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

DECLARATION OF DR. TIMOTHY JOHN ROY HARRIS UNDER 37 C.F.R. § 1.132

I, Timothy Harris, do hereby declare and state

1. I am a citizen of the United Kingdom, and reside in San Diego, California.

2. I am the same Timothy John Roy Harris who provided a Declaration in Reexamination No. 90/007,542 on November 25, 2005 ("First Declaration").

3. As I indicated in my First Declaration, I have been retained by Genentech and City of Hope to provide my views on certain issues that have been raised in the reexamination of U.S. Patent No. 6,331,415 ("the '415 patent"). I also note that I have been, and am being, compensated for my time at a rate of $500 per hour.

4. My credentials and experience are essentially as I indicated in paragraphs 1 to 3 of my First Declaration. I also note that the company of which I was Chief Executive Officer, Novasite Pharmaceuticals, recently ceased operations.

5. For the reasons set forth in my First Declaration, I believe, based on my educational training and work experience, I am able to report views that would be representative of a person of ordinary skill in the art in early April of 1983 (*i.e.*, just prior to April 8, 1983). I believe a person of ordinary skill in the field of the '415 patent claims would have a doctorate in molecular biology or a similar scientific discipline, along with about two years of post-doctoral experience.

6. In addition to all of the patents and printed publications I previously reviewed in preparing my First Declaration, I reviewed the following publications:

   - Deacon et al., Biochemical Society Transactions, 4:818-20 (1976) ("Deacon");

- Valle et al., Nature, 291:338-340 (1981) ("Valle 1981");
- Valle et al., Nature, 30:71-74 (1982) ("Valle 1982");
- Dallas, WO 82/03088 ("Dallas");
- Ochi et al., Nature, 302:340-342 (1983) ("Ochi"); and
- Oi et al., Proc. Nat'l. Acad. Sci., 80:825-829 (1983) ("Oi").

7. I also reviewed the following documents (in addition to the materials I identified in paragraph 6 of my First Declaration):

- A PTO Office Action in Reexamination Nos. 90/007,542 and 90/007,859, dated August 16, 2006 ("Second Office Action");
- A PTO Order Granting *ex parte* reexamination of the '415 patent, dated January 23, 2006 ("Second Reexamination Order"); and
- A Request for Ex Parte Reexamination, dated December 23, 2005 ("Second Request for Reexamination"), including attachments to that Request.

8. In addition, I reviewed relevant literature from that general time period (*i.e.*, before April 8, 1983), as I had indicated in paragraph 7 of my First Declaration.

9. In my First Declaration, I explained why certain scientific findings or observations of the Office were inaccurate. I also explained why certain comments in the Office Action were inconsistent with how a person of ordinary skill in the art would have read certain references. In forming these opinions, I evaluated not only what each reference individually taught, but whether and how that reference would be considered in combination with U.S. Patent No. 4,816,567 ("the '567 patent") claims and the other references cited by the Office.

Observations on the New Rejections

10. Beginning on page 22 of the Second Office Action, there is a discussion of the '567 patent claims and various references. In this section, the Office identifies two specific reasons why claims of the '415 patent are believed to be obvious in view of prior art.

- At page 22, the Office states "(i) One of ordinary skill in the art would have been motivated to express, in a single host, light and heavy immunoglobulin chains (using one or two vectors) when viewing the reference Cabilly 1 patented invention in light of the prior art." The Office cites the Axel, Rice, Kaplan and Dallas references to support this point.
- At page 25, the Office states "(ii) The prior art provides further motivation to make active antibody with a reasonable expectation of success." The Office cites

the Deacon, Valle 1981 and Ochi papers to support this point. I note that the Office has also found the information in another paper by Valle (Valle 1982) to be cumulative to what is taught by the Deacon paper, and information in the Oi paper to be cumulative to what is taught by the Ochi paper.

11. I do not believe the Second Office Action accurately portrays what these references actually teach. I also do not believe these references would have been considered, individually or collectively, in the way the Second Office Action suggests they would have by a person of ordinary skill in the art in early April of 1983.

12. Certainly, by early April of 1983, there was interest within the industry of using recombinant DNA technology to produce proteins with known commercial value, including functional immunoglobulin molecules. However, the state of the art at that time and the experiences of those working in the recombinant DNA field, coupled with the information in the references cited by the Office, would not have led people to be particularly optimistic about achieving this goal, and did not provide any clear direction as to how to do so.

Overview of the Relevant Technological Field in April of 1983

13. In early April of 1983, the field of genetic engineering was still developing. It was nothing like the mature field it is today, over two decades later. A relatively small number of proteins had been made by recombinant DNA technology. Almost all of those were relatively simple monomeric (*i.e.*, one polypeptide chain) proteins.

14. In a review article I wrote that was published in April of 1983, I provided a list of eukaryotic proteins that had been produced in E. coli using recombinant methods. *See* Harris, Genetic Engineering, 4:127-85 (1983), attached as Exhibit B to my previous declaration, at pages 164 to 169. All but one of these examples concerned production of relatively simple monomeric proteins. The exception was insulin, which I reported had been produced by individually expressing each of the two chains of the insulin protein in different E. coli cell lines, or by expressing "preproinsulin" (a single polypeptide) which was enzymatically processed *in vitro* to form mature insulin. *See*, pages 137 to 138.

15. My 1983 review article provides a perspective on the types of recombinant DNA projects that had been published by early April of 1983 concerning the expression of recombinant proteins in E. coli. I note that all of the examples described in the review article involved production of one polypeptide in one transformed host cell.

16. I was not aware of any published reports as of early April of 1983 documenting production of a multimeric protein by independently expressing in a single cell recombinant DNA sequences corresponding to the constituent polypeptides of the multimeric protein. I also was not aware of any published reports at that time of production of a multimeric protein of the size (~150 kD) or structural complexity of an immunoglobulin tetramer.

17. As the '415 patent explains, the immunoglobulin tetramer is a large, complex multimeric protein made up of four polypeptides: two light chains and two heavy chains. The

structure of the tetrameric immunoglobulin molecule is generally maintained by a series of disulfide bonds between pairs of cysteine residues and non-covalent interactions between the four polypeptides. For example, in an IgG (depicted in Figure 1 of the '415 patent, reproduced below), pairs of heavy and light chains are linked through inter-chain disulfide bonds, and each pair is linked to the other pair through three disulfide bonds formed between cysteine residues within the heavy chains. In addition to these inter-chain disulfide bonds, each polypeptide subunit (*i.e.*, each heavy and each light chain) is stabilized by two or four intra-chain disulfide bonds. *See, e.g.*, '415 patent at col. 3, lines 19-38.

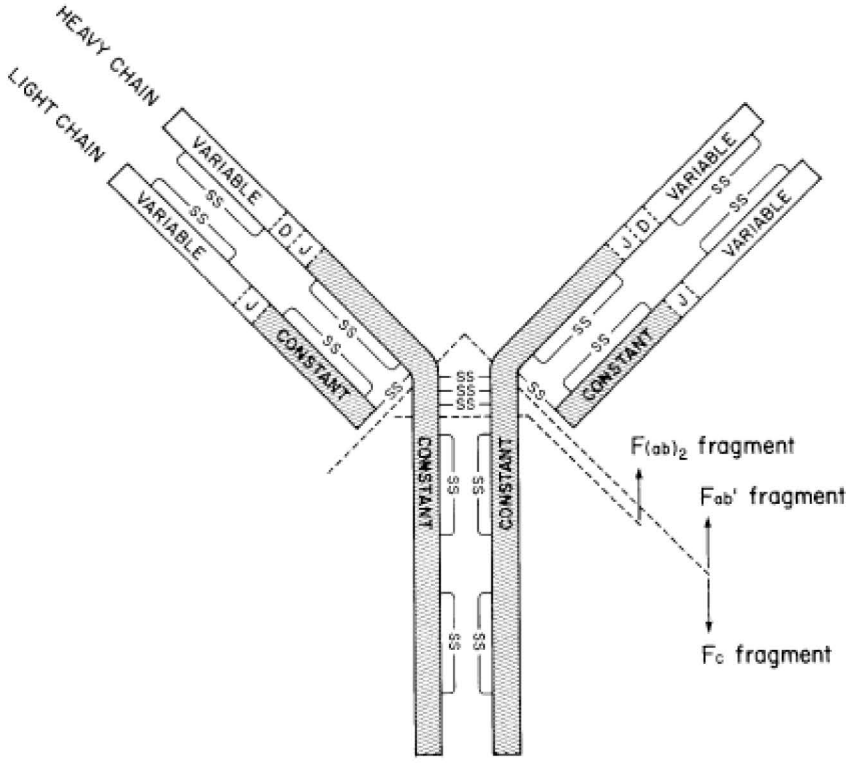

Fig. 1.

18. Based on these known structural characteristics of the tetrameric immunoglobulin molecule, I believe a person of ordinary skill in the art, in early April of 1983, would have expected that the production of an immunoglobulin tetramer using recombinant DNA techniques would have been a significantly more challenging undertaking than the types of projects described in my review article or the molecules described in Axel *et al.*, U.S. Patent No. 4,399,216 ("Axel") (*i.e.*, β–globin) and Rice & Baltimore, Proc. Nat'l.

Acad. Sci., 79:7862-7865 (1982) ("Rice") (*i.e.*, a recombinant immunoglobulin light chain gene).

The Reasonable Expectations of a Person of Ordinary Skill in the Art in Early April of 1983

19. I believe many of the scientific observations in the Second Office Action reflect an inaccurate description of the expectations of a person of ordinary skill in the art in early April of 1983.

20. As I previously indicated, in early April of 1983, I was not aware of any literature reporting the successful production of a multimeric protein by independently expressing in a single host cell recombinant DNA sequences encoding the constituent polypeptides of the multimeric protein. I also do not believe a person having ordinary skill at that time would have many expectations regarding a project of the scale of the '415 patent process based solely on their knowledge of general techniques for producing polypeptides in host cells transformed with recombinant DNA sequences.

21. The Office refers to the transfection experiments conducted in B-lymphoid cell lines in the Second Office Action. The Office apparently considers these types of experiments to be relevant to the '415 patent claims. In my view, these experiments provide little insight into the questions that would have influenced the expectations of a person of ordinary skill in the art contemplating production of an immunoglobulin tetramer or a fragment derived from it through expression of recombinant DNA sequences encoding the heavy and light chains in a single transformed host cell. However, to the extent that the Office does consider these experiments, they should also consider how a person of ordinary skill would have evaluated them in the context of what else was known about B-cells.

22. By early April of 1983, there was an extensive amount of literature documenting research on how B-lymphocytes produce immunoglobulins. That literature had shown that the native processes that govern immunoglobulin production in cells of the B-lymphocyte lineage were complicated and involved many variables.

23. For example, the literature had shown that the processes that govern the assembly and expression of immunoglobulin genes were unique compared to other types of genes. Immunoglobulin genes are assembled by rearrangement of gene fragments in the B-cell incidental to the cell's development into mature, immunoglobulin secreting plasma B-cells. The factors that controlled or influenced the processes of B-cell development as well as the assembly and expression of immunoglobulin genes, however, were not understood by early April of 1983.

24. For example, as Drs. Rice and Baltimore explained in the introduction of their 1982 *PNAS* paper:

> B-cell differentiation proceeds from the "pre-B" lymphocyte, which synthesizes μ immunoglobulin (Ig) heavy chains but no light chains, to the mature B lymphocyte, which synthesizes both heavy and light chains and expresses surface Ig, and finally to the Ig-secreting plasma cell (1-5). The availability of transformed cell analogs has allowed biochemical

characterization of these stages of cellular differentiation (6-11). Recently such studies have contributed greatly to our understanding of the structure of Ig gene segments and the joining of these segments to produce a functionally rearranged Ig gene (12-17).

Although much is now known about Ig gene structure, relatively little is known about the molecular mechanisms that control Ig gene expression.

25. It was also known then that a variety of factors affect the ability of B-lymphocytes to produce and secrete immunoglobulin tetramers. Some of these insights came from the study of lymphocyte cell lines, such as hybridomas and myeloma cells lines. For example, several groups had reported that mutant hybridoma cell lines that produce excess heavy chain often would die, leading to the view that the presence of excess free heavy chain polypeptides in these cells was toxic to the cells. *See,* Wilde & Milstein, Eur. J. Immunol., 10:462-467 (1980); Kohler, Proc. Nat'l. Acad. Sci., 77:2197-2199 (1980) (attached as Exhibits A and B, respectively). This would have caused a person of ordinary skill to question whether unbalanced or uncontrolled production of heavy chain and light chain polypeptides in a transformed host cell would be toxic to the cell.

26. Similarly, it was assumed that mature B-lymphocytes had unique features or attributes that gave these cells the specialization required to produce, properly fold, assemble and secrete immunoglobulins. This was supported by findings in the literature. For example, Wabl and Steinberg reported on the existence of a protein, called "BiP," which they reported bound to free heavy chain in pre-B cells. *See* Wabl & Steinberg, Proc. Nat'l. Acad. Sci., 79:6976-6978 (Nov. 1982) (attached as Exhibit C). They suggested this "helper" protein might be involved in the assembly and expression of immunoglobulin genes or the production of the immunoglobulin molecule.

27. Thus, research that had been done by early April of 1983 had shown that a number of independent but interrelated factors could affect the successful production of the immunoglobulin by the B-lymphocyte including:

(i) The timing and levels of expression of messenger RNA from the native immunoglobulin genes in the B-lymphocytes,

(ii) The amount of heavy and light chain polypeptides present in the cell at various times and locations (*i.e.*, the "stoichiometry" of polypeptides in the cellular environment where the immunoglobulin tetramer might be formed),

(iii) The developmental state of the B-lymphocyte (*e.g.*, whether it had gained the capacity through its development to express the immunoglobulin genes at appropriate levels, or could process the gene expression products to form the immunoglobulin tetramer), and

(iv) The presence of agents in B-lymphocytes that facilitated proper assembly and secretion of the tetrameric immunoglobulin molecule (so-called "helper" proteins).

28. The picture all this work painted was that the expression of immunoglobulin genes in B-lymphocytes, and the natural processes governing production and secretion of tetrameric immunoglobulin molecules by these cells, was complicated and influenced by many interrelated variables. To the extent that a person of ordinary skill in the art turned to the work involving B-lymphocytes for guidance, that person would have reached the conclusion that successful co-expression of recombinant heavy and recombinant light chain DNA sequences in a single host cell, and assembly of immunoglobulin tetramers following co-expression of the introduced sequences, would have been dependent on many interrelated factors. Such a person would not have expected the task to have been as straightforward or predictable as the Office suggests it was in early April of 1983.

The '567 Patent Claims

29. There are three significant differences between the claims of the '415 patent and the claims of the '567 patent.

30. First, the '567 patent claims require that a host cell be transformed with a DNA sequence encoding only one immunoglobulin polypeptide, either a light or a heavy chain. The '415 patent claims, however, require that a single host be transformed with two DNA sequences, one encoding at least the variable domain of a heavy chain polypeptide, and one encoding at least the variable domain of a light chain polypeptide.

31. Second, the '567 patent claims do not require the production of heavy and light immunoglobulin chains as separate molecules in a single transformed host cell, which is required by the '415 patent claims. The '567 claims only refer to the production of either a heavy or a light chimeric immunoglobulin chain polypeptide.

32. Third, unlike the '415 patent claims, the '567 patent claims do not refer to, or require, the assembly of multiple immunoglobulin heavy and light chain polypeptides into an immunoglobulin molecule or immunologically active fragment. Instead, the claims only refer to the isolation of an individual heavy or light chimeric immunoglobulin chain polypeptide after it has been produced by the cell.

33. These are significant distinctions. As I explained above, the challenges of achieving co-expression of two eukaryotic genes in the same cell were different in character than the challenge of expressing only one eukaryotic gene. A person of skill in the art would have expected the approach of expressing recombinant heavy and light chain DNA sequences in the same cell to present a distinct challenge.

34. The Second Office Action also addresses some of the language used in the '567 patent claims and what that language would have meant to a person of ordinary skill. Specifically, at page 33, the Office appears to suggest that a person of ordinary skill in the art would have read the phrase "specificity for a particular known antigen" in the '567 claims as indicating that one should perform the '567 process only to produce a fully assembled and functional immunoglobulin molecule. This is not how a person of ordinary skill in the art would have read that phrase in early April of 1983. As I explained in paragraphs 11 to 14 of my First Declaration, a person of ordinary skill in the

art in early April of 1983 would have understood this phrase in the '567 patent claims as referring to the amino acid sequences found within the variable domain of the individual light or heavy chain being produced as it is these sequences that determine the specificity of antibody. Such a person would not have read these passages in the claims as being a requirement that a functional, antigen-binding immunoglobulin molecule containing heavy and light chains be produced.

The Axel Patent

35. At page 23, the Second Office Action states:

> Axel describes the process as particularly suited for transformation of DNA into eukaryotic cells for making antibodies (*see* col. 3, lines 31-36). Axel discloses and claims the expression of antibodies in mammalian host cells as intact (assembled) proteins. See *Axel*: abstract; col. 5, lines 3-7 and 24-28; patent claims 1, 7, 22-24, 28 and 29.

36. The Second Office Action (page 34) also states:

> Additionally, the Axel reference suggests expressing two immunoglobulin chains in a single cell, since Axel discloses and claims (e.g. claim 7) DNA (i.e. DNA1) encoding an antibody that necessarily possesses both light and heavy immunoglobulin chains. In this respect, the Axel reference clearly encompasses one or more genes which encode one or more proteins: e.g. "… DNA which includes a gene **or genes** coding for desired proteinaceous materials…" (Abstract lines 1-4, with emphasis). Accordingly, although Axel lacks an antibody example, Axel nonetheless suggests recombinant antibody production in a suitable host (e.g. eukaryote).

37. These observations are factually incorrect, and inconsistent with how a person of ordinary skill would have read the Axel patent in early April of 1983.

38. I addressed the Axel patent in my First Declaration at paragraphs 20 to 30. I explained that the Axel patent literally describes a process that uses only two recombinant DNA sequences. "DNA I" encodes a desired "proteinaceous material not associated with a selectable phenotype" that is to be isolated from the transformed host cell. "DNA II" encodes a selectable marker which, when expressed by the transformed cell line, allows a scientist to add an agent into the cell culture where the transformed cells are growing to select out those that have been successfully transformed with DNA II. The polypeptide encoded by DNA II is not recovered from the host cell. I found no examples or other information in the Axel patent describing a process in which a "DNA III" was included, or in which a DNA I was constructed encoding more than one polypeptide chain. *See, e.g.*, paragraph 25 of my First Declaration.

39. The Axel patent cotransformation experiments only involve one instance in which the DNA I is a mammalian gene -- rabbit β-globin protein. This is a relatively simple monomeric polypeptide with a molecular weight of about 16 kD, unlike the large (~150 kD) complex immunoglobulin tetramer. I believe the examples in the Axel patent would

have provided little or no insight into the expression of an immunoglobulin tetramer in a single cell, consistent with my opinion that the Axel patent is addressing production of one polypeptide by one transformed host cell.

40. I also pointed out that the Axel patent disclosure does not contain any suggestions regarding the desirability of expressing both heavy and light immunoglobulin chains in a single mammalian cell line. *See,* paragraphs 27 and 28.

41. The passages of the Axel patent referred to in the Second Office Action do not change my opinion. For example, the Office points to column 3, lines 31 to 36 of the Axel patent, which reads:

> The process of this invention is particularly suited for the insertion into eukaryotic cells of DNA which codes for proteinaceous materials which are not associated with a selectable phenotype such as interferon protein, insulin, growth hormones clotting factors, viral antigens, antibodies and certain enzymes.

42. Contrary to what the Second Office Action states, this passage does not suggest that the Axel patent "DNA I + DNA II" method is particularly suited for making assembled antibodies any more than it suggests that the method is "particularly suited" for making any other type of polypeptide listed along with "antibodies." Instead, in my opinion, the reference to "interferon protein, insulin, growth hormones clotting factors, viral antigens, antibodies and certain enzymes" would have been viewed by a person of ordinary skill in the art as simply being a laundry list of types of proteins having economic value at the time the Axel patent was filed. I believe a person of ordinary skill would view these and other references throughout the patent to "antibodies" as simply identifying an antibody polypeptide (*i.e.*, a heavy or a light chain polypeptide) as a type of polypeptide that can be produced by the Axel process.

43. The Second Office Action also refers to claim 7 of the Axel patent. Claim 7 is consistent with my opinion that the Axel patent is not making any suggestion particularly relevant to production of tetrameric antibodies. This is because the claim is referring in the alternative to "a viral antigen" and to "an antibody." Viral antigens do not have a characteristic or uniform physical structure like a tetrameric immunoglobulin. I do not believe a person of ordinary skill in the art would have referred to these classes of polypeptides in the alternative if they were intending to convey a particular observation about the multimeric nature of the antibody molecule.

44. In my opinion, the Second Office Action is incorrect when it suggests that these various references to the word "antibody" or "antibodies" mean that the Axel patent is specifically describing methods for transforming a single mammalian cell line with recombinant DNA sequences encoding both the heavy and the light immunoglobulin chains. The Axel patent certainly does not provide any specific guidance or suggestion how to do so. *See,* paragraphs 27 and 28 of my First Declaration. It also does not provide an indirect suggestion to do so because the Axel DNA I plus DNA II method contemplates a one protein-one transformed host cell process.

45. The passage in the Axel patent Abstract cited in the Second Office Action as referring to "gene or genes" does not change my opinion. This passage is referring to the concept of inserting multiple copies of the same gene coding for a single polypeptide of interest in order to enhance the yields of the desired polypeptide. It is not referring to insertion of multiple distinct genes coding for different proteins, such as distinct genes coding for the heavy and light chains of an immunoglobulin molecule.

46. The Axel patent explains that the point of introducing multiple copies of DNA I is to increase the number of copies of DNA I that can be expressed by the cell, by which process "multiple copies of proteinaceous or other desired molecules can be produced within eukaryotic cells." Axel at column 7, lines 32-34; *see also* column 14, lines 16 through 18 ("[t]he number of rabbit globin genes integrated into these transformants is variable" – note that there is only one rabbit B-globin gene being referred to in this passage). The body of the Axel patent explains two approaches for obtaining eukaryotic host cells with multiple copies of a gene encoding a desired recombinant protein; namely: (i) inserting multiple copies of the same gene coding for a single polypeptide of interest, or (ii) inserting a construct that will amplify with increasing selection agent (*e.g.*, methotrexate) concentrations. *See,* column 6, line 44 to column 7, line 45.

47. I also disagree with the statement in the Second Office Action that the Axel patent "discloses and claims the expression of antibodies in mammalian host cells as intact (assembled) proteins." (Emphasis added.) Certainly, the mere use of the word "antibody" does not convey this suggestion. In addition, after a thorough review of the Axel patent, I have been unable to locate any description concerning "intact" or "assembled" antibodies. In my opinion, if the Axel patent were describing techniques particularly suited for expressing recombinant DNA sequences encoding light and heavy chain polypeptides in a single host cell, and their subsequent assembly into an immunoglobulin tetramer, there would have been some discussion in the patent about how assembled antibodies consist of multiple discrete polypeptides. There is none.

48. Finally, I do not believe the Axel patent would have suggested to a person of ordinary skill in the art in early April of 1983 that they modify a process of producing one immunoglobulin chain in one cell (*i.e.*, the '567 patent process) by producing both heavy and light immunoglobulin chains in one host cell (i.e., the '415 patent process). As I explained above, the Axel patent is concerned with transformation of a eukaryotic cell with a DNA encoding a single recombinant protein (or multiple copies of that single DNA) in one transformed host cell.

The Rice Paper

49. The Second Office Action also provides an inaccurate description of what the Rice paper would have taught to a person of ordinary skill in the art in early April of 1983. At page 23, the Second Office Action states:

> *Rice* inserted the light chain gene into a plasmid, transfected the cells, and then examined the polypeptides as well as the RNA produced by the cells (*see* pages 7863-7864 and Figures 2 and 3). Lastly, since the cells were

> producing both immunoglobulin light and heavy chain, the cells were examined for the ability to assemble the chains into IgG molecules, leading to the observation that "[e]ssentially all of the κ chain produced in the K-2 cells appear to be assembled into IgG2b" (*see* page 7864 and Abstract penultimate sentence). Thus, *Rice* demonstrates the successful expression of both heavy and light chains in a host with subsequent assembly into immunoglobulins. (Emphasis added.)

50. It then states, at page 35, that:

> Further, the *Rice* reference 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. Thus, in light of this teaching it would be reasonable for one of ordinary skill in the art to expect that expressing a light and heavy chain of the *same* antigen specificity (e.g. derived from a known antibody) in a competent host would result in assembly of a functional antibody. (Underlined emphasis added.)

51. This description of the Rice paper and what it would have suggested to a person of ordinary skill in the art in early April of 1983 is factually inaccurate and inconsistent with how I believe a person of ordinary skill in the art would have actually interpreted the paper at that time.

52. As I explained in my First Declaration (paragraphs 33 to 39), the Rice paper acknowledges that the factors responsible for control of immunoglobulin gene expression in lymphoid cells were not known at the time of the publication. *See,* page 7862 ("relatively little is known about the molecular mechanisms that control Ig gene expression.") The Rice paper (at pages 7864-5) also states that:

> The major result of these studies is the demonstration that a functional κ gene can be introduced into a lymphoid cell line in which it will be continuously expressed. This opens the possibility of examining control and rearrangement mechanisms in lymphoid cells by using inserted genetic elements.

53. Based on this, a person of ordinary skill in the art would view the Rice paper as teaching a technique that could be useful in designing experiments to deduce the mechanisms that control expression of endogenous immunoglobulin genes.

54. Even with respect to the work they actually performed, the authors of the Rice paper were careful to point out what they did not know about their experimental system. In Rice, the authors did not insert a well-defined DNA sequence encoding only the immunoglobulin light chain into the cell. Instead, they inserted a piece of genomic DNA that contained several uncharacterized sequences beyond the sequence of the light chain. These included the intervening sequence between the sequences coding for the variable (Vk), joining (Jk) and constant (Ck) regions, and about 1-1.5 kb of DNA on either side of the

light chain sequence. The authors noted that "any of this extra DNA could be involved in promoter and control functions," suggesting that more than just the DNA sequence of the light chain is required for expression. (*See,* page 7865.) They also noted that the rearranged light chain gene "apparently" used its own promoter to control expression, rather than the standard promoter selected and inserted by the authors of the Rice paper. The promoter that caused expression was not identified or characterized. (*See,* page 7865.)

55. The Rice authors also indicated that it was "an open question" as to whether the endogenous heavy chain expression controlled expression of the recombinant light chain, and suggest further investigation. (*See,* page 7865.)

56. These quotes are consistent with my opinion that a person of ordinary skill in the art in early April of 1983 would not have characterized the Rice paper as the Office does; namely, that it generally established that one could express recombinant DNA sequences encoding heavy and light immunoglobulin chains in a single host cell, and that these chains would be expected to form immunoglobulin tetramers.

57. I do not believe a person of ordinary skill in the art would have extended the results observed in Rice to expression of DNA sequences encoding both immunoglobulin chains in any type of host cell. For example, Rice provides very little information about the physical characteristics of the rearranged kappa light chain gene they used (*e.g.*, they did not identify the presence, nature and location of any promoter, regulatory or control elements). It is evident that the focus of the Rice work is the study of B-lymphocytes, and how these types of cells assemble and express their own endogenous immunoglobulin genes.

58. I also believe the absence of information about the physical characteristics of the rearranged kappa light chain gene (*e.g.*, presence, nature and location of any promoter, regulatory or control elements) to have been a significant omission that would have limited the ability of such a person to extend the experimental methods described in the Rice paper as the Office suggests.

59. In early April of 1983, I also do not believe a person of skill in the art would have equated the experimental results in Rice with results that might be obtained in experiments using recombinant heavy and light chain genes, let alone experiments involving DNA sequences that were not "genes" derived from a native B-cell. As Rice acknowledged, the successful expression of the recombinant light chain gene may have been linked to the ongoing ability of the cell to express its endogenous heavy chain gene. The questions associated with achieving successful expression of two recombinant genes in a B-cell – something not addressed in any way in the Rice paper –would not have been answered by the experimental results provided in Rice.

60. I also disagree with the statement at page 35 of the Second Office Action that the Rice paper "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" or that this would have

led a person of skill "to expect that expressing a light and heavy chain of the same antigen specificity (*e.g.*, derived from a known antibody) would result in assembly of a functional antibody."

61. In my opinion, a person of ordinary skill in the art in early April of 1983 would have distinguished successful expression of a recombinant DNA sequence introduced into a lymphoid cell from "natural" expression of an endogenous gene by a B-cell. Expressing a recombinant DNA sequence in a host cell requires that one transfect the cell and successfully cause the cell to express the inserted sequence. The natural expression by a cell of one of its endogenous genes does not require one to perform these steps.

62. This observation in Rice also does not suggest what might happen if their transformed lymphoid cell were to be transformed with a recombinant DNA sequence encoding a heavy chain polypeptide in addition to the recombinant light chain gene. Given the uncertainty expressed in the paper about what factors contributed to the successful expression of the recombinant light chain gene, I do not believe a person of skill in the art would have jumped to the conclusion that one could introduce two immunoglobulin genes into the same cell and achieve successful expression of both sequences. The authors of the paper, for example, did not make any suggestion along these lines.

63. A person of skill also would have expected, based on general knowledge of B-cell behavior, that the timing, sequence, and levels of expression of light and heavy chain genes would have been important factors that would affect the ability of a B-cell to produce and secrete immunoglobulin, and indeed to the survival of the B-cell. The variable expression levels of recombinant light chain genes reported in Rice, as well as in the Ochi and Oi papers, would have caused a person of ordinary skill to question whether controlled expression of the recombinant heavy and light chain genes could be achieved. It also would have raised questions as to whether unbalanced or uncontrolled expression would have caused the co-transformed B-cell to stop expressing the sequences, not assemble the immunoglobulin tetramer or to die in culture.

64. The Rice paper also did not clearly demonstrate that the endogenous heavy chain and the recombinant light chain assembled into a proper tetramer made up of two heavy chains and two light chains. Instead, the Rice paper merely observed the disappearance of a band on a gel corresponding to the light chain and attributed it to "assembly" of an IgG2b. The Rice paper does not include the actual data used to reach this conclusion in the paper. *See,* page 7864, right column ("In other experiments (not shown) no free κ chain was found in the κ-2 cells, although a significant amount was present in MPC11 cells." (emphasis added)).

65. These observations are consistent with statements in the Ochi and Oi papers. The Ochi paper describes the Rice and Oi papers as only showing "the expression of cloned κ light chain genes in transformed lymphoid cells." (Ochi at page 342).

66. In view of these observations, I do not believe a person of ordinary skill in the art, in early April of 1983, would have viewed the Rice paper as being any more informative about co-expression of recombinant DNA sequences encoding immunoglobulin heavy

and light chains than what the '567 patent claims already require. This is because the Rice paper only teaches expression of a single recombinant light chain gene in a cell line that was already expressing an endogenous heavy chain gene. It says nothing about expressing two recombinant immunoglobulin chains in the same host cell.

67. Thus, like the Axel patent, the Rice paper would not have encouraged a person of ordinary skill in the art to modify the '567 patent claims by taking the additional step of transforming the host cell already transformed with recombinant DNA sequence encoding one of the immunoglobulin chains with a recombinant DNA sequence coding for the other immunoglobulin chain.

Kaplan

68. As to Kaplan, European Patent No. 0 044 722 ("Kaplan"), I refer to paragraphs 40-41 of my First Declaration where I point out that nothing in the Kaplan patent disclosure suggests production of heavy and light immunoglobulin chains in a single bacterial or yeast host cell. Rather, Kaplan presents only a hypothetical plan for production of one immunoglobulin chain in one bacterial or yeast host cell. As I read the discussions of the Kaplan patent in the Second Office Action, it appears that the Examiner does not disagree with this characterization.

69. I also pointed out that the Kaplan patent does not provide any experimental results, examples or specific guidance regarding production of heavy or light immunoglobulin chains in a host cell.

70. As such, I believe that the Kaplan patent does not provide any particular suggestion to a person of ordinary skill in the art to modify the '567 patent claims to transform a host cell with recombinant DNA sequence(s) encoding immunoglobulin light and heavy chains. This is because Kaplan does not propose to express DNA sequences corresponding to both immunoglobulin chains in one transformed host cell.

Dallas

71. The Second Office Action states that the Dallas patent publication "teaches that two different proteins (in addition to a selectable marker) can be expressed in a single cell and such expression may be accomplished by the use of two vectors, each containing DNA sequences encoding one of the proteins, or by use of a single vector that contains DNA sequences encoding each of the proteins." The Office apparently views the Dallas publication in combination with the '567 patent claims and the Axel, Rice and Kaplan references as providing a specific suggestion to a person of ordinary skill in the art in early April of 1983 to transform a single bacterial host cell with DNA encoding for two or more distinct eukaryotic proteins, which supposedly would then specifically suggest the idea of producing the heavy and light chains of an immunoglobulin in a single transformed host cell. I disagree.

72. The Dallas publication describes methods for transforming an E. coli with bacterial genes encoding one or more bacterial cell surface proteins. There is no disclosure in the Dallas publication concerning production of eukaryotic proteins, such as immunoglobulin light

or heavy chains. A person of ordinary skill in the art in early April of 1983 would have understood that there were less significant challenges associated with expressing bacterial genes in an E. coli cell as compared to producing two large complex eukaryotic immunoglobulin proteins using recombinant DNA sequences alien to the E. coli cell.

73. I believe that one of ordinary skill in the art would have considered the cited portions of the Dallas publication to be a restatement of what was already well known at the time; namely, that one could introduce and express multiple bacterial genes (*e.g.*, two different antibiotic resistance genes) using the same or different plasmids in a single bacterial host cell.

74. The examples in the Dallas publication also are small, relatively simple, monomeric polypeptides (*i.e.*, the k88, k99, and LT-B antigens) having molecular weights between ~11 kD and ~30 kD. By contrast, an assembled immunoglobulin tetramer is a complex eukaryotic protein having an approximate molecular weight of 150 kD. A demonstration of expression of bacterial genes encoding simple bacterial polypeptides would have provided no relevant insights to a person of ordinary skill in the art in early April of 1983 attempting to transform and achieve expression in a bacterial or eukaryotic cell line of DNA sequences encoding the heavy and light chains of an immunoglobulin.

75. Other aspects of the Dallas publication illustrate why this reference would not have suggested modifying the '567 patent claims after reading any of the Axel, Rice or Kaplan references as the Office suggests.

76. For example, Dallas does not describe processes where proteins encoded by recombinant DNA sequences are recovered from the host cell. Dallas explains that the transformed cell – not proteins isolated from it – is administered to an animal as the vaccine. *See, e.g.*, page 3 ("Another object of the invention is to provide an improved vaccine employing toxoids or adhesins as the antigenic determinants but which does not require purification and isolation of such substances." (emphasis added)); page 10 ("It may be seen, therefore, that the invention provides improved vaccines and an improved vaccination procedure in which live or attenuated or killed non-pathogenic microorganisms are used." (emphasis added)).

77. Dallas also reports variable levels of expression of the introduced bacterial genes. For example, it indicates that an E. coli transformed with two plasmids was not stable (*see,* Example III, page 8), and that the expression levels were lower in an E. coli transformed with a single plasmid containing two genes relative to expression levels in individually transformed cells. *See,* page 8, lines 21-24 ("The composite plasmid produced both antigens as assessed by agglutination tests, but the level of antigen production was lower than with each gene in a separate bacterium.").

78. As such, I do not believe a person of ordinary skill in early April of 1983 would have considered these examples of bacterial gene expression in Dallas as being relevant to the Axel patent, the Rice paper or the Kaplan publication, each of which concerned eukaryotic genes and proteins. I do not see how Dallas could thus have suggested to a

person of ordinary skill in the art that they modify the '567 patent process, alone or in combination with either Axel, Rice or Kaplan.

Ochi and Oi Papers

79. The Second Office Action states that the Ochi and Oi papers are "cumulative in their teaching of restoring hybridoma cell antibody expression by vector transformation with a light chain gene." Based on this statement, I am limiting my comments to the Ochi paper. I note, however, that both of these papers describe comparable experiments involving insertion of cloned light chain genes into differentiated lymphocyte cell lines. I do not believe either of these papers provides much beyond what is provided in the Rice paper.

80. The Ochi paper describes an experiment in which a cloned light chain gene encoding an anti-TNP antibody light chain was used to transfect a mutant hybridoma cell line (the igk-14 line) that was expressing the endogenous heavy chain gene encoding an anti-TNP heavy chain but had lost its ability to express the endogenous light chain gene for the anti-TNP antibody light chain. The Ochi paper (at page 340) notes that:

> "[b]ecause the igk-14 cells still produce the TNP-specific μ heavy chain, it would be expected that the expression of the [recombinant light chain] κ_{TNP} gene in these cells would restore the production of TNP-specific IgM."

81. This passage provides significant insight into the thinking of highly skilled researchers in the art as of early April of 1983. I would anticipate that the reason why the Ochi group "expected" that the expression of the recombinant light chain gene for the TNP antibody would restore antibody production was because (i) the hybridoma cell line being transfected was derived from a hybridoma that was successfully expressing both heavy and light chain genes, and producing assembled immunoglobulin, (ii) the light chain gene used to transfect the mutant hybridoma line was obtained from the parental (normally functioning) hybridoma, and (iii) the mutant cell line was continuing to express its other heavy and light chain genes.

82. The Ochi paper did not discuss the expression of recombinant DNA sequences encoding heavy chain and light chain polypeptides in a cell that was not expressing either beforehand.

83. The authors of the Ochi paper also did not suggest that their transfection and expression results would be broadly extendable to any type of cell line or situation. Instead, they chose to employ very limited experimental conditions to test a basic hypothesis — whether one could restore gene expression in a cell line that, due to a random mutation, lost its ability to express the same gene. They describe the limited nature of their experimental design in their abstract at page 340, where they state:

> The mechanisms responsible for the regulation of the expression of rearranged immunoglobulin genes is poorly understood. The technique of modifying cloned genes *in vitro* and transferring the modified genes to cells in culture provides a tool for identifying the structural features required for

> gene expression. … To analyse immunoglobulin genes in this manner, however, it is first necessary to use, as recipients, cells that normally permit immunoglobulin production. (Emphasis added.)

84. I believe a person of ordinary skill in the art would not broadly extend the observations and findings in the Ochi paper as the Office suggests; namely, to reasonably expect that one could transform a single B-cell with recombinant DNA sequences encoding light and heavy chain polypeptides, achieve successful expression of the introduced genes, and achieve assembly of functional immunoglobulin tetramers.

85. As I explained above, a person of ordinary skill in the art in early April of 1983 would have expected that successful production of an immunoglobulin tetramer would depend, at least in part, on "correct" levels of expression of the immunoglobulin light and heavy chain genes. The Ochi authors indicated they did not know why they observed varying levels of expression of the recombinant light chain gene, and did not explain how to control expression levels of even the recombinant light chain gene they introduced.

86. As a result, I do not believe the Ochi paper would have encouraged persons of ordinary skill in the art to modify the '567 patent claims, directly or after reading Axel, Kaplan, Rice and Dallas, as the Office suggests. In other words, I do not believe a person of skill would have found any additional guidance in Ochi that would have affected their expectations as to whether one could achieve production of an immunoglobulin tetramer by modifying the '567 patent process by transforming the "singly" transformed host cell with a second recombinant DNA sequence encoding the other immunoglobulin chain. Like Axel, Rice and Kaplan, the Ochi publication documents experiments where only one recombinant DNA sequence (*i.e.*, a recombinant light chain gene) was expressed. This is already required by the '567 claims.

Deacon and Valle Publications

87. The Second Office Action cites the Deacon, Valle 1981 and Valle 1982 papers. These publications relate to experiments where messenger RNA (mRNA) fractions isolated from immunoglobulin producing cells were extracted and injected to unfertilized eggs from *Xenopus laevus* (the South African clawed toad).

88. The Second Office Action, at page 5, states:

> The 1982 Valle and Deacon references are cumulative in their teaching of microinjection of mRNA encoding light and heavy immunoglobulin chains into Xenopus oocyte cells to produce secreted active antibody. Accordingly, only the Deacon reference will be used in the obviousness double patenting rejection(s) recited below.

89. Based on this statement, I will address the Deacon and Valle 1981 papers in this declaration.

90. Both papers concern use of an experimental model system for translating mRNA and studying protein production and assembly. The model system used the *Xenopus* oocyte

cell, which is an undifferentiated egg cell. Upon fertilization, the *Xenopus* oocyte will begin dividing and ultimately differentiate into all the different types of cells in the *Xenopus* frog.

91. At the outset, the Examiner should recognize that experiments performed in *Xenopus* oocytes are not representative of what might be observed in a differentiated cell, such as the "host cells" that are described in the '567 and '415 patent specifications. For example, most differentiated cells do not contain the same diverse range of translational and other cellular elements that enable the *Xenopus* oocyte to perform its unique translational functions on diverse mRNA types. In other words, *Xenopus* oocytes, because they are unique undifferentiated cells, have a special capacity to translate mRNA relative to differentiated cells.

92. I do not believe any person of ordinary skill in the art in April of 1983 –or today– would consider a *Xenopus* oocyte to be a "host cell" within the meaning of the '415 patent. A host cell is a cell that can make copies of itself and can pass on to those copies genetic material that has been introduced into it. Unfertilized frog eggs do not satisfy either of these criteria. An unfertilized frog egg cannot replicate and will eventually die. As a result, it does not pass on any genetic information.

93. As a result of the substantial differences between "host cells" and *Xenopus* oocytes that would have been known by a person of ordinary skill in the art in early April of 1983, I do not believe such a person would have considered the *Xenopus* oocyte model predictive of what would occur in a host cell transformed with recombinant DNA sequences, as required by the '415 patent claims.

94. I also do not believe a person of ordinary skill in the art in early April of 1983 would agree with what the Office states at page 26 of the Second Office Action; namely, that:

> ... once the mRNA or vector DNA is expressed, the ability of the two chains to assemble into an immunoglobulin does not depend on the genetic material used for such expression. Accordingly, the difference between using vector DNA vs. mRNA for host transformation is not substantive.

95. For example, as explained above, a *Xenopus* oocyte is not a "host cell" as I understand that term from the '415 and '567 patent specification. The injection of mRNA into a *Xenopus* oocyte thus is not "host transformation." Rather, mRNA is itself the product of the expression of genes or an introduced DNA sequence by the transcriptional processes of the cell. There is a considerable difference between expressing a DNA in a transformed cell as opposed to producing a protein by translating mRNA in a microinjected *Xenopus* oocyte.

96. In my opinion, in early April of 1983, a person of ordinary skill in the art would consider any of the following to be substantial distinctions that would lead them to question whether the results seen in the *Xenopus* oocytes microinjection experiments would be observed in transformed host cell systems required by the '415 patent claims:

(i) In early April of 1983, many believed the timing and levels of expression of mRNA from the transcription of the immunoglobulin genes in B-cells to be important to successful production of immunoglobulin tetramer. This view derived from observations of B-cell mutants that expressed only one immunoglobulin chain gene.

(ii) The Valle 1981 paper points to the importance of the order and timing of production of the individual heavy and light chain polypeptides as being an important factor affecting production of immunoglobulins in *Xenopus* oocytes. The types of manipulations described in the Valle 1981 paper (*e.g.*, production of one, then the other, immunoglobulin chain in the *Xenopus* oocyte through direct injection of corresponding mRNA over the course of hours or days) could not have been achieved with recombinant DNA host cell transformation techniques known in early April of 1983.

97. Accordingly, I do not agree that a person of ordinary skill would have considered the differences between using mRNA fractions in *Xenopus* experiments and the processes of transforming host cells with recombinant DNA sequences specified in the '415 patent claims to be non-substantive. I also do not agree that the *Xenopus* experiments made predictable or otherwise taught a person of ordinary skill how to achieve in a host cell the results observed in these oocyte experiments, because these experiments do not address the significant challenge of producing a transformed host cell that functions as required by the '415 patent claims (*i.e.*, by inserting recombinant DNA sequences encoding heavy and light chain polypeptides and obtaining the successful transcription and translation of those DNA sequences).

Builder and Accolla

98. As I noted in paragraphs 42 and 43 of my First Declaration, the Builder, U.S. Patent No. 4,511,502 ("Builder") and Accolla, Proc. Nat'l. Acad. Sci., 77:533-536 (1980) ("Accolla") references do not concern expression of recombinant DNA sequences encoding heavy and light chain polypeptides in a single host cell.

99. The Builder patent concerns general reconstitution strategies for recovering expressed polypeptides from bacterial cells. There is no discussion in the Builder patent of processes particularly suited to production of immunoglobulin tetramers. As explained above, the tetrameric structure of an immunoglobulin is complex, and depends on numerous correctly formed disulfide bonds. There is nothing in the Builder patent that specifically suggests applying its techniques to the task of assembling an immunoglobulin tetramer.

100. The Accolla reference also is not relevant to the '415 processes. This reference simply describes production in a hybridoma cell line of a murine monoclonal antibody that binds to the carcinoembryonic antigen (CEA). There is nothing in Accolla that discusses the idea of producing monoclonal antibodies that bind to the CEA antigen through recombinant DNA techniques.

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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.

Timothy John Roy Harris

26 October 2006
Date