DECLARATION OF HARVEY F. LODISH, Ph.D. IN SUPPORT OF AMGEN INC.'S OPPOSITION TO ROCHE'S MOTION FOR SUMMARY JUDGMENT OF INVALIDITY FOR DOUBLE PATENTING OVER CLAIM 10 OF THE '016 PATENT

PART 1

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 INC.'S OPPOSITION TO ROCHE'S MOTION FOR SUMMARY JUDGMENT OF INVALIDITY FOR DOUBLE PATENTING OVER CLAIM 10 OF THE '016 PATENT

I, Harvey F. Lodish, declare under penalty of law:

1. I am submitting this declaration in support of Amgen's opposition to Roche's motion for summary judgment that the claims-in-suit[1] are invalid for obviousness-type double patenting over claim 10 of U.S. Patent No. 4,667,016 ("the '016 patent").

2. Before making this declaration, I reviewed Roche's summary judgment motion and accompanying documents (Docket Items 490-95), and the other documents attached to or referred to herein.

3. On June 14, 2007, I submitted a declaration in support of Amgen's Motion for Summary Judgment of No Obviousness-Type Double Patenting (Docket Item 502), which I incorporate herein in its entirety. A summary of my professional experience, affiliations, and work, including a copy of my curriculum vitae, is provided in that prior declaration.

I. A PERSON OF ORDINARY SKILL IN THE ART IN 1983-84 WOULD NOT HAVE FOUND THE CLAIMS-IN-SUIT TO BE OBVIOUS IN LIGHT OF CLAIM 10 OF THE LAI '016 PATENT

4. 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 the claims-in-suit to be obvious in light of claim 10 of the '016 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 the claims-in-suit to be ***not*** obvious over '016 claim 10.

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[1] I understand that the claims-in-suit are: claims 3, 7-9, 11-12, and 14 of U.S. Patent No. 5,547,933 ("the '933 patent"); claim 1 of U.S. Patent No. 5,955,422 ("the '422 patent"); claim 7 of U.S. Patent No. 5,756,349; claims 4-9 of U.S. Patent No. 5,618,698 ("the '698 patent"); and

5. The question that resolves the issue of whether the claims of Dr. Lin's patents-in-suit are patentably distinct from '016 claim 10 is: would '016 claim 10 render an ordinarily skilled artisan in 1983-84 — operating without the benefit of the teachings of the Lin or Lai patents — any closer to achieving the products claimed in the '933 and '422 patents, or the processes claimed in the '868, '698, and '349 patents than she would have been otherwise? For the reasons explained below, it is my opinion that '016 claim 10 clearly would not. The mere recitation of the phrase "recombinant erythropoietin," without knowledge of the product's complete amino acid sequence, or the possession of an isolated DNA encoding said sequence, would perhaps at most convey an unachieved hope or goal with no guidance whatsoever how to achieve that goal. Since the '016 patent claims could not provide an ordinarily skilled artisan with the limitations of the claims-in-suit, nor instruct the artisan how to practice the claims-in-suit, it simply cannot render the claims-in-suit invalid for obviousness-type double patenting under the one-way double patenting test.

A. CLAIM 10 OF THE LAI/STRICKLAND '016 PATENT

6. Claim 10 of the '016 patent states:

> 10. A process for the efficient recovery of recombinant erythropoietin from a mammalian cell culture supernatant fluid, said process comprising the following steps in sequence:
>
> (1) subjecting the fluid to ion exchange chromatographic separation at about pH 7.0, thereby to selectively bind erythropoietin in said sample to a DEAE agarose cationic resin;
>
> (2) stabilizing materials bound to said resin against degradation by acid activated proteases through treatment with urea;
>
> (3) selectively eluting bound materials having a pKa greater than that of erythropoietin by treatment with aqueous acid at a pH of about 4.3.

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claims 1-2 of U.S. Patent No. 5,441,868 ("the '868 patent").

(4) selectively eluting erythropoietin by treatment with an aqueous salt at a pH of about 7.0;

(5) subjecting erythropoietin-containing eluent fractions to reverse phase liquid chromatographic separation involving an immobilized C4 resin, thereby to selectively bind erythropoietin in said fluid to said resin;

(6) selectively eluting bound erythropoietin from said resin with an aqueous ethanol solution of about 60 percent at a pH of about 7.0; and,

(7) isolating erythropoietin-containing fractions of the eluent.

7. Importantly, '016 claim 10 recites a process for the efficient recovery of some thing from the culture supernatant fluid of a mammalian cell: recombinant erythropoietin. The claim does not instruct one skilled in the art how to produce that product in cell culture, nor does it describe what the structure and composition of that product are. In short, '016 claim 10 is not itself a process for the production of erythropoietin. Therefore, a person of ordinary skill in the art would not have looked to Claim 10 of the '016 patent to prepare an *in vivo* biologically active erythropoietin because it provides no indication of how to do so. For this reason, it is nonsensical to consider the purification procedure of '016 claim 10 as rendering obvious a process of producing an erythropoietin product, let alone an *in vivo* biologically active erythropoietin product. In fact, '016 claim 10 does not even acknowledge the importance of the *in vivo* biological activity of human erythropoietin, let alone recite how to make an erythropoietin glycoprotein possessing it.

8. I also note that '016 claim 10 does not mention many of the characteristics of Dr. Lin's inventions claimed in the patents-in-suit. '016 claim 10 does not mention: the steps to take for the production of an *in vivo* biologically active erythropoietin glycoprotein; the structure of any mammalian host cells that might be used for such production; any *in vivo* biological active erythropoietin glycoprotein products; any pharmaceutical compositions prepared from those

products; or any methods of treatment using those products. '016 claim 10 simply describes a seven-step procedure for the purification of recombinant erythropoietin from a cell culture supernatant.

9. The '016 patent was filed in June of 1985 and claims a method for purifying EPO. This method is just one of many ways to purify EPO. For example, Miyake, Kung, and Goldwasser published a technique to substantially purify urinary EPO in 1977.[2] Likewise, the Lin patent specification also teaches a method for purifying recombinant EPO:

> The productivity of the EPO producing CHO cell lines described above can be improved by appropriate cell culture techniques. The propagation of mammalian cells in culture generally requires the presence of serum in the growth media. A method for production of erythropoietin from CHO cells in media hat does not contain serum greatly facilitates the purification of erythropoietin from the culture medium. The method described below is capable of economically producing erythropoietin in serum-free media in large quantities sufficient for production.[3]
>
> Mammalian cell expression products may be readily recovered in substantially purified form from culture media using HPLC (C4) employing an ethanol gradient, preferably at pH7.[4]

10. Serum-free production as disclosed by Dr. Lin was an important advance for purifying EPO for use in pharmaceutical compositions. Mammalian cells are usually grown in growth medium containing about ten percent serum from animals (e.g., fetal calf serum). The serum in the growth medium contains a complex mix of proteins and other biomolecules, including particular growth factors that are necessary for the health and growth of the cultured cells. The presence of these contaminants in cell culture greatly complicates the ability to isolate

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[2] Miyake *et al.* "Purification of Human Erythropoietin," *J. Biol. Chem.* Aug. 10 252(15):5558-64 (1977) (Exhibit A).

[3] '933 Patent, col. 27:8-16.

[4] '933 Patent, col. 28:29-32.

pure EPO from cell culture supernatant, and their elimination through serum-free growth conditions rendered the purification of EPO from cell culture much more efficient and simple.

11. The high-pressure liquid chromatography "HPLC (C4)" step disclosed in Dr. Lin's specification is one of the steps in the multi-step procedure claimed in the '016 patent.

12. Roche suggests that the term "recombinant erythropoietin from a mammalian cell culture supernatant," which appears in '016 claim 10, ***standing on its own*** — without the benefit of Dr. Lin's enabling patent specification — would have taught skilled artisans how to produce recombinant EPO in mammalian cell culture. This argument is absurd. Many entities, including Genetics Institute, Biogen, Genentech, and academic scientists had the best technical talent, strong desire and the financial backing to produce "recombinant erythropoietin" by any means possible, yet all failed to achieve the goal until Lin showed the way. In order for Roche's assertion to hold, it would also have to be true that Dr. Lin's cloning of the EPO gene and production of a glycoprotein product possessing the *in vivo* biological activity of human EPO was obvious in light of the prior art. As the Patent Office determined, and as has been found by many courts, Dr. Lin's inventions were not obvious.

13. In my opinion, the analogy made by Amgen's counsel during examination of the '933 patent is particularly apt: "a method of purifying recombinant EPO cannot be modified to produce recombinant EPO any more than a method of washing a car can be modified to make a car." The method of purifying EPO claimed in claim 10 of the Lai '016 patent presumes the availability of recombinant erythropoietin produced in mammalian cell culture as a starting material, just as the invention of the automated car wash presumed the availability of cars to wash. Neither method teaches how to make the necessary starting material, whether EPO

polypeptide or cars. Instead, they both presume and rely on the fact that someone else will invent and provide what is needed to practice the method.

14. Without the teachings of Dr. Lin's patents, the Lai '016 claim 10 method for recovering "recombinant erythropoietin from a mammalian cell culture supernatant fluid" is a mere hope or wish, as is "a method for purifying a drug which cures all forms of cancer" or "a method for washing a car that gets 200 miles per gallon." In order to practice claim 10 of the '016 patent one must have:

- Mammalian cells;
- Capable of growth in culture;
- That have been genetically altered;
- To produce and secrete a recombinant protein having an amino acid sequence of erythropoietin.

That in turn required:

- Isolation of a DNA encoding erythropoietin;
- Construction of transformed or transfected mammalian cells capable of expressing that DNA upon growth in culture; and
- A means for growing the cells

15. None of this knowledge was known to those skilled in the art prior to Dr. Lin's inventions, and none of this knowledge is taught by, or even implicit in, the '016 claim 10.

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

16. 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.

17. 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

18. 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.

19. 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) (Exhibit 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.

20. 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.

21. 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, carbonyl 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 body, 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.

22. 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, the glycosylation of erythropoietin was known to be important for biological function, but its structure was unknown

23. 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. Bio.l Chem.* 249:4202-6 (1974) (Exhibit B); *see also* Lowy, *et al.*, "Inactivation of Erythropoietin by Neuraminidase and by Mild Substitution Reactions," *Nature* 186:102 (1960) (Exhibit C); Briggs, *et al.*, "Hepatic clearance of intact and desialylated erythropoietin," *Amer. J. of Physiology* 227:1385-1388 (1974) (Exhibit D) ("These results indicate that desialylation of ESF causes its rapid hepatic clearance from the circulation . . .").

24. 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 (Exhibit B).

25. 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. (Exhibit E, at 984).[5]

26. 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.

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[5] Dr. Lin and colleagues confirmed the result found for urinary EPO using recombinant EPO in early 1984. *See* Exhibit 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.").

27. 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 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) (Exhibit G); Sasaki, H., *et al.*, "Site Specific Glycosylation of Recombinant Human Erythropoietin," *Biochemistry* 275361 8-8626 (1988) (Exhibit 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) (Exhibit 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 19-822 (1989) (Exhibit J).

28. 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: 12059-1 2076 (1987) (Exhibit 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) (Exhibit K).

29. 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) (Exhibit L).

3. The cellular source of erythropoietin was unknown

30. 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) (Exhibit M) ("The exact cellular source for erythropoietin production in the kidney is still unknown.").

31. 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) (Exhibit 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) (Exhibit 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.").

32. 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

33. 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 (e.g., 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*.

34. 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.

35. Such mammalian proteins made in bacterial cells, however, general will not undergo any of the post-translational modifications such as glycosylation that would normally occur if the protein were made in mammalian cells.[6] This is because the enzymes that catalyze these modifications are generally not found in bacteria.

36. 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.

37. 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) (Exhibit 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)

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

(Exhibit Q). Proteins thus can require proper glycosylation for either *in vitro* or *in vivo* activity or both.

38. 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 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.

39. 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 directedly 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."[7] 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

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under certain conditions.

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

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.

40. 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 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.

41. 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.

42. 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) (Exhibit 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.

43. 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) (Exhibit 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 that 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.

44. Likewise, Goeddel's patent application filed in 1983 (U.S. Patent No. 4,766,075 (Exhibit 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).

45. 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).

46. 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) (Exhibit 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.

47. 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."[8]

48. 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.

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

49. 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 asantibodies, 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 illicit 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.

50. 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. CLONING A DNA ENCODING HUMAN EPO WAS NOT OBVIOUS IN 1983

51. Roche contends that cloning rEPO from humans would have been obvious to one skilled in the art in 1983. I disagree.

52. One of ordinary skill in 1983 would not have had a reasonable expectation of successfully obtaining a DNA encoding human EPO by screening a cDNA library with degenerate oligonucleotide probes designed using amino acid sequences obtained from human EPO.

1. The failure of others to clone the human EPO gene powerfully demonstrates that cloning the gene would not have been obvious to one skilled in the art in 1983

53. The combination of steps required to clone a DNA encoding human EPO was neither simple nor obvious, nor was it made so by the prior successful cloning of DNAs encoding other human or non-human proteins. When one looks at the genes that were cloned in the relevant time frame, each one presented circumstances that were unique to the particular protein for which a DNA was sought. The fact that a unique combination of circumstances led to an occasional and infrequent success for one protein did not mean that the same approach would work for an entirely different protein. Each presented unique challenges that made the efforts to clone them neither obvious nor readily applicable to other, unrelated genes. This is why the occasional success in cloning and characterizing a gene for a given protein was then and is now published in first-rank scientific journals.

54. In the early 1980s, many highly skilled groups were working diligently to clone the EPO gene, including, among others, Genetics Institute, Biogen, and Genentech.[9] All of these groups pursued the approach described by Roche as well as other approaches, yet none of them succeeded. In my opinion, this fact is compelling contemporaneous evidence that the cloning of DNA encoding human EPO was not, in fact, "obvious."

2. In 1983 it was not obvious to those skilled in the art how to obtain accurate protein sequences from naturally-occurring human EPO, nor was it obvious what sequences of the EPO

[9] *See Amgen Inc. v. Chugai Pharm. Co. Ltd.*, 13 U.S.P.Q.2d 1737, 1750-53 (D. Mass. 1989).

protein would be useful to design oligonucleotide probes to screen DNA libraries for a clone of the EPO gene or a cDNA encoding EPO

55. In 1983, it was neither routine nor obvious for one of ordinary skill in the art to obtain accurate amino acid sequences that would be useful for making useful sets of degenerate probes to screen a library of DNA clones for DNA encoding human EPO. Indeed, before and even after Lin's inventions, several world-class laboratories at Cal-Tech, Harvard, Biogen, Genetics Institute and even Amgen struggled for years to obtain the N-terminal sequence of human EPO without success.

56. As a threshold matter, obtaining the human EPO protein in sufficient amounts and purity to conduct accurate protein sequencing was not easy – one was apparently limited to obtaining this glycoprotein from the urine of patients with aplastic anemia. Even if sufficient amounts of protein had been obtained, one still faced the difficulty of obtaining accurate sequences of the amino acids in this EPO glycoprotein. In 1983, the science of sequencing minute amounts of proteins was still in its infancy. As a result, one would frequently identify more than one amino acid at a single position during peptide sequencing, meaning one could not determine the "correct" amino acid at that position. At that time, glycosylated residues and other modified amino acids could not generally be identified by protein sequencing, leaving gaps in the amino acid sequence where one could not identify the original residue that was modified. This was true, for example, for glycosylated asparagines (three of which we now know are present in human EPO), hydroxylated prolines or lysines, and cysteines involved in disulfide bonds (four of which are also now known to be present in human EPO).

3. In 1983, designing useful sets of degenerate oligonucleotide probes corresponding to human EPO amino acid sequences was not obvious

57. In 1983, the uncertainty and difficulty in screening DNA libraries did not reside in deducing the set of DNA sequences that would encode a given amino acid sequence. At that time, those skilled in the art were able to use published tables that listed the different DNA codons that encoded each amino acid, and it was reasonably straight-forward to use such tables to specify the differing sequences of probes that could encode a given sequence of amino acids.

58. Rather, the uncertainty and difficulty in DNA cloning resided in selecting the particular length and sequence of amino acids to target for probe screening, in selecting the hybridization conditions to be used in screening the library, in building a library of DNA clones that actually contained an intact copy of the DNA being sought, and in confirming that the targeted amino acid sequence was in fact based on a sequence actually within the protein being sought.

59. Due to the degeneracy of the genetic code, skilled artisans knew in 1983 that almost every amino acid sequence would be encoded by more than one DNA sequence (only two of the 20 amino acids are encoded by a single 3- nucleotide sequence). Indeed, some amino acids are encoded by up to six different DNA codons per amino acid. Consequently, a sequence of six amino acids comprised entirely of these highly degenerate amino acids would require 46,656 different DNA probes to encode every possible sequence of DNA nucleotides that could possibly encode those six, highly degenerate amino acids.[10] If the probe were shortened to target only 5 of those same 6 amino acids, the number of different DNA probes required to encode

[10] Some other amino acids are encoded by 4 different codons, some by 3 different codons, some by 2, and a very few by only one codon. A sequence of 6 amino acids comprised entirely of amino acids encoded by 2 codons each would still require 64 (2x2x2x2x2x2) different DNA sequences to encode every possible DNA sequence for those 6 amino acids. Such low

every possible variation of the sequence of 5 amino acids could be reduced from 46,656 different DNA probes to 7,776 different DNA probes (still an impossibly large number). In either case the unique DNA encoding the desired amino acid sequence will be a minute fraction of all of the DNA sequences in the probe — 1 per 7,776 or 1 per 46,656 — and thus the likelihood that any DNA clone detected by the set of DNA probes would encode a segment of the desired protein would be very low. In other words, the noise-to-signal ratio and thus the frequency of false positive clones will be very high.

60. The selection of an appropriate sequence of amino acids within a protein to target for DNA probe screening was further complicated by the fact that the optimal conditions, especially the salt concentration and temperature, under which a given DNA sequence will hybridize with a complementary DNA sequence will vary depending on the composition of nucleotides comprising the sequence. Double- stranded sequences of DNA rich in A and T nucleotides are less stable (that is, the two strands of DNA will dissociate easier) than those rich in G and C nucleotides. Because a mixture of different probes must be used to screen for a DNA encoding a given amino acid sequence, some of those probes will have more G and C nucleotides than A and T nucleotides, and some of those probes will have more A and T nucleotides than G and C nucleotides. Achieving the right hybridization conditions to eliminate false positives, while avoiding false negatives, entails great skill, experienced judgment, and much experimentation for each set of probes.

61. Moreover, given the state-of-the-art in oligonucleotide synthesis in 1983, one could not have great confidence that what one thought was a complete set of partially- or fully-degenerate oligonucleotide probes actually contained each of the intended probes, or contained

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degeneracy sequences are very uncommon in human proteins, however.