EXHIBIT E

6/C. 4
B. 4/4
12/9/

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of:

FU-KUEN LIN

Serial No: 113,178

Filed: October 23, 1987

For: "PRODUCTION OF ERYTHROPOIETIN"

Group Art Unit: 183

Examiner: H. E. Schain

I hereby certify that this paper is being deposited with the United States Postal Service as first class mail, postage prepaid, in an envelope addressed to: Commissioner of Patents and Trademarks, Washington, D.C. 20231, on this date:

December 1 1988
(Date)

Jeffrey S. Sharp (31,879)
Attorney for Applicant(s)

APPLICANT'S AMENDMENT AND REPLY UNDER 37 C.F.R. §1.111 AND 1.115

and

DECLARATION OF THOMAS W. STRICKLAND UNDER 37 C.F.R. §1.132

Hon. Commissioner of Patents
and Trademarks
Washington, D.C. 20231

Sir:

This is in response to the Office Action dated June 2, 1988 in the above-identified application wherein claims 1-13, 16, 39-41, 47-49 and 55-57 were variously rejected under 35 U.S.C. §112, 102(b) and 103. Reconsideration and allowance is respectfully requested in view of the following amendments and remarks.

AMENDMENTS

Please amend the above-identified application as follows:

IN THE SPECIFICATION

Page 7, line 27, "32 member" should be --32-member--.

Page 8, line 22, please delete the second occurrence of "the".

Page 11, line 3, "Expt.Hematol." should be --Exp.Hematol.--.

Page 11, line 4, "(1980:" should be --(1980);--.

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

Page 13, line 13, please insert --"-- after "effects".

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

Page 22, line 4, "Tables V and VI" should be --Figures 5 and 6--.

Page 22, line 22, "Table VI" should be --Figure 6--.

Page 27, line 24, "Example" should be --Examples--.

Page 32, line 35, please delete the comma (,) after "Springs".

Page 48, line 15, please delete "glutamine" and insert in place thereof --glutamic acid--.

Page 48, line 29, "Table VI" should be --Figure 6--.

Page 54, line 36, "EcoR1" should be --EcoRI--.

Page 55, line 13, "BamH1" should be --BamHI--.

Page 55, line 15, "BamH1" should be --BamHI--.

Page 61, line 25, "hemogeneous" should be --homogeneous--.

Page 88, line 36, "lablled" should be --labelled--.

Page 91, line 29, please delete "a".

Page 92, line 10, "Table VI" should be --Figure 6--.

Page 95, line 10, "membrances" should be --membranes--.

IN THE CLAIMS

Please cancel claims 1-13, 16, 39-40 and 47-49 without prejudice to Applicant's right to pursue claims of the same or similar scope in a duly filed continuing application.

Please amend claims 41, 55 and 56 as follows:

C1

--41. (Amended) A glycoprotein product having a primary structural conformation and glycosylation sufficiently duplicative of that of a naturally occurring human erythropoietin to allow possession of [one or more of the biological properties thereof] the in vivo biological property of causing bone marrow cells to increase production of reticulocytes and red blood cells and having an average carbohydrate composition which differs from that of naturally occurring human erythropoietin.

C2

55. (Amended) A pharmaceutical composition comprising an effective amount of a [polypeptide] glycoprotein product according to [claims 1, 16, 39, 40 or] claim 41 and a pharmaceutically acceptable diluent, adjuvant or carrier.

C2 cont

56. (Amended) A method for providing erythropoietin therapy to a mammal comprising administering an effective amount of a [polypeptide] glycoprotein product according to [claims 1, 16, 39, 41 or] claim 41.--

Please insert new claims 61 through 66.

--61. A glycoprotein product according to claim 41 further characterized by being the product of expression of an exogenous DNA sequence in a eucaryotic host cell.

C3

62. A glycoprotein product according to claim 61 wherein the exogenous DNA sequence is a cDNA sequence.

63. A glycoprotein product according to claim 61 wherein the exogenous DNA sequence is a genomic sequence.

64. A glycoprotein according to claim 61 wherein the host cell is a mammalian cell.

65. A glycoprotein product according to claim 64 wherein the host cell is a COS cell.

66. A glycoprotein product according to claim 64 wherein the host cell is a CHO cell.--

Add D1

REMARKS

Upon entry of the above-requested amendments, amended claims 41, 55 and 56 will be pending in the application along with original claim 57 and new claims 62 through 66.

Applicant acknowledges with thanks the interview kindly granted by the Examiner to Mr. Steven Odre on July 20, 1988 and wherein Applicant's intent to pursue claims as presented herein was noted. No agreement on allowability of claims was reached.

The Claimed Subject Matter

For ease of consideration, the full text of independent claim 41 and dependent claims 55-57 and 61-66 are set out immediately below.

> 41. A glycoprotein product having a primary structural conformation and glycosylation sufficiently duplicative of that of a naturally occurring human erythropoietin to allow possession of the in vivo biological property of causing bone marrow cells to increase production of reticulocytes and red blood cells and having an average carbohydrate composition which differs from that of naturally occurring human erythropoietin.
>
> 61. A glycoprotein product according to claim 41 further characterized by being the product of expression of an exogenous DNA sequence in a eucaryotic host cell.
>
> 62. A glycoprotein product according to claim 61 wherein the exogenous DNA sequence is a cDNA sequence.
>
> 63. A glycoprotein product according to claim 61 wherein the exogenous DNA sequence is a genomic sequence.
>
> 64. A glycoprotein according to claim 61 wherein the host cell is a mammalian cell.
>
> 65. A glycoprotein product according to claim 64 wherein the host cell is a COS cell.
>
> 66. A glycoprotein product according to claim 64 wherein the host cell is a CHO cell.
>
> 55. A pharmaceutical composition comprising an effective amount of a glycoprotein product according to claim 41 and a pharmaceutically acceptable diluent, adjuvant or carrier.

56. A method for providing erythropoietin therapy to a mammal comprising administering an effective amount of a glycoprotein product according to claim 41.

57. A method according to claim 56 wherein the therapy comprises enhancing hematocrit levels.

As is apparent from consideration of independent claim 41, the subject matter herein claimed is seen to comprise Applicant's novel glycoprotein preparations having amino acid sequence characteristics in common with naturally occurring human erythropoietin isolated from urine, having carbohydrate composition characteristics different from those of naturally occurring erythropoietin and nonetheless having the glycosylation-requiring in vivo biological activity (promoting reticulocyte and red blood cell production) characteristics of naturally occurring human erythropoietin.

Dependent claims 61-66 further characterize products of the present invention in terms of their derivation from eucaryotic host cell expression (61) of exogenous cDNA (62) or genomic DNA (63) sequences, particularly in mammalian host cells (64) such as COS (65) and CHO (66) cells.

Pharmaceutical composition claim 55 recites the incorporation of claim 41 products in effective amounts with suitable diluents and the like, while therapeutic method claims (56 and 57) are directed to the use of claim 41 products in hematopoietic therapy such as the increasing of hematocrit.

The Outstanding Rejections

Applicant notes that the decision to cancel and refrain from pursuing claims 1-13, 16, 39-40 and 47-49 in the present application effectively moots a number of the outstanding rejections set out in the June 2, 1988 Office Action. More specifically, it appears that all grounds for rejection (including the provisional double patenting rejection) are mooted with the possible exception of the rejection under Section 112 applied to original claims 41 and 55-57 beginning at page 3 of the Action, the Section 102(b)/103 rejection of claim 41 based on Miyake et al. set out at page 8 of the Action, and the Section 103 rejection of claims 41 and 55-57 based on Miyake et al., Takezawa et al., Chiba et al., or Sugimoto et al., in view of Papayannopoulo et al. as set out at page 9 of the Action.

Briefly summarized, it was the Examiner's position that the claim terms, "sufficiently duplicative of" and "average carbohydrate composition which differs from" are inadequate for purposes of the second paragraph of Section 112 and that the therapeutic method claims do not appear to be enabled by the specification.

In rejecting original claim 41 under 35 U.S.C. 102(b)/103, it was the Examiner's position that the Miyake et al. publication's notation of differing forms of glycosylated urinary erythropoietin as well as in vivo inactive asialo erythropoietin anticipated or rendered obvious the claimed subject matter. The Examiner specifically noted that he "believes the native and recombinant forms of human EPO to be inherently identical".

Finally, the rejection of original claims 41 and 55-57 under 35 U.S.C. §103 was based on the notation that

the primary references teach preparations of erythropoietin from natural sources while Papayannopoulo et al. describe in vivo effects of erythropoietin therapy, rendering Applicant's products and methods obvious.

For the following reasons, Applicant respectfully submits that the outstanding rejections may properly be withdrawn.

Patentability Arguments

1. The Section 112 Rejections May Be Withdrawn

Applicant submits that the pending claims, especially product claim 41 and therapeutic method claim 56 as amended, are in conformity with all requirements of Section 112 with regard to enablement and specificity of terminology. Applicant notes at the outset that issues of specificity and enablement must always take into account the state of the art with regard to the subject matter being claimed.

At the time of Applicant's invention, nothing was known of the primary structural conformation (amino acid sequence) of human erythropoietin except the identity of a few of the amino terminal residues. It was the present Applicant who first developed knowledge of the full amino acid sequence of erythropoietin and first generated the presently claimed glycoprotein products which allowed for full scale clinical application to provide therapeutic effects consistent with the in vivo activity of naturally occurring erythropoietin.

Having provided the public with its first knowledge of naturally occurring erythropoietin's primary structural conformation. Applicant has provided the pubic with

the wherewithal for determining the extent to which any sequence of amino acids may function, in vivo, as though it were the sequence encoded by the erythropoietin gene in the human genome. No impermissible vagueness attends claiming glycoproteins which share that amino acid sequence to an extent sufficient to allow the products to function, in vivo, as erythropoietin hematopoietic agents. Thus, the term "sufficiently duplicative of" as applied to amino acid sequence needed for the specified in vivo biological activity is not violative of the requirements of the second paragraph of 35 U.S.C. §112.

The same reasoning applies to reference in claim 41 to glycosylation sufficiently duplicative of that of naturally occurring erythropoietin. At the time of the invention, the art knew that erythropoietin isolated from urine was a glycoprotein and that treatment to remove its carbohydrate would destroy in vivo biological activity. Applicant was the first to provide for a glycoprotein which is both different from previously isolated urinary erythropoietin in its glycosylation and yet sufficiently like the natural product (previously isolated in the art) in terms of its glycosylation to allow it to fill the long-felt need (unsatisfiable by urinary isolates) for life-sustaining human therapeutic agents for, e.g., the anemia associated with dialysis in renal failure patients.

The precise nature of the differences in the carbohydrate structures of products of the present invention and urinary-derived human erythropoietin are only now starting to be understood, as evidenced by the results of the experimental procedures detailed in the attached Declaration of Thomas W. Strickland. Briefly put, the procedures demon-

strate that the urinary erythropoietin is heterogeneous in terms of glycosylation, that the same is true of recombinant erythropoietin preparations of the present invention, and that, most importantly, the two products are clearly distinct from each other in terms of glycosylation.

Having provided the public with its first knowledge concerning the fact that a glycoprotein can exist which is simultaneously different in carbohydrate composition from urinary source erythropoietin and yet sufficiently like it in glycosylation to allow for in vivo biological activity, no impermissible vagueness attends the recitation of these unique and readily ascertainable characteristics in a patent claim.

As for the enablement requirements of paragraph one of Section 112, Applicant believes that conformity with such requirements is manifest in the specification. Specifically, illustrated in Example 10, commencing at page 63, line 36, are the very first therapeutic procedures ever practiced with erythropoietin products of the invention which demonstrated (using crude cell conditioned medium of transformed CHO cells) that these products possess the capacity for generating in vivo hematocrit elevating biological activity in mice. Beginning at page 86, line 15, the specification goes on to provide extensive descriptions of erythropoietin therapy. Against this background and upon consideration of the prior art which the Examiner acknowledges has established the in vivo effects of erythropoietin, Applicant submits that all enablement requisites of Section 112 have been met.

The above remarks are believed to establish that no proper basis exists for rejection of the pending claims under 35 U.S.C. §112.

2. The Rejections Under Sections 102 and 103 May Be Withdrawn

Applicant submits that neither the Miyake et al. reference nor the other asserted "primary" or secondary references cited by the Examiner operate to anticipate or render obvious the presently claimed subject matter.

As stated in the specification at page 19, lines 26-32, it has consistently been the thrust of Applicant's endeavors to provide the novel glycoproteins herein claimed.

> "Novel glycoprotein products of the invention includes those having a primary structural conformation sufficiently duplicative of that of a naturally-occurring (e.g., human) erythropoietin to allow possession of one or more of the biological properties thereof and having an average carbohydrate composition which differs from that of naturally-occurring (e.g., human) erythropoietin."

Confirmation of these assertions of novelty is found in the attached Declaration of Thomas Strickland which provides detailed description and analysis of the differences in carbohydrate structure between FDA clinical lot preparations of recombinant erythropoietin according to the present invention and human urinary erythropoietin isolates as represented by samples actually obtained by Miyake et al. in the work forming the basis for the publication, as well as urinary erythropoietin samples obtained by means of a specified modification of the Miyake et al. procedure.

Briefly summarized, recombinant and urinary erythropoietin samples were first subjected to isoelectric focusing and the urinary isoforms consistently displayed lower (more acidic) isoelectric points than the recombinant product isoforms. Next, recombinant and urinary products were subjected to enzymatic digestion with N-glycanase or

mixtures of that enzyme with a sialic acid residue-removing enzyme. The results of this analysis revealed that the differences in isoelectric points first noted above were not attributable to amino acid composition but rather to differences in carbohydrate composition. Finally, digestion of both recombinant and urinary erythropoietin was carried out using a sialidase which removes both the projected types of sialic acid linkages (2 - 3 and 2 - 6). Isoelectric focusing again revealed differences in charge between the urinary and recombinant isoforms.

The work described in the Strickland Declaration and that of the publication cited by Strickland, as well as the results set out in the Sasaki et al. publication noted by the Examiner, stands as testimony to the differences between Applicant's products and those of Miyake et al. In sum, Applicant's products are indeed novel.

Against a background wherein the prior art had noted the essential nature of sialic acid residues for in vivo biological activity, it could hardly be characterized as within the reasonable expectation of an ordinarily skilled artisan (i.e., obvious) that Applicant could call into existence the glycoprotein products herein claimed -- glycoproteins which have a carbohydrate composition conspicuously different from that of human urinary erythropoietin glycoprotein isolates, but which nonetheless have sufficient amino acid sequence and glycosylation similarities to allow them to possess the essential in vivo biological activity of naturally occurring erythropoietin. In the face of an indisputable and long-felt need in the art for the present invention, the grant of patent protection commensurate in scope with Applicant's remarkable contribu-

tion as reflected by the presently pending claims is clearly warranted.

CONCLUSION

The foregoing amendments and remarks are believed to establish that claims 41, 55-57 and 61-66 are in conformity with all requirements of 35 U.S.C. §112 and are directed to subject matter which is novel and unobvious under 35 U.S.C. §102 and 103. An early notice of allowance thereof is solicited.

Respectfully submitted,

MARSHALL, O'TOOLE, GERSTEIN, MURRAY & BICKNELL

By _____
Jeffrey S. Sharp (Reg. No. 31,879)
Attorneys for Applicants
Two First National Plaza
Chicago, Illinois 60603
(312) 346-5750

Chicago, Illinois
December 1, 1988