

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

CELLTRION, INC.,
Petitioner,

v.

REGENERON PHARMACEUTICALS, INC.,
Patent Owner.

Case No. IPR2023-00620
Patent No. 10,406,226

**DECLARATION OF DR PETER TESSIER IN SUPPORT OF PETITION
FOR *INTER PARTES* REVIEW OF U.S. PATENT NO. 10,406,226**

TABLE OF CONTENTS

I.	INTRODUCTION	1
II.	BACKGROUND AND QUALIFICATIONS	2
III.	LEGAL STANDARDS	4
	A. Claim Construction.....	4
	B. Prior Art and Earliest Effective Filing Date.....	7
	C. Obviousness.....	7
	D. Burden of Proof.....	9
	E. The POSA.....	9
IV.	A BRIEF OVERVIEW OF THE '226 PATENT	11
V.	SCIENTIFIC BACKGROUND	13
	A. Recombinant Manufacturing of Therapeutic Proteins	13
	B. Protein Purification.....	15
	C. VEGF Antagonists	18
	D. Aflibercept.....	20
	E. VEGF Flt1D2.Flk1D3.FcΔC1	24
	F. Expression and purification of VEGF antagonists.....	26
	G. Glycosylation.....	28
VI.	ANALYSIS OF THE CHALLENGED CLAIMS	30
	A. Claims 1-4 are Obvious over the '319 <i>Publication</i>	30
	1. Claim 1	30
	2. Claim 2	36

3.	Claim 3	38
4.	Claim 4	40
B.	Claims 1-4 are Obvious over the combination of the '319 <i>Publication</i> , and <i>Fahrner</i>	42
1.	Claims 1 and 3	43
2.	Claim 2	46
3.	Claim 4	47
C.	Claims 3-4 are Obvious over the Combination of the '319 <i>Publication</i> , <i>Holash</i> , <i>Wulff</i> , and <i>Fahrner</i>	47
1.	Claim 3	48
2.	Claim 4	52
VII.	SECONDARY EVIDENCE OF NON-OBVIOUSNESS	53
VIII.	AUTHENTICATION OF EXHIBITS	53
IX.	CONCLUDING STATEMENTS	54
X.	APPENDIX - LIST OF EXHIBITS CITED	55

I, Peter M. Tessier, Ph.D., declare as follows:

I. INTRODUCTION

1. I have been retained by counsel for Celltrion, Inc. (“Celltrion”) as a technical expert in connection with the above-identified proceeding. I have been asked to provide my opinions concerning the “obviousness” of the claims of U.S. Patent No. 10,406,226 (“the ’226 patent”) (EX1001), which relate to methods of manufacturing the protein aflibercept and another Vascular Endothelial Growth Factor (“VEGF”) antagonist, in view of prior publications concerning such methods. I understand my declaration will be submitted in support of Celltrion’s Petition for *Inter Partes* Review of the ’226 patent against Regeneron Pharmaceuticals, Inc. (“Regeneron” or “Patent Owner”).

2. My opinions are based on my review of the ’226 patent (EX1001), its prosecution history (EX1013), the relevant prior art that I have identified and of which I have been informed, other documents referred to in this declaration, and my many years of experience in the industry with the development of therapeutic proteins, including during the relevant time period for the questions I have been asked to consider.

3. I am being compensated for my time at my usual consulting rate of \$650 per hour. I am also being reimbursed for reasonable travel expenses related to my consultation. I do not have a personal or financial interest in the outcome of this proceeding nor is my compensation affected by its outcome.

II. BACKGROUND AND QUALIFICATIONS

4. I am currently an Albert M. Mattocks (Endowed) Professor in the Departments of Pharmaceutical Sciences and Chemical Engineering and Biomedical Engineering at the University of Michigan. My work focuses on protein engineering, and my laboratory aims to develop best-in-class therapeutic antibodies to address key biomedical challenges. My work is focused in three main areas: protein engineering and directed evolution; biomolecular screening and high-throughput characterization; and machine learning and computational predictions.

5. I have a Bachelor of Science in Chemical Engineering from the University of Maine, where I was Co-Valedictorian, and a Ph.D. in Chemical Engineering from the University of Delaware.

6. After graduating with my Ph.D., I was an American Cancer Society Post-Doctoral Fellow at the Whitehead Institute for Biomedical Research, at MIT, from 2003 to 2007. After completing the fellowship, I joined Rensselaer Polytechnic Institute (“RPI”) in 2007, first as an Assistant Professor, then as an Associate Professor and then as a Richard Baruch M.D. Career Development (Endowed) Associate Professor and Professor. I was at RPI until 2017, when I joined the University of Michigan in my current role.

7. I have extensive experience related to the discovery, engineering, expression, purification, biophysical characterization, and formulation of protein therapeutics, including antibodies and Fc-fusion proteins. In particular, my lab routinely expresses antibodies and Fc-fusion proteins in mammalian cells, purifies them using standard methods (including Protein A, size-exclusion chromatography and ion-exchange chromatography), and formulates them in standard pharmaceutical formulations.

8. I have authored or co-authored over 80 published, peer-reviewed articles, abstracts, and presentations, as well as a book chapter. I am also named as an inventor on three patent applications and one issued patent, all relating to antibodies and protein aggregation.

9. I have held positions on the Editorial Board of the *Journal of Biological Chemistry*, as a Review Editor for *Frontiers in Molecular Biosciences*, and as a Membership Chair for the American Chemical Society. I have acted as co-chair for a number of conferences on biotherapeutics and biomolecular engineering, and am a Panelist for the National Science Foundation and the National Institutes of Health.

10. I have received over 50 grants to my laboratory since 2008, totaling over \$10 million. Members of my lab, including at the postdoctoral, graduate, and undergraduate level, have won a number of awards from organizations including

the American Chemical Society, the National Institutes of Health, and the National Science Foundation.

11. I have been invited to give over 100 seminars since 2007, by a number of organizations, universities and pharmaceutical and biotechnology companies, including Regeneron.

12. My curriculum vitae accompanies this declaration as EX1003.

III. LEGAL STANDARDS

13. I do not hold any legal qualifications; however, Celltrion's counsel has informed me of a number of legal principles to consider when formulating my opinions relating to the patentability of the '226 patent. These principles are set forth below.

A. Claim Construction

14. I understand that in an IPR proceeding like this one, the terms in a patent claim are generally given their ordinary and customary meaning as understood by a "person of ordinary skill in the art" ("POSA") in the context of the entire patent disclosure. If, however, an inventor defines a claim term in the patent disclosure, then the inventor's definition applies. But if the inventor chooses to define a claim term, they must do so with reasonable clarity, deliberateness, and precision.

15. I further understand that claims can be “independent” or “dependent.”

An independent claim does not refer to or incorporate the elements of any other claim. A dependent claim refers to another claim and includes all of its limitations while also adding additional limitation(s). The '226 patent contains two independent claims: claims 1 and 3. The remaining claims, claims 2 and 4, are dependent on these two claims respectively.

16. Claims 1 and 3 claim a polynucleotide encoding a VEGF antagonist fusion protein that “comprises amino acids 25-457” of SEQ ID NO: 2 (claim 1) or SEQ ID NO: 4 (claim 3). I have been informed by counsel that the term “comprises” means “includes”, and therefore, the protein includes those amino acids but may also include others. Thus, I construe the claims as requiring a VEGF antagonist fusion protein that has a sequence that includes the same amino acids at locations 27 to 457 as those in SEQ ID Nos. 2 or 4, but the sequence can also include additional amino acids, so long as it still binds VEGF to some degree. Accordingly, the claims encompass a VEGF antagonist fusion protein with a sequence that matches the full amino acid sequence of SEQ ID Nos 2 or 4, *i.e.* amino acids 1 to 458 of SEQ ID Nos. 2 or 4.

17. I note that the named inventors have stated that the phrase “substantially free of protein contaminants,” which appears in claim 2, means “preferably, that at least 90% of the weight of protein of the VEGF-specific fusion

protein antagonist preparation used for making a formulation is VEGF fusion protein antagonist protein, more preferably at least 95%, most preferably at least 99%.” EX1001, 5:45-53. I have been informed by counsel that the inventor’s use of the term “preferably” does not require that the preparation used to make the formulation be at least 90% free of protein contaminants. My opinion does not change whichever way the phrase “substantially free of protein contaminants” is construed.

18. I also note that the inventors stated that “[s]ubstantially free of aggregates” means that “at least 90% of the weight of fusion protein is not present in an aggregate at the time the fusion protein is used to prepare the pharmaceutically effective formulation,” EX1001, 5:53-58, *i.e.* this phrase in claims 1 and 3 appears to be the equivalent of stating that the protein is “substantially free of aggregates” at the time the protein is used to prepare a formulation.

19. Given these definitions, a POSA would understand that the claimed levels of aggregates and contaminants were intended to be measured at the time the protein was being used to prepare a formulation, *i.e.* after purification.

20. I did not find that the named inventors in this case provided their own definition of any other claim terms in the disclosure of the ’226 patent.

Accordingly, I interpreted the claim terms consistent with their ordinary and customary meaning to a POSA.

B. Prior Art and Earliest Effective Filing Date

21. I have been asked to assume that the earliest possible filing date for the '226 patent is March 22, 2006, which I will sometimes refer to as the “priority date.” I have also been asked to assume that any printed publication published prior to that date should be considered “prior art” to the patent. I have been informed by counsel that the '226 patent claims priority to a provisional application filed on March 25, 2005. My analysis and the challenges to the claims I discuss below apply whether the '226 patent is accorded a filing date of March 22, 2006, or March 25, 2005.

22. I further understand that a document is considered a “printed publication” if it was published or otherwise made available such that a POSA exercising reasonable diligence could have located it.

C. Obviousness

23. I understand from counsel that for a patent claim to be unpatentable, it must be “non-obvious” over the prior art.

24. I understand that a patent claim is obvious if the differences between the claims and the prior art are such that the subject matter as a whole would have been obvious to a POSA at the time the invention was made.

25. I understand that when assessing whether a claim is obvious, I should consider: (i) the scope and content of the prior art; (ii) the level of ordinary skill in the art; (iii) the differences between the prior art and what is claimed; and (iv) any “secondary” evidence that may indicate that the claims are not obvious.

26. I understand that a claimed invention may be obvious when, for example, there is some motivation, reason, or suggestion that would have led a POSA to modify a prior-art reference or to combine prior-art teachings to arrive at the claimed invention.

27. I also understand that when combining or modifying prior-art references to arrive at the claimed invention, the POSA must also have a reasonable expectation of success, given their general knowledge and what has been disclosed in the prior art. I understand that this success need only be reasonably expected and does not need to be guaranteed or proven.

28. I understand that “secondary” evidence may weigh against a finding that a claimed invention would have been obvious. Such secondary evidence may include: (i) commercial success of a product due to the merits of the features of the product that are the subject of the patent claims; (ii) a long-felt but unresolved need for the solution provided by the patent claim; and (iii) unsuccessful attempts by others to find the solution provided.

D. Burden of Proof

29. I understand that in an IPR, a claim is not patentable if a “preponderance of the evidence” establishes that the claim is rendered obvious by the prior art. I understand that “a preponderance of the evidence” means “more likely than not.”

E. The POSA

30. I understand that claim construction and obviousness must be judged from the perspective of the POSA to which the patent is directed. I understand that the POSA is a hypothetical person or persons that is presumed to have a perfect knowledge of all of the relevant prior art.

31. I understand that in determining who a POSA is the following may be considered: (1) the educational level of the inventor(s); (2) the type of problems encountered in the relevant technical field; (3) prior-art solutions to those problems; (4) rapidity with which innovations are made; and (5) the sophistication of the technology and educational level of active workers in the field.

32. Here, the '226 patent concerns methods of manufacturing VEGF antagonist fusion proteins, including expression in CHO cells and purifying to reach a desired low level of aggregates and contaminants. Based on my extensive experience in protein development, including first-hand knowledge of how therapeutic proteins were manufactured around 2005-2006, the POSA at the time

of invention would have had a Ph.D. in Chemical, Biological or Biomedical Engineering or a similar field, including Biology, Biochemistry or Chemistry, with at least several, i.e. more than 2, years of experience in the development, manufacture and characterization of therapeutic proteins, including for example, fusion proteins or antibodies. The POSA may also have had less education but substantially more relevant practical experience. This individual would have understood how to design and engineer therapeutic proteins, how to express them in host cells systems, and how to implement and control purification of proteins in order to optimize their use in therapeutic products. This individual also would have been able to use state-of-the-art purification and analytical methods, such as Protein A affinity chromatography, size exclusion chromatography, and anion and cation exchange chromatography.

33. This POSA would have had access to other individuals typically employed in developing therapeutic proteins, including those involved in analytical chemistry, pharmacokinetics, formulation development, clinical testing, pharmaceutical packaging, and regulatory affairs. Those diversely-qualified individuals would have worked together as needed during the manufacturing and development process.

34. My opinions would not change even if a different definition of POSA is adopted.

IV. A BRIEF OVERVIEW OF THE '226 PATENT

35. The '226 patent is entitled “Method of Manufacturing VEGF Antagonist Fusion Proteins.” The '226 patent, based on the information on the face of the patent, is assigned to Regeneron Pharmaceuticals, Inc. EX1001.

36. I have reviewed the claims, specification, and prosecution history of the '226 patent.

37. Claims 1 and 3 of the '226 patent describe a method of manufacturing a particular VEGF antagonist protein, identified by part of the amino acid sequence that encodes the protein (the sequence for aflibercept in the case of claim 3, and the sequence for another VEGF antagonist in claim 1). The method of claims 1 and 3 includes expressing the protein in CHO cells and purifying it, such that at least 90% of the weight of the protein is not present as an aggregate. EX1001, 19:35-46; 20:34-44. Dependent claims 2 and 4, which depend from claims 1 and 3 respectively, further specify that the fusion protein is “purified until substantially free of protein contaminants” (claim 2) and “posttranslationally glycosylated at one or more asparagine residues” (claim 4).¹ *Id.*, 19:47-78; 20:45-48.

¹ I note that the '226 patent includes a certificate of correction dated September 10, 2019, in which claim 4 was corrected to depend from claim 3, instead of claim

38. Although the '226 patent claims methods of manufacturing VEGF antagonists, the '226 patent does not describe these methods in any of its examples, nor does it discuss the manufacturing process in any detail in the specification. The specification mentions CHO cells only once, noting that “[i]n specific embodiments, the VEGF antagonist is expressed in a mammalian cell line such as a CHO cell and may be modified posttranslationally,” and that the protein can be prepared by “any suitable method known in the art.” *Id.*, 5:37-58. It does not describe any culture conditions or media used, nor does it describe purification methods or conditions. The '226 patent specifies that the protein is preferably substantially free of contaminants and aggregates, but does not describe any methods for achieving those results. *Id.* I note that during prosecution of the patent, the examiner noted as much, stating “[t]he specification does not offer even a paragraph about obtaining the claimed products in CHO and not even a single method step is detailed.” EX1013 (*'226 prosecution history*), 169.

39. The specification is otherwise focused on formulations of VEGF antagonists, including aflibercept, and their stability. The examples in the patent describe the stability of formulations of the protein over time, but none of the

1, and the word “post-transitionally” was corrected to “posttranslationally.”

EX1001, Certificate of Correction.

examples provide any detail on how the proteins were expressed or purified, the level of aggregates or contaminants in the protein prior to being formulated into the compositions in the examples, or the glycosylation properties of the proteins. *See id.*, Examples 1-5.

40. Based on my review of the specification, there is nothing in the '226 patent to suggest that anything other than the ordinary skill of a POSA at the time was required to produce the fusion proteins described, including purifying to ensure at least 90% of the protein is not present as an aggregate, that the protein is substantially free of contaminants, and is glycosylated at one or more asparagine residues, as required by the challenged claims.

V. SCIENTIFIC BACKGROUND

A. Recombinant Manufacturing of Therapeutic Proteins

41. Production of therapeutic proteins through recombinant technology has been around since as early as the 1980s. EX1009 (*Wurm*), 1393.

Recombinant technology refers to bringing together genetic material from multiple sources to create DNA sequences that are naturally not found in the genome.

42. The basic process for developing a recombinant manufacturing process typically follows a number of standard steps, including transferring the relevant recombinant gene to cells, selection of cells, cultivation and analysis, and choosing the most productive cell line. *Id.* The basic concepts of this process have not changed since the 1980s; however, the productivity of recombinant cell lines

by the mid 2000s had “dramatically” increased following many years of research into the technology. *Id.*, 1393-94.

43. By the priority date of the '226 patent, production of therapeutic proteins by this technology was common and around 60-70% of all recombinant protein pharmaceuticals were produced via mammalian cells, given expression in these cells was shown to be superior to other host systems. *Id.*, 1393. CHO cells in particular “dominate[d]” the mass production of proteins by 2004 (*id.*, 1396), and presented distinct advantages for expressing complex proteins over other expression systems like *E. coli*. For example, the use of CHO cells allowed for streamlined control of product-related variants during the cell culture phase, allowing for a more streamlined purification process. EX1005 (*Fahrner*), 302. As such, CHO cells became the preferred choice amongst manufacturers of therapeutic proteins by the early 2000s.

44. Following production by recombinant technology in CHO cells, the resulting mixture includes the desired protein and other products produced by the process, including misfolded, degraded, or denatured forms of the same protein. EX1010 (*Chang*), 2. Because developers of a protein, particularly proteins intended for therapeutic use, aim to have as pure a form of the protein as possible to work with, the mixture produced by the cell culture process is further purified to remove as many of these other product impurities as possible, as discussed further

below. *Id.* The resulting purified form of the protein is often referred to as the “bulk” and can then be used to formulate the protein in a therapeutic product. *Id.*, 3.

B. Protein Purification

45. As of the priority date, it was standard practice to purify proteins like aflibercept and other VEGF-antagonist variants during the manufacturing process. Purification helped to reduce impurities that could form during the production process, which could cause problems for the therapeutic use of the protein. *See* EX1005 (*Fahrner*) 301-302. The prior art acknowledged that there were “extreme purity requirements” for therapeutic proteins and that the purification process must reliably produce highly purified proteins for therapeutic approval. *Id.*

46. Aggregation, which refers to the presence of aggregated forms of a protein, was a well-known product-related impurity prior to the filing date of the ’226 patent. Prior art at the time, including publications by the FDA, taught the potential negative effects of aggregation and other impurities, including immunogenicity and altered pharmacokinetics. EX1005 (*Fahrner*), 304; EX1020 (*FDA PTC 1997*), 14 (noting that “[l]oss of specific immunoreactivity due to denaturation or formation of aggregates ... may lead to altered pharmacokinetics and/or binding to non-target tissues.”).

47. As a result, skilled artisans developing proteins for therapeutic use used the purification process to reduce aggregation to the lowest levels possible, typically well below 10%, and sometimes, to amounts as low as less than 0.5%, in the final bulk protein. See EX1005 (*Fahrner*), 304, 306, Table 12.1. Regulatory authorities also required that the manufacturer demonstrate the structural integrity of an antibody, including its purity, as part of the approval process. See EX1020 (*FDA PTC 1997*), 12. It was also well-known that other contaminants, including animal proteins and materials, DNA, endotoxin, other pyrogens, and culture media constituents, could be produced when manufacturing proteins and therefore should be controlled and reduced for similar reasons to those for aggregation. EX1020 (*FDA PTC 1997*), 10-11; EX1005 (*Fahrner*), 302.

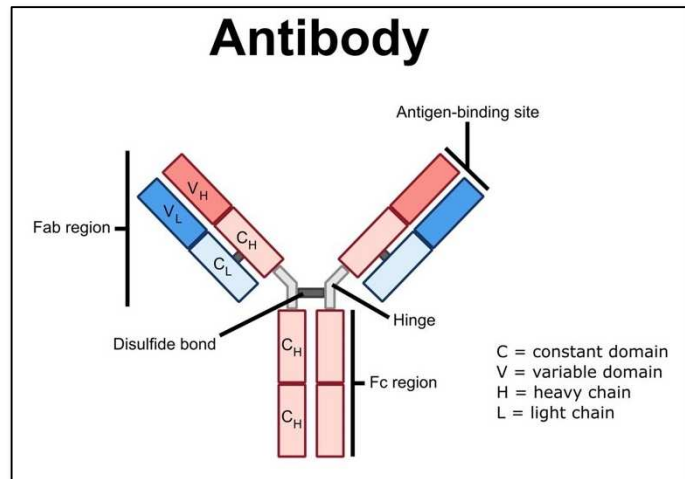
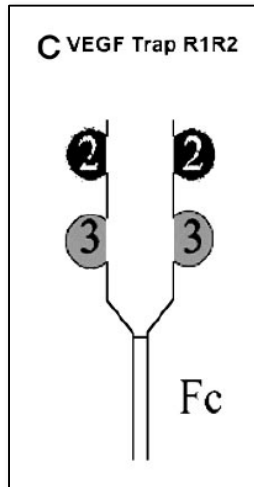
48. As such, persons of skill in the art at the time were very familiar with ways to reduce aggregates and other contaminants to very low levels and would not have developed a protein for therapeutic use without doing so. Standard processes for purifying proteins to reduce these impurities in large scale commercial manufacturing included Protein A affinity chromatography, cation exchange chromatography, and anion exchange chromatography. EX1005 (*Fahrner*) 302, 305; EX1004 (*'319 Publication*), 69:21-71:5, Example 22. Protein A affinity chromatography was typically used as the first step in the purification process, while the other techniques (cation exchange, anion exchange) were used

as further “polishing” steps to remove further impurities. *See* EX1005 (*Fahrner*), 305. Another purification technique, size exclusion chromatography, could be used as a second step after Protein A purification; however, size exclusion chromatography was and is typically more common in smaller scale production processes, including experimental production of proteins. Size exclusion chromatography is less common in the purification of proteins produced in large scale, commercial manufacturing.

49. Further, as discussed above, it was well-known at the time of the priority date that an advantage of expressing proteins in CHO cells in particular was that the level of product-related impurities could be well-controlled during the cell culture process, allowing for streamlined purification. *See supra* ¶43.

50. I note that while some of the references I cite to may refer to “proteins” while others refer to “antibodies,” aflibercept (and similar VEGF Trap variants) and antibodies are both proteins with similar structures, *i.e.*, they are large, complex, multi-domain, recombinant proteins that are produced for therapeutic use in cell culture (usually CHO cells).

51. Further, both are “Y” shaped and have two arms, which specifically bind to a target ligand and are linked together at their bases by an Fc immunoglobulin domain:²



52. As such, a POSA would understand that the expression and purification techniques and approaches being taught when a reference refers to antibodies would also apply to fusion proteins like aflibercept.

C. VEGF Antagonists

53. VEGF is a naturally-occurring protein that regulates “angiogenesis,” the process by which new blood vessels are formed. *See EX1006 (Holash)*, 11393.

² Antibody diagram obtained from

<https://step1.medbullets.com/immunology/105060/antibodies>.

VEGF binds to specific VEGF receptors on the surfaces of cells responsible for angiogenesis, initiating the VEGF pathway. *Id.* Two of the most well-known and best-characterized VEGF receptors are VEGF receptor 1 (VEGFR1), also known as Flt1, and VEGF receptor 2 (VEGFR2), also known as Flk1. *Id.*, 11393; EX1007 (*Wulff*), 2798, Fig. 1. VEGFR1 and VEGFR2 both have an extracellular region consisting of seven domains to which VEGF binds. EX1007 (*Wulff*), 2798, Fig. 1. Once they bind to VEGF via these extracellular domains, the VEGF receptors combine to form dimers, which are the active, cell-signaling form. EX1012 (*Rudge*), 412.

54. As early as 2002, VEGF had been identified as playing a key role in angiogenesis, which is necessary for tumor growth. EX1006 (*Holash*) 11393; EX1011 (*Ferrara*), 968. By 2005-2006, a number of VEGF inhibitors had been developed and explored as anti-cancer therapies given their potential to inhibit tumor growth. EX1011 (*Ferrara*), 971. One such example is bevacizumab, a humanized monoclonal antibody that binds to VEGF and blocks its activity. *Id.*, 967, 971.

55. VEGF inhibitors had also been developed to treat age-related macular degeneration (wet AMD), a disease characterized by proliferation of blood vessels in the retina of the eye. An example developed for this purpose is ranibizumab, a modified fragment of the bevacizumab antibody. EX1012 (*Rudge*), 411.

D. Aflibercept

56. Aflibercept is a VEGF inhibitor developed by Regeneron, and marketed under the name EYLEA®. Aflibercept is a fusion protein of domain 2 of the human VEGFR1 receptor and domain 3 of the human VEGFR2 receptor, linked via the Fc domain of a human IgG antibody as shown below:

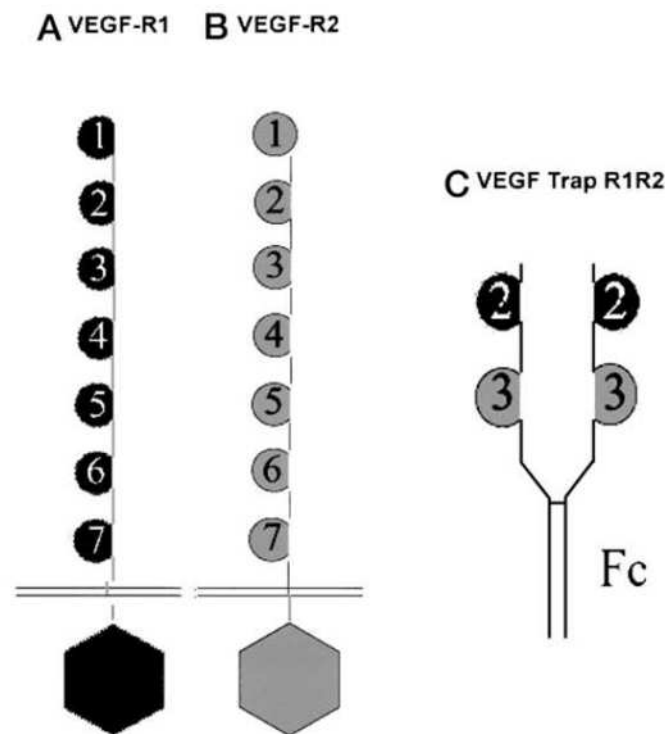


FIG. 1. Structure of VEGF receptors and the VEGF Trap. VEGF-R1 (A) and VEGF-R2 (B) both contain seven extracellular domains, which differ between the two receptors. These extracellular domains are responsible for VEGF binding. The soluble VEGF Trap R1R2 (C) was created by fusion of domain 2 of VEGF-R1 and domain 3 of VEGF-R2 with the FC portion of IgG.

EX1007 (*Wulff*), 2798, Fig. 1. Aflibercept, like bevacizumab and ranibizumab, works by binding to VEGF and “trapping” it before it can bind to cell-surface VEGF receptors, therefore preventing it from triggering angiogenesis. EX1006 (*Holash*), 11393.

57. Regeneron published detailed descriptions of its development of aflibercept as early as 2002, along with information regarding its *in vivo* experiments, which demonstrated aflibercept's superior therapeutic promise over other known VEGF inhibitors at the time, including older VEGF-trap fusion proteins that Regeneron had also been studying. *See generally* EX1007 (*Wulff*); EX1006 (*Holash*). For example, early in development, Regeneron had experimented with a recombinant "parental VEGF-trap" fusion protein in which the first three extracellular domains of the human VEGFR1 receptor were fused to the Fc region of human IgG1. EX1006 (*Holash*), 11393. That parental VEGF-trap had poor pharmacokinetics, which Regeneron's scientists reasoned was due to the fact that some of the extracellular domains of VEGFR1 were positively charged, which can lead to non-specific binding to negatively-charged components of the extracellular matrix in the tissue at the site of injection. *Id.*, 11394-95, Fig. 1. As a result of this finding, Regeneron removed or replaced positively-charged domains to create less positively-charged variants, finding that this significantly improved pharmacokinetic performance. *Id.*, 11395. As such, aflibercept emerged as the variant that had the best combination of anti-VEGF activity and *in vivo* pharmacokinetics, and which compared favorably to other VEGF antagonists such as monoclonal antibodies. *Id.*, 11397. As Regeneron's scientists put it, "[t]he combination of high-affinity and improved pharmacokinetics apparently

contributes toward making VEGF-Trap_{R1R2} [aflibercept] one of the most, if not the most, potent and efficacious VEGF blocker available.” *Id.*, 11397.

58. Regeneron noted in its prior art publications that aflibercept had the additional advantage of being composed of “entirely human sequences,” which would “hopefully minimiz[e] the possibility that it might prove immunogenic in human patients.” *Id.*, 11397. In comparison to existing antibody VEGF antagonists, “far lower circulating levels of VEGF-Trap_{R1R2} [aflibercept] are required for similar efficacy” and its “safety has recently been confirmed in toxicological studies in cynomologus monkeys.” *Id.* As a result of these superior properties, Regeneron had noted in its prior art publications as early as 2002 that “the [aflibercept] VEGF-Trap is currently in human clinical trials for several different types of cancer.” *Id.*

59. Regeneron had also published initial positive data for its initial human clinical trials of aflibercept by 2005. EX1012 (*Rudge*), 414-15. Regeneron characterized the initial trial results as “quite promising” and noted that aflibercept was “now entering more advanced clinical trials in vascular eye diseases.” *Id.*, 414-15.

60. Regeneron had also widely published the sequence of aflibercept prior to the priority date, although over the years it had referred to the protein by its earlier scientific names, VEGF-Trap_{R1R2}, VEGFR1R2-FcΔC1(a), and “VEGF

Trap-Eye” rather than the non-proprietary name “aflibercept” it coined later as the molecule neared regulatory approval.³ As early as 2004, Regeneron had published the amino acid sequence of VEGFR1R2-FcΔC1(a) (aflibercept) in at least three earlier patent publications. *See* EX1004 (‘319 *Publication*), 22:1-2, Figs. 24A-24C; EX1017 (‘667 *Publication*), ¶89, SEQ ID No. 10; EX1018 (‘309

³ When obtaining a patent term extension for its patent 7,374,758 (“the ‘758 Patent”) based on the regulatory approval of EYLEA®, Regeneron represented to the Patent Office that aflibercept is “also known as VEGF trap, VEGF-trap, VEGF Trap-Eye and VEGF-Trap_{R1R2}.” EX1015 (‘758 *patent term extension*), 2, 6-7. Regeneron also represented that “aflibercept is described in [*Holash*] as VEGF-Trap_{R1R2}”, and that the amino acid sequence of aflibercept is set forth in Figures 24A-24C of the ‘758 patent. *Id.*, 5, 6-7 (noting that the “Flt1 Ig domain 2” of aflibercept “spans amino acid residues 27 through 129,” “Flk1 Ig domain 3 spans amino acid residues 130 through 231,” and “the Fc multimerizing component” spans amino acid residues 232 through 458”). The ‘758 patent is a divisional of the PCT application published as the ‘319 *Publication*, and Figures 24A-24C of the ‘758 patent are the same as Figures 24A-24C in the ‘319 *Publication*. *See* EX1004; EX1014.

Publication), ¶5, SEQ ID Nos. 1 and 2 (disclosing that VEGFR1R2-FcΔC1(a) is “also termed VEGF-Trap_{R1R2}”).

E. VEGF Flt1D2.Flk1D3.FcΔC1

61. In its prior art publications Regeneron also disclosed its development of another VEGF-antagonist, which, like aflibercept, is also a fusion protein of domain 2 of the human VEGFR1 receptor and domain 3 of the human VEGFR2 receptor, linked via the Fc domain of a human IgG antibody. *See generally* EX1004 ('319 *Publication*); EX1017 ('667 *Publication*). Regeneron referred to this variant as “Flt1D2.Flk1D3.FcΔC1” and likewise published the amino acid sequence for this protein. EX1004 ('319 *Publication*), Figs. 21A-21C, 58:6-64:22, Example 17; EX1017 ('667 *Publication*), ¶31, SEQ ID Nos. 7 and 8. Just like with aflibercept, Regeneron detailed how it produced this VEGF variant and how it performed *in vivo* in publications before the priority date of the '226 patent. *See generally* EX1004 ('319 *Publication*). Regeneron expressed this VEGF variant in CHO cells, grew the cells in bioreactors and then purified the protein by affinity and size exclusion chromatography. EX1004 ('319 *Publication*), 67:7-12, Example 20. Aflibercept differs from the Flt1D2.Flk1D3.FcΔC1 expressed protein as shown in Figure 36 of the '319 *Publication* in that three amino acids have been added between amino acids 26 and 27 of Flt1D2.Flk1D3.FcΔC1 (Figures 21A-21C)—SDT—and the three amino acids corresponding to amino acids 229-231 of

Flt1D2.Flk1D3.FcΔC1—GPG— have been removed. *Id.*, 67:7-12.⁴ Regeneron also published results of various tests on this variant, including its binding stoichiometry, peptide mapping, and pharmacokinetics. *Id.*, 74:15-79-6, 80-20, 83:19, Examples 25, 26, 28, 29. Regeneron disclosed that this protein had five possible N-linked glycosylation sites on asparagine residues, all glycosylated to varying degrees. *Id.*, 80:20-82:2, Example 28 (“Complete glycosylation is observed at Asn33, Asn193, and Asn282. Partial glycosylation is observed on Asn65 and Asn120.”), Fig. 36.

⁴ I also conducted a comparison of the full sequences for SEQ ID NO: 2 and SEQ ID NO: 4 in the '226 patent, which as I note below are the same as Figs. 21A-21C and 24A-24C in the '319 *Publication* respectively, and the sequence alignment is shown in EX1025. I conducted this comparison by extracting the sequences from the patent and aligning them using an online tool available from the European Bioinformatics Institute. As expected, the only differences between these two sequences are that SEQ ID NO: 4 has three amino acids (SDT) added between amino acids 26 and 27 of SEQ ID NO: 2 and the three amino acids corresponding to amino acids 229-231 (GPG) of SEQ ID NO: 2 have been removed. These are the same differences as shown with the corresponding sequences in the '319 *Publication*. See *supra* ¶61.

62. Regeneron's testing of this protein's pharmacokinetics and ability to inhibit tumor growth were also disclosed in the prior art, showing that the protein significantly reduced the growth of tumors *in vivo* and showed a similar pharmacokinetic profile to aflibercept. *Id.*, 82:4-84:17, Examples 29-30, Figs. 37, 39 and 40. As a result, Regeneron characterized this variant, along with aflibercept, as "appropriate . . . for use in therapies in which antagonism of VEGF is a desired result." *Id.*, 29:3-6.

F. Expression and purification of VEGF antagonists

63. In a number of prior art publications, Regeneron disclosed that it used a CHO cell host vector system to express aflibercept and other VEGF-antagonist variants it was developing, including Flt1D2.Flk1D3.FcΔC1. *See, e.g.*, EX1004 ('319 Publication), 12:14-26, 15:19-27, 67:19-69:19, Figs. 21A-21C; 24A-24C, claims 9, 20, Example 21; EX1017 ('667 Publication), ¶¶15, 31, 98, Example 5; Figs. 7-10; *see also* EX1018 ('309 Publication) ¶¶22 and 26 (noting CHO cells were a suitable host cell and pointing the skilled artisan to the '319 Publication for "a complete description of VEGF-receptor based antagonists including VEGFR1R2-FcΔC1(a)" and incorporating the '319 Publication into the '309 Publication "by reference in its entirety."). Wulff also disclosed that Regeneron made the VEGF-Trap_{R1R2} (aflibercept) used in its *in vivo* experiments by expressing it in CHO cells. EX1007 (Wulff), 2798 and n.1 (explaining that "[t]he VEGF Trap

R1R2 used in these experiments ... was expressed in CHO cells” and pointing the reader to the ‘319 *Publication* for a description of “the detailed molecular structure” of VEGF-Trap_{R1R2} and how it was created.”). *Holash* likewise disclosed that the VEGF-Trap variants described in its *in vivo* experiments, including aflibercept, were produced from expression in CHO cells. EX1006 (*Holash*), 11393-94.

64. It is not surprising that Regeneron chose to use CHO cells to express the VEGF antagonists it was developing at that time. As discussed above, CHO cells “dominate[d]” the mass production of proteins by 2004, EX1009 (*Wurm*), 1396, and were a preferred choice for manufacturers at the time, including Regeneron. *See supra* ¶43.

65. Regeneron’s own prior art references demonstrate that the VEGF-antagonists it developed and produced in CHO cells, including aflibercept and VEGF Flt1D2.Flk1D3.FcΔC1, were purified during the manufacturing process using standard purification techniques. *See, e.g.*, EX1004 (‘319 *Publication*), 69:21-71:5, Example 22 (describing purification of Flt1D2.Flk1D3.FcΔC1 using Protein A and size exclusion chromatography); EX1017 (‘667 *Publication*), ¶¶ 39, 50 (teaching that the VEGF traps may be purified by, *inter alia*, ion exchange chromatography and Protein A chromatography). A POSA at the time would have understood the purpose of purifying these proteins was to reduce aggregation and

other contaminants to ensure the protein could be approved and used safely and effectively for therapeutic use.

G. Glycosylation

66. Glycosylation refers to the process where sugars or “glycans” are created, altered, and attached to proteins or other biomolecules. Protein glycosylation is one of the most common forms of post-translation modifications that can occur in a protein, particularly proteins expressed in CHO cells. There are two main types of glycosylation: N-linked glycosylation and O-linked glycosylation. N-linked glycosylation refers to the process where glycans are attached to the side-chain nitrogen atoms of asparagine residues, whereas O-linked glycosylation refers to when glycans are attached to the side-chain oxygen atoms of hydroxyl amino acids, particularly serine and threonine residues. In particular, N-linked glycosylation typically occurs by attaching a glycan to the amine of an asparagine residue for the sequence motif “asparagine-X-serine/threonine,” where the first residue after asparagine (“X”) can be any residue except proline and the second residue after asparagine can be either serine or threonine. EX1022 (*Liu*), 1867; *see also* EX1021 (*Jefferis*), 13.

67. The glycosylation of IgGs was well-characterized by the priority date, and N-linked glycosylation was known to occur in the IgG at a conserved asparagine residue in the C_H2 (also known as C_γ2) domain (located in the Fc region)

at position 297. *See* EX1021 (*Jefferis*), 12; EX1023 (*Krapp*), 980; EX1024 (*Mimura*), 45539. This same IgG-Fc region of antibodies is commonly used for producing Fc fusion proteins, including for aflibercept and similar VEGF Traps. *See* EX1007, 2798, Fig. 1; *supra* ¶¶50-51, 56. Because the conserved Fc glycosylation site is maintained in any Fc fusion protein, such as in aflibercept and similar VEGF Traps, these fusion proteins will contain at least one N-linked glycan in the Fc region. In addition, Fc fusion proteins can also contain N-linked glycans in the non-Fc portion of the fusion protein, and these sites can be readily identified based on the sequence motif “asparagine-X-serine/threonine”, as discussed above. *See supra* ¶66.

68. Glycosylation of asparagine residues of the Fc region of IgG was also shown to be essential to the functions of the Fc region. EX1021 (*Jefferis*), 11-13; EX1023 (*Krapp*), 980-81; EX1024 (*Mimura*), 45539. For example, glycosylation of the Fc region is important for IgG and Fc-fusion protein binding to FcγRs, which results in various effector functions, such as Antibody-Dependent Cellular Cytotoxicity (ADCC) and Complement-Dependent Cytotoxicity CDC. EX1021 (*Jefferis*), 14-15; EX1023 (*Krapp*), 980-81; EX1024 (*Mimura*), 45539.

69. Regeneron’s own prior art publications showed that the VEGF traps it was developing at the time contained glycosylation at five asparagine residues, which were located in the sequence motif “asparagine-X-serine/threonine.” *See*

EX1004 (*'319 Publication*), 80:20-82:2, Example 28; *see also* EX1017 (*'667 Publication*), ¶98. In particular, there were two expected N-linked glycosylation sites in hFLT1 IgG Domain 2, two in the hFLK1 IgG Domain 3, and one in the Fc portion of the fusion protein. EX1004 (*'319 Publication*), 80:20-82:2, Example 28; *see also* EX1017 (*'667 Publication*), ¶98 (stating that “when expressed as secreted proteins in CHO [cells]” the VEGF Traps were glycosylated “as expected.”).

VI. ANALYSIS OF THE CHALLENGED CLAIMS

A. Claims 1-4 are Obvious over the *'319 Publication*

70. Claims 1-4 are obvious in view of Regeneron’s own prior art patent publication the *'319 Publication* (EX1004), which was published on December 14, 2000, and therefore was a printed publication available prior to the *'226 patent’s* filing date of March 22, 2006.

1. Claim 1

71. Independent claim 1 covers a method of manufacturing a VEGF antagonist fusion protein where the method includes the following: (1) expressing the protein in a CHO cell; (2) the CHO cell includes a polynucleotide encoding the VEGF protein identified by amino acids 27-457 of SEQ ID NO: 2; (3) the fusion protein binds VEGF; (4) purifying the protein; and (5) wherein at least 90% of the weight of the protein is not present as an aggregate.

72. Example 21 in the *'319 Publication* discloses a method of manufacturing used to produce modified Flt1 receptors. *Id.*, 67:19-69:20. One of

those modified receptors is the VEGF antagonist Flt1D2.Flk1D3.FcΔC1(a), which binds VEGF and as discussed above shares a similar structure to aflibercept. *See supra* ¶61, n.4. Thus, to the extent the preamble is limiting, it is taught by the '319 *Publication*.

73. The sequence for Flt1D2.Flk1D3.FcΔC1(a) is disclosed in Figs. 21A-21C of the '319 *Publication*. Amino acids 27-457 of the sequence correspond to the fusion protein, while amino acids 1-26 correspond to the Flt1 signal sequence. *Compare* Figs. 21A-21C with Fig. 36. I conducted a sequence comparison of this sequence against SEQ ID NO. 2 of the '226 Patent and confirm that they match in their entirety, including amino acids 27-457.⁵ EX1026. Therefore, the '319 *Publication* meets this limitation of claim 1.

74. The '319 *Publication* clearly teaches that the method for producing Flt1D2.Flk1D3.FcΔC1(a) involves expression in CHO cells and purification. Specifically, the '319 *Publication* states:

The process for production of Flt1D2.Flk1D3.FcΔC1(a) protein using the expression plasmid pFlt1D2.Flk1D3.FcΔC1(a) described *supra* in Example

⁵ I conducted this comparison by extracting the sequences from the patent and aligning them using an online tool available from the European Bioinformatics Institute.

1 involves suspension culture of recombinant Chinese hamster ovary (CHO K1/E1A) cells which constitutively express the protein product. The cells are grown in bioreactors and the protein product is isolated and purified by affinity and size exclusion chromatography.

Id., 67:25-68:5.

75. The '319 *Publication* also provides detailed methods and conditions to expand the Flt1D2.FlklD3.FcΔC1(a) expressing cell lines, as well as methods of transferring the cell lines to a bioreactor. *Id.*, 68:8-69:13.

76. The '319 *Publication* states that the chimeric polypeptide molecules (i.e., the VEGF antagonist proteins) “may be purified by any technique which allows for the subsequent formation of a stable biologically active chimeric polypeptide molecule.” *Id.*, 34:9-11.

77. Example 22 of the '319 *Publication* describes harvest and purification of modified Flt1 receptors, and in particular, Flt1D2.FlklD3.FcΔC1(a). *Id.*, 69:21-71:5. The '319 *Publication* states that the protein product was aseptically harvested from the bioreactor, loaded onto a Protein A column, and the eluted protein was frozen. *Id.*, 70:3-13. The frozen protein was thawed and concentrated, and the concentrated protein was loaded onto a size exclusion column packed with Superdex 200 resin to remove aggregates and other contaminants. *Id.*; *see also id.*, 39:15-19. Therefore, the '319 *Publication* meets these limitations of claim 1.

78. The '319 *Publication* investigated the binding stoichiometry of both Flt1D2.Flk1D3.FcΔC1(a) and VEGFR1R2-FcΔC1(a) with human VEGF165. *Id.*, 74:15-76:10. The binding stoichiometry was determined by measuring with the level of VEGF saturation binding to Flt1D2.Flk1D3.FcΔC1(a) or VEGFR1R2-FcΔC1(a) surfaces, or by measuring the concentration of VEGF165 to prevent binding of Flt1D2.Flk1D3.FcΔC1(a) or VEGFR1R2-FcΔC1(a) to a VEGF BIAcore chip surface. *Id.*, 74:20-25. The '319 *Publication* disclosed that binding stoichiometry was one VEGF165 dimer molecule per one Flt1D2.Flk1D3.FcΔC1(a) or VEGFR1R2-FcΔC1(a). *Id.*, 75:12-15; 76:7-10, Fig. 28; *see also id.*, 90:5-10 (noting that Flt1D2.Flk1D3.FcΔC1(a) has a high affinity for VEGF165). The '319 *Publication* thus discloses that the VEGF antagonist protein Flt1D2.Flk1D3.FcΔC1(a) binds VEGF, meeting this limitation of the claim.

79. Based on all of the above, the '319 *Publication* teaches all of the process steps required by claim 1; however, the '319 *Publication* does not explicitly state that 90% of the weight of the protein is not present as an aggregate. Given the purification steps taught by the '319 *Publication*, a POSA would have expected this limitation to have been met given a target of less than 10% aggregates is generous, and allows for more aggregation than a POSA would typically aim for when purifying a protein for therapeutic use.

80. But, if not, reaching a target of at least 90% of the weight of the protein not being present as an aggregate, or in other words, less than 10% aggregation, after purifying a protein produced by the method disclosed in the '319 *Publication* would not have been difficult for a POSA at the time, and was well within the level of ordinary skill. Further, as noted above, the specification itself does not describe or exemplify how the proteins were purified to reach the claimed level of aggregation, which shows that Regeneron considered such methods to be within the ordinary skill of a POSA reading the '226 patent. *See supra* ¶¶38, 40.

81. As discussed in ¶¶45-52, *supra*, at the time of the priority date it was common to purify proteins to as low levels of aggregation as possible if intending to use them for therapeutic use. A POSA would have had general knowledge that targeted aggregation levels were typically less than 10%, including targets as low as less than 0.5%, in order to avoid adverse effects caused by impurities and in order to achieve regulatory approval. EX1005 (*Fahrner*), 304, 306, Table 12.1.

82. The '319 *Publication* specifically states that the disclosed chimeric molecules were appropriate for therapeutic use targeting VEGF antagonism. EX1004 ('319 *Publication*), 29:3-6. The '319 *Publication* teaches that the Flt1D2.Flk1D3.FcΔC1(a) (the protein of SEQ ID NO:2) is considerably less sticky to the extracellular matrix than other proteins tested. *Id.*, 65:15-19, Fig. 23. Further, the '319 *Publication* demonstrates that fusion proteins such as

Flt1D2.Flk1D3.FcΔC1(a) had longer half lives in serum (*id.*, 83:15-19, Fig. 38), and inhibited tumor growth *in vivo* (*id.*, 84:14-17, Figs. 39-40).

83. A POSA at the time of invention would have understood from these disclosures that the Flt1D2.Flk1D3.FcΔC1(a) protein produced by Regeneron according to the disclosed method was intended for and suitable for therapeutic use. Accordingly, the claimed level of protein aggregation of less than 10% for use in a therapeutic product would have been well above what was required for therapeutic purposes or regulatory approval. Rather, a POSA would have aimed for a much lower amount of aggregation than the 10% required by the claims. *See supra* ¶¶45-47.

84. The claimed level of less than 10% aggregation was therefore well above what a POSA would typically be targeting at this time, and thus to the extent it may not have been achieved by the '319 *Publication*, it would not have been difficult to achieve. This is further demonstrated by the fact that the specification of the '226 patent itself does not describe how to purify the protein and achieve this claimed result, because this was a matter of routine skill for a POSA at the time.

85. The production and purification techniques disclosed in the '319 *Publication* are consistent with typical techniques used at the time by a POSA, as it teaches the use of a Protein A affinity chromatography and size exclusion

chromatography to remove aggregates and other contaminants, techniques that were well-known and commonly used by a POSA at the time. EX1004 ('319 *Publication*), 70:3-13; *see also id.*, 39:15-19; *supra* ¶¶45-52. As discussed above in paragraph 48, size exclusion chromatography was more commonly used in purification of proteins produced on a small, experimental scale, such as the production being described in the '319 *Publication*.

86. Based on all of the above points, a POSA would have had motivation and a reasonable expectation of success of achieving the method of claim 1, including wherein at least 90% of the weight of the protein is not present as an aggregate.

2. Claim 2

87. Claim 2 depends from claim 1, and adds the limitation that the “fusion protein is purified until substantially free of protein contaminants.” I note that the '226 patent does not describe methods or conditions to achieve this limitation.

88. The '226 patent notes that the phrase “substantially free of protein contaminants” means “preferably, that at least 90% of the weight of protein of the VEGF-specific fusion protein antagonist preparation used for making a formulation is VEGF fusion protein antagonist protein.” EX1001, 5:48-52. I understand the use of the term “preferably” to mean that the amounts were

intended to be in this range, but that it could also encompass amounts lower than 90%.

89. As discussed in paragraphs 77 and 85, *supra*, the '319 *Publication* teaches purification of the Flt1D2.Flk1D3.FcΔC1(a) protein using Protein A affinity chromatography and size exclusion chromatography. A POSA would understand that Protein A affinity chromatography would remove protein contaminants as Protein A binds to the Fc portion of the VEGF antagonist. *See, e.g.* EX1004 ('319 *Publication*), 38:15-17. The POSA would also understand that the size exclusion chromatography would remove any remaining protein contaminants. Thus, a POSA would understand that the purification methods of the '319 *Publication* would result in a preparation substantially free of protein contaminants as required by claim 2.

90. As discussed above, these purification methods were well-known and commonly used at the time of the priority date, and use of these techniques to remove contaminants was well within the routine skill of a POSA at the time. *See supra* ¶48. As with aggregation, a POSA would be motivated to remove as many contaminants as possible from a protein intended to be used in a therapeutic product to reduce potential adverse effects and to obtain regulatory approval. *See, supra* ¶¶47-48, 65. A target of less than 10% contaminants allows for more

contamination than a POSA would typically be aiming for when purifying a protein for therapeutic use.

91. Further, as noted above, the specification itself does not describe or exemplify how the proteins were purified to reach the claimed level of contaminants, which shows that Regeneron considered such methods to be within the ordinary skill of a POSA reading the '226 patent. *See supra* ¶¶38, 40.

92. As such, a POSA would have had motivation and a reasonable expectation of success of achieving the method of claim 2.

3. Claim 3

93. The only difference between claim 1 and claim 3 is that in claim 1 the CHO cell includes a polynucleotide encoding the VEGF protein comprising amino acids 27-457 of SEQ ID NO:2, and in claim 3, the CHO cell includes a polynucleotide encoding the VEGF protein comprising amino acids 27-457 of SEQ ID NO:4.

94. My analysis above as to how claim 1 is rendered obvious by the '319 *Publication* is set forth above in Section VI.A.1, and applies equally to claim 3.

95. The '319 *Publication* discloses a VEGF antagonist having the sequence of SEQ ID NO:2, *i.e.*, aflibercept, which it refers to by one of Regeneron's other names for this protein: VEGFR1R2.FcΔC1(a). EX1004 ('319 *Publication*), 11:14-12:1, 15:19-27, Figs. 24A-24C. Example 20 of the '319

Publication describes the construction of an expression vector for the protein, and the amino acid sequence for the protein is disclosed in Figs. 24A-24C. I conducted a sequence comparison of this sequence against SEQ ID NO. 4 of the '226 Patent and confirm that they match in their entirety, including amino acids 27-457.⁶ EX1027.

96. I note that the '319 *Publication* does not provide a specific example of preparing this VEGF antagonist in CHO cells and purifying it; however, the '319 *Publication* describes its processes as producing modified Flt1 receptors (*id.*, 67:19-20), as well as harvesting and purifying them (*id.*, 69:21-22). The '319 *Publication* teaches that the same methods are being used to prepare and purify the unmodified Flt(1-3)-Fc using CHO cells. *Id.*, 36:21-39:19. Additionally, the '319 *Publication* specifically discloses CHO cells that transiently express VEGFR1R2-FcΔC1(a) (*id.*, 82:11-13) and as discussed above, a POSA would have been aware at the time that Regeneron had used CHO cells to prepare aflibercept, *i.e.*, the protein in claim 3, as well as purifying it. *See supra* ¶¶63-65.

⁶ I conducted this comparison by extracting the sequences from the patent and aligning them using an online tool available from the European Bioinformatics Institute.

97. For these reasons and those discussed above for claim 1, a POSA therefore would have had a reasonable expectation of success of producing VEGFR1R2-FcΔC1(a) (SEQ ID NO: 4, aflibercept) in accordance with the method of claim 3.

4. Claim 4

98. Claim 4 is dependent on claim 3, and adds the limitation that the “VEGF antagonist is posttranslationally glycosylated at one or more asparagine residues.” The ’226 patent does not disclose any methods of glycosylating a protein beyond producing the VEGF antagonist fusion protein in CHO cells.

99. At the time of invention, the POSA understood that expression of proteins in CHO cells provided for N-linked glycosylation of the protein. *See supra* ¶¶66-69. That understanding is also consistent with the disclosures in the ’226 patent specification, which states that “the VEGF antagonist is expressed in a mammalian cell line such as CHO cells and may be modified posttranslationally... [and in an] embodiment the fusion protein ... is glycosylated at Asn residues 62, 94, 149, 222, and 308.” EX1001, 5:37-42. In other words, glycosylation is achieved by expressing Fc fusion proteins in CHO cells.

100. Although the ’319 *Publication* does not expressly disclose glycosylation at asparagine residues on the aflibercept protein, it does disclose that glycosylation was observed at five asparagine residues on the

Flt1D2.Flk1D3.FcΔC1 protein, which as discussed above has a similar structure to aflibercept. *Id.*, Example 28; *see supra* ¶61, n.4. “Complete glycosylation is observed at Asn33, Asn193, and Asn282. Partial glycosylation is observed on Asn65 and Asn 120.” *Id.*, 26:6-9; 81:20-82:2; Fig. 36. One site of complete glycosylation is Asn282, which has the sequence NST, as shown in Fig. 36. *Id.*, 81:20-82:2; Fig. 36. The first amino acid in Figure 36 of the ‘319 *Publication* starts at the amino acid “G” of the sequence “GRPFV,” corresponding to the “G” at amino acid 27 of Figure 21A. *Compare* Fig. 36 with Fig. 21A. Thus, Asn (N) 282 of Figure 36 corresponds to Asn (N) 308 of Fig. 21B.

101. A POSA would have had a reasonable expectation from the disclosure of the similar structures of this variant and aflibercept in the ‘319 *Publication*, along with both proteins being expressed in CHO cells and purified by the same methods, that aflibercept would similarly be post-translationally glycosylated at one or more asparagine residues. The Fc portion of Flt1D2.Flk1D3.FcΔC1(a) is the same Fc portion of VEGFR1R2-FcΔC1(a), accordingly, the POSA would also reasonably expect to also obtain N-linked glycosylation at this location in VEGFR1R2-FcΔC1(a) (SEQ ID NO: 4) grown in CHO cells.

102. Further, as discussed above, a POSA would have known from his or her general knowledge at the time that Fc fusion proteins almost always had some level of glycosylation, particularly when expressed in CHO cells, and that

glycosylation of proteins was essential for certain functions of the Fc region. *See supra* ¶¶66-69. This knowledge, combined with the fact that the very similar Flt1D2.Flk1D3.FcΔC1 protein produced by the same methods showed glycosylation at a number of asparagine residues, would have given a POSA motivation to produce a protein with similar glycosylation at asparagine residue sites with a reasonable expectation of success.

103. The mapping techniques described in the *'319 Publication* to identify glycosylation sites for Flt1D2.Flk1D3.FcΔC1 (*e.g.*, EX1004, Example 28) were well within the ordinary skill of a POSA at the time and could have been easily applied to aflibercept.

104. As such, a POSA would have had a reasonable expectation of success of producing VEGFR1R2-FcΔC1(a) (SEQ ID NO: 4, aflibercept), wherein at least one of the asparagine residues is glycosylated, in accordance with the method of claim 4.

B. Claims 1-4 are Obvious over the combination of the *'319 Publication*, and *Fahrner*.

105. As discussed above, it is my opinion that the *'319 Publication* (EX1004) renders the methods of claims 1-4 obvious. But to the extent that the *'319 Publication* fails to render obvious the limitation that at least 90% of the weight of the protein is not present as an aggregate, or that the protein is substantially free of contaminants, any deficiency is remedied by *Fahrner*

(EX1005). *Fahrner* was published in July 2001, and therefore was a printed publication available prior to the '226 patent's filing date of March 22, 2006.

1. Claims 1 and 3

106. As discussed above, the '319 *publication* discloses the VEGF antagonist produced by a polynucleotide encoding the VEGF protein including amino acids 27-457 of SEQ ID NO: 2 (Flt1D2.Flkl1D3.FcΔC1, claim 1), which is the same as the sequence as disclosed in Figs. 21A-21C. *See supra* Section VI.A.1. The '319 *Publication* also discloses the VEGF antagonist produced by a polynucleotide encoding the VEGF protein including amino acids 27-457 of SEQ ID NO: 4 (aflibercept, claim 3), which is the same as the sequence as disclosed in Figs. 24A-24C. *See supra* Section VI.A.3. As discussed above, the '319 *Publication* also teaches that these VEGF antagonists are produced in CHO cells and purified using standard purification techniques. *See supra* Sections VI.A.1 and VI.A.3.

107. *Fahrner* discloses that the most clinically significant antibodies are produced in CHO cells, as the level of product-related variants can be effectively controlled during cell culture to ensure a streamlined purification process where little or no variants are required to be removed. EX1005 (*Fahrner*), 301-302.

108. *Fahrner* would have not only motivated a POSA to purify the VEGF antagonist fusion protein to at least a level wherein at least 90% of the weight of

the purified fusion protein is not present as an aggregate, but a POSA would in actuality aim for a much lower level of aggregates. According to *Fahrner*, unless the stringent purity requirements for biological pharmaceuticals are met, there can be no approved therapeutic product, and as such, the primary consideration of purification is purity. *Id.*, 303. *Fahrner* states that aggregates in particular are the most common product-related variant that must be removed during the purification process because of possible immunogenicity. *Id.*, 304. *Fahrner* notes that the typical aggregate content of harvested cell culture fluid (“HCCF”) is 5-15% for most antibodies, and is then typically reduced through purification to below 0.5% in the final bulk. *Id.*, 304, 306, Table 12.1.

109. *Fahrner* specifically teaches a purification process using Protein A affinity chromatography to remove host cell proteins, DNA, and endotoxins, followed by cationic exchange chromatography to remove aggregates and host cell protein, and anionic exchange chromatography to remove DNA and residual host cell proteins. *Id.*, 305-308.

110. Although the '319 *Publication* and *Fahrner* disclose somewhat different purification methods (both use Protein A affinity chromatography (protein A binds to the Fc region), but the '319 *Publication* then uses size exclusion chromatography, whereas *Fahrner* then uses a combination of anion and cation exchange chromatography), all of these methods were well-known and

commonly used at the time, and the POSA would understand that each purification method has the same goal—removing aggregated forms of the proteins and reducing contaminants. As discussed in paragraph 48, *supra*, a POSA would understand that size exclusion chromatography was more common in purification on a smaller scale, while the techniques described in *Fahrner* were more commonly used for large scale manufacturing.

111. Because *Fahrner* teaches that the primary consideration of purification is purity, the POSA would not only have purified the VEGF antagonist protein to at least wherein 90% of the weight of the protein is not present as an aggregate, i.e. less than 10% aggregates, but in actuality, as taught by *Fahrner*, a POSA would aim to reduce it even further to ensure very low levels of aggregation in the bulk product to be used to formulate a product. EX1005 (*Fahrner*), 302, 304.

112. As discussed above, the '226 specification does not teach any particular methods or conditions for the purification process, and the purification methods in *Fahrner* would have been well-known to the POSA at the relevant time period and well within their ordinary skill. *See supra* ¶¶38, 40, 45-52. As such a POSA would have had a reasonable expectation of success of achieving a preparation wherein at least 90% of the weight of the protein is not present as an aggregate.

113. Based on all of the above, and my analysis outlined in Sections VI.A.1 and VI.A.3 above, claims 1 and 3 would have been obvious over the combination of the '*319 Publication* and *Fahrner*.

2. Claim 2

114. As discussed in Section VI.A.2, it is my opinion that the '*319 Publication* renders claim 2 obvious. However, to the extent that the '*319 Publication* may be seen as not rendering obvious the limitation that the VEGF antagonist protein is substantially free of protein contaminants, *Fahrner* renders this limitation obvious.

115. My opinion set forth above in Section VI.B.1 as to why the POSA would have been motivated by *Fahrner* to reduce aggregates of the VEGF antagonist fusion protein, applies equally as to why the POSA would have been motivated to reduce protein contaminants. That is, given the overarching goal of purity and the strict requirements for the same imposed by regulatory authorities, a POSA would have understood that the protein contaminants in the purified protein preparation intended to be used to make a therapeutic product should be as low as possible. EX1005 (*Fahrner*), 303. As *Fahrner* notes, Protein A chromatography, a step which both *Fahrner* and the '*319 Publication* teach, removes host cell proteins. *Id.*, 306. The '*319 Publication* teaches that its use of size exclusion chromatography also removes contaminants along with aggregates (EX1004,

39:15-18), and *Fahrner* teaches that the cation exchange chromatography and anion exchange chromatography purification steps also remove protein contaminants (EX1005, 307-308). Thus, *Fahrner*, along with the '319 *Publication*, provides a motivation to purify the VEGF antagonist protein until it is substantially free of protein contaminants, and a reasonable expectation of success in doing so.

116. Accordingly, claim 2 is obvious over the combination of the '319 *Publication* and *Fahrner*.

3. Claim 4

117. As set out above in Section VI.A.4, it is my opinion that claim 4 is rendered obvious by the '319 *Publication* given its teachings regarding glycosylation of the VEGF antagonists and the POSA's knowledge at the time. The same reasoning set out for claim 4 above, as well as the analysis of claim 3 above, applies equally here.

118. Accordingly, claim 4 is obvious over the combination of the '319 *Publication* and *Fahrner*.

C. Claims 3-4 are Obvious over the Combination of the '319 *Publication*, *Holash*, *Wulff*, and *Fahrner*

119. As discussed above, it is my opinion that the '319 *Publication* alone, as well as in combination with *Fahrner* renders the methods of claims 1-4 obvious. *See supra* Sections VI.A. and VI.B. To the extent that the '319 *Publication* is seen

not to provide a reason to produce SEQ ID NO:4 (VEGFR1R2-FcΔC1(a), aflibercept) by expression in CHO cells and purification, any deficiency is remedied by *Holash* (EX1006) and *Wulff* (EX1007). *Holash* was published in August 2002 and *Wulff* was published in July 2002; therefore both were printed publications available prior to the '226 patent's filing date of March 22, 2006.

1. Claim 3

120. Independent claim 3 recites a method of manufacturing a VEGF antagonist fusion protein where the method includes the following: (1) expressing the protein in a CHO cell; (2) the CHO cell includes a polynucleotide encoding the VEGF protein identified by amino acids 27-457 of SEQ ID NO: 4 (i.e. aflibercept); (3) the fusion protein binds VEGF; (4) purifying the protein; and (5) at least 90% of the weight of the protein is not present as aggregates.

121. As discussed above, the '319 *Publication* and *Fahrner* together disclose all the elements of claim 3 and render it obvious. *See supra* Sections VI.A.3 and VI.B.1.

122. Additionally, both *Holash* and *Wulff* teach that aflibercept was produced by expression in CHO cells and purified before administration *in vivo*, and disclose its superior binding activity and pharmacokinetic profile over other VEGF antagonists. EX1006 (*Holash*), 11394-95; EX1007 (*Wulff*), 2798, 2804-06. The disclosures in these two publications would have further motivated a POSA to

develop aflibercept for therapeutic use by using the method described in the '319 *Publication*.

123. *Wulff* teaches the administration of aflibercept to marmoset monkeys to assess its effects on angiogenesis.⁷ *Id.*, 279. *Wulff* states that the VEGF trap used in the experiments, which is VEGF Trap R1R2 (aflibercept), “was expressed in CHO cells and was purified by Protein A affinity chromatography followed by size-exclusion chromatography.” EX1007 (*Wulff*), 2798. *Wulff* further cites to the '319 *Publication* (EX1004) to describe the VEGF antagonist used, stating that “the detailed molecular structure and how it was created are described in the patent REG 710-A-PCT, VEGF Trap Application published December 2000, Publication WO 00/75319 A1.” EX1007 (*Wulff*), 2798, n.1.

124. *Wulff* teaches that administration of aflibercept resulted in “a significant decrease in thecal proliferation and endothelial cell area, demonstrating the suppression of thecal angiogenesis.” *Id.*, 2797. It also discloses that

⁷ A POSA would have understood that the “vehicle” referred to in *Wulff* contained aflibercept for the test animals and no aflibercept for the control animals, since the aflibercept could not have been injected into the animals unless it was dissolved within the vehicle.

aflibercept “may be more efficient in inhibiting VEGF” than previously tested VEGF antagonists. *Id.*, 2804.

125. *Holash* details Regeneron’s *in vivo* experiments with VEGF Trap R1R2 (aflibercept) in mice. It discloses that “[a]ll of the VEGF-Trap variants were produced and purified from Chinese hamster ovary cells.” EX1006 (*Holash*) 11393-94. *Holash* explains that VEGFTrap_{R1R2} (aflibercept) “was designed to overcome the poor *in vivo* pharmacokinetic properties of an earlier soluble VEGF decoy receptor,” and that the VEGF Trap has “markedly enhanced pharmacokinetic properties” and “effectively suppresses tumor growth and vascularization *in vivo*.” *Id.*, 11393.

126. In *Holash*, Regeneron’s scientists had expressly acknowledged aflibercept’s promising potential and that it was “one of the most, if not the most, potent and efficacious VEGF blocker available” and that it was in human clinical trials as a result. *Id.*, 11397. Further, *Holash* teaches that aflibercept compared favorably to other non-Regeneron VEGF antagonist human therapeutics that had been approved by FDA, such as monoclonal antibodies like bevacizumab and ranibizumab. *Id.* As Regeneron’s scientists put it, “[t]he combination of high-affinity and improved pharmacokinetics apparently contributes toward making VEGF-Trap_{R1R2} [aflibercept] one of the most, if not the most, potent and efficacious VEGF blocker available.” *Id.*

127. Further, Regeneron noted in *Holash* that aflibercept had the additional advantage of being composed of “entirely human sequences,” which would “hopefully minimiz[e] the possibility that it might prove immunogenic in human patients.” *Id.* In comparison to existing antibody VEGF antagonists, “far lower circulating levels of VEGF-Trap_{R1R2} [aflibercept] are required for similar efficacy” and its “safety has recently been confirmed in toxicological studies in cynomologus monkeys.” *Id.*

128. As a result of these superior properties, Regeneron disclosed in *Holash* that “the [aflibercept] VEGF-Trap is currently in human clinical trials for several different types of cancer.” *Id.*

129. A POSA seeking to reproduce the performance of aflibercept that Regeneron observed in *Holash* and *Wulff* would also have used CHO cells, not only given their known benefits at the time, but since (1) there would have been no reason to choose a different mammalian cell type than the one Regeneron had used to produce the promising results; (2) expression in a different mammalian cell type carried at least some risk that there might be some different post-translational processing of the aflibercept than in the CHO cells, which in turn could lead to changes in the protein’s performance, and (3) expression in a different mammalian cell type carried at least some risk of producing additional host cell protein and/or DNA impurities, and a POSA would wish to avoid this risk as much as possible by

sticking to a proven method that was already shown to have worked (*i.e.*, cultivation in CHO cells).

130. Given the clear potential and promising results for aflibercept that Regeneron had published in both *Holash* and *Wulff*, a POSA would have been motivated to manufacture aflibercept for use in a therapeutic product in humans and animals by the same process it described in these references and the '*319 Publication*, *i.e.* by expression in CHO cells and purification using common techniques.

131. Accordingly, a POSA would have been motivated to combine the teachings in the '*319 Publication*, *Holash*, *Wulff*, and *Fahrner*, to arrive at the claimed manufacturing method of claim 3 with a reasonable expectation of success. Claim 3 is therefore obvious over this combination.

2. Claim 4

132. Claim 4 depends from claim 3 and further requires that the protein is post-translationally glycosylated at one or more asparagine residues.

133. I provide my opinion in Section VI.A.4 above as to why the '*319 Publication* renders obvious the additional limitation of claim 4, as well my analysis of claim 3 above in Section VI.C.1, and that analysis applies equally here.

134. For those reasons, a POSA would have been motivated to combine the teachings in the '*319 Publication*, *Holash*, *Wulff*, and *Fahrner*, to arrive at the

claimed manufacturing method of claim 4 with a reasonable expectation of success. Claim 4 is therefore obvious over this combination.

VII. SECONDARY EVIDENCE OF NON-OBVIOUSNESS

135. I am not aware of any relevant “secondary” evidence that would relate to the particular elements of the claims of the ’226 patent. I may respond to any secondary evidence I may become aware of in the future.

VIII. AUTHENTICATION OF EXHIBITS

136. In preparing my declaration I downloaded a guidance document that was available publicly on the FDA’s website. In my experience, the dates that these types of FDA documents were publicly available corresponds with the publication dates given on FDA’s website. EX1020 is a copy of the publicly available FDA Guidance Document titled “Points to Consider in the Manufacture and Testing of Monoclonal Antibody Products for Human Use.”⁸ The FDA website includes a date of “February 1997” below the title of the document, and the document itself shows a date of February 28, 1997, on its title page.

⁸ Available at <https://www.fda.gov/regulatory-information/search-fda-guidance-documents/points-consider-manufacture-and-testing-monoclonal-antibody-products-human-use>

137. Therefore, this guidance document was available more than a year prior to the '226 patent priority date.

IX. CONCLUDING STATEMENTS

138. In signing this declaration, I understand that the declaration will be filed as evidence in a contested case before the Patent Trial and Appeal Board of the United States Patent and Trademark Office. I acknowledge that I may be subject to cross-examination in this case and that cross-examination will take place within the United States. If cross-examination is required of me, I will appear for cross-examination within the United States during the time allotted for cross-examination.

139. I declare that all statements made herein of my knowledge are true, and that all statements made on information and belief are believed to be true, and that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code.

Dated: February 23, 2023

By: 
Peter M Tessier, Ph.D.

X. APPENDIX - LIST OF EXHIBITS CITED

Exhibit No.	Description
1001	U.S. Patent No. 10,406,226 (“226 Patent”)
1003	Dr. Peter Tessier <i>curriculum vitae</i>
1004	International Publication No. WO 00/75319 A1 to Papadopoulos et al. (“319 Publication”)
1005	Robert Fahrner et al., <i>Industrial Purification of Pharmaceutical Antibodies: Development, Operation, and Validation of Chromatography Processes</i> , 18:1 Biotechnology and Genetic Engineering Reviews 301-327 (Jul. 2001) (“Fahrner”)
1006	Jocelyn Holash et al., <i>VEGF-Trap: A VEGF Blocker with Potent Antitumor Effects</i> , 99 (17) PNAS 11393-11398 (Aug. 20, 2002) (“Holash”)
1007	Christine Wulff et al., <i>Prevention of Thecal Angiogenesis, Antral Follicular Growth, and Ovulation in the Primate by Treatment with Vascular Endothelial Growth Factor Trap R1R2</i> , 143(7) Endocrinology 2797-2807 (Jul. 2002) (“Wulff”)
1009	Florian Wurm, <i>Production of recombinant protein therapeutics in cultivated mammalian cells</i> , 22 (11) Nature Biotechnology 1393-1398 (Nov. 2004) (“Wurm”)
1010	Chang, B.S. and Hershenson, S., <i>Practical Approaches to Protein Formulation</i> , Integritybio (2002) in “Rationale Design of stable formulations-theory and practices” (J.F. Carpenter and M.C. Manning eds.) Kluwer Academic/Plenum publishers, New York, pp. 1-25 (“Chang”)
1011	Napoleone Ferrara and Robert Kerbel, <i>Angiogenesis as a therapeutic target</i> , 438 (15) Nature Publishing Group 967-974 (2005) (“Ferrara”)
1012	J.S. Rudge et al., <i>VEGF Trap as a Novel Antiangiogenic Treatment Currently in Clinical Trials for Cancer and Eye Diseases, and VelociGene®-based Discovery of the Next</i>

	<i>Generation of Angiogenesis Targets</i> , LXX (70) Cold Spring Harbor Laboratory Press 411-418 (2005) (“Rudge”)
1013	Portions of prosecution history for U.S. Patent No. 10,406,226 (“’226 prosecution history”)
1014	U.S. Patent No. 7,374,758 (“’758 Patent”)
1015	U.S. Patent No. 7,374,758 patent term extension correspondence (“’758 patent term extension”)
1017	U.S. Publication No. 2004/0014667 A1 (“’667 Publication”)
1018	U.S. Publication No. 2004/0265309 A1 (“’309 Publication”)
1020	<i>Points to Consider in the Manufacture and Testing of Monoclonal Antibody Products for Human Use</i> , U.S. Department of Health and Human Services Food and Drug Administration Center for Biologic Evaluation and Research 1-50 (Feb. 28, 1997) (“FDA PTC 1997”)
1021	Royston Jefferis <i>Glycosylation of Recombinant Antibody Therapeutics</i> , 21 Biotechnology Progress 11-16 (2005) (“Jefferis”)
1022	Liming Liu <i>Antibody Glycosylation and Its Impact on the Pharmacokinetics and Pharmacodynamics of Monoclonal Antibodies and Fc-Fusion Proteins</i> , 104 Journal of Pharmaceutical Sciences 1866–1884 (2015) (“Liu”)
1023	S Krapp et al, <i>Structural Analysis of Human IgG-Fc Glycoforms Reveals a Correlation Between Glycosylation and Structural Integrity</i> , 325 (5) Journal of Molecular Biology 979-989 (2003) (“Krapp”)
1024	Y Mimura et al, <i>The role of oligosaccharide residues of IgG1-Fc in FcγIIb binding</i> , 276 Journal of Biological Chemistry 45539-45547 (2001) (“Mimura”)
1025	Sequence alignment of SEQ ID NO: 2 and SEQ ID: NO 4 from ’226 patent

1026	Sequence alignment of SEQ ID NO: 2 from '226 patent and Figs. 21A-21C of '319 Publication
1027	Sequence alignment of SEQ ID NO: 4 from '226 patent and Figs. 24A-24C of '319 Publication