EXHIBIT
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PATENT
ATTORNEY DOCKET NO. 11009/32021

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of:	)	For: PRODUCTION OF ERYTHROPOIETIN
Fu-Kuen Lin	)	
	)	Group Art Unit: 1804
Serial No: 08/468,381	)	
	)	Examiner: James Martinell, Ph.D.
Filed: June 6, 1995	)	

SECOND 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 07/113,179 filed October 23, 1987, and issued as U.S. Patent No. 5,441,868 on August 15, 1995, 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 06/665,841, 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.--

C'

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].

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8D

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.
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.--

C2 cont.

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--.

C3

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--.

82

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--.

C4

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--.

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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 drawing FIGURES 1 through 21.

In the Claims

Please cancel claims 64 through 69.

Please amend claim 62 as set out below.

C5

62. (Amended) A process for the preparation of an *in vivo* biologically active erythropoietin product comprising the steps of transforming or transfecting a host cell with an isolated DNA sequence [such that said host cell expresses *in vivo* biologically active erythropoietin polypeptide] encoding the mature erythropoietin amino acid sequence of Figure 6 and isolating said erythropoietin product from said host cell or the medium of its growth.

Please enter new claims 70 through 75.

C6

--70. A process for the production of a glycosylated erythropoietin polypeptide having the *in vivo* biological property of causing bone marrow cells to increase production of reticulocytes and red blood cells comprising the steps of:

a) growing, under suitable nutrient conditions, vertebrate cells comprising promoter DNA, other than human erythropoietin promoter DNA, operatively linked to DNA encoding the mature erythropoietin amino acid sequence of Figure 6; and

b) isolating said glycosylated erythropoietin polypeptide expressed by said cells.

~~71.~~ The process of claim ~~70~~ 4 wherein said promoter DNA is viral promoter DNA.

~~72.~~ A process for the production of a glycosylated erythropoietin polypeptide having the *in vivo* biological property of causing bone marrow cells to increase production of reticulocytes and red blood cells comprising the steps of:

a) growing, under suitable nutrient conditions, vertebrate cells comprising amplified DNA encoding the mature erythropoietin amino acid sequence of Figure 6; and

b) isolating said glycosylated erythropoietin polypeptide expressed by said cells.

6b Cont.

~~73.~~ 7 The process of claim ~~72~~ 6 wherein said vertebrate cells further comprise amplified marker gene DNA.

~~74.~~ 8 The process of claim ~~73~~ 7 wherein said amplified marker gene DNA is Dihydrofolate reductase (DHFR) gene DNA.

~~75.~~ 9 The process according to claims ~~62~~ 2, ~~70~~ 4 ~~and 72~~ or 6 wherein said cells are mammalian cells.

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[redacted]

REMARKS

Upon entry of the above-requested amendments, claims 61, 62 (amended) and 70 through 75 will be pending in the present application. To facilitate examination, the totality of claims pending after amendment is set out in Exhibit 1 hereto.

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. 7) agreement on allowability was not reached. Examiner Martinell did indicate, however, that he was favorably impressed with applicant's proposal to cancel claims 65 through 69, amend claim 62 to recite the sequence of Figure 6 and insert claims to the subject matter of claims 70 and 72 along with appropriate dependent claims.

The above-requested amendments to the specification are consistent with those entered in parent application Serial No. 07/113,179 prior to issuance as U.S. Patent No. 5,441,868.

Claims 70 through 72 do not include new matter. Support for reference to use of a non-EPO promoter, and particularly a viral promoter, for expression of polypeptides of the invention is found in Examples 7B and 10. See, *e.g.*, Example 7B, specification page 56, lines 6 and 7 which note: "In the following construction, the EPO gene is altered so that it is expressed using the SV40 late promoter." In the construction, the SV40 promoter is inserted 5′ to EPO-encoding DNA so that transcription of the DNA is controlled by the SV40 promoter. Similarly, Example 11 describes EPO-encoding DNA designed for expression in *E. coli* operatively linked to the lambda P_L promoter and EPO-encoding DNA designed for expression in yeast operatively linked to the α-factor promoter. Example 12 reports the results of experiments in which the Example 11 constructs were used to express erythropoietin. General characteristics of promoters and their regulatory elements are discussed in the paragraph spanning pages 2 and 3 of the application.

Support for reference to DNA amplification and use of an amplified marker gene is found in Example 10. See, *e.g.*, page 58, lines 33-35, stating: "The

[redacted]

present example describes expression systems employing Chinese hamster ovary (CHO) DHFR- cells and the selectable marker, DHFR." See also, page 60, lines 9-23, which note:

> The quantity of EPO produced by the cell strains described above can be increased by gene amplification giving new cell strains of greater productivity. The enzyme dihydrofolate reductase (DHFR) which is the product coded for by the DHFR gene can be inhibited by the drug methotrexate (MTX). More specifically, cells propagated in media lacking hypoxanthine and thymidine are inhibited or killed by MTX. Under the appropriate conditions, (e.g., minimal concentrations of MTX) cells resistant to and able to grow in MTX can be obtained. These cells are found to be resistent to MTX due to an amplification of the number of their DHFR genes, resulting in increased production of DHFR enzyme. The surviving cells can, in turn, be treated with increasing concentrations of MTX, resulting in cell strains containing greater numbers of DHFR genes. 'Passenger genes' (e.g., EPO) carried on the expression vector along with the DHFR gene or transformed with the DHFR gene are frequently found also to be increased in their gene copy number.

Attached hereto is a Terminal Disclaimer under 37 C.F.R. §1.321 with reference to U.S. Patent No. 5,441,868.

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. 07/113,179 (which issued as U.S. Patent No. 5,441,868) and ir related application Serial No. 08/487,774 (which issued as U.S. Patent No. 5,547,933).

Applicant respectfully submits that claims 62, 63 and 70 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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Chicago, Illinois
December 20, 1996

[redacted]

EXHIBIT 1

61. A process for the preparation of an *in vivo* biologically active erythropoietin product comprising the steps of:

a) growing, under suitable nutrient conditions, host cells transformed or transfected with an isolated DNA sequence selected from the group consisting of (1) the DNA sequences set out in FIGS. 5 and 6, (2) the protein coding sequences set out in FIGS. 5 and 6, and (3) DNA sequences which hybridize under stringent conditions to the DNA sequences defined in (1) and (2) or their complementary strands; and

b) isolating said erythropoietin product therefrom.

62. A process for the preparation of an *in vivo* biologically active erythropoietin product comprising the steps of transforming or transfecting a host cell with an isolated DNA sequence encoding the mature erythropoietin amino acid sequence of Figure 6 and isolating said erythropoietin product from said host cell or the medium of its growth.

70. A process for the production of a glycosylated erythropoietin polypeptide having the *in vivo* biological property of causing bone marrow cells to increase production of reticulocytes and red blood cells comprising the steps of:

a) growing, under suitable nutrient conditions, vertebrate cells comprising promoter DNA, other than human erythropoietin promoter DNA, operatively linked to DNA encoding the mature erythropoietin amino acid sequence of Figure 6; and

b) isolating said glycosylated erythropoietin polypeptide expressed by said cells.

71. The process of claim 70 wherein said promoter DNA is viral promoter DNA.

72. A process for the production of a glycosylated erythropoietin polypeptide having the *in vivo* biological property of causing bone marrow cells to increase production of reticulocytes and red blood cells comprising the steps of:

a) growing, under suitable nutrient conditions, vertebrate cells comprising amplified DNA encoding the mature erythropoietin amino acid sequence of Figure 6; and

b) isolating said glycosylated erythropoietin polypeptide expressed by said cells.

73. The process of claim 72 wherein said vertebrate cells further comprise amplified marker gene DNA.

74. The process of claim 73 wherein said amplified marker gene DNA is Dihydrofolate reductase (DHFR) gene DNA.

75. The process according to claims 62, 70 and 72 wherein said cells are mammalian cells.