Patent

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

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Patent Owner:	Genentech, Inc. and City of Hope			

For: Merged Reexaminations of U.S. Patent No. 6,331,415 (Cabilly *et al.*)

DECLARATION OF STEVEN LANIER MCKNIGHT UNDER 37 C.F.R. § 1.132

I, Steven Lanier McKnight, do hereby declare and state

1. I am a citizen of the United States and reside in Dallas, Texas. My c.v. is attached as Exhibit A.

2. I have been retained by Genentech and City of Hope to provide my opinion on certain issues in the patent reexamination proceedings involving U.S. Patent No. 6,331,415 ("the '415 patent"). I am being compensated for my time at a rate of $750.00 per hour.

3. I have reviewed the following documents in the course of preparing this declaration:

- U.S. Patent No. 5,840,545 ("the '545 patent");
- U.S. Application No. 06/358,414 ("the '414 application");
- The '415 patent;
- U.S. Patent No. 4,816,567 ("the '567 patent");
- U.S. Patent No. 4,399,216 ("Axel");
- Deacon & Ebringer, BIOCHEMICAL SOCIETY TRANSACTIONS 4: 818-820 (1976) ("Deacon");
- European Patent No. 0 044 722 ("Kaplan");
- Ochi *et al.*, NATURE 302: 340-342 (1983) ("Ochi");

- Oi *et al.*, PROC. NATL. ACAD. SCI. 80: 825-829 (1983) ("Oi");
- Rice & Baltimore, PROC. NATL. ACAD. SCI. 79: 7862-7865 (1982) ("Rice");
- Valle *et al.*, NATURE 300: 71-74 (1982) ("Valle 1982");
- Valle *et al.*, NATURE 291: 338-340 (1981) ("Valle 1981");
- WO 82/03088 ("Dallas");
- The Declaration of Richard Axel filed during prosecution of U.S. Application No. 08/422,187;
- Opposition Request of European Patent No. 0120694 filed in the European Patent Office on behalf of Genentech, Inc.

4. I have also reviewed documents associated with the two reexamination proceedings, including:

- The PTO Office Action dated February 16, 2007;
- The PTO Office Action dated August 16, 2006;
- A Request for Ex Parte Reexamination dated December 23, 2005, including attachments to that Request;
- The Declaration of David Baltimore submitted in connection with the December 23, 2005 Request for Ex Parte Reexamination;
- The Declarations of Dr. Rice, Dr. Colman, and Dr. Harris filed with the responses of the patent owner to the two office actions

5. I understand that patentability is evaluated using the perspective of a person of ordinary skill in the technical field of the invention just prior to the filing date of the patent (*i.e.*, early April of 1983). A person of ordinary skill in the field of the '415 patent would have had a Ph.D. in molecular biology or a comparable scientific discipline, and two to three years of practical experience, such as that gained through a post-doctoral appointment or comparable assignment. I believe I am well-qualified to express an opinion on what a person of ordinary skill in the art of the '415 patent would have believed or expected in early April of 1983, because at that time I was a person who had a level of experience in line with this definition and worked with people who met this definition.

6. I understand that the '545 patent issued from an application filed on June 5, 1995. I also understand that there were several earlier applications filed between 1982 and 1995 related to the '545 patent. I understand that the first of these applications was the '414 application filed in March of 1982, and that the contents of this application are to be the

focus of my analysis. In particular, I have been asked to determine if the '414 application describes a host cell that produces two different immunoglobulin chain polypeptides or a process where two different polypeptides are expressed in a single host cell. I understand that the requirements of the host cell and process are outlined in the claims of the '545 patent.

7. I also have been asked to determine if there is any description in the '414 application of procedures for coexpressing two different polypeptides in a single host cell. Finally, I have been asked to provide my views on the observations of the PTO contained in the Final Office Action dated February 17, 2007.

General Observations On The '414 Application

8. The '414 application describes procedures for making what it calls an rFv binding composition, or rFv. An rFv consists of two polypeptides, each with an amino acid sequence that corresponds to the variable region sequence of an immunoglobulin chain. An "L-rFv" polypeptide contains a variable region sequence from a light chain immunoglobulin, and an "H-rFv" contains a variable region sequence from a heavy chain immunoglobulin.

9. The '414 application indicates that an rFv can contain two polypeptides with the same amino acid sequence, or with different amino acid sequences. *See*, p. 3, line 37 to p. 4, line 2 ("the L- and H- designations will normally mean light and heavy respectively, but in some instances the two chains [of the rFv] may be the same and derived from either the light or heavy chain sequences.").

10. Pages 5 to 18 of the '414 application provide a general description of procedures for producing L-rFv and H-rFv polypeptides, and rFv binding compositions. The '414 application also provides an example of using these procedures on pages 19-42 ("Example 1"). These procedures can be summarized as follows:

    a. Produce a hybridoma that makes an antibody with a desired specificity. *See*, p. 5, line 32 to p. 6, line 18.

    b. Prepare a purified whole cell mRNA extract from the hybridoma, and use this to prepare a cDNA library using a reverse transcriptase. *See*, p. 6, line 19 to p. 8, line 7. This produces cDNA molecules with sequences that are complementary to each of the discrete mRNA sequences (mRNA transcripts) in the mRNA extract.

    c. Amplify the cDNA library. This is done by inserting the cDNA molecules into plasmids, transforming a bacterial host cell culture with the plasmids, and growing the transformed bacterial host cells under selective pressure (*i.e.*, in the presence of an agent that causes bacterial cells that did not incorporate a plasmid to die). This produces a collection of bacterial clones, each containing a plasmid with one of the cDNA molecules from the cDNA library in it. *See*, p. 8, line 12 to p. 9, line 1.

d. Identify colonies of transformed bacterial cells that contain plasmids with cDNA encoding either the heavy or the light chain using a nucleotide probe corresponding to the constant domain of the heavy or the light chain. Then, select these colonies and grow the colonies under selective pressure to produce a population of identical copies (clones) of the bacterium with the desired heavy or light chain cDNA sequence. *See*, p. 9, lines 1-19.

e. Extract the cDNA from the individual clone selected by colony hybridization, and use it to produce a "tailored" cDNA that encodes the variable region of either the heavy or the light chain polypeptide. *See*, p. 9, line 20 to p. 14, line 15.

f. Insert the modified cDNA into an expression vector (*i.e.*, a plasmid containing an origin of replication, a promoter, and an insertion site), and transform another bacterial host (*E. coli*) with the plasmid. *See*, p. 14, line 16 to p. 16, line 23.

g. Express either the light or heavy chain variable region polypeptide by growing a transformed bacterial host cell, and then isolate, purify, and renature the polypeptide. *See*, p. 17, line 1 to p. 18, line 14. Repeat the process with the other immunoglobulin chain.

h. Combine the individually produced chains *in vitro* to form the rFv binding composition. *See*, p. 16, lines 24-28.

11. If these procedures are followed as they are written, individual L-rFv and H-rFv polypeptides will be produced in separate cell cultures and these individually prepared polypeptides will be isolated, renatured, and combined in a test tube to form an rFv. I did not find any description of procedures in the '414 application of a "coexpression" strategy (*i.e.*, where two polypeptides with different amino acid sequences would be produced in a single transformed cell culture).

12. All of the processes described in the '414 application relate to bacterial expression systems. There are some references to the use of yeast cell cultures to amplify DNA sequences, but there are no procedures described in the '414 application for expressing proteins in yeast-based systems. There is also no description of using mammalian cell lines to produce rFv polypeptides in the '414 application.

The '414 Application Does Not Describe Or Suggest Coexpression Of L-rFv And H-rFv Polypeptides In A Single Host Cell

13. I could find no description in the '414 application of a single host cell that produces two different polypeptides, or a process where two different polypeptides are expressed in a single host cell. As such, I do not believe there is any description in the '414 application of a host cell meeting the requirements of claim 1 of the '545 patent, or a process meeting the requirements of claim 2 of the '545 patent as these claims have been interpreted by the PTO.

14. The '414 application clearly states that an rFv is to be made by producing the L-rFv and H-rFv polypeptides in separate cells and combining them in a test tube after expression and purification. For example, on page 16, lines 24-28, the '414 application plainly states:

> The resulting construct [*i.e.*, a cDNA insert encoding the L-rFv or H-rFv polypeptide in an appropriate expression vector] is then introduced into an appropriate host to provide expression of the heavy or light polypeptide members of the rFv and the polypeptides isolated. The heavy and light polypeptide members of the rFv are then combined in an appropriate medium to form the rFv. (emphasis added).

15. This clearly indicates that each of the L-rFv and H-rFv polypeptides will be produced in separate cells. The "appropriate medium" being referred to is the test tube environment where the two expressed and purified polypeptide chains are finally mixed together after they have been separately produced and isolated. An appropriate medium is not referring to a transformed bacterial host cell.

16. All of the techniques and options in the '414 application for producing L-rFv and H-rFv polypeptides are consistent with this approach. For example, page 17, lines 35-38, indicates that "[w]here the light or heavy chain is not secreted, the transformed microorganisms containing the appropriate ds cDNA for either light or heavy chains are grown in liquid culture and cleared lysates prepared." (emphasis added). This again makes clear that each of the L-rFv and H-rFv polypeptides is being produced in a separate cell culture.

17. Similarly, page 18, lines 4-7, indicates that the "eluates from each of the heavy and light chain isolations are pooled, followed by treatment to renature the polypeptides to form L-rFv and H-rFv respectively." (emphasis added). These references to multiple isolations clearly indicate that separate cell cultures are being used to produce the two different polypeptides. A single isolate would be the result of lysing a single host cell that was producing both the L-rFv and H-rFv polypeptides.

18. As such, in my opinion, it is absolutely clear that, if the procedures described in the '414 application are followed as they are written, each of the polypeptides will be produced in separate cells. I do not believe any other reading of these sections of the '414 application would be rational, logical, or scientifically accurate.

The Procedures Described In The '414 Application Will Not Yield Genetic Constructs Encoding More than One Polypeptide Or Host Cells That Contain Multiple Plasmids

19. The procedures in the '414 application produce a "tailored" cDNA sequence by starting with a cDNA obtained from a cDNA library that encodes a full length heavy or full length light immunoglobulin chain polypeptide. *See*, p. 6, lines 19-34. These starting cDNA sequences are produced using mRNA transcripts isolated from a hybridoma that is producing an antibody with a desired specificity (*i.e.*, a cDNA library is produced

through reverse transcription of the mRNA transcripts in a purified mRNA extract from the hybidoma).

20. By 1982, it was well known that the heavy and light chains of an immunoglobulin are encoded by separate genes, and that these genes are located on different chromosomes. *See, e.g.*, Hood *et al.*, ANN. REV. GENET. 9: 305-353 (1975). When these genes are transcribed by the hybridoma cell, discrete mRNA transcripts will be produced – one associated with transcription of the light chain gene, and a different one from transcription of the heavy chain gene. Because the technique for producing the cDNA library makes cDNAs that are complementary to each of these individual mRNA sequences or transcripts, none of the cDNAs in the library will contain sequences corresponding to both heavy and light immunoglobulin chains.

21. The amplification procedures described in the '414 application produce copies of the cDNAs in the cDNA library. These procedures use simple transformations of bacterial cells using a common vector/plasmid. *See, e.g.*, p. 8, lines 11-31; p. 27, lines 14-27. When this process is followed as it is described in the application, each plasmid will incorporate one cDNA from the cDNA library. None of the plasmids will contain sequences from both the heavy and the light chain immunoglobulin chains, so none of the amplified cDNA sequences will contain both heavy and light chain sequences.

22. The "tailored" cDNAs made by the '414 procedures use these amplified cDNA sequences as the "starting material" for the tailored cDNA sequence. The individual cDNAs are sequenced and subjected to restriction mapping. *See*, p. 9, lines 20-25 ("these analyses insure that the isolated cDNA clones completely encode the variable region and, optionally, the leader sequences for the light or heavy chain of the desired immunoglobulin."). This source cDNA from the cDNA library is also used to prepare the tailored cDNA sequence encoding the L-rFv or the H-rFv.[1]

23. There are no steps described in the '414 application where different cDNAs from the cDNA library are ligated together before they are inserted into a plasmid for

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[1] The process of producing the tailored cDNA sequence also does not create cDNAs that contain both heavy and light chain sequences. *See, e.g.*, '414 application at pages 9-14, 28-39. The first step in that process involves sequencing the cDNA clones encoding the light and heavy chain polypeptides. Once the sequence information is in hand, an oligomer (a short DNA sequence) is synthesized that will hybridize to a portion of variable region sequence in either the heavy or the light chain. The oligomer also has a stop codon at the end of its variable region sequence. The oligomer is then incubated with a single strand of amplified cDNA from the cDNA library, and treated with enzymes to prepare a double stranded DNA. The '414 application refers to this double-stranded DNA as a "heteroduplexed" ds cDNA because it contains two strands of DNA that are not 100% complementary (*i.e.*, the one grown from the oligomer contains a stop codon). When this heteroduplexed ds DNA is amplified, it produces "homoduplexed" ds DNA (*i.e.*, where the two DNA strands are 100% complementary). This homoduplexed ds DNA containing the introduced stop codon is then hybridized with a second synthesized oligomer that contains a start codon at the beginning of the variable region sequence. The oligomer is then incubated with a single strand of amplified cDNA from the cDNA library and treated with enzymes to prepare a double stranded DNA. This double stranded DNA is also a "heteroduplexed" ds cDNA, since it contains two strands of DNA that are not 100% complementary (the first strand contains a stop codon, but no start codon). When this heteroduplexed ds DNA is amplified, it produces the tailored homoduplexed ds DNA. This homoduplexed ds DNA is then prepared for insertion into a plasmid for expression to produce the L-rFv or H-rFv polypeptide.

amplification. As such, none of the steps outlined in the '414 application for preparing "tailored" cDNA sequences produce a starting cDNA that contains both heavy and light chain sequences.

24. None of the procedures described in the '414 application insert two different cDNA inserts into one genetic construct, either for amplification or expression purposes. Instead, all of the procedures and techniques described in the application insert one cDNA into each plasmid.

25. I could find no description in the '414 application of a procedure which produces a genetic construct containing either a single cDNA insert encoding two different sequences (*e.g.*, encoding both L-rFv and the H-rFv polypeptides), or two different cDNA inserts each encoding one of the two polypeptides.

The Techniques Described In The '414 Application Clearly Call For Production Of Individual Polypeptides In Separate Bacterial Host Cells

26. If the transformation procedures described in the application are followed as they are written, they will not produce host cells that contain two different plasmids. As such, the '414 application, in my opinion, cannot be read as describing host cells that produce two different polypeptides.

27. The procedures and techniques described in the '414 application for preparing genetic constructs and host cells are all consistent with this one-clone/one-plasmid/one-polypeptide approach.

28. First, the procedures use hybridization techniques to select transformed bacterial colonies and confirm that these colonies contain the cDNA of interest. *See, e.g.*, p. 9, lines 1-9; p. 12, lines 19-25. *See also* Grunstein & Hogness, PROC. NATL. ACAD. SCI. 72(10): 3961-3965 (1975). For example, on page 9, lines 10-13, the '414 application states that "the host colonies, usually bacterial, which have DNA which hybridizes to either the light or heavy chain probes are picked and then grown in culture under selective pressure." (emphasis added). This indicates to me that the cells being probed contain only one cDNA insert corresponding to either the heavy or the light immunoglobulin chain.

29. Second, only simple expression vectors and procedures are described in the '414 application and in its example. *See, e.g.*, p. 14, line 22 to p. 15, line 6; p. 40, lines 3-9. These expression vectors contain a single transcription/translation cassette. A plasmid with only one such cassette will direct a transformed cell to transcribe and translate only one inserted cDNA sequence. This clearly shows that only a single cDNA insert will be expressed by each transformed cell.

30. Third, the example in the '414 application closely tracks this one-plasmid/one-cell approach. On page 41, lines 6-15, the '414 application specifies that individual cDNA inserts encoding the light or heavy chain variable polypeptides are incorporated into separate plasmids, and these separate plasmids are used to transform host cells:

> The "tailored" pGM1 is isolated, partially restricted with PstI and the DNA sequences coding for the light and heavy chain variable regions prepared above inserted individually into the tailored site to provide two plasmids having DNA sequences coding for the light (pGM1L) and heavy (pGM1H) chains, in accordance with the procedure described previously for insertion. (emphasis added)

31. This passage clearly indicates that only one cDNA insert, encoding either the L-rFv or the H-rFv polypeptide, will be inserted into each plasmid. In my opinion, this description cannot be read as suggesting that a single cDNA sequence encoding both of the polypeptides will be inserted into a single plasmid, or that multiple cDNA inserts encoding different polypeptides will be inserted into one pGM1 plasmid.

The Techniques Described In The '414 Application Are Consistent With Only Individual Polypeptide Expression

32. I do not believe someone familiar with basic molecular biology techniques could read the '414 application as describing procedures where a single bacterial host cell is transformed with two different plasmids, or where a single host cell is being engineered to produce two different polypeptide sequences.

33. For example, the use of the same starting plasmid to produce the pGM1L and the pGM1H plasmids suggests that different cell cultures are being produced – one transformed with the pGM1L plasmid, and the other with the pGM1H plasmid. This is also consistent with the indication in this section of the application that clones containing either the pGM1L plasmid or the pGM1H plasmid are to be identified using restriction mapping techniques. *See*, p. 41, lines 13-15.

34. Restriction mapping techniques compare the enzyme digest of a plasmid extracted from a clone to a reference map of the digest of the plasmid produced before the transformation step or expected from the genetic engineering process leading to the construction of the plasmid. Restriction mapping was, and remains, a standard procedure, and is straightforward when applied to comparisons involving a single plasmid. It becomes far more complicated when two plasmids are involved, because there will be a mixture of the two enzyme digests. This complication makes it inordinately difficult to use restriction mapping to confirm the outcome of gene cloning experiments. In my opinion, if the bacterial colonies under study each contained two different plasmids, the application would have mentioned something about how one should perform the restriction mapping procedures.

35. I also do not believe a scientist familiar with molecular biology would read the description as indicating that individual bacterial clones are being transformed with two plasmids having the same selectable marker. Even assuming one cell incorporated both plasmids during the transformation process, the resulting cell colony and culture would not retain any of these doubly-transformed cells within a matter of hours. This is because the cells in the culture would need only one plasmid to exhibit antibiotic resistance, and there is strong evolutionary pressure in the cell culture against maintenance of clones

with multiple plasmids having the same drug resistance marker. These facts would have been very familiar to a scientist working in this field in early April of 1983, as well as when the '414 application was written in 1982.

36. In my opinion, if the authors of the '414 application were intending to produce bacterial clones containing two different plasmids, they would have included a very clear description of a procedure that could be used to produce and to maintain a stable culture of these bacterial double transformants. The absence of that description indicates to me that they did not intend to make double transformants.

37. The '414 application also describes procedures which use a single antibiotic resistance gene. In particular, Example 1 uses the same pGM1 plasmid to produce both expression plasmids. The pGM1 plasmid contains a single antibiotic resistance gene. *See*, Miozzari & Yanofsky, J. BACTERIOL. 133(3): 1457-1466 (1978). Antibiotic resistance genes, or marker genes, allow scientists to differentiate bacterial cells that have successfully incorporated a plasmid, from cells that have not, by growing the culture in the presence of the antibiotic. Those cells that do not contain and express the antibiotic resistance gene in the plasmid die, thereby producing a culture which only contains bacterial clones that have successfully incorporated the plasmid.

38. The use of the single-marker pGM1-based plasmid also would not make sense if the '414 application was describing procedures for transforming bacterial clones with two different plasmids. For example, culturing the transformed cells in the presence of the marker antibiotic would not differentiate clones that successfully incorporated only the pGM1L plasmid from those that successfully incorporated only the pGM1H plasmid, or from clones that incorporated both plasmids. This would also make it impossible to use the antibiotic as a source of selective pressure to prepare and maintain a homogenous culture of clones that maintained both plasmids.

39. Considering all of these points, I believe the '414 application can only be read as describing procedures that produce only one polypeptide in one host cell (*i.e.*, either the L-rFv polypeptide or the H-rFv polypeptide).

There Is No Description Of Host Cells Meeting The Requirements Of Claim 1 Of The '545 Patent In The '414 Application

40. As written, claim 1 seems to cover a scenario where the two polypeptides of the rFv have an identical amino acid sequence. Specifically, the claim states "[a] host cell which expresses a recombinant double-chain antibody fragment (rFv) comprising two polypeptide chains having substantially the same amino acid sequence of at least a portion of the variable region . . . of a mammalian immunoglobulin . . . ." (emphasis added). This reading seems to be the scenario described on pages 2-3 of '414 application, which indicates that the L-rFv and H-rFv polypeptides can actually have the same sequence. *See*, p. 3, line 32 to p. 4, line 2. A host cell meeting the requirements of claim 1 read in this way would only have to be transformed with one plasmid containing one cDNA insert encoding either the L-rFv or H-rFv polypeptide.

41. Despite this, the PTO states that claim 1 of the '545 patent defines a host cell that produces two different polypeptides. This means that the PTO is reading this claim to require the host cell to be transformed with cDNA sequences encoding two different polypeptides, and that the host cell express those two sequences.

42. In my opinion, as I explained above, a scientifically correct reading of the description in the '414 application makes it clear that it is not describing at any point a host cell that will produce both heavy and light immunoglobulin chain variable region polypeptides. Instead, the '414 disclosure very clearly describes procedures in which each variable region polypeptide is produced in a separate host cell culture and is then isolated, renatured, and combined in a test tube to form an rFv.

43. In the February 16, 2007 Office Action, the PTO identified a number of sections of the '545 patent that it believes are describing a host cell that produces both heavy and light chain variable region polypeptides.

44. First, on page 20 of the Office Action, the PTO states that the '545 patent describes:

> a "host cell" transformed with a single genetic construct (e.g. including pBR322; see e.g. Moore at col. 5, lines 32-35 and col. 7, lines 39-50) . . . encoding variable light and heavy chains . . .. (emphasis in original).

45. I disagree. There is no description in these sections of the patent of a host cell has been transformed with a single genetic construct that encodes two different polypeptide sequences.

- Col. 5, lines 32-34 states that "a wide variety of vectors may be employed for amplification or expression of the ds cDNA to produce the light and heavy chains of the immunoglobulin." This simply indicates that many vectors were available in 1982 for amplification and expression of cDNA sequences. It does not suggest that a single cDNA encoding two immunoglobulin chains should be expressed in a single host cell.

- In addition, this section of the patent (*i.e.*, col. 5, lines 35-47) describes amplification of cDNA in the cDNA library, not expression of cDNA. This is done by incorporating the ds cDNA from the cDNA library into plasmids and transforming a host to incorporate each plasmid. The statements in this section make it clear to me that individual cDNAs are being inserted into individual plasmids for amplification. For example, col. 5, lines 33-36, makes references to vectors that contain "an appropriate restriction site" (emphasis added), which indicates that each plasmid will incorporate only one cDNA insert from the cDNA library. And, col. 5, lines 36-37 states that "[t]he ds cDNA obtained from the reverse transcription of the mRNA" is being used. This indicates that a single ds cDNA is inserted into each vector, not multiple distinct ds cDNAs, and each cDNA being amplified will encode only one of the two immunoglobulin chains. As I explained earlier, there will be no cDNA molecules in the cDNA library

produced by reverse transcription of the mRNA extract that contain sequences from both the heavy and light immunoglobulin chains.

- Col. 7, lines 39-50 is describing amplification procedures. The first sentence of this section (lines 39-41) indicates that the vectors will have a single ds cDNA insert encoding only one polypeptide (*i.e.*, "the vector which is employed provides for amplification and convenient isolation of transformants having the variable region coding sequence insert."). The references to "hybrid plasmid" and mismatched sequences refers to double-stranded DNA, where one strand contains the "native" cDNA produced from the cDNA library (and, ultimately, from the mRNA extract) and the other strand contains a cDNA grown from the synthetic strand that incorporates a stop codon at the end of the variable region coding sequence. When transformants containing a plasmid with this mismatched ds cDNA divides, one cell will contain a copy of the native sequence and the other cell will contain a copy of the "site mutated" sequence. Both of these sequences still encode only a heavy chain sequence or a light chain sequence, not both. Also, these transformed cells will not produce the polypeptides because the plasmid containing the cDNA insert does not contain regulatory elements that will direct the host cell to transcribe and translate (*i.e.*, express) the inserted cDNA.

46. Next, the PTO, at pages 20-21, states that the '545 patent describes "a 'host cell' transformed with . . . two separate constructs comprising DNA (e.g. ds cDNA derived from a hybridoma as in instant claim 14: see Moore patent claim 2) encoding variable light and heavy chains [E.g. see Moore patent claim 1; col. 10, lines 1-5; col. 23, lines 35-45 (pBR322); and col. 24, lines 50-60 (pGM1L and pGM1H); col. 11, lines 5-12] . . . ." (emphasis in original). Again, the PTO is mistaken in its interpretation of these sections of the patent.

- As I explained above, there is no description in the original '414 application of a process having the steps listed in claim 2 of the '545 patent or a host cell that produces heavy and light immunoglobulin chain polypeptides.

- The sentence at col. 10, lines 1-5, does not describe expression of two different polypeptides in a single host cell. This sentence is in a section of the '545 patent that is reviewing the overall procedure for producing rFv binding compositions. The quoted sentence indicates that the primer repair step (col. 9, line 67) is "repeated twice to provide ds cDNA coding for the variable region with translational regulatory signals at predetermined sites." In other words, since each cDNA has to have regulatory signals inserted at each end of the cDNA, the primer repair steps must be repeated twice. This section is not indicating that a host cell is being transformed twice, or that two different cDNA inserts are being incorporated into a plasmid for expression. The sentence also immediately precedes the passage in the patent (*i.e.*, col. 10, lines 8-13) that clearly states that two different genetic constructs were produced and introduced into separate host cells to provide for expression of either the L-rFv or H-rFv polypeptide.

- Column 23, lines 35-45 illustrates an amplification procedure that tracks the procedure described at col. 7, lines 39-50. It states that the "heteroduplexes having the 'tailored' sequences of the variable regions of the light and heavy chains are then ligated to PstI linkers, restricted with PstI endonuclease and inserted into the PstI site of pBR322." (emphasis added). The next sentence indicates that "the plasmids containing the tailored ds cDNA with the stop codons at the end of the variable regions are isolated and sequences coding for the variable regions . . . are excised . . . ." As I read these sections, they are referring to two sets of heteroduplexed ds cDNA and two plasmids, which is in line with the procedure described at col. 7, lines 39-50 (*i.e.*, one plasmid for each mismatched ds cDNA, and each mismatched ds cDNA encodes either a light or heavy chain sequence only). The use of the single restriction site (*i.e.*, the PstI site) also clearly indicates that one plasmid with one cDNA insert is being described.

- Column 24, lines 50-60, of the '545 patent describes the example where the pGM1L and pGM1H plasmids are prepared. As I explained above, each of these plasmids contains only one cDNA insert encoding either the L-rFv or the H-rFv polypeptide. Each plasmid is used to prepare a separate transformed host cell culture that will produce either the L-rFv or the H-rFv polypeptide, but not both. This is because each bacterial clone in the culture will contain one plasmid and each plasmid will contain one cDNA sequence encoding one polypeptide.

- Column 11, lines 5-12 is describing the properties of rFv's. It does not concern genetic constructs or expression procedures.

47. My review of the '414 application leads me to conclude that it does not describe anywhere a host cell that will produce both light and heavy variable region polypeptides. Instead, every description of host cells and methods for expressing L-rFv and H-rFv cDNA sequences in the '414 application calls for expression of a single cDNA insert encoding only one polypeptide in a separate bacterial cell culture. As such, I do not believe the '414 application describes host cells that meet the requirements of claim 1 of the '545 patent, contrary to the PTO's interpretation of this claim.

There Is No Description Of A Process Meeting the Requirements Of Claim 2 Of The '545 Patent In The '414 Application

48. I also did not find any description in the '414 application of a process for expressing two different tailored DNA molecules in a single transformed host cell. As I explained above, there is no description of procedures that produce a single plasmid that contains two different cDNA inserts. There is also no description in the '414 application of procedures that will create a single genetic construct that contains a cDNA sequence encoding both heavy and light chain sequences, especially if the tailored cDNA is produced using cDNA from a cDNA library made from an mRNA extract from a hybridoma.

49. The steps listed in claim 2 of the '545 patent for producing an rFv are:

(1) cloning first and second DNA molecules respectively encoding heavy and light chains from a hybridoma producing an antibody to a predetermined ligand;

(2) tailoring the cloned DNA molecules to express fragments comprising 95-125 amino acids of the heavy and light chain variable regions, without constant regions, in a host cell;

(3) inserting the tailored DNA molecules into an expression vector in proper relationship with transcriptional and translational regulatory signals in the vector;

(4) transforming the host cell with the expression vector and growing the host cell, whereby the light and heavy variable region polypeptides are expressed and associate to form an rFv having substantially the same binding specificity for the predetermined ligand as the antibody from the hybridoma.

50. Like the host cell claim, the process steps in this claim are confusing. My natural reading of the process would call for expression of each polypeptide fragment in a separate host cell. However, I understand that the PTO has interpreted these steps to require expression of both the L-rFv and H-rFv fragments in one host cell.

51. I could not find any description in the '414 application of a process having steps 2, 3, and 4, as they are being interpreted by the Office. As I explained earlier, if the methods for inserting a cDNA into a plasmid are followed as they are written in the '414 application, they will create a single cDNA insert encoding one of either the L-rFv polypeptide or the H-rFv polypeptide, and this one cDNA insert will be incorporated into one plasmid. Host cells transformed with this plasmid will contain one plasmid and will produce either the L-rFv or the H-rFv polypeptide, not both polypeptides.

52. Also, as I explained above, there is no description in the '414 application of a process where a single cDNA encoding both heavy and light chain sequences is produced or called for. If the procedures in the '414 application are followed as they are written, they will not produce a single cDNA containing both heavy and light chain sequences.

53. The PTO cites certain passages of the '545 patent that it believes are describing a procedure in which "an immunologically functional immunoglobulin fragment" is made which includes the steps of "independently expressing in a host cell variable heavy and light chain domains ... lacking constant regions." *See*, February 16, 2007 Office Action, p. 20. The passages identified by the PTO do not even mention expression of foreign cDNA sequences.

- Col. 1, lines 33-42 describes the properties of an immunoglobulin. It says nothing about expression of light and heavy chain variable region polypeptides.

- Col. 3, lines 59-63 describes a way to obtain DNA sequences encoding antibodies (*i.e.*, by immunizing non-human hosts with an antigen and then obtaining antibody producing cells). It does not discuss expression of cDNA sequences encoding light and heavy immunoglobulin chain variable region polypeptides.

- Col. 17, lines 4-8, indicates that the sequences listed in the figure below this section of the patent encode the κ-chain of MOPC41 (a hybridoma-produced antibody). There is nothing mentioned in this section of the patent about expression of heavy and light chain variable region polypeptides.

54. As such, I do not believe there is any description of a process that meets the requirements of claim 2 of the '545 patent, as the PTO has interpreted the steps in this claim.

There Are No Original Claims In The '414 Application That Require Coexpression

55. I reviewed the claims that were filed with the '414 application, which appear on pages 44-50 of the application. These claims clearly state that only one cDNA encoding either a heavy or a light chain variable region (not both) is to be inserted into a plasmid and that plasmid is to be used to transform a host cell for production of either the L-rFv or the H-rFv polypeptide.

- Claim 1 reads: "a method of preparing a binding polypeptide which consists essentially of the amino acid sequence of at least a portion of the variable region of a light or heavy chain of an immunoglobulin . . . ." (emphasis added). The claim is describing a process for making one polypeptide encoding the variable region of the heavy or light immunoglobulin chain (*i.e.*, an L-rFv or an H-rFv polypeptide) in one host cell.

- Claim 7 describes a method for preparing an rFv fragment. It generally follows the procedures outlined in the specification. For example, the claim indicates that the source cDNA is obtained from reverse transcription of an mRNA extract from a hybridoma to "produce ds cDNA having a coding strand coding for said light or heavy chain . . . ." (emphasis added). On page 45, lines 25-30, the cDNA is to be tailored by removing at least portions of "the regions flanking said variable regions of said light or heavy chains . . . ." (emphasis added). On page 46, lines 22-25, the claim states that "N- and C-terminus tailored ds cDNA is obtained coding for the variable region of the light or heavy chain free of the constant region of said immunoglobulin." (emphasis added). Then, on page 46, lines 30-34, the vector containing the ds cDNA encoding either the L-rFv or H-rFv polypeptide is used to transform a host, "whereby the light or heavy variable region polypeptides are expressed." (emphasis added). Finally, the rFv is produced by "combining said light and heavy region polypeptides to form said rFv" (p. 46, lines 33-34). All of this is consistent with my reading of the '414 application, which calls for individual production of the L-rFv or H-rFv polypeptides in separate host cell cultures, followed by combination of those polypeptides in a test tube.

- Claim 18 is similar to claim 7, but indicates which residues from the source light or heavy chain the L-rFv or H-rFv polypeptides are supposed to be retained (p. 48, lines 3-4). Like claim 7, it specifies production of cDNAs that encode "said light or heavy chains" (p. 48, lines 16-17) (emphasis added); obtaining "N- and C-terminus tailored ds cDNA coding for the variable region of the light or heavy

chain . . ." (p. 49, lines 12 to 15) (emphasis added); transformation and growing of a host cell "whereby the light or heavy variable region polypeptides are expressed" (p. 49, lines 21-22) (emphasis added); and then "combining said light and heavy region polypeptides to form said rFv" (p. 49, lines 23-24). Again, in my opinion, this claim is clearly calling for production of the L-rFv or H-rFv polypeptides in separate host cell cultures.

56. As such, in my opinion, the claims that were filed with the '414 application clearly call for production of individual heavy or light chain variable region polypeptides in separate host cell cultures.

The '414 Application Does Not Discuss Or Suggest Chimeric Antibodies

57. I disagree with the PTO's conclusions on page 21 of the February 16, 2007 Office Action regarding chimeric immunoglobulins. The PTO suggests that the contents of the '414 application would make obvious to a person of ordinary skill in the art the production of chimeric immunoglobulins as an alternative to producing variable region polypeptides from light and heavy immunoglobulin chains. I disagree because the PTO appears to indicate that a strategy that requires removal of one polypeptide sequence is essentially the same as one that requires addition of a new and different polypeptide sequence. These are two completely different strategies.

58. The '414 application focuses on the elimination of constant region sequences from immunoglobulin chains. The only procedures described in the '414 application start with a native source immunoglobulin sequence, and then selectively delete sequences that do not correspond to the variable region of the source immunoglobulin chain. In my opinion, no reasonable scientist would make the same jump the PTO has made from the information actually contained in the '414 application. This would require the scientist to ignore the explicit directions in the '414 application.

59. For example, the '414 application identifies benefits of "variable-region only" polypeptides that are dependent on the complete removal of any constant region sequence of the immunoglobulin chains (e.g., their significantly reduced size relative to intact immunoglobulins). See, p. 1, line 25 to p. 2, line 18. Chimeric immunoglobulins contain constant region sequences, and would not provide these benefits because they would be equivalent in size to the source immunoglobulins that the '414 patent are modifying.

60. Chimeric immunoglobulins also have functional attributes that are linked to their constant region sequences, such as complement fixation and antibody-dependent cellular cytotoxity. An rFv contains no constant region sequences and could not trigger these types of immune responses. I do not see any scientific basis for the PTO's opinion that a person of ordinary skill would connect these two very different concepts.

The '414 Application Contains Nothing That Would Make Obvious The Coexpression Of Heavy And Light Chain Sequences In A Single Transformed Host Cell

61. In my opinion, the PTO is not accurately interpreting the '545 patent and the other patents and publications it refers to in the February 16, 2007 Office Action. I believe these inaccurate interpretations have led it to incorrectly conclude that coexpression of heavy and light immunoglobulin chains in a single host cell would have been obvious to a person of ordinary skill in the art in the early April 1983 time frame.

62. The PTO is simply incorrect when it reads the portion of the '545 patent disclosure that was filed in 1982 (*i.e.*, the contents of the '414 application) as describing procedures for coexpressing heavy and light chain variable region polypeptides in a single transformed host cell. As I explained above, the '414 application does not describe or suggest coexpression procedures. Instead, it very clearly calls for expression of each immunoglobulin variable region polypeptide in a separate host cell, and then indicates that these individually prepared light and heavy chain variable region polypeptides be mixed together *in vitro* (*i.e.*, in a test tube) to form an rFv.

63. I disagree with the conclusions of the PTO in the February 16, 2007 Office Action that are based on this incorrect reading of the patent description:

- The analysis on pages 19-21 of the Office Action incorrectly states that the '545 patent "provides motivation to co-express (using one or two vectors) the Cabilly 1 patented light and heavy antibody chains in a single host cell." As I explain above, the portions of the '545 patent filed in 1982 do not describe or suggest coexpression. Because it actually indicates that individual immunoglobulin chain polypeptides should be produced in separate host cells, the '545 patent is clearly suggesting the opposite of what the PTO has stated.

- The statement on pages 32-33 of the Office Action incorrectly suggests that the '545 patent "provides further motivation to co-express (using one or two vectors) the Cabilly 1 patented light and heavy antibody chains comprising variable regions in a host cell with a reasonable expectation of producing an assembled functional antibody." The explanation provided by the PTO on pages 32-33 is based on an incorrect reading of the parts of the '545 patent being quoted. I have explained the errors in this analysis above.

- The statement on page 34 of the Office Action improperly equates production of variable region only-polypeptides with production of chimeric antibodies containing foreign constant region sequences. A person of skill in the art in early April of 1983 would not have been motivated to produce chimeric immunoglobulin chains based on the '545 concept of entirely removing constant domains from immunoglobulins.

- The statement on page 34 of the Office Action states that the '545 patent describes use of "eukaryotic secreting hosts . . . from which functioning rFv is

recovered." There is only a single passing reference to yeast cells used for amplification in the '414 application. I do not believe a person of skill would equate this with what the PTO states. The part of the '545 patent cited by the PTO is referring to secretion of the polypeptide being produced from the transformed host cell, which could occur in a bacterial cell line.

- The statement on page 36, incorrectly interprets the '545 patent as describing "use of the vector(s) comprising variable regions of the heavy and light chain expressed in a host cell to achieve active single chain antibodies provides *further motivation* that additionally including the constant regions would result in the *successful production* of an antibody (*e.g.* chimeric)." As I have already explained, the 1982-version of the description of the '545 patent very clearly indicates that each individual heavy or light variable region chain polypeptide is to be produced in a separate host cell. After that is done, the two polypeptides are combined in a test tube. There is nothing I see in this description that would provide any motivation or lead someone to expect that production of two full-length antibody chains in one host cell would lead to the successful production of a chimeric antibody.

- The statement on page 54 that the '545 patent "does expressly teach the independent expression of variable light and variable heavy chains in a single host to produce a functional single chain immunoglobulin fragment" is incorrect, as I explained above.

64. As such, I do not agree with the PTO that the description in the '414 application, when it is considered with the other documents referred to by the PTO, would motivate or otherwise suggest to a person of ordinary skill in the art that existing procedures for producing individual immunoglobulin chains in a single host cell could or should be modified to produce heavy and light chain polypeptides in a single host cell.

The Axel Patent Does Not Make Obvious Coexpression

65. I did not find information in the Axel patent that would support the PTO's conclusion that this patent provides a motivation or suggestion to coexpress immunoglobulin heavy and light chains in a single mammalian host cell.

66. The Axel patent describes procedures for producing individual polypeptides of interest in a transformed mammalian cell. These procedures use a two DNA concept – one DNA encoding a marker gene that introduces a selectable phenotype into the cell and one DNA encoding the desired polypeptide that is to be produced and isolated. The marker gene introduces a selectable phenotype into the mammalian cells, which enables these cells to be differentiated from cells that have not incorporated it or are not expressing the incorporated marker DNA. The Axel procedure assumes that some of the cells that express the marker gene will express one or more copies of the other DNA sequence encoding the desired polypeptide.

67. The Axel patent indicates that the polypeptides that can be produced by its method include antibody polypeptides. I read the word "antibody" as it is used in the Axel patent to mean individual heavy or light chain polypeptides, not assembled antibody tetramers because of the way the Axel patent refers to antibodies – as one of many types of polypeptides that can be produced. *See*, *e.g.*, Axel at col. 3, lines 31-36.

68. Axel refers in the identical way to enzymes as another type of protein that can be made. Enzymes often contain many discrete subunits. For example, a well known enzyme in early April of 1983, RNA polymerase, has ten discrete subunits. Under the PTO's logic, the Axel patent is describing how to express all ten subunits of RNA polymerase in one host cell because RNA polymerase is an enzyme, and enzymes are listed in the patent specification and claims.

69. I did not find any discussion in the Axel patent of issues that logically would have been addressed if it was describing procedures for introducing and expressing a gene encoding more than one new protein in a cell. For example, the Axel patent does not indicate how to extend the DNA I plus DNA II procedure to produce an *additional* desired polypeptide in the transformed host cell.

70. I also note that the Axel patent process is based on probabilities – at least some of the cells subjected to the transformation process will stably incorporate and express not only the first DNA encoding the marker gene, but also the second DNA encoding the polypeptide of interest. A person trained in molecular biology techniques would understand this. If the Axel patent were describing procedures for producing two or more polypeptides of interest in one cell, there would have been some discussion in Axel of the more complex probabilities of incorporating and expressing two different DNAs encoding different polypeptides of interest. The absence of any discussion of these concepts in the Axel patent again indicates to me that it is describing procedures for producing only a single desired polypeptide in each host cell.

71. As to antibodies, I believe the Axel patent would have mentioned something about assembly of the light and heavy chains after they have been produced in a host cell if it were describing coexpression of heavy and light chains in one host cell. The absence of any discussion of this issue tells me that the Axel patent is only describing procedures for production of only individual antibody polypeptides in each mammalian cell line.

72. Dr. Axel explained in his declaration that the PTO mentions on pages 46 and 47 of the February 16, 2007 Office Action that the Cabilly disclosure adds this missing information. As he indicates, the combination of the procedures described in the Axel patent and the guidance and information in the Cabilly patent would explain how to produce heavy and light immunoglobulin chains in a single transformed mammalian cell line. *See*, Axel Declaration, paragraph 7. In my opinion, the information that Dr. Axel refers to about coexpression in his declaration is the information contained in the Cabilly patent, not the Axel patent. Also, I do not read his statements as indicating that the Axel patent is describing procedures that produce both heavy and light immunoglobulin chains in one mammalian host cell.

73. I also do not agree with the PTO's interpretation of the references in the Axel patent to "genes" on page 51 of the February 16, 2007 Office Action. The PTO states that the Axel abstract's reference to "a DNA, including gene or genes encoding proteineaceous material' means that the Axel patent is describing a procedure for producing multiple desired polypeptides in a single host cell. I think the PTO is reading this passage incorrectly. The more plausible and correct reading of this sentence is that it is referring to expression of multiple copies of the same gene – which is explained at col. 6, lines 44 to 66, of the Axel patent.

74. The PTO indicates (on pages 29-30 of the Office Action) that it believes the Axel patent is describing procedures of expressing "antibodies in mammalian host cells as intact (assembled) proteins." The PTO identifies the Axel abstract, col. 5, lines 3-7 and 24-28, and claims 1, 7, 22-24, 28, and 29 as the basis for its belief this is what the Axel patent is describing. I have reviewed these parts of the Axel patent. None of these sections of the Axel patent discuss "intact" or "assembled" antibodies.

- The Axel abstract simply outlines the concept described in the Axel patent of transforming a eukaryotic host cell with a first DNA that encodes a selectable marker and a second DNA encoding a "desired proteinaceous material" (*i.e.*, a protein or polypeptide of interest). There is no mention in the abstract of producing assembled or intact antibodies.

- Col. 5, lines 3-7 indicates that the experiments being described in the patent can be conducted in a wide variety of types of eukaryotic cells. Nothing in this passage is discussing production of assembled or intact antibodies or other types of multimeric proteins.

- Col. 5, lines 24-28 is a laundry list of types of polypeptides that can be produced using the process described in the Axel patent. There is nothing in this section of the patent that indicates that the Axel process is intended to produce intact or assembled antibodies or other types of multimeric proteins.

- Claim 1 of the patent outlines in general language the process of co-transforming a host cell with two DNA sequences, one encoding the selectable phenotype (DNA II) and the other (DNA I) encoding desired polypeptide.

- Claim 7 lists antibody polypeptides as one type of protein that can be produced. There is nothing in this claim that mentions intact or assembled antibodies.

- Claim 22 indicates that a foreign proteinaceous material is produced by the procedure outlined in claim 1. It also indicates that the transformed eukaryotic cells are cultured in a manner that will create a "multiplicity" of these cells, and that the protein made by these cells is recovered.

- Claim 23 again lists "antibody" as a type of protein that can be made by the procedures outlined in claims 1 and 22. It does not indicate that the antibody is to be produced as an intact or assembled antibody.

- Claim 24 specifies that the eukaryotic cell is a mammalian cell. There is no indication that the mammalian cell is to produce an intact or assembled antibody.

- Claim 28 outlines a procedure where foreign DNA I "coding for proteinaceous material which is not associated with a selectable phenotype" is inserted into a suitable eukaryotic cell. The insertion is to be done by co-transformation with an unlinked DNA II that codes for proteinaceous material associated with a selectable phenotype. The procedure very clearly tracks the DNA I plus DNA II concept outlined in the body of the patent. There is no mention of intact or assembled antibodies in this claim.

- Claim 29 again provides the list of types of proteins that can be produced by the procedure outlined in the claim 28. Again, this identifies antibody proteins as one of several different types of proteins that can be made, but does not mention anything about the production of intact or assembled antibodies.

75. The PTO also states that a person of ordinary skill would have combined the information in the '545 and Axel patents and conclude they together make obvious producing immunoglobulin proteins in mammalian cell lines because immunoglobulins are mammalian proteins. *See*, February 16, 2007 Office Action at pp. 12-13. The mammalian origin of the polypeptides seems to be irrelevant to the question of whether the Axel patent would suggest coexpression of immunoglobulin heavy and light chains in a single mammalian cell line. This PTO comment also ignores the fact that many mammalian proteins in the 1983-time frame were being produced in bacterial host cells, and that the '545 patent itself describes the production of a mammalian-origin polypeptide in bacterial host cells. This was because bacterial expression systems were simpler, cheaper, and easier to use than mammalian cultures, which is also a reason why I do not believe the combination that the PTO suggests would have been obvious to someone working in this field in early April of 1983.

76. Considering all of these points, I disagree with the PTO's opinion that the '545 patent, the Axel patent, and the single immunoglobulin chain expression procedures of the '567 patent claims would have made the coexpression procedures of the '415 patent obvious to a person of ordinary skill in the art in early April of 1983. I do not see how a person of ordinary skill could find the motivation for "coexpression" that the PTO suggests from these patents because they do not describe or suggest coexpression procedures.

77. The PTO seems to be overlooking the very important point that there are no procedures outlined in the descriptions of the Axel patent, the '545 patent, or in the '567 patent claims that are clearly devoted to coexpression of heavy and light immunoglobulin chains in a single transformed host cell of any type. This gap of information is crucial. The state of expression techniques in early April of 1983 was such that a scientist working in this field would have skeptically viewed the idea that coexpression of heavy and light chains was so predictable that it could be described without any mention of a strategy for accomplishing this task.

78. My opinions are not altered by the information in the Rice, Kaplan, Dallas, Ochi, Oi, Valle 1981, and Deacon publications (as I explain below). Since there is no indication in the Axel patent, the '545 patent, or the '567 patent claims that coexpression should even be attempted, I fail to see how the additional work being described in these other publications would somehow suggest that coexpression would be obvious to a person of ordinary skill in the art in early April of 1983.

The Rice Publication Does Not Make Obvious Coexpression Of Heavy And Light Immunoglobulin Chains In A Single Host Cell

79. The PTO also states that the 1982 publication of Rice and Baltimore would make coexpression of heavy and light chains obvious because it teaches coexpression of light and heavy immunoglobulin chains in a single host cell. I disagree.

80. The Rice publication describes an experiment where a rearranged light chain gene was introduced into a mature lymphoid cell line that was expressing its endogenous heavy chain gene, but had lost its ability to produce an endogenous light chain protein. *See*, Rice, p. 7862.

81. It is very clear that the lymphoid cell line was not transformed with two different exogenous genes. The experimental design is plainly focused on introduction of only one gene encoding only one protein (the light chain). There is nothing in Rice which discusses issues that would be mentioned if it was describing expression of more than one exogenous protein.

82. In my opinion, the Rice publication does not show "successful expression of both heavy and light immunoglobulin chains in a host with subsequent assembly into immunoglobulins," as the PTO states on page 30 of the February 16, 2007 Office Action. A scientist working in this field would not equate a cell's expression of its own endogenous genes to be equivalent to the introduction and expression of a foreign DNA sequence in that organism. If this were the case, genetic engineering would have been a trivial task. Thirty years of experience demonstrates the contrary.

83. I also do not believe a person of ordinary skill in the art would have made any significant assumptions about expression of antibody proteins based on the work reported Rice. For example, the rearranged light chain gene used in the experiments was not characterized. Instead, the paper reports that it was unclear what enabled the lymphoid cell to express the introduced gene, and that expression may have been due to the presence of uncharacterized regulatory elements in the light chain gene. *See*, Rice at p. 7865. It also suggests that expression may have been controlled or regulated by the continued expression of the endogenous heavy chain gene. *See, id.* The observations in Rice would not have led a person of ordinary skill in early April of 1983 to extrapolate the findings in Rice in the way the PTO has. Instead, they demonstrate that expression of immunoglobulin genes in lymphoid cells was not well understood, was regulated by unknown and uncharacterized factors and processes, and that additional research was needed to identify and understand what actually controlled expression in lymphoid cells. Rather than making coexpression "obvious," these comments also would have made it

clear to a person of ordinary skill in the art in early April of 1983 that expression of even a single rearranged gene (*i.e.*, isolated from another lymphoid cell) was an uncertain and unpredictable process.

84. The PTO also states that Rice "clearly teaches to one of ordinary skill in the art that an exogenous immunoglobulin light chain assembles with a heavy chain endogenously produced by the cell even though both chains possess different antigen specificity" and that this would have led a person of ordinary skill to "expect that expressing a light and heavy chain of the same antigen specificity . . . in a competent host would result in the assembly of a functional antibody." February 16, 2007 Office Action, p. 53. I disagree.

85. In my opinion, the Rice paper clearly articulates the uncertainty that existed in early April of 1983 about the mechanisms and processes for immunoglobulin gene expression and antibody production by B cells. It was not known at that time how lymphoid cells rearrange and regulate expression of immunoglobulin genes. It also was not known whether gene expression was linked to assembly and secretion of immunoglobulin molecules. This is why Rice and Baltimore suggested on page 7865 that:

> Whether the introduced κ gene is responding directly to LPS or to the product of the heavy chain allele is an open question. The possibility that transcription of the light chain gene is controlled by a product of the heavy chain locus is an interesting possibility and needs further investigation.

86. This indicates that the authors of Rice themselves thought that the expression of the exogenous light chain gene and its subsequent association of its expression product with the endogenous heavy chain were somehow linked. That would have been consistent with the thinking at the time that not only the expression of the two genes was linked, but that expression of immunoglobulin genes and assembly of immunoglobulins were linked. *See, e.g.*, Valle 1982 at p. 71, right col. (citing Roth & Koshland, BIOCHEMISTRY 20: 6594-6595 (1981) (indicating that disulfide interchange enzyme "plays a critical role in the formation of intramonomer bonds common to all immunoglobulin molecules."); Wabl & Steinberg, PROC. NATL. ACAD. SCI. 79: 6976-6978 (1982) (suggesting that BiP might be involved in the assembly and expression of immunoglobulin genes or the production of the immunoglobulin molecule).

87. In my opinion, the showing in Rice that an exogenous light chain gene produces an expression product that may associate with the endogenously produced heavy chain would not have led a person of ordinary skill in the art to expect that a B cell would express two exogenous genes and that the expression products of these two genes "in a competent host would result in the assembly of a functional antibody." There is simply no foundation for this within the Rice paper, or in the literature in that time period relating to immunoglobulin gene expression.

88. It should be kept in mind that during this time frame (1982-1983), how lymphoid cells expressed their light and heavy chain genes or how that process of expression related to assembly and secretion of the immunoglobulin was not known. *See*, Second Declaration of Dr. Douglas Rice at ¶¶ 13-14. During the 1982-1983 time frame, a person of ordinary

skill would have thought that a lymphoid cell capable of expressing the introduced light chain gene and processing the light chain gene product would largely owe its ability to the continued expression of the endogenous heavy chain gene. *See, e.g.*, Rice Second Declaration at ¶ 31.

89. I reviewed Dr. Baltimore's statement that he expected, without evaluating any experimental results, that he would have expected that two exogenous immunoglobulin chains, if expressed in a co-transformed lymphoid cell line, would form a functional antibody. *See, e.g.*, Declaration of David Baltimore at ¶¶ 4, 5. I do not believe any person of ordinary skill in the art would have made this statement in early April of 1983.

90. There is a very big "if" in his statement – if the cells produced the two proteins. Whether a lymphoid cell could be transformed and induced to express two exogenous immunoglobulin genes is something that is not even speculated upon in Rice. Instead, like other papers at that time, Rice raises many questions about what has to be done to cause a B-cell to express even one foreign immunoglobulin gene. Dr. Baltimore's statement also seems inconsistent with the skepticism reflected in papers by other scientists working in the field of immunoglobulin gene expression. These other scientists were reporting findings that suggested that not only was the way immunoglobulin genes were expressed important to the ability of the B-cell to produce properly formed antibodies, it was even important to the viability of the B-cell. For example, Dr. Kohler had reported in 1980 that that excess heavy chain production could be toxic to the cell, and others reported that many hybridomas spontaneously lose the ability to secrete immunoglobulins or stop expression of one of the two immunoglobulin chains. *See*, Kohler, PROC. NATL. ACAD. SCI. 77(4): 2197-2199 (1980) ("It is therefore proposed that free immunoglobulin heavy chain is toxic for the [mammalian hybridoma] cells"); Coffino *et al.*, PROC. NATL. ACAD. SCI. 68: 219-223 (1971).

91. As such, I do not believe a person of ordinary skill who evaluated the entire contents of the Rice publication in early April of 1983 would have concluded that Rice successfully demonstrated coexpression of exogenous heavy and light immunoglobulin chains, or that the results Rice reports concerning assembly of the expression products of the exogenous light chain gene and the endogenous heavy chain gene could be extended as the PTO states. Instead, I believe a person of ordinary skill would have expected immunoglobulin gene expression and assembly to be interrelated and would not have made the assumptions about assembly of immunoglobulins that the PTO has. As such, I do not believe Rice would have motivated a person to modify the procedures for expressing one immunoglobulin chain as the PTO states.

Kaplan Does Not Make Coexpression Obvious

92. The Kaplan patent describes procedures for producing light or heavy immunoglobulin chains in separate bacterial or yeast cell cultures. Under the Kaplan procedure, these individually produced human immunoglobulin chains can then be combined *in vitro* to reconstitute the immunoglobulin.

93. The Kaplan procedure is outlined on pages 9-10 of the document. Like the '545 patent, the process starts with production of a cDNA library from an mRNA extract by using reverse transcriptase treatment of the mRNA transcripts. *See*, Kaplan at p. 9, lines 27-37. Then, "the ds DNA for the heavy and light chains may be joined to any conventional vector by conventional means." *See, id.* at p. 10, lines 1-2. Since the light and heavy chains are encoded in separate genes on separate chromosomes, there will not be any mRNA transcripts that contain heavy and light chain sequences in the mRNA extract used to produce this ds DNA.

94. The procedure outlined on page 10 follows a conventional procedure for transforming a host cell with a single plasmid. The way the procedure is described makes it clear that each clone contains a single DNA insert encoding either a light chain sequence or a heavy chain sequence. For example, on p. 10, lines 24-28, "individual clones are tested for production of the desired light and heavy chains." The "clones" are individual bacterial cells that have incorporated one plasmid that contains a DNA sequence encoding a light chain or a heavy chain, but not both.

95. Also, like the '545 patent, the Kaplan patent calls for assembly of the immunoglobulin by combining the light and heavy chains in a test tube. On p. 10, lines 31-33, it states that "the purified light and heavy chains are then combined under mildly oxidizing conditions to provide for folding of the chains together and disulfide formation." This plainly is indicating that the two separately produced and purified chains are to be combined in a test tube.

96. The PTO states that "although Kaplan fails to specifically exemplify the recombinant making of antibodies, Kaplan nevertheless provides a road map to one of ordinary skill in the art how to do so." In my opinion, the road map that Kaplan is providing is at most the same one provided by the '567 patent claims, the Axel patent, and the '414 application, which is to make the light and heavy immunoglobulin chains in separate host cells. I fail to see how Kaplan would influence the opinion of a person of ordinary skill in the art in the way the PTO suggests – *i.e.*, to modify a procedure where light and heavy immunoglobulin polypeptides are produced in separate cultures to produce both chains in a single host cell.

Dallas Does Not Make Coexpression Obvious

97. The PTO states that the Dallas publication teaches coexpression procedures using one or two plasmids. *See*, February 16, 2007 Office Action, pp. 31-32. It also states that a person of ordinary skill would combine this information with the information in Axel, Rice, Kaplan, and the '567 patent claims. I do not agree.

98. The Dallas publication concerns production of a bacterial vaccine. The goal in Dallas is to make the bacterial cell express one or two new bacterial genes that encode bacterial cell surface proteins. It indicates that this can be done by transforming an *E. coli* with two different plasmids, each containing an inserted bacterial gene encoding a different bacterial protein, or by a plasmid that has been designed to contain the two bacterial genes.

99. There are many significant differences between the Dallas procedures and the coexpression procedures in the '415 patent. For example, the proteins of Dallas (e.g., adhesins) must remain associated with the bacterial cell to create the vaccine. *See, e.g.*, Dallas at p. 3, lines 13-16. Bacterial genes encoding adhesins "necessary for adherence of the pathogenic microorganism being vaccinated against," or toxins "causative of the disease" are introduced to bacterial host cells for production. *See*, Dallas at pp. 3-4. Dallas explains that in order to function as a vaccine and provide lasting immunity, the employed adhesin must be expressed as a surface antigen and adhere to cell surfaces so it can multiply. *See, e.g.*, Dallas at pp. 1-4, 10. The Dallas procedures explicitly do not isolate these proteins from the bacterial hosts. *See*, Dallas at p. 3, lines 13-16. This is important because the cell, rather than the isolated proteins, is administered as the vaccine. Dallas indicates that this use of the bacterial host presents an improvement over vaccines that existed in the art at the time, which utilized killed pathogens or isolated toxins or adhesins. *See*, Dallas at pp. 1-3. Complex mammalian proteins such as heavy and light chain antibody polypeptides, which do not associate in any permanent manner with the cell surface of the producing cell, are not taught or suggested and would have had no place in the Dallas vaccines.

100. The bacterial proteins being expressed also are simple, small polypeptides. Immunoglobulins are large, complex proteins that are foreign to the bacterial cells of Dallas. The ability of these bacterial cells to express the introduced bacterial genes and produce these small bacterial polypeptides would not have lead one of skill to expect that the same procedures could be used to produce one or two complex immunoglobulin proteins in these cells.

101. I do not believe any or all of this information, even when evaluated with the '567 patent claims, would have led a person of ordinary skill in the art to conclude that the coexpression processes of the '415 patent were obvious in early April of 1983. They certainly would not have led that person to assume that coexpression of heavy and light chains was predictable. The Dallas publication and the other documents simply leave too many questions unanswered.

102. As such, I do not believe that coexpression of heavy and light chain variable region polypeptides in a single transformed host cell would have been obvious or predictable as of April of 1983 based on what I find in the Axel, Rice, Kaplan, and Dallas publications, including when that information is combined with the process of producing a heavy or light chain from the '567 patent claims.

Work In Xenopus Ooctyes Does Not Make Production Of Immunoglobulin Tetramers Obvious

103. The PTO states that the mRNA injection experiments described in Valle 1981 and Deacon would lead one of skill to believe that two chains, however produced, will assemble properly and be secreted from a eukaryotic host cell in which they are produced. The PTO also states that a person of ordinary skill in the art would attach no significance to the fact that these experiments involved injection of an mRNA extract rather than genetic transformation of host cells with DNA. *See*, February 16, 2007 Office Action, pp. 62-64. I disagree.

104. The pre-April 1983 literature that studied expression of immunoglobulin genes in B-lymphocytes indicated that *how* immunoglobulin genes are expressed was extremely important in determining whether the cells could successfully produce and secrete functional immunoglobulins. Specifically, that literature was reporting that:

- the successful production of immunoglobulins in lymphocytes was highly dependent on the sequence of expression, and the levels at which the two immunoglobulin genes were expressed (*see, e.g.*, Colman *et al.*, "The oocyte as a secretory cell," MOLECULAR BIOLOGY OF EGG MATURATION, CIBA FOUNDATION SYMPOSIUM 98: 249-267 (Pitman Books, London 1983));

- the levels of expression of each immunoglobulin gene could affect production of the other chain or production of the immunoglobulin (*see, e.g.*, Rice);

- the stage in the development of the B cell was important to expression of the genes and production or secretion of the immunoglobulin (*see, e.g.*, Siden *et al.*, PROC. NATL. ACAD. SCI. 78: 1823-27 (1981));

- the presence of helper proteins in the cell was important (*see* Wabl & Steinberg, PROC. NATL. ACAD. SCI. 79: 6976-6978 (1982)); and

- unbalanced expression could have significant consequences for the viability of the cell (*see* Kohler, PROC. NATL. ACAD. SCI. 79: 2197-2199 (1980)).

105. None of these questions are answered by the work with *Xenopus* oocytes because the use of mRNA from a viable antibody-producing cell that is successfully expressing its immunoglobulin genes entirely sidesteps these questions. The work with *Xenopus* oocytes also is not analogous to the work that would have to be done to produce a transformed host cell. *See, e.g.*, Declaration of Dr. Alan Colman. A host cell must incorporate the foreign DNA and pass this foreign DNA on to its progeny, otherwise it will not be a transformed host cell. *See, id.* Injecting mRNA into a *Xenopus* oocyte does not genetically transform the cell, and the oocyte – even if fertilized – will not pass the foreign mRNA onto its progeny. *See, id.*

106. On page 63-64 of the February 16, 2007 Office Action, the PTO quotes a statement made by a lawyer for Genentech in a European Patent Office proceeding. The statement reads:

> Valle clearly teaches production of an immunologically functional heterologous immunoglobulin molecule in eukaryotic cell transfected by separate DNA molecules encoding its heavy and light chains, respectively. In view of the broad implications evidenced by the Abstract, the fact that the actual experiment was performed with microinjected mRNAs is not relevant. In any event, because the messenger RNA carries the information from DNA to the ribosomal sites of protein synthesis, it is functionally equivalent to DNA.

107. Several aspects of this statement are plainly incorrect as a scientific matter. First, Valle 1981 did not describe any experiment where a eukaryotic host cell was transfected with DNA. Second, mRNA is not equivalent to DNA in its function or role in a cell. While both contain genetic information, they serve very different functions. DNA is capable of replication and stores information that is meant to be passed on to progeny cells. DNA also incorporates regulatory control sequences upstream (such as a transcription promoter and start site) and downstream (such as a transcription stop site) of the region encoding the mRNA. Eukaryotic DNA also contains introns (regions not expressed in the form of mRNA) that must be spliced out to form a functional mRNA. On the other hand, mRNA is a transient molecule not capable of replication that carries transcribed genetic information from the nucleus to the cytoplasm for expression and assembly of polypeptides. One of skill in the art, in my opinion, would not equate the two types of molecules.

108. As such, I do not think any practicing scientist in this field would have considered the translation of microinjected mRNA in a *Xenopus* oocyte and transformation of a host cell with DNA to be equivalent in any respect. This would be particularly true for immunoglobulins, based on what was known in early April of 1983 about the sensitivity of B cells to proper expression of their immunoglobulin genes, both for viability of the cell and for the successful production of antibodies. As such, I do not think anyone of ordinary skill in the art would have agreed with PTO's conclusion that any difference between the mRNA work done in *Xenopus* oocyte and procedures for coexpressing heavy and light chains from a host cell transformed with DNA would be insignificant.

The Ochi And Oi Work Would Not Change Expectations

109. The work reported in Oi and Ochi would not cause a person of ordinary skill working in this field to conclude that coexpression of exogenous heavy and light immunoglobulin chains in a single host cell would be likely to produce assembled immunoglobulin. These experiments, if anything, demonstrate that transformation of lymphocyte cells with exogenous DNA was not well-understood or predictable. The results reported by these authors showed widely varying results despite the relative simplicity of the experimental design of these experiments.

110. Oi reported significantly varying results in their paper. First, they stated that the "frequency at which stable transformed lymphoid cell lines were generated was influenced by every parameter tested." They also reported widely varying results on the ability of the transformed cell line to produce light chain protein from the introduced gene. For example, they reported that "twelve independently transformed Y3 and seven BW5147 cell lines did not produce detectable amounts of the S107 light chain." Oi at p. 827. They also saw varying results on production and secretion of the light chain protein in other cell lines (*e.g.*, production with no secretion, secretion of light chain only, and secretion of light chain-heavy chain aggregates). On page 828, they point out that even in the cell lines that did successfully produce the introduced light chain, the amounts of produced light chain protein varied widely (*i.e.*, "amounts varied from barely detectable to quantities equal to endogenous light chain").

111. The work reported in Ochi also shows that expression of only one light chain gene in one type of cell line was not predictable. Like Oi, Ochi stated that "the mechanisms responsible for the regulation of the expression of rearranged immunoglobulin genes are poorly understood." *See*, Ochi at p. 340. Ochi also reported that the varying levels of expression of their introduced light chain gene "raises the question of whether all the regulatory elements of the normal KTNP gene are present or functioning in the cloned fragment." Ochi at p. 342.

112. These experimental results would have raised more questions than they answer about whether B cells could be made to express two exogenous immunoglobulin genes or could be made to produce assembled immunoglobulins. If anything, one of ordinary skill would assume that only those B cells that express one or more of their endogenous immunoglobulin genes would have the necessary cellular machinery for the expression, folding, and assembly of the two chains.

113. Considering all these points, I do not agree with the PTO's conclusions that coexpression would have been reasonably predictable, or that proper assembly and secretion of immunoglobulins would have been expected in a co-transformed host cell, based on the work reported in the Ochi and Oi papers. Instead, I believe a person of ordinary skill in the art, in early April of 1983, would have thought that successful expression of two immunoglobulin proteins in one transformed host cell would have been unpredictable and that assembly of the two proteins into an immunoglobulin tetramer would have been even more unpredictable.

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Patent

Control Nos. 90/007,542; 90/007,859 Attorney Docket No. 22338-10230

I declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the patent subject to this reexamination proceeding.

May 18, 2007

Date