Amgen Inc. v. F. Hoffmann-LaRoche LTD et al Doc. 503

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

DECLARATION OF THOMAS W. STRICKLAND, Ph.D.
IN SUPPORT OF AMGEN'S MOTION FOR SUMMARY JUDGMENT OF NO OBVIOUSNESS-TYPE DOUBLE PATENTING

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1. I am a Principal Scientist at Amgen Inc. ("Amgen") and currently work at the company's Longmont, Colorado facilities. My current duties involve support of the commercial production of therapeutic proteins and the development of purification processes for protein molecules in development.

2. I was awarded my doctorate degree in Biochemistry from Vanderbilt University in 1981. The focus of my studies in earning my Ph.D. and writing my thesis was the glycoprotein hormones of the pituitary and placenta. After receiving my degree in 1981, I was an Assistant Research Biological Chemist at the University of California, Los Angeles (UCLA), Department of Biological Chemistry. I remained at UCLA through March 1984. My research activities at UCLA included studies on the biosynthesis and protein folding of glycoprotein hormones and the chemical modification, isolation, and receptor cross-linking of glycoprotein hormones.

3. Between April 1984 and July 1995, I was a research scientist in the Protein Chemistry Department at Amgen in Thousand Oaks, California. During my tenure at Amgen, my job responsibilities included research, development, and optimization of the purification procedures related to recombinant human erythropoietin.

4. Between July 1995 and March 1999, I worked as a consultant for Amgen and undertook further study at the University of Northern Arizona. In March 1999, I returned to Amgen in the position of Manufacturing Process Engineer.

5. A copy of my most recent *curriculum vitae* is attached hereto as Exhibit A.

6. I am submitting this Declaration in support of Amgen's Motion for Summary Judgment of No Obviousness-Type Double Patenting. Specifically, I am providing a chronology of the work conducted leading up to the processes claimed in U.S. Patent No. 4,667,016.

7. Starting about September 1984, one of my job responsibilities was to work on developing an economical and efficient protocol for purifying large quantities of recombinant human erythropoietin for use in what was to become Amgen's EPOGEN® product. At the time I started my work, I was aware of the information contained in the prior art, and particularly, Miyake *et al., J. Biol. Chem. 252*:5558-5564 (1977). By November, I was also aware of the use of a C_4 RP-HPLC purification at neutral pH using an ethanol gradient to purify recombinant erythropoietin from culture media (this one-step purification method is the same as disclosed in Dr. Lin's patents). Although the procedures described in Miyake *et al.* and Dr. Lin's patent are sufficient to obtain a highly purified recombinant erythropoietin preparation, each has its drawbacks for use in a large-scale, commercial process.

8. For example, the procedure described by Miyake *et al.* involves seven basic purification steps (as set forth in Table V of Miyake et al.) for purifying uEPO from the urine of aplastic anemia patients:

- DEAE cellulose
- Phenol extraction
- Ethanol precipitation
- DEAE-agarose
- Sulfopropyl-Sephadex
- Sephadex G-100
- Hydroxyapatite

This procedure was cumbersome in the sense that many steps were required. It was also not well-suited for commercial scale production of a product. For example, the second step of the Miyake *et al.* procedure is a phenol extraction step. While perfectly adequate for its stated purpose – the inactivation of enzymes that degrade erythropoietin – the procedure can be difficult to perform at a large commercial scale.

9. Similarly, Dr. Lin's disclosed method was not an economical way to purify EPO from cell culture material. In order to run large quantities of EPO containing cell culture media through a C_4 RP-HPLC column to practice Dr. Lin's purification method, a very large column size would be needed. This type of column, among other things, is very costly and difficult to re-use.[1]

10. I therefore worked to optimize a purification protocol that would be used to purify recombinant human erythropoietin for use in a pharmaceutical composition at large scale. At the time, Dr. Por-Hsung Lai was leader of the group within Amgen responsible for protein analytics. That group also assisted in developing the reverse phase chromatography steps defined in the process described in the '016 Patent.

11. As a consequence of the work conducted at Amgen from late 1984 through early 1985, an improved method was developed for purifying recombinant EPO for use in a pharmaceutical composition. This method, as set forth in claim 10 of my '016 patent, is a multiple step process that relies in large measure on an ion exchange chromatography step and a reverse phase chromatography step.

12. The first ion exchange chromatography step of claim 10 of the '016 patent uses a DEAE agarose column. While Miyake *et al.* used a similar column in the fourth step of his procedure, the '016 process differs significantly in at least one way: the column (and bound materials from the preparation) is washed with a low pH solution to elute contaminants that are

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[1] I note that Amgen uses a C_4 reverse phase column at neutral pH using an ethanol gradient in its recombinant EPO purification process today. This column, however, is not used as a first step in the procedure and by the time the column is used, significantly less mass is run through the column, allowing for the use of a much more manageable column size. Also, due to the difficulty of cleaning this resin, it is disposed of after its single use.

less negatively charged than EPO. Urea present in this wash helps to protect the EPO from degradation by proteases that are active under these low pH conditions.

13. The composition of this wash is important to purification on this column. The low pH of this wash makes many of the contaminants positively charged so that they lose affinity and elute from the positively charged resin. Chinese hamster ovary (CHO) cells used to manufacture the EPO produce acid activated proteases. Urea is used to protect the product from these proteases during the low pH wash.

14. I did not appreciate the need to mitigate against proteolytic degradation of crude recombinant EPO containing solutions at low pH until sometime in December 1984. As I first learned in the December 1984 time frame, loading and washing the DEAE column under low pH conditions without urea resulted in an increase in the appearance of EPO breakdown products. *See* page 65 of my Laboratory Notebook No. 1115 (Exhibit B). As I also learned in late December 1984, these breakdown products were not readily separated from EPO using a subsequent HPLC or gel filtration chromatography step. *See* page 79 of my Laboratory Notebook No. 1115 (Exhibit B). At this time, I did not load the column at neutral pH, use urea to protect against proteolytic degradation at low pH, or elute the product at neutral pH following the low pH wash as defined in claim 10 of the '016 patent.

15. I identified the utility of urea as a protective agent in January 1985. Based on this information, beginning in January 1985, I started adding urea to the load material, as well as to the low pH wash. *See* page 38 of my Laboratory Notebook No. 1135 (Exhibit C). I did not, however, load or elute the column at neutral pH at this time.

16. Based on information garnered from the experiments that were performed, including the failures, by late February 1985, I developed what I considered to be the satisfactory

initial steps of a purification procedure for use in a commercial scale process. It was not until this time (late February 1985), when I had actually conducted the steps disclosed in claim 10 of the '016 patent, that I was reasonably certain that such a process would actually work to yield an erythropoietin preparation that could be used in a pharmaceutical composition. These steps resulted in the significant separation and purification of EPO from CHO cell contaminants, as evidenced by the gel at, for example, page 91 of my Laboratory Notebook No. 1135 (AM-ITC-00137783). In fact, as shown by the gel, substantial purification occurs during the DEAE ion exchange chromatography step.

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

Executed this 12th day of June, 2007 at Longmont, Colorado.

Thomas W. Strickland

THOMAS W. STRICKLAND, PH.D.