

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

XENCOR, INC.,

Petitioner

v.

MERUS N.V.,

Patent Owner

IPR2025-00605
Patent No. 11,926,859

**DECLARATION OF BRIAN J. SUTTON, Ph.D. IN SUPPORT OF PATENT
OWNER'S PRELIMINARY RESPONSE**

I. INTRODUCTION

1. I, Dr. Brian J. Sutton, Ph.D., declare as follows:

2. I have been retained by Merus N.V., (“Patent Owner”) as an independent expert in this proceeding brought by Xencor, Inc. (“Petitioner”) before the United States Patent and Trademark Office regarding U.S. Patent No. 11,926,859 (“the ’859 Patent”) (Ex. 1001).

3. I was asked to provide my opinions regarding whether: (1) the disclosure of the specification relied upon, namely the ’935 Provisional Application, reasonably conveys to a POSA that the inventors had possession at that time, 20th April 2012, of all the elements of the later claimed subject matter; and (2) Lazar (Ex. 1004) alone or in combination with Kannan (Ex. 1007) discloses or suggests all of the limitations of claims 1-7 of the ’859 Patent.

4. I am compensated at my normal and customary hourly consulting rate of £350 for the time that I spend on this matter. My opinions are independent and my compensation is not contingent on my opinions in this matter. I have no interest in the outcome of this proceeding.

II. BACKGROUND AND QUALIFICATIONS

5. Below, I summarize my qualifications, which can be found in more detail in my *curriculum vitae*, which I understand is being submitted as Exhibit 2011.

6. I received both a B.A. in Chemistry and a Ph.D. in Molecular Biophysics from the University of Oxford in 1976 and 1980, respectively.

7. My doctoral work and early publications concerned the structure of the antibody combining site for antigen, the structure of the receptor-binding Fc region of IgG, and the mechanism of antibiotic resistance enzymes. I used spin-labels, ESR and NMR to probe the structure and mobility of antibody combining sites, and applied X-ray crystallography to determine the structure of the Fc region of IgG, at the same time initiating X-ray studies of the bacterial metallo- β -lactamases that are responsible for bacterial resistance to antibiotics.

8. The structure of the *B. cereus* zinc-dependent enzyme was one of the first of this class to be determined, and I worked with SmithKline Beecham (now GSK); this and other enzyme structures subsequently led to a programme of inhibitor design.

9. I completed my post-doctoral training at the Oxford University Laboratory of Molecular Biophysics in 1986, where I received the Royal Society Howe Junior Research Fellowship and a Royal Society University Research Fellowship. In 1994, I was awarded a Nuffield Foundation Research Fellowship at King's College London.

10. I have spent more than 40 years in structural biology research, mainly using X-ray crystallography and studying proteins, principally antibodies, and with a focus on the Fc regions of IgE, IgG and other antibody classes.

11. Presently, I am Emeritus Professor of Molecular Biophysics at the Randall Centre for Cell & Molecular Biophysics, King's College London. Previously, I served as Head of Structural Biology and Director of the Centre for Biomolecular Spectroscopy at King's College London.

12. My current research focuses on IgE-Fc/receptor interactions and their inhibition, and tracing the evolution of antibody structure using X-ray crystallography and X-ray solution scattering.

13. I was a founding member and Programme Leader for *IgE Structure, Function and Regulation* in the MRC & Asthma UK Centre for Allergic Mechanisms of Asthma. I was also a member of the Asthma UK Research Grants Committee, and I was the co-organizer of the Federation of American Societies for Experimental Biology (FASEB) meeting IgE and Allergy – 50 Years and Onward.

14. I have received multiple major research grants, including from the UKRI Medical Research Council (including two Programme grants) and the Biotechnology and Biological Sciences Research Council, the Wellcome Trust (including two Programme grants), and Asthma UK.

15. Over the course of my career, I have authored or co-authored about 175 peer-reviewed journal papers and 7 book chapters.

16. I am a named inventor on two patent families related to novel anti-IgE inhibitors.

III. PERSON OF ORDINARY SKILL IN THE ART

17. In my opinion, given the nature of the technology at issue, a person of ordinary skill in the art (“POSA”) would have a Ph.D. in biochemistry, chemistry, molecular or structural biology, molecular biophysics, antibody engineering, immunology, or related discipline and at least 2 years of related experience in academia or industry or a Master’s degree in any of the above fields with at least 4 years of related experience in academia or industry.

18. I am informed that all of my opinions in this Declaration should be from the perspective of a POSA as of April 20, 2012. None of my opinions in this Declaration would change if I used Dr. Presta’s definition of a POSA instead of the one set forth above.

IV. TECHNOLOGICAL OVERVIEW

19. Antibodies, which are also referred to as immunoglobulins or Igs, are naturally generated by the body and are an important defense for fighting infections; they are a key component of an immune response directed against a “foreign”

invading organism or for eliminating cancer cells. Antibodies or Igs are complex proteins that are made from combinations of 20 amino acids.

20. Each amino acid has distinct properties based on their side chains. Some amino acids are positively charged because the side chains can accept protons at physiological pH (approximately pH 7.4). Some amino acids have negatively charged side chains which donate protons at physiological pH. Other amino acids are neutral, without any charge.

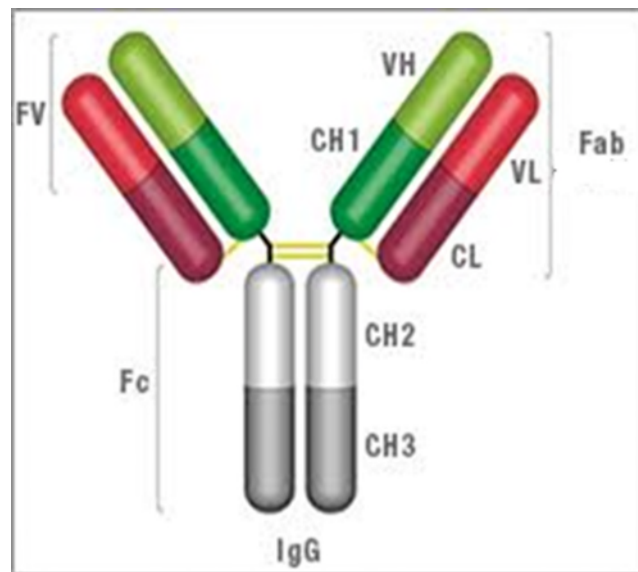
21. Additionally, some amino acids are hydrophobic because their side chains are mostly constituted of carbon and hydrogen atoms and cannot interact with water. Others are hydrophilic because their side chains can form hydrogen bonds with surrounding water molecules.

22. The charge, hydrophilicity and hydrophobicity of the amino acids are crucial to determining, among other things, the folding of a protein structure.

23. Natural or wild-type Igs have a Y shape that consists of two identical light (L) chains and two identical heavy (H) chains; this is typical of an IgG class of antibody, and is the basic unit of antibodies of the other human classes (IgM, IgA, IgE and IgD) (See figure below).

24. Each IgG heavy chain consists of one variable domain (VH) and three constant domains (CH1, CH2, and CH3). Each light chain consists of one variable

domain (VL) and one constant domain (CL). The CH2 and CH3 domains of the heavy chains interact to form the “fragment crystallizable” or Fc region. The VH and VL domains form the Fv fragment, which binds antigen, and together with the CL and CH1 domains forms the “fragment antigen-binding” or Fab region (See figure below).



25. The heavy chains are held together (dimerized) through covalent disulphide bridges in the “hinge” region between the Fab and Fc regions (See figure above), and by strong non-covalent interactions such as hydrophobic interactions, salt bridges, van der Waals forces and hydrogen bonds between interacting surfaces of the two CH3 domains. The CH2 domains do not interact directly with each other and contain sites of glycosylation (not shown in figure).

26. The various Ig domains fold independently, and for Fc dimerization to occur, each CH3 domain must first fold. Once properly folded, the CH3 domain of one heavy chain can then interact with the CH3 domain of the other heavy chain to form a non-covalent dimer and maintain the dimeric structure of the antibody.

27. The folding of the CH3 domain is largely controlled by hydrophobic amino acid residues clustering together in the core of the protein domain to avoid contact with water. The surface of the CH3 domain is principally hydrophilic, the amino acid side chains interacting with water molecules, but with a hydrophobic “patch”. The amino acids of the hydrophobic patch on the surface of the folded CH3 region will form hydrophobic and other non-covalent interactions with the amino acids of the hydrophobic patch on the CH3 domain of the other, identical heavy chain. These amino acids interact to form a principally hydrophobic CH3-CH3 dimer interface.

28. Nature has evolved the formation of four salt bridges, partially or almost fully buried around the edge of the principally hydrophobic CH3-CH3 dimer interface; these electrostatic interactions between oppositely charged amino acid side chains (salt bridges) enhance the formation of the CH3 domain dimer. The salt bridges must remain at least partially buried within the hydrophobic CH3-CH3

dimer interface because the salt bridges will be weaker or may not form at all if exposed to water.

29. In addition, the amino acid residues in the CH3-CH3 dimer interface make van der Waals contact with each other. These van der Waals interactions occur only when side chains from different amino acids across the interface make very close contact, and this commonly occurs because of the high degree of shape complementarity at the CH3:CH3 interface.

30. Making modifications to the amino acids that make up the hydrophobic core of the CH3 domains may negatively affect their ability to fold, and this may also be the case for amino acids at the hydrophobic interface, which may affect folding and/or dimerization. Generally, it was well known in the art by a POSA at the relevant time period, that one should not make modifications to the hydrophobic core of the CH3 to introduce hydrophilic or charged residues, due to the likelihood of disrupting the CH3 interface and destabilizing the antibody's Fc.

31. Gunasekaran discloses to a POSA not to modify the neutral residues in the hydrophobic core of the CH3 region or in the hydrophobic region of the CH3-CH3 interface. According to Gunasekaran, “[i]t has long been established that the hydrophobic core of protein domains plays an important role in protein folding and stability,” accordingly, it was understood to modify charged residues, not

hydrophobic residues, to improve heterodimerization, as Gunasekaran stated. See Gunasekaran (EX1012) at 4.

32. Wild-type Igs can only bind one kind of antigen and are “monospecific” (although bivalent—meaning that they have two binding domains—by virtue of their two identical Fab regions) while bispecific Igs are engineered to bind two different antigens by bringing together two different heavy chains from two different wild-type antibodies, *i.e.*, heterodimerization.

33. Engineering a bispecific Ig with two different heavy chains is challenging for many reasons. For example, it is difficult to overcome the strong, natural affinity for the CH3 domains of identical heavy chains to interact with each other, as discussed above. Conversely, it is difficult to promote the interaction of the CH3 domains from two different heavy chains.

34. As of April 20, 2012, POSAs were aware of techniques such as “knobs-into-holes” and “charge reversal” or “electrostatic steering” to try to create bispecific antibodies; these approaches enhance heterodimerization of different heavy chains and reduce the homodimerization that occurs in the formation of the wild-type antibodies. However, POSAs also understood that these techniques had shortcomings because they often resulted in insufficient yield of the desired heterodimer compared with that of the two homodimers, as well as reduced protein

stability and consequent impaired functionality of the heterodimeric antibody, resulting in unstable proteins with poor half-life and shelf life, making them unsuitable for development.

35. Merus pursued a different approach that went against conventional wisdom by modifying a neutral residue in the hydrophobic interface of the CH3 dimer, one on each heavy chain, from an amino acid having a neutral side chain to an amino acid having a charged side chain, to create an interaction between two oppositely charged residues to enhance the stability of the CH3-CH3 dimer interface.

36. Surprisingly, Merus' heterodimerization technique achieves heterodimeric antibodies capable of higher yield and increased stability. Merus was granted the '859 patent for its novel invention.

V. THE '859 PATENT

37. I am informed that Xencor challenges Claims 1-7 of the '859 Patent in its Petition.

38. Independent Claim 1 is directed to a heterodimeric antibody.

Independent Claim 1 of the '859 Patent recites:

A heterodimeric antibody comprising a first human CH3 domain comprising a positively charged amino acid residue at position 364 according to the EU numbering system, and a second human CH3 domain comprising a negatively charged amino acid residue at position 368 according to the EU numbering system.

39. Dependent Claims 2-7 depend from Independent Claim 1. Dependent Claims 2-7 include all of the elements of Independent Claim 1 and further require additional limitations, including that the said positively charged amino acid residue at position 364 comprises a lysine (K) or an arginine (R) residue, and that the negatively charged residue at position 368 comprises an aspartic acid (D) or glutamic acid (E) residue (Claim 2), and that the said positively charged amino acid residue at position 364 comprises a lysine (K) residue, and that the negatively charged residue at position 368 comprises an aspartic acid (D) residue (Claim 3), and a bispecific antibody (Claim 4), a human IgG (Claim 5), a human IgG 1 (Claim 6), and a pharmaceutically acceptable carrier (Claim 7).

VI. THE CLAIMED SUBJECT MATTER OF THE '859 PATENT IS DISCLOSED IN THE APRIL 20, 2012 PROVISIONAL APPLICATION

40. I am informed that the disclosure of the specification relied upon should reasonably convey to a POSA that the inventors had possession at that time, 20th April 2012, of all the elements of the later claimed subject matter.

41. It is my opinion that the inventors of the '859 Patent had possession of all the necessary and sufficient elements of a heterodimeric antibody with CH3-CH3 substitutions of “a positively charged amino acid residue at position 364” and “a negatively charged amino acid residue at position 368,” on April 20, 2012. The disclosures of the '935 Provisional Application reasonably convey to a POSA that the inventors had possession of both of these elements of the invention.

42. The Specification of the '935 Provisional Application discloses the claimed “heterodimeric antibody” and states:

Bispecific antibodies based on the IgG format, consisting of 2 heavy and two light chains have been produced by a variety of methods. For instance, bispecific antibodies may be produced by fusing two antibody-secreting cell lines to create a new cell line or by expressing two antibodies in a single cell using recombinant DNA technology. These approaches yield multiple antibody species as the respective heavy chains from each antibody may form monospecific dimers (also called homodimers), which contain two identical paired heavy chains with the same specificity, and **bispecific dimers (also called heterodimers) which contain two different paired heavy chains with different specificity.**

See '935 Provisional Application (EX1030) at p. 4, lines 5-13 (emphasis added).

43. The Specification of the '935 Provisional Application also discloses the claimed “CH3 domain” and states:

The term '**CH3 domain**' is well known in the art. The IgG structure has four chains, two light and two heavy chains; each light chain has two domains, the variable and the constant light chain (VL and CL) and **each heavy chain has four domains, the variable heavy chain (VH) and three constant heavy chain domains (CH1, CH2, CH3).**

See '935 Provisional Application (EX1030) at p. 15, lines 28-31.

44. Further, the Specification of the '935 Provisional Application also discloses the claimed positively charged amino acid residue and negatively charged amino acid residue in the CH3 domain. The '935 Provisional Application states:

The present invention provides novel engineered CH3 domains as well as novel combinations of CH3 mutations. Before the present invention, charged contact amino acids of CH3 domains that were known to be involved in CH3-CH3 pairing were substituted by amino acids of opposite charge, thereby influencing the CH3-CH3 pairing. **The mutations according to the present invention are an inventive alternative to this approach, because now CH3 amino acids that are non-charged or neutral in wildtype CH3 are substituted with charged residues.** The present invention in this embodiment does not exchange charged contact amino acids by amino acids of opposite charge but substitutes non-charged CH3 amino acids for charged ones. The approach of the present invention provides not only a method for efficiently steering the dimerization of CH3 domains but also has the advantage that at least one additional charge-charge interaction in the CH3 interface is created. In view of this additional charge-charge interaction on top of the existing

charge-pairs in the CH3-CH3 interface, the dimers according to the invention are generally more stable as compared to the wild type dimers. Moreover, it has surprisingly become possible to increase the proportion of one or more Ig-like molecules of interest in a mixture even further. As described herein before, methods known in the art for preferential production of a bispecific antibody typically involves the production of some undesired dimeric side products.

See '935 Provisional Application (EX1030) at p. 23, line 28 – at p. 24, line 13 (emphasis added).

45. Based on this disclosure in the '935 Provisional Application, a POSA would understand that substituting neutral residues in the CH3 domain with charged residues would result in the production of the claimed heterodimeric antibody. The '935 Provisional Application's disclosure further *specifies* the *exact* substitutions that a POSA may make. The Specification states:

The term “**charged amino acid residue**” or “**charged residue**” as used herein means amino acid residues with electrically charged side chains. These can either be **positively charged side chains**, such as present in arginine (Arg, R), histidine (His, H) and lysine (Lys, K) or can be **negatively charged side chains**, such as present in aspartic acid (Asp, D) and glutamic acid (Glu, E). The term “**neutral amino acid residue**” or **neutral residue** as used herein refers to all other amino acids that do not carry electrically charged side chains. These neutral residues include serine (Ser, S), threonine (Thr, T), asparagine (Asn, N), glutamine (GLu, Q),

Cysteine (Cys, C), glycine (Gly, G), proline (Pro, P), alanine (Ala, A), valine (Val, V), isoleucine (Ile, I), leucine (Leu, L), methionine (Met, M), phenylalanine (Phe, F), tyrosine (Tyr, Y), and tryptophan (Trp, T).

See '935 Provisional Application (EX1030) at p. 17 (emphasis added).

46. The Specification of the '935 Provisional Application further specifies the exact CH3 residues on each heavy chain that a POSA could target to achieve heterodimerization through neutral to charge substitutions. In fact, in Table A and Table 7 of the '935 Provisional Application, the inventors list the CH3 residues that a POSA may substitute with the exact substitutions. Indeed, CH3 residues 364 and 368 are listed in both Table A and Table 7.

47. Table A of the '935 Provisional Application (reproduced below) discloses specific neutral residues located in the CH3 domain, defining them as “interface residues,” which interact on chain A with chain B. Indeed, the precise residues recited by the claimed invention are identified as interface residues for forming heterodimers, which I have highlighted below. A POSA would understand that “contacting residues” are known as “any amino acid residue present in the CH3 domain that can be involved in interdomain contacts.” See the '935 Provisional Application, p. 16, lines 17-18.

Table A: List of CH3 domain interface residues

Interface residue in chain A	Contacting residues in chain B
Q347	K360
Y349	S354, D356, E357, K360
T350	S354, R355
L351	L351, P352, P353, S354, T366
S354	Y349, T350, L351
R355	T350
D356	Y349, K439
E357	Y349, K370
K360	Q347, Y349
S364	L368, K370
T366	L351, Y407
L368	S364, K409
K370	E357, S364
N390	S400
K392	L398, D399, S400, F405
T394	T394, V397, F405, Y407
P395	V397
V397	T394, P395
D399	K392, K409
S400	N390, K392
F405	K392, T394, K409
Y407	T366, T394, Y407, K409
K409	L368, D399, F405, Y407
K439	D356

48. Table 7 of the '935 Provisional Application discloses specific positions for “[i]dentification of novel charge pair mutants.” See '935 Provisional Application, Example 13, at pp. 51-54. Here, there is a specific reference that the positively charged residue at position 364 is a lysine (K) or an arginine (R) residue, and that the negatively charged residue at position 368 is an aspartic acid (D) or glutamic acid (E) residue. Furthermore, there is a specific reference that the neutral

residue at position 364 is substituted with a positive residue (K), and that the neutral residue at position 368 is substituted with a negative residue (D).

Table 7: list of amino acid substitutions in the various constructs that were made (EU numbering)

AA substitutions in CH3	construct #	Effect on homodimer formation (- = no effect; +++ = max. inhibition; NT= not tested on gel)
Q347K	8	-
Y349D	9	+.
Y349K	10	+.
T350K	11	-
T350K, S354K	12	+.
L351K, S354K	13	+.
L351K, T366K	14	++
L351K, P352K	15	+.
L351K, P353K	16	++
S354K, Y349K	17	++
D356K	18	-
E357K	19	-
S364K	20	++
T366K, L351K	21	++
T366K, Y407K	22	+++
L368K	23	NT
L368K, S364K	24	++
N390K, S400K	25	+.
T394K, V397K	26	+

AA substitutions in CH3	construct #	Effect on homodimer formation (- = no effect; +++ = max. inhibition; NT= not tested on gel)
T394K, F405K	27	+++
T394K, Y407K	28	+++
P395K, V397K	29	+.
S400K	30	-
F405K	31	+++
Y407K	32	++
Q347K, V397K, T394K	33	+
Y349D, P395K, V397K	34	+
T350K, T394K, V397K	35	NT
L351K, S354K, S400K	36	+
S354K, Y349K, Y407K	37	+.
T350K, N390K, S400K	38	+.
L368K, F405K	39	++
D356K, T366K, L351K	40	+++
Q347K, S364K	41	+++
L368D, Y407F	42	+
T366K	43	+
L351K, S354K, T366K	44	+
Y349D, Y407D	45	+
Y349D, S364K,	46	+

49. In my opinion, a POSA would reasonably understand from the disclosure of the Specification of the '935 Provisional Application that substituting what the patent refers to as "interfacing residues" 364 and 368 from neutral to positive with a lysine (K) or an arginine (R) residue at position 364 and an aspartic acid (D) or glutamic acid (E) residue at position 368 would produce the claimed heterodimeric antibody in Claims 1-3 of the '859 Patent, and that the inventors were in possession of such an invention. The heterodimeric antibody of Claims 1-3 of the '859 Patent is sufficiently disclosed in the '935 Provisional Application.

50. Dependent Claim 4 of the '859 Patent adds the limitation that the heterodimeric antibody is a bispecific antibody. The Specification of the '935 Provisional Application discloses that the claimed antibodies are bispecific. See, *e.g.*, '935 Provisional Application (EX1030) at p. 19, lines 8-14. The Specification of the '935 Provisional Application states "[a]s described herein below in more detail, the present invention provides novel CH3 mutations which enable the production of certain bispecific Ig-like molecules of interest without a significant amount of undesired (dimeric) by-products." See '935 Provisional Application (EX1030) at p. 19. The Specification of the '935 Provisional Application further states "[f]or instance, Example 17 discloses a method using mutations according to the present invention, wherein the proportion of a bispecific antibody of interest was

raised to such extent that no dimeric by-product was detectable in the resulting mixture at all.” See ’935 Provisional Application (EX1030) at p. 24, lines 19-22.

51. Dependent Claims 5 and 6 of the ’859 Patent add the limitations that the heterodimeric antibody is human IgG (Claim 5), and further human IgG1 (Claim 6). The Specification of the ’935 Provisional Application discloses that the heterodimeric antibody is human IgG1. The Examples in the Specification of the ’935 Provisional Application disclose “amino acid substitutions to create various different CH3-domains” using the “construct vector MV1057,” which contains “nucleic acid sequences encoding the normal wildtype IgG 1 Fc part.” See ’935 Provisional Application (EX1030) at pp. 38 – 40 (Examples 1 – 6). A POSA would further understand that the description of human IgG1 is human IgG.

52. Dependent Claim 7 of the ’859 Patent adds the limitation that the heterodimeric antibody is in a pharmaceutically acceptable carrier. The Specification of the ’935 Provisional Application discloses a pharmaceutical composition of the heterodimeric antibody and a pharmaceutically acceptable carrier. The Specification of the ’935 Provisional Application states that “[h]ence, with such mutations according to the present invention, a bispecific Ig-like molecule can be produced in a single cell with a high proportion with essentially no contaminating dimeric by-products being present, which is particularly suitable for

the production of a pharmaceutical composition.” See ’935 Provisional Application (EX1030) at p. 24, lines 25-29. The Specification of the ’935 Provisional Application further states that:

Also provided is a pharmaceutical composition comprising a mixture of at least two Ig-like molecules obtainable by a method according to the invention. Said at least two Ig-like molecules according to the invention are preferably antibodies. Said pharmaceutical composition may comprise a mixture comprising monospecific or bispecific Ig-like molecules, or a combination of monospecific and bispecific Ig-like molecules.

See ’935 Provisional Application (EX1030) at p. 36, lines 14-19. A POSA would understand from this disclosure that this description includes an acceptable “pharmaceutical carrier.”

VII. LAZAR ALONE OR IN COMBINATION WITH KANNAN DOES NOT DISCLOSE OR SUGGEST EVERY ELEMENT OF CLAIMS 1-7 OF THE ’859 PATENT

53. Independent Claim 1 of the ’859 Patent requires a heterodimeric antibody with a positively charged amino acid residue at position 364 in one CH3 domain and a negatively charged amino acid residue at position 368 in the other CH3 domain. The elements of Independent Claim 1 are not disclosed or suggested by Lazar alone or in combination with Kannan. Rather, Lazar discloses the opposite, *i.e.*, a negatively charged amino acid residue at position 364 and a positively charged

amino acid residue at position 368. Kannan also does not disclose a positively charged amino acid residue at position 364 in one CH3 domain and a negatively charged amino acid residue at position 368 in the other CH3 domain. Therefore, a POSA would not be able to arrive at the heterodimeric antibody of Independent Claim 1 by combining the disclosures of Lazar and/or Kannan.

A. Lazar Does Not Disclose Or Suggest The Claimed Heterodimeric Antibody

54. The Specification of the '859 Patent defines the claimed heterodimeric antibody:

Bispecific antibodies based on the IgG format, consisting of 2 heavy and two light chains have been produced by a variety of methods. For instance, bispecific antibodies may be produced by fusing two antibody-secreting cell lines to create a new cell line or by expressing two antibodies in a single cell using recombinant DNA technology. These approaches yield multiple antibody species as the respective heavy chains from each antibody may form monospecific dimers (also called homodimers), which contain two identical paired heavy chains with the same specificity, and **bispecific dimers (also called heterodimers) which contain two different paired heavy chains with different specificity.**

See '859 Patent (EX1001), Col. 3, lines 35-46, (emphasis added)

55. With respect to the CH3 domain, the Specification of the '859 Patent states that:

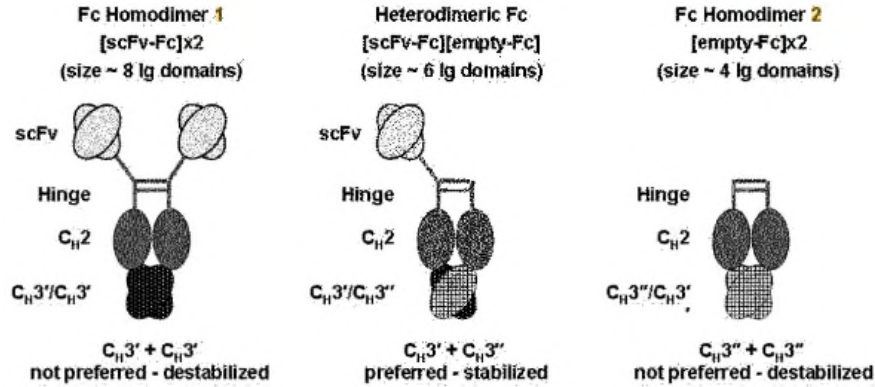
The term “**CH3 domain**” is well known in the art. The IgG structure has four chains, two light and two heavy chains; each light chain has two domains, the variable and the constant light chain (VL and CL) and **each heavy chain has four domains, the variable heavy chain (VH) and three constant heavy chain domains (CH1, CH2, CH3).**

See '859 Patent (EX1001), Col. 12, lines 22-27 (emphasis added).

56. A POSA would understand that the '859 Patent is directed to a heterodimeric antibody with two heavy chains each having VH, CH1, CH2, and CH3, and two Fab arms each with a different binding site specificity.

57. In contrast, Lazar discloses a binding moiety for screening purposes, namely scFv-Fc/empty-Fc format, which does not have two different heavy chains consisting of four domains and with different binding site specificities as defined in the Specification of the '859 Patent. Lazar also discloses an alternative type of bispecific moiety in which the interaction with the first antigen [Ag 1] is bivalent and the interaction with the second antigen [Ag 2] is monovalent. In this construct, the two chains are identical with respect to their antigen specificity [Ag 1]. The second specificity [Ag 2] is generated by fusion of Fv or Fab domains to the C termini of the two chains, separated by a linker region. One chain carries a fusion of VH or VH-CH1 while the other carries VL or VL-CL: these constructs are referred to as mAb-Fv and mAb-Fab, respectively.

58. The scFv-Fc/empty-Fc format of Lazar is not the claimed heterodimeric antibody with two different binding specificities as defined in the Specification of the '859 Patent. According to the disclosed sequences and Figure 1 in Lazar, empty-Fc does not have any antigen-binding site and the chain scFv-Fc has only one binding site (*i.e.*, a single-chain variable fragment (scFv)). Thus, the scFv-Fc/empty-Fc format is a monospecific moiety.



59. The chains of the scFv-Fc/empty-Fc format are not heavy chains with four domains as defined in the Specification of the '859 Patent. According to Lazar, the scFv-Fc has a VH-linker-Vκ-Hinge-CH2-CH3' and the empty-Fc has a Hinge-CH2-CH3''. Such sequences clearly show that the scFv-Fc lacks a CH1 domain, and the empty-Fc lacks VH and CH1 domains. See Lazar (EX1004) at ¶ 238 and Figure 1.

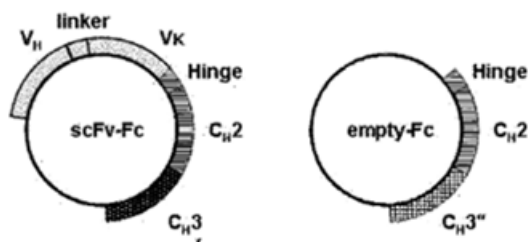


Figure 1

60. Therefore, Lazar's scFv-Fc/empty-Fc formats do not have "two different paired heavy chains with different specificity" as required by the Specification of the '859 Patent.

61. Dr. Presta relies on variants disclosed in Table 1 and Figures 5-7 of Lazar to argue that Lazar discloses or suggests every limitation of Claim 1. Dr. Presta states "Table 1 identifies a heterodimer incorporating CH3 domains with amino acid substitutions at those very positions—*i.e.*, position 364 and position 368" and "[e]qually, Figures 5-7 of *Lazar* depict various heterodimers with corresponding negative/positive and positive/negative amino acid substitutions at contact residues to measure impact on heterodimer formation." See Presta Declaration (EX1002), at ¶ 198.

62. However, all of the variants in Table 1 and Figures 5-7 were tested in the scFv-Fc/empty-Fc format. Lazar states:

A DNA library encoding variant immunoglobulin polypeptides, designed as described above, was constructed in the scFv-Fc and empty-Fc constructs. Pairs of scFv-Fc and empty-Fc constructs were cotransfected into HEK293E cells for expression, and resulting proteins were purified using protein A affinity chromatography. Relative concentrations of the expressed protein species were determined using the Agilent 2100 Bioanalyzer microfluidics-based platform, as described above. Resulting gels are shown in FIG. 4. A quantitative summary of the results from this library is provided in FIG. 5. A number of designed variants increased the content of heterodimer relative to that of the native parent Fc region. Using the information obtained from the previous library, a new library was designed and screened as described above. Quantitative electrophoretic results are shown in FIGS. 6 and 7. **A number of designed variants increased the content of heterodimer relative to that of the native parent Fc region. Preferred and most preferred variants from this screen are listed in Tables 1 and 2 respectively.**

See Lazar (EX1004), at ¶¶ 240-241 (emphasis added).

63. The scFv-Fc/empty-Fc construct in Lazar was designed for testing the variants in Figures 5-7. Lazar made clear that the variants in Figures 5-7 were tested in the empty-Fc/scFv-Fc format by the use of the headers: “Empty-Fc” and “scFv-Fc”, as shown below:

Figure 5

Lane	Empty-Fc	scFv-Fc	Empty -M	Empty -D	Hetero	scFv -D	scFv -M
1	IgG1 WT	IgG1 WT	2%	33%	42%	23%	0%
2	S364D	Y349K	0%	6%	73%	21%	0%
3	S364E	Y349K	0%	7%	75%	18%	0%

Figure 6

Empty-Fc	scFv-Fc	Empty -M	Empty -D	Hetero	scFv -D	scFv -M
F405A	T394F	3%	26%	66%	5%	0%
F405A	T394W	3%	31%	64%	2%	0%

Figure 7

Empty-Fc	scFv-Fc	Empty -M	Empty -D	Hetero	scFv -D	scFv -M
F405A	T394F	1%	19%	72%	8%	0%
F405S	T394Y	0%	3%	40%	57%	0%
K370C	S364C	0%	24%	0%	76%	0%

64. Lazar's scFv-Fc/empty-Fc format is not the claimed heterodimeric antibody with different specificity as defined in the '859 Patent. Therefore, the variants in Table 1 and Figures 5-7, which were used only for scFv-Fc/empty-Fc formats, do not disclose or suggest the subject matter claimed in Independent Claim 1.

65. Lazar's alternative bispecific moieties (*i.e.*, mAb-Fv, mAb-Fab, Fab-Fv, and Fab-Fab) are also not the claimed heterodimeric antibody with different specificity as defined in the '859 Patent.

66. According to Lazar, the alternative moieties are created by adding a Fv or Fab at the C termini of wild-type IgG. See Lazar (EX1004) at ¶ 46. As shown in Figure 8 of Lazar, the addition of a Fv or a Fab does not change the fact that the two chains have the same antigen specificity (for Ag1) in the Fab regions.

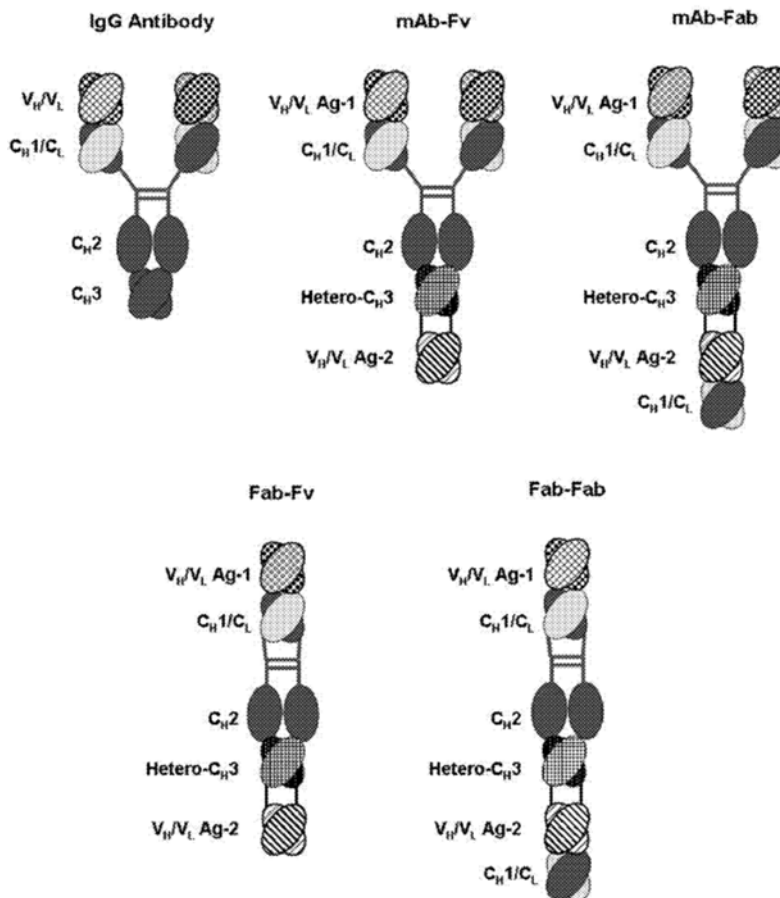


Figure 8

67. As shown in Fig 8, Fab-Fv and Fab-Fab are “analogues of the mAb-Fv and mAb-Fab that bind both antigen-1 and antigen-2 monovalently.” See Lazar (EX1004) at ¶ 48. Therefore, the alternative bispecific moieties are not the antibodies of the '859 Patent that have heavy chains as required by the Patent (“each heavy chain has four domains, the variable heavy chain (VH) and three constant heavy chain domains (CH1, CH2, CH3)”) with different binding specificities.

68. Furthermore, Lazar incorporated only one combination of substitutions (*i.e.*, Y349T/T394F and S364H/F405A) into the alternative bispecific format.

69. Therefore, it is my opinion that Lazar does not disclose or suggest the claimed heterodimeric antibody with heavy chains having different specificities as defined in the Specification of the '859 Patent and required by Independent Claim 1.

B. Lazar Does Not Disclose Or Suggest The Claimed Amino Acid Residues

70. Lazar does not disclose or suggest the features of “a positively charged amino acid residue at position 364” and “a negatively charged amino acid residue at position 368” as required by Independent Claim 1 of the '859 Patent.

71. Rather, Lazar suggests the opposite, *i.e.*, a negatively charged amino acid residue at position 364 and a positively charged amino acid residue at position 368; the mutations S364E and L368K are shown in Table 1 “Preferred CH3 domain variants that favor Fc heterodimerization”. See Lazar (EX1004) at ¶ 241 and Table 1. Dr. Presta admits that “Table 1 of Lazar identifies a heterodimer incorporating CH3 domains substituting in 364E (negatively charged) on a first CH3 domain and 368K (positively charged) on a second CH3,” whereas the '859 Patent claims are for the opposite charges. See Presta Declaration (EX1002), ¶198.

72. However, Dr. Presta is of the opinion that a POSA would reverse Lazar's preferred substitutions to arrive at the claimed residue pair. *Id.* But, Dr.

Presta fails to explain *why* a POSA would reverse the substitutions disclosed by Lazar.

73. Dr. Presta is of the opinion that:

Based on Lazar's teachings in paragraph 123, a person of ordinary skill in the art would understand that either a complementary charge pair of amino acid residues could be substituted in at positions 364 and 368 of a first and second CH3 domain – positive/negative or negative/positive. Therefore, while Lazar identifies a “[p]referred” “S364D” / “L368K” (negatively / positively charged) heterodimer, a person of ordinary skill in the art would appreciate that the “preferabl[e]” amino acid substitutions of paragraph 123 could equally be applied to those positions to form a “S364H / L368E” or “S364R / L368E” (positively / negatively charged).

See Presta Declaration (EX1002), ¶ 189. Dr. Presta offers further evidence from substitutions at positions 364 and 370 as examples that show Lazar tested “both S364D/K370R (negative/positive substitution) and S364R/K370D (positive/negative substitution).” See Presta Declaration (EX1002), ¶ 198.

74. Firstly, these are different kinds of substitutions, distinct from the approach in the '859 Patent. They are *not* neutral to charged on each chain, but neutral to negative (S364D) and positive to positive (K370R); and neutral to positive (S364R), and positive to negative (K370D).

75. Secondly, positions 364 and 370 are self-evidently not the same as positions 364 and 368. The example variants at positions 364 and 370 that Dr. Presta selected cannot be directly compared to variants at positions 364 and 368 because they have different residue location and surrounding environment that are critical for heterodimerization. Thirdly, the data in Figure 5 of Lazar (reproduced below) reveals that reversing the substitutions causes a substantial effect upon the degree of heterodimerization. S364R and K370D lead to a high fraction of heterodimer (*i.e.*, 59%, lane 17) whereas the reversed pair of substitutions leads to a low fraction of heterodimer (*i.e.*, 38%, lane 14). A further example of charge reversal is shown in Figure 5, lanes 20 and 22; here again reversal of the charged substitutions causes a substantial effect upon the fraction of heterodimerization. From the data presented in Lazar, a POSA would therefore understand that charge reversal will have a significant effect upon heterodimerization. Lastly, I note that a POSA would see that substitutions of these same residues highlighted by Dr. Presta, namely S364F and K370G [Figure 5, lane 15], yield an even higher fraction of heterodimer (*i.e.*, 65%) suggesting to the POSA that the substitution of neutral residues to neutral residues is preferred to neutral to charged substitutions.

76. Lazar first used “computational structure-based methods (PDA® technology) to “evaluate possible amino acid substitutions in the CH3 region for

their ability to stabilize Fc heterodimers and destabilize Fc homodimers.” See Lazar (EX1004) at ¶ 236. Selected variants were then screened using the scFv-Fc and empty-Fc constructs. See Lazar (EX1004) at ¶ 240. A quantitative summary of the variants is provided in Figures 5-7, and “Preferred” and “Most preferred” variants from this screen are listed in Tables 1 and 2 respectively. See Lazar (EX1004) at ¶¶ 240-241.

77. Thus the “Preferred CH3 domain variants” in Table 1 represent the most promising amino acid residue substitutions for heterodimerization at the positions considered by Lazar. For example, Figure 5 of Lazar (reproduced below) shows the different degree of heterodimerization between the charge-swapped variants T411E and K370R (41%) and T411K and K370E (61%) (Figure 5, lanes 20 and 22). Only the T411K and K370E variant, which provides the higher degree of heterodimerization, and therefore the preferred variant, is listed in Table 1 (reproduced below). This suggests to a POSA that swapping charges for any of the preferred variants in Table 1 will only decrease the degree of heterodimerization.

78. Figure 5 of Lazar (reproduced below) shows the degree of heterodimerization for the variants that Dr. Presta selected to support his arguments, namely, S364D and K370R (38%) compared with S364R and K370D (59%). However, neither of these pairs is listed in Table 1 (reproduced below). Rather, a

variant with substitutions to neutral residues (*i.e.*, S364F and K370G) is listed as a preferred variant because it provides a higher degree of heterodimerization (65%) than the two variants that Dr. Presta highlighted. Based on the degree of heterodimerization of the variants selected by Dr. Presta, a POSA would rather pick the variant with substitutions to neutral residues for further development.

Figure 5

Lane	Empty-Fc	scFv-Fc	Empty -M	Empty -D	Hetero	scFv -D	scFv -M
1	IgG1 WT	IgG1 WT	2%	33%	42%	23%	0%
2	S364D	Y349K	0%	6%	73%	21%	0%
3	S364E	Y349K	0%	7%	75%	18%	0%
4	S364F	Y349A	0%	13%	59%	29%	0%
5	S364G	Y349W	0%	26%	52%	23%	0%
6	S364H	Y349T	0%	0%	65%	35%	0%
7	S364Y	Y349A	0%	2%	51%	47%	0%
8	S364Y	Y349S	0%	2%	56%	42%	0%
9	S364D	L368K	6%	31%	51%	12%	0%
10	S364E	L368S	4%	37%	54%	5%	0%
11	S364E	L368K	3%	19%	64%	14%	0%
12	K409D	L368K	0%	4%	73%	23%	0%
13	K409E	L368K	4%	24%	70%	1%	0%
14	S364D	K370R	0%	24%	38%	38%	0%
15	S364F	K370G	0%	22%	65%	13%	0%
16	S364H	K370S	0%	0%	35%	65%	0%
17	S364R	K370D	19%	11%	59%	11%	0%
18	S364R	K370E	22%	12%	50%	17%	0%
19	S364Y	K370G	0%	4%	50%	46%	0%
20	T411E	K370R	0%	29%	41%	29%	0%
21	T411K	K370D	0%	10%	53%	37%	0%
22	T411K	K370E	0%	12%	61%	27%	0%
23	Y349I/S364F	Y349E/S364D	0%	29%	48%	23%	0%
24	Y349W/S364H	Y349T/S364G	0%	12%	45%	43%	0%
25	S364D/K370G	S364Y/K370R	2%	16%	71%	11%	0%
26	S364Y/K370V	S364T/K370G	5%	35%	38%	22%	0%
27	L368E/K409E	L368K	6%	33%	61%	0%	0%

TABLE 1

Preferred CH3 domain variants that favor Fc heterodimerization.	
Variant 1	Variant 2
F405A	T394F
S364D	Y349K
S364E	L368K
S364E	Y349K
S364F	K370G
S364H	Y349K
S364H	Y349T
S364Y	K370G
T411K	K370E
V397S/F405A	T394F
K370R/T411K	K370E/T411E
L351E/S364D	Y349K/L351K
L351E/S364E	Y349K/L351K
L351E/T366D	L351K/T366K
P395T/V397S/F405A	T394F
S364D/K370G	S364Y/K370R
S364D/T394F	Y349K/F405A
S364E/F405A	Y349K/T394F
S364E/F405S	Y349K/T394Y
S364E/T411E	Y349K/D401K
S364H/D401K	Y349T/T411E
S364H/F405A	Y349T/T394F
S364H/T394F	Y349T/F405A
Y349C/S364E	Y349K/S354C
L351E/S364D/F405A	Y349K/L351K/T394F
L351K/S364H/D401K	Y349T/L351E/T411E
S364E/T411E/F405A	Y349K/T394F/D401K
S364H/D401K/F405A	Y349T/T394F/T411E
S364H/F405A/T411E	Y349T/T394F/D401K

79. The list of preferred variants in Table 1 includes the variant S364E and L368K. The charge-swapped version of this (*i.e.*, a positively charged amino acid

residue at position 364 and a negatively charged amino acid residue at position 368) is not even listed in Figure 5-7.

80. Therefore, a POSA would not reverse the substitutions of the “Preferred” (Table 1) and “Most preferred” (Table 2) variants based on the data of Lazar.

81. In particular, a POSA looking at the data presented by Lazar would not conclude that substituting residues at positions 364 and 368 with positively or negatively charged residues, respectively, would be advantageous for the formation of heterodimers; other types of substitutions at different locations, or indeed other approaches, would be more attractive to a POSA.

82. The 230 variants (including some repeats and wild-type controls) reported in Lazar Figures 5-7 include examples of neutral to neutral, charged to charged, neutral to charged, and charged to neutral substitutions, with some as single substitutions, others as double, and yet others as triple substitutions. As discussed above, these were all screened only using Lazar’s scFv-Fc/empty-Fc format, and none include the specific substitutions required by the ’859 Patent claims of a positive substitution at 364 and negative substitution at 368. Lazar neither highlights, nor do the data support, any particular advantage of neutral to charged substitutions. Dr. Presta notes that the variant S364E and L368K is disclosed in Table 1, but a POSA

would not have any cause to choose those mutations over others, and in fact the data suggest that other mutations, not involving substitutions at positions 364 and 368, would be preferred.

83. Lazar reports in Figures 5-7 the heterodimerization yield fractions for each of the variants. The variant with the best heterodimerization yield fraction, indeed the only variant with 100% yield of heterodimer, involves three simultaneous charged to charged substitutions in each chain (*i.e.*, K370D/K392D/K409D in one chain and E356K/E357K/D399K in the other chain). Furthermore, the only substitution that was taken into the alternative bispecific construct (with the next highest heterodimer yield of up to 85%) involved only neutral to neutral substitutions (*i.e.*, S364H/F405A in one chain and Y349T/T394F in the other chain). None of the variants involved with residue substitutions at positions 364 and 368 in Table 1 and Figures 5-7 provide higher heterodimeric yield fractions than these mutations. See Lazar (EX1004) at Table 1 and Figures 5-7. A POSA would not substitute residues with charged residues at positions 364 and 368 based on Lazar's disclosures that suggest other substitutions at different locations with higher yield fractions of heterodimer.

84. Lazar further states that CH3 domain modifications to yield “[h]eterodimeric Fc variants are not a necessity” for generation of the alternative

bispecific moieties, since the VH and VL domains attached to the chains provide specificity for Ag-2 might themselves preferentially associate with each other or at least provide a means to purify the heterodimer utilizing its affinity for Ag-2. See Lazar (EX1004) at ¶ 108.

85. Accordingly, it is my opinion that a POSA looking at the data presented by Lazar would conclude that other types of substitutions at different locations, or indeed other approaches, would be more advantageous for the formation of heterodimers than the claimed residues at positions 364 and 368.

C. Lazar In Combination With Kannan Does Not Disclose Or Suggest Claimed Amino Acid Residues

86. It is my opinion that a POSA would not arrive at the inventions of Independent Claim 1 based on the disclosures of Lazar in combination with Kannan. Like Lazar, Kannan also does not disclose or suggest “a positively charged amino acid residue at position 364” and “a negatively charged amino acid residue at position 368” as required by Independent Claim 1 of the ’859 Patent. Dr. Presta relies on Kannan for its disclosure of the charge reversal technique to argue that a POSA would reverse the charges of Lazar’s preferred CH3 domain variants. In my opinion, applying Kannan’s charge reversal technique to the preferred CH3 domain variants in Lazar is without scientific basis.

87. As discussed above, there is no disclosure in Lazar that would direct a POSA to substitute the neutral amino acid with a positively charged amino acid residue at position 364 (364(+)) and the neutral amino acid with a negatively charged amino acid residue at position 368 (368(-)). In fact, Dr. Presta admits that Lazar actually discloses the opposite, *i.e.*, substituting to a negatively charged amino acid residue at position 364 and a positively charged amino acid residue at position 368.

88. Dr. Presta is of the opinion that a POSA would reverse the charges disclosed in Lazar at positions 364 and 368 based on Kannan because Kannan generally discloses changing a negatively charged residue to a positively charged residue at the CH3 domain interface. See Presta Declaration, ¶¶ 191-192.

89. Dr. Presta is thus of the opinion that a POSA would re-engineer variants that had already been exhaustively engineered through Lazar's substitution selection processes and thorough screening and go through another step of reversing the charges of those variants made by Lazar. In the absence of any additional data, a POSA would not consider generating further substitutions altering the variants generated by Lazar, because those variants had already been thoroughly explored by Lazar. Unlike Kannan's variants that were substituted from wild-type, the engineered variants of Lazar already have modified structures and surrounding environments. Indeed, Kannan is highly selective

regarding the locations of substitutions. Kannan states “[i]t must be stated here that different combinations will have diverse effects on the quaternary (homodimer/heterodimer) structure formation depending on surrounding residues at the mutation site and role of water molecules.” See Kannan (EX1007) at p. 10, lines 6-10. A POSA would therefore not consider reversing charges according to Kannan’s disclosure for any of Lazar’s preferred variants with any expectation of success.

90. Moreover, as discussed above, the “Preferred CH3 domain variants” in Table 1 represent the most promising amino acid residue substitutions for heterodimerization amongst those residue positions tested by Lazar. A POSA would have no basis for applying any modifications suggested in Kannan because the data in Lazar show that making such modifications to Lazar’s “Preferred CH3 domain variants” would only reduce the heterodimerization yield.

91. Gunasekaran Kannan, who is listed as a first named inventor of Kannan (EX1007), suggested not to modify the hydrophobic core residues for heterodimerization in his publication (EX1012). Both publications are directed to the same heterodimerization technique (*i.e.*, electrostatic steering through charge swapping). In Gunasekaran (EX1012), it is stated that “[i]t has long been established that the hydrophobic core of protein domains plays an important role in protein

folding and stability” and “exploiting charged residues as opposed to hydrophobic residues at the CH3 domain interface may have benefits in terms of retaining the generally favorable biophysical properties of the Fc.” See Gunasekaran (EX1012) at page 4. Based on such disclosures of Kannan (EX1007) and Gunasekaran (EX1012), a POSA would not be motivated to modify hydrophobic core residues, which comprises primarily neutral, nonpolar amino acids.

92. Therefore, it is my opinion that Lazar alone or in combination with Kannan does not disclose or suggest “a positively charged amino acid residue at position 364” and “a negatively charged amino acid residue at position 368” as required by Independent Claim 1 of the ’859 Patent. Lazar alone or in combination with Kannan also does not disclose the limitations in Dependent Claims 2-7 of the ’859 Patent because Dependent Claims 2-7 of the ’859 Patent depend from Independent Claim 1.

VIII. CONCLUSION

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

Date: 28th June 2025

A handwritten signature in black ink, appearing to read "B. J. Sutton", written in a cursive style.

Brian J. Sutton, Ph.D.