UNITED STATES DISTRICT COURT
DISTRICT OF MASSACHUSETTS

AMGEN INC., Plaintiff, v. F. HOFFMANN-LAROCHE LTD., a Swiss Company, ROCHE DIAGNOSTICS GmbH, a German Company and HOFFMANN LAROCHE INC., a New Jersey Corporation, Defendants.	Civil Action No.: 05-12237 WGY

DECLARATION OF HARVEY F. LODISH, PH.D. IN SUPPORT OF AMGEN'S BENCH MEMORANDUM AND OFFER OF PROOF REGARDING NO OBVIOUSNESS-TYPE DOUBLE PATENTING

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I. BACKGROUND

1. I am a Professor of Biology and Professor of Bio Engineering at the Massachusetts Institute of Technology (MIT) and a Member of the Whitehead Institute for Biomedical Research. I am submitting this declaration in support of Amgen's Bench Memorandum and Offer of Proof Regarding Obviousness-Type Double Patenting. If called to testify as to the truth of the matters stated herein, I could and would do so competently.

2. A copy of my curriculum vitae, reflecting my professional experience, affiliations, and work has previously been filed as Docket Item ("D.I.") 502, Ex. A.

3. I received an A.B. degree summa cum laude from Kenyon College in 1962, and a Ph.D. from the Rockefeller University in 1966. I was a post-doctoral Fellow at the Medical Research Council Laboratory of Molecular Biology in Cambridge, England from 1966 to 1968. I held the positions of Assistant Professor and Associate Professor at the Massachusetts Institute of Technology (MIT) during the years 1968-71 and 1971-76, respectively. Since 1976, I have been a full Professor of Biology at MIT and since 1999 Professor of Bioengineering. In 1982, I became a Founding Member of the Whitehead Institute for Biomedical Research.

4. Since 1961, I have authored or co-authored more than 500 scientific publications, in a variety of peer-reviewed scientific journals, as detailed in D.I. 502, Ex. A.

5. I was elected to the National Academy of Sciences in 1987. In 2004, I was President of the American Society for Cell Biology, an international organization of more than 10,000 scientists. I have also served on a variety of external advisory boards and grant review panels. A complete list is provided in D.I. 502, Ex. A.

6. As described in detail in my curriculum vitae, I have been a researcher, a teacher, a writer, and an editor in the fields of molecular and cellular biology for over 35 years. Adherence to the scientific method is the common thread that runs through all the aspects of my

career. Excellence is achieved in the field of science through application of the logical principles and philosophies shared by the scientific community. Like other scientists, through study and practice I have collected a set of analytical tools that I use to address all scientific problems. For example, I apply these tools when I evaluate others' work. If others do not rigorously apply scientific methodology, I properly discount their assertions.

7. In the course of my career, I have taught many M.I.T. undergraduates, Ph.D. students, and post-doctoral fellows. Imparting an understanding of proper scientific method is one of my major goals. More specifically, I teach students how to formulate testable hypotheses, how to design and perform well-controlled, repeatable experiments to validate hypotheses, and to evaluate experimental outcomes objectively. It is only by understanding and applying the scientific method rigorously that students can develop into scientists whose work will withstand the scrutiny of the scientific community and advance scientific knowledge.

8. I have served on the Editorial Boards for many peer-reviewed scientific journals. For example, I was a member of the Board of Reviewing Editors of the journal *Science* from 1991 to 1999, and a Member of the Editorial Board of the journal *Proceedings of the National Academy of Sciences* from 1996 to 2000. Furthermore, I have reviewed hundreds of articles for publication in many different journals. When I review papers for potential publication, I must consider critically whether the work is well conceived, controlled, and performed in order to establish whether its scientific conclusions are correct. Additionally, I consider whether the work is sufficiently described such that other workers in the field can repeat, confirm, and extend the reported findings.

9. I am the principal editor and author of the textbook *MOLECULAR CELL BIOLOGY*, now in its Fifth Edition.[1] The Sixth Edition has just been published. In addition to

[1] *See* Lodish *et al.*, *Molecular Cell Biology*, 5th Ed. W.H. Freeman Co., New York.

my seven co-authors, literally dozens of our scientific colleagues from around the world have contributed to chapters, and reviewed and commented on the manuscripts. This textbook has been relied upon by scientific researchers, undergraduate students, and graduate and medical students all around the world since the publication of our First Edition in 1986. The Fifth Edition has been translated into six languages. It is considered one of the most authoritative resources in the fields of molecular and cellular biology. The textbook presents a comprehensive, authoritative review of the fields of molecular and cellular biology, and is intended for advanced undergraduates and graduate and medical students. In the course of preparing my book over the past 20 years, I have comprehensively studied, in detail, the published literature to determine what experimental work is new, significant, and sufficiently credible to merit reliance by the scientific community at large.

10. In the course of my career as a researcher, I have personally applied the scientific method to many different avenues of research, including cell signaling, protein synthesis, cell membranes and their formation, cell death, fat cell biology, and, most relevant here, blood cell differentiation. One example of my experience in blood cell differentiation is my work concerning the characterization of the murine erythropoietin ("EPO") receptor, the protein on the surface of red blood cell precursors that binds to EPO and that mediates the activity of EPO in cells and *in vivo* (in the body).

11. I have been studying glycoprotein synthesis and function in mammalian cells since about 1976. My laboratory has made several significant contributions to the understanding of the glycosylation process. Prominent examples of our work include first establishing that the addition of oligosaccharides (or "glycans") to asparagines on glycoproteins occurs during the synthesis of the polypeptide and its translocation into the endoplasmic reticulum, and purifying

and characterizing the hepatocyte asialoglycoprotein receptor, a major component of the system of clearance of glycoproteins from the circulation.

12. Representative examples of my pre-1983 publications in the field of glycosylation include: Lodish, H.F., *et al.*, "Membrane assembly: synthesis and intracellular processing of the vesicular stomatitis viral glycoprotein," *Birth Defects Orig Artic Ser.* 14(2):155-75 (1978); Rothman, J.E., and Lodish, H.F., "Synchronised transmembrane insertion and glycosylation of a nascent membrane protein," *Nature* 269(5631):775-80 (1977); Lingappa, V.R. *et al.*, A signal sequence for the insertion of a transmembrane glycoprotein. Similarities to the signals of secretory proteins in primary structure and function," *J Biol Chem.* 253(24):8667-70 (1978); Rothman, J.E. *et al.*, "Glycosylation of a membrane protein is restricted to the growing polypeptide chain but is not necessary for insertion as a transmembrane protein," *Cell* 15(4):1447-54 (1978); Schwartz, A.L. *et al.*, "Difficulties in the quantification of asialoglycoprotein receptors on the rat hepatocyte," *J Biol Chem.* 255(19):9033-6 (1980); Schwartz, A.L., *et al.*, "Identification and quantification of the rat hepatocyte asialoglycoprotein receptor," *Proc Natl Acad Sci U S A.* 78(6):3348-52(1981); and Lodish, H.F., and Kong, N., "Glucose removal from *N*-linked oligosaccharides is required for efficient maturation of certain secretory glycoproteins from the rough endoplasmic reticulum to the Golgi complex," *J Cell Biol.* 98(5):1720-9. I have continued to research and publish in this field to the present day.

13. I was also very involved in cloning genes from several eukaryotic cells, including human and other mammalian cells, beginning in 1980 and continuing throughout the 1980s to the present day.

14. Moreover, in the early 1980s, I was also particularly interested in the production of recombinant proteins for therapeutic and industrial purposes. In particular, I was interested in how it would be possible to recapitulate the complex processing of mammalian proteins in

heterologous expression systems. In 1981, I published a review article on this subject. Lodish, H.F., "Post-translational modification of proteins," *Enzyme Microb Technol.* 3(3):177-188 (1981). This article demonstrates that I am uniquely qualified to opine on the knowledge and understanding of an ordinarily skilled artisan in the fields pertinent to the claims-at-issue during the 1983-84 time period.

15. During the course of prior litigation involving the patents-in-suit between Amgen and Transkaryotic Therapies and Hoechst Marion Roussel, I reviewed in detail the patents-in-suit, portions of the prosecution histories, and related scientific publications. I testified at trial in connection with that action and prepared several expert reports.

16. Earlier in the present litigation between Amgen and Roche, I submitted two declarations explaining my opinions regarding certain obviousness-type double patenting issues. (*See* D.I. 502, D.I. 578.) My prior declarations included as exhibits a number of prior art references, patent documents, and other publications. To the extent I rely on those same documents in this declaration, I have cited the versions previously filed with the Court. In other instances herein, I have cited the trial exhibit ("TX") versions of documents. Documents that have not previously been filed with the Court, either in my prior declarations or as trial exhibits, are attached to this declaration and identified as follows: "9/26/07 Lodish Decl., Ex. __."

A. LEVEL OF SKILL IN THE ART

17. I have been asked to consider whether a person of ordinary skill in the art in 1983-84 (i.e., at the time just before the inventions taught and claimed in Dr. Lin's patents-in-suit) would have found claims 1 and 2 of the '868 patent and claims 6-9 of the '698 patent to be obvious over claims 2, 4, 6, 7, 25 and/or 27 of the '008 patent. A "person of ordinary skill" or "ordinarily skilled artisan" in the field relevant to Dr. Lin's claims would have been a research scientist with a Ph.D. or M.D. and at least two years of postdoctoral research experience in the

field of molecular biology, cellular biology, or protein expression. As discussed below, it is my opinion that a person of ordinary skill in the art in 1983-84 would have found each of claims 1 and 2 of the '868 patent and claims 6-9 of the '698 patent to be ***not*** obvious over each of claims 2, 4, 6, 7, 25 and 27 of the '008 patent.

B. BEFORE DR. LIN'S INVENTIONS, A PERSON OF ORDINARY SKILL IN THE ART ATTEMPTING TO PRODUCE *IN VIVO* BIOLOGICALLY ACTIVE RECOMBINANT EPO WOULD HAVE BEEN CONFRONTED WITH MANY POSSIBLE APPROACHES, GREAT UNCERTAINTY AS TO EACH, AND NO REASONABLE EXPECTATION THAT ANY PARTICULAR APPROACH WOULD SUCCEED

18. When Dr. Lin began his efforts to produce *in vivo* biologically active EPO using recombinant DNA techniques, he faced a daunting array of competing choices and difficult problems. The amino acid sequence of EPO was unknown. The DNA sequence of EPO was unknown. The particular cell type(s) in the human body that naturally produce EPO was unknown. The cellular receptor(s) with which human EPO interacts in the human body to produce red blood cells was unknown, and consequently what, if any, recombinant EPO products would interact effectively with the EPO receptor(s) *in vivo* was unknown. Because the human cell type(s) that naturally produce EPO was unknown, the set of post-translational modifications that are made to EPO polypeptides by those cells was also unknown. Whether any such post-translational modifications were needed to produce a man-made product that would perform the desired function of human EPO *in vivo* and, if so, which modifications were needed, which if any cell types would in fact produce those modifications — and only those modifications — correctly, and, if so, how to identify cells that would reliably do so, were all unknown and unknowable until empirically tested and proven. To the extent that minute amounts of human EPO protein had been isolated from urine, the available product was insufficient to characterize the complete amino acid sequence and carbohydrate structures of the purified product. Even then, such excreted urinary products were necessarily exposed to conditions that would be

expected to alter their composition from natural, biologically active EPO protein found in the bloodstream, and therefore could not be relied upon to predict with confidence the actual structure and composition of EPO products needed to achieve EPO's *in vivo* function in the body.

19. Recombinant expression of biologically active human glycoproteins in cultured, mammalian cells was still in its infancy. In fact, prior to 1984, I am not aware of and Roche has not cited any report of any *in vivo* biologically active recombinant human glycoprotein successfully produced in cultured, mammalian cells. While scientists did understand that glycosylation potentially played an important role in the function of glycoproteins like EPO, they did not know or understand what function(s) it performed, how naturally occurring EPO was glycosylated when it was produced and circulated in the body, or whether differences in glycosylation caused by production in different cell types would affect the biological activity of EPO and, if so, how. Only as a result of Dr. Lin's successful production of an *in vivo* biologically active recombinant human EPO glycoprotein in CHO and COS cells, were scientists then able to explore and begin to resolve these uncertainties.

1. Little was known about the structure of erythropoietin

20. Erythropoietin as it is produced in the body is a glycoprotein hormone that stimulates progenitor cells in the bone marrow to multiply and to differentiate into reticulocytes (immature red blood cells) and then mature red blood cells. This *in vivo* biological function had been established by the work of many researchers over the course of almost 100 years.

21. Before Dr. Lin's ground-breaking inventions provided an abundant source of high-quality EPO glycoprotein, only minute amounts of human urinary EPO were available. Much of the pre-1984 research on erythropoietin was performed with crude, unpurified material. The primary source of purified EPO before Dr. Lin was from the urine of aplastic anemia

patients. Miyake, *et al.,* "Purification of human erythropoietin," *J. Biol. Chem*. 252(15):6538-64. (1977) (D.I. 578, Ex. A). Because there is so little EPO in urine, even from these patients who have much higher levels than normal, very little urinary EPO was available. Moreover, because EPO could not be isolated directly from the blood, there was no way to know whether EPO purified from urine accurately reflected the structure of naturally produced EPO prior to its removal from circulation and excretion in the urine. Because excreted urinary EPO is exposed to a different environment than EPO in the bloodstream, one skilled in the art at the time would have understood that urinary EPO is exposed to different enzymes that could either remove or damage structures normally present on naturally occurring plasma EPO, or could impart structures to the excreted urinary EPO molecule that are not present on plasma EPO.

22. Because only vanishingly small amounts of EPO could be obtained from urine, researchers before Lin were actively searching for other sources of EPO, but failed to find any adequate source. For example, extensive searches by Goldwasser and others for tumor cells or other cultured cells that produced EPO were largely unsuccessful. Potential cells produced crude extracts that showed infinitesimal erythropoietic activity in biological assays. However, the ability to sustain such activity quickly declined over time, and no one ever succeeded in isolating EPO from these extracts. Consequently, it was simply unknown whether the faint erythropoietic activity detected in the biological assays of these extracts was attributable to the presence of human EPO in the extract, or to some other agent or combination of agents.

23. Given the minute amounts of EPO that were available, very little was known about the structure and function of erythropoietin prior to Lin's inventions. Prior to 1983, a partial amino acid sequence was reported for the N-terminus of the human EPO protein, but that reported sequence subsequently proved to be not only incomplete but incorrect in several important respects. While it was known that EPO was a glycoprotein, the specific number,

location and structures of its glycans had not been investigated. Nor did skilled artisans in 1983 have any insight or knowledge about whether or the extent to which a biologically active EPO glycoprotein would require any number of post-translational cellular modifications, such as (1) proteolytic cleavage; (2) formation of disulfide bonds; (3) particular glycosylation; or (4) covalent addition of other molecules such as sulfate, phosphate, carboxyl or acetyl groups. Because the actual structure of EPO was unknown, it was impossible to know which, if any, such post-translational modifications would prove to be necessary to produce a recombinant EPO product that would perform the biological function of human EPO *in vivo*. Indeed, since it was known that naturally occurring EPO was apparently produced by very few, highly specialized cells in the kidney, the likelihood that such cells used special or unique enzymes to process and modify the final, secreted structure and composition of the naturally occurring EPO glycoprotein was very real.

24. It was known by 1983 that mammalian cells perform many post-translational modifications that impact biological function in a species, cell-type, and protein specific manner. Moreover, an ordinarily skilled artisan would have appreciated that any of these potential modifications could have been critical for function. And, the ordinarily skilled artisan would have understood that every cultured cell had its own particular properties and capacity to impart any or all of these post-translational modifications to an expressed protein. Whether EPO had any such modifications was unknown. Thus, expression of EPO in a mammalian cell that did not normally produce EPO could easily result in different post-translational modifications of the EPO protein in ways that would disrupt or destroy the intended biological function of the protein. A worker at the time would have been doubtful that cells that did not normally produce human EPO would properly make any of these modifications, and would therefore expect that EPO

expressed in such heterologous cells could be non-functional, absent proof of successful expression of *in vivo* biologically active EPO from any mammalian host cell.

2. In 1983-84, the glycosylation of erythropoietin was known to be important for biological function, but its structure was unknown

25. Some of the studies performed before Dr. Lin's inventions indicated that certain carbohydrate structures appeared to be necessary for the *in vivo* biological function of EPO. For example, Dr. Goldwasser's 1974 article described how the sialic acids on sheep plasma EPO are necessary for *in vivo* but not *in vitro* biological function: "Desialation (decrease in sialic acids) results in complete loss of biological activity when it is assayed *in vivo.* When the assay is done *in vitro* asialoerythropoietin has full activity, or when assayed at low levels of hormones is about three times more active that the native hormone. The loss of activity can be explained by the hepatic removal of asialogylcoproteins from the circulation." Goldwasser, *et al.*, "On the mechanism of erythropoietin-induced differentiation. The role of sialic acid in erythropoietin action," *J. Biol. Chem.* 249:4202-6 (1974) (D.I. 578, Ex. B); s*ee also* Lowy, *et al.*, "Inactivation of Erythropoietin by Neuraminidase and by Mild Substitution Reactions," *Nature* 186:102 (1960) (D.I. 578, Ex. C); Briggs, *et al.*, "Hepatic clearance of intact and desialylated erythropoietin," *Amer. J. of Physiology* 227:1385-1388 (1974) (D.I. 578, Ex. D) ("These results indicate that desialylation of ESF causes its rapid hepatic clearance from the circulation . . .").

26. Dr. Goldwasser hypothesized that the higher *in vitro* activity observed for desialylated EPO was a result of relieving repulsion between the sialic acids on EPO and the target cell surface: "This increase [*in vitro* activity] may reflect the fairly large reduction in negative charge that accompanies desialation. If the target cells are negatively charged, the presence of 16 to 18 strong anionic groups on the native hormone may retard interaction with the cells; the asialohormone might then have easier access to the cells. A similar situation obtains with human chorionic gonadotropin where the asialo form of the hormone has a higher affinity

for receptor sites than for the native hormone." Goldwasser, *et al., J. Biol. Chem.* 249:4202-6 (1974) at 4205 (D.I. 578, Ex. B).

27. Moreover, Dordal demonstrated that complete deglycosylation of EPO had similar effects to desialylation:

> Digestion of the hormone with *S. pneumoniae* mixed glycosidases reduces the apparent molecular weight from 39,000 to 28,500. The glycosidase-treated epo retains 50-70% of its activity *in vitro* but is inactive *in vivo*. . . . These results suggest that deglycosylated epo may retain its intrinsic ability to stimulate erythropoiesis but may lack the stability *in vivo* required for successful hormone replacement therapy.

Dordal, M., "The Function and Composition of the Carbohydrate Portion of Human Urinary Erythropoietin." Thesis, University of Chicago, 7/27/82. (D.I. 578, Ex. E, at 984).[2]

28. Thus, it was known in 1983 that the presence of sialic acids on the termini of the carbohydrates attached to EPO appeared to play an important role in the *in vivo* biological function of EPO. It was also known that the complete elimination of glycosylation from EPO protein apparently led to the loss of *in vivo* biological activity. It was not known why these carbohydrates were required for *in vivo* function, nor was it known whether changes or differences in the location, number or type of carbohydrate structures attached to an EPO polypeptide would affect or impair its *in vivo* activity.

29. By the end of 1984, no specific analysis of the glycan structures of either urinary EPO or recombinant EPO had been published. In the late 1980s, the glycosylation structures found on urinary and recombinant EPO were studied in depth. It was confirmed that both

[2] Dr. Lin and colleagues confirmed the result found for urinary EPO using recombinant EPO in early 1984. *See* D.I. 578, Ex. F ("Determine the effect of deglycosylation of EPO on its *in vivo* and *in vitro* biological activity. Deglycosylated EPO has full *in vitro* activity but no *in vivo* activity.").

urinary EPO and recombinant EPO have three *N*-linked and one *O*-linked oligosaccharides. It was further found that the *N*-linked carbohydrate chains attached at positions 24, 38, and 83 of EPO are heterogeneous with respect to sugar composition and structure. Sasaki, H., *et al.*, "Carbohydrate Structures of Erythropoietin Expressed in Chinese Hamster Ovary Cells by a Human Erythropoietin cDNA," *J. Biol. Chem.* 262:12059-12076 (1987) (D.I. 578, Ex. G); Sasaki, H., *et al.*, "Site Specific Glycosylation of Recombinant Human Erythropoietin," *Biochemistry* 27, 8618-8626 (1988) (D.I. 578, Ex. H). As a consequence, different molecules of EPO will have different numbers of attached sialic acid residues. Egrie, J. and Browne, J., "Development and Characterization of Novel Erythropoiesis Stimulating Protein (NESP)," *Nephrol. Dial. Transplant* 16 [suppl]:3-13 (2001) (D.I. 578, Ex. I); Takeuchi, M., *et al.*, "Relationship Between Sugar Chain Structure and Biological Activity of Recombinant Human Erythropoietin Produced in Chinese Hamster Ovary Cells," *Proc. Natl. Acad. Sci. USA*, 86:78 19-7822 (1989) (D.I. 578, Ex. J).

30. Further, it was found that the glycosylation of recombinant human EPO produced by CHO cells differs from human urinary EPO. Sasaki, H. *et al.*, "Carbohydrate Structures of Erythropoietin Expressed in Chinese Hamster Ovary Cells by a Human Erythropoietin cDNA," *J. Biol. Chem.* 262: 12059-12076 (1987) (D.I. 578, Ex. G); Takeuchi, M., *et al.*, "Comparative Study of the Asparagine-linked Sugar Chains of Human Erythropoietins Purified from Urine and the Culture Medium of Recombinant Chinese Hamster Ovary Cells," *J. Biol. Chem.* 263(8):3657-3663 (1988) (D.I. 578, Ex. K).

31. In fact, it is only because the carbohydrates attached to urinary EPO characteristically differ from those attached to recombinant EPO that sporting authorities, such as the International Olympic Committee and the Tour de France, are able to test for the illicit use of recombinant EPO by athletes. The differences in carbohydrate chains attached to naturally

occurring urinary EPO and recombinant human EPO are detected using an assay called isoelectric focusing gel electrophoresis, which is used to perform the urine analyses for EPO "doping" in the Olympic games and the Tour de France. Lasne, F. and de Ceaurriz, J., "Recombinant Erythropoietin in Urine," *Nature* 405:635 (2000) (D.I. 578, Ex. L).

3. The cellular source of erythropoietin was unknown

32. While the work of Goldwasser and others had demonstrated that the principal site of erythropoietin production appeared to be the kidney, as of 1983-84 the specific cell type(s) within the kidney that naturally produce EPO were unknown. *See, e.g.*, Erslev, A.J., and Caro, J., "Physiologic and molecular biology of erythropoietin," *Med. Oncol. Tumor. Pharmacother.* 3(3-4):159-64 (1986) (D.I. 578, Ex. M) ("The exact cellular source for erythropoietin production in the kidney is still unknown.").

33. Indeed, the cell type(s) that naturally produce human EPO is still subject to debate. Some believe that tubular cells of the kidney are responsible for EPO production. Mujais SK, *et al.*, "Erythropoietin is produced by tubular cells of the rat kidney," *Cell Biochem Biophys*. 30(1):153-66 (1999) (D.I. 578, Ex. N). Others, including Roche's expert Dr. Fisher, have stated that interstitial cells are the primary site of EPO production in the kidney. Fisher, J.W., *et al.*, "Erythropoietin production by interstitial cells of hypoxic monkey kidneys," *Br. J. Haematol.* 9527-32 (1996) (D.I. 578, Ex. O) ("The present finding that interstitial cells produce Epo in hypoxic monkey kidneys suggests that interstitial cells in the kidneys of other primates such as human are likely to be the primary site of Epo productions as well.").

34. Because the specific cell type(s) that produce EPO in the human body were unknown as of the date of Dr. Lin's inventions, it was not possible to identify the specific post-translational modifications that such cells make to the EPO polypeptide before it is secreted from the cells for circulation in the bloodstream. Thus, there was no way to know what carbohydrate

and other structure(s) would be required to replicate biologically functional EPO, and thus what cell type(s) could be transformed with DNA encoding human EPO and grown in culture to produce such glycoprotein products.

4. Recombinant expression of glycoproteins was in its infancy in 1983-84

35. Early experiments in the field focused primarily on expression in bacterial cells like *E. coli*, which were incapable of glycosylation. Prior to Dr. Lin's inventions, expression of heterologous proteins in mammalian cells was still in the earliest stages of development. Most importantly, there were no published reports of the successful production of a recombinant human glycoprotein in mammalian host cells that was biologically active *in vivo*. Even if there had been such a report, successful production of one particular *in vivo* biologically active glycoprotein would not have led a person of ordinary skill in the art in 1983 to believe that production of biologically active EPO was predictable. While a person of ordinary skill in the art may have had a reasonable expectation of success in achieving some expression (i.e., production of a protein), they would not have had, prior to Dr. Lin's work, a reasonable expectation of success that the human glycoprotein produced in a mammalian host cell would be biologically active *in vivo*.

36. Techniques for the recombinant expression of proteins were first developed using bacteria, principally *E. coli*, as host cells. These techniques were adequate for the production of some mammalian proteins in functional form. Examples of functional *E. coli*-produced recombinant proteins include human insulin, G-CSF, hGH, and certain interferons.

37. Such mammalian proteins made in bacterial cells, however, generally will not undergo any of the post-translational modifications such as glycosylation that would normally

occur if the protein were made in mammalian cells.[3] This is because the enzymes that catalyze these modifications are generally not found in bacteria.

38. The bacterial expression approach proved adequate for some proteins including the ones I mentioned above because they do not require mammalian-specific modifications such as glycosylation for *in vivo* biological function in animals.

39. Some mammalian proteins were functionally expressed in *E. coli* even though the native molecules are modified by glycosylation. Thus, not all glycoproteins need be glycosylated for function. One example is interferon α ("leukocyte A interferon"). Gutterman, *et al.*, "Recombinant Leukocyte A Interferon: Pharmacokinetics, Single Dose Tolerance, and Biological Effects in Cancer Patients," *Annals of Internal Medicine* 96:549-566 (1982) (D.I. 578, Ex. P). On the other hand, some glycoproteins do require glycosylation for their function *in vitro* or *in vivo*. "The retention of biological activity by glycoproteins void of carbohydrate is variable and unpredictable. In some instances, the absence of carbohydrate results in no loss of functional activity as is the case of the antiviral activity associated with the α- and β-interferons (Kelker, *et al.*, 1983; Knight & Fahey, 1982). In other cases, murine C4 loses hemolytic activity (Karp, *et al.*, 1983) or the von Willebrand-VIIIC complex appears inactive upon partial deglycosylation (Gralnick, *et al.*, 1983)." Little, S.P., *et al.*, "Functional Properties of Carbohydrate Depleted Tissue Plasminogen Activator," *Biochemistry* 23, 6191-6195 (1984) (D.I. 578, Ex. Q). Proteins thus can require proper glycosylation for either *in vitro* or *in vivo* activity or both.

40. In 1983-84, the field was just beginning to explore the use of mammalian host cells for the expression of mammalian glycoproteins. At that time, an ordinarily skilled artisan would, in my opinion, have understood that the use of cells from different species, or the use of

[3] With the possible exception of the disulfide bond formation, which can occur in bacterial cells

different cell types from the same species, frequently resulted in differences in post-translational modification of an expressed protein, and that such differences could prevent expression of *in vivo* biologically active glycoproteins.

41. In my opinion, my 1981 review article accurately captured the uncertainty that ordinarily skilled artisans would have experienced prior to Lin's inventions when expressing secreted mammalian proteins in recombinant systems: "Most proteins, secreted proteins in particular, are extensively modified after their synthesis by proteolytic cleavages, S-S bond formation, and glycosylation. The roles of each of these modifications in the structure, function or stability of any particular protein must be determined directly as it is not yet possible to make any generalizations or predictions concerning the physiological importance of these post-translational alterations of any specific glycoprotein or secreted protein."[4] I know of no research between 1981 and 1984 that would have altered the uncertainty to express a secreted protein by recombinant techniques as I stated in my 1981 article. Therefore, while ordinarily skilled artisans were often motivated to express newly cloned genes for complex glycoproteins in cells other than those from which the proteins naturally originated, they would not reasonably expect to succeed in doing so until they had empirically demonstrated that the expressed glycoprotein protein exhibited the *in vivo* biological activity of the native polypeptide.

42. By 1983, the field of recombinant expression of glycoproteins in mammalian host cells was still in its infancy. Expression of only a handful of mammalian glycoproteins had been attempted. Of those attempted, some were proteins that were previously known not to require glycosylation for biological activity. In many cases, the biological activity of the recombinant proteins was not measured. Likewise, in no case had analysis of the specific oligosaccharide

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under certain conditions.

[4] Lodish, "Post-Translational Modification of Proteins," *Enzyme Microb Technol.* 1981 Jul; 3(3):177-280, at 186 (D.I. 578, Ex. R).

structures imparted by heterologous host cells onto any recombinant glycoprotein been performed. Clearly by 1983 — or 1984 for that matter — there was insufficient experience with the expression of recombinant glycoproteins in heterologous host cells for ordinarily skilled artisans to be able to generalize any principles that would enable one to predict whether any given glycoprotein could be successfully expressed in an *in vivo* biologically active form, absent proof of successful prior production.

43. By 1983, it was well-recognized that different cell types and different species could impart different structures to a single protein. Thus, an ordinarily skilled artisan would have been well aware that expressing a recombinant mammalian glycoprotein in a cultured cell line that was a different cell type or species than that from which the desired protein originated would likely result in a novel glycoprotein with different oligosaccharides than the native molecule expressed from its normal environment.

44. Also, by 1983, there was a great deal of scientific interest concerning the roles specific glycan structures play in the function of the glycoprotein to which they are attached. Many different functions had been identified or postulated for the oligosaccharides on glycoproteins, including stability, protein-protein interaction, clearance rate, and self-recognition. Nonetheless, the field's understanding of the function of glycosylation was rudimentary in 1983. Because of this limited knowledge base and the recognized importance of glycosylation, the next decade saw an enormous amount of research regarding the functions of glycosylation, which confirmed the complexity and importance of this biological phenomenon. Varki, A., "Biological roles of oligosaccharides: all of the theories are correct," *Glycobiology* 3:97-130 (1993) (D.I. 578, Ex. S). Thus, an ordinarily skilled artisan in 1983 would have been aware that the glycosylation structures found on any given glycoprotein could contribute one or more of a wide array of different functions. However, the particular functions of glycosylation

on a given glycoprotein and the tolerance for variation in oligosaccharide structure would have been highly unpredictable in the absence of proof that a particular glycoprotein had been successfully expressed in a heterologous host cell and demonstrated *in vivo* biological activity.

45. In 1983, Konrad and his colleagues noted that glycosylation of recombinant glycoproteins would depend on the host cell chosen. Konrad, M. *et al.*, "Applications of genetic engineering to the pharmaceutical industry," *Ann N Y Acad Sci.* 413:12-22 (1983) (D.I. 578, Ex. T).

- "Hopefully one of the contributions of genetic engineering will be to make experiments possible that will more completely elucidate the role of the carbohydrate residues. IFNs are certainly not unique in being glycosylated. Of the major proteins in the blood, only serum albumin is not glycosylated." *Id. at* 17.

- "However even in its present form [expressed in CHO cells] this cell line produces levels that are an order of magnitude higher than that produced from regular fibroblasts. It is unlikely that the pattern of sugar residues will be exactly the same as that produced by human fibroblasts, although it may be quite close. It will enable us to proceed more rapidly in investigations of just what the sugar means to the biochemical properties of this kind of IFN." *Id.* at 21.

46. Likewise, Goeddel's patent application filed in 1983 (U.S. Patent No. 4,766,075 (D.I. 578, Ex. U)) also anticipated the dependence of glycosylation on the host cell: "depending upon the host cell, the human tissue plasminogen activator hereof may contain associated glycosylation to a greater or lesser extent compared with native material." ('075 Col. 4:10-14). "In addition, the location of and degree of glycosylation will depend on the nature of the host cellular environment." ('075 Col. 5:18-20).

47. Dr. Lin's patent specification also identified this issue. Lin's patent specification explicitly recognized the differences between different species' glycosylation: "Depending upon the host employed, polypeptides of the invention may be glycosylated with mammalian or other eukaryotic carbohydrates or may be non-glycosylated." ('933 Patent at 10:28-31).

48. The authors of the Colby paper noted that differences in glycosylation between CHO cell material and native material have immunological consequences. Colby, C.B., *et al.*, "Immunologic differentiation between *E. coli* and CHO cell-derived recombinant and natural human beta-interferons," *J. Immunol.* 133(6):3091-5 (1984) (D.I. 578, Ex. V).

- "Because the HuIFN-β gene is expressed in a hamster cell environment, it is possible that the CHO cells glycosylated HuIFN-β differently than do human fibroblast cells. Such differences in glycosylation may result in an unmasking of the anti-viral site on the CHO cell produced IFN molecule, with the site becoming form accessible to the anti HuIFN-β antibody while the overall conformation of the protein molecule remains unchanged. Alternatively, differences in glycosylation could produce overall conformational differences between the molecules such that the anti-viral site of the IFN molecule cross-reacts with higher affinity with the neutralizing antibody. In either case, CHO-rHuIFN-β would be preferentially neutralized by anti-HuIFN-β, as reported in Table I." *Id.* at 3094.

- "In view of the immunological non-identity of the __ IFN, it is important to know whether these *in vitro* immunologic differences are significant enough for the host's immune system to perceive the recombinant IFN as foreign. If so, the recombinant HuIFN-β could elicit an antigenic response *in vivo.* Recently it was reported that recombinant HuIFN-α was antigenic in several human cancer patients treated i.v. with recombinant HuIFN-α (27), whereas an antigenic response is rarely observed in human cancer patients treated with either natural HuIFN-α, HuIFN-β, or both." *Id.* at 3094.

49. Furthermore, a 1984 review article by Roche's expert Dr. Gaylis is particularly revealing as to the uncertainty and confusion of the field in the 1983-84 timeframe as to whether recombinantly produced EPO would be biologically active *in vivo*. In his assessment of the state of the art as of 1984, Dr. Gaylis stated:

> "It is hoped that with new advances in genetic engineering, the Ep gene will be cloned and transferred to a different organism such as E. coli, as this would facilitate production of the hormone in quantities adequate for clinical use. Clearly, then, the production of EP by 1411H is of significant biological interest and may be of clinical value if the gene controlling Ep synthesis can be cloned and used for the manufacture of the hormone."[5]

[5] Gaylis, F.D., *et al.*, "*In vitro* models of human testicular germ-cell tumors." *World J. of Urol.* 2:2-5, 5 (1984) (D.I. 578, Ex. W).

50. Thus, in 1984, Dr. Gaylis proffered the hope that if EPO could be cloned it could be inserted into *E. coli* cells for "production of the hormone in quantities adequate for clinical use." But, of course, as we know now, the EPO material produced from *E. coli* by Dr. Lin, although active *in vitro*, turned out to be inactive *in vivo*. That is because *E. coli.*, a bacterial cell, is incapable of glycosylating the proteins it produces — a fact known to the skilled artisan in 1984. That in 1984 Dr. Gaylis suggested to his peers that EPO for clinical use could be made from *E. coli.* reflects the truly unsettled state of affairs as to whether recombinantly produced EPO would in fact be *in vivo* biologically active. In particular, Dr. Gaylis' suggestion that *E. coli* was the route for making recombinant EPO for clinical use shows that, it certainly was not obvious that recombinant EPO made from mammalian cells in culture would be *in vivo* active and therapeutically effective.

51. It would be incorrect to assume that any oligosaccharide structures added by host cells to a protein requiring glycosylation would confer *in vivo* biological activity. Because there are many known, and even more unknown, *in vivo* interactions between the carbohydrate chains on glycoproteins and other proteins (such as antibodies, and receptor proteins) and cells, it was simply not possible in 1983 (and today still is not possible) to successfully predict *a priori* how a differently glycosylated glycoprotein will behave and perform *in vivo*. It may interact with its intended receptor or it may not. It may be removed from the blood or from other body tissues faster or slower. It may prove antigenic and elicit an immune reaction or it may not. It may interact with a different and unintended receptor or it may not. These are just some of the uncertainties that result from changes made to the carbohydrate structure of a glycoprotein. Until one makes and empirically tests how a glycoprotein actually behaves *in vivo*, one cannot successfully predict whether it will behave as desired.

52. To summarize, by 1983 one could not have predicted which specific oligosaccharides a host cell would add to a given protein. Moreover, the field could not predict the tolerance of a particular glycoprotein to changes in its oligosaccharide structure. Today, we know that certain recombinant glycoproteins will function despite significant changes in their oligosaccharide structure as compared to their native structure, but even this knowledge regarding specific glycoproteins does not allow those skilled in the art to successfully predict *a priori* how changes made to a different glycoprotein can and will affect its ability to perform its intended *in vivo* function.

C. A PERSON OF ORDINARY SKILL IN THE ART IN POSSESSION OF A DNA SEQUENCE ENCODING EPO IN 1983-84 WOULD STILL HAVE LACKED A REASONABLE EXPECTATION OF SUCCESS IN PRODUCING *IN VIVO* BIOLOGICALLY ACTIVE EPO OUTSIDE THE HUMAN BODY ABSENT PROOF OF SUCCESSFUL PRIOR PRODUCTION OF SUCH EPO

53. A central premise of Roche's obviousness-type double patenting argument is that once Dr. Lin was in possession of a DNA sequence encoding EPO, he could predictably expect that he would produce *in vivo* biologically active EPO by merely inserting the DNA into mammalian cells using known techniques and waiting for production. I disagree.

54. While some of the tools and techniques for producing recombinant glycoproteins in mammalian cells were known prior to October 1983, the field had not progressed to a state in which one of ordinary skill in the art could reasonably expect success, particularly where the protein of interest (EPO) had never been successfully produced in a recombinant cell.[6] Moreover, in my opinion, a person of ordinary skill in the art at that time would have reasonably believed that it was just as likely that *in vivo* biologically active EPO could not be successfully produced. A person of ordinary skill in the art in October 1983 would have expected that differences in post-translational modifications like glycosylation between the cells in the human

[6] My opinion would be the same if the relevant date of analysis were prior to November 30, 1984.

body that naturally produce EPO and the selected recombinant cells could prevent production of EPO glycoprotein in a form that was biologically active *in vivo* absent experimental validation.

55. The following facts are apparent from the literature published before (or, in some instances, contemporaneous with) Lin's inventions:

- Erythropoietin is a glycoprotein, and at least the sialic acids attached to the carbohydrate chains are important for biological activity.
- Oligosaccharide chains added to proteins by eukaryotic cells, specifically including mammalian and other vertebrate cells, have an extremely large variety of different carbohydrate substituents, structures, and properties.
- Specific oligosaccharide structures are required for the function of many glycoproteins.
- The host cell species and cell type can determine the oligosaccharide structures attached to a particular glycoprotein.
- Mammalian cells perform many post-translational modifications in addition to glycosylation that impact function in a species and cell-type and protein specific manner. Whether EPO had any such modifications was unknown.
- At the time of Lin's inventions only a handful of recombinant glycoproteins had been expressed in vertebrate cells, and prior to November 1984 only one (tPA) may have been shown to have *in vivo* biological activity.
- It was assumed that recombinant proteins produced in host cells from the homologous cell types and species would be more likely to have *in vivo* biological activity and be useful than would recombinant proteins produced in cells of a different type or different species.
- Given the art of expression of recombinant proteins in mammalian cells in 1983-84, there was no reasonable expectation that any given glycoprotein could be produced in any specific mammalian or other vertebrate host cell in an amount sufficient to have an *in vivo* biological or therapeutically effective activity.

56. In addition, the 1983-1984 time period was the very birth of the technology of recombinant expression of glycoproteins in mammalian cells. As I discussed above, even by the end of 1984, there was insufficient experience with this technology to draw any conclusions about whether any particular recombinant glycoprotein could be expressed in an *in vivo* biologically active form. Only a few proteins had been expressed, and in no case had reasonable

fidelity of glycosylation as compared to the native glycoprotein been established. Moreover, most of the earliest proteins produced did not require glycosylation to be biologically active *in vivo*, and in almost all instances, the researchers had not even tested whether their recombinant products had any *in vivo* biological activity.

57. Prior to Dr. Lin's successful expression of *in vivo* biologically active recombinant human EPO, there were few, if any, reports of glycoproteins that had been produced by recombinant means and demonstrated to possess *in vivo* biological activity. I understand that during prosecution of the patents-in-suit, Amgen's attorneys characterized EPO as an "obligate glycoprotein."

58. As discussed above, earlier experiments by Goldwasser and Dordal demonstrated that naturally occurring EPO that lacked sialic acids or was deglycosylated lacked *in vivo* biological activity. Therefore, a person skilled in the art in 1983 would have expected that EPO likely required some form of glycosylation in order to be *in vivo* biologically active.

59. Two implications followed from this expectation. First, it meant that some structure in addition to EPO's amino acid sequence was required and must be present on the protein in order for the protein to have *in vivo* biological activity. What those precise structures were for EPO and whether any recombinant cell would predictably produce such structures as were needed for EPO's *in vivo* biological activity was not known or obvious prior to Lin's work, although for EPO it evidently entailed some form of glycosylation. Whether differences in the type, amount, or structure of the required glycosylation would affect the protein's *in vivo* biological activity was not known, and what if anything else in addition to glycosylation might also be needed, was not known. Second, it was not known whether a recombinant cell would add other unwanted or unneeded structures to the protein, or change the protein in some way that would impair biological activity *in vivo*. In sum, until a particular protein was

actually expressed in a cultured cell and tested for bioactivity *in vivo*, it was not possible to predict with reasonable confidence whether the recombinantly produced glycoprotein would have the desired *in vivo* biological activity. Once Lin demonstrated that EPO could be produced in vertebrate cells, e.g., CHO and COS, and have *in vivo* bioactivity, efforts to produce recombinant EPO in other vertebrate cells became much more predictable.

60. These facts establish that at the time Lin's inventions were made, it was highly unpredictable whether EPO could be produced in an *in vivo* biologically active glycosylated form from recombinant host cells.

61. I understand that it is improper to use hindsight to determine whether a patent claim would have been obvious at the time of invention. In my opinion, Roche's argument that the successful outcome of Lin's plan to produce *in vivo* biologically active material in heterologous recombinant host cells was expected or predictable is an exercise in hindsight.

62. A priori, in 1983-84 an ordinarily skilled artisan would have had no way of knowing whether CHO host cells would add appropriate glycans to human EPO and, if they did, would add them efficiently enough to produce a population of EPO glycoproteins of sufficient quality to provide detectable *in vivo* biological activity. Thus, in 1984 there was no reason to believe that a transformed CHO cell would modify EPO with the same or similar sugars as a human cell that naturally makes EPO, or that the sugars added by the non-human cell would impart the claimed biological effect.

63. Today we know that CHO cells are a good host for the production of recombinant human glycoproteins. But in 1983, the field had no experience with expression of *in vivo* bioactive glycoproteins on which to draw.

64. Post-1984 publications concerning the glycosylation of recombinant glycoproteins, including EPO, reinforce the surprising nature of Lin's successful expression of *in*

vivo functional EPO from heterologous cells. For example, a 1991 review article emphasized that researchers were ***pleasantly surprised*** that when they examined the glycosylation of recombinant proteins expressed in CHO cells that the CHO oligosaccharides were as similar as they are to the native glycosylation of these human proteins:

> "Detailed *N*-linked and *O*-linked oligosaccharide structures have been determined for several glycoproteins produced using recombinant CHO cells, including EPO, t-PA, interferon-βl and IL-2. ***A pleasant surprise from these recent analyses*** has been the remarkable degree to which the oligosaccharide structures from the CHO-produced glycoproteins correspond to the structures of those same proteins isolated from human urine or produced using normal human diploid cells. As a result, Chinese hamster ovary cells have emerged as the cell line of first choice for the synthesis of recombinant human therapeutic glycoproteins, although CHO cells do possess deficiencies that may limit their applicability in specific cases, such as limited capability for γ-carboxylation and inability for oligosaccharide sulfation." Gooche *et al.*, "The Oligosaccharides Of Glycoproteins: Bioprocess Factors Affecting Oligosaccharide Structure And Their Effect On Glycoprotein Properties" *BioTechnology* 9:1347-1355 (1991) (emphasis added) (D.I. 578, Ex. X).

65. Similarly, a scientific 1988 publication analyzing the glycosylation patterns found on EPO states that "[t]his paper proved, ***for the first time***, that recombinant technique can produce glycoprotein hormone whose carbohydrate structures are common to the major sugar chains of the native one." Takeuchi et al., "Comparative Study of the Asparagine-linked Sugar Chains of Human Erythropoietins Purified from Urine and the Culture Medium of Recombinant Chinese Hamster Ovary Cells," *J. Biol. Chem.* 263(8):3657-3663 (1988) (emphasis added) (D.I. 578, Ex. K).

66. A 1993 review article by Lis and Sharon ("Protein glycosylation, structural and functional aspects," *Eur. J. Biochem*. 218:1-27 (1993) (D.I. 578, Ex. Y)) is particularly compelling evidence of the inventive significance of Dr. Lin's process and EPO product inventions.

> Also, genetic engineering makes it possible to produce glycoproteins in heterologous systems on a large scale, both for research purposes and for therapeutic use (Table 1). We are indeed witnessing the emergence of glycotechnology [70], a branch of biotechnology that uses novel approaches to manipulate carbohydrates or related materials, with the aim of creating new products or new procedures for the betterment of our lives. ***An impressive example is erythropoietin, a circulating glycoprotein hormone that stimulates erythropoiesis, which has the distinction of being the first recombinant glycoprotein produced industrially for clinical use.*** It is being employed on a wide scale for the treatment of anemia in patients on haemodialysis [71]; its sales in 1991 reached $645 million. Another clinically important recombinant glycoprotein is the thrombolytic agent, tissue plasminogen activator (tPA), with sales of close to $200 million in the same year. Still, ***the manifold effects of carbohydrates on the stability and biological activities of glycoproteins are a source of much concern in the biotechnological production of pharmacologically useful glycoproteins*** [72-75]. (emphasis added).

67. This passage from Lis and Sharon is significant in a number of respects. First, it acknowledges that "glycotechnology" was still an emerging field 10 years ***after*** Dr. Lin's inventions. This statement makes plain that the field of glycoprotein production was new and unpredictable in the 1983-84 time period. Second, Lis and Sharon rightly describe Lin's work as "an impressive example" of glycotechnology, given that it "has the distinction of being the first recombinant glycoprotein produced industrially for clinical use." Lastly, the authors note that "the manifold effects of carbohydrates on the stability and biological activities of glycoproteins" remained "a source of much concern in the biotechnological production of pharmacologically useful glycoproteins."

68. Successful heterologous expression of *in vivo* biologically active EPO from recombinant host cells was unexpected and surprising. Thus the claimed production of *in vivo* biologically active EPO is not obvious in light of EPO DNA-containing cells. EPO was the one of the first two glycoproteins requiring glycosylation for *in vivo* function to be successfully produced by recombinant means in mammalian cells. Therefore, ordinarily skilled artisans

would not have expected recombinant EPO produced in non-natural cell types and species to have proper *in vivo* biological function until after Dr. Lin's successful experiments.

69. Because of the uncertainties in the art I described above, in my opinion the ordinarily skilled artisan could not have reasonably expected to have produced *in vivo* biologically active EPO until he actually received the positive *in vivo* test results. Thus, Dr. Lin did not have possession of the inventions of the claims-in-suit until he actually successfully transformed and tested heterologous mammalian cells for the production of *in vivo* biologically active EPO, which I understand to have occurred in early March 1984.[7]

70. Until Dr. Lin proved that *in vivo* biologically active EPO could be made in cells outside of the body, no one could predict whether it would ever work. Once Dr. Lin was successful, persons skilled in the art knew that EPO could be produced in an *in vivo* biologically form outside the body. Future efforts to produce EPO under different conditions might require some additional experimentation, but the expectation of success changed dramatically. By proving *in vivo* biologically active EPO could be produced in hamster cells and monkey cells in addition to the natural production from human cells in the body, Dr. Lin's teachings would have led one of ordinary skill in the art to believe that *in vivo* biologically active EPO could be expressed in a broad range of different vertebrate or mammalian host cells, albeit with some additional experimentation required.

[7] *Amgen Inc. v. Chugai Pharm. Co. Ltd.*, 13 U.S.P.Q.2d 1737, 1748 (D. Mass. 1989).

D. THE REFERENCES CITED BY ROCHE'S EXPERTS DURING DISCOVERY DO NOT ESTABLISH THAT AN ORDINARILY SKILLED ARTISAN IN 1983-84 WOULD HAVE HAD A REASONABLE EXPECTATION OF SUCCESS IN EXPRESSING *IN VIVO* BIOLOGICALLY ACTIVE EPO

71. Taken collectively, Roche's experts cited many articles from the pre-1985 time frame that relate to the recombinant expression of proteins in mammalian cells. Although many articles are cited, the sum total of the information known to ordinarily skilled artisans would not have lead to a reasonable expectation of success in expressing *in vivo* biologically active EPO.

1. Articles relating to general tools or methods for protein expression in mammalian cells

72. In their expert reports, Roche's experts cite many articles that relate to "tools" or methods for expressing proteins in mammalian cells. None of these articles actually show the expression of a recombinant glycoprotein in mammalian host cells:

- Axel U.S. Pat. No. 4,399,216
- Axel U.S. Pat. No. 5,149,636
- Gluzman *et al.* SV40-transformed simian cells support the replication of early SV40 mutants. Cell. (1981) Jan;23(1):175-82
- Graf *et al.* Transformation of the gene for hypoxanthine phosphoribosyltransferase. Somatic Cell Genetics 5: 1031-1044 (1979)
- Graham *et al.* A new technique for the assay of infectivity of human adenovirus 5 DNA. Virology. (1973) Apr;52(2):456-67
- Kaufman *et al.* Growth-dependent expression of dihydrofolate reductase mRNA from modular cDNA genes. Mol Cel Bio. Sept (1983); 1598-1608
- Kaufman and Sharp, "Amplification and Expression of Sequences Cotransfected with a Modular Dihydrofolate Reductase Complementary DNA Gene," J.J. Mol Biol. 159: 601-621 (1982)
- McBride *et al.* Transfer of Genetic Information by Purified Metaphase Chromosomes (1973)

- Merril *et al.* Bacterial virus gene expression in human cells. Nature. (1971) Oct 8;233(5319):398-400
- Pellicer *et al.*, The transfer and stable integration of the HSV thymidine kinase gene into mouse cells. Cell 14:133-141 (1978)
- Schimke *et al.* "Gene amplification and drug resistance in cultured murine cells," SCIENCE, 202: 1051- 1055 (1978)
- Subramani *et al.* Expression of the mouse dihydrofolate reductase complementary deoxyribonucleic acid in simian virus 40 vectors. Mol Cell Biol. (1981) Sep;1(9):854-64
- Urlaub and Chasin, "Isolation of Chinese hamster cell mutants deficient in dihydrofolate reductase activity," PROC. NAT'L ACAD. SCl., 77: 42 I 6-4220 (1980)
- Wigler *et al.*, "Transformation of mammalian cells with an amplifiable dominant-acting gene," PROC. NATL. ACAD. SCI., 77:3567-3570 (1980)
- Wigler *et al.* Transfer of purified herpes virus thymidine kinase gene to cultured mouse cells. Cell. (1977) May;11(1):223-32
- Wigler *et al.* Transformation of mammalian cells with genes from procaryotes and eucaryotes. Cell. (1979) Apr;16(4):777-85
- Willecke et al, Cotransfer of two linked human genes into cultured mouse cells. PNAS 75:1274-1278 (1976)
- Wullems *et al.* Expression of human hypoxanthine Phosphoribosyl transferase in Chinese hamster cells treated with isolated human

73. One of the cited method references that merits particular attention is U.S. Patent No. 4,399,216 ("the Axel Patent") (Trial Exhibit ("TX") 2024), which is one example of an early technique intended for expression of recombinant proteins in mammalian cells. Generally, the claims of the '216 patent relate to methods for generating recombinant eukaryotic cells that produce desired recombinant proteins. The Axel patents focus on two process steps to achieve expression: cotransformation and coamplification.

74. The Axel patent specification itself does not provide any explicit guidance regarding the production of glycoproteins. The Axel specification merely states:

> Still another aspect of the present invention involves the preparation of materials normally produced within eucaryotic cells in minute amounts such as glycoproteins including interferon, which are in part protein but additionally include other chemical species such as sugars, ribonucleic acids, histones and the like. ***Although the method or methods by which cells synthesize complicated cellular materials such as the glycoproteins are poorly understood***, it is anticipated that by using the process of the present invention it will be possible to synthesize such materials in commercially useful quantities. Specifically, ***it is anticipated*** that after inserting a gene or genes for the protein portion of a cellular material such as a glycoprotein, which includes a non-protein portion, into a eucaryotic cell of the type, which normally produces such material, the cell will not only produce the corresponding proteinaceous material but will utilize already existing cellular mechanisms to process the proteinaceous materials, if and to the extent necessary, and will also add the appropriate non-proteinaceous material to form the complete, biologically active material. Thus, for example, the complete biologically active glycoprotein, interferon, could be prepared by first synthesizing interferon protein in the manner described and additionally permitting the cell to produce the non-proteinaceous or sugar portion of interferon and to synthesize or assemble true interferon therefrom. The interferon so prepared could then be recovered using conventional techniques. (TX 2024, at col. 7, lines 31-58 (emphasis added)).

75. Thus, the Axel patent merely conveys the hope that expressing a recombinant protein in a mammalian host cell will result in "the complete biologically active material." But, none of the experiments reported in the Axel patent disclose a transformed cell where a gene of interest has been successfully translated into a protein or glycoprotein. Thus, none of the Axel experiments demonstrated the isolation or functional assay of any recombinant foreign protein encoded by a gene of interest by the transformed cells. Moreover, there is no discussion of any structure, composition, or sequence of oligosaccharides that might be attached to any natural or recombinant protein. Furthermore, there is no disclosure of what specific culture conditions, if any, would be required to achieve such a result.

2. Articles discussing recombinant expression of some mammalian glycoproteins in mammalian cells

76. During discovery, Roche's experts also cited articles that discuss the recombinant expression of a small number of mammalian glycoproteins in mammalian cells. In my opinion, none of these articles would have provided the ordinarily skilled artisan with a reasonable expectation of success in expressing *in vivo* biologically active erythropoietin protein in a mammalian cell expression system. The references identified by Roche's experts that disclose the expression of mammalian glycoproteins in mammalian cells follow:

MAMMALIAN GLYCOPROTEIN	**PUBLICATION CITED BY ONE OR MORE ROCHE EXPERTS DURING DISCOVERY**
Interferon γ ("immune interferon")	Haynes, J. and Weissman, C., "Constitutive, long-term production of human interferons by hamster cells containing multiple copies of a cloned interferon gene," *Nucleic Acids Res.*, 11:687-706 (1983) Devos, R. *et al.*, "Purification of recombinant glycosylated human gamma interferon expressed in transformed Chinese hamster ovary cells," *J Interferon Res.* 4:461-8 (1984 October) Fiers EP Published Application No. 0088540 (TX 2028) Gray and Goeddel. Cloning and Expression of Murine Immune Interferon. cDNA," *Proc. Nat'l Acad. Scl.*, 80:5842-5846 (1983) Scahill, *et al.*, "Expression and characterization of the product of a human immune interferon cDNA gene in Chinese hamster ovary cells," *Proc. Nat'l Acad. Scl.* 80: 4654-4658 (1983)
Interferon β (fibroblast interferon")	Canaani *et al.,* "Regulated expression of human interferon beta 1 gene after transduction into cultured mouse and rabbit cells," *Proc Natl Acad Sci U S A.* 79(17):5166-70 (1982) Sept. McCormick *et al.*, "Inducible expression of amplified human beta interferon genes in CHO cells," *Mol. & Cell. Biol.*, 4: 166-172(1984) McCormick US Pat. No. 4,966,843 Zinn *et al.,* "Regulated expression of an extrachromosomal human beta-interferon gene in mouse cells," *Proc Natl Acad Sci U S A.* 79(16):4897-901 (1982) Aug. Higashi *et al.*, "Structure and expression of a cloned cDNA for mouse interferon," *J. Biol. Chem.* 258: 9522-29 (1983) McCormick US Pat. App. 438,991

MAMMALIAN GLYCOPROTEIN	PUBLICATION CITED BY ONE OR MORE ROCHE EXPERTS DURING DISCOVERY
	Taniguchi *et al.*, "Construction and Identification of a Bacterial Plasmid Containing the Human Fibroblast Interferon Gene Sequence," *Proc. Japan Acad.* 55:464-469 (1979)
IL-2	Taniguchi *et al.*,"Structure and expression of a cloned cDNA for human interleukin-2," *Nature* 302: 305-310 (1983)
Multi-CSF (IL-3)	Yokota *et al.*, "cDNA clones coding for polypeptides exhibiting multi-linage cellular growth factor activity," U.S. Patent 4,695,542, application filed 10/4/83, continuation-in-part filed 3/19/84
Immunoglobulins	Neuberger, "Expression and regulation of immunoglobulin heavy chain gene Transfected into lymphoid cells," *EMBO J.* 2:1373-1378 (1983) Oi, *et al.*, "Immunoglobulin gene expression in transformed lymphoid cells," *Proc. Nat'l. Acad. Sci.* 80:825-829 (1983) Sidman C., "Differing Requirements for Glycosylation in the Secretion of Related Glycoproteins is Determined Neither by the Producing Cell Nor by the Relative Number of Oligosaccharide units" *Journ. Biol. Chem.* 256:9374-9376 (1981) Seidman, *et al.*, "Antibody Diversity," Science, 202: 11 (1978)
Pig major transplantation antigen (SLA)	Singer *et al.*, "Characterization of a porcine clone encoding a major histrocompatibility antigen: expression in mouse L cells," *PNAS* 79:1403-1407 (1982)
Factor VIII	Toole US Pat. No. 4,757,006
Viral antigens	Sveda and Lai. Functional expression in primate cells of cloned cDNA coding for the hemagglutinin surface glycoprotein of influenza virus. Proc. Natl. Acad. Sci. 1981 Sept. Vol. 78, No. 9:5488-5492 Gething and Sambrook. Construction of influenza haemagglutinin genes that code for intracellular and secreted forms of the protein. Nature. 1982 Dec.Vol. 300:598-603 Levinson, *et al.*, U.S. Patent No. 4,741,901
tPA	Goeddel *et al.*, "Human Tissue Plasminogen Activator," U.S. Patent 4,766,075 filed 4/7/83 (TX 2030) Collen *et al.*, "Biological Properties of Human tissue-type plasminogen activator obtained by expression of recombinant DNA in mammalian cells," *J. Pharm. & Expt. Therapeutics*, 231, 146-152 (1984) Goeddel *et al.*, EP 0 093 619

MAMMALIAN GLYCOPROTEIN	PUBLICATION CITED BY ONE OR MORE ROCHE EXPERTS DURING DISCOVERY
	Levinson *et al.*, EPO 0117059 (1984) Levinson *et al.*, EPO 0117060 (1984)

77. Before addressing the specifics of the prior art references cited by Roche's experts, I will make some general comments concerning their analyses. First, none of Roche's experts who render opinions regarding obviousness-type double patenting seriously address whether an ordinarily skilled artisan would have had a reasonable expectation of success in practicing Lin's claimed inventions given the state of the art at the time. I understand that this is an essential part of any obviousness analysis. It is my opinion that just because an approach may be obvious to try, it does not necessarily mean there is any likelihood of predictability or success.

78. Second, Roche's experts do not acknowledge the very significant difference between a protein's activity as measured in an ***in vitro*** (in a test tube or Petri dish) assay as compared to ***in vivo*** (in an animal) assay. As I discuss below, there can be meaningful and important differences between the functions of glycoproteins as measured by these assays. Because almost all of Dr. Lin's claims concern ***in vivo*** biologically active erythropoietin, Roche's experts' unstated generalizations from the results of ***in vitro*** biological assays are very misleading, and contribute to my conviction that the prior art at the time of Dr. Lin's inventions did not provide a reasonable expectation of success of producing ***in vivo*** biologically active human erythropoietin, even after Dr. Lin cloned the EPO DNA.

79. By the end of 1984, while some mammalian glycoproteins had been expressed in mammalian host cells, the field's experience was still very limited. In fact, I know of no reports of the successful expression of glycosylated mammalian glycoproteins in mammalian cells before 1982.

80. I will address each glycoprotein identified as prior art by Roche's experts in turn.

Interferon γ

81. In 1983, Haynes and Weissman disclosed transfection and expression of human interferon γ ("IFN-γ") in mammalian tissue culture cells. Haynes, J. and Weissman, C., "Constitutive, long-term production of human interferons by hamster cells containing multiple copies of a cloned interferon gene," *Nucleic Acids Res.*, 11:11(3):687-706 (1983) (TX 2001). The authors reported that the transfected host cells supernatant had interferon activity in an *in vitro* assay, measuring its effect on cells growing in culture. No assay for *in vivo* biological activity — that is in an animal — was performed. The authors also performed some preliminary physical characterization of the protein produced by the transformed cells. They found that the observed molecular weight and purification properties were consistent with glycosylation of the expressed protein. But no effort was made to compare the oligosaccharides imparted by the rodent host cells side-by-side with the native oligosaccharide structures of human IFN-γ. Nonetheless, the authors noted that their observed molecular weights for IFN-γ (22,400 and 23,400) did not perfectly agree with the values for native human IFN-γ reported in the literature, and that the difference might have been due to differences in post-translational modification of recombinant human IFN-γ made in hamster cells and normal IFN-γ made by human leukocytes. *Id.* at 702-703.

82. The authors of the Haynes article recognized that the use of heterologous host cells — hamster cells for production of a human protein — may lead to undesirable results because the glycosylation imparted by hamster ovary cells might not recapitulate the glycosylation imparted by the leukocytes that naturally produce IFN–γ in the human body:

"[P]osttranslational modification in leukocytes and hamster cells could be different." Therefore the authors suggested that a human host cell line might be more appropriate: "As it is not known whether glycosylation in hamster and human cells leads to identical structures it may be more appropriate to generate and use a human dhfr$^-$ cell line for the production of human glycoprotein." This statement is a strong indication that an ordinarily skilled artisan would not have reasonably expected a recombinant glycoprotein expressed in a heterologous cell such as a CHO cell to have *in vivo* biological activity.

83. None of the other IFN-γ references discussed by Roche's experts (the Fiers '540 patent application, the Gray and Goeddel paper, and the Scahill paper) disclose any *in vivo* biological activity of the recombinant human IFN-γ.

84. Even after 1983 there was uncertainty concerning the role of the carbohydrate chains attached to human gamma interferon. "The production of fully active γ-interferon demonstrated that the lack of a glycosylation system in *E. coli* would not necessarily affect the product quality." Vehar *et al.*, "Characterization studies of human tissue-type plasminogen activator produced by recombinant DNA technology." *Cold Spring Harbor Symp. On Quant. Biol.* 51:551-562 (1986) (AM-ITC 01059954-65) (9/26/07 Lodish Decl., Ex. A); Kelker *et al.*, "Effects of Glycosidase Treatment on the Physiochemical Properties and Biological Activity of Human Interferon-γ" *J. Biol. Chem.* 258:8010-13 (1983) (AM-BER-ERB001045-48) (9/26/07 Lodish Decl., Ex. B) ("Although treatment with glycosidases did not result in a marked change in the degree or the pattern of antiviral activity at the tissue culture level, alterations in the carbohydrate moiety might significantly influence biological activity in the intact organism. Studies with other glycoproteins showed that the sugar moiety can exert a marked effect on plasma clearance rates and tissue distribution (27, 28). Removal of carbohydrate also might affect susceptibility to proteolytic degradation (29, 30), stability (31),

and solubility of the molecule (32). Additional studies will be required to determine the effect of selective deglycosylation on these parameters of IFN-γ.").

Interferon β (IFN–β)

85. McCormick, F. *et al.*, probably the most detailed of several references cited by the Roche experts, reports the expression of glycosylated human IFN beta in heterologous cells. McCormick, F. *et al.*, "Inducible expression of amplified human beta interferon genes in CHO cells," *Mol Cell Biol.* 4(1):166-72 (1984) (AM-ITC 00007576-82) (9/26/07 Lodish Decl., Ex. C) (*see also* the McCormick '843 patent and '991 patent application). McCormick *et al.* determined that the expressed protein was glycosylated because radioactive monosaccharides could be incorporated into it during synthesis. The authors do not report any results of *in vivo* biological assays. Moreover, the authors do not report any structural comparison of the carbohydrates attached to the recombinant material to that on the native human IFN beta to assess the fidelity of the recombinant expression process.

86. It is interesting that McCormick *et al.* report that interferon β requires modification in order for *in vitro* bioactivity. This result appears to contradict others' research on deglycosylated native material. The authors themselves cite publications where there was no loss in activity after treatment of the beta interferon with glycosidases. The weight of opinion at the time was that carbohydrates could be removed from IFN-β with no loss of functional activity: "The retention of biological activity by glycoproteins void of carbohydrate is variable and unpredictable. In some instances, the absence of carbohydrate results in no loss of functional activity as is the case of the antiviral activity associated with the α- and β-interferons." Little, S. P. *et al.*, "Functional Properties of Carbohydrate Depleted Tissue Plasminogen Activator," *Biochemistry* 23: 6191-6195 (1984) (D.I. 578, Ex. Q). Moreover, it is absolutely clear now that IFN-β does not require glycosylation because there is an *E. coli* produced IFN-β that has been

approved as safe and efficacious for human therapy (Betaseron®, Chiron Corp.). Thus, it is my opinion that an ordinarily skilled artisan in 1983-84 would not have considered the literature concerning IFN-β expression — which did not appear to require glycosylation for function — to be particularly analogous to the expression of *in vivo* biologically active EPO — which does not require glycosylation for function — and thus this art is not relevant to the central question here.

87. I note that Roche's experts Drs. Kellems and Lowe quote a statement from the McCormick '991 patent application which they both characterize as acknowledging the "desirability of producing recombinant IFN-β through mammalian host cell expression over production in bacterial cells." However, had Drs. Kellems and Lowe quoted the entire passage, an entirely different message emerges:

> Although at least some IFNs are believed to be glycoproteins, IFN-β is the only interferon that has been shown to be a glycoprotein by chemical measurement of its carbohydrate content. It has one N-glycosidyl attachment site (E. Knight, Jr., Proc. Natl. Acad. Sci., 73 520 (1976), E. Knight, Jr., and D. Fahey, J. Interferon Res., 2 (3) 421 (1982)). Even though not much is known about the kinds of sugars which make up the carbohydrate moiety of IFN-β it has been shown that the carbohydrate moiety is not essential for its antigenicity, biological activity or hydrophobicity. (T. Taniguchi *et al.*, supra; E. Knight, Jr. and E. Knight Jr. [sic] and D. Fahey supra). *E. coli*, which has been used for the expression of the IFN-β gene, has no mechanism for attachment of carbohydrates to proteins. The IFN-β produced in *E. coli* by DNA technology has *in vitro* anti-viral activity similar to that of native IFN-β indicating that glycosylation is probably not essential for full biological activity. However, studies of *E. coli* produced IFN-β suggests that although it retains biological activity similar to that of the native human IFN-β even with 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 to 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 in the conformation closest to that of native human IFNs. There are however, technical problems involved with introducing DNA fragments into animal tissue culture cells

> which are quite impermeable to nucleic acids. ***Other problems relating to the production of the host IFN which may be antigenic to other species need to be addressed and solved as these samples would not be suitable for clinical and therapeutic uses.***[8]

88. The full passage from the McCormick patent application is revealing because it establishes that, unlike erythropoietin, IFN-β was thought not to need glycosylation for biological function. Second, the passage makes clear that expression in "animal hosts" would be expected to provide the "conformation ***closest*** to that of native human IFNs," not ***identical*** to the native human proteins. And third, the passage conveys the very real concern that the expression of human IFN-β in non-human cells may lead to antigenicity issues that would preclude clinical use of the recombinantly produced IFN-β materials.

IL-2

89. Roche's experts also cite the Taniguchi *et al.* paper as an example of "expression of a known glycoprotein, [where] 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." Like each of the interferon papers described above, the Taniguichi *et al.*, paper does not test the activity of the expressed protein in an *in vivo* assay. The only assay for IL-2 function that is disclosed in the paper involves incorporation of radiolabel into cloned cytotoxic T lymphocytes (CTTL-2 cells) grown in culture (*see* Fig. 5). Moreover, and very tellingly, the Taniguichi paper clearly suggests that a bacterial, not mammalian, expression system is appropriate for IL-2: "Expression of the IL-2 cDNA in *E. coli* is in progress and it will soon become possible by used of cloned cDNA to produce this immunoregulatory molecule in large quantity for various purposes." Taniguchi *et al.*,

[8] McCormick '991 Patent Application, at 2-3. (TX 2026) (emphasis added).

"Construction and Identification of a Bacterial Plasmid Containing the Human Fibroblast Interferon Gene Sequence," *Proc. Japan Acad.* 55:464-469 (1979) (TX 2012.1410-15) (AM-ITC 000764-69).

Multi-CSF/IL-3

90. The Yokota *et al.* patent (U.S. Patent No. 4,695,542, application filed 10/4/83, continuation-in-part filed 3/19/84) (AM-ITC 00449814-30) (9/26/07 Lodish Decl., Ex. D) describes the cloning of a gene for a lymphokine then known as "multi-CSF" or "multi-lineage cellular growth factor."[9] The multi-CSF gene was then expressed in COS (monkey fibroblast) cells and assayed for ***in vitro*** biological activity. The Yokota patent does not disclose whether multi-CSF requires glycosylation for either *in vitro* or *in vivo* function, whether the material expressed in COS cells is actually glycosylated, how, if glycosylated, the oligosaccharide structures of the recombinant protein compare to those of native multi-CSF, and critically, whether COS-expressed multi-CSF had any ***in vivo*** biological activity.

91. I understand that the examiner of Dr. Lin's '868 patent cited Yokota *et al.* along with the '008 claims in his rejection for obviousness-type double patenting which mirrors Roche's current arguments.[10] I further understand that in successfully overcoming this obviousness-type double patenting rejection, among other things, Amgen argued that the Yokota patent was not scientifically relevant to the question because multi-CSF is not a human "obligate glycoprotein" (in other words, that multi-CSF does not require glycosylation for *in vivo* bioactivity). Amgen relied on later work of Metcalfe *et al.*, (Blood 68:46-47 (1986) and Exp. Hematol. 15:288-295 (1987)) which demonstrate that non-glycosylated *E. coli*-produced multi-CSF is *in vivo* biologically active.[11] I agree with Amgen's argument that Yokota's multi-CSF

[9] This protein is now known as interleukin 3.

[10] '179 Application 8/11/94 Office Action (TX 2012.818-21) (AM-ITC 00455242-45).

[11] '179 Application 10/7/94 Applicant's Amendment and Remarks under 37 C.F.R. 1.111 and 1.115 at 10 (TX 2012.832) (AM-ITC 00455256); *see also* '179 Application 9/27/88 Applicant's

does not require glycosylation for *in vivo* biological function, and Yokota's disclosure would not have provided a reasonable expectation of success in producing erythropoietin, which was known at the time to require proper glycosylation for biological function, although the structural details of what that glycosylation needed to be were still unknown.

Viral antigens

92. Roche's experts Drs. Kellems and Lowe cite Sveda and Gething and Sambrook for the proposition that COS cells recombinantly-expressed a glycosylated influenza virus hemagglutinin surface glycoprotein (HA protein). The HA protein is a viral membrane protein, unlike the secreted human glycoproteins that were being pursued as potential therapeutics. These references do not test or demonstrate *in vivo* activity for the recombinantly expressed HA protein. These references do not perform glycosylation characterization nor do they suggest that glycosylation is required for biological activity. Most importantly, HA protein is expressed using a host cell (COS or CV-1 cell) that normally expresses that protein. There is a considerable difference between recombinantly expressing a glycoprotein in a host cell that does not normally express that glycoprotein (like COS or CHO cells for EPO) and recombinantly expressing a protein that a cell usually produces. One would expect in the latter case that the cell would have the right machinery and perform the correct processing to produce a functional protein; the same cannot be said in the former case.

Tissue plasminogen activator (tPA)

93. tPA is a secreted glycosylated serine protease enzyme that converts the serum protein plasminogen to the enzyme plasmin. While plasminogen is enzymatically inactive, plasmin functions to dissolve the accumulations of the fibrin that constitute blood clots.

Reply under 37 C.F.R. 1.111 at 4-5 (TX 2012.497-98) (AM-ITC 000265-66).

Genentech's tPA drug (known as "Alteplase" or "Activase®") is used to treat clots in the coronary arteries associated with heart attacks, and clots in the brain associated with strokes.[12] One of the key features of tPA is its short half-life in the body. One half of the administered Alteplase drug is destroyed within five minutes of injection: "The clearance of Alteplase in AMI patients has shown that it is rapidly cleared from the plasma with an initial half-life of less than 5 minutes."[13] This short half-life was known before 1984. Korninger, C., *et al.*, "Turnover of Human Extrinsic Plasminogen Activator in Rabbits" (1981) *Thromb. Haemostasis* 46, 658-661 (AM-BER-ERB001263-68) (9/26/07 Lodish Decl., Ex. E); Nilsson, T., *et al.*, "*In vivo* metabolism of human tissue-type plasm" *Scand. J. Haematol*. 33, 49-53 (1984) (AM-LOD-ERB000869-75) (9/26/07 Lodish Decl., Ex. F); Collen, D. *et al.*, "Biological Properties of Human Tissue-Type Plasminogen Activator Obtained by Expression of Recombinant DNA in Mammalian Cells" *J. Pharmacol. and Exp. Therap*. 231:146-152 (1984) (TX 2012.260-66) (AM-ITC 00270500-07). It was also shown not long after that clearance of tPA is not mediated by glycosylation specific mechanisms.[14] The short-half life of tPA is important for its usefulness as a therapeutic, because tPA interferes with clotting, and its persistence would lead to unchecked bleeding if surgery were required:

> Turnover studies in rabbits indicated that rt-PA and mt-PA disappear equally rapidly (T1/2 = 3 min) from the circulation, apparently by specific clearance in the liver. The short T1/2 of t-PA probably necessitates its continuous infusion in order to obtain *in vivo* thrombolysis. Base-line hemostasis will, however, be restored rapidly after discontinuation of the infusion, which may be advantageous if invasive procedures should then be required.[15]

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[12] Activase® Full Prescribing Information. http://www.gene.com/gene/products/information/pdf/activase-prescribing.pdf.

[13] *Id.*

[14] Emeis, J. J., *et al.*, "Hepatic clearance of tissue type plasminogen activator in rats," *Thromb. Haemostasis* 54, 661-664 (1985) (AM-BER-ERB000544-49) (9/26/07 Lodish Decl., Ex. G).

[15] Collen, D. *et al.*, "Biological Properties of Human Tissue-Type Plasminogen Activator

94. The tPA development program at Genentech was contemporaneous with, and is in some ways parallel to, the development of EPO by Amgen. There are a series of publications regarding tPA published by Genentech scientists in the 1983-1984 time frame. First, Pennica *et al.*, *Nature* 301:214 (1983) (TX 2012.268-75) (AM-ITC 00454399-406), published the cloning of the tPA gene and expression in *E. coli*. *In vitro* biological activity was observed, but no *in vivo* biological activity was measured.

95. Second, in April 1983 Genentech filed a patent application (U.S. Patent 4,766,075 (D.I. 578, Ex. U)) concerning the recombinant expression of tPA, including the expression of tPA in mammalian host cells. The Goeddel patent discloses, in addition to the subject matter disclosed in Pennica *et al.*, *in vitro* activity of tPA (determined in a colony assay) produced recombinantly in CHO cells. ***The patent does not address whether either bacterially or mammalian-produced recombinant tPA has any in vivo biological activity.*** Nor does the patent compare the structure or activity of the native material to either the *E. coli* or CHO-produced recombinant proteins. The patent does not determine whether the mammalian-produced recombinant tPA is actually glycosylated. In fact, the patent does not disclose any biochemical characterization of CHO-produced recombinant tPA at all. The published European counterpart to this U.S. patent is EP0093619 (TX 2029). The '619 application has no additional disclosure as compared to the U.S. '075 patent. I understand that Genentech made two earlier patent filings concerning (EP0117059 and tPA EP0117060), neither of which disclosed recombinant expression of *in vivo* biologically active tPA in mammalian host cells. Thus, Amgen accurately stated that neither the '619 publication nor the other Genentech publications (the EP0117059 and EP0117060) demonstrate "the production of an obligate human

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Obtained by Expression of Recombinant DNA in Mammalian Cells" *J. Pharmacol. and Exp. Therap*. 231:146-152 (1984) (TX 2012.260-66) (AM-ITC 00454391-97).

glycoprotein such as might give rise, by analogy, to any reasonable expectation of success in the practice of the methods of present claims 65-69."[16]

96. In fact, Genentech's scientists working on tPA made clear that the field was still unpredictable in 1983. In the European '619 application, they explained:

> Molecular biologists are able to recombine various DNA sequences with some facility, creating new DNA entities capable of producing copious amounts of exogenous protein product in transformed microcubes and cell cultures. The general means and methods are in hand for the *in vitro* litigation of various blunt ended or "sticky" ended fragments of DNA, producing potent expression vehicles useful in transforming particular organisms, thus directing their efficient synthesis of desired exogenous product. ***However, on an individual product basis, the pathway remains somewhat tortuous and the science has not advanced to a stage where regular predictions of success can be made. Indeed, those who portend successful results without the underlying experimental basis, do so with considerable risk of inoperability.***

(TX 2012.281-82) (AM-ITC 00454412-13) (emphasis added). Thus, in the context of their work on tPA, the Genentech scientists recognized that it was not possible to predict in advance whether production would be successful for a particular glycoprotein prior to experimental validation.

97. In addition, the Goeddel/Genentech '075 patent recognizes that the specific glycan structures of recombinant tPA produced by mammalian cells will be dependent on the specific host cell chosen, and will not necessarily be equivalent to that of native tPA:

- "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 native material."[17]
- "In addition, the location of and degree of glycosylation will depend on the nature of the host cellular environment."[18]

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[16] '868 File History, 5/26/88 2nd Prelim. Amend., at 18-19 (TX 2012.231-32) (AM-ITC 000208-09).

[17] U.S. Patent 4,766,075 col. 4:10-14; (D.I. 578, Ex. U).

98. The prosecution histories of Genentech's Goeddel tPA patents also evidence Goeddel's and Genentech's own contemporaneous and subsequent belief that the work and inventions described in Goeddel's patents and patent application did not render obvious, as of 1989 or later, the production of an *in vivo* biologically active recombinant glycoprotein in transformed host cells that did not naturally produce the protein.

99. Indeed, in overcoming various rejections by the Patent Office based on the prior purification and characterization of naturally occurring human tPA from human cells grown in culture, Genentech extensively and repeatedly explained and argued why the production of an *in vivo* biologically active recombinant glycoprotein in mammalian cells that do not naturally produce such proteins would have been novel and non-obvious to ordinarily skilled artisans, even as of 1998.

100. During prosecution of the '075 patent, Genentech made a series of points that are relevant to the state of recombinant production of glycoproteins. For example, in an April 22, 1985 Office Action, the PTO rejected the DNA, plasmid, cell and process claims as obvious based on a combination of Rijken (purified human tPA), Bolen (isolation of tPA mRNA) and Hung (cloning of a tPA DNA with expression of plasminogen activator protein).[19] In arguing the non-obviousness of these claims, Genentech made the following points, which are wholly consistent with the points I have made about the state of the art at the time of Lin's inventions, and apply directly to the situation confronting the art at the time of Dr. Lin's inventions:

> The desirability of producing human tissue plasminogen activator does not ensure its achievability or enable one to predict with a reasonable degree of confidence that it can be achieved by "a person having ordinary skill in the art." The fact is that there is nothing in the art of record or in the state of the art at the time of the present invention was made that

[18] *Id.* at col. 5:18-20.

[19] '075 File History, 4/22/85 Office Action, at 4-5 (9/26/07 Lodish Decl., Ex. H).

> would have taught anyone how to prepare recombinant t-PA except in the most general terms that would constitute the definition of an objective rather than the means for attaining that objective.
>
> The principal flaw in the Examiner's rejection, even as applied to the non-elected claims, is that ***there is no basis in the art of record for predicting with reasonable certainty that human t-PA could be expressed in a recombinant system, that it would be compatible with recombinant host cells,*** or that bioactive t-PA of a degree of purity enabled by the present invention could be produced by any practical means.
>
> It would have been appreciated by those skilled in the art at the time this invention was made that the expression of human t-PA in transformed cells would be fraught with many potential difficulties. The art of recombinant DNA technology appears to be deceptively straightforward but is inherently unpredictable. A case in point is the Hung et al. patent, which appears to be based only on predictability and is clearly a prophetic disclosure but is, in fact, inoperative.
>
> One of the reasons for not being able to reasonably predict the ability of a recombinant cell to successfully produce by expression a heterologous protein concerns the fate of foreign DNA in a host cell system. For example, it is not predictable that mRNA, if produced at all from such DNA, will be stable or that it will be accurately translated into a full-length protein. Even if it is, one cannot be certain that the protein will not be degraded by enzymes, either within the cell or extracellularly, or that the recombinant cell will properly fold the molecule conformationally so that it will exhibit its desired biological activity. The human t-PA of the present invention contains some 527 amino acids, with many potential cleavage sites and some essential conformational requirements for biological activity. Thus, ***it would certainly have been unpredictable before the fact that one could obtain by recombinant DNA technology a biologically active protein such as the one forming the basis of the present invention.*** At the same time, it is clear that only recombinant DNA technology can assure that a human t-PA absolutely free of unrelated proteins of human origin can be obtained. [20]

101. After these statements by Genentech, the DNA claims and host cell claims were finally allowed as the '075 patent on March 21, 1988.

[20] '075 File History, 10/21/85 Amendment at 24-26 (emphasis added) (9/26/07 Godfrey Decl., Ex. G).

102. Second, arguing non-obviousness in a related application, Genentech again emphasized the unpredictability of glycosylation of recombinant glycoproteins and reiterated the arguments that it had made in the '259 prosecution, stating:

> The applicants submit that at the time the invention was made, and even today, it would not have been predictable whether such glycosylation differences would in fact produce intact, functionally biologically active glycoprotein. In support of this position, the applicants submit remarks made in originally filed USSN 07/012,694. These remarks include arguments, based on three subsequent publications concerning the pronounced unpredictability of glycosylation on the biological activity of a particular glycoprotein. These articles are not prior art, but rather are powerfully instructive as to the contemporary state of the art, emphasizing the patentable difference glycosylation makes, especially in 1982 when this application was effectively filed.[21]

103. Genentech then went on to repeat the specific arguments it had made during the '259 prosecution.

104. Lastly, in the '314 prosecution Genentech again argued the unpredictability of functional glycoprotein expression:

> At the time this invention was made, it was unknown (a) what effect glycosylation differences would have on the biological activity of a protein, and (b) whether the cell type used for expression of the protein would effect the glycosylation pattern.[22]
>
> *****
>
> It would not have been predictable whether glycosylation differences would, in fact, produce intact, functionally and biologically active glycoprotein. On this point, even later published papers reiterate this uncertainty. For example, three back-to-back papers published in 1989 show both uncertainty.[23]
>
> *****
>
> Again, this indicates that even in 1989, the scientific community

[21] '486 File History, 11/12/96 Amendment at 3-4 (9/26/07 Lodish Decl., Ex. I).

[22] '314 File History, 7/2/1996 Amendment at 5 (9/26/07 Lodish Decl., Ex. J).

[23] *Id*. at 6.

> continued to speculate as to what the resultant properties of a glycoprotein would be, if the N-glycosylation patterns would be altered, once again suggesting that the state of glycosylation of a given glycoprotein could not be predicted, or if it were, the biological profile whether viewed in terms of rank biological activity or immunogenicity, is not predictable or reasonably foreseeable to one skilled in the art.
>
> Thus, at the time this invention was made, it could not have been predicted with reasonable certainty that the recombinant t-PA products having glycosylation structure different from that disclosed by the prior art, would be useful in the manner that they have proved to be, namely, in therapeutic application in a safe manner to human beings.[24]
>
> *****
>
> As argued previously, at the time the invention was made it was unknown (a) what effect glycosylation differences would have on the biological activity of a protein, and (b) whether the cell type used for expression of the protein would effect the glycosylation pattern. Thus, it would not have been predictable whether such glycosylation differences would, in fact, produce intact, functionally biologically active glycoprotein.[25]
>
> *****
>
> Thus, at the time this invention was made, it could not have been predicted with reasonable certainty that the recombinant tPA products having glycosylation structure different from that disclosed by the prior art, or native material, would be useful in the manner that they have proved to be, namely, in therapeutic application in a safe manner to human beings.[26]

105. I must reiterate that evidence of successful *in vivo* biological activity (which, I have explained previously, the Genentech patents do not provide) for a single recombinant glycoprotein would not be readily generalizable to other unrelated human glycoproteins. The uncertainties in sufficiently recapitulating the native post-translational

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[24] *Id*. at 8.

[25] *Id*. at 3.

[26] *Id*. at 5.

modifications of a highly complex glycoprotein to afford *in vivo* biological activity are profoundly linked to the particular glycoprotein under investigation. Thus even if Genentech had disclosed the successful expression of *in vivo* biologically active tPA from recombinant mammalian cells, it would not have provided ordinarily skilled artisans with a reasonable expectation of success in producing other, unrelated glycoproteins like EPO. In fact, the expectation of success in producing *in vivo* biologically active EPO from mammalian cells grown in culture was far lower than that for tPA. As is clear from the tPA file histories, others had successfully obtained tPA from non-recombinant human cells grown in culture (the Rijken and Collen references mentioned herein). As I discussed above, in the case of EPO there had been no successful isolation of human erythropoietin from mammalian cells grown in culture. In fact, the art believed that the erythropoietic activity that had been reported from some cell lines had "physiochemical properties different from native Ep." Hagiwara, et al., "Erythropoietin production in a primary culture of human renal carcinoma cells maintained in nude mice." *Blood* 63(4):828-835 at 832 (Apr. 1984) (AM-ITC 00451162-69) (9/26/07 Lodish Decl., Ex. K). Thus, the non-obviousness arguments that Genentech made during prosecution of the tPA patents reflect a broader state of the art that further supports the conclusion that it would not have been obvious to produced *in vivo* biologically active EPO the moment the EPO gene was cloned.

106. In summary, I conclude that Genentech's scientific arguments concerning the unpredictability in the art of expressing *in vivo* biologically active human glycoproteins, which were accepted by the PTO and led to the issuance of multiple patents to tPA proteins long after the issuance of the patent to the tPA DNA, are consistent with and serve to confirm the opinions I set forth above.

107. Roche's experts cite a 1984 report suggesting that mammalian-produced tPA had *in vivo* biological activity: Collen, D. *et al.*, "Biological Properties of Human Tissue-

Type Plasminogen Activator Obtained by Expression of Recombinant DNA in Mammalian Cells" *J. Pharmacol. and Exp. Therap.* 231:146-152 (1984) (TX 2012.260-66) (AM-ITC 00454391-97). Collen, *et al.* reported the administration of "recombinant t-PA obtained by expression of cDNA of t-PA in a mammalian cell system" to rabbits to measure the dissolution of artificially induced blood clots. The Collen paper does not indicate the source of the recombinant tPA, or even the type of cells in which the protein was produced. Moreover, this reference provides no characterization of the structure or glycosylation of mammalian-produced recombinant tPA. It merely reports that a recombinantly-produced tPA was active in animal studies. Lastly, the Collen study was not published until July 1984, after Dr. Lin's invention of a process for the production of *in vivo* biologically active EPO. Consequently, I understand that it cannot be considered as prior art to the claims-in-suit.

108. Roche's experts Drs. Kellems and Lowe both misleadingly state that "[in] 1984, Genentech began clinical trials using the CHO cell produced protein." This statement is misleading because Genentech did not publish any information about the clinical trials for several years, so this information was not part of the knowledge in the art before Dr. Lin's inventions. Drs. Kellems and Lowe cite a 1987 press release to support their statement. I am not aware of any statement from Genentech in 1984 admitting that it was using CHO-cell produced tPA in clinical trials. To further demonstrate the confusion over the source of recombinant tPA, I note that at least one research article concerning pre-clinical testing of Genentech's tPA erroneously identified Genentech's product as "produced by cloning and expression of human tPA in *E. coli*."[27] In this context I also note the failure of the Collen paper to identify the source of the recombinant tPA used in their studies.

[27] Agnelli, *et al.*, "Sustained Thrombolysis with DNA-Recombinant Tissue Type Plasminogen Activator in Rabbits" *Blood* 66:399-401 (1985) (AM-LOD-ERB000005-07) (9/26/07 Lodish Decl., Ex. L).

109. In 1984, the role of glycosylation in tPA function was unclear. No one had yet compared the *in vivo* biological activity of mammalian-cell produced recombinant tPA to *E. coli* produced recombinant tPA. The Little *et al.*, "Functional Properties of Carbohydrate Depleted Plasminogen Activator," *Biochemistry* 23, 6191-6195 (1984) (D.I. 578, Ex. Q) paper demonstrates that little was known about the importance of glycosylation for bioactive recombinant tPA in 1984:

> Tissue plasminogen activator (TPA) is a protein containing approximately 6.8% (w/w) carbohydrate (Rangy *et al.*, 1982). TPA enzymatically converts plasminogen into plasmin only at the site of the fibrin clot. Because it activates only fibrin-associated plasminogen, it is expected to be a useful thrombolytic agent and has been expressed in Escherichia coli as a recombinant DNA product (Pennica *et al.*, 1983). The recombinant molecule contains no carbohydrate and is fibrinolytically active. However, it is not known whether the lack of the carbohydrate moiety affects the specific activity of TPA or the kinetics of plasminogen activation. We have sought to examine the functional properties of TPA depleted of carbohydrate by *in vivo* and *in vitro* approaches to better assess the potential problems associated with recombinant derived TPA.
>
> Knowing that TPA essentially void of carbohydrate retains the fibrin-dependent properties allows one to speculate on other problems that may be addressed by examining recombinant derived nonglycosylated TPA. To date, the specific activity of the TPA expressed in *E. coli* has not been documented. Our study suggests that if the TPA molecule is allowed to form in the native conformation without carbohydrate, the specific activity should be similar to native TPA.
>
> It has been demonstrated that terminal sialic acid and galactose residues are important in determining the hepatic clearance and plasma half-life of some glycoproteins (Zahlten *et al.*, 1981). How nonglycosylated TPA will react as an antigen, its biological half-life, and its interaction with extracellular inhibitors await further study.

110. An assessment of the glycosylation of CHO-produced tPA was not published until 1985. Kaufman, R.J. *et al,* "Coamplification and Coexpression of Human Tissue-Type Plasminogen Activator and Murine Dihydrofolate Reductase Sequences in Chinese

Hamster Ovary Cells," *Mol. Cell. Biol,* 5(7): 1750-1759 (1985) (AM-BER-ERB001035-44) (9/26/07 Lodish Decl., Ex. M); *see also* Vehar *et al.*, "Characterization studies of human tissue-type plasminogen activator produced by recombinant DNA technology," *Cold Spring Harbor Symp. On Quant. Biol.* 51:551-562 (1986) (AM-ITC 01059954-65) (9/26/07 Lodish Decl., Ex. A).

111. Opdenakker *et al.* also published a study on the impact of glycosylation on tPA function in 1986. They stated: "The biological significance of the carbohydrate moiety has until now not been documented. Here we describe experiments which demonstrate that alterations in the carbohydrate can affect *in vitro* enzymatic activity of tissue-type plasminogen activator." Opdenakker *et al.*, "Influence of Carbohydrate Side Chains on Activity of Tissue-Type Plasminogen Activator," *Proc. Soc. Experimental Biology and Medicine* 182:248-257 (1986) at 248 (AM-LOD-ERB000897-906) (9/26/07 Lodish Decl., Ex. N).

112. Parekh *et al.* demonstrated that two recombinant tPAs are glycosylated differently than native tPA: "Our results show that both CHO and murine rt-PA are N-glycosylated differently with respect to each other and to t-PA secreted by normal and transformed human cell lines (Parekh *et al.*, 1989). They support our previous conclusion that both the occupancy and nature of the oligosaccharides at N-glycosylation sites of t-PA can influence its fibrin-dependent activation of plasminogen." Parekh *et al.*, "N-Glycosylation and *in vitro* Enzymatic Activity of Human Recombinant Tissue Plasminogen Activator Expressed in Chinese Hamster Ovary Cells and a Murine Cell Line," *Biochemistry* 28:7670-7679 (1989) at 7671 (AM-ITC 00015346) (9/26/07 Lodish Decl., Ex. O). "For both the CHO and murine C127 cell derived t-PA, the incidence of sialylated structures is very similar to that of complex-type oligosaccharides possessing outer arm nonreducing terminal galactose residues (Tables I and II). This indicates that almost all such structures are at least monosialylated on the rt-PA

polypeptide, unlike the t-PA isolated from the normal human colon fibroblast cell strain CDD-l8Co (Parekh *et al.*, 1989). Both the CHO rt-PA and murine rt-PA possess a significant number of oligosaccharides whose outer arms terminate in N-acetylglucosamine (8.8% and 10.4%, respectively). This was also found to be so for Bowes melanoma t-PA, but not human colon fibroblast t-PA." *Id.* at 7677. "[B]ut our results indicate that the t-PA's described to date are N-glycosylated differently, supporting the view that N-glycosylation is cell-type-specific. Each t-PA analyzed was associated with certain unique oligosaccharide structures." *Id.*

113. Parekh *et al.* also noted that CHO-produced tPA could vary greatly from the native human protein: "Such systems are generally assumed to be faithful with respect to transcription and translation of the recombinant gene [see, however, Opdenakker *et al.* (1988)]. However, with respect to posttranslational modifications, ***recombinant DNA derived t-PA could vary greatly from the naturally occurring form.***" *Id.* (emphasis added).

Summary of state of the art regarding expression of mammalian glycoproteins in mammalian host cells

114. In 1984, with the possible exception of tPA, the field had yet to produce by recombinant production in mammalian cells a glycoprotein that required proper glycosylation for *in vivo* biological function and to test whether the recombinant glycoprotein possessed the desired *in vivo* biological activity. The following table summarizes my analysis of this literature.

MAMMALIAN GLYCOPROTEIN	GLYCOSYLATION REQUIRED FOR *IN VIVO* BIOLOGICAL FUNCTION?	COMPARISON TO NATIVE GLYCOPROTEIN?	DEMONSTRATION OF *IN VIVO* BIOLOGICAL ACTIVITY?
IFN γ	No	No	No
IFN β	No?	No	No
IL-2	No	No	No
Multi-CSF	No	No	No
Factor VIII	Not known	No	No
Immunoglobulins	In some cases	No	No
tPA	Maybe	No	First published in 1984

115. Even assuming tPA had been expressed and shown to be *in vivo* biologically active before Dr. Lin's inventions, I do not believe that this single example would have given an ordinarily skilled artisan any confidence or reasonable expectation that ***any other*** glycoprotein that required proper glycosylation for *in vivo* biological activity could be expressed in heterologous host cells in an *in vivo* biologically active form. I do not believe that this single example is easily generalizable to the expression of EPO. Simply because the Collen paper suggests that unnamed cells express tPA in a form sufficient to allow *in vivo* biological activity of the tPA enzyme does not mean that mammalian host cells could impart functional glycosylation to the EPO hormone. This is particularly so since the role of glycosylation in tPA function had not yet been determined in 1983-84.

116. In my opinion, knowledge of the significant differences in the nature of tPA as compared to the nature of EPO would have led the ordinarily skilled artisan to discount the tPA results when considering whether expression of EPO in heterologous mammalian cells could reasonably be expected to lead to the production of *in vivo* biologically active EPO. In particular, as I explained above, the ordinarily skilled artisan would have understood that while tPA is an incredibly short-lived enzyme that would be deleterious if it persisted *in vivo*, EPO is a

hormone that must persist in the body for extended periods of time before any *in vivo* biological activity can occur and be observed. Therefore, the requirements for proper post-translational modifications, particularly glycosylation, would have been understood to be very different between tPA and EPO. One would not have expected tPA to have the same acute requirement for proper glycosylation in order to function in the few minutes it needs to persist in the blood stream, whereas an ordinarily skilled artisan would have understood that proper glycosylation would be necessary to allow EPO to discharge its function as a hormone, to escape removal from the blood by cell surface receptors that bind abnormal carbohydrates (such as galactose — or mannose — terminated oligosaccharides), and to elicit *in vivo* biological activity.

E. DR. LIN'S PATENT APPLICATIONS DESCRIBE A SUCCESSION OF DIFFERENT INVENTIONS

117. To successfully produce *in vivo* biologically active recombinant EPO for the first time in history, in the midst of all the uncertainty described above, it was necessary to, among other things: (a) "clone" the EPO gene by discovering its DNA sequence, (b) discover and select cell types that could successfully produce *in vivo* biologically active EPO in sufficient quantities for administering to patients, (c) genetically engineer and modify such cells to express *in vivo* biologically active EPO, and (d) validate that the cells actually produced sufficient quantities of *in vivo* biologically active EPO. Each of these successive inventions was necessary to develop a protein that could be administered to patients to treat anemia. None of these inventions, standing alone, was sufficient to achieve that result.

118. In the 1983-84 time period, Amgen's Dr. Fu-Kuen Lin made a series of path-breaking inventions which he documented in a succession of four patent applications filed on December 13, 1983 (Ser. No. 561,024), February 2, 1984 (Ser. No. 582,185), September 28, 1984 (Ser. No. 655,841), and November 30, 1984 (Ser. No. 675,298). The later applications each build on the information that was included in the preceding applications. I find that the

successive disclosures of these applications closely track the progression of Dr. Lin's experiments at Amgen, with only the early cloning information present in the first application, and the complete description of all of his inventions set forth in the fourth application. Thus, the successive patent applications illustrate the progression of Dr. Lin's work over time and highlight the fact that Dr. Lin made multiple, significant scientific discoveries, not a single scientific discovery.

119. Magistrate Judge Saris's factual findings regarding Amgen's inventions are found at pages 1746-1751 of her opinion in *Amgen Inc. v. Chugai Pharm. Co. Ltd.*, 13 U.S.P.Q.2d 1737 (D. Mass 1989). The Judge found that: (a) "[t]he successful cloning of the EPO gene took place in September or early October, 1983"; (b) "[o]n February 13 and 14, 1984, Amgen conducted experiments to show that the recombinant human EPO produced in the COS cell was biologically active" (I understand that these were *in vitro* tests); and (c) "[f]rom March 1-9, 1984, Amgen conducted an *in vivo* bioassay and determined that the recombinant EPO was biologically active." *Id.* at 1748.

120. The last of Dr. Lin's four applications contains a breadth of information and teachings relating to EPO that are set forth in the patents-in-suit. For example, the patents-in-suit provide the following information:

- The precise full-length amino acid sequence of the human EPO polypeptide, including the signal peptide and C-terminal arginine residue, which are subsequently removed from the EPO polypeptide (*see* Figure 6 of Amgen's Patents);
- The precise sequence of the human EPO gene as it exists in the genome of human cells, including both the regulatory and structural regions of the gene (*see* Figure 6);
- The sequence, number and arrangement of exons and introns in the human EPO structural gene, including all of the EPO intron splice donor and splice acceptor sites;
- Methods for isolating the human EPO gene from a human genomic DNA library;

- Methods for making cells that produce human EPO;
- Methods for producing biologically active human EPO glycoprotein products in genetically manipulated vertebrate cells;
- Methods for amplifying EPO DNA within cells;
- Variants and analogs of EPO polypeptides and methods of making them;
- DNA sequences that encode human EPO, but differ from the natural EPO DNA sequences by including preferred codons for expression in prokaryotic and yeast cells;
- Demonstrations of immunological, *in vitro*, and *in vivo* biological properties of EPO produced by genetically manipulated cells; and
- Methods for treating anemic patients by EPO therapy.

121. Particularly as of 1983-84, the breadth and quality of Dr. Lin's experiments and the description of his methods and results were impressive, reflecting a series of truly breakthrough discoveries that garnered significant attention, respect, and acclaim when they were reported to the scientific community.

II. THE INVENTIONS CLAIMED IN DR. LIN'S '868 AND '698 PATENTS ARE PATENTABLY DISTINCT FROM THE INVENTIONS CLAIMED IN DR. LIN'S '008 PATENT

A. LEGAL STANDARD

122. I understand that the issue is whether the invention defined by a subsequently issued claim would have been obvious to one skilled in the art at the time of the invention in light of the invention claimed in an earlier-issued claim. In making this determination, I am to assume that the ordinarily skilled artisan had knowledge of the complete state of the art prior to the invention, as well as the invention defined in the earlier-issued claim, but nothing else. In particular, she does not have access to disclosures made in the patent specification that accompanies the earlier-issued claim.

123. I further understand that the relevant time for assessing the state of the art is the time just before the inventions claimed in Dr. Lin's '868 and '698 patents, which I am told is no later than November 30, 1984.

124. I have been informed that the Court will apply the following methodology, on a claim-by-claim basis, to determine whether the inventions claimed in Dr. Lin's '868 and '698 patents would have been obvious over the inventions claimed in Dr. Lin's '008 patent to an ordinarily skilled artisan in 1983-84:

- Determine the scope of each relevant claim.[28]
- Identify the differences between each invention claimed in the '008 patent and each invention claimed in the '868 and '698 patents.
- Assess the ordinarily skilled artisans' knowledge as of 1983-84 regarding the subject matter of the Dr. Lin's inventions.
- Assess whether the ordinarily skilled artisan would have had a reasonable expectation of success in practicing the inventions claimed in the '868 and '698 patents, given the state of the art in 1983-84, and the invention claimed in the '008 patent.
- Assess whether the inventions claimed in the '868 and '698 patents reflect results that would have been unexpected in light of the inventions claimed in the '008 patent.
- Relying on this information, assess whether the inventions claimed in the '868 and '698 patents would have been obvious over the inventions claimed in the '008 patent.

B. SUMMARY OF NON-OBVIOUSNESS OF '868 AND '698 CLAIMED INVENTIONS OVER '008 CLAIMED INVENTIONS

125. Roche's experts contend that the claims of the '008 patent (directed to DNA sequences encoding EPO and host cells transformed with such DNA) rendered Amgen's later-issued claims in the '868 and '698 patents obvious to one of ordinary skill in the art. The central thrust of Roche's argument is that once a person of ordinary skill in the art had the isolated EPO

[28] I understand that the Court has issued a claim construction Order in this case (D.I. 613) that I have reviewed and applied as part of my analysis.

DNA sequence, it would have been obvious to such person, in 1983-84, how to make cells capable of producing an EPO glycoprotein, and then to further culture those cells and isolate an EPO glycoprotein that had *in vivo* biological activity. I disagree.

126. It is my understanding that the relevant inquiry is whether any of the inventions claimed in the '008 claims, in combination with the knowledge of a person of ordinary skill in the art in 1983-84, would have rendered the later-issued '868 and '698 claimed inventions obvious. A critical aspect of this inquiry is that the detailed text of Dr. Lin's specification describing his experiments cannot be considered. Rather, the comparison is only between the '008 claims and the '868 and '698 claims.

127. I provide a more detailed explanation of my analysis in the sections that follow. However, I wish to highlight what I consider to be the most significant differences between the claims of the '008 patent and the claims of the '868 and '698 patents, and the reasons why possession of an isolated EPO DNA sequence was not enough, by itself, to render the '868 and '698 claims obvious.

128. First, the '008 claims were directed to EPO DNA and host cells with certain capabilities that were transfected with EPO DNA with certain capabilities. The '008 claims did not require production of EPO with *in vivo* biological activity, or the processes necessary for making such biologically active EPO. In my opinion, therefore, there are important distinctions between the inventions claimed in the '008 claims and the inventions claimed in the '868 and '698 claims.

129. Second, the fact that certain '008 claims recite host cells transformed or transfected with DNA encoding EPO or EPO analogs in a manner "allowing the host cell to express erythropoietin" or "capable of glycosylating" does not alter my opinion. In 1983-84, inserting DNA into a cell in a manner that ***could*** allow the cell to express EPO was very distinct

from claiming a process that ***will*** lead to the production of an *in vivo* biologically active EPO from a cell. Saying "I have a bat that is capable of hitting a 100 mph fastball" is far different than saying "I will swing the bat at a 100 mph fastball in a particular way which will lead to a home run."

130. Third, in 1983-84, without the benefit of Dr. Lin's disclosures, a person of ordinary skill in the art would not have had a reasonable expectation of success that isolated EPO DNA would necessarily lead to the production of EPO with *in vivo* biological activity.

131. Too much was unknown at that time regarding the structure and function of EPO, the role of glycosylation in EPO's function, and the possibility of differences for EPO produced in cells of different types, or from different species, to lead to a reasonable expectation of success. For example, a person of ordinary skill in the art would not have known whether the particular human kidney cells that make EPO in the human body imparted special glycosylated structures on the EPO molecule that were critical to its biological activity. A person of ordinary skill in the art would not have known whether production of EPO in a cultured mammalian cell might create a form of EPO that would trigger a severe immunological reaction when injected into humans.

132. Before Dr. Lin's work, a person of ordinary skill in the art would have known that there were many different reasons why a human glycoprotein might not be produced in a biologically active form in cultured cells. Before Lin, there were no reports of successful production of human glycoproteins in mammalian cells with *in vivo* biological activity, with at best, one possible exception. This uncertainty was exacerbated by the absence of any experiment demonstrating that *in vivo* biologically active EPO had actually been successfully made and isolated from recombinant cells. It was only after Dr. Lin's work demonstrating that a biologically active human EPO glycoprotein could be successfully produced in at least CHO

cells that one of ordinary skill in the art could begin to expect success for producing *in vivo* biologically active EPO going forward. Dr. Lin's development of methods of producing biologically active EPO glycoprotein, and proof that such methods actually worked were important experimental validations. To say that everything followed predictably once the DNA sequence encoding EPO was isolated ignores the substantial, subsequent work performed by Dr. Lin and his colleagues as well as the unpredictability of the art prior to Dr. Lin's success.

C. THE CLAIMS OF DR. LIN'S '008 PATENT ARE DIRECTED TO EPO DNA AND HOST CELLS TRANSFECTED WITH EPO DNA THAT HAVE CERTAIN DESIRED CAPABILITIES, BUT THE '008 CLAIMS DO NOT DESCRIBE EPO WITH *IN VIVO* BIOLOGICAL FUNCTION, OR PROCESSES FOR MAKING SAME

133. I understand that U.S. Patent No. 4,703,008 ("the '008 patent") was awarded to Amgen's Dr. Fu-Kuen Lin on October 27, 1987. The '008 patent was based on a series of four patent applications, the first filed on December 13, 1983 and the last filed on November 30, 1984.

134. I understand that Roche contends that claims 2, 4, 6, 7, 25, and/or 27 of the '008 patent render the asserted claims of the '868 and '698 patents invalid because of double patenting.[29] In their initial expert reports submitted during discovery, Roche's experts Dr. Blobel (¶¶ 25-48), Dr. Kellems (¶¶ 160-192), and Dr. Lowe (¶¶ 173-204) each compare claims of the '008 patent to those of the patents-in-suit, and conclude that each of the claims-in-suit is obvious in light of the claims of the '008 patent. I have considered the analyses of Drs. Blobel, Kellems, and Lowe, and I disagree with their conclusion that the claims of the patents-in-suit are invalid for obviousness-type double patenting over the claims of the '008 patent.

135. The claims of the '008 patent can be divided into two groups. The first group, claims 1-6, concerns erythropoietin DNA and host cells transformed with such DNA. The second group, from claim 7 onward, concerns DNAs encoding analogs of erythropoietin and host

[29] Roche's Pre-Trial Brief (D.I. 919), at 43, 45.

cells transformed with such DNA. I understand that Claims 7, 8, 23-27, and 29 of this second group were found by the Federal Circuit Court of Appeals to be invalid as not enabled.[30] Unlike '008 claims 2, 4, and 6, which were held not invalid by the Federal Circuit in the *Amgen v. Chugai* case,[31] '008 claim 7 and its dependants are not restricted to DNAs encoding the single polypeptide human erythropoietin.

136. I am informed that in order to determine whether the claims of a patent are patentably distinct from the claims of another patent, it is first necessary to ascertain the meaning and scope of each claim. I will address each group of claims in turn. The language of representative claims, and examples of the claims from which they depend, follows:

Claim 4. A procaryotic or eucaryotic host cell transformed or transfected with a DNA sequence according to claim 1, 2 or 3 in a manner allowing the host cell to express erythropoietin.
Claim 2. A purified and isolated DNA sequence consisting essentially of a DNA sequence encoding human erythropoietin.
Claim 27. A transformed or transfected CHO cell according to claim 25.

[30] *Amgen, Inc. v. Chugai Pharmaceutical Co.*, 927 F.2d 1200, 1214 (Fed. Cir. 1991) ("Considering the structural complexity of the EPO gene, the manifold possibilities for change in its structure, with attendant uncertainty as to what utility will be possessed by these analogs, we consider that more is needed concerning identifying the various analogs that are within the scope of the claim, methods for making them, and structural requirements for producing compounds with EPO-like activity. It is not sufficient, having made the gene and a handful of analogs whose activity has not been clearly ascertained, to claim all possible genetic sequences that have EPO-like activity. Under the circumstances, we find no error in the court's conclusion that the generic DNA sequence claims are invalid under Section 112.").

[31] *Amgen, Inc. v. Chugai Pharmaceutical Co.*, 927 F.2d 1200, 1209 (Fed. Cir. 1991). Note: I am referring only to claims 4 and 6 as they depend from claim 2, since that appears to be what the Federal Circuit considered in *Amgen v. Chugai*.

Claim 25. A transformed or transfected mammalian host cell according to claim 24.

Claim 24. A transformed or transfected host cell according to claim 23 which host cell is capable of glycosylating said polypeptide.

Claim 23. A procaryotic or eucaryotic host cell transformed or transfected with a DNA sequence according to claim 7, 8, or 11 in a manner allowing the host cell to express said polypeptide.

Claim 7. A purified and isolated DNA sequence consisting essentially of a DNA sequence encoding a polypeptide having an amino acid sequence sufficiently duplicative of that of erythropoietin to allow possession of the biological property of causing bone marrow cells to increase production of reticulocytes and red blood cells, and to increase hemoglobin synthesis or iron uptake.

137. All of the claims of the '008 patent are directed to either DNA sequences, or host cells into which DNA sequences have been introduced. No '008 claims are directed to either EPO compositions, or to processes for making such compositions.

138. The first group of claims ('008 claims 1-6) is directed towards EPO DNAs and host cells transformed or transfected with such DNAs. I will specifically address Claims 2 and 4 as representative of this group.

139. **Claim 2.** Claim 2 refers to a "purified and isolated" DNA molecule "consisting essentially of a DNA sequence encoding human erythropoietin." A literal reading of claim 2 suggests that the DNA does not contain any sequences other than the coding sequences for the erythropoietin polypeptide, in view of the term "consisting essentially of." In other words, the DNA of claim 2 literally excludes, for example, transcriptional control sequences or any other type of regulatory sequences operatively linked to the EPO coding sequence.

140. **Claim 4.** Claim 4 refers to a prokaryotic or eukaryotic host cell "transformed or transfected" with the "purified and isolated" DNA molecule of claim 2. A literal reading of this clause of claim 4 suggests that the DNA would not be expressed in the cell, since the transfected DNA cannot have any of the transcriptional regulatory sequences required for expression.

However, claim 4 further states that the DNA is transfected "in a manner allowing the host cell to express erythropoietin." This implies that the transformed or transfected DNA is linked to some transcriptional regulatory sequences. There is nothing in the claim that says that the erythropoietin protein is actually produced by the host cell, only that the cell has the potential, under unstated conditions, to do so. This is a significant distinction because there are many circumstances in which a transfected DNA might have the potential to express an encoded protein, but are missing other important prerequisites to expression. For instance, if DNA encoding EPO were operatively linked to an inducible promoter, then transfection of such a DNA construct would not result in expression of the EPO protein unless inducing conditions were present.

141. Claim 4 encompasses every possible host cell in the biological universe. It was known that the ability of various cells to express and properly process exogenous proteins varied dramatically across the universe. Claim 4 does not suggest which cells, if any, might be used to express EPO so that the protein would in fact be expressed and would have the *in vivo* biological activity of erythropoietin.

142. Claim 4 does not require that any transfected cell be capable of producing more than a minute amount of EPO protein.

143. Claim 4 does not require that any EPO produced by the host cell have the secondary or tertiary protein structure of native EPO.

144. Claim 4 does not require that any EPO produced by the host cell be glycosylated. In fact, by explicitly including prokaryotic host cells, which in 1983 were known to lack the capability to glycosylate, it is clear that this claim cannot require, or even suggest, glycosylation.

145. Claim 4 does not require that any EPO expressed have the *in vivo* biological function of stimulating erythropoiesis, or be therapeutically effective for treating any human disease state.

146. To summarize, I conclude that Claim 4 does not require: (1) that the host cell actually express EPO; (2) that the host cell be capable of producing any more than a minute amount of EPO; (3) that any EPO expressed be properly folded; (4) that any EPO expressed be glycosylated; or (5) that any EPO expressed be functional.

147. **Claim 7.** Like claim 2, claim 7 is directed to an "isolated and purified DNA" that encodes a polypeptide. Unlike claim 2 which is directed only to DNA encoding a single protein — human erythropoietin — claim 7 is directed to a genus of DNAs encoding a genus of polypeptides (EPO analogs[32]), that if made and properly processed, would have the *in vivo* biological activity of "causing bone marrow cells to increase production of reticulocytes and red blood cells, and to increase hemoglobin synthesis or iron uptake."

148. Because Claim 7 is directed merely to DNA sequences, and DNA sequences do not have erythropoietic activity, there is nothing in the claim that suggests that the protein encoded by any of the DNAs of the claim be actually made, or that such protein be functionally active. Achieving such activity would require, at a minimum, engineering a DNA construct with appropriate regulatory sequences that could be introduced into an appropriate host cell to achieve adequate expression of a protein, followed by appropriate protein folding, appropriate post-translational modifications, secretion into the medium, and isolation therefrom. Given the use of the "consisting essentially of" language, this claim explicitly excludes such engineering.

149. With respect to the claim language "purified and isolated DNA sequence consisting essentially of a DNA sequence encoding a polypeptide having an amino acid sequence

[32] In this context "analog" means an artificial protein that is similar to a natural protein, but

sufficiently duplicative of that of erythropoietin to allow possession of the biological property of causing bone marrow cells to increase production of reticulocytes and red blood cells, and to increase hemoglobin synthesis or iron uptake," I note that this language is considerably different in meaning from "DNA sequence encoding human erythropoietin" as used in claims 2 and 4. As noted by the Federal Circuit Court of Appeals, because the claim is directed to DNA sequences encoding analogs of EPO, the number of different DNAs implicated by this language is potentially enormous: "The district court found that over 3,600 different EPO analogs can be made by substituting at only a single amino acid position, and over a million different analogs can be made by substituting three amino acids. The patent indicates that it embraces means for preparation of 'numerous' polypeptide analogs of EPO. Thus, the number of claimed DNA encoding sequences that can produce an EPO-like product is potentially enormous."[33]

150. **Claim 23.** Claim 23 refers to a prokaryotic or eukaryotic host cell "transformed or transfected" with the DNA molecule of, among other claims, claim 7. As for claim 4, a literal reading of this clause of claim 23 suggests that the DNA would not be expressed in the cell, since the transfected DNA does not have any transcriptional regulatory sequences. However, claim 23 further states that the DNA is transfected "in a manner allowing the host cell to express said polypeptide." This implies that the transformed or transfected DNA is linked to some transcriptional regulatory sequences. There is nothing in the claim that says that the polypeptide is actually produced by the host cell, only that the cell has the potential, under unstated conditions, to do so. This is a significant distinction because there are many circumstances in which a transfected DNA might have the potential to express an encoded protein, but other important prerequisites to expression are missing. For instance, if DNA encoding the polypeptide were operatively linked to an inducible promoter, then transfection of such a DNA

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which has a different amino acid sequence than EPO.

construct would not result in expression of the polypeptide unless inducing conditions were present.

151. Claim 23 encompasses every possible host cell ("prokaryotic or eukaryotic") in the biological universe. It was known that the ability of various cells to express and properly process exogenous proteins varied dramatically across this biological universe. Claim 23 does not suggest which cells, if any, might be used to express an EPO analog to ensure that the protein would have the *in vivo* biological activity recited in claim 7. Thus, claim 23 allows a situation where the DNA encodes a potentially active EPO analog, but that the particular host cell could not possibly express an *in vivo* biologically active protein, e.g. the entire portion of the claim directed towards prokaryotic hosts.

152. Claim 23 does not require that any transfected cell be capable of producing more than a minute amount of EPO protein.

153. Claim 23 does not require that any polypeptide produced by the host cell have the appropriate secondary or tertiary protein structure for function.

154. Claim 23 does not require that any polypeptide produced by the host cell be glycosylated. In fact, by expressly including prokaryotic host cells, which in 1983 were known to lack the capability to glycosylate, it is apparent that this claim cannot require, or even suggest, glycosylation.

155. Claim 23 does not require that any polypeptide expressed have *in vivo* biological function of stimulating erythropoiesis, or be therapeutically effective for treating any human disease state.

156. To summarize, I conclude that claim 23 does not require: (1) that the host cell actually express any polypeptide; (2) that the host cell be capable of producing any more than a

[33] *Amgen, Inc. v. Chugai Pharmaceutical Co*., 927 F.2d 1200, 1213 (Fed. Cir. 1991).

minute amount of such a polypeptide; (3) that any polypeptide expressed be properly folded; (4) that any polypeptide expressed be glycosylated; and (5) that any polypeptide expressed be functional.

157. **Claim 24.** Claim 24 further specifies that the host cell, according to claim 23 is further "capable of glycosylating" the described EPO analog polypeptides if produced by the host cell. Like the "allowing" language of claim 23, the language of claim 24 does not actually require that any glycosylated protein actually be produced.

158. "Capable of glycosylating" is not synonymous with capable of providing glyscosylation sufficient to allow for *in vivo* biological function. This language covers ***any*** glycosylation: single monosaccharides, glycosylation at only some of the glycosylation sites; glycosylation that is foreign to mammals, etc. It is in no way limited to glycosylation that is sufficiently duplicative of the natural erythropoietin glycosylation to allow *in vivo* biological activity.

159. In accordance with what I have stated about claim 23, I conclude that claim 24 does not require: (1) that the host cell actually express any polypeptide; (2) that the host cell be capable of producing any more than a minute amount of such a polypeptide; (3) that any polypeptide expressed be properly folded; (4) that any polypeptide expressed be functional; (5) that any glycosylation imparted to the protein, if expressed, be sufficient for *in vitro* biological function; or (6) that any glycosylation imparted to the protein, if expressed, be sufficient for *in vivo* biological function.

160. **Claim 25.** Claim 25 further specifies that the host cell, according to claim 24, is a "mammalian host cell." With respect to the claim language "mammalian host cell," as I explained above, no one had systematically compared the structures of glycans attached to glycoproteins across the entire class of mammals by 1983. It was known that different

mammalian species, and different cell types within a single mammalian species could attach different carbohydrate chains to glycoproteins. Furthermore, it was expected that specific glycan structures would be necessary for the function of particular glycoproteins. Therefore, it was unknown whether any particular mammalian cell would be capable of imparting glycosylation sufficient to allow for the functional expression of any human polypeptide or analog.

161. In accordance with what I have stated about claim 23, I conclude that claim 25 does not require: (1) that the host cell actually express any EPO analog polypeptide; (2) that the host cell be capable of producing any more than a minute amount of such a polypeptide; (3) that any polypeptide expressed be properly folded; (4) that any polypeptide expressed be functional; or (5) that any glycosylation imparted to the protein be sufficient for *in vitro*, or *in vivo* biological function.

162. **Claim 27.** With respect to the claim language "CHO cell," as I discussed above, as of 1983, no one had systematically compared the structures of glycans attached to glycoproteins in CHO cells versus human cells. It was known that different mammalian species, and different cell types within a single mammalian species could attach very different carbohydrate chains to glycoproteins. Furthermore, it was expected that specific glycan structures would be necessary for the function of particular glycoproteins. Thus it was unknown whether Chinese Hamster Ovary cells would be capable of imparting glycosylation sufficient to allow for the functional expression of heterologous proteins like human EPO.

163. In accordance with what I have stated about claim 23, I conclude that claim 27 does not require: (1) that the CHO cell actually express any polypeptide; (2) that the CHO cell be capable of producing any more than a minute amount of such a polypeptide; (3) that any polypeptide expressed be properly folded; or (4) that any polypeptide expressed have *in vitro* or *in vivo* biological function.

164. For each of these claims, one skilled in the art would not have been able to *a priori* predict which, if any, host cells would actually produce functional EPO analog. Rather a skilled artisan would not know whether such an EPO analog could be successfully produced until he or she actually tried.

165. At most, the language of claims 7, 23, 24, 25, and 27 would be an invitation to try to produce *in vivo* biologically active EPO analogs by expression in mammalian cells. But an ordinarily skilled artisan in 1983 would have perceived at least the following uncertainties with such expression: (1) whether a particular EPO analog would have the potential to encode an active polypeptide; (2) whether the cells would impart the required post-translational modifications to allow for *in vivo* biological activity; and (3) whether expression would reach the minimum threshold to allow for *in vivo* biological activity.

166. Only the invalid '008 claims depending on '008 claim 7 make any reference to the *in vivo* biological activity of any protein. I consider the fact that claim 7 and its dependent claims were found to be non-enabled to be highly material to the present analysis. With claims 7, 23-27, and 29, Amgen attempted to claim a class of DNAs, encoding a large number of different polypeptides:

> "Claim 7 is a generic claim, covering all possible DNA sequences that will encode any polypeptide having an amino acid sequence "sufficiently duplicative" of EPO to possess the property of increasing production of red blood cells. As claims 8, 23-27, and 29, dependent on claim 7, are not separately argued, and are of similar scope, they stand or fall with claim 7."[34]

Because it could not set forth a particular amino acid sequence to delimit the enormous set of claimed DNAs, Amgen attempted to limit the DNAs by a common function: encoding a polypeptide "sufficiently duplicative of that of erythropoietin to allow possession of the biological property of causing bone marrow cells to increase production of reticulocytes and red

blood cells, and to increase hemoglobin synthesis or iron uptake." The CAFC found that the Lin patent specification did not enable this functional definition of sequence because the specification did not provide enough teachings "concerning identifying the various analogs that are within the scope of the claim, methods for making them, and structural requirements for producing compounds with EPO-like activity. It is not sufficient, having made the gene and a handful of analogs whose activity has not been clearly ascertained, to claim all possible genetic sequences that have EPO-like activity."[35] Because the specification does not enable this claim term, claim 7 does not adequately disclose DNA sequences encoding proteins with erythropoietic biological activity. Because these claims are not enabled, even if they suggest the claims-in-suit, they would not provide a reasonable expectation of success in practicing the claims of the patents-in-suit.

167. The principal difference between the asserted '868 and '698 claims, and the '008 claims, is that each of the asserted '868 and '698 claims recite a ***positive requirement*** for the product of the claimed process to ***have*** the *in vivo* biological activity of causing bone marrow cells to increase production of reticulocytes and red blood cells. As explained above, the '008 claims lack this critical requirement.

168. Additional discussion of the differences between the asserted process claims of the '868 and '698 patents on the one hand and the '008 claims on the other hand can be found below.

169. In my opinion, the significant differences between the '868 and '698 asserted claims and the '008 claims preclude a determination that the '868 and '698 asserted claims are invalid for obviousness-type double patenting over the '008 claims.

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[34] *Amgen, Inc. v. Chugai Pharmaceutical Co.*, 927 F.2d 1200, 1212 (Fed. Cir. 1991).
[35] *Id.* at 1214.

D. '868 CLAIMS 1 AND 2 ARE PATENTABLY DISTINCT FROM '008 CLAIMS 2, 4, 6, 7, 25, AND 27

170. The differences between claims 2, 4, 6, 7, 25, and 27 of the '008 patent and claims 1-2 of the '868 patent are shown in the following chart:

'008 Claims 2, 4, 6, 7, 25, 27	'868 Claims 1 and 2
2. A purified and isolated DNA sequence consisting essentially of a DNA sequence encoding human erythropoietin. 4. A procaryotic or eucaryotic host cell transformed or transfected with a DNA sequence according to claim 1, 2 or 3 in a manner allowing the host cell to express erythropoietin. 6. A procaryotic or eucaryotic host cell stably transformed or transfected with a DNA vector according to claim 5. 7. A purified and isolated DNA sequence consisting essentially of a DNA sequence encoding a polypeptide having an amino acid sequence sufficiently duplicative of that of erythropoietin to allow possession of the biological property of causing bone marrow cells to increase production of reticulocytes and red blood cells, and to increase hemoglobin synthesis or iron uptake. 25. A transformed or transfected mammalian host cell according to claim 24. 27. A transformed or transfected CHO cell according to claim 25.	1. A process for the production of 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. 2. The process according to claim 1 wherein said host cells are CHO cells.

171. '868 claim 1 recites a process for producing and isolating *in vivo* biologically active EPO glycoprotein in a mammalian host cell to which exogenous EPO DNA has been introduced. '868 claim 2 is a similar process performed using CHO host cells only. These claims have not been previously construed by any court.

172. As described just above, there are at least two material distinctions between the claims of the '008 patent and claims 1 and 2 of the '868 patent.

173. First, the asserted claims of the '868 patent positively require that the product of the claimed process to have the *in vivo* biological activity of causing bone marrow cells to increase production of reticulocytes and red blood cells, while the '008 claims do not. The unexpected capacity to replicate the functional contribution of post-translational modifications such as glycosylation is what makes the glycoprotein invention claimed in the '868 patent non-obvious over the DNA invention claimed in the '008 patent. It is one thing to have a DNA that will cause a cell to produce a glycoprotein; it is a very different thing to produce a glycoprotein that will have a desired *in vivo* activity.

174. Second, the '008 claims are to DNA products and host cell products, while '868 claims 1 and 2 are to processes for producing *in vivo* biologically active erythropoietin glycoproteins.

175. In my opinion, for the reasons explained in this declaration, each of the inventions as a whole claimed in '868 claims 1 and 2 would ***not*** have been obvious to a person of ordinary skill in the art in 1983-84, even in light of claims 2, 4, 6, 7, 25 and/or 27 of the '008 patent.

E. '698 CLAIMS 6-9 ARE PATENTABLY DISTINCT FROM '008 CLAIMS 2, 4, 6, 7, 25, AND 27

176. The differences between claims 2, 4, 6, 7, 25, 27 of the '008 patent and claims 6-9 of the '698 patent are shown in the following chart:

'008 Claims 2, 4, 6, 7, 25, 27	'698 Claims 6-9
2. A purified and isolated DNA sequence consisting essentially of a DNA sequence encoding human erythropoietin. 4. A procaryotic or eucaryotic host cell transformed or transfected with a DNA sequence according to claim 1, 2 or 3 in a manner allowing the host cell to express erythropoietin. 6. A procaryotic or eucaryotic host cell stably transformed or transfected with a DNA vector according to claim 5.	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

7. A purified and isolated DNA sequence consisting essentially of a DNA sequence encoding a polypeptide having an amino acid sequence sufficiently duplicative of that of erythropoietin to allow possession of the biological property of causing bone marrow cells to increase production of reticulocytes and red blood cells, and to increase hemoglobin synthesis or iron uptake. 25. A transformed or transfected mammalian host cell according to claim 24. 27. A transformed or transfected CHO cell according to claim 25.	b) isolating said glycosylated erythropoietin polypeptide expressed by said cells. 7. The process of claim 6 wherein said vertebrate cells further comprise amplified marker gene DNA. 8. The process of claim 7 wherein said amplified marker gene DNA is Dihydrofolate reductase (DHFR) gene DNA. 9. The process according to claims 2, 4 and 6 wherein said cells are mammalian cells.

177. The asserted '698 claims recite processes for producing and isolating *in vivo* biologically active EPO glycoprotein in a vertebrate host cell with defined structural attributes.

178. Some of the claim terms of the '698 claims, including "vertebrate cells" (all '698 claims) and "mammalian cells" (claim 9) were interpreted by the Court in the HMR/TKT matter.[36]

179. The asserted '698 claims are patently distinct from the '008 claims for at least the same reasons as the '868 claims. Moreover, there are additional material distinctions between claims 6-9 of the '698 patent and the claims of the '008 patent.

180. First, '698 claim 6 recites the term "comprising amplified DNA encoding the mature erythropoietin amino acid sequence of FIG. 6." There is no equivalent limitation in any '008 claim. It would have been particularly unexpected in 1983-84 that *in vivo* biologically active recombinant EPO could be produced using a process involving amplified DNA, such as that claimed in '698 claim 6, because the ordinarily skilled artisan would have been concerned that engineering host cells to produce very large quantities of a foreign protein like EPO

[36] *Amgen Inc. v. Hoechst Marion Roussel, Inc.*, 126 F. Supp. 2d 69, 83-90 (D. Mass. 2001) *aff'd in pertinent part* 314 F.3d (Fed. Cir.2003); *Amgen Inc. v. Hoechst Marion Roussel, Inc.,* 339 F. Supp. 2d 202, 245-258 (D. Mass. 2004), *aff'd in pertinent part* 457 F.3d 1293, 1308 (Fed. Cir.

increases the likelihood of misfolding or mislocalization of the desired recombinant protein.

181. Second, '698 claim 7 recites the term "further comprise amplified marker gene DNA." There is no equivalent limitation in any '008 claim.

182. Third, '698 claim 7 recites the term "wherein said amplified marker gene DNA is Dihydrofolate reductase (DHFR) gene DNA." There is no equivalent limitation in any '008 claim.

183. In my opinion, for the reasons explained in this declaration, each of the inventions as a whole claimed in '698 claims 6-9 would ***not*** have been obvious to a person of ordinary skill in the art in 1983-84, even in light of claims 2, 4, 6, 7, 25 and/or 27 of the '008 patent.

F. MY OPINIONS IN THE *IN RE COLUMBIA UNIVERSITY PATENT LITIGATION* CASE ARE CONSISTENT WITH MY OPINIONS IN THIS CASE

184. The patents involved in the *In Re Columbia University Patent Litigation* case (Columbia case) contain claims that broadly encompass various aspects of cotransformation and coamplification and involve DNAs that encode proteinaceous material and glycoproteins.

185. One of my opinions in the Columbia case was that later claims that recite glycoproteins generally are obvious in view of the recital of particular glycoproteins in the earlier claims. A glycoprotein is simply a protein that has a least one sugar residue attached to it. As I explained in my Rebuttal Expert Report in the Columbia case none of the later claims requires that the glycoprotein be functional or therapeutically useful following administration to humans or animals. None of the later claims requires that the protein be glycosylated in the same manner as in the donor species or that the cells reproduce any specific pattern of glycosylation. None requires any particular post-translational modification. None requires that the protein be

2006).

"properly" modified following translation. All that is required is that the stated protein be a glycoprotein, *i.e.*, that it have at least one sugar attached to it.[37]

186. Roche contends that my opinions in the Columbia case support their argument that Lin's claimed inventions would have been obvious as of 1983. This is not true. In my Columbia report, I surveyed the state of the art as it related to certain subject matter at issue in the Columbia case. I did not attempt to survey or characterize the complete state of the art, nor did I address the complex choices and uncertainties that would have confronted one, such as Lin, who wished to produce a specific human glycoprotein having a specific *in vivo* biological function.

187. In Columbia, the issue was whether previously issued claims to production of proteins in CHO cells rendered obvious subsequently issued claims to production of glycoproteins in CHO cells. In Columbia, the only difference between the earlier claims and the later claims was a distinction between proteins and ***glyco***proteins, without any regard to whether the glycoproteins needed to be functional. Mammalian cells, such as CHO cells, were known to glycosylate certain proteins they produced. To one skilled in the art at the time, the production of proteins in CHO cells would have implied the production of glycosylated proteins, and thus a later claim to production of ***glyco***proteins in CHO cells added nothing significantly different than the earlier claim to production of proteins in CHO cells. Since the later claim to glycoproteins did not specify a particular carbohydrate structure, or any functional difference between the earlier claimed proteins and the later claimed glycoproteins to distinguish themselves from the earlier protein claims, there was no patentable distinction between the earlier protein claim and the later glycoprotein claim. In other words, a skilled artisan would reasonably have predicted that the expression of a normally glycosylated protein in CHO cells would produce a protein

[37] *See* D.I. 578, Exhibit Z (Rebuttal Expert Report of Harvey F. Lodish, Ph.D. (Sept. 17, 2004))

having at least some glycosylation, and that prediction would likely have proved to be true once the experiment was actually performed.

188. Here, however, the issue is very different. CHO cells do not normally produce erythropoietin, and before Lin's inventions, it was not known whether CHO cells could and would produce an erythropoietin glycoprotein that would perform the specific *in vivo* biological functions of human EPO: stimulating the production of red blood cells. Indeed, as detailed above and in my expert reports, there were then many reasons for skilled artisans to doubt whether recombinant CHO cells growing in culture could produce a glycoprotein product that performed the *in vivo* function of human EPO. The fact that a cell type, such as CHO, can glycosylate a protein it produces, does not mean that the glycosylated protein it produces will have the specific glycosylation and other post translational modifications that EPO requires in order to perform its specific biological function *in vivo*. Before Lin's inventions, in 1983-84, a skilled artisan would not have reasonably expected that the expression of an EPO protein in CHO cells grown in culture would successfully produce a glycoprotein that performed the biological function of human EPO *in vivo*. Until the experiment was actually performed, and empirical proof obtained to show that the product produced and isolated from CHO cells grown in culture actually performed the biological function of EPO *in vivo*, the most that a skilled artisan would have said at the time was they hoped it would do so.

189. Roche contends that portions of my expert report in the Columbia case confirm that the following techniques used in the field were obvious and well known:

- Transformation of mammalian cells with exogenous DNA
- The use of CHO cells for producing recombinant proteins
- The amplification of genes in mammalian cell cultures

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at ¶ 17.

- The use of dihydrofolate reductase (DHFR)
- The use of viral promoters

190. The fact that various techniques were known and practiced in the art hardly means that Lin's particular combination of techniques to solve several long-standing and highly challenging problems that others repeatedly tried but failed to solve would have been obvious. The notion is akin to the argument that a Monet painting would have been obvious because others before Monet had used paint brushes, paint, and canvas to paint water lilies.

It is true that workers of ordinary skill in the art had various types of cultured cells that could be used as host cells in transformation experiments and that CHO cells were among the different cell types that could be used as host cells for DNA transformation and recombinant protein production. It was also known that amplified genes could be selected by exposing cells to selection pressure and that the dihydrofolate reductase (DHFR) gene was one of several approaches that could have been used as an amplifiable selectable phenotype. Exogenous promoters, including viral promoters, were known to function in many types of cultured mammalian cells. My opinion that these techniques could be used to express recombinant proteins generally is consistent with my opinions in this case.

III. THE CLAIMS IN DR. LIN'S '933, '349 AND '422 PATENTS FALL WITHIN GROUPS I, IV AND V OF THE 1986 RESTRICTION REQUIREMENT IN DR. LIN'S '298 APPLICATION

191. Earlier in this litigation, I submitted a declaration explaining my opinions that the claims in Dr. Lin's '933, '349, and '422 patents fall within restriction Groups I, IV and V of the Patent Office's 1986 restriction requirement. (*See* Docket Item 502, at ¶¶ 17-34.) I hereby incorporate those opinions into this declaration in their entirety.

192. I declare that all statements made herein of my own knowledge are true, that all statements made on information and belief are believed to be true, and that these statements were

made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both (18 U.S.C. § 1001).

Executed this 25th day of September, 2007.

HARVEY F. LODISH, PH.D.