EXHIBIT 2

IN THE UNITED STATES DISTRICT COURT
FOR THE 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

CONTAINS RESTRICTED ACCESS BLA/IND CONFIDENTIAL INFORMATION

SUBJECT TO PROTECTIVE ORDER

NON-INFRINGEMENT EXPERT REPORT OF RICHARD A. FLAVELL, PH.D.

directed to the production and purification of proteins produced by cells. I have been informed that Roche maintains no such cells in the United States. Nor does Amgen in its expert reports suggest that this process exists in the United States. I have nevertheless reviewed portions of Roche's Biologics License Application ("BLA") for CERA that state that Roche uses cells to produce a starting material in the CERA manufacturing process in Germany.[78] According to my understanding of the Patent Laws, practicing any method outside the United States is not itself an act of infringement.

b. Roche Does Not Transform or Transfect Mammalian Host Cells with an Isolated DNA Sequence Encoding Human Erythropoietin

61. In my opinion, Roche does not employ host cells that have been "transformed or transfected with an isolated DNA sequence encoding human erythropoietin" as that phrase is properly interpreted. I understand this claim limitation to require that the DNA sequence inserted into the cell be "an isolated DNA sequence." This is clear from the claim language which does not say that the genetic modification is done simply with DNA encoding human erythropoietin. Instead, the claim is quite specific: the transformation or transfection is "with" an **isolated** DNA sequence encoding human erythropoietin.

62. I have reviewed Amgen's claim construction submissions and note that Amgen's position is consistent with my own understanding. Amgen has told this Court that this claim limitation covers "purified DNA":

[78] *See e.g.*, ITC-R-BLA 00004662.

> Cells transformed and transfected. And bear in mind that we're talking about a claim term that says cells transformed or transfected with a DNA, an isolated DNA sequence encoding human erythropoietin, I believe is the claim term, or something close to that. What we are proposing is cells that have received purified DNA encoding the genetic instructions for human EPO – I guess what we should say is, because I think it's getting a little ambiguous when we say and their offspring, we should say and succeeding generations of those cells.[79]

Consistently throughout prosecution, Amgen sought allowance for claims that specified sequences encoding erythropoietin as being "purified and isolated DNA."[80] Amgen's insistence on the use of purified DNA in this claim – then and now – highlights the importance of understanding the methods of transformation and transfection in historical context. As shown below, the use of purified and isolated DNA is a critical element of DNA transfer according to the claims.

63. As of October 1983, there were four generally-accepted methods for transferring genetic information to a mammalian host cell: (1) somatic cell hybridization, (2) protoplast fusion, (3) chromosome-mediated gene transfer, and (4) DNA-mediated gene transfer.

64. Somatic cell hybridization, also called cell fusion, involves the fusion of two cells to form a combined cell with two nuclei that eventually fuse together after mitosis. In this process, all DNA from one cell is introduced into the host cell. The principal advantage of somatic cell hybridization is that it is not necessary to isolate or purify the DNA transferred to the host cell.

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[79] 4/17/07 Markman Hrg. Tr. at 101 (emphasis added); *see also* Amgen's Rebuttal Markman Brief. I understand also that Amgen is currently negotiating with Roche to agree that, in fact, that host cells according to the claims of the Amgen patents have been "genetically modified with isolated and purified DNA containing genetic instructions for human erythropoietin." Email from Suh to Day, dated Apr. 20, 2007. I reserve the right to amend my opinion pending an agreed-upon claim construction or order of the Court.

[80] *See e.g.*, U.S.S.N. 675,298 at 100 (claim 34) (AM-ITC 00873443).

(In fact, this cannot be done.) This lack of purity is the main disadvantage of the technique. Because the entire genome gets transferred to a host cell, the transformation/transfection system lacks specificity and is difficult to use selection methods to obtain pure cell populations.[81] Therefore, for somatic cell hybridization, isolated DNA is not transferred to the host cell.

65. Protoplast fusion is another form of cell fusion.[82] According to this method, bacterial protoplasts – bacteria without their cell walls – containing cloned DNA, were fused to host cells by centrifugation. DNA contents of the bacterial protoplast are incorporated into the host cell in an event akin to somatic cell hybridization.[83] The bacterial chromosome is transferred into the host cells, and there is a real possibility that some bacterial DNA sequences are incorporated into transformed cells.[84] Like somatic cell hybridization, this process lacks purity, but it is not necessary to isolate or purify cloned DNA prior to transformation of a host cell. Therefore, in bacterial protoplast fusion, isolated DNA is not transferred to the host cell.

66. Chromosome-mediated gene transfer involves the isolation of chromosomes – packed DNA molecules in the nucleus of cells – and incubating them with other cells. Under appropriate conditions, these isolated chromosomes can be taken up by cells, and genes borne by the chromosomes are sometimes expressed.[85] Isolated DNA is not transferred to the host cell using this technique.

67. Finally, DNA-mediated gene transfer involves the transfer of isolated and purified DNA fragments to host cells using one of several methods, including calcium phosphate precipitation,

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[81] Tischfield 1974.
[82] Schaffner, W., Proc. Natl. Acad. Sci. USA, 77: 2163-67 (1980); Sandri-Goldin (1981).
[83] Sandri-Goldin 1981; Sandri-Goldin 1983.
[84] Sandri-Goldin 1984 at 136.
[85] McBride, O.W., et al. (1978) *Proc. Natl. Acad. Sci. USA* 75: 914-18.

electroporation or microinjection.[86] DNA-mediated gene transfer, working with purified DNA, enables greater ease in manipulating DNA with complicated enzymatic reactions, such as those disclosed in the Amgen patent specification. In contrast to the other three methods for transformation or transfection, **this method transfers isolated DNA to a host cell**.

68. Methods for transformation and transfection are distinguished on their requirement for transfer of isolated DNA to a host cell. However, claim 1 of the '868 patent expressly requires that host cells be transformed or transfected **with an isolated DNA sequence**. Coupled with my analysis of transformation methods known at the time of the invention, I believe that the full limitation in claim 1 of the '868 patent was conceived because of the use of DNA-mediated gene transfer as set forth in the specification, and an isolated DNA sequence was therefore a specified claim feature of claim 1 and its dependent claim 2. Roche has never and does not now practice this method.

69. The claim feature for an "isolated DNA sequence" carries its own inherent limitation. In my opinion, its inclusion in the claim indicates that steps should be taken to purify DNA prior to introduction into a host cell. I understand that this Court heard testimony on the proper meaning of the term "isolated" as used in a claim element in a previous litigation concerning these patents:

> After considering these excerpts from the patent, Dr. Lodish testified that <u>one of ordinary skill in the art would understand the term "isolate" to mean "to recover in pure form.</u>" The Court agrees.[87]

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[86] U.S. Patent No. 4,399,216 (filed Feb. 25, 1980); Graham, F.L. & van der Eb, A.J. (1973) *Virology* 52: 456-67.

[87] *Amgen v. Hoechst Marion Roussel, Inc.*, 126 F.Supp.2d. 69, 132 (D. Mass. 2001) (internal citations removed) (emphasis added).

70. The definition of "isolate" adopted by the Court is essentially the same as its ordinary meaning, "to set apart from others," or "an individual, population, or kind obtained by or resulting from selection or separation."[88] In my experience as a molecular biologist, the term "isolate" has no special meaning when applied to either proteins or DNA. As with proteins, purification of DNA involves a number of techniques that were well known to those of skill in the art in October 1983, including phenol-chloroform extraction, gel electrophoresis, cesium chloride density gradient centrifugation, and ethanol precipitation.[89] By the time of the invention, it was possible to use these techniques to isolate an essentially pure sample of DNA from a heterogenous mixture of DNA having a variety of sequences.

71. All examples of the specification further sheds light on Amgen's intended meaning for the term "isolated" as it appears in the claims. The patent discloses five examples of host cell transformation and transfection with an isolated DNA sequence, including introduction of purified and isolated DNA into COS cells, CHO cells and *E. coli* via DNA-mediated gene transfer.

72. In my opinion, one of skill in the art at the time of the invention reading the patent specification would have understood that the claimed mammalian host cells transformed or transfected with an isolated DNA sequence envisioned the use of DNA-mediated gene transfer, and not any of the other three methods which are not taught in the patents. I note that Amgen has not argued that a claim construction to the contrary should apply.[90] Cell fusion,

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[88] MERRIAM WEBSTER'S COLLEGIATE DICTIONARY 621 (10th ed. 1999).

[89] *See* T. Maniatis, E. F. Fritsch & J. Sambrook, MOLECULAR CLONING, A LABORATORY MANUAL §§ 3, 5 (1982).

[90] However, should Amgen change course and argue otherwise, I reserve the right to amend my report as necessary to deal with any new claim construction Amgen should raise.

chromosome-mediated gene transfer, and protoplast fusion were all known and practiced at the time of the invention, however did not involve transfer of genetic material along with other cellular components both genetic and non-genetic. This transfer does not involve "isolated DNA sequences" according to the claims in light of the specification.

73. In my opinion, Roche does not infringe claims 1 or 2 of the '868 patent because the cells Roche uses to create its epoetin beta reagent were not "transformed or transfected with an isolated DNA sequence encoding human erythropoietin."[91] In my opinion, the cells used by Roche in the manufacture of CERA were not created using isolated and purified of DNA according to the claim limitation, therefore, Roche does not infringe claims 1 and 2 of the '868 patent.

74. CERA also does not infringe under the doctrine of equivalents because Amgen surrendered matter encompassing cell fusion methods in representations Lin made to the Patent Office during prosecution in which he argued that his claims were patentably different than the prior art, including a Sugimoto patent (U.S. 4,377,513) which related to the use of cell fusion methods. For example, to overcome a rejection by the examiner during prosecution of U.S. 4,703,008 -- which is the parent of the '868 patent – Lin told the Patent Office that "Under no circumstances can the claims be urged to 'read on' non-isolated DNA ..." of the Sugimoto reference.[92] By setting forth this explanation of the claimed feature in the related '008 patent file history, one of skill in the art would understand that the "isolated DNA" does not include cell fusion methods, including protoplast fusion techniques used to transfect the cells used to make

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[91] *See* ITC-R-BLA 00004987-92.

[92] File History of the '008 Patent, Paper 12, 10/2/86 Amendment and Reply at 13 discussing pending claim 14 ("A purified and isolated DNA sequence ...") and claim 34 ("A purified and isolated DNA sequence encoding for a polypeptide ... of naturally occurring erythropoietin.")

Roche's harvested cell-free culture supernatant from which it ultimately derives its erythropoietin beta starting material.

75. Even if Amgen were not estopped from asserting protoplast fusion as an equivalent, CERA still would not infringe this claim limitation under the doctrine of equivalents because protoplast fusion is not insubstantially different than transformation/transfection with isolated DNA. Indeed, there are substantial differences. Protoplast fusion requires that cells literally "smush" together, rather than having isolated DNA enter through transient openings in the plasma membrane of the host cell. This is quite a different way of achieving DNA transfer not compatible with the plain words of the claim limitation. For at least these reasons, Roche's protoplast fusion method is not an equivalent to the transformation or transfection with an isolated DNA sequence specified by claim 1 of the '868 patent.

c. Importation of MIRCERA™ Does Not Infringe Any of the Asserted Process Claims Because CERA Is Materially Changed from the Product of the Claimed Process In Ways That Are Not Insubstantial

76. I have been told that the law creates an act of infringement of a process claim by the importation of the product of that process into the United States. Such an act will not be infringement, however, if that product has been materially changed. This being the case, I am prepared to offer my opinion at trial that CERA is materially changed from any product properly within the scope of the process covered in claims 1 and 2 of the '868 patent. The bases I will focus on to prove a material and not insubstantial change are:

(i) Roche's epoetin beta starting material used to synthesize CERA is materially changed from the glycosylated erythropoietin polypeptide produced by host cells;

(ii) difference in molecular weight between CERA and Roche's epoetin beta starting material;

(iii) CERA's demonstrated lower binding affinity to the EPO receptor versus epoetin beta;

(iv) measured differences in receptor-mediated metabolism;

(v) CERA's higher potency than epoetin beta, both *in vitro* and *in vivo*;

(vi) CERA's longer *in vivo* half-life versus epoetin beta; and

(vii) observed differences in intracellular signaling properties between CERA and epoetin beta.

I will also explain that CERA's material differences with a product produced according to the claims demonstrate non-infringement under the doctrine of equivalents.

(i) The Epoetin Beta Starting Material is Materially Changed from the Crude Isolate Recovered from the Host Cell Medium In A Way That Is Not Insubstantial

77. The process claimed in the '868 patent covers the creation of a glycosylated erythropoietin polypeptide produced by host cells. This product, at best, is a crude heterogeneous mixture of cell-derived materials and cell culture medium. During Interference proceedings pertaining to the process claims of the '868 patent, Lin argued successfully to the Patent Office that "isolating" polypeptides was <u>not</u> a purification step stating that:

> <u>The isolation step (b) means nothing more than separating the expressed product from the cells</u> (LR 229) and would obviously be necessary to determine the in vivo biological activity of the expression product. Any effort by Fritsch et al to argue that the isolation step of the Count means purification is nothing more than an afterthought which is inconsistent with Fritsch et al's own disclosure, as noted earlier.[93]
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> * * *

[93] Amgen Brief for Party Lin, Interference No. 102,097 at 48 (AM-ITC 00337649 - AM-ITC 00337715 at 00337700) (emphasis added).

> As for the isolating step, there is clearly nothing separatively inventive in this. Fritsch et al again try to equate isolation with purification, but as noted earlier, these two are not the same ....[94]

Based on Lin's argument, the Patent Office agreed that "isolating said glycosylated erythropoietin" did not require purification, instead acknowledging that there is

> no evidence suggesting that the work done at Amgen relating to the expression of the EPO gene in mammalian host cells and isolation of the resulting glycoprotein product involved anything other than the exercise of ordinary skill by practitioners in that field.[95]

I understand that the Court in this case determined that "isolating said glycosylated erythropoietin polypeptide expressed by said cells" as used in the asserted claims of the '868 patent does not include purification but merely isolation.[96] Therefore, it is the crude harvested cell-free culture supernatant extracted and separated from the cells which produced it that is the subject of the "isolating" step (b) of the asserted claims. In fact, Amgen scientist Dr. Strickland confirmed that such a crude substance required subsequent purification to remove contaminants and other undesired products.[97]

78. The crude isolate is not the starting material or reagent used in the chemical reaction to create CERA, and I doubt that it could be used that way. The steps that Roche takes to convert the crude protein supernatant recovered from its cell fermentation tanks into a homogenous product useful as a reagent in its specific chemical reaction to create CERA is not insubstantial and constitutes a material change. In my experience, purification of any protein is not a trivial

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[94] Amgen Brief for Party Lin, Interference No. 102,097 at 58 (AM-ITC 00337649- AM-ITC 00337715 at 00337710) (emphasis added).

[95] *Fritsch v. Lin*, Interference No. 102,097, 1991 WL 332571, *3 (Bd. Pat. App. & Interf. 1991) (emphasis added).

[96] 4/17/07 Markman Hr'g Tr. at 97-98.

[97] Strickland Depo. Tr., Mar. 9, 2007 at 109-111.

product present in MIRCERA™ has been materially changed by subsequent processes. Therefore in my opinion, for the same reasons as articulated above for the asserted claims of the '868 and '698 patents, Roche does not infringe claim 7 of the '349 patent. In my opinion, however, Roche does not infringe claim 7 for the additional reasons outlined below.

a. The Claim Limitation "U of erythropoietin per 10^6 cells in 48 hours as determined by radioimmunoassay" Cannot Be Used to Determine Whether Claimed Vertebrate Cells Exist

123. CERA does not meet each and every limitation present in claim 7 of the '349 patent. Claim 7 refers to the vertebrate cells described in claims 1 through 6 capable of producing EPO in the medium of its growth "in excess of 100 U of erythropoietin per 10^6 cells in 48 hours as determined by radioimmunoassay."[156]

124. The "U" in the claims I understand to mean Units of biological activity, and I don't believe there is any disagreement on this.[157] I have reviewed portions of the Expert Report of Charles G. Zaroulis, M.D., and understand that several international standards for EPO have existed, each having a different specific activity equating biological activity with an amount of EPO.[158] In addition, several informal standards have been used to quantify EPO, each having different specific activities.[159] I have also read portions of the Expert Report of Dr. Thomas Kadesch (the "Kadesch report") and agree with his conclusion that different standards can yield different "readouts" for Units of bioactivity. Standards have been used in a number of different

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[156] '349 patent, col. 38, ll. 9-12. Other claims specify that the vertebrate cells produce either 500 U or 1000 U.

[157] *See* Goldwasser Feb. 14, 2007 Depo. Tr. at 50-54; Lin Mar. 28, 2007 Depo. Tr. at 73-74.

[158] *See* Expert Report of Charles G. Zaroulis, M.D., dated April 6, 2007 (the "Zaroulis report") ¶¶ 34-44; Sytkowski, A.J., (2004) ERYTHROPOIETIN: BLOOD, BRAIN AND BEYOND 5.

[159] Zaroulis report, ¶¶ 41-42.

types of assays – *in vitro* bioassays, *in vivo* bioassays, and radioimmunoassays, to name a few.[160] Specific activity is particularly important for assays that rely on antibodies because antibody binding cannot measure the biological activity of either a standard or an unknown sample. I have reviewed documents that suggest that at least in Amgen's hands, different standards when used in the same assay gave very different results for samples of EPO measured.[161]

125. In my opinion, Roche cannot be shown to meet this limitation. The limitation specifically requires the use of a radioimmunoassay to determine human EPO production by amount of biological activity. A radioimmunoassay measures amounts and not Units of biological activity. Therefore Roche cannot be shown to have cells making EPO of this activity as determined by radioimmunoassay because radioimmunoassay cannot determine biological activity. In addition, the patent specification does not disclose sufficient information regarding the specific activity of standards or unknown samples to determine Units, if one took the radioimmunoassay result and attempted to infer activity. This means that the outcome of the assay could change depending on the variables chosen, raising the possibility that Roche could meet the limitation under certain conditions, but not under others conditions. Without guidance as to the proper assay conditions to use, one cannot determine with any certainty that the limitation has or has not been met even if it did not mean what it actually says.

b. Roche's Documents Do Not Establish that the Limitation "U of erythropoietin per 10^6 cells in 48 hours as determined by radioimmunoassay" Has Been Met

126. I understand that Dr. Lodish has performed calculations based on assorted documents relating to Roche's regulatory filings for CERA and NeoRecormon®, its duly licensed epoetin

[160] *Id.* at ¶ 65.
[161] AM-ITC 00550986-1044.

synthesized product is somehow equivalent to the claimed "glycoprotein product of the expression in a mammalian host cell" of claim 3 and its dependent claims.

148. Furthermore, because the EPO beta Roche uses as a starting material used to synthesize CERA is indistinguishable from urinary EPO, Roche is using a product that is found in the disclosures that predate Amgen's claimed invention, which I understand is referred to as prior art. I have been informed that using something from the prior art cannot be found to be an equivalent to a claimed invention because a patentee may not include the prior art within the scope of its claims. For this reason also, CERA cannot be found to infringe the claims of the '933 patent under the doctrine of equivalents.

c. CERA Is Not A "Non-Naturally Occurring" Glycoprotein

149. In addition, claim 3 of the '933 patent (and its dependent claims discussed below) claim a "non-naturally occurring" glycoprotein. I understand that the "non-naturally occurring" limitation means that the claimed erythropoietin glycoprotein has unique glycosylation patterns that differ from EPO products that occur in nature such as urinary EPO and EPO derived from plasma. The term "non-naturally occurring" in the '933 patent requires the claimed EPO product be structurally different in some way from naturally occurring EPO because the asserted claims of these patents recite glycoproteins having the "in vivo biological activity of causing bone marrow cells to increase production of reticulocytes and red blood cells" – in other words the same activity as natural EPO.

150. For example, Amgen has argued with respect to the asserted claims of the '933 patent that the expression of DNA encoding EPO in a mammalian cell imparts "essential structural

characteristics".[189] Amgen also made similar arguments to the Patent Office to secure the '933 patent claims. I have reviewed these arguments Lin made in order to distinguish the claimed EPO products from naturally occurring EPO based on putative differences in their glycosylation patterns. For example, Amgen's original claims were rejected based on a 1977 article by Miyake which discloses the isolation and purification of naturally derived human urinary erythropoietin.[190] In response, Amgen submitted a declaration from its scientist Dr. Thomas Strickland which purported to show that uEPO and Amgen's claimed EPO have different glycosylation as shown by isoelectric focusing.[191] Thus, Amgen relied on this argument to persuade the Patent Office that its claimed EPO was different from natural EPO and to obtain issuance of its claims.

151. CERA exists as a set of isomers each with complete molecular structure. None of these structures have discrete "parts" or "components." There is no legitimate basis for separating CERA into parts as Amgen's experts suggest. However, even assuming Amgen's flawed deconstruction of the molecule were proper, in my opinion, the epoetin beta starting reagent used in the synthesis of CERA is not a "non-naturally occurring" EPO glycoprotein as required by the asserted claims of the '933 patent. There is no reliable evidence that the epoetin beta starting reagent has glycosylation that differs from that of any naturally occurring species of EPO, including urinary EPO and EPO in plasma. Indeed, in my opinion, documents such as Roche's

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[189] See Amgen Inc.'s Claim Construction Br., March 5, 2007 at p. 14. Amgen has also argued that the "non-naturally occurring erythropoietin glycoprotein" products of the '080 patent, discussed in Section __ are defined by reference to "their distinct glycosylation." *See id.* at 13.

[190] AM-ITC 00941097-98.

[191] AM-ITC 00941119-1135.

BLA support the conclusion that its starting material includes molecules that are the same as naturally occurring EPO.

152. I understand that in a prior litigation on the '933 patent, this Court found that "(1) the glycosylation of urinary erythropoietin has 'enormous heterogeneity'; (2) different purification techniques, several of which were known by one skilled in the art in 1984, result in differing glycosylated erythropoietin populations; (3) despite referring to at least two purification methods, the patent does not identify which human urinary erythropoietin preparation ought to be used as a standard, nor would a skilled person know which urinary EPO preparation should be used; and (4) different urinary erythropoietin samples have different glyosylation" and therefore "the glycosylation of human urinary erythropoietin is a standardless standard."[192] The Court concluded that "[a]s a result, making comparisons between the glycosylation of recombinant EPO and that of human urinary EPO is virtually impossible."[193] I agree.

153. In my opinion, Roche's epoetin beta starting material used in the synthesis of CERA cannot be distinguished from urinary EPO or other natural EPO because there is too much variability in the different types of glycosylation observed from sample to sample in urinary EPO and, thus, there is no reliable standard available which is necessary in order to draw a distinction between natural and "non-naturally occurring" EPO.[194] EPO produced by host cells also displays this variability in glycosylation from sample to sample. For example, different methods of isolating or purifying EPO result in different populations of EPO glycoforms (glycoprotein

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[192] *Amgen Inc. v. Hoechst Marion Roussel, Inc*., 126 F. Supp. 2d 69, 155 (D. Mass. 2001)

[193] *Id.*

[194] *See* Wide, L. et al. (1990) *Br. J. Haematol.*, 76:121-7; Tam, R.C. et al. (1991) *Br. J. Haematol.*, 79:504-11.

molecules that differ only in the structures of sugar residues).[195] Also, glycoproteins produced by different cell types may exhibit different distributions of glycoforms.[196]. Moreover, the conditions of the media in which cells are grown may influence the types of glycoforms produced.[197]

154. Each of these factors creates microheterogeneity in EPO glycosylation which the Court recognized previously. Thus, although there may be observable differences in distributions of glycoforms when comparing specific samples of EPO, the differences in distribution are entirely dependent upon the specific EPO samples used, and are influenced by factors including the source of the EPO, intracellular and extracellular environment, degree of purity and other conditions. Variations between specific populations of epoetin alfa or beta and uEPO cannot be broadened to draw a general conclusion that epoetin alfa or beta is different from of all naturally occurring EPO, let alone uEPO.

155. I have reviewed literature that suggests that epoetin alfa or beta "is indistinguishable from urinary EPO" in terms of glycosylation.[198] Studies by Takeuchi[199]and Sasaki[200] used various assaying techniques to compare epoetin alfa or beta and uEPO both before and after treatment with enzymes to remove their carbohydrate and both reached the conclusion that every

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[195] Goldwasser Depo. Tr. at 304; AM-ITC 00913913-919; AM-ITC 00914115-118; Browne Depo. Tr. at 224-227; s*ee also See also* Miyazaki, *J. of Imm. Methods*, 113 (1988) 261-167; Inoue, *Biol. Pharm. Bull.,* Vol. 17, No. 2 (1994) 180-184; Storring, *J. of Endoc.,* Vol. 134, No. 1 (1992) 459-484; Imai, *J. Biochem.,* 107 (1990) 352-359.

[196] *See, e.g.*, Choppin, *Exp. Hemo.,* Vol. 15 No. 2 (1987) 171-176; Wojchowski, *Biochim. et Biophys. Acta,* 910 (1987) 224-232; Takeuchi, M (1988), *J. of Biol. Chemistry*, vol 263, no. 8, 3657-3663

[197] See Cumming, (1992) Develop. biol. Standard., vol. 76, pp. 83-94; Yuen, C., (2003), Br. J. of Haematology, 121, 511-526.

[198] *See* Imai, *J. Biochem.,* 107 (1990) 352-359.

[199] Takeuchi, M (1988), *J. of Biol. Chemistry*, vol 263, no. 8, 3657-3663.

[200] Sasaki, H., (1987), *J. of Biol. Chemistry*, vol. 262, no. 25, pg. 12059-12076.

carbohydrate structure identified in epoetin alfa or beta was also observed in uEPO. Amgen's Dr. Strickland admitted in his recent deposition that all of the glycoforms found in epoetin alfa are also found in urinary EPO.[201]

156. In any event, Amgen has not pointed to any evidence that shows that Roche's epoetin beta possesses glycosylation that can be distinguished from that of urinary EPO or plasma EPO, as it must in order to demonstrate that epoetin beta is "non-naturally occurring" as set forth in the claims of the '933 patent. Indeed, Roche's regulatory information regarding CERA shows that epoetin beta possesses glycosylation patterns that cannot be differentiated from that of natural urinary EPO, and in other regards cannot be distinguished from it. Roche's EPO beta starting reagent and natural urinary EPO as now known, are both polypeptides consisting of 165 amino acid residues.[202] Roche's BLA shows that the primary structure of EPO correlates with the predicted amino acid sequence derived from EPO cDNA (with the exception of the C-terminal arginine, as explained below) and to be identical to the primary structure of urinary EPO as determined by protein sequencing.[203] Further, both Roche's EPO beta starting reagent and urinary EPO are shown to lack the C-terminal arginine 166 predicted from the cDNA sequence, ending instead in an aspartate residue at position 165. As Roche's BLA correctly observes:

> All EPO products analyzed so far – either from human urine or recombinant production – only contain 165 amino acids, missing the last arginine residue. This has been shown by various methods . . . ."[204]

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[201] Strickland Depo. Tr. (3/9/07) at 261-66 ("all of the structures found on recombinant EPO are also found on urinary EPO").

[202] See ITC-R-BLA-00004029, 5580; Recny, M.A., et al. (1987) *J. Biol. Chem.* 262: 17156-63.

[203] ITC-R-BLA-00005582-83, ITC-R-BLA-00005596-15, ITC-R-BLA-00005618-32

[204] ITC-R-BLA-0005616; *see also* ITC-R-BLA-00004029; Recny, M.A., et al. (1987) *J. Biol. Chem.* 262: 17156-63.

157. Roche's BLA also shows no difference between the glycosylation of the epoetin beta starting reagent and urinary EPO. For example, they are both glycosylated at the same positions: 24, 38 and 83, and at the serine residue at position 126.[205] Furthermore, the BLA states that "no other modifications were detected than the natural occurring ones."[206] Additionally the total amounts of all carbohydrates were determined for Roche's EPO beta starting reagent in relative molar amounts by carbohydrate composition analysis using gas chromatography.[207]

158. Roche's BLA also shows no differences in the content of sialic acid sugars between epoetin beta and urinary EPO. Roche conducted a study with the objective of quantifying the sialic acids per molecule of EPO beta starting reagent applying chromatographic techniques to determine the total amount of sialic acids in 11 samples.[208] With regard to EPO beta the study's findings "revealed comparable molar amounts of sialic acids in the range of 11-12 mol/mol EPO," i.e., 11 to 12 sialic acids per molecule EPO.[209] The study also found that "[i]t has been shown for the human urinary EPO as well as for the recombinantly produced EPO in CHO cells that the number of sialic acids is in the range of 10 to 13 per molecule EPO."[210] In my opinion, this study supports the conclusion that the sialic acid content of Roche's EPO beta starting reagent cannot be distinguished from that of urinary EPO.

159. Thus, it is my opinion that the data submitted on Roche's EPO beta starting reagent does not demonstrate any difference in glycosylation from urinary EPO. Nor is there any data to

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[205] See ITC-R-BLA-00004029; ITC-R-BLA-00005647-55.
[206] ITC-R-BLA-00005599
[207] *See* ITC-R-BLA-0005656-57
[208] ITC-R-BLA-0005658-60 at 5658
[209] *Id.*; *see also* ITC-R-BLA-00005669
[210] *Id.*; *see also* Sasaki (1987), *supra* (reporting 10.4 moles of sialic acid per mole of urinary EPO)

support a contention that Roche's EPO beta starting reagent differs in glycosylation from naturally occurring plasma or serum EPO. For all anyone knows there may be other naturally occurring EPOs that are indistinguishable from epoetin beta. For all the reasons noted above, it is my opinion that Roche's epoetin beta starting reagent used in the synthesis of CERA is not a "non-naturally occurring" EPO glycoprotein according to the claim 3 of the '933 and its dependent claims. And therefore, under Amgen's mistaken logic, the "EPO in" CERA is not non-naturally occurring.

d. MIRCERA™ Is Not a Pharmaceutical Composition According to Claims 9, 11, 12 and 14 of the '933 Patent

160. Claim 9 of the '933 patent, is dependent from claim 3 (discussed above) and requires:

> A pharmaceutical composition comprising an effective amount a glycoprotein product effective for erythropoietin therapy according to claim 1, 2, 3, 4, 5 or 6 and a pharmaceutically acceptable diluent, adjuvant or carrier.

Claims 12 is also directed to pharmaceutical compositions comprising an effective amount of a glycoprotein product as claimed in claim 3 (through claim 7). Claims 11 and 14 cover a method of treating a kidney dialysis patient comprising administering the pharmaceutical compositions of claims 9 and 12, respectively. In my opinion, MIRCERA™ is not pharmaceutical composition within the scope of these claims because MIRCERA™ does not contain a glycoprotein product of the expression in a mammalian host cell of an exogenous DNA sequence encoding human erythropoietin. Therefore, the asserted claims of the '933 patent are not (and will not be) literally infringed or infringed under the doctrine of equivalents.

161. As a preliminary matter, I note that Amgen's counsel told the Court during the April 17, 2007 Markman Hearing that I understand the term "pharmaceutical composition" to require human administration: