# EXHIBIT 8



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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

FRITSCH ET AL. ) Interference No. 102,334
v. )
LIN ) Examiner-in-Chief: Marc L. Caroff

DECLARATION OF JOAN C. EGRIE

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

Sir:

I, Joan C. Egrie, residing at 561 Los Vientos Drive, Newbury Park, California 91320, declare:

(1) I received a B.S. degree in biochemistry from Cornell University in 1969 and a Ph.D. degree in physiological chemistry in 1975 from the University of Wisconsin, Madison, Wisconsin. I subsequently did post-doctoral work at the University of California, Berkeley, Department of Zoology, in 1976-1977 and served as a post-graduate research zoologist and lecturer in that department of the University of California, Berkeley, from January, 1978 to August, 1981.

(2) I have authored or co-authored the publications shown in the attached curriculum vitae Lin Exhibit 110, (Document Nos. L00920-L00924).

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(3) I joined Amgen, Inc. as a research scientist on September 21, 1981 and I am still employed by Amgen. My present position is that of Head, Stem Cell Biology Laboratory.

(4) I am aware of the decision of the United States District Court, Massachusetts District, in Amgen, Inc. v Chugai Pharmaceutical Co., Ltd., 13 USPQ 2d 1737 (1989) ("Massachusetts decision") and the more recent decision of the United States Court of Appeals for the Federal Circuit dated March 5, 1991 ("Federal Circuit" decision), affirming the Massachusetts decision in relevant part and holding that Dr. Fu-Kuen Lin of Amgen was the first to clone the human EPO gene.

(5) As background for this declaration, I note that the Massachusetts decision reads in relevant part as follows in describing Dr. Lin's work and the work done on his behalf:

> "The successful cloning of the EPO gene took place in September or early October 1983. (Tr. 4,64-66; 5, 123-124). This was the first time that Lin ever designed, ordered and used two sets of probes, both fully degenerate, from two different regions of the EPO gene to screen a genomic library. (Tr. 5, 91, 124). Amgen (someone other than Dr. Lin) sequenced the gene to confirm it was the EPO gene. (Tr. 4, 74).
>
> In late October, 1983, Lin cloned the monkey cDNA EPO sequence. (Tr. 4, 72). On December 3, 1983, Lin also hybridized the human EPO gene to monkey EPO cDNA so that he could determine from an electron micrograph which area of the human DNA consisted of

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introns; and what the sizes of the exons and introns were. (Tr. 4, 68-72; PX 63-38).'

* * * * * * * * * * * * * * *

'By January 10, 1984, Amgen had expressed human EPO in human embryonic kidney cells called "293" cells and in COS cells, which are monkey kidney cells. (Tr. 4-75-77; PX 63-39; PX 63-41). Someone other than Dr. Lin did the work with the mammalian expression system. (Tr. 5, 51-52).
Lin was personally involved in the E. Coli expression of EPO. (Tr. 5,52). On February 13 and 14, 1984, Amgen conducted experiments to show that the recombinant human EPO produced in the COS cell was biologically active. (Tr. 4, 80).' II

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'From March 1-9, 1984, Amgen conducted an *in vivo* bioassay and determined that the recombinant EPO was biologically active. (Tr. 4, 82-83). II

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By May 2, 1984, human rEPO had been expressed in CHO cells. (Tr. 4, 86). Jeff Browne and Ralph Smalling worked together on the EPO project team, which Lin continued to head through 1984 to develop a cell line in 1984."

(6) I am the person at Amgen who was responsible for determining the biological activity of recombinant EPO referred to in the sections which are marginally designated by (II) opposite the quotation from the Massachusetts decision appearing in paragraph (5). I was also responsible for additional tests to characterize mammalian and non-mammalian cell expressed recombinant EPO including in vivo and in vitro biological assays, immunological assays, Western analysis, and

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determination of the effect of recombinant EPO on the hematocrit of mice. Experiments directed towards determining in vivo biological activity in the exhypoxic polycythemic mouse assay of recombinant EPO expressed in COS 1 and CHO cells were carried out in conjunction with Dr. Peter Dukes at the Children's Hospital, Los Angeles, California. The in vivo biological activity so determined for recombinant EPO expressed in both COS 1 and CHO cells as well as the ability of these preparations to increase the hematocrit of mice established that the recombinant EPO had the in vivo biological property of causing bone marrow cells to increase the production of reticulocytes and red blood cells. Jeri Lane, working under my direction and supervision, assisted in conducting radioimmunoassays and Western analyses. In addition, Cheryl Bradley conducted EPO in vitro bone marrow culture assays at my request and under my direction and supervision.

(7) I was aware in late 1983, probably near the end of October and certainly by the first week of November 1983, that Dr. Lin's investigations had resulted in the isolation of several clones which he maintained included the EPO gene. I was also personally aware, from my contact with Dr. Lin and Dr. Browne, that Dr. Browne's group, which included Mr. Ralph Smalling and Ms. Geri Trail, was asked to use the positive clones which Dr. Lin had isolated, in mammalian cell transfection experiments, for the expression of recombinant EPO. I understood Dr. Browne was responsible for the mammalian cell expression work with Mr. Smalling and Ms. Trail working under his supervision and direction. Starting in November

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1983, I was involved in work directed towards characterizing recombinant human and monkey EPO expressed by mammalian cells, bacteria and yeast. In particular, I worked with Dr. Browne's group to characterize mammalian cell expressed recombinant monkey and human EPO. Those experiments involved transfecting mammalian cells with nucleic acid sequences believed to encode monkey or human EPO and culturing those cells under conditions such that recombinant EPO was produced.

(8) On January 18, 1984, I received from Dr. Browne's group 4 lyophilized samples color-coded Blue (E3), Orange (E5), Red (E7) and Green (M7). I was advised that Dr. Browne's group had transfected a human EPO genomic clone obtained from Dr. Lin into human embryonic kidney cells (293 cells). Conditioned medium from these cells (5.0 ml) was harvested at 3, 5, and 7 days post-transfection and medium (5.0 ml) from mock-transfected 293 cells was harvested at 7 days post-transfection. The 4 harvested samples were dialyzed vs. 10mM Tris-HCl, pH 7.4, for 5 hours and then lyophilized to dryness. The samples color-coded Blue (E3), Orange (E5), Red (E7) and Green (M7) corresponded to the supernatants collected from the EPO gene-transfected cells at days 3, 5, and 7 and the mock-transfected cells on day 7, respectively. These samples were each resuspended in 0.7 ml of water, and assayed in an EPO RIA using antiserum raised against a crude preparation of human urinary EPO. The results of this assay, which were obtained on January 19, 1984, indicated that recombinant human EPO was expressed by those cells transfected

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with the human EPO gene, but not by the mock-transfected cells. Levels of recombinant human EPO were estimated to be 15.9, 23.7 and 35.4 mU/ml for the samples collected on days 3, 5, and 7 post-transfection. (Lin Exhibit 112, Document Nos. L00982-L00985).

(9) The results of the experiment referred to in paragraph (8) were confirmed on January 25-26, 1984 when the above samples E7 and M7 (7 day cell-conditioned medium from EPO gene or mock-transfected 293 cells) were assayed in a second RIA system, using a rabbit antiserum which was raised against a peptide corresponding to the first 20 amino acids of human EPO. Using this RIA, no EPO was detected in the mock-transfected sample, whereas recombinant human EPO at a level of approximately 22 mU/ml was detected in the E7 sample. Jeri Lane, working under my direction and supervision, assisted me in carrying out this work and the assay was recorded in her notebook. (See Lin Exhibit 116, Document Nos. L01101-L01104).

(10) On January 25, 1984, I received two additional sets of samples from Dr. Browne's group. I understood that these samples corresponded to 2 different transfections where an expression vector containing a BamHI-Hind III fragment with the human EPO genomic gene was introduced into both COS 1 and 293 cells. I was informed that for each of these transfections, cell-conditioned medium was collected at 5 and 7 days post-transfection for the experimental samples, and at 5 days (COS 1) or

5 and 7 days (293)-for the mock-transfections (293 or COS 1 cells transfected with mouse carrier DNA). All samples were dialyzed and lyophilized to dryness. I resuspended the samples in water at 5 times their original concentration and assayed them in an RIA using antiserum raised against a crude preparation of human urinary EPO. Results obtained on January 27, 1984, indicated that the 293 cells transfected with this expression vector containing the human EPO gene were producing recombinant human EPO with levels of 3.3 and 6.56 mU/ml being detected for the unconcentrated 5 and 7 day samples, respectively.

Samples of media from the COS 1 cells transfected with the same expression vector were also shown to contain recombinant human EPO, at levels of approximately 392 and 566 mU/ml for the day 5 and 7 samples respectively. As expected, the mock-transfected COS 1 cell-conditioned medium was negative. (Lin Exhibit 112, Document Nos. L01001-L01005.)

The level of recombinant human EPO in the day 7 COS 1 cell sample referred to above was confirmed in a second RIA determination which I performed on March 13-14, 1984 where a value of 490 mU/ml was obtained. (Lin Exhibit 113, Document Nos. L01080-L01086.)

(11) After establishing that the transfected COS 1 cells were expressing recombinant human EPO, and quantifying the expression levels by immunological assays as shown in paragraph (10) above, I next wanted to determine if the secreted recombinant EPO was biologically active, using both in vitro and in vivo bioassays.

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In order to determine if the COS 1 cell-expressed recombinant human EPO was biologically active in vitro, aliquots of the 7 day post-transfection medium (samples described in paragraph 10 above) were assayed in a bioassay according to the method of Goldwasser, Eliason and Sikkema, Endocrinology 97, 315-323, 1975, copy attached as Lin Exhibit 118 (Document Nos. L01448-L01456). This assay measures the uptake of $^{59}$Fe into the heme of rat bone marrow cells cultured in the presence of varying concentrations of EPO.

Ms. Cheryl Bradley, working under my direction and supervision, was responsible for performing the in vitro bioassays described in this Declaration. I outlined the assay to Ms. Bradley and where appropriate I did all of the necessary preparation work for the assay.

On February 13, 1984, the 5x concentrated day 7 COS 1 cell medium samples (mock and human EPO gene transfected) referred to in paragraph (10) were assayed. A second objective of this experiment was to determine if antiserum raised to a crude human urinary EPO preparation could neutralize the biological activity of the recombinant human EPO. For this purpose, samples of the RIA positive day 7 medium were incubated with immune or preimmune rabbit serum for approximately 2 1/2 hours, prior to being assayed for biological activity. The results of the in vitro bioassay experiment indicated that the recombinant human EPO in the day 7 COS 1 cell conditioned medium sample was biologically active and that this activity could be neutralized by an antibody raised to a partially-purified preparation of human urinary EPO, but not by preimmune serum from the same rabbit. From this assay, I

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calculated that there were approximately 986 mU/ml of biologically active recombinant human EPO in the sample. As expected, media from mock-transfected cells assayed at near background levels in this assay, confirming the results obtained in the RIAs. (See Lin Exhibit 119, Document Nos. L01274-L01288.)

In a second in vitro bioassay, begun on February 20, 1984, the same day 7 COS 1 cell conditioned medium sample once again was shown to contain biologically active recombinant human EPO at a level of approximately 1,140 mU/ml. The biological activity of this sample was neutralized by immune serum from a rabbit immunized with a peptide corresponding to the first 20 amino acids of human EPO. (See Lin Exhibit 119, Document Nos. L01161-L01173.)

(12) Another series of COS 1 cell-conditioned medium samples from cells transfected with the human EPO gene were generated by Mr. Smalling. These samples were of a sufficient volume to permit their assay in four different EPO assays in order to characterize the recombinant human EPO. The assays used were RIA, in vitro bioassay, exhypoxic polycythemic mouse in vivo bioassay and an assay to determine the effect on the hematocrit of injected animals.

Mr. Smalling transfected COS 1 cells with the pSV4ST-human EPO gene construct on February 13 and February 14, 1984, respectively. Day 3 and day 7 media were collected from each transfection experiment on the appropriate date and brought to my laboratory. I combined the two day 3 cell-conditioned medium samples (E3) from the two transfections, and similarly, I also combined the day 7 media

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samples (E7). The day 3 and day 7 pools were then sterile-filtered, aliquoted and frozen at -20°C. In my notebooks, this pooled day 3 cell conditioned medium is generally referred to as E3, February 16-17, 1984, and the pooled day 7 sample is referred to as E7, February 20-21, 1984. On February 21, 1984, I assayed each of these samples in an RIA using an antibody raised against a crude preparation of human urinary EPO. A titer of approximately 4.0 and 8.2 U/ml was determined for the day 3 and day 7 media samples, respectively. (Lin Exhibit 113, Document Nos. L01024-L01028.)

These values were confirmed in two subsequent RIAs initiated on February 24, 1984 and March 13, 1984. The 3 day post-transfection COS 1 cell-conditioned medium assayed at levels of 2.8 and 3.4 U/ml in these two assays, and the 7 day post-transfection COS 1 cell sample assayed at levels of 5.5 and 8.3 U/ml in the February 24, 1984 and March 13, 1984 assays, respectively. (Lin Exhibit 113, Document Nos. L01029-L01035 and Document Nos. L01080-L01086.)

When assayed in the in vitro bioassay of Goldwasser et al (Lin Exhibit 118) I determined that these two samples had levels of biologically active recombinant human EPO which were comparable to the levels determined by the RIA. The first in vitro bioassay, initiated on these samples on March 7, 1984, estimated approximately 4.0 and 5.6 U/ml of in vitro biologically active recombinant human EPO in the day 3 and day 7 post transfection COS 1 cell conditioned media samples, respectively. (See Lin Exhibit 119, Document Nos. L01174-L01182, L01186-L01192.)

At my direction, these samples were reassayed together in the in vitro bioassay

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on 2 additional occasions. These assays were initiated on March 13, 1984 and March 19, 1984. Values of 3.4 and 5.75 U/ml in vitro bioactive recombinant EPO were obtained for the day 3 COS 1 cell conditioned media sample in the March 13 and March 19, 1984 assays, respectively. Values of 8.8 and 8.7 U/ml of in vitro bioactive recombinant EPO were obtained for the day 7 COS 1 cell conditioned media sample in the March 13, 1984 and March 19, 1984 bioassays. (See Lin Exhibit 119, Document Nos. L01193-L01217.)

(13) The in vivo bioactivity of recombinant human EPO expressed in COS 1 cells and in CHO cells was determined by assaying samples of cell conditioned medium in the exhypoxic polycythemic mouse in vivo bioassay, essentially according to the method of Cotes and Bangham (Nature 191:1065-1067, 1961). A copy of this reference being attached as Lin Exhibit 2, (Document Nos. L00629-L00631). In this assay, mice are made polycythemic by exposure to reduced pressure of about 1/2 atmosphere for two weeks. Upon return to normal atmospheric conditions, the endogenous EPO production and hence the rate of erythropoiesis of these animals is suppressed. These mice respond to the administration of exogenous EPO by increasing their production of red blood cells. Following the injection of EPO standard or test solution, radiolabelled iron ($^{59}Fe$) is administered. Two days after the injection of radioactive iron, the animals are sacrificed and the $^{59}Fe$ incorporated into circulating red blood cells is determined. The extent of incorporation of $^{59}Fe$ is a measure of the rate of erythropoiesis and is related to the amount of injected EPO.

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This assay is a widely accepted assay for measuring the in vivo biological activity of EPO since it is influenced by both the ability of the molecule to interact with its receptor and its serum half-life.

At the time Dr. Lin cloned the human EPO genomic gene it was known that the carbohydrate moieties of human urinary EPO were important to its in vivo biological activity. I expected that the carbohydrate composition of recombinant human EPO would likewise be important to its in vivo biological activity. I also knew at the time Dr. Lin cloned the human EPO gene that the pattern of glycosylation of a protein varied from species to species and from cell type to cell type within a species. Therefore, it was my expectation that recombinant human EPO produced in a non-human eucaryotic host cell would probably have a different carbohydrate composition from that of human urinary EPO.

The carbohydrate portion of EPO, particularly its sialic acid content, substantially affects its in vivo bioactivity. In contrast, EPO molecules without any oligosaccharide can still possess in vitro biological activity, but have virtually no activity in vivo.

(14) On February 28, 1984, I prepared and made dilutions of the 2 recombinant EPO samples referenced in paragraph 13 above and a control sample for shipment to Dr. Dukes of Children's Hospital of Los Angeles for assay in the exhypoxic polycythemic mouse in vivo bioassay. The samples sent to Dr. Dukes consisted of 4 dilutions (1:1, 1:2, 1:4, and 1:8) for the 3 day post-transfection COS 1 cell conditioned medium, (E3, February 16-17, 1984), 5 dilutions (1:2, 1:4, 1:8, 1:16,

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and 1:32) of the 7 day post-transfection COS 1 cell conditioned medium (E7, February 20-21, 1984), and 2 dilutions (1:1 and 1:4), of mock-transfected COS 1 cell conditioned medium (control). The assay was begun on March 5, 1984 and completed on March 9, 1984. On March 12, 1984 Dr. Dukes sent me a letter with the results and final report for this assay. The results indicated that the samples coded E3 and E7 contained in vivo biologically active recombinant human EPO, whereas the control sample did not. The level of in vivo biologically active recombinant human EPO was estimated to be 0.37 and 0.82 U/ml for samples E3 and E7, respectively. See Lin Exhibit 113, Document Nos. L01053-L01055 as well as my letter to Dr. Dukes dated February 28, 1984 which accompanied the package with the samples (Exhibit 6, Document No. L00652) and Dr. Duke's letter to me dated March 12, 1984 accompanying the final assay results (Exhibit 7, Document Nos. L00653-L00655). The values obtained in the in vivo bioassay were lower than those obtained in the immunological and in vitro bioassay methods (i.e., the in vivo activity was about 10% of that determined by the immunological and in vitro bioassays).

(15) Another in vivo bioassay was performed on the E7 sample referenced in paragraph (14) above. On March 22, 1984, I prepared samples to be delivered to Dr. Dukes' laboratory for in vivo bioassay. Two of the samples, coded Group 5, A and B were undiluted aliquots of E7 without and with the addition of rabbit anti-EPO antiserum (raised against a partially purified preparation of human urinary EPO), respectively. The purpose of the experiment was to confirm the previously obtained

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in vivo bioassay result and to determine if the anti-EPO antiserum would neutralize the in vivo biological activity of the recombinant human EPO produced by the COS 1 cells. The assay was initiated on March 26, 1984 and completed on March 30, 1984. The results, which were mailed to me by Dr. Dukes on April 2, 1984, indicated that the in vivo biological activity of E7 was 0.48 U/ml and that this activity could be neutralized by the anti-human urinary EPO antiserum. (See Exhibit 10, Document No. L00660 which is my letter of March 22, 1984 to Dr. Dukes and Exhibit 11, Document Nos. L00661-L00663, which is Dr. Dukes' reporting letter to me of April 2, 1984, with attachments.)

(16) One additional experiment was performed to determine the effect of the COS 1 cell-expressed recombinant human EPO on the hematocrit of mice.

Beginning on March 5, 1984, nude mice received 0.5 ml intraperitoneal injections of the COS 1 cell expressed recombinant human EPO (n=3), or cell conditioned media from mock-transfected COS 1 cells (n=3) according to the following schedule:

3/5-3/9 - 0.5 ml of E3 COS 1 cell conditioned medium.
(~0.185 U of in vivo bioactive recombinant EPO)
(Referred to in paragraphs 12-14 above)

3/12, 3/13 - 0.5 ml of 4.9x concentrated E3 sample referred to above

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3/21, 22, 23,
26-30, 4/2-4 - 0.5 ml of 7 day COS 1 cell-conditioned medium from EPO gene-transfected cells received from Dr. Browne on March 20, 1984. By RIA, the sample titered at 11.7 U/ml.

On March 19, 1984 and April 6, 1984 the hematocrits of both the control and the recombinant human EPO-treated groups were determined. When compared to the control group, the hematocrit of the recombinant human EPO treated group was found to be elevated (55.7% vs. 51.8% and 57.2% vs. 50.5% for the recombinant EPO vs. control groups on March 19, 1984 and April 6, 1984, respectively), indicating that the COS 1 cell-expressed recombinant human EPO possessed the in vivo biological property of causing bone marrow cells to increase production of reticulocytes and red blood cells. (See Lin Exhibit 113, Document Nos. L01064-L01068.)

(17) I was aware that Dr. Browne's group also focused efforts on constructing a CHO cell line which consitutively produced recombinant human EPO. On May 23, 1984, I received the first cell-conditioned medium samples from transfected CHO cells (the human rEPO expressed in CHO cells as referred to in the last paragraph of the portion of the Massachusetts decision quoted in paragraph (5) above) from Dr. Browne's assistant, Mr. Smalling. Mr. Smalling informed me that the samples were

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obtained by transfecting CHO cells with the human EPO genomic gene and collecting cell-conditioned media from two different transfections: 3 1/2 day cell-conditioned media sample from cells encoded H3 and 5 1/2 day cell conditioned media sample from cells encoded B11. On May 23, 1984, I assayed these samples in an RIA using antiserum raised against a crude preparation of human urinary EPO, and on May 24, 1984 titers of 5.7 and 18.2 U/ml were determined for H3 and B11, respectively. (See Lin Exhibit 114, Document Nos. L01149-L01154.) For confirmation, these two samples were assayed again on July 3, 1984 in the RIA described immediately above. Values of 5.55 and 17.8 U/ml were obtained for H3 and B11, respectively, which values were in good agreement with the results of the first RIA determination. (See Lin Exhibit 114, Document Nos. L01155-L01160.)

(18) The CHO cell-conditioned medium samples H3 and B11, described in paragraph (17) above, were assayed under my direction and supervision in a series of in vitro bioassays, beginning on May 28, 1984. In this assay, levels of in vitro bioactive recombinant human EPO were found to be 10.22 and 27.55 U/ml, for H3 and B11, respectively. (See Lin Exhibit 119 Document Numbers L01251-L01256.) Data for other assays on these samples were recorded by me in the period June 4, 1984 to July 11, 1984. (See Lin Exhibit 119, Document Nos. L01257-L01273 and L01289-L01298.) When the results of these replicate assays were averaged the mean value obtained for sample H3 was 5.3±1.4 U/ml and for B11 was 15.8±4.6 U/ml in vitro bioactive recombinant human EPO.

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(19) To determine the in vivo biological activity of the H3 and B11 CHO cell conditioned media samples referenced in paragraphs (16) and (17) above, on May 31, 1984, my research associate, Ms. Jeri Lane, under my direction and supervision, prepared 4 dilutions of H3 (1:2, 1:4, 1:8, and 1:16), 5 dilutions of B11 (1:4, 1:8, 1:16, 1:32, and 1:64) and 2 dilution of a control sample (cell conditioned medium from mock-transfected CHO cells; 1:2 and 1:4) and sent them at my request to Dr. Dukes. (See Exhibit 16, Document No. L00672.) On June 4, 1984 these samples were assayed in the exhypoxic polycythemic mouse bioassay. The assay was completed on June 8, 1984 and on June 11, 1984 Dr. Dukes sent a letter and the final assay report to me. (See Exhibit 17, Document Nos. L00673-L00675.) The results indicated that the level of in vivo biologically active recombinant human EPO in these samples was equivalent to the EPO levels obtained for these samples in both the RIA and the in vitro bioassay. Samples H3 and B11 were determined to have 5.6 and 16.8 U/ml of in vivo biologically active recombinant EPO, respectively. As expected, the control sample assayed negative for EPO in vivo biological activity. (See Lin Exhibit 114, Document No. L01154a.)

(20) I was also informed by Dr. Browne that, in parallel with the efforts to transfect the human genomic EPO sequences into mammalian cells, his group was performing experiments under his direction to obtain cells expressing the monkey EPO cDNA obtained from Dr. Lin. On December 6, 1983, I obtained two batches of 6

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cell-conditioned media samples (coded F, G, H, L, M, and X) from Mr. Smalling who informed me that he had transfected the monkey EPO cDNA or control DNA into COS 1 cells. I assayed these samples in a blinded fashion in an EPO RIA using an antiserum raised against a crude preparation of human urinary EPO. The results of this experiment obtained on December 8, 1983, showed that 2 of the samples, H and L, were able to strongly inhibit the binding of tracer $^{125}$I-EPO to the antibody, indicating that these samples contained recombinant monkey EPO. The inhibition seen was near or above the limits of sensitivity of the assay, precluding a reliable determination of the amount of recombinant monkey EPO present. The remaining four samples, F, G, M, and X did not produce any inhibition in the RIA indicating the absence of recombinant monkey EPO in the samples. Following the conclusion of the RIA and exchange of results, Mr. Smalling indicated that samples F, G, and X were obtained from cells which contained the monkey EPO cDNA in a reverse orientation, whereas samples H and L were from cells containing the monkey EPO sequence in the correct orientation, appropriate to allow expression of recombinant monkey EPO. The sample labeled M was obtained from the mock-transfected cells. It was thus evident that only samples from cells in which the vector was inserted in the correct orientation (H and L) appeared to be producing recombinant monkey EPO. (Lin Exhibit 112, Document Nos. L00936-L00940.)

(21) On December 10-12, 1983 another EPO RIA, similar to the one noted in paragraph (20) above, was initiated to determine the level of recombinant monkey

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EPO expressed in Mr. Smalling's COS 1 cell supernatants. In this experiment samples H, L and G were assayed over a wide range of dilutions. In agreement with the previous experiment, sample G was shown to contain no recombinant monkey EPO. Samples H and L, however, were found to have 2.7 and 2.5 U of recombinant monkey EPO/ml, respectively. (Lin Exhibit 112, Document Nos. L00941-L00943.)

(22) Having established that COS 1 cell-conditioned media samples H and L contained recombinant monkey EPO by RIA, I next wanted to determine if the expressed recombinant monkey EPO was biologically active, using both in vitro and in vivo biological activity assays.

On December 15, 1983, I prepared and sent Dr. Dukes six samples for assaying in the exhypoxic polycythemic mouse in vivo bioassay. The six samples sent to Dr. Dukes consisted of the second of two COS 1 cell conditioned medium samples prepared by Mr. Smalling (described in paragraph 21 above) and transferred to me on December 6, 1983 coded $H_2$ and $L_2$ (each at a 1:1 and 1:7 dilution), and undiluted aliquots of $F_2$ and $X_2$. (See Exhibit 4, Document Nos. L00647-L00648.) The letter designations were used to indicate a correspondence with samples F, X, H and L in paragraphs 21 and 22 above. The samples were assayed essentially according to the procedure of Cotes and Bangham (See Exhibit 2, Document Nos. L00629-L00631) beginning on December 19, 1983. The assay was completed on December 23, 1983 and although all of the final calculations had not been performed, sufficient data were available for Dr. Dukes and I to make some preliminary estimates of the EPO

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content of the samples. On the afternoon of December 23, 1983, Dr. Dukes and I discussed the data on the telephone and assigned preliminary estimates reflecting biological activity of EPO to samples $H_2$ and $L_2$, respectively. $F_2$ and $X_2$ gave responses which were indistinguishable from those of the in vivo assay negative control sample.

(23) On December 29, 1983 Dr. Dukes sent me a final report of the assay results from which potencies of 1.24 and 0.94 U/ml of recombinant monkey EPO were assigned to samples $H_2$ and $L_2$, respectively; $X_2$ and $F_2$ were confirmed to contain no EPO. (See Lin Exhibit 112, Document Nos. L00964-L00965 as well as Exhibit 5, Document Nos. L00649-L00651, the latter being a copy of Dr. Dukes' December 29, 1983 reporting letter to me with attachments.) The results with $H_2$ and $L_2$ indicated that the recombinant monkey EPO expressed by the COS 1 cells was active in vivo, having the ability to increase the rate of erythropoiesis, as determined by the extent of incorporation of $^{59}Fe$ into red blood cells. This indicated to me that the recombinant monkey EPO which had been obtained would have the biological properties of EPO in vivo to cause bone marrow cells to increase production of reticulocytes and red blood cells.

(24) A second in vivo bioassay was performed on samples $H_2$ and $L_2$. Dilutions of each sample (1:3, 1:5 and 1:7) were prepared and I sent these to Dr. Peter Dukes on March 7, 1984. The exhypoxic polycythemic mouse in vivo bioassay

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was begun on March 12, 1984 and completed on March 16, 1984. On March 19, 1984, Dr. Dukes sent me a letter along with the final assay results indicating levels of recombinant monkey EPO of 1.1 ± 0.12 and 0.93 ± 0.27 U/ml for samples $H_2$ and $L_2$, respectively, thus confirming the results of the first in vivo bioassay for these samples. (See Lin Exhibit 113, Document Number L01069, as well as Exhibit 8, Document No. L00656 which is my letter to Dr. Dukes that accompanied the samples I sent him on March 7, 1984 and Exhibit 9, Document Nos. L00657-L00659 which is a copy of the letter, with attachments, that Dr. Dukes sent to me providing the in vivo bioassay results.

(25) In order to determine whether the recombinant monkey EPO-containing samples $H_2$ and $L_2$ referred to above, were also biologically active in vitro, a bioassay using rat bone marrow cells was performed following the procedure of Goldwasser et al (Lin Exhibit 118). For the purposes of these experiments samples $X_2$, $F_2$, and $G_2$ which corresponded to the second of the two batches of control samples prepared by Mr. Smalling, (previously described in paragraph 20 above), were also assayed.

These in vitro bioassay experiments were initiated on December 19, 1983 and December 20, 1983, with results being obtained on December 21, 1983 and December 22, 1983 for the two assays, respectively. In the first experiment (in which samples $F_2$, $H_2$, $X_2$, and $L_2$ were assayed) no in vitro biologically active recombinant monkey EPO was found in samples $F_2$ and $X_2$, confirming the previously obtained RIA results on these samples. Samples $H_2$ and $L_2$ were found to contain 4.8 and 3.6 U/ml of in

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vitro biologically active recombinant monkey EPO, respectively. In the second experiment (in which samples $G_2$, $L_2$, and $H_2$ were assayed), sample $G_2$ was found not to contain any recombinant monkey EPO, whereas samples $H_2$ and $L_2$ were determined to have 3.8 and 2.9 U/ml of in vitro biologically active recombinant monkey EPO, respectively. From these two experiments, I concluded that the recombinant monkey EPO obtained from the COS 1 cells was biologically active in vitro. (See Lin Exhibit 112, Document Nos. L00954-L00963.)

(26) RIA determinations (using an antiserum raised against a crude preparation of urinary EPO) were also performed directly on sample $H_2$ on January 6, 1984 and March 13, 1984. Levels of recombinant monkey EPO of 3.12 and 2.94 U/ml were determined in these two assays, respectively (Lin Exhibit 112, Document Nos. L00966-L00970 and Lin Exhibit 113, Document Nos. L01080-L01086). In a similar fashion, sample $L_2$ was also assayed directly in the above-described RIA on January 6, 1984, January 18, 1984, February 21, 1984, and February 24, 1984 where levels of the recombinant monkey EPO were determined to be 2.92, 2.91, 2.64 and 2.86 U/ml, respectively. (Lin Exhibit 112, Document Nos. L00966-L00970, L00982-L00985, and Lin Exhibit 113, Document Nos. L01024-L01028 and L01029-L01034.) The values obtained by RIA for recombinant monkey EPO expressed in samples $H_2$ and $L_2$ were in close agreement with those determined by the in vitro bioassay. Values obtained in the in vivo bioassay were lower than those obtained by the

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immunological and in vitro bioassay methods (i.e., the in vivo activity was about 30% of that determined by the immunological and in vitro bioassays).

(27) In addition to expressing the recombinant monkey EPO cDNA in COS 1 cells, I was also advised by Dr. Browne that his group transfected this sequence into CHO cells. On February 15, 1984 I obtained from Mr. Smalling a sample of cell-conditioned medium from CHO cells transfected with the monkey EPO cDNA (labeled $C_4$) and a sample from mock-transfected CHO cells. These samples were assayed for their ability to inhibit the binding of $^{125}$I-EPO to an antiserum raised against a crude preparation of human urinary EPO. On February 16, 1984 I determined that the sample $C_4$ strongly inhibited the binding of tracer $^{125}$I-EPO to the antibody indicating the presence of expressed recombinant monkey EPO in the supernatant. The mock-transfected control sample, however, showed no inhibition of binding, indicating the absence of EPO in this sample. (See Lin Exhibit 113, Document No. L01023.)

(28) Subsequently, Mr. Smalling gave me two additional samples of medium from CHO cells transfected with the monkey EPO cDNA and grown for 5 days in the presence of 10 nM methotrexate ("MTX"), coded Line 3 and Line 6. On March 13, 1984, April 5, 1984, and April 25, 1984 these samples were assayed in RIAs using an antiserum raised against a crude preparation of human urinary EPO to quantify the levels of expressed recombinant monkey EPO. Estimates of 39.7, 32.8 and 37.9 U/ml

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were obtained for Line 3 in each of the three assays, respectively, and estimates of 40.2, 40.5, and 42.8 U/ml were obtained for Line 6 in each of the three assays, respectively. (Lin Exhibit 113, Document Nos. L01080-L01086 and L01091-L01096; and Exhibit No. 114, Document Nos. L01140-L01143.)

(29) Aliquots of Line 6-produced cell conditioned medium also were assayed in the in vitro bioassay on April 3, 1984, April 9, 1984, April 23, 1984 and April 30, 1984. The results of these assays indicated that the CHO cell-produced recombinant monkey EPO was biologically active in vitro, at a level consistent with that found by RIA. (See Lin Exhibit 119, Document Nos. L01218-L01250.)

(30) In order to determine the level of in vivo biological activity of the recombinant monkey EPO in the sample coded Line 6, an aliquot was dialyzed for 2 hours to remove the methotrexate, and on April 5, 1984, dilutions of (A1-5) of 1:1, 1:2, 1:4, 1:8, and 1:16 were prepared and sent to Dr. Dukes. A second sample, cell conditioned media from CHO cells transfected with the nucleic acid encoding $\alpha$-FSH, obtained from Dr. Browne's group, was diluted (B1-2) 1:1 and 1:2 and also sent to Dr. Dukes to serve as a negative control for the experiment. At my request, these samples were assayed by Dr. Dukes in the exhypoxic polycythemic mouse bioassay beginning on April 9, 1984 and terminating April 13, 1984. On April 16, 1984, Dr. Dukes sent me a letter with the final assay results indicating that the titer of recombinant monkey EPO in sample A (line 6) was approximately 42.0 U/ml as

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measured in the in vivo bioassay. Sample (B), both dilutions, was inactive as expected. (See Lin Exhibit 113, Document No. L01090, as well as Exhibit 12, Document No. L00664, which is the letter written to Dr. Dukes and Exhibit 13, Document Nos. L00665-L00667, which is Dr. Dukes' letter to me, with attachments.)

(31) The indicated level of in vivo biological activity found in the line 6 CHO cell expressed recombinant monkey EPO was confirmed in a second assay, which was performed from April 23-27, 1984 in Dr. Dukes laboratory. Dilutions of the dialyzed Line 6 sample, corresponding to 1:20, 1:40, 1:80, and 1:160 were prepared and sent to Dr. Dukes on April 19, 1984, in advance of the assay. On April 30, 1984 Dr. Dukes sent me a letter and the final assay results indicating a potency of 42.0 U/ml for the in vivo biological activity of the recombinant monkey EPO in the Line 6 sample. This set of experiments performed on the CHO cell-produced recombinant monkey EPO, indicated that levels of biologically active recombinant EPO, as measured by both the in vivo and in vitro bioassays were comparable to the levels of EPO determined by RIA. In addition, subsequent experiments indicated that the recombinant monkey EPO was able to increase the hematocrit of normal mice (see paragraphs 32-35 below). See Lin Exhibit 114, Document No. L01138, as well as Exhibit 14, Document No. L00668 which is the cover letter written by me to Dr. Dukes which accompanied the samples sent to Dr. Dukes and Exhibit 15, Document Nos. L00669-L00671 which is Dr. Dukes' reporting letter to me, with attachments.

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(32) In addition to demonstrating biological activity in in vitro and in vivo bioassay systems, I wanted to directly assess the ability of recombinant monkey EPO to increase the red blood cell mass in mice. Beginning on March 5, 1984, 6 nude mice were injected with either preparations of recombinant monkey EPO (n=3) or a control preparation (n=3) consisting of either cell-conditioned media from mock-transfected cells or cells which had the monkey EPO cDNA integrated in a reverse orientation. The following injection schedule was used:

| | |
|---|---|
| 3/5-3/8 | Inject mice intraperitoneally with 0.5 ml of COS 1 cell expressed recombinant monkey EPO containing approximately 3.65 U/ml by RIA. |
| 3/27-3/30, 4/2-4/4 | Inject mice intraperitoneally with 0.5 ml of CHO cell-derived recombinant monkey EPO containing approximately 90 U/ml by RIA. |

(33) On April 6, 1984 hematocrit determinations indicated that those animals receiving recombinant monkey EPO had increased their red blood cell mass, whereas those animals receiving control preparations did not. The group average hematocrit of the three mice administered recombinant monkey EPO was 68.3%, compared to a pre-treatment value of 47.5%. In contrast, the group average hematocrit of the two surviving mice receiving the control preparation was 52.1% compared to their pre-treatment hematocrit of 47.4%. Following withdrawal of the recombinant EPO

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administration, the hematocrit of the treated mice returned to baseline. (See Lin Exhibit 113, Document Nos. L01064-L01067.) This indicated to me that the recombinant monkey EPO was biologically active in vivo and able to increase the production of red blood cells.

(34) In addition, on March 6, 1984, in conjuction with Dr. Browne's group, 6 nude mice were injected with either $10^7$ CHO cells containing the recombinant monkey EPO cDNA (n=3, treated group) or $10^7$ CHO cell not containing the recombinant monkey EPO cDNA (n=3 control group). On March 19, 1984 hematocrit determinations performed on both groups of animals indicated that those animals receiving the CHO cells transfected with the recombinant monkey EPO cDNA had an elevation in their red blood cell mass. Group average hematocrits of 72.7% vs. 51.2% were obtained for the treated and control groups, respectively. (Lin Exhibit 113, Document Nos. L01064-L01068.)

(35) An additional experiment was performed to determine the ability of CHO cell-derived recombinant monkey EPO to increase the hematocrit of normal BalbC mice. Beginning on May 1, 1984 mice were injected with either cell conditioned media from CHO cells (n=3, control) or with media from CHO cells expressing recombinant monkey EPO (n=5, treated group)). Mice in the treated group received either 4.4 units (n=2), 11.0 units (n=1), or 44.2 units (n=2) of recombinant monkey EPO as determined by RIA. A total of 18 intraperitoneal injections were

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administered from May 1, 1984 until June 6, 1984, and hematocrit determinations were performed three times per week. The results indicated that the hematocrit of the control mice did not change significantly during the treatment period, whereas the hematocrit of the recombinant monkey EPO treated mice increased in a dose-dependent manner. Peak hematocrits were determined to be approximately 70%, 65%, and 60% for the animals receiving 44, 11 and 4.4 units of recombinant monkey EPO, respectively, compared to approximately 50% for the control mice. (Lin Exhibit 114, Document Numbers, L01144-L01146 and L01457).

The three experiments referred to in paragraphs 32 through 35 above, indicate that the expressed recombinant monkey EPO is biologically active in vivo having the property of increasing the red blood cell mass of treated animals.

(36) In parallel with the experiments performed on the COS 1 and CHO cell-expressed recombinant human and monkey EPO to determine its biological and immunological activity and effect on hematocrit of normal animals, I also determined the size of the recombinant EPO in relation to the EPO in a partially purified pooled source human urinary EPO preparation provided by Dr. Eugene Goldwasser.

On February 26, 1984 the recombinant human EPO from sample E7, (cell-conditioned medium collected 7 days post-transfection of COS 1 cells with a vector containing the human EPO gene, referred to as E7, February 20-21, 1984 above) and the partially purified human urinary EPO preparation were immunoprecipitated. The resulting immunoprecipitates were subjected to SDS-PAGE,

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transferred to nitrocellulose, and the EPO in each sample was specifically recognized by reaction with an anti-EPO monoclonal antibody. The results of the experiment indicated that the COS 1 cell-expressed recombinant human EPO migrated to the same extent as the EPO in the partially purified human urinary EPO preparation. No EPO was detected in cell conditioned media from mock-transfected COS 1 cells, as expected. (See Lin Exhibit 113, Document Nos. L01036-L01050.)

This comigration on SDS-PAGE of the EPO in the partially purified human urinary preparation and the COS 1 cell expressed recombinant human EPO was also noted in two similar experiments performed on March 5-8, 1984. (See Lin Exhibit 113, Document Nos. L01056-L01062 and attached pages), and on May 7, 1984 (see Lin Exhibit 117, Document Nos. L01114-L01125.)

In addition, the May 7, 1984 experiment, which was conducted by my associate Ms. Lane, under my direction and supervision, also showed that the COS 1 cell-expressed recombinant human EPO and the pooled source human urinary EPO migrated identically after treatment with ENDO F, an enzyme which removes asparagine-linked carbohydrate chains. This suggested that the carbohydrate portion of the COS 1 cell-expressed recombinant human EPO and the pooled source human urinary EPO were of approximately the same size. (See Lin Exhibit 117, Document Nos. L01114 to L01125).

In contrast to these results, on July 10, 1984, in an experiment similar to the one described above, my assistant, Ms. Lane working under my direction and supervision, showed that the recombinant human EPO expressed by CHO cells

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migrated more slowly than the partially purified human urinary EPO. (Lin Exhibit 117, Document Nos. L01126-L01135.) Upon digestion of the CHO cell-expressed recombinant human EPO with ENDO F, it migrated to the same extent as the ENDO F digestion products of the partially purified human urinary EPO. These results obtained on September 21, 1984 indicate that the difference in apparant molecular weight observed for the CHO-derived recombinant human EPO and Dr. Goldwasser's partially purified pooled source human urinary EPO is a result of differences in the carbohydrate portion of the molecules. (Lin Exhibit 115, Document Nos. L01070-L01077.) These results are similar to those obtained for CHO cell derived recombinant monkey EPO which was shown to migrate more slowly than the pooled source human urinary EPO prior to treatment with ENDO F. Following treatment with ENDO F, however, the CHO cell derived recombinant monkey EPO comigrated with a similarly treated pooled source human urinary EPO preparation. (See Exhibit 117, Document Nos. L01114-L01125).

(37) The experimental work referred to in paragraph (36) above showed that the CHO cell derived recombinant human EPO had a different apparent molecular weight from that of the pooled source of human urinary EPO, provided by Dr. Goldwasser. This difference in molecular weight appeared to be due to differences in the carbohydrate portion of the molecules.

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(38) I am aware that Interference No. 102,334, in which this declaration is headed, defines the invention at issue as follows:

Count 1

A non-naturally occurring glycoprotein product of the expression in a non-human eucaryotic host cell of an exogenous DNA sequence consisting essentially of a DNA sequence encoding human erythropoietin said product possessing the in vivo biological property of causing human 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.

(39) I confirm that the results of the exhypoxic polycythemic mouse in vivo bioactivity assays and mouse hematocrit studies referred to in this declaration, which were performed with the recombinant human EPO expressed by Dr. Browne's group, indicated that the expression products so obtained possessed the characteristics recited in the count of Interference No. 102,334, of the indicated biological properties of causing bone marrow cells to increase production of reticulocytes and red blood cells. In addition, as noted in paragraphs 36 and 37, the glycoprotein had an average carbohydrate composition for the CHO cell-expressed recombinant EPO which differed from the partially purified pooled source human urinary EPO provided by Dr. Goldwasser.

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(40) Lin Exhibits 112, 113, 114 and 115 referred to above represent portions of my notebook records (Notebook Nos. 540, 569, 723 and 633 respectively) which I was required to keep as part of my duties as an employee of Amgen. The entries which I made in these notebooks accurately reflect work conducted on the dates indicated.

(41) My assistant, Jeri Lane, also kept notebook records for work she did under my direction and supervision. Her relevant notebooks are Notebook Nos. 315 and 717. Cover pages and notebook pages which are referred to in this Declaration constitute Lin Exhibits 116 and 117, Document Nos. L01099-L01111 and L01112-L01135, respectively. Lin Exhibit 119 is a compilation of in vitro bioassay data wherein experiments were performed by Ms. Cheryl Bradley under my direction and supervision.

(42) I declare that all statements made herein of my own knowledge are true and that all statements made or information are believed to be true. I declare further that these statements were made with the knowledge that willful false

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statements and the like so made are punishable by fine or imprisonment, or both under Section 1101 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the Lin patent and applications of patents issued therein.

Date: 3-18-91

Joan C. Egrie

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