Exhibit C

Roche Parties
EF Fritsch
2nd Witness Statement and Expert Report
Exhibit EFF 4
11th December 2000

IN THE HIGH COURT OF JUSTICE **CH 1993-K-No. 937**
CH 1993 B-No. 4552

CHANCERY DIVISION

PATENTS COURT

In the matter of European Patents (UK) Nos. 148,605 and 411,678 and in the matter of actions for infringement and counterclaims for revocation thereof by inter alia Kirin-Amgen Incorporated, Janssen-Cilag Limited and Roche Diagnostics GmbH

SECOND WITNESS STATEMENT AND EXPERT REPORT OF EDWARD F FRITSCH

I, **EDWARD F FRITSCH** of 35 Cambridge Park Drive, Cambridge, Massachusetts, will say as follows:-

1. INTRODUCTION

1.1. I am the same Edward Fritsch who signed a witness statement dated 8th November 2000 in these proceedings. A copy of my CV, which includes a list of my publications, is attached at EFF 4.

1.2. I have been asked by the Roche parties' solicitors to reply to certain aspects of the report of Professor Wall submitted on behalf of the Kirin-Amgen parties. In doing so, I give details of the work that I carried out at Genetics Institute ("GI") that led to the identification and cloning of the human EPO ("huEPO") gene and huEPO cDNA. I have attempted to be as accurate as possible, given that the events took place 16-18 years ago. I have also been asked to give expert evidence on the availability of tissue sources which could have been used to make a cDNA library for use in the isolation of the huEPO cDNA.

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1.3. I was a post-doctoral fellow in the laboratory of Dr Tom Maniatis at California Institute of Technology ("Cal Tech") from April 1978 to April 1980. During this period I worked with Tom on the cloning and characterisation of the human globin gene family. This work involved the construction of a genomic DNA ("gDNA") library that is referred to as the Maniatis or Lawn library. We had calculated that there was a greater than 99% probability that a given DNA sequence would be present in a library of this size. Part of our work was reported in a paper that I co-authored (Lawn *et al.* (1978)).

1.4. As this library was generally regarded to be an excellent library, it was the subject of many requests to the Maniatis laboratory for aliquots. Many aliquots were supplied and this was the library subsequently used by both myself and by Dr Lin at Amgen to isolate the huEPO gene. By about the end of 1982, however, the original library had been substantially exhausted and was no longer being supplied. An amplification of the library was still available at this time, but this was not of the same quality as the original library. The probability that any given sequence would be represented in the amplified library had been significantly reduced.

1.5. Between 1980 and 1983 I was a co-instructor in the Cold Spring Harbor course in Molecular Cloning along with Tom Maniatis. The director of Cold Spring Harbor was Professor James Watson who, along with Professor Francis Crick, received the Nobel prize for his work on the double helix structure of DNA. For many years, Cold Spring Harbor laboratories sponsored courses in state of the art technologies, such as molecular cloning, for leading research scientists around the world. The Molecular Cloning course was highly regarded and attracted numerous applications from all over the world.

1.6. Following the 1980 course (which was the first Molecular Cloning course), Tom Maniatis, Joe Sambrook (a leading research scientist at Cold Spring Harbor) and I co-authored the standard laboratory textbook "Molecular Cloning – A Laboratory

Manual" which was published in 1982. A second edition, significantly expanded, was published in 1989.

2. GI'S EPO PROJECT

2.1. In paragraph 94(c) of his report, Professor Wall says that "*none of the teams of investigators who were endeavoring to clone the epo gene chose to use the route which Dr Lin adopted.*" In fact, as I explain below, the route that GI used to clone the huEPO gene was the same as that used by Dr Lin. I firmly believe that the only reason that Dr Lin succeeded in cloning the huEPO gene before GI was because Amgen had access to sufficient quantities of urinary EPO ("uEPO") sooner than GI. As I explain below, GI also succeeded in cloning huEPO cDNA. We reported this work in Nature (Jacobs *et al* (1985)). This was the first time that either the huEPO cDNA or gDNA sequences had been published. We were also the first group to identify a tissue source from which a cDNA library containing huEPO cDNA could be made.

2.2. I discussed the possibility of establishing a project to isolate and clone the huEPO gene with Dr Maniatis, in December 1980, prior to joining GI as a full time employee. Dr Maniatis was one of the founders of GI. We decided that this would be a suitable project for me because of my prior work in the field of erythroid cell gene expression at Cal Tech and Michigan State University. We saw huEPO as a suitable candidate protein for production by recombinant DNA technology and recognized that it was a potentially valuable product.

2.3. Only very small amounts of uEPO had been isolated from human urine, collected mainly from Japanese patients suffering from aplastic anemia. European and North American physicians would treat patients to prevent them from remaining highly anemic. In Japan, however, patients were allowed to remain more anemic, and this meant that their urine contained very much higher levels of uEPO.

2.4. At the outset, we identified two potential difficulties with the project. Firstly, there was no known tissue source from which a human cDNA library containing huEPO cDNA could be constructed. Prior to this time all efforts to clone DNA based on protein sequence information had been carried out using cDNA libraries.

2.5. The second potential difficulty was that only a very small quantity of uEPO had ever been isolated. Correct protein sequence information was needed in order to design probes that could be used to screen any DNA library. GI had access to only limited amino acid sequence information from Dr Rodney Hewick. Before joining GI from Cal Tech in 1981, Dr Hewick had developed a prototype of the gas phase sequenator, an improved protein sequencing machine, which became commercially available in 1982. While at Cal Tech, Dr Hewick was asked to demonstrate the capabilities of his prototype machine by conducting an N-terminal sequence analysis on a very small quantity of uEPO that had been purified by Dr Goldwasser's group. The sequence information obtained was limited to the first 26 amino acids and included two unassigned residues.

2.6. I decided that the approach we should follow would be to screen a DNA library with at least two sets of fully degenerate oligonucleotide probes. I believed that the use of two sets of probes which were complementary to different parts of the sequence would enable us to identify huEPO DNA in either a gDNA library or a suitable cDNA library (if one could be found).

2.7. I identified two approaches that we could use to design and construct the probes. The first relied on the N-terminal amino acid sequence information obtained from Dr Hewick. The second approach relied on obtaining more uEPO, which could be sequenced to obtain additional amino acid sequence information. This would allow us to design more specific probes based on the least degenerate region of the sequence. Because of the very significant difficulty of obtaining sufficient purified uEPO (and its cost), we recognized that substantial additional financial resources

would be needed for this approach. As I explain below, this certainly proved to be the case.

2.8. I concluded that there was a distinct advantage in screening a gDNA library because there was a very high probability that the huEPO gene would be present in the gDNA library whereas there was no firm evidence that any particular cDNA library would include the huEPO cDNA.

2.9. Although I was aware of the potential difficulties of screening the gDNA library with mixed oligonucleotide probes, I believed that the project stood a reasonably high chance of success given accurate sequence information to design probes. When I joined, in April 1982, GI was a small start-up company with only 28 full time employees. I would not have recommended that we commit my time and the company's resources to the EPO project without being reasonably confident that we would succeed in obtaining a recombinant biologically active EPO.

2.10. I estimated that it would take about six months to verify whether the first approach (using the existing Hewick sequence) could be successful and about 6-18 months to complete the second approach (obtaining uEPO for sequencing). In the end, we were not able to isolate the huEPO gene using the existing Hewick sequence because of an error in the sequence. As I will explain below, once we had obtained uEPO in April 1984, I was able to isolate the huEPO gene from a gDNA library within 3 months.

3. POTENTIAL SOURCES OF uEPO

3.1. Dr Miyake, through his contacts in Japan, controlled the major source of Japanese aplastic anemic human urine. Such urine was generally acknowledged to be the only viable source of uEPO.

3.2. Dr Miyake had worked in the laboratory of Dr Goldwasser on a project funded by the National Institutes of Health (NIH) relating to the purification of uEPO. During this time, and for a while afterwards, Dr Miyake supplied Dr Goldwasser with the

crude urine. The results of this collaboration led to the publication of their paper on uEPO purification in 1977.

3.3. We discounted Dr Goldwasser as a potential source of uEPO from the beginning because we knew he had an arrangement to supply uEPO to Amgen and that it was unlikely that he would supply us with uEPO. Dr Goldwasser had released some purified uEPO for distribution via the NIH, but the amounts being distributed were not sufficient for sequencing.

3.4 Having identified Dr Miyake, who had left Dr Goldwasser's laboratory and was working at Wright State University, as a potential source of uEPO, GI entered into discussions with Dr Miyake for the supply of purified uEPO in 1982. Unfortunately, these discussions were not fruitful as Dr Miyake was requesting terms which were untenable for GI as a small start-up company.

4. GI'S INITIAL SCREENING ATTEMPTS

4.1. The sequence provided to me by Dr Hewick was as follows:

Ala Pro ??? Arg Leu Ile ??? Asp Ser Arg Val Leu Glu Arg Tyr Leu Leu Glu Ala Lys Glu Ala Glu Lys Ile Thr

4.2. I designed and ordered a number of sets of fully degenerate probes based on this sequence information. These ranged from 32 14-mers to 256 19-mers. I used these probes to screen the gDNA library but failed to isolate EPO DNA. In retrospect, this was because of the error at residue 24 of the Hewick sequence – in each case I used at least one probe based around this residue.

4.3. At the same time as screening the gDNA library using these probes, I was also using the same probes to screen cDNA libraries.

4.4. My work on screening libraries using probes based on the Hewick sequence started in October 1982 and continued until March 1984. By about April 1983, after a number of unsuccessful screens using these probes, we concluded that there was

probably an error in the sequence (the probes were hybridizing well and picking out clones but they were not huEPO clones) and we decided that we needed to obtain further sequence information.

4.5. We attempted to find other sources of huEPO for sequencing, but were unsuccessful. One of these potential sources was Dr Judith Sherwood of the Albert Einstein College of Medicine who claimed to have a renal carcinoma cell line producing huEPO. Dr Sherwood provided us with a sample of this cell line in November 1983, but it did not produce enough EPO for sequencing.

4.6. In January/February 1984 it became apparent that we were not going to obtain further huEPO without the help of Dr Miyake and GI re-entered negotiations with him. An agreement was subsequently reached for the supply of uEPO by Dr Miyake. We received the first shipment of uEPO from Dr Miyake in April 1984 and this was followed by three more shipments.

5. DESIGNING PROBES USING THE NEW SEQUENCE INFORMATION

5.1. Dr. Hewick passed the first sample received from Dr Miyake through a reverse phase HPLC column. He then digested the EPO with trypsin, separated the fragments using reverse phase HPLC and determined the sequence of a number of the fragments using a gas phase sequenator.

5.2. We were particularly interested in the sequence of the fragments designated as T-35 and T-30[1] as their UV absorbance indicated the presence of aromatic residues which are coded for by low degeneracy codons and which are therefore useful for making probes.

5.3. I designed a series of three different sets of probes from the T-35 fragment sequence information:

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[1] The fragments are named according to the time it takes them to come off the reverse phase HPLC e.g.. T-35 describes the fragment that came off the reverse phase HPLC after approximately 35 minutes.

(i) T35A 17-mer - 32 fold degenerate from amino acid residues 47-52;

(ii) T35B 18-mer - 128 fold degenerate from amino acid residues 46-51; and

(iii) T35C 20-mer - 128 fold degenerate from amino acid residues 46-52.

5.4. I also designed a set of 14-mer probes from the T-30 fragment sequence. These were 96 fold degenerate and were from amino acid residues 145 to 149. I received the 17-mer and 14-mer probes on 25th May 1984 and the 18-mer and 20-mer probes on 25th June 1984.

5.5. We first used the T35A 17-mer to screen a cDNA library made from the renal carcinoma cell line obtained from Dr Sherwood. This was not successful. I believe this was because the library did not contain EPO cDNA (as shown later by Northern analysis of the mRNA from the renal carcinoma cell line – see below).

5.6. We also used these probes to carry out a further screen of the Maniatis gDNA library. This work began on 31st May 1984. We used the T35A 17-mer probe followed by the T30 14-mer probe. We also used the T35B 18-mer and T35C 20-mer probes (when they became available) to provide more rapid confirmation of the correct clone.

5.7. We identified 2 phages that hybridized to these probes and these were designated "KSG-1" and "KSG-8".

5.8. These positive clones were sequenced by Dr Charles Shoemaker in GI's sequencing group in July. From this I concluded that we had isolated at least part of the huEPO gene. We subsequently confirmed that we had, in fact, isolated the entire huEPO gene.

5.9. We were therefore successful in isolating the huEPO gene within three months of obtaining the uEPO from Miyake. I believe that we would have been successful at a very much earlier stage in the project if we had a supply of uEPO from which we could obtain sequence information.

6. OBTAINING THE CDNA SEQUENCE FOR EPO

6.1. After successfully isolating the huEPO gene from the gDNA library, I continued my work on obtaining a cDNA clone for the expression of huEPO.

6.2. We had always wanted to express huEPO using the cDNA sequence rather than the genomic clone. Using a cDNA clone for expression has the advantage that the cell used to express the protein does not have to remove the introns and correctly splice the message. The splicing of introns imposes an extra burden on the cell and there is no guarantee that a cell that does not normally express the huEPO gene will remove the introns and splice the message in the same way or as efficiently as the cells naturally expressing the gene. There was also evidence that expressing heterologous genes from genomic clones in non-human mammalian cells could result in incorrect processing.

6.3. Isolating and sequencing a huEPO cDNA clone would also enable us to determine the intron/exon borders for the genomic huEPO clone.

6.4. I had several cDNA libraries available for screening, including ones made from fetal liver, adult liver and the renal carcinoma cell line obtained from Dr Sherwood. By this time, I also had available to me a 20 week old fetal liver phage library that had been constructed by Dr Jay Toole. Dr Toole was working on another project for GI and had constructed this library in the course of that project. It seemed to be a very good library, with a large number of clones.

6.5. I could have continued to use the probes based on the T-35 and T-30 sequences to screen these libraries (the only one I had screened, using these probes, was the renal carcinoma cDNA library) but this would have taken more time. Instead, I carried out Northern analyses[2] of mRNA from the tissue sources from which the libraries were made using an 87-mer primer-extended probe that corresponded to an 87 base

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[2] A Northern analysis involves separating mRNAs based on size in agarose gels and then transferring the mRNA to a nitro-cellulose or nylon filter, followed by hybridisation with a probe.

pair exon of the huEPO gene. We designated this probe "PE 87" or "PE". The point of doing this Northern analysis was to identify which, if any, of the cDNA libraries to focus the screening for the huEPO cDNA on.

6.6. In July 1984, I detected a single band in the lane on the Northern blot that corresponded to 20 week old human fetal liver mRNA. I concluded from this that 20 week old fetal liver was a source of huEPO mRNA and I decided to screen the 20 week old human fetal liver cDNA library to attempt to detect and isolate a full length huEPO cDNA.

6.7 I then began screening the 20 week old human fetal liver cDNA library using the PE probe. A number of positives showed up on the autoradiograms of the screenings. I rescreened the cDNA library and detected four positive phage. The DNA from these phage were sequenced (by Liz Orr) in August 1984 and I confirmed from this that we had isolated 3 independent clones containing sequence corresponding to huEPO mRNA.

6.8 Further characterization of the huEPO clones was carried out in August and September 1984. This involved additional sequencing experiments. Clone FL13 was shown to be a full length huEPO cDNA.

7. **POTENTIAL TISSUE SOURCES FOR huEPO mRNA**

7.1 I have been asked to explain what was known about potential tissue sources for huEPO mRNA in the early 1980s.

7.2 When I started work on the EPO project at GI, it was well established that in adults, kidney defects could result in decreased EPO production. But it was not known that EPO was actually produced in the kidney until the work of Farber *et al* (1983) showed that mRNA from the kidney of phenylhydrazine treated baboons could be translated in oocytes *in vitro* into biologically active EPO. Further, it was known

that EPO production was only significant in hypoxic conditions. Obtaining human kidney expressing the EPO gene was impractical. Various cell lines (mainly renal carcinoma cell lines) had been mentioned in the literature with claims that they produced EPO. Dr Sherwood's cell line was one of these – as we discovered, it did not produce detectable amounts of EPO mRNA.

7.3 I was aware that human fetal liver had been suggested as a possible source of EPO mRNA. However, as far as I can recall, the papers which made these suggestions were inconclusive. None of these papers are referred to in the '605 patent. I do not know whether other molecular biologists working in the field were aware of these papers or not.

7.4 It was certainly not known that fetal liver would be a source of EPO mRNA to enable the construction of a cDNA library which contained EPO cDNA. It was not clear that EPO was actually produced in the fetal liver as opposed to being produced elsewhere and eventually released from the liver. In the latter case, mRNA would not be present in the liver. Even if EPO were produced in the liver, it was not known how much EPO mRNA would be present. As in adults the levels of EPO production in the fetus are likely to be affected by the level of oxygen in the blood. This would have meant that the production of EPO mRNA might only be switched on in response to certain physiological conditions.

7.5 There were also uncertainties arising out of the fact that gene expression in fetal development can be a complex, time-dependent event. First, it was known that, in several cases, the genes expressed during fetal development are different from those expressed in the adult. The globin genes are perhaps the best-known example of this phenomenon. The human genome has a number of different, homologous globin genes which are expressed at different times during development. If EPO expression had been similar, a human fetal liver cDNA library might have contained a fetal huEPO cDNA which was not homologous enough to adult huEPO probes to be isolated. Alternatively, the fetal huEPO cDNA could have been mistakenly

isolated in the place of the desired adult huEPO cDNA. The problem of differential gene expression in the adult and fetus in the context of probing a human fetal liver cDNA library for huEPO cDNA was recognized by Browne *et al.* (1986) at p.700.

7.6 Secondly, it was known that expression of a gene during development could be time-dependent. In other words, a gene might not need to be expressed early during fetal development, may be switched on for a short period during fetal development and eventually become dormant as the production switches to another tissue. If this were so for EPO, then success in obtaining EPO mRNA from the fetal liver would critically depend on the age of the fetus. It is now known that EPO expression in the fetal liver of some species is indeed time-dependent. It is believed that the same is true of human fetal EPO expression, though even now it is not known quite what the time-dependence is.

7.7 As I have said, the 20 week old fetal liver library from which I isolated the huEPO cDNA and the mRNA which I used in the Northern analysis had been prepared in Jay Toole's laboratory when working on another project and just happened to be available to me at the time. In retrospect, having liver from this particular stage of fetal development was an extremely lucky break. So far as I am aware, no one has ever succeeded in isolating huEPO cDNA from any cDNA library other than one made from 20 week old fetal liver.

7.8 Also, because of the low abundance of huEPO mRNA it was fortunate that the library prepared by Dr Toole contained a large number of independent clones. Based on our screening of the 20 week old fetal liver library, the abundance of the huEPO cDNA clones was one in 300,000. This is comparable to the abundance of a single copy gene in a human gDNA library.

7.9 In paragraph 111 of his report, Professor Wall says that "*armed with exact probes it was then possible to identify cell or tissue sources of EPO such as fetal liver cells and to obtain cDNA copies from them when this had previously not been feasible.*"

7.10 Professor Wall makes it sound as if, once one had the huEPO gene sequence and could design an exact probe, finding a cell or tissue source for huEPO and then huEPO cDNA would have been easy. I do not agree. Finding a suitable tissue source was still a research project. After all, the lack of a suitable tissue source had been one of the major obstacles to cloning huEPO and the '605 patent does not identify a suitable tissue source.

7.11 In fact, only 20 week old fetal liver has proved to be a viable source of huEPO from which a suitable cDNA library can be constructed. We were very fortunate to have both the tissue source and the library available. Had I not had the luck of having a good 20 week old fetal liver cDNA library at hand, I might have spent months looking for a suitable tissue source without any success.

7.12 Secondly, I disagree with Professor Wall's statement that it had previously not been feasible to isolate an EPO cDNA clone in the absence of an exact probe. In my view a skilled worker could have obtained huEPO cDNA from a good 20 week old fetal liver cDNA library using mixed oligonucleotide probes based on correct huEPO amino acid sequence. The key was not having an exact probe but having a good cDNA library from the correct tissue source.

7.13 Obviously, in screening a cDNA library an exact probe, if available, would be preferable to using mixed oligonucleotide probes. That is why I screened the 20 week old fetal liver library with the PE probe. But in my view it was not necessary and mixed oligonucleotide probes based on the correct amino acid sequence would have also led to success as this approach had been successful in isolating other cDNAs and the genomic huEPO clone.

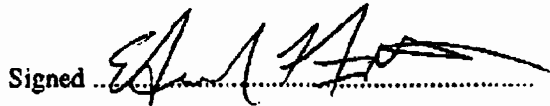
8. THE CELL EXPRESSION ROUTE TO cDNA

8.1 In paragraph 109 of his report, Professor Wall claims that it was "*well known in 1983 that a gene of interest could be sub-cloned into an expression vector and transfected into a host cell to produce mRNA from which cDNA could be prepared.*" In paragraph 111 of his report Professor Wall says that by this method a skilled worker could "easily" obtain a cDNA clone for expression of EPO.

8.2 To the best of my recollection, by 1984, I had not heard of this technique being used successfully. Expression of a genomic clone in a heterologous cell was not a technique that I would have or did adopt as a way of obtaining huEPO cDNA once I had isolated the genomic clone. Instead, as I explained above, I continued to try to isolate huEPO cDNA from cDNA libraries made from natural tissue sources.

8.3 The cell expression route to cDNA is not mentioned in the 1982 or 1989 editions of the Molecular Cloning Manual and, to the best of my recollection, is not a method we ever taught to our students in the Cold Spring Harbor course.

I understand my duty to the Court and have complied with that duty. I believe that the facts I have stated in this statement and report are true and that the opinions I have expressed are correct.

Signed 

EDWARD F FRITSCH

Dated 12/11/00

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