head-to-tail manner. There are some 20 amino acids that can be used to make proteins, and variations in the order in which they are added and the length of the chains allows each amino acid sequence to take on its own particular shape and activity and thus for each protein to have its own role. I may give examples

[1] References thought to 1983 refer to the period prior to the first filing date of the patents-in-suit, December 13, 1983.

to illustrate this principle. Individual proteins are remarkably different, and they provide both the major structural units and the molecules that perform the work of the cell. For reference, a typical mammalian cell contains roughly 200 million protein molecules representing some 10,000 to 20,000 different protein sequences. The variations in amino acid sequence and length that give each protein its unique character are encoded in the DNA. The code of the DNA is used to synthesize a messenger RNA (mRNA), a single-stranded nucleic acid that is complementary to the sequence of one of the DNA strands. This process of making mRNA from the information present in DNA is known as transcription. The mRNA is then "read" by another group of molecules made up of proteins and other types of RNA that turn the message into proteins. The process of synthesizing proteins from the information contained in the sequence of the mRNA is known as translation. Thus, the central dogma of molecular biology started with a DNA code, which transcribed into a mRNA, which translated into a protein. Finally, many proteins are modified by post-translational processes that may include induction of the correct folding of the amino acid chain into its proper final three-dimensional shape; the transport of the protein to its correct location within the cell, on the cell surface, or secretion into extracellular space; the association with other proteins or other cellular molecules to form multimeric complexes; and the modification of specific amino acid residues in the protein with chemicals that help control protein function or processing. The chemical modification includes such events as phosphorylation or glycosylation. I may provide examples to explain these basic scientific concepts.

17. Using the techniques of recombinant DNA, cells can be manipulated to make proteins of interest to biologists and others. To create a cell that synthesizes a new protein not normally present in that cell, DNA sequences that code for the protein of interest, often known as

a gene, would need to be inserted into the cell. First, of course, the DNA that codes for the protein of interest must be identified and then isolated or synthesized. If the sequence of the protein of interest is not known, some method to identify the gene must be found. In 1983, one approach to identify the DNA that codes for that protein is by sequencing some or all of the protein of interest and using that information to find a DNA sequence that encodes the protein of interest. Thus, a small portion of a protein's amino acid sequence information is used to identify the gene for the protein of interest, and then after sequencing all of the DNA that encodes that protein, one is able to determine the full protein sequence and know the exact DNA sequence that codes for the protein of interest. Using standard DNA methods known in 1983, one can insert that identified gene into a cell that does not normally contain the DNA or protein coded by the gene. If the coding region of interest is surrounded by other DNA elements that tell the cell to accurately transcribe a mRNA from the coding region, then the cell can make the protein of interest. The process of identifying and propagating the exogenous DNA that codes for a protein of interest is called cloning that gene.

18. The cloned gene is then produced by the host cell. This encompasses transcription of the mRNA, translation of the mRNA into a protein, and then the newly synthesized protein undergoes the normal post-translational processing events of the host cell.

19. The techniques used to transfer exogenous DNA into a host cell are termed, generally, transfection and transformation. The terms "transformation" and "transfection" refer to the transfer of exogenous DNA into a cell. For the purposes of the work under consideration here, transfection is the more important process, because this refers to the transfer of DNA into a eukaryotic cell, which would include cells of mammalian origin used in these patents.

20. DNA fragments of interest are often maintained on a circular piece of double-stranded DNA known as a plasmid. Plasmids are small extra-chromosomal double-stranded DNA molecules (i.e., not part of the normal chromosome DNA) typically found in bacteria that contain only a few genes, relative to the number of genes present in the complete bacterial chromosomal DNA. They have their own origins of replication and often carry a drug resistance gene that can be used to select for the continued propagation of the plasmid in a host bacteria. These small circular DNA plasmids can be purified from bacterial cultures by standard biochemical methods, and thus researchers isolated DNA molecules that can contain fragments of interest for a variety of further tests and manipulations.

21. Plasmids and other suitable DNA molecules, such as the lambda DNA (a molecule of DNA from the bacteriophage lambda) that are used to carry and propogate DNA fragments of interest are commonly known as vectors. Inserted DNA fragments are used for a vast array of purposes. When the inserted DNA fragments contain the coding instructions for full length proteins or fragments of proteins along with flanking sequences that allow the coding region to be transcribed and translated in cells, the constructs are known as expression vectors. Vectors typically contain a second gene known as a genetic marker conferring a physically identifiable trait, e.g. a phenotype, that can be selected for or against, thereby creating cells that express the easily identified phenotype if the second gene – the genetic marker – is present, or do not express the phenotype if the second gene, and thus also the desired gene, is not present. Often the genetic marker confers a trait that allows the host cell that has successfully incorporated the plasmid with the gene marker, and thus also the protein of interest, to grow under conditions in which the cell without the plasmid with the genetic marker and desired gene will not grow. This made it easy to select cells that have the gene of interest, because cells

without the gene of interest die. Plasmids and other vectors served as important tools in genetics and biochemistry labs, where they were commonly used to amplify (make many copies of) or express particular genes and to make large amounts of proteins. To do this, one grew a host cell containing a plasmid or other suitable vector harboring the gene of interest. The host cell could be induced to produce large amounts of the vector, and thus large amounts of the protein whose gene has been inserted into the vector DNA. This was a cheap and easy way of mass-producing a gene or the protein it then codes for, for example, insulin or in the present case, EPO. I intend to present this tutorial using demonstratives, such as drawings showing cells, DNA and proteins, but may use other examples to clarify if I feel it necessary to teach these concepts.

B. Vertebrate cells, in particular mammalian cells, and more particularly CHO cells were typical cells used to synthesize proteins for human use

22. As of 1983, three major classes of host cells were typically used by those skilled in the art to produce recombinant proteins: bacterial cells, such as *E. coli*, yeast cells, such as *Saccharomyces cerevisiae*, and cells derived from mammals, such as Chinese Hamster Ovary (CHO) cells.

23. It was also known at that time and before, that CHO cells were a cell line that proliferates under suitable nutrient conditions. Suitable nutrient conditions vary according to the cell type, but there were numerous published papers and other references, such as those supplied by the companies who sold the nutrient medium (e.g., Gibco, and others) that provided standard recipes for the cell media that could be used to grow standard cells of interest. The recipes provided in the various papers and manuals included recipes comprising the minimal required nutrients for a given cell type (minimal media), media enriched with certain ingredients (rich media) and other variations useful for particular cell growth conditions. Of course, all cells need suitable nutrient conditions to proliferate. While suitable nutrient conditions for a bacterial cell

are fairly flexible, and typically comprise a powdered medium dissolved in water that contains some simple salts and sugars, a mammalian cell culture requires a richer medium, as was well known in 1983. Suitable nutrient conditions for mammalian cells typically use media based on a much richer recipe, and one that also includes some quantity of mammalian sera, typically from newborn or fetal calf, to keep the cells reproducing in culture. By 1983, the conditions for growing any of these cells were easily available, both from the manufacturers of the media ingredients, and from a variety of published articles and manuals.

24. Further it was known at that time, CHO cells, like other host cells derived from mammals suitable for expression of foreign DNA, could produce properly glycosylated polypeptides, also known as glycoproteins.[2]

25. It was well known in the art that production of glycoproteins could be accomplished with mammalian derived cell lines because bacterial cells typically lack the enzymes required for glycosylation of proteins.[3] CHO cells were well known by 1983 to express foreign glycoproteins having their known *in vivo* biological activity.[4]

26. Moreover, by 1983, it was well understood that CHO cells were especially useful host cells. In addition to their ability to express a wide variety of foreign polypeptides, it was

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[2] *See also,* Li et al., "Biosynthesis of lipid-linked oligosaccharides," J. BIOL. CHEM., 254: 1600-1605 (1979); Scahill, et al., "Expression and characterization of the product of a human immune interferon cDNA gene in Chinese hamster ovary cells," PROC. NAT'L ACAD. SCI. 80: 4654-4658 (1983); Singer et al., "Characterization of a porcine genomic clone encoding a major histocompatibility antigen: Expression in mouse L cells," PROC. NAT'L ACAD. SCI. 79:1403-1407 (1982); Haynes and Weissman, "Constitutive, long-term production of human interferons by hamster cells containing multiple copies of a cloned interferon gene," NUCL. ACIDS RES., 11: 687-706 (1983).

[3] *See* Sherwood and Goldwasser, "A radioimmunoassay for erythropoietin," BLOOD, 54: 885-893 (1979).

[4] *See e.g.* EPO 0 093 619 (Goeddel et al, 1983); Scahill et al., "Expression and characterization of the product of a human immune interferon cDNA gene in Chinese hamster ovary cells," PROC. NAT'L ACAD. SCI. 80: 4654-4658 (1983); Haynes et al, "Constitutive, long-term production of human interferons by hamster cells containing multiple copies of a cloned interferon gene," NUCL. ACIDS RES., 11: 687-706 (1983). See also, EPO 0 117 059, 0 117 060 (Levinson et al, 1984); McCormick et al., "Inducible expression of amplified human beta interferon genes in CHO cells," MOL. & CELL. BIOL., 4: 166-172 (1984); Devos et al., "Purification of recombinant glycosylated human gamma interferon expressed in transformed Chinese hamster ovary cells," J. INTERFERON RES., 4: 461-468 (1984).

known at the time that foreign DNA could be stably integrated into the CHO host cells to produce stably transfected cell lines.[5] It was known that CHO cells could express and secrete exogenous proteins, if the correct signals for secretion were included in the coding regions of the foreign protein. It was also known that these secreted proteins were glycosylated. Furthermore, at that time, there was a reasonable expectation that the secreted proteins would have their expected biochemical activities.

27. The use of CHO cells as host cells was well-known prior to the conception and reduction to practice of the claims-in-suit.[6]

C. Scientists routinely sought amplification of protein production through use of promoter DNA and amplified marker gene and DHFR in suitable nutrient conditions

28. DNA that carries the coding sequence for a protein of interest requires additional sequences including a promoter in order to express the corresponding polypeptide in a host cell.

29. The promoter is the controlling region that directs the host cell transcriptional machinery to produce a mRNA corresponding to the coding sequence in the vector. It must be

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[5] *See e.g.* EPO 0 093 619 (Goeddel et al, 1983); Li et al, "Biosynthesis of Lipid-linked Oligosaccharides," J. BIOL. CHEM., 254: 1600-1605 (1979); Scahill et al., "Expression and characterization of the product of a human immune interferon cDNA gene in Chinese hamster ovary cells," PROC. NAT'L ACAD. SCI. 80: 4654-4658 (1983); Haynes et al, "Constitutive, long-term production of human interferons by hamster cells containing multiple copies of a cloned interferon gene," NUCL. ACIDS RES., 11: 687-706 (1983). See also, Devos et al., "Purification of recombinant glycosylated human gamma interferon expressed in transformed Chinese hamster ovary cells," J. INTERFERON RES., 4: 461-468 (1984); EPO 0 117 059, 0 117 060 (Levinson et al, 1984); McCormick et al., "Inducible expression of amplified human beta interferon genes in CHO cells," MOL. & CELL. BIOL., 4: 166-172 (1984).

[6] *See e.g.* EPO 0 093 619 (Goeddel et al, 1983); Li et al, "Biosynthesis of Lipid-linked Oligosaccharides," J. BIOL. CHEM., 254: 1600-1605 (1979); Scahill et al., "Expression and characterization of the product of a human immune interferon cDNA gene in Chinese hamster ovary cells," PROC. NAT'L ACAD. SCI. 80: 4654-4658 (1983); Haynes et al, "Constitutive, long-term production of human interferons by hamster cells containing multiple copies of a cloned interferon gene," NUCL. ACIDS RES., 11: 687-706 (1983). See also, EPO 0 117 059, 0 117 060 (Levinson et al, 1984); Devos et al., "Purification of recombinant glycosylated human gamma interferon expressed in transformed Chinese hamster ovary cells," J. INTERFERON RES., 4: 461-468 (1984); McCormick et al., "Inducible expression of amplified human beta interferon genes in CHO cells," MOL. & CELL. BIOL., 4: 166-172 (1984).

operably linked to the DNA encoding the desired polypeptide in order to express the polypeptide.

30. As of 1983, the promoters of choice were specially selected promoters that were known to express large quantities of the desired mRNA in the host cell.

31. Some of the first promoters to be found and characterized were viral promoters. In general, promoters isolated from mammalian cells or from viruses that grow in mammalian cells will work effectively in most mammalian cells. Bacterial promoters were also used to express proteins in bacterial cells, and yeast promoters were used to express proteins in yeast cells. Thus, many promoters from various mammalian sources, typically non-human, were likely to work in CHO cells. The use of promoter DNA to direct the synthesis of foreign mRNAs within a host cell was well known in the art by 1983.[7]

32. By 1983, it was known how to insert a coding region for a protein of interest behind an active and efficient promoter capable of expressing in mammalian cells large quantities of mRNA from the inserted gene. The preferred method to introduce the promoter–coding region construct into a host cell at this time was transfection. The transfected DNA entered the cell and was inserted into the host cell DNA. There it was replicated along with the host DNA at every cell division and thus was maintained in each daughter cell in the population.

33. Even better, if one linked the promoter-coding region construct of interest to another gene, a selectable marker gene such as dihydrofolate reductase (DHFR), there were ways to manipulate the host cells into making even more target protein. After the DNA containing the two genes are transfected into cells, the DNA is inserted into the host chromosomal DNA, commonly in one location and commonly with only few copies linked together. Then the cells

[7] *See* U.S. Pat. Nos. 4,766,075 (to Goeddel); 4,356,270 (to Itakura); *see also* U.S. Pat. Nos. 4,264,731 (to Shine); 4,273,875 (to Manis); 4,293,652 (to Cohen); EPO 0 093 619 (Goeddel et al, 1983).

harboring the foreign DNA are grown in low concentrations of a drug that inhibits cells without the selectable marker gene. In this case, the selectable marker gene is present; it encodes a protein that blocks the action of the drug. When cells are then grown in slightly higher concentrations of the drug, only a minor population of the cells that happen to have more copies of the foreign DNA (and thus make more of the selectable drug resistance marker) can grow. This process is repeated with increasing drug concentration, and the resulting cells eventually have a large number of copies of the foreign DNA. Although there was no selection of the original promoter-coding region of interest, the copy number of this DNA construct is also amplified along with the drug resistance. The outcome is a population of cells that have very high numbers of the gene of interest.

34. The end result of using a marker gene is a host cell that (1) has amplified DNA (i.e., multiple copies of the DNA vector having the desired gene for the target protein), (2) has a promoter that makes abundant copies of the target protein, and (3) expresses an amplifiable marker (DHFR), which prevents the cell line from losing the desired gene. Thus, resultant cells frequently synthesize high quantities of the target protein. All this was known by 1983.

35. The use of DHFR with CHO cells was known prior to the conception and reduction to practice of this claim-in-suit.[8]

[8] Srinivasan and Lewis, "Transfer of the Dihydrofolate Reductase Gene into Mammalian Cells Using Metaphase Chromosomes or Purified DNA," INTRODUCTION OF MACROMOLECULES INTO VIABLE MAMMALIAN CELLS, 27-45 (1980); Milbrandt et al., "Organization of a Chinese Hamster Ovary Dihydrofolate Reductase Gene Identified by Phenotypic Rescue," MOL. CELL BIOL. 3: 1266-1273 (1983); Nunberg et al., "Amplified dihydrofolate reductase genes are localized to a homogeneously staining region of a single chromosome in a methotrexate-resistant Chinese hamster ovary cell line," PROC. NAT'L ACAD. SCI., 75: 5553-5556 (1978); *See also*, U.S. Pat. No. 4399216 (Axel et al., filed 1980); Urlaub and Chasin, "Isolation of Chinese hamster cell mutants deficient in dihydrofolate reductase activity," PROC. NAT'L ACAD. SCI., 77: 4216-4220 (1980); Schimke et al. "Gene amplification and drug resistance in cultured murine cells," SCIENCE, 202: 1051-1055 (1978).

D. Recombinant proteins were typically sought after for use in pharmaceutical compositions

36. After production and purification of any therapeutic substance, including small molecule drugs, natural proteins and recombinant proteins, these substances must be formulated into pharmaceutical compositions suitable for administration into a human.

37. Known formulations in 1983 included a variety of known diluents, adjuvants and carriers.

IV. ERYTHROPOIETIN HAD BEEN EXTENSIVELY STUDIED AS OF 1983

A. Human urinary EPO had been isolated and used to treat kidney failure patients

38. One of ordinary skill in the art in December 1983 would have understood the following, as articulated in Judge Young's opinion in *Amgen, Inc. v. Hoechst Marion Roussel, Inc.*, 339 F. Supp. 2d 202, 214 (D. Mass 2004):

> EPO is a naturally occurring hormone that controls erythropoiesis, the production of red blood cells in bone marrow. ... Erythropoiesis occurs continuously to offset cell destruction.... It enables a sufficient (but not excessive) amount of red blood cells to be available in the blood to provide tissue oxygenation...Hemoglobin is the protein in the red blood cells that actually transports the oxygen. ...The amount of hemoglobin correlates to the amount of oxygen... Hematocrit, which indicates the relative proportion of red blood cells to the total volume of blood, measures the ability of the blood to supply oxygen to the body...Thus, generally an increase or decrease in hematocrit equates with an increase or decrease in hematocrit equates with an increase or decrease in the ability to supply oxygen to the body... Under normal conditions, a person has a hematocrit of about forty-five to fifty, which means forty-five to fifty percent of the blood is made up of red blood cells.
>
> EPO is produced in the kidney and liver. Therefore, patients with chronic renal failure lack normal levels of EPO and suffer from anemia... Introduction of additional EPO into the patient's body can increase a patient's hematocrit level and sustain it at or near normal levels... In other words, the blood is able to provide a steady supply of sufficient oxygen to the tissues...

39. A procedure for isolating human EPO from urine was published as of 1977.[9] This procedure was in fact cited in the patents-in-suit[10] as the procedure used to isolate urinary EPO (uEPO).

40. As far back as the 1960s, EPO had been associated with the *in vivo* biological activity of stimulating of reticulocytes and red blood cell production.

41. Because it became clear that the production of EPO from human urine would be economically unfeasible, when recombinant DNA technology became available, various groups set out to clone the EPO gene and use the cloned EPO gene to express and purify EPO in a suitable cell line. Because EPO is naturally made in the kidney, kidney damage, such as the type that creates the necessity for dialysis, was known to cause low serum levels of EPO and resultant anemia.

42. By 1983 there were various *in vitro* and *in vivo* assays known[11] for detecting EPO in various cellular fractions, fluids and media.

43. Some of these assays relied on antibody-EPO interactions (e.g., the *in vitro* RIA referred to in the patents-in-suit), among other things, to quickly follow and quantify the levels of EPO protein. The RIA was the assay used to determine how much EPO was produced, and *in vivo* activity assays were used to determine how active the produced EPO was compared to isolated human uEPO. These *in vivo* assays could be used to confirm the EPO-containing

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[9] *See* Miyake, Goldwasser et al, "Purification of Human Erythropoietin," J. BIOL. CHEM., 252: 5558-5564 (1977).

[10] *See, e.g.*, Lin '008, col. 9, lines 13-21 (referencing an Amgen patent application to Egrie for a mouse-mouse hybridoma cell line that produces mAb to hEPO made from a 20-aa EPO sequence) and the Lin 561024 application, p. 17, lines 1-6 referencing the same Amgen patent application.

[11] *See* Goldwasser et al., "An assay for erythropoietin *in vitro* at the milliunit level," ENDOCRINOLOGY, 97: 315-323 (1975); Sherwood and Goldwasser, "A radioimmunoassay for erythropoietin," BLOOD, 54: 885-893 (1979); Cotes and Bangham, "Bio-assay of erythropoietin in mice made polycythæmic by exposure to air at a reduced pressure," NATURE, 191: 1065-1067 (1961); Hammond et al., "Production, utilization and excretion of erythropoietin," ANN. N.Y. ACAD. SCI., 169: 516-527 (1968).

fractions in the EPO purification methods described by the patents-in-suit, and to assess the amount, and relative activity, of the isolated and purified EPO.[12]

44. Without an assay such as the RIA and a biological activity assay for EPO, the technology in 1983 did not allow one of ordinary skill in the art to follow the purification of a polypeptide such as EPO from other proteins present in the cellular source. A robust assay method to find and qauntify the EPO protein in each fraction of a purification protocol was required, and a biological activity assay was needed to determine the relative activity of EPO. By 1983, various activity assays for tracking EPO were readily available to one having ordinary skill in the art, and biological activity assays such as using mice designed to look at EPO-stimulated increases in red blood cell production were similarly available.[13]

45. In addition, hybridoma cell lines producing mAbs to EPO were known at least by 1982, such as Goldwasser's report of a rat-mouse hybridoma cell line that produced monoclonal antibody directed to human EPO.[14]

B. Mammalian cells were generally preferred for seeking glycosylation of expressed proteins

46. At least since 1979, it was known that EPO was glycosylated (i.e., containing sugar) when isolated from naturally occurring sources and that the asialo form was not active.[15]

47. In the case of EPO, it was known by 1979 that naturally occurring isolated human EPO was glycosylated.[16]

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[12] *See* Lin '008, col. 33, lines 30-51, particularly lines 44-51 "Radioimmunoassay activity for the isolates ...; in vitro assay activity ranged from ...; and in vivo assay activity ranged from ..."

[13] *See* Hammond et al., "Production, utilization and excretion of erythropoietin," ANN. N.Y. ACAD. SCI., 169: 516-527 (1968).

[14] *See* Weiss, et al., "Characterization of a monoclonal antibody to human erythropoietin," BIOCHEMISTRY, 79: 5465-5469 (1982).

[15] *See* Sherwood and Goldwasser, "A radioimmunoassay for erythropoietin," BLOOD, 54: 885-893 (1979).

[16] *See* Sherwood and Goldwasser, "A radioimmunoassay for erythropoietin," BLOOD, 54: 885-893 (1979).

48. Human EPO was later found to be a 165-amino-acid-long, glycosylated protein. Effective glycosylation of the protein (i.e., addition of particular sugars to the protein, in a particular arrangement) was known to be necessary for *in vivo* activity of EPO. Note that glycosylated proteins are also referred to as glycoproteins or glycosylated polypeptides.

49. It had been well understood from before 1983 that a cell system that would produce a glycosylated EPO would be needed to allow the production and purification of biologically active EPO. It was also known that the most likely source of glycosylated EPO would be from mammalian cells. Other expression systems such as yeast cells might succeed, but mammalian cell culture would be highly likely to produce appropriately glycosylated proteins. Other secreted glycoproteins had been successfully produced and purified at this time. These proteins were known to be active. Among the glycosylated and active proteins produced by 1983 were some of the interferons,[17] immunoglobulins,[18] tPA[19] and other glycosylated proteins. It would have been reasonable to expect that in making biologically active recombinant EPO (rEPO), one would prefer to use mammalian cells to achieve desired glycosylation.

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[17] Scahill, et al., "Expression and characterization of the product of a human immune interferon cDNA gene in Chinese hamster ovary cells," PROC. NAT'L ACAD. SCI. 80: 4654-4658 (1983); Gray and Goeddel, "Cloning and Expression of Murine Immune Interferon cDNA," PROC. NAT'L. ACAD. SCI., 80:5842-5846 (1983); Taniguchi et al., "Construction and Identification of a Bacterial Plasmid Containing the Human Fibroblast Interferon Gene Sequence," PROC. JAPAN ACAD., 55: 464-469 (1979); Haynes and Weissman, "Constitutive, Long-Term Production of Human Interferons by Hamster Cells Containing Multiple Copies of a Cloned Interferon Gene," NUCL. ACIDS RES., 11: 687-706 (1983). See also, McCormick et al., "Inducible expression of amplified human beta interferon genes in CHO cells," MOL. & CELL. BIOL., 4: 166-172 (1984).

[18] *See* Oi, et al., "Immunoglobulin gene expression in transformed lymphoid cells," PROC. NAT'L. ACAD. SCI. 80:825-829 (1983); Neuberger, "Expression and regulation of immunoglobulin heavy chain gene transfected into lymphoid cells," EMBO J. 2:1373-1378 (1983); Sidman, "Differing requirements for glycosylation in the secretion of related glycoproteins is determined neither by the producing cell nor by the relative number of oligosaccharide units," J. BIOL. CHEM., 256: 9374-9376 (1981)*; see also* Seidman et al., "Antibody Diversity," SCIENCE, 202: 11 (1978).

[19] *See* US 4,766,075 to Goeddel et al.

C. An exogenous DNA sequence is needed to produce a corresponding amino acid sequence

50. Like other recombinant proteins, recombinant erythropoietin (rEPO), is produced by the cloning of an exogenous DNA sequence into a host cell line.

51. EPO was cloned from humans because it was well known in the art in and prior to 1983 that proteins with human sequences were more likely to be active in humans than proteins with nonhuman sequences and non-immunogenic. Thus, once human EPO was cloned, one of ordinary skill in the art would have known to use a rEPO expressing cell line to produce human EPO.

52. The coding region for the human EPO protein would be cloned behind a promoter of interest, and then this construct would be introduced by a method such as transfection into a mammalian cell line. One of ordinary skill in the art would have known that it would have been useful to have included with the promoter–EPO DNA sequences an amplifiable drug resistance marker such as DHFR to facilitate the generation of cells with multiple copies of both the promoter–EPO gene and the DHFR gene.

53. A natural consequence of cloning the human EPO gene into a host cell line is that the cell line will use the exogenous DNA to encode an amino acid sequence, such as shown in FIG. 6 of the patents-in-suit; this would be entirely expected to one of ordinary skill in the art at the time of the cloning.

54. The investigative and development activity surrounding EPO in the 1970s and early 1980s was directed ultimately to an EPO treatment for humans.[20] Goldwasser and Miyake, for example, focused on isolating and investigating human EPO. And the goal of the various groups looking to develop a recombinant EPO in the early 1980s was precisely to develop a

[20] See Background section of patents-in-suit, e.g. '933 col. 5:39 – col. 6:59.

recombinant human EPO. Thus, the recombinant EPO that was being developed was repeatedly compared to the human EPO isolated from urine.

V. ORDINARY SKILL IN THE ART

55. Throughout this report, I refer to one of ordinary skill in the art and what that person would know or understand from reading the patents and patent claims at issue. By this I mean a person with an advanced degree, such as a Ph.D. in biochemistry, immunology, genetics, molecular biology, medicine or related scientific fields, and two years or more of post-graduate or professional laboratory experience. I believe I can speak to what this person of ordinary skill would have known in 1983, when the original patent applications were filed, because, at that time, I had been working since 1974 with the then-current methods for molecular biology, including my work on the cloning of the p53 gene. Indeed, I was working in the early 1980's at two of the leading institutions in the field of molecular biology, the Imperial Cancer Research Fund Laboratories in London, England and Cold Spring Harbor Laboratory in Cold Spring Harbor, New York. Accordingly, I am well aware of the range of knowledge and understanding by young scientists and veterans during the relevant time period. My testimony set forth herein is thus based on my own substantial experience and knowledge in the field and on my opinion of how one of ordinary skill in the art of cloning technology would view and understand the patents and prior art.

VI. '016 PATENT CLAIM 10

56. Generally speaking, claim 10 of the '016 patent is directed to production of purified recombinant EPO. The process begins with culturing mammalian cells transfected with the EPO gene sequence in a suitable nutrient environment to express recombinant EPO that is purified through a series of steps.