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 ROYSTON JEFFERIS, PhD, DSc, MRCP, FRCPath

TABLE OF CONTENTS

Page

I, Royston Jefferis, PhD, DSc, MRCP, FRCPath, Emeritus Professor of the University of Birmingham, United Kingdom, 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 a host cell that expresses antibodies with altered sugar chains (i.e. glycosylation patterns)—without fucose—that result in more effective antibodies (measured by the ADCC standard) because the sugar fucose is not bound to their 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 could provide enhanced Antibody Dependent Cellular Cytotoxicity:

> The fucose residue may be of particular interest" . . . [fucose is] near the Fcγ receptor binding site and could influence binding by the receptor.

(Ex. 1003 at 1592.)

3. 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

[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.)

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

I. Credentials and Expertise

4. I am currently Emeritus Professor in the Institute of Immunology and Immunotherapy, College of Medical & Dental Sciences, University of Birmingham, UK.

5. After receiving BSc and PhD degrees in Chemistry, I moved to the Medical School (Birmingham, UK) to initiate research into the structure and function of antibody molecules, in health and disease. Our studies revealed the profound influence that glycosylation of the IgG class of antibodies has on the activation of effector mechanisms of the antibodies, *in vitro* and *in vivo*. Extension to the engineering and design of recombinant antibody therapeutics led to consultancies with the biopharmaceutical industry. I have been an active academic for more than half a century at the University of Birmingham.

6. My activities are reflected in over 300 publications with more than 200 referenced on NCBI PubMed. In consideration of published research, I was awarded the degree of Doctor of Science (DSc) and elected a Member of the Royal

College of Physicians (MRCP) and Fellow of the Royal College of Pathologists (FRCPath). A copy of my Curriculum Vitae is attached as **Exhibit B**.

II. Legal Standards

7. 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.

8. 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.

9. 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 suggested 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.

10. 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.

11. 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

12. 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 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 with working on the genetics of antibodies.

13. This definition conforms to level of skill and knowledge that the '446 patent applicant itself noted had been reached by October 6, 2000. During prosecution of the '446 patent's grandparent application, the applicant characterized the basic enabling techniques described in the patent claims. As the applicant stated to the patent examiner in order to get the '292 patent:

> In fact, the 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.)

14. 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 to no-fucose antibodies (U.S. Patent No. 7,214,775, claiming priority to 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 Arts in 2000

15. In this section, I provide some background information and an introduction to antibody structure and function 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.

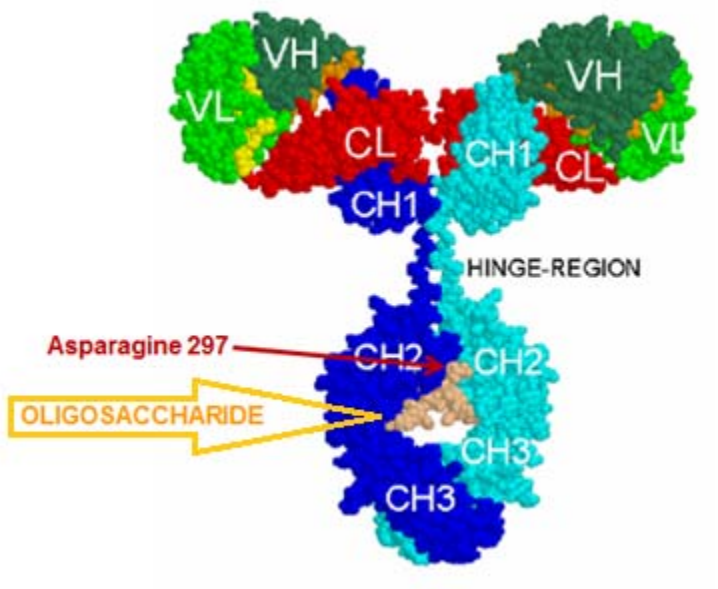
A.) Antibody Structure and Function

16. The germ theory of disease, proposed in the middle of the 19th century (Robert Koch & Louis Pasteur), held that disease results from invasions of the animal (including human) by a foreign body (microorganism, bacterium, virus etc.). The animal’s immune system responds by producing anti-bodies (antibodies). The generic term for a substance provoking and immune response is antigen.

17. Antibodies (also called immunoglobulins, ‘Ig’ for short) are water-soluble glycoproteins molecules comprising sugars/carbohydrates (=glyco) and protein (all antibodies are glycosylated at conserved positions in their constant regions (Jefferis and Lund, CHEM. IMMUNOL. 65:111·128 (1997)) that specifically recognise and bind to antigens. Antibodies are present in blood and tissue fluids. Antibodies function by binding antigens to trigger mechanisms that can neutralise and/or eliminate the antigen. There are a number of classes of antibodies in humans. The most important therapeutically—and the best studied—are immunoglobulins G (‘IgG’).

18. The basic structure of IgG is four polypeptide chains: two identical “light chains” and two identical “heavy chains.” These chains fold to generate

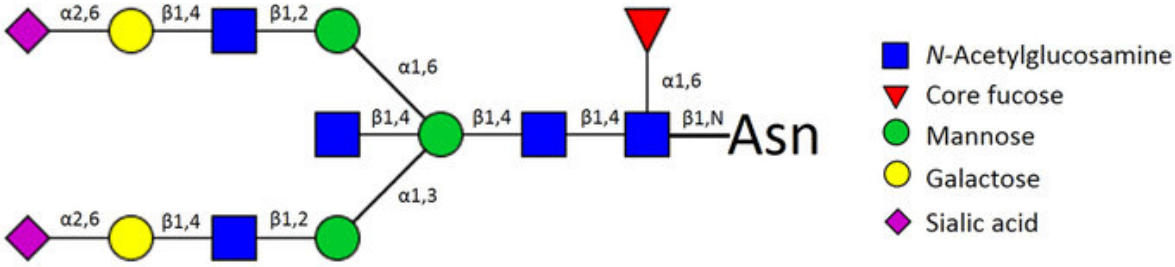
three-dimensional variable regions (VH & VL) and constant regions (CH & CL). These features of an IgG molecule are shown in the below figure.



19. The molecule may be cleaved at the "hinge" region to release two antigen binding fragments: (1) a Fab region (VHCH1/VLCL) and (2) an Fc region (CH2CH3/CH2CH3). Each CH2 region bears an oligosaccharide (oligo: few; saccharide: sugar) attached at the asparagine 297 amino acid residue, as shown above.

20. The Fc region of a single IgG molecule can bind weakly (with low affinity) to various cellular receptors without activating them; however, when antibody binds antigen it forms large complexes that can bind strongly (with high avidity) and simultaneously to multiple receptors on the surface of an immune cell to activate that cell. As has been known for decades, the presence of oligosaccharide on the Fc is essential for Fc receptor (FcγR) binding and

activation, and these vary with the precise oligosaccharide sugars present. The figure below shows a typical oligosaccharide chain with bound fucose.

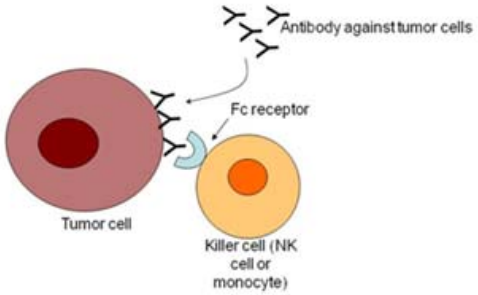


21. The above figure shows a representative IgG complex diantennary oligosaccarhide. The "core" heptasaccharide residues—(GlcNAc)2Man3(GlcNAc)2—are shown in blue. Fucose is shown in red. The enzyme fucosyltransferase is responsible for adding fucose to the sugar chain.

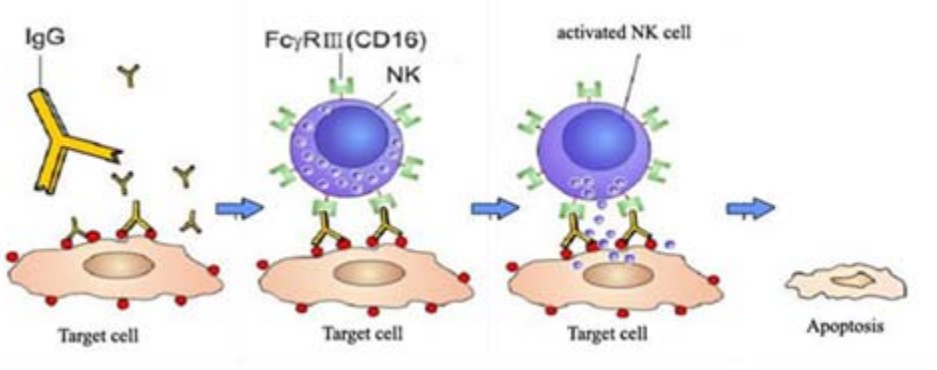
B.) The Correlation Between Sugar Chain Modification and Antibody Effector Function

22. The correlation between sugar content and antibody binding and immune-cell activation (i.e., antibody effector function) was a hot area of investigation in the prior art well before the priority date of the '446 patent, including in my own lab. (*See, e.g.*, Exs. 1027-1029, 1033.)

23. The effector function of IgG specifically involves activity mediated by the NK (natural killer) cells to which IgG binds, which is central to the killing of cancer cells. IgG effector function is depicted in the figure below.



24. The effect of the antibody binding to the NK cell is called antibody-dependent cellular cytotoxicity ('ADCC'). The endpoint of the action of the antibody's binding to an NK cell is the death of the target cell (hence 'cytotoxicity'), depicted below.



25. A POSA would have known that ADCC is an inflammatory response mediated by NK cells to kill cancer cells and is enhanced for non-fucosylated IgG; however, killing by polymorphonuclear cells is favoured for fucosylated IgG; anti-inflammatory forms of IgG (bearing fucose, galactose and sialic acid) are selected to treat patients with certain autoimmune diseases.

26. As I discuss below, the ’446 patent itself describes in detail how it was known in the prior art that changes to the sugars on the antibody oligosaccharide improve or lessen an antibody’s ADCC effect. The prior art also describes how antibody ADCC improved when fucose sugars in particular are removed from the Fc region oligosaccharides on the antibodies.

27. As of the year 2000, my research of a decade or more had been focused on the influence that glycosylation (the addition of sugars) of the IgG class of antibodies has on the activation of effector mechanisms—i.e., the avidity with which their constant region binds to cells and effects an immunological response (especially antibody-dependent cellular cytotoxicity/ADCC)—and the significance for human therapy.

28. By October 6, 2000, as a result of research in the field, including my own, it was well known that the binding of the constant region of an antibody, as measured by ADCC, could be profoundly affected by modifications in the sugar chain attached at Asn297 to the constant portion of the antibody. Indeed, in the year 2000, I was aware of numerous investigations into the sugar-modification/ADCC connection. The ’446 patent specification describes some of these in detail:

> Regarding the sugar chain, Boyd et al. have examined effects of a sugar chain on the ADCC activity and CDC activity by treating a human CDR-grafted antibody CAMPATH-lH (human IgGl subclass) produced by a Chinese hamster ovary cell (CHO cell) or a mouse

> myeloma NSO cell (NSO cell) with various sugar hydrolyzing enzymes, and reported that elimination of the non-reducing end sialic acid did not have influence upon both activities, but the CDC activity alone was affected by further removal of galactose residue and about 50% of the activity was decreased, and that complete removal of the sugar chain caused disappearance of both activities [*Molecular Immunol*., 32, 1311 (1995)]. Also, Lifely et al. have analyzed the sugar chain bound to a human CDR-grafted antibody CAMP ATH-lH (human IgG 1 subclass) which was produced by CHO cell, NSO cell or rat myeloma YO cell, measured its ADCC activity, and reported that the CAMPATH-lH derived from YO cell showed the highest ADCC activity, suggesting that N-acetylglucosamine (hereinafter referred also to as "GlcNAc") at the bisecting position is important for the activity [*Glycobiology*, 5, 813 (1995); WO 99/54342]. These reports indicate that the structure of the sugar chain plays an important role in the effector functions of human antibodies of IgGl subclass and that it is possible to prepare an antibody having increased effector function by changing the structure of the sugar chain.

(Ex. 1001 at 2:11-35.)

29. I recognise these published investigations as coeval with my group's efforts, and agree that their results—as with ours—indicated that as of the alleged Priority Date of the '446 patent it was known that the structure of the sugar chain plays an important role in the effector functions of human antibodies of IgGl subclass and that it is possible to modulate antibody effector function (e.g., ADCC) "by changing the structure of the sugar chain." (*See* Ex. 1001 at 2:11-3:4.)

30. The '446 patent specification, however, draws a conclusion that I do not agree with:

> However, actually, structures of sugar chains are various and complex, and it cannot be said that an actual important structure for the effector function was identified.

(Ex. 1001 at 2:35–38.)

31. Later, the ’446 patent reiterates this conclusion, with which I disagree:

> 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.)

32. In fact, published research at the time expressly specified such a sugar structure important for effector function, found to enhance the critical ADCC effector function—the removal of the fucose sugar normally bound to N-acetyl glucosamine:

> 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).) All of this calls out the absence of fucose as important for IgG binding and ADCC enhancement.

33. Rothman and his team showed that a castanospermine-induced IgG antibody phenotype generally enhanced antibody-dependent cellular cytotoxicity [ADCC] mediated by natural killer cells. (*Id.* at 1113.) Castanospermine was a known inhibitor of glycosylation (the process creating the sugar chain). (*See Id.* at 1114) (“Recently, a series of carbohydrate processing inhibitors have become available. These inhibitors interfere with discrete steps involved in the maturation

of protein-bound oligosaccharides Castanospermine (Cs), ... ").) Rothman's study indicated that Castanospermine blocked the addition of fucose from the antibody sugar chain. Thus, Rothman attributed the enhanced antibody effector function to this removal of fucose. (Ex. 1002 at 1122.)

34. Rothman also noted other research that had pointed to the absence of fucose as an ADCC enhancer:

> Enhancement of NK cell-mediated ADCC correlates with the expression of phenotypes characterized by IgG glycopeptides which bind to Con A but not to LcH. Glycopeptides from human myeloma IgG with similar lectin-binding properties have been identified as complex-type oligosaccharides in which core fucosylation is absent (Kornfield *et al.*, 1981). In addition to these complex-type structures, high mannose-type structures also would be expected to lack fucosylation, as these oligosaccharides are not substrates for the core fucosyl transferase (Hubbard and Ivatt, 1981).
>
> The mere exposure of peripheral mannosyl residues, however, seems to be insufficient to enhance ADCC, as the SW-induced phenotype did not alter lymphocyte-mediated ADCC even though peripheral mannosyl residues are expressed in this phenotype.

(Ex. 1002 at 1122.)

35. *Rothman* thus taught a POSA specifically about a no-fucose/increased ADCC correlation: "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 1113, 1122.) This is directly contrary to the '446 patent's assertion that an actual important structure for the effector function had not yet been identified, and a POSA would have concluded the same.

36. Additional prior art, based on the structure of the antibody sugar chain, expressly supports the teachings of *Rothman*—that fucose affected antibody binding and thus ADCC:

> ***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).)

37. In view of these teachings, and based upon my own knowledge in the art, it is my opinion that a POSA as of October 6, 2000, having common knowledge of antibody glycosylation would have expected that a sugar chain attached at Asn297 of an antibody, without an α1,6-fucose sugar, would have shown enhanced effector function, e.g., enhanced ADCC.

V. Introduction to the '446 Patent

38. 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 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.)

39. The sole independent claim of the ’446 patent recites a “host cell” that has “decreased or no α1,6-fucosyltransferase activity for adding fucose,” 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.)

40. 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.

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

42. Consistent with the state of the art as of the alleged Priority Date of the ’446 patent, the ’446 patent details specific prior-art knowledge about 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.)

43. The '446 patent specification also cites 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 addition of 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-afucosyltransferase into mouse L cell [Science, 252, 1668 (1991)]." (Ex. 1001 at 4:65-5:2.)

44. As discussed above, 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.

45. In my opinion, based on my direct knowledge of the area of immunology as of October 6, 2000, as well as a review of relevant prior art (prior to October 6, 2000), a POSA would have known that modification of sugar chain structure (and removal of fucose, in particular) would influence effector function. The importance of glycosylation was established throughout the 1980's. (*See, e.g.*, Exs. 1030-1032.)

46. Further, in my opinion, a POSA as of October 6, 2000, knowing of the afucosylation-enhanced antibody effector function, would have been strongly motivated to obtain therapeutic antibodies with enhanced ADCC. While there were other ways to achieve antibodies having a sugar chain without fucose, the most direct way to consistently obtain such an antibody would have been the creation of a host cell to express these modified antibodies. The standard approach would have been to import the antibody genes into a host cell to express the antibody, and to genetically "knock out" the enzyme that added α1,6-fucose to the sugar chain (i.e., the α1,6-fucosyltransferase enzyme). (*See* Ex. 1007 at ¶¶ 21-42.) The skilled person would have reasonably expected this to work, as explained more fully in the Declaration of Brian G. Van Ness, Ph.D (Ex. 1007), which I incorporate herein.

47. 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 take this approach with α1,6-fucosyltransferase. As the Patent Owner stated in bringing *Malý* to the Examiner's attention:

> [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.)

48. 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

49. 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.

50. 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."

51. 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).)

52. 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.

53. 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.

54. 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

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

56. 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.

57. *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 the 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.)

58. *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

59. As discussed above, *supra* **Section IV**, 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. The prior art (represented by *Rothman*) describes the correlation between sugar chain modification—including the removal of fucose, particularly—and improved ADCC. I believe that the known correlation between removal of fucose and improved ADCC (represented by *Rothman*) would have motivated a POSA to

utilise 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"

60. *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.)

61. *Rothman,* which is also representative of the state of the art as of the alleged Priority Date of the '446 patent, teaches 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.)

62. As discussed above, *supra* **Section IV**, the teachings of *Rothman* are coeval with my group's research efforts, and I believe that *Rothman's* results—as with ours—indicated that as of the alleged Priority Date of the '446 patent it was known that the structure of the sugar chain plays an important role in the effector

functions of human antibodies of IgGl subclass and that it was possible to modulate antibody effector function (e.g., ADCC) "by changing the structure of the sugar chain." (*See* Ex. 1001 at 2:11-3:4.) In my opinion, a POSA as of October 6, 2000, having common knowledge of antibody glycosylation would have expected that a sugar chain attached at Asn297 of an antibody, without an α1,6-fucose sugar, would have shown enhanced/altered effector function, e.g., ADCC.

63. Given this understanding, 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." I agree with Professor Van Ness' analysis that the knowledge of a POSA as of the alleged Priority Date would have rendered the act of "fucosyltransferase knock-out" routine. (*See* Ex. 1007 at ¶¶ 21-81.)

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,"

64. *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.) Further, I agree with Professor Van Ness' analysis as to the state of genetic engineering technologies as of the alleged Priority Date of the '446 patent and incorporate his analysis herein. (*See* Ex. 1007 at ¶¶ 21-81.) Accordingly, in my opinion, a POSA would consider routine "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." 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.)

65. 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."

66. *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.) *Umaña* further describes engineering such host cells to "produce 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 3:6-11; *see also* Ex. 1004 at 1:11-13.)

67. *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.)

68. Accordingly, in my opinion, a POSA would have found the creation of the "mammalian host cell" set forth in claim 1 to be obvious.

69. In view of the above, in my opinion, claim 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]."

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

71. Dependent claims 2–5 of the '446 patent recite creation of a host cell with "decreased or no α1,6-fucosyltransferase activity for adding fucose" 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 commonly used animal cell line. (*See* Ex. 1006.) And *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.)

72. 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. (Ex. 1004 at 2:10-16; Ex. 1006.) I have reviewed and agree with Professor Van Ness' analysis that 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. (*See* Ex. 1007 at ¶¶ 25, 82-84.) 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: "[t]he isolated host cell of claim 1, wherein said antibody molecule is an IgG antibody."

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

74. 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

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

76. 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.

77. *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.)

78. *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

79. As discussed above, *supra* **Section IV**, 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. The prior art (represented by *Harris*) describes the correlation between sugar chain

modification—including the removal of fucose, particularly—and improved ADCC. I believe that the known correlation between removal of fucose and improved ADCC (represented by *Harris*) would have motivated a POSA to utilise 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"

80. *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.)

81. *Harris,* which is also representative of the state of the art as of the alleged Priority Date of the '446 patent, teaches 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.)

82. As discussed above, *supra* **Section IV**, the teachings of *Harris* are coeval with my group's research efforts, and I believe that *Harris'* results—as with ours—indicated that as of the alleged Priority Date of the '446 patent it was known that the structure of the sugar chain plays an important role in the effector functions of human antibodies of IgGl subclass and that it was possible to modulate antibody effector function (e.g., ADCC) "by changing the structure of the sugar chain." (*See* Ex. 1001 at 2:11-3:4.) In my opinion, a POSA as of October 6, 2000, having common knowledge of antibody glycosylation would have expected that a sugar chain attached at Asn297 of an antibody, without an α1,6-fucose sugar, would have shown enhanced effector function, e.g., enhanced ADCC.

83. Given this understanding, 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." I agree with Professor Van Ness' analysis that the knowledge of a POSA as of the alleged Priority Date would have rendered the act of "fucosyltransferase knock-out" routine. (*See* Ex. 1007 at ¶¶ 21-42, 60-81.)

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,"

84. *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.) Further, I agree with Professor Van Ness' analysis as to the state of genetic engineering technologies as of the alleged Priority Date of the '446 patent and incorporate his analysis herein. (*See* Ex. 1007 at ¶¶ 21-42, 54-59, 87-108.) Accordingly, in my opinion, a POSA would consider routine "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." 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.)

85. 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."

86. *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.) *Umaña* further describes engineering such host cells to "produce 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 3:6-11; *see also* Ex. 1004 at 1:11-13.)

87. *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.)

88. Accordingly, in my opinion, a POSA would have found the creation of the "mammalian host cell" set forth in claim 1 to be obvious.

89. In view of the above, in my opinion, claim 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]."

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

91. 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 commonly used animal cell line. (*See* Ex. 1006.) And *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.)

92. 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. (Ex. 1004 at 2:10-16; Ex. 1006.) I have reviewed and agree with Professor Van Ness’ analysis that 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. (*See* Ex. 1007 at ¶¶ 15, 109-111.) 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: “[t]he isolated host cell of claim 1, wherein said antibody molecule is an IgG antibody.”

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

94. 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 Introduction and the *Malý* Reference

95. 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.

96. 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.

97. 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

98. As discussed above, *supra* **Section IV**, 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. The

prior art (represented by *Rothman*) describes the correlation between sugar chain modification—including the removal of fucose, particularly—and improved ADCC. I believe that the known correlation between removal of fucose and improved ADCC (represented by *Rothman*) would have motivated a POSA to utilise 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"

99. *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.)

100. *Rothman,* which is also representative of the state of the art as of the alleged Priority Date of the '446 patent, teaches 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.)

101. As discussed above, *supra* **Section IV**, the teachings of *Rothman* are coeval with my group's research efforts, and I believe that *Rothman's* results—as with ours—indicated that as of the alleged Priority Date of the '446 patent it was known that the structure of the sugar chain plays an important role in the effector functions of human antibodies of IgGl subclass and that it was possible to modulate antibody effector function (e.g., ADCC) "by changing the structure of the sugar chain." (*See* Ex. 1001 at 2:11-3:4.) In my opinion, a POSA as of October 6, 2000, having common knowledge of antibody glycosylation would have expected that a sugar chain attached at Asn297 of an antibody, without an α1,6-fucose sugar, would have shown enhanced/altered effector function, e.g., ADCC.

102. Given this understanding, 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." I agree with Professor Van Ness' analysis that the knowledge of a POSA as of the alleged Priority Date would have rendered the act of "fucosyltransferase knock-out" routine. (*See* Ex. 1007 at ¶¶ 21-42, 114-134.) I also agree that the "knock-out" performed by *Malý* demonstrates the routine nature of completing the a "knock-out" of α1,6-fucosyltransferase in host cells as of the alleged Priority Date.

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,"

103. *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.) Further, I agree with Professor Van Ness' analysis as to the state of genetic engineering technologies as of the alleged Priority Date of the '446 patent and incorporate his analysis herein. (*See* Ex. 1007 at ¶¶ 21-42, 54-59, 114-134.) Accordingly, in my opinion, a POSA would consider routine "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." 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.)

104. 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"

105. *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.) *Umaña* further describes engineering such host cells to "produce 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 3:6-11; *see also* Ex. 1004 at 1:11-13.)

106. *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.)

107. Accordingly, in my opinion, a POSA would have found the creation of the "mammalian host cell" set forth in claim 1 to be obvious.

108. In view of the above, in my opinion, claim 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]."

109. The combination of *Rothman*, *Umaña*, *Malý*, 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 commonly used animal cell line. (*See* Ex. 1006.) And *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. (Ex. 1004 at 2:10-16; Ex. 1006.) I have reviewed and agree with Professor Van Ness’ analysis that 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. (*See* Ex. 1007 at ¶¶ 25, 135-137.) 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: “[t]he isolated host cell of claim 1, wherein said antibody molecule is an IgG antibody.”

112. The combination of *Rothman*, *Umaña*, *Malý*, 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.) *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 Introduction and the *Malý* Reference

114. 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.

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 *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

117. As discussed above, *supra* **Section IV**, 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. The prior art (represented by *Harris*) describes the correlation between sugar chain modification—including the removal of fucose, particularly—and improved ADCC. I believe that the known correlation between removal of fucose and improved ADCC (represented by *Harris*) would have motivated a POSA to utilise known, routine genetic engineering techniques (represented by *Umaña*) to create the "host cell" recited in claims 1-6 of the '446 patent.

1. <u>Claim 1</u> 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. *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.)

119. *Harris,* which is also representative of the state of the art as of the alleged Priority Date of the '446 patent, teaches 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.)

120. As discussed above, *supra* **Section IV**, the teachings of *Harris* are coeval with my group's research efforts, and I believe that *Harris'* results—as with ours—indicated that as of the alleged Priority Date of the '446 patent it was known that the structure of the sugar chain plays an important role in the effector functions of human antibodies of IgGl subclass and that it was possible to modulate antibody effector function (e.g., ADCC) "by changing the structure of the sugar chain." (*See* Ex. 1001 at 2:11-3:4.) In my opinion, a POSA as of October 6, 2000, having common knowledge of antibody glycosylation would have expected that a sugar chain attached at Asn297 of an antibody, without an α1,6-fucose sugar, would have shown enhanced effector function, e.g., enhanced ADCC.

121. Given this understanding, 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." I agree with Professor Van Ness' analysis that the knowledge of a POSA as of the alleged Priority Date would have rendered the act of "fucosyltransferase knock-out" routine. (*See* Ex. 1007 at ¶¶ 21-42, 140-160.) I also agree that the "knock-out" performed by *Malý* demonstrates the routine nature of completing the a "knock-out" of α1,6-fucosyltransferase in host cells as of the alleged Priority Date.

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,"

122. *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.) Further, I agree with Professor Van Ness' analysis as to the state of genetic engineering technologies as of the alleged Priority Date of the '446 patent and incorporate his analysis herein. (*See* Ex. 1007 at ¶¶ 21-42, 54-59, 140-160.) Accordingly, in my opinion, a POSA would consider routine "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." 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.)

123. 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"

124. *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.) *Umaña* further describes engineering such host cells to "produce 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 3:6-11; *see also* Ex. 1004 at 1:11-13.)

125. *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.)

126. Accordingly, in my opinion, a POSA would have found the creation of the "mammalian host cell" set forth in claim 1 to be obvious.

127. In view of the above, in my opinion, claim 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]."

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

129. 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 commonly used animal cell line. (*See* Ex. 1006.) And *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.)

130. 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. (Ex. 1004 at 2:10-16; Ex. 1006.) I have reviewed and agree with Professor Van Ness' analysis that 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. (*See* Ex. 1007 at ¶¶ 25, 161-163.) 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: "[t]he isolated host cell of claim 1, wherein said antibody molecule is an IgG antibody."

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

132. 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 *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

133. In my opinion, claim 5 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.

134. 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 claim 5 of the '446 patent.

135. 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

136. 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* Ex. 1006.) I have reviewed and agree with Professor Van Ness’ analysis that 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. (*See* Ex. 1007 at ¶¶ 25, 166-171.) *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.)

137. 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. (*See* Ex. 1007 at ¶¶ 25, 166-171.) *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.)

138. 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.

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 Introduction and the *Gao* Reference

139. In my opinion, claim 1 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.

140. 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 claim 5 of the '446 patent.

141. 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

142. 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* Ex. 1006.) I have

reviewed and agree with Professor Van Ness' analysis that 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. (*See* Ex. 1007 at ¶¶ 25, 172-177.) *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.)

143. 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. (*See* Ex. 1007 at ¶¶ 25, 172-177.) *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.)

144. 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.

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

145. 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.

146. 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.

147. 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 the 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.

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

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

/ Royston Jefferis /
Royston Jefferis, PhD, DSc, MRCP, FRCPath

EXHIBIT A

EXHIBIT A – MATERIALS CONSIDERED

REFERENCES

1. Jefferis, R., Lund, J., Goodall, M. (1995) *Recognition of sited on human IgG fo Fcγ receptors: the role of glycosylation*. Immunol.Letters 44, 111-117. (Ex. 1027)

2. Sarmay, G, Lund, J, Gergely, J. & Jefferis, R. (1992) *Mapping and comparison of the interaction sites on the Fc region of IgG responsible for triggering antibody dependent cellular cytotoxicity (ADCC) through different types of Fcγ receptor*. Mol. Immunol. 29, 633-639. (Ex. 1028)

3. Jefferis, R, Lund, J, Mizitani, H, Nakagawa, H, Kawazoe, Y, Arata, Y & Takahashi, N. (1990) *A comparative study of the N-linked Oligosaccharide structures of human IgG subclass proteins*. Biochem. J. 268, 529-537. (Ex. 1029)

4. Nose M., Wigzell H. *Biological significance of carbohydrate chains on monoclonal antibodies.* PROC NATL ACAD SCI USA. 1983 Nov; 80(21):6632–6636 (Ex. 1030)

5. Leatherbarrow R.J., Rademacher T.W., Dwek R.A., Woof J.M., Clark A., Burton D.R., Richardson N., Feinstein A. *Effector functions of a monoclonal aglycosylated mouse IgG2a: binding and activation of complement component C1 and interaction with human monocyte Fc receptor.* MOL IMMUNOL. 1985 Apr; 22(4):407-15 (Ex. 1031)

6. M. R. Walker, J. Lund, K. M. Thompson, R. Jefferis. *Aglycosylation of human IgG1 and IgG3 monoclonal antibodies can eliminate recognition by human cells expressing Fc gamma RI and/or Fc gamma RII receptors.* BIOCHEM J. 1989 Apr 15; 259(2): 347–353. (Ex. 1032)

7. Kornfeld K., Reitman, M.L., Kornfeld, R. *The Carbohydrate-binding Specificicity of Pea and Lentil Lectins*, J. Biological Chemistry. 1981 July. No. 13, 6633-6640 (Ex. 1033)

ADDITIONAL MATERIALS CONSIDERED

8. WO 99/54342, Umaña et al., publ. Oct. 28, 1999 ("*Umaña*") (Ex. 1004) and all references cited therein

9. 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
10. Harris et al., *Refined Structure of an Intact IgG2a Monoclonal Antibody*, 36 Biochemistry 1581 (1997) ("Harris") (Ex. 1003.) and all references cited therein
11. 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)
12. 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
13. Declaration of Brian G. Van Ness and all materials cited therein (Ex. 1007)
14. Information referenced in this Declaration
15. U.S. Patent No. 6,946,292 – Kanda et al.
16. U.S. Patent No. 6,946,292 – PTO File History
17. U.S. Patent No. 7,425,446 – Kanda et al.
18. U.S. Patent No. 7,425,446 – PTO File History
19. U.S. Patent No. 8,067,232 – Kanda et al.
20. U.S. Patent No. 8,067,232 – PTO File History
21. U.S. Patent No. 7,214,775 – Hanai et al.
22. U.S. Patent No. 7,214,775 – PTO File History
23. Patents or other references cited in the '292, '232, and '446 patents

EXHIBIT B

CURRICULUM VITAE

Name:	Royston Jefferis
Position held:	Professor Emeritus: Molecular Immunology University of Birmingham
Date of Birth:	28 November 1938
Undergraduate Education:	Department of Chemistry, University of Birmingham, 1958 -1961
Qualification:	**BSc**
Postgraduate Education:	Department of Chemistry, University of Birmingham, 1961 - 1964
Qualification:	**PhD**
	Thesis:Aspects of Haloformate and Cyclic Ketal Chemistry
Further Qualifications:	**CChem:** Chartered Chemist 1992
	FRSC: Fellow of the Royal Society of Chemistry 1992
	FLS: Fellow of the Linean Society 1993
	MRCPath: Member of the Royal College 1989
	DSc: Doctor of Science 1987
	FRCPath: Fellow of the Royal College 1997 of Pathology.
	MRCP: Member of the Royal College of Physicians 2007
Membership:	British Society for Immunology Biochemical Society American Association of Immunology

Postdoctoral Experience:

1964-1965	Nuffield Research Associate. Department of Experimental Pathology, University of Birmingham
1965 – 1967	Honorary Research Fellow. MRC Group for Basic Immunology, Director PGH Gell: within Department of Experimental Pathology, University of Birmingham

May 1966	Awarded Fellowship of International Laboratory of Genetics and Biophysics, Naples, to attend course on DNA - RNA hybridization.
1967-1979	Lecturer, Department of Experimental Pathology,University of Birmingham
1968– 1969	Awarded American Arthritis Foundation Fellowship and Wellcome Research Travel Grant to spend a year at the University of California, San Diego in the laboratory of Dr R F Doolittle.
1979-1987	Senior Lecturer, Department of Experimental Pathology; later Department of Immunology
1987-1992	Reader in Immunology, Department of Immunology
1988-98	Deputy Head: Department of Immunology
1992 -	Professor of Molecular Immunology
1998 – 2002	Head: Department of Immunology
2006 -	Professor Emeritus

Professional Appointments

1974-1980	Secretary/Treasurer: Biochemical Immunology Group.
1982– 1997	Chairman: Human Immunoglobulins Sub-committee of the IUIS Standardisation Committee.
1971-1985	Ordinary Committee Member: British Society for Immunology
1982-1985	British Society for Immunology Representative: British Co-ordinating Committee for Biotechnology
1983-1984	Acting Treasurer: British Society for Immunology
1984-1985	Assistant Treasurer: British Society for Immunology
1986-1995	Treasurer: British Society for Immunology
1985-	Assessor: Health Research Council of New Zealand
1985 - 2005	Editorial Board: Journal of Immunological Methods
1985 - 2004	Editorial Board: Clinical and Experimental Immunology

1988 - 2004	Editorial Board: Immunology
2007 -	Editorial Board: Open Biotechnology
2008 -	Editorial Board: mAbs

University Appointments

Faculty of Science and Engineering Board of Postgraduate Studies

Faculty of Science and Engineering Board of Postgraduate Studies Applications Panel

Postgraduate Mitigation's Committee (Science and Engineering)

Scientific Projects Committee, Faculty of Medicine and Dentistry

1981-1984	Faculty of Medicine and Dentistry Board
1983-1985	University Biotechnology Management Group
1985-1988	Biotechnology Advisory Group
1992 - 1998	Board of Studies Bachelor of Medical Sciences
1991 - 2002	Curriculum Development Committee for the degree of Bachelor of Medical Sciences.
1999 -	Member of First Level Ethical Review Process (FLERP)
1999 -	Member of Birmingham Ethical Review Sub-Committee (BERSC)
2002 - 2013	Chair: BERSC
2002 - 2010	School of Medicine Senior Library Representative;
	Chair: School of Medicine Library Committee
2006 - 2008	Member: Information and Learning Resource Policy Committee (ILRPC)
2009 - 2011	Member of University: Research Governance & Ethics Group.

Consultancies:

Have acted as a consultant to pharmaceutical companies, both major and small, specialising in the development of antibody therapeutics.

I have worked with law firms advising on patent applications and/or challenges

Invited speaker at conferences 2004 - 2014:

Cell Culture Engineering Conference IX: – Mexico, March 2004

3rd International Congress on Recombinant Antibodies: - Germany, May 2004

Comparability & Immunogenicity of Biologicals: - Germany, June 2004

7th Jenner Glycobiology & Medicine Symposium: - UK, September 2004

Cell Culture and Upstream Processing: Germany, September 2004

Bioproduction: - Germany, October 2004

3rd International Conference on Post-translational Modifications: - USA, November 2004

Plant made Pharmaceuticals – Montreal – January, 2005

Antibody Production – San Diego – March, 2005

BioProcess International – Berlin – April, 2005

Tumour Immunotargeting – Tours, May 2005

Recombinant Antibodies – Berlin, June 2005

American Association of Pharmaceutical Scientists: – 2005 National Biotechnology Conference: USA, June, 2005

Glycoproteomics – Dubrovnik – June - 2005

Comparability & Immunogenicity of Biologicals – Lisbon, October 2005

Antibody Production & Downstream Processing – Amsterdam, October - 2005

BioProduction – Prague – February - 2006

Pharmaceutical Analytical Science Group – UK - April 2006

Recombinant Antibodies: Expression workshop – Zurich – June 2006

Post-translational Modifications – Washington – September 2006

BioProduction 2006 – Dublin – October 2006

Biotechnologia Habana – November - 2006

BioProduction 2006: Expression workshop – Dublin – 2006

BioProduction 2006: Antibodies – Dublin – 2006

Post-translational Modifications – Basle – 2006

Antibody therapeutics – San Diego – December 2006

ESACT-UK – January - 2007

Keystone Symposia – February 2007

AAPS National Biotech Conference – San Diego – June 2007.

PEACE (Protein expression in animal cells) – Brazil – September 2007.

Immunogenicity for Biologics – Budapest – September 2007

Early Develoment of biologics – Hamburg – October 2007

8th Jenner: Glycobiology & Medicine – Dublin – October 2007

Post-translational Modifications – Prague – November 2007

Antibody Engineering – Singapore – February 2008

Molecular Biology & Biotechnology, Sheffield – February 2008

Cell Development & Engineering – San Diego – June 2008

Immunogenicity for Biologics – Hamburg – September 2008

Bio-Production – Düsseldorf – October 2008

Bio-analytical Method Development – San Francisco – October 2008

Post translational Modifications – Munich – November 2008

British Pharmaceutical Society – Brighton – December 2008

BioProcess International – Düsseldorf – April, 2009

Glycoscience Ireland – Galway – May 2009

Inflammation and Immunology (IAI) Workshop – Vienna – May 2009

Signalling in the immune system – Balatonozod – September – 2009

Post-translational modifications – Prague - September – 2009

Smi Biological Production – London – September 2009

Immunogenicity for Biologics – Prague – September – 2009

Bioproduction – Barcelona – November 2009

State University of New York: Buffalo – November – 2009

Congreso Biotecnología Habana – Cuba - November – 2009

BioConference Live – November 2009

ESACT Scientific Committee – Vienna – December 2009

Cell Culture XII – Banff – April 2010

PEGS – Boston May 2010

Paul Erhlich Immunogenicity – Langen, Germany June 2010

Recombinant Antibodies – Berlin – June 2010

Comparability – Berlin – June 2010

Mass spectrometry applications in Biotechnology – California – September 2010

Immunogenicity – Brussels – September 2010

Post-translational modifications – Brussels- September – 2010

Regulatory Affairs – Berlin – November 2010

Pre-clinical safety & Efficacy testing of biological products – Berlin – December 2010

Immunogenicity – Munich – March 2011

Biopharmaceutical Development & production – Bellevue USA – March 2011

4th Annual Proteins Congress – London – April 2011

ESACT 2011: Cell based Technologies – Vienna – May 2011

Recombinant Antibodies – Barcelona – May 2011

Canceropole – Tours – May 2011

Cell development & engineering – Boston – June 2011

Bioprocessing, Biologics & Biotherapeutics – Edinburgh – July 2011

Antibody discovery & development – Amsterdam – September – 2011

Immunogenicity & PK/PD – Berlin – September 2011

French Society for Immunology – Montpelier – November - 2011

European Antibody Congress – Geneva – November – 2011

European Immunogenicity Platform – Copenhagen – February – 2012

Antibodies Asia (IBC) – Shanghai - February 2012

Biotherapeutics Analytic Summit – Baltimore – March 2012

8th Monoclonal Antibodies – London – May 2012

Biotherapeutics – London – June 2012

Bioprocessing – London – June 2012

Antibody manufacturing – London – September 2012

Post-translational Modifications – Berlin – September 2012

Immunogenicity Summit – Bethesda – October 2012

High order structure of Biomolecules – London – October 2012

Bioproduction 2012 – Berlin – October 2012

Antibody Engineering & Discovery – Frankfurt – February 2013

Immunogenicity – London – March - 2013

New cells – New Vaccines – Wilmington, USA – March 2013

Biotherapeutics – Stevenage – May - 2013

Glycoimmunology Workshop – Harvard, USA – May 2013

MabImprove – Montpelier, France – June – 2013

BITC Summer Symposium – University of New Hampshire – USA - July 2013

Bioproduction – Dublin – October 2013

Protein aggregation and immunogenicity – Workshop – Colorado – USA – 2014

Protein Characterisation and Post Translational Modifications IBC. – Berlin - September 2014

MIBio 2014 – Cambridge – September 2014

Bio-production IBC. – Barcelona – October 2014

Bioinnovation Leaders GBX Summit – London – February 2015

EULAR – AbbVie - Rome – Biosimilars - June 2015

Empowered Antibodies – Barcelona – June 2015

NIBRT/Abbvie – Dublin "Recombinant Biologics: Immunogenicity issues" June 2015

European Immunology Congress – September 2015

ESDR – Abbvie – Rotterdam – Biosimilars - September 2015

Immunogenicity Workshop – IBC Berlin September 2015

Bioanalytical Formulation – IBC – Berlin – September 2015

Nottingham – MSc Lecture: IgG Glycoforms – November 2015

Nottingham – MSc Lecture: Antigen/antibody immune complexes – November 2015

Bioanalytical Formulation Summit – Vonlanthen Group - Berlin – February 2016

EIP Symposium – Vilamoura – February 2016

PEGS Europe – Lisbon – November 2016

Invited seminars delivered to companies and institution:

National Institute for Biologicals Standards and Control: UK, February 2004.

Pierre-Fabre – France, June 2004

Novimmune – Switzerland, June 2004

Cambridge Antibody Technology – UK, September 2004

Roche, Penzberg – Germany, October 2004

CovX – La Jolla, California – November 2004

Pfizer – St Louis – November 2004

Novartis – Basle – January 2005

Pfizer – St. Louis – March 2005

University of Kent – April, 2005

Aeres Biomedical – London, May 2005

Genentech – San Fransisco - June 2005

Lilly – Indianapolis – September 2005

Novartis – Basle – September 2005

Amgen – Los Angeles – November 2005

Millipore – UK December 2005

Glycoform – Abingdon – January 2006

Serano – Vevey – April 2006

Novartis – Basle – May 2006

Cambridge Antibody Technology/AstraZenica – June 2006

Xoma – San Francisco – September 2006

Immunobiology – Cambridge – November 2006

Novartis – Basle - January 2007

Lonza – Slough – February – 2007

Pierre-Fabre – France – May 2007

CSL Behring – Switzerland – May 2007

Merck – Gwyedd, USA – September 2007

Lonza – Washington – October 2007

Novartis – Basle – May 2008

Gilde (Ozyrane) Review of investment opportunity – June 2008

Merck – Gwyedd, USA July 2008

Novartis - Basle – September 2008

Bayer – Wuppertal, Germany September 2008

Genentech – San Fransisco – October 2008

Takeda – San Fransisco – October 2008

Novartis – Basel – February – 2009

Roche – Penzberg – March – 2009

Symphogen – Denmark – June 2009

Life Technologies – New York – October – 2009

Merck – Gwynedd, Philadelphia USA – November 2009

Merck – Gwynedd, Philadelphia – February 2010
Three x 2 hr telephone conferences

Oxyrane – Manchester – April 2010

Oxyrane – Gent – July 2010

Oxyrane – Gent – September 2010

Manchester University – June 2011

Suppremol – Munich – July 2011

Highfield Sciences Specialist School – Wolverhampton – March 2012

CIIC Cancer studies – Birmingham – June 2012

MedImmune – Cambridge – March – 2013

Lonza – Slough – July 2013

Roche – Penzberg – March – 2014

Merck Serano – Switzerland July 2014

Boehringer Ingelheim - 2014

Merck – Switzerland – October 2015

NIBRT – Dublin – June 2016

NIBRT – Dublin – September 2016

LimmaTech biologics – Zurich – August 2016

PUBLICATIONS

~ 300 publications; 207 listed on PubMed (excluding 5 that are another Jefferis R)

Baggett N., Buck K.W., Foster A.B., Jefferis R., Rees B.H. & Webber J.M., (1965) Aspects of stereochemistry. Part XIX. Isopropylidene derivatives of some polyhydric alcohols. Observations on the hydrolytic behaviour of migration of cyclic ketals. *J. Chem. Soc*., **641**, 3382-3388.

Buck K.W., Foster A.B., Jefferis R. & Webber J.M. (1966) Some approaches to the synthesis of deoxyfluorosugars. Abstract papers. *Am Chem Soc Meeting*, **152**, 250.

Baggett N., Buck K.W., Foster A.B., Jefferis R. & Webber J.M. (1967) Mode of decomposition of chloro- and fluor-formates of some carbohydrates and related compounds. *Carbohydrate Res*., **4**, 3413-351.

Foster A.B, Jefferis R. & Webber J. M. (1967) Pyridine-catalysed decarboxylation of cis and trans-4-tert-butycyclohexl chloro-formate. *Carbohydrate Res*., **4**, 352-354.

Gergely J., Stanworth D.R., Jefferis R., Normansell D.E., Henney C.S. & Pardoe G.I. (1967) Structural Studies of immunoglobulins. I. The role of cysteine in papain proteolysis. *Immunochem.,* **4**, 101-111.

Jefferis R. & Stanworth D.R. (1967) Structural differences within the gamma G class of immunoglobulins. *Nature*, **215**, 276-277.

Jefferis R. & Stanworth D.R. (1967) Studies on low molecular weight peptides derived from human gamma G globulin. I. Application of gel-filtration with Gilford monitoring to their isolation and preliminary characterisation. *Proc Technicon 5th Colloquium on Amino Acid Analysis*, p84.

Henney C.S., Jefferis R. & Stanworth D.R. (1968) Studies on low molecular weight peptides derived from human gamma G globulin. II. Identification of rheumatoid factor reactive peptides in proteolytic digests. *Biochem. Biophys. Acta*, **154**, 295-304.

Jefferis R., Weston P.D., Stanworth D.R. & Clamp J. R. (1968) Relationship between papain sensitivity of human gamma G immunoglobulins and their heavy chain subclass. *Nature*, **219**, 646-649.

Jefferis R. & Stanworth D.R. (1969) Relationship between papain sensitivity of human G globulins and their heavy chain subclass. *FEBS Symposium*, **15**, 213-219.

Jefferis R. & Drew R, (1974) Rabbit b4 light chain fragments bearing isotypic and allotypic specificities. *Clin. Exp. Immunol*., **16**, 89-98.

Jefferis R. (1974) Identification of two antigenically distinct b-negative light chains in rabbits undergoing total b-locus suppression. *J. Immunogenetics*, **1**, 393. 12

Jefferis R. & Butwell A.J. (1975) Isolation of a human IgD myeloma protein by isotachophoresis. *Science Tools*, **22**, 1.

Bradwell A.R., Deverill I. & Jefferis R. (1975) Bisalbuminaemia Birmingham - a new variant in an Indian family. *Vox Sang*., **28**, 383-388.

Jefferis R., Butwell A.J. & Clamp J.R. (1975) Studies of human IgD myeloma proteins. Carbohydrate composition of intact protein and some proteolytic fragments. *Clin. Exp. Immunol.,* **22**, 311-315.

Jefferis R. (1975) Isolation of a V_HC_L fragment on tryptic digestion of human IgD myeloma proteins. *Immunol. Commun*., **4**, 477-482.

Jefferis R. (1975) The application of Ultragel AcA 34 to the isolation of human IgD proteins. *Science Tools*, **22**, 33-34.

Jefferis R. (1976) A rapid technique for the isolation of human IgD myeloma proteins employing Ultragel AcA 34. *J. Immunol. Methods*, **9**, 231-234.

Jefferis R., Matthews J.B. & Bayley P. (1977) Structural studies of human immunoglobulin D myeloma proteins: Circular dichroic studies of two intact proteins and enzymatically derived fragments. *Biochem. Soc. Trans.,* **5**, 279-282.

Jefferis R. & Matthews J.B. (1977) Studies of human IgD myeloma proteins. Proteolytic digestion patterns. *Immunochem*., **14**, 171-178.

Jefferis R., Matthews J.B. & Bayley P. (1977) Studies of human IgD myeloma proteins. Circular dichroism of intact protein and some proteolytic fragments. *Immunochem*., **14**, 383-396.

Ling N.R., Bishop S. & Jefferis R. (1977) Use of antibody-coated red cells for sensitive detection of antigen and in rosette tests for cells bearing surface immunoglobulin. *J. Immunol. Methods*, **15**, 279.

Matthews J.B. & Jefferis R. (1977) A human kappa Bence-Jones protein (K_I) showing new amino acid substitutions. *Immunochem*., **14**, 793-797.

Matthews J.B. & Jefferis R. (1977) The effects of chemical modification on the antigenicity of a human kappa Bence-Jones protein. *Immunochem*., **14**, 799-807.

Jefferis R. & Matthews J.B. (1977) Structural studies of human IgD paraproteins. *Immunol. Rev*., **37**, 25-47.

Sewell H.F., Chambers L., Maxwell V., Matthews J.B. & Jefferis R. (1978) The natural antibody response to E. coli includes antibodies of the IgD class. *Clin. Exp. Immunol.,* **51**, 104-110.

Jefferis R., Matthews J.B. & Bayley B., (1978) Studies of human IgD myeloma proteins. Conformational changes induced in the Fc fragment on heating or exposure to acid pH. *Immunochem*., **15**, 19-25. 27

Jefferis R., Drew R. & Haire M. (1979) Immunoglobulin G subclasses in cerebrospinal fluid of patients with multiple sclerosis and in patients without neurological disease. In: *Protein Transmission through Living Membranes*. (ed. W.A. Hemmings) p323-327 Elsevier, North Holland

Sewell H.F., Matthews J.B., Flack V. & Jefferis R. (1979) Human immunoglobulin D in colostrum, saliva and amniotic fluid. *Clin Exp Immunol*., **36**, 183-188.

Jefferis R., Chambers L. & Jehanali A. (1979) Pronase digestion of human IgG and Fc. *Mol. Immunol.* **16**, 231-236.

Matthews J.B. & Jefferis R. (1979) Studies of intact variable regions and variable region sub-fragments isolated from a human kappa Bence-Jones protein (sub-group 1). *Mol. Immunol*., **16**, 401-408.

Shaala A.Y., Hodgson C., Ling N.R. & Jefferis R. (1980) B lymphocytes in contact dermatitis. *Br J Dermatol*., **103**, 159-165.

Matthews J.B. & Jefferis R. (1980) Isolation and characterisation of the Cγ2 and Cγ3 homology regions from the Fc fragment of immunoglobulin D. IRCS - *Medical Science*, **8**, 688-689.

Steensgard J., Jacobsen C., Lowe J., Hardie D., Ling N.R. & Jefferis R. (1980) The development of difference turbidimetric analysis for monoclonal antibodies to human IgG. *Mol Immunol*., **17**, 1315-1318.

Jefferis R. (1980) The activity and possible significance of anti-immunoglobulins. In: *Recent Advances in Clinical Immunology (*ed. R.A. Thompson), Vol. 2. pp 65-89 Churchill Livingstone, Edinburgh London and New York.

Jefferis R. Deverill I., Ling N.R. & Reeves W.G. (1980) Quantitation of human total IgG, Kappa IgG and Lambda IgG in serum using monoclonal antibodies. *J Immunol Methods,* **39**, 355-362.

Lowe J., Hardie D., Jefferis R., Ling N.R., Drysdale P., Richardson P., Raykundalia C., Catty D., Appleby P., Drew R. & MacLennan I.C.M. (1981) Properties of antibodies to human immunoglobulin kappa and lambda chains. *Immunology*, **42**, 649-659.

Jefferis R. (1981) Immunoglobulin D - antibody or receptor? Part I. Structural features. *Trends in Biochemical Sciences*, **6**, 111-113.

Calvert J.E. & Jefferis R. (1981) Immunoglobulin D - antibody or receptor? Part II. Functional Aspects. *Trends in Biochemical Sciences* **6**, 125-127.

Calvert J.E., Ling N.R. & Jefferis R. (1981) Study of proteolytic removal of Fab and Fc determinants of lymphocyte membrane IgD using a direct rosette assay. *Immunol*., **44**, 89-95.

Jefferis R., Deverill I. & Steensgaard J. (1981) Characteristics of monoclonal antibody/ antigen interactions and their application to quantitative techniques. *Biochem. Soc. Trans*., **8**, 116-117. 42

Prior M., Ling N.R., Evans S., May K. & Jefferis R. (1981) Quantitation of IgG kappa and IgG lambda in normal and pathological sera. *Protides of the Biological Fluids*, **29**, 785.

May K., Senior S., Gani H.M., Porter P., Jefferis R. & Ling N.R. (1981).Use of monoclonal antibodies in the characterisation of immunoglobulin classes and sub-classes in animal species. *Protides of the Biological Fluids*, **29**, 789-791.

Deverill I., Jefferis R., Ling N. R. & Reeves W.G. (1981) Monoclonal antibodies to human IgG: Reaction characteristics in the centrifugal fast analyser. *Clin. Chem*., **27**, 2044-2047.

Jefferis R., Lowe J., Ling N.R., Porter P. & Senior S. (1982) Immunogenic and antigenic epitopes of immunoglobulin. I. Cross-reactivity or murine monoclonal antibodies to human IgG with immunoglobulins of certain animal species. *Immunol*., **45**, 71-77.

Partridge L., Lowe J., Hardie D., Ling N.R. & Jefferis R. (1982) Immunogenic and antigenic epitopes of immunoglobulins. II. Antigenic differences between secreted and membrane IgG demonstrated using monoclonal antibodies. *J. Immunol*., **128**, 1-6.

Ling N.R., Lowe J. & Jefferis R. (1982) Monoclonal antibodies to idiotypic determinants of a human IgG paraprotein: evidence for the complexity of idiotype. *Biochem. Soc. Trans.*, **10**, 98-99.

Steensgaard J., Jacobsen C., Lowe J., Ling N.R. & Jefferis R. (1982) Theoretical and ultracentrifugal analysis of immune complex formation between monoclonal antibodies and human IgG. *Immunol*., **46**, 751-760.

Ling N.R., Lowe J. & Jefferis R. (1982) Monoclonal antibodies to the idiotypic determinants of a human IgG paraprotein: evidence for complexity of the anti-idiotype response. *Clin. Exp. Immunol*., **48**, 1-7.

Nik Jaffar M.I. & Jefferis R. (1982) Immunogenic and antigenic epitopes of immunoglobulins III. Studies with monoclonal antibodies to the C3 domain of human IgG. *Biochem. Soc. Trans.*, **10**, 225-226.

Evans S.W., Jefferis R. & Ling N.R. (1982) Immunogenics and antigenic epitopes of immunoglobulin IV. Evidence for related variable region epitopes in a panel of B J proteins. *Biochem. Soc. Trans*., **10**, 226-227.

Lowe J., Bird P., Hardie D., Jefferis R & Ling N.R. (1982) Monoclonal antibodies to determinants on human gamma chains: Properties of antibodies showing sub-class restriction or sub-class specificity. *Immunol*., **47**, 329-336.

Walker M., Lowe J., Bird P., Ling N.R. & Jefferis R. (1982) Immunogenic and antigenic epitopes of immunoglobulins VI. Application of monoclonal antibodies to human IgG sub-classes in quantitative assays. *Protides of the Biological Fluids*, **30**, 559-561.

Nik Jaffar M.I., Lowe J.A., Ling N.R. & Jefferis R. (1983) Immunogenic and antigenic epitopes of immunoglobulin V. Reactivity of a panel of monoclonal antibodies with sub-fragments of human Fc and paraproteins having deletions. *Mol. Immunol*., **20**, 679-686. 55

Walker M. & Jefferis R. (1983) Quantitation of sub-populations of human IgG employing monoclonal antibodies in the Multistat III. *"Spin Off" Instrumental Laboratories*, No**5**, 12-13.

Jefferis R. (1983) Introns, exons and immunoglobulins. *Trends in Biochemical Sciences*, **8**, 124.

Jefferis R. (1983) Monoclonal antibodies and British Industry - Too little and too late? *Immunology Today*, **4**, 128-133.

Ling N.R., Lowe J.A., Hardie D., Evans S. & Jefferis R. (1983) Detection of free light chains in human serum and urine using pairs of monoclonal antibodies reacting with C kappa epitopes not available on whole immunoglobulins. *Clin. Exp. Immunol*., **52**, 234-240.

Godal T., Rudd E., Heikkila R., Funderud S., Michaelsen T., Jefferis R., Ling N.R. & Hildrum K. (1983) Triggering of monoclonal human lymphoma B cells with antibodies to IgM heavy chains: differences of response obtained with monoclonal as compared with polyclonal antibody. *Clin Exp. Immunol*., **54**, 756-764.

Nik Jaffar M.I., Ling N.R., Lowe J. & Jefferis R. (1984) Immunogenic and antigenic epitopes of immunoglobulins VII. Spatial groups of human Fc epitopes and the influence of histidine 435 on monoclonal antibody specificity. *Mol. Immunol*., **21**, 137-143.

Ling N.R. & Jefferis R. (1984) Monoclonal antibodies. In: *Practical Immunoassay* (ed. W Butt), p199-215.

Jefferis R. & Ling, N.R. (1984) More pitfalls in the use of monoclonal antibodies. *Immunol. Today*, **5**, 127.

Jefferis R., Steinitz M. & Nik Jaffar M.I.B. (1984) VIII. A monoclonal rheumatoid factor having specificity for a discontinuous epitope determined by Histidine/Arginine interchange at residue 435 of immunoglobulin G. *Immunol. Letters*, **7**, 191-194.

Partridge L., Jefferis R., Hardie D., Ling N.R. & Richardson P. (1984) Sub-classes of IgG on the surface of human lymphocytes: A study with monoclonal antibodies. *Clin.Exp. Immunol*., **56**, 167-174.

Smeland E., Jefferis R. & Godal T. (1984) Surface immunoglobulin G sub-classes in B cell lymphomas as revealed by monoclonal antibodies. *Clin. Exp. Immunol*., **57**, 163-170.

Woof J., Nik Jaffar M.I.B., Jefferis R. & Burton D. R. (1984) The monocyte binding domain(s) on human immunoglobulin G. *Mol Immunol*., **21**, 523-527.

Zouali M., Jefferis R. & Eyquem A. (1984) Ig sub-class distribution of autoantibodies to DNA and to nuclear ribonucleoproteins in autoimmune disease. *Immunol*., **51**, 595-600.

Bird P., Lowe J., Jefferis R., de Lange G., van Loghem E. & Ling N.R. (1984) Monoclonal antibodies to an immunoglobulin allotype marker G1m (f). *Vox Sang*. **47**, 366-372. 70

Bird P. ,Lowe J., Stokes R.P., Bird A.G., Ling N.R. & Jefferis R. (1984) The separation of human IgG into subclass fractions by immunoaffinity chromatography and assessment of specific antibody activity. *J. Immunol. Methods*, **71**, 97-105.

Jefferis R., Nik Jaffar M.I.B., McAdam R. & Walker M. (1984) Immunogenecity and antigenecity of immunoglobulin IX. Antigenicity and effector functions of human IgG1 proteins having Cγ2 or Cγ3 deletions. *Biochem. Soc. Trans*., **12**, 737-738.

Farris M.A., Hardie D., de Lange G. and Jefferis R, (1984) Immunogenicity and antigenicity of immunoglobulin X. Monoclonal antibodies specific for human IgA; the IgA1 and IgA2 sub-classes and an nAm(2) isoallotypic epitope. *Vox Sang*., **48**, 116-121.

Jefferis R., Walker M., Nik Jaffar M.I.B. Czech C. & William K. (1985) Immunogenicity and antigenicity of immunoglobulin XI. Effector sites of human IgG probed with monoclonal antibodies to Fc. *Biochem. Soc. Trans*., **13**, 108.

Walker M.A., Hardie D., Lowe J., Ling N.R., de Lange G. & Jefferis R. (1985) Immunogenicity and antigenicity of immunoglobulins XII. Intact light chain and heavy chain isotype restricted V associated epitopes. *Immunol*., **55**, 205-211.

Jefferis R. & Ambler D., (1985) Immunogenicity and antigenicity of immunoglobulins XIII Balb/c monoclonal antibodies to the human G1m(f) allotype. A possible germ line specificity. *Biochem. Soc. Trans*., **13**, 911.

Sarmay G., Jefferis R., Klein E., Benzcur M. & Gergely J. (1985) Mapping the functional topography of Fc with monoclonal antibodies: localisation of epitopes interacting with binding sites of Fc receptor on human K cells. *Eur. J. Immunol*., **15**, 1037-1042.

Jefferis R., Reimer C.B., Skvaril F., de Lange G., Ling N.R., Lowe J., Walker M.R., Phillips D. J., Aloisio C.H., Wells T.W., Vaerman J.P., Magnusson C.G., Kubagawa H., Cooper M., Vartdal F., Vandvik B., Haaijman J.J., Makela O., Sarnesto A., Lando Z., Gergely J., Radl J. & Molinaro G.A. (1985) Evaluation of monoclonal antibodies having specificity for human IgG subclasses: results of IUIS/WHO collaborative study. *Immunol Letters*, **10**, 223-252.

Woof J.M., Partridge L.J., Jefferis R. & Burton D. R. (1986) Localisation of the monocyte binding region on immunoglobulin G. *Mol. Immunol*., **23**, 319-330.

J. Gergely, G. Sármay, D.R. Stanworth and R. Jefferis (1985) Mapping of epitopes on IgG Fc interacting with Fc receptors on K cells Proceedings of the 16th FEBS Congress. Part A

Lavery M., Kearns M.J. Price D. G., Emery A. N., Jefferis R. & Nienow A.W. (1985) Physical conditions during batch culture of hybridomas in laboratory scale, stirred tank reactors. *Develop. Biol. Standard*., **60**, 199-206.

Biewenga J., Faber A., de Lange G., Van Leewen F., Van der Eeden P., Jefferis R., Haaijman J. J. & Vlug A. (1986) Monoclonal antibodies against different domains of IgA: Specificities determined by immunoblotting and haemagglutination inhibition. *Mol. Immuno*l., **23**, 761-767.
82
Walker M.R., Bird P., Ulaeto D.O., Vartdal F., Goodall D.M. & Jefferis R. (1986) Immunogenic and antigenic epitopes of immunoglobulins XIV: Antigenic variants of IgG4 proteins revealed with monoclonal antibodies. *Immunol*., **57**, 25-28. 82

Martini A., Plebani A., Ravelli A., Avanzini A., Jefferis R., Zonta L., Notarangelo L. D. & Ugazio A. G. (1986) IgG sub-class serum levels in juvenile chronic arthritis. *Ann. of the Rheum. Dis*., **45**, 400-404.

Burton D.R., Gregory L. & Jefferis R. (1986) Aspects of the molecular structure of IgG subclasses. *Monographs in Allergy*, **19**, 7-35.

Jefferis R. (1986) Immunogenic and antigenic epitopes of immunoglobulins XV: Human IgG subclass epitopes recognised by murine monoclonal antibodies. *Monographs in Allergy*, **19**, 71-85.

Jefferis R. (1986) Immunogenic and antigenic epitopes of immunoglobulins XVI: Polyclonal and monoclonal antibody reagents specific for the human IgG subclasses. *Monographs in Allergy*, **20**, 26-33.

Mageed R.A., Dearlove M., Goodall D.M. & Jefferis R. (1986) Immunogenic and antigenic epitopes of immunoglobulins XVII. Monoclonal antibodies reactive with common and restricted idiotopes on the heavy chain of human rheumatoid factors. *Rheumatology International*, **6**, 179-183.

Walker M.R., Solomon A., Ling N.R., Brown B., Lowe J.A., Hardie D. & Jefferis R. (1986) Immunogenic and antigenic epitopes of immunoglobulins XVIII. Subpopulations of human lambda chains defined with a panel of monoclonal antibodies. *Immunology*, **59**, 467-472.

Mageed R.A., Walker M.R. & Jefferis R. (1986) Immunogenic and antigenic epitopes of immunoglobulins XIX. Restricted light chain subgroup expression on human rheumatoid factor paraproteins determined by monoclonal antibodies. *Immunology*, **59**, 473-478.

Partridge L.J., Woof J.M., Jefferis R. & Burton D.R. (1986) The use of anti-IgG monoclonal antibodies in mapping the monocyte receptor site on IgG. *Mol Immunology*, **23**, 1365-1372.

Sarmay G., Jefferis R. & Gergely J. (1986) CH2 and CH3 domain deleted IgG1 paraproteins inhibit differently Fc receptor mediated binding and cytolysis. *Immunol Letters*, **12**, 307-312.

Mageed R. A. & Jefferis R. (1987) The molecular specificity and idiotype of human rheumatoid factors. *Monographs in Allergy*, **22**, 24-30.

Calvert J.E., Proctor S.J. & Jefferis R. (1987) Activation of B chronic lymphocytic cells by Branhamella Catarrhalis. *Immunology*, **60**, 45-50.

Takahashi N., Ishii I., Ishihara H., Mori M., Tejima S., Jefferis R., Endo S. & Arata Y. (1987) Comparative structural study of the N-linked oligosaccharides of human normal and pathological immunoglobulin G. *Biochemistry*, **26**, 1137-1144.

Jose S.A., Griffiths H., Lunec J., Mageed R.A. & Jefferis R. (1987) Immunogenic and antigenic epitopes of immunoglobulins XX. Denaturation of human IgG3 by free radicals. *Mol Immunol*., **24**, 1145-1150. 95

Jefferis R. & Hodgson L. (1987) Immunogenic and antigenic epitopes of immunoglobulins XXI. The effects of carbamylation on the antigenicity of human IgG probed with monoclonal antibodies. *Biochem. Trans.*, **15**, 472-473.

Newkirk M.M., Mageed,R.A., Jefferis R., Chen P.P. & Capra J.D, (1987) The complete amino acid sequences of the variable regions of two human IgM rheumatoid factors, BOR and KAS of the Wa idiotypic family reveals a restricted usage of heavy and light chain variable and joining region gene segments. *J. Exp. Med.* **166**, 550-564.

Gregory L., Davis K.G., Sheth B., Boyd J., Jefferis R., Nave C., & Burton D.R. (1987) The solution conformation of the subclasses of IgG deduced from sedimentation and small angle X-ray scattering studies. *Mol Immunol*., **24**, 821-829.

Jefferis R., Walker M.R., Hodgson L. & Burton D.R. (1987) Immunogenic and antigen epitopes of immunoglobulin XXII. Human Fc epitopes and interaction sites for effector molecules. *Protides of the Biological Fluids*, **35**, 371-374.

Mageed R.A., Carson D.A. & Jefferis R. (1987) Immunogenic and antigenic epitopes of immunoglobulins XXIII. Idiotypy and molecular specificity of human rheumatoid factors: analysis of cross-reactive idiotype of rheumatoid factor paraproteins from the Wa idiotype group in relation to their IgG subclass specificity. *Scand J Immunol*., **28**, 233-240.

Walker M.R., Lee J. & Jefferis R. (1987) Immunogenic and antigenic epitopes of immunoglobulins XXIV. Detection of human immunoglobulin light chain carbohydrate using Concanavalin A. Biochem et Biophys Acta **915**, 314-320.

Jefferis, R, Jose, S, Mageed, RA, Griffiths, HR & Lunec, J. (1987) Free radical induced denaturation of human IgG. Clin. Rheum. **6**, 117-118.

Mageed, RA & Jefferis, R. (1987) Analysis of rheumatoid factor idiotype in relation to molecular specificity. Clin. Rheum. **6**, 135-136.

Smeland, EB, Beiske, K, Ohlsson, R, Holte, H, Rudd, E, Blomhoff HK, Jefferis, R & Godal, T, (1987) Activation of human B cells: Alternate options for initial triggering and effects on nonmitogenic concentrations of anti IgM antibodies on resting and activated cells. J. Immunol. **138**, 3179-3184.

Bruggemann, M, Wiliams, GT, Bindon, CI, Teale, C, Clark, MR, Frewin, M, Walker, MR, Jefferis, R, Waldman, H & Neuberger, MS. (1987) Comparison of the effector function of human immunoglobulins using a matched set of chimeric antibodies. J. Exp. Med. **166**, 1351-1361.

Mageed, RA, Tunn, EJ, Shokri, F, Bacon, PA & Jefferis, R. (1987) Structural analysis of rheumatoid factor in an early synovitis clinic. Br. J. Rheumatol. **25**, 959.

Mageed, RA & Jefferis, R. (1987) Analysis of variable region gene usage in rheumatoid factors: possible relationship to genetic predisposition to rheumatoid arthritis. Workshop Proceedings: Disease Markers **6**, 57. 107

Walker, MR, Solomon, A, Deutsch,H & Jefferis,R. (1988) Immunogenic and antigenic epitopes of immunoglobulins XXV. Monoclonal antibodies that differentiate the Mcg+/Mcg- and Oz+/Oz- C region isotypes of human lambda chains. J Immunol. **140**, 1600-1604.

Jefferis, R & Walker, MR. (1988) The biological significance of specific antibody IgG subclass profiles. Monogr. in Allergy **23**, 73-77.

Griffiths, HR, Lunec, J, Jefferis, R, Blake, DR, Wilson, RL. (1988) A study of ROS induced denaturation of IgG3 using monoclonal antibodies: implications for inflamatory joint disease. Basic Life Sci., **49**, 361-364.

Crowley, JJ, Goldfien, RD, Shrohenloher, RE, Mageed, RA, Jefferis, R, Koopman, WJ, Carson, DA & Fong, S. (1988) Incidence of three cross-reactive idiotypes defines two mutually exclusive kappa light chain variable region groups for human rheumatoid factor paraprotein. J. Immunol. **140**, 3411-3418.

Burton, DR, Jefferis, R, Partridge, LJ & Woof, JM. (1988) Molecular recognition of antibody (IgG) by cellular Fc receptor (FcRI). Mol. Immunol. **25**, 1175-81.

Jefferis, R, Cranmer, S, Arata, Y & Takahashi, N. (1988) Glycosylation heterogeneity in human IgG subclass proteins. Biochem. Soc. Trans. **16**, 339-341.

Walker, MR, Kumpel, BM, Thompson.,K, Woof, JM, Burton,DR, & Jefferis, R. (1988) Immunogenic and antigenic epitopes of immunoglobulins. Binding of human anti-D antibodies to FcRI on the monocyte-like U937 cell line. Vox Sang. **55**, 222-228.

Thompson, KM, Randen, I, Natvig, JB, Waalen, K, Forre, O, Jefferis, R & Carson, D. (1988) The production and characterisation of human monoclonal rheumatoid factor (RF) secreting hybridomas derived from rheumatoid synovial tissue. Scand. J. Immunol. **28**, 501.

Harmer, I & Jefferis, R. (1988) Structural and idiotypic relationships between monoclonal antibodies specific for IgG1 (Fc) subclass epitopes. Biochem. Soc. Trans. **16**, 747-748.

Jefferis, R. (1988) Paraproteins in lymphoproliferative disease. In: *B lymphocytes in human disease* (ed. G Bird and Jane E Calvert) pp 380-391. Oxford Medical Publications, Oxford, New York, Tokyo.

Walker, MR, Woof, JM, Burton, DR, Bruggemann, M & Jefferis, R. (1988) Immunoglobulin subclass specificity of Fc receptor II determined using human chimeric anti-5-iodo--4-hydroxy-3-nitrophenacetyl monoclonal antibodies. Biochem. Soc. Trans. **16**, 725-726.

Silverman, GJ, Goldfien, RD, Chen, P, Mageed, RA, Jefferis, R, Goni, F, Frangione, B, Fong, S & Carson DA. (1988) Idiotypic and subgroup analysis of human monoclonal rheumatoid factors: Implications for structural and genetic basis of autoantibodies in humans. J. Clin. Invest. **82**, 469-475.

Jefferis, R & Mageed, RA. (1989) The epitope specificity and idiotypy of monoclonal rheumatoid factors. Scand. J. Rheum. Suppl. **75**, 89-92.

Crowley, JJ, Mageed, RA, Silverman, GS, Jefferis, R, Chen, PP & Carson, DA. (1988) Structural and genetic analysis of a new cross-reactive idiotype (CRI) associated with human rheumatoid factor heavy chain. Arthr.and Rheum. **31,** S47.

Mageed, RA, Carson, DA & Jefferis, R. (1989) Analysis of rheumatoid factor autoantibodies in patients with essential mixed cryoglobulin and rheumatoid arthritis. Scand. J. Rheum. Suppl. **75**, 172-178.

Rozsnyay, Z, Sarmay, G, Walker, M, Maslanka, K, Valasek, Z, Jefferis, R & Gergely J, (1989) ADCC of red blood cells sensitized with human monoclonal anti-D (Rh) antibodies: distinctive roles for FcRI and FcRII receptor. Immunol. **66**, 491-498.

Walker, MR, Woof, JM, Bruggemann, M, Burton, DR & Jefferis, R, (1989) Interaction of human chimeric antibodies with human FcRI and FcRII receptors: Requirement for antibody-mediated host cell-target cell interactions. Mol. Immunol. **26**, 403-411.

Walker, MR, Lund, J, Thompson, KM & Jefferis, R. (1989) Aglycosylation of human IgG1 and IgG3 monoclonal antibodies eliminates recognition by human cells expressing FcRI and/or FcRII receptors. Biochem. J. **259**, 347-353.

Jefferis, R & Mageed, RA. (1989) The specificity and reactivity of Rheumatoid Factors with human IgG. Monographs in Allergy **26**, 45-60.

Abderrazik, M, Moynier, M, Couret, M, Combe, B, Mageed, RAK, Jefferis, R, Sany, J & Brochier, J. (1989) Frequency of rheumatoid factor producing precursor B cells in rheumatoid arthritis. Idiotypic studies (ed. Clot, J, Sany, J and Brochier) . pp.143-147. Septieme Symposium International D'Immuno-Rheumatologie.

Jefferis, R. (1989) Structure/function relationships within the human IgG subclasses. Protides of the Biological Fluids **36**, 21-35.

Shokri, F, Mageed, RA, Kitas, GD, Kitsakis, P, Moutsopoulos, HM & Jefferis, R. (1989) Monoclonal anti-cross reactive idiotype antibodies as possible probes for lymphoproliferation - Primary Sjogrens Syndrome. Br. J. Rheum. **28**, 458-459.

Crowley, JJ, Mageed, RA, Silverman, GJ, Chen, PP, Kozin, F, Erger, S, Jefferis, R & Carson DA. (1990) The incidence of a new human cross-reactive idiotype linked to subgroup VHIII heavy chains. Mol. Immunol. **27**, 87-94.

Shokri, F, Mageed, RA, Tunn, E, Bacon, P & Jefferis, R. (1990) Qualitative and quantitative expression of VHI-associated cross-reactive idiotopes within IgMRF of patients with early synovitis. Ann. Rheum. Diseases. **49**, 150-154.

Mageed, RA, Goodall, DM & Jefferis, R. (1990) A highly conserved conformational idiotope on human IgM rheumatoid factor paraproteins of the Wa cross-reactive idiotype family defined by a monoclonal antibody. Rheumatol. Int. **10**, 57-63.

Lydyard, PM, Quartey-Papafio, R, Broker, BM, Hay, F, Jefferis, R & Mageed, RA. (1990) The antibody repertoire of early B cells. III. Comparisons with chronic lymphocytic leukaemic cells in the expression of cross-reactive idiotopes characteristic of certain rheumatoid factors and identifying VK and VH gene family products. Scand. J. Immunol. **32**, 709-716.

Brown, CHS, Plater-Zyberk, C, Mageed, RA, Jefferis, R & Maini, RN. (1990) Analysis of immunoglobulins secreted by hybridomas derived from rheumatoid synovia, evidence of an oligoclonal B cell response. Clin. Exp. Immunol. **80**, 366-372.

Nelson, PN, Fletcher, SM, de Lange, GG, Van Leeuwen, AM, Goodall, DM & Jefferis, R. (1990) Evaluation of McAB with putative specificity for human IgG allotype Vox Sang. **59**, 190-197.

Jefferis, R, (1990) Molecular structure of human IgG subclasses. In: The Human IgG subclasses, *Molecular analysis of structure, function and regulation*. (ed. F Shakib) Pergamon Press, Oxford. pp15-30

Jefferis, R. (1990) Structure/function relationships for human IgG subclasses. *In: Molecular Aspects of Immunoglobulin Subclasses*. (ed. F Shakib) Pergamon Press, Oxford. pp93-108.

Lund, J, Tanaka, T, Takahashi, N, Sarmay, G, Arata, Y, & Jefferis, R. (1990) A protein structural change in aglycosylated IgG3 correlates with loss of huFcγRI and huFcγRIII binding and/or activation. Mol. Immunol. **27**, 1145-1154.

Jefferis, R. Lund, J & Pound, JD. (1990) Molecular definition of interaction sites on human IgG for Fc receptors. Molec. Immunol. **27**, 1237-1240.

Jefferis, R, Lund, J, Mizitani, H, Nakagawa, H, Kawazoe, Y, Arata, Y & Takahashi, N. (1990) A comparative study of the N-linked Oligosaccharide structures of human IgG subclass proteins. Biochem. J. **268**, 529-537.

Thompson, K, Randen, I, Natvig, JB, Mageed, RA, Jefferis, R, Carson, DA, Tighe, H & Forre, O. (1990) Human monoclonal rheumatoid factors derived from the polyclonal repertoire of rheumatoid synovial tissue: incidence of cross reactive idiotypes and expression of V_H and V_K subgroups. Eur. J. Immunol. **20**, 863-868.

Jefferis, R. & Kumararatne, DS. (1990) Selective IgG subclass Deficiency: Quantification and Clinical Relevance. Clin. Exp. Immunol. **81**, 357-368.

Kumararatne, DS, Hazelwood, MA, Bird, P, Forte, M, Joyce, H, Misbah, S, Goodall, DM, Jefferis, R & Chapel, H. (1990) The physiology of human TI-2 antibody responses: Lessons from patients with selective failure of carbohydrate antibody responses. In: *J. Gooi & H. Chapel Clinical immunology - A Practical Approach*. IRL Press, pp1-22.

Shokri, F, Mageed, RA, Maziak, BR, Jefferis, R. (1990). Expression of V_HIII-associated cross-reactive idiotype (CRI) on human B-lymphocytes: association with Staphylococcal protein A (SPA) binding and Staphylococcus aureus Cowan I (SAC) stimulation. J. Immnol. **146**, 936-940.

Deacon, EM, Matthews, JM, Potts, AJC., Hamburger, J, Mageed, RA & Jefferis, R. (1990) Expression of rheumatoid factor associated cross-reactive idiotyptes by glandular B cells in Sjögren's syndrome. Clin. Exp. Immunol. **83**, 280-285. 145

Natvig, JB, Randen, I, Thompson, K, Forre, O, Mageed, RA, Jefferis, R, Carson, DA, Tighe, H, Pascual, V, Victor, KD & Capra, JD (1990) Probing of the Rheumatoid Factor V Gene Repertoire in Rheumatoid Arthritis by Hybridoma Clones. Clin.Exp.Rheum., **8**, (Suppl. 5), 75-80.

Lund J. Winter G. Jones PT. Pound JD. Tanaka T. Walker MR. Artymiuk PJ. Arata Y. Burton DR. Jefferis R. (1991) Human FcγRI and FcγRII interact with distinct but overlapping sites on human IgG. J.Immunol. 147 2657 - 2662.

Shokri,F., Mageed, R.A.K., Kitas, G.D., Katsikis, P., Moutsopoulos, H.M. and Jefferis, R. (1991) Quantitation of Cross-Reactive Idiotype Positive Rheumatoid Factor Produced in Autoimmune Diseases. An Indicator of Clonality and B-Cell proliferative Mechanisms. Clin. Exp. Immunol. **85**, 20-27.

Goodall, M, Hazelwood, MA, Kumararatne, D.S & Jefferis, R. (1991) The quantitation of anti-polysaccharide antibodies in human sera. In: *Progress in Immune deficiency III* (ed. Chapel H, Levinsky R J and Webster ADB). pp130-131.Royal Society of Medicine Services Ltd.

Kumararatne, DS, Joyce, HJ, Hazelwood, MA, Goodall, M, Jefferis, R, Misbath, S, & Chapel H. (1991) Selective deficiency of anti-polysaccharide antibodies as a risk factor for invasive disease caused by capsulated bacterial pathogens. Progress in Immune Deficiency III. pp132 -133. Ed: Capel H, Levinsky R J and Webster A D B. Royal Society of medicine Services Ltd.

Jefferis, R & Deverill, I. (1991) The antigen:antibody reaction. In: *Principles and Practice of Immunoassay* (ed. C P Price and D J Newman) Chapter 1 pp 1-18. Macmillan Publishers Ltd. London.

Nelson, PN, Fletcher, SM, MacDonald, D, Goodall, DM, Jefferis, R. (1991) Assay restriction profiles ot three monoclonal antibodies recognizing the G3m(u) allotype. J. Immunol. Meth. **138**, 57-64.

Jefferis, R. (1991) Structure - function relationships in human immunoglobulins. The Netherlands Journal of Medicine, **39**, 188-198.

Mageed, RA, Mackenzie, LB, Stephenson, FK, Yulsel, B, Shokri, F, Maziak, BR, Jefferis, R & Lydyard, PM. (1991) Selective expression of immunoglobulin genes in human $CD5^+$ B lymphocytes from cord blood. J. Exp. Med. **174**, 109-113.

Shokri, F, Mageed, RA, Maziak, BR, Talal, N, Williams, B & Jefferis, R. (1991) Restricted expression of cross-reactive idiotype as a marker for lymphoproliferation in primary Sjögren's Syndrome. Clin. Exp. Rheum. **9**, 335.

Abderrazik, M., Moynier, M., Jefferis, R., Mageed, R.A.K., Combe, B., Sany, J. and Brochier, J. (1992) Analysis of monoclonal rheumatoid factors obtained from the B cell repertoire in rheumatoid arthritis. Scand.J.Immunol. **35**, 149 - 157.

Jefferis, R & Pound, J. (1991) Immunoglobulins In: Inflammation. In: *Basic principles and clinical correlates. 2nd edition* (ed. Gallin JI, Goldstein IM. Snyderman R) Raven Press.

Jefferis, R. (1992) Allotypes. In: *Encyclopaedia of Immunology*. (eds, IM Roitt and PJ Delves) Saunders, Florida. **1**, 55-57.

Lund, J, Pound, JD, Jones, PT, Duncan, AR, Bentley, T, Goodall, M, Levine, B, Jefferis, R & Winter, G. (1992) Multiple binding sites on the C_H2 domain for mouse $Fc_\gamma RII$. Mol. Immunol. **29**, 53-60.

Jefferis, R. (1992) Antibody Subclasses. *In: Structure of Antigens* (ed. M.H.V. Van Regenmortel) pp321-338. CRC Press. Boca Raton.

Mackenzie, LM, Mageed, RA, Youinou, PY, Yuksel, B, Jefferis, R & Lydyard, PM (1992) Repertoire of $CD5^+$ and $CD5^-$ cord blood B cells: specificity and expression of VHI and VHIII associated idiotopes. Clin. Exp. Immunol. **88**, 107-111.

Jefferis R., Reimer C.B., Skvaril F., de Lange G., Goodall D.M., Bentley D.J., Phillips D. J., Vlug A., Harada S., Radl J., Classen E., Boersma J.A. & Coolen J. (1992) Evaluation of monoclonal antibodies having specificity for human IgG subclasses: results of the 2nd IUIS/WHO collaborative study. *Immunol Letters*, **31**, 143-168.

Sarmay, G, Lund, J, Gergely, J. & Jefferis, R. (1992) Mapping and comparison of the interaction sites on the Fc region of IgG responsible for triggering antibody dependent cellular cytotoxicity (ADCC) through different types of Fcγ receptor. Mol. Immunol. **29**, 633-639.

Jefferis, R, Takahashi, N, Lund, J, Tyler, R & Hindley, S. (1992). Does an antibody molecule act as a template directing (determining) its glycosylation? Biochem. Soc. Trans. **21**, 337S.

Lund, J, Takahashi, N, Goodall, M, Nkagawa, H, Bentley, T, Tyler, R & Jefferis, R. (1992) Glycosylation profiles of chimeric human/mouse antibodies *in vivo* and *in vitro*. Annals Rheum. Dis. **51**, 1272-1273.

Mierau, R, Gause, A, Kuppers, R, Michels, M, Mageed, RA, Jefferis, R & Genth, E. (1992) A human monoclonal IgA rheumatoid factor uses a VKIV light chain and a VHI heavy chain. Rheum. Int. **12**, 23-31.

Downham, M, Busby, S, Jefferis, R & Lydiatt, A. (1992) Immunoaffinity chromatography in biorecovery: An application of recombinant DNA technology to generic adsorption processes. J.Chromatography (Biomedical Applications) **584**, 59-68.

Jefferis, R (1992) Standardization: Who wants it? Clin. Exp. Immunol. **89**, 500-501.

Goodall, M., Bentley TL., Jefferis R. (1992) Purification of monoclonal antibody from culture supernatant produced in hollow fibre bioreactors. Animal Cell Technology: Developments, Processes and Products. 621–623

Pound, J, Lund, J & Jefferis, R. (1992) Immune complex recognition by leucocyte Fc receptors. IMLS Triennial Congress.

Lydyard PM. MacKenzie LE. Youinou PY. Deane M. Jefferis R. Mageed RA. (1992) Specificity and idiotope expression of IgM produced by CD5+ and CD5- cord blood B-cell clones. Annals of the New York Academy of ciences. **651**, 527-39

171

Lund, J, Takahashi, N, Hindley, S, Tyler, R, Goodall, M. & Jefferis, R. (1993) Glycosylation of human IgG subclass and mouse IgG2b heavy chains secreted by mouse J558L transfectoma cell lines as chimeric antibodies. Human Antibodies and Hybridomas. **4**, 20-25.

Pound, JD, Lund, J, Jones, PT, Winter, G & Jefferis, R (1993) Fc$_\gamma$RI mediated triggering of the human mononuclear phagocyte respiratory burst. Molec. Immunol. **30**, 233-241.

Pound, JD, Lund, J & Jefferis, R. (1993) Aglycosylated chimeric human IgG3 can trigger the human phagocyte respiratory burst. Molec. Immunol. **30**, 469-478.

Lund, J, Takahashi, N, Nakagawa, H, Bentley, T, Hindley, S, Tyler, R, Goodall, M & Jefferis, R. (1993) Control of IgG/Fc glycosylation: a comparison of oligosaccharides from chimeric human/mouse and mouse subclass immunoglobulin Gs. Molec. Immunol. **30**, 741-748.

Jefferis, R. (1993) What is an idiotype? Immunology Today. **14**, 119-120.

Jefferis, R. (1993) Idiotypes and idiotypic networks: a time to re-define concepts. Clin. Exp. Immunol. **91**, 193-195

Newkirk, M, Rauch, J, Mageed, RA, Jefferis, R, Posnet, D & Silverman, G. (1993) Restricted immunoglobulin variable gene usage by hybridoma rheumatoid factors from patients with systemic lupus erythematosus and rheumatoid arthritis. Molec. Immunol. **30**, 255-263.

Jefferis, R. (1993) Glycosylation of antibody molecules: functional significance. Glycoconjugate J. **10**, 357-361.

Shokri, F, Mageed, R, Maziak, R, Talal, N, Amos, N, Williams, B & Jefferis, R. (1993). Lymphoproliferation in primary Sjögren's Syndrome: Evidence for selective expansion of a B-cell subset characterized by the expression of cross-reactive idiotypes. Arth. & Rheum. **36**, 1128-1136.

Shokri, F, Mageed, R, Richardson, P & Jefferis, R. (1993). Modulation and high frequency expression of autoantibody associated cross-reactive idiotypes linked to the VH1 subgroup in CD5-expressing B lymphocytes from patients with chronic lymphocytic leukemia (B-CLL). Scand. J. Immunol. **37**, 673-679

Jefferis, R & Lund, J. (1993) Molecular characterisation of IgG antibody effector sites. In: *Protein engineering of antibody molecules for prophylactic and therapeutic applications in man* (ed. MR Clark.) pp 115-126, Academic Titles, Nottingham.

Wheeler, K, Pound, J, Gordon, J & Jefferis, R. (1993) Engagement of CD40 lowers the threshold for activation of resting B cells via antigen receptor. Eur. J. Immunol. **23**, 1165-1168.

Shokri, F, Mageed, R & Jefferis, R. (1993) A quantitative ELISA for measurement of rheumatoid factor associated cross-reactive idiotypes in serum from patients with rheumatic diseases. Brit. J. Rheum. **32**, 862-869.

Hindley, SA, Gao, Y, Nash, PH, Sautes, C, Lund, J, Goodall, M & Jefferis, R. (1993) The interaction of IgG with FcRII: Involvement of the lower hinge binding site as probed with NMR. Bichem. Soc. Trans. **21**, 337s 185

Shokri, F., Mageed, R.A., Richardson, P., & Jefferis, R. (1993) Immunophenotypic and idiotypic characterisation of the leukemic B-cells from patients with prolymphocytic leukemia - evidence for a selective expression of immunoglobulin variable region gene products. Leukemia Res. **17**, 669-676.

Wheeler, K, Pound, J, Gordon, J & Jefferis, R. (1993) Stimulation of resting B lymphocytes with picomolar concentrations of anti-immunoglobulin on engagement of CD40. J. Immunol. 150, 98.

Lebeque, S, Galibert, L, Vardoorer, J, Jefferis, R, Martinez-Valdez, B, Rousset, F & Banchereau, J. (1993) Molecular confirmation of isotype switching in human lymphocytes-B cultures with IL-4 in the CD40 system. J. Cell. Biochem. S17B, 181.

Gilhespy-Muskett, AM, Partridge, J, Jefferis, R & Homans, SW. (1994) A novel ^{13}C isotopic labelling strategy for probing the structure and dynamics of glycan chains *in situ* on glycoproteins. J Glycobiology 4, 485-490.

Pound, J, Lund, J & Jefferis, R. (1994) Structural basis of IgG effector triggering: Functional sites and the role of carbohydrate. In: Proceedings of the International Conference "Modern Enzymology: Problems and Trends" pp643-668. Nova Science Publishers Inc. New York.

Jefferis, R, Pound, J, Lund, J & Goodall, MG. (1994) Effector mechanisms activated by human IgG subclass antibodies: Clinical and molecular aspects. Ann Biol Clin (Paris). **52**, 57-65.

Jefferis, R & Goodall, M. (1994) Selective human IgG subclass antibody deficiencies. Polish J. Immunol. 19, 111-119.

Nelson, PN, Goodall, M & Jefferis, R. (1994) Characterization of putative monoclonal anti-G3m(u) amd anti-G3m(g) reagents and their antigenic determinants. Immunol. Investigations, **23**, 39-45.

Jefferis, R. (1994) Idiotypes as markers for immunoglobulin genes contributing to the production of rheumatoid factor: Theoretical and practical implications. Clin.Exp.Rheum. 12, 571-574.

Lund J, Takahashi N, Goodall M, Pound J & Jefferis R. (1994) Oligosaccharide-protein interactions in IgG antibody molecules: structural and functional consequences. Biochem.Soc.Trans. 22, 102S.

Berberian, L, Shukla J, Jefferis R & Braun J. (1994) Selective activation and deletion of V_H3 B cells in HIV infection. J.AIDS. **7**, 641- 646.

Jefferis, R., (1994) Antibodies. In: Encyclopedia of Molecular Biology. Ed. J. Kendrew. pp54-57. Blackwell Science Ltd., Oxford.

Jefferis, R. (1995) Rheumatoid factors, B cells and immunoglobulin genes. Brit.Med.Bull. **51**, 332-345. 198

R Jefferis. (1995) Nomenclature of V-region serological markers. Immunology Today. **16**, 207-208.

Galibert, L., vanDooren, J., Durand, I., Rousset, F., Jefferis, R., Banchereau, J., Lebeque, S. (1995) Anti-CD40 plus interleukin-4-activated human naive B cell lines express unmutated immunoglobulin genes with intraclonal heavy chain isotype variability. Eur.J.Immunol. **25**, 733-737.

Rajnavolgyi, E., Fazekas, G., Lund, J., Dearon, M., Teillaud, J-L., Jefferis, R., Fridman, W., Gergely, G. (1995) Activation of effector functions by immune complexes of mouse IgG2a with isotype specific autoantibodies. Immunology, **84**, 645-652.

Jefferis, R., Lund, J., Goodall, M. (1995) Recognition of human IgG by Fcγ receptors: the role of glycosylation. Immunol.Letters **44**, 111-117.

Jefferis, R., Goodall, M., Tishchenko, V., Nash, P., Lund, J. (1995) Glycosylation of antibody molecules: A small step for structure, a leap for function. Adv.Exp.Med.&Biol. Glycoimmunology, **376**, 153.

Lund, J., Takahashi, N., Pound, J., Goodall, M., Nakagawa, H. and Jefferis, R. (1995) Oligosaccharide-protein interactions in IgG can modulate recognition by Fcγ receptors. FASEB J. **9**, 115-119.

Mestecky, J., Hamilton, RG., Magnusson, C., Jefferis, R., Vaerman, JP., Goodall, MG., de Lange, GG., Moro, I., Aucouturier, P., Radl, J., Cambiaso, C., Silvain, C., Preud'homme, JL., Kusama, K., Carlone, G., Biewenga, J., Kobayashi, K., Skvaril, F., Reimer, CB,. (1996) Evaluation of monoclonal antibodies with specificity for human IgA, IgA subclasses and secretory component: Results of an IUIS/WHO collaborative study. J.Immumnol.Methods. **193**, 103-148.

Jefferis, R. (1996) Immunoglobulins: Allotypes. In: *Encyclopaedia of Immunology*. (eds, IM Roitt and PJ Delves) Saunders, Florida.

Sohi MK., Corper AL., Wan T., Steinitz M., Jefferis R., Beale D., He M., Feinstein A., Sutton BJ. and Taussig MJ. (1996) Crystallisation of a complex between the Fab fragment of a human IgM rheumatoid factor (RF-AN) and the Fc fragment of human IgG4. Immunology, **88**, 636-641.

Lund J., Takahashi N., Pound J., Goodall M. and Jefferis R. (1996) Multiple interactions of IgG with its core oligosaccharide can modulate recognition by complement and human FcγRI and influence the synthesis of its oligosaccharide chains. J.Immunol. **157**, 4963-4969.

Jefferis R., Takahashi N., Lund J., Goodall M., Farooq M., Pound J. and Church S. (1996) Glycosylation of antibody molecules: in vivo and in vitro. Glycoconjugate J. 13:888.

Jefferis R., Lund J., Goodall M. (1996) Modulation of FcγR and human complement activation by IgG3-core-oligosaccharide-interactions. Immunol. Lett. **54**, 101-104.

Nelson PN., Westwood OMR., Jefferis R., Goodall M. and Hay FC. (1996) Monoclonal antibodies with dual specificity corroborated by epitope mapping. Immunology 86 (Supp 1):151.

Corper AL., Sohi MK., Bonagura VR., Steinitz M., Jefferis R., Feinstein A., Beale D., Taussig MJ. And Sutton BJ. (1997) Structure of a human IgM rheumatoid factor Fab in complex with its autoantigen IgG Fc. Nature; Structural Biology. 4:374-381.

Arrol H. and Jefferis R. (1997) Antibody and protein glycosylation in health and disease. In: Immunoregulation in health and disease. Ed: Lukic ML., Colic M., Stojkovic MM. And Cuperlovic K., pp115-138. Academic Press, London..

Jefferis R. and Lund J. (1997) Glycosylation of antibody molecules: structural and functional significance. In: Chemical Immunology, **65**. Ed: JD Capra. Karger, Basel.

Abadeh, S., Church, S., Dong, S., Lund, J., Goodall, M. and Jefferis R. (1997) Remodelling the oligosaccharide of human IgG antibodies: effects on biological activities. Biochem.Soc.Trans. 25:S661

Farooq, M., Takahashi N., Arrol, H., Drayson, M. and Jefferis, R. (1997) Glycosylation of antibody molecules in multiple myeloma. Glycoconjugate J. 14:489-492.

Nelson, PN., Westwood, OM., Jefferis, R., Goodall, M. and Hay, FC. (1997) Characterisation of anti-IgG monoclonal antibody A57H by epitope mapping. Biochem.Soc.Trans. 25(2):373S.

Routier, FH., Hounsell, EF., Rudd, PM., Takahashi, N., Bond A., Axford, J., Hay, F. and Jefferis R. (1998) Quantitation of human IgG glycoforms isolated from rheumatoid sera: A critical evaluation of chromatographic methods. J.Immunol.Methods. 213:113-130.

Farooq M., Takahasi N., Drayson M., Lund J. and Jefferis R. A longitudinal study of glycosylation of a human IgG3 paraprotein in a patient with multiple myeloma. In: *Glycoimmunology 2* (Axford JS. ed). pp95-104. Plenum Press, New York 1998

Jefferis R., Lund J., Church S., Mimura Y. and Goodall M. (1998) Single amino acid replacements resulting in high galactosylation and sialylation of antibody molecules. ABSTR PAP AM CHEM S 216: 242-BIOT Part 1

Sutton BJ., Corper AL., Sohi MK., Jefferis R., Beale D. and Taussig MJ. The structure of a human rheumatoid factor bound to IgG Fc. In: *Glycoimmunology 2* (Axford JS. Ed) pp 41-50, Plenum Press, New York, 1998.

Jefferis R., Lund J. and Pound JD. 1998. IgG-Fc mediated effector functions: molecular definition of interaction sites for effector ligands and the role of glycosylation. Immunol.Rev. 163:59-76.

Abadi J, Freidman J, Mageed RA, Jefferis R, Rodriguez-Barradas M, Pirofski L-a. (1998) Human antibodies elicited by a 23-valent pneumococcal polysaccharide vaccine express idiotypic determinants indicative of V_H3 segment usage. J.Infect.Dis. 178:707-716.

Melero J, Aguilera I, Mageed RA, Jefferis R, Tarrago D, Nunez-Roldan A, Sanchez B. (1998) The frequent expansion of a subpopulation of B cells that express RF-associated cross-reactive idiotypes: Evidence from analysis of a panel of autoreactive monoclonal antibodies. Scand.J.Immunol. 48:152-158.

Jefferis, R. (1998) Allotypes, Immunoglobulin. In: Roitt IM, Delves PJ, eds. Encyclopaedia of Immunology. 2nd edition. Florida: Saunders, 1998:74-77

Shah P., Reece-Ford M., Dong S., Goodall M., Pidaparthi S., Jefferis R., and Jenkins N. (1998) Physiological influences on recombinant IgG glycosylation. Biochem.Soc.Trans. 26:S114.

Tishchenko V., Lund J., Goodall M. and Jefferis R. (1998). Co-operative structures within glycosylated and aglycosylated mouse IgG2b. In: Biocalorimetry: Application of calorimetry in the Biological Sciences. Ed: JE. Lanchbury and BZ. Chowdhry. pp267-275. John Wiley & Sons. London.

Potter KN, Yucheng L, Mageed RA, Jefferis R, Capra JD (1998). Anti-idiotypic antibody D12 and superantigen SpA both interact with human VH3 encoded antibodies on the external face of the heavy chain involving FR1, CDR2 and FR3. Molec.Immunol. 35:1170-1187.

Potter KN, Yucheng L, Mageed RA, Jefferis R, Capra JD (1999). Molecular characterisation of the VH1 variable region specific determinants recognised by anti-idiotypic monoclonal antibodies G6 and G8. Scandinavian J. Immunol. 50:14-20.

Ayatollahi M, Malekhossini Z, Mortazavi H, Jefferis R, Ghaderi A. (1999) Development of an ELISA based method for the detection of pemphigus autoantibodies. Iran Biomedical J, 3: 99-101.

Tse WY, Abadeh S, McTiernan A, Jefferis R, Savage COS., Adu D (1999). No association between neutrophil FcγRIIa allelic polymorphism and ANCA-positive systemic vasculitis. Clin.Exp.Immunol. 117:198-205.

Ghirlando R., Lund J., Goodall M., Jefferis R. (1999) Glycosylation of human IgG-Fc: influences on structure revealed by differential scanning micro-calorimetry. Immunol.Lett. 68:47-52

Jefferis R., Farooq M., Abadeh S., Church S., Mimura Y., Goodall M., Lund J., Takahashi N. (1999) Glycosylation of human IgG antibodies: Influence of galactosylation on structure and function. FASEB 13: (4) A331-A331 Part 1 Suppl. S.

Tse WY., Abadeh S., McTiernan A., Jefferis R., Savage COS., Adu D. (2000) Neutrophil FcγRIIIb allelic polymorphism in anti-neutrophil cytoplasmic antibody positive systemic vasculitis. Clin.Exp.Immunol. 119:574-577.

MacLennan I, Jefferis R, Turner M (2000) Noel Ling - 1922-2000 – Obituary J.Immunol.Methods. 238:1-2.

Mimura Y., Ghirlando R., Lund J., Goodall M. and Jefferis R. (2000) The contribution of glycosylation to the stability and function of antibody molecules. Biochem.Soc.Trans.28:pt.3, 75

Izad M., Jefferis R. and Shokri F. (2000) IgG Subclass analysis of anti-HBs antibodies produced in response to vaccination with recombinant HbsAg and infection with the wild hepatitis B virus. Iranian J Med Sci. 25(1&2):42-49

Lund J., Takahashi N., Popplewell A., Goodall M., Pound J., Tyler R., King D and Jefferis R. (2000) Expression and characterisation of truncated glycoforms of humanised L243 IgG1: architectural features can influence synthesis of its oligosaccharide chains and affect superoxide production triggered through human FcγRI. Eur.J.Biochemistry. 267:7246-7257.

Mimura Y., Church S., Ghirlando R,. Dong S., Goodall M., Lund J. and Jefferis R. (2000) The influence of glycosylation on the thermal stability and effector function expression of human IgG1-Fc: properties of a series of truncated glycoforms. Molecular Immunology. 37:697-706.

Mimura Y., Lund J., Church S., Goodall M., Dong S. and Jefferis R. (2001) Butyrate increases production of human chimeric IgG in CHO-K1 cells whilst maintaining function and glycoform profile. J.Immunol.Meth. 247:205-216.

Lund, J., Goodall, M., Takahashi, N., Tyler, R. and Jefferis, R. (2001) Engineering IgG antibodies to control their glycosylation. Proceedings of 14th Meeting of the European Federation of Immunological Societies.

Mimura, Y., Ghirlando, R., Sondermann, P., Lund, J. and Jefferis, R. (2001) The molecular specificity of IgG-Fc interactions with Fcγ receptors. Progress in basic and clinical immunology. Ed. A. Mackiewicz. Adv Exp Med Biol 2001;495:49-53. Kluwer Academic/Plenum, New York

Jefferis, R. (2001) Glycosylation of human IgG antibodies: relevance to therapeutic applications. Biopharm. 14:19–26.

Kumararatne, D.S., Joyce H.J., Jefferis R. (2001) The utility of IgG subclass measurements for investigating infection prone patients CPD Bulletin Immunology & Allergy 2:44-47

Jassal, R., Jenkins, N., Charlwood, J., Camilleri, P., Jefferis, R. and Lund, J. (2001) Sialylation of human IgG-Fc carbohydrate by transfected rat α(2 – 6) sialyltransferase. Biochem.Biophys.Res.Comm. 286:243-249.

Mimura, Y., Sondermann, P., Ghirlando, R., Lund, J., Young, S.P., Goodall, M. and Jefferis, R. (2001). The role of oligosaccharide residues of IgG1-Fc in FcγIIb binding. J.Biol.Chem. 276:45539-45547.

Holland, M., Takada, K., Okomoto, T., Takahashi, N., Kato, K., Adu, D., Ben-Smith, A., Harper, L., Savage, C.O.S. and Jefferis, R. (2002) Hypogalactosylation of serum IgG in patients with ANCA-associated systemic vasculitis. Clin.exp.Immunol. 129:183-190.

Watt, G., Levens, M., Lund, J., Goodall, M., Jefferis, R., and Boons, GJ. (2002) Synthesis of homogeneous neoglycoforms of IgG-Fc molecules and their functional properties. Human Antibodies 11:29-30.

Jefferis, R. and Lund, J. (2002) Interaction sites on human IgG-Fc for FcγR: Current models. Immunol. Lett. 82:57-65.

Jefferis, R. (2002) Glycosylation of recombinant IgG antibodies and its relevance for therapeutic applications. (a soupcon of sugar helps the medicine work) Cell Engineering Volume 3. Ed: M Al-Rubeai. Pp 93 – 108. Kluwer, Dordrecht..

Krapp, S., Mimura, Y., Jefferis, R., Huber, R. and Sondermann, P. (2003) Structural analysis of human IgG glycoforms reveals a correlation between oligosaccharide content, structural integrity and Fcγ-receptor affinity. J.Mol.Biol. 325:979-989.

Nelson, PN., Westwood, OMR., Soltys, A., Jefferis, R., Goodall, DM., Baumforth, KRN., Frampton, G., Tribbick, G., Roden, D., Astley SJ., and Hay, FC. (2003) Characterisation and molecular display of target epitopes of pan-IgG/anti-G3m(u) and anti-Fc specific monoclonal antibodies using pepscan. Immunol.Lett. 88: 77-83.

Watt, G.M., Lund, J., Levens, M., Kolli, K., Jefferis, R.. and Boons, G-J. (2003) Site-specific glycosylation of an aglycosylated human IgG1-Fc antibody protein generates neoglycoprotein with enhanced function. Chemistry & Biology. 10:807-814.

Ayatollahi, M., Joubeh, S., Mortazavi H., Jefferis, R., and Ghaderi A. (2004) IgG4 as the predominant autoantibody in sera from patients with active state of pemphigus vulgaris. J Eur Acad Dermatol Venereol. 18:241-2.

Holland, M., Hewins, P., Goodall, M., Adu, D., Jefferis, R., and Savage, C.O.S. (2004) Anti-neutrophil cytoplasm antibody IgG subclasses in Wegener's granulomatosis: A possible pathogenic role for the IgG4 subclass. Clin.Exp.Immunol. 138:183-192.

Jefferis, R. (2005) Glycosylation of Recombinant Antibody Therapeutics. Biotechnology Progress. 21:11-16.

Mihai, S; Herrero-Gonzalez, JE; Goodall, M, Jefferis R, Savage CO, Zillikens D, Sitaru C. (2005) Non-complement fixing IgG4 autoantibodies from bullous pemphigoid patients activate leukocytes and induce blisters in cryosections of human skin J Invest Derm 125:A45-A45

Goodall, M. and Jefferis, R. (2005) The impact of glycosylation on monoclonal antibody potency and stability. Bioforum Europe 9:30-31.

Jefferis, R. (2005) Glycosylation of normal and recombinant antibody molecules. Advances in Experimental Medicine and Biology. 564:143-148.

Gomord, V., Chamberlain, P., Jefferis, R. and Foye, L. (2005) Biopharmaceutical production in plants: problems, solutions and opportunities. Trends Biotechnol. 23:559-65.

Holland, M., Yagi, H., Takahashi, N., Kato, K., Savage, C.O.S., Goodall, D.M. and Jefferis, R. (2006) Differential glycosylation of polyclonal IgG, IgG-Fc and IgG-Fab isolated from the sera of patients with ANCA associated systemic vasculitis. Biochim Biophys Acta. 1760:669-77.

Jefferis, R. Antibodies. In: Immunology 7th edition. Eds: Roitt, Brostoff, Roth & Male. Mosby, London 2006.

Jefferis, R. Post-translational modifications of recombinant proteins. In: Cell Culture and Upstream Processing. Ed: M.Butler. Taylor and Francis. 2006.

Jefferis, R. (2006) Criteria for selection of IgG isotype and glycoform of antibody therapeutics. In Touch Briefings: Bioprocessing & Biopartnering 2006. 2-5.

Jefferis, R. (2006) Criteria for selection of IgG isotype and glycoform of antibody therapeutics. Bioprocess International. 4:40-43.

Jefferis, R. (2006) A sugar switch for anti-inflammatory antibodies. News & Views. Nat Biotechnol. 24:1230-1231.

Walsh, G. and Jefferis, R. (2006) Post-translational modifications in the context of therapeutic proteins. Nat Biotechnol. 24:1241-52.

Jefferis, R. Human IgG glycosylation in inflammation and inflammatory disease. In: Comprehensive Glycoscience: from Chemistry to Systems Biology. Editor-in-Chief: Kammerling, J.P.; Elsevier 2007.

Pos, W; Luken, BM; Kremer-Hovinga, J, Johanna, Turenhout EAM, Jefferis R, Dong JF, Fijnheer R, Voorberg J. (2007) Human antibodies with specificity for the spacer domain of ADAMTS13 are derived from VH1-69 germline genes. Blood 110:642A-642A.

Colman, R., Hussain, A., Goodall, M., Young, S.P., Pankhurst, T., Lu, X., Jefferis, R. and Savage, C.O.S. (2007) Chimeric antibodies to proteinase 3 of IgG1 and IgG3 subclasses induce different magnitudes of functional responses in neutrophils. Ann Rheum Dis. 66:676-82.

Mihai, S., Chiriac, M.T., Herrero-González, J.E., Goodall, M., Jefferis, R., Savage, C.O.S., Zillikens, D.and Sitaru, C. (2007) Non-complement fixing IgG4 autoantibodies activate leucocytes and induce dermal-epidermal separation. J Cell Mol Med. 11:1117-28.

Magdelaine-Beuzelin, C., Kaas, Q., Wehbi, V., Ohresser, M., Jefferis, R., Lefranc, M-P., Watier, H. (2007) Structure-function relationships of the variable domains of monoclonal antibodies approved for cancer treatment. Critical Reviews in Oncology/Hematology. 64:210-225.

Mimura, Y., Ashton, P.R., Takahashi, N., Harvey, D.J. and Jefferis, R. (2007) Contrasting glycosylation profiles between Fab and Fc of a human IgG protein studied by electrospray ionization mass spectrometry. J Immunol Methods. 326:116-26.

Jefferis R. (2007) Antibody therapeutics: isotype and glycoform selection. Expert Opin. Biol. Ther. 7:1401-13.

Jefferis R. (2007) Glycosylation and the demands of antibody engineering.
http://www.biopharminternational.com/biopharm/Downstream+Processing/Glycosylation-and-the-Demands-of-Antibody-Engineer/ArticleStandard/Article/detail/462764

Jefferis, R. (2009) Glycosylation of antibody therapeutics: optimisation for purpose. Methods in Molecular Biology. 483, 223-38.

Jefferis, R. (2009) Aglycosylated antibodies and the methods of making and using those antibodies: WO2008030564. Expert Opin. Ther. Pat. 19:101-105.

Mimura, Y., Jefferis, R., Mimura-Kimura, Y., Abrahams, J., Rudd, P. M. Glycosylation of therapeutic IgGs, In Therapeutic antibodies: From theory to practice, (Ed) An, Z., Wiley, 2009. p67-89.

Magdelaine-Beuzelin, C., Vermeire, S., Goodall, M., Baert, F., Ohresser, M., Degenne, D., Dugoujon, J.M., Jefferis, R., Rutgeerts, P., Lefranc, M-P.,Watier, H. (2009) IgG1 heavy chain-coding gene polymorphism, marker of G1m allotypes, and development of antibodies-to-Infliximab. Pharmacogenet Genomics. 19, 383-7.

Jefferis, R. (2009) Glycosylation as a strategy to improve antibody-based therapeutics. Nature Reviews: Drug Discovery. 8, 226-234.

Jefferis R. (2009) Recombinant antibody therapeutics: the impact of glycosylation on mechanisms of action. Trends Pharmacol Sci. 30, 356-62

Jefferis, R. (2009) Glycoforms of human IgG in health and disease. Trends in Glycoscience and Glycotechnology. 21, 105-17.

Hussain, A., Pankhurst, T., Goodall, M., Colman, R., Jefferis, R., Savage, CO., Williams, JM. (2009) Chimeric IgG4 PR3-ANCA induces selective inflammatory responses from neutrophils through engagement of Fc gamma receptors. Immunology, 128, 236-44.

Jefferis, R. and Lefranc, M-P. (2009) Human immunoglobulin allotypes: possible implications for immunogenicity. mAb 1, 332-38.

Jefferis, R. (2009) Antibody therapeutics: New models for old. BioForum Europe

Jefferis, R. The antibody paradigm: present and future development as a scaffold for biopharmaceutical drugs. Biotechnology and Genetic Engineering Reviews. Ed: S. Harding; 26: 1-42. Nottingham University Press (2010).

Masuda K, Yamaguchi Y, Takahashi N, Jefferis R, Kato K. (2010) Mutational deglycosylation of the Fc portion of IgG causes O-sulfation of tyrosine adjacently preceding the originally glycosylated site. FEBS Lett. 584:3474-3479

Büttel IC, Chamberlain P, Chowers Y, Ehmann F, Greinacher A, Jefferis R, Kramer D, Kropshofer H, Lloyd P, Lubiniecki A, Krause R, Mire-Sluis A, Platts-Mills T, Ragheb JA, Reipert BM, Schellekens H, Seitz R, Stas P, Subramanyam M, Thorpe R, Trouvin JH, Weise M, Windisch J, Schneider CK. (2011) Taking immunogenicity assessment of therapeutic proteins to the next level. Biologicals. 39:100-109.

Jefferis R. (2011) Aggregation, immune complexes and immunogenicity. mAb 3: 503-4

Jefferis R. (2012) Isotype and glycoform selection for antibody therapeutics. Arch. Biochem. Biophys. 526: 159-166.

Jefferis R. (2012) Ch. 3 Antibodies. Immunology, 8th Edition. Ed: Male D., Brostoff J., Roth D., Roitt I. Saunders , London 2012

Campbell JP, Cobbold M, Wang Y, Goodall M, Bonney SL, Chamba A, Birtwistle J, Plant T, Jefferis R, Drayson MT. (2013) Development of a highly-sensitive multi-plex assay using

monoclonal antibodies for the simultaneous measurement of kappa and lambda immunoglobulin free light chains in serum and urine. J Immunol Methods. 391:1-13.

Jefferis R. (2013) IgG-Fc protein engineering. In: Antibody Fc, Linking adaptive and Innate Immunity. Editors: F. Nimmerjahn & M. Ackerman. Elsevier Amsterdam 2013

Jefferis R. (2013) Review of Glycosylation Engineering of Biopharmaceuticals: Methods and Protocols: A book edited by Alain Beck MAbs. 5:1-3.

Davies AM, Rispens T, Ooijevaar-de Heer P, Gould HJ, Jefferis R, Aalberse RC, Sutton BJ. (2014) Structural determinants of unique properties of human IgG4-Fc. J Mol Biol. 426:630-644

Jefferis R (2014) Glycosylation of Antibody Molecules. In: Handbook of Antibody Therapeutics. 2nd Edition. Ed: S. Dubel & J. Reichert. Wiley-VCH 2014

Hayes JM., Cosgrave FJ., Struwe WB., Wormald M., Davey GP., Jefferis R., Rudd PM. (2014) Glycosylation of immunoglobulins and their Fc receptors. Curr Top Microbiol Immunol. 382:165-199.

Davies AM., Jefferis R., Sutton BJ (2014). Crystal structure of deglycosylated human IgG4-Fc .Mol Immunol. 62:46-53.

Jefferis R. (2014) Monoclonal Antibodies: Mechanisms of action. In: Current state of the art & emerging technologies for the characterisation of monoclonal antibodies Volume 1. Monoclonal Antibody Therapeutics: Structure, Function, and Regulatory space. Editors: D.L. Davis, J. Schiel & Borisov O. ACS Symposium Series; American Chemical Society: Washington, DC, 2014.

Wuhrer M., Stavenhagen K., Koeleman CAM. Selman MHJ., Harper L., Jacobs BJ., Savage COS., Jefferis R., Deelder AM., Morgan M. (2015) Skewed Fc glycosylation profiles of anti-proteinase 3 immunoglobulin G1 autoantibodies from granulomatosis with polyangiitis patients show low levels of bisection, galactosylation and sialylation. J Proteome Res. 14:1657-65

Jefferis R (2016) Post-translational modifications and the Immunogenicity of Biotherapeutics. J. Immunological Research 2016;2016:5358272

Yusuke Mimura, Ronan M Kelly, Louise Unwin, Simone Albrecht, Roy Jefferis, Margaret Goodall, Yoichi Mizukami, Yuka Mimura-Kimura, Tsuneo Matsumoto, Hiroshi Ueoka, Pauline M Rudd. (2016) Enhanced sialylation of a human chimeric IgG1 variant produced in human and rodent cell lines. J Immunol Meth. 428:30-36.

De-Kuan Chang, Vinodh B. Kurella, Subhabrata Biswas, Jianhua Sui, Xueqian Wang, Jiusong Sun, Yuval Avnir, Yanyan Wang, Madhura Panditrao, Eric Peterson, Aimee Tallarico, Stacey Fernandes, Margaret Goodall, Quan Zhu, Jennifer R. Brown, Roy Jefferis and Wayne A. Marasco. (2016) Humanized mouse G6 anti-idiotypic monoclonal antibody has therapeutic potential against *IGHV1-69* germline gene based B-CLL. mAbs 8:787-798.

Jefferis R. "Recombinant Proteins and mAbs" in "Glycosylation and Protein Function"; a book in the series "Advances in Glycobiotechnology" Ed. Erdmann Rapp; Springer. In preparation.

Jefferis R. Immunogenicity of biologicals: the role of post-translational modifications. GaBi Posted 17/06/2016 http://www.gabionline.net/Biosimilars/Research/Immunogenicity-of-biologicals-the-role-of-post-translational-modifications

Campbell JP., Heaney JLJ., Shemar M., Baldwin D., Griffin AE., Oldridge E., Goodall M., Afzal Z., Plant T., Cobbold M. Jefferis R., Jacobs JFM., Hand C., Drayson MT. Development of a rapid and quantitative lateral flow assay for the simultaneous measurement of serum kappa and lambda immunoglobulin free light chains (FLC): inception of a new near-patient FLC screening tool. Clin Chem Lab Med. On line Aug 9th

Jefferis R. (2016) Glyco-engineering of human IgG-Fc to modulate biologic activities. Current Pharmaceutical Biotechnology, 17(15): 1333-1347.

Jefferis R. (2017) Antibody post-translational modifications, pp:151-200. In: Biosimilars of Monoclonal Antibodies. A Practical Guide to Manufacturing and Preclinical Development. Ed. Liu C. & Morrow J. Wiley 2017.

Fisher B., Jefferis R., Gordon C. Biologic agents: Monoclonal Antibodies and Receptor antagonists. Rheumatology 7th Edition 2017

Jefferis R. (2017) Characterization of recombinant biologics: the link between structure and function. In: Biosimilar Drug Product Development. Ed: Laszlo Endrenyi, Paul Declerck, Shein-Ghung Chow. Taylor & Francis, Boca Raton, Florida 2017

308 publications; 211 listed on PubMed (excluding 5 that are another Jefferis R)

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).)