EXHIBIT 5

UNITED STATES DISTRICT COURT
DISTRICT OF MASSACHUSETTS

AMGEN, INC., Plaintiff, v. F. HOFFMANN-LA ROCHE, LTD., ROCHE DIAGNOSTICS GMBH, and HOFFMANN-LA ROCHE, INC. Defendants.	Civil Action No. 05-CV-12237 WGY

EXPERT REPORT OF DR. ROBERT LANGER

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

22. Claim 1 of the '349 patent, from which claim 7 depends, reads:

> Vertebrate cells which can be propagated in vitro and which are capable upon growth in culture of producing erythropoietin in the medium of their growth in excess of 100 U of erythropoietin per 10^6 cells in 48 hours as determined by radioimmunoassay, said cells comprising non-human DNA sequences which control transcription of DNA encoding human erythropoietin.

23. Claim 1 of the '422 patent reads:

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

B. Amgen construction of the claims

24. I understand that the court has not yet construed the claims of the Lin patents. If the court construes the claims as Amgen has proposed -- to encompass PEG modified proteins having in vivo erythropoietin-like activity that are therapeutically useful -- it is my opinion that these claims are not enabled and have insufficient written description and are thus invalid.

VIII. OPINION

A. Factual background

(1) Chemical modification of proteins with polyethylene glycol

25. Proteins are made up of strings of amino acids. Human erythropoietin ("HuEPO") is a protein with a primary sequence of 165 amino acids (AM-ITC 000546627).

26. A variety of chemical modifications have been attempted on proteins. For the purposes of this report, Roche's Counsel has asked me to focus on the science of polyethylene glycol-protein modifications, as they stood in the 1983-1984 timeframe.

27. The term "PEGylation" is known in the art to refer to the "process by which polyethylene glycol chains are attached to protein and peptide drugs." (Harris & Chess (2003) at 214). "Polyethylene glycols are pH-neutral, nontoxic, water-soluble polymers that consist of repeating ethylene oxide subunits, each with a molecular weight of 44, and two terminal hydroxyl groups (Figure 1) (7). They are either linear (5 to 30 kd) or branched (40 to 60 kd) chain structures." (Molineux (2002) at 14). PEGylation is typically performed on protein and peptide drugs to shield them "from proteolytic enzymes" and to "improve pharmacokinetics." (Harris & Chess (2003) at 214).

28. PEGylation typically refers to the covalent modification of proteins. Covalent bonds are stable bonds that result from the sharing of one or more pairs of electrons.

29. It is understood that the covalent attachment of PEG to a protein results in a new chemical entity. For example a scientific text reported in 2006 that "the organic and polymer chemistry of PEG activation has now matured, and protein pegylation is becoming viable commercially. However, the technique needs significant know-how, and the modified protein is considered to be a new chemical entity from a regulatory point of view." (Banga (2006) at 205).

30. An Amgen scientist, Graham Molineux, has described how PEGylated molecules are different from their unPEGylated counterparts. Specifically, he stated:

> As might be expected, the physicochemical properties of pegylated molecules are different from those of their nonpegylated counterparts. Conformational changes, steric interference, and changes in electrostatic binding properties and hydrophobicity may

> affect their binding affinity to receptors and result in reduced in vitro activity (7). (Molineux (2002) at 14; *see also* Molineux Tr. (3/28/07) 76:17 - 77:14).

31. Dr. Molineux also references some of the technical problems associated with early attempts at pegylation (Molineux, (2002) at 14). In another article, he discussed the optimization that is necessary to PEGylate proteins (Molineux, (2003) at 75; *see also* Molineux Tr. (3/28/07) 72:21 - 73:22).

32. The first chemical steps of PEGylation that enabled the protection of proteins from destruction during drug delivery were developed in the late 1970's by Frank Davis and his colleagues at Rutgers University (Harris & Chess (2003) at 215).

33. As mentioned above, I have been asked to generate an opinion as to whether the Lin patents would enable a person of skill in the art in 1983-1984 timeframe to generate a PEG modified protein having in vivo erythropoietin-like activity that is therapeutically useful, through routine experimentation. In my opinion, the knowledge and experimentation required to generate a PEG modified protein having in vivo erythropoietin-like activity that is therapeutically useful was not trivial in 1983-1984. This understanding is supported by several documents. For example, in 1983 a scientific article concerning protein PEGylation stated:

> Enzymes used as pharmacological agents for systemic therapy offer promise in the treatment of several diseases, but have considerable limitations because of problems of protein immunogenicity, instability and, often, rapid elimination. One method overcoming these difficulties appears to be the masking of the polypeptide structure by linking polymers to the protein surface (Holcenberg 1982). **This technique is still in its early stages concerning choice of polymer, method of coupling and long-term toxicity**. (emphasis added) (Veronese et al. (1983) at 757).

34. Control over the amount of PEGylation, and the locations of PEG attachment, and PEG molecular weight are important factors in the generation of a PEG

modified protein having in vivo erythropoietin-like activity that is therapeutically useful. For example in 1985 it was reported that "A prime practical consideration when using PEG-protein conjugates is to determine the optimum degree of substitution and PEG molecular weight needed to give the desired effect (on immunogenicity or partitioning, for example) without producing excessive protein deactivation. **There appear to be no general rules in this regards, as effects vary greatly from system to system**." (emphasis added) (Harris (1985) at 351).

35. One of the first publications of PEGylation was in 1977 (Abuchowski et al. (1977)). This early work used cyanuric chloride to PEGylate the target protein. This chemistry was the most frequently used method of PEGylation in 1983-1984. For example, in 1985, it was reported that "The most frequently applied route" of preparing protein-PEG conjugates "is that of Abuchowski and Davis in which the PEG-cyanuric chloride derivative (refer to Electrophilic Derivatives) is reacted with the protein". (Harris (1985) at 350).

36. One potential problem with cyanuric chloride-based PEGylation of proteins is the potential for PEG attachment at different sites on the protein. In their 1977 paper, Abuchowski et al. discuss the potential for conjugation at a variety of protein groups using this chemistry, as follows:

> Johnson et al., report that cyanuric chloride reacts with amino, imino, and hydroxyl groups to form stable linkages… The coupling is conveniently followed by measuring reduction in primary amino groups in the protein, although other nucleophiles undoubtedly react also…. Nucleophiles on the protein other than amino groups may react with the activated PEG so that the size of the modified albumins is probably somewhat larger than that calculated from loss of primary amine groups. (Abuchowski et al. (1977) at 3579).

37. As noted above by Abuchowski's group, cyanuric chloride-based PEGylation can affect several different sites of the target protein. The potential for multiple sites

of PEGylation makes control of attachment difficult, and can lead to inactivation of the protein.

For example a scientific article published in 1983 studying PEGylation techniques states:

> Two interesting characteristics of some PEG-protein adducts, as compared to the unmodified proteins, are an increase in blood circulation time and a decrease in antigenicity and immunogenicity (1,6). **Unfortunately, the coupling of PEG to proteins often results in substantial loss of activity.** The coupling of cyanuric chloride activated PEG to SOD results in an approximate loss of 50% of the activity. Modification of L-glutaminase-L-asparaginase results in loss of almost 90% of its activity (6,10). (emphasis added) (Beauchamp et al. (1983) at 31).

38. Cyanuric chloride based PEGylation has the potential to reduce activity in a variety of proteins. For example, another scientific article published in 1984 stated:

> Cyanuric chloride has routinely been used as the coupling agent to attach PEG to enzymes (Abuchowski et al 1977). However, this agent substantially inactivates asparaginases from *Escherichia coli, Vibrio succinogenes, Erwinia carotovora* and *Acinetobacter glutaminasificans* (Abuchowski et al. 1979). Ashihara et al. (1978) modified an asparaginase from *E. coli* A-1-3 with cyanuric chloride-activated PEG and obtained only 7% activity. (Abuchowski (1984) at 175).

39. Other problems associated with Abuchowski's chemical methods were appreciated in the literature in 1983, and research on additional chemical means for protein PEGylation was ongoing. For example, a scientific article published in 1983 states that:

> … we found certain disadvantages in coupling the polymer through trichloro-s-triazine according to the method reported in the literature (3): a. **the modified enzyme presents species at higher molecular weight than expected on the base of bound PEG; this probably due to the fact that the third chloride of the reagent reacted further giving a cross-linked protein**; b. the modification is unsuitable for enzymes possessing reactive –SH groups in the active site; and c. the modified enzyme presents significant absorption in the UV region due to the s-triazine ring and, therefore, spectroscopic characterizations are greatly hampered. **Furthermore, the presence of chlorotriazine derivatives in compounds for clinical use is dangerous according to the regulations given by Food and Drugs Administration**. (emphasis added) (Boccu et al. (1983) at 94).

40. In my opinion, it is possible that the PEGylation methods available in 1983-1984, and in particular the most commonly used method based on cyanuric chloride, would inactivate EPO as well. For example, in 1990 it was reported that:

> the activity of rHuEPO (recombinant human EPO) was sensitive to chemical modifications of the lysine, arginine and tyrosine residues, as well as carboxyl groups. Among these, modification of the lysine residues with various lysine specific reagents resulted in a wide range of changes in the activity of modified rHuEPO. Modifications that changed the positive charges of the lysine residues to neutral or negative charges, such as carbamylation, trinitrophylation, acetylation or succinylation, caused a substantial loss in the vitro activity, whereas amidination which left the total number of positive charges of lysine unchanged did not effect the activity. **It is clear that the positive charges of the lysine residues are essential for the activity of rHuEPO** in vitro. (emphasis added) (Satake et al. (1990) at 128).

41. Importantly, cyanuric chloride mediated conjugation of PEG to lysine could diminish the lysine positive charge at physiological pH.

42. The effects of first generation PEGylation methods on lysines has been appreciated as a potential problem. For example, a scientific article from 2003 addresses this as follows:

> The first-generation pegylation methods were fraught with difficulties. With first-generation pegylation, the PEG polymer was generally attached to the ε amino groups of lysine. This resulted in the modification of multiple lysines, and gave mixtures of PEG isomers with different molecular masses[25]. The existence of these isomers makes it difficult to reproduce drug batches, and can contribute to the antigenicity of the drug and poor clinical outcomes. (Harris & Chess (2003) at 215).

43. The possibility that EPO could be inactivated by PEGylation is further supported by published work from Amgen's scientist, Steven Elliott. In 2003, Dr. Elliot published a book chapter on erythropoietin that stated:

> Many pegylation chemistries have been tried to reduce undesirable by-products, improve the specificity and efficacy of PEG

> attachments, and minimize immunogenicity risk of the protein conjugate while maximizing the *in vitro* and *in vivo* activity of the resultant molecule [41]. The current chemistries typically target the reactive amino groups on Lys or the amino terminal amine. rHuEPO has eight Lys, some of which are part of the active site [17]. Therefore, some pegylated EPO molecules conjugated to Lys may have low activity because PEG may interfere with receptor binding and activation. Other pegylated EPO molecules may have low activity because attached polymer results in structural alterations that interfere with receptor interaction. (Elliott (2003) at 247).

(2) The science of PEGylation was not fully developed in 1983-1984

44. In my opinion, the science of PEGylation was not fully developed in 1983-1984, and the generation of a PEG modified protein having in vivo erythropoietin-like activity that is therapeutically useful, was not something that could be done by routine experimentation. For example, the challenges associated with the PEG coupling methods used to generate therapeutically effective PEGylated proteins were discussed in 1992:

> **Despite the well-established advantages of PEG-proteins over their unmodified counterparts, this technology has not yet been very widely exploited commercially because until recently most methods were suboptimal; they use conditions which are likely to reduce biological activity, they are not readily controlled with respect to the number of PEG molecules attached, and they often require purification steps which themselves endanger labile proteins.** They also leave part of the coupling moiety attached to the protein, where it may serve as an antigen… (emphasis added) (Delgado (1992) at 274).

45. Even some of the relatively modern methods for PEGylation, developed after 1983-1984, had the potential to induce protein inactivation. For example, the potential for PEGylation-mediated protein inactivation was discussed in a scientific article in 1996, where it was reported that:

> The most often used modifiers for the attachment of PEG to proteins are methoxy-PEG (Gotoh et al., 1992; Inada et al., 1984 and 1995, N-hydroxysuccinimide-PEG (Inada et al., 1995) and succinimidyl carbonate-PEG (Chiu et al., 1993; Zalipsky et al.,

> 1992 and 1993). The amphipathic character of these activated PEG molecules allows modification of the protein amino groups in aqueous solutions under slightly alkaline conditions. This type of modification often leads to inactivation of proteins containing amino groups important for their biological activity or of proteins unstable in alkaline conditions." (Kynclova (1996) at 644).

46. In fact, in 2003, nearly 20 years after the filing date of the Lin patents, it was reported that "PEGylation has taken 20 years to emerge as a viable pharmaceutical tool. During this time there have been important advances in the chemistry of PEGylation, in the generation of biomolecule therapeutics and in understanding PEG-biomolecule conjugates." (Harris & Chess (2003) at 220).

47. Even today it is appreciated that protein PEGylation requires significant know-how. For example a scientific text reports in 2006 that "the organic and polymer chemistry of PEG activation has now matured, and protein PEGylation is becoming viable commercially. However, the technique needs significant know-how, and the modified protein is considered to be a new chemical entity from a regulatory point of view." (Banga (2006) at 205).

(3) Amgen's work on PEGylated EPO

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Redacted

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Dated April 6, 2007

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