

EXHIBIT H-20

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

20/2
um.
7/17/87
RECEIVED
JUL 13 1987
GROUP 120

Application of:) "PRODUCTION OF
FU-KUEN LIN) ERYTHROPOIETIN"
Serial No: 675,298) Group Art Unit 127
Filed: November 30, 1984) Examiner - A. Tanenholtz

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

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

Sir:

This is in response to the Office Action dated June 21, 1987 in the above-identified application. Reconsideration and allowance of all claims is respectfully requested in view of the following amendments and remarks.

AMENDMENTS

IN THE SPECIFICATION

At page 25, following line 5 of the original text, delete the entire text of the insertion requested in the amendment dated October 3, 1986 and insert in place thereof the following: --Reference is made to FIGURES 1 through 21, wherein: FIGURE 1 is a graphic representation of a radio-immunoassay 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 according to the invention.--

21

At page 37 line 6, please delete the entire text of the insertion requested in the amendment dated October 3, 1982 [i.e., delete "duplicated as FIGURE 5 comprising portions 5A, 5B and 5C"].

- x -

11A

At page 37, line 6, please delete "Table V" and insert --FIGURE 5--.

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 42, line 25, please delete the entire text of the insertion requested in the amendment dated October 3, 1986 [i.e., ", duplicated as FIGURE 6 comprising portions 6A, 6B, 6C, 6D and 6E"].

At page 42, line 25, please delete "Table VI" and insert in place thereof --FIGURE 6--.

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 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 6" and insert in place thereof --FIGURE 6--.

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--.

- 7 -
415

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

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

Please delete the entire text of page 50.

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

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 the entire text of the insertion requested in the amendment of October 3, 1986 [i.e., "duplicated as Figure 7"].

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

- 7 -

416

424

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

At page 75, line 28, please delete the entire text of the insertion requested in the amendment dated October 3, 1986 [i.e., "(the last-mentioned Table being duplicated as FIGURE 8)"].

At page 75, line 28, please delete "Tables XV through XXI" and insert in place thereof --FIGURES 16 through 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 89, line 16, please delete "Table VI" and insert in place thereof -- FIGURE 6--.

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 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--.

- 4 -

417

425

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

IN THE DRAWING

Please enter drawing Figures 5 through 21 in accordance with the "Letter" submitted concurrently herewith.

IN THE CLAIMS

Please cancel claims 73-103 without prejudice.
Please insert new claims 104-134.

102

~~104~~. A purified and isolated DNA sequence encoding erythropoietin, said DNA sequence selected from the group consisting of:

- (a) the DNA sequences set out in Figures 5 and 6 or their complementary strands; and
- (b) DNA sequences which hybridize under stringent conditions to the DNA sequences defined in (a).

²
~~105~~. A purified and isolated DNA sequence consisting essentially of a DNA sequence encoding human erythropoietin.

³
~~106~~. A purified and isolated DNA sequence consisting essentially of a DNA sequence encoding monkey erythropoietin.

⁴
~~107~~. A procaryotic or eucaryotic host cell transformed or transfected with a DNA sequence according to claim ¹~~104~~, ²~~105~~ or ³~~106~~ in a manner allowing the host cell to express erythropoietin.

418

102
Contd

⁵
108. A biologically functional circular plasmid or viral DNA vector including a DNA sequence according to claim ¹104, ²105, or ³106.

⁶
109. A procaryotic or eucaryotic host cell stably transformed or transfected with a DNA vector according to claim ⁵108.

9

⁷
110. A purified and isolated DNA sequence consisting essentially of a DNA sequence encoding a polypeptide having ^{an amino acid sequence} ~~a primary structural conformation~~ sufficiently duplicative of that of erythropoietin to allow possession of the biological property of causing bone marrow cells to increase production of reticulocytes and red blood cells, and to increase hemoglobin synthesis or iron uptake.

⁸
111. A cDNA sequence according to claim ⁷110.

⁹
112. A monkey species erythropoietin coding DNA sequence according to claim ⁸111.

¹⁰
113. A DNA sequence according to claim ⁹112 and including the protein coding region set forth in Figure 5.

¹¹
114. A genomic DNA sequence according to claim ⁷110.

¹²
115. A human species erythropoietin coding DNA sequence according to claim ¹¹114.

-87
419

102
Contd

¹³
~~116~~. A DNA sequence according to claim ¹²~~115~~ and including the protein coding region set forth in Figure 6.

¹⁴
~~117~~. A DNA sequence according to claim ⁷~~110~~ and including one or more codons preferred for expression in E.coli cells.

¹⁵
~~118~~. A DNA sequence according to claim ¹⁴~~117~~, coding for expression of human species erythropoietin.

¹⁶
~~119~~. A DNA sequence according to claim ¹⁵~~118~~ including the protein coding region set forth in Figure 7.

¹⁷
~~120~~. A DNA sequence according to claim ⁷~~110~~ and including one or more codons preferred for expression in yeast cells.

¹⁸
~~121~~. A DNA sequence according to claim ¹⁷~~120~~, coding for expression of human species erythropoietin.

¹⁹
~~122~~. A DNA sequence according to claim ¹⁸~~121~~ including the protein coding region set forth in Figure 8.

²⁰
~~123~~. A DNA sequence according to claim ⁷~~110~~ covalently associated with a detectable label substance.

²¹
~~124~~. A DNA sequence according to claim ²⁰~~123~~ wherein the detectable label is a radiolabel.

- 7 -

420

102
Contd 1

²²
~~125~~. A single-strand DNA sequence according to
claim ²⁰~~123~~.

²³
~~126~~. A procaryotic or eucaryotic host cell trans-
formed or transfected with a DNA sequence according to claim
⁷~~110~~, ⁸~~111~~ or ¹¹~~114~~ in a manner allowing the host cell to
express said polypeptide.

²⁴
~~127~~. A transformed or transfected host cell
according to claim ²³~~126~~ which host cell is capable of
glycosylating said polypeptide.

²⁵
~~128~~. A transformed or transfected mammalian host
cell according to claim ²⁴~~127~~.

²⁶
~~129~~. A transformed or transfected COS cell
according to claim ²⁵~~128~~.

²⁷
~~130~~. A transformed or transfected CHO cell
according to claim ²⁵~~128~~.

²⁸
~~131~~. A biologically functional circular plasmid
or viral DNA vector including a DNA sequence according to
claim ⁷~~110~~.

²⁹
~~132~~. A procaryotic or eucaryotic host cell stably
transformed or transfected with a DNA vector according to
claim ²⁸~~131~~.

³⁰
~~133~~. A DNA sequence according to claim ⁷~~110~~ coding
for [Phe¹⁵]hEPO, [Phe⁴⁹]hEPO, [Phe¹⁴⁵]hEPO, [His⁷]hEPO,

- 91 -
421

103
Cont'd

[Asn² des-Pro² through Ile⁶]hEPO, [des-Thr¹⁶³ through Arg¹⁶⁶]hEPO, or [427-55]hEPO.

³¹
134. A purified and isolated DNA sequence as set out in Figures 5 or 6 or the complementary strand of such a sequence.

REMARKS

Upon entry of the above-requested amendments to the specification, all of the original "Tables" in the specification, except for original Tables I through IV, will have been "converted" to Figures.

Upon entry of the above-requested amendments to the claims, claims 104 through 134 will remain in the application and will be correlated to prior claims 73 through 103 in the manner indicated in the following Table.

- 8 -
422

<u>Prior Claim</u>	<u>New Claim</u>	<u>Prior Claim</u>	<u>New Claim</u>
73	104, 105, 106	90	122
74	107, 126-130	91	123
75	108	92	124
76	109	93	125
77	110	94	131
78	111	95	132
79	112	96	110
80	113	97	--
81	114	98	--
82	115	99	--
83	116	100	133
84	--	101	--
85	117	102	--
86	118	103	134
87	119		
88	120		
89	121		

The amendments to the claims are being made without prejudice to Applicant to pursue claims of the same or similar scope as those cancelled in a duly-filed continuing application.

A. The Claimed Subject Matter

As related in Applicant's prior communications, the claims remaining in this application relate to DNA sequences, DNA vectors, transformed and transfected host cells useful in the preparation of biologically active erythropoietin products including, e.g., polypeptide fragments and analogs of erythropoietin.

Independent claim 104 is thus directed to purified and isolated DNA sequences substantially defined by reference to the DNA sequences revealed in Figures 5 and 6 and independent claims 105 and 106 are specifically directed to

- 18 -

423

purified and isolated DNA sequences encoding human and monkey erythropoietin. Dependent claims 107-109 respectively relate to host cells transformed or transfected with erythropoietin expressing DNA, to vectors including such DNA and to hosts transformed with such vectors.

Independent claim 110 is directed generally to DNA sequences which code for procaryotic or eucaryotic host expression of polypeptides having amino acid sequences sufficiently duplicative of that of erythropoietin to allow for specified erythropoietin properties. Dependent claims 111-134 are directed to presently preferred forms of DNA sequences, vectors, and transformed or transfected hosts based on the claim 110 DNA sequences. Claims 127-130 specifically relate to the host cell systems exemplified by Examples 7, 8 and 10.

B. The Outstanding Office Action, The Rejections Of The Claims And Applicant's Responses Thereto

In the communication dated March 12, 1987, Applicant responded to numerous outstanding objections to the specification and rejections to the claims under §§112, 101, 102 and 103. Applicant understands that the amendments and remarks set out in the March 12, 1987 communication have resulted in reconsideration and withdrawal of the following rejections propounded in the Action dated February 5, 1987:

- (1) The Section 112 (2nd paragraph) rejection of certain claims on grounds of their reference to drawings;
- (2) The "provisional" Section 101 rejection of claims based on copending parent patent applications;
- (3) The rejection of certain claims under Section 112 (2nd paragraph) for incorporation of brackets;

- 4 -

424

432

(4) The rejection of certain claims under Section 112 (1st paragraph) due to alleged lack of specificity of reference to "hybridization";

(5) The rejection of certain claims under Section 103 based on the Sue et al. reference when considered with the Breslow et al. or Woods et al. references;

(6) The rejection of certain claims under Section 103 based on the Sue et al. reference and other specified secondary references; and

(7) The rejection of at least certain claims under Section 102(b) based on the Talmadge et al. reference.

Due to the number and variety of new objections and rejections set forth in the Action dated June 21, 1987, Applicant submits that the issues raised therein are best treated by means of responses which "track" the order of their appearance in the Action.

1. The Renewed Objections To The Disclosure
Based On Reference To Drawings And
Figures May Properly Be Withdrawn

As indicated above, application has now "converted" all Tables in the specification (other than Tables I through IV) into Figures. Applicant disagrees that the submission of these Figures 5 through 8 in a separate envelope labelled as an Exhibit was an inappropriate or improper way of presenting the drawings. Notwithstanding this disagreement, Applicant is concurrently submitting a complete set of new Figures 5-21 under cover of a "Letter" requesting entry of the same. Despite the fact that Applicant maintains that reference to Tables, in sequence in the Figures, would facilitate reading of the specification, none of the Figures include notations of their historical basis in Tables of the specification.

- 12 -

425

433

2. No Basis Exists For Rejection
Of The Claims Under 35 U.S.C. §112
(First or Second Paragraphs)

In the Action dated June 21, 1987, certain new rejections of prior claims 73 through 102 were propounded. More specifically, the Examiner first objected to element "(c)" of claim 73 as indefinite and unduly broad, stating that the recitation, "but for the degeneracy of the genetic code" was unclear, and that the recitation, "would hybridize" was improperly "permissive". Applicant respectfully disagrees with the Examiner's position. As clearly delineated in the specification (see, e.g., page 94, lines 15-24), element "(c)" of claim 73 (which has its origins in original claim 14) clearly delineates as within the scope of Applicant's invention those naturally-occurring allelic or manufactured or mutagenized genomic and cDNA sequences which, but for the degeneracy of the genetic code, would hybridize to the Figure 5 or Figure 6 sequences. Applicant submits that the specification fully "enables" such sequences (see, e.g., the ECEPO and SCEPO, E.coli and yeast preference codon DNA sequences) and that a person of ordinary skill in the art would clearly understand what was embraced by the recitations of claim. Clearly if one manufactures or mutagenizes a gene to include, e.g., a position 4 arginine-specifying codon AGG rather than CGC as in Figure 6, the prospects for hybridization between the manufactured sequence and the Figure 6 sequence are diminished. The same amino acid is encoded, however, and but for the degeneracy of the genetic code the three-base codon provided would hybridize.

Despite this position, Applicant acknowledges the Examiner's notation of equivalence in scope of alternatively worded claims:

- 12 -

426

434

"In order to embrace the subject matter of that Markush member (c) a separate claim drawn to an isolated DNA sequence encoding an erythropoietin selected from the group consisting of human and monkey erythropoietin would be acceptable."

In order to expedite prosecution of this application has reconstituted prior claim 73 as new independent claims 104, 105 and 106.

It was the Examiner's further position that:

"...the sequence of claim 77 and 96 appear to embrace substantially all known DNA sequences since the isolated DNA sequence is not designated as coding for erythropoietin. One that encodes 'a' therapeutic activity of erythropoietin is not the same thing."

This position was taken despite the notation in the Examiner's Interview Summary dated March 4, 1987 to the effect that amendment of the claims "to recite a therapeutic activity of EPO" would result in obviation or dropping of prior Section 112 rejections. Applicant disagrees with the Examiner's new position objecting to reference to "a therapeutic activity" of erythropoietin and notes that the therapeutic activities of erythropoietin are clearly disclosed in the present specification and cited background prior art in a manner meeting all requirements of Section 112.

Despite this position, Applicant acknowledges the Examiner's notation of equivalence in scope of alternatively worded claims:

"The embodiments of claims 77 and 96 could properly be expressed as for example an isolated DNA sequence consisting of a DNA sequence encoding a polypeptide having the structure sufficiently duplicative of a naturally-occurring erythropoietin to allow possession of the biological properties of being able to cause bone marrow cells to increase hemoglobin synthesis and iron uptake and stimulate reticulocyte response."

- 14 -

437

435

In order to expedite prosecution of this application has reconstituted prior claims 77 and 96 as new claim 110.

It was the Examiner's further new position that claim 74 (which replaced prior claim 15 with only a change in dependency) "appears to read on naturally-occurring erythropoietin producing cells". Applicant disagrees. Claim 74 (which is now replaced by claim 107) clearly recites that the subject host cells have had erythropoietin product encoding DNA inserted into them, with or without use of vectors, in a manner allowing the inserted DNA (or future "generations" of such DNA replicated in the host's progeny) to be expressed as an erythropoietin product. The Examiner's attention is respectfully drawn to the text spanning specification page 94, line 25 through page 95, line 15.

It was also the Examiner's further new position that claims 84, 86, 87, 89, 90 and 99 specifying manufactured DNA sequences are redundant of claim 77 or 96 "since the DNA sequence has no memory of its creation". In order to expedite prosecution of this application, Applicant has cancelled claims 84 and 99 and has reconstituted prior claims 85, 86, 87, 88, 89 and 90 to delete reference to the "manufactured" characterization of the DNA sequence in view of the Examiner's notation of redundancy and equivalence.

Finally, the Examiner has objected to claim 100, holding that absent the designation, "isolated", it reads on "naturally-occurring mutant" erythropoietin sequences with the additions, replacements or deletions specified in the claim. Applicant disagrees, noting that the specific analogs recited in prior claim 100 are not in any way known to correspond to any naturally-occurring mutants of erythro-

- 15 -

438

436

poietin and are entirely of Applicant's creation. The Examiner's position therefore constitutes mere speculation. Applicant notes, however, that prior independent claim 100 has been reconstituted as claim 128 which in turn, is dependent on claim 110 which includes the term, "isolated". The rejection is thus mooted.

The foregoing remarks are believed to establish that claims 104-134 are in compliance with all requirements of Section 112.

3. No Basis Exists For Rejection
Of The Claims Under Section
102(b)/103 Based on Talmadge et al.

It was the Examiner's position that the grounds asserted for rejection of prior claims under Section 112 allowed for claims 73-79, 81, 84, 85, 88, 91-99 to be "deemed to embrace the recombinant materials disclosed by Talmadge et al.". Applicant strenuously disagrees but believes that the issue is entirely mooted in view of the requested amendments to the claims.

4. No Basis Exists For Rejection
Of The Claims Under Section 103

It was the Examiner's position that the subject matter of all claims was rendered statutorily obvious upon consideration of the disclosures of Goldwasser et al., Weiss, et al. and Egrie taken in view of either Young et al. or Broome et al. and in further view of Ullrich et al. or Martial et al. The Examiner's specific argument was as follows:

"Goldwasser, Weiss et al and Egrie teach the preparation of a monoclonal antibody to human erythropoietin. Young et al and Broome et al teach a process of isolating genes using

antibody probes. More specifically, Young et al teach isolating unknown foreign antigenic proteins encoded by antigen producing clones of a lambda gt11 recombinant cDNA library by using antibody probes. The foreign gene is inserted into the galactosidase gene of the lambda phage so as to result in a fused protein. Ullrich et al and Martial teach a basic process for isolating mRNA and converting it into a cDNA library for use in cloning and expressing mammalian genes. It would be obvious to prepare erythropoietin as a fused peptide by extracting the messenger RNA for erythropoietin from kidney cells known to be rich therein and converting that mRNA to a cDNA library in a manner taught by Ullrich et al or Martial. If desired, substituting the lambda gt 11 phage library vector of Young et al for its advantages would be an obvious choice. It would further be obvious to use the Young et al. or Broom et al gene isolating technique together with erythropoietin antibody of the primary references as a probe for isolating a clone producing erythropoietin. At best only routine genetic engineering techniques would be involved." (Emphasis supplied.)

For the reasons which follow, Applicant respectfully disagrees and submits that the subject matter herein claimed is patentably distinct from the disclosures and suggestions of the cited references.

Applicant notes at the outset that the presently claimed subject matter involves novel DNA, not a novel method for obtaining it.

Applicant is in agreement with the Examiner's characterization of the Goldwasser et al., Weiss et al. and Egrie references. They do "teach the preparation of a monoclonal antibody to human erythropoietin". Applicant simply notes that the antibody of Weiss et al. is the same as that of Goldwasser et al. which, in turn, differs from that of Egrie. The Egrie antibody recognizes an epitope presented in the amino terminal of erythropoietin. The epitope recognized by the Goldwasser antibody is not identified in the references. Applicant disagrees with the Examiner's charac-

- 17 -

430

438

terizations of the remaining references and with the Examiner's "combination" of the reference with those of Goldwasser et al., Weiss et al. and Egrie as allegedly allowing the claimed invention to be obtained by an ordinarily skilled worker through practice of "at best only routine genetic engineering techniques".

Applicant first disagrees with the Examiner's position that the Ullrich et al. and Martial et al. references would place in the hands of an ordinarily skilled worker the wherewithal for "extracting messenger RNA for erythropoietin from kidney cells known to be rich therein and converting that mRNA to a cDNA library". In the work reported by Ullrich et al., mRNA for insulin was isolated from cells only upon the solution of a problem in the low proportion of insulin-producing B cells in the pancreas. Specifically, Ullrich et al. report at page 1313 that:

"The isolation of insulin mRNA is complicated by the low proportion of the endocrine B cells (which produce insulin) in the pancreas and the high levels of ribonuclease in the dominant acinar cells. These problems have been circumvented by adapting procedures for the relatively large scale isolation of islets of Langerhans (5) (in which the majority of the cells are B cells) from the rat pancreas, and by using a method that allows the extraction of intact, translatable mRNA from sources rich in ribonuclease (6)."

The workpieces for the Ullrich et al. cDNA preparation work were highly specialized cells within a specific tissue type within an organ known for decades to be the source of the protein whose DNA was sought.

Likewise, Martial et al. isolated growth hormone messenger RNA from a well defined, overproducing tissue source -- human pituitary tumor cells. Note that in testing these cells for the relative abundance of growth hormone

- 18 -

430

439

mRNA, the messenger RNA preparations were subjected to in vitro translation with the result that: "...hGH mRNA appears to be the most abundant species in the acromegalic tumors". (See page 603, left column.)

Against this background, it is proper to note that the Examiner has provided no support for his conclusions that human kidney cells are "known to be rich [in erythropoietin messenger RNA]" and that a kidney cDNA library would include multiple cDNA's encoding erythropoietin. One can make a cDNA library from the mRNA of essentially any cell. This does not, however, allow the conclusion that the library will include even one translatable copy of the DNA sought. Indeed the art previously considered and relied upon by the PTO belies such a conclusion. Farber et al. (References C31, 32 and 33) and Lee-Huang (PTO reference R; Applicant's reference C58) constructed kidney cell cDNA libraries which did not allow for erythropoietin gene isolation. Lee-Huang affirmatively states with respect to erythropoietin that:

"The biogenesis of this hormone is still not clearly defined, but the kidney is known to play a key role." (Page 2708, left column.)

Lee-Huang goes on to describe the difficulties of obtaining viable human kidney samples (most healthy tissue is reserved for transplants) and then the rigors involved in securing kidney tumor cells which could be characterized as potential high producers of erythropoietin (and hence, arguably likely to have high levels of erythropoietin messenger RNA). At page 2709, Lee-Huang states:

- 18 -
432

440

"The scarcity of viable human kidney samples and their low level of Ep make effective cellular fractionation and accurate Ep estimation extremely difficult. It was therefore necessary for us to search for sources with elevated Ep levels and greater tissue availability. It has been well documented that erythrocytosis may be associated with various renal tumors. Tumor extracts from some renal carcinoma patients have shown an increased level of EP, and such tumors were often considered as Ep-producing tumors. We have conducted an extensive search for surgical renal carcinomas with Ep-producing activity. Ep activity was assayed in vivo by the exhypoxic polycythemic mouse method. Of 36 renal cell carcinoma extracts examined, 2 demonstrated high Ep activity (0.9 and 3.0 Ep units/ml), 6 showed moderate activity (0.1 to 0.7 Ep units/ml), and the rest had either marginal (<0.1 Ep units/ml) or undetectable Ep activity. The samples with high Ep bio-activity were used for mRNA preparation."
(Citations omitted)

Thus, as pointed out in Applicant's submission of October 3, 1986, there was, at the time of the invention, a serious problem securing what could be recognized as erythropoietin-producing cells, much less cells producing high levels of the protein or cells "known to be rich" in erythropoietin messenger RNA such as would provide a cDNA library with multiple copies of erythropoietin-encoding DNA.

For the Examiner to characterize the publications of Ullrich et al. and Martial et al. as readily enabling the preparation of a library including translatable human erythropoietin cDNA by an ordinarily skilled worker is unsupported and in fact contradicted by other references comprising the totality of the art.

Applicant vigorously disagrees with the Examiner's conclusion that Young et al "teach isolating unknown foreign antigenic proteins encoded by antigen producing clones of a lambda gt11 recombinant cDNA library by using antibody probes". Young et al. did no work with a "cDNA library" in

the sense that such might actually be generated from a collection of RNA's according to Ullrich et al and Martial et al. The authors employed two specific "model" DNA's encoding mouse α -amylase and chicken albumin. (See "construction of Model Recombinants" at page 1196). With such model recombinant materials in hand and with a "model" high affinity polyclonal antibody to the encoded polypeptide in hand,* to the encoded polypeptide in hand, Young et al. were able to demonstrate the general operability of their model system. This is a far cry from a "teaching" of a procedure that could be straightforwardly applied to isolate genes for any "unknown foreign antigenic proteins" such as erythropoietin.

The disclosures of the Broome et al. reference add nothing to disclosures of Young et al. that as would elevate their demonstration of a model system to a "teachings of reasonably successful means for isolating erythropoietin from a cDNA library derived from erythropoietin mRNA enriched cells. Apparently aware of the inherent limitations of their two antibody "model" system (employed to detect a model gene for beta-galactosidase) Broome et al. conclude on page 2749 that:

"Uses for this immunological screening procedure include direct identification of clones containing specific foreign DNA segments, if they

* There is at least some basis for arguing that polyclonal antibodies could be better for practicing Young et al. or Broome et al. procedures in a true cDNA library screen. Note, for example, that a high affinity polyclonal antibodies would, in principle, be more likely to recognize an expression product of a cDNA fragment encoding a carboxy terminal region than, for example, a monoclonal antibody to an amino terminal sequence. Because cDNA is made from mRNA by "backward" copying, the least abundant species of cDNA made would be one encoding a full length polypeptide including the amino terminal.

- 24 -

434

442

express a translation product either fortuitously or after in vitro manipulations to that end".
(Emphasis supplied.)

When properly viewed in the context of their limited teachings with respect to use of known cell sources of high levels of mRNA and use of model systems designed to optimize "identification" of genes, the combined disclosures of Ullrich et al., Martial et al., Broome et al., and Young et al. provide no true scientific basis for the Examiner's conclusion that their composite methodologies could routinely be applied to obtain the claimed subject matter.

One further point is highly pertinent in this regard. Lee-Huang tried to isolate an erythropoietin gene by antibody screening methods and, by all objective criteria presently available, failed to do so. (See Applicant's communication of October 3, 1986, at page 29 through 37 wherein restriction mapping of erythropoietin-encoding human DNA and size analysis of in vitro mRNA expression products strongly supports the conclusion that erythropoietin-encoding DNA was never isolated by Lee-Huang, despite her access to an allegedly erythropoietin-rich message source).

Applicant respectfully submits that the combined teachings of products and procedures of the art references now relied upon by the Examiner simply do not provide a basis for characterizing the subject matter of the claims as "obvious" within the context of Section 103. At best, the Examiner can only state that the invention might have been achieved by means other than those employed by Applicant. Such speculation is not an appropriate basis for a conclusion of obviousness. Were Applicant claiming an invention in an antibody screening method for isolating erythro-

- 27 -

138

biotin-encoding DNA, the art relied upon might be pertinent. Applicant here claims specific new products never before in the possession of the public. That he might have brought them into existence by some other, untested, means alluded to by the prior art references is not pertinent to patentability.

CONCLUSION

The foregoing remarks are believed to establish that claims 104-134 are in condition for allowance and an early notice thereof is solicited.

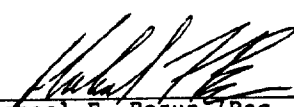
Consistent with Applicant's position, it is noted that Applicant's March 12, 1987 communication included a proper authorization under 37 C.F.R. §1.311(b) and that upon allowance this application may be "tagged" for priority in printing according to M.P.E.P. §1309.

In the event that contact with Applicant's counsel is deemed appropriate, Applicant requests that the Examiner telephone associate counsel, Steven M. Odre, Esq. (Reg. No. 29,094), at (805) 499-5725.

Respectfully submitted,

MARSHALL, O'TOOLE, GERSTEIN,
MURRAY & BICKNELL

By


Michael F. Borun (Reg. No. 25,447)
A Member of the Firm
Attorneys for Applicants
Two First National Plaza
Chicago, Illinois 60603
(312) 346-5750

Chicago, Illinois

July 10, 1987

~~- 28 -~~

438

444

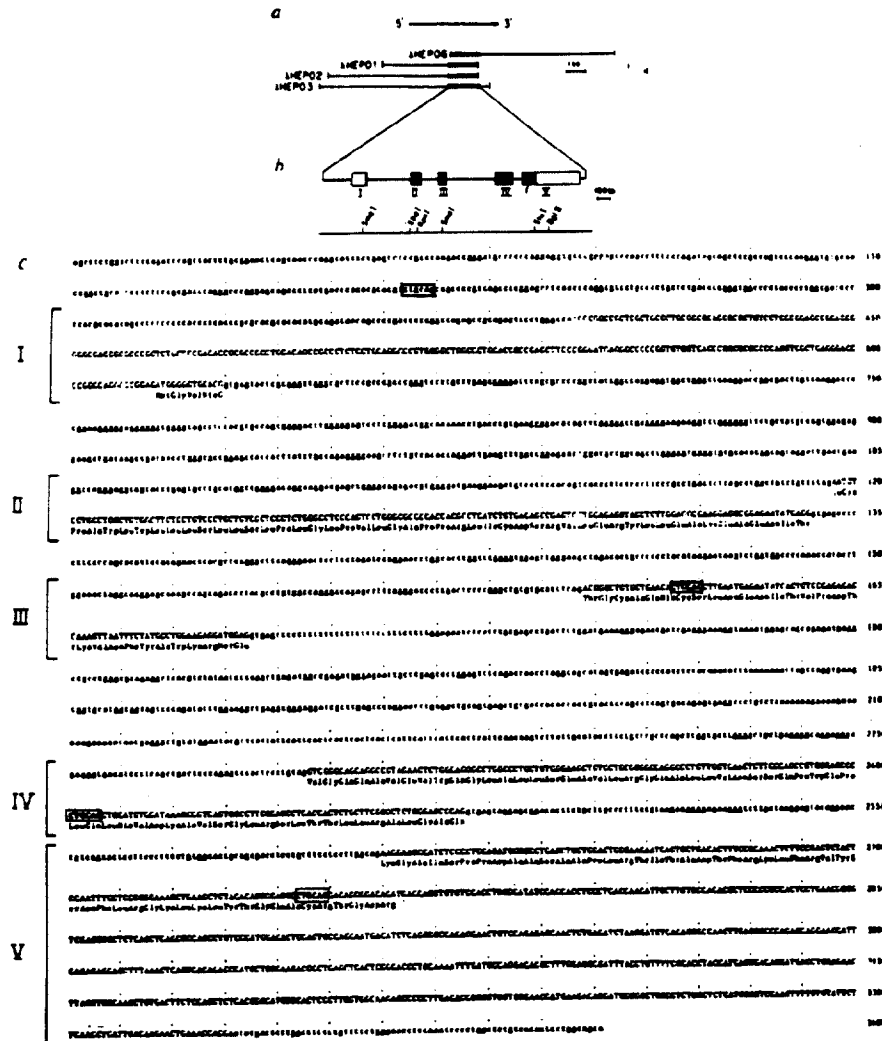


Fig. 3 Structure of the erythropoietin gene. The relative sizes and positions of four independent genomic clones (Δ HEPO1, 2, 3, and 4) described in the text are illustrated by the overlapping lines in a. The thickened line indicates the position of the erythropoietin gene. The region containing the gene was sequenced completely from both strands using an exonuclease III generated series of deletions (C.S., unpublished observations) through this region. b, A schematic representation of five exons coding for erythropoietin mRNA. The precise 5' boundary of exon I is unknown (indicated by the broken box). The 5' boundary of exon I shows here is derived from Δ HEPOFL1, which has a 5' untranslated region 39 nucleotides longer than that of Δ HEPOFL3. The protein coding portions of the exons are darkened. c, Complete nucleotide sequences of the region. Exon sequences are given in capital letters; intron sequence in lower case letters. The location of exons I-V are indicated by the bars with numerals on the left. Because of difficulties in interpreting sequencing gel data from the very G+C-rich regions of exon I, the level of certainty for exon I sequence is reduced slightly.

438