EXHIBIT 29

PART TWO

A. I don't know the individual who we obtained from. But this is obtained from – I believe so – through Columbia University, yes.

Q. Okay. And now, the – you see where it talks about a DHFR negative? Do you see that?

A. Yes.

Q. That technology of using DHR [sic] negative as a selectable marker, was that known to you?

A. Yeah, it was known to – to the scientists at the time.

Q. All right. And so who did the work of putting together the EPO gene with the DHFR gene?

A. In this case, it's – again, Jeff Browne's group.

Q. Okay. And did you have to instruct them or give them any guidance on how to do this?

A. No, I don't have to. Just like I give the piece DNA to my associate, and tell them to like it or something, they wouldn't know what to do.

Q. I'm going to ask you about who did the work and the use of methotrexate just to focus you for when you read.

A. (Examining document) Yeah. This – again, this is done by Jeff Browne's group.

Q. Okay. Now, this paragraph talks about inhibiting using methotrexate. Do you see that?

A. Where is it?

Q. It's line 45, 46, 47.

A. Yes.

Q. Did you learn about how to do that from Dr. Browne?

A. This is known at the time, when you want to select cells which – let me put it this way: This methotrexate is long – is known – is known to – to inhibit dihydrofolate reductase. This is known. This is the way the people use to amplify the DHFR gene, which is known at the time.

A. The assay aspect was carried out by Joan Egrie's groups. And in terms of amplification, carry out culture, this I think is carried out by Jeff Browne's group.

Q. And did you give either of those groups instructions how to do what's in this paragraph of?

A. They already been doing this so long. I don't need to give them any instruction.

Q. And there, it says on line 25, "Standard screening procedures are being employed in an attempt to isolate genetically homogeneous clones with the highest production capacity." Do you see that?

A. Yes.

Q. Was that accurate?

A. Yes. That's what it says here, yes.

Q. And who did that work?

A. Again, this is done by Jeff Browne's group, I think.

(*See also* Lin Interference Testimony cited *infra* in paragraphs 211-16).

103. As further illustrated by the prior art described below, to express recombinant human erythropoietin Lin adopted essentially the same approach that many researchers at that time had used to express numerous mammalian glycoproteins, including a variety of human glycoproteins. Nothing in the methods Lin used to express a human glycoprotein was novel or departed in any significant way from the prior art. A skilled scientist would have found the approach Lin adopted the natural and obvious choice for expressing a human glycoprotein, and would have had a reasonable expectation that such an approach would work to express a functional *in vivo* biologically active human EPO. Dr. Lin himself held such an expectation. (*See* AM-ITC 00174334-35 at 17:12-15 ("Q. Did you accept the fact that if the gene could be cloned that it could be successfully incorporated into host cells for the purpose of expressing erythropoietin? A. Yes, I would think so. Yes."); Lin Depo. Tr. (3/29/07) at 368 ("Q. My question was, whether you had the expectation when you had the genomic EPO gene that when put into a mammalian cell and expressed, that the resulting EPO would be biologically active? [objection interposed] A. Of course, we would expect that it – to be – to have that activity – in the biological activity.")).

B. Prior Use of Recombinant DNA Technology To Express Mammalian Proteins

104. One of the first widespread applications of recombinant DNA technology was in the process of "cloning" or making identical copies of a particular DNA fragment, most commonly by using bacterial cells, as was described above. Soon after, this technology was also applied to using cells as "host cells" or cellular factories to express foreign proteins by introducing DNA encoding foreign proteins into the cells under circumstances, outlined below, that caused the protein expression machinery of the host cell to be applied to the DNA sequence that was introduced.

105. To achieve the goal of expressing mammalian proteins in bacterial systems, researchers, by modifying the vectors used for DNA cloning, designed expression vectors that one could use both to introduce and express foreign DNA encoding such desired proteins. Such expression vectors ordinarily incorporated appropriate bacterial DNA regulatory sequences linked to the inserted gene that would direct the bacterial transcription machinery to transcribe the coding sequence into mRNA, and a selectable marker gene that could be used to identify and isolate recombinant cells carrying the expression vector. Such expression strategies allowed expression of several mammalian genes using bacterial host cell systems. (Itakura 1977, Goeddel 1979, Martial 1979).

106. At the same time, researchers recognized that when made in bacterial cells, many mammalian proteins would lack the post-translational modifications found in mature mammalian proteins, such as glycosylation. While experience with bacterially expressed proteins had demonstrated that certain mammalian proteins could retain biological function, it was known that post-translational modifications carried out in mammalian cells, especially for many secreted mammalian proteins, could contribute to the proper folding, stability or biological function of

such proteins. In addition, production of a biologically active mammalian (human) protein from bacteria often required additional steps after isolating the protein, which were required to renature or refold the protein and restore biological activity. (Goeddel 1979, Marston 1986). For these reasons, researchers developed analogous methods for directly expressing mammalian (human) proteins in mammalian cells.

107. Prior to October 1983, the prior art described the successful expression of various human and other mammalian glycoproteins in a functional and biologically active form from suitable mammalian host cells, including CHO cells. Methodology was widely available to one of skill for carrying out the various steps necessary to express a cloned mammalian gene in a recombinant mammalian host cell. As detailed below, the skilled scientist would have had knowledge of suitable host cells for expressing glycosylated proteins, expression vectors for use in such host cells, techniques for introducing foreign or exogenous DNA into mammalian cells, amplification of the introduced DNA in the mammalian host cell, and techniques for isolating and purifying the expressed recombinant protein from host cell cultures.

108. Methods for transforming mammalian host cells, that is, changing the cell genome by introducing foreign DNA, had been in use even before recombinant DNA technology was routinely used to introduce cloned DNA fragments into mammalian cells. Numerous examples of transformation had been described where such methods were carried out by isolating chromosomes from human cells and introducing them into cells of another species, or by introduction of specific fragments of viral DNAs. Several different mammalian cell lines, including human cells, several mouse cell lines and Chinese Hamster Ovary (CHO) cells were routinely used in such studies. (Merril 1971, Willecke 1976, Wullems 1976, Pellicer 1978, Graf 1979).

109. Prior to October 1983, cloned DNA was routinely used for transformation of mammalian and other vertebrate cells. Several widely used vertebrate cell lines were known to be suitable for use as host cells for expressing foreign proteins, including mouse, human, monkey and hamster cell lines such as CHO, BHK, COS, MDCK, VERO and HeLa cells, and one could routinely propagate and culture such cell lines. (Canaani 1982, Gething 1981, Goeddel 1979, Scahill 1983, Sveda 1981, Zinn 1982; Goeddel US Pat. No. 4,766,075; McCormick US Pat. No. 4,966,843; McCormick US Pat. App. 438,991). Expression vectors suitable for use with such host cells were also well known in the art. (Gething 1981, Kaufman 1982, Wigler 1977; Levinson US Pat. No. 4,741,901; Axel US Pat. No. 5,149,636). For example, expression vectors would ordinarily include a promoter located in front of the gene to be expressed, and any necessary ribosome binding sites, RNA splice sites, polyadenylation site, and transcriptional termination sequences, an appropriate selectable marker and if intended for use as an autonomously replicating vector in the mammalian host cell, an origin of replication functional in the host cell. (Levinson US Pat. No. 4,741,901; Toole US Pat. No. 4,757,006 at cols. 7-9; Goeddel US Pat. No. 4,766,075; Axel US Pat. No. 5,149,636).

110. Prior to October 1983, each of these common features of an expression vector was well described in the art. Numerous suitable promoters were well known and included various known viral promoters, such as those derived from SV40, adenovirus and polyoma viruses, as well as various cellular promoters. (Mayo 1982, Gething 1981, Kaufman 1982, Wigler 1977; Levinson US Pat. No. 4,741,901). Further, it was well known prior to October 1983 how to position such promoters in an expression vector such that one could control expression of an adjacent coding sequence in the host cell. (Kaufman 1982). In addition to promoter sequences, such vectors could also include enhancer sequences, such as those obtained from animal viruses

such as SV40, polyoma, bovine papilloma, retrovirus, or adenovirus. (Toole '006 patent, col. 8). Additionally, the prior art described a number of suitable selectable markers that one could use to facilitate selection and isolation of stably transformed recombinant clones. (Chang 1976, McBride 1973, Shimke 1978, Urlaub 1980, Wigler 1977, Wigler 1979).

111. While long term expression of recombinant proteins would ordinarily be carried out by stably transforming host cells with an appropriate expression vector, transient transformation of mammalian host cells was also commonly used as a convenient method to quickly express sufficient amounts of recombinant proteins for initial characterization and study. A number of COS monkey cell lines were known as particularly suitable host cells for transient expression. The COS genome contains a stably integrated functional SV40 T antigen gene. As a consequence, plasmid expression vectors bearing an SV40 replication origin will autonomously replicate in these cells, due to the continuous presence of T antigen. (Gluzman 1981, Gray 1983, Higashi 1983).

112. As exemplified by the following studies, COS cells, as well as similar transient expression systems were widely used for short-term amplified expression of various cloned genes for human and other glycoproteins in sufficient quantities to allow initial characterization and evaluation of a functional, glycosylated form of the protein. For example, primary African Green monkey cells were used to express the cloned gene for the influenza virus hemagglutinin surface glycoprotein (HA). (Sveda 1981). The COS cell produced product was glycosylated and was functionally active as assayed by an erythrocyte agglutination assay, in which functional activity depends on glycosylation of the HA protein. (*Id.*).

113. Gething and Sambrook similarly used a recombinant SV40 based virus carrying an HA encoding cDNA to infect either CV-1 or COS-1 cells. (Gething 1981). HA expressed in

the recombinant host cells was characterized by radioimmunoassay, Western blot analysis (Fig. 3), indirect immunoflourescent staining (Fig. 4) and by a heamagglutination assay (Fig 5). (*Id.* at 622-23). The recombinant HA expressed from both CV-1 and COS-1 at high levels appeared normal in all respects, was glycosylated and displayed on the cell surface in an antigenic and biologically active form. (*Id.* at 624). In another example involving expression of a known glycoprotein, biologically active human interleukin-2 was expressed from COS-7 cells by transfecting the cells with an expression vector bearing an IL-2 cDNA fused to an SV40 promoter. (Taniguchi 1983). Similarly, a biologically active mouse IFN-β "indistinguishable from the authentic mouse interferon-β" was synthesized and secreted from COS-7 cells transfected with a cDNA encoding mouse IFN-β fused to an SV40 promoter. (Higashi 1983).

114. Another example of the use of mammalian host cells to express a glycoprotein prior to October 1983 is provided by U.S. Patent No. 4,741,901 (Levinson et al.), which describes recombinant expression of the Hepatitis surface antigen (HBsAG) glycoprotein. Hepatitis B is a viral disease which is transmitted through a virion or viral particle, which encloses and carries a DNA molecule encoding viral functions. A major glycoprotein component of the virion, HBsAG can be found in infected plasma in the form of small non-infectious spherical particles. Because antibodies against HBsAG are protective against Hepatitis B infection, these non-infectious particles can effectively be used as a vaccine.

115. Levinson describes expression of the coding sequence for HBsAG using two different transient expression systems using monkey cell lines, CV-1 and COS-7. In addition to describing preparation of a suitable vector for expressing the HBsAG gene, Levinson describes routine techniques for maintaining, culturing and transforming the monkey host cells. HBsAG was expressed in CV-1 cells using a viral vector consisting of a recombinant SV40 genome with

the coding region of the SV40 capsid protein replaced by that of the HBsAG gene. The vector was first introduced through a standard transfection technique in CV-1 cells co-infected with a temperature sensitive virus carrying the VP gene (tsA28), allowing for production of infectious particles carrying the recombinant SV40 genome. CV-1 cells infected with recombinant SV40 virus and tsA28 were used for expression of HBsAG. As characterized by sedimentation velocity, sedimentation rate and electron microscopic analysis, the HBsAG synthesized and secreted from the infected CV-1 cell cultures was indistinguishable from authentic 22 nm hepatitis surface antigen particles isolated from the medium of a Hepatitis virus infected liver cell line.

116. HBsAG was also transiently expressed in COS-7 cells using recombinant plasmid expression vectors bearing an SV40 replication origin. U.S. Patent No. 4,741,901 (Levinson et al.) As discussed above, such plasmid expression vectors will replicate in the COS-7 host cells due to the presence of SV40 T antigen, thereby amplifying expression of the recombinant protein encoded by the vector, in this case HBsAG. A comparison of COS-7 produced HBsAG with that of authentic HBsAg demonstrated that the recombinant product made in monkey host cells exhibited similar, if not identical, immunogenicity to authentic HBsAg, which had previously demonstrated effectiveness as a vaccine in humans. The kinetics and titers of anti-HBsAg antibody appearance in mice immunized with tissue culture derived HBsAg were indistinguishable from those observed in mice immunized with authentic HBsAg. (Levinson '901 patent col. 14).

117. In addition, prior to October 1983, it was known that one could achieve high levels of stable expression in mammalian cells by use of amplifiable expression vectors. (*See* Axel U.S. Pat. No. 4,399,216). One system particularly suited for expressing mammalian

proteins at high levels used a particular mutant CHO cell line (DHFR$^-$ CHO K1), and the DHFR coding sequence as a selectable marker. (Urlaub 1980, Kaufman 1982). To survive, DHFR$^-$ CHO cells require the presence of glycine, hypoxanthine (a purine source) and thymidine in the culture medium, due to a mutation in the DHFR gene, which renders the DHFR$^-$ CHO cells deficient in dyhydrofolate reductase (DHFR) enzyme activity. By incorporating the DHFR coding sequence into an appropriate expression vector, the DHFR coding sequence serves as a selectable marker for cells that stably integrate the vector into their chromosomes. When DHFR$^-$ CHO cells are grown in selective media lacking hypoxanthine and thymidine, only those cells stably transformed with an exogenous DHFR gene survive. (*See* Axel U.S. Pat. No. 4,399,216; Kaufman 1982).

118. Moreover, in cells that had stably integrated an expression vector containing the DHFR coding sequence, it was demonstrated that subsequent selection in increasing levels of methotrexate (MTX), an inhibitor of dihydrofolate reductase could be used to obtain MTX resistant cells containing a high copy number of the DHFR sequence, as well other DNA sequences carried on the expression vector. In this manner, one could select recombinant cell lines expressing substantial quantities of a mammalian protein through co-amplification of the DNA coding sequence for that protein. (*See* Kaufman 1982; Axel U.S. Pat. No. 4,399,216). Initially, such an approach was used for amplification of genes for the bacterial protein, XGPRT, and for SV40 virus T antigen protein. Subsequent studies demonstrated the utility of such methods for the amplification and expression of cloned genes for human proteins, including various glycoproteins such as interferons and tPA. (Kaufman 1982, Hayes 1983, McCormick 1984, Scahill 1983; Goeddel US Pat. No. 4,766,075).

119. Prior to October 1983, mammalian host cell systems as described above had been used to express a variety of mammalian (including human) glycoproteins. Much of this work related to an interest in using these glycoproteins as therapeutic agents. (Toole US Pat. No. 4,757,006; Goeddel 4,766,075; Goeddel US Pat. App. 438,991 at 1; Fiers Euro. Pat. App. EP0088540). As an example, interferons (IFNs) are secreted polypeptides that protect cells from virus infection. Prior to October 1983, two classes had been described: Type I IFN (IFN-α, IFN-β) induced by viral infections and Type II IFN (IFN-γ) induced after stimulation of T lymphocytes. (McCormick 1984). Both human IFN-β and IFN-γ were known glycoproteins. (Yip 1981). Prior to October 1983, several groups had independently expressed recombinant IFN-γ using mammalian host cells.

120. One such group at Biogen first isolated a cDNA clone for human IFN-γ from a library of mitogen-stimulated human splenocytes. (Fiers Euro. App. No. EP0088540; Scahill 1983). A mammalian host cell system, DHFR$^-$ CHO cells, was chosen in order to express a glycosylated form of the protein from the IFN cDNA, thereby allowing a comparison of its pharmacokinetic properties with the unglyscosylated form. (Fiers '540 app. at 49; Scahill 1983 at 4654). Fiers describes preparation of suitable expression vectors, as well as routine methodology for maintaining, culturing and stably transforming the cells, and for selection and screening of stable transformants. (Graham 1973, Kaufman 1982, Kaufman 1983, Subramani 1981, Urlaub 1980). To select stable transformants carrying the recombinant gene, Fiers used a co-transformation strategy, in which a co-transformed plasmid served as a selectable marker for selection and isolation of stable transformants. As described in the application, the DHFR$^-$ CHO host cell system provided an efficient approach for expressing large quantities of the recombinant protein in glycosylated form. (Fiers '540 app. at 52-53). In this regard, even

though it was known that the unglycosylated E. coli produced IFN-γ had biological activity, it was desirable to express a glycosylated form for comparative animal studies and clinical trials because of possible differences in the pharmacokinetic properties of the glycosylated protein. (Scahill 1983 at 4654).

121. Haynes and Wiessmann describes an additional example of using the DHFR$^-$ CHO cell line as a host cell for expression of both IFN-α and IFN-γ. This report additionally describes methods for obtaining high level expression of the recombinant IFN proteins by subjecting the transformed cells to methotrexate selection to amplify the introduced recombinant IFN genes. (Haynes 1983). Such an approach was successful in increasing expression of the recombinant IFN-γ by as much as several hundred-fold and allowed for production of large amounts of functionally active and glycosylated protein. (Haynes 1983 at 697-699, 702).

122. Prior to October 1983, the DHFR$^-$ CHO cell system had also been used to express the cloned gene for Interferon-β (IFN-β). (McCormick US Patent App. 438,991; McCormick US Patent No. 4,966,843; McCormick 1984). In particular, McCormick describes use of this host cell system with expression vectors for IFN-β utilizing either a constitutive (SV40) promoter or its own inducible promoter allowing increased expression through a superinduction protocol. Higher levels of expression could also be obtained through methotrexate selection of recombinant DHFR$^-$ CHO cell clones bearing increased copy number of IFN-β encoding DNA. (McCormick 1984).

123. Prior to October 1983, the DHFR$^-$ CHO cell system had also been used to express the cloned gene for Interferon-β (IFN-β). (McCormick US Patent App. 438,991; McCormick US Patent No. 4,966,843; McCormick 1984). In particular, McCormick describes use of this host cell system with expression vectors for IFN-β utilizing either a constitutive (SV40) promoter or

its own inducible promoter allowing increased expression through a superinduction protocol. Higher levels of expression could also be obtained through methotrexate selection of recombinant DHFR$^-$ CHO cell clones bearing increased copy number of IFN-β encoding DNA. (*Id.*). Two distinct major expression products were observed with apparent molecular weights of 23 kD and 18.5 kD corresponding to glycosylated and unglycosylated forms of IFN-β. Specific biological activity of the CHO produced product was determined by two assay methods. The 23kD glycosylated form was highly active with an approximate specific activity of 10^9 U/mg, while the unglycosylated 18.5 kD form was 300 times less active. (McCormick 1984 at 171). I note the authors refer to work from another group at Cetus (that at the time was unpublished) reporting that wild type recombinant interferon expressed in E. coli had significantly lower specific activity (10^7 U/mg) than the CHO cell produced material. (McCormick 1984 at 171, Mark 1984 at 5662).

124. In this regard, McCormick notes the desirability of producing recombinant interferon-β through mammalian host cell expression over production in bacterial cells. While studies of E. coli-produced IFN-β suggested that "it retains biological activity similar to that of native human IFN-β even without the glycosyl moieties, it exhibits altered physical properties which may be due in part to the absence of glycosyl residues. In order to correctly characterize IFNs and study their efficacy as therapeutic agents, it would be desirable to produce them in animal hosts where the protein would be expected to be glycosylated and the conformation closest to that of native human IFNs." (McCormick '991 app. at 3).

125. In summary, both the '843 patent and the '991 priority application disclose that human IFN-β is a glycoprotein as indicated by its carbohydrate content and remark that the recombinant human proteins produced in the host cell systems used were "expected to be

glycosylated and in conformation closest to that of native human IFNs." (McCormick '991 app. at 2-3; McCormick '843 patent at col. 1:49-50, 2:3-8). Moreover, the '991 application claims a method for production of interferon "where in said interferon is glycosylated" (McCormick '991 app., claims 13-14; McCormick '843 patent, claim 15). In particular, the '991 application describes use of mammalian cells ('991 app. at 4), CHO cells ('991 app. at 10), CHO cells deficient in DHFR activity ('991 app. at 9, 11-12), use of methotrexate with CHO cells ('991 app. at 15), viral promoters in mammalian cells, including SV40 ('991 app. at 8-9), amplification with methotrexate ('991 app. at 15), transfecting DHFR deficient CHO cells ('991 app. at 12-14), suitable growth conditions for transfected cells ('991 app. at 14-15), pharmaceutical compositions of interferon ('991 app. at 10), and that the disclosed recombinant techniques produce glycosylated products "substantially identical in structure, properties and confirmation to native IFNs" ('991 app. at 17) unlike prior art interferons that "exhibit[] altered physical properties which may be due in part to the absence of glycosyl residues." ('991 app. at 3; '843 patent at col. 2:1-3).

126. The utility of mammalian host cells for expressing functional recombinant human glycoproteins was further demonstrated by use of DHFR$^-$ CHO to express human tissue type plasminogen activator (tPA), a glycoprotein involved in regulating blood clotting in the body. Scientists in Belgium in 1979 first purified and characterized tPA and showed that it could dissolve large clots in experimental animals. Genentech in 1981 undertook development of the drug using recombinant biotechnology techniques to produce sufficient quantities of recombinant human tPA to be tested therapeutically. In 1984, Genentech began clinical trials using the CHO cell produced protein. (*See* TPA Approval Blood Clot Dissolver, http://www.fda.gov/bbs/topics/NEWS/NEW00191.html). Goeddel et al. U.S. Patent No.

4,766,075, filed April 7, 1983, describes the cloning of the human tPA gene and expression of the DNA encoding the human gene in CHO cells, which the patent explains was to produce the human protein to provide a human tPA suitable for "prophylactic or therapeutic treatment of human beings for various cardiovascular conditions or diseases" and to produce the human protein in "sufficient amounts to initiate and conduct animal and clinical testing as prerequisites to market approval." ('075 patent at col. 3). Essentially the same description of the cloning and expression of recombinant human tPA was published in European Patent Application Publication EP0093619, dated November 9, 1983.

127. The Goeddel '075 patent describes construction of expression vectors containing the DNA sequence encoding human tPA protein, introduction of the vectors into DHFR$^-$ and DHFR+ CHO cells, and selection and isolation of stably transformed recombinant CHO cell clones expressing human tPA. In particular, a sequence encoding human tissue plasminogen activator was inserted into an expression plasmid containing a mutant DHFR with low binding affinity for MTX. ('075 patent at cols. 24-25). The patent further describes the use of this expression plasmid and recombinant CHO cells stably transformed with the expression plasmid to generate recombinant CHO cell clones carrying amplified copies of the recombinant human tPA gene for high level expression of the recombinant protein. ('075 patent at cols. 25-28). The amplified CHO cell clones all showed increased levels of tPA production, on the order of 100-fold over that exhibited by the unamplified cell cultures, resulting in levels approaching 50pg/cell/day. ('075 patent at col. 27).

C. Amgen's Flawed Arguments During Prosecution of its '868 and '698 Process Patent Claims

128. During the prosecution of Ser. No. 113,179 (which led to the '868 and '698 patents-in-suit), Amgen submitted a Second Preliminary Amendment in which it presented new

claims 65 through 69 generally directed to a process for making an in vivo biologically active erythropoietin protein by expressing a DNA sequence in a mammalian host cell, in particular CHO cells (claim 66) and COS cells (claim 67). (AM-ITC 00953205-225 (Second Preliminary Amendment, dated May 24, 1988)). Amgen argued these claims were patentable and non-obvious in view of prior art describing host cell expression of recombinant proteins because the process recited by the claims to make erythropoietin was one of the first instances (if not the first instance) of producing in vivo biologically active human glycoprotein by expression in a recombinant host cell. (*See* AM-ITC 00953210; *see also* AM-ITC 00953223, AM-ITC 00953277).

129. Amgen argued that none of the prior art was relevant because erythropoietin was what it termed an "obligate glycoprotein," that is one requiring proper glycosylation for in vivo biological activity. In particular, Amgen argued that: "Unlike other human glycoproteins such as the interferons and Interleukin-2, human erythropoietin was conspicuously known to be an obligate glycoprotein and no hope at all existed for isolating in vivo active material from recombinant host cells unless, at a minimum, both the issues of required polypeptide sequence and of required glycosylation could be successfully attended to." (AM-ITC 00953214). Amgen acknowledged that tissue plasminogen activator (t-PA) was also a human "obligate glycoprotein" ("Naturally occurring tPA is believed by applicant to share with erythropoietin the characteristic of being an obligate human glycoprotein."), but represented that none of the prior art described expression of recombinant tPA in a mammalian host cell.

130. In particular, Amgen conducted a computer-assisted prior art search and reported that of the references discovered during the prior art search "[t]he only reference located which appeared to relate to recombinant production of an in vivo biologically active obligate human

glycoprotein was Collen et al., J. Pharm. & Expt. Therapeutics, 231, 146-152 (1984) relating to tissue plasminogen activator." (AM-ITC 00953220-221). Amgen asserted that the Collen reference was "accepted for publication and published well after Applicant's initial description of COS cell expression and in vivo biological activity reported in parent application Serial Nos. 561,024 and 582,185" but that "[t]he reference does not describe how the recombinant mammalian host cell expression was prepared." (AM-ITC 00953221).

131. Amgen stated that "[i]n a subsequent attempt to determine whether published patent applications might exist concerning mammalian cell production of recombinant tPA, a search was conducted for applications regarding tPA in the Derwent World Patent Index data base." (AM-ITC 00953222). Amgen argued that three applications located were not relevant to patentability of the pending claims. (AM-ITC 00953222). In particular, Amgen cited EP 0 093 619 ("EP '619") by Goeddel et al., which as discussed above, describes the cloning and recombinant expression of tPA. (AM-ITC 0095322; EP '619 Application). Amgen however argued that EP '619 "contains no description of [the] use of mammalian host cell expression systems for tPA production." (AM-ITC 00953222 (emphasis in original)). In fact, Amgen stated "that the only clear mention of such systems was entirely speculative and appears in the 'Summary of Invention' at page 7." In addition, depending upon the host cell, the human tissue plasminogen activator hereof may contain associated glycosylation to a greater or lesser extent compared with the native material." (AM-ITC 00953222).

132. Notably, Amgen continued to argue that none of the prior art relating to recombinant expression of glycosylated human proteins was relevant to the subject matter of its pending process claims would have been obvious. (*See, e.g.*, AM-ITC 00953233 ("urges that EPO is an obligate glycoprotein and that the Yokota et al. multi CSF is not an obligate

protein...."); AM-ITC 00953277 ("it appears that Applicant may have been the first to have successfully produced a human obligate glycoprotein by recombinant methods"); AM-ITC 00953699-700 ("To the extent that Yokota et al. might have been cited as prior art under 35 U.S.C. §102(e)/103 on the issue of obviousness of the claimed subject matter, it is also irrelevant because human M-CSF is not an obligate human glycoprotein.").

133. As discussed below, Amgen's arguments to the Examiner were seriously flawed. Most significantly, Amgen inaccurately characterized the Goeddel EP '619 application. The EP '619 reference in fact describes using the same mammalian host cells (COS cells and CHO cells) as disclosed in Amgen's patent to express an "obligate" human glycoprotein. Additionally, I disagree with Amgen's argument that the prior art relating to recombinant expression of human glycoproteins was irrelevant. As discussed elsewhere herein, the prior art expression of recombinant human glycoproteins, including tPA, would have provided the skilled scientist with a reasonable expectation of success in using mammalian host cells such as COS and CHO cells to produce an in vivo biologically active human erythropoietin.

134. Among the many details of using mammalian host cells to express a recombinant human tPA, the EP '619 application describes the use of mammalian cells and other vertebrate cells, in particular CHO cells (EP '619 at 15-16), as well as CHO cells deficient in DHFR activity (EP '619 at 17), use of methotrexate for amplification of foreign DNA introduced into the host cell (EP '619 at 17, 21 43, 48), viral promoters in mammalian cells, including SV40 (EP '619 at 16), techniques for transfecting CHO cells (EP '619 at 48), suitable growth conditions for transfected cells (EP '619 at 49) and pharmaceutical compositions of tPA (EP '619 at 6, 50). Significantly, the Goeddel application indicates that the recombinant techniques described in the application enable "the production of sufficient quality and quantity material to initiate and

conduct animal and clinical testing" (EP '619 at 1) unlike prior art tPA "isolated from various human tissue, e.g., uterine tissue, blood, serum ... and from cell culture." (EP '619 at 3; *see also id.* at 4, 7). In this regard, the application claims a "composition comprising a therapeutically effective amount of human tissue plasminogen activator according to Claims 1-5 in admixture with a pharmaceutically acceptable carrier." (EP '619, claim 11; *see also id.*, claims 12-15).

D. The Prior Art Contradicts Amgen's Arguments During the '008 Patent Prosecution That Cloning the EPO Gene From a cDNA Library Would Not Have Been Obvious

135. Lin Application Ser. No. 06/675,298 ("the '298 application") issued as US 4,703,008 on October 27, 1987. The '298 application was the parent application to all the asserted Lin patents, as indicated on the face of these patents. During prosecution of the '008 patent, the examiner rejected the pending claims to DNA sequences encoding human erythropoietin as obvious over the prior art. In particular, Examiner Tanenhotz noted that:

> "Ullrich et al and Martial teach a basic process for isolating mRNA and converting it into a cDNA library for use in cloning and expressing mammalian genes. It would be obvious to prepare erythropoietin as a fused peptide by extracting the messenger RNA for erythropoietin from kidney cells known to be rich therein and converting that mRNA to a cDNA library in the manner taught by Ullrich et al or Martial." (AM-ITC 00873694-95).

136. In response, Amgen's attorney argued that by the Lin patent filing date, one of skill could not have identified a suitable cDNA library from which to isolate a human erythropoietin cDNA clone:

> Thus, as pointed out in Applicant's submission of October 3, 1986, there was, at the time of the invention, a serious problem securing what could be recognized as erythropoietin-producing cells, much less cells producing high levels of the protein or cells "known to be rich" in erythropoietin messenger RNA such as would provide a cDNA library with multiple copies of erythropoietin-encoding DNA.

> For the Examiner to characterize the publications of Ullrich et al. and Martial et al. as readily enabling the preparation of a library including translatable human erythropoietin cDNA by an ordinarily skilled worker is unsupported and in fact contradicted by other references comprising the totality of the art. (AM-ITC 00873748).

137. As evident from the prior art described above, prior to October 1983, a number of EPO producing cell lines had been described or had been developed that one of skill in the art would have considered as obvious sources for EPO mRNA. As discussed above, in view of this art, it would have been obvious to use such EPO producing cells to construct a cDNA library, and one of skill would have had a reasonable expectation of success in isolating an erythropoietin cDNA clone from such a library. In fact, in the case of the 1411-H cells, prior to October 1983, Amgen itself had generated data indicating that the 1411-H yolk sac carcinoma cells produced significant amounts of erythropoietin over a prolonged period of time. (*See* Egrie Depo. Tr. (3/27/07) at 270-280; AM-ITC 00052045; AM-ITC 00057704; AM-ITC 00057723; AM-ITC 00057735; AM-ITC 00057708-18, AM-ITC 0057689-701; AM-ITC 00057687; AM-ITC 00057688; *see also* Lin Depo. Tr. (3/28/07) at 19:7-30:20; AM-ITC 00174810-14; AM-ITC 00175694-95; AM-ITC 00174790-95; AM-ITC 00168328-33).

VII. The Asserted Lin Patent Claims Would Have All Been Obvious to One of Ordinary Skill prior to October 1983 in View of the Prior Art Discussed Above

138. As discussed above, in my opinion, it would have been obvious to one of skill prior to October 1983 to isolate a cDNA encoding human erythropoietin and then to produce an *in vivo* biologically active recombinant human erythropoietin by expressing such a cDNA in a mammalian host cell, such as a CHO cell. As a consequence, prior to October 1983, each of the claimed processes, products and pharmaceutical compositions recited by each of the asserted Lin patent claims would have been obvious. As explained below, none of the particular limitations

found in any of the asserted claims define any further distinction that would have rendered the particular claimed products or processes non-obvious to one of skill in the art at the time.

139. I have reviewed the proposed claim constructions provided to the Court by Roche and by Amgen, which are attached to this report as Ex. D. For the purpose of my analysis, I have applied the construction decided previously by the Court or the narrower of the two constructions proposed by the parties unless otherwise indicated. To the extent the Court adopts a claim construction that differs in any assumption I have relied upon, I intend to supplement my analysis to reflect that claim construction.

A. Asserted Process Claims

(i) '868 patent claims 1 and 2

140. The only asserted independent claim of the '868 patent, claim 1 reads:

> 1. A process for the production of a glycosylated erythropoietin polypeptide having the *in vivo* biological property of causing bone marrow cells to increase production of reticulocytes and red blood cells comprising the steps of:
>
> (a) growing, under suitable nutrient conditions, mammalian host cells transformed or transfected with an isolated DNA sequence encoding human erythropoietin; and
>
> (b) isolating said glycosylated erythropoietin polypeptide therefrom.

141. I understand '868 patent claim 1 as reciting "a process for the production of a glycosylated erythropoietin" comprising two steps: (1) "growing…mammalian host cells transformed or transfected with an isolated DNA sequence encoding human erythropoietin" and (2) isolating said glycosylated erythropoietin polypeptide therefrom." The glycosylated erythropoietin has the "in vivo biological property of causing bone marrow cells to increase

production of reticulocytes and red blood cells." Dependent claim 2, also asserted is directed to the process of claim 1 where "said host cells are CHO cells."

142. As described above, prior to October 1983, it would have been obvious to isolate a cDNA clone encoding human erythropoietin from a cDNA library. As also described above, it would have been obvious to use a mammalian host cell as Amgen defines it, such as a COS cell or CHO cell to express the encoded human erythropoietin protein, such that it would have *in vivo* biological activity. Mammalian cell lines such as COS cells and CHO cells had been widely used for recombinant expression. Methods for culturing such cells, as well as for transforming such cells with foreign DNA were well described and routine. It further would have been obvious to use methods for amplification to express the recombinant human erythropoietin protein at high levels. Moreover, based on routine methods in the art for protein purification, it would have been obvious to isolate the biologically active erythropoietin from the media of the transformed mammalian host cells and thereby carry out the second required step in the process of '868 claims 1 and 2. The limitation to using CHO cells according to claim 2 in my opinion fails to provide any non-obvious distinction over such a method as CHO cells would have been an obvious choice to use for recombinant expression of human glycoproteins. Indeed, as noted above, the use of CHO cells was suggested to Dr. Lin by his friend, a curator at the ATCC. (*See* Lin Depo. Tr. (3/28/07) at 63-67).

(ii) '698 Patent Claims 4-9

143. The two asserted independent claims of the '698 patent read as follows:

> 4. A process for the production of a glycosylated erythropoietin polypeptide having the in vivo biological property of causing bone marrow cells to increase production of reticulocytes and red blood cells comprising the steps of:

> a) growing, under suitable nutrient conditions, vertebrate cells comprising promoter DNA, other than human erythropoietin promoter DNA, operatively linked to DNA encoding the mature erythropoietin amino acid sequence of FIG. 6; and
>
> b) isolating said glycosylated erythropoietin polypeptide expressed by said cells.
>
> 6. A process for the production of a glycosylated erythropoietin polypeptide having the in vivo biological property of causing bone marrow cells to increase production of reticulocytes and red blood cells comprising the steps of:
>
> a) growing, under suitable nutrient conditions, vertebrate cells comprising amplified DNA encoding the mature erythropoietin amino acid sequence of FIG. 6; and
>
> b) isolating said glycosylated erythropoietin polypeptide expressed by said cells.

144. I understand independent claims 4 and 6 of the '698 patent are both directed to "a process for the production of a glycosylated erythropoietin polypeptide" comprising two steps: (1) "growing . . . vertebrate cells" comprising certain DNA sequences; and (2) "isolating said glycosylated erythropoietin polypeptide expressed by said cells." The glycosylated erythropoietin polypeptide exhibits the "in vivo biological property of causing bone marrow cells to increase production of reticulocytes and red blood cells." Both claims specify that the cells comprise "DNA encoding the mature erythropoietin amino acid sequence of FIG. 6." Such DNA would include a sequence encoding human erythropoietin. Claim 6 specifies that the DNA sequence encoding human erythropoietin is "amplified." Claim 4 specifies that the cells also comprise "promoter DNA, other than human erythropoietin promoter DNA" which is "operatively linked" to the "DNA encoding the mature erythropoietin amino acid sequence of FIG. 6." Such cells would therefore include a sequence encoding human erythropoietin.

145. In my opinion, both these claimed processes would have been obvious to one of skill prior to October 1983. As described above, prior to October 1983, it would have been obvious to isolate a cDNA clone encoding human erythropoietin from a cDNA library. Mammalian cells are vertebrate cells, as recited by the claim. As further described above, it would have been obvious to use a mammalian host cell such as a COS cell or CHO cell to express the encoded human erythropoietin protein, such that it would have *in vivo* biological activity. To do so, it would have been obvious to use one of several amplifiable expression vectors in which the sequence encoding human erythropoietin was placed under the transcriptional control (that is, operably linked) of suitable non-human erythropoietin promoter DNA sequences, such as SV40 or other viral promoters. It would have been obvious to introduce such an expression vector into one of several mammalian cells routinely used for recombinant expression of glycoproteins, such as a COS or CHO cells, using routine and well described methodology for transformation of mammalian host cells, including transfection and infection, as explained above. It would have been obvious to carry out these processes by growing such mammalian host cells under suitable nutrient conditions, as methods for culturing such cells were well known and routine.

146. It further would have been obvious to use methods for amplification, thereby generating cells comprising amplified DNA encoding human erythropoietin, in order to express the recombinant human erythropoietin protein at high levels. Moreover, based on routine methods in the art for protein purification, it would have been obvious to isolate the biologically active erythropoietin from the transformed mammalian host cell cultures and thereby carry out the second required step of the method recited by '698 claims 4 or 6.

147. Dependent claim 5 further limits the process of claim 4 by specifying the promoter DNA be "viral promoter DNA." Dependent claim 7 further limits the process of claim 6 to using vertebrate cells that "further comprise amplified marker gene DNA." Dependent claim 8 further limits claim 7 by specifying that the amplified marker gene DNA is "Dihydrofolate reductase (DHFR) gene DNA." Dependent claim 9 is directed to the process of claims 4 and 6 where "said cells" used in the process "are mammalian." In my opinion, these further limitations to the processes recited by '698 claims 4 or 6 would have all been obvious and routine, as discussed above. Prior to October 1983, numerous viral promoters were known and it was well known how to position such promoters in an expression vector to drive transcription of an adjacent coding sequence. As discussed previously, it would have been obvious to use a selectable marker, including a gene for DHFR, to amplify DNA introduced into the host cell and thereby generate cells comprising amplified marker DNA, in order to express the recombinant human erythropoietin protein at high levels. Lastly, as also noted above, use of mammalian host cells, such as COS or CHO cells would have been an obvious choice for expressing a recombinant human glycoprotein.

(iii) '349 Patent Claim 7

148. The only '349 patent claim asserted is dependent Claim 7, which depends from any of '349 patent claims 1-6. Incorporating those claims, claim 7 can be read as follows:

> Claim 7. A process for producing erythropoietin comprising the step of culturing, under suitable nutrient conditions, vertebrate cells [which can be propagated in vitro and which are capable upon growth in culture of producing erythropoietin in the medium of their growth in excess of 100, 500, or 1000 U of erythropoietin per 10^6 cells in 48 hours as determined by radioimmunoassay, said cells comprising non-human DNA sequences which control transcription of DNA encoding human erythropoietin].

149. I understand dependent claim 7 of the '349 patent therefore describes "a process for producing erythropoietin" where a required step is culturing, "under suitable nutrient conditions" vertebrate cells described by claims 1, 2, 3, 4, 5 or 6, all of which include a DNA sequence encoding human erythropoietin. These claims specify that the cells can be propagated *in vitro* and are capable of producing erythropoietin in excess of 100, 500 or 1000 U per 10^6 cells in 48 hours. Claim 7 however does not require a specific rate of EPO production to be achieved when using these cells in the process.

150. As described above, prior to October 1983, it would have been obvious to isolate a cDNA clone encoding human erythropoietin from a cDNA library. Mammalian cells are vertebrate cells, as recited by the claim. As further described above, it would have been obvious to use a mammalian host cell such as a COS cell or CHO cell to express the encoded human erythropoietin protein, such that it would have *in vivo* biological activity. To do so, it would have been obvious to use one of several amplifiable expression vectors in which the sequence encoding human erythropoietin was placed under the transcriptional control (that is, operably linked) of suitable non-human promoter DNA sequences, such as SV40 or other viral promoters. It would therefore have been obvious to then introduce the vector into one of several mammalian cells routinely used for recombinant expression of glycoproteins, such as a COS or CHO cells. To do so, it would have been obvious to use routine and well described methodology for transformation of mammalian host cells, to select for transformants as explained above, and then to culture the recombinant host cells to produce a human erythropoietin. Moreover, based on routine methods in the art for protein purification, it would have been obvious to isolate the biologically active erythropoietin from the transformed mammalian host cell cultures.

151. As described above in section, prior to October 1983, the prior art described numerous examples of using amplification to achieve high level transient and stable expression of human glycoproteins in host cells such as COS cells or CHO cells, and expression vectors for use in such methods. For example, the Goeddel '075 patent describes use of amplification to generate recombinant host cells capable of expressing tPA in amounts of 28 to 98μg per 10^6 cells/48 hours. ('075 patent at col. 27, table 3).

152. Based on my review, the '349 patent does not define the standard against which the radioimmunoassay units recited in the '349 patent claims are to be defined, therefore leaving this term indefinite. The first mention of units however refers to erythropoietin with a specific activity of 70,400 units/mg of protein. ('349 patent at col. 7). Assuming production of erythropoietin with this specific activity, it would have been obvious to use known expression vectors and amplification methods to generate recombinant host cells capable of expressing in culture erythropoietin in the range of 100-1000 units (approximately 1.4 to 14 μg) per 10^6 cells/48 hours, as recited by '349 claim 7. (If one assumes a higher specific activity, the required level of protein expression would be correspondingly less).

153. In particular, it would therefore have been obvious to one of skill, by transforming a mammalian host cell with an expression vector provided with a DNA sequence encoding human erythropoietin, to generate mammalian host cells capable of expressing erythropoietin at levels recited by any of the various '349 patent claims 1-6, and to culture such recombinant host cells under suitable nutrient conditions to produce erythropoietin.

B. Asserted Claim to a Pharmaceutical Composition

(i) '422 Patent Claim 1

154. The only asserted '422 patent claim, independent claim 1 reads as follows:

> Claim 1. A pharmaceutical composition comprising a therapeutically effective amount of human erythropoietin and a pharmaceutically acceptable diluent, adjuvant or carrier, wherein said erythropoietin is purified from mammalian cells grown in culture.

155. I understand the '422 patent claim 1 as being directed to a pharmaceutical composition comprising a therapeutically effective amount of human erythropoietin. I understand that the Federal Circuit recently interpreted "therapeutically effective amount" as used in the '422 patent to mean an amount "that elicits any one or all of the effects often associated with in vivo biological activity of natural EPO, such as those listed in the specification, column 33, lines 16 through 22: stimulation of reticulocyte response, development of ferrokinetic effects (such as plasma iron turnover effects and marrow transit time effects), erythrocyte mass changes, stimulation of hemoglobin C synthesis and, as indicated in Example 10, increasing hematocrit levels in mammals." (*Amgen Inc. v. Hoechst Marion Roussel, Inc.*, 457 F.3d 1293, 1303 (Fed. Cir. 2006) ("*Amgen VII*")).

156. As described above, prior to October 1983, it would have been obvious to isolate a cDNA clone encoding human erythropoietin from a cDNA library. As further described above, it would have been obvious to use a mammalian host cell such as a COS cell or CHO cell to express the encoded human erythropoietin protein, such that one would expect the expressed recombinant human erythropoietin to exhibit the *in vivo* biological activity of the naturally occurring glycoprotein. For example, it would have been obvious to use one of several suitable expression vectors described in the prior art, including expression vectors suitable for use in amplification of the introduced erythropoietin cDNA and thereby allowing high level expression of the human erythropoietin.

157. It would therefore have been obvious to insert a cDNA encoding human erythropoietin into a suitable expression vector, and then introduce the vector into one of several mammalian cells routinely used for recombinant expression of glycoproteins, such as a COS or CHO cells. To do so, it would have been obvious to use routine and well described methodology for transformation of mammalian host cells, including transfection or infection, and to select transformants as explained above, and then to culture the recombinant host cells to produce a human erythropoietin. Moreover, based on routine methods in the art for protein purification, it would have been obvious to isolate the biologically active erythropoietin from the transformed mammalian host cell cultures.

158. In my opinion, the further limitation of '422 patent claim 1 that the human erythropoietin is in a pharmaceutical composition comprising a pharmaceutically acceptable diluent, adjuvant or carrier would have been obvious and routine. As indicated by the prior art cited elsewhere in this report, suitable pharmaceutical vehicles, that is diluents, adjuvants and carriers, and their formulation, were well known in the art and described in standard treatises, for example, Remington's Pharmaceutical Sciences by E.W. Martin. (*See also, e.g.*, Goeddel '075 patent at cols. 27-28). Much of the interest at the time in recombinant DNA technology was in using such an approach to produce recombinant human proteins in useful quantities in order to initiate and conduct animal and clinical testing. (*Id.*). Having expressed and isolated recombinant human erythropoietin, it would have been obvious to formulate a suitable pharmaceutical composition containing a recombinant human glycoprotein such as human erythropoietin, and comprising a well known suitable diluent, adjuvant or carrier for use in an animal or human subject. (*Id.*).

C. Asserted Product Claims and Claims Dependent on Those Product Claims

(i) '933 Patent Claims 3, 7-9, 11-12 and 14

159. The single asserted independent claim of the '933 patent reads as follows:

> Claim 3. A non-naturally occurring glycoprotein product of the expression in a mammalian host cell of an exogenous DNA sequence comprising a DNA sequence encoding human erythropoietin said product possessing the in vivo biological property of causing bone marrow cells to increase production of reticulocytes and red blood cells.

160. I understand '933 patent claim 3 is directed to a human erythropoietin product characterized as being a "non-naturally occurring glycoprotein product of the expression in a mammalian host cell of an exogenous DNA sequence comprising a DNA sequence encoding human erythropoietin." In this regard, the product is one that can be obtained from recombinant host cell transformed with foreign DNA encoding human erythropoietin, such as a cDNA sequence encoding human erythropoietin. The claim further recites that the product possesses the *in vivo* biological activity of causing bone marrow cells to increase production of reticulocytes and red blood cells. A recombinant protein does not occur in nature without human intervention. As such I would understand a "non-naturally occurring glycoprotein product" to include a recombinant glycoprotein product expressed from a mammalian host cell, such as a COS cell or CHO cell.

161. As described above, prior to October 1983, it would have been obvious to isolate a cDNA clone encoding human erythropoietin from a cDNA library. As further described above, it would have been obvious to use a mammalian host cell such as a COS cell or CHO cell to express the encoded human erythropoietin protein, such that one would expect the expressed recombinant human erythropoietin to exhibit the *in vivo* biological activity of the naturally occurring glycoprotein. In particular, it would have been obvious to insert a cDNA encoding human erythropoietin into a suitable expression vector, as described in the prior art, and then

introduce the vector into one of several mammalian cells routinely used for recombinant expression of glycoproteins, such as a COS or CHO cells. To do so, it would have been obvious to use routine and well described methodologies for transformation of mammalian host cells, including transfection or infection, and to select transformants as explained above, and then to culture the recombinant host cells to produce a human erythropoietin.

162. As evident from the previously discussed prior art [cite to section], it would have been obvious to use non-human mammalian host cells, including CHO cells to express a recombinant human glycoprotein that exhibited *in vivo* biological activity, with a reasonable expectation of success. The use of a non-human mammalian host cell to produce the human erythropoietin glycoprotein product of '933 patent claim 3, as recited in '933 patent claim 7, or specifically the use of a CHO cell as recited in '933 patent claim 8, therefore would have been obvious.

163. Dependent '933 patent claims 9 and 12 read as follows:

> 9. 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.
>
> 12. A pharmaceutical composition comprising an effective amount of a glycoprotein product effective for erythropoietin therapy according to claim 7 and a pharmaceutically acceptable diluent, adjuvant or carrier.

164. I understand these dependent claims describe pharmaceutical compositions containing an erythropoietin glycoprotein, including a human erythropoietin glycoprotein, where the erythropoietin is characterized as being non-natural or further characterized as being the product of expression of a mammalian host cell, including a non-human mammalian host cell.

Both claims require that the pharmaceutical composition includes a pharmaceutically acceptable diluent, adjuvant or carrier.

165. In my opinion, it would have been obvious to use the recombinant human erythropoietin product claimed by the '933 patent claims in a pharmaceutical composition containing a pharmaceutically acceptable diluent, adjuvant or carrier as recited in '933 patent dependent claims 9 and 12. As indicated by the prior art cited elsewhere in this report, suitable pharmaceutical vehicles, that is diluents, adjuvants and carriers, and their formulation, were well known in the art and described in standard treatises, for example, Remington's Pharmaceutical Sciences by E.W. Martin. (*See also, e.g.*, Goeddel '075 patent at cols. 27-28). Much of the interest at the time in recombinant DNA technology was in using such an approach to produce recombinant human proteins in useful quantities in order to initiate and conduct animal and clinical testing. (*Id.*). Having expressed and isolated recombinant human erythropoietin, it would have been obvious to formulate a suitable pharmaceutical composition containing a recombinant human glycoprotein such as human erythropoietin, and comprising a suitable diluent, adjuvant or carrier in order to use the human erythropoietin in an animal or human subject. (*Id.*).

166. Dependent '933 patent claims 11 and 14 read as follows:

> 11. A method for treating a kidney dialysis patient which comprises administering a pharmaceutical composition of claim 9 in an amount effective to increase the hematocrit level of said patient.
>
> 14. A method for treating a kidney dialysis patient which comprises administering a pharmaceutical composition of claim 12 in an amount effective to increase the hematocrit level of said patient.

167. I understand that both these dependent claims are directed to use of the pharmaceutical compositions of dependent claims 9 and 12 to treat a kidney dialysis patient by administering a sufficient amount of the pharmaceutical composition to increase the patient's hematocrit level. In my opinion, the recited methods would have been obvious to one of skill in the art. Having a source of recombinant human EPO, it would have been obvious to provide the recombinant EPO in a pharmaceutical composition and to use that pharmaceutical composition in treating a kidney dialysis patient. As early as 1971, it was appreciated that human EPO could be important for "possible therapeutic use in some types of refractory anemia . . . ." (Goldwasser 1971). The hypothesis that chronic renal failure, which typically requires dialysis, is associated with a refractory anemia due to insufficient renal production of erythropoietin was confirmed by studies demonstrating a dose-dependent correction of anemia in uremic sheep by parenteral administration of erythropoietin-enriched plasma. (Eschbach 1984 (original submission date July 5, 1983; published August 1984). Thus, these studies and others implied that EPO therapy should correct the hypoproliferative anemia observed in patients with chronic renal failure on maintenance dialysis. Thus, prior to October 1983, the desirability of treating dialysis patients with human EPO was widely recognized and appreciated. Moreover, as I noted above, it would have been obvious to use methods for amplification to generate host cells capable of expressing human erythropoietin at sufficiently high levels to allow one to isolate human erythropoietin in an amount sufficient to elicit any one or all of the effects often associated with *in vivo* biological activity of natural EPO, either in an animal, or in a human subject. I further note that in the specification of the patents-in-suit there is no example of use in a human. The patents are premised on the same expectation which renders them obvious.

(ii) '080 Patent Claims 3, 4 and 6

168. The single asserted independent claim of the '080 patent reads as follows:

> Claim 3. A non-naturally occurring erythropoietin glycoprotein having the in vivo biological activity of causing bone marrow cells to increase production of reticulocytes and red blood cells, wherein said erythropoietin glycoprotein comprises the mature erythropoietin amino acid sequence of FIG. 6.

169. Claim 3 of the '080 patent, the only asserted independent claim is directed to a "non-naturally occurring erythropoietin glycoprotein," which "comprises the mature erythropoietin amino acid sequence of Fig. 6." The claim further recites that the product possesses the *in vivo* biological activity of causing bone marrow cells to increase production of reticulocytes and red blood cells.

170. I note that Fig. 6 depicts the amino acid sequence of the primary expression product of human erythropoietin. When expressed in a mammalian cell, the N-terminal residue at position 166 is ordinarily cleaved during cellular processing of the protein, leaving the mature human erythropoietin protein which contains only 165 amino acids. (Lai 1986, Jacobs 1985). I note that the Lin patents provide no evidence regarding this specific post-translational modification. Moreover, the Lin patents provide no evidence indicating whether cleavage of the residue at position 166 has any effect on the claimed biological activity of human EPO. In this regard, to the extent that Amgen claims that the asserted '080 patent claims would cover a 165 amino acid human erythropoietin, there can be no patentable distinction between the 166 amino acid protein of '080 patent claim 3 and the 165 amino acid protein of '933 patent claim 3, which is also directed to a non-naturally occurring erythropoietin glycoprotein expressed from a mammalian host cell.

171. Therefore, to the extent that Amgen asserts that '080 patent claim 3 covers a recombinant 165 amino acid human erythropoietin, for example one expressed by a mammalian

host cell such as a COS cell or CHO cell, the claimed recombinant product would have been obvious for the same reasons as described above for '933 patent claim 3. Similarly for the same reasons as discussed for '933 patent claims, it would have been obvious to use a therapeutically effective amount of the recombinant human erythropoietin product claimed by '080 patent claim 3 in a pharmaceutical composition as recited in '080 patent claim 4, and consequently to use such a pharmaceutical composition in a method for treating a dialysis patient, as recited in '080 patent claim 6.

VIII. Opinions as to Invalidity of the Claims of the Patents-in-Suit In View of Obviousness-Type Double Patenting Over the Claims of the Lin '008 Patent

172. For the purpose of my scientific analysis, I have been informed that a claim which encompasses an obvious variant of an invention claimed in a previously issued patent is invalid for obviousness-type double patenting. I understand that to analyze a claim for obviousness-type double patenting, one first compares the claims of the earlier patent to determine the differences between the later claim and the earlier claims. One then determines whether the any of the differences between the later claim and the earlier claims, alone or in combination, render the later claim patentably distinct. I understand that later claims are not patentably distinct if the later claims are either anticipated by the earlier claims, or at the time the later claims were invented would have been obvious to one of ordinary skill in the art in view of the earlier claims and the available prior art.

A. Claims 1 and 2 of the '868 Patent are Not Patentably Distinct from Claims 2, 4, 6, 7, 25 and 27 of the '008 Patent

173. '868 patent independent claim 1 is directed to "a process for the production of a glycosylated erythropoietin" comprising two steps: (1) "growing . . . mammalian host cells transformed or transfected with an isolated DNA sequence encoding human erythropoietin" and

(2) isolating said glycosylated erythropoietin polypeptide therefrom." The glycosylated erythropoietin has the "in vivo biological property of causing bone marrow cells to increase production of reticulocytes and red blood cells." Dependent claim 2 is directed to the process of claim 1 where "said host cells are CHO cells."

174. Claims 25 and 27 of the '008 patent are directed to a recombinant mammalian host cell, transformed with a DNA sequence, including a DNA sequence encoding the amino acid sequence of erythropoietin, as to allow the host cell to express an erythropoietin with the "biological property of causing bone marrow cells to increase production of reticulocytes and red blood cells." In my opinion, there is no patentable distinction between the claimed process comprising use of host cells to produce a glycosylated erythropoietin as recited by the '866 claims 1 and 2, and the host cells of claims 25 and 27 of the '008 patent, wherein the host cells are transformed with so as to allow expression of a biologically active glycosylated erythropoietin with the "biological property of causing bone marrow cells to increase production of reticulocytes and red blood cells."

175. As described above, prior to October 1983, mammalian cell lines such as COS cells and CHO cells had been widely used for recombinant expression. Conditions for culturing such cells were well described and routine. Also, as further described above, it would have been obvious to express a human glycoprotein, including human EPO, such that it would have *in vivo* biological activity, by expressing it in mammalian host cells such as COS cells or CHO cells. Moreover, it would have been obvious to use methods for amplification to express the recombinant protein at high levels. In particular, it would therefore have been obvious to one of skill to grow a mammalian host cells as recited by '008 patent claim 25, or the CHO host cells of

'008 patent claim 27 under suitable nutrient conditions to produce a biologically active glycosylated erythropoietin as recited by the process of '868 patent claim 1 or 2.

176. The limitation to a "transformed or transfected mammalian host cell" which has been "transformed or transfected with a DNA sequence encoding a polypeptide having an amino acid sequence sufficiently duplicative of that of erythropoietin" ('008 patent claims 25 and 27) is implicit in the recited limitation of '868 patent claim 1 to "growing . . . mammalian host cells transformed or transfected with an isolated DNA sequence encoding human erythropoietin." Moreover, based on routine methods in the art for protein purification, it would have been obvious to one of skill to isolate the biologically active erythropoietin from the host cells of claims 25 and 27 of the '008 patent and thereby carry out the second required step in the process of '868 patent claims 1 and 2.

177. Similarly, in my opinion there is no patentable distinction between claim 2 of the '008 patent to a DNA sequence "consisting essentially of a DNA sequence encoding human erythropoietin, and the claimed process as recited by '868 patent claims 1 or 2, comprising use of host cells to produce a biologically active glycosylated erythropoietin capable of causing bone marrow cells to increase production of reticulocytes and red blood cells. As discussed above, having a DNA sequence encoding human erythropoietin, it would have been obvious to one of skill to choose a mammalian host cell such as a CHO cell to express the human EPO protein encoded by such a DNA sequence in a glycosylated and biologically active form, to grow such cells under suitable nutrient conditions, and to isolate the expressed human EPO protein. Moreover, for the same reasons, there is no patentable distinction between claims 4 or 6 of the '008 patent to recombinant host cells, in particular host cells transformed with the DNA sequence of '008 patent claim 2, and the claimed process as recited by '868 patent claims 1 or 2,

comprising use of host cells to produce a biologically active glycosylated erythropoietin capable of causing bone marrow cells to increase production of reticulocytes and red blood cells. Specifically, it would be obvious to one of skill that possession of the recombinant host cells of claims 4 or 6 of the '008 patent would enable the use of such cells to achieve the '868 patent claims 1 or 2 to produce " a biologically active glycosylated erythropoietin capable of causing bone marrow cells to increase production of reticulocytes and red blood cells."

B. Claims 4-9 of the '698 Patent Are Not Patentably Distinct from Claims 2, 4, 6, 7, 25 or 27 of the '008 Patent

178. Independent claims 4 and 6 of the '698 patent are both directed to "a process for the production of a glycosylated erythropoietin polypeptide" comprising two steps: (1) "growing . . . vertebrate cells" comprising certain DNA sequences; and (2) "isolating said glycosylated erythropoietin polypeptide expressed by said cells." The glycosylated erythropoietin polypeptide has the "in vivo biological property of causing bone marrow cells to increase production of reticulocytes and red blood cells." Both claims specify that the cells comprise "DNA encoding the mature erythropoietin amino acid sequence of FIG. 6." Claim 4 specifies that the cells also comprise "promoter DNA, other than human erythropoietin promoter DNA" which is "operatively linked" to the "DNA encoding the mature erythropoietin amino acid sequence of FIG. 6." Claim 6 specifies that the "DNA encoding the mature erythropoietin amino acid sequence of FIG. 6" is "amplified." Dependent claim 5 further limits the process of claim 4 by specifying the promoter DNA be "viral promoter DNA." Dependent claim 7 further limits the process of claim 6 to using vertebrate cells that "further comprise amplified marker gene DNA." Dependent claim 8 further limits claim 7 by specifying that the amplified marker gene DNA is "Dihydrofolate reductase (DHFR) gene DNA." Dependent claim 9 is directed to the process of claims 4 and 6 where "said cells" used in the process "are mammalian."

179. I note that claim 9 is also dependent on independent claim 2, which is not being asserted. Claim 2 is directed to "a process for the preparation of an in vivo biologically active erythropoietin product" comprising two steps (1) transforming or transfecting a host cell" and (2) isolating said erythropoietin product from said host cell or the medium of its growth. Both the host cells used in the process of claim 2, and the host cells used in the process of claim 4 comprise a "DNA sequence encoding the mature amino acid sequence of FIG. 6." In my opinion, there is no meaningful distinction between the limitation to vertebrate cells comprising a "DNA sequence encoding the mature amino acid sequence of FIG. 6," as used in claims 4 and 6 and the limitation in claim 2 to "transforming or transfecting a host cell with a DNA sequence encoding the mature amino acid sequence of FIG. 6." Prior to October 1983, the skilled scientist would have known of routine methods for transforming or transfecting DNA into mammalian host cells and isolating and selecting the transformed recombinant cells.

180. Claims 25 and 27 of the '008 patent are directed to a recombinant mammalian host cell, transformed in such a manner as to allow the host cell to express an erythropoietin with the "biological property of causing bone marrow cells to increase production of reticulocytes and red blood cells." In my opinion, there is no patentable distinction between the host cell claims 25 or 27 of the '008 patent, and the process recited by either '698 claim 4 or 6, comprising use of vertebrate cells, including host cells into which one has introduced DNA encoding the mature erythropoietin amino acid sequence of FIG. 6, to produce a glycosylated erythropoietin having the *in vivo* biological property of causing bone marrow cells to increase production of reticulocytes and red blood cells.

181. As described above, prior to October 1983, mammalian cell lines such as COS cells and CHO cells had been widely used for recombinant expression. Conditions for culturing

such cells were well described and routine. Also, as further described above, it would have been obvious to express a human glycoprotein, including human EPO, such that it would have *in vivo* biological activity, by expressing it in mammalian host cells such as COS cells or CHO cells. Moreover, it would have been obvious to use methods for amplification to express the recombinant protein at high levels. In particular, it would therefore have been obvious to one of skill to grow a mammalian host cells as recited by '008 claim 25, or the CHO host cells of '008 claim 27 under suitable nutrient conditions to carry out the process recited by either '698 claim 4 or 6 and produce a biologically active glycosylated erythropoietin.

182. Similarly, in my opinion there is no patentable distinction between claim 2 of the '008 patent to a DNA sequence "consisting essentially of a DNA sequence encoding human erythropoietin, and the process recited by either '698 patent claim 4 or 6, comprising use of vertebrate cells, including host cells into which one has introduced DNA encoding the mature erythropoietin amino acid sequence of FIG. 6. As discussed above, having a DNA sequence encoding human erythropoietin, it would have been obvious to one of skill to choose a mammalian host cell such as a CHO cell to express the human EPO protein encoded by such a DNA sequence in a glycosylated and biologically active form, to grow such cells under suitable nutrient conditions, and to isolate the expressed human EPO protein. Moreover, for the same reasons, there is no patentable distinction between claims 4 or 6 of the '008 patent to recombinant host cells, in particular host cells transformed with the DNA sequence encoding human erythropoietin of '008 claim 2 and the claimed process as recited by recited by either '698 patent claim 4 or 6.

183. The use of promoter DNA, other than erythropoietin promoter DNA in the process of '698 patent claim 4, or specifically a viral promoter as recited by '698 patent claim 5

does not provide any patentable distinction over the DNA and host cell claims 2, 4, 6, 7, 25 and 27 of the '008 patent. As described above, the prior art described numerous examples of promoters and various expression vectors including such promoters where such promoters were operably linked to drive expression of exogenous genes, and use of viral promoters, such as promoters from the SV40 virus. It would have been obvious to one of skill in using either the claimed DNA or host cells expressing such DNA to use such promoters to express DNA encoding human erythropoietin in a mammalian or other vertebrate cell, such as a COS cell or CHO cell.

184. Moreover, the use of cells comprising amplified marker DNA as recited by '698 patent claim 7, and specifically, amplified maker DNA corresponding to the DHFR gene, does not provide any patentable distinction over the DNA and host cell claims 2, 4, 6, 7, 25 and 27 of the '008 patent. As described above, prior to October 1983, the prior art described numerous examples of using amplification for transient and for stable expression of human glycoproteins in host cells such as COS cells or CHO cells, and expression vectors encoding various marker genes such as the DHFR gene for use in such methods. It would have been obvious to use such expression vectors and amplification methods to express human erythropoietin in such host cells, resulting in host cells comprising amplified marker DNA, including amplified DHFR marker gene DNA.

C. Claim 7 of the '349 Patent is not Patentably Distinct from Claims 2, 4, 6, 7 and 25 of the '008 Patent

185. Dependent claim 7 of the '349 patent is directed to "a process for producing erythropoietin" comprising culturing, "under suitable nutrient conditions" vertebrate cells described by claims 1, 2, 3, 4, 5 or 6. These claims specify that the cells can be propagated in vitro and are capable of producing human erythropoietin in excess of 100, 500 or 1000 U per 10^6

cells in 48 hours. Claim 7 however does not require a specific rate of EPO production to be achieved when using these cells in the process.

186. Claims 25 and 27 of the '008 patent are directed to a recombinant mammalian host cell, transformed in such a manner as to allow the host cell to express an erythropoietin with the "biological property of causing bone marrow cells to increase production of reticulocytes and red blood cells." In my opinion, there is no patentable distinction between the host cell claims 25 or 27 of the '008 patent, and the process recited by '349 patent claim 7 of producing erythropoietin by culturing, "under suitable nutrient conditions" vertebrate cells capable of producing human erythropoietin in excess of 100, 500 or 1000 U per 10^6 cells in 48 hours.

187. As described above, prior to October 1983, mammalian cell lines such as COS cells and CHO cells had been widely used for recombinant expression. Conditions for culturing such cells were well described and routine. Also, as described above, it would have been obvious to express a human glycoprotein, including human EPO by expressing it in mammalian host cells such as COS cells or CHO cells. As further described above, prior to October 1983, the prior art described numerous examples of using amplification to achieve high level transient and stable expression of human glycoproteins in host cells such as COS cells or CHO cells, and expression vectors for use in such methods. For example, the Goeddel '075 patent describes use of amplification to generate recombinant host cells capable of expressing tPA in amounts of 28 to 98 μg per 10^6 cells/48 hours. ('075 patent at col. 27, table 3).

188. Based on my review, the '349 patent does not define the standard against which the radioimmunoassay units recited in the '349 patent claims are to be defined, therefore leaving this term indefinite. The first mention of units however refers to erythropoietin with a specific activity of 70,400 units/mg of protein. '349 patent, col. 7. Assuming production of

erythropoietin with this specific activity, it would have been obvious to use known expression vectors and amplification methods to generate recombinant host cells capable of expressing in culture erythropoietin in the range of 100-1000 units (approximately 1.4 to 14 μg) per 10^6 cells/48 hours, as recited by '349 patent claim 7. (If one assumes a higher specific activity, the required level of protein expression would be correspondingly less). In particular, it would therefore have been obvious to one of skill to use a mammalian host cell as recited by '008 claim 25, or specifically the CHO host cell recited by '008 patent claim 27, transformed with an appropriate expression vector to allow one to generate a host cell capable of expressing human EPO at levels recited by '349 claim 7.

189. Similarly, in my opinion there is no patentable distinction between claim 2 of the '008 patent to a DNA sequence "consisting essentially of a DNA sequence encoding human erythropoietin, and the process recited by '349 patent claim 7 for producing a human erythropoietin. Having a DNA sequence encoding human erythropoietin, as discussed above, it would have been obvious to one of skill to choose a mammalian host cell such as a CHO cell to express the human EPO protein encoded by such a DNA sequence, to use known expression vectors and amplification techniques to generate cells capable expressing human EPO at levels recited by the '349 claim 7, and to culture such cells under suitable nutrient conditions in order to produce human EPO as recited by '349 patent claim 7. Moreover, for the same reasons, there is no patentable distinction between claims 4 or 6 of the '008 patent to recombinant host cells, in particular host cells transformed with the DNA sequence encoding human erythropoietin of '008 claim 2 and the claimed process as recited by '349 patent claim 7.

D. Claim 1 of the '422 Patent is not Patentably Distinct from Claims 2, 4, 6, 7, 25 and 27 of the '008 Patent

190. Claim 1 of the '422 patent is directed to a pharmaceutical composition comprising a therapeutically effective amount of human erythropoietin. I understand that the Federal Circuit recently interpreted "therapeutically effective amount" as used in the '422 patent to mean an amount "that elicits any one or all of the effects often associated with in vivo biological activity of natural EPO, such as those listed in the specification, column 33, lines 16 through 22: stimulation of reticulocyte response, development of ferrokinetic effects (such as plasma iron turnover effects and marrow transit time effects), erythrocyte mass changes, stimulation of hemoglobin C synthesis and, as indicated in Example 10, increasing hematocrit levels in mammals." (*Amgen VII*, 457 F.3d at 1303).

191. Claims 25 and 27 of the '008 patent are directed to a recombinant mammalian host cell, transformed in such a manner as to allow the host cell to express an erythropoietin with the "biological property of causing bone marrow cells to increase production of reticulocytes and red blood cells." In my opinion, based on the definition of "therapeutically effective" provided by the Federal Circuit, there is no patentable distinction between the host cell claims 25 or 27 of the '008 patent and a pharmaceutical composition comprising a therapeutically effective amount of human erythropoietin as recited by '422 patent claim 1.

192. As described above, prior to October 1983, mammalian cell lines such as COS cells and CHO cells had been widely used for recombinant expression. Conditions for culturing such cells were well described and routine. Also, as described above, it would have been obvious to express a human glycoprotein, including human EPO, such that it would have *in vivo* biological activity, by expressing it in mammalian host cells such as COS cells or CHO cells. In particular, it would therefore have been obvious to one of skill to grow a mammalian host cells

as recited by '008 patent claim 25, or the CHO host cells of '008 claim 27 to produce a therapeutically effective amount of human erythropoietin. Moreover, as I note above, it would have been obvious to use methods for amplification to generate host cells capable of expressing human erythropoietin at sufficiently high levels to allow one to isolate human erythropoietin in an amount sufficient to elicit any one or all of the effects often associated with *in vivo* biological activity of natural EPO, either in an animal, or in a human subject.

193. Similarly, in my opinion there is no patentable distinction between claim 2 of the '008 patent to a DNA sequence "consisting essentially of a DNA sequence encoding human erythropoietin, and a pharmaceutical composition comprising a therapeutically effective amount of human erythropoietin as recited by '422 patent claim 1. As discussed above, having a DNA sequence encoding human erythropoietin, it would have been obvious to one of skill to choose a mammalian host cell such as a CHO cell to express the human EPO protein encoded by such a DNA sequence in a glycosylated and biologically active form, to grow such cells under suitable nutrient conditions, and to isolate the expressed human EPO protein. Moreover, for the same reasons, there is no patentable distinction between claims 4 or 6 of the '008 patent to recombinant host cells, in particular host cells transformed with the DNA sequence encoding human erythropoietin of '008 patent claim 2 and the claimed pharmaceutical composition recited by '422 patent claim 1.

194. In my opinion, the requirement in claim 1 of the '422 patent of a "pharmaceutically acceptable diluent, adjuvant or carrier" does not provide any patentable distinction over the DNA and host cell claims 2, 4, 6, 7, 25 and 27 of the '008 patent. As indicated by the prior art cited elsewhere in this report, suitable pharmaceutical vehicles, that is diluents, adjuvants and carriers, and their formulation, was well known in the art and described

in standard treatises, for example, Remington's Pharmaceutical Sciences by E.W. Martin. (*See also, e.g.*, Goeddel '075 patent at cols. 27-28). Much of the interest at the time in recombinant DNA technology was in using such an approach to produce recombinant human proteins in useful quantities in order to initiate and conduct animal and clinical testing. (*Id.*). Having expressed and isolated recombinant human erythropoietin, it would have been obvious to formulate a suitable pharmaceutical composition containing a recombinant human glycoprotein such as human erythropoietin, and comprising a suitable diluent, adjuvant or carrier in order to use the human erythropoietin in an animal or human subject. (*Id.*).

E. Claims 3, 7-9, 11-12 and 14 of the '933 Patent are Not Patentably Distinct from Claims 2, 4, 6, 7 and 25 of the '008 Patent

195. Claim 3 of the '933 patent, the only asserted independent claim is directed to a "non-naturally occurring glycoprotein product of the expression in a mammalian host cell of an exogenous DNA sequence comprising a DNA sequence encoding human erythropoietin." The claim further recites that the product possesses the in vivo biological activity of causing bone marrow cells to increase production of reticulocytes and red blood cells. I would understand such a product to include human erythropoietin recombinantly expressed in a mammalian host cell, such as a COS cell or CHO cell, transformed with an expression vector containing the DNA sequence encoding human erythropoietin.

196. Claims 25 and 27 of the '008 patent are directed to a recombinant mammalian host cell, transformed in such a manner as to allow the host cell to express an erythropoietin with the "biological property of causing bone marrow cells to increase production of reticulocytes and red blood cells." In my opinion, there is no patentable distinction between the host cell claims 25 or 27 of the '008 patent, wherein the host cells are transformed with so as to allow expression of a biologically active glycosylated erythropoietin with the "biological property of causing bone

marrow cells to increase production of reticulocytes and red blood cells, and the human erythropoietin glycoprotein product of '933 claim 3 possessing the in vivo biological property of causing bone marrow cells to increase production of reticulocytes and red blood cells.

197. As described above, prior to October 1983, mammalian cell lines such as COS cells and CHO cells had been widely used for recombinant expression. Conditions for culturing such cells were well described and routine. As further described above, it would have been obvious to express a human glycoprotein, including human EPO, in mammalian host cells such as COS cells or CHO cells such that it would exhibit the *in vivo* biological activity of the protein produced in the body. Moreover, it would have been obvious to use methods for amplification to express the recombinant protein at high levels. In particular, it would therefore have been obvious to one of skill to grow a mammalian host cells as recited by '008 claim 25, or the CHO host cells of '008 claim 27 to produce an *in vivo* biologically active recombinant human erythropoietin as recited by '933 claim 3.

198. Moreover, the recitation of a "mammalian host cell...comprising a "DNA sequence encoding human erythropoietin" of '933 claim 3 does not provide any patentable distinction over the host cell '008 claims 25 and 27, as this limitation is implicit and obvious in the recitation of a "transformed or transfected mammalian host cell" which has been "transformed or transfected with a DNA sequence encoding a polypeptide having an amino acid sequence sufficiently duplicative of that of erythropoietin," appearing in '008 claims 25 and 27.

199. Similarly, in my opinion there is no patentable distinction between claim 2 of the '008 patent to a DNA sequence "consisting essentially of a DNA sequence encoding human erythropoietin," and the human erythropoietin glycoprotein product of '933 claim 3 possessing the *in vivo* biological property of causing bone marrow cells to increase production of

reticulocytes and red blood cells. As discussed above, having a DNA sequence encoding human erythropoietin, it would have been obvious to express the DNA encoding human EPO in a mammalian host cell such as a COS or CHO cell, such that it would exhibit the *in vivo* biological activity of the protein produced in the body. Moreover, for the same reasons, there is no patentable distinction between the recombinant host cells of '008 patent claims 4 or 6, in particular host cells transformed with the DNA sequence encoding human erythropoietin of '008 patent claim 2, and the human erythropoietin glycoprotein product of '933 claim 3.

200. As evident from the above discussion, it would have been obvious to use mammalian host cells, including CHO cells to express a recombinant human erythropoietin. The use of a non-human mammalian host cell to produce the human erythropoietin glycoprotein product of '933 claim 3, as recited in '933 claim 7, or specifically the use of a CHO cell as recited in '933 claim 8, therefore does not provide any patentable distinction over the DNA and host cell claims 2, 4, 6, 7, 25 and 27 of the '008 patent.

201. Moreover, the requirement of a pharmaceutically acceptable diluent, adjuvant or carrier as recited in '933 dependent claims 9 and 12 does not provide any patentable distinction over the DNA and host cell claims 2, 4, 6, 7, 25 and 27 of the '008 patent. As indicated by the prior art cited elsewhere in this report, suitable pharmaceutical vehicles, that is diluents, adjuvants and carriers, and their formulation, was well known in the art and described in standard treatises, for example, Remington's Pharmaceutical Sciences by E.W. Martin. (*See also, e.g.*, Goeddel '075 patent at cols. 27-28). Much of the interest at the time in recombinant DNA technology was in using such an approach to produce recombinant human proteins in useful quantities in order to initiate and conduct animal and clinical testing. (*Id.*). Having expressed and isolated recombinant human erythropoietin, it would have been obvious to

formulate a suitable pharmaceutical composition containing a recombinant human glycoprotein such as human erythropoietin, and comprising a suitable diluent, adjuvant or carrier in order to use the human erythropoietin in an animal or human subject. (*Id.*).

202. Dependent '933 patent claims 11 and 14 are directed to methods for treating a kidney dialysis patient with the pharmaceutical compositions of claims 9 and 11. Having a source of recombinant human EPO, such as that produced using the DNA and host cells recited by '008 claims 2, 4, 6, 7, 25 and 27, it would have been obvious to provide the recombinant EPO in a pharmaceutical composition and to use that pharmaceutical composition in treating a kidney dialysis patient. As early as 1971, it was appreciated that human EPO could be important for "possible therapeutic use in some types of refractory anemia (Goldwasser 1971). The hypothesis that chronic renal failure, which typically requires dialysis, is associated with a refractory anemia due to insufficient renal production of erythropoietin was confirmed by studies demonstrating a dose-dependent correction of anemia in uremic sheep by parenteral administration of erythropoietin-enrichd plasma (Eschbach 1984 (original submission date July 5, 1983; published August 1984). Thus, these studies, and others implied that EPO therapy should correct the hypoproliferative anemia observed in patients with chronic renal failure on maintenance dialysis. Thus, prior to October 1983, the desirability of treating dialysis patients with human EPO was widely recognized and appreciated.. Moreover, as I note above, it would have been obvious to use methods for amplification to generate host cells capable of expressing human erythropoietin at sufficiently high levels to allow one to isolate human erythropoietin in an amount sufficient to elicit any one or all of the effects often associated with *in vivo* biological activity of natural EPO, either in an animal, or in a human subject. The limitation to using the recombinant human EPO

in a method for treating a dialysis patient therefore does not provide any patentable distinction over the DNA and host cells recited by '008 patent claims 2, 4, 6, 7, 25 and 27.

F. Claims 3-4 and 6 of the '080 Patent are Not Patentably Distinct from Claims 2, 4, 6, 7, 25 or 27 of the '008 Patent

203. Claim 3 of the '080 patent, the only asserted independent claim is directed to a "non-naturally occurring erythropoietin glycoprotein," which "comprises the mature erythropoietin amino acid sequence of Fig. 6." The claim further recites that the product possesses the *in vivo* biological activity of causing bone marrow cells to increase production of reticulocytes and red blood cells.

204. As discussed above, to the extent that Amgen contends that '080 claim 3 covers a non-naturally occurring 165 amino acid human erythropoietin, there can be no patentable distinction between '080 patent claim 3 and '933 claim 3, which also is directed to a non-naturally occurring human erythropoietin glycoprotein expressed from a mammalian host cell. Therefore, to the extent that Amgen asserts that '080 patent claim 3 covers such a product, for example one expressed by a mammalian host cell such as a COS cell or CHO cell, for the same reasons as described above for '933 claim 3, there would be no patentable distinction between '080 patent claim 3 and the host cell claims 25 or 27 of the '008 patent, wherein the host cells are transformed with so as to allow expression of a biologically active glycosylated erythropoietin with the "biological property of causing bone marrow cells to increase production of reticulocytes and red blood cells." Similarly for the same reasons as discussed for '933 patent claims, the recitation of a pharmaceutical composition in '080 patent claim 4, or the method of using the recombinant human EPO in a method for treating a dialysis patient, as recited in '080 claim 6 does not provide any patentable distinction over the DNA and host cells recited by '008 patent claims 2, 4, 6, 7, 25 and 27.

IX. Amgen's Representations During the *Fritsch v. Lin* Interference

A. Amgen's Statements Confirm that Using Mammalian Host Cells To Express a Glycosylated Biologically Active Erythropoietin Would Have Been Obvious

205. During the prosecution of the '868 patent, the '868 application was involved in an interference proceeding, No. 102,097, between Amgen (senior party) and Genetics Institute. (*Fritsch v. Lin*, 21 U.S.P.Q. 2d 1737 (Bd. Pat. App. & Interf. 1992) (Interference No. 102,097)). There was count (interference claim) involved in this interference, which was directed to a process for making a glycosylated, *in vivo* biologically active erythropoietin. Comparing this count to the process claims below that I understand have been asserted against Roche, in my opinion, the '097 interference count contains all the essential elements recited by the asserted process claims, as indicated below in bold:

> '097 Interference Count: A **process for the preparation** of an **in vivo biologically active glycosylated polypeptide** comprising steps of 1. **growing mammalian cells transformed with DNA encoding a polypeptide sufficiently duplicative of human EPO** to have the **in vivo biological properties of increasing red blood cells and reticulocytes**, 2. transcribing the DNA to mRNA, 3. translating the mRNA into a polypeptide, 4. glycosylating the polypeptide in a manner sufficiently duplicative of the glycosylation of natural human EPO to effect the recited biological activity and 5. **isolating the glycosylated polypeptide**. Id. at 1738.
>
> '868 claim 1: A **process for the production** of a **glycosylated erythropoietin** having the **in vivo biological property of causing bone marrow cells to increase production of reticulocytes and red blood cells** comprising the steps of (a) **growing**, under suitable nutrient conditions, **mammalian host cells transformed or transfected with an isolated DNA sequence encoding human erythropoietin**; and (b) **isolating said glycosylated erythropoietin polypeptide** therefrom.
>
> '698 claim 4: A **process for the production** of a **glycosylated erythropoietin polypeptide** having the **in vivo biological property of causing bone marrow cells to increase production of reticulocytes and red blood cells** comprising the steps of: a) growing, under suitable nutrient conditions, **vertebrate cells**

> **comprising** promoter DNA, other than human erythropoietin promoter DNA, operatively linked to **DNA encoding the mature erythropoietin amino acid sequence of FIG. 6**; and b) **isolating said glycosylated erythropoietin polypeptide** expressed by said cells.
>
> '349 claim 7: A **process for producing erythropoietin** comprising the step of **culturing**, under suitable nutrient conditions... [**vertebrate cells** which can be propagated in vitro and which are capable upon growth in culture of **producing erythropoietin** in the medium of their growth in excess of 100, 500, or 1000 U of erythropoietin per 10^6 cells in 48 hours as determined by radioimmunoassay, said **cells comprising** non-human DNA sequences which control transcription of **DNA encoding human erythropoietin**].

206. After the Lin '008 patent issued, it was involved in an interference proceeding No. 102,096 between Amgen (senior party) and Genetics Institute. (*See Fritsch v. Lin*, 21 U.S.P.Q.2d 1731 (Bd. Pat. App. & Interf. 1991) (Interference No. 102,096)). There was also one count involved in this interference, which was identical to claim 2 of the '008 patent:

> '096 interference count: A purified and isolated DNA sequence consisting essentially of a DNA sequence encoding human erythropoietin.

207. During the 102,097 interference, Amgen argued that the Board should adopt the findings of the District Court and the Federal Circuit that Amgen had invented an isolated DNA sequence encoding human erythropoietin and host cells transformed with such a sequence, as claimed in its '008 patent, before Genetics Institute had invented the same subject matter. (citing *Amgen, Inc. v. Chugai Pharms.*, 13 U.S.P.Q.2d 1737 (D. Mass. 1989), *aff'd in relevant part*, 927 F.2d 1200 (Fed. Cir. 1991)):

> In doing so, the Court rejected a Section 102(g) anticipation attack by Fritsch based on Fritsch's own work at Genetics Institute, and upheld claims of the Lin '008 patent including the following:
>
> 2. A purified and isolated DNA sequence consisting essentially of a DNA sequence encoding a human erythropoietin.