

EXHIBIT 2

IN THE UNITED STATES DISTRICT COURT
FOR THE DISTRICT OF MASSACHUSETTS

Certified Copy

AMGEN INC.,)	
)	
Plaintiff,)	
)	
vs.)	No. 05-12237 WGY
)	
F. HOFFMANN-LA ROCHE LTD., a)	
)	
Swiss Company, ROCHE DIAGNOSTICS)	
)	
GmbH, a German Company, and)	
)	
HOFFMANN-LA ROCHE INC., a New)	
)	
Jersey Corporation,)	
)	
Defendants.)	

Continued Videotaped Deposition of
 EUGENE GOLDWASSER, Ph.D., taken before GREG S.
 WEILAND, CSR, RMR, CRR, Notary Public, pursuant to
 the Federal Rules of Civil Procedure for the United
 States District Court pertaining to the taking of
 depositions, at Suite 4100, Three First National
 Plaza, in the City of Chicago, Cook County,
 Illinois, commencing at 9:08 o'clock a.m., on the
 26th day of February, 2007.

VOLUME 2

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1 containing cells are grown, and we purified mouse
2 erythropoietin by that same method.

3 Q. And is your testimony with respect to
4 recombinant erythropoietin being purified in a
5 manner different from that of urinary erythropoietin
6 based on that work that you've done?

7 A. Yes.

8 Q. Now, do you have an understanding as to
9 what the specific activity was as to the urinary
10 erythropoietin that you obtained from Kumamoto?

11 MS. BEN-AMI: Objection.

12 THE WITNESS: We published the number of,
13 if I remember right, 70,000 units per milligram of
14 protein. That was a rounded off number. It varied
15 from somewhat lower than that to almost twice that.

16 BY MR. MADRID:

17 Q. Do you have an understanding of what
18 specific activity is?

19 A. Yes.

20 Q. What is it?

21 A. Units of activity per milligram of protein
22 as we defined it.

23 Q. And are those units of activity in a
24 biological sense or in some other sense?

25 A. The units are biological activity as

1 referred to a standard reference preparation.

2 Q. Now, do you know what the specific
3 activity is as to the recombinant epo produced by
4 AMGen from CHO cells?

5 MS. BEN-AMI: Objection.

6 THE WITNESS: I think I can remember the
7 number. I think it's on the order of 160,000 units
8 per milligram, something like that.

9 BY MR. MADRID:

10 Q. Do you know whether or not urinary and
11 recombinant epo have the same or different specific
12 activities?

13 A. Yes, I do.

14 Q. What do you know?

15 A. They're different.

16 Q. And how do they differ?

17 A. The urinary erythropoietin has a lower
18 specific activity.

19 Q. When did you first come to this
20 understanding?

21 A. Understanding of the difference?

22 Q. Yes.

23 A. When I learned what the specific activity
24 of the recombinant erythropoietin was.

25 Q. Did you examine -- let me withdraw that.

1 Did you ever examine why the specific
2 activity of urinary erythropoietin and recombinant
3 erythropoietin differ?

4 A. Yes.

5 Q. When was that?

6 A. I can't tell you the year, but the paper
7 was published in about '97, '98, something like
8 that.

9 Q. And why did you make such examination?

10 A. I was curious to see what the difference
11 was, why there was such a difference.

12 Q. Did you make any scientific findings with
13 respect to why urinary erythropoietin differs from
14 recombinant erythropoietin --

15 A. Yes.

16 Q. -- in specific activity?

17 A. Yes, I did.

18 Q. And what were your findings?

19 MS. BEN-AMI: Objection, calls for expert
20 testimony.

21 THE WITNESS: Our conclusion was that
22 there was a probable conformational difference in
23 the two preparations, and we had evidence to
24 substantiate or at least justify that conclusion.

25

1 BY MR. MADRID:

2 Q. What do you mean by conformational
3 difference?

4 A. The way the molecule is folded up.

5 Q. Is that a difference in structure?

6 A. Yes.

7 Q. I want to show you what I believe is a
8 grant proposal of yours.

9 This is Goldwasser Exhibit 41, and it
10 bears the Bates 00146339 and it continues through to
11 00146346, Goldwasser Exhibit 41.

12 (Exhibit 41 marked as
13 requested.)

14 MR. MADRID: Counsel, I'm going to need
15 that notebook in a few seconds, so if you can finish
16 taking a look at it.

17 MS. BEN-AMI: I'm not done inspecting it.
18 I need to take a break to inspect it. I'll be happy
19 to give it to you, but I need it back.

20 MR. MADRID: When will you be returning it
21 to us?

22 MS. BEN-AMI: Today.

23 MR. MADRID: What time?

24 MS. BEN-AMI: I don't know. I don't know
25 when the deposition is over. I don't know when

1 you're going to take a break. I'm not going to tear
2 apart your notebook.

3 BY MR. MADRID:

4 Q. So take a moment to take a look at that
5 exhibit.

6 Have you had an opportunity to look at
7 Goldwasser 41?

8 A. Yes.

9 Q. What is it?

10 A. It's an application for a continuation
11 grant, which in essence is essentially a formality
12 at NIH.

13 Q. Did you prepare this document?

14 A. Yes.

15 Q. I want to direct your attention to the
16 page that has the Bates number AM-ITC 00146344. If
17 you look on this page, there's a series of numbered
18 entries, and then there's a lettered entry D. It's
19 about midpoint on the page.

20 Do you see that?

21 A. Yes.

22 Q. And I'll read it for the record. It says,
23 our study of the structure function relationship of
24 both urinary and recombinant epo has shown a very
25 clear difference between them.

1 Do you see that?

2 A. Yes.

3 Q. Did you believe this to be a true and
4 correct statement when you wrote it?

5 A. Yes.

6 Q. Does it remain true and correct as far as
7 you know?

8 A. Yes.

9 MS. BEN-AMI: Objection.

10 BY MR. MADRID:

11 Q. Now, the reference in this sentence to
12 urinary erythropoietin, is this the same urinary
13 erythropoietin that was given to the three patients
14 in the three-patient study?

15 A. I'd have to check the notebook to know.

16 Q. Was it produced by the Miyake purification
17 method?

18 A. Yes.

19 Q. That is the urinary erythropoietin that's
20 referred to in this Goldwasser Exhibit 41?

21 A. Yes.

22 Q. Now, what did you mean when you wrote our
23 study of the structure function relationship of both
24 urinary and recombinant epo has shown a very clear
25 difference between them?

1 A. I meant that there was a very clear
2 difference between them. We found a number of
3 differences, structural, chemical differences
4 between the two of them.

5 Q. And after the term for this grant, did you
6 continue to study the structure function
7 relationship of both the urinary and recombinant
8 erythropoietin?

9 A. I think so. The dates are not clear in my
10 mind, but I think we published a paper on it
11 sometime after this, the date of this proposal.

12 Q. When was this proposal prepared?

13 A. When was it prepared? Well, it was signed
14 in April '88.

15 Q. Okay. So did you submit GW 41 to the NIH
16 in or about the spring of 1988?

17 A. Yes. It was sent in that month.

18 MR. MADRID: All right. So let's take a
19 break, ten-minute break.

20 THE VIDEOGRAPHER: The time is now 3:31.
21 We are going off the record.

22 (Whereupon, a short recess was
23 taken.)

24 THE VIDEOGRAPHER: This is the end of
25 Videotape Number 4, Volume 2, in the deposition of

1 Eugene Goldwasser.

2 (Whereupon, a short recess was
3 taken.)

4 THE VIDEOGRAPHER: This marks the
5 beginning of Videotape Number 5, Volume 2, in the
6 deposition of Eugene Goldwasser. The time is now
7 3:45 p.m.

8 Please continue.

9 BY MR. MADRID:

10 Q. Dr. Goldwasser, in your deposition, in
11 this deposition on February 14, you referred to a
12 publication from 1997, and just before the break
13 earlier, you referred to a publication from 1997. I
14 want to show you a document and see if you recognize
15 this.

16 The document is marked Goldwasser 42, and
17 it bears the Bates numbers AM-ITC 00991084 through
18 88. It bears the title A Probable Conformational
19 Difference Between Recombinant and Urinary
20 Erythropoietins, Goldwasser 42.

21 (Exhibit 42 marked as
22 requested.)

23 BY MR. MADRID:

24 Q. Would you please take a look at that and
25 tell me if you recognize that document.

1 A. I do.

2 Q. What is it?

3 A. It's a paper we published in 1997.

4 Q. Now, is this the paper you were referring
5 to earlier in your testimony when you talked about
6 publishing in 1997?

7 A. I think so, the paper about the difference
8 between the two forms, yeah.

9 Q. Okay. Did you participate in authoring
10 this publication?

11 A. I wrote it.

12 Q. And were you involved in doing the
13 experimental work that underlies this publication?

14 A. I designed the experiments, and
15 Charles Kung did the actual handling of it.

16 Q. Were you involved in supervising
17 Charles Kung in handling those experiments?

18 A. Supervising is a strong word. He knew
19 what to do. I didn't have to tell him what to do.

20 Q. Okay. How long have you been working with
21 Mr. Kung?

22 A. About 35 years.

23 Q. Now, with respect to this publication,
24 GW 42, what was the source of the urinary
25 erythropoietin material that's discussed in that

1 publication?

2 A. It was -- do you mind if I check in here?

3 Q. Sure, please go ahead.

4 A. It's somewhere in here.

5 Q. Let me ask you a separate question and
6 direct your attention to the first page of GW 42,
7 the second paragraph where it says, in the present
8 paper, we demonstrate the differences between u-epo,
9 open paren, the beta form, and there's a footnote
10 there, and r-epo with a cross symbol with respect to
11 ease of iodination and to inactivation by iodine.

12 Do you see that?

13 A. Yes.

14 Q. Okay. Now, does that refresh your
15 recollection as to what the source of the urinary
16 erythropoietin was that was examined with respect to
17 the publication G 42?

18 A. Yes. It is the beta fraction or
19 fraction 3 from a hydroxyl appetite column of the
20 original preparation of urinary epo.

21 Q. Now, is this the same urinary
22 erythropoietin that was administered to the three
23 patients in the three-patient experiment we've
24 talked about today?

25 A. Yes.

1 MS. BEN-AMI: Objection.

2 BY MR. MADRID:

3 Q. How do you know that?

4 A. It's the only one we called beta epo.

5 Q. Now, what was the source of the
6 recombinantly produced human epo that are discussed
7 in this publication, Goldwasser 42?

8 A. It came from AMGen.

9 Q. Now, in the experimental work for this
10 publication, did you compare the urinary and
11 recombinant erythropoietins by means of
12 accessibility to iodination?

13 A. Yes.

14 Q. What does accessibility to iodination
15 measure?

16 A. It's an indirect indication of something
17 about the structure and the environment of the
18 tyrosines that get labeled with iodine.

19 Q. Based on your work, did you make any
20 scientific findings as to whether or not urinary
21 erythropoietin and recombinant erythropoietin differ
22 with respect to accessibility to iodination?

23 A. We did.

24 MS. BEN-AMI: Objection, expert testimony.

25

1 BY MR. MADRID:

2 Q. What were your findings?

3 A. The urinary epo had much more, or the
4 tyrosines, tyrosine 15 to be specific of urinary
5 epo, was much more available or accessible from the
6 solvent than the recombinant epo.

7 Q. Now, I want to direct your attention to
8 Figure 1 of this paper, which would appear on Bates
9 00991085.

10 Do you see that?

11 A. Yes.

12 Q. Did you make any findings as to the data
13 reflected in Figure 1 of the publication?

14 A. Those data again indicate -- it's hard
15 to -- that the urinary epo would only be iodinated
16 to the extent of about one iodine per molecule
17 whereas the recombinant went up much higher than
18 that.

19 Q. Based on your findings and your work
20 that's reflected in Goldwasser 42, do you know
21 whether or not a difference in accessibility to
22 iodination is consistent or inconsistent with a
23 difference in structural conformation as between
24 urinary erythropoietin and recombinant
25 erythropoietin?

1 MS. BEN-AMI: Objection, calls for expert
2 testimony.

3 THE WITNESS: The inference you draw from
4 those experiments is that the conformation is
5 different.

6 BY MR. MADRID:

7 Q. Did you draw that inference?

8 A. Yes.

9 Q. Do you continue to believe that the
10 conformation is different on the basis of
11 iodination?

12 MS. BEN-AMI: Objection, calls for expert
13 testimony.

14 THE WITNESS: I used all the data to draw
15 the conclusion about the difference in conformation,
16 not the one experiment.

17 BY MR. MADRID:

18 Q. Okay, fair enough. Now, in this
19 experimental work that's in GW 42, did you compare
20 urinary and recombinant erythropoietin with respect
21 to inactivation by iodination?

22 A. Yes.

23 Q. And what does inactivation by iodination
24 measure?

25 A. Loss of biological activity.

1 Q. And based on your work, the work that was
2 done for Goldwasser 42, did you make any scientific
3 findings as to whether or not urinary erythropoietin
4 and recombinant erythropoietin differ with respect
5 to inactivation by iodination?

6 A. Yes.

7 MS. BEN-AMI: Objection, calls for expert
8 testimony.

9 BY MR. MADRID:

10 Q. What were your findings?

11 A. The urinary erythropoietin was almost
12 completely inactivated by substitution of two
13 iodines. Recombinant erythropoietin was much
14 more -- much less affected by substitution of
15 iodine.

16 Q. Do you draw -- did you -- I'm sorry, let
17 me withdraw that.

18 Did you draw any inference from that
19 finding?

20 MS. BEN-AMI: Objection, calls for expert
21 testimony.

22 THE WITNESS: We inferred that there was
23 at least one tyrosine in urinary erythropoietin
24 which was essential for its biological activity
25 which was -- and that biological activity was

1 destroyed by putting that iodine on there.

2 BY MR. MADRID:

3 Q. Now, based on your finding, the findings
4 in Goldwasser 42, do you know whether a difference
5 in inactivation by iodination is consistent or
6 inconsistent with a difference in structural
7 conformation as between urinary erythropoietin and
8 recombinant erythropoietin?

9 MS. BEN-AMI: Objection, calls for expert
10 testimony.

11 THE WITNESS: To put it the other way
12 around, we inferred the conformation difference from
13 the experimental results.

14 BY MR. MADRID:

15 Q. In the experimental work for this
16 publication, Goldwasser 42, did you compare the
17 urinary and recombinant erythropoietin products by
18 means of trypsin inactivation?

19 A. Yes.

20 Q. What does trypsin inactivation measure?

21 A. The sensitivity of the protein backbone,
22 the peptide bonds of the protein to a proteolytic
23 enzyme, trypsin.

24 Q. And did you make any scientific findings
25 with respect to whether or not urinary

1 erythropoietin and recombinant erythropoietin differ
2 with respect to trypsin inactivation?

3 A. We did.

4 Q. What were those findings?

5 MS. BEN-AMI: Objection, calls for expert
6 testimony.

7 THE WITNESS: Urinary erythropoietin was
8 much more sensitive to tryptic hydrolysis than
9 recombinant.

10 BY MR. MADRID:

11 Q. Did you draw any inferences from that,
12 from those findings?

13 MS. BEN-AMI: Same objection.

14 THE WITNESS: Once again, it suggests that
15 there's a difference in conformation between those
16 two molecules.

17 BY MR. MADRID:

18 Q. Between urinary erythropoietin and
19 recombinant erythropoietin?

20 A. Yes.

21 Q. In the experimental work for
22 Goldwasser 42, did you compare urinary and
23 recombinant erythropoietin by means of circular
24 dichroism?

25 A. Yes.

1 MS. BEN-AMI: Objection. And I would
2 point out we have gone well beyond the scope of
3 direct for quite some time. This is your direct
4 exam, but I mean you've gone well beyond the scope
5 of direct.

6 MR. MADRID: First of all, you're making a
7 speech. Secondly, that's not correct. In point of
8 fact, the subject of differences was raised in the
9 examination on the 14th.

10 BY MR. MADRID:

11 Q. Doctor --

12 MS. BEN-AMI: Could you point that out to
13 me?

14 MR. MADRID: I'll be happy to, but now is
15 not the proper time.

16 BY MR. MADRID:

17 Q. Doctor, what does circular dichroism
18 measure?

19 MS. BEN-AMI: Objection, beyond the scope
20 of direct, it's calls for expert testimony.

21 THE WITNESS: It's a crude measure of the
22 folding up of the molecule.

23 BY MR. MADRID:

24 Q. Based on your work, did you make any
25 scientific findings as to whether or not urinary

1 erythropoietin and recombinant epo differ with
2 respect to circular dichroism?

3 A. We did.

4 Q. And what were those findings?

5 A. They differed.

6 Q. Did you draw any inferences on those, from
7 those findings?

8 A. That there was a probable conformation --

9 MS. BEN-AMI: Objection, expert testimony.
10 I'm sorry.

11 THE WITNESS: That there was a probable
12 conformational difference.

13 BY MR. MADRID:

14 Q. When you say that there was a probable
15 conformational, conformational difference, was that
16 as between the urinary erythropoietin and the
17 recombinant erythropoietin?

18 A. Yes.

19 Q. Doctor, I'd like to direct your attention
20 to the portion of your deposition testimony that was
21 taken on February 14 on Page 178 of the transcript.
22 Now, there's a question there on 178 at Line 3, and
23 I'm going to read the testimony that follows:

24 "QUESTION: Okay. So what was your
25 understanding of why the iodination of the epo

1 inactivated it?

2 "ANSWER: Because as we published some
3 years later, the tyrosine in urinary
4 erythropoietin at position 15 was very much
5 involved with the binding to the receptor and
6 therefore the biological activity, and by
7 putting the bulky iodine in, you got -- you
8 changed the structure so that it no longer had
9 any biological activity.

10 "QUESTION: Did you know that in 1983?

11 "ANSWER: No.

12 "When did you learn that?

13 "ANSWER: '97 or something like that.

14 "QUESTION: 1997?

15 "ANSWER: '97 I think.

16 "QUESTION: Yeah, okay.

17 "ANSWER: Whenever we published that
18 paper."

19 Doctor, can you tell me whether or not the
20 reference in your testimony, published that paper,
21 is Goldwasser Exhibit 42 that we've been looking at
22 a reference to the '97 paper that's being referred
23 to in your testimony?

24 A. Yes.

25 Q. Now, getting back to Goldwasser 42,

1 looking at the experimental work for that
2 publication, did you compare urinary and recombinant
3 erythropoietin by means of second derivative
4 spectra?

5 A. Yes.

6 Q. And what does second derivative spectra
7 measure?

8 A. That too is a crude indicator of the
9 environment of the amino acid side chains that
10 absorb ultraviolet light, which in this case are
11 mostly tyrosines.

12 Q. Did you make any findings with respect to
13 second derivative spectra?

14 A. Yes.

15 Q. What were those findings?

16 MS. BEN-AMI: Objection, calls for expert
17 testimony, beyond the scope of direct.

18 THE WITNESS: There was a small difference
19 in the exposure of tyrosines to the solvent.

20 BY MR. MADRID:

21 Q. And did you draw any inferences on the
22 basis of those findings?

23 A. Once again, that there was a difference
24 between those two molecules.

25 MS. BEN-AMI: Objection, calls for expert