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PATENT
ATTORNEY DOCKET NO. 11009/32685

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of:	)	For: PRODUCTION OF ERYTHROPOIETIN
Fu-Kuen Lin	)	
Serial No: 08/468,556	)	Group Art Unit: 1804
Filed: June 6, 1995	)	Examiner: James Martinell, Ph.D.

THIRD PRELIMINARY AMENDMENT
AND TERMINAL DISCLAIMER PURSUANT TO 37 C.F.R. §1.321

Assistant Commissioner for Patents
Washington, DC 20231

Sirs:

Please enter the following amendments.

In the Specification

At page 1, first paragraph please delete and insert

--This is a continuation of my co-pending U.S. Patent Application 08/202,874 filed February 28, 1994, now abandoned, which was a continuation of U.S. Patent Application 07/113,178, filed October 23, 1987, now abandoned, which was a continuation of U.S. Patent Application 06/675,298 filed November 30, 1984 and issued as U.S. Patent No. 4,703,008 on October 27, 1987, which was a continuation-in-part of U.S. Patent Application 06/655,841, filed September 28, 1984, now abandoned, which was a continuation-in-part of U.S. Patent Application 06/582,185, filed February 21, 1984, now abandoned, and which was a continuation-in-part of U.S. Patent Application 06/561,024, filed December 13, 1983, now abandoned,--

At page 7, line 27, please delete [32 member] and insert in place thereof --32-member--.

At page 8, line 22, please delete the second occurrence of [the].

12/20/96
3rd Preliminary Amendment

At page 11, line 3, please delete [Expt. Hematol.] and insert in place thereof --Exp. Hematol.--.

At page 11, line 4, please delete [(1980:] and insert in place thereof --(1980)--.

At page 11, line 6, please insert a space before "1832".

At page 13, line 13, please insert --"-- after "effects".

At page 13, lines 20-21, please insert --)-- after "propagation".

At page 22, line 4, please delete [Tables V and VI] and insert in place thereof --FIGURES 5 and 6--.

At page 22, line 22, please delete [Table VI] and insert in place thereof --FIGURE 6--.

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

At page 25, following line 5 of the original text, 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.

C2 cont. 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 27, line 24, please delete [Example] and insert in place thereof --Examples--.

At page 30, lines 21, please delete [Asn] and insert --Asn-- in place thereof.

At page 31, line 5, please delete [and RIA Analysis].

At page 32, line 35, please delete the comma[,] after "Springs".

At page 34, line 32, after "83" please insert --deposited with the American Type Culture Collection, 12301 Parklawn Drive, Rockville, Maryland, under deposit accession No. A.T.C.C. 67545 on October 20, 1987--.

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At page 37, line 6, please delete [Table V] and insert --FIGURE 5, comprising portions 5A, 5B and 5C.--.

At page 37, line 6, please delete [Table] and insert --FIGURE--.

Please delete the entire text of pages 38 through 40.

At page 41, line 1, please delete [Table V] and insert in place thereof --FIGURE 5--.

At page 41, line 20, please delete [18, pp. 533-543 (1979] and insert --supra--.

At page 41, line 29, please delete [NEF-976] and insert --NEF-972--.

At page 42, line 24, after "λhE1]" please insert --,deposited with the American Type Culture Collection, 12301 Parklawn drive, Rockville, Maryland, under deposit accession No. A.T.C.C. 40381 on October 20, 1987--.

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At page 42, line 25, please delete [Table VI] and insert in place thereof --FIGURE 6, comprising portions 6A, 6B, 6C, 6D and 6E--.

Please delete the entire text of pages 43 through 47.

At page 48, line 1, please delete [Table VI] and insert in place thereof --FIGURE 6--.

At page 48, line 15, please delete [glutamine] and insert in place thereof --glutamic acid--.

At page 48, line 29, please delete [Table VI] and insert in place thereof --FIGURE 6--.

At page 48, line 34, please delete [Table VI] and insert in place thereof --FIGURE 6--.

At page 49, line 1, please delete [Table] and insert in place thereof --FIGURE--.

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

At page 49, line 8, please delete [Table VI] and insert in place thereof --FIGURE 6--.

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

At page 49, line 15, please delete [Table] and insert in place thereof --FIGURE--.

At page 49, line 16, please delete [Table VII, below] and insert in place thereof --FIGURE 9--.

At page 49, line 18, please delete [Table] and insert in place thereof --FIGURE--.

At page 49, line 27, please delete [Table VI] and insert in place thereof --FIGURE 6--.

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

Please delete the entire text of page 50.

Page 53, line 13, after "orientation" please insert --(vectors F, X and G).--.

At page 54, line 36, please delete [EcoR1] and insert in place thereof --EcoRI--.

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 55, line 13, please delete [BamH1] and insert in place thereof --BamHI--.

At page 55, line 15, please delete [BamH1] and insert in place thereof --BamHI--.

At page 61, line 25, please delete [hemogeneous] and insert in place thereof --homogeneous--.

At page 63, line 35, please delete [Table 6] and insert in place thereof --FIGURE 6--.

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

At page 65, line 34, please delete [Table 6] and insert in place thereof --FIGURE 6--.

At page 66, line 12, please delete [Tables VIII through XIV below] and insert in place thereof --FIGURES 10 through 15 and 7--.

Please delete the entire text of pages 67 through 72.

At page 73, line 1, please delete [Table VIII] and insert in place thereof --FIGURE 10--.

At page 73, lines 6 and 7, please delete [Table IX] and insert in place thereof --FIGURE 11--.

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

At page 73, line 23, please delete [Tables X and XII] and insert in place thereof --FIGURES 12 and 14--.

At page 73, line 26, please delete [Table XI] and insert in place thereof --FIGURE 13--.

At page 73, line 32, please delete [Table XIV] and insert in place thereof --FIGURE 7--.

At page 74, line 9, after "1984", insert --(Published EPO Application No. 136,490)--.

At page 74, line 29, please delete [Table XIV] and insert in place thereof --FIGURE 7--.

At page 75, line 28, please delete [Tables XV through XXI] and insert in place thereof --FIGURES 16 trough 21 and 8--.

At page 75, lines 30 and 31, please delete [Tables XV, XVII and XIX] and insert in place thereof --FIGURES 16, 18 and 20--.

At page 75, line 32, please delete [Tables XVI, XVIII and XX] and insert in place thereof --FIGURES 17, 19 and 21--.

Please delete the entire text of pages 77 through 82.

At page 83, line 21, please delete [Table XXI] and insert in place thereof --FIGURE 8--.

At page 86, line 2, please delete [33932, 33934 and 33933] and insert in place thereof --39932, 39934 and 39933--.

At page 88, line 36, please delete [lablled] and insert in place thereof --labelled--.

At page 89, line 16, please delete [Table VI] and insert in place thereof --FIGURE 6--.

At page 89, line 21, please delete [118] and insert --128-- in place thereof.

At page 90, line 15, please delete [Table V] and insert in place thereof --FIGURE 5--.

At page 90, line 16, please delete [Table VI] and insert in place thereof --FIGURE 6--.

At page 90, lines 29 and 30, please delete [Table V and VI] and insert in place thereof --FIGURES 5 and 6--.

At page 91, line 29, please delete [a].

At page 92, line 10, please delete [Table VI] and insert in place thereof --FIGURE 6--.

At page 94, line 6, please delete [Tables V and VI] and insert in place thereof --FIGURES 5 and 6--.

At page 94, line 14, please delete [Tables V and VI] and insert in place thereof --FIGURES 5 and 6--.

At page 94, line 33, please delete [mammlain] and insert in place thereof --mammalian--.

At page 95, line 10, please delete [membrances] and insert in place thereof --membranes--.

In the Drawings

Please add the enclosed formal drawings FIGURES 1 through 21.

In the Claims

Please cancel claims 64 through 68 and insert new claims 69 through 75.

--69. An isolated 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 Figure 6 and has glycosylation which differs from that of human urinary erythropoietin.

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70. An isolated 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 Figure 6 and is not isolated from human urine.

71. 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 Figure 6.

72. A pharmaceutical composition comprising a therapeutically effective amount an erythropoietin glycoprotein product according to claim ~~69~~, ~~70~~ or ~~71~~.

73. A method for providing erythropoietin therapy to a mammal comprising administering an effective amount of a pharmaceutical composition of claim ~~72~~.

74. A method for treating a kidney dialysis patient which comprises administering a pharmaceutical composition of claim ~~72~~ in an amount effective to increase the hematocrit level of said patient.

75. An isolated polypeptide product characterized by being the product of the expression by a procaryotic host cell of an exogenous DNA sequence encoding the mature erythropoietin amino acid sequence of Figure 6.

REMARKS

Upon entry of the above-requested amendments, claims 69 through 75 will be pending in the application. The requested amendments to the specification bring its text in line with the text of U.S. Patent No. 5,547,933 and do not introduce any new matter.

Applicant acknowledges with thanks the interview kindly granted by Examiner Martinell to the undersigned counsel of record and Mr. Stuart Watt on December 11, 1996. As reflected in the Interview Summary (PTO-413, Paper No. 4), agreement on allowability was not reached. Examiner Martinell did indicate, however, that he was favorably impressed with the Applicant's proposal to prosecute claims identical to independent claims 69 and 70 set out above, along with dependent pharmaceutical composition claims corresponding to canceled claims 65 through 67.

Applicant notes that claims 69, 70 and 71 all differ in scope from glycoprotein claim 1 of U.S. 5,547,933 in specifying that the claimed subject matter comprises the mature[1] human erythropoietin sequence of Figure 6. Claim 69 (like glycoprotein claim 1) recites carbohydrate differences in comparison to human urinary erythropoietin and claim 70 recites a negative limitation with respect to isolation from human urine. No discussion was had during the interview concerning the specific subject matter of newly-submitted claims 71 and 75.

Applicant attaches hereto a Terminal Disclaimer pursuant to 37 C.F.R. §1.321 which, *inter alia*, disclaims the terminal portion of any patent issuing on the present application which extends beyond the term of U.S. Patent 5,547,933.

Submitted concurrently herewith is an Information Disclosure Statement and associated PTO-1449, along with copies of all prior art of record in parent application Serial No. 08/487,774 (which issued as U.S. Patent No. 5,547,933) and in related patent application Serial No. 07/113,179 (which issued as U.S. Patent No. 5,441,868).

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[1] Support for reference to the "mature" sequence is found in the specification at page 48, lines 33-35.

The following remarks address the patentability of the procaryotic host cell expression products which constitute the subject matter of new claim 75.

Applicant notes at the outset that this claimed subject matter has its origins in great-grandparent application U.S. Serial No. 06675,298. Illustrative claims of that application include the following:

> 1. A purified and isolated polypeptide having part or all of the primary structural conformation and one or more of the biological properties of naturally-occurring erythropoietin and characterized by being the product of procaryotic or eucaryotic expression of an exogenous DNA sequence.
>
> 7. A polypeptide according to claim 1 possessing part or all of the primary structural conformation of human erythropoietin as set forth in Table VI [Figure 6] or any naturally occurring allelic variant thereof.
>
> 10. A polypeptide according to claim 1 which has the in vitro biological activity of naturally-occurring erythropoietin.
>
> 58. A purified and isolated DNA sequence as set out in Table V or VI [Figure 6] or a fragment thereof or the complementary strand of such a sequence or fragment.
>
> 59. A polypeptide product of the expression of a DNA sequence according to claim 58 in a procaryotic or eucaryotic host cell.

In the Office Action mailed July 3, 1986 in Serial No. 06/675,298, a six-way restriction requirement was imposed wherein groups I and II were set out as follows:

> I. Claims 1-13, 16, 39-41, 47-54 and 59, drawn to polypeptide, classified in Class 260, subclass 112.
>
> II. Claims 14, 15, 17-36, 58 and 61-72, drawn to DNA, classified in Class '536, subclass 27.

It was the Examiner's position with respect to invention groups I and II that:

> In this case, the product as claimed may be made by a materially different product, such as isolation from a naturally occurring source.

Clearly embraced by the original claims of group I were the procaryotic cell expression products now recited in new claim 75, shown in Example 12 of the specification to possess the in vitro biological activity of naturally-occurring human urinary erythropoietin.

Consistent with the restriction requirement, prosecution in Serial No. 06/675,298 was limited to the DNA subject matter eventually resulting in issuance of U.S. 4,703,008. The polypeptide subject matter of Group I was the subject of application Serial No. 07/113,178. Separate Interferences were declared by the Patent Office concerning the subject matter of U.S. 4,703,008 and Serial No. 07/113,178 and the present inventor was awarded priority in each case. Continuation of prosecution of Serial No. 07/113,178 led to issuance of product claims in U.S. Patent 5,547,933 and to prosecution of the present application.

Against this background, Applicant respectfully submits that the above-noted Terminal Disclaimer beyond the term of U.S. Patent 5,547,933 is appropriate to moot any possible obviousness-type double patenting consideration for any of the claims now pending, and particularly procaryotic expression product claim 75.

Applicant submits that the subject matter of the pending claims is conspicuously patentable over the prior art of record. Applicant specifically notes that, in the European counterpart of the present application, the assertion was made that the human urinary erythropoietin product subjected to de-glycosylation processing as described in a 1982 abstract authored by Dordal *et al.*, *Experimental Hematology*, **10**, Supp. 11, p. 133 Abstract No. 222 (1982) (1449 Reference C-60, a copy of which is attached as Exhibit 1 hereto) was anticipatory of non-glycosylated erythropoietin products of expression in procaryotic host cells (which cells, of course, are incapable of effecting glycosylation). Applicant submits that this position is clearly incorrect. The Dordal *et al.* abstract, while disclosing 1982 attempts to remove carbohydrate from urinary erythropoietin, fails to disclose any product that is free of carbohydrate (*i.e.* a non-glycosylated product) such as is produced by procaryotic host cells.

Briefly summarized, the Abstract describes application of two different methods directed to removal of carbohydrate: (1) treatment with mixed glycosidases

of *Streptococcus pneumoniae*; and (2) treatment with 70% hydrogen fluoride. The mixed glycosidase treatment resulted in a molecular weight reduction from 39,000 to 28,500, clearly reflecting an incomplete removal of carbohydrate. Compare specification page 48, line 33 through page 49, line 1 wherein the mature amino acid sequence of human erythropoietin was noted to provide an estimated molecular weight of about 18,400 for the polypeptide alone. Had all carbohydrate been removed, the molecular weight of the treated product would be expected to approach 18,000. That the mixed glycosidase treatment was incapable of removing all carbohydrate is confirmed by consideration of existing knowledge of the mode of action of the bacterial glycosidases revealed in Glasgow *et al.*, *J. Biol. Chem.*, *252(23)*:8615-8623 (1977) attached as Exhibit 2 hereto. As noted in the right hand column of page 8615, the enzymes are not capable of removing an N-acetylglucosamine sugar which attaches to asparagine residues in N-linked glycosylation.

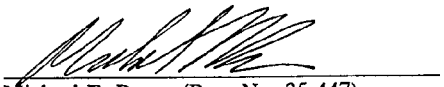
Turning next to the treatment of human urinary erythropoietin with hydrogen fluoride, the Dordal *et al.* abstract, on its face, states that such processing effected removal of "...75% of the carbohydrate found in the original material..."

Because the Dordal *et al.* abstract does not reveal the complete de-glycosylation of urinary erythropoietin, it cannot be maintained to provide a disclosure or suggestion of the generation of non-glycosylated products of procaryotic host cell expression recited in claim 75.

Applicant respectfully submits that claims 69 through 75 are in condition for allowance and an early notice thereof is respectfully solicited.

Respectfully Submitted,

MARSHALL, O'TOOLE, GERSTEIN,
MURRAY & BORUN

BY: 

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