UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

CELLTRION, INC.,

Petitioner,

v.

REGENERON PHARMACEUTICALS, INC.,

Patent Owner.

Patent No. 10,464,992

Inter Partes Review No. IPR2023-00462

DECLARATION OF DR. ALEXANDER M. KLIBANOV

I, Dr. Alexander M. Klibanov, make this declaration in connection with the proceeding identified above.

I. INTRODUCTION

1. I have been retained by counsel for Patent Owner, Regeneron Pharmaceuticals, Inc. ("Regeneron"), as a technical expert in connection with the proceeding identified above. I submit this declaration in support of Patent Owner's Preliminary Response to the Petition for *Inter Partes* Review of United States Patent No. 10,464,992 ("the 992 patent").

2. I am being paid at an hourly rate of $950 for my time spent working on this matter. I have no personal or financial stake or interest in the outcome of the present proceeding.

II. QUALIFICATIONS

3. I am currently a Professor Emeritus of Chemistry and Bioengineering at the Massachusetts Institute of Technology ("M.I.T."), where I taught and conducted research for over 40 years. From 2014 to 2019 (and also from 2007 to 2012), I held the Novartis Endowed Chair Professorship at M.I.T. From 2012 to 2014, I held the Roger and Georges Firmenich Endowed Chair Professorship in Chemistry. Prior to that, I was a Professor of Chemistry and a Professor of Bioengineering at M.I.T., positions I held from 1988 and 2000, respectively. From 1979 to 1988, I was an Assistant Professor, then Associate Professor, and

thereafter a Full Professor of Applied Biochemistry in the Department of Applied Biological Sciences (formerly the Department of Nutrition and Food Science) at M.I.T.

4. I obtained my M.S. degree in Chemistry from Moscow University in Russia in 1971 and Ph.D. in Chemical Enzymology from the same University in 1974. Thereafter, I was a Research Chemist at Moscow University's Department of Chemistry for three years. From 1977 to 1979, following my immigration to the United States, I was a Post-Doctoral Associate at the Department of Chemistry, University of California in San Diego.

5. Over the last 50+ years as a practicing chemist, I have extensively researched, published, taught, and lectured in many areas of chemistry, including biological, protein, medicinal, and formulation chemistry.

6. I have earned numerous prestigious professional awards and honors. For example, I was elected to the U.S. National Academy of Sciences (considered among the highest honors that can be given to an American scientist) and also to the U.S. National Academy of Engineering (considered among the highest honors that can be given to an American engineer). I am also a Founding Fellow of the American Institute for Medical and Biological Engineering and a Corresponding Fellow of the Royal Society of Edinburgh (Scotland's National Academy of Science and Letters). In addition, I have received the Arthur C. Cope Scholar

Award, the Marvin J. Johnson Award, the Ipatieff Prize, and the Leo Friend Award, all from the American Chemical Society, as well as the International Enzyme Engineering Prize.

7. I currently serve on the Editorial Boards of over a dozen scientific journals, including "Open Journal of Pharmacology," "Applied Biochemistry and Biotechnology," "Nanocarriers," "Open Access Academic Books in Chemistry," "Biotechnology and Bioengineering," "Journal of Biological Chemistry and Molecular Pharmacology," "Recent Patents in Biotechnology," "Archives of Medical Biotechnology," "Current Pharmaceutical Biotechnology," and "International Journal of Drug Design, Delivery, and Safety."

8. I have published over 315 scientific papers in various areas of chemistry. Of particular relevance to this proceeding are my numerous publications specifically dealing with protein chemistry, including therapeutic proteins and their stability. I am a named inventor of 32 issued United States patents and of a number of pending ones. I have given over 370 invited lectures, including many distinguished named lectureships, at professional conferences, universities, and corporations all over the world, many dealing with formulation, stability, stabilization, and biological evaluation of pharmaceutically active proteins (including enzymes and their inhibitors, as well as antibodies and growth

factors). According to a recent Stanford University-led study, the overall impact of my published work places me in the top 0.01% of all scientists in the world.

9. In addition to my research and teaching activities at M.I.T., I have consulted widely for pharmaceutical, medical device, and biotechnology companies. Those consulting relationships have included both innovator and generic pharmaceutical companies.

10. I also have founded six pharmaceutical companies and have been on the scientific advisory boards and/or boards of directors of those companies and of many others. Of particular relevance to the present proceeding, one of the pharmaceutical companies founded by me focused on intravenous and subcutaneous delivery of therapeutic antibodies and fusion proteins.

11. A number of the aforementioned consulting, advisory, and directorship activities have dealt specifically with formulation (such as liquid), stabilization, administration (including parenteral), posttranslational modification, testing, and medical applications of therapeutically active proteins.

12. My *curriculum vitae*, attached as Ex. 2002, summarizes my education and academic experience. Included in it is a list of all my professional publications and patents.

13. My opinions and views set forth in this declaration are based on my education, training, and professional experience, as well as the materials I

reviewed (see the next paragraph) in preparing this declaration and the state of scientific knowledge in the art pertaining to the subject matter of the ’992 patent at the time of its earliest priority date of June 16, 2006.

14. In forming my opinions expressed herein, I have reviewed the following materials: (a) the Petition for *Inter Partes* review of the ’992 patent, IPR2023-00462 (the “Petition”); (b) the Declaration of Dr. Ralph Tarantino submitted in support of the Petition (the “Tarantino Declaration”); (c) all exhibits cited in both documents; and (d) all articles, papers, and patent documents cited herein.

III. SUMMARY OF OPINIONS

15. In this declaration, I address scientific assumptions made by Dr. Tarantino about whether the prior art on which he relies discloses the use of a particular engineered fusion protein for which Petitioner has submitted a reference (“Dix”) that is not prior art[1] containing data on the percentage of the protein

[1] Dr. Tarantino notes that he has been asked to assume that the priority date for the ’992 patent is June 16, 2006. Ex.1002, ¶ 25. I am not an attorney or an expert in U.S. patent law. I understand from counsel for Regeneron that Petitioner does not contend that this Dix reference is prior art but relies on it in order to purportedly describe what was in the prior art. *See* Petition, 24 n. 3

remaining in its native conformation after storage in an aqueous formulation at 5°C. As explained below, Dr. Tarantino's assumptions in this regard are unsupported and unjustified because the prior art references use terms like "VEGF Trap" or "VEGFTrap$_{R1R2}$" (where "VEGF" stands for vascular endothelial growth factor) that do not describe just one specific VEGF antagonist protein, but instead refer to a genus of such VEGF antagonists. Nothing in the cited prior art or the non-prior art Dix reference indicates that the same specific VEGF antagonist protein was used in pharmaceutical formulations, nor does Dr. Tarantino contend that all VEGF antagonists in the genus would have the properties, including a certain 2-month storage stability, required by the '992 patent claims. Dr. Tarantino's critical assumption that the VEGF antagonist protein in the non-prior art Dix reference is necessarily the same as used in the cited prior art is thus unjustified.

IV. RELEVANT TECHNICAL BACKGROUND

A. Protein Structure Generally

("Petitioner does not offer Dix as prior art here but instead offers Dix 'to elucidate what the prior art consisted of.'"); Ex. 1002, ¶ 96. Therefore, I will also cite to other non-prior art references below regarding the stability properties of proteins.

16. Amino acids are the building blocks of all proteins. There are some 20 "standard" amino acids (whose names can be referred to by their respective unique three-letter or one-letter abbreviations), and proteins are formed by covalently linking amino acids to each other by means of peptide bonds to form a polypeptide chain. Ex. 2043 (Berg), 4-5.[2] Each protein "has a unique, precisely defined amino acid sequence," which refers to the order in which the individual amino acid residues are arranged therein.[3] *Id*. at 6. The sequence of amino acids defining a protein is referred to as the protein's primary structure. But the overall protein structure is much more complex than the primary structure alone. That is because the polypeptide chain defining a protein exists not in linear two-dimensional form but instead is folded into a complex three-dimensional shape. For example, portions of the polypeptide chain will spontaneously fold into defined regions (such as helices), which are referred to as the secondary structure of the protein. And these primary and secondary structures spontaneously combine to form the overall three-dimensional folded form of the protein, called its tertiary structure. Within a given protein tertiary structure, there may be structural

---

[2] Excerpts from J.M. Berg et al., BIOCHEMISTRY (5th edn., 2002) ("Berg").

[3] The term "amino acid residues" refers to the unique portion of each amino acid left over after the amino acids are chemically linked to form a protein.

segments or "domains," which can be thought of as the portions of the protein that can fold up and function almost independently of their neighboring regions. Ex. 2027 (Doolittle), 2.[4] As an example, the diagram below shows the three domains of the protein argininosuccinate lyase (ASL), numbered consecutively:

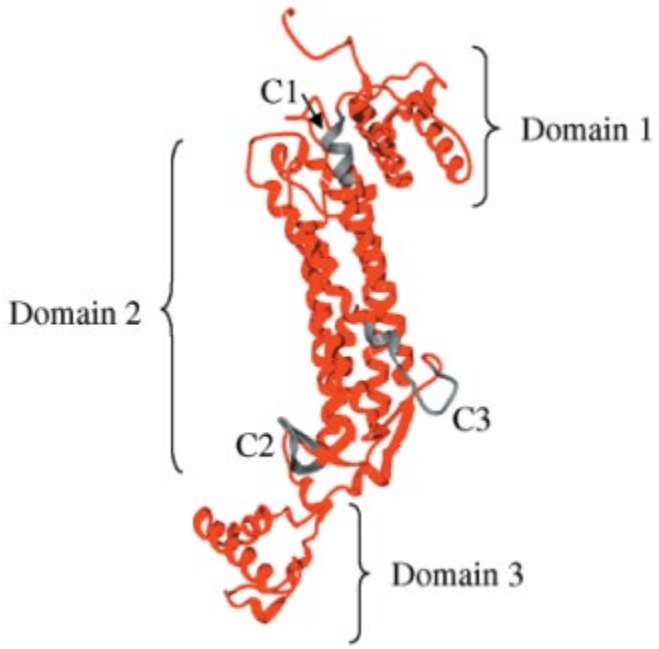


Figure 1: Three structural domains of ASL protein according to Sampaleanu et al.

[4] Doolittle, R. F. (1995). *The Multiplicity of Domains in Proteins.* ANNUAL REVIEW OF BIOCHEMISTRY, 64(1), 287-314. doi:10.1146/annurev.bi.64.070195.001443 ("Doolittle").

Ex. 2028 (Sampaleanu), 5.[5]

B. VEGF Receptor Proteins

17. Vascular endothelial growth factor ("VEGF") is a type of protein that has been implicated in various diseases ranging from vascular disease in the retina of the eye to cancer. The human VEGF family consists of five related proteins that occur naturally in the human body: VEGFA, VEGFB, VEGFC, VEGFD, and PIGF (placental growth factor). Ex. 2029 (Pandey), 1.[6] These VEGF proteins interact with the corresponding portions of one of three distinct "VEGF receptor" ("VEGFR") proteins (*see* Figure 2 below): VEGFR1, VEGFR2, and VEGFR3 (VEGF receptors 1, 2, and 3, respectively). *Id.* VEGFR1 is also known as Flt1 (Fms-like tyrosine kinase 1, where Fms is yet another protein), and VEGFR2 is

---

[5] Sampaleanu, L. M., Vallée, F., Thompson, G. D., & Howell, P. L. (2001). *Three-Dimensional Structure of the Argininosuccinate Lyase Frequently Complementing Allele Q286R*. Biochemistry, 40(51), 15570-15580, at 15574. doi:10.1021/bi011525m ("Sampaleanu").

[6] Pandey, A. K., Singhi, E. K., Arroyo, J. P., Ikizler, T. A., Gould, E. R., Brown, J., … Moslehi, J. (2017). *Mechanisms of VEGF (Vascular Endothelial Growth Factor) Inhibitor–Associated Hypertension and Vascular Disease.* Hypertension, 71(2), e1-e8. doi:10.1161/hypertensionaha.117.10271 ("Pandey").

also known as KDR (kinase insert domain receptor) or Flk1 (fetal liver kinase 1). *Id.*

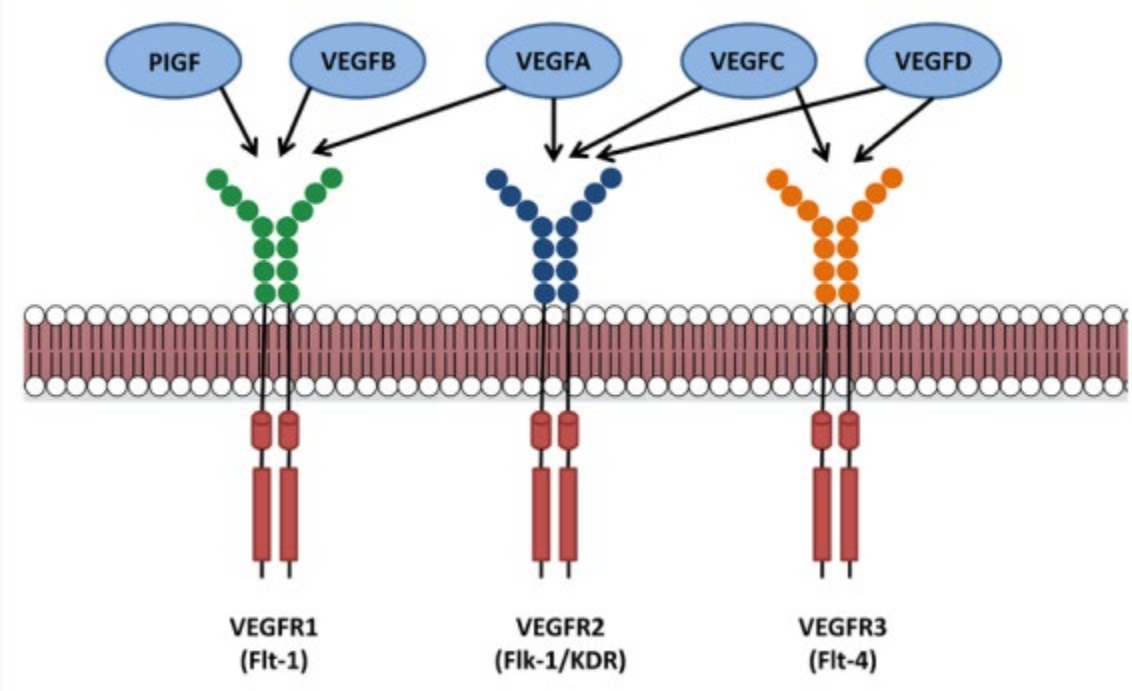


Figure 2: VEGF ligands and receptors, drawn from Fig. 1 of Pandey.

Id. at 2.

18. Structurally, the VEGFR1, VEGFR2, and VEGFR3 receptor proteins each has 7 distinct domains that extend from the surface of the cell (depicted in the figure above as individual green, blue, and orange circles) that are referred to numerically (*e.g.*, "VEGFR1 domain 1" or "VEGFR2 domain 2").

C. Fusion Proteins

19. As their name suggests, fusion proteins are engineered in the laboratory by chemically combining different parts of distinct proteins together to obtain a "hybrid" protein that does not naturally occur as such. Ex. 2017,[7] 6.

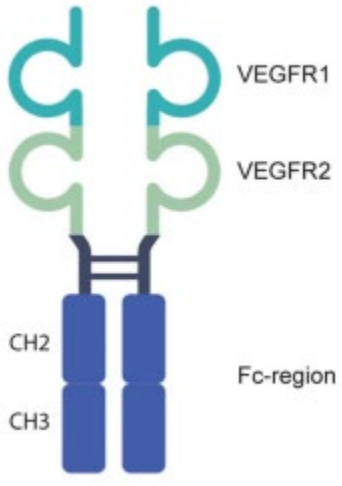
20. In 2006, fusion proteins were an emerging class of therapeutic proteins. Only three fusion proteins were approved by the U.S. Food and Drug Administration ("FDA") by then: Enbrel (in 1998), Amevive (in 2003), and Orencia (in 2005). Ex. 2030,[8] 2 (Table 4.1). In 2011, Regeneron obtained its first FDA approval for aflibercept (or EYLEA®), which is a fusion protein now approved in the United States and in the European Union to treat certain diseases,

---

[7] Excerpts from ANTIBODY FUSION PROTEINS (Chamow, S. M., & Ashkenazi, A. (eds.), 1999).

[8] Shukla, A. A., & Gottschalk, U., *Downstream Processing of Fc-Fusion Proteins.* In THERAPEUTIC FC-FUSION PROTEINS (Chamow, S. M., et al. (eds.), 2014).

like wet macular degeneration and metastatic colorectal cancer. A schematic representation of the general architecture of the aflibercept protein is shown below:



21. As seen above, aflibercept is formed via a fusion of parts from VEGF receptor proteins (specifically, domain 1 from VEGFR1 ("VEGFR1") and domain 2 from VEGFR2 ("VEGFR2")) combined with parts from an antibody protein (specifically, the CH2 and CH3 domains of the "Fc region" of the antibody). But this generalized structural description of aflibercept is unavoidably simplistic and incomplete, for example, because typically fusion proteins contain bridging (or "hinge") regions, which are amino acid sequences that hold protein chains together, and "signal" sequences, which are short chains of amino acid residues found at the end of a protein sequence that act like a "flag" to signal to the transport mechanism of the cell where the protein should go. Thus, importantly,

even if they comprise the same structural domains, two fusion proteins may still have quite different amino acid sequences overall.

D. Protein Stability

22. A protein's structure and function (*i.e.*, properties) are typically closely linked (hence the classical adage "structure begets function"). If changes to the protein structure occur, whether at the primary, secondary, or tertiary structural level, changes in the protein's function usually follow. One important and relevant herein manifestation of a protein's function is stability, which refers to ability of a protein to retain its native properties (such as native conformation or shape) over time under given conditions. In the case of pharmaceutical proteins, for example, if the protein is not stable and degrades in some way (*i.e.*, its structure changes), then there may be a partial or even complete loss of therapeutic efficacy. Seemingly minor structural differences among proteins often can result in major stability differences and marked variations in which degradation pathways are prevalent for a given protein formulation.

23. For example, one common way in which proteins degrade (deteriorate) is through their aggregation. Aggregation generally occurs when

proteins first partially unfold and then clump together due to hydrophobic interactions.[9] Ex. 2015 (Frokjaer), 62-63; Ex. 1047, 21.

24. Factors affecting the formation of aggregates vary widely based on the nature of the protein itself and on the additional ingredients typically present in the formulation (often referred to as "excipients"). As the prior art explained, protein "unfolding is primarily caused by the solvent environment (pH, salt, co-solvents, etc.), temperature, or surface interactions," while "[t]he initial stages of aggregation are quite specific in the sense that they involve the interaction of specific structural subunits of one molecule with 'matching' hydrophobic surface areas of structural subunits of a neighboring molecule." Frokjaer, 100-101. Notably, "[e]very protein has unique physicochemical characteristics and the

---

[9] Aggregation is one common physical degradation pathway for proteins. Denaturation, surface adsorption, deamidation, polypeptide chain cleavage, and oxidation are other common degradation pathways that may result in the degradation of a protein in a given pharmaceutical formulation. Ex. 2015 at 33-34, Excerpts from PHARMACEUTICAL FORMULATION DEVELOPMENT OF PEPTIDES AND PROTEINS 70-71 (Frokjaer, S., & Hovgaard, L. (eds.), 2000) ("Frokjaer").

potential to behave differently stability-wise in the presence of a particular excipient." Ex. 1013.

25. While it may seem that a pharmaceutical formulator's goal should be to have a protein that remains intact, *i.e.*, perfectly stable, in a formulation throughout its shelf-life, the goal of stability must be balanced against several competing concerns. For example, the route of administration may impose constraints on the volume of a therapeutic dose. For intravitreous injections, a medication is injected into the small space in the back of the eye called the vitreous cavity. But there are practical limitations on the volume of a liquid that can be injected intravitreously; therefore, higher protein concentrations may be used for intravitreal formulations in order to provide a therapeutically effective amount of a protein drug in a single dose. *Compare* Ex. 1013, 141 (upper limit of at least 10-20 mL for intravenous ("IV") administration); *with* Ex. 2018 (Hollands), 4[10] (finding elevated intraocular pressure even with a dose volume of just 0.05 mL). But raising the concentration of a protein in a liquid formulation typically also increases the likelihood of aggregation. In other words, having a higher protein

[10] Hollands, H., et al. (2007). *Short-term Intraocular Pressure Changes After Intravitreal Injection of Bevacizumab*. Can. J. Ophthalmol., 42, 807-811. doi:10.3129/i07-172.

concentration (*i.e.*, a more "crowded" aqueous formulation) is in tension with long-term stability because, all else being equal, a more concentrated protein solution generally tends to exhibit more aggregation over time. Ex. 1002, ¶ 102 ("formulations with lower protein concentrations tend to exhibit ***less*** aggregation than ones with higher concentrations.") (citing Ex. 1006, Table 6); *see also* Ex. 1013, 146 ("rates of degradation typically vary as a function of protein concentration.").

26. As another example, it may be desirable to include a preservative in the pharmaceutical formulation to prevent microbial contamination and growth. Ex. 1006, 10. But this extra excipient introduces additional stability concerns because "[i]n general, the addition of preservative(s), regardless of the preservative used, significantly changes the stability profiles of proteins," sometimes leading to aggregation and other modes of protein degradation. Ex. 1006, 10.

27. Thus, what constitutes an acceptable stability profile for a protein formulation is specific to that protein, its formulation, and its use.

V. THE CITED PRIOR ART

28. Dr. Tarantino primarily relies on two references as disclosures of the VEGF antagonist formulated as required by the claims of the '992 patent: Fraser

(Ex. 1009)[11] and Wulff (Ex. 1016).[12] Both of these references, in turn, cite the Holash reference (Ex. 1010),[13] and Wulff additionally cites the '319 Publication (Ex. 1029).[14] The relevant disclosures in these four prior art references are summarized below.

---

[11] Fraser, H. M., et al. (2005). *Single Injections of Vascular Endothelial Growth Factor Trap Block Ovulation in the Macaque and Produce a Prolonged, Dose-Related Suppression of Ovarian Function*. J. Clin. Endocrinol. & Metab., 90(2),1114-1122 ("Fraser").

[12] Wulff, C., et al. (2002). *Prevention of Thecal Angiogenesis, Antral Follicular Growth, and Ovulation in the Primate by Treatment with Vascular Endothelial Growth Factor Trap R1R2*. Endocrinology, 143(7), 2797-2807 ("Wulff").

[13] Holash, J., et al. (2002). *VEGF-Trap: A VEGF Blocker with Potent Antitumor Effects*. Proc. Natl. Acad. Sci. USA, 99(17), 11393-11398 ("Holash").

[14] International Publication No. WO 00/75319 to Papadopoulos et al. ("'319 Publication").

2. Fraser

29. Fraser studied the effects on ovarian function in macaques resulting from doses of a VEGF antagonist that the paper refers to variously as "VEGF Trap" or "VEGFTrap$_{R1R2}$." Ex. 1009 at 1114, 1115, 1121-22. According to Fraser, the protein was provided by "Regeneron Pharmaceuticals, Inc., Tarrytown, NY." *Id.*, 1115. Apart from these names, Fraser states that the fusion protein used "compris[es] Ig [*i.e.*, immunoglobulin] domain 2 of human VEGF-R1 and Ig domain 3 of human VEGF-R2, expressed in sequence with the human Fc." *Id.* Fraser presents no stability data for the protein formulations it describes and thus no native conformation data.

3. Wulff

30. Wulff, like Fraser, variably describes the VEGF antagonist as "VEGF Trap," "VEGF Trap R1R2," "VEGF R1R2," or just "R1R2." Ex. 1016, 2797, 2801-04, 2805, 2806. Wulff also provides a functional description of the protein as "compris[ing] 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." *Id.*, 2798. Wulff illustrates the fusion protein used therein with the following cartoon drawing, that I have annotated with green arrows, showing the fusion protein on the right-hand side:

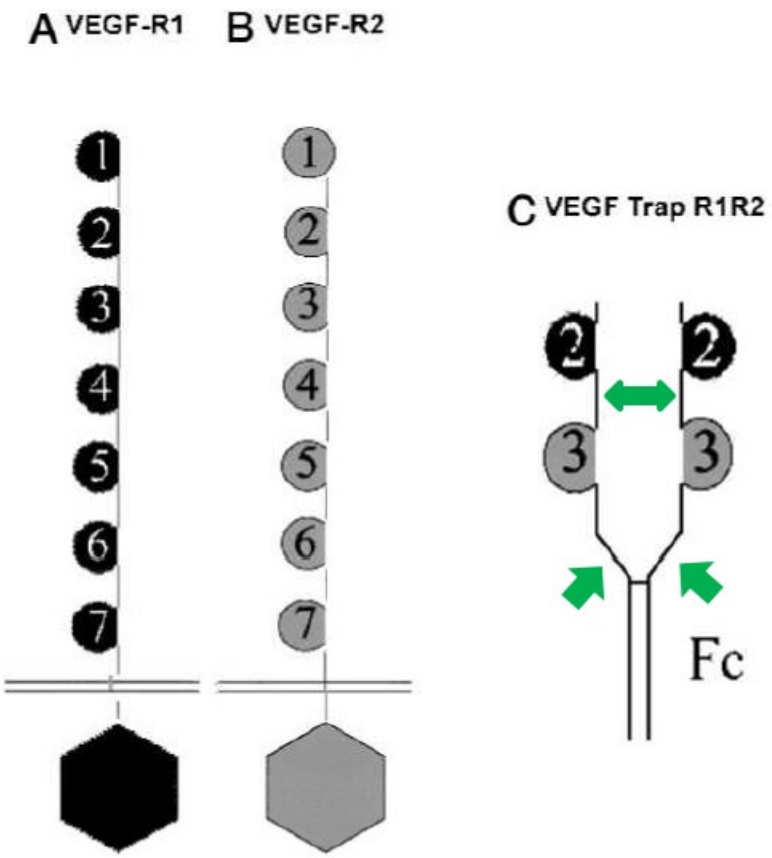


Figure 3: Structures of VEGF-R1 (A) and VEGF-R2 (B), along with VEGF Trap R1R2 (C), as shown in Fig. 1 of Wulff.

31. Figure 3 above (which is Figure 1 in Wulff) is a schematic diagram, of a type common in the scientific literature, and it shows VEGF receptors 1 and 2, as well as their respective binding domains 2 and 3, that form the VEGF Trap R1R2 molecule. Unlike an amino acid sequence, where one particular amino acid unambiguously follows a certain other amino acid along the polypeptide chain, a schematic diagram like Figure 3 is inevitably imprecise. For example, Wulff's pictorial diagram connects the binding domains (depicted as equally-sized, incomplete, black and grey numbered circles) and the Fc fragment of human IgG

(depicted as a long three-sided column) with straight lines, representing some sort of peptide linkers between the domains and the Fc fragment, which I have marked with green arrows. The amino acid residues that encompass these linkers are not specified in any way. Like Fraser, Wulff presents no stability data for the protein formulations it describes.

4. Holash

32. Holash (Ex. 1010) is a Regeneron-authored article published in 2002. Ex. 1010, 11393. Holash discloses results of pre-clinical trials that assessed the pharmacokinetic and pharmacodynamic properties of a genus of VEGF antagonists known as "VEGF Traps." *Id.*, 11393-94. Specifically, in a section entitled "Engineering VEGF-Traps," Holash describes modifying a "parental VEGF-Trap" to create "VEGF-Trap$_{\Delta B1}$," "VEGF-Trap$_{\Delta B2}$," and other fusion proteins, yielding various "VEGF-Trap variants." *Id*. Holash then reports on the performance of these various "VEGF-Traps" (*i.e.*, plural). *Id.*, 11394-95 (referring to "tes[ing] these various VEGF-Traps" and "[u]sing blockade of VEGF-induced acute hypotension to pharmacodynamically compare VEGF-Traps"). Therefore, Holash makes clear that the term "VEGF Traps" refer to a ***group*** of proteins.

33. Holash discusses properties of a VEGF trap protein that it refers to as "R1R2" (Ex. 1010, 11395, Fig. 2) or VEGF-Trap$_{R1R2}$ (*id.*, 11395). Holash explains

that this fusion protein "possesses the second Ig domain of VEGFR1 and the third Ig domain of VEGFR2 fused to the Fc portion of human IgG1." *Id.*, 11394.

34. Like Wulff above, Holash provides what it calls "[s]chematics" of various VEGF Traps, including the R1R2 trap. I have excerpted and annotated one such schematic (Fig. 1A from Holash) here:

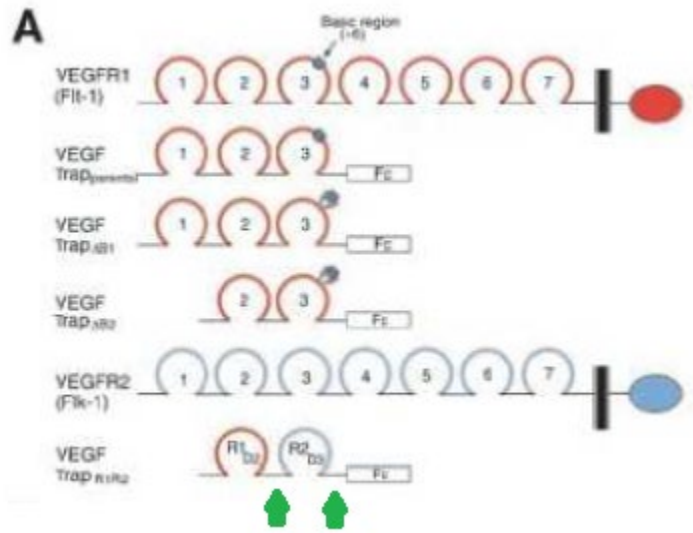


Figure 4: Schematics of VEGF-Traps, drawn from Fig. 1 of Holash.

35. Again, the binding domains (depicted as equally-sized incomplete, open numbered circles) and the Fc fragment (depicted as an open horizontal rectangle) are connected by straight lines, representing peptide linkers between the domains and the Fc fragment, which linkers I have marked with green arrows. The amino acid residues that form the linking sequences are not provided. And, like Fraser and Wulff, Holash presents no stability data for the protein formulations it describes.

5. '319 Publication

36. The '319 Publication (Ex. 1029) is a PCT patent publication referenced in Wulff. Ex. 1016, 2798 at n. 1. The '319 Publication discloses two different VEGF antagonist proteins (denoted Flt1.D2.Flk1D3.FcΔC1(a) and VEGFR1R2-FcΔC1(a)). Both proteins include Fc regions from human antibody fused to domain 2 from VEGR1 and domain 3 from VEGFR2. Ex. 1029, 58:22-59:10. Both of these proteins are generally referred to in the '319 Publication as "R1R2" proteins because they include the same general structural domains. Despite these similarities, the '319 Publication also confirms that these two proteins have different amino acid sequences and are, in fact, distinct proteins (as discussed in further detail below). Like, Fraser, Wulff, and Holash, the '319 Publication presents no stability data for the protein formulations it describes.

VI. DETAILED BASES FOR OPINIONS

37. Dr. Tarantino contends that a non-prior art Regeneron patent, namely the Dix patent issued in 2012 (Ex. 1021),[15] discloses data about the same fusion proteins and protein formulations as described in Fraser and Wulff; Dix's disclosures about the percentage of the protein remaining in the native conformation after certain periods of time and under certain conditions are thus

[15] U.S. Patent No. 8,110,546 to Dix et al. issued on February 7, 2012 ("Dix").

ostensibly directly relevant to what Fraser and Wulff disclosed. Dr. Tarantino relies in particular on the data found in Table 9 of Dix.

38. Dr. Tarantino assumes that Fraser and Wulff are referring unambiguously to a single protein—the same protein whose remaining native conformation data are reported in Dix. Ex. 1002, ¶¶ 61, 62, 64. That critical assumption, however, is not supported. The relied-upon prior art references, as pointed out above, use various terms like "VEGF Trap," "$VEGFTrap_{R1R2}$," "VEGF Trap R1R2," or "R1R2." As shown below, these terms refer to a group or a ***genus*** of fusion proteins with certain characteristics in common and not necessarily to the single protein whose remaining native conformation data are reported in Table 9 of Dix, which protein is unambiguously identified in Dix by its amino acid sequence.

A. "R1R2" and Similar Prior Art Terms and Descriptions Refer to a Genus of VEGF Antagonists With Different Structures, Not to a Single, Specific Protein

39. As described above, the prior art relied upon by Dr. Tarantino uses distinct terms, such as "VEGF Trap," "VEGF Trap R1R2," "VEGF R1R2," "$VEGFTrap_{R1R2}$," or just "R1R2," and provides a description of certain domains contained in such fusion protein. However, the prior art also indicates that "VEGF Trap" refers to a general category of VEGF antagonists and that the "R1R2" terms likewise refer to a group of fusion proteins that feature certain domains of VEGFR

fused to the Fc portions of an antibody but that can also differ from each other in several ways. There are at least three reasons for these differences.

40. First, even proteins that contain the same domains (such as VEGFR1 domain 2 and VEGFR2 domain 3, as denoted by "R1R2") may still contain different linking sequences of amino acid residues in between those domains and/or different signal sequences in addition to those domains.

41. Second, the relied-upon prior art refers to proteins using the designation "R1R2" or similar terms even if those proteins undergo amino acid sequence modifications that do not appreciably affect their binding efficacy to VEGF.

42. Third, the relied-upon prior art discloses different amino acid sequences for VEGFR1 domain 2 and VEGFR2 domain 3, and a description in terms of these domains does not specify which amino acid sequences those domains correspond to.

43. I discuss each of these aspects separately in more detail below.

1. "R1R2" Proteins May Have Different Linking Sequences or Signal Sequences

44. Although "R1R2" and similar terms refer to a group of proteins that contain VEGFR1 domain 2 and VEGFR2 domain 3, they may have differences in

their linking and signal sequences. The '319 Publication (Ex. 1029)[16] and the Daly reference (Ex. 2013)[17] both refer to proteins that differ in this manner as "R1R2" proteins.

45. The '319 Publication is particularly relevant to the use of the term "R1R2" in Wulff (and other relied-upon prior art references) because it is a Regeneron patent publication that is directly referenced in Wulff. Wulff states that "[t]he detailed molecular structure and how it was created are described in the patent REG710-A-PCT, VEGF Trap Application published December 2000, Publication WO 00/75319 A1." Ex. 1016, 2798 at n. 1.

46. As discussed above, the '319 Publication discloses two specific fusion proteins that are referred to as "R1R2" proteins: Flt1.D2.Flk1D3.FcΔC1(a) and VEGFR1R2-FcΔC1(a), which I color-code herein for ease of reference. The '319 Publication provides the sequences for both Flt1.D2.Flk1D3.FcΔC1(a) and VEGFR1R2-FcΔC1(a) in Figures 21A-21C, *id.* at 21:16-17, and Figures 24A-24C, *id.* at 22:1-2, respectively. I have aligned these sequences, as shown below, which demonstrates that these two "R1R2" proteins have distinct primary structures due

[16] International Publication No. WO 00/75319 to Papadopoulos et al. ("'319 Publication").

[17] U.S. Patent No. 8,110,546 to Dix et al. ("Dix").

to different amino acid residues in the linking sequence of each protein (the portion of the protein between two domains).[18]

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    MVSYWDTGVLLCALLSCLLLTGSSSG---GRPFVEMYSEIPEIIHMTEGRELVIPCRVTS  57
            MVSYWDTGVLLCALLSCLLLTGSSSG   GRPFVEMYSEIPEIIHMTEGRELVIPCRVTS
Sbjct  1    MVSYWDTGVLLCALLSCLLLTGSSSGSDTGRPFVEMYSEIPEIIHMTEGRELVIPCRVTS  60

Query  58   PNITVTLKKFPLDTLIPDGKRIIWDSRKGFIISNATYKEIGLLTCEATVNGHLYKTNYLT  117
            PNITVTLKKFPLDTLIPDGKRIIWDSRKGFIISNATYKEIGLLTCEATVNGHLYKTNYLT
Sbjct  61   PNITVTLKKFPLDTLIPDGKRIIWDSRKGFIISNATYKEIGLLTCEATVNGHLYKTNYLT  120

Query  118  HRQTNTIIDVVLSPSHGIELSVGEKLVLNCTARTELNVGIDFNWEYPSSKHQHKKLVNRD  177
            HRQTNTIIDVVLSPSHGIELSVGEKLVLNCTARTELNVGIDFNWEYPSSKHQHKKLVNRD
Sbjct  121  HRQTNTIIDVVLSPSHGIELSVGEKLVLNCTARTELNVGIDFNWEYPSSKHQHKKLVNRD  180

Query  178  LKTQSGSEMKKFLSTLTIDGVTRSDQGLYTCAASSGLMTKKNSTFVRVHEKGPGDKTHTC  237
            LKTQSGSEMKKFLSTLTIDGVTRSDQGLYTCAASSGLMTKKNSTFVRVHEK   DKTHTC
Sbjct  181  LKTQSGSEMKKFLSTLTIDGVTRSDQGLYTCAASSGLMTKKNSTFVRVHEK---DKTHTC  237

Query  238  PPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHN  297
            PPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHN
Sbjct  238  PPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHN  297

Query  298  AKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREP  357
            AKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREP
Sbjct  298  AKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREP  357

Query  358  QVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFL  417
            QVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFL
Sbjct  358  QVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFL  417

Query  418  YSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK  458
            YSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
Sbjct  418  YSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK  458
```

[18] The blastp suite of the BLAST® computer program with default alignment parameters was used to create the sequence comparison. It is available at https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE=Proteins ("Align two or more sequences").

47. Specifically, as the yellow highlighting above indicates, Flt1.D2.Flk1D3.FcΔC1(a) (shown as the "Query" sequence above) (1) lacks three amino acid residues (serine-aspartic acid-threonine, denoted "SDT") present in VEGFR1R2-FcΔC1(a) (shown as the "Sbjct" sequence above); and (2) contains three additional amino acid residues (glycine-proline-glycine, denoted "GPG") that are not present in VEGFR1R2-FcΔC1(a).

48. The sequence alignment presented above does not show where in the VEGF antagonists described in the '319 Publication the different sequences are located, but this information can be obtained by identifying the different sequences above in the Figures of the '319 Publication, which include domain information. Fig. 24A, which gives the nucleic acid and amino acid sequences for VEGFR1R2-FcΔC1(a), shows the additional "SDT" (grey) that is not found in Flt1.D2.Flk1D3.FcΔC1(a). In the excerpt below, the end of the signal sequence[19] is shown in pink, the beginning of the Flt1 (VEGR1) domain 2 in orange, and the additional "SDT" sequence is part of the linking sequence between the domains:

[19] A signal sequence enables a protein to find its correct location outside the cell membrane by tagging the protein for transport through the cell membrane and out of the cell. Ex. 2015, 23.

```
            70          80          90          100         110         120
             *           *           *           *           *           *
ACA GGA TCT AGT TCC GGA AGT GAT ACC GGT AGA CCT TTC GTA GAG ATG TAC AGT GAA ATC
TGT CCT AGA TCA AGG CCT TCA CTA TGG CCA TCT GGA AAG CAT CTC TAC ATG TCA CTT TAG
 T   G   S   S   S   G>
21_hFLT1 SIGNAL SEQ_26>
                        S   D   T   G   R   P   F   V   E   M   Y   S   E   I>
                        _27_________30___hFLT1 IG DOMAIN 2_____________________40>
```

Ex. 1029, Fig. 24A (annotations added); *see also* Ex. 1029, 51:5-12.

49. Figure 21B from the ’319 Publication shows the nucleic acid and amino acid sequences for Flt1.D2.Flk1D3.FcΔC1(a), which include a GPG sequence not present in VEGFR1R2-FcΔC1(a). The end of the Flk1 (VEGFR2) domain 3 is highlighted in violet, the beginning of the human Fc domain is highlighted in gold, and the GPG sequence highlighted in green bridges the two domains (hence the label “Srf_Bridge_”) without being a part of either:

```
                                >Srf_Bridge_
                                     |
        730       740       750    |    760       770       780       790       800
AGAACAGCACATTTGTCAGGGTCCATGAAAAGGGCCCGGGCGACAAAACTCACACATGCCCACCGTGCCCAGCACCTGAA
TCTTGTCGTGTAAACAGTCCCAGGTACTTTTCCCGGGCCCGCTGTTTTGAGTGTGTACGGGTGGCACGGGTCGTGGACTT
K  N  S  T  F  V  R  V  H  E  K>
___________HFLK1 D3_____________>
                                G  P  G>
                                _________>
                                          D  K  T  H  T  C  P  P  C  P  A  P  E>
                                                                              244
                                          ____________FCΔC1(A)_______________>
```

Ex. 1029, Fig. 21B (color annotations added).

50. As reflected in the above-quoted excerpts from the ’319 Publication, a fusion protein that, like this one, is accurately described as being comprised of

VEGFR1 domain 2 and VEGFR2 domain 3 expressed in sequence with a human Fc antibody constant region, may also contain additional amino acid sequences, such as a signal sequence, as well as bridging sequences that do not form part of these domains. Thus, the explicit disclosures of the '319 Publication confirm that a "R1R2" protein is not a single, particular, unique protein, but instead refers to a ***group*** of several proteins having different amino acid sequences.

51. Additional prior art references further confirm that the term "R1R2" is used as a general structural description for a group of proteins, not as a specific description of a particular protein's sequence. For example, Daly (Ex. 2013), a published Regeneron patent application, explains that there are a variety of fusion proteins comprising "a fusion polypeptide comprising receptor components R1-R2-F, wherein R1 is vascular endothelial cell growth factor (VEGF) receptor component Ig domain 2 of Flt-1 (Flt1D2), R2 is VEGF receptor component Ig domain 3 of Flk-1 (Flk1D3) (also known as KDR), and F is a fusion component." *Id.*, ¶ 5.

52. Consistent with the '319 Publication, Daly explains that "R1R2" proteins can differ in their bridging sequences. It describes a protein called "pTE115" that "w[as] constructed from a precursor of the parent VEGF trap, VEGFR1R2.FcΔC1(a) (SEQ ID Nos:7-8) [one of the two fusion proteins described in the '319 Publication], in which the three amino acid residues glycine-alanine-

proline served as a [peptide] linker between the Flk1D3 and FcΔC1(a)" but which "[i]n all other respects" was "identical to that of the VEGF trap, VEGFR1R2.FcΔC1(a)." This pTE115 protein was used to construct additional VEGF trap proteins, all of which satisfy the structural definition of a R1R2 protein. But as the Daly reference makes clear, pTE115 and VEGFR1R2.FcΔC1(a) (which are both R1R2 proteins) have distinct amino acid sequences due to differences between the linker/bridging sequences in each protein. *Id.*, ¶¶ 29-30.

2. "R1R2" Fusion Proteins May Feature Variants of the Domains

53. Moreover, when discussing these VEGF trap proteins, Daly revealingly states that "[t]he term 'Ig domain' of Flt-1 or Flk-1 is intended to encompass not only the complete wild-type domain, but also insertional, deletional, and/or substitutional variants thereof which substantially retain the functional characteristics of the intact domain. . . . The term 'functional equivalents' when used in reference to R1 and R2, is intended to encompass an R1 and R2 domain with at least one alteration, e.g., a deletion, addition, and/or substitution, which retains substantially the same functional characteristics as does the wild type R1 and R2, that is, a substantially equivalent binding to VEGF. It will be appreciated that various amino acid substitutions can be made in R1 and R2 without departing from the spirit of the invention with respect to the ability of these receptor components to bind and inactivate VEGF." *Id.*, ¶¶ 29-30. Thus,

multiple VEGF antagonist proteins may properly be characterized as "R1R2" despite their structural differences.

54. Indeed, Daly discloses multiple possible amino acid sequences for R1 and R2. For example, it discloses that "[i]n the specific embodiments, R1 is amino acids 27-126 of SEQ ID NO:6, or 1-126 of SEQ ID NO:6 (including the signal sequence 1-26); or amino acids 27-129 of SEQ ID NO:8, or 1-129 of SEQ ID NO:8 (including the signal sequence at 1-26). *Id.*, ¶ 32.

55. Daly goes on to disclose that "[i]n the most specific embodiments, R2 is amino acids 127-228 of SEQ ID NO:6, or amino acids 130-231 of SEQ ID NO:8." By specifying multiple sequences for R1 and R2, Daly teaches that there are distinct amino acid sequences that are nonetheless all R1R2 VEGF Trap fusion proteins.

3. "R1R2" Proteins Can Differ Because the Fusion Domains that Make Up an "R1R2" Protein Correspond to Multiple Amino Acid Sequences

56. The third reason for difference among "R1R2" proteins stems from the fact that each of the components of a "R1R2" protein—VEGFR1 domain 2 and VEGFR2 domain 3—was not understood to correspond to a single amino acid sequence.

57. The prior art further confirms that more than one amino acid sequence may be generally characterized as VEGFR1 domain 2*, i.e.*, "R1." Below is a chart

summarizing various amino acid sequences of VEGFR1 domain 2, as defined by different prior art literature sources. I show how these sequences were derived in Appendix A.

Literature Source	VEGFR1 (Flt-1) domain 2		
	Starting Amino Acid	**Ending Amino Acid**	**Domain Length**
Davis-Smyth et al. 1996 (Ex. 2013)[20]	134 Pro (P)	226 Thr (T)	93 residues
UNIPROT P17948 (Ex. 2032)[21] (Ex. 2033)[22]	151 Gly (G)	214 His (H)	64 residues
'319 Publication (EX1029)[23]	27 Gly (G)	126 Asp (D)	100 residues

[20] Davis-Smyth, T., et al. (1996). *The Second Immunoglobulin-like Domain of the VEGF Tyrosine Kinase Receptor Flt-1 Determines Ligand Binding and May Initiate a Signal Transduction Cascade*. EMBO JOURNAL, 15, 4919 ("Davis-Smyth").

[21] P17948 VGFR1_Human, available at https://www.uniprot.org/uniprotkb/P17948/entry ("UNIPROT P17948").

[22] P17948 VGFR1_Human, Entry Version 140 (txt) (Jan. 11, 2011), available at https://rest.uniprot.org/unisave/P17948?format=txt&versions=140 ("UNIPROT P37948 v140").

[23] U.S. Patent No. 7,374,758 B2 ("Papadopoulos").

58. Similarly, the prior art confirms that more than one amino acid sequence may be generally characterized as VEGFR2 domain 3, *i.e.*, "R2." Below is a chart summarizing various amino acid sequences of VEGFR2 domain 3, as defined by different literature sources. I show how these sequences were derived in Appendix B.

Literature Source	**VEGFR2 (KDR) domain 3**		
	Starting Amino Acid	**Ending Amino Acid**	**Domain Length**
Davis-Smyth (Ex. 2013)	226 Val (V)	327 Lys (K)	102 residues
Lu et al. 2000 (Ex. 2082)[24]	221 Tyr (Y)	334 Ser (S)	114 residues
Franklin et al. 2011 (Ex. 2035)[25]	220 Gly (G)	328 Ser (S)	109 residues
UNIPROT P35968 (Ex. 2036)[26]	224 Tyr (Y)	320 Thr (T)	97 residues

[24] Lu, D., et al. (2000). *Identification of the Residues in the Extracellular Region of KDR Important for Interaction with Vascular Endothelial Growth Factor and Neutralizing Anti-KDR Antibodies*. J. BIOL. CHEM., 275, 14321 ("Lu").

[25] Franklin, M. C., et al. (2011). *The Structural Basis for the Function of Two Anti-VEGF Receptor 2 Antibodies*. STRUCTURE, 19, 1097 ("Franklin").

[26] P35968 VGFR2_Human, available at https://www.uniprot.org/uniprotkb/P35968/entry ("UNIPROT P35968").

(Ex. 2037)[27]			

59. In sum, a general description of a VEGF antagonist in terms of its domains (*e.g.*, VEGFR1 domain 2, VEGFR2 domain 3, and a human Fc expressed in sequence) does not specifically identify the amino acid sequence of the protein. Different VEGF antagonists may satisfy the requirements of a "R1R2" protein, but nonetheless have different amino acid sequences overall, based on differences in bridging or signal sequences or in the particular R1 and/or R2 sequence present.

60. Thus, the structural information from Fraser that the fusion protein used "compris[es] Ig domain 2 of human VEGF-R1 and Ig domain 3 of human VEGF-R2, expressed in sequence with the human Fc" (Ex. 1009, 1115) refers to a genus of potential proteins, not necessarily to any one, specific VEGF antagonist.

61. The same is true for Wulff and Holash, both of which provide general structural definitions of the VEGF antagonists, but not of a specific amino acid sequence. *See*, *e.g.*, Wulff (Ex. 1016, 2798) (disclosing proteins "compris[ing] 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

[27] P35968 VGFR2_Human, Entry Version 127 (txt) (Jan. 11, 2011), available at https://rest.uniprot.org/unisave/P35968?format=txt&versions=127 ("UNIPROT P35968 v127").

sequence with the Fc portion of human IgG"); Holash ((Ex. 1010, 11393) "VEGF-$Trap_{R1R2}$ was created by fusing the second Ig domain of VEGFR1 with the third Ig domain of VEGFR2").

62. As a result, I do not believe that Dr. Tarantino has proven that any one of Fraser, Wullff, Holash, or the '319 Publication is necessarily describing the same VEGF antagonist as used in the Dix formulations, for which data on the percentage of protein remaining in the native conformation after storage at 5°C as measured by size exclusion chromatography ("SEC") are reported. As such, Dr. Tarantino's reliance on Dix to provide stability data missing from the relied-upon prior art is improper and unsupported because it was well known that even minor differences in proteins' primary structure may result in different stability profiles. Thus, Dix's disclosure of the percentage of one VEGF antagonist that remains in the native conformation under certain circumstances and periods of time does not necessarily mean that formulations of other VEGF antagonists would exhibit similar, let alone the same, properties for the reasons discussed in further detail below. Accordingly, given the merely schematic domain structural information provided in the four prior art references discussed above, there is no way scientific identify a single protein from it, let alone the specific protein, certain storage stability properties of which are provided in Table 9 of Dix.

B. Structural Differences Among "R1R2" Proteins May Result in Stability Differences

63. By 2006, it was known that even small structural differences between two proteins of the same class can lead to significant stability differences. For example, the Robinson reference (Ex. 2038)[28] reports that a "critical element" in efforts to construct hybrid (such as fusion) proteins "is the design of the peptide linkers that serve to connect different protein domains or subunits" and further explains that "***[a]lterations in linker regions have been found to affect the stability***, oligometric state, proteolytic resistance, and solubility of single-chain proteins." Ex. 2038, 1 (emphasis added). Robinson constructed a library of linkers, varying both the linker length and composition, and found that stability changed substantially as a function of both. *Id.*, 5929, 5930-33.

[28] Robinson, C. R., & Sauer, R. T. (1998). *Optimizing the Stability of Single-chain Proteins by Linker Length and Composition Mutagenesis*. PROC. NATL. ACAD. SCI. USA, 95, 5929-5934 ("Robinson").

64. The Tong reference (Ex. 2039)[29] examined the effect of varying the inter-domain linker's amino acid sequence between various domains of the human protein titin (Ex. 2039, 1), and reported significant differences in stability among the different resulting protein variants. *Id.*, 2-6.

65. The Chen reference (Ex. 2040)[30] further reviews examples of different linker amino acid sequences that can improve protein stability or increase intrinsic biological activity of proteins that otherwise have the same functional domains. *See* Ex. 2040, Table 3.

66. Notably, changing even a single amino acid residue in a protein can sometimes significantly alter the stability. For example, my own pre-2006

---

[29] Tong, B., Tian, F. & Zheng, P. (2022). *Interdomain Linker Effect on the Mechanical Stability of Ig Domains in Titin*. INT. J. MOL. SCI., 23, 9836, doi:10.3390/ijms23179836 ("Tong").

[30] Chen, X., Zaro, Z., & Shen, W. (2013). *Fusion Protein Linkers: Property, Design and Functionality*. ADV. DRUG DELIV. REV., 65(10), 1357-1369. doi: https://doi.org/10.1016/j.addr.2012.09.039 ("Chen").

publication (Ex. 2041)[31] reported that replacing asparagine ("N") with aspartic acid ("D") at position 78 of the enzyme triosephosphate isomerase markedly altered, namely reduced, this protein's stability. In particular, the mutant protein was less resistant than its wild-type predecessor to denaturation and inactivation caused by elevated temperature, denaturants, alkaline pH, and proteolysis.

67. Similarly, the Sanfelice reference (Ex. 2019)[32] reported that the protein frataxin typically undergoes cold denaturation at 16.8° C. However, changing just a single amino acid residue resulted in a mutant protein undergoing cold denaturation at 3-4º C, *i.e.*, over 10º C lower. (Ex. 2019, 2-3, Table 1.)

---

[31] Casal, J. I., Ahern, T. J., Davenport, R. C., Petsko, G. A., & Klibanov, A.M. (1987). *Subunit Interface of Triosephosphate Isomerase: Site-Directed Mutagenesis and Characterization of the Altered Enzyme*. Biochemistry, 26, 1258-1264 ("Casal").

[32] Sanfelice, D., Morandi, E., Pastore, A., Niccolai, N., & Temussi, P. A. (2015). *Cold Denaturation Unveiled: Molecular Mechanism of the Asymmetric Unfolding of Yeast Frataxin*. CHEMPHYSCHEM, 16, 3599-3602. doi: 10.1002/cphc.201500765.

68. Likewise, the Yang reference (Ex. 2042)[33] reported that a single-point mutation in SARS-CoV-2 virus's spike protein (replacing aspartic acid ("D") with glycine ("G") at position 614) significantly increases the thermal stability of the protein under physiological pH. This mutant protein, in contrast to its wild-type parent, "also exhibited exceptional long-term stability when stored at 37° C for up to 2 months." *Id.*, Abstract.

69. To summarize, the references in the relied-upon prior art to "R1R2" proteins (and similar terminology) demonstrably encompass a multi-species genus of proteins, which may, and generally would have been expected to, have different stability profiles from one protein to another. Dr. Tarantino has failed to prove that Dix necessarily refers to the same specific protein as described in any of the prior art references cited as disclosing the claimed formulation (*i.e.*, Fraser or Wulff). Therefore, and in view of the well-documented differences in protein stability that can be caused even by minor changes in the primary structure, I do not agree with Dr. Tarantino's and Petitioner's key premise that the prior art

---

[33] Yang, T.-J., Yu, P.-Y., Chang, Y.-C., Hsu, S.-T. D. (2021). *D614G Mutation in the SARS-CoV-2 Spike Protein Enhances Viral Fitness by Desensitizing It to Temperature-Dependent Denaturation*, JOURNAL OF BIOLOGICAL CHEMISTRY, 297(4), 101238. doi: 10.1016/j.jbc.2021.101238 ("Yang").

protein formulations would necessarily exhibit similar, let alone the same, native conformation properties as those reflected in Table 9 of Dix.

* * *

I declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and that these statements were made with 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: April 25, 2023

Alexander M. Klibanov, Ph.D.

Appendix A – Amino Acid Sequences for VEGFR1 Domain 2

70. I provide in this Appendix the details behind my determination of the first and last amino acid residues for VEGFR1 domain 2 in various prior art literature sources.

71. In determining the amino acid sequence comprising VEGFR1 domain 2 disclosed by Davis-Smyth (Ex. 2031), I reviewed the sequence information provided by Davis-Smyth's Figure 1, which is an "[a]lignment of the E[xtracellular]C[ellular]D[omain]s of Flt-1, KDR and Flt-4" (where "Flt-1" refers to VEGFR1, "KDR" stands for kinase domain receptor and refers to VEGFR2, and "Flt-4" refers to VEGFR3). Ex. 2031, 2. An excerpt of Figure 1 of Davis-Smyth is reproduced below starting at position 1:

```
Flt-1     1 MVSYWDTGVLLC-ALLSCLLLTGSS---SGSKLKDPELSLKGTQHIMQAGQTLHLQCRGEAAHKWSLPEMV------SKESERLSITKSACGRNGKQFCS
KDR       1 M----ESKVLLAVALWLCVETRAASVGLPSVSLDLPRLSIQKDILTIKANTTLQITCRGQRDLDWLWPNNQ-------SGSEQRVEVTECSDG---LFCK
Flt-4     1 M----QRGAALCLRLWLCLGLLDGLV--SGYSMTPPTLNITEESHVIDTGDSLSISCRGQHPLEWAWPGAQEAPATGDKDSEDTGVVRDCEGTDARPYCK

Flt-1    91 TLTLNTAQANHTGFYSCKYLAVPTSKKKETESAIYIFISDTGRPFVEMYSEIPEIIHM--TEGRELVIPCRVTSPNITVTL-KKFPLDTLIPDGKRIIWD
KDR      87 TLTIPKVIGNDTGAYKCFYR------ETDLASVIYVYVQDYRSPFIASVSDQHGVVYITENKNKTVVIPCLGSISNLNVSLCARYPEKRFVPDGNRISWD
Flt-4    95 VLLLHEVHANDTGSYVCYYKYIKARIEGTTAASSYVFVRDFEQPFI----NKPDTLLV--NRKDAMWVPCLVSIPGLNVTL--RSQSSVLWPDGQEVVWD

Flt-1   188 SRKGFIISNATYKEIGLLTCEATVNGHLYKTN-YLTHRQTNTIIDVQISTPRPVKLLRGHTLVLNCTATTPLNTRVQMTWSYPDEKNKRAS--VRRRIDQ
KDR     181 SKKGFTIPSYMISYAGMVFCEAKINDESYQSIMYIVVVVGYRIYDVVLSPSHGIELSVGEKLVLNCTARTELNVGIDFNWEYPSSKHQHKKLVNRDLKTQ
Flt-4   187 DRRGMLVSTPLLHDALYLQCETTWGDQDFLSNPFLVHITGNELYDIQLLPRKSLELLVGEKLVLNCTVWAEFNSGVTFDWDYPGKQAERGKWVPERRSQQ
```

Figure 3: Alignment of the CDs of Flt1, KDR and Flt4, taken from Fig. 1 of Davis-Smyth.

72. As expressly denoted in the figure caption, each of the "boxed areas" represents one of "seven Ig-like domains" of the three proteins aligned, with "Flt-1" representing VEGFR1. Therefore, the stretch of the amino acid sequence comprising VEGFR1 domain 2 is the "Flt-1" sequence contained in the second box (annotated by my addition of green highlight above). To determine where

VEGFR1 domain 2 begins starting from Flt-1 position 91, a threonine ("T" using a one-letter code for amino acids henceforth unless stated otherwise), I counted the position of the first amino acid residue of Flt-1 contained in this second box. An enlarged and annotated excerpt below shows that the first amino acid residue of VEGFR1 domain 2, a proline ("P"), is located at position 134.

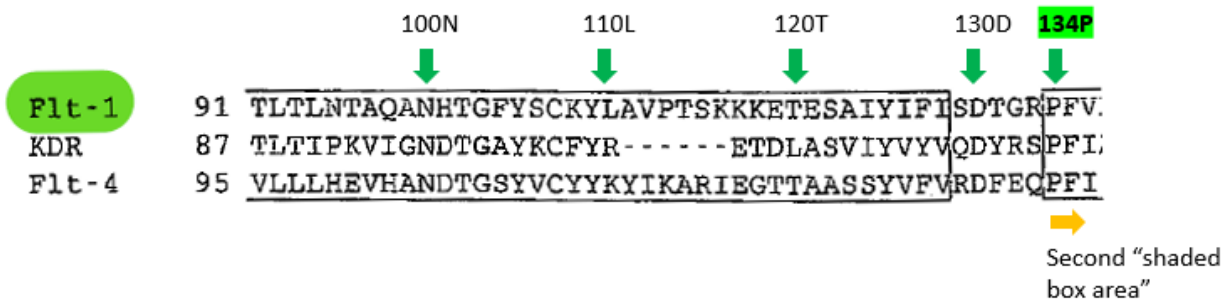


Figure 4: Inset showing first amino acid residue of VEGFR1 domain 2 according to Davis-Smyth.

73. To determine where VEGFR1 domain 2 ends starting from Flt-1 position 188, a serine ("S"), I counted the position of the last amino acid residue contained in this second box. The "-" was not counted as an amino acid residue position. An enlarged and annotated excerpt below shows that the last amino acid

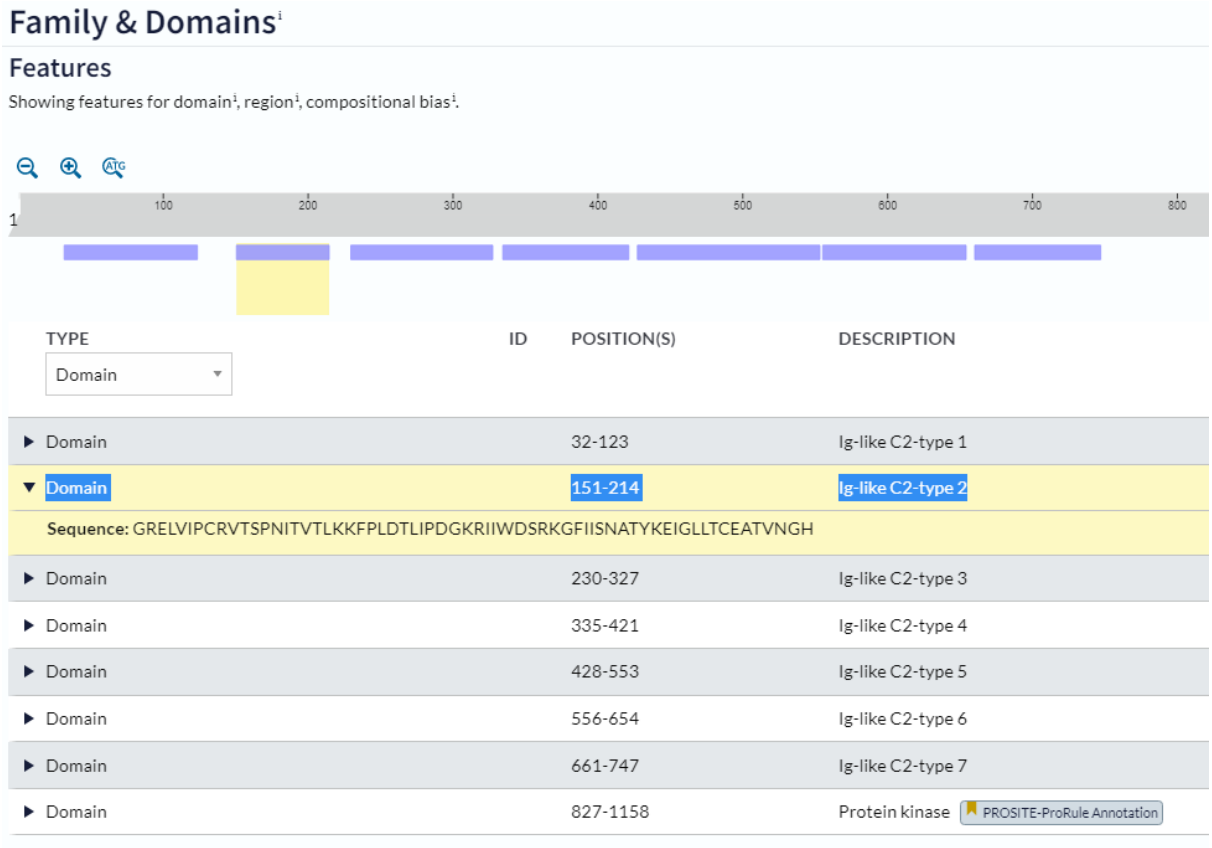
residue of VEGFR1 domain 2, a threonine ("T"), is located at position 226.

Figure 5: Inset showing last amino acid residue of VEGFR1 domain 2 according to Davis-Smyth.

74. Based on the starting and ending positions extracted from Davis-Smyth, I calculated the length of the stretch of amino acid sequence comprising VEGFR1 domain 2 to be 93 amino acid residues (226 - 134 + 1).

75. In determining the amino acid sequence comprising VEGFR1 domain 2 disclosed by UNIPROT P17948 (Ex. 2032), I reviewed the "Family & Domains" section of the website. This section annotates the seven domains of VEGFR1 as "Ig-like C2-type" 1 through 7. When I expanded the description for VEGFR1 domain 2 ("Ig-like C2 type 2"), the annotation indicates that the domain spans amino acid positions "151-214" and provides the corresponding amino acid sequence of the domain. Taken together, this annotation indicates that the VEGFR1 domain 2 starts at position 151, a glycine ("G"), and ends at position 214, a histidine ("H"). An excerpt of the domain annotation information from

UNIPROT P17948 is reproduced below.



Family & Domains

Features

Showing features for domain, region, compositional bias.

TYPE	ID	POSITION(S)	DESCRIPTION
Domain		32-123	Ig-like C2-type 1
Domain		151-214	Ig-like C2-type 2
Sequence: GRELVIPCRVTSPNITVTLKKFPLDTLIPDGKRIIWDSRKGFIISNATYKEIGLLTCEATVNGH			
Domain		230-327	Ig-like C2-type 3
Domain		335-421	Ig-like C2-type 4
Domain		428-553	Ig-like C2-type 5
Domain		556-654	Ig-like C2-type 6
Domain		661-747	Ig-like C2-type 7
Domain		827-1158	Protein kinase PROSITE-ProRule Annotation

Figure 6: Domain annotation information from UNIPROT P17948.

76. Based on the starting and ending positions extracted from UNIPROT P17948, I calculated the length of the stretch of amino acid sequence comprising VEGFR1 domain 2 to be 64 amino acid residues (214 - 151 + 1).

77. I further reviewed the amino acid sequence comprising VEGFR1 domain 2 disclosed by the ’319 Publication (Ex. 1029). In the ’319 Publication, the relevant amino acid sequence information is presented as a part of a fusion protein “Flt1D2.Flk1D3.FcΔC1(a)” with sequence information given in Fig. 21A-

C. Ex. 1029, 21:16-17. I have annotated and reproduced this amino acid sequence below:

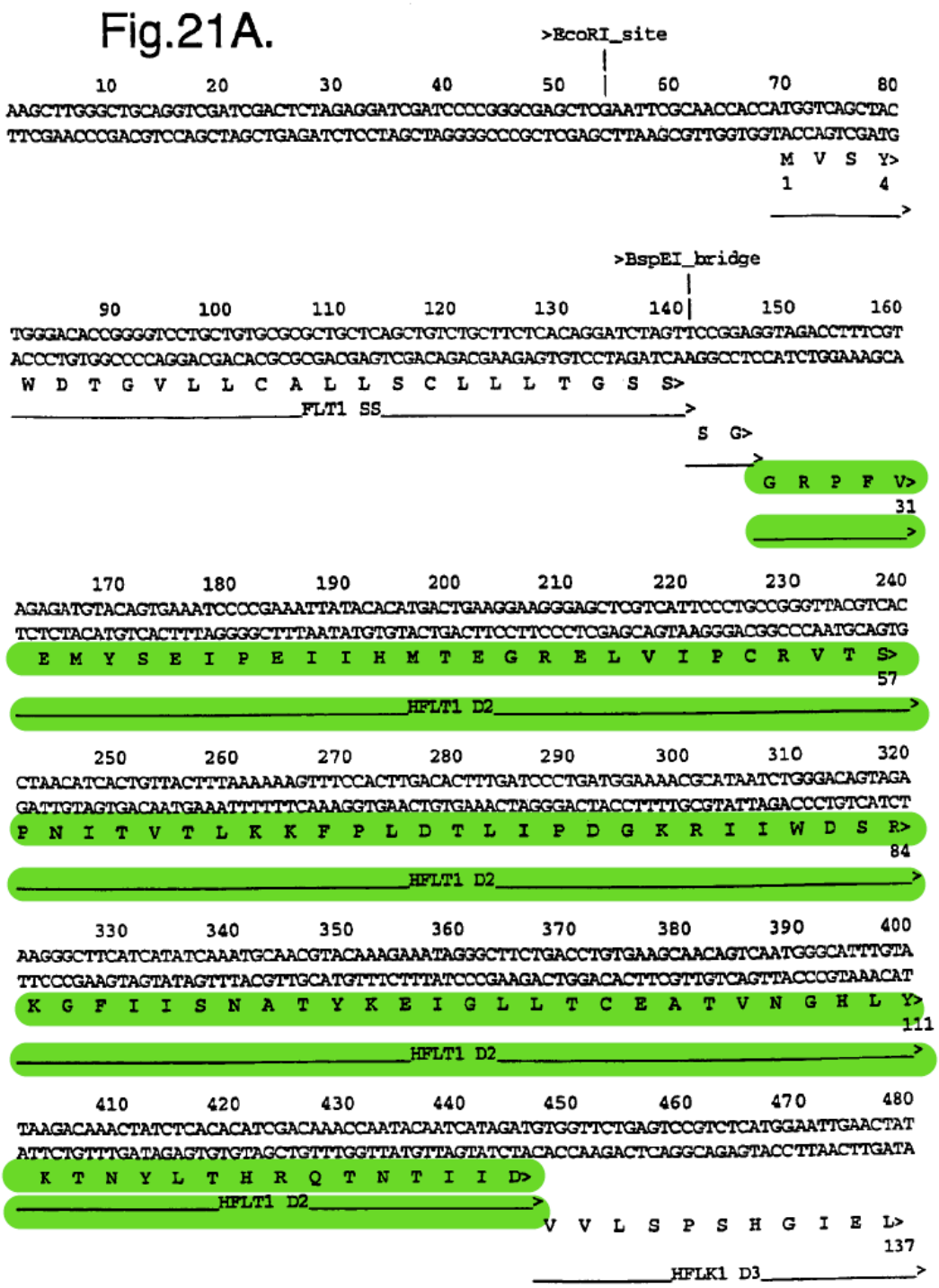


Figure 7: Excerpt from Fig. 21 of the '319 Publication.

78. In the excerpt depicted above, "HFLT1 D2" represents VEGFR1 domain 2. The corresponding stretch of amino acid sequence relevant to my analysis is highlighted in green above. To determine where VEGFR1 domain 2 begins starting from HFLT1 D2 position 31, a valine ("V"), I reverse-counted the position of the first amino acid residue in the domain. An enlarged and annotated excerpt below shows that the first amino acid residue of VEGFR1 domain 2, a glycine ("G"), is located at position 27.

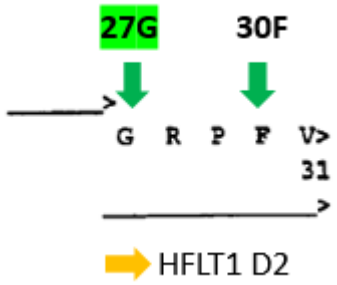


Figure 8: Detail of first amino acid residue of VEGFR1 domain 2 according to the '319 Publication.

79. To determine where VEGFR1 domain 2 ends starting from HFLT1 D2 position 112, a lysine ("K"), I counted the position of the last amino acid residue in the domain.[34] An enlarged and annotated excerpt below shows that the

[34] I understand that the above-starting lysine ("K") is in position 112 because the immediately prior amino acid residue from the previous row is marked as position 111.

last amino acid residue of VEGFR1 domain 2, an aspartic acid ("D"), is located at position 126.

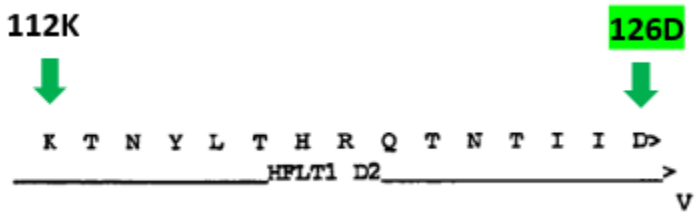


Figure 9: Detail of last amino acid residue of VEGFR1 domain 2 according to the '319 Publication.

80. Based on the starting and ending positions extracted from Papadopoulos, I calculated the length of the stretch of amino acid sequence disclosed as encoding VEGFR1 domain 2 to be 100 amino acid residues (126 - 27 + 1).

Appendix B – Amino Acid Sequences for VEGFR2 Domain 3

81. In determining the amino acid sequence comprising VEGFR2 domain 3 disclosed by Davis-Smyth (Ex. 2031), I reviewed the sequence information provided by the same Figure 1 I discussed above in paragraph 71, which is an "[a]lignment of the E[xtracellular]C[ellular]D[omain]s of Flt-1, KDR and Flt-4." Ex. 2031, 2. An excerpt of Figure 1 of Davis-Smyth is reproduced below starting at position 1:

```
Flt-1     1 MVSYWDTGVLLC-ALLSCLLLTGSS---SGSKLKDPELSLKGTQHIMQAGQTLHLQCRGEAAHKWSLPEMV------SKESERLSITKSACGRNGKQFCS
KDR       1 M----ESKVLLAVALWLCVETRAASVGLPSVSLDLPRLSIQKDILTIKANTTLQITCRGQRDLDWLWPNNQ-------SGSEQRVEVTECSDG---LFCK
Flt-4     1 M----QRGAALCLRLWLCLGLLDGLV--SGYSMTPPTLNITEESHVIDTGDSLSISCRGQHPLEWAWPGAQEAPATGDKDSEDTGVVRDCEGTDARPYCK

Flt-1    91 TLTLNTAQANHTGFYSCKYLAVPTSKKKETESAIYIFISDTGRPFVEMYSEIPEIIHM--TEGRELVIPCRVTSPNITVTL-KKFPLDTLIPDGKRIIWD
KDR      87 TLTIPKVIGNDTGAYKCFYR------ETDLASVIYVYVQDYRSPFIASVSDQHGVVYITENKNKTVVIPCLGSISNLNVSLCARYPEKRFVPDGNRISWD
Flt-4    95 VLLLHEVHANDTGSYVCYYKYIKARIEGTTAASSYVFVRDFEQPFI----NKPDTLLV--NRKDAMWVPCLVSIPGLNVTL--RSQSSVLWPDGQEVVWD

Flt-1   188 SRKGFIISNATYKEIGLLTCEATVNGHLYKTN-YLTHRQTNTIIDVQISTPRPVKLLRGHTLVLNCTATTPLNTRVQMTWSYPDEKNKRAS--VRRRIDQ
KDR     181 SKKGFTIPSYMISYAGMVFCEAKINDESYQSIMYIVVVVGYRIYDVVLSPSHGIELSVGEKLVLNCTARTELNVGIDFNWEYPSSKHQHKKLVNRDLKTQ
Flt-4   187 DRRGMLVSTPLLHDALYLQCETTWGDQDFLSNPFLVHITGNELYDIQLLPRKSLELLVGEKLVLNCTVWAEFNSGVTFDWDYPGKQAERGKWVPERRSQQ

Flt-1   285 SNSHANIFYSVLTIDKMQNKDKGLYTCRVRSGPSFKSVNTSVHIYDKAFITVKHRKQQVLETVAGKRSYRLSMKVKAFPSPEVVWLKDGLPATEKSARYL
KDR     281 SGSEMKKPLSTLTIDGVTRSDQGLYTCAASSGLMTKKNSTFVRVHEKPFVAFGSGMESLVEATVGER-VRIPAKYLGYPPPEIKWYKNGIPLESN--HTI
Flt-4   287 THTELS---SILTIHNVSQHDLGSYVCKANNGIQRFRESTEVIVHENPFISVEWLKGPILEATAGDELVKLPVKLAAYPPPEFQWYKDGKALSGR--H--
```

Figure 10: Figure 1 of Davis-Smyth.

82. Each of the "boxed areas" represents one of "seven Ig-like domains" of the three proteins aligned. In the excerpt above, the "KDR" (kinase domain receptor) represents VEGFR2. Therefore, the stretch of amino acid sequence comprising VEGFR2 domain 3 is the "KDR" amino acid sequence contained in the third box (annotated by my addition of green highlight above). *Id.*, Figure 1. To determine where VEGFR2 domain 3 begins starting from KDR position 181, a serine ("S"), I counted the position of the first amino acid residue of KDR

contained in this third box. An enlarged and annotated excerpt below shows that the first amino acid residue of VEGFR2 domain 3, a valine ("V"), is located at position 226.

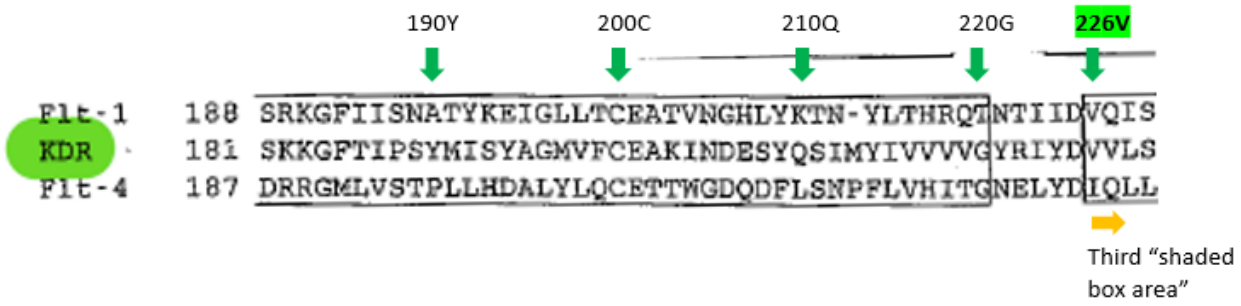


Figure 11: Detail of first amino acid residue of VEGFR2 domain 3 according to Davis-Smyth.

83. To determine where VEGFR2 domain 3 ends starting from KDR position 281, a serine ("S"), I counted the position of the last amino residue contained in this third box. An enlarged and annotated excerpt below shows that the last amino acid residue of VEGFR2 domain 3, a lysine ("K"), is located at position 327.

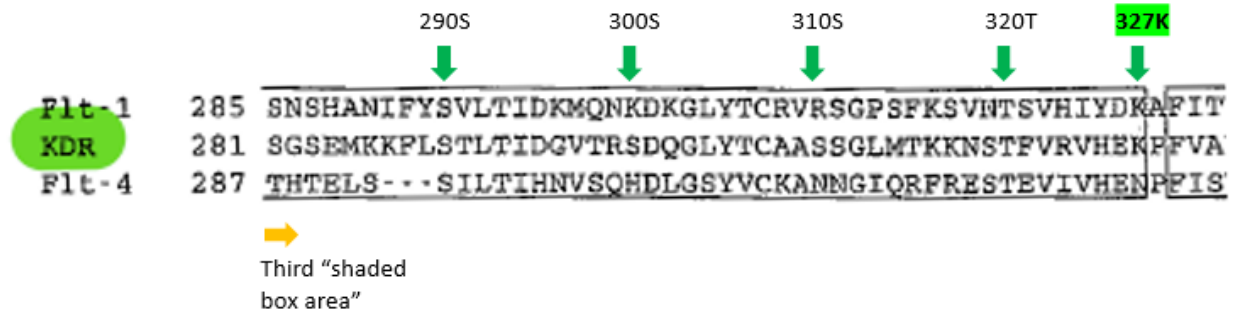


Figure 12: Detail of last amino acid residue of VEGFR2 domain 3 according to Davis-Smyth.

84. Based on the starting and ending positions extracted from Davis-Smyth, I calculated the length of the stretch of amino acid sequence comprising VEGFR2 domain 3 to be 102 amino acid residues (327 - 226 + 1).

85. Lu (Ex. 2034) discloses a different set of starting and ending amino acid residues of VEGFR2 domain 3. Under the "experimental procedures" section, Lu provides a "KDR domain 3 (from Tyr^{221} to Ser^{334})." Ex. 2034, 2. Based on this disclosure, the first amino acid residue of VEGFR2 domain 3 is a tyrosine ("T") located at position 221 and the last amino acid residue is a serine ("S") located at position 334. Therefore, Lu's amino acid sequence comprising VEGFR2 domain 3 has a length of 114 amino acid residues (334 - 221 + 1).

86. Franklin (Ex. 2035) discloses yet another set of starting and ending amino acid residues of VEGFR2 domain 3. Under the "experimental procedures" section, Franklin provides a "KDR domain 3[] containing residues Gly220-Ser328 of human KDR." Ex. 2035, 8. Based on this disclosure, the first amino acid residue of VEGFR2 domain 3 is a glycine ("G") located at position 220 and the last amino acid residue is a serine ("S") located at position 328. Therefore, Franklin's amino acid sequence comprising VEGFR2 domain 3 has a length of 109 amino acid residues (328 - 220 + 1).

87. In determining the amino acid sequence comprising VEGFR2 domain 3 disclosed by UNIPROT P35968 (Ex. 2036), I reviewed the "Family & Domains"

section of the website. This section annotates the seven domains of VEGFR2 as "Ig-like C2-type" 1 through 7. When I expanded the description for VEGFR2 domain 3 ("Ig-like C2 type 3"), the annotation indicates that the domain spans amino acid positions "224-320" and provides the corresponding amino acid sequence of the domain. Taken together, this annotation indicates that the VEGFR2 domain 3 starts at position 224, a tyrosine ("Y"), and ends at position 320, a threonine ("T"). An excerpt of the domain annotation information from UNIPROT P35968 is reproduced below.

Family & Domains

Features

Showing features for domain, region, compositional bias.

TYPE	ID	POSITION(S)	DESCRIPTION
Domain		46-110	Ig-like C2-type 1
Domain		141-207	Ig-like C2-type 2
Domain		224-320	Ig-like C2-type 3
Sequence: YDVVLSPSHGIELSVGEKLVLNCTARTELNVGIDFNWEYPSSKHQHKKLVNRDLKTQSGSEMKKFLSTLTIDGVTRSDQGLYTCAASSGLMTKKNST			
Domain		328-414	Ig-like C2-type 4
Domain		421-548	Ig-like C2-type 5
Domain		551-660	Ig-like C2-type 6
Domain		667-753	Ig-like C2-type 7
Domain		834-1162	Protein kinase PROSITE-ProRule Annotation

88. Based on the starting and ending positions extracted from UNIPROT P35968, I calculated the length of the stretch of amino acid sequence comprising VEGFR2 domain 3 to be 97 amino acid residues (320 - 224 + 1).