

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

SAMSUNG BIOEPIS CO., LTD.,
Petitioner,

v.

REGENERON PHARMACEUTICALS, INC.,
Patent Owner.

Case No: IPR2025-00176

U.S. Patent No. 11,084,865

**PETITION FOR *INTER PARTES* REVIEW
OF U.S. PATENT NO. 11,084,865**

DECLARATION OF DR. MICHAEL BUTLER

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TABLE OF EXHIBITS

Exhibit	Description
1001	U.S. Patent No. 11,084,865
1010	Jocelyn Holash et al., VEGF-Trap: A VEGF Blocker with Potent Antitumor Effects, 99 (17) PNAS 11393-11398 (Aug. 20, 2002) (“Holash”)
1015	J.S. Rudge et al., VEGF Trap as a Novel Antiangiogenic Treatment Currently in Clinical Trials for Cancer and Eye Diseases, and VelociGene®-based Discovery of the Next Generation of Angiogenesis Targets, 70 COLD SPRING HARBOR SYMPOSIA ON QUANTITATIVE BIOLOGY 411-418 (2005) (“Rudge”)
1016	Christine Wulff et al., Prevention of Thecal Angiogenesis, Antral Follicular Growth, and Ovulation in the Primate by Treatment with Vascular Endothelial Growth Factor Trap R1R2, 143(7) ENDOCRINOLOGY 2797-2807 (Jul. 2002) (“Wulff”)
1019	U.S. Patent No. 7,374,758 to Papadopoulos et al. (“’758 Patent”)
1027	U.S. Patent Publication No. 2004/0265309 to Kandel et al. (“’309 Publication”)
1028	U.S. Patent Publication No. 2005/0112061 to Holash et al. (“’061 Publication”)
1029	International Publication No. WO 00/75319 to Papadopoulos et al. (“’319 Publication”)
1039	US2006/0058234 (“Daly”)
1040	Butler <i>Curriculum Vitae</i>
1079	Application for Extension of Patent Term to U.S. Patent No. 7,374,758. (“The ’758 PTE”)
1080	US2004/0014667 (“’667 Publication”)
1081	US20050175610 (“Wiegand”)
1082	Drickamer K, Taylor ME. Evolving views of protein glycosylation. Trends Biochem Sci. 1998 Sep;23(9):321-4. (“Drickamer”)

Exhibit	Description
1083	Kimura R, Miller WM. Glycosylation of CHO-derived recombinant tPA produced under elevated pCO ₂ . <i>Biotechnol Prog.</i> 1997 May-Jun;13(3):311-7. (“Kimura”)
1084	Baker KN, Rendall MH, Hills AE, Hoare M, Freedman RB, James DC. Metabolic control of recombinant protein N-glycan processing in NS0 and CHO cells. <i>Biotechnol Bioeng.</i> 2001 May 5;73(3):188-202. (“Baker”)
1085	Kobata A. A journey to the world of glycobiology. <i>Glycoconj J.</i> 2000 Jul-Sep;17(7-9):443-64. (“Kobata”)
1086	Dwek RA. Glycobiology: “towards understanding the function of sugars”. <i>Biochem Soc Trans.</i> 1995 Feb;23(1):1-25. (“Dwek”)
1087	L. Liu, Antibody Glycosylation and Its Impact on the Pharmacokinetics and Pharmacodynamics of Monoclonal Antibodies and Fc-Fusion Proteins, 104 <i>Journal of Pharmaceutical Sciences</i> 1866–1884 (2015). (“Liu 2015”)
1088	R. Jefferis, Glycosylation of Recombinant Antibody Therapeutics, 21 <i>Biotechnology Progress</i> 11-16 (2005). (“Jefferis”)
1089	Patent Owner Response filed in <i>Celltrion, Inc. v. Regeneron Pharmaceuticals, Inc.</i> , PTAB-IPR2023-00462 (“’992 IPR POR”)
1090	Ronin C, Granier C, Caseti C, Bouchilloux S, Van Rietschoten J. Synthetic substrates for thyroid oligosaccharide transferase. Effects of peptide chain length and modifications in the Asn-Xaa-Thr-region. <i>Eur J Biochem.</i> 1981 Aug;118(1):159-64. doi: 10.1111/j.1432-1033.1981.tb05499.x. PMID: 6793364. (“Ronin”)
1091	Imperiali B, Hendrickson TL. Asparagine-linked glycosylation: specificity and function of oligosaccharyl transferase. <i>Bioorg Med Chem.</i> 1995 Dec;3(12):1565-78. doi: 10.1016/0968-0896(95)00142-5. PMID: 8770382. (“Imperiali”)
1092	Anguita R, Tasiopoulou A, Shahid S, Roth J, Sim SY, Patel PJ. A Review of Aflibercept Treatment for Macular Disease. <i>Ophthalmol Ther.</i> 2021 Sep;10(3):413-428. doi: 10.1007/s40123-021-00354-1. Epub 2021 Jun 13. Erratum in: <i>Ophthalmol Ther.</i> 2021 Sep;10(3):429. (“Anguita”)

Exhibit	Description
1093	Hart GW. Glycosylation. Curr Opin Cell Biol. 1992 Dec;4(6):1017-23. ("Hart")

I. INTRODUCTION

1. I have been retained by Quinn Emanuel Urquhart & Sullivan on behalf of Samsung Bioepis Co., Ltd. (“Petitioner”) to provide expert opinions regarding the production of aflibercept.

2. My opinions in this declaration are based on the documents I cite along with my professional training, experience and knowledge that I have acquired working in the field of microbiology and bioprocessing. I am being compensated for my services as an expert at my standard consulting rate of \$600 per hour. My compensation is in no way contingent on the substance of my opinions or the outcome of this case.

3. My opinions and their underlying reasoning are explained in detail below.

II. BACKGROUND AND QUALIFICATIONS

4. I am a Principal Investigator at the National Institute for Bioprocessing Research and Training (NIBRT) in Ireland with over 30 years of experience in biochemical engineering and the production of biopharmaceuticals.

5. I have a Ph.D. in Biochemistry from King’s College at the University of London, U.K. I am currently a Principal Investigator in Cell Technology at NIBRT in Ireland, an Adjunct Professor at the University College Dublin, and a

Distinguished Professor Emeritus in the Department of Microbiology at the University of Manitoba, Canada. I previously served as the Chief Scientific Officer for NIBRT from 2016 to 2019, and was the Director of Mabnet, a national network for monoclonal antibody production, from 2010 to 2017.

6. I lead a laboratory where our current research focuses on Mammalian Cell Technology for the production of biopharmaceuticals, including antibodies. My research concerns many areas of drug development and manufacture, including (1) protein glycosylation, (2) development of cell culture media to maximize cell growth and production of biopharmaceuticals, (3) dielectrophoretic analysis of cells to monitor cell metabolism, (4) bioprocess development such as cell culture conditions and media formulations to optimize the production of recombinant proteins, (5) metabolic management and engineering to understand intracellular requirements during protein production, (6) cell robustness to identify cell culture factors that improve cell viability during the manufacturing process, and (7) production of viruses from anchorage-dependent cells.

7. As part of my research, I have also previously collaborated with numerous industrial entities for projects related to recombinant protein and drug manufacturing. For example, from 2010 to 2016, I collaborated with 10 different Canadian companies as part of my role as Director of MabNet to help develop and manufacture antibodies. From 2007 to 2010, I also collaborated with the National

University & National Health Research Institute of Taiwan to help develop and manufacture dengue vaccines. In fact, in 2004, I was awarded the NSERC Synergy Award for innovative research involving academic and industry collaborations for my work with Cangene Corporation.

8. I founded a company called Biogro Technologies Inc., which develops serum-free media formulations for the growth of commercially important mammalian cell lines.

9. I have served as a consultant for at least 24 companies, governments with interests in biopharmaceuticals, and other organizations, including LH Fermentation (1990-1993), Gibco (1991), Biomira (1993), Amersham Biosciences (1996-2001), Vallee/Brazil (1995 & 2003), SmithKline Beecham (1997-1998), Bayer (1998), Merck (2000, 2014), GeneMedix (2003), Industry Canada/ Federal Government (2003), M&K Engineering (2004), Serologicals (2004), Serono (2004), BayerHealthcare (2004), ML Laboratories (2004-present day), Genzyme (2005), Pfizer (2007), Novartis (2009), Spimaco (2009), Taylor-Wessing (2011), CoherusBiosciences (2014), Dechert (2015), Abbvie (2015), and Patterson Belknap (2015-2018).

10. I am also a past or current member of a number of scientific societies that focus on the production and manufacture of biopharmaceuticals, including antibodies. I am a member of the Biochemical Society, European Society for Animal

Cell Technology, Canadian Society of Microbiology, and Canadian Society of Chemical Engineering. I am also a founding member and past President of the International Society for Protein Expression.

11. I have published over 200 articles, book chapters, and textbooks related to mammalian cell culture, production of proteins and antibodies, and manufacturing processes. Several of my publications are directly relevant to the subject matter of the '205 patent and the biosimilar denosumab manufacturing process. For example, I have published on fed-batch protein production processes in CHO cells, mammalian cell metabolism, strategies for enhancement of recombinant protein production in mammalian cells, and supplements to cell culture media. To date, my work has been cited over 6000 times.

12. I have also been invited to chair numerous meetings related to the manufacturing of recombinant proteins and antibodies. For instance, I chaired the Upstream Processing session at the 2022 BioProcess International conference in Boston. I was also session chair for the "Cellular Mechanisms of protein synthesis, processing & secretion" at the 2011 ESACT in Vienna. Other session chairs I have held were directed to "Recent Development in Animal Cell Culture," "Advances in media development," and "Protein Expression in Animal Cells (PEACe)."

13. I am a named inventor on several patent applications related to methods of producing monoclonal antibodies and enhancing protein production and cell growth.

14. In sum, I have over 30 years of research and industry experience in biochemical engineering and the production of biopharmaceuticals. I have included a copy of my curriculum vitae in support of my opinions. Ex.1040, Butler CV.

III. INFORMATION RELIED UPON

15. In addition to my general knowledge gained as a result of my education and experience, I have reviewed and considered, among other things, the prior art of record discussed herein.

16. The full list of information, in addition to my professional, academic and industry experience, that I have relied upon in forming my opinions for this report is set forth throughout the report and in the Table of Exhibits.

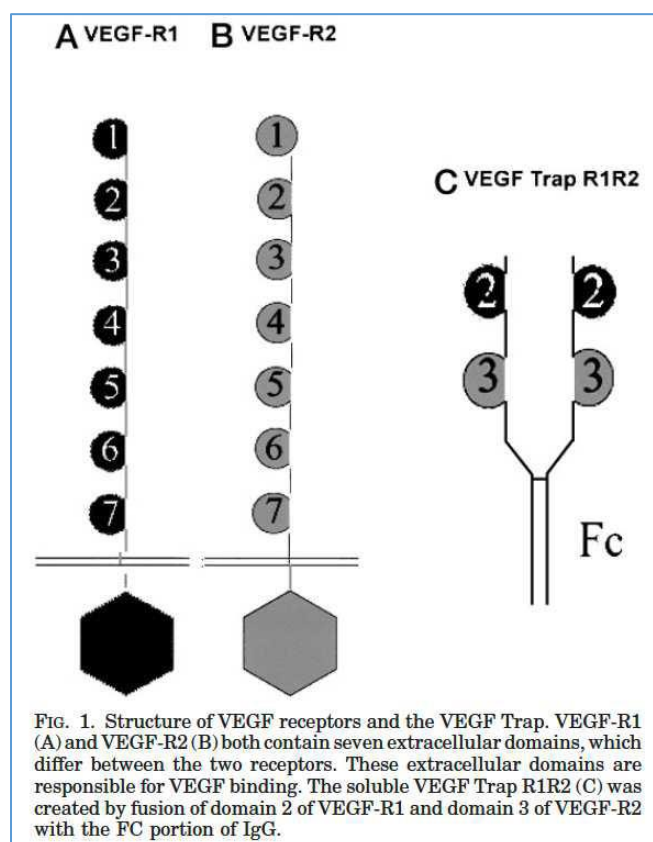
17. Where my conclusions are based on certain assumptions that I have adopted, I have stated such assumptions in this Declaration.

IV. GLYCOSYLATION OF AFLIBERCEPT

A. The Aflibercept Sequence Was Known in the Art Before March 2006

18. Aflibercept is a VEGF antagonist developed by Regeneron, and now marketed under the trade name EYLEA. Ex.1092, Anguita. Aflibercept was known at the time as a VEGF antagonist consisting of a fusion of domain 2 of the human

VEGFR1 receptor and domain 3 of the human VEGFR2 receptor. *See*, Ex.1016, Wulff, 2798, Fig. 1; Ex.1010, Holash, 11393-94. Those domains were linked via the Fc domain of a human IgG antibody as shown below:



See Ex.1016, Wulff, 2798, Fig. 1; *see also* Ex.1010, Holash, 11393-394. Aflibercept, like other anti-VEGF agents such as bevacizumab and ranibizumab known at the time, works by binding to VEGF and “trapping” it (hence the name, VEGF Trap) before it can bind to cell-surface VEGF receptors, thereby downregulating angiogenesis. *See* Ex.1010, Holash, 11393. I understand aflibercept was known

before March 2006 by several names, including “VEGF Trap” or “VEGF Trap R1R2.”¹ *See, e.g.*, Ex.1010, Holash; Ex.1016, Wulff.²

19. Regeneron widely published its development of aflibercept and related molecules as early as 2002, with details on their *in vivo* experiments, molecular structures, pharmacokinetics, and manufacturing processes. *See, e.g.*, Ex.1016,

¹ 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}.” *See* Ex.1079, ‘758 PTE, 2, 6-7. I understand that 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. The ‘758 patent is a divisional of the PCT application published as the ‘319 Publication, and Figures 24A-24C of the ‘758 patent are identical to Figures 24A-24C in the ‘319 Publication. *See*, Exs.1019 and 1029.

² Wulff is titled “Prevention of Thecal Angiogenesis, Antral Follicular Growth, and Ovulation in the Primate by Treatment with Vascular Endothelial Growth Factor Trap R1R2.” Ex.1016. It was published in the Journal of Endocrinology in July 2002, prior to the ‘865 patent’s alleged priority date of March 2006.

Wulff; Ex.1010, Holash. For example, Wulff investigated the ability of aflibercept to inhibit thecal angiogenesis. Ex.1016, 2798. Wulff noted that “[t]he VEGF Trap R1R2 used in these experiments is a recombinant chimeric protein comprising portions of the extracellular, ligand binding domains of the human VEGF receptors Flt-1 (VEGF-R1, Ig domain 2) and KDR (VEGF-R2, Ig domain 3) expressed in sequence with the Fc portion of human IgG (Fig. 1).” *Id.* Wulff further noted that “[t]he VEGF trap was expressed in CHO cells and was purified by protein A affinity chromatography followed by size-exclusion chromatography.” *Id.*

20. Wulff further states that aflibercept’s 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.” Ex.1016, 2798.

21. I have reviewed the WO00/75319 A1 (“’319 Publication”).³ The ‘319 Publication describes the development of aflibercept in more detail, including

³ The ‘319 Publication is a PCT publication published on December 14, 2000, prior to the ‘865 patent’s alleged priority date. Ex.1029, ‘319 Publication. The ‘319 Publication is therefore prior art to the ‘865 patent.

expression in CHO cells, consistent with the discussion in Wulff. *See* Ex.1029, ‘319 Publication, Examples 17-21.

22. The ‘319 Publication refers to aflibercept as VEGFR1R2-Fc Δ C1(a), another name for VEGF-TrapR1R2. *Id.*; *see also* Ex.1027, [0005], SEQ ID Nos. 1 and 2 (disclosing that VEGFR1R2-Fc Δ C1(a) is “also termed VEGF-TrapR1R2”). The ‘319 Publication discloses the amino acid sequence and structure of VEGFR1R2-Fc Δ C1(a) (aflibercept). Ex.1029, 11:14-12:1, 15:19-27, Fig. 24A-24C; *see also* Ex.1080, US2004/0014667 (“‘667 Publication”), 89, SEQ ID No. 10; *see also* Ex.1081, US20050175610 (“Wiegand”), [0085], Example 20. When aligned,⁴ the VEGFR1R2-Fc Δ C1(a) (aflibercept) sequence disclosed in the ‘319 Publication is identical to SEQ ID NO: 4 of the ‘865 patent with signal sequence at 1-26 as shown below:

⁴ The sequence comparisons included in this declaration were generated using the blastp suite of BLAST® with default alignment parameters. The tool is available at <https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE=Proteins> under the “Align two or more sequences” option.

Score	Expect	Method	Identities	Positives	Gaps
949 bits(2452)	0.0	Compositional matrix adjust.	458/458(100%)	458/458(100%)	0/458(0%)
Query 1	MVSYWDTGVLLCALLSCLLLTGSSSGSDTGRPFVEMYSEIPEIIHMTGRELVIPCRVTS				60
Sbjct 1	MVSYWDTGVLLCALLSCLLLTGSSSGSDTGRPFVEMYSEIPEIIHMTGRELVIPCRVTS				60
Query 61	PNITVTLKKFPLDTLIPDGKRIIWSRKGFIIISNATYKEIGLLTCEATVNGHLYKTNLYT				120
Sbjct 61	PNITVTLKKFPLDTLIPDGKRIIWSRKGFIIISNATYKEIGLLTCEATVNGHLYKTNLYT				120
Query 121	HRQTNTIIDVVLSPSHGIELSVGEKLVLNCTARTELVNGIDFNWEYPSSKHQHKLVNRD				180
Sbjct 121	HRQTNTIIDVVLSPSHGIELSVGEKLVLNCTARTELVNGIDFNWEYPSSKHQHKLVNRD				180
Query 181	LKTQSGSEMKKFLSTLTIDGVTRSDQGLYTCAASSGLMTKKNSTFVRVHEKDKTHTCPPC				240
Sbjct 181	LKTQSGSEMKKFLSTLTIDGVTRSDQGLYTCAASSGLMTKKNSTFVRVHEKDKTHTCPPC				240
Query 241	PAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKT				300
Sbjct 241	PAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKT				300
Query 301	KPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVY				360
Sbjct 301	KPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVY				360
Query 361	TLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSK				420
Sbjct 361	TLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSK				420
Query 421	LTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK		458		
Sbjct 421	LTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK		458		

Compare Ex.1029, 11:14- 12:1, 15:19-27, Fig. 24A-24C with Ex.1001, '865 patent, SEQ ID NO: 4. The "Query" sequence corresponds to SEQ ID NO: 4 in the '865 patent, and "Sbjct" sequence corresponds to VEGFR1R2-FcΔC1(a) listed in Figures 24A-24C of the '319 Publication. The first amino acids 1-26 form the signal sequence, which guides the protein to the endoplasmic reticulum for processing and is then cleaved by a signal peptidase to allow further maturation of the protein. Ex.1029, '319 Publication, Fig. 24A-24C. Accordingly, the mature aflibercept protein comprises amino acids 27-457 of SEQ ID NO: 4 of the '865 patent as disclosed by the '319 Publication. See Ex.1029, '319 Publication, Fig. 24A-24C.

B. The Glycosylation Sites of Aflibercept Were Disclosed and Known in the Art

23. I understand that claims 14, 22, 39, and 47 recite that aflibercept “is glycosylated at asparagine residues corresponding to asparagine residues 62, 94, 149, 222 and 308 of SEQ ID NO: 4.” These five glycosylation sites of aflibercept claimed by the ‘865 patent were well known in the art by at least March 16, 2006.

24. Specifically, US2006/0058234 (“Daly”)⁵ disclosed the glycosylation sites of SEQ ID NO: 8, which is identical to the aflibercept sequence SEQ ID NO: 4 recited by the ‘865 patent. *Compare* Ex.1039, Daly, SEQ ID NO:8 with Ex.1001, ‘865 patent, SEQ ID NO:4; *see also* Ex.1039, Daly, [0013]. As shown below, I have aligned the Daly sequence with SEQ ID NO: 4 of the ‘865 patent and confirmed their identity.

⁵ I understand that Daly published on March 16, 2006 before the alleged priority date of the ‘865 patent and constitutes prior art.

Score	Expect	Method	Identities	Positives	Gaps
949 bits(2452)	0.0	Compositional matrix adjust.	458/458(100%)	458/458(100%)	0/458(0%)
Query 1		MVSYWDTGVLLCALLSCLLLTGSSSGSDTGRPFVEMYSEIPEIIHMTGRELVIPCRVTS			60
Sbjct 1		MVSYWDTGVLLCALLSCLLLTGSSSGSDTGRPFVEMYSEIPEIIHMTGRELVIPCRVTS			60
Query 61		PNITVTLKKFPLDTLIPDGKRIIWDNRKGFIIISNATYKEIGLLTCEATVNGHLYKTNLYT			120
Sbjct 61		PNITVTLKKFPLDTLIPDGKRIIWDNRKGFIIISNATYKEIGLLTCEATVNGHLYKTNLYT			120
Query 121		HRQNTIIDVVLSPSHGIELSVGEKLVNCTARTELVGIDFNWEYPSSKHQHKLVNRD			180
Sbjct 121		HRQNTIIDVVLSPSHGIELSVGEKLVNCTARTELVGIDFNWEYPSSKHQHKLVNRD			180
Query 181		LKTQSGSEMKKFLSTLTIDGVTRSDQGLYTCAASSGLMTKKNSTFVRVHEKDKTHTCPPC			240
Sbjct 181		LKTQSGSEMKKFLSTLTIDGVTRSDQGLYTCAASSGLMTKKNSTFVRVHEKDKTHTCPPC			240
Query 241		PAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKT			300
Sbjct 241		PAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKT			300
Query 301		KPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVY			360
Sbjct 301		KPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVY			360
Query 361		TLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSK			420
Sbjct 361		TLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSK			420
Query 421		LTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK	458		
Sbjct 421		LTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK	458		

See, e.g., Ex.1039, Daly, SEQ ID NO:8, ‘865 patent, SEQ ID NO: 4. The “Query” sequence corresponds to SEQ ID NO: 4 of the ’865 patent, and “Sbjct” sequence corresponds to SEQ ID NO: 8 in Daly.

25. Daly teaches that when this sequence is expressed in a mammalian cell line such as a CHO cell, it “comprises amino acids 27-457 of SEQ ID NO: 8 and is glycosylated at Asn residues 62, 94, 149, 222 and 308.” Ex.1039, Daly, More specifically, Daly describes in Example 8 that the aflibercept sequence was expressed in CHO cells and “analyzed further to determine glycosylation patterns and oligosaccharide content.” *Id.*, at [0064]. As reported, the results of that analysis confirmed that the sequence was “glycosylated at the Asn residues of positions 62, 94, 149, 222, and 308.” *Id.*; see also [0080-0084]. These are the same five

glycosylation sites recited by the claims of the '865 patent. Therefore, the five glycosylation sites claimed by the '865 patent were disclosed and known in the art by at least March 16, 2006.

C. A POSA Would Have Expected That Aflibercept Expressed In CHO Cells Would Be Glycosylated at Positions 62, 94, 149, 222, and 308

26. Even assuming that the glycosylation sites of aflibercept were not explicitly disclosed in the prior art, the glycosylation sites of aflibercept expressed in CHO cells as described in Wulff would have been obvious to a POSA.

27. First, glycosylation refers to the process by which “glycans” are created, altered, and attached to proteins. *See, e.g.,* Ex.1082, Drickamer. Among post-translation modifications, protein glycosylation is common, particularly for proteins expressed in CHO cells. *See, e.g.,* Ex.1083, Kimura; Ex.1084, Baker.

28. There are two primary forms of glycosylation: O-linked glycosylation and N-linked glycosylation. *See generally,* Ex.1085, Kobata. O-linked glycosylation refers to glycans attached to the side-chain oxygen atoms of hydroxyl amino acids, particularly serine and threonine residues. O-linked glycosylation does not have a strict consensus sequence, but it commonly occurs in mucin-type domains or serine/threonine-rich regions, where sugars like N-acetylgalactosamine (GalNAc) are first attached to serine or threonine residues, followed by the addition of other sugar units to form more complex structures. *See generally,* Ex.1086, Dwek; Ex.

1093, Hart. Because the claims of the '865 patent specifically require glycosylation at asparagine residues, and not serine or threonine residues, I have been asked to focus my analysis on N-linked glycosylation.

29. N-linked glycosylation refers to glycans attached to the side-chain nitrogen atoms of asparagine residues. *See*, Ex.1086, Dwek, 688-689. N-linked glycosylation occurs based on a specific motif in which a glycan is attached to the amine of an asparagine (Asn) residue in 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. *See Id.*; *see also* Ex.1087; Liu, 1867; *see also* Ex.1088, Jefferis, 13.

30. In other words, a POSA familiar with the aflibercept sequence, which as noted above was well-known, would have been able to identify the N-linked glycosylation sites by recognizing the “asparagine-X-serine/threonine” motif within the sequence. There are, in total, five positions in the aflibercept sequence satisfying the N-linked glycosylation motif – asparagine residues at 62, 94, 149, 222, and 308 of SEQ NO: 4 recited in the '865 patent claims. In the figure below, I have highlighted the N-linked glycosylation motifs within the known aflibercept sequence, where a POSA would have expected at least some level of glycosylation.

Sbjct	1	MVSYWDTGVLLCALLSCLLLTGSSSGSDTGRPFVEMYSEIPEIIHMTGRELVIPCRVTS	60
Sbjct	61	P ^{NIT} VTLKKFPLDTLIPDGKRIIWDSRKGFIIS ^{NAT} YKEIGLLTCEATVNGHLYKTNYLT	120
Sbjct	121	HRQTNTIIDVVLSPSHGIELSVGEKLV ^{NCT} ARTELVGIDFNWEYPSSKHQHKLVNRD	180
Sbjct	181	LKTQSGSEMKKFLSTLTIDGVTRSDQGLYTCAASSGLMTKK ^{NST} FVRVHEKDKTHTCPPC	240
Sbjct	241	PAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKT	300
Sbjct	301	KPREEQY ^{NST} YRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVY	360
Sbjct	361	TLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPVLDSDGSFFLYSK	420
Sbjct	421	LTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK	458

31. The glycosylation sites of a related VEGF trap molecule with an amino acid sequence nearly identical to aflibercept, as reported in the ‘319 Publication, would have further reinforced a POSA’s expectation of glycosylation of aflibercept at the five identified sites.

32. For example, in addition to describing VEGFR1R2-FcΔC1(a) (aflibercept), the ‘319 Publication details the characterization of a related VEGF trap molecule called Flt1D2.Flk1D3.FcΔC1(a). Ex.1029, ‘319 Publication. Flt1D2.Flk1D3.FcΔC1(a) differs from VEGFR1R2-FcΔC1(a) (aflibercept) in that three amino acids corresponding to amino acids 27-29 of VEGFR1R2-FcΔC1(a) (aflibercept) were removed from Flt1D2.Flk1D3.FcΔC1(a) (Figures 21A-21C)—SDT—and three amino acids have been added between amino acid positions 228 and 232 of Flt1D2.Flk1D3.FcΔC1(a). *See*, Ex.1029, at 67:7-12. As acknowledged by Regeneron, “[t]he POSA would recognize that these differences are in the linking sequences between the R1 and R2 domains.” Ex.1089, ‘992 IPR Patent Owner Response, 25. I provide below an alignment of Flt1D2.Flk1D3.FcΔC1(a) and

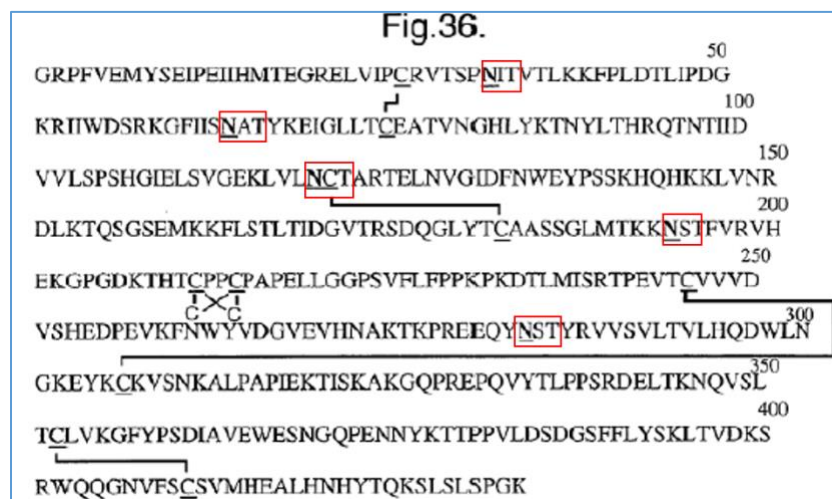
VEGFR1R2-FcΔC1(a) (afibercept) submitted by Regeneron with the differences highlighted in yellow, where the “Query 1” sequence corresponds to Flt1D2.Flk1D3.FcΔC1(a) and the “Sbjct 1” sequence corresponds to VEGFR1R2-FcΔC1(a) (afibercept):

Score	Expect	Method	Identities	Positives	Gaps
930 bits(2404)	0.0	Compositional matrix adj.	455/461(99%)	455/461(98%)	6/461(1%)
Query 1	MVSYWDTGVLLCALLSCLLLTGSSSG--GRPFVEMYSEIPEIIHMTGRELVIPCRVTS				57
Sbjct 1	MVSYWDTGVLLCALLSCLLLTGSSSGSDTGRPFVEMYSEIPEIIHMTGRELVIPCRVTS				60
Query 58	PNITVTLKKFPLDTLIPDGKRIIWSRKGFIISNATYKEIGLLTCEATVNGHLYKTNLYT				117
Sbjct 61	PNITVTLKKFPLDTLIPDGKRIIWSRKGFIISNATYKEIGLLTCEATVNGHLYKTNLYT				120
Query 118	HRQTNTIIDVWLSPSHGIELSVGEKLVNCTARTELNVGIDFNWEYPSSKHQHKLVNRD				177
Sbjct 121	HRQTNTIIDVWLSPSHGIELSVGEKLVNCTARTELNVGIDFNWEYPSSKHQHKLVNRD				180
Query 178	LKTQSGSEMKKFLSTLTIDGVTRSDQGLYTCAASSGLMTKKNSTFVRVHEKGPGDKHTHC				237
Sbjct 181	LKTQSGSEMKKFLSTLTIDGVTRSDQGLYTCAASSGLMTKKNSTFVRVHEKDKHTHC				237
Query 238	PPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHN				297
Sbjct 238	PPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHN				297
Query 298	AKTKPREEQYNSTYRVSVLTVLHQDWLNGKEYCKVSNKALPAPIEKTISKAKGQPREP				357
Sbjct 298	AKTKPREEQYNSTYRVSVLTVLHQDWLNGKEYCKVSNKALPAPIEKTISKAKGQPREP				357
Query 358	QVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFL				417
Sbjct 358	QVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFL				417
Query 418	YSKLTVDKSRWQQGNVFCSCVMHEALHNHYTQKSLSLSPGK			458	
Sbjct 418	YSKLTVDKSRWQQGNVFCSCVMHEALHNHYTQKSLSLSPGK			458	

Ex.1089, 25.

33. The '319 Publication further discloses that “[t]here are five possible N-linked glycosylation sites in Flt1D2.Flk1D3.FcΔC1(a). All five of them are found to be glycosylated to varying degrees. Complete glycosylation was observed at Asn33 (amino acid sequence NIT), Asn193 (amino acid sequence NST), and Asn282 (amino acid sequence NST). In addition, partial glycosylation is observed on Asn65

and Asn120.” Ex.1029, ’319 Publication, 81-82 (Example 28); Ex.1081, Wiegand at ¶¶108-110, Example 28.⁶ Fig. 36 of the ’319 Publication, reproduced below, shows a peptide mapping sequence that identifies the glycosylation sites for Flt1D2.Flk1D3.FcΔC1(a) with red boxes indicating the glycosylated asparagine residues.



Ex.1029, ’319 Publication, Figure 36.

34. These map to the *same* five glycosylation sites expected for the aflibercept sequence. I provide below an alignment of Flt1D2.Flk1D3.FcΔC1(a) and VEGFR1R2-FcΔC1(a) (aflibercept) with the overlapping glycosylation sites

⁶ According to the ’319 Publication, Flt1D2.Flk1D3.FcΔC1(a) was “CHO cell transiently expressed.” Ex.1029, 77:10-15. Accordingly, the signal sequence at 1-26 is cleaved, as reflected in Figure 36 below.

noted in red boxes, where the “Query 1” sequence corresponds to Flt1D2.Flk1D3.FcΔC1(a) and the “Sbjct 1” sequence corresponds to VEGFR1R2-FcΔC1(a) (aflibercept):

Score	Expect	Method	Identities	Positives	Gaps
930 bits(2404)	0.0	Compositional matrix adjust.	455/461(99%)	455/461(98%)	6/461(1%)
Query 1	MVSYNDTGVLICALLSCLLTGSSSG--GRPFVEMYSEIPEIIMTEGRELVIPCRVTS				57
Sbjct 1	MVSYNDTGVLICALLSCLLTGSSSGSDGRPFVEMYSEIPEIIMTEGRELVIPCRVTS				60
Query 58	PNITVTLLKKFPLDTLIPDGKRIWDSRKGFIIISNATYKEIGLLTCEATVNGHLYKTNVLT				117
Sbjct 61	PNITVTLLKKFPLDTLIPDGKRIWDSRKGFIIISNATYKEIGLLTCEATVNGHLYKTNVLT				120
Query 118	HRQTNTIIDVVLSPSHGIELSVGEKLVLCARTELNVGIDFNWEYPSSKHQHKLVNRD				177
Sbjct 121	HRQTNTIIDVVLSPSHGIELSVGEKLVLCARTELNVGIDFNWEYPSSKHQHKLVNRD				180
Query 178	LKTQSGSEMKKFLSTLTIDGVTSDQGLYTCAASSGLMTKKNSTFVRVHEKGPDKTHTC				237
Sbjct 181	LKTQSGSEMKKFLSTLTIDGVTSDQGLYTCAASSGLMTKKNSTFVRVHEK--DKTHTC				237
Query 238	PPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHN				297
Sbjct 238	PPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHN				297
Query 298	AKTKPREEQYNISTYRVVSVLTVLHQDWLNGKEYCKVSNKALPAPIEKTISKAKGQPREP				357
Sbjct 298	AKTKPREEQYNISTYRVVSVLTVLHQDWLNGKEYCKVSNKALPAPIEKTISKAKGQPREP				357
Query 358	QVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFL				417
Sbjct 358	QVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFL				417
Query 418	YSKLTVDKSRWQGNVFCSCVMHEALHNIHYTQKSLSLSPGK			458	
Sbjct 418	YSKLTVDKSRWQGNVFCSCVMHEALHNIHYTQKSLSLSPGK			458	

35. Because glycosylation primarily depends on the presence of conserved N-linked motifs, and the variations in linker sequences do not implicate these motifs, a POSA would have considered the difference in amino acid sequence between aflibercept and Flt1D2.Flk1D3.FcΔC to be insignificant with respect to glycosylation and, at a minimum, would have reasonably expected aflibercept’s glycosylation sites to match those of Flt1D2.Flk1D3.FcΔC1(a). *See, e.g.,* Ex.1090, Ronin, 164; Ex.1091, Imperiali, 1567. A POSA would have recognized that as long as the key glycosylation motifs remain accessible, the glycosylation sites would remain consistent between these molecules.

36. Accordingly, based on the teachings of prior art, the glycosylation sites of aflibercept expressed in CHO cells as recited by the '865 patent claims would have been obvious to a POSA.

V. CONCLUSION

37. This declaration, along with the attached appendices, is based on my present assessment of material and information currently available to me.

38. I hereby declare that all statements made herein of my own knowledge are true and that all statements made herein on information and belief are believed to be true. Further, these statements were made with the knowledge that willful false statements and the lie so made are punishable by fine, imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the above-identified patent.

November 18, 2024

Date

A handwritten signature in black ink, appearing to read "M. Butler", with a long, sweeping horizontal stroke underneath.

Michael Butler, Ph.D