Filed on behalf of: Celltrion, Inc.
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UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

CELLTRION, INC.,
Petitioner,

v.

REGENERON PHARMACEUTICALS, INC.,
Patent Owner.

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

PETITION FOR INTER PARTES REVIEW OF U.S. PATENT NO. 10,406,226

TABLE OF CONTENTS

Page

I. INTRODUCTION AND SUMMARY

Celltrion, Inc. ("Petitioner") petitions for *inter partes* review ("IPR") under 35 U.S.C. §§ 311-319 and 37 C.F.R. §§ 42 *et seq.*, seeking cancellation of claims 1-4 (the "challenged claims") of U.S. Patent No. 10,406,226 ("'226 patent," EX1001), currently assigned to Regeneron Pharmaceuticals, Inc. ("Regeneron" or "Patent Owner"). This petition demonstrates a reasonable likelihood that claims 1-4 of the '226 are unpatentable, and respectfully requests institution of trial.

The challenged claims are drawn to a method of manufacturing VEGF antagonists, including aflibercept specifically, by expressing the protein in a Chinese hamster ovary ("CHO") cell, followed by purifying the expressed proteins. According to the claims, the protein is purified such that at least 90% of the weight of the protein is not present as an aggregate, and in dependent claims, until the protein is substantially free of protein contaminants, and where the protein is post-translationally glycosylated at one or more asparagine residues.

The '226 patent, however, mentions CHO cells only once, and does not specify any methods for the production or purification of the VEGF antagonist proteins in CHO cells, leaving it to the ordinary artisan to fill in the steps and conditions needed to achieve the claimed levels of aggregation, protein contaminants, and glycosylation.

Filling in those steps and conditions would have required only routine skill. Regeneron itself had disclosed its method of expressing aflibercept in CHO cells and purifying the expressed protein to reduce aggregation and contaminants before the priority date. Aflibercept and other VEGF antagonist proteins produced by this method were disclosed in Regeneron's *'319 Publication* (EX1004), *Holash* (EX1006), and *Wulff* (EX1007), all published more than one year before the effective filing date of the '226 patent. Thus, as explained below, the '226 patent relies on well-known methods to produce and purify the VEGF antagonist proteins to achieve levels of aggregation, contaminants, and glycosylation that were all easily achievable by the ordinary artisan. Reducing aggregation and contaminants in a protein produced in a CHO cell and purifying to the levels claimed in the patent was well within the skill of a POSA at the time, and in fact a POSA would have targeted aggregate and contaminant levels well below those claimed to avoid adverse effects and to ensure regulatory approval. Further, glycosylation was known to occur on the Fc portion of fusion proteins like aflibercept and a POSA would have expected a VEGF antagonist produced in a CHO cell to be glycosylated according to the claims. None of the resulting features of the proteins claimed in the '226 patent would have been unexpected when expressing in a CHO cell and purifying according to well-known and standard techniques. The fact that the inventors did not include

any description of how the proteins were expressed or purified in the patent shows it was well within the skill of a POSA to do so.

Petitioner files this Petition and supporting expert declaration from Dr Peter Tessier (EX1002), a protein engineering expert, with over 20 years' experience in the development of therapeutic proteins, to apprise the Board of invalidating prior art. *See* EX1002 ¶¶1-12; *see generally* EX1003. Claims 1-4 should be found unpatentable and cancelled.

A. Brief Overview of the '226 Patent

The '226 patent is titled "Method Of Manufacturing VEGF Antagonist Fusion Proteins." Independent claim 3 is representative and is reproduced below:

> 3. A method of manufacturing a VEGF antagonist fusion protein, said method comprising:
>
> a. expressing said VEGF antagonist fusion protein in a Chinese hamster ovary (CHO) cell comprising a polynucleotide encoding the VEGF antagonist fusion protein which comprises amino acids 27-457 of SEQ ID NO:4 wherein said fusion protein binds vascular endothelial growth factor (VEGF); and
>
> b. purifying said VEGF antagonist fusion proteins;
>
> wherein at least 90% of the weight of the purified fusion protein is not present as an aggregate.

EX1001, 20:34-44. Independent claim 1 is identical to claim 3, with the exception that it claims the "polynucleotide encoding the VEGF antagonist fusion protein which comprises amino acids 27-457 of SEQ ID NO:2", instead of SEQ ID NO:4. *Id.*, 19:35-46.

Dependent claims 2 and 4, which depend from claims 1 and 3 respectively, further specify that the fusion protein is "purified until substantially free of protein contaminants" (claim 2) and "posttranslationally glycosylated at one or more asparagine residues" (claim 4). *Id.*, 19:47-78; 20:45-48; EX1002 ¶37.

The '226 patent states that the protein "can be prepared by any suitable method known in the art." EX1001, 5:44-45. The disclosure of the '226 patent, however, mentions CHO cells only once. Thus, despite claiming methods of manufacturing VEGF antagonists in CHO cells, the '226 patent does not describe or exemplify these methods. Rather, the disclosure only notes that "[i]n specific embodiments, the VEGF antagonist is expressed in a mammalian cell line such as a CHO cell and may be modified posttranslationally," without any discussion of culture conditions, media, purification methods, purification conditions etc. *Id.*, 5:37-39; EX1002 ¶¶38-39. The '226 patent disclosure further claims the protein is preferably substantially free of protein contaminants and purified to achieve less than 10% aggregation, but it does not disclose or exemplify any methods for achieving those limitations. EX1001, 5:48-62; *see also* EX1013, 169 (The examiner

noting "[t]he specification does not offer even a paragraph about obtaining the claimed products in CHO and not even a single method step is detailed.").

Rather, the '226 patent is focused on formulations of these proteins and their stability. All of the examples describe the stability of formulations of the protein over time. *See* EX1001, Examples 1-5. There is nothing in the disclosure of the '226 patent to suggest that there was anything outside the level of skill of the ordinary artisan required to produce and purify the VEGF antagonist fusion proteins of SEQ ID NOS. 2 and 4, including purifying to ensure at least 90% of the protein is not present as an aggregate, that the protein is substantially free of contaminants, and is glycosylated at one or more asparagine residues, as required by the challenged claims. EX1002 ¶40.

B. Prosecution History

The application that issued as the '226 patent was filed on August 31, 2017, claiming benefit through a chain of applications to a provisional application filed more than 12 years earlier, on March 25, 2005. EX1001, 1. The '226 patent's priority chain includes six issued patents, two abandoned applications, and the 2005 provisional application. *Id.* Although the '226 patent claims priority to the provisional application, that application did not disclose expression in CHO cells, as required by the challenged claims. *See generally*, EX1008. As such, for the purposes of this IPR, Petitioner assumes the earliest effective filing date to which

the claims are entitled is March 22, 2006, the filing date of the first non-provisional application in the priority chain. EX1001, 1; EX1002 ¶21. Petitioner submits that regardless of which priority date is used this Petition demonstrates the unpatentability of the claims. Other than Application No. 15/692,893, which led to the allowance of the '226 patent, none of the claims cited in the priority applications were drawn to methods of manufacturing VEGF antagonist fusion proteins as in the challenged claims. *See e.g.*, EX1016.

The originally filed independent claims of the '226 patent included a claim to the mammalian cell that encodes the fusion protein, as well as a claim to the method of manufacturing the protein by expressing and purifying the protein as claimed in the issued patent; however, the independent process claim covered a broader group of proteins, namely those comprising "in order from the N-terminus to the C-terminus an immunoglobulin-like (Ig) domain 2 of a first VEGF receptor, an Ig domain 3 of a second VEGF receptor and a multimerizing component" and not comprising "a signal peptide or C-terminal lysine." EX1013, 42-44. The limitations requiring the protein to comprise amino acids 27-457 of SEQ ID NO:2 and NO:4 were included as dependent claims. *Id.* Additionally, the independent claims did not include a limitation requiring at least 90% of the weight of the protein not be present as an aggregate – this was instead included as a dependent claim. *Id*.

In the first Office Action, in September 2018, the Examiner rejected all pending claims (1-20) under 35 U.S.C § 112. EX1013, 95. First, a number of the claims were rejected as indefinite because they referred to glycosylation of particular residues and the examiner argued that without the sequence identification, the position of those residues isn't actually known. *Id.*, 96. All claims were also rejected for lacking written description because they claimed a genus, but there was insufficient description of that genus because the specification did not describe a representative number of species, or structural features common to the genus. *Id.*, 97-101. The examiner noted that there was insufficient information in the specification about how to make and use the protein, particularly without sequence information. *Id.* Finally, all claims were also rejected as failing to comply with the enablement requirement because the specification did not provide adequate description of the polynucleotide and the specification did not address manufacturing aspects at all, instead being focused on formulations. *Id.*, 101-103. As such, the examiner considered the amount of experimentation that would be required to be "immense." *Id*.

In response to these rejections, the applicant requested a telephone interview and submitted amended claims in January 2019. EX1013, 151-56. In doing so, the applicant cancelled original claims 1-20, and submitted 22 new claims, some of which claimed the cell line and others claimed the manufacturing process. *Id.* In

the new claims, the sequence ID was included in the independent claims. *Id.* The examiner issued a second office action in March 2019, rejecting some claims but allowing others. *Id.*, 163. The rejections included an indefiniteness rejection of a claim that required glycosylation at asparagine residues, but where the examiner noted the residues were not located at the claimed positions, and enablement rejections of cell line claims. *Id.*, 164-69. The examiner again noted the lack of guidance in the specification, stating that "[t]he specification does not offer even a paragraph about obtaining the claimed products in CHO and not even a single method step is detailed." *Id.*

In response, in May 2019, Regeneron cancelled all claims except four, two of which were amended. EX1013, 183-87. All of the claims previously rejected were cancelled, while the amendments added limitations from previously pending but allowed claims, (sequence IDs, CHO cells, and the requirement for at least 90% to not be present as an aggregate). *Id.* After these amendments were submitted, the claims were allowed. *Id.*, 189.

C. Brief Overview of the Scope and Content of the Prior Art

The publications below reflect invalidating disclosures of the claimed formulations, together with knowledge that skilled artisans would bring to bear in reading the prior art at the time, i.e., March 22, 2006. *See Ariosa Diagnostics v. Verinata Health, Inc.*, 805 F.3d 1359, 1365-68 (Fed. Cir. 2015). As established in

KSR, the knowledge of a skilled artisan is part of the store of public knowledge that must be consulted when considering whether a claimed invention would have been obvious. *KSR Int'l Co. v. Teleflex Inc.*, 550 U.S. 398, 415-22 (2007).

1. Background

a. *Manufacturing of Recombinant Therapeutic Proteins*

By 2006, the production of therapeutic proteins by recombinant technology (bringing together genetic material from multiples sources to create DNA sequences) was common, including the use of mammalian cells, and in particular, CHO cells, as an expression system. EX1009, 1393; EX1002 ¶¶41, 43. The basic process for producing therapeutic proteins by recombinant technology began as early as the 1980s, and followed a standard set of steps. EX1009, 1393; EX1002 ¶42. That process developed over the following two decades, resulting in the productivity of recombinant cell lines being "dramatically" increased by the early to mid-2000s. EX1009, 1393; EX1002 ¶42. By the priority date of the '226 patent, around 60-70% of all recombinant protein pharmaceuticals were produced via mammalian cells, because expression in these cells was shown to be superior to other host systems. EX1009, 1393; EX1002 ¶43. By 2004, CHO cells in particular "dominate[d] the domain of mass production of recombinant protein products." EX1009, 1396; EX1002 ¶43. This was because CHO cells presented distinct advantages for expressing complex proteins over other expression systems like *E. coli*. For

example, the use of CHO cells allowed for streamlined control of product-related variants during the cell culture phase, allowing for a more streamlined purification process. EX1005, 302; EX1002 ¶43. Given its advantages, CHO cells became the preferred expression system for protein production by the early 2000s. EX1002 ¶43, 49.

Following production via the recombinant process in CHO cells, the desired protein is typically present in a mixture with other products produced by the process, including misfolded, degraded, denatured, or aggregated forms of the same protein. EX1010, 2; EX1002 ¶44. Developers of proteins for therapeutic use required as pure a form of the protein as possible to avoid adverse effects that can be caused from these other products and contaminants of the manufacturing process. EX1002 ¶¶44-45. As such, proteins produced by the cell culture process are purified to remove as much of these impurities as possible. EX1010, 2; EX1002 ¶44. The resulting purified form of the protein is often referred to as the "bulk" and can then be used to formulate the protein into a therapeutic formulation. EX1010 (*Chang*), 3; EX1002 ¶44.

b. *Protein Purification*

As of the priority date, it was common to purify expressed proteins during the manufacturing process to reduce impurities that could form during the manufacturing process and that might create safety and efficacy concerns for the

therapeutic use of the protein. *See* EX1005, 301-302; EX1002 ¶45. It was well-known that the regulatory approval process imposed "extreme purity requirements" for therapeutic proteins and that the purification process needed to reliably produce highly purified proteins for therapeutic approval. EX1005, 301-302; EX1002 ¶45. Regulatory authorities also required characterization of the antibody's structural integrity, including purity, as part of the approval process. *See* EX1020, 12; EX1002 ¶¶36-37, 47.

Aggregation was a well-known product-related impurity prior to the filing date of the '226 patent. EX1002 ¶46. Prior art at the time taught the potential negative effects of aggregation, including immunogenicity, and as a result, aggregation was typically reduced during the purification process to low levels in the final bulk, usually much less than 10%, before the protein was formulated for therapeutic use. EX1005, 304, 306, Table 12.1 (noting possible immunogenicity from aggregates and that "[t]he aggregate content in the [harvested cell culture fluid] is about 5-15% for many antibodies, and it is typically reduced to below 0.5% in the final bulk."); *see also* EX1020, 14 (noting that "[l]oss of specific immunoreactivity due to denaturation or formation of aggregates … may lead to altered pharmacokinetics and/or binding to non-target tissues"); EX1002 ¶¶46-47. It was also well-known that other contaminants, including DNA, endotoxins, and animal proteins, could be produced when manufacturing proteins and needed to be

controlled and reduced for safe and effective therapeutic use. EX1005, 302-305; EX1020, 10-11, 14; EX1002 ¶47.

At the time, there were a number of common purification techniques employed by those developing therapeutic proteins. These included protein A affinity chromatography, size exclusion chromatography ("SEC"), cation exchange chromatography, and anion exchange chromatography. EX1005, 302, 305; EX1004, 69:21-71:5, Example 22; EX1002 ¶48. A POSA at this time would have known and been comfortable with ways to reduce aggregates and other contaminants to very low levels using these purification processes and would not have developed a protein for therapeutic use without purifying the expressed protein. EX1002 ¶48.

As Dr Tessier explains, purification typically involved multiple steps, with Protein A affinity chromatography used as the first step, while the other techniques discussed in the prior art (cation exchange, anion exchange) were used as further "polishing" steps to remove further impurities. EX1002 ¶48. SEC could also be used as a second step after Protein A purification; however, SEC was and is typically used with smaller scale production processes, including experimental production of proteins, and is less common on a large commercial manufacturing scale. EX1002 ¶48. As Dr Tessier also points out, some prior art references cited refer to "proteins" while others refer to "antibodies"; however, proteins like aflibercept share a similar structure with antibodies, including the Fc region that appears in both. EX1002

¶¶50-51. A POSA reading the prior art references would understand that the approaches taught for expression and purification of antibodies would also apply to proteins like aflibercept. EX1002 ¶52.

Further, as discussed above, it was well-known at the time that an advantage of expressing proteins in a CHO cell in particular was that the level of product-related impurities could be well-controlled during a streamlined production and recovery process. *See supra* Section I.C.1.a.

c. *VEGF Antagonists*

VEGF is a naturally-occurring protein that regulates "angiogenesis," the process by which new blood vessels are formed. EX1006, 11393; EX1002 ¶53. VEGF binds to specific VEGF receptors on the surfaces of cells responsible for angiogenesis, thereby increasing their activity. EX1006, 11393; EX1002 ¶53. Two of the best-characterized VEGF receptors are VEGF receptor 1 (VEGFR1) also known as Flt1, and VEGF receptor 2 (VEGFR2) also known as Flk1. EX1006, 11393, EX1007, 2798, Fig. 1; EX1002 ¶53. VEGFR1 and VEGFR2 both have an extracellular region consisting of seven domains to which VEGF binds. EX1007, 2798, Fig. 1; EX1002 ¶53. Once they bind to VEGF via these extracellular domains, the VEGF receptors combine to form a dimer that is the active, cell-signaling form. EX1012, 412; EX1002 ¶53.

VEGF had been identified as playing a role in angiogenesis in tumors, which is necessary for tumor growth, as early as 2002. EX1006, 11393; EX1011, 968; EX1002 ¶54. By 2005-2006, a number of VEGF inhibitors had been developed as anti-cancer therapies given their potential to inhibit tumor growth. EX1011, 971; EX1002 ¶54. One such example was bevacizumab, a humanized monoclonal antibody that binds to VEGF and blocks its activity. EX1011, 967, 971; EX1002 ¶54.

VEGF inhibitors had also been developed to treat age-related macular degeneration (wet AMD), a disease characterized by proliferation of blood vessels in the retina of the eye. EX1002 ¶55. An example developed for this purpose was ranibizumab, a modified fragment of the bevacizumab antibody. EX1012, 411; EX1002 ¶55.

d. *Aflibercept*

Aflibercept is a VEGF inhibitor developed by Regeneron and marketed under the name EYLEA®. It is also known as "VEGF-Trap$_{R1R2}$", "VEGFR1R2-FcΔC1(a)," and "VEGF Trap-Eye." EX1002 ¶60. Aflibercept is a fusion protein of domain 2 of the human VEGFR1 receptor and domain 3 of the human VEGFR2 receptor, linked via the Fc domain of a human IgG antibody as shown below:

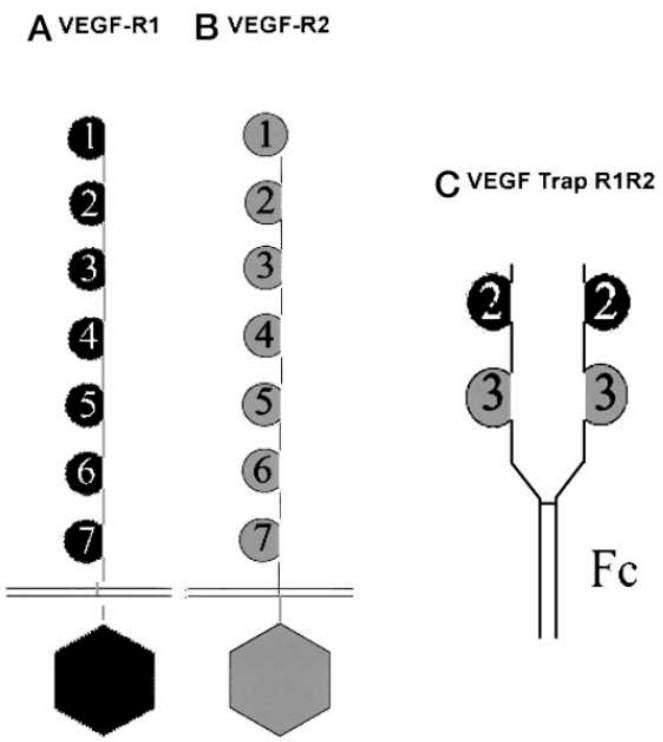

FIG. 1. Structure of VEGF receptors and the VEGF Trap. VEGF-R1 (A) and VEGF-R2 (B) both contain seven extracellular domains, which differ between the two receptors. These extracellular domains are responsible for VEGF binding. The soluble VEGF Trap R1R2 (C) was created by fusion of domain 2 of VEGF-R1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

EX1007, 2798, Fig. 1; EX1002 ¶56. Aflibercept, like bevacizumab and ranibizumab, works by binding to VEGF and "trapping" it before it can bind to cell-surface VEGF receptors, preventing it from triggering angiogenesis. EX1006, 11393; EX1002 ¶56.

Regeneron had published detailed descriptions of its development of aflibercept as early as 2002, along with its *in vivo* experiments, which demonstrated aflibercept's superior therapeutic promise over other known VEGF inhibitors at the time, including older VEGF-trap fusion proteins that Regeneron had also been

studying. *See generally* EX1007; EX1006; EX1002 ¶57. For example, early in development, Regeneron had experimented with a recombinant "parental VEGF-trap" fusion protein in which the first three extracellular domains of the human VEGFR1 receptor were fused to the Fc region of human IgG1. EX1006, 11393; EX1002 ¶57. That parental VEGF-trap had poor pharmacokinetics, which Regeneron's scientists reasoned was due to the fact that certain of the extracellular domains of VEGFR1 were positively charged, which can lead to non-specific binding to negatively-charged components of the extracellular matrix in the tissue at the site of injection. EX1006, 11394-95, Fig. 1; EX1002 ¶57. As a result of this finding, Regeneron removed or replaced positively-charged domains to create less-positively-charged variants, finding that this significantly improved pharmacokinetic performance. EX1006, 11395; EX1002 ¶57. Aflibercept thus emerged as the variant with the best combination of anti-VEGF activity and *in vivo* pharmacokinetics, and which compared favorably to other VEGF antagonists such as monoclonal antibodies. EX1006, 11397; EX1002 ¶57. As Regeneron's scientists put it, "[t]he combination of high-affinity and improved pharmacokinetics apparently contributes toward making VEGF-Trap$_{R1R2}$ [aflibercept] one of the most, if not the most, potent and efficacious VEGF blocker available." EX1006, 11397; EX1002 ¶57. Regeneron noted that aflibercept had the additional advantage of being composed of "entirely human sequences," which would "hopefully minimiz[e] the

possibility that it might prove immunogenic in human patients." EX1006, 11397; EX1002 ¶58. In comparison to existing antibody VEGF antagonists, "far lower circulating levels of VEGF-Trap$_{R1R2}$ [aflibercept] are required for similar efficacy" and its "safety has recently been confirmed in toxicological studies in cynomologus monkeys." EX1006, 11397; EX1002 ¶58. As a result of these superior properties "the [aflibercept] VEGF-Trap is currently in human clinical trials for several different types of cancer." EX1006, 11397; EX1002 ¶58.

Regeneron had also published initial positive data for its initial human clinical trials of aflibercept by 2005. EX1012, 414-15; EX1002 ¶59. Regeneron characterized the initial trial results as "quite promising" and noted that aflibercept was "now entering more advanced clinical trials in vascular eye diseases." EX1012, 414-15; EX1002 ¶59.

Regeneron had also widely published the sequence of aflibercept prior to the priority date, although it referred to the protein by its earlier scientific names, VEGF-Trap$_{R1R2}$ and VEGFR1R2-FcΔC1(a), rather than the non-proprietary name "aflibercept" it coined as the molecule neared regulatory approval.[1] EX1002 ¶60.

[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 Office that aflibercept is "also known as VEGF trap, VEGF-trap, VEGF Trap-

As early as 2004, Regeneron had published the amino acid sequences of VEGFR1R2-FcΔC1(a) in at least three earlier patent publications. *See* EX1004, 22:1-2, Figs. 24A-24C; EX1017, ¶89, SEQ ID No. 10; EX1018, ¶5, SEQ ID Nos. 1 and 2 (disclosing that VEGFR1R2-FcΔC1(a) is "also termed VEGF-Trap$_{R1R2}$); EX1002 ¶60.

e. *VEGF Flt1D2.Flk1D3.FcΔC1*

In its prior art publications Regeneron also disclosed its development and manufacture of another VEGF-antagonist, which, like aflibercept, is also a fusion protein of domain 2 of the human VEGFR1 receptor and domain 3 of the human

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Eye and VEGF-Trap$_{R1R2}$." EX1015, 2, 6-7; EX1002 ¶60, n.3. 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. EX1015, 5, 6-7 (noting that the "Flt1 Ig domain 2" of aflibercept "spans amino acid residues 27 through 129," "Flk1 Ig domain 3 spans amino acid residues 130 through 231," and "the Fc multimerizing component" spans amino acid residues 232 through 458"); EX1002 ¶60, n.3. The '758 patent is a divisional of the PCT application published as the '*319 Publication*, and Figures 24A-24C of the '758 patent are the same as Figures 24A-24C in the *'319 Publication*. *See* EX1014; EX1004; EX1002 ¶60, n.3.

VEGFR2 receptor, linked via the Fc domain of a human IgG antibody. *See generally* EX1004; EX1017; EX1002 ¶61. Regeneron had likewise published the sequence for this protein, which it referred to as Flt1D2.Flk1D3.FcΔC1. EX1004, Figs. 21A-21C, 58:6-64:22, Example 17; EX1017, ¶31, SEQ ID Nos. 7 and 8; EX1002 ¶61. Just like with aflibercept, Regeneron detailed its development and production of this VEGF variant and its *in vivo* performance, in publications before the priority date of the '226 patent. *See generally* EX1004; EX1002 ¶61. Regeneron also expressed this VEGF variant in CHO cells, grew the cells in bioreactors and then purified the protein by affinity and size exclusion chromatography. EX1004, 67:7-12, Example 20; EX1002 ¶61.

Aflibercept differs from the Flt1D2.Flk1D3.FcΔC1 expressed protein as shown in Figure 36 of the *'319 Publication* in that three amino acids have been added between amino acids 26 and 27 of Flt1D2.Flk1D3.FcΔC1 (Figures 21A-21C)—SDT—and the three amino acids corresponding to amino acids 229-231 of Flt1D2.Flk1D3.FcΔC1—GPG— have been removed. EX1004, 67:7-12; EX1002 ¶61, n.4. Regeneron also detailed the properties of this protein, including its binding stoichiometry, peptide mapping, and pharmacokinetics. EX1004, 74:15-79-6, 80:20-83:19, Examples 25, 26, 28, 29; EX1002 ¶61. Regeneron disclosed that this variant had five possible N-linked glycosylation sites on asparagine residues, all glycosylated to varying degrees. EX1004, 80:20-82:2, Example 28; Fig. 36;

EX1002 ¶61. Regeneron also demonstrated that this variant significantly reduced the growth of tumors *in vivo* and showed a similar pharmacokinetic profile to aflibercept. EX1004, 82:4-84:17, Examples 29-30, Figs. 37, 39 and 40; EX1002 ¶62. As a result, Regeneron characterized this variant, along with aflibercept, as "appropriate … for use in therapies in which antagonism of VEGF is a desired result." EX1004, 29:3-6; EX1002 ¶62.

f. *Expression and purification of VEGF antagonists*

In a number of prior art publications, Regeneron disclosed that it used a CHO cell host vector system to express aflibercept and other VEGF-antagonist variants, including Flt1D2.Flk1D3.FcΔC1. *See, e.g.*, EX1004, 12:14-26,15:19-27, Figs. 21A-21C, 24A-24C, claims 9, 20, 67:19-69:19, Example 21 (describing and claiming the production of VEGF antagonists in CHO cells); EX1017, ¶¶15, 31, 98 Example 5; Figs. 7-10; *see also* EX1018 ¶¶22, 26 (noting that CHO cells were a suitable host cell and pointing the skilled artisan to the *'319 Publication* for "a complete description of VEGF-receptor based antagonists including VEGFR1R2-FcΔC1(a)" and incorporating the *'319 Publication* into the *'309 Publication* "by reference in its entirety."); EX1002 ¶¶63. *Wulff* disclosed that Regeneron made VEGF-Trap$_{R1R2}$ by expressing it in CHO cells. EX1007, 2798 and n.1 (explaining that "[t]he VEGF Trap R1R2 used in these experiments … was expressed in CHO cells" and pointing the reader to the *'319 Publication* for a description of "the detailed molecular

structure" of VEGF-Trap$_{R1R2}$ and how it was created."); EX1002 ¶63. *Holash* also disclosed that the VEGF-Trap variants described in its experiments, including aflibercept, were produced from CHO cells. EX1006, 11393-94; EX1002 ¶63.

As discussed above, CHO cells "dominated" the mass production of proteins by 2004, EX1009, 1396, and were a preferred choice for manufacturers of therapeutic proteins at the time, including Regeneron. EX1002 ¶¶43, 64. Unsurprisingly, Regeneron disclosed in a number of prior art references that the VEGF-antagonists it developed and produced, including aflibercept and *VEGF* Flt1D2.Flk1D3.FcΔC1, were produced via CHO cells and purified as part of the manufacturing process. *See, e.g.*, EX1004, 69:21-71:5, Example 2 (describing purification of Flt1D2.Flk1D3.FcΔC1 using Protein A and size exclusion chromatography); EX1017 ¶¶ 39, 50 (teaching that the VEGF traps may be purified by, *inter alia*, ion exchange chromatography and Protein A chromatography); EX1002 ¶65. Regeneron's purification of VEGF antagonists it produced used standard purification techniques, as discussed in Section I.C.1.b. above. EX1002 ¶65. A POSA at the time would have understood the purpose of purifying these proteins via these methods was to reduce aggregation and other contaminants to ensure the protein could be used safely and effectively for therapeutic use. EX1002 ¶65.

g. *Glycosylation*

Glycosylation refers to the process where sugars or "glycans" are created, altered and attached to proteins or other biomolecules. EX1002 ¶65. Protein glycosylation is one of the most common forms of post-translation modifications that can occur in a protein, particularly proteins expressed in CHO cells. EX1002 ¶66. There are two main types of glycosylation: N-linked glycosylation, which refers to glycans being attached to the side-chain nitrogen atoms of asparagine residues, and O-linked glycosylation, which refers to glycans being attached to the side-chain oxygen atoms of hydroxyl amino acids, particularly serine and threonine residues. EX1002 ¶66. N-linked glycosylation typically occurs by attaching a glycan to the amine of an asparagine residue for the sequence motif "asparagine-X-serine/threonine," where the first residue after asparagine ("X") can be any residue except proline and the second residue after asparagine can be either serine or threonine. EX1022, 1867; *see also* EX1021, 13; EX1002 ¶66.

By 2005-2006, the glycosylation of IgGs was well-characterized, and N-linked glycosylation was known to occur in the IgG at a conserved asparagine residue in the C_H2 (also known as $C_\gamma2$) domain (in the Fc region) at position 297. *See* EX1021, 12; EX1023, 980; EX1024, 45539; EX1002 ¶67. This same IgG-Fc region of antibodies is commonly used for producing Fc fusion proteins, including for aflibercept and similar VEGF Traps. *See* EX1007, 2798, Fig. 1; EX1002 ¶¶50-

51, 56, 67; *supra* I.C.1.b-d. The conserved Fc glycosylation site is maintained in any Fc fusion protein, such as in aflibercept and similar VEGF Traps, and therefore these fusion proteins will contain at least one N-linked glycan in the Fc region. EX1002 ¶67. In addition, Fc fusion proteins can also contain N-linked glycans in the non-Fc portion of the fusion protein, and these sites can be readily identified based on the sequence motif "asparagine-X-serine/threonine", as discussed above. EX1002 ¶¶66-67.

It had also been shown prior to the priority date that glycosylation of asparagine residues of the Fc region of IgG was essential to the functions of the Fc region. EX1021, 12; EX1023, 980-81; EX1024, 45539; EX1002 ¶68. For example, glycosylation of the Fc region is important for IgG and Fc-fusion protein binding to FcγRs, which results in various effector functions, such as Antibody-Dependent Cellular Cytotoxicity (ADCC) and Complement-Dependent Cytotoxicity CDC. EX1021, 14-15; EX1023, 980-81; EX1024, 45539; EX1002 ¶68.

Regeneron's own prior art publications showed that the VEGF Traps it was developing contained glycosylation at a number of asparagine residues, which were located in the sequence motif "asparagine-X-serine/threonine." EX1004, 8-:20-82:2, Example 28; EX1017 ¶98; EX1002 ¶69. In particular, there were two expected N-linked glycosylation sites in hFLT1 IgG Domain 2, two in the hFLK1 IgG Domain 3, and one in the Fc portion of the fusion protein. EX1004, 80:20-82:2, Example

28; *see also* EX1017 ¶98 (stating that "when expressed as secreted proteins in CHO [cells]" the VEGF Traps were glycosylated "as expected."); EX1002 ¶69.

2. Key Prior Art[2]

a. *'319 Publication (EX1004)*

WO 00/75319 A1 ("*'319 Publication"*, EX1004) is a PCT publication that was published on December 14, 2000, which is more than one year prior to the '226 patent's earliest possible priority date of March 22, 2006, and thus qualifies as prior art under pre-AIA 35 U.S.C. 102(b) and post-AIA 35 U.S.C. 102(a)(1). It is entitled "Modified Chimeric Polypeptides with Improved Pharmacokinetic Properties" and states on its face that it is assigned to Regeneron. The *'319 Publication* is the "Publication WO 00/75319 A1" referred to in *Wulff*, discussed below, as disclosing the structure of VEGF-Trap$_{R1R2}$.

The *'319 Publication* describes its invention as "Flt1 receptor polypeptides that have been modified in such a way as to improve their pharmacokinetic profile," and as having "improved pharmacokinetic properties." EX1004, 1:14-16; 10:3-4. It discloses the specific steps Regeneron took to develop such Flt1 receptor

[2] The asserted prior art references all qualify as publications that were available to—and indeed cited by—interested, skilled artisans before March 22, 2006. EX1002 ¶¶22, 70, 105, 119.

polypeptides, including aflibercept, describing that the Flt1 receptor polypeptides can be produced by expression in CHO cells and purified to produce a stable, biologically active protein. *See id.*, 34:9-18, 58:6-69:19, Examples 17-21; *see also id.*, 12:19-26; claims 20, 21. According to the *'319 Publication*, the Flt1 receptor polypeptides "may be purified by any technique which allows for the subsequent formation of a stable, biologically active chimeric polypeptide molecule." *Id.*, 34:9-11. For example, the *'319 Publication* teaches the use of Protein A affinity chromatography, which binds the Fc portion of Flt1 receptor polypeptides, to initially purify the molecules obtained from CHO cells. *Id.*, 38:12-20, Example 2; *see also id.*, 39:6-19 (more detailed description of the purification process); *id.*, 70:1-25, Example 22 (same).

The *'319 Publication* teaches that the Flt1D2.Flk1D3.FcΔC1(a), a modified Flt protein, is considerably less sticky to the extracellular matrix than are Flt1(1-3)-Fc unmodified proteins. EX1004, 65:15-19, Fig. 23. The *'319 Publication* demonstrates further that fusion proteins such as Flt1D2.Flk1D3.FcΔC1(a) had longer half lives in serum (*id.*, 83:15-19, Fig. 38), and inhibited tumor growth *in vivo* (*id.*, 84:14-17, Figs. 39-40).

Amino acids 27-457 of the sequence for Flt1D2.Flk1D3.FcΔC1(a) correspond to amino acids 27-457 of SEQ ID NO:2 of challenged claim 1. *Id.*, Figs. 21A-21C; EX1002 ¶73, n.5. Figure 36 shows the expressed protein, including glycosylation

sites (underlined "N") and cysteine bonds. EX1004, 25:18-26:4. Amino acids 27-457 of the sequence correspond to the fusion protein, while amino acids 1-26 correspond to the Flt1 signal sequence. *Compare* Fig. 21A with Fig. 36; EX1002 ¶73.

The *'319 Publication* refers to aflibercept as VEGFR1R2-FcΔC1(a) (SEQ ID NO:4 of the challenged claims), which Regeneron has represented is another name for VEGF-Trap$_{R1R2}$. *See supra* n.1. It also discloses the amino acid sequence and structure of VEGFR1R2-FcΔC1(a) (aflibercept). EX1004, 11:14-12:1, 15:19-27, Figs. 24A-24C; EX1002 ¶95, n.6. The *'319 Publication* teaches that a VEGFR1R2-FcΔC1(a) expression vector was constructed by starting with the Flt1D2.Flk1D3.FcΔC1(a), and inserting DNA encoding amino acids SDT between amino acids 26 and 27 of Flt1D2.Flk1D3.FcΔC1(a) as shown in Figures 21A-21C (which corresponds to amino acids 27-29 of Figs. 24A-24C (the amino acid sequence of aflibercept)) and removal of DNA encoding amino acids GPG corresponding to amino acids 229-231 of Figures 21A-21C. EX1004, 67:7-12. Thus, amino acids 27-457 of Figures 24A-24C were expressed in the *'319 Publication*. *E.g. id.*, 82:10-13.

b. *Holash (EX1006)*

Holash is titled "VEGF-Trap: A VEGF blocker with potent antitumor effects" and was published in the scientific journal Proceedings of the National Academy of

Sciences on August 20, 2002. August 2002 is more than one year prior to the '226 patent's earliest possible priority date of March 22, 2006, and thus Holash qualifies as prior art under pre-AIA 35 U.S.C. 102(b) and post-AIA 35 U.S.C. 102(a)(1). *Holash* lists Regeneron as an employer of at least one of the authors. EX1006, 11393.

Holash describes Regeneron's development of aflibercept. In particular, it discloses that an earlier, positively-charged "parental VEGF Trap" fusion protein had poor pharmacokinetic properties that Regeneron postulated "might be due to the high positive charge of this protein (pI 9.4), which in turn may result in its deposition at the site of s.c. injection because of nonspecific adhesion to highly negatively charged proteoglycans that comprise the extracellular matrix." *Id.*, 11395. To test that theory, Regeneron "engineered several variants of the parental VEGF-Trap with reduced positive charges." *Id.* One of the variants was named "VEGFTrap$_{R1R2}$." *Id.* This variant "was created by fusing the second Ig domain of VEGFR1 with the third Ig domain of VEGFR2" and had a lower positive charge. *Id.*, 11393, 11395. Beyond having a lower charge, Regeneron reasoned from prior structural studies that this structure would result in superior binding: "Previous structural analyses indicated that VEGFR1 might make greater use of its second Ig domain in contacting VEGF, whereas VEGFR2 instead makes greater use of its third Ig domain (26), raising the

interesting and useful possibility that VEGFTrap$_{R1R2}$ might actually bind more tightly to VEGF than the parental versions." *Id.*, 11395.

Subsequent in vitro and in vivo testing confirmed Regeneron's hypotheses. VEGFTrapR1R2 proved to be the best of all of the variants Regeneron created:

> The combination of high-affinity and improved pharmacokinetics apparently contributes toward making VEGF-TrapR1R2 one of the most, if not the most, potent and efficacious VEGF blocker available. An additional advantage is that VEGF-TrapR1R2 is composed of entirely human sequences, hopefully minimizing the possibility that it might prove immunogenic in human patients. Despite its wholly human nature, VEGF-TrapR1R2 binds all species of VEGF tested, from human to chicken VEGF (not shown), making it a very versatile reagent that can be used in almost any experimental animal models.

Id., 11397. *Holash* also disclosed that the VEGF-TrapR1R2 was prepared using CHO cells. *Id.*, 11394 ("All of the VEGF-Trap variants were produced and purified from Chinese hamster ovary cells.").

c. *Wulff (EX1007)*

Wulff is titled "Prevention of Thecal Angiogenesis, Antral Follicular Growth, and Ovulation in the Primate by Treatment with Vascular Endothelial Growth Factor Trap R1R2" and was published in the scientific journal Endocrinology in July 2002, which is more than one year prior to the '226 patent's earliest possible priority date

of March 22, 2006. *Wulff* thus qualifies as prior art under pre-AIA 35 U.S.C. 102(b) and post-AIA 35 U.S.C. 102(a)(1). *Wulff* lists Regeneron as an employer of at least one of the authors. EX1007, 2797.

Wulff conducted *in vivo* tests to investigate the ability of aflibercept to inhibit thecal angiogenesis in marmoset monkeys. *Id.*, 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.* *Wulff* refers the reader to the *'319 Publication* (EX1004) for the structure of VEGF-Trap$_{R1R2}$, stating that "the detailed molecular structure and how it was created are described in the patent REG 710-A-PCT, VEGF Trap Application published December 2000, Publication WO 00/75319 A1." *Id.*, n.1.

Wulff notes that "four marmosets were treated with VEGF trap at a dose of 25 mg/kg, injected sc on d 0, 2, 4, 6, and 8 of the follicular phase" and that "[c]ontrol animals were treated with vehicle containing 5mM phosphate, 5 mM citrate, 100 mM sodium chloride, 0.1% (wt/vol) Tween 20, and 20% (wt/vol) sucrose)." *Id.* A POSA would have understood that the "vehicle" referred to in *Wulff* contained

aflibercept for the test animals and no aflibercept for the control animals, since the aflibercept could not have been injected into the animals unless it was dissolved within the vehicle. EX1002 ¶123, n.7.

d. *Fahrner (EX1005)*

Fahrner is a review article discussing methods of purifying antibodies at industrial scale. EX1005, 301. *Fahrner* was published in the scientific journal Biotechnology and Genetic Engineering Reviews in July 2001, which is more than one year prior to the '226 patent's earliest possible priority date of March 22, 2006, and thus *Fahrner* qualifies as prior art under pre-AIA 35 U.S.C. 102(b) and post-AIA 35 U.S.C. 102(a)(1). *Fahrner* teaches that that the most clinically significant antibodies are produced in CHO cells. *Id.* The main advantage of using CHO cells include that "the level of product-related variants can be effectively controlled during cell culture so that little or no variants must be removed during recovery," which allows for a streamlined recovery process using protein A affinity chromatography and cationic and anionic exchange chromatography. *Id.*, 302. According to *Fahrner*, the primary consideration of purification is purity, because, unless the purity requirement for biological pharmaceuticals is met, there may be no product. *Id.*, 303.

As for aggregates, *Fahrner* states that they are the most product-related variant that must be removed because of their possible immunogenicity. EX1005

304. *Fahrner* notes that the typical aggregate content of harvested cell culture fluid ("HCCF") is 5-15% for most antibodies and is then typically reduced to below 0.5% in the final bulk. *Id.*, 304, 306, Table 12.1. Cation exchange chromatography is used to remove the aggregates. *Id.*

The process disclosed by *Fahrner* is Protein A chromatography, cationic exchange chromatography, and then anionic exchange chromatography. *Id.*, 305. Protein A chromatography clears host cell proteins, DNA, and endotoxin, but does not clear aggregates and may add Protein A. *Id.*, 306. Cation exchange chromatography clears host cell proteins, aggregate, and leached protein A. *Id.*, 307. Anion exchange chromatography removes DNA and residual host cell proteins. *Id.*, 308.

3. Level of Ordinary Skill in the Art

A person of ordinary skill in the art (referred to herein as a "skilled artisan") is presumed to be aware of all pertinent art, think along the lines of conventional wisdom, and possess common sense and ordinary creativity in the pertinent field. A skilled artisan here would have would have had a Ph.D. in Chemical, Biological or Biomedical Engineering or a similar field, including Biology, Biochemistry or Chemistry, with at least several, *i.e.*, more than 2, years of experience in the development, manufacture and characterization of therapeutic proteins, including for example, fusion proteins or antibodies. EX1002 ¶¶30-32, 34. The POSA may

also have had less education but substantially more practical relevant experience. *Id*. This individual would have understood how to design and engineer therapeutic proteins, how to express them in host cells systems, and how to implement and control purification of proteins in order to optimize their use in therapeutic products. *Id*. This individual also would have been able to use then state-of-the-art purification and analytical methods, such as protein A affinity chromatography, size exclusion chromatography, and anion and cation exchange chromatography. *Id*. This POSA would have had access to other individuals typically employed in developing therapeutic proteins, including those involved in analytical chemistry, pharmacokinetics, formulation development, clinical testing, pharmaceutical packaging, and regulatory affairs. *Id.* ¶33. Those diversely-qualified individuals would have worked together as needed during the manufacturing and development process. *Id*.

II. THE BOARD SHOULD DECLINE TO EXERCISE ITS DISCRETION TO DENY INSTITUTION

A. The Board Should Not Exercise Its Discretion Under Section 325(d) to Deny Institution

Patent Owner may urge the Board to deny institution because "the same or substantially the same prior art or arguments previously were presented to the Office," but the Board should decline to exercise its discretion to deny institution. 35 U.S.C. §325(d).

In determining whether to exercise its discretion to deny institution under §325(d), the Board applies a two-part framework. *Advanced Bionics, LLC v. MED-EL Elektromedizinische Geräte GmbH*, IPR2019-01469, Paper 6 (Feb. 13, 2020) (precedential). The first part assesses "whether the same or substantially the same art previously was presented to the Office or whether the same or substantially the same arguments previously were presented to the Office." *Id.*, 8. "[I]f either condition of [the] first part of the framework is satisfied," the second part assesses "whether the petitioner has demonstrated that the Office erred in a manner material to the patentability of [the] challenged claims." *Id.* The following factors help inform whether the first part of the framework is satisfied: "(a) the similarities and material differences between the asserted art and the prior art involved during examination; (b) the cumulative nature of the asserted art and the prior art evaluated during examination; (c) the extent to which the asserted art was evaluated during examination, including whether the prior art was the basis for rejection; (d) the extent of the overlap between the arguments made during examination and the manner in which Petitioner relies on the prior art or Patent Owner distinguishes the prior art; (e) whether Petitioner has pointed out sufficiently how the Examiner erred in its evaluation of the asserted prior art; and (f) the extent to which additional evidence and facts presented in the petition warrant reconsideration of the prior art or

arguments." *Id.*, 9-10; *see also Becton, Dickinson & Co. v. B. Braun Melsungen AG*, IPR2017-01586, Paper 8, 17-18 (Dec. 15, 2017) (precedential).

The '226 patent claims priority to a series of applications: U.S. Application Nos. 15/342,989, 15/064,343, 14/550,385, 13/909,745, 13/428,510, 13/343,214, 12/835,065, and 11/387,256. However, it was not until the application that led to the '226 patent, 15/692,893, that claims to a manufacturing process using CHO cells were examined. *See* EX1016.

In addition, this petition presents art and arguments that are materially different than those presented to the Office during prosecution of the '226 patent. *Fahrner* and *Wulff* were not listed on the front of the '226 patent, nor were they applied by the Office during prosecution. And although the *'319 Publicatio*n and *Holash* are listed on the face of the patent as cited references, these references were not discussed or applied during prosecution, and there is no evidence that these references were considered by the Office in context of the claimed method of producing VEGF antagonist fusion proteins, especially as both references discuss using CHO cells and known purification methods to produce VEGF antagonist fusion proteins.

The only rejections made during prosecution of the application that led to the '226 patent were rejections based on 35 U.S.C. § 112, due to certain claims not specifying the sequence of the protein, and claiming a genus without disclosing a

representative number of species. *See supra* Section I.B. In order to overcome the rejections, Regeneron cancelled and amended claims to add limitations to the sequence, as well as to recite the limitation requiring less than 10% aggregates. *See generally* EX1013. No rejections were made on the basis of obviousness or anticipation over any prior art references, and Regeneron did not argue any unexpected results for the claimed manufacturing method. Thus, it is apparent that the Office did not consider the *'319 Publication* or *Holash* references.

In addition, it was error for the *'319 Publication* and *Wulff* not to be applied against the claims. Regeneron did not make the Examiner aware of its own *'319 Publication*, which disclosed the same VEGF antagonist comprising amino acids 27-457 of both SEQ ID NO: 2 and SEQ ID NO: 4 that is described in the challenged claims. *The '319 Publication* also disclosed a method producing those VEGF antagonist proteins using CHO cells and purifying the proteins using Protein A and size exclusion chromatography. EX1004, 67:25-68:5. Nor did Regeneron make the Examiner aware of *Wulff*, which disclosed that Regeneron's VEGF $Trap_{R1R2}$ protein was made using CHO cells, as required by the challenged claims, and directed the reader to the *'319 Publication* and its disclosure that the VEGF $Trap_{R1R2}$ protein is aflibercept. This petition therefore raises new arguments about the *'319 Publication* and *Wulff* that are based on new evidence and prior art that were not before the Office during prosecution.

A review of the *Becton Dickinson* factors supports institution. As discussed, although the *'319 Publication* was cited as a reference on the face of the patent, it was not cited during examination, and it was not similar to any prior art involved during prosecution. The examiner did not make a prior art rejection in the application leading to the '226 patent, the only application in priority chain that contains claims drawn to methods of producing VEGF antagonist fusion proteins using CHO cells. Thus, factor (a) supports institution. As no prior art rejections were made, the *'319 Publication* is not cumulative to the prior art involved during examination, and as it was not the basis for any rejection, factors (b), (c), and (d) support institution. And since Petitioner has explained why failing to apply the *'319 Publication* against the claims, including the failure of Regeneron to bring *Wulff* to the attention of the Examiner, factors (e) and (f) support institution.

Both parts of the Board's two-part framework are satisfied, and the '226 patent claims would not have been allowed had the Office considered the evidence and arguments presented herein. The Board should thus decline to exercise its discretion under §325(d).

B. The Board Should Not Exercise Its Discretion Under Section 314(a) to Deny Institution

Regeneron may argue that the Board should exercise its discretion under 314(a) and the Board's precedential decision in *Apple Inc. v. Fintiv, Inc.*, IPR2020-00019, Paper 11 (PTAB Mar. 20, 2020) (precedential), to deny institution based on

the co-pending district court litigation in *Regeneron Pharmaceuticals, Inc. v. Mylan Pharmaceuticals Inc.*, Case No. 1-22-cv-00061 (N.D.W.V), filed on August 2, 2022.

Under *Fintiv*, the Board looks at the following factors:

1. whether the court granted a stay or evidence exists that one may be granted if a proceeding is instituted;

2. proximity of the court's trial date to the Board's projected statutory deadline for a final written decision;

3. investment in the parallel proceeding by the court and the parties;

4. overlap between issues raised in the petition and in the parallel proceeding;

5. whether the petitioner and the defendant in the parallel proceeding are the same party; and

6. other circumstances that impact the Board's exercise of discretion, including the merits.

The most important factor here which counsels against the Board exercising its discretion to deny institution is 5—whether the petitioner and defendant in the parallel proceeding are the same party. Petitioner here, Celltrion, is not a party to that litigation. Factors 1 and 3 are thus neutral, as Celltrion has no input as to whether a stay will be pursued at the district court, and has no investment in the district court proceeding.

As to the other factors, the District Court has entered a scheduling order. EX1028. However, in the scheduling order, Regeneron was ordered to identify six patents from three patent families to which the deadlines would apply. *Id.* The '226 patent is not one of those patents. EX1029. Thus, factor 2 does not favor exercise of discretion not to institute, as the '226 patent is not at issue in the first stage of litigation. And as the '226 patent is not at issue, overlap of issues in the IPR and the District Court is not at play.

Accordingly, a review of the *Fintiv* factors counsels against the Board exercising its discretion to deny institution.

Finally, under the interim guidance provided by the Director[3], the "PTAB will not rely on the *Fintiv* factors to discretionarily deny institution in view of parallel district litigation where a petition presents compelling evidence of unpatentability." Interim Guidance, 2. Therefore, the Board should decline to exercise its discretion to deny institution based on the compelling evidence of unpatentability provided by this Petition.

[3] Interim Procedure for Discretionary Denials in AIA Post-Grant Proceedings with Parallel District Court Litigation (June 21, 2022), available at https://www.uspto.gov/sites/default/files/documents/interim_proc_discretionary_denials_aia_parallel_district_court_litigation_memo_20220621_.pdf.

As discussed below in the challenges, the *'319 Publication* teaches all of the limitations of the challenged claims except for the limitation of "wherein at least 90% of the weight of the purified fusion protein is not present as an aggregate." But as noted throughout this Petition, the '226 patent provides no guidance of how to achieve the claimed aggregate amount, and thus relies on the skill of the ordinary artisan to achieve that claimed result. Moreover, Dr. Tessier's declaration establishes that "wherein at least 90% of the weight of the purified fusion protein is not present as an aggregate" is a generous target for protein purification and that in fact the POSA would understand that one would want as little amount of aggregated protein as possible when formulating a therapeutic. EX1002 ¶¶79-83. Thus, there is nothing inventive about the claimed methods.

Regeneron may also argue that the '226 patent is also currently the subject of *ex parte* reexamination, Control No. 90/014,449, and thus the Board should exercise its discretion not to institute based on that co-pending proceeding. The reexamination request, however, is based on a different theory of unpatentability, that is, obviousness-type double patenting. *See* February 11, 2020 Request for *Ex Parte* Reexamination. And as of the date of filing of this petition, no action has been taken since September 10, 2020. Accordingly, the Board should decline to exercise its discretion to deny institution.

III. GROUNDS FOR STANDING (37 C.F.R. § 42.104(A))

Petitioner certifies that the '226 patent—which issued on September 10, 2019—is available for IPR and that Petitioner is not barred or estopped from requesting an IPR challenging any claim of the '226 patent on the grounds identified herein. Petitioner has not filed a civil action challenging the validity, or been served with a complaint alleging infringement of the '226 patent, more than one year prior to this Petition's filing. *See Motorola Mobility LLC v. Arnouse*, IPR2013-00010, Paper 20 (PTAB Jan. 30, 2013), 5-6.

IV. MANDATORY NOTICES UNDER 37 C.F.R. § 42.8

Pursuant to 37 C.F.R. §§ 42.8(a)(1) and 42.8(b), the following mandatory notices are provided as part of this Petition.

A. Real-Parties-in-Interest (37 C.F.R. § 42.8(b)(1))

Celltrion, Inc. is the real party-in-interest.

B. Related Matters (37 C.F.R. § 42.8(b)(2))

Petitioner recently filed *Celltrion, Inc. v. Regeneron Pharms., Inc.*, IPR2023-00462 (PTAB), challenging U.S. Patent No. 10,464,992 ("'992 patent"). The '226 and '992 patents are currently being asserted in *Regeneron Pharms, Inc. v. Mylan Pharms Inc.*, Case No. 1-22-cv-00061 (N.D.W.V), filed on August 2, 2022. The '992 patent was also challenged in *Chengdu Kanghong Biotechnology Co., Ltd. v. Regeneron Pharms, Inc.*, IPR2021-00402 (PTAB), which the parties terminated due

to settlement on June 25, 2021. Additionally, the '992 patent is currently the subject of an *ex parte* reexamination, Control No. 90/014,448, wherein the requestor challenges the claims on obviousness-type double-patenting. While the reexamination was ordered, no substantive action has been taken. To the best of Petitioner's knowledge, there are no other judicial or administrative matters that would affect, or be affected by, a decision in this proceeding.

C. Lead and Back-Up Counsel and Service Information (37 C.F.R. § 42.8(b)(3), (4))

Lead Counsel: Lora M. Green (Reg. No. 43,541)

Back-Up Counsel: Yahn Lin Chu (Reg. No. 75,946)

Robert Cerwinski (to be admitted *pro hac vice*)

Aviv Zalcenstein (to be admitted *pro hac vice*)

David Kim (to be admitted *pro hac vice*)

Brigid Morris (to be admitted *pro hac vice*)

Petitioner hereby consents to electronic service. Please direct all correspondence to lead and back-up counsel at the contact information below. A power of attorney accompanies this petition.

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D. Payment of Fees Under 37 C.F.R. § 42.15(a) and § 42.103

The required fees are submitted herewith. If any additional fees are due at any time during this proceeding, the Office is authorized to charge such fees to Deposit Account No. DA604962.

V. THRESHOLD REQUIREMENT FOR *INTER PARTES* REVIEW

This Petition meets and exceeds the threshold required under 35 U.S.C. § 314(a). As explained below, for each ground, there is a reasonable likelihood that Petitioner will prevail with respect to at least one of the challenged claims.

VI. OVERVIEW OF CHALLENGE AND PRECISE RELIEF REQUESTED

A. Challenged Claims

Petitioner requests IPR of claims 1-4 of the ’226 patent, and cancellation of these claims as unpatentable.

B. Statutory Grounds of Challenge

Each of the following prior art references and/or combinations of references renders the challenged claims invalid:

Ground	35 U.S.C.	References	Claims
1	§103	Obvious over the *’319 publication* (EX1004)	1-4

2	§103	Obvious over the combination of the *'319 Publication* and *Fahrner* (EX1005)	1-4
3	§103	Obvious over the combination of the *'319 Publication*, *Fahrner, Holash* (EX1006) , and *Wulff* (EX1007)	3-4

Petitioner's full statement of the reasons for the relief requested is set forth in greater detail below, and in the supporting declaration of Dr. Tessier.

VII. CLAIM CONSTRUCTION

In accordance with 37 C.F.R. § 42.100(b), the challenged claims must be "construed using the same claim construction standard that would be used to construe the claim in a civil action under 35 U.S.C. § 282(b)," *i.e.*, the *Phillips* standard. 83 Fed. Reg. 197, 51340-51359 (Oct. 11, 2018); *Phillips v. AWH Corp.*, 415 F.3d 1303, 1312 (Fed. Cir. 2005).

Petitioner has not proposed any terms for construction because no constructions are necessary to resolve the disputes identified in this Petition. *See Vivid Techs., Inc. v. Am. Sci. & Eng'g, Inc.*, 200 F.3d 795, 803 (Fed. Cir. 1999). Petitioner relies on the definitions provided by the patentee and the plain language of the claims in the '226 patent to demonstrate that the claims are anticipated and/or obvious in light of the prior art. EX1002 ¶14-20. Accordingly, formal claim

construction is unnecessary. *See Hakim v. Cannon Avent Grp., PLC*, 479 F.3d 1313, 1318-19 (Fed. Cir. 2007) ("When there is no dispute as to the meaning of a term that could affect the disputed issues of the litigation, 'construction' may not be necessary."); *Vivid Techs.*, 200 F.3d at 803 (only those terms that are in controversy need to be construed and only to the extent necessary to resolve the controversy).[4]

Claims 1 and 3 claim a polynucleotide encoding a VEGF antagonist fusion protein that "comprises amino acids 25-457" of SEQ ID NO: 2 (claim 1) or SEQ ID NO: 4 (claim 3). The accepted legal meaning of the transitional phrase "comprises" is "includes," and therefore the claims are not limited to a protein comprising only amino acids 25-457 of SEQ ID Nos: 2 or 4. In other words, the claims encompass proteins including amino acids 27 to 457 of SEQ ID Nos. 2 or 4, but there can also be additional amino acids on either end of the protein, so long as it still binds VEGF to some degree. EX1002 ¶16. Accordingly, the claims encompass a fusion protein with a sequence that matches the full sequence of SEQ ID Nos. 2 or 4, *i.e.* amino acids 1-458. *Id*.

[4] Petitioner reserves the right to propose constructions for claim terms in this proceeding in response to arguments raised by Patent Owner in any future submission.

The ’226 specification also provides express definitions for some of the claim terms. Specifically, “substantially free of protein contaminants,” which appears in claim 2, has been defined as “preferably, that at least 90% of the weight of protein of the VEGF-specific fusion protein antagonist preparation used for making a formulation is VEGF fusion protein antagonist protein, more preferably at least 95%, most preferably at least 99%.” EX1001, 5:45-53; EX1002 ¶17. Because the inventors of the ’226 patent chose to use the term “preferably” the claims would encompass amounts lower than 90%.

The ’226 specification also states that “[s]ubstantially free of aggregates” means that “at least 90% of the weight of fusion protein is not present in an aggregate at the time the fusion protein is used to prepare the pharmaceutically effective formulation.” EX1001, 5:53-58, EX1002 ¶18.

As Dr Tessier explains, a POSA would understand that the claimed levels of aggregates and contaminants were intended to be measured at the time the protein was being used to prepare a formulation, *i.e.,* after purification. EX1002 ¶19.

VIII. GROUNDS FOR UNPATENTABILITY – DETAILED ANALYSIS

A. Obviousness

1. Legal Standards

A patent claim is invalid under 35 U.S.C. § 103(a) if the differences between the claims and the prior art are such that the subject matter as a whole would have

been obvious at the time the invention was made to a person having ordinary skill in the pertinent art. *KSR*, 550 U.S. at 406. Furthermore, "[w]hen there is a design need or market pressure to solve a problem and there are a finite number of identified, predictable solutions, a person of ordinary skill has good reason to pursue the known options within his or her technical grasp. If this leads to the anticipated success, it is likely the product not of innovation but of ordinary skill and common sense." *Id.*, 421.

The obviousness inquiry is "expansive and flexible," and the motivation to combine teachings found in separate prior art references can come from many sources, including: "[the] interrelated teachings of multiple patents; the effects of demands known to the design community or present in the marketplace; and the background knowledge possessed by a person having ordinary skill in the art." *Id.*, 415; *see also id.*, 418.

When relying on secondary considerations—including long-felt need, failure of others, unexpected results, commercial success, copying, licensing, and industry praise—as evidence of non-obviousness, a patentee must establish a nexus between the secondary considerations and the claimed invention. *Ormco Corp. v. Align Tech., Inc.*, 463 F.3d 1299, 1311-12 (Fed. Cir. 2006). There is no nexus unless the offered secondary consideration actually results from something that is both claimed

and novel in the claim. *In re Huai-Hung Kao*, 639 F.3d 1057, 1068-70, 1072 (Fed. Cir. 2011).

2. Ground 1: Claims 1-4 are obvious over the *'319 Publication* (EX1004)

Challenged claim 1 is drawn to a method of manufacturing a VEGF antagonist fusion protein where the method includes the following: (1) expressing the protein in a CHO cell; (2) the CHO cell includes a polynucleotide encoding the VEGF protein identified by amino acids 27-457 of SEQ ID NO: 2; (3) the fusion protein binds VEGF; (4) purifying the protein; and (5) at least 90% of the weight of the protein is not present as an aggregate. The only difference between claim 1 and claim 3 is that claim 3 is drawn to amino acids 27-457 of SEQ ID NO:4, which is the sequence for aflibercept.

Claims 3 and 4 depend from claims 1 and 2 respectively, and require that the "fusion protein is purified until substantially free of protein contaminants" and that the "VEGF antagonist is posttranslationally glycosylated at one or more asparagine residues."

The disclosure of the *'319 Publication* is discussed in Section I.C.2.a, *supra*. The *'319 Publication* renders claims 1-4 obvious.

a. *Claim 1*

i) A method of manufacturing a VEGF antagonist protein

Example 21 of the *'319 Publication* discusses a cell culture process used to produce modified Flt1 receptors. EX1004, 67:19-69:20; EX1002 ¶72. Specifically, the *'319 Publication* states:

> The process for production of Flt1D2.Flk1D3.FcΔC1(a) protein using the expression plasmid pFlt1D2.Flk1D3.FcΔC1(a) described *supra* in Example 1 involves suspension culture of recombinant Chinese hamster ovary (CHO K1/E1A) cells which constitutively express the protein product. The cells are grown in bioreactors and the protein product is isolated and purified by affinity and size exclusion chromatography.

EX1004, 67:25-68:5; EX1002 ¶74.

Thus, to the extent the preamble is limiting, it is taught by the *'319 Publication.* EX1002 ¶¶71-72.

ii) Expressing the protein in a CHO cell

As discussed above as to the preamble in Section VIII.A.2.a, the *'319 Publication* teaches expression in CHO cells. *E.g.*, EX1004, 67:25-68:3; EX1002 ¶74. The *'319 Publication* then provides more detailed methods and conditions to expand the Flt1D2.Flk1D3.FcΔC1(a) expressing cell lines, as well as methods of transferring the cell lines to a bioreactor. EX1004, 68:8-69:13; EX1002 ¶75.

The *'319 Publication* thus clearly teaches expressing the protein in a CHO cell. EX1002, ¶77.

iii) the CHO cell includes a polynucleotide encoding the VEGF protein identified by amino acids 27-457 of SEQ ID NO: 2

As Dr. Tessier explains, the sequence of the Flt1D2.Flk1D3.FcΔC1(a) disclosed by the *'319 Publication* is the same as SEQ ID NO: 2 of claim 1. EX1004, Figs. 21A-21C; EX1002, ¶73, n.5; EX1026.

iv) the fusion protein binds VEGF

The *'319 Publication* expressly looked at the binding stoichiometry of Flt1D2.Flk1 D3.FcΔC1(a) and VEGFR1R2-FcΔC1(a) with human VEGF165. EX1004, 74:15-76:10; EX1002 ¶78. The binding stoichiometry was determined by measuring with the level of VEGF saturation binding to Flt1D2.Flk1 D3.FcΔC1(a) or VEGFR1R2-FcΔC1(a) surfaces, or by measuring the concentration of VEGF165 to prevent binding of Flt1D2.Flk1 D3.FcΔC1(a) or VEGFR1R2-FcΔC1(a) to a VEGF BIAcore chip surface. EX1004, 74:20-25; EX1002 ¶78. It was found that the binding stoichiometry was one VEGF165 dimer molecule per one Flt1D2.Flk1 D3.FcΔC1(a) or VEGFR1R2-FcΔC1(a). EX1004, 75:12-15; 76:7-10, Fig. 28; *see also id.*, 90:5-10 (noting that Flt1D2.Flk1 D3.FcΔC1(a) has a high affinity for VEGF165); EX1002 ¶78.

The *'319 Publication* thus discloses that the VEGF antagonist protein binds VEGF, meeting this limitation of this claim. EX1002 ¶78.

v) purifying the protein

The *'319 Publication* discloses that the chimeric polypeptide molecules (i.e., the VEGF antagonist protein) "may be purified by any technique which allows for the subsequent formation of a stable biologically active chimeric polypeptide molecule." EX1004, 34:9-11; EX1002 ¶76.

Example 22 of the *'319 Publication* describes harvest and purification of modified Flt1 receptors, and in particular, Flt1D2.Flk1D3.FcΔC1(a). EX1004, 69:21-71:5; EX1002 ¶77. The *'319 Publication* states that the protein product was aseptically harvested from the bioreactor, loaded onto a Protein A column, and the eluted protein was frozen. EX1004, 70:3-13; EX1002 ¶77. The frozen protein was thawed and concentrated, and the concentrated protein was loaded onto a size exclusion column packed with Superdex 200 resin to remove aggregates and other contaminants. EX1004, 70:3-13*; see also id.*, 39:15-19; EX1002 ¶77.

vi) at least 90% of the weight of the protein is not present as an aggregate

As discussed above, Regeneron's own publication, the *'319 Publication*, teaches all of the process steps required by claim 1. *See supra* VIII.A.2.a.i-v. The *'319 Publication*, however, fails to explicitly state that 90% of the weight of the protein is not present as an aggregate. As discussed below, to the extent the purification method of the *'319 Publication* may not have achieved that, a POSA would have had motivation to reduce the amount of aggregates, as well as a

reasonable expectation of success of achieving this level of aggregation required by the claim.

As Dr. Tessier notes, the POSA would have understood that 90% of the weight of the protein not being present as an aggregate would not have been a hard target to achieve at the relevant time frame. EX1002, ¶¶79-80. Dr. Tessier also notes that the POSA would have also understood that it was not unusual to target and achieve much lower levels of aggregation when developing a therapeutic protein, including targets as low as less than 0.5 by weight of aggregate. EX1002 ¶¶45-52, 81. The teachings of the *'319 Publication* are consistent with the understanding of the POSA, as it teaches the use of a Protein A column and a size exclusion column packed with Superdex 200 resin to remove aggregates and other contaminants, both of which were common purification techniques at the time. EX1004, 70:3-13*; see also id.*, 39:15-19; EX1002 ¶85.

Moreover, the *'319 Publication* specifically states that the disclosed chimeric molecules "provide for the first time appropriate molecules for use in therapies in which antagonism of VEGF is a desired result." EX1004, 29:3-6; EX1002 ¶82. Accordingly, the *'319 Publication* teaches that the Flt1D2.Flk1D3.FcΔC1(a) (protein of SEQ ID NO:2) is considerably less sticky to the extracellular matrix than are Flt1(1-3)-Fc proteins. EX1004, 65:15-19, Fig. 23; EX1002 ¶82. The *'319 Publication* demonstrates further that fusion proteins such as

Flt1D2.Flk1D3.FcΔC1(a) had longer half lives in serum (EX1004, 83:15-19, Fig. 38), and inhibited tumor growth *in vivo* (*id.*, 84:14-17, Figs. 39-40); EX1002 ¶82. A POSA therefore would have understood that the Flt1D2.Flk1D3.FcΔC1(a) protein produced by Regeneron as described in the *'319 Publication* was intended for therapeutic use. EX1002 ¶83.

A POSA at the time of invention would have understood that a protein preparation wherein at least 90% of the weight of the protein is not present as an aggregate would not have been sufficient for therapeutic purposes, rather, a POSA would want a preparation that had significantly lower levels of aggregates to avoid negative effects and to ensure regulatory approval. EX1002 ¶¶83-84.

The disclosure of the '226 patent is consistent with the understanding of the POSA at the time of invention, as explained by Dr. Tessier. As explained above in Section I.A, the '226 patent only mentions CHO cells once, and does not provide any methods or conditions for producing SEQ ID NO: 2 or SEQ ID NO: 4 using said CHO cells. Nor does the disclosure of the '226 patent discuss methods or conditions for purifying the protein to achieve at least 90% of the weight of the protein is not present as an aggregate. *E.g.*, EX1001, 5:37-58. The specification of the '226 patent thus presumes that preparing the protein in CHO cells and purifying it such at least 90% of the weight of the protein is not present as an aggregate was well within the level of skill of the POSA. Thus, a POSA would have had motivation for and a

reasonable expectation of success of achieving the method of claim 1 wherein at least 90% of the weight of the protein is not present as an aggregate. EX1002 ¶86.

Accordingly, claim 1 is obvious over the *'319 Publication*. EX1002 ¶¶70, 86.

b. *Claim 2*

Claim 2 depends on claim 1, and adds the limitation that the "fusion protein is purified until substantially free of protein contaminants." Again, the '226 patent does not describe methods or conditions to achieve this limitation. EX1002 ¶87.

The '226 patent states that "preferably, that at least 90% of the weight of protein of the VEGF-specific fusion protein antagonist preparation used for making a formulation is VEGF fusion protein antagonist protein." EX1001, 5:48-52. Because the '226 patent uses "preferably" in the specification, the claim encompasses amounts lower than 90%. EX1002 ¶88.

As discussed above in Section VIII.A.2.a.v, the *'319 Publication* teaches purification of the protein using Protein A chromatography and size exclusion chromatography. As Dr. Tessier explains, a POSA would understand that Protein A chromatography would remove protein contaminants as Protein A binds to the Fc portion of the VEGF antagonist. *See, e.g.,* EX1004, 38:15-17; EX1002 ¶89. The POSA would also understand that the size exclusion chromatography would remove any remaining protein contaminants. EX1002 ¶89. Thus, a POSA would understand that the purification methods of the *'319 Publication* would result in a preparation

substantially free of protein contaminants, including wherein at least 90% of the weight of protein of the VEGF-specific fusion protein antagonist preparation used for making a formulation is VEGF fusion protein antagonist protein, as required by claim 2. EX1002 ¶89.

The POSA would also have had a reason to reduce protein contaminants, as well as a reasonable expectation of success of preparing a VEGF antagonist fusion protein, wherein the fusion protein is purified until substantially free of protein contaminants as defined by the '226 patent. As Dr. Tessier testifies, the POSA would have understood that a protein preparation that is to be used in a therapeutic protein formulation should have a low level of protein contaminants. EX1002 ¶90. The POSA would have also understood that at least 90% of the weight of the protein being VEGF fusion protein antagonist protein, or in other words less than 10% contaminants, would not have been a hard target to achieve at the relevant time frame, and was in fact much more generous than a POSA would typically target to reduce adverse effects and to ensure regulatory approval. EX1002 ¶90. This is consistent with the teachings of the specification, which does not teach any methods or conditions for removing protein contaminants from the CHO produced VEGF antagonist fusion protein. EX1002 ¶91.

Claim 2 is thus also obvious over the *'319 Publication*. EX1002, ¶¶70, 92.

c. *Claim 3*

The analysis of how claim 1 is rendered obvious by the *'319 Publication* is set forth above in Section VII.A.2.a, and applies equally to claim 3. The only difference between claim 1 and claim 3 is that in claim 1 the CHO cell includes a polynucleotide encoding the VEGF protein identified by amino acids 27-457 of **SEQ ID NO: 2**, and in claim 3, the CHO cell includes a polynucleotide encoding the VEGF protein identified by amino acids 27-457 of **SEQ ID NO: 4**. EX1002 ¶93. The claimed method as it applies to SEQ ID NO: 4 is also rendered obvious by the *'319 Publication. Id.* ¶94.

The *'319 Publication* discloses a VEGF antagonist having the sequence of SEQ ID NO: 4 (VEGFR1R2.FcΔC1(a)), and exemplifies the construction of an expression vector for the protein. EX1004, 67:4-17 (Example 20), Figs. 24A-24C; EX1002 ¶95. The sequence provided for VEGFR1R2.FcΔC1(a) in the *'319 Publication* is the same sequence as SEQ ID NO: 4 in the '226 patent. EX1002 ¶95, n.6; EX1027. Although the *'319 Publication* does not provide a specific example of preparing this VEGF antagonist in CHO cells and purifying it, the *'319 Publication* describes its processes as producing modified Flt1 receptors (EX1004, 67:19-20), as well as harvesting and purifying them (*id.*, 69:21-22); EX1002 ¶96. The *'319 Publication* teaches that the same methods are being used to prepare and purify the unmodified Flt(1-3)-Fc using CHO cells. EX1004, 36:21-39:19; EX1002 ¶96.

Moreover, the *'319 Publication* specifically exemplifies CHO cells that transiently express VEGFR1R2-FcΔC1(a) (EX1004, 82:11-13) and a POSA also would have been aware that Regeneron had used CHO cells to prepare proteins of SEQ ID NO: 4, as well as purifying it. *See supra* I.C.1.f, EX1002 ¶¶96, 63-65. The POSA would thus have had a reasonable expectation of success of expressing VEGFR1R2-FcΔC1(a) (SEQ ID NO: 4, aflibercept) in CHO cells as required by claim 3. EX1002 ¶97.

For these reasons and those discussed above for claim 1, claim 3 is therefore rendered obvious by the *'319 Publication.*

d. *Claim 4*

Claim 4 is dependent on claim 3 and adds the limitation that the "VEGF antagonist is posttranslationally glycosylated at one or more asparagine residues." The '226 patent does not disclose any methods of glycosylating a protein beyond producing the VEGF antagonist fusion protein in CHO cells. EX1002 ¶98. This type of glycosylation was known to occur in the Fc region of fusion proteins and was shown to be essential to the functions of that region. *See supra* 1.C.1.g; EX1002 ¶99.

At the time of invention, the POSA understood that expression of proteins in CHO cells provided for N-linked glycosylation of the protein. EX1002 ¶99; *see also* EX1017, ¶98 (stating that "[w]hen expressed as secreted proteins in CHO [cells]"

the VEGF Traps were glycosylated "as expected."). That understanding is consistent with teachings in the '226 patent specification, which states that "the VEGF antagonist is expressed in a mammalian cell line such as CHO cells and may be modified posttranslationally…. [and in an] embodiment the fusion protein … is glycosylated at Asn residues 62, 94, 149, 222, and 308." EX1001, 5:37-42. That is, glycosylation is achieved by expressing Fc fusion proteins in CHO cells. EX1002 ¶99.

Moreover, although the *'319 Publication* does not expressly disclose glycosylation at asparagine residues on the aflibercept protein, the *'319 Publication* states that there are "five possible N-linked glycosylation sites in Flt1D2.Flk1D3.FcΔC1(a) and are found to be glycosylated to varying degrees. Complete glycosylation is observed at Asn33, Asn193, and Asn282. Partial glycosylation is observed on Asn65 and Asn 120." EX1004, 26:6-9; 81:20-82:2; Fig. 36; EX1002 ¶100. One site of complete glycosylation is Asn282, which has the sequence NST, as shown in Fig. 36. EX1004, 81:20-82:2; Fig. 36; EX1002 ¶100. The first amino acid in Figure 36 of the *'319 Publication* starts at the amino acid "G" of the sequence "GRPFV," corresponding to the "G" at amino acid 27 of Figure 21A. *Compare* Fig. 36 with Fig. 21A; EX1002 ¶100. Thus, Asn (N) 282 of Figure 36 corresponds to Asn (N) 308 of Fig. 21B. EX1002 ¶100. The Fc portion of Flt1D2.Flk1D3.FcΔC1(a) is the same Fc portion of VEGFR1R2-FcΔC1(a),

accordingly, the POSA would also reasonably expect obtaining N-linked glycosylation at this position in VEGFR1R2-FcΔC1(a) (SEQ ID NO: 4) grown in CHO cells. EX1002 ¶101. Further, as Dr Tessier explains, the structures and sequences of Flt1D2.Flk1D3.FcΔC1(a) and aflibercept were very similar and therefore a POSA would expect similar glycosylation with aflibercept. EX1002 ¶¶91, n.4, 102; EX1025.

Additionally, the mapping techniques described in the *'319 Publication* to identify glycosylation sites for Flt1D2.Flk1D3.FcΔC1 (*e.g.*, EX1004, Example 28) were well within the ordinary skill of a POSA at the time and could have been easily applied to aflibercept to identify glycosylation. EX1002 ¶103. Based on the above, the POSA would have had motivation to produce a glycosylated protein, and the POSA would have had a reasonable expectation of success of obtaining a VEGF antagonist that was glycosylated at one or more asparagine residues. EX1002 ¶104.

Thus, claim 4 is rendered obvious by the *'319 Publication*.

3. Ground 2: Claims 1-4 are obvious over the *'319 Publication* (EX1004) and *Fahrner* (EX1005)

As discussed above in Section VII.A.2, the *'319 Publication* renders obvious the methods of claims 1-4. But to the extent that the *'319 Publication* fails to render obvious the limitation that at least 90% of the weight of the protein is not present as an aggregate as recited in claims 1 and 3, any deficiency is remedied by *Fahrner*. EX1002 ¶105-06.

a. *Claims 1 and 3*

Fahrner is discussed above in Section I.C.2.d. *Fahrner* notes that the most clinically significant antibodies[5] are produced in CHO cells, as the level of product-related variants can be effectively controlled during cell culture so that little or no variants must be removed during recovery, allowing for a streamlined purification process. EX1005, 301-302; EX1002 ¶107.

Fahrner would have motivated a POSA to purify the VEGF antagonist fusion protein to at least a level wherein at least 90% of the weight of the purified fusion protein is not present as an aggregate. EX1002 ¶108. According to *Fahrner*, the primary consideration of purification is purity, because, unless the purity

[5] As Dr Tessier notes in his declaration, although some references refer to "antibodies" while others refer to "proteins," aflibercept and similar VEGF antagonist fusion proteins share similar structures to antibodies, i.e. they are large, complex, multi-domain, recombinant proteins produced for therapeutic use in cell culture and both structures are "Y" shaped and have two arms that bind to a target ligand and are linked together at the base by an Fc immunoglobulin domain. EX1002 ¶¶50-51. As such, a POSA would understand the teachings of this reference relating to purification of therapeutic antibodies apply equally to fusion proteins intended for therapeutic used. *Id.* ¶52.

requirement for biological pharmaceuticals is met, there may be no product. EX1005, 303; EX1002 ¶108. *Fahrner* states that aggregates are the most product-related variant that must be removed because of possible immunogenicity. EX1005, 304; EX1002 ¶108. *Fahrner* notes that the typical aggregate content of harvested cell culture fluid ("HCCF") is 5-15% for most antibodies and is then typically reduced through purification to below 0.5% in the final bulk. EX1005, 304, 306, Table 12.1; EX1002 ¶108.

Fahrner specifically teaches a purification process using protein A affinity chromatography to remove host cell proteins, DNA, and endotoxin, followed by cationic exchange chromatography to remove aggregates and host cell protein, and anionic exchange chromatography to remove DNA and residual host cell proteins. EX1005, 305-308; EX1002 ¶109.

Although the *'319 Publication* and *Fahrner* disclose somewhat different purification methods (both use Protein A affinity chromatography, the *'319 Publication* then uses size exclusion chromatography, whereas *Fahrner* then uses a combination of anion and cation exchange chromatography), the POSA would understand that each purification method has the same goal—removing aggregated VEGF antagonist fusion protein and reducing contaminants, such as protein contaminants. EX1002 ¶110. Because *Fahrner* teaches that the primary consideration of purification is purity, the POSA would have purified the VGFR

antagonist protein to at least wherein 90% of the weight of the protein is not present as an aggregate, and in fact, as taught by *Fahrner*, would want to reduce it even further in the final preparation used in making the pharmaceutical formulation. EX1005, 302, 304; EX1002 ¶111. And, as discussed above in Section I.A, the '226 specification does not teach any particular methods or conditions for the purification, such purification methods would have been well known to the POSA at the relevant time period, and the POSA would have had a reasonable expectation of success of achieving a preparation wherein at least 90% of the weight of the protein is not present as an aggregate. EX1002 ¶112.

Thus, the combination of the *'319 Publication* and *Fahrner* renders claims 1 and 3 obvious. EX1002 ¶113.

b. *Claim 2*

For the reasons set forth in Section VIII.A.2.b, the *'319 Publication* renders claim 2 obvious. To the extent that the *'319 Publication* may be seen as not rendering obvious the limitation that the VEGF antagonist protein is substantially free of protein contaminants, *Fahrner* renders this limitation obvious. EX1002 ¶114.

The reasoning discussed above in Section VIII.A.3.a as to why *Fahrner* would motivated the POSA to reduce aggregates of the VEGF antagonist fusion protein applies equally to the reason why the POSA would have wanted to reduce protein contaminants. EX1002 ¶115. That is, given the overarching goal of purity, the

POSA would have understood that the protein contaminants in the purified protein preparation used to make the pharmaceutical preparation should be as low as possible. EX1005, 303; EX1002, ¶115. As *Fahrner* notes, Protein A chromatography, a step which both *Fahrner* and the *'319 Publication* share, removes host cell proteins. EX1005, 306; EX1002 ¶115. The *'319 Publication* teaches that the use of size exclusion chromatography also removes contaminants along with aggregates (EX1004, 39:15-18), and *Fahrner* teaches that the cation exchange chromatography and anion exchange chromatography purification steps also remove protein contaminants (EX1005, 307-308). EX1002 ¶115. Thus, *Fahrner*, along with the *'319 Publication*, provide motivation, as well as a reasonable expectation of success of purifying the VEGF antagonist protein until it is substantially free of protein contaminants. EX1002 ¶115. Accordingly, the combination of the *'319 Publication* and *Fahrner* renders claim 2 obvious. *Id.* ¶116.

c. *Claim 4*

As discussed above in Section VIII.A.2.d, claim 4 is rendered obvious by the *'319 Publication* given its disclosure of glycosylated VEGF antagonist protein as well as the POSA's knowledge at the time. The same reasoning set out for claim 4 in ground 1, as well as the reasoning above as to claim 3 in Section XX, applies equally here. EX1002 ¶117.

The combination of the *'319 Publication* and *Fahrner* render claim 4 obvious. EX1002 ¶118.

4. Ground 3: Claims 3 and 4 are obvious over the combination of the '319 *Publication*, *Fahrner, Holash* and *Wulff*

As discussed above in Sections VIII.A.2 and VIII.A.3, claims 3 and 4 are rendered obvious by the *'319 Publication* alone, as well as the *'319 Publication* as combined with *Fahrner*. To the extent that the *'319 Publication* fails to provide a reason to express SEQ ID NO: 4 (VEGFR1R2-FcΔC1(a), aflibercept) in CHO cells, any deficiency is remedied by *Holash* (EX1006) and *Wulff* (EX1007). EX1002 ¶119.

a. *Claim 3*

As discussed above in Sections VIII.A.2.c and VIII.A.3.a, claim 3 is the same method as set forth in claim 1, except that the CHO cell includes a polynucleotide encoding the VEGF protein identified by amino acids 27-457 of ***SEQ ID NO: 4 (i.e., aflibercept)***. EX1002 ¶120. The combination of the *'319 Publication* and *Fahrner* render obvious all of the limitations of claim 3. *See supra*, Section VIII.A.3.a; EX1002 ¶121.

To the extent that it may be determined that the *'319 Publication* fails to provide a sufficient reason to produce a VEGF antagonist fusion protein of having amino acids 27-457 of SEQ ID NO: 4 (i.e., aflibercept), any deficiency is remediated by *Holash* and *Wulff*, both Regeneron publications. EX1002 ¶120.

Both *Holash* and *Wulff* disclose aflibercept's superior binding activity and pharmacokinetic profile over other VEGF antagonists. EX1006, 11394-95; EX1007, 2804-06; EX1002 ¶122. Moreover, both *Holash* and *Wulff* teach production of aflibercept in CHO cells followed by purification. EX1006, 11394; EX1007, 2798; EX1002 ¶122. Thus, *Holash* and *Wulff* would have motivated a POSA to express and purify SEQ ID NO: 4 (aflibercept) produced in CHO cells for use in therapeutic formulations. EX1002 ¶122.

Wulff looked at the administration of aflibercept to marmoset monkeys to assess its effects on angiogenesis. EX1007, 2797; EX1002 ¶123. *Wulff* states that the VEGF trap used in the experiments, VEGF Trap R1R2 (aflibercept), "was expressed in CHO cells and was purified by protein A affinity chromatography followed by size-exclusion chromatography." EX1007, 2798; EX1002 ¶123. *Wulff* specifically cites to the *'319 Publication* (EX1004) to describe the VEGF antagonist used, noting that "the detailed molecular structure and how it was created are described in the patent REG 710-A-PCT, VEGF Trap Application published December 2000, Publication WO 00/75319 A1." EX1007, 2798, n.1; EX1002 ¶123. According to *Wulff*, aflibercept "may be more efficient in inhibiting VEGF" than previously tested VEGF antagonists. EX1007, 2804; EX1002 ¶124.

Holash details Regeneron's *in vivo* experiments with VEGF Trap R1R2 (aflibercept) in mice, in which that "[a]ll of the VEGF-Trap variants were produced

and purified from Chinese hamster ovary cells." EX1006, 11393-94; EX1002 ¶125. Holash found that aflibercept had "markedly enhanced pharmacokinetic properties" and "effectively suppresses tumor growth and vascularization *in vivo*." EX1006, 11393; EX1002 ¶125. "The combination of high-affinity and improved pharmacokinetics apparently contributes toward making VEGF-Trap$_{R1R2}$ [aflibercept] one of the most, if not the most, potent and efficacious VEGF blocker available." EX1006, 11397; EX1002 ¶126. Further, Regeneron noted in *Holash* that aflibercept had the additional advantage of being composed of "entirely human sequences," which would "hopefully minimiz[e] the possibility that it might prove immunogenic in human patients." EX1006, 11397; EX1002 ¶127. In comparison to existing antibody VEGF antagonists, "far lower circulating levels of VEGF-Trap$_{R1R2}$ [aflibercept] are required for similar efficacy" and its "safety has recently been confirmed in toxicological studies in cynomologus monkeys." EX1006, 11397; EX1002 ¶127. *Holash* noted further "the [aflibercept] VEGF-Trap is currently in human clinical trials for several different types of cancer." EX1006, 11397; EX1002 ¶128.

Accordingly, *Holash* and *Wulff* would have motivated a POSA at the relevant time period to express amino acids 27-457 of SEQ ID NO: 4 (i.e., aflibercept) using the method of the *'319 Publication* and *Fahrner* to an VEGF antagonist fusion protein to obtain a protein having the performance as described in *Holash* and *Wulff*.

EX1002 ¶129. A POSA would have had a reasonable expectation of success as all of the *'319 Publication*, *Fahrner, Holash,* and *Wulff* teach expression of proteins in CHO cells, and each of the *'319 Publication*, *Holash,* and *Wulff* teach the expression of VEGF antagonist fusion proteins in CHO cells. EX1002 ¶130. Moreover, there would have been no reason to choose a different mammalian cell type than the one the Regeneron *Holash* and *Wulff* publications had used to produce the promising results. EX1002 ¶129.

Accordingly, the combination of the *'319 Publication*, *Fahrner, Holash,* and *Wulff* renders claim 3 obvious. EX1002 ¶131.

b. *Claim 4*

As discussed above in Section VIII.A.2.d, claim 4 is rendered obvious by the *'319 Publication* given its disclosure of glycosylated VEGF antagonist protein as well as the POSA's knowledge at the times. EX1002 ¶132-33. The same reasoning set out for claim 4 in ground 1, as well as the reasoning set forth above as to claim 3 in Section VIII.A.4.a above, applies equally here. EX1002 ¶133.

The combination of the *'319 Publication*, *Fahrner, Holash,* and *Wulff* render claim 4 obvious. EX1002 ¶134.

5. Secondary Evidence of Non-Obviousness

Petitioner is not aware of any relevant secondary considerations that have a nexus to, or are commensurate in scope, with any of the challenged claims. EX1002,

¶135. Petitioners reserves the right to respond to any allegations of secondary considerations.

IX. CONCLUSION

For the reasons set forth above, claims 1-4 of the ’226 patent are unpatentable. Petitioners therefore request that a *inter partes* review of these claims be instituted.

Respectfully submitted,

Dated: February 28, 2023

/Lora M. Green/
Lora M. Green, Lead Counsel
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X. CERTIFICATE OF COMPLIANCE

Pursuant to 37 C.F.R. §42.24(d), the undersigned certifies that this Petition complies with the type-volume limitation of 37 C.F.R. §42.24(a). The word count application of the word processing program used to prepare this Petition indicates that the Petition contains 13,931 words, excluding the parts of the brief exempted by 37 C.F.R. §42.24(a).

Respectfully submitted,

Dated: February 28, 2023

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XI. APPENDIX – LIST OF EXHIBITS

Exhibit No.	Description
1001	U.S. Patent No. 10,406,226 ("'226 Patent")
1002	Declaration of Dr. Peter M Tessier ("Tessier")
1003	Dr. Peter Tessier *curriculum vitae*
1004	International Publication No. WO 00/75319 A1 to Papadopoulos et al. ("'319 Publication")
1005	Robert Fahrner et al., *Industrial Purification of Pharmaceutical Antibodies: Development, Operation, and Validation of Chromatography Processes,* 18:1 Biotechnology and Genetic Engineering Reviews 301-327 (Jul. 2001) ("Fahrner")
1006	Jocelyn Holash et al., *VEGF-Trap: A VEGF Blocker with Potent Antitumor Effects*, 99 (17) PNAS 11393-11398 (Aug. 20, 2002) ("Holash")
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1026	Sequence alignment of SEQ ID NO: 2 from ’226 patent and Figs. 21A-21C of ’319 Publication
1027	Sequence alignment of SEQ ID NO: 4 from ’226 patent and Figs. 24A-24C of ’319 Publication
1028	Scheduling order in *Regeneron Pharmaceuticals, Inc. v. Mylan Pharmaceuticals Inc.*, Case No. 1-22-cv-00061 (N.D.W.V)
1029	Regeneron’s Stipulation Regarding Case Narrowing And Injunctive Relief in *Regeneron Pharmaceuticals, Inc. v. Mylan Pharmaceuticals Inc.*, Case No. 1-22-cv-00061 (N.D.W.V)

CERTIFICATE OF SERVICE

Pursuant to 37 C.F.R. §§ 42.6(e) and 42.105(a), this is to certify that I caused to be served a true and correct copy of the foregoing (and accompanying Exhibits 1001-1029) by overnight courier (Federal Express or UPS), on this 28th day of February 2023, on the Patent Owner at the correspondence address of the Patent Owner as follows:

A&P – Regeneron
Attn: IP Docketing
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Respectfully submitted,

Dated: February 28, 2023

/Lora M. Green/
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