EXHIBIT 12

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of: Fu-Kuen Lin
Serial No: 08/100,197
Filed: August 2, 1993
For: PRODUCTION OF ERYTHROPOIETIN

Group Art Unit: 1633
Examiner: James Martinell, Ph.D.

AMENDMENT

Assistant Commissioner for Patents
Washington, DC 20231

Sir:

Please enter the following amendments.

IN THE SPECIFICATION

At page 22, line 24, please delete [Example] and insert in place thereof --Examples--.

At page 25, following line 5 of the original text and the amendment following this line dated 11/6/90, please delete [Reference is made to FIGURES 1 through 21, wherein: FIGURE 1 is a graphic representation of radioimmunoassay analysis of products of the invention; FIGURES 2 through 4 illustrate vector constructions according to the invention; and, FIGURES 5 through 21 are DNA and polypeptide sequences] and please insert the following:

--Reference is made to FIGURES 1 through 21, wherein: FIGURE 1 is a graphic representation of a radioimmunoassay analysis of products of the invention;
Figure 2 shows vector pDSVL-MkE.

Figure 3 shows vector pSVgHuEPO.

Figure 4 shows vector pDSVL-gHuEPO.

Figure 5A, 5B and 5C (collectively referred to as Figure 5) show the sequence of monkey EPO cDNA and the encoded EPO.

Figures 6A, 6B, 6C, 6D and 6E (collectively referred to as Figure 6) show the sequence of human genomic EPO DNA and the encoded EPO.

Figure 7 shows the sequence of the ECEPO gene.

Figure 8 shows the sequence of the SCEPO gene.

Figure 9 shows a comparison of the human and monkey EPO polypeptides.

Figure 10 shows the ECEPO section 1 oligonucleotides.

Figure 11 shows section 1 of the ECEPO gene.

Figure 12 shows the ECEPO section 2 oligonucleotides.

Figure 13 shows section 2 of the ECEPO gene.

Figure 14 shows the ECEPO section 3 oligonucleotides.

Figure 15 shows section 3 of the ECEPO gene.

Figure 16 shows the SCEPO section 1 oligonucleotides.

Figure 17 shows section 1 of the SCEPO gene.

Figure 18 shows the SCEPO section 2 oligonucleotides.

Figure 19 shows section 2 of the SCEPO gene.

Figure 20 shows the SCEPO section 3 oligonucleotides.

Figure 21 shows the section 3 of the SCEPO gene.--

At page 49, line 13, please delete [therin] and insert in place thereof --therein--.

At page 49, line 29, please delete [Table VI] and insert in place thereof --FIGURE 6--.

At page 55, line 1, please delete [SalI] and insert in place thereof --SalI--.

At page 55, line 4, please delete [SalI] and insert in place thereof --SalI--.

At page 63, line 20, please delete [membranc] and insert in place thereof --membrane--.

At page 64, line 30, please correct the spelling of "recombinant".

At page 73, line 21, please delete [(Tables XI and XIII)] and insert in place thereof --(FIGURES 13 and 15)--.

At page 90, line 4, please delete [resopnse] and insert in place thereof --response--.

IN THE CLAIMS

Please cancel claims 61 - 63.

Please add the following claims:

-- 64. 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.

65. A pharmaceutically-acceptable preparation containing a therapeutically effective amount of erythropoietin wherein human serum albumin is mixed with said erythropoietin. --

IN THE DRAWINGS

Please add the enclosed formal drawings FIGURES 1 through 21.

REMARKS

Upon entry of the above-requested amendments, claims 64 and 65 will be pending in the present application. Amendments requested for the specification bring the text of the specification in line with that of parent application Serial No. 07/113,179 which issued as U.S. Patent No. 5,441,868 and do not introduce any new matter.

Applicant expresses his appreciation to Examiner Martinell and Supervisory Examiner Stanton for the interview held on April 21, 1999, concerning this application. For the record, Applicant notes that prosecution of this application was suspended by the Office on March 31, 1995 for consideration of a potential interference and has remained suspended since that time until recently when the application was returned from the Board to the Examining group. Applicant appreciated Examiner Stanton's prompt responses to his numerous status inquiries throughout this period.

As indicated at the interview, Applicant is filing herewith terminal disclaimers for U.S. Patent Nos. 5,547,933; 5,621,080; and 5,856,298, the earliest expiration date of which is August 20, 2013.

Claims 61-63 were pending in the application and are hereby cancelled. Newly added Claim 64 is directed to a pharmaceutical composition of human erythropoietin which is obtained from mammalian cells grown in culture. Newly added Claim 65 is directed to a pharmaceutical preparation containing erythropoietin mixed with human serum albumin and coincides with the subject matter of previously allowable Claims 61 and 62.

Human erythropoietin as recited in Claim 64 is disclosed in several examples of the application. Example 1 discloses the use of human erythropoietin isolated from the urine of patients afflicted with aplastic anemia ("urinary EPO") to produce tryptic fragments and the amino acid sequencing of those fragments. Examples

7 and 10 disclose the production of human erythropoietin in COS-1 and CHO cells respectively. Thus, human erythropoietin is understood to include any polypeptide having the amino acid sequence of EPO isolated from human urine and may be produced in human cells or in other mammalian cells. The application further discloses that the glycosylation of human erythropoietin may differ depending upon the host cell used for production. Claim 64, however, excludes EPO that is isolated from human urine by the phrase "purified from mammalian cells grown in culture." This phrase is intended to include any EPO produced by mammalian cells (human, CHO, COS, etc.) that are grown in culture, which means in vitro.

In contrast to Claim 64, newly added Claim 65 does not limit the source of the EPO but does specify that the EPO is mixed with human serum albumin in the preparation.

As discussed at the interview, Applicant believes that the subject matter of Claims 64 and 65 is novel and non-obvious over the prior art and is fully supported by the disclosure of the application. With respect to Claim 65, the two Goldwasser references reviewed at the interview disclose the use of bovine serum albumin to stabilize partially purified erythropoietin preparations obtained from sheep plasma. These references do not disclose a pharmaceutically acceptable preparation, and there is no indication that BSA or other stabilizing additive would be necessary once the purified EPO was obtained. In fact, the Chapter 10, Erythropoietin, Goldwasser reference states: "It is obvious that, when further purification or protein determinations are being carried out, exogenous protein cannot be added. Loss of activity can then be minimized by keeping solutions as concentrated and as cold as possible."

The present application was the first disclosure to provide a pharmaceutical composition of a therapeutically effective amount of erythropoietin. Applicant reiterates his position that the Sugimoto reference, U.S. 4,377,513, is not repeatable and is non-enabling because no information is provided concerning how to

obtain or select the starting kidney tumor cells or the hybridoma resulting from fusion with a leukemic lymphoblastoid cell. No deposit of any cells is referenced in the Sugimoto patent. Further, the reference does not disclose the isolation or characterization of any EPO product, but only reports that a relatively low level of EPO activity was detected. In any event, Applicant submits that the present claims define patentable subject matter over Sugimoto even if the disclosure is considered to be repeatable. First, there is no disclosure of a pharmaceutical composition in the reference and it cannot be assumed that it was straightforward to purify the EPO from the suspension of disaggregated tumor cells. Second, the purported method of producing EPO disclosed in Sugimoto cannot be viewed as viable for providing sufficient amounts of EPO to prepare a composition having a therapeutically effective amount of EPO. Consequently, it is submitted that both Claims 64 and 65 are novel and non-obvious over the Sugimoto disclosure. It is believed that the other relevant references have been previously distinguished.

Applicant therefore respectfully submits that claims 64 and 65 are in condition for allowance and an early notice thereof is respectfully solicited.

Respectfully Submitted,

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