Amgen Inc. v. F. Hoffmann-LaRoche LTD et al Doc. 526 Att. 2

Exhibit D

Declaration of Howard S. Suh in Support of Roche's Motion for Summary Judgment that the Asserted Claims of the '933 Patent are Invalid for Indefiniteness and Lack of Written Description

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UNITED STATES DISTRICT COURT
DISTRICT OF MASSACHUSETTS

AMGEN INC.,

Plaintiff,

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.

Civil Action No.: 05-12237 WGY

SUPPLEMENTAL EXPERT REPORT OF AJIT VARKI, MD

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beta revealed significant variation in elution times indicating unique glycosylation patterns."[45]

27. Dr. Imperiali's statement that it has not been shown that "***all*** recombinant EPO has glycosylation which differs from ***all*** naturally occurring EPO"[46] sets an unrealistically high and practically impossible standard. It is not possible to test all hypothetically possible recombinant EPOs. However, all of the comparison experiments that have been performed on real recombinant EPOs that have actually been produced support my opinion that such differences do exist. Neither Dr. Bertozzi nor Dr. Imperiali has identified any data on any rEPO that contradicts my conclusions.

28. Dr. Imperiali states "although different preparations of uEPO and rEPO can have different distributions of EPO glycoforms, they will have glycoforms in common as well."[47] First, whether rEPO and uEPO have glycoforms in common has no bearing on whether there are differences between rEPO, such as EPO beta, and naturally occurring EPO. Two products can have some components in common, but if their overall composition is different, the products are different. Both rEPO preparations and preparations of naturally occurring EPO are complex mixtures of different glycoforms, even if uEPO and rEPO have some glycoforms in common, that does mean they are the same, or cannot be distinguished from each other. Second, Dr. Imperiali fails to point to any convincing evidence that any rEPO preparation is identical to any naturally occurring preparation. Indeed, Dr. Imperiali fails to present any unequivocal proof that recombinant EPO, such as EPO beta, has ***any*** glycoforms that are identical to glycoforms found in urinary or plasma EPO.

29. Instead, Dr. Imperiali argues that "[t]he reason that different types of cells

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[45] *Id.* at 3629.

[46] Imperiali Report ¶ 102 (emphasis added).

[47] *Id.* ¶ 51.

make the same glycoforms is that the glycosylation pathway is clearly defined" and "since the enzymes themselves are closely conserved ... any particular glycoform distribution is a subset of the total spectrum of glycoforms that occurs as a result of this pathway."[48] I disagree. First, it is not the case that every cell type contains the same complement of enzymes. Second, as discussed above and in my Initial Expert Report, experimental evidence supports my position that there are glycoforms in recombinant EPO that are not present in urinary EPO.

30. Dr. Imperiali herself admits that "due to the inherent microheterogeneity of EPO glycoforms" it is a fact that "all EPO samples (urinary and recombinant) differ from one to the next in glycosylation depending on, for example, their source, how they were collected, and the purification methods used."[49] However, it appears to be Dr. Imperiali's position that "though there may be observable differences in distributions of glycoforms (e.g. how many of each one) when comparing a sample of uEPO to a sample of rEPO, the differences in distribution are entirely dependent upon the particular EPO samples used, and are based on factors such as source, intracellular and extracellular environment, degree of purity and other conditions. Thus, these observed variations cannot be used to define any generalized and absolute difference between glycosylation of uEPO versus rEPO."[50] First, I disagree that recombinant EPO and urinary EPO solely differ in the relative distribution of glycoforms. Second, to the extent that Dr. Imperiali is suggesting that the reported differences in glycosylation are function of the particular sample of EPO tested and do not reflect an absolute difference between rEPO and uEPO, I also disagree. As I discuss in my initial report, the structure of the co- and post-translational modifications imparted to EPO depends on both the inherent EPO-protein

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[48] *Id.* ¶ 52.

[49] *Id.* ¶ 102.

[50] *Id.* ¶ 55.

modifying properties of the particular cells that make EPO in the body and the particular environment in which they are grown. Because these cells are not available, and because their microenvironment in the kidney cannot be reproduced in culture, it is very unlikely that scientists can ever develop an EPO production system using cells grown in culture that accurately duplicates the structure of urinary EPO

III. ROCHE'S DEGLYCOSYLATION EXPERIMENTS DO NOT ESTABLISH A DIFFERENCE FROM DR. LIN'S CLAIMED ERYTHROPOIETIN

A. THE EXPERIMENTS RELIED UPON BY DR. IMPERIALI ARE NOT PROPERLY DOCUMENTED

31. To support her opinion that peg-EPO does not depend on *N*-linked glycosylation for *in vivo* activity, Dr. Imperiali relies on experiments performed by Roche comparing the bioactivity of peg-EPO and EPO beta in normocythemic mouse ("normo-mouse") bioassays.[51] In particular, Dr. Imperiali relies on experiments performed by another Roche expert, Dr. Sven-Michael Cords, who compared the effects of various recombinant EPO preparations on reticulocyte counts over a period of 48 to 120 hours.[52] The recombinant EPO preparations include "EPO beta; N-deglycosylated and desialylated EPO beta; MIRCERA™; N-deglycosylated and desialylated MIRCERA™ and a control solution."[53]

32. Dr. Imperiali states that "samples were also analyzed to confirm deglycosylation using various analytical protocols set forth in the Dembowski declaration including SDS Page and HPLC protocols."[54] However, I have reviewed the expert reports of Dr. Imperiali and Dr. Cords, including the attached declarations and I see no actual data to support

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[51] *Id.* ¶¶ 128-144.

[52] *Id.* ¶¶ 141-144.

[53] *Id.*¶ 141.

[54] *Id.* ¶ 141.

translational glycosylation, immature proteins may misfold, aggregate, and be degraded before leaving the ER."[59] Dr. Imperiali stated that unglycosylated glycoproteins may be produced in a variety of ways, but that these "may fold either correctly or incorrectly from nonglycosylated folding intermediates."[60] Further, Dr. Imperiali acknowledged that "carbohydrates may be removed from a secreted, folded glycoprotein by enzymatic deglycosylation" but noted that "[t]his process probes the effect on protein conformation of removing the saccharide from a glycoprotein that is already folded."[61] Thus, the deglycosylation experiments that Dr. Imperiali relies upon, even assuming they were properly executed, do not establish that glycosylation is not important for the biological activity of peg-EPO. Even if glycosylation can be removed post-folding and pegylation with something less than a complete destruction of EPO function, one cannot conclude from these experiments that glycosylation is not critical for the production and function of peg-EPO.

Executed this 1st day of June, 2007 at La Jolla, California.

AJIT VARKI, MD

[59] O'Connor, *et al.*, "Modulation of protein structure and function by asparagine-linked glycosylation," *Chem. Biol.* 3:803-812 at 803 (1996).

[60] *Id.* at 805.

[61] *Id.* at 805.