UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

ARAGEN BIOSCIENCE, INC.
AND
TRANSPOSAGEN BIOPHARMACEUTICALS, INC.,

Petitioners,

v.

KYOWA HAKKO KIRIN CO., LTD.
Patent Owner

Patent No. 7,425,446
Issued: September 16, 2008
Filed: November 28, 2005
Inventors: Yutaka Kanda, Mitsuo Satoh, Kazuyasu Nakamura, Kazuhisa Uchida, Toyohide Shinkawa, Naoko Yamane, Emi Hosaka, Kazuya Yamano, Motoo Yamasaki, Nobuo Hanai
Title: Antibody Composition-Producing Cell

Inter Partes Review No. _____

DECLARATION OF BRIAN G. VAN NESS, PhD

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I, Brian G. Van Ness, PhD, Professor, Department of Genetics, Cell Biology & Development, University of Minnesota, hereby depose and say:

1. I have been retained by counsel for Aragen Bioscience, Inc. and Transposagen Biopharmaceuticals, Inc. in connection with the above-captioned proceeding. If called upon as a witness, I could competently testify to the truth of each statement herein. I have been asked to provide an opinion concerning U.S. Patent No. 7,425,446 (the "'446 patent") (Ex. 1001) and to render an opinion as to whether the subject matter recited in the claims of '446 patent would have been obvious as of October 6, 2000 to a person of ordinary skill, in light of certain prior-art references and the common knowledge at the time. References that I have relied on in my analysis are attached as **Exhibit A**.

2. As set forth below, I conclude that all claims of the '446 patent are invalid as obvious. The alleged invention is host cells that express antibodies with altered sugar chains (i.e. glycosylation patterns)—without fucose—that result in more effective antibodies (measured by the Antibody Dependent Cell Cytotoxicity (ADCC) standard)) because the sugar fucose is not bound/present on the antibody sugar chains. Apart from the "quite advanced" state of the enabling technology (as the Patent Owner put it during prosecution), three references specifically teach the alleged invention. *Umaña*[1] teaches:

[1] WO 99/54342, Umaña et al., publ. Oct. 28, 1999 ("*Umaña*") (Ex. 1004)

> methods for producing in a host cell an antibody which has an altered glycosylation pattern resulting in an enhanced antibody dependent cellular cytotoxicity (ADCC).
>
> (Ex. 1004 at 8:24-28.)

Rothman[2] points out removal specifically of fucose will provide that enhanced Antibody Dependent Cellular Cytotoxicity (ADCC):

> [t]hus, absence of core fucosylation [i.e. no fucose] itself would appear to be a likely candidate as a structural feature necessary for enhancement of NK cell-mediated ADCC.
>
> (Ex. 1002 at 1122.)

Similarly, *Harris*[3] points out removal specifically of fucose will provide enhanced ADCC. (Ex. 1003 at 1592.)

3. The grounds discussed herein make the challenged claims obvious to the skilled person. In support of my conclusions, I set forth below the reasons and bases in several sections which provide: (1) a summary of my credentials and expertise—**Section I**; (2) legal standards on which my opinion is based—**Sections II, III**; (3) a discussion of the background technology related to the '446 patent and an introduction to the '446 patent—**Sections IV-V**; (4) an identification of the claim constructions that I apply for my analysis—**Section VI**; (5) my specific

[2] Rothman et al., *Antibody-dependent cytotoxicity mediated by natural killer cells is enhanced by castanospermine*-2015-present *induced alterations of IgG glycosylation*, 26 Mol. Immunol. 1113 (1989) ("Rothman") (Ex. 1002.)
[3] Harris et al., *Refined Structure of an Intact IgG2a Monoclonal Antibody*, 36 Biochemistry 1581 (1997) ("Harris") (Ex. 1003.)

analysis that the claims of the ’446 patent are invalid as obvious—**Sections VII-XIII**.

I. Credentials and Expertise

4. I am currently Professor, Department of Genetics, Cell Biology & Development & Cancer Center at the University of Minnesota in Minneapolis.

5. In 1973, I earned my B.S. in biology from the Indiana University of Pennsylvania. In 1975, I earned my M.S. in chemistry from the same University. In 1979, I earned my Ph.D. in biochemistry from the University of Minnesota.

6. From 1980 to 1982 I had a post-doctoral appointment in Molecular Immunology at the Institute for Cancer Research in Fox Chase, Pennsylvania. Since then, I have had many academic, administrative, and scientific appointments. These appointments are listed below:

- **1982-87** – Asst. Professor Biochemistry, University of Iowa;
- **1983-87** – Asst. Professor Genetics Program, University of Iowa;
- **1987** – Assoc. Professor Biochemistry, University of Iowa;
- **1987** – Assoc. Professor Biochemistry and Institute of Human Genetics, University of Minnesota;
- **1992-1998** – Professor Biochemistry and Institute of Human Genetics, University of Minnesota;
- **1998-Present** – Professor Genetics, Cell Biology & Development Department, University of Minnesota;
- **2015-Present** – Adjunct Professor Dept. Clinical & Experimental Pharmacy, University of Minnesota.

7. I provide a list of my consulting/advisory positions:

- **2001-2003** – Scientific Advisory Board & Vice President for Research, Blizzard Genomics, Inc.
- **2004-Present** – Consultant: Cell Signaling Technologies (Boston, MA) Scientific Advisory Board, International Myeloma Foundation,
- **2004-2011** – Research Co-Director, Bank On A Cure
- **2012-2013** – Senior Fellow of Commercialization, MN BioBusiness Alliance
- **2013-Present** – Life Science Alley Institute Advisory Board
- **2014-Present** – Advisory Board, EruditeEDU (an education company),
- **2013-Present** – Founder, CEO: Target Genomics Solutions, LLC
- **2015-Present** - Scientific Advisory Board, GeneSpark Foundation
- **2015-Present** - Scientific Advisory Board, Waldenstrom Macroglobulinemia Foundation

8. I have received other awards in the industry:

- Chi Beta Phi (National Honorary Science fraternity), **1981-82**
- Damon Runyon-Walter Winchell Cancer Fund Fellowship, **1984-87**
- Searle Scholar Award, **2006**
- International Myeloma Foundation Honoree for Directing Bank On A Cure Program

9. I have received scores of substantial grant awards to support my work, which are set forth over multiple pages of my Curriculum Vitae, attached as **Exhibit B**.

10. My administrative appointments have included:

- **1997-2001** – Program Director Cancer Genetics, University of Minnesota Cancer Center;
- **2000-2009** – Department Head, Genetics, Cell Biology & Development Department;
- **2009-2012** – Director Institute of Human Genetics, University of Minnesota;
- **2010-2014** – Co-PI/Director PUMA-Pharmacogenomics, University of Minnesota Alliance. Human Genetics, University of Minnesota;

11. Other appointments and a list of my published research can be found in my Curriculum Vitae, attached as **Exhibit B**.

12. I am a member of a number of professional organizations, including those listed below, with dates indicating my date of membership:

- **1983** - American Immunological Society
- **1988** - Eastern Cooperative Oncology Group
- **1992** - American Association for the Advancement of Science
- **1999** - American Society of Hematology
- **2008** - Pharmacogenomics Research Network (PGRN)

II. Legal Standards

13. I have been informed by counsel and understand that determining whether a patent claim is invalid is made from the perspective of a person of ordinary skill in the art. That determination is made as of the date of priority applicable to the patent claims. For my analysis, I have used the date of the foreign application (JP 2000-308526) to which the '446 patent claims priority—October 6,

2000 (hereinafter, "Priority Date"). This date may change should there be other information disclosing a different invention date, but at this time I have not seen such information.

14. I have been informed by counsel and understand that the terms of the '446 patent claims should be interpreted according to their broadest reasonable construction in light of the specification. I further understand that the claim terms should be construed from the perspective of a person of ordinary skill in the art at the time of the filing of the '446 patent. For the purposes of this analysis, I have applied and analyzed the '446 patent claims according to their meaning in light of the intrinsic patent record as viewed from the perspective of a person of ordinary skill in the art as of the alleged Priority Date.

15. I have been informed by counsel and understand that a claim may be invalid as obvious if the differences between a claim and one or more prior art references are such that the claim as a whole would have been obvious to a person of ordinary skill in the art at the time the invention was made. I understand that assessing which prior art references to combine and how they may be combined to match the challenged claim may not be based on hindsight reconstruction or *ex-post* reasoning. Hindsight reconstruction is using the patent itself as a road map for recreating the invention. In assessing obviousness, only what was known before the invention was made can be considered. I also understand that one important

guard against such hindsight reconstruction is a determination whether a person of ordinary skill in the art would have been motivated, taught, or given the suggestion to combine the relevant teachings of the prior art to duplicate the challenged claim at the time of the filing of the application on the patented invention.

16. In addition to demonstrating obviousness by the combination of prior art references, I understand that a patent may also be obvious if the variation of the prior art is in a manner that is predictable. A patent may also be obvious if the variation from the prior art constitutes a combination of familiar elements according to their known methods or functions. Further, a design need or market pressure for which there is a finite number of identifiable, predictable solutions may provide appropriate motivation to a person of ordinary skill in the art to modify the prior art. In other words, if a combination is obvious to try then the combination may be obvious.

17. I have been informed by counsel and understand that various objective signs of nonobviousness, secondary considerations, may impact the determination of obviousness, provided there is some link between the claimed invention and the secondary factors considered. I have been informed that examples of these secondary considerations include commercial success of a product using the invention, a long-felt but unsolved need for the invention, evidence of copying the claimed invention, industry acceptance of the invention, skepticism or disbelief by

those skilled in the art, failure of others, near-simultaneous invention by multiple parties, and praise of the invention.

III. Level of Ordinary Skill in the Art

18. With respect to the ’446 patent (Ex. 1001), a person of ordinary skill in the art (hereinafter, “POSA”) would have had knowledge of the scientific literature no later than October 6, 2000 concerning the means and methods for creating cells in which the gene for the fucose-adding enzyme fucosyltransferase was removed or “knocked-out,” resulting in a modified sugar chain giving improved antibodies. The POSA would have a doctorate in molecular immunology or biochemistry of glycoproteins including antibodies, knowledge of routine genetic procedures including gene “knock-outs,” and a few years’ practical experience working on the genetics of antibodies.

19. This definition of a POSA conforms to level of skill and knowledge that the ’446 patent applicant noted had been reached by October 6, 2000. During prosecution of the ’446 patent’s grandparent application, the applicant characterized the basic enabling techniques to create the modified-antibody-producing mammalian cells as “quite advanced”:

> [T]he state of the art in the field of, for example, genetic manipulation techniques, at the time of the present invention, were quite advanced. Moreover, the knowledge in the art relating to antibody production from CHO cells, manipulation of CHO cells and enzymes relating to the synthesis of an intracellular sugar nucleotide, GDP-fucose and/or modification of a sugar chain in which fucose is bound to the 6-

position of N-acetylglucosamine in the reducing end through an α(1-6)glycosyl bond in a complex N-glycoside-linked sugar chain, were advanced at the time of the present invention.

* * *

One of ordinary skill in the art will appreciate that in order to obtain a knock-out cell, the intron and exon structures of the target gene should be, advantageously, recognized. One of ordinary skill in the art would appreciate the intron and exon structures of, for example, α1,6-fucosyltransferase, by using a method similar to the method described in Example 12 of the present specification, if the cDNA of the target gene is known. The following references (copies of which are attached) describe that the relevant structures can be determined based on the cDNA:

(i) Glycobiology, vol.9, 323-334 (1999) and

(ii) Glycobiology, vol.8, 87-94 (1998).

In reference (i), the structure motif which is important to the activity of the fucosyltransferase was expected from fucosyltransferases derived from various species (see Figs. 2, 3, 4 and 6). In the reference (ii), the structure which is important to the activity of the fucosyltransferase was similarly expected (Fig. 3).

As for the region of the gene to be deleted, one of ordinary skill will appreciate that after the determination of the structure, any region can be deleted, so long as the activity of the gene is deleted. Generally, one of the ordinary skill in the art would delete the following regions.

(1) ATG site,

(2) promoter region, and/or

(3) active site of protein.

The deletion of these region[sic] is apparent for one of ordinary skill in the art based on the following references (copies of which are attached), for example:

(iii) The EMBO Journal, vol.16, 1850-1857, (1997), and

(iv) Cell, vol.86, 643-653, (1996)

In reference (iii), the exon I containing ATG of β1,4-garalactosyltransferase is deleted to prepare a knock-out mouse (p. 1851, right column, Fig. 1A). Also, in the reference (iv), the region containing a catalyst domain of fucosyltransferase VII is deleted (p. 644, right column, Fig. 2A).

The inventors of the presently claimed invention found cDNA encoding α1,6-fucosyltransferase in CHO cells and the exon 2 genomic region, as described in Example 12 of the present specification. Since the exon 2 contains ATG site, this selection was carried out according to ordinary, well-known methods in the production of knock-out cells. It will be apparent for one of ordinary skill in the art that a knock-out cell could be prepared, without an undue amount of experimentation, by deleting [I'd point out, through homologous recombination driven by the knock-out construct;], for example, regions containing an ATG site, a promoter region, and/or an active site of a protein of interest in addition to or in place of the exon 2 region exemplified in the present application.

(Ex. 1036 (selected pages), Aug. 12, 2004 Amend. at 32–35.)

20. The definition of a POSA I have applied also comports with the statements made by Patent Owner during prosecution of an earlier related patent application (U.S. Patent No. 7,214,775, claiming priority of April 9, 1999), where, in the May 2, 2006 Shitara Declaration, Patent Owner stated that construction of gene constructs and knock-out CHO cells were "standard methods" in the prior art. (Ex. 1035 (selected pages), May. 2, 2006 Shitara Decl. at 5 (citing presentation slides nos. 22–26 and 30–32) ("Following papers provide standard methods of gene knock-out of CHO cells: Zheng, H. & Wilson, J.H., *Gene targeting in normal and amplified cell lines*, 344 Nature 170–73 (1990); Rolig et al., *Survival,*

mutagenesis and host cell reactivation in a Chinese hamster ovary cell ERCC1 knock-out mutant, 12(4) Mutagenesis 277–83 (1997)).)

IV. The State of Antibody and Genetic-Engineering Arts in 2000

A.) Development of "Knock-Out" Genetic-Engineering Techniques

21. Recall the alleged invention is host cells that express antibodies with altered sugar chains (i.e. glycosylation patterns)—without fucose—that result in better antibodies: more effective (measured by the ADCC standard) because the sugar fucose is not bound to their sugar chains. Both host cells to produce antibodies as well as the correlation between sugar-changes and antibody effectiveness (ADCC) were well known in the prior art and involved routine procedures. The only allegedly inventive aspect is discovering the no-fucose/better ADCC correlation. But that too was well known in the prior art. And, as I discuss below, a POSA would have found it obvious, with a reasonable expectation of success, to create such host cells by knocking out the gene for the enzyme that puts fucose on the antibody—using only routine, "standard" (as the Patent Owner puts it) knock-out methods.

22. In this light, I provide here some background information relating to the state of antibody and genetic-engineering arts to provide better context for assessing the '446 patent in view of the prior art. In my opinion, the concepts embodied in the claims of the '446 patent are not new as of the alleged Priority

Date of the ’446 patent. The claims also do not represent the combination of known elements that resulted in an unexpected and unanticipated result. Instead, the alleged inventions of the ’446 patent were already known.

23. I consider first the overall state of the art as of October 6, 2000 in antibody structure and function, genetic engineering, and antibody therapeutics. Based upon my own experience and my review of the state of the art as of the alleged Priority Date of the ’446 patent, in my opinion, the enabling technology for the creation of mammalian cells to produce improved antibodies lacking fucose on their F_c-region sugar chains was standard and, indeed, routine as of the October 6, 2000. The only allegedly non-obvious step was to correlate a lack of fucose with enhanced ADCC. But *Rothman*, *Harris*, and others had already shown this correlation, as explained more fully in the Declaration of Royston Jefferis, PhD, DSc, MRCP, FRCPath. (*See* Ex. 1026.) Wanting to produce such improved antibodies, in my opinion, a POSA would have found it obvious to employ known genetic engineering techniques to express antibodies having modified, no-fucose, sugar chains to get enhanced ADCC function.

24. In conjunction with my opinion in this matter, I have reviewed and rely on the Declaration submitted by Professor Royston Jefferis concerning the state of the art as of this date specifically concerning antibody sugar-chain/ADCC correlations of structure and function. I agree that a POSA would have known of

the ADCC improvement gained by IgG afucosylation (i.e. loss of fucose) taught in the prior art, and would have been motivated to employ the prior-art teaching of mammalian cells genetically engineered—including by gene knock-outs—to express antibodies having modified sugar chains and enhanced ADCC function.

25. In general, genetic engineering, whereby DNA sequences are introduced that modify an organism's genome and may result in novel changes in gene expression, protein expression, and phenotypes (i.e., traits) was standard practice in molecular biology labs in the 1980's and 1990's. Mammalian cell targets of genetic engineering included CHO cells, NSO cells, SP2/0 cells, YB2/0 cells, among many others. *See* Maniatis, T, Fritsch, EF, and Sambrook, S, *Molecular Cloning*. Cold Spring Harbor, First edition, 1983, Second Edition, 1990 (hereinafter, "*Maniatis*"). The source of cells was not a restriction in gene modification, the only requirement being the ability to maintain and grow cells of interest in laboratory cultures. Introducing the DNA to achieve novel sequence expression was referred to as transfection; and various technologies were well developed to transfect virtually any DNA sequence into target cells. *Id.*

26. One important question that could be addressed in genetic engineering was the consequence of a targeted genetic loss of function for a trait or biologic activity of a cell or products produced by the cell (e.g., antibodies)—"knocking out" the targeted gene. In this context, "knocking out" a gene means using genetic

engineering techniques to disrupt the DNA sequence that regulates or codes for the gene product so that the cell can no longer generate a functional protein from that altered sequence. The gene is structurally and functionally "knocked out."

27. In the context of this case, the gene target to "knock out" would have been the enzyme that puts fucose on the antibody sugar chain. Loss of function for that enzyme could reasonably be expected to result in an antibody lacking fucose sugar residues on the Fc sugar chains. An effective approach described in 1987 by Dr. Mario Capecchi—which could be described as a landmark study—described site directed mutagenesis in mammalian stem cells. (Ex. 1008.) In this published work, Dr. Capecchi outlined a protocol "useful for targeting mutations into any gene." (*Id.*)

28. In 1989, Dr. Capecchi described "knock-out" methodology in a classic paper entitled "*Altering the genome by homologous recombination*." (Ex. 1009.) Over the next several years, the technology improved with better understanding of vector construction that allowed for efficient targeting of genomes. For example, innovations in the field enabled specific and efficient targeting by lengths of DNA homologous to the target sequence that could find and base pair with the target sequence and thus introduce mutations to disrupt and silence the gene—the "knock-out." (*See*, e.g., Ex. 1010.)

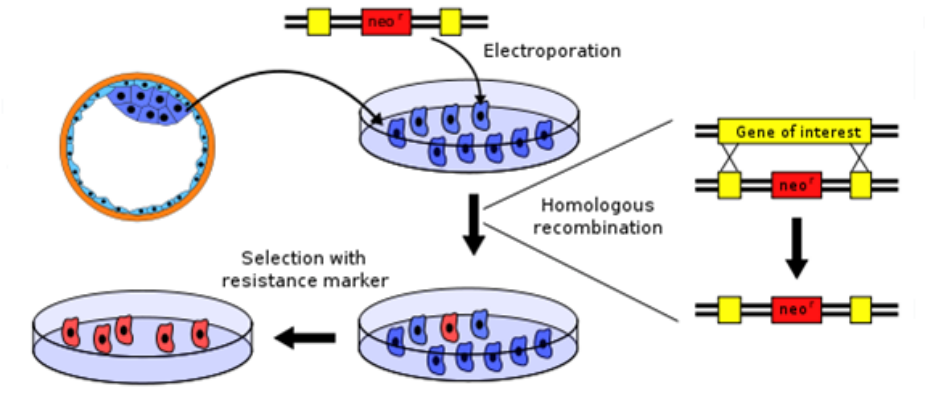
29. Dr. Capecchi concluded in a 1989 paper: "Homologous recombination between DNA sequences residing in the chromosome and newly introduced, cloned DNA sequences (gene targeting) allows the transfer of any modification of the cloned gene into the genome of a living cell. This article discusses the current status of gene targeting with particular emphasis on germ line modification … and describes the different methods so far employed to identify those … cells in which the desired targeting event has occurred." (Ex. 1009.)

30. The technique of homologous recombination also allowed a working copy of a gene (e.g. an antibody) to be inserted into a cell genome, as well as knocking out a functioning gene already in the cell. Mammalian cells were routinely used as a "factories" to produce molecules—especially antibodies—by inserting the gene for the antibody of interest into the host cell (CHO, COS, etc.). Thus a POSA, wanting to make an antibody that is afucosylated, would obtain a host cell with an antibody already in place or else insert the antibody gene. To then produce antibodies that lack the fucosyltransferase enzyme, the POSA would knock out the gene that encodes it. With the gene non-functional, this modified cell would act as the host cell for the production of afucosylated antibodies.

31. Subsequent "knock-out" approaches then expanded greatly. By 1999, over 3,000 publications reported what became standard technology for "knock-outs," each publication distinct—not in the methods employed, rather, the target

gene and cells of that particular study. A full review of the 3,000-plus publications that detail the development of "knock-out" techniques in the 1980s and 1990s—a task of immense scope—would reveal that the ability to genetically modify a target cell to inactivate or "knock-out" an existing gene by replacing it or disrupting it with an artificial piece of DNA, introduced by standard transfection protocols, was standard technology by the mid-1990s. The technology was used to create a knockout in the genome of every cell in a live organism (mouse), in 1989, for which Mario Capecchi, Martin Evans, and Oliver Smithies were awarded a Nobel Prize.

32. The figure below schematically shows how a gene knock-out is accomplished through a combination of techniques that were standard by 1995.



33. The above figure shows a homologous recombination to knock-out a target gene (e.g, α1,6-fucosyltransferase, shown in red) in a cell. Cells are grown in a culture dish, and a DNA construct is made that contains a selectable marker (in

this case an antibiotic resistance gene, NEO, shown in red), flanked by sequences that will base pair with the target gene. Enzymatic machinery in the cell catalyzes the exchange of the vector DNA into the host genome DNA by homologous recombination. The host gene (e.g. α1,6-fucosyltransferase) is disrupted ("knocked-out") and the selectable marker (NEO) confers resistance of cells that have incorporated the NEO gene to the antibiotic, neomycin. The only cells that survive are the red cells that, in our example, (1) have had their α1,6-fucosyltransferase genes knocked out and (2) the NEO antibiotic-resistance gene inserted.

34. Typically, a gene-containing "vector" is constructed in the lab—usually a bacterial plasmid or other DNA construct that is transfected into target cells in culture (e.g., CHO cells). The construct is engineered to recombine with the target gene, which is accomplished by incorporating DNA sequences identified from the target gene itself into the construct. A base pairing occurs between the vector and the cells' genomic sequence; recombination then occurs in the region of that sequence within the gene, resulting in the insertion of a foreign sequence to disrupt the gene (termed homologous recombination). (*See* Ex. 1011.) Often, a selectable marker is included that is incorporated into the host genome at the target site (*e.g.*, an antibiotic resistance gene), as illustrated in the example above. With its sequence interrupted, the altered gene in most cases was translated into a

nonfunctional protein, if it was translated at all; whereas the intact selectable marker was expressed and cells selected (in this case by the ability to grow in the presence of an antibiotic). This technology was routine in the 1990's to a POSA, with thousands of published publications. (*See, e.g.,* Exs. 1012, 1013.) Indeed, as stated in the opening line of one paper from 1995: "[i]ntroduction of defined modifications at a genomic level by gene targeting ha[d] become a widely used technique." (Ex. 1013.)

B.) Genetic Engineering in the Field of Immunology

35. In the field of immunology, the 1980's and 1990's saw an explosion of genetic modifications that influenced immunoglobulin (Ig) production in a variety of target cells engineered by transfection to produce novel antibodies, or genetic modifications to improve antibody function. (*See*, *e.g.*, Exs. 1014, 1015, 1016, 1017.) These innovations included new approaches that allowed scientists to modify sugar chains normally attached to amino acid residues in antibody molecules, and these innovations occurred hand-in-hand with new discoveries in antibody structure and function, antibody engineering, and antibody therapeutics.

36. As early as 1989, chimeric mouse-human IgG genes were constructed, and after DNA insertion into target cells could produce the IgG. One of the targets for subsequent modification was the amino acids that link to sugars. These modifications allowed scientists to examine effects of sugar chain modification on

IgG functions. (Ex. 1018.) Indeed, as early as 1982, the role of the sugar fucose was found to effect antibody dependent cytotoxicity. (Ex. 1019.)

37. As Prof. Jefferis confirms in his Declaration, research into antibody structure and function, antibody engineering, and antibody therapeutics revealed a correlation between modification of an antibody sugar chain and the efficiency ("effector function") of the action of the antibodies to cause a strong immune response, measured as Antibody-Dependent Cellular Cytotoxicity ("ADCC"). (*See* Ex. 1026 at ¶¶ 15-37.)

38. As also confirmed by Prof. Jefferis, published research explained that the removal of a particular sugar (the fucose sugar normally bound to N-acetyl glucosamine) would enhance critical ADCC effector function. (*See* Ex. 1026 at ¶¶ 15-37; Ex. 1002 at 1122; Ex. 1003 at 1592.)

39. Given the state of genetic engineering technology, methods that would allow a POSA to knock-out genes in host cells were well within the ability of those skilled in the art, as taught by the widely practiced methods of DNA transfection. *See Maniatis*; and homologous recombination as taught by Cappecchi in 1987 (Ex. 1008), and subsequently applied in thousands of reports between 1989-1999. The methodology provided the opportunity to transfect any gene in any host cell grown in culture, including immunoglobulin genes into COS and CHO cells (Ex. 1020), as well as knock out genes responsible for the transfer of sugars to

immunoglobulin proteins (e.g., sugar transferases). Specifically, both transfection of mammalian cells with antibody genes and knock-outs of fucosyltransferase could be—and were—routinely accomplished. (*See, e.g.*, Ex. 1005.)

40. As of the alleged Priority Date of the ’446 patent, fucosyltransferase was known to be the enzyme that puts fucose on the antibody sugar chain. The human fucosyltransferase gene sequence had been cloned in 1994 by Sasaki et al.(269(20) J. Biol. Chem. 14730–37 (1994)). Indeed, during prosecution of the ’446 patent’s grandparent application, the patentee cited specific prior-art articles that confirm that sufficient information of the gene sequence for α1,6-fucosyltransferase had already been published. (*See* Ex. 1036 (selected pages), Aug. 12, 2004 Amend. at 33–34 (“In reference (i), the structure motif which is important to the activity of the fucosyltransferase was expected from fucosyltransferases derived from various species (see Figs. 2, 3, 4 and 6). In the reference (ii), the structure which is important to the activity of the fucosyltransferase was similarly expected (Fig. 3).”)

41. Knowing this sequence—which a POSA could have determined independently and routinely—would have allowed a POSA to target this gene and disable it by using known knock-out techniques.

42. By 2000, the technologies of transfection and gene knock-out were routine and well known to a POSA. Thus, as set forth more fully below, the core

alleged novelty of the '446 patent claims—removing fucose from antibodies to get antibodies of enhanced effector function—would have been obvious to a POSA as of the alleged priority date of the '446 patent.

V. Introduction to the '446 Patent

43. I have reviewed the '446 patent entitled "*Antibody Composition-Producing Cell*" and the related prosecution history, including the prosecution history of the '446 patent's parent applications. As stated in the Summary of Invention (and Abstract):

> [the] object of the present invention is to provide a host cell which produces an antibody composition and can control a sugar chain structure bound to the antibody molecule…a production method of an antibody composition using the cell and an antibody composition produced by the production method.

(Ex. 1001 at 5:35-42.)

44. The sole independent claim of the '446 patent recites a "host cell" that has "decreased or no α1,6-fucosyltransferase activity," as shown below:

> **1**. An isolated mammalian host cell which has decreased or no α1,6-fucosyltransferase activity for adding fucose to N-acetylglucosamine of a reducing terminus of N-glycoside-linked sugar chains by deleting a gene encoding α1,6-fucosyltransferase or by adding a mutation to said gene to reduce or eliminate the α1,6-fucosyltranferase activity, wherein said mammalian host cell produces an antibody molecule.

(Ex. 1001 at Cl. 1.)

45. Stated another way, the sole independent claim of the ’446 patent is directed to creating a mammalian host cell that has the cells’ α1,6-fucosyltransferase gene knocked out in order to express afucosylated antibodies with enhanced effector (ADCC) function.

46. The dependent claims in the ’446 patent are simply directed to particular mammalian cells—all routinely used—and the IgG antibody molecule itself.

47. Consistent with the state of the art as of the alleged Priority Date of the ’446 patent, the ’446 patent acknowledges the already-known connection between sugar-structure/modification and its effect on antibody-effector-function. (Ex. 1001 at 2:1–38.) For instance, the specification of the ’446 patent notes that prior research established that “the structure of the sugar chain plays an important role in the effector functions of human antibodies of IgG subclass and that it is possible to prepare an antibody having more higher [sic] effector function by changing the structure of the sugar chain.” (Ex. 1001 at 2:31–35.)

48. The ’446 patent specification also cites known, prior-art examples of techniques for modifying the structure of the IgG antibody sugar chain. A notable example of a known genetic modification discussed in the ’446 patent is the technique of adding the fucose sugar to the “non-reducing” end (as opposed to the usual, reducing, end) of the sugar chain “by introducing human β-galactoside-2-

αfucosyltransferase into mouse L cell [Science, 252, 1668 (1991)]." (Ex. 1001 at 4:65-5:2.) The skilled person would have reasonably expected to do the converse: knock out the fucosyltransferase gene to prevent α1,6 addition of fucose at the reducing end of the sugar chain.

49. The '446 patent specification describes the alleged problem in the art not as one of available techniques, but as a lack of knowledge as to the specific structures on the sugar chain that are "important structure for the effector function[.] (Ex. 1001 at 2:35–38.) Later in the specification, the '446 patent reiterates this conclusion:

> Particularly, although it has been revealed little by little that the effector function of antibodies is greatly influenced by the sugar chain structure, a truly important sugar chain structure has not been specified yet.

(Ex. 1001 at 5:25-29.) I disagree with both of these statements.

50. As set forth in greater detail herein, I agree with the opinion of Prof. Jefferis that the description in the '446 patent as to the knowledge of a POSA regarding the correlation between sugar chain modification and enhanced ADCC as of the alleged Priority Date is incorrect. (*See* Ex. 1026.) I agree with Prof. Jefferis' analysis that a POSA would have known (and would have been expressly taught by the prior art) the sugar structure important for improved effector function: i.e. one lacking fucose. (*Id.* at ¶¶ 15-48.)

51. In my opinion, based on my direct knowledge of the area of molecular immunology and genetics as of October 6, 2000, as well as a review of relevant prior art (prior to October 6, 2000), a POSA knowing of the correlation between afucosylation and enhanced antibody effector function, would have been strongly motivated to obtain cells to produce therapeutic antibodies with enhanced ADCC.

52. Indeed, others had already successfully knocked out a fucosyltransferase gene, specifically the α1,3-fucosyltransferase enzyme, from mouse embryo cells. (*See* Ex. 1005.) In my opinion, this success would have only emboldened the skilled person to "knock-out" α1,6-fucosyltransferase. As the Patent Owner stated in bringing *Malý* to the Examiner's attention during prosecution of the '446 patent's grandparent application:

> [I]n the reference (iv) [*Malý*], the region containing a catalyst domain of fucosyltransferase VII is deleted (p. 644, right column, Fig. 2A).
>
> The inventors of the presently claimed invention found cDNA encoding α1,6-fucosyltransferase in CHO cells and the exon 2 genomic region, as described in Example 12 of the present specification. Since the exon 2 contains ATG site, this selection was carried out according to ordinary, well-known methods in the production of knock-out cells. It will be apparent for one of ordinary skill in the art that a knock-out cell could be prepared, without an undue amount of experimentation, by deleting, for example, regions containing an ATG site, a promoter region, and/or an active site of a protein of interest in addition to or in place of the exon 2 region exemplified in the present application.

(Ex. 1036 (selected pages), Aug. 12, 2004 Amend. at 32–35.)

53. As such, in my opinion, the methods claimed in the ’446 patent would have been obvious to, and indeed were well within the abilities of, a POSA.

VI. Claim Construction

54. I understand that claim terms should be interpreted according to their broadest reasonable construction in light of the specification, and that the words of the claims should be given their plain meaning unless that meaning is inconsistent with the specification. I further understand that the claim terms should be construed from the perspective of a person of ordinary skill in the art at the time of the filing of the ’446 patent.

55. In this regard, I have reviewed the intrinsic patent record and conclude that a POSA would understand the claim language “which has decreased or no α1,6-fucosyltransferase activity for adding fucose” to mean “which has zero or no α1,6-fucosyltransferase activity for adding fucose.” Similarly, I conclude that a POSA would understand the claim language “deleting a gene encoding α1,6-fucosyltransferase or by adding a mutation to said gene to reduce or eliminate the α1,6-fucosyltranferase activity” to mean “deleting a gene encoding α1,6-fucosyltransferase or by adding a mutation to said gene to remove or eliminate the α1,6-fucosyltranferase activity.”

56. During prosecution of the ’446 patent’s grandparent application, the Examiner rejected pending claims as non-enabled for only a mere “***decrease***” in

α1,6-fucosyltransferase activity, but enabled only for a 100% loss of α1,6-fucosyltransferase activity:

> because ***the specification, while being enabling for a CHO cell comprising a deletion of at least exon 2 of one FUT8 gene, which deletion produces a non-functional enzyme***, into which a gene encoding an antibody is introduced, such antibody gene being expressed and producing antibodies having complex N-glycoside-linked sugar chains bound to the Fc region, wherein among the total complex N-glycoside-linked sugar chains bound to the Fc region in the composition, ***the ratio of a sugar chain in which fucose is not bound to N-acetylglucosamine at the 6 position is 20% or more, does not reasonably provide enablement for any CHO cell or any CHO cell comprising any deletion of a gene encoding FUT8 that produces any decrease in such enzyme***,

(Ex. 1036 (selected pages), Feb. 13, 2004 Off. Act. at 7 (emphasis added).) And later the Examiner explained:

> It is maintained that Applicant still has not provided an enabling disclosure based on even one single enzyme mutation that ***decreases the activity of such enzyme*** to the proper amount, in CHO cells and thereby allows such cells to produce the claimed characteristic glycosylations (e.g., Official Action of 13 February 2004, p. 7, first paragraph, "... that produces any decrease in such enzyme [activity] . . ."). Applicant has ***only demonstrated the ability to completely remove activity*** in a reasonably predictable manner[.]

(*Id.*, Nov. 3, 2004 Off. Act. at 11 (emphasis added).)

57. In response to the Examiner's rejections, the applicant amended the claims to remove "decreased." (Ex. 1036 (selected pages), Dec. 17, 2004 Resp. to Off. Act.) Finally, the same Patent Owner in an earlier-filed patent family

(claiming a priority date of April 9, 1999) directed to no-fucose antibodies, argued in slides presented to the Examiner that its knock-out invention produced 100% fucose-free antibodies:

> Further examples of the invention
>
> -Establishment of FUT8 Knock-out CHO/DG44 cells can be made according to the standard methods reported before the patent application:
>
> α1,6-Fucosyltransferase: (FUT8) Knock Out to produce 100% fucose (-) antibodies

(Ex. 1035 (selected pages), May. 2, 2006 Shitara Decl., slide 4.) This confirms the all-or-nothing effect of knocking out the fucosyltransferase genes.

58. I see no support in the patent specification for the creation of finessed knockouts to create fucosyltransferase enzymes having varying particular levels of functionality, in line with the Examiner's same finding based on the intrinsic record.

59. Accordingly, in my opinion, the broadest reasonable interpretation for "which has decreased or no α1,6-fucosyltransferase activity for adding fucose" is "which has zero or no α1,6-fucosyltransferase activity for adding fucose." And the broadest reasonable interpretation of "deleting a gene encoding α1,6-fucosyltransferase or by adding a mutation to said gene to reduce or eliminate the α1,6-fucosyltranferase activity" is "deleting a gene encoding α1,6-

fucosyltransferase or by adding a mutation to said gene to remove or eliminate the α1,6-fucosyltranferase activity."

VII. GROUND 1: Claims 1-6 of the '446 patent are Obvious over *Rothman* in view of *Umaña* and the knowledge of a POSA.

A.) Opinion Introduction and the *Rothman* and *Umaña* References

60. In my opinion, claims 1-6 of the '446 patent are obvious over *Rothman* in view of *Umaña* and the knowledge of a POSA.

61. My analysis is set forth below. I also incorporate into my analysis the accompanying claim chart (**Exhibit C**), which sets forth portions of the cited prior art references corresponding to claims 1-6 of the '446 patent.

62. *Umaña*, which is prior art to the '446 patent, is directed "generally, to methods for the glycosylation engineering of proteins to alter and improve their therapeutic properties" and "describes methods for producing in a host cell an antibody which has an altered glycosylation pattern resulting in an enhanced antibody dependent cellular cytotoxicity (ADCC)." (Ex. 1004 at 8:24-28.) *Umaña* further explains that such methods include "the use of gene knockout technologies or the use of ribozyme methods" that "tailor the host cell's glycosyl transferase and/or glycosidase expression levels[.]" (*Id.* at 15:20-22.)

63. *Rothman,* which is also prior art to the '446 patent, describes how the "absence of core fucosylation itself would appear to be a likely candidate as a

structural feature necessary for enhancement of NK cell-mediated ADCC." (Ex. 1002 at 1222.)

B.) Obviousness over *Rothman* in view of *Umaña* and the knowledge of a POSA

64. I agree with the analysis of Prof. Jefferis that there was a well-known correlation between antibody sugar chain modification and the efficiency ("effector function") of an antibody as of the alleged Priority Date of the '446 patent. (*See* Ex. 1026 at ¶¶ 15-37, 55-74.) And, more specifically, I agree with Prof. Jefferis that the prior art (represented by *Rothman*) describes the correlation between sugar chain modification—including the removal of fucose, particularly—and improved ADCC. (*Id.* at ¶¶ 55-74.) Based upon my review of the prior art and knowledge gained over my 35-plus-year career in genetic immunology, I believe that the known correlation between removal of fucose and improved ADCC (represented by *Rothman*) would have motivated a POSA to utilize known, routine genetic engineering techniques (represented by *Umaña*) to create the "host cell" recited in claims 1-6 of the '446 patent.

1. Claim 1 limitation a: "[a]n isolated mammalian host cell which has decreased or no α1,6-fucosyltransferase activity for adding fucose to N-acetylglucosamine of a reducing terminus of N-glycoside-linked sugar chains"

65. I have reviewed *Rothman*, which explains that the "absence of core fucosylation itself would appear to be a likely candidate as a structural feature

necessary for enhancement of NK cell-mediated ADCC." (Ex. 1002 at 1122; *see also* Ex. 1002 at 1114.) I have also reviewed and agree with Prof. Jefferis' analysis that *Rothman*'s teachings would have motivated a POSA to utilize known, routine genetic engineering techniques (represented by *Umaña*) to create the "host cell" recited in claims 1-6 of the '446 patent. (*See* Ex. 1026 at ¶¶ 55-74.) Moreover, in my opinion, creating such a cell would have been obvious and routine to a POSA.

66. Given the teachings of *Rothman*, and considering the state of genetic engineering technology as of the alleged Priority Date, in my opinion, a POSA would have found it obvious to create "[a]n isolated mammalian host cell which has decreased or no α1,6-fucosyltransferase activity for adding fucose to N-acetylglucosamine of a reducing terminus of N-glycoside-linked sugar chains[.]" As discussed above, *supra* **Section IV**, the knowledge of a POSA as of the alleged Priority Date would have rendered the act of fucosyltransferase knock-out routine.

67. *Umaña,* which is representative of the state of the art as of the alleged Priority Date of the '446 patent, is directed to the creation of a host cell using "genetic knockout techniques" to "tailor the host cell's glycosyl transferase and/or expression levels[.]" (Ex. 1004 at 3:9-11, 15:20-22.) *Umaña* further describes engineering such host cells by transfecting nucleic acid "encoding a whole antibody molecule," which "produce[s] altered glycoforms of proteins having

improved therapeutic values, e.g., an antibody which has an enhanced antibody dependent cellular cytotoxicity (ADCC) in a host cell." (*Id.* at 15:24-28, 3:6-9.)

68. *Umana's* disclosure is consistent with the state of the art as of the alleged Priority Date of the '446 patent. As discussed above, *supra* **Section IV**, the knowledge of a POSA as of the alleged Priority Date would have rendered the act of creating a host cell which has "decreased or no α1,6-fucosyltransferase activity" routine.

69. In this regard, I note that as of the alleged Priority Date of the '446 patent, I was working with both antibodies and genetic engineering. In particular, I employed the technique of homologous recombination of genes called "knock-ins," a refinement of the original technique of "knockouts," both of which targeted particular genes to disable their expression. (*See, e.g.*, Exs. 1021, 1022, 1023.)

70. The knock-out and knock-in techniques were routinely used as of the alleged Priority Date of the '446 patent, in order to obtain genetically modified cells to produce modified molecules—modified because a gene involved in their usual production had been disabled, i.e., knocked out or knocked in. (*See, e.g.*, Ex. 1009.) The routine techniques allowed scientists to completely remove certain genetic traits.

71. The removal of certain genetic traits and the resulting production of modified molecules was useful for a variety of purposes, including, for instance to:

- Identify the impact on a cellular physiology by a loss of function. *See, e.g.,* Bernstein, A and Brietman, M. 1989. *Genetic ablation in transgenic mice*. Mol Biol Med 6:523-530; Berke G. 1994. *The binding and lysis of target cells by cytotoxic lymphocytes: molecular and cellular aspects*. Ann Rev Immunol 12:735-773; Bachman MF and Kundig TM. 1994. *In vivo versus in vitro assays for assessment of T- and B- cell function*. Curr Opin Immunol. 6:320-326.

- Identify how a loss of function affects protein-protein interactions. *See, e.g.,* Ahmed, M , et al. 1994. *A protein that activates expression of a multidrug efflux transporter upon binding the transporter substrates*. J Biol Chem. 269:28506-28513; Cosgrove, D. et al. 1992. *Evaluation of the functional equivalence of major histocompatibility complex class II A and E complexes*. J Exp Med. 176:629-634.

- Identify how proteins function when genes that modify their structure are deleted (e.g., fucosyltranferase deletion impact on antibody functions, such as ADCC). *See, e.g.*, Baba, T. et al. 1994. *A cytoplasmic domain is important for the formation of a SecY-SecE translocator complex*. Proc Natl Acad Sci. 91:4539-4543; Cowan, PJ, et al. 1998. *Knock out of alpha 1.3-galatosyltransferase or expression of alpha 1,2 fucosyltransferase further protects CD55 and CD59-expressing mouse hearts in an ex vivo model of xenograft rejection*.

- Identify how functions change if novel genes or genes resulting in novel proteins are engineered into cell by knock-in techniques. *See, e.g.*, Metzger, H. 1994. *Immunoglobulin receptors. Handicapping the immune response*. Curr Biol. 4:644-646.

- Identify how knock-in or knock-out affects cellular functions, such as immune response pathways. *See, e.g.*, Wiman KG. 1993. *The retinoblastoma gene: role in cell cycle control and cell differentiation*. FASEB J. 10:841-845; Declerck, PJ, et al. 1995. *Generation of monoclonal antibodies against autologous proteins in gene-inactivated mice*. J Biol Chem. 270:8397-8400; Yang, Y, et al. 1994. *MHC class I-restricted cytotoxic T lymphocytes to viral antigens destroy hepatocytes in mice infected with E1-deleted recombinant adenovirus*. Immunity 5:433-442.

72. Accordingly, in my opinion, a POSA would have been highly motivated to create host cells with their fucosyltransferase genes knocked out to acquire antibodies with improved ADCC. The expected therapeutic benefits were obvious and considerable: lower doses of antibodies, more effective immune responses, and fewer side effects.

73. In light of the specific motivation to remove fucose from IgG antibodies to improve their ADCC effector function, and the known genetic sequence of the α1,6-fucosyltransferase gene (and the ability to determine this independently), in my opinion a POSA as of the alleged Priority Date of the '446 patent would have found it obvious—with at least a reasonable expectation of success—to apply routine knock-out techniques to create mammalian cells with "decreased or no α1,6-fucosyltransferase activity."

2. <u>Claim 1</u> limitations b/c: "deleting a gene encoding α1,6-fucosyltransferase or by adding a mutation to said gene to reduce or eliminate the α1,6-fucosyltranferase activity,"

74. *Umaña*, which is representative of the state of the art as of the alleged Priority Date of the '446 patent, explains that "the use of gene knockout technologies or the use of ribozyme methods may be used to tailor the host cell's glycosyl transferase and/or glycosidase expression levels, and is therefore within the scope of the invention." (Ex. 1004 at 15:20–22; *see also* Ex. 1004 at 7:15-18.) In my opinion, a POSA would consider "gene knockout technologies," as

described in *Umaña* to encompass "deleting a gene" or "adding a mutation to said gene." A POSA would view these routine techniques as allowing for the elimination of "α1,6-fucosyltranferase activity."

75. As discussed above, *supra* **Section IV**, the knowledge of a POSA as of the alleged Priority Date would have rendered the act of "deleting a gene encoding α1,6-fucosyltransferase" or "adding a mutation to said gene to reduce or eliminate the α1,6-fucosyltransferase activity" routine. And given the known correlation between the lack of fucose and ADCC, a POSA would have been motivated to perform such a deletion or mutation. (*See* Ex. 1002 at 1114, 1122.)

76. Accordingly, in my opinion, a POSA would have found "deleting a gene encoding α1,6-fucosyltransferase" or "adding a mutation to said gene to reduce or eliminate the α1,6-fucosyltransferase activity" to be obvious.

3. Claim 1 limitation d: "wherein said mammalian host cell produces an antibody molecule.

77. *Umaña*, which is representative of the state of the art as of the alleged Priority Date of the '446 patent, is directed to the creation of a host cell using "genetic knockout techniques" to "tailor the host cell's glycosyl transferase and/or expression levels[.]" (Ex. 1004 at 3:6-11, 15:20-22.) *Umaña* notes that "[m]ammalian cells are the preferred hosts for production of therapeutic glycoproteins, due to their capability to glycosylate proteins in the most compatible form for human application." (Ex. 1004 at 2:4–6.) And *Umaña* states that host cells

according to disclosed method can be made from a variety of "cultured cells, e.g., mammalian cultured cells, such as CHO cells, BHK cells, NSO cells, SP2/0 cells, or hybridoma cells, yeast cells, and insect cells, to name only few, but also cells comprised within a transgenic animal or cultured tissue." (Ex. 1004 at 7:31–8:1.) Indeed, "[a]ny type of cultured cell line can be used as background to engineer the host cell lines of [*Umaña's*] invention." (Ex. 1004 at 15:23-24.)

78. *Umaña* relays "a method for enhancing the ADCC activity of therapeutic antibodies," which is "achieved by engineering the glycosylation pattern of the Fc region of such antibodies[.]" (Ex. 1004 at 23:23-33.) *Umana*'s disclosed method "provides alternative glycoforms of proteins having improved therapeutic properties. The proteins of the invention include antibodies with an enhanced antibody-dependent cellular cytotoxicity (ADCC), which have been generated using the disclosed methods and host cells." (Ex. 1004 at 3:17-20; *see also* Ex. 1004 at 8:24-28.)

79. *Umaña* discloses an embodiment "directed to host cells that have been engineered such that they are capable of expressing a preferred range of a glycoprotein-modifying glycosyl transferase activity which increases complex N-linked oligosaccharides carrying bisecting GlcNAc." (Ex. 1004 at 2:28-31.) And *Umaña* discloses other embodiments "directed to methods for the generation of modified glycoforms of glycoproteins, for example antibodies, including whole

antibody molecules, antibody fragments, or fusion proteins that include a region equivalent to the Fc region of an immunoglobulin, having an enhanced Fc-mediated cellular cytotoxicity, and glycoproteins so generated." (*Id.* at 2:31–3:3.)

80. Accordingly, in my opinion, a POSA would have found the production of an antibody molecule in the "mammalian host cell" of claim 1 to be obvious.

81. In view of the above, in my opinion, claims 1 of the '446 patent would have been obvious over *Rothman* in view of *Umaña* and the knowledge of a POSA.

4. Dependent Claims 2-5: "[t]he isolated host cell of claim 1, wherein said host cell is a [CHO cell / NS0 cell / SP2/0 cell / YB2/0 cell]."

82. The combination of *Rothman*, *Umaña*, and the knowledge of a POSA renders obvious all elements of claim 1, as set forth above.

83. Dependent claims 2–5 of the '446 patent recite creation of a host cell with "decreased or no α1,6-fucosyltransferase activity" in different types of mammalian cells, all of which were well known in the prior art as of the alleged Priority Date of the '446 patent. *Umaña,* which is representative of the state of the art as of the alleged Priority Date of the '446 patent, explains that while "[c]hinese hamster ovary (CHO) cells have been most commonly used during the last two decades. . . . Other commonly used animal cells include baby hamster kidney

(BHK) cells, NSO- and SP2/0-mouse myeloma cells." (Ex. 1004 at 2:10–16.) YB2/0 was also a long-established cell line. (*See* Ex. 1006.) Indeed, *Umaña* is clear that "[a]ny type of cultured cell line can be used as background to engineer the host cell lines of [*Umaña's*] invention." (Ex. 1004 at 15:23-24.)

84. Thus, as of the alleged Priority Date of the '446 patent, mammalian cell targets of genetic engineering routinely included CHO cells, NSO cells, SP2/0 cells, YB2/0 cells, among many others. *See Maniatis*; *see also* Ex. 1006. The source of cells was not a restriction in gene modification, the only requirement being the ability to maintain and grow cells of interest in laboratory cultures. Introducing the DNA to achieve novel sequence expression was referred to as transfection; and various routine technologies were well developed to transfect virtually any DNA sequence into a variety of target cells. Thus, in my opinion, dependent claims 2-5 would have been obvious over *Rothman* in view of *Umaña* and the knowledge of a POSA.

5. Dependent Claim 6: "the isolated host cell of claim 1, wherein said antibody molecule is an IgG antibody."

85. The combination of *Rothman*, *Umaña*, and the knowledge of a POSA renders obvious all elements of claim 1, as set forth above.

86. Dependent claim 6 of the '446 patent simply identifies the "antibody molecule" produced by the host cell as IgG. *Umaña* specifically investigated the glycosylation pattern of the sugar chain of an "IgG" antibody. (Ex. 1004 at 34:20–

21.) *Rothman* too specifically investigated the glycosylation pattern of the sugar chain of an "IgG" antibody: "[i]n this report, we describe the functional effects of alterations in IgG glycosylation induced by inhibitors of glycosylation and carbohydrate processing. (Ex. 1002 at 1114.) Thus, in my opinion, dependent claim 6 would have been obvious over *Rothman* in view of *Umaña* and the knowledge of a POSA.

VIII. GROUND 2: Claims 1-6 of the '446 patent are Obvious over *Harris* in view of *Umaña* and the knowledge of a POSA.

A.) Opinion Introduction and the *Harris* and *Umaña* References

87. In my opinion, claims 1-6 of the '446 patent are obvious over *Harris* in view of *Umaña* and the knowledge of a POSA.

88. My analysis is set forth below. I also incorporate into my analysis the accompanying claim chart (**Exhibit C**), which sets forth portions of the cited prior art references corresponding to claims 1-6 of the '446 patent.

89. *Umaña*, which is prior art to the '446 patent, is directed "generally, to methods for the glycosylation engineering of proteins to alter and improve their therapeutic properties" and "describes methods for producing in a host cell an antibody which has an altered glycosylation pattern resulting in an enhanced antibody dependent cellular cytotoxicity (ADCC)." (Ex. 1004 at 8:24-28.) *Umaña* further explains that such methods include "the use of gene knockout technologies

or the use of ribozyme methods" that "tailor the host cell's glycosyl transferase and/or glycosidase expression levels[.]" (*Id.* at 15:20-22.)

90. *Harris,* which is also prior art to the '446 patent, describes how the "[t]he fucose residue may be of particular interest," explaining that fucose is "near the Fcγ receptor binding site and could influence binding by the receptor." (Ex. 1003 at 1592.)

B.) Obviousness over *Harris* in view of *Umaña* and the knowledge of a POSA

91. I agree with the analysis of Prof. Jefferis that there was a well-known correlation between antibody sugar chain modification and the efficiency ("effector function") of an antibody as of the alleged Priority Date of the '446 patent. (*See* Ex. 1026 at ¶¶ 15-37, 75-94.) And, more specifically, I agree with Prof. Jefferis that the prior art (represented by *Harris*) describes the correlation between sugar chain modification—including the removal of fucose, particularly—and improved ADCC. (*Id.* at ¶¶ 75-94.) Based upon my review of the prior art and knowledge gained over my 35-plus-year career in genetic immunology, I believe that the known correlation between removal of fucose and improved ADCC (represented by *Rothman*) would have motivated a POSA to utilize known, routine genetic engineering techniques (represented by *Umaña*) to create the "host cell" recited in claims 1-6 of the '446 patent.

1. Claim 1 limitation a: "[a]n isolated mammalian host cell which has decreased or no α1,6-fucosyltransferase activity for adding fucose to N-acetylglucosamine of a reducing terminus of N-glycoside-linked sugar chains"

92. I have reviewed *Harris*, which explains that the "[t]he fucose residue may be of particular interest," explaining that fucose is "near the Fcγ receptor binding site and could influence binding by the receptor." (Ex. 1003 at 1592.) I have also reviewed and agree with Prof. Jefferis' analysis that *Harris*'s teachings would have motivated a POSA to utilize known, routine genetic engineering techniques (represented by *Umaña*) to create the "host cell" recited in claims 1-6 of the '446 patent. (*See* Ex. 1026 at ¶¶ 75-94.) Moreover, in my opinion, creating such a cell would have been obvious and routine to a POSA.

93. Given the teachings of *Harris*, and considering the state of genetic engineering technology as of the alleged Priority Date, in my opinion, a POSA would have found it obvious to create "[a]n isolated mammalian host cell which has decreased or no α1,6-fucosyltransferase activity for adding fucose to N-acetylglucosamine of a reducing terminus of N-glycoside-linked sugar chains[.]" As discussed above, *supra* **Section IV**, the knowledge of a POSA as of the alleged Priority Date would have rendered the act of fucosyltransferase knock-out routine.

94. *Umaña,* which is representative of the state of the art as of the alleged Priority Date of the '446 patent, is directed to the creation of a host cell using "genetic knockout techniques" to "tailor the host cell's glycosyl transferase and/or

expression levels[.]" (Ex. 1004 at 3:9-11, 15:20-22.) *Umaña* further describes engineering such host cells by transfecting nucleic acid "encoding a whole antibody molecule," which "produce[s] altered glycoforms of proteins having improved therapeutic values, e.g., an antibody which has an enhanced antibody dependent cellular cytotoxicity (ADCC) in a host cell." (*Id.* at 15:24-28, 3:6-9.)

95. *Umana's* disclosure is consistent with the state of the art as of the alleged Priority Date of the '446 patent. As discussed above, *supra* **Section IV**, the knowledge of a POSA as of the alleged Priority Date would have rendered the act of creating a host cell which has "decreased or no α1,6-fucosyltransferase activity" routine.

96. In this regard, I note that as of the alleged Priority Date of the '446 patent, I was working with both antibodies and genetic engineering. In particular, I employed the technique of homologous recombination of genes called "knock-ins," a refinement of the original technique of "knockouts," both of which targeted particular genes to disable their expression. (*See, e.g.*, Exs. 1021, 1022, 1023.)

97. The knock-out and knock-in techniques were routinely used as of the alleged Priority Date of the '446 patent, in order to obtain genetically modified cells to produce modified molecules—modified because a gene involved in their usual production had been disabled, i.e., knocked out or knocked in. (*See, e.g.*, Ex.

1009.) The routine techniques allowed scientists to completely remove certain genetic traits.

98. The removal of certain genetic traits and the resulting production of modified molecules was useful for a variety of purposes, including, for instance to:

- Identify the impact on a cellular physiology by a loss of function. *See, e.g.,* Bernstein, A and Brietman, M. 1989. *Genetic ablation in transgenic mice*. Mol Biol Med 6:523-530; Berke G. 1994. *The binding and lysis of target cells by cytotoxic lymphocytes: molecular and cellular aspects*. Ann Rev Immunol 12:735-773; Bachman MF and Kundig TM. 1994. *In vivo versus in vitro assays for assessment of T- and B- cell function*. Curr Opin Immunol. 6:320-326.

- Identify how a loss of function affects protein-protein interactions. *See, e.g.,* Ahmed, M , et al. 1994. *A protein that activates expression of a multidrug efflux transporter upon binding the transporter substrates*. J Biol Chem. 269:28506-28513; Cosgrove, D. et al. 1992. *Evaluation of the functional equivalence of major histocompatibility complex class II A and E complexes*. J Exp Med. 176:629-634.

- Identify how proteins function when genes that modify their structure are deleted (e.g., fucosyltranferase deletion impact on antibody functions, such as ADCC). *See, e.g.,* Baba, T. et al. 1994. *A cytoplasmic domain is important for the formation of a SecY-SecE translocator complex*. Proc Natl Acad Sci. 91:4539-4543; Cowan, PJ, et al. 1998. *Knock out of alpha 1.3-galatosyltransferase or expression of alpha 1,2 fucosyltransferase further protects CD55 and CD59-expressing mouse hearts in an ex vivo model of xenograft rejection*.

- Identify how functions change if novel genes or genes resulting in novel proteins are engineered into cell by knock-in techniques. *See, e.g.,* Metzger, H. 1994. *Immunoglobulin receptors. Handicapping the immune response*. Curr Biol. 4:644-646.

- Identify how knock-in or knock-out affects cellular functions, such as immune response pathways. *See, e.g.*, Wiman KG. 1993. *The retinoblastoma*

gene: role in cell cycle control and cell differentiation. FASEB J. 10:841-845; Declerck, PJ, et al. 1995. *Generation of monoclonal antibodies against autologous proteins in gene-inactivated mice*. J Biol Chem. 270:8397-8400; Yang, Y, et al. 1994. *MHC class I-restricted cytotoxic T lymphocytes to viral antigens destroy hepatocytes in mice infected with E1-deleted recombinant adenovirus*. Immunity 5:433-442.

99. Accordingly, in my opinion, a POSA would have been highly motivated to create host cells with their fucosyltransferase genes knocked out to acquire antibodies with improved ADCC. The expected therapeutic benefits were obvious and considerable: lower doses of antibodies, more effective immune responses, and fewer side effects.

100. In light of the specific motivation to remove fucose from IgG antibodies to improve their ADCC effector function, and the known genetic sequence of the α1,6-fucosyltransferase gene (and the ability to determine this independently), in my opinion a POSA as of the alleged Priority Date of the '446 patent would have found it obvious—with at least a reasonable expectation of success—to apply routine knock-out techniques to create mammalian cells with "decreased or no α1,6-fucosyltransferase activity."

2. <u>Claim 1</u> limitations b/c: "deleting a gene encoding α1,6-fucosyltransferase or by adding a mutation to said gene to reduce or eliminate the α1,6-fucosyltranferase activity,"

101. *Umaña*, which is representative of the state of the art as of the alleged Priority Date of the '446 patent, explains that "the use of gene knockout technologies or the use of ribozyme methods may be used to tailor the host cell's

glycosyl transferase and/or glycosidase expression levels, and is therefore within the scope of the invention." (Ex. 1004 at 15:20–22; *see also* Ex. 1004 at 7:15-18.) In my opinion, a POSA would consider "gene knockout technologies," as described in *Umaña* to encompass "deleting a gene" or "adding a mutation to said gene." A POSA would view these routine techniques as allowing for the elimination of "α1,6-fucosyltranferase activity."

102. As discussed above, *supra* **Section IV**, the knowledge of a POSA as of the alleged Priority Date would have rendered the act of "deleting a gene encoding α1,6-fucosyltransferase" or "adding a mutation to said gene to reduce or eliminate the α1,6-fucosyltransferase activity" routine. And given the known correlation between the lack of fucose and ADCC, a POSA would have been motivated to perform such a deletion or mutation. (*See* Ex. 1003 at 1592.)

103. Accordingly, in my opinion, a POSA would have found "deleting a gene encoding α1,6-fucosyltransferase" or "adding a mutation to said gene to reduce or eliminate the α1,6-fucosyltransferase activity" to be obvious.

3. Claim 1 limitation d: "wherein said mammalian host cell produces an antibody molecule.

104. *Umaña*, which is representative of the state of the art as of the alleged Priority Date of the '446 patent, is directed to the creation of a host cell using "genetic knockout techniques" to "tailor the host cell's glycosyl transferase and/or expression levels[.]" (Ex. 1004 at 3:6-11, 15:20-22.) *Umaña* notes that

"[m]ammalian cells are the preferred hosts for production of therapeutic glycoproteins, due to their capability to glycosylate proteins in the most compatible form for human application." (Ex. 1004 at 2:4–6.) And *Umaña* states that host cells according to disclosed method can be made from a variety of "cultured cells, e.g., mammalian cultured cells, such as CHO cells, BHK cells, NSO cells, SP2/0 cells, or hybridoma cells, yeast cells, and insect cells, to name only few, but also cells comprised within a transgenic animal or cultured tissue." (Ex. 1004 at 7:31–8:1.) Indeed, "[a]ny type of cultured cell line can be used as background to engineer the host cell lines of [*Umaña's*] invention." (Ex. 1004 at 15:23-24.)

105. *Umaña* relays "a method for enhancing the ADCC activity of therapeutic antibodies," which is "achieved by engineering the glycosylation pattern of the Fc region of such antibodies[.]" (Ex. 1004 at 23:23-33.) *Umana*'s disclosed method "provides alternative glycoforms of proteins having improved therapeutic properties. The proteins of the invention include antibodies with an enhanced antibody-dependent cellular cytotoxicity (ADCC), which have been generated using the disclosed methods and host cells." (Ex. 1004 at 3:17-20; *see also* Ex. 1004 at 8:24-28.)

106. *Umaña* discloses an embodiment "directed to host cells that have been engineered such that they are capable of expressing a preferred range of a glycoprotein-modifying glycosyl transferase activity which increases complex N-

linked oligosaccharides carrying bisecting GlcNAc." (Ex. 1004 at 2:28-31.) And *Umaña* discloses other embodiments "directed to methods for the generation of modified glycoforms of glycoproteins, for example antibodies, including whole antibody molecules, antibody fragments, or fusion proteins that include a region equivalent to the Fc region of an immunoglobulin, having an enhanced Fc-mediated cellular cytotoxicity, and glycoproteins so generated." (*Id.* at 2:31–3:3.)

107. Accordingly, in my opinion, a POSA would have found the production of an antibody molecule in the "mammalian host cell" of claim 1 to be obvious.

108. In view of the above, in my opinion, claims 1 of the '446 patent would have been obvious over *Harris* in view of *Umaña* and the knowledge of a POSA.

4. Dependent Claims 2-5: "[t]he isolated host cell of claim 1, wherein said host cell is a [CHO cell / NS0 cell / SP2/0 cell / YB2/0 cell]."

109. The combination of *Harris*, *Umaña*, and the knowledge of a POSA renders obvious all elements of claim 1, as set forth above.

110. Dependent claims 2–5 of the '446 patent recite creation of a host cell with "decreased or no α1,6-fucosyltransferase activity" in different types of mammalian cells, all of which were well known in the prior art as of the alleged Priority Date of the '446 patent. *Umaña,* which is representative of the state of the art as of the alleged Priority Date of the '446 patent, explains that while "[c]hinese

hamster ovary (CHO) cells have been most commonly used during the last two decades. . . . Other commonly used animal cells include baby hamster kidney (BHK) cells, NSO- and SP2/0-mouse myeloma cells." (Ex. 1004 at 2:10–16.) YB2/0 was also a long-established cell line. (*See* Ex. 1006.) Indeed, *Umaña* is clear that "[a]ny type of cultured cell line can be used as background to engineer the host cell lines of [*Umaña's*] invention." (Ex. 1004 at 15:23-24.)

111. Thus, as of the alleged Priority Date of the '446 patent, mammalian cell targets of genetic engineering routinely included CHO cells, NSO cells, SP2/0 cells, YB2/0 cells, among many others. *See Maniatis*; *see also* Ex. 1006. The source of cells was not a restriction in gene modification, the only requirement being the ability to maintain and grow cells of interest in laboratory cultures. Introducing the DNA to achieve novel sequence expression was referred to as transfection; and various routine technologies were well developed to transfect virtually any DNA sequence into a variety of target cells. Thus, in my opinion, dependent claims 2-5 would have been obvious over *Harris* in view of *Umaña* and the knowledge of a POSA.

5. Dependent Claim 6: "the isolated host cell of claim 1, wherein said antibody molecule is an IgG antibody."

112. The combination of *Harris*, *Umaña*, and the knowledge of a POSA renders obvious all elements of claim 1, as set forth above.

113. Dependent claim 6 of the ’446 patent simply identifies the “antibody molecule” produced by the host cell as IgG. *Umaña* specifically investigated the glycosylation pattern of the sugar chain of an “IgG” antibody. (Ex. 1004 at 34:20–21.) Thus, in my opinion, dependent claim 6 would have been obvious over *Harris* in view of *Umaña* and the knowledge of a POSA.

IX. GROUND 3: Claims 1-6 of the ’446 patent are Obvious over *Rothman* in view of *Umaña, Malý*, and the knowledge of a POSA.

A.) Opinion and Introduction to the *Malý* Reference

114. In my opinion, claims 1-6 of the ’446 patent are obvious over *Rothman* in view of *Umaña*, *Malý*, and the knowledge of a POSA.

115. My analysis is set forth below. I also incorporate into my analysis the accompanying claim chart (**Exhibit C**), which sets forth portions of the cited prior art references corresponding to claims 1-6 of the ’446 patent.

116. I incorporate herein the background of the *Rothman* and *Umaña* references discussed above. As the Patent Owner stated in bringing *Malý* to the Examiner’s attention during prosecution of the ’446 patent’s grandparent application:

> [I]n the reference (iv) [*Malý*], the region containing a catalyst domain of fucosyltransferase VII is deleted (p. 644, right column, Fig. 2A).
>
> The inventors of the presently claimed invention found cDNA encoding α1,6-fucosyltransferase in CHO cells and the exon 2 genomic region, as described in Example 12 of the present specification. Since the exon 2 contains ATG site, this selection was

> carried out according to ordinary, well-known methods in the production of knock-out cells. It will be apparent for one of ordinary skill in the art that a knock-out cell could be prepared, without an undue amount of experimentation, by deleting, for example, regions containing an ATG site, a promoter region, and/or an active site of a protein of interest in addition to or in place of the exon 2 region exemplified in the present application.

(Ex. 1036 (selected pages), Aug. 12, 2004 Amend. at 32–35.)

B.) Obviousness over *Rothman* in view of *Umaña, Malý,* and the knowledge of a POSA

117. I agree with the analysis of Prof. Jefferis that there was a well-known correlation between antibody sugar chain modification and the efficiency ("effector function") of an antibody as of the alleged Priority Date of the '446 patent. (*See* Ex. 1026 at ¶¶ 15-37, 95-113.) And, more specifically, I agree with Prof. Jefferis that the prior art (represented by *Rothman*) describes the correlation between sugar chain modification—including the removal of fucose, particularly—and improved ADCC. (*Id.* at ¶¶ 95-113.) Based upon my review of the prior art and knowledge gained over my 35-plus-year career in genetic immunology, I believe that the known correlation between removal of fucose and improved ADCC (represented by *Rothman*) would have motivated a POSA to utilize known, routine genetic engineering techniques (represented by *Umaña*) to create the "host cell" recited in claims 1-6 of the '446 patent. Indeed, the "knock-out" performed by *Malý* demonstrates the routine nature of completing the "knock-out" of α1,6-fucosyltransferase in a variety of cells as of the alleged Priority Date.

1. Claim 1 limitation a: "[a]n isolated mammalian host cell which has decreased or no α1,6-fucosyltransferase activity for adding fucose to N-acetylglucosamine of a reducing terminus of N-glycoside-linked sugar chains"

118. I have reviewed *Rothman*, which explains that the "absence of core fucosylation itself would appear to be a likely candidate as a structural feature necessary for enhancement of NK cell-mediated ADCC." (Ex. 1002 at 1122; *see also* Ex. 1002 at 1114.) I have also reviewed and agree with Prof. Jefferis' analysis that *Rothman*'s teachings would have motivated a POSA to utilize known, routine genetic engineering techniques (represented by *Umaña*) to create the "host cell" recited in claims 1-6 of the '446 patent. (*See* Ex. 1026 at ¶¶ 95-113.) Moreover, in my opinion, creating such a cell would have been obvious and routine to a POSA.

119. Given the teachings of *Rothman*, and considering the state of genetic engineering technology as of the alleged Priority Date, in my opinion, a POSA would have found it obvious to create "[a]n isolated mammalian host cell which has decreased or no α1,6-fucosyltransferase activity for adding fucose to N-acetylglucosamine of a reducing terminus of N-glycoside-linked sugar chains[.]" As discussed above, *supra* **Section IV**, the knowledge of a POSA as of the alleged Priority Date would have rendered the act of fucosyltransferase knock-out routine.

120. *Umaña,* which is representative of the state of the art as of the alleged Priority Date of the '446 patent, is directed to the creation of a host cell using "genetic knockout techniques" to "tailor the host cell's glycosyl transferase and/or

expression levels[.]" (Ex. 1004 at 3:9-11, 15:20-22.) *Umaña* further describes engineering such host cells by transfecting nucleic acid "encoding a whole antibody molecule," which "produce[s] altered glycoforms of proteins having improved therapeutic values, e.g., an antibody which has an enhanced antibody dependent cellular cytotoxicity (ADCC) in a host cell." (*Id.* at 15:24-28, 3:6-9.)

121. *Umana's* disclosure is consistent with the state of the art as of the alleged Priority Date of the '446 patent. As discussed above, *supra* **Section IV**, the knowledge of a POSA as of the alleged Priority Date would have rendered the act of creating a host cell which has "decreased or no α1,6-fucosyltransferase activity" routine. Indeed, the "knock-out" performed by *Malý* demonstrates the routine nature of completing the a "knock-out" of α1,6-fucosyltransferase in a variety of cells as of the alleged Priority Date.

122. In this regard, I note that as of the alleged Priority Date of the '446 patent, I was working with both antibodies and genetic engineering. In particular, I employed the technique of homologous recombination of genes called "knock-ins," a refinement of the original technique of "knockouts," both of which targeted particular genes to disable their expression. (*See, e.g.*, Ex. 1021, 1022, 1023.)

123. The knock-out and knock-in techniques were routinely used as of the alleged Priority Date of the '446 patent, in order to obtain genetically modified cells to produce modified molecules—modified because a gene involved in their

usual production had been disabled, i.e., knocked out or knocked in. (*See, e.g.*, Ex. 1009.) The routine techniques allowed scientists to completely remove certain genetic traits.

124. The removal of certain genetic traits and the resulting production of modified molecules was useful for a variety of purposes, including, for instance to:

- Identify the impact on a cellular physiology by a loss of function. *See, e.g.,* Bernstein, A and Brietman, M. 1989. *Genetic ablation in transgenic mice*. Mol Biol Med 6:523-530; Berke G. 1994. *The binding and lysis of target cells by cytotoxic lymphocytes: molecular and cellular aspects*. Ann Rev Immunol 12:735-773; Bachman MF and Kundig TM. 1994. *In vivo versus in vitro assays for assessment of T- and B- cell function*. Curr Opin Immunol. 6:320-326.

- Identify how a loss of function affects protein-protein interactions. *See, e.g.,* Ahmed, M , et al. 1994. *A protein that activates expression of a multidrug efflux transporter upon binding the transporter substrates*. J Biol Chem. 269:28506-28513; Cosgrove, D. et al. 1992. *Evaluation of the functional equivalence of major histocompatibility complex class II A and E complexes*. J Exp Med. 176:629-634.

- Identify how proteins function when genes that modify their structure are deleted (e.g., fucosyltranferase deletion impact on antibody functions, such as ADCC). *See, e.g.,* Baba, T. et al. 1994. *A cytoplasmic domain is important for the formation of a SecY-SecE translocator complex*. Proc Natl Acad Sci. 91:4539-4543; Cowan, PJ, et al. 1998. *Knock out of alpha 1.3-galatosyltransferase or expression of alpha 1,2 fucosyltransferase further protects CD55 and CD59-expressing mouse hearts in an ex vivo model of xenograft rejection*.

- Identify how functions change if novel genes or genes resulting in novel proteins are engineered into cell by knock-in techniques. *See, e.g.,* Metzger, H. 1994. *Immunoglobulin receptors. Handicapping the immune response*. Curr Biol. 4:644-646.

- Identify how knock-in or knock-out affects cellular functions, such as immune response pathways. *See, e.g.*, Wiman KG. 1993. *The retinoblastoma gene: role in cell cycle control and cell differentiation*. FASEB J. 10:841-845; Declerck, PJ, et al. 1995. *Generation of monoclonal antibodies against autologous proteins in gene-inactivated mice*. J Biol Chem. 270:8397-8400; Yang, Y, et al. 1994. *MHC class I-restricted cytotoxic T lymphocytes to viral antigens destroy hepatocytes in mice infected with E1-deleted recombinant adenovirus*. Immunity 5:433-442.

125. Accordingly, in my opinion, a POSA would have been highly motivated to create host cells with their fucosyltransferase genes knocked out to acquire antibodies with improved ADCC. The expected therapeutic benefits were obvious and considerable: lower doses of antibodies, more effective immune responses, and fewer side effects.

126. In light of the specific motivation to remove fucose from IgG antibodies to improve their ADCC effector function, and the known genetic sequence of the α1,6-fucosyltransferase gene (and the ability to determine this independently), in my opinion a POSA as of the alleged Priority Date of the '446 patent would have found it obvious—with at least a reasonable expectation of success—to apply routine knock-out techniques to create mammalian cells with "decreased or no α1,6-fucosyltransferase activity."

2. Claim 1 limitations b/c: "deleting a gene encoding α1,6-fucosyltransferase or by adding a mutation to said gene to reduce or eliminate the α1,6-fucosyltranferase activity,"

127. *Umaña*, which is representative of the state of the art as of the alleged Priority Date of the '446 patent, explains that "the use of gene knockout

technologies or the use of ribozyme methods may be used to tailor the host cell's glycosyl transferase and/or glycosidase expression levels, and is therefore within the scope of the invention." (Ex. 1004 at 15:20–22; *see also* Ex. 1004 at 7:15-18.) In my opinion, a POSA would consider "gene knockout technologies," as described in *Umaña* to encompass "deleting a gene" or "adding a mutation to said gene." A POSA would view these routine techniques as allowing for the elimination of "α1,6-fucosyltranferase activity."

128. As discussed above, *supra* **Section IV**, the knowledge of a POSA as of the alleged Priority Date would have rendered the act of "deleting a gene encoding α1,6-fucosyltransferase" or "adding a mutation to said gene to reduce or eliminate the α1,6-fucosyltransferase activity" routine. And given the known correlation between the lack of fucose and ADCC, a POSA would have been motivated to perform such a deletion or mutation. (*See* Ex. 1002 at 1114, 1122.)

129. Accordingly, in my opinion, a POSA would have found "deleting a gene encoding α1,6-fucosyltransferase" or "adding a mutation to said gene to reduce or eliminate the α1,6-fucosyltransferase activity" to be obvious.

3. Claim 1 limitation d: "wherein said mammalian host cell produces an antibody molecule.

130. *Umaña*, which is representative of the state of the art as of the alleged Priority Date of the '446 patent, is directed to the creation of a host cell using "genetic knockout techniques" to "tailor the host cell's glycosyl transferase and/or

expression levels[.]" (Ex. 1004 at 3:6-11, 15:20-22.) *Umaña* notes that "[m]ammalian cells are the preferred hosts for production of therapeutic glycoproteins, due to their capability to glycosylate proteins in the most compatible form for human application." (Ex. 1004 at 2:4–6.) And *Umaña* states that host cells according to disclosed method can be made from a variety of "cultured cells, e.g., mammalian cultured cells, such as CHO cells, BHK cells, NSO cells, SP2/0 cells, or hybridoma cells, yeast cells, and insect cells, to name only few, but also cells comprised within a transgenic animal or cultured tissue." (Ex. 1004 at 7:31–8:1.) Indeed, "[a]ny type of cultured cell line can be used as background to engineer the host cell lines of [*Umaña's*] invention." (Ex. 1004 at 15:23-24.)

131. *Umaña* relays "a method for enhancing the ADCC activity of therapeutic antibodies," which is "achieved by engineering the glycosylation pattern of the Fc region of such antibodies[.]" (Ex. 1004 at 23:23-33.) *Umana*'s disclosed method "provides alternative glycoforms of proteins having improved therapeutic properties. The proteins of the invention include antibodies with an enhanced antibody-dependent cellular cytotoxicity (ADCC), which have been generated using the disclosed methods and host cells." (Ex. 1004 at 3:17-20; *see also* Ex. 1004 at 8:24-28.)

132. *Umaña* discloses an embodiment "directed to host cells that have been engineered such that they are capable of expressing a preferred range of a

glycoprotein-modifying glycosyl transferase activity which increases complex N-linked oligosaccharides carrying bisecting GlcNAc." (Ex. 1004 at 2:28-31.) And *Umaña* discloses other embodiments "directed to methods for the generation of modified glycoforms of glycoproteins, for example antibodies, including whole antibody molecules, antibody fragments, or fusion proteins that include a region equivalent to the Fc region of an immunoglobulin, having an enhanced Fc-mediated cellular cytotoxicity, and glycoproteins so generated." (*Id.* at 2:31–3:3.)

133. Accordingly, in my opinion, a POSA would have found the production of an antibody molecule in the "mammalian host cell" of claim 1 to be obvious.

134. In view of the above, in my opinion, claims 1 of the '446 patent would have been obvious over *Rothman* in view of *Umaña*, *Malý*, and the knowledge of a POSA.

4. Dependent Claims 2-5: "[t]he isolated host cell of claim 1, wherein said host cell is a [CHO cell / NS0 cell / SP2/0 cell / YB2/0 cell]."

135. The combination of *Rothman*, *Umaña*, *Malý*, and the knowledge of a POSA renders obvious all elements of claim 1, as set forth above.

136. Dependent claims 2–5 of the '446 patent recite creation of a host cell with "decreased or no α1,6-fucosyltransferase activity" in different types of mammalian cells, all of which were well known in the prior art as of the alleged

Priority Date of the ’446 patent. *Umaña,* which is representative of the state of the art as of the alleged Priority Date of the ’446 patent, explains that while “[c]hinese hamster ovary (CHO) cells have been most commonly used during the last two decades. . . . Other commonly used animal cells include baby hamster kidney (BHK) cells, NSO- and SP2/0-mouse myeloma cells.” (Ex. 1004 at 2:10–16.) YB2/0 was also a long-established cell line. (*See* Ex. 1006.) Indeed, *Umaña* is clear that “[a]ny type of cultured cell line can be used as background to engineer the host cell lines of [*Umaña’s*] invention.” (Ex. 1004 at 15:23-24.)

137. Thus, as of the alleged Priority Date of the ’446 patent, mammalian cell targets of genetic engineering routinely included CHO cells, NSO cells, SP2/0 cells, YB2/0 cells, among many others. *See Maniatis*; Ex. 1006. The source of cells was not a restriction in gene modification, the only requirement being the ability to maintain and grow cells of interest in laboratory cultures. Introducing the DNA to achieve novel sequence expression was referred to as transfection; and various routine technologies were well developed to transfect virtually any DNA sequence into a variety of target cells. Thus, in my opinion, dependent claims 2-5 would have been obvious over *Rothman* in view of *Umaña*, *Malý*, and the knowledge of a POSA.

5. Dependent Claim 6: "the isolated host cell of claim 1, wherein said antibody molecule is an IgG antibody."

138. The combination of *Rothman*, *Umaña*, *Malý*, and the knowledge of a POSA renders obvious all elements of claim 1, as set forth above.

139. Dependent claim 6 of the '446 patent simply identifies the "antibody molecule" produced by the host cell as IgG. *Umaña* specifically investigated the glycosylation pattern of the sugar chain of an "IgG" antibody. (Ex. 1004 at 34:20–21.) *Rothman* too specifically investigated the glycosylation pattern of the sugar chain of an "IgG" antibody: "[i]n this report, we describe the functional effects of alterations in IgG glycosylation induced by inhibitors of glycosylation and carbohydrate processing. (Ex. 1002 at 1114.) Thus, in my opinion, dependent claim 6 would have been obvious over *Rothman* in view of *Umaña, Malý*, and the knowledge of a POSA.

X. GROUND 4: Claims 1-6 of the '446 patent are Obvious over *Harris* in view of *Umaña, Malý*, and the knowledge of a POSA.

A.) Opinion and Introduction to the *Malý* Reference

140. In my opinion, claims 1-6 of the '446 patent are obvious over *Harris* in view of *Umaña*, *Malý*, and the knowledge of a POSA.

141. My analysis is set forth below. I also incorporate into my analysis the accompanying claim chart (**Exhibit C**), which sets forth portions of the cited prior art references corresponding to claims 1-6 of the '446 patent.

142. I incorporate herein the background of the *Harris* and *Umaña* references discussed above. As the Patent Owner stated in bringing *Malý* to the Examiner's attention during prosecution of the '446 patent's grandparent application:

> [I]n the reference (iv) [*Malý*], the region containing a catalyst domain of fucosyltransferase VII is deleted (p. 644, right column, Fig. 2A).
>
> The inventors of the presently claimed invention found cDNA encoding α1,6-fucosyltransferase in CHO cells and the exon 2 genomic region, as described in Example 12 of the present specification. Since the exon 2 contains ATG site, this selection was carried out according to ordinary, well-known methods in the production of knock-out cells. It will be apparent for one of ordinary skill in the art that a knock-out cell could be prepared, without an undue amount of experimentation, by deleting, for example, regions containing an ATG site, a promoter region, and/or an active site of a protein of interest in addition to or in place of the exon 2 region exemplified in the present application.

(Ex. 1036 (selected pages), Aug. 12, 2004 Amend. at 32–35.)

B.) Obviousness over *Harris* in view of *Umaña, Malý,* and the knowledge of a POSA

143. I agree with the analysis of Prof. Jefferis that there was a well-known correlation between antibody sugar chain modification and the efficiency ("effector function") of an antibody as of the alleged Priority Date of the '446 patent. (*See* Ex. 1026 at ¶¶ 15-37, 114-130.) And, more specifically, I agree with Prof. Jefferis that the prior art (represented by *Harris*) describes the correlation between sugar chain modification—including the removal of fucose, particularly—and improved

ADCC. (*Id.* at ¶¶ 114-130.) Based upon my review of the prior art and knowledge gained over my 35-plus-year career in genetic immunology, I believe that the known correlation between removal of fucose and improved ADCC (represented by *Rothman*) would have motivated a POSA to utilize known, routine genetic engineering techniques (represented by *Umaña*) to create the "host cell" recited in claims 1-6 of the '446 patent. Indeed, the "knock-out" performed by *Malý* demonstrates the routine nature of completing the "knock-out" of α1,6-fucosyltransferase in a variety of cells as of the alleged Priority Date.

1. Claim 1 limitation a: "[a]n isolated mammalian host cell which has decreased or no α1,6-fucosyltransferase activity for adding fucose to N-acetylglucosamine of a reducing terminus of N-glycoside-linked sugar chains"

144. I have reviewed *Harris*, which explains that the "[t]he fucose residue may be of particular interest," explaining that fucose is "near the Fcγ receptor binding site and could influence binding by the receptor." (Ex. 1003 at 1592.) I have also reviewed and agree with Prof. Jefferis' analysis that *Harris*'s teachings would have motivated a POSA to utilize known, routine genetic engineering techniques (represented by *Umaña*) to create the "host cell" recited in claims 1-6 of the '446 patent. (*See* Ex. 1026 at ¶¶ 114-130.) Moreover, in my opinion, creating such a cell would have been obvious and routine to a POSA.

145. Given the teachings of *Harris*, and considering the state of genetic engineering technology as of the alleged Priority Date, in my opinion, a POSA

would have found it obvious to create "[a]n isolated mammalian host cell which has decreased or no α1,6-fucosyltransferase activity for adding fucose to N-acetylglucosamine of a reducing terminus of N-glycoside-linked sugar chains[.]" As discussed above, *supra* **Section IV**, the knowledge of a POSA as of the alleged Priority Date would have rendered the act of fucosyltransferase knock-out routine.

146. *Umaña,* which is representative of the state of the art as of the alleged Priority Date of the '446 patent, is directed to the creation of a host cell using "genetic knockout techniques" to "tailor the host cell's glycosyl transferase and/or expression levels[.]" (Ex. 1004 at 3:9-11, 15:20-22.) *Umaña* further describes engineering such host cells by transfecting nucleic acid "encoding a whole antibody molecule," which "produce[s] altered glycoforms of proteins having improved therapeutic values, e.g., an antibody which has an enhanced antibody dependent cellular cytotoxicity (ADCC) in a host cell." (*Id.* at 15:24-28, 3:6-9.)

147. *Umana's* disclosure is consistent with the state of the art as of the alleged Priority Date of the '446 patent. As discussed above, *supra* **Section IV**, the knowledge of a POSA as of the alleged Priority Date would have rendered the act of creating a host cell which has "decreased or no α1,6-fucosyltransferase activity" routine. Indeed, the "knock-out" performed by *Malý* demonstrates the routine nature of completing the a "knock-out" of α1,6-fucosyltransferase in a variety of cells as of the alleged Priority Date.

148. In this regard, I note that as of the alleged Priority Date of the '446 patent, I was working with both antibodies and genetic engineering. In particular, I employed the technique of homologous recombination of genes called "knock-ins," a refinement of the original technique of "knockouts," both of which targeted particular genes to disable their expression. (*See, e.g.*, Ex. 1021, 1022, 1023.)

149. The knock-out and knock-in techniques were routinely used as of the alleged Priority Date of the '446 patent, in order to obtain genetically modified cells to produce modified molecules—modified because a gene involved in their usual production had been disabled, i.e., knocked out or knocked in. (*See, e.g.*, Ex. 1009.) The routine techniques allowed scientists to completely remove certain genetic traits.

150. The removal of certain genetic traits and the resulting production of modified molecules was useful for a variety of purposes, including, for instance to:

- Identify the impact on a cellular physiology by a loss of function. *See, e.g.,* Bernstein, A and Brietman, M. 1989. *Genetic ablation in transgenic mice*. Mol Biol Med 6:523-530; Berke G. 1994. *The binding and lysis of target cells by cytotoxic lymphocytes: molecular and cellular aspects*. Ann Rev Immunol 12:735-773; Bachman MF and Kundig TM. 1994. *In vivo versus in vitro assays for assessment of T- and B- cell function*. Curr Opin Immunol. 6:320-326.

- Identify how a loss of function affects protein-protein interactions. *See, e.g.,* Ahmed, M , et al. 1994. *A protein that activates expression of a multidrug efflux transporter upon binding the transporter substrates*. J Biol Chem. 269:28506-28513; Cosgrove, D. et al. 1992. *Evaluation of the functional equivalence of major histocompatibility complex class II A and E complexes*. J Exp Med. 176:629-634.

- Identify how proteins function when genes that modify their structure are deleted (e.g., fucosyltranferase deletion impact on antibody functions, such as ADCC). *See, e.g.*, Baba, T. et al. 1994. *A cytoplasmic domain is important for the formation of a SecY-SecE translocator complex*. Proc Natl Acad Sci. 91:4539-4543; Cowan, PJ, et al. 1998. *Knock out of alpha 1.3-galatosyltransferase or expression of alpha 1,2 fucosyltransferase further protects CD55 and CD59-expressing mouse hearts in an ex vivo model of xenograft rejection*.

- Identify how functions change if novel genes or genes resulting in novel proteins are engineered into cell by knock-in techniques. *See, e.g.*, Metzger, H. 1994. *Immunoglobulin receptors. Handicapping the immune response*. Curr Biol. 4:644-646.

- Identify how knock-in or knock-out affects cellular functions, such as immune response pathways. *See, e.g.*, Wiman KG. 1993. *The retinoblastoma gene: role in cell cycle control and cell differentiation*. FASEB J. 10:841-845; Declerck, PJ, et al. 1995. *Generation of monoclonal antibodies against autologous proteins in gene-inactivated mice*. J Biol Chem. 270:8397-8400; Yang, Y, et al. 1994. *MHC class I-restricted cytotoxic T lymphocytes to viral antigens destroy hepatocytes in mice infected with E1-deleted recombinant adenovirus*. Immunity 5:433-442.

151. Accordingly, in my opinion, a POSA would have been highly motivated to create host cells with their fucosyltransferase genes knocked out to acquire antibodies with improved ADCC. The expected therapeutic benefits were obvious and considerable: lower doses of antibodies, more effective immune responses, and fewer side effects.

152. In light of the specific motivation to remove fucose from IgG antibodies to improve their ADCC effector function, and the known genetic sequence of the α1,6-fucosyltransferase gene (and the ability to determine this

independently), in my opinion a POSA as of the alleged Priority Date of the '446 patent would have found it obvious—with at least a reasonable expectation of success—to apply routine knock-out techniques to create mammalian cells with "decreased or no α1,6-fucosyltransferase activity."

2. Claim 1 limitations b/c: "deleting a gene encoding α1,6-fucosyltransferase or by adding a mutation to said gene to reduce or eliminate the α1,6-fucosyltranferase activity,"

153. *Umaña*, which is representative of the state of the art as of the alleged Priority Date of the '446 patent, explains that "the use of gene knockout technologies or the use of ribozyme methods may be used to tailor the host cell's glycosyl transferase and/or glycosidase expression levels, and is therefore within the scope of the invention." (Ex. 1004 at 15:20–22; *see also* Ex. 1004 at 7:15-18.) In my opinion, a POSA would consider "gene knockout technologies," as described in *Umaña* to encompass "deleting a gene" or "adding a mutation to said gene." A POSA would view these routine techniques as allowing for the elimination of "α1,6-fucosyltranferase activity."

154. As discussed above, *supra* **Section IV**, the knowledge of a POSA as of the alleged Priority Date would have rendered the act of "deleting a gene encoding α1,6-fucosyltransferase" or "adding a mutation to said gene to reduce or eliminate the α1,6-fucosyltransferase activity" routine. And given the known

correlation between the lack of fucose and ADCC, a POSA would have been motivated to perform such a deletion or mutation. (*See* Ex. 1003 at 1592.)

155. Accordingly, in my opinion, a POSA would have found "deleting a gene encoding α1,6-fucosyltransferase" or "adding a mutation to said gene to reduce or eliminate the α1,6-fucosyltransferase activity" to be obvious.

3. Claim 1 limitation d: "wherein said mammalian host cell produces an antibody molecule.

156. *Umaña*, which is representative of the state of the art as of the alleged Priority Date of the '446 patent, is directed to the creation of a host cell using "genetic knockout techniques" to "tailor the host cell's glycosyl transferase and/or expression levels[.]" (Ex. 1004 at 3:6-11, 15:20-22.) *Umaña* notes that "[m]ammalian cells are the preferred hosts for production of therapeutic glycoproteins, due to their capability to glycosylate proteins in the most compatible form for human application." (Ex. 1004 at 2:4–6.) And *Umaña* states that host cells according to disclosed method can be made from a variety of "cultured cells, e.g., mammalian cultured cells, such as CHO cells, BHK cells, NSO cells, SP2/0 cells, or hybridoma cells, yeast cells, and insect cells, to name only few, but also cells comprised within a transgenic animal or cultured tissue." (Ex. 1004 at 7:31–8:1.) Indeed, "[a]ny type of cultured cell line can be used as background to engineer the host cell lines of [*Umaña's*] invention." (Ex. 1004 at 15:23-24.)

157. *Umaña* relays "a method for enhancing the ADCC activity of therapeutic antibodies," which is "achieved by engineering the glycosylation pattern of the Fc region of such antibodies[.]" (Ex. 1004 at 23:23-33.) *Umana*'s disclosed method "provides alternative glycoforms of proteins having improved therapeutic properties. The proteins of the invention include antibodies with an enhanced antibody-dependent cellular cytotoxicity (ADCC), which have been generated using the disclosed methods and host cells." (Ex. 1004 at 3:17-20; *see also* Ex. 1004 at 8:24-28.)

158. *Umaña* discloses an embodiment "directed to host cells that have been engineered such that they are capable of expressing a preferred range of a glycoprotein-modifying glycosyl transferase activity which increases complex N-linked oligosaccharides carrying bisecting GlcNAc." (Ex. 1004 at 2:28-31.) And *Umaña* discloses other embodiments "directed to methods for the generation of modified glycoforms of glycoproteins, for example antibodies, including whole antibody molecules, antibody fragments, or fusion proteins that include a region equivalent to the Fc region of an immunoglobulin, having an enhanced Fc-mediated cellular cytotoxicity, and glycoproteins so generated." (*Id.* at 2:31–3:3.)

159. Accordingly, in my opinion, a POSA would have found the production of an antibody molecule in the "mammalian host cell" of claim 1 to be obvious.

160. In view of the above, in my opinion, claims 1 of the ’446 patent would have been obvious over *Harris* in view of *Umaña*, *Malý*, and the knowledge of a POSA.

4. Dependent Claims 2-5: “[t]he isolated host cell of claim 1, wherein said host cell is a [CHO cell / NS0 cell / SP2/0 cell / YB2/0 cell].”

161. The combination of *Harris*, *Umaña*, *Malý*, and the knowledge of a POSA renders obvious all elements of claim 1, as set forth above.

162. Dependent claims 2–5 of the ’446 patent recite creation of a host cell with “decreased or no α1,6-fucosyltransferase activity” in different types of mammalian cells, all of which were well known in the prior art as of the alleged Priority Date of the ’446 patent. *Umaña,* which is representative of the state of the art as of the alleged Priority Date of the ’446 patent, explains that while “[c]hinese hamster ovary (CHO) cells have been most commonly used during the last two decades. . . . Other commonly used animal cells include baby hamster kidney (BHK) cells, NSO- and SP2/0-mouse myeloma cells.” (Ex. 1004 at 2:10–16.) YB2/0 was also a long-established cell line. (*See* Ex. 1006.) Indeed, *Umaña* is clear that “[a]ny type of cultured cell line can be used as background to engineer the host cell lines of [*Umaña’s*] invention.” (Ex. 1004 at 15:23-24.)

163. Thus, as of the alleged Priority Date of the ’446 patent, mammalian cell targets of genetic engineering routinely included CHO cells, NSO cells, SP2/0

cells, YB2/0 cells, among many others. *See Maniatis*; Ex. 1006. The source of cells was not a restriction in gene modification, the only requirement being the ability to maintain and grow cells of interest in laboratory cultures. Introducing the DNA to achieve novel sequence expression was referred to as transfection; and various routine technologies were well developed to transfect virtually any DNA sequence into a variety of target cells. Thus, in my opinion, dependent claims 2-5 would have been obvious over *Harris* in view of *Umaña*, *Malý*, and the knowledge of a POSA.

5. Dependent Claim 6: "the isolated host cell of claim 1, wherein said antibody molecule is an IgG antibody."

164. The combination of *Harris*, *Umaña*, *Malý*, and the knowledge of a POSA renders obvious all elements of claim 1, as set forth above.

165. Dependent claim 6 of the '446 patent simply identifies the "antibody molecule" produced by the host cell as IgG. *Umaña* specifically investigated the glycosylation pattern of the sugar chain of an "IgG" antibody. (Ex. 1004 at 34:20–21.) Thus, in my opinion, dependent claim 6 would have been obvious over *Harris* in view of *Umaña, Malý*, and the knowledge of a POSA.

XI. GROUND 5: Claim 5 of the '446 patent is obvious over *Rothman* in view of *Umaña, Gao*, and the knowledge of a POSA.

A.) Opinion Introduction and the *Gao* Reference

166. In my opinion, dependent claim 5 of the '446 patent is obvious over *Rothman* in view of *Umaña* and the knowledge of a POSA for the reasons set forth

above. *See supra* **Section VII**. It is my opinion that claim 5 is also obvious over *Rothman* in view of *Umaña, Gao*, and the knowledge of a POSA.

167. My analysis is set forth below. I also incorporate into my analysis the accompanying claim chart (**Exhibit C**), which sets forth portions of the cited prior art references corresponding to claims 1-6 of the ’446 patent.

168. I incorporate herein the background of the *Rothman* and *Umaña* references discussed above. *Gao*, which is prior art to the ’446 patent and is otherwise representative of the state of the art as of the alleged Priority Date of the ’446 patent, explicitly describes the “[c]haracterization of YB2/0 cell line by counterflow centrifugation elutriation[.]” (Ex. 1006 at Title.)

B.) Obviousness over *Rothman* in view of *Umaña, Gao*, and the knowledge of a POSA

169. As discussed above, by the alleged Priority Date of the ’446 patent, mammalian cell targets of genetic engineering routinely included CHO cells, NSO cells, SP2/0 cells, and YB2/0 cells, among many others. *See Maniatis*; Ex. 1006. The source of cells was not a restriction in gene modification, the only requirement being the ability to maintain and grow cells of interest in laboratory cultures. *Umaña*, for instance, states that “[a]ny type of cultured cell line can be used as background to engineer the host cell lines of [*Umaña’s*] invention.” (Ex. 1004 at 15:23-24.)

170. Introducing the DNA to achieve novel sequence expression was referred to as transfection; and various routine technologies were well developed to transfect virtually any DNA sequence into a variety of target cells. *Gao* explicitly describes the "[c]haracterization of YB2/0 cell line by counterflow centrifugation elutriation[.]" (Ex. 1006 at Title.) Gao further discloses that "[t]he YB2/0 cell line and its derivatives, moreover, can be propagated in (LOUxAO)Fl hybrid rats, making it a useful, model for the study of neoplasms of the immune system." (Ex. 1006 at 435.)

171. In my opinion, the creation of the isolated host cell of claim 1 in a YB2/0 cell would have been obvious to a POSA. The use of YB2/0 cells was routine in the art, and a POSA would have been motivated to use YB2/0 cells (as with any available mammalian cell targets) to suit their particular research needs. Thus, in my opinion, claim 5 of the '446 patent is obvious.

XII. GROUND 6: Claim 5 of the '446 patent is obvious over *Harris* in view of *Umaña, Gao*, and the knowledge of a POSA.

A.) Opinion and Introduction to the *Gao* Reference

172. In my opinion, dependent claim 5 of the '446 patent is obvious over *Harris* in view of *Umaña* and the knowledge of a POSA for the reasons set forth above. *See supra* **Section VIII**. It is my opinion that claim 5 is also obvious over *Harris* in view of *Umaña, Gao*, and the knowledge of a POSA.

173. My analysis is set forth below. I also incorporate into my analysis the accompanying claim chart (**Exhibit C**), which sets forth portions of the cited prior art references corresponding to claims 1-6 of the ’446 patent.

174. I incorporate herein the background of the *Harris* and *Umaña* references discussed above. *Gao*, which is prior art to the ’446 patent and is otherwise representative of the state of the art as of the alleged Priority Date of the ’446 patent, explicitly describes the “[c]haracterization of YB2/0 cell line by counterflow centrifugation elutriation[.]” (Ex. 1006 at Title.)

B.) Obviousness over *Harris* in view of *Umaña, Gao*, and the knowledge of a POSA

175. As discussed above, by the alleged Priority Date of the ’446 patent, mammalian cell targets of genetic engineering routinely included CHO cells, NSO cells, SP2/0 cells, and YB2/0 cells, among many others. *See Maniatis*; Ex. 1006. The source of cells was not a restriction in gene modification, the only requirement being the ability to maintain and grow cells of interest in laboratory cultures. *Umaña*, for instance, states that “[a]ny type of cultured cell line can be used as background to engineer the host cell lines of [*Umaña’s*] invention.” (Ex. 1004 at 15:23-24.)

176. Introducing the DNA to achieve novel sequence expression was referred to as transfection; and various routine technologies were well developed to transfect virtually any DNA sequence into a variety of target cells. *Gao* explicitly

describes the "[c]haracterization of YB2/0 cell line by counterflow centrifugation elutriation[.]" (Ex. 1006 at Title.) Gao further discloses that "[t]he YB2/0 cell line and its derivatives, moreover, can be propagated in (LOUxAO)Fl hybrid rats, making it a useful, model for the study of neoplasms of the immune system." (Ex. 1006 at 435.)

177. In my opinion, the creation of the isolated host cell of claims 1 in a YB2/0 cell would have been obvious to a POSA. The use of YB2/0 cells was routine in the art, and a POSA would have been motivated to use YB2/0 cells (as with any available mammalian cell targets) to suit their particular research needs. Thus, in my opinion, claim 5 of the '446 patent is obvious.

XIII. There Are No Indicia of Nonobviousness that Would Overcome The Strong Evidence of Obviousness Discussed Above

178. It is my understanding that secondary considerations of non-obviousness should be considered in any obviousness consideration. Secondary considerations include such items as commercial success, copying, prior failure, licensing, long felt unfulfilled need, unexpected results and skepticism. I have not been provided with any evidence of any of these secondary considerations. Should Patent Owner provide such information, I may amend or supplement my report.

179. I have been asked to opine as to the whether the '446 patent claims subject matter that is unexpected in view of the prior art. As discussed at length above, I believe the subject matter of the '446 patent would have been obvious as

of the alleged Priority Date. In view of at least the prior art discussed herein (*Rothman*, *Harris,* and *Umaña*), in my opinion, a POSA would understand the removal of fucose ("knock-out" of α1, 6-fucosyltransferase genes) to encompass an obvious and routine use of known technologies. More specifically, given the known correlation between removal of fucose and improved ADCC, a POSA would have been motivated and capable of utilizing routine genetic engineering techniques to create the "host cell" recited in claims 1-6 of the '446 patent.

180. I have also been asked to opine as to whether experts in the field would have expressed skepticism as to the invention claimed in the '446 patent. Again, for the reasons discussed at length above, I do not believe experts in the field would have expressed skepticism. As discussed above, the '446 patent describes the alleged problem in the art not as one of available techniques, but as a lack of knowledge as to the specific structures on the sugar chain that are "important structure for the effector function[.]" (Ex. 1001 at 2:35–38, 5:25-29.) However, the prior art—*Rothman* or *Harris*—gives every reason to expect that a knockout cell for fucosyltransferase would produce an improved antibody, and the state of the art establishes that it would be an obvious and routine exercise to do so. Patent Owner itself said that the enabling state of the art was "quite advanced," which is confirmed by state of the art discussed above. *Supra* **Sections III-V**. In my opinion, the record does not—and would not—show skepticism by experts.

Indeed, I believe that the prior art and background knowledge of a POSA as of the alleged Priority Date show the opposite. Experts in the field would expect to see improved antibody effector function with the "knock-out" of α1,6 fucosyltransferase genes, and they would have been more than capable of engineering mammalian cell lines having zero or no α1,6-fucosyltransferase activity.

181. I reserve the right to supplement this report, and to use additional demonstrative aids in presenting testimony at hearing or trial.

182. I declare under penalty of perjury that the foregoing is true and correct.

/ Brian G. Van Ness /
Brian G. Van Ness, PhD

EXHIBIT A

EXHIBIT A – MATERIALS CONSIDERED

REFERENCES

1. Maniatis, T, Fritsch, EF, and Sambrook, S, *Molecular Cloning*. Cold Spring Harbor, First edition, 1983, Second Edition, 1990.

2. Thomas, KR and Cappechi , MR. 1987. Site-directed mutagenesis by gene targeting in mouse embryo-derived stem cells. *Cell.* 51:503-512. (Ex. 1008.)

3. Cappecchi, MR. 1989. Altering the genome by homologous recombination. *Science* 244:1299-1292. (Ex. 1009.)

4. Hasty, P, Rivera-Perez, J, Bradley, A. 1991. The length of homology required for gene targeting in embryonic stem cells. *Mol Cell Biol* 11:5586-5591. (Ex. 1010.)

5. Koller, BH, Hagemann, LJ, Doetschman, T, Hagaman, JR, Huang, S, Williams, PJ, First, NL, Maeda, N, Smithies, O. 1989. Germ-line transmission of planned alteration made in a hypoxanthine phsophoribosytransferase gene by homologous recombination in embryonic stem cells. *Proc Natl Acad Sci USA* 86:8927-8931. (Ex. 1011.)

6. Walsman AS. 1992 Targeted homologous recombination in mammalian cells *Crit Rev Oncol Hematol* 12:49-64. (Ex. 1012.)

7. Galli-Taliadaoros, LA< Sedgwick, JD, Wood, SA, Korner, H. 1995 Gene knock-out technology: a methodological overview for the interested novice. J *Immunol Methods* 181:1-15. (Ex. 1013.)

8. Morrison, SL. And Oi, VT. 1984. Transfer and expression of immunoglobulin genes. *Ann Rev Immunol* 2:239-256. (Ex. 1014.)

9. Morrison, SL 1985 Transfectomas provide novel chimeric antibodies. *Science* 229:1202-1207. (Ex. 1015.)

10. Gritzmacher, CA. 1986 Expression of recombinant immunoglobulin genes to produce novel molecules with specific functions. *Immunol Res* 5:210-220. (Ex. 1016.)

11. Morrison, SL, Wims, L, Wallick, S, Tan, L, Oi, V. 1987. Genetically engineered antibody molecules and their application. *Ann NY Acad Sci* 507:187-198. (Ex. 1017.)

12. Tao, MH, and Morrison, SL. Studies of aglycosylated chimeric mouse-human IgG. 1989. Role of carbohydrate in the structure and effector functions mediated by the human IgG constant region. *J Immunol* 143:2595-2601. (Ex. 1018.)

13. Nair, MP and Schwatrz, SA. 1982. Suppression of human natural and antibody dependent cytotoxicity by soluble factors from unstimulated normal lymphocytes. *J Immunol* 129:2511-2518. (Ex. 1019.)

14. Morton, HC, Atkin, JD, Owens, RJ, Woof, JM. 1993. Purification and characterization of chimeric human IGA1 and IgA2 expressed in COS and Chinese hamster ovary cells. *J Immunol* 151:4743-4752. (Ex. 1020.)

15. Rowley, M., Liu, P., and Van Ness, B. 2000. Heterogeneity in therapeutic response of genetically altered myeloma cell lines to IL-6, dexamethasone, doxorubicin, and melphalan. *Blood.* 96:3175-3180. (Ex. 1021.)

16. Liu, X. and Van Ness, B. 1999. Gene targeting of the KI-KII sequence elements in a model pre-B cell line: Effects on germline transcription and rearrangement of the k locus. *Molecular Immunol*. 36:461-469. (Ex. 1022.)

17. Billadeau, D. Jelinek, D., Shah, N., LeBien, T.W. and Van Ness, B. 1995 Introduction of an activated N-ras oncogene alters the growth characterization of the IL-6 dependent myeloma cell line ANBL6. *Cancer Research*. 55:3640-3646. (Ex. 1023.)

ADDITIONAL MATERIALS CONSIDERED

18. WO 99/54342, Umaña et al., publ. Oct. 28, 1999 ("*Umaña*") (Ex. 1004) and all references cited therein
19. Rothman et al., *Antibody-dependent cytotoxicity mediated by natural killer cells is enhanced by castanospermine*-2015-present *induced alterations of IgG glycosylation*, 26 Mol. Immunol. 1113 (1989) ("Rothman") (Ex. 1002) and all references cited therein
20. Harris et al., *Refined Structure of an Intact IgG2a Monoclonal Antibody*, 36

Biochemistry 1581 (1997) ("Harris") (Ex. 1003) and all references cited therein

21. Maly et al., *The α(1,3)Fucosyltransferase Fuc-TVII Controls Leukocyte Trafficking through an Essential Role in L-, E-, and P-selectin Ligand Biosynthesis*, Cell, 1996; 83: 643-653 ("Maly") (Ex. 1005)
22. Gao et al., *Characterization of YB2/O Cell Line by counterflow centrifugation elutriation*, Exp. Toxic Pathol. 1992; 44: 435-438 ("Gao") (Ex. 1006) and all references cited therein
23. Declaration of Royston Jefferis, PhD, DSc, MRCP, FRCPath (Ex. 1026) and all materials cited therein
24. Information referenced in this Declaration
25. U.S. Patent No. 6,946,292 – Kanda et al.
26. U.S. Patent No. 6,946,292 – PTO File History
27. U.S. Patent No. 7,425,446 – Kanda et al.
28. U.S. Patent No. 7,425,446 – PTO File History
29. U.S. Patent No. 8,067,232 – Kanda et al.
30. U.S. Patent No. 8,067,232 – PTO File History
31. U.S. Patent No. 7,214,775 – Hanai et al.
32. U.S. Patent No. 7,214,775 – PTO File History
33. Patents or other references cited in the '292, '232, and '446 patents

EXHIBIT B

CURRICULUM VITAE

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IDENTIFYING INFORMATION

Education

Degree	Institution	Date Degree Granted
B.S., Biology	Indiana University of Pennsylvania Indiana, PA	1973
M.S., Chemistry	Indiana University of Pennsylvania Indiana, PA	1975
Ph.D., Biochemistry [Advisor: Dr. James Bodley.]	University of Minnesota Minneapolis, MN	1979
Postdoctoral appointment, Molecular Immunology [Advisor: Drs. Martin Weigert & Robert Perry]	Institute for Cancer Research, Fox Chase, PA	1980-82

Academic Appointments

1982-87	Assistant Professor	Biochemistry, University of Iowa
1983-87	Assistant Professor	Genetics Program, University of Iowa
1987	Associate Professor	Biochemistry, University of Iowa
1987	Associate Professor	Biochemistry and Institute of Human Genetics University of Minnesota
1992-1998	Professor	Biochemistry and Institute of Human Genetics University of Minnesota
1998-present	Professor	Genetics, Cell Biology & Development Department University of Minnesota
2015-present	Adjunct Professor	Dept. Clinical & Experimental Pharmacy, U of MN

Academic Administrative Appointments

1997-2001	Program Director	Cancer Genetics University of Minnesota Cancer Center
2000-2009	Department Head	Genetics, Cell Biology & Development Department
2009-2012	Director	Institute of Human Genetics, U of MN
2010-2014	co-PI/Director	PUMA-Pharmacogenomics, University of MN Alliance

Consulting / Advisory Positions

Scientific Advisory Board & Vice President for Research,
Blizzard Genomics, Inc. 2001-2003
Consultant: Cell Signaling Technologies (Boston, MA)
Scientific Advisory Board, International Myeloma Foundation, 2004-present
Research Co-Director, Bank On A Cure, 2004-2011
Senior Fellow of Commercialization, MN BioBusiness Alliance, Aug 2012-2013
Life Science Alley Institute Advisory Board, 2013-present
Advisory Board, EruditeEDU (an education company), 2014-present
Expert consultant to Robins, Kaplan, Miller & Ciresi L,L,P. Law Firm 2010-present
FOUNDER, CEO: Target Genomics Solutions, LLC. 2013-present
Scientific Advisory Board, GeneSpark Foundation 2015-present
Scientific Advisory Board, Waldenstrom Macroglobulinemia Foundation 2015-present

Professional Organizations

1983-	American Immunological Society
1988-	Eastern Cooperative Oncology Group
1992-	American Association for the Advancement of Science
1999-	American Society of Hematology
2008-	Pharmacogenomics Research Network (PGRN)

Honors and Awards

1973	B.S. - cum laude
1972-73	Chi Beta Phi (National Honorary Science fraternity)
1981-82	Damon Runyon-Walter Winchell Cancer Fund Fellowship
1984-87	Searle Scholar Award,
2006	International Myeloma Foundation Honoree for Directing Bank On A Cure Program

RESEARCH AND SCHOLARSHIP

Grant/Research Awards

1982	University of Iowa (Biochemistry) Start-up funds	$20,000
1983	Iowa American Cancer Society "Rearrangement and Expression of Immunoglobulin Genes"	7,500
1983	University of Iowa Medical School Dean's Research Award "Immunoglobulin Genes"	5,000
1983	Iowa Diabetes-Endocrinology Research Center Award "Requirements and Regulation of Immunoglobulin Gene Transcription"	12,000

1983-86	NIH RO1 AI 20034 "Rearrangement and Expression of Immunoglobulin Genes"	203,397
1984-86	Basil O'Connor Starter Research Grant #5-480 "Regulation of Immunoglobulin Genes"	45,000
1984-87	Searle Scholar Award 84-F-102 "Expression and Regulation of Immunoglobulin Genes"	157,500
1986-91	NIH RO1 GM37687 "Rearrangement and Regulation of Immunoglobulin Genes"	551,450
1987-89	Institute of Human Genetics Research Funds	40,000 (+2-year postdoctoral salary)
1988-89	Minnesota Medical Foundation CRF-115-88 "Rearrangement of Human Immunoglobulin Genes"	6,000
1988-89	Institutional grant, American Cancer Society "Molecular Methods for Detecting Lymphoid Malignancies"	3,500
1987-89	NCI Eastern Cooperative Oncology Group (Molecular Reference Lab)	65,000
1989-94	NCI Eastern Cooperative Oncology Group (Molecular Reference Lab)	288,500
1990-91	Bone Marrow Transplant Committee Award	7,050
1990-92	Minnesota Leukemia Task Force, "Immune Reconstitution in Leukemia Patients After Bone Marrow Transplantation"	33,000
1991-92	University of Minnesota Graduate School	24,000
1991-92	MN Medical Foundation, "Regulated Expression of Immunoglobulin Genes"	9,600
1991-92	Schering Laboratories	7,000
1993-94	ECOG Pilot Grant "T Cell Receptor Usage in Multiple Myeloma"	5,000
1991-95	NIH GM37687 "Rearrangement and Regulation of Immunoglobulin Genes"	385,555
1992-97	NCI Bone Marrow Transplant Program Project (J. Kersey, P.I.) Project 6 (T. LeBien, P.I.; BVN, Co-investigator) "Immune Dysfunction Post BMT" Current year: $58,300	297,000(to BVN)
1997-1998	Leukemia Task Force, p53 and Rb in Myeloma	40,000

1994-1999 NCI CA21115, to the Eastern Cooperative Oncology Group 190,826
Molecular Genetics of Multiple Myeloma

1994-1999 NIH CA-62242 Studies on Monoclonal Gammopathies
Program Project Grant - Project 4: Detection and Characterization of
Myeloma Precursors 158,000
Current year: $76,959

1995-2000 PO1 CA65493 Biology and Transplantation of the Human Stem Cell;
Project 4; Current year: $44,400 222,000

1998-99 Academic Health Center Seed Grant, Transgenic expression of Bcl-xL
25,000

1998-1999 Minnesota Medical Foundation, The influence of stromal cells on
therapeutic response in multiple myeloma. 10,000

1999-2000Eastern Cooperative Oncology Group Pilot Project: Genetic
Polymorphisms that influence detoxification in myeloma $15,000

2000-2002Multiple Myeloma Research Foundation, Profiling IL-6 induced
gene expression and signal transduction in myeloma $200,000

1999-2004 NIH CA62242 Studies on Monoclonal Gammopathies 744,149
Current year: $127,752

1994-2004 Schering Laboratories Grant for Laboratory studies associated with
SWOG, CALGB, ECOG Myeloma Intergroup Clinical Trial 105,000
Current year: $25,000 (projected, based on patient accrual)

1994-2004 Amgen Grant for Analysis of Tumor Mobilization in SWOG,
CALGB, ECOG Intergroup Clinical Trial 120,000
Current yeart: $90,000 (projected, based on patient accrual)

1999-2002NIH CA78620 Impact of Donor Lymphocyte Infusions on Myeloma Patients
(Co-investigator; Neil Kay, PI) $17,241 annual direct to BVN $ 35,000

2002-2004 NCI, R21 CA96609 Genetic polymorphisms in Myeloma $200,000

2002-2004 Leukemia Research Foundation: Mouse model of myeloma $35,000

2003-2007 International Myeloma Foundation, Bank On A Cure (equipment) $243,500

2007- 10 University of Minnesota-Mayo Clinic Partnership in Faculty Recruiting
$2,487,000

2003-2008 Fund to Cure Myeloma $198,000

2008-2009 Donor to Mn Medical Foundation $150,000

2009-2010 Millenium Pharmaceuticals (S. Janz PI, BVN co-I)
(Funded large mouse breeding program of transgenic model for myeloma)

		$92,000
2012-2013	Millenium Pharmaceuticals (BVN PI) Transgenic mouse modeling of therapeutic response in myeloma (pending 2013 renewal)	$101,000
1999-2015	NCI CA21115, to the Eastern Cooperative Oncology Group Molecular Genetics of Multiple Myeloma (Robert Comis PI; BVN co-I ECOG Myeloma DNA Bank Director)	$337,755
2009-2015	AHC Translational award, Lung cancer genomics (BVN PI)	$238,000
2011-2015	NIH R01 CA154517-01A1 Disclosing Genomic Incidental Findings in a Cancer BioBank: An ELSI Experiment" (Susan Wolf, PI)	$220,000
2012-2015	Onyx Pharnaceuticals (BVN PI) Drug response in a mouse model of myeloma	$75,000
2015-2016	International Myeloma Foundation fellowship to Dr. Amit Mitra Drug resistance in myeloma (BVN: mentor)	$50,000
2015-2017	Minnesota Partnership for Biotechnology and Medical Genomics Predicting response and resistance to proteasome inhibitors in multiple Myeloma (B Van Ness, U of MN; S. Kumar, Mayo – co-PIs)	$850,000
2015-2016	U of MN Cancer Center Brainstorm Award Targeting Deubiquinating Enzymes (DUBs) as therapeutic targets to overcome drug resistance in diverse cancers (B Van Ness, M Bazzaro, Co-PIs)	$50,000

PENDING

Leukemia Lymphoma Society
Epigenetic Therapies in Myeloma

NIH
Expression Profiling to predict therapeutic response in myeloma

International Myeloma Foundation
Targeting the epigenome in myeloma

Royalty Payments to University

2003-present	LICENSE #L1507 from Cell Signaling Technologies, Boston, MA Tissue and Developmental Specific expression of a Bcl-xL transgenic mouse (Use in monoclonal antibody production) Ongoing to the University	As of October, 2016	$833,000

Publications

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117. **Van Ness**, B, Ramos, C, Haznadar, M, Hoering, A,Haessler, J, Crowley, J, Jacobus, S, Oken, M, Rajkumar, V, Greipp, P, Barlogie, B, Durie, B, Katz, M, Atluri, G, Ganf, G, Gupta, R, Steinbach, M, Kumar, V, Mushlin, R, Johnson, D, and Morgan, G. (2008) Genomic Variation in Myeloma: Design, content, and initial application of the Bank On A Cure SNP Panel to analysis of survival. BMC Medicine. **6**:26.

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119. Wee Joo Chng, Tammy Price-Troska, Natalia Gonzalez-Paz, Scott Van Wier, Emily Blood, Susanna Jacobus, Kim Henderson, Martin Oken, Brian **Van Ness**, Philip Greipp [2], S. Vincent Rajkumar, Rafael Fonseca. 2007. Clinical significance of p53 mutation in myeloma. Leukemia. 21:582-584.

120. Wolf, S..**Van Ness**, B, and 20 authors. (2008). Managing incidental findings in human subjects research: Analysis and recommendations. Journal of Law, Medicine & Ethics. 36:219-248.

121. **Van Ness**, B. (2008) Genomic research and incidental findings. Journal of Law, Medicine & Ethics 36:292-297.

122.]W Chng , N Gonzalez-Paz, T Price-Troska. S Jacobus , S V Rajkumar , M M Oken, R A Kyle, K J Henderson, S Van Wier, P Greipp, B **Van Ness**, R Fonseca. (2008) Clinical and Biological Significance of RAS mutations in Multiple Myeloma. Leukemia 21:582-584.

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138. Wolf, S., Crock, B, **Van Ness**, B, et al. (2012) Managing incidental findings and research results in genomic research involving biobanks and archived datasets. Genetics in Med. 14:361-384. PMID: 22436882

139. Ferriere, M, and **Van Ness**, B. (2012) Return of research results and incidental findings in the clinical trials cooperative group setting. Genetics in Med. 14:411-416. PMID: 22382800

140. Bemmels, H and **Van Ness**. (2012) Mapping the inputs, analyses, and outputs of biobank research systems to identify sources of incidental findings and individual research results for potential return. Genetics in Med. 14:385-392. PMID: 22382801

141. Johnson D.C[1], Haznadar M,[2] Ramos C[2], Gregory W.M,[3] Walker B.A[1], Boyd K.D[1], Davies F.E[1], Child.J.A[3], Crowley J[4], Bartlogie B[5], Durie B.G.M[6] , **Van Ness** B[2], Morgan G.J[1]. Impact of Inherited Genetic Variation on Bone disease and Skeletal Related Events in Multiple Myeloma Patients. Submitted.

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146. Stessman, H, Mansoor, A, ***Van Ness, B,** and *Baughn, L. (2013) Stabilization of AID by bortezomib does not confer increased drug target mutation frequency. Leukemia & Lymphoma**.** In press. **(***co-corresponding authors) PMID: 23734619

147. Stessman, H, Mansoor, BS, Zhan, F, Janz, S, Lindens, MA, *Baughn LB, ***Van Ness, B.** (2013) Reduced CXCR4 expression is associated with extramedullary disease in a mouse model of myeloma and predicts poor survival in multiple myeloma patients treated with bortezomib. Submitted. **(***co-corresponding authors) Leukemia. 27:2075-2077. PMID: 23728080

148. Mitra, A, Stessman, H, Shaughnessy, J, and **Van Ness, B** (2014) Profiling bortezomib resistance in multiple myeloma: implications in personalized pharmacotherapy. In Resistance to Proteasome Inhibitors in Cancer: Molecular Mechanisms and Reversal Strategies. (Ping Dou, ed., Springer publisher.)

149. Stessman, H, Lulla, A, Xia, T, Mitra, A, Harding, T, Mansoor, A, Myers, C, **Van Ness, B***, Dolloff, N* (*Co-senior corresponding authors) (2014) High throughput drug screening identifies compounds and molecular strategies for targeting proteasome inhibitor resistant multiple myeloma. Leukemia. 28:2263-2267.

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154. Petersen, GM and **Van Ness, B**. (2015) Returning a research participant's genomic results to relatives: Perspectives from mangers of two distinct research biobanks. J Law, Medicine & Ethics, 43:523-528.

155. **Van Ness, B.** (2016) Applications and limitations in translating genomics to clinical practice. Translational Res. 168:1-5.

156. Mitra, A, Mukherjee, U, Jang, J, Harding, T, Stessman, H, Li, Y, Abyzov, A, Jen, J, Kumar, S, Rajkumar, V, **Van Ness, B**. (2016) Single-cell analysis of targeted transcriptome (SCATTome) predicts drug sensitivity of single cells within human myeloma tumors. Leukemia. 30(5):1094-10102.

157. A. K. Mitra, H. A. Stessman, R. J. Schaefer, W. Wang, C. L. Myers, **B. G. Van Ness**, S. Beiraghi. (2016) Identification of single nucleotide polymorphisms associated with familial nonsyndromic cleft lip with or without cleft palate. Frontiers in Genetics. In press.

158. Wolf, SM, Scholtes, E, Koenig, BA, Petersen, GM, Berry, SA, Beskow, LM, Daly, MB, Fernandez, CV, Green, RC, LeRoy, BS, Lindor, NM, O'ROurke, PO, Breitkopf, CR, Rothstein, MA, **Van Ness, B**, and Wilford BS. (2016) Pragmatic recommendations & implementation tools for return of genomic research results to relative, including after the proband's death. Genetics in Med. In press.

159. Rabellino, A, Melegari, M, Tompkins, VS, Chen, W, **Van Ness, BG**, TGeruya-Feldstein J. Conacci-Sorrell, M, Janz, S, Scaglioni, PP. (2016) PIAS1 promotes lymphomagenesis through MYC upregulation. Cell Rep. Epub ahead of print. PMID: 27239040.

160. Baughn, LB, Mitra, A, **Van Ness, BG**, Noble-Orcutt, K, Sachs, Z, Linden, M. (2016) Phenotypic and functional characterization of a bortezomib resistant multiple myeloma cell line by flow and mass cytometry. Leuk Lymphoma 16:1-10.

161. Mitra, A, Dodge, J, Van Ness, J, Sokeye, I, and **Van Ness, B**. (2016) A novel splice site mutation in EHMT1 resulting in Kleefstra Syndrome with pharmacogenomics screening and behavior therapy for regressive behaviors. Mol Gen & Genomic Med. In press.

162. Mitra, AK, Harding, T, Muherjee UK, Jang, JS, Li, Y, HongZen, R, Jen, J, Sonneveld, P, Kumar, S, Kuehl WM, Rajkumar, V, **Van Ness, B**. (2017) A gene expression signature distinguishes innate response and resistance to proteasome inhibitors in multiple myeloma. Blood Cancer J In press.

Submitted / In preparation

1. Amit Kumar Mitra, Ujjal Kumar Mukherjee, **Brian Van Ness** Extra-ordinary response/resistance prediction (ExtRRa) algorithm: demonstration in multiple myeloma. In preparation.

2. Jen, J, Jang, JS, Abyzov, A, Kumar, V., **Van Ness, B**, and Mitra, A. Single cell gene expression analysis of MGUS, smouldering myeloma , and active myeloma reveals subclonal populations. In Preparation.

6. Harding, A, Swanson, J, Nolan, M, **Van Ness, B**. Combining epigenetic inhibitors to EZH2 and HDACs shows synergistic response in multiple myeloma. In preparation.

Patents

- 6,610,470 B2 **Apparatus for generating a temperature gradient and methods for using the gradient to characterize molecular interactions** (Aug. 26, 2003)
- Pending: **3'KE-Bcl-xL transgenic mouse**
- **Transcription Classification and Prediction of Drug Response (T-CAP DR)** (U.S. Provisional Patent Application Serial No.: 62/262,709, filed December 3, 2015).

Invited Presentations

Invited Speaker, Gordon Conference on Immunobiology and Immunochemistry, 1981
Invited Speaker, Gordon Conference on Nucleic Acids, 1982
Invited Seminar, University of Minnesota, Department of Biochemistry, November 1983
Invited Seminar, University of Illinois Medical Center, Chicago, October 1985
Invited Seminar, University of Minnesota, Institute of Human Genetics, October 1986
Invited Seminar, University of Minnesota, Laboratory Medicine & Pathology, November 1987
Invited Seminar, University of Minnesota, Genetics and Cell Biology, January 1988
Invited Seminar, University of Minnesota, Microbiology, February 1988
Invited Seminar, Ohio University, Division of Molecular Biology, May 1988
Invited Seminar, University of California, San Francisco, Pathology, March 1989
Invited Seminar, University of Missouri, Microbiology, April 1989
Invited Speaker, Animal Cell Reactor Engineering, Minneapolis, May 1989
Invited Seminar, Mayo Clinic, Immunology Division, June 1989
Invited Speaker, Bloomington Rotary Club, June 1989
Speaker, 7th International Congress of Immunology, Berlin, July 1989
Invited Seminar, University of Kentucky, Microbiology, September 1989
Lecturer, CME course, "Molecular Biology for Clinicians," Hennepin County Medical Center, October 1989
Invited Seminar, Mayo Clinic, Immunology Division, October 1989
Seminar, University of Minnesota Bone Marrow Transplant Conference, January 1990
Invited Speaker, 7th Annual Advances in Cancer Treatment Research, Bone Marrow Transplant Symposium, March 1990
Invited Seminar, Fox Chase Cancer Center, Philadelphia, March 1990
Invited Seminar, University of Minnesota, Veterinary Medicine, May 1990
Invited Seminar, The College of St. Catherine, April 1991
Invited Seminar, Hybritech, CA, January 1992

Invited Seminar, University of California, San Diego, January 1992
Invited Seminar, Continuing Medical Education, Hennepin County Medical Center, March 1992
Invited Seminar, University of Pennsylvania, April 1992
Invited Seminar, University of Arkansas Cancer Center, March 1993
Invited Speaker, Myeloma Conference, Vancouver Hospital, June 1993
Invited Speaker, International Myeloma Conference, Rochester, MN, October, 1993
Invited Seminar, University of Iowa, Department of Biochemistry, November, 1993
Invited Speaker, Conference on Neoplasia, NIH, April, 1994
Invited Speaker, American Society of Hematology, Nashville, TN, December, 1994
Invited Seminar, Vanderbilt University, Nashville, TN, December, 1994
Invited Speaker, International Myeloma Conference, Le Baule, France, September, 1995
Speaker, American Society of Hematology, Seattle, WA, Dec. 1995
Invited Seminar, Univ. Texas Southwestern Medical Center, April, 1996
Invited Seminar, University of North Carolina, September, 1996
Chair of Workshop on Circulating Cells in Myleoma, International Myeloma Conference, Boston, June 1997
Invited seminar, University of Alberta / Cross Cancer Center. Feb. 1997
Invited seminar, University of Kansas Medical School, Feb., 1998
Invited seminar, Human Genome Systems, Inc., April, 1998
Invited seminar, University of Arkansas Cancer Center, April, 1998.
Invited speaker, 3M, Minneapolis, MN, July 1999
Invited speaker, Expert's Roundtable of the Myeloma Research Foundation, September, 1999.
Invited speaker, VII International Myeloma Workshop, Stockholm, Sweden, September, 1999.
Invited speaker, Relay for Life, Cancer Survivors; Allentown, PA 4/01
Invited speaker (Organizing Committee), VIII Int'l Myeloma Workshop, Banf, Canada, 5/01
Invited speaker, British Society of Hematology, Leeds, England, 4/01
Invited speaker, 3M; Minneapolis, MN, 12/01
Invited speaker. University of Arkansas, Patient & Professional Forum of MMRF; 3/02
Invited participant (group leader) Int'l Myeloma Fdn Workshop, St. Johns, VI; 5/02
Session chair, American Society of Hematology, Preclinical models of myeloma 12/02
Co-organizer, NCI Workshop on Mouse models of plasma cell malignancies, 3/03
Invited speaker, Minnesota Academy of Medicine, 3/03
Invited speaker, 9th International Myeloma Workshop, Salamenca, Spain, 5/03
Invited speaker, Minnesota Socciety of Clinical Oncology, Minneapolis, MN 2/04
Invited speaker, European Myeloma Conference, Torino, Italy 3/04
Invited speaker, 10th International Myeloma Workshop, Sydney, Australia, 4/05
Invited speaker, National Cancer Institute, 7/04
Invited speaker, Southwest Oncology Group national meeting, Kansas City, MO 10/04
Invited speaker, Post Genomics Summit, Beijing China 5/06
Invited speaker, Genomics in the Clinic, CHI, San Francisco, 5/06
Invited speaker, AACR Clinical Symposium, 10/06
Invited speaker, Family Medicine CME, 6/06
Invited speaker, Myeloma Genetics Expert Roundtable, Madonna-Camplignio, Italy 6/06
Invited Speaker, International Myeloma Workshop, Kos, Greece, 6/07
Invited Speaker, Symposium: Using Genomics as a Guide to Cancer Therapeutics, Mayo Clinic 9/07
Invited speaker, University of Iowa, January, 2008.
Invited Speaker, Children's Hospital of Michigan / Wayne State University, Detroit, MI 2/08
Invited Speaker, International Myeloma Foundation Scientific retreat, Bermuda, April 2008
Invited Speaker, Millenium Pharmaceuticals, Boston, August, 2008
Invited Speaker, Int'l Myeloma Foundation Founder's Circle, Los Angeles, 9/08
Invited Speaker, Myeloma Patient Support Groups (Mpls, Stillwater) multiple dates, 2006-2008
Session chair, International Myeloma Workshop, Washington DC, 2/09
Invited Speaker, Waldenstroms Macroglobulinemia Patient Support Group, Memphis, 4/09

Invited speaker, St Judes Children's Research Hospital, 4/09
Invited speaker, Breast Cancer Survivor Conference, Personalized Medicine, Minneapolis, 10/09
Invited Speaker, IVth International Workshop on Myeloma Therapies, Cape Cod, MA 9/10
Invited Speaker, Midwest Forum on Personalized Medicine (Life Science Alley) Minneapolis, 10/10
Invited Speaker, (session chair) BIT Int'l Forum on Personalized Medicine, Shanghai, China, 11/10
Invited Speaker, 4th National Conference on Genomics and Public Health, Bethesda, MD 12/10
Invited speaker, Wisconsin Institute for Discovery, UW Madison, 3/11
Invited speaker, Session co-chair Czech Republic Hematology Conference, Olomouc, CZ 6/11
Invited speaker, Session co-chair 5th Int'l Workshop - Pharmacogenomics in Myeloma, London 8/11
Invited Co-chair, Session at the American Society of Hematology, San Diego, CA 12/11
Invited Speaker, Marshfield Clinic, Marshfield, WI 4/12
Invited Speaker, Int'l Waldenstrom Macroglobulinemia Foundation Conference, Phila, PA 6/12.
Invited Speaker, Vth International Workshop on Myeloma Therapies, Scottsdale, Az 10/12
Invited speaker, Dana Farber Cancer Institute, Boston, MA 3/13
Invited Speaker, Millenium Pharmaceuticals, Boston, MA 3/13
Invited Speaker, University of Iowa Cancer Center, Iowa City, IA, 8/13
Invited Speaker, Onyx Phramaceutical (now Amgen), San Francisco, CA 9/13
Organizer, Gene Patent Forum, Minneapolis, MN 10/13
Invited Speaker, Multiple Myeloma Patient Support Group, Stillwater, MN 11/13, 11/14
Invited Speaker, Pat's Myeloma Beach Party (Patient Support Group) Amelia Island, FL 3/15
Invited Speaker, Multiple Myeloma Expert Forum, Integrating New Targets; Atlanta, GA 8/15
Invited Speaker, Minnesota Venture Conference, Minneapolis, MN 10/15
Invited Speaker, Launching startups around U Research, Minneapolis, MN, 12/15
Invited Speaker, Myeloma Patient Support Group, Stillwater, MN 1/16
Co-organizer, Pharmacogenomics: Research to Implementation Conference 6/16
Keynote Speaker Hematologic Malignancies Summit, Whistler, Canada 3/4/16
Invited Speaker 2016 Myeloma Beach Party (Patient Forum), FL 4/1/16
Invited Speaker, City of Hope, Duarte, CA 5/18/16
Invited Speaker, Optum Health, Minneapolis, 10/06/16
Invited Seminar, University of Minnesota Autism Initiative 9/16
Invited Seminar, Dept Genetics, Cell Bio & Dev, U of MN 10/20/16

TEACHING AND CURRICULUM DEVELOPMENT

Courses Taught

Year	Course Title and Number	Students	Lectures	% Resp.
Iowa				
1983	Seminar 99:282	10	14	100
1983	Metabolism 99:130	60	12	25
1984-Spr	Metabolism 99:130	65	12	25
1984-Fall	Metabolism 99:130	65	12	25
1984-Fall	Topics in Molecular Biology 99:237	8	15	100
1985-Spr	Molecular Biology Lab 99:151	8	16	50
1985-Fall	Metabolism 99:130	60	12	30
1986-Spr	Molecular Biology Lab 99:151	12	16	40
1985-Fall	Molecular Biology Minicourse	17	8	40
Minnesota				
1987-92	Advanced Topics in Molecular Biol. Bc/GCB/MdBc 8214	40	8	20

1987-95	Advanced Human Genetics GCB 5073	15	2	10
1991-94	Clinical Immunology	25	1	5
1991-97	Biochem. Mol. & Cell. Biology MdBc 5100 (Course Director, 1994-97)	180	10	15
1997-98	Biochem. Mol. & Cell. Biology MdBc 5100	180	10	15
1999-present	Biochem. Mol. & Cell. Biology MdBc 5100	180	10	15
	Advanced Molecular Genetics	25	15	30
2001-2013	Cancer Biology MiCaB8004	25	4	10
2012-present	Ethics, Public Policies and Careers In Molecular Biology MCB 8124	20-25	10	50
2010-present	Law and Genetics GCD9708 (Co-Developer, course director)	15-20	10	100
2015	Pharmacogenomics, PHAR6224	12	4	20
2016	Molecular Biology & Society GCD3020	125	15	50
2016	Genetics & Society GCD 8073	120	8	35

ADVISING AND MENTORING

Student Degrees Awarded

Rodney Feddersen, M.S., 1986 Daniel Fitzsimmons, post-doc
Mziwandile Madizekela, M.S., 1987
David Lowery, Ph.D., 1988
Adriana Marcuzzi, Ph.D., 1988
Rodney Feddersen, Ph.D., 1989
Debra Martin, Ph.D., 1990
Keats Nelms, Ph.D., 1991
Susan Christian, Ph.D. 1993
Judy Schanke, Ph.D., 1994
Regan Fulton, Ph.D., 1994
Daniel, Billadeau, Ph.D., 1996
Darin O'Brien, Ph.D., 1997
Pocheng Liu, Ph.D., 1998
Xiangdong Liu, Ph.D., 1998
Matthew Rowley, Ph.D., 2001
Wancheung Cheung, Ph.D., Ph.D. 2001
Jessica Niemi, M.S., 2001
Michael Linden, Ph.D. 2004
Paula Croonquist, Ph.D. 2004
Mary Gosse, MS, 2008
Majda Haznadar, Ph.D., 2010
Heather Zierhut, Ph.D., 2012 (co-advisor)
Holly Stessman, PhD. 2013

Other

Robert Hromas, Medical Fellow
Elizabeth King, Principal Lab Technician
Yue Wang, Postdoctoral Associate
Ambika Mathur, Postdoctoral Research Associate
Clifford Kashtan, Assistant Professor, Pediatrics
Georgia Wiesner, Medical Fellow

Lynn Quam, Assistant Scientist
Carrie Kaiser, Senior Lab Technician
Tina Lyons, Student Lab Technician
Wendy Taylor, Lab Technician
Anila Prabhu, Post-doctoral fellow
Richard Nho, Post-doctoral fellow
Theresa Faltesek, Principle Lab Tech
Debra McWilliam Principle Lab Tech
Fangyi Zhao, post-doctoral fellow
Tim Nice, undergraduate
Piradeep Suntharalingam, Lab tech
Kristin Boylan, Post doc
Christine Ramos, Lab tech
Wei Wang, Lab tech
Sal Abdulah, undergraduate
Anthony Day, Medical student
Greg Wu, Research Associate
Patrick Day, undergraduate
Vishal Lamba, Ph.D., post doc res assoc
Jiri Minarcik, M.D., Visiting Fulbright Scholar
Samantha Quandahl, undergraduate, research assist
Jessica Swanson, undergraduate UROP
Marissa Nolan, undergraduate

Current

Amit Mitra, Ph.D. - Res Assoc
Taylor Harding – Graduate student
Monica Akre – Graduate student
Marissa Nolan – undergraduate
Emily Rankine - undergraduate

PROFESSIONAL SERVICE

Committees

Departmental

Biochemistry Graduate Student Admissions Committee, University of Iowa, 1986-87
Cell Biology Training Grant Steering Committee, University of Iowa, 1985-87
Genetics Program Steering Committee, University of Iowa, 1985-87
Chair, Biochemistry Graduate Student Admissions Committee, 1987-96
Institute of Human Genetics Executive Committee, 1987-present
Department of Biochemistry Coordinating Committee, 1992-1999
Director of Graduate Studies, 1996-1998
Department of Biochemistry Chair Advisory Committee 1996-1998
Department of Genetics, Cell & Developmental Biology, Promotions and Tenure Committee

University

University Biosafety Committee, University of Iowa, 1984-86
Faculty Search Committee - Institute of Human Genetics, Medical Oncology, University of Minnesota, 1988
Ad hoc Committee on Medical School Curriculum
Minnesota Medical Foundation Grant Review Board, 1988-1994
Faculty Search Committee, Pediatric Genetics, 1989
Faculty Search Committee, Dentistry, 1989

Microchemical Facility Committee, 1989-
Medical School Committee to Develop First Year Integrated Course, 1989-
Institutional American Cancer Society Grant Review Board, 1989-1994
Faculty Search Committee, Pathology, 1990
Faculty Search Committee, Institute of Human Genetics (Committee Chair), 1990-91
Faculty Search Committee, Chair Pediatrics Developmental Genetics, 1990-91
Harrison Chair in Developmental Genetics, search 1991-93
Biochemistry Head Search Committee, 1992-93
Medical School Promotions and Tenure Committee, 1994-1998
Medical Task Force on Grading Policies, 1995
Cancer Center Executive Committee, 1996-present
Cancer Center Faculty Search Committee, 1996
Program Leader for Cancer Genetics in Cancer Center, 1996-2001
Chair, Cancer Genetics Task Force, 1997
Chair, Search Committee for Schering Endowed Chair in Cancer Genetics, 1998-99
Head, Department Genetics, Cell Biology & Development, 2000-
University Genomic Advisory Committee, 2001-
CBS and Medical School Basic Science Council 2000-
Medical School Budget & Finance Committee 2003-
University Autism Center Task Force 2003-
University Task Force for the Office of Business Development
Consortium on *Law and Values in Health, Environment and Life Sciences (2006-)*
Chair, Search Committee, Faculty in Pharmacogenomics, 2007
Chair, BioMedical Genomics Center Steering Committee 2009-present
Organizing Committee IHG Annual Symposium*, Medical Genomics and Personalized Medicine* April 2008
Chair, Search Committee, Director BioMedical Genomics Center 2008
Chair, BioMedical Genomics Task Force Committee, 2008
Leader, AHC Committee of Translational Technology Cores, 2009-
Chair, Biomedical Genomics Center Steering Committee, 2008-
Member, AHC Finance Committee 2009-
Member, Co-PI Pharmacogenomics, Univeristy of MN Alliance (PUMA) 2009-
Member, Minnesota Super Computer Data Management Advisory Committee, 2009-2010
Member, Academic Health Center Program Review Committee, 2011
Member, Executive Committee Consortium on Law and Science, 2011-
Multidisciplinary Advisory Committee, Clinical Translational Science Institute, 2011-
Director, Translational Science Graduate Program Development, 2011- 2013
Director, Technology Resources, CTSI, 2011-2012
Member, Genomics Cluster Hiring Committee in College of Biological Sciences, 2012-
Member, IRB Advisory Committee on Genomics, 2015
Member, Faculty Consultative Committee, Medical School, 2015-

National

Reviewer, Journal of Immunology; Nucleic Acids Research; P.N.A.S.; Nature; Science; Molecular and Cell Biology, Journal of Clinical Investigation, Blood, Leukemia
Chair, Immunogenetics, Gene Regulation Session at Midwest Immunology Conference, September 1985
Ad hoc Member, Allergy and Immunology Study Section, NIH, June 1987
Ad hoc Editor, Gene, 1988
Co-chair, ECOG (Eastern Cooperative Oncology Group) Myeloma Committee, 1988-
Minnesota Arthritis Grant Review Board, 1988
Member, Ad hoc Study Section, NIH, June 1989

Iowa Research Council Grant Review, 1990
Member, Special NIH Study Section - Program Project Review, Immunity and Cancer, November 1990
Member, ECOG Scientific Advisory Committee, 1991-92
Consultant, Hybritech Inc., San Diego, CA 1992
Cochair, ECOG National Laboratory Committee, 1992-present
Ad hoc Member, Allergy and Immunology Study Section, NIH, July, 1993
Reviewer, VA Merit Grants, 1994
Member, American Cancer Society Immunology Study Section, 1995-1998
Cochair, ECOG Laboratory Retreat, Boston, May, 1996
Chair, Myeloma Workshop at the International Myeloma Conference, 1997
Consultant, Virginia Piper Cancer Institute, Minneapolis, MN 1993-present
Editorial Board, BLOOD, 1997-2002
Editorial Board, Nucleic Acids Research 1995-2000
Consultant, Human Genome Sciences, Bethesda, MD. 1999-2000
Member, NCI Progress Review Group in Leukemia, Lymphoma, Myeloma 1/01
Member, Pharmacogenomics Research Network 2007-present
Member, Committee to review Intramural NCI Genetics Branch 10/08
Member, Committee to review Intramural NCI Metabolism Branch 3/09
Member, Cell Mediated Clinical Oncology Emphasis Panel, NIH 6/09 – 5/12
Member, Committee of Life Science Law and Public Policy (NIH supported) 2007-present
Co-chair, Myeloma preclinical studies, American Society of Hematology, New Orleans, 12/13
Member, Expert Advisory Group, Advancing Collaborative Genetic Research; Ethical and Policy Challenges (NIH), Bethesda, MD 9/22-23/14

International

Member, Scientific Advisory Board of the International Myeloma Foundation
Member, Scientific Advisory Board of the Multiple Myeloma Research Foundation
Organizing Committee, VIII Int'l Workshop in Myeloma, Banf, Canada, May, 2001
Organizing Committee, IX Int'l Workshop in Myeloma, Salamenca, Spain. June 2003
Co-director Bank On A Cure for the International Myeloma Foundation, 2003-
Chair, Genetics/Proteomics Core, Multiple Myeloma Research Foundation Consortium, 2003
Co-organizer, 1st Workshop Mouse plasma cell malignancies, NIH 2003
Scientific Advisory Committee, 10th International Myeloma Workshop, 2005
Scientific Program Committee, 11th International Myeloma Workshop. 2007
Scientific Advisory Committee, GeneSpark, 2015-
Editorial Board, Journal of Human Genetics & Genomic Medicine 2017-

Community Outreach

2003-present Active participant in Patient Support Groups (Myeloma, Cancer)
2012 Senior Fellow, Life Science Alley Program in Commercialization
2014-present Co-director, Twin Cities Cancer Genomics Committee

COMMERCIALIZATION

2014 Founder, CEO Target Genomics, LLC
2016 Consultant, OneOme

EXHIBIT C

Ground 1: *Rothman* in view of *Umaña* and the Common Knowledge Renders Claims 1–6 Obvious

Claim Language	Evidence & Corresponding Disclosure
[1.a] An isolated mammalian host cell which has decreased or no α-1,6-fucosyltransferase activity for adding fucose to N-acetylglucosamine of a reducing terminus of N-glycoside-linked sugar chains	"The invention provides ***host cells*** which harbor a nucleic acid encoding the protein of interest, e.g., an antibody, ***and at least one*** nucleic acid encoding a glycoprotein-modifying ***glycosyl transferase***." (Ex. 1004 at 3:9–11 (emphasis added).) "***Also the use of gene knockout technologies*** or the use of ribozyme methods ***may be used to tailor the host cell's glycosyl transferase*** and/or glycosidase ***expression levels***, and is therefore within the scope of the invention." (Ex. 1004 at 15:20–22 (emphasis added).) "Our data suggests a possible involvement of core fucosylation of IgG in NK cell-mediated ADCC." (Ex. 1002 at 1114.) "Thus, ***absence of core fucosylation*** itself would appear to be a likely candidate as a structural feature necessary for enhancement of NK cell-mediated ADCC." (Ex. 1002 at 1122 (emphasis added).)
[1.b] by deleting a gene encoding α-1,6-fucosyltransferase or	"Also ***the use of gene knockout technologies or the use of ribozyme methods may be used to tailor the host cell's glycosyl transferase and/or glycosidase expression levels***, and is therefore within the scope of the invention." (Ex. 1004 at 15:20–22 (emphasis added).) "Examples of glycoprotein-modifying glycosyl transferases include, ***but are not limited to*** glycosyl transferases such as GnT III, GnT V, GalT, and Man II." (Ex. 1004 at 7:15–18 (emphasis added).) "Our data suggests a possible involvement of core fucosylation of IgG in NK cell-mediated ADCC."

Claim Language	Evidence & Corresponding Disclosure
	(Ex. 1002 at 1114.) "Thus, ***absence of core fucosylation*** itself would appear to be a likely candidate as a structural feature necessary for enhancement of NK cell-mediated ADCC." (Ex. 1002 at 1122 (emphasis added).)
[1.c] by adding a mutation to said gene to reduce or eliminate the α-1,6-fucosyltranferase activity,	"Also ***the use of gene knockout technologies or the use of ribozyme methods may be used to tailor the host cell's glycosyl transferase and/or glycosidase expression levels***, and is therefore within the scope of the invention." (Ex. 1004 at 15:20–22 (emphasis added).) "Examples of glycoprotein-modifying glycosyl transferases include, ***but are not limited to*** glycosyl transferases such as GnT III, GnT V, GalT, and Man II." (Ex. 1004 at 7:15–18 (emphasis added).) "Our data suggests a possible involvement of core fucosylation of IgG in NK cell-mediated ADCC." (Ex. 1002 at 1114.) "Thus, ***absence of core fucosylation*** itself would appear to be a likely candidate as a structural feature necessary for enhancement of NK cell-mediated ADCC." (Ex. 1002 at 1122 (emphasis added).)
[1.d] wherein said mammalian host cell produces an antibody molecule.	"…the present invention relates to glycosylation engineering to generate proteins with improved therapeutic properties, ***including antibodies*** with enhanced antibody-dependent cellular cytotoxicity." (Ex. 1004 at 1:11-13 (emphasis added).) "More specifically, the present invention is directed to a ***method for producing altered glycoforms of proteins having improved therapeutic values, e.g., an antibody*** which has an enhanced antibody dependent cellular cytotoxicity (ADCC), ***in a host***

Claim Language	Evidence & Corresponding Disclosure
	cell. The invention provides ***host cells*** which harbor a nucleic acid encoding the protein of interest, e.g., an antibody, ***and at least one*** nucleic acid encoding a glycoprotein-modifying ***glycosyl transferase***." (Ex. 1004 at 3:6–11 (emphasis added).) "Furthermore, the present invention provides alternative glycoforms of proteins having improved therapeutic properties. ***The proteins of the invention include antibodies with an enhanced antibody-dependent cellular cytotoxicity (ADCC), which have been generated using the disclosed methods and host cells***." (Ex. 1004 at 3:17-20 (emphasis added); *see also* Ex. 1004 at 8:24-28.) "***Host cells include cultured cells, e.g., mammalian cultured cells***…" (Ex. 1004 at 7:31–8:1 (emphasis added); *see also* Ex. 1004 at 2:4–6.) "Any type of cultured cell line can be used as background to engineer the host cell lines of the present invention." (Ex. 1004 at 15:23-24.)

Claim 2	Evidence & Corresponding Disclosure
The isolated host cell of [claim 1], wherein said host cell is a CHO cell.	"Among mammalian cells, Chinese hamster ovary ***(CHO) cells have been most commonly used during the last two decades***…" (Ex. 1004 at 2:10–16 (emphasis added).)

Claim 3	Evidence & Corresponding Disclosure
The isolated host cell of [claim 1], wherein said host cell is a NSO cell.	"***Other commonly used animal cells include*** baby hamster kidney (BHK) cells, ***NSO-*** and SP2/0-mouse myeloma cells." (Ex. 1004 at 2:10–16 (emphasis added).)

Claim 4	**Evidence & Corresponding Disclosure**
The isolated host cell of [claim 1], wherein said host cell is a SP2/0 cell.	"***Other commonly used animal cells include*** baby hamster kidney (BHK) cells, NSO- and ***SP2/0-mouse myeloma cells***." (Ex. 1004 at 2:10–16 (emphasis added).)

Claim 5	**Evidence & Corresponding Disclosure**
The isolated host cell of [claim 1], wherein said host cell is a YB2/0 cell.	"***Host cells include cultured cells, e.g., mammalian cultured cells***[.]" (Ex. 1004 at 7:31–8:1 (emphasis added).) "***Any type of cultured cell line can be used as background to engineer the host cell lines of the present invention***." (Ex. 1004 at 15:23-24 (emphasis added).) *As of the alleged Priority Date of the '446 patent, mammalian cell targets of genetic engineering routinely included CHO cells, NSO cells, SP2/0 cells, YB2/0 cells, among many others.*

Claim 6	**Evidence & Corresponding Disclosure**
The isolated host cell of [claim 1], wherein said antibody molecule is an IgG antibody.	"In this report, ***we describe the functional effects of alterations in IgG glycosylation*** induced by inhibitors of glycosylation and carbohydrate processing. (Ex. 1002 at 1114 (emphasis added).) "[t]his [antibody] vector design was based on reports of reproducible high-level expression of recombinant ***IgG*** genes in CHO cells." (Ex. 1004 at 34:20–21 (emphasis added).)

Ground 2: *Harris* in view of *Umaña* and the Common Knowledge Renders Claims 1–6 Obvious

Claim Language	Evidence & Corresponding Disclosure
[1.a] An isolated mammalian host cell which has decreased or no α-1,6-fucosyltransferase activity for adding fucose to N-acetylglucosamine of a reducing terminus of N-glycoside-linked sugar chains	"The invention provides ***host cells*** which harbor a nucleic acid encoding the protein of interest, e.g., an antibody, ***and at least one*** nucleic acid encoding a glycoprotein-modifying ***glycosyl transferase***." (Ex. 1004 at 3:9–11 (emphasis added).) "***Also the use of gene knockout technologies*** or the use of ribozyme methods ***may be used to tailor the host cell's glycosyl transferase*** and/or glycosidase ***expression levels***, and is therefore within the scope of the invention." (Ex. 1004 at 15:20–22 (emphasis added).) "***The fucose residue may be of particular interest***. In both this antibody and the human Fc it interacts with Tyr313, but the interactions are quite different in the two cases. This ***fucose is also near the Fcγ receptor binding site and could influence binding by the receptor***." (Ex. 1003 at 1592 (emphasis added).)
[1.b] by deleting a gene encoding α-1,6-fucosyltransferase or	"Also ***the use of gene knockout technologies or the use of ribozyme methods may be used to tailor the host cell's glycosyl transferase and/or glycosidase expression levels***, and is therefore within the scope of the invention." (Ex. 1004 at 15:20–22 (emphasis added).) "Examples of glycoprotein-modifying glycosyl transferases include, ***but are not limited to*** glycosyl transferases such as GnT III, GnT V, GalT, and Man II." (Ex. 1004 at 7:15–18 (emphasis added).) "***The fucose residue may be of particular interest***. In both this antibody and the human Fc it interacts with Tyr313, but the interactions are quite different in the two cases. This ***fucose is also near the Fcγ receptor***

Claim Language	Evidence & Corresponding Disclosure
	binding site and could influence binding by the receptor." (Ex. 1003 at 1592 (emphasis added).)
[1.c] by adding a mutation to said gene to reduce or eliminate the α-1,6-fucosyltranferase activity,	"Also ***the use of gene knockout technologies or the use of ribozyme methods may be used to tailor the host cell's glycosyl transferase and/or glycosidase expression levels***, and is therefore within the scope of the invention." (Ex. 1004 at 15:20–22 (emphasis added).) "Examples of glycoprotein-modifying glycosyl transferases include, ***but are not limited to*** glycosyl transferases such as GnT III, GnT V, GalT, and Man II." (Ex. 1004 at 7:15–18 (emphasis added).) "***The fucose residue may be of particular interest***. In both this antibody and the human Fc it interacts with Tyr313, but the interactions are quite different in the two cases. This ***fucose is also near the Fcγ receptor binding site and could influence binding by the receptor***." (Ex. 1003 at 1592 (emphasis added).)
[1.d] wherein said mammalian host cell produces an antibody molecule.	"…the present invention relates to glycosylation engineering to generate proteins with improved therapeutic properties, ***including antibodies*** with enhanced antibody-dependent cellular cytotoxicity." (Ex. 1004 at 1:11-13 (emphasis added).) "More specifically, the present invention is directed to a ***method for producing altered glycoforms of proteins having improved therapeutic values, e.g., an antibody*** which has an enhanced antibody dependent cellular cytotoxicity (ADCC), ***in a host cell.*** The invention provides ***host cells*** which harbor a nucleic acid encoding the protein of interest, e.g., an antibody, ***and at least one*** nucleic acid encoding a glycoprotein-modifying ***glycosyl transferase***." (Ex. 1004 at 3:6–11 (emphasis added).)

Claim Language	Evidence & Corresponding Disclosure
	"Furthermore, the present invention provides alternative glycoforms of proteins having improved therapeutic properties. ***The proteins of the invention include antibodies with an enhanced antibody-dependent cellular cytotoxicity (ADCC), which have been generated using the disclosed methods and host cells***." (Ex. 1004 at 3:17-20 (emphasis added); *see also* Ex. 1004 at 8:24-28.) "***Host cells include cultured cells, e.g., mammalian cultured cells***…" (Ex. 1004 at 7:31–8:1 (emphasis added; *see also* Ex. 1004 at 2:4–6.) "Any type of cultured cell line can be used as background to engineer the host cell lines of the present invention." (Ex. 1004 at 15:23-24.)

Claim 2	Evidence & Corresponding Disclosure
The isolated host cell of [claim 1], wherein said host cell is a CHO cell.	"Among mammalian cells, Chinese hamster ovary ***(CHO) cells have been most commonly used during the last two decades***..." (Ex. 1004 at 2:10–16 (emphasis added).)

Claim 3	Evidence & Corresponding Disclosure
The isolated host cell of [claim 1], wherein said host cell is a NSO cell.	"***Other commonly used animal cells include*** baby hamster kidney (BHK) cells, ***NSO-*** and SP2/0-mouse myeloma cells." (Ex. 1004 at 2:10–16 (emphasis added).)

Claim 4	Evidence & Corresponding Disclosure
The isolated host cell of [claim 1], wherein said host cell is a SP2/0 cell.	"***Other commonly used animal cells include*** baby hamster kidney (BHK) cells, NSO- and ***SP2/0-mouse myeloma cells***." (Ex. 1004 at 2:10–16 (emphasis added).)

Claim 5	**Evidence & Corresponding Disclosure**
The isolated host cell of [claim 1], wherein said host cell is a YB2/0 cell.	"***Host cells include cultured cells, e.g., mammalian cultured cells***[.]" (Ex. 1004 at 7:31–8:1 (emphasis added).) "***Any type of cultured cell line can be used as background to engineer the host cell lines of the present invention***." (Ex. *Umaña* at 15:23-24 (emphasis added).) *As of the alleged Priority Date of the '446 patent, mammalian cell targets of genetic engineering routinely included CHO cells, NSO cells, SP2/0 cells, YB2/0 cells, among many others.*

Claim 6	**Evidence & Corresponding Disclosure**
The isolated host cell of [claim 1], wherein said antibody molecule is an IgG antibody.	"[t]his [antibody] vector design was based on reports of reproducible high-level expression of recombinant ***IgG*** genes in CHO cells." (Ex. 1004 at 34:20–21 (emphasis added).)

Ground 3: *Rothman* in view of *Umaña, Malý*, and the Common Knowledge Renders Claims 1–6 Obvious

Claim Language	Evidence & Corresponding Disclosure
[1.a] An isolated mammalian host cell which has decreased or no α-1,6-fucosyltransferase activity for adding fucose to N-acetylglucosamine of a reducing terminus of N-glycoside-linked sugar chains	"The invention provides ***host cells*** which harbor a nucleic acid encoding the protein of interest, e.g., an antibody, ***and at least one*** nucleic acid encoding a glycoprotein-modifying ***glycosyl transferase***." (Ex. 1004 at 3:9–11 (emphasis added).) "***Also the use of gene knockout technologies*** or the use of ribozyme methods ***may be used to tailor the host cell's glycosyl transferase*** and/or glycosidase ***expression levels***, and is therefore within the scope of the invention." (Ex. 1004 at 15:20–22 (emphasis added).) "Our data suggests a possible involvement of core fucosylation of IgG in NK cell-mediated ADCC." (Ex. 1002 at 1114.) "Thus, ***absence of core fucosylation*** itself would appear to be a likely candidate as a structural feature necessary for enhancement of NK cell-mediated ADCC." (Ex. 1002 at 1122 (emphasis added).) "Southern blot analysis identified embryonic stem (ES) **cell transfectants** containing homologous integration . . . approximately 26% of the progeny **were Fuc-TVII (-/-)**." (Ex. 1005 at 644.)
[1.b] by deleting a gene encoding α-1,6-fucosyltransferase or	"Also ***the use of gene knockout technologies or the use of ribozyme methods may be used to tailor the host cell's glycosyl transferase and/or glycosidase expression levels***, and is therefore within the scope of the invention." (Ex. 1004 at 15:20–22 (emphasis added).) "Examples of glycoprotein-modifying glycosyl transferases include, ***but are not limited to*** glycosyl

Claim Language	Evidence & Corresponding Disclosure
	transferases such as GnT III, GnT V, GalT, and Man II." (Ex. 1004 at 7:15–18 (emphasis added).) "Our data suggests a possible involvement of core fucosylation of IgG in NK cell-mediated ADCC." (Ex. 1002 at 1114.) "Thus, ***absence of core fucosylation*** itself would appear to be a likely candidate as a structural feature necessary for enhancement of NK cell-mediated ADCC." (Ex. 1002 at 1122 (emphasis added).)
[1.c] by adding a mutation to said gene to reduce or eliminate the α-1,6-fucosyltranferase activity,	"Also ***the use of gene knockout technologies or the use of ribozyme methods may be used to tailor the host cell's glycosyl transferase and/or glycosidase expression levels***, and is therefore within the scope of the invention." (Ex. 1004 at 15:20–22 (emphasis added).) "Examples of glycoprotein-modifying glycosyl transferases include, ***but are not limited to*** glycosyl transferases such as GnT III, GnT V, GalT, and Man II." (Ex. 1004 at 7:15–18 (emphasis added).) "Our data suggests a possible involvement of core fucosylation of IgG in NK cell-mediated ADCC." (Ex. 1002 at 1114.) "Thus, ***absence of core fucosylation*** itself would appear to be a likely candidate as a structural feature necessary for enhancement of NK cell-mediated ADCC." (Ex. 1002 at 1122 (emphasis added).)
[1.d] wherein said mammalian host cell produces an antibody molecule.	"…the present invention relates to glycosylation engineering to generate proteins with improved therapeutic properties, ***including antibodies*** with enhanced antibody-dependent cellular cytotoxicity." (Ex. 1004 at 1:11-13 (emphasis added).)

Claim Language	Evidence & Corresponding Disclosure
	"More specifically, the present invention is directed to a ***method for producing altered glycoforms of proteins having improved therapeutic values, e.g., an antibody*** which has an enhanced antibody dependent cellular cytotoxicity (ADCC), ***in a host cell.*** The invention provides ***host cells*** which harbor a nucleic acid encoding the protein of interest, e.g., an antibody, ***and at least one*** nucleic acid encoding a glycoprotein-modifying ***glycosyl transferase***." (Ex. 1004 at 3:6–11 (emphasis added).) "Furthermore, the present invention provides alternative glycoforms of proteins having improved therapeutic properties. ***The proteins of the invention include antibodies with an enhanced antibody-dependent cellular cytotoxicity (ADCC), which have been generated using the disclosed methods and host cells***." (Ex. 1004 at 3:17-20 (emphasis added); *see also* Ex. 1004 at 8:24-28.) "***Host cells include cultured cells, e.g., mammalian cultured cells***…" (Ex. 1004 at 7:31–8:1 (emphasis added); *see also* Ex. 1004 at 2:4–6.) "Any type of cultured cell line can be used as background to engineer the host cell lines of the present invention." (Ex. 1004 at 15:23-24.)

Claim 2	Evidence & Corresponding Disclosure
The isolated host cell of [claim 1], wherein said host cell is a CHO cell.	"Among mammalian cells, Chinese hamster ovary ***(CHO) cells have been most commonly used during the last two decades***..." (Ex. 1004 at 2:10–16 (emphasis added).)

Claim 3	Evidence & Corresponding Disclosure
The isolated host cell of [claim 1], wherein said host cell is a NSO cell.	"***Other commonly used animal cells include*** baby hamster kidney (BHK) cells, ***NSO-*** and SP2/0-mouse myeloma cells." (Ex. 1004 at 2:10–16 (emphasis added).)

Claim 4	Evidence & Corresponding Disclosure
The isolated host cell of [claim 1], wherein said host cell is a SP2/0 cell.	"***Other commonly used animal cells include*** baby hamster kidney (BHK) cells, NSO- and ***SP2/0-mouse myeloma cells***." (Ex. 1004 at 2:10–16 (emphasis added).)

Claim 5	Evidence & Corresponding Disclosure
The isolated host cell of [claim 1], wherein said host cell is a YB2/0 cell.	"***Host cells include cultured cells, e.g., mammalian cultured cells***[.]" (Ex. 1004 at 7:31–8:1 (emphasis added).) "***Any type of cultured cell line can be used as background to engineer the host cell lines of the present invention***." (Ex. 1004 at 15:23-24 (emphasis added).) *As of the alleged Priority Date of the '446 patent, mammalian cell targets of genetic engineering routinely included CHO cells, NSO cells, SP2/0 cells, YB2/0 cells, among many others.*

Claim 6	Evidence & Corresponding Disclosure
The isolated host cell of [claim 1], wherein said antibody molecule is an IgG antibody.	"In this report, ***we describe the functional effects of alterations in IgG glycosylation*** induced by inhibitors of glycosylation and carbohydrate processing. (Ex. 1002 at 1114 (emphasis added).) "[t]his [antibody] vector design was based on reports of reproducible high-level expression of recombinant ***IgG*** genes in CHO cells." (Ex. 1004 at 34:20–21 (emphasis added).)

Ground 4: *Harris* in view of *Umaña, Malý*, and the Common Knowledge Renders Claims 1-6 Obvious

Claim Language	Evidence & Corresponding Disclosure
[1.a] An isolated mammalian host cell which has decreased or no α-1,6-fucosyltransferase activity for adding fucose to N-acetylglucosamine of a reducing terminus of N-glycoside-linked sugar chains	"The invention provides ***host cells*** which harbor a nucleic acid encoding the protein of interest, e.g., an antibody, ***and at least one*** nucleic acid encoding a glycoprotein-modifying ***glycosyl transferase***." (Ex. 1004 at 3:9–11 (emphasis added).) "***Also the use of gene knockout technologies*** or the use of ribozyme methods ***may be used to tailor the host cell's glycosyl transferase*** and/or glycosidase ***expression levels***, and is therefore within the scope of the invention." (Ex. 1004 at 15:20–22 (emphasis added).) "***The fucose residue may be of particular interest***. In both this antibody and the human Fc it interacts with Tyr313, but the interactions are quite different in the two cases. This ***fucose is also near the Fcγ receptor binding site and could influence binding by the receptor***." (Ex. 1003 at 1592 (emphasis added).) "Southern blot analysis identified embryonic stem (ES) **cell transfectants** containing homologous integration . . . approximately 26% of the progeny **were Fuc-TVII (-/-)**." (Ex. 1005 at 644.)
[1.b] by deleting a gene encoding α-1,6-fucosyltransferase or	"Also ***the use of gene knockout technologies or the use of ribozyme methods may be used to tailor the host cell's glycosyl transferase and/or glycosidase expression levels***, and is therefore within the scope of the invention." (Ex. 1004 at 15:20–22 (emphasis added).) "Examples of glycoprotein-modifying glycosyl transferases include, ***but are not limited to*** glycosyl transferases such as GnT III, GnT V, GalT, and Man II." (Ex. 1004 at 7:15–18 (emphasis added).)

Claim Language	Evidence & Corresponding Disclosure
	"***The fucose residue may be of particular interest***. In both this antibody and the human Fc it interacts with Tyr313, but the interactions are quite different in the two cases. This ***fucose is also near the Fcγ receptor binding site and could influence binding by the receptor***." (Ex. 1003 at 1592 (emphasis added).)
[1.c] by adding a mutation to said gene to reduce or eliminate the α-1,6-fucosyltranferase activity,	"Also ***the use of gene knockout technologies or the use of ribozyme methods may be used to tailor the host cell's glycosyl transferase and/or glycosidase expression levels***, and is therefore within the scope of the invention." (Ex. 1004 at 15:20–22 (emphasis added).) "Examples of glycoprotein-modifying glycosyl transferases include, ***but are not limited to*** glycosyl transferases such as GnT III, GnT V, GalT, and Man II." (Ex. 1004 at 7:15–18 (emphasis added).) "***The fucose residue may be of particular interest***. In both this antibody and the human Fc it interacts with Tyr313, but the interactions are quite different in the two cases. This ***fucose is also near the Fcγ receptor binding site and could influence binding by the receptor***." (Ex. 1003 at 1592 (emphasis added).)
[1.d] wherein said mammalian host cell produces an antibody molecule.	"…the present invention relates to glycosylation engineering to generate proteins with improved therapeutic properties, ***including antibodies*** with enhanced antibody-dependent cellular cytotoxicity." (Ex. 1004 at 1:11-13 (emphasis added).) "More specifically, the present invention is directed to a ***method for producing altered glycoforms of proteins having improved therapeutic values, e.g., an antibody*** which has an enhanced antibody dependent cellular cytotoxicity (ADCC), ***in a host cell***. The invention provides ***host cells*** which harbor a

Claim Language	Evidence & Corresponding Disclosure
	nucleic acid encoding the protein of interest, e.g., an antibody, ***and at least one*** nucleic acid encoding a glycoprotein-modifying ***glycosyl transferase***." (Ex. 1004 at 3:6–11 (emphasis added).) "Furthermore, the present invention provides alternative glycoforms of proteins having improved therapeutic properties. ***The proteins of the invention include antibodies with an enhanced antibody-dependent cellular cytotoxicity (ADCC), which have been generated using the disclosed methods and host cells***." (Ex. 1004 at 3:17-20 (emphasis added); *see also* Ex. 1004 at 8:24-28.) "***Host cells include cultured cells, e.g., mammalian cultured cells***…" (Ex. 1004 at 7:31–8:1 (emphasis added); *see also* Ex. 1004 at 2:4–6.) "Any type of cultured cell line can be used as background to engineer the host cell lines of the present invention." (Ex. 1004 at 15:23-24.)

Claim 2	Evidence & Corresponding Disclosure
The isolated host cell of [claim 1], wherein said host cell is a CHO cell.	"Among mammalian cells, Chinese hamster ovary ***(CHO) cells have been most commonly used during the last two decades***..." (Ex. 1004 at 2:10–16 (emphasis added).)

Claim 3	Evidence & Corresponding Disclosure
The isolated host cell of [claim 1], wherein said host cell is a NSO cell.	"***Other commonly used animal cells include*** baby hamster kidney (BHK) cells, ***NSO-*** and SP2/0-mouse myeloma cells." (Ex. 1004 at 2:10–16 (emphasis added).)

Claim 4	Evidence & Corresponding Disclosure
The isolated host cell of [claim 1], wherein said host cell is a SP2/0 cell.	"***Other commonly used animal cells include*** baby hamster kidney (BHK) cells, NSO- and ***SP2/0-mouse myeloma cells***." (Ex. 1004 at 2:10–16 (emphasis added).)

Claim 5	Evidence & Corresponding Disclosure
The isolated host cell of [claim 1], wherein said host cell is a YB2/0 cell.	"***Host cells include cultured cells, e.g., mammalian cultured cells***[.]" (Ex. 1004 at 7:31–8:1 (emphasis added).) "***Any type of cultured cell line can be used as background to engineer the host cell lines of the present invention***." (Ex. 1004 at 15:23-24 (emphasis added).) *As of the alleged Priority Date of the '446 patent, mammalian cell targets of genetic engineering routinely included CHO cells, NSO cells, SP2/0 cells, YB2/0 cells, among many others.*

Claim 6	Evidence & Corresponding Disclosure
The isolated host cell of [claim 1], wherein said antibody molecule is an IgG antibody.	"[t]his [antibody] vector design was based on reports of reproducible high-level expression of recombinant ***IgG*** genes in CHO cells." (Ex. 1004 at 34:20–21 (emphasis added).)

<u>Ground 5</u>: *Rothman* in view of *Umaña, Gao,* and the Common Knowledge Renders Dependent Claim 5 Obvious

<u>Claim 5</u>	<u>Evidence & Corresponding Disclosure</u>
The isolated host cell of [claim 1], wherein said host cell is a YB2/0 cell.	"*Characterization of **YB2/0 cell line** by counterflow centrifugation elutriation*[.]" (Ex. 1006 at Title (emphasis added).) "The non-secreting rat myeloma clone ***YB 2/0 is a highly efficient fusion partner for the production of hybridomas***. ***YB 2/0*** was initially derived from the hybrid myeloma YB 2/3 HL cell line after cloning in soft agar multiple times and selecting for the absence of immunoglobulin secretion. ***The YB2/0 cell line*** and its derivatives, moreover, can be propagated in (LOU X AO)F1 hybrid rats, ***making it a useful, model for the study of neoplasms of the immune system***." (Ex. 1006 at 435 (emphasis added).)

Ground 6: *Harris* in view of *Umaña, Gao,* and the Common Knowledge Renders Dependent Claim 5 Obvious

Claim 5	Evidence & Corresponding Disclosure
The isolated host cell of [claim 1], wherein said host cell is a YB2/0 cell.	"*Characterization of **YB2/0 cell line** by counterflow centrifugation elutriation*[.]" (Ex. 1006 at Title.) "The non-secreting rat myeloma clone ***YB 2/0 is a highly efficient fusion partner for the production of hybridomas***. ***YB 2/0*** was initially derived from the hybrid myeloma YB 2/3 HL cell line after cloning in soft agar multiple times and selecting for the absence of immunoglobulin secretion. ***The YB2/0 cell line*** and its derivatives, moreover, can be propagated in (LOU X AO)F1 hybrid rats, ***making it a useful, model for the study of neoplasms of the immune system***." (Ex. 1006 at 435 (emphasis added).)