EXHIBIT 14

UNITED STATES DISTRICT COURT
DISTRICT OF MASSACHUSETTS

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

DECLARATION OF HARVEY F. LODISH, Ph.D. IN SUPPORT OF AMGEN INC.'S REPLY TO DEFENDANTS' CLAIM CONSTRUCTION BRIEF

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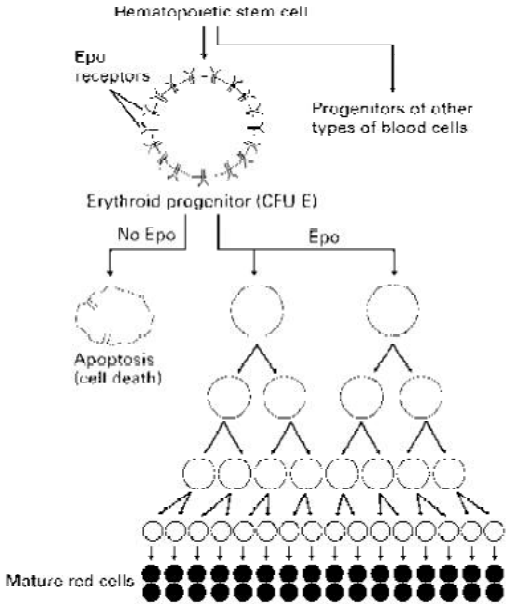
25. When EPO binds to the EPO receptors on the target erythroid progenitor cells, it initiates a signal transduction process that ultimately causes the progenitor cells to survive, proliferate and differentiate into mature red blood cells. Figure 14-7 from my textbook, *Molecular Cell Biology*, depicts this process:



26. A glycoprotein hormone like EPO cannot function effectively in the body unless it possesses the appropriate three-dimensional structure (also known as "conformation") required to survive its passage through the bloodstream to the target erythroid progenitor cells in the bone marrow, and then bind and activate the EPO receptor on those cells. The amino acid sequence of a protein, and, in addition, post-translational modifications (such as glycosylation) determine the three-dimensional structure of a protein like EPO. The amino acid sequence (or primary structure) dictates the secondary structure (localized folding of parts of the polypeptide chain), and tertiary structure (long-range folding of the polypeptide), as well as the sites of post-translational modification of the protein. However, as discussed below, the particular post-translational modifications (such as oligosaccharide structures) that are imparted to the

polypeptide are determined by the host cell. Based upon a protein's sequence of amino acid residues, many noncovalent bonds between the amino acids and with their surrounding medium will further determine the protein's conformation. These include ionic interactions that are based on electrical charge, hydrogen bonds, and hydrophobic interactions. Additionally, in many secreted proteins such as EPO, covalent bonds between two cysteine residues, termed disulfide bonds, are important for stabilizing the protein's conformation. EPO has two such disulfide bonds in its normal conformation.

27. Human EPO has a primary structure of 165 amino acids linked to one another in a unique and specific order. The amino acid sequence for human EPO is depicted at positions +1 through +165 in Figure 6 of Amgen's Patents. EPO's two disulfide bonds (bridges) link the sulfur atom on the cysteine residue at position 7 with the sulfur atom on the cysteine residue at position 161, and the sulfur atom on cysteine residue 29 with the sulfur atom on cysteine residue 33 in the polypeptide sequence. EPO has a secondary and tertiary structure (four long conserved alpha helices folded together in a particular arrangement) that is very similar to that of other cytokines. This structural homology suggests that cytokines evolved from a common ancestral protein.

28. Long after Lin's inventions, and as a direct result of having the EPO DNA and protein products his inventions made possible, the three-dimensional structure of a human EPO analog was determined using x-ray crystallography and nuclear magnetic resonance ("NMR"). (Syed et al., "Efficiency of signaling through cytokine receptors depends critically on receptor orientation." *Nature* 395:511-516 (1998); Cheetham et al., "NMR structure of human erythropoietin and a comparison with its receptor bound conformation," *Nat. Struct. Biol.* 5:861-66).

AN ORDINARILY SKILLED ARTISAN'S UNDERSTANDING OF "HUMAN ERYTHROPOIETIN"

29. I agree with the interpretation of the term "human erythropoietin" in Dr. Lin's patent specification to mean a protein having the amino acid sequence of human EPO, such as the amino acid sequence of EPO isolated from human urine. I do not believe that "human erythropoietin" requires that the EPO be glycosylated, or have the same structure as would be manufactured by mammalian cells as of 1984.[3] These additional requirements are not supported by the analysis of Lin's patent specification or the knowledge in the art as of 1984.

30. First, I do not agree that the term "human erythropoietin" necessarily requires that the human erythropoietin protein be glycosylated. First, Lin's patent specification clearly states the "polypeptides of the invention," in other words, erythropoietin, may be "non-glycosylated": "Depending upon the host employed, ***polypeptides of the invention*** may be glycosylated with mammalian or other eucaryotic carbohydrates or ***may be non-glycosylated***."[4] The various examples of the Lin patent further confirm that Lin contemplated non-glycosylated human erythropoietin. For example, Examples 11 and 12 of the patent are directed, among other things, at the expression of human erythropoietin (comprising the exact 165 amino acid sequence of human EPO set forth in Lin's Figure 6) in bacterial cells of the species *Escherichia coli*.[5] It was well-known before 1983 that prokaryotic cells like *E. coli* are incapable of adding carbohydrates to proteins.[6] Thus, the Lin patent specification makes clear that human erythropoietin can be

[3] *See* Defendants' Opening Memorandum in Support of Their Proposed Claim Construction at 1 (Docket No. 311).

[4] Amgen Brief, Appendix B, '933 Patent at 10:28-33 (Docket No. 312) (emphasis added).

[5] *Id.* at columns 29-32.

[6] *See, e.g.*, Lodish, H.F., "Post-translational modification of proteins," *Enzyme Microb Technol.*, 3(3):177-188 (1981) (attached as Exhibit D).

glycosylated or unglycosylated. The addition of carbohydrates to the 165-amino-acid polypeptide backbone via the formation of covalent bonds at the point of attachment creates a ***glycosylated*** erythropoietin polypeptide. But both glycosylated forms and the unglycosylated form are human erythropoietin polypeptides. Specifically, in an N-glycosylated protein, the amide $-NH_2$ of asparagine is chemically modified in that a hydrogen atom is replaced by a sugar, but nonetheless the amino acid's identity as asparagine is unchanged. Likewise, the identity of the polypeptide — here, human erythropoietin — is also unchanged by the addition of an oligosaccharide.

31. Second, I do not agree that the Lin patent specification or the knowledge in the art as of 1984 would require that "human erythropoietin" have a "structure that would be produced in mammalian cells as of the invention date." Again, Lin's patent examples directed towards production of human erythropoietin from non-mammalian cells make it plain that Roche's interpretation is incorrect. In addition to *E. coli* expression, Examples 11 and 12 also disclose production of human erythropoietin in the baker's yeast, *Saccharomyces cerevisiae*. It was well known in 1983 that while *S. cerevisiae* cells are capable of glycosylation, the particular glycans that they impart to polypeptides are considerably different than those imparted by mammalian cells.[7] I illustrated precisely this phenomenon in a review article I published in 1981:

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[7] *See id.*

[9] Amgen Brief, Appendix B at 10:28-33 (Docket No. 312) (emphasis added).

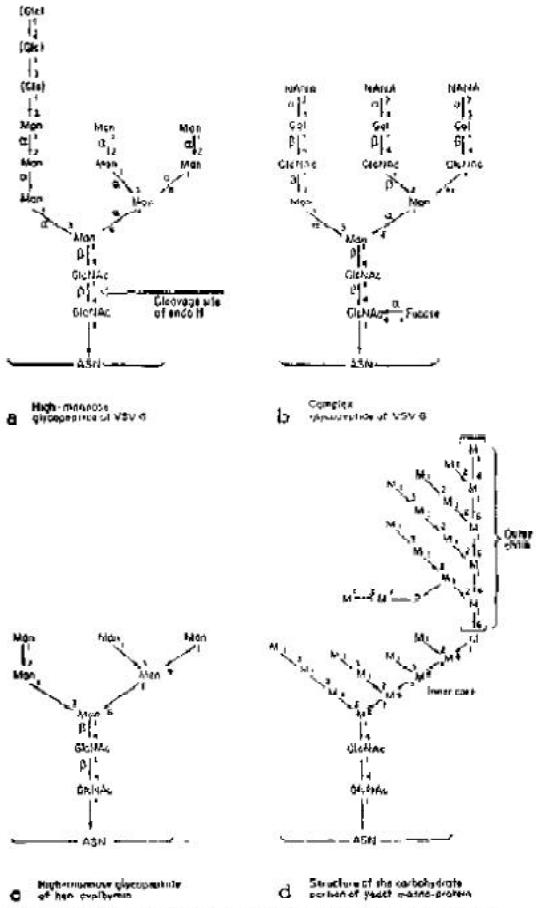


Figure 10 Structures of asparagine-linked oligosaccharides. (a) Structure of the two high mannose carbohydrate chains found on the microsomal form of VSV G protein (drawn from refs 76 and 15); including the site of cleavage by endoglycosidase H. (b) Structure of the asparagine-linked oligosaccharide found on the VSV glycoprotein[77]. (c) Structure of one of the related high-mannose glycopeptides of hen ovalbumin[78]. (d) Structure of the carbohydrate portion of yeast mannoprotein[79].

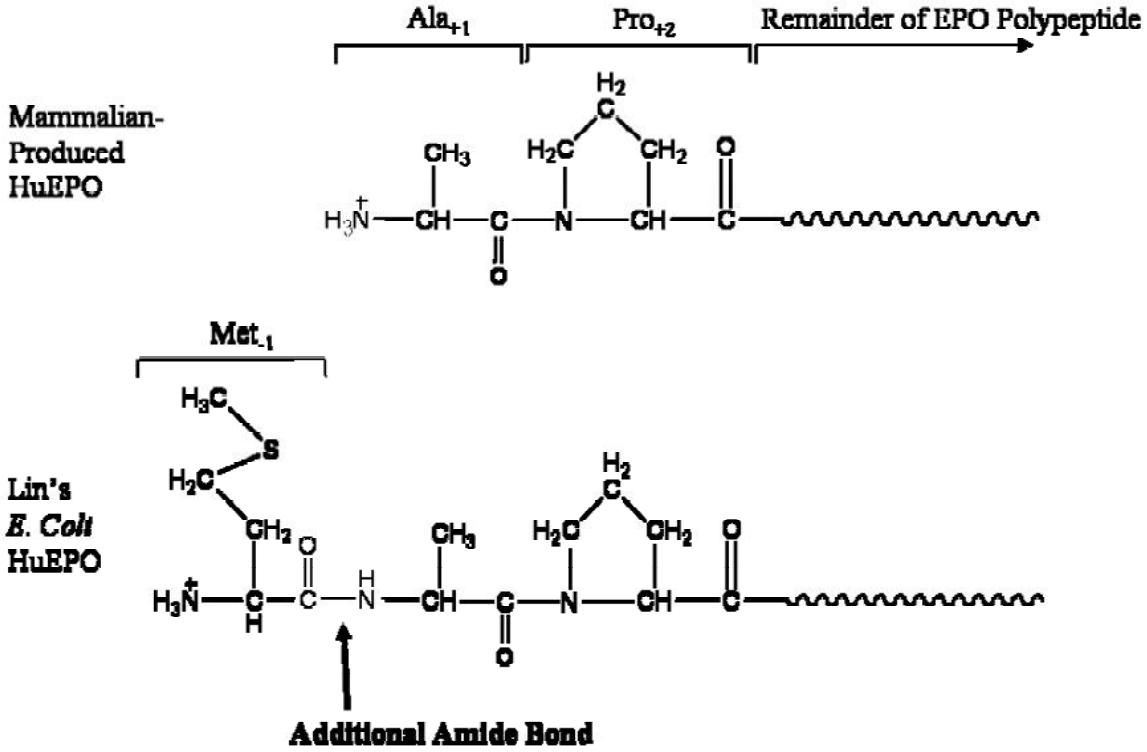
As shown in Figure 10 of my review article, the N-linked oligosaccharides of vertebrate cells (panels a-c) are significantly different than those found in yeast proteins (panel d).

32. In addition to differences in glycosylation state, the patent specification also contemplates human erythropoietin with other differences in structure as compared to the "structure that would be produced in mammalian cells as of the invention date." In particular, the patent specification states that human erythropoietin can include a methionine residue linked to the amino-terminus of human erythropoietin by an amide bond: "Polypeptides of the

invention may also include an initial methionine amino acid residue (at position -1)."[9] In the context of Example 11, the patent specification further states: "FIGS. 10 through 15 and 7 illustrate the design and assembly of a manufactured gene encoding a human EPO translation product lacking any leader or presequence but including an initial methionine residue at position -1." This passage in particular makes plain that according to Lin (as well as the common understanding at the time), that even when an additional molecule, here methionine, is added to the polypeptide sequence of human EPO, it is still a "human EPO." Again, like glycosylation, if a bond to a hydrogen atom from the nitrogen atom in the amino group of Ala_{+1} is replaced by an amide bond to a methionine, this does not change the identity of that amino acid as alanine, nor does it change the identity of the polypeptide as human erythropoietin.

33. Example 11 describes construction of a synthetic gene for human erythropoietin that has been optimized for production in *E. coli* by, among other things, the replacement of the codons for the normal 23 amino acid signal sequence[10] found in the native EPO gene with a methionine codon. Upon expression in *E. coli*, this would result in the synthesis of a human erythropoietin with an addition methionine amino acid at position –1. I set forth below a comparison of the chemical structures between the human EPO produced by mammalian cells (such as the CHO cells of Lin's Example 10) and the human EPO produced by *E. coli* cells as described in Examples 11 and 12, which has an additional amide bond between Ala+1 and Met-1 which is not present in human EPO produced by mammalian cells:

[10] In mammalian cells, the signal sequence is cleaved from the nascent erythropoietin polypeptide in the endoplasmic reticulum. Prokaryotes like *E. coli* often lack the ability to cleave off a signal sequence from a mammalian protein.



34. I note that Dr. Kadesch addresses the structure of amino acids and the amide bonds in polypeptides in his declaration at paragraphs 19-22. I must make a few corrections and clarifications with respect to Dr. Kadesch's descriptions. First, Dr. Kadesch incorrectly represents the terminal amino group of amino acids as the structure $-NH_2$ and the terminal carboxylate group as COOH. In fact, the terminal amino group is comprised of a mixture of the structures $-NH_2$ and $-NH_3^+$, where the terminal nitrogen atom of the polypeptide chain is covalently linked to either two or three hydrogen atoms. In a solution of amino acids (or the EPO protein), these two structures are present in equilibrium, meaning that the forms can freely exchange by the addition or subtraction of a proton (a hydrogen atom with a positive charge, "H^+") from the medium. The average proportion of the $-NH_2$ and $-NH_3^+$ species is dependant on the pH of the medium in which the EPO is suspended. The same equilibrium situation exists for the carboxylate group on amino acids, which are a mixture of COOH and COO^-. Contrary to Dr.

immune cells of mammals, the genes for immunoglobulins (antibodies) are rearranged into many different structures, which differ from cell to cell within the immune system and from all the other cells of the organism.

52. In describing the process of "protein synthesis" (see paragraphs 24-34), Dr. Kadesch omits the important step of mRNA splicing. Like almost all mammalian mRNAs, the EPO mRNA is produced as a precursor comprised of both exons and introns, and then is spliced into its final form in the nucleus before it is transported to the cytoplasm and translated by ribosomes on the rough endoplasmic reticulum.

53. In paragraph number 28 of Dr. Kadesch's report, he incorrectly states that serine is encoded by four codons. In fact, serine is encoded by six codons.[16]

FURTHER TESTIMONY

54. If requested by the Court, I may provide oral testimony at a hearing or at trial consistent with the statements made in this Declaration.

I declare under penalty of perjury under the laws of the United States of America that the foregoing is true and correct.

Executed this 19th day of March, 2007 at Cambridge, Massachusetts.

/s/ Harvey F. Lodish, Ph.D.

HARVEY F. LODISH, PH.D.

[16] Molecular Cell Biology, 5th Edition at 120.