EXHIBIT A

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

SECOND SUPPLEMENTAL EXPERT REPORT OF AJIT VARKI, MD

Contains Amgen/Roche Confidential Material and Restricted Access BLA/IND

REDACTED

Confidential Information Subject to Protective Order

B. THE PROCESS AND SOURCE LIMITATIONS OF DR. LIN'S PRODUCT CLAIMS DEFINE THE STRUCTURE OF DR. LIN'S NOVEL EPO PREPARATIONS.

10. It appears to be Dr. Bertozzi's opinion that claim language of Dr. Lin's asserted '933 and '422 product claims does not distinguish them from the prior art.[8] I disagree. It is my opinion that the process and source limitations confer specific structures to the claimed products and that those specific compositions are different from the structure of the EPO that was purified from human urine before Dr. Lin made his inventions.[9]

11. '933 claim 3 recites:

> A non-naturally occurring glycoprotein product of the expression in a mammalian host cell of an exogenous DNA sequence comprising a DNA sequence encoding human erythropoietin said product possessing the *in vivo* biological property of causing bone marrow cells to increase production of reticulocytes and red blood cells.

12. As I explained in my Initial Expert Report, "expression in a mammalian host cell of an exogenous DNA sequence comprising a DNA sequence encoding human erythropoietin" will inevitably lead to the production of populations of erythropoietin molecules that are different in the structures of their constituent EPO molecules as compared to prior art urinary EPO preparations. This is because when a gene for a secreted glycoprotein is removed from its normal cellular environment, and inserted into a different type of cell — often from a different species — which is grown under far different conditions than its *in situ* environment in the body, the glycoprotein that is produced will have, among other things, different glycan structures than the naturally-occurring glycoprotein.[10] As Dr. Bertozzi herself has observed in a scientific publication, "protein glycosylation in living tissues can differ drastically from that in

---

[8] *See, e.g.*, Bertozzi Supplemental Report ¶ 31.

[9] Initial Expert Report ¶ 58.

[10] *See, id.* at ¶¶ 80-85 for a more detailed discussion.

Lin's claimed EPO that are lacking from prior art urinary EPO (and *vice versa*), even if there were not any molecular species in Dr. Lin's claimed EPO that were completely absent from prior art urinary EPO, the populations of molecules found in prior art urinary EPO and Dr. Lin's claimed EPO are clearly distinct from each other. Dr. Bertozzi agrees. Because the populations of molecules found in prior art urinary EPO and Dr. Lin's claimed EPO products are different, prior art urinary EPO does not anticipate or render obvious Dr. Lin's EPO product claims.

24. The ***absence*** from Dr. Lin's claimed EPO of molecular species found in prior art urinary EPO also renders Dr. Lin's EPO a novel composition which has different chemical and functional properties as compared to the prior art. The removal of undesired molecular species can be just as significant as the addition of new molecular species. Thus if a patent claim is to preparations each comprised of a heterogenous mixture of molecules (such as claim 3 of '933 patent and claim 1 of the '422 patent), then a prior art mixture significantly and measurably different in composition and distribution would not anticipate the claimed invention. Thus, even assuming if Dr. Bertozzi were correct that all of the molecular species found in a preparation of Dr. Lin's claimed EPO could be found in prior art urinary EPO, which they are not, those prior art preparations would not render Dr. Lin's claims anticipated or obvious. This is because, as Dr. Bertozzi admits, their glycoform distributions are measurably different.

25. In light of the above discussion, I will recap the differences between Dr. Lin's claimed human EPO products and the prior art urinary EPO, which I have previously explained in depth in my two previous reports. No reports demonstrate that any prior art urinary EPO preparation possesses all of the glycoforms present in Lin's claimed products.[22] Indeed, no reports demonstrate that any urinary EPO preparation prepared at any time, or even whole urine

[22] Even if a particular analytical technique fails to identify a difference between two preparations, it does not mean that no differences exist. *See* Initial Expert Report ¶135.

possesses all of the glycoforms present in Lin's claimed products. Instead, many different experimental approaches from many different laboratories around the world have demonstrated measurable and reproducible differences between urinary EPO and recombinant EPO covered by Dr. Lin's product claims. Preparations of EPO obtained from mammalian cells grown in culture differ from prior art EPO purified from urine in a number of different ways, including:

- Isoelectric points of glycoforms;[23]
- Sulfation;[24]
- Polylactosamine repeat content;[25]
- O-glycan structure;[26]
- Presence of N-glycolylneuraminic acid;[27] and
- Absence of α2-6 sialic acid linkages.[28]

26. As I have previously explained, these differences exist because the cellular source of human EPO (*i.e.* whether endogenously produced in the human body or produced in mammalian cells grown in culture) dictates its glycoform distribution in terms of both the identity of the components in an individual composition or mixture and the relative distribution of those components. Despite Dr. Bertozzi's arguments, even today in 2007 it is not possible to accurately recreate the glycoform distribution of a complex glycoprotein like human EPO by expressing a recombinant form in a mammalian host cell. I will address this fact in further detail below.

---

[23] Initial Expert Report ¶¶ 93-124.
[24] *Id.* ¶¶ 125-183
[25] *Id.* ¶¶ 201-204.
[26] *Id.* ¶¶ 205-210.
[27] *Id.* ¶¶ 225-236.
[28] *Id.* ¶¶ 237-244.