

BISPECIFIC ANTI-*PSEUDOMONAS* ANTIBODIES WITH MODIFIED FC
REGIONS AND METHODS OF USE THEREOF

CROSS-REFERENCE TO RELATED APPLICATIONS

- [0001] This application claims the priority benefit of U.S. Provisional Application No. 63/501,036, filed May 9, 2023, which is herein incorporated by reference in its entirety.

REFERENCE TO SEQUENCE LISTING SUBMITTED ELECTRONICALLY

- [0002] The content of the electronically submitted sequence listing (Name: PSEUD-110-WO-PCT_Seqlisting_ST26.xml; Size: 58,580 bytes; and Date of Creation: April 23, 2024) is herein incorporated by reference in its entirety.

FIELD OF THE DISCLOSURE

- [0003] This disclosure relates to anti-*Pseudomonas* Psl and PcrV bispecific antibodies. Such antibodies can be used, for example, in the prevention and treatment of *Pseudomonas* infections. Advantageously, the anti-*Pseudomonas* Psl and PcrV bispecific antibodies exhibit reduced aggregation during manufacture and have a clinically useful half-life. Furthermore, the disclosure provides compositions useful in such therapies.

BACKGROUND OF THE DISCLOSURE

- [0004] *Pseudomonas aeruginosa* (*P. aeruginosa*) is a gram-negative opportunistic pathogen that causes both acute and chronic infections in compromised individuals (Ma et al., *Journal of Bacteriology* 189(22):8353-8356 (2007)). This is partly due to the high innate resistance of the bacterium to clinically used antibiotics, and partly due to the formation of highly antibiotic-resistant biofilms (Drenkard E., *Microbes Infect* 5:1213-1219 (2003); Hancock & Speert, *Drug Resist Update* 3:247-255 (2000)).
- [0005] *P. aeruginosa* is a common cause of hospital-acquired infections in the Western world. It is a frequent causative agent of bacteremia in burn victims and immune compromised individuals (Lyczak et al., *Microbes Infect* 2:1051-1060 (2000)). It is also the most common cause of nosocomial gram-negative pneumonia (Craven et al., *Semin Respir Infect* 11:32-53 (1996)), especially in mechanically ventilated patients, and is the most

prevalent pathogen in the lungs of individuals with cystic fibrosis (Pier et al., *ASM News* 6:339-347 (1998)).

[0006] Furthermore *P. aeruginosa* is known to colonize the airway in patients with non-cystic fibrosis bronchiectasis. Non-cystic fibrosis bronchiectasis is a chronic disease characterized by abnormal and permanent dilation of the bronchi resulting in chronic cough, sputum production, and recurrent bacterial infections of the airway. Patients with bronchiectasis suffer from a high morbidity due to frequent exacerbations impairing quality of life and facilitating resistance to antibiotics, leading to reduced lung function.

[0007] *Pseudomonas* Psl exopolysaccharide is reported to be anchored to the surface of *P. aeruginosa* and is thought to be important in facilitating colonization of host tissues and in establishing/maintaining biofilm formation (Jackson, K. D., et al., *J Bacteriol* 186, 4466-4475 (2004)). Its structure comprises mannose-rich repeating pentasaccharide (Byrd, M. S., et al., *Mol Microbiol* 73, 622-638 (2009)).

[0008] PcrV is a component of the type III secretion system. PcrV appears to be an integral component of the translocation apparatus of the type III secretion system mediating the delivery of the type III secretory toxins into target eukaryotic cells (Sawa T., et al. *Nat. Med.* 5, 392-398 (1999)). Active and passive immunization against PcrV improved acute lung injury and mortality of mice infected with cytotoxic *P. aeruginosa* (Sawa et al. 2009). The major effect of immunization against PcrV was due to the blockade of translocation of the type III secretory toxins into eukaryotic cells.

[0009] Due to increasing multidrug resistance, there remains a need in the art for the development of improved agents for targeting *Pseudomonas*.

BRIEF SUMMARY

[0010] Provided herein are anti-*Pseudomonas aeruginosa* PcrV protein and Psl exopolysaccharide bispecific antibodies comprising modifications to the Fc region that can result in increased half-life.

[0011] In some aspects provided herein, the present disclosure relates to a bispecific antibody that specifically binds to *Pseudomonas aeruginosa* PcrV protein and Psl exopolysaccharide, wherein the antibody comprises a modified IgG Fc region, the modified IgG Fc region comprising amino acid substitutions at two or more of positions 432 to 437,

numbered according to the EU numbering index of Kabat, relative to a wild-type IgG Fc region; wherein

- (i) positions 432 and 437 are each substituted with cysteine;
- (ii) position 433 is histidine or is substituted with arginine, proline, threonine, lysine, serine, alanine, methionine, or asparagine;
- (iii) position 434 is asparagine or is substituted with arginine, tryptophan, histidine, phenylalanine, tyrosine, serine, methionine or threonine;
- (iv) position 435 is histidine; and
- (v) position 436 is tyrosine or phenylalanine or is substituted with leucine, arginine, isoleucine, lysine, methionine, valine, histidine, serine, or threonine; and

wherein the antibody has an increased half-life compared to the half-life of a corresponding antibody having the wild-type IgG Fc region.

[0012] In some aspects, the modified IgG Fc region is a modified IgG1 Fc region. In some aspects, the modified IgG Fc region is a modified human IgG Fc region (e.g. a modified human IgG1 Fc region).

[0013] In some aspects, the bispecific antibody exhibits less aggregation in solution than an antibody comprising a heavy chain comprising the amino acid sequence of SEQ ID NO:19 and a light chain comprising the amino acid sequence of SEQ ID NO:20. In some aspects, the bispecific antibody exhibits less aggregation in a shake plate overgrow screen than an antibody comprising a heavy chain comprising the amino acid sequence of SEQ ID NO:19 and a light chain comprising the amino acid sequence of SEQ ID NO:20.

[0014] In some aspects, the bispecific antibody promotes opsonophagocytic killing activity of *P. aeruginosa*, optionally wherein the bispecific antibody mediates similar *in vitro* opsonophagocytic killing activity of *P. aeruginosa* as an antibody comprising a heavy chain comprising the amino acid sequence of SEQ ID NO:19 and a light chain comprising the amino acid sequence of SEQ ID NO:20.

[0015] In some aspects, the bispecific antibody further comprises an amino acid insertion after position 437, optionally wherein the amino acid insertion is glutamic acid.

[0016] In some aspects, the binding affinity of the bispecific antibody for FcRn at pH 6.0 is higher than the binding affinity for FcRn of a corresponding antibody having the wild-type human IgG1 Fc region at pH 6. In some aspects, the binding affinity of the bispecific antibody for FcRn at pH 7.4 is higher than the binding affinity for FcRn of a corresponding

antibody having the wild-type human IgG1 Fc region at pH 7.4. In some aspects provided herein, the modified human IgG1 Fc region exhibits increased pH dependence on binding affinity for FcRn compared to a corresponding antibody having the wild-type human IgG1 Fc region.

- [0017] In some aspects, the modified human IgG1 Fc region has amino acid substitutions at three of positions 432, 433, 434, 435, 436, and 437. In some aspects, the modified human IgG1 Fc region has amino acid substitutions at four of positions 432, 433, 434, 435, 436, and 437. In some aspects, the modified human IgG1 Fc region has amino acid substitutions at five of positions 432, 433, 434, 435, 436, and 437. In some aspects, the modified human IgG1 Fc region has amino acid substitutions at six of positions 432, 433, 434, 435, 436, and 437.
- [0018] In some aspects, the modified human IgG1 Fc region comprises the amino acid sequence of SEQ ID NO:44 or the amino acid sequence of SEQ ID NO:33.
- [0019] In some aspects, the bispecific antibody is not a HexaBody.
- [0020] In some aspects, the bispecific antibody competitively inhibits binding to PcrV of an antibody comprising a heavy chain variable region (VH) comprising the amino acid sequence of SEQ ID NO:13 and a light chain variable region (VL) comprising the amino acid sequence of SEQ ID NO:14.
- [0021] In some aspects, the bispecific antibody binds to the same epitope of PcrV as an antibody comprising a VH comprising the amino acid sequence of SEQ ID NO:13 and a VL comprising the amino acid sequence of SEQ ID NO:14.
- [0022] In some aspects, the bispecific antibody comprises an antigen-binding domain that binds to *Pseudomonas aeruginosa* PcrV protein and comprises a VH-CDR1 comprising the amino acid sequence of SEQ ID NO:1, a VH-CDR2 comprising the amino acid sequence of SEQ ID NO:2, a VH-CDR3 comprising the amino acid sequence of SEQ ID NO:3, a VL-CDR1 comprising the amino acid sequence of SEQ ID NO:4, a VL-CDR2 comprising the amino acid sequence of SEQ ID NO:5, and a VL-CDR3 comprising the amino acid sequence of SEQ ID NO:6.
- [0023] In some aspects, the antigen-binding domain that binds to *Pseudomonas aeruginosa* PcrV protein comprises a VH comprising the amino acid sequence of SEQ ID NO:13 and/or a VL comprising the amino acid sequence of SEQ ID NO:14.

- [0024] In some aspects, the antigen-binding domain that binds to *Pseudomonas aeruginosa* PcrV protein comprises a heavy chain variable region and a light chain variable region on separate polypeptides.
- [0025] In some aspects, the bispecific antibody competitively inhibits binding to Psl of an antibody comprising a VH comprising the amino acid sequence of SEQ ID NO:15 and a VL comprising the amino acid sequence of SEQ ID NO:16.
- [0026] In some aspects, the bispecific antibody binds to the same epitope of Psl as an antibody comprising a VH comprising the amino acid sequence of SEQ ID NO:15 and a VL comprising the amino acid sequence of SEQ ID NO:16.
- [0027] In some aspects provided herein, the antibody comprises an antigen-binding domain that binds to *Pseudomonas aeruginosa* Psl exopolysaccharide and comprises a heavy chain variable region VH-CDR1 comprising the amino acid sequence of SEQ ID NO:7, a VH-CDR2 comprising the amino acid sequence of SEQ ID NO:8, a VH-CDR3 comprising the amino acid sequence of SEQ ID NO:9, a light chain variable region VL-CDR1 comprising the amino acid sequence of SEQ ID NO:10, a VL-CDR2 comprising the amino acid sequence of SEQ ID NO:11, and a VL-CDR3 comprising the amino acid sequence of SEQ ID NO:12.
- [0028] In some aspects provided herein, the antigen-binding domain that binds to *Pseudomonas aeruginosa* Psl exopolysaccharide comprises a VH comprising the amino acid sequence of SEQ ID NO:15 and/or a VL comprising the amino acid sequence of SEQ ID NO:16.
- [0029] In some aspects, the antigen-binding domain that binds to *Pseudomonas aeruginosa* Psl exopolysaccharide comprises a VH and a VL on the same polypeptide. In some aspects, the antigen-binding domain that binds to *Pseudomonas aeruginosa* Psl exopolysaccharide comprises a linker between the VH and the VL, optionally wherein the linker comprises the amino acid sequence of SEQ ID NO:18.
- [0030] In some aspects, the antigen-binding domain that binds to *Pseudomonas aeruginosa* Psl exopolysaccharide comprises an scFv. In some aspects, the scFv comprises a linker, optionally wherein the linker comprises the amino acid sequence of SEQ ID NO:18. In some aspects, the scFv is in the orientation VH-linker-VL. In some aspects, the scFv comprises the amino acid sequence of SEQ ID NO:17. In some aspects, the scFv is on the same polypeptide chain as the VH of the antigen-binding domain that binds to

Pseudomonas aeruginosa PcrV protein. In some aspects, the scFv is C-terminal to the VH of the antigen-binding domain that binds to *Pseudomonas aeruginosa* PcrV protein.

[0031] In some aspects, the bispecific antibody comprises (i) a heavy chain of the formula VH-CH1-H1-L1-S-L2-H2-CH2-CH3, wherein VH is an anti-*Pseudomonas aeruginosa* PcrV heavy chain variable domain; CH1 is a heavy chain constant region domain 1; H1 is a first heavy chain hinge region fragment; L1 is a first linker; S is an anti-*Pseudomonas aeruginosa* Psl scFv molecule; L2 is a second linker; H2 is a second heavy chain hinge region fragment; CH2 is a heavy chain constant region domain-2; and CH3 is a heavy chain constant region domain-3; and (ii) a light chain of the formula VL-CL, wherein VL is an anti-*Pseudomonas aeruginosa* PcrV light chain variable domain, and CL is an antibody light chain kappa constant region or an antibody light chain lambda constant region.

[0032] In some aspects, CH1 comprises the amino acid sequence of SEQ ID NO:21. In some aspects, H1 comprises the amino acid sequence of SEQ ID NO:22. In some aspects, L1 comprises the amino acid sequence of SEQ ID NO:28. In some aspects, L2 comprises the amino acid sequence of SEQ ID NO:28. In some aspects, H2 comprises the amino acid sequence of SEQ ID NO:23. In some aspects, CH2-CH3 comprises the amino acid sequence of SEQ ID NO:30. In some aspects, CL is an antibody light chain kappa constant region. In some aspects, CL comprises the amino acid sequence of SEQ ID NO:24.

[0033] In some aspects, the bispecific antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:31 and/or a light chain comprising the amino acid sequence of SEQ ID NO:20.

[0034] In some aspects provided herein, the present disclosure relates to an isolated polynucleotide comprising a nucleic acid molecule encoding the heavy chain of the bispecific antibody described herein. In some aspects, the isolated polynucleotide further comprises a nucleic acid molecule encoding the light chain of the bispecific antibody described herein.

[0035] In some aspects provided herein, the present disclosure relates to a vector comprising (i) a nucleic acid molecule encoding the heavy chain of the bispecific antibody, or (ii) a nucleic acid molecule encoding the heavy chain of the bispecific antibody described herein and a nucleic acid molecule encoding the light chain of the bispecific antibody described herein. In some aspects, the present disclosure relates to a pair of vectors, wherein the first vector of the pair of vectors comprises a nucleic acid molecule encoding the heavy

chain of the bispecific antibody described herein, and the second vector of the pair of vectors comprises a nucleic acid molecule encoding the light chain of the bispecific antibody described herein.

[0036] In some aspects provided herein, the present disclosure relates to a host cell comprising (i) an isolated polynucleotide comprising a nucleic acid molecule encoding the heavy chain of the bispecific antibody described herein, (ii) a vector comprising a nucleic acid molecule encoding the heavy chain of the bispecific antibody described herein or a nucleic acid molecule encoding the heavy chain of the bispecific antibody described herein and a nucleic acid molecule encoding the light chain of the bispecific antibody described herein, or (iii) a nucleic acid molecule encoding the heavy chain of the bispecific antibody described herein and a nucleic acid molecule encoding the light chain of the bispecific antibody described herein. In some aspects provided herein, the present disclosure relates to a host cell comprising a pair of vectors, wherein the first vector of the pair of vectors comprises a nucleic acid molecule encoding the heavy chain of the bispecific antibody described herein, and the second vector of the pair of vectors comprises a nucleic acid molecule encoding the light chain of the bispecific antibody described herein.

[0037] In some aspects provided herein, the present disclosure relates to a method of producing a bispecific antibody, the method comprising culturing the host cell described herein, and optionally isolating the bispecific antibody. In some aspects, the present disclosure relates to bispecific antibody produced by the method described herein.

[0038] In some aspects provided herein, the present disclosure relates to a composition comprising the bispecific antibody described herein, and a pharmaceutically acceptable carrier.

[0039] In some aspects provided herein, the present disclosure relates to a method of treating or preventing a *Pseudomonas* infection in a subject in need thereof, the method comprising administering the bispecific antibody described herein, or the composition comprising the bispecific antibody described herein to the subject. In some aspects provided herein, the present disclosure relates to use of the bispecific antibody described herein or the composition comprising the bispecific antibody described herein in the preparation of a medicament for use in treating or preventing a *Pseudomonas* infection in a subject in need thereof. In some aspects provided herein, the present disclosure relates to the bispecific antibody described herein or the composition comprising the bispecific

antibody described herein for use in treating or preventing a *Pseudomonas* infection in a subject in need thereof.

[0040] In some aspects, the infection is a lung infection, a respiratory tract infection, pneumonia, bacteremia, a bone infection, a joint infection, a skin infection, a burn infection, a wound infection, or any combination thereof.

[0041] In some aspects provided herein, the present disclosure relates to a method of treating bronchiectasis in a subject in need thereof, the method comprising administering the bispecific antibody described herein or the composition comprising the bispecific antibody described herein. In some aspects provided herein, the present disclosure relates to use of the bispecific antibody described herein or the composition comprising the bispecific antibody described herein in the preparation of a medicament for use in treating bronchiectasis in a subject in need thereof. In some aspects provided herein, the present disclosure relates to the bispecific antibody described herein or the composition comprising the bispecific antibody described herein for use in treating bronchiectasis in a subject in need thereof.

[0042] In some aspects provided herein, the present disclosure relates to a method of improving pre-bronchodilator forced expiratory volume 1 (FEV₁) in a subject with bronchiectasis, the method comprising administering the bispecific antibody described herein or the composition comprising the bispecific antibody described herein. In some aspects provided herein, the present disclosure relates to use of the bispecific antibody described herein or the composition comprising the bispecific antibody described herein in the preparation of a medicament for use in improving pre-bronchodilator forced expiratory volume 1 (FEV₁) in a subject with bronchiectasis. In some aspects provided herein, the present disclosure relates to the bispecific antibody described herein or the composition comprising the bispecific antibody described herein for use in improving pre-bronchodilator forced expiratory volume 1 (FEV₁) in a subject with bronchiectasis.

[0043] In some aspects provided herein, the present disclosure relates to a method of reducing *Pseudomonas aeruginosa* load in a subject with bronchiectasis, the method comprising administering the bispecific antibody described herein or the composition comprising the bispecific antibody described herein to the subject. In some aspects provided herein, the present disclosure relates to use of the bispecific antibody described herein or the composition comprising the bispecific antibody described herein in the

preparation of a medicament for use in reducing *Pseudomonas aeruginosa* load in a subject with bronchiectasis. In some aspects provided herein, the present disclosure relates to the bispecific antibody described herein or the composition comprising the bispecific antibody described herein for use in reducing *Pseudomonas aeruginosa* load in a subject with bronchiectasis.

[0044] In some aspects provided herein, the present disclosure relates to a method of reducing bronchiectasis exacerbations in a subject in need thereof, the method comprising administering the bispecific antibody described herein or the composition comprising the bispecific antibody described herein to the subject. In some aspects provided herein, the present disclosure relates to use of the bispecific antibody described herein or the composition comprising the bispecific antibody described herein in the preparation of a medicament for use in reducing bronchiectasis exacerbations in a subject in need thereof. In some aspects provided herein, the present disclosure relates to the bispecific antibody described herein or the composition comprising the bispecific antibody described herein for use in reducing bronchiectasis exacerbations in a subject in need thereof.

[0045] In some aspects provided herein, the present disclosure relates to a method of reducing the need for intravenous antibiotics in a subject with bronchiectasis, the method comprising administering the bispecific antibody described herein or the composition comprising the bispecific antibody described herein to the subject. In some aspects provided herein, the present disclosure relates to use of the bispecific antibody described herein or the composition comprising the bispecific antibody described herein in the preparation of a medicament for use in reducing the need for intravenous antibiotics in a subject with bronchiectasis. In some aspects provided herein, the present disclosure relates to the bispecific antibody described herein or the composition comprising the bispecific antibody described herein for use in reducing the need for intravenous antibiotics in a subject with bronchiectasis.

[0046] In some aspects provided herein, the present disclosure relates to a method of stabilizing lung function in a subject with bronchiectasis, the method comprising administering the bispecific antibody described herein or the composition comprising the bispecific antibody described herein to the subject. In some aspects provided herein, the present disclosure relates to use of the bispecific antibody described herein or the composition comprising the bispecific antibody described herein in the preparation of a

medicament for use in stabilizing lung function in a subject with bronchiectasis. In some aspects provided herein, the present disclosure relates to the bispecific antibody described herein or the composition comprising the bispecific antibody described herein for use in stabilizing lung function in a subject with bronchiectasis.

- [0047] In some aspects, the bronchiectasis is non-cystic fibrosis bronchiectasis.
- [0048] In some aspects, the method or use or antibody for use or composition for use further comprises administering an antibiotic.
- [0049] In some aspects, the subject is colonized with *Pseudomonas aeruginosa*, optionally wherein the respiratory tract of the subject is colonized with *Pseudomonas aeruginosa*.

BRIEF DESCRIPTION OF THE DRAWINGS/FIGURES

- [0050] **FIG. 1A** shows the opsonophagocytic (OPK) killing activity of AZD0292 and gremubamab (MEDI3902) in an opsonophagocytosis assay. The data demonstrate that AZD0292 exhibits equivalent opsonophagocytic killing activity as gremubamab. (See Example 1.)
- [0051] **FIG. 1B** shows the anti-cytotoxic killing activity of AZD0292 and gremubamab (MEDI3902) in an anti-cytotoxicity assay. The data demonstrate that AZD0292 exhibits equivalent anti-cytotoxic activity as gremubamab. (See Example 1.)
- [0052] **FIG. 2** shows that the opsonophagocytic activity of AZD0292, gremubamab (MEDI3902), afucosylated gremubamab (gremubamab-AFuc), gremubamab-AFuc additionally comprising the YTE half-life extension mutations in its Fc region (gremubamab-AFuc-YTE), and negative control IgG mAb. The data demonstrates that AZD0292, comprising N3Y half-life extension mutations, is more active in an opsonophagocytic assay in comparison to a gremubamab YTE half-life extension derivative.
- [0053] **FIG. 3** shows increased serum exposure of AZD0292 in mice, in comparison to gremubamab following 10 mg/kg intravenous administration (IV). The study was performed in Tg32 human FcRn transgenic mice (n = 4 per time point). (See Example 2.)
- [0054] **FIG. 4A** shows the opsonophagocytic killing activity of AZD0292 and gremubamab when exposed to temperature variations (4°C and 45°C) and light exposure,

compared to control groups. There was no difference observed between AZD0292 and gremubamab under these stressed conditions. (See Example 3.)

[0055] **FIG. 4B** shows the anti-cytotoxicity activity between AZD0292 and gremubamab when exposed to temperature variations (4°C and 45°C) and light exposure, compared to control groups. There was no difference observed between AZD0292 and gremubamab under these stressed conditions. (See Example 3.)

[0056] **FIG. 5A** shows the percent monomer in AZD0292 (hashed bars) and MEDI3902 (empty bars) compositions. (See Example 4.)

[0057] **FIG. 5B** shows the percent aggregates in AZD0292 (hashed bars) and MEDI3902 (empty bars) compositions. (See Example 4.)

[0058] **FIG. 6** shows the percent of aggregation for AZD0292 and MEDI3902 for each clone tested. (See Example 4.) Y-axis labelling “A-H” and X-axis labelling “1-12” indicate grid positions in a 96-well plate, with each position representing a different expressing clone.

[0059] **FIG. 7** shows a scatter plot matrix of assessing the correlation between the percent high molecular weight species, the mean concentration of the PhyTip Protein A purified sample, and the titer on the final day from the fed batch 96-well plate bioreactor. (See Example 4.)

[0060] **FIGs. 8A-8B** show the normality test results for AZD0292 (**FIG. 8A**) and MEDI3902 (**FIG. 8B**) aggregates using Anderson-Darling and Shapiro-Wilk tests. (See Example 4.)

[0061] **FIG. 8C** shows the t-test results comparing the average percent aggregation between clones expressing MEDI3902 and AZD0292. (See Example 4.)

[0062] **FIGs. 9A-9B** show the percent aggregation (**FIG. 9A**) and end-of-culture titre (**FIG. 9B**) and associated with each clone expressing AZD0292 (hashed bars) and MEDI3902 (empty bars). The clones are plotted based on percent aggregation values in ascending order from left to right. (See Example 4.)

[0063] **FIG. 10** shows the percent aggregate over months of storage at 40°C. Data points for MEDI3902 are shown as squares (■) and AZD0292 are shown as triangles (▲). (See Example 4.)

DETAILED DESCRIPTION

I. Definitions

[0064] The headings provided herein are not limitations of the various aspects or aspects of the disclosure, which can be had by reference to the specification as a whole. Accordingly, the terms defined immediately below are more fully defined by reference to the specification in its entirety.

[0065] It is to be noted that the term "a" or "an" entity refers to one or more of that entity; for example, "an antibody," is understood to represent one or more antibodies. As such, the terms "a" (or "an"), "one or more," and "at least one" can be used interchangeably herein.

[0066] Furthermore, "and/or" where used herein is to be taken as specific disclosure of each of the two specified features or components with or without the other. Thus, the term and/or" as used in a phrase such as "A and/or B" herein is intended to include "A and B," "A or B," "A" (alone), and "B" (alone). Likewise, the term "and/or" as used in a phrase such as "A, B, and/or C" is intended to encompass each of the following aspects: A, B, and C; A, B, or C; A or C; A or B; B or C; A and C; A and B; B and C; A (alone); B (alone); and C (alone).

[0067] As used herein, the terms "about" and "approximately," when used to modify a numeric value or numeric range, indicate that deviations of up to 10% above and down to 10% below the value or range remain within the intended meaning of the recited value or range, and includes the exact number these terms are modifying. It is understood that wherever aspects are described herein with the language "about" or "approximately" a numeric value or range, otherwise analogous aspects referring to the specific numeric value or range are also provided.

[0068] It is understood that wherever aspects are described herein with the language "comprising," otherwise analogous aspects described in terms of "consisting of" and/or "consisting essentially of" are also provided. In this disclosure, "comprises," "comprising," "containing" and "having" and the like can mean "includes," "including," and the like; "consisting essentially of" or "consists essentially of" are open-ended, allowing for the presence of more than that which is recited so long as basic or novel characteristics of that which is recited is not changed by the presence of more than that which is recited, but excludes prior art aspects.

- [0069] Units, prefixes, and symbols are denoted in their *Système International de Unites* (SI) accepted form. Numeric ranges are inclusive of the numbers defining the range.
- [0070] Unless otherwise indicated, amino acid sequences are written left to right in amino to carboxy orientation.
- [0071] Unless defined otherwise, technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure is related. For example, the *Concise Dictionary of Biomedicine and Molecular Biology*, Juo, Pei-Show, 2nd ed., 2002, CRC Press; *The Dictionary of Cell and Molecular Biology*, 3rd ed., 1999, Academic Press; and the *Oxford Dictionary Of Biochemistry And Molecular Biology*, Revised, 2000, Oxford University Press, provide one of skill with a general dictionary of many of the terms used in this disclosure.
- [0072] As used herein, the terms "antibody" and "immunoglobulin" are used interchangeably and refer to an antibody molecule that recognizes and specifically binds to a target, such as a protein, polypeptide, peptide, carbohydrate, polynucleotide, lipid, or combinations of the foregoing (*e.g.*, a glycoprotein), through at least one antigen recognition site within the variable region of the immunoglobulin molecule. The term "antibody" encompasses monoclonal antibodies, chimeric antibodies, humanized antibodies, human antibodies, bispecific antibodies, and any other immunoglobulin molecule so long as the antibodies exhibit the desired biological activity. An antibody can be of any the five major classes of immunoglobulins: IgA, IgD, IgE, IgG, and IgM, or subclasses (isotypes) thereof (*e.g.*, IgG1, IgG2, IgG3, IgG4, IgA1, and IgA2), based on the identity of their heavy-chain constant domains referred to as alpha, delta, epsilon, gamma, and mu, respectively. The different classes of antibodies have different and well known subunit structures and three-dimensional configurations. For the structure and properties of the different classes of antibodies, *see, e.g., Basic and Clinical Immunology*, 8th Ed., Daniel P. Stites, Abba I. Terr and Tristram G. Parslow (eds.), Appleton & Lange, Norwalk, CT, 1994, page 71 and Chapter 6.
- [0073] The term "antibody fragment" refers to a portion of an antibody. An "antigen-binding fragment" of an antibody refers to a portion of an antibody that binds to an antigen. An antigen-binding fragment of an antibody can comprise the antigenic determining regions of an antibody (*e.g.*, the complementarity determining regions (CDRs)). Examples of antigen-binding fragments of antibodies include, but are not limited to Fab, Fab', F(ab')₂,

and Fv fragments, linear antibodies, and single chain antibodies. An antigen-binding fragment of an antibody can be monovalent or multi-valent (*e.g.*, bivalent). An antigen-binding fragment of an antibody can be monospecific or multi-specific (*e.g.*, bispecific). An antigen-binding fragment of an antibody can be derived from any animal species, such as rodents (*e.g.*, mouse, rat, or hamster) and humans or can be artificially produced.

[0074] An “antigen-binding domain” or “antigen-binding region” refers to a monovalent portion of an antibody that binds to an antigen. An “antigen-binding domain” can comprise the antigenic determining regions of an antibody (*e.g.*, the complementarity determining regions (CDRs)). An antibody or antigen-binding fragment thereof (including monospecific and multi-specific (*e.g.*, bispecific) antibodies or antigen-binding fragments thereof can comprise an antigen-binding domain.

[0075] The term “Fc region,” sometimes referred to as “Fc” or “Fc domain”, as used herein refers the portion of an IgG molecule that correlates to a crystallizable fragment obtained by papain digestion of an IgG molecule. The Fc region consists of the C-terminal half of the two heavy chains of an IgG molecule that are linked by disulfide bonds. It has no antigen-binding activity but contains the carbohydrate moiety and the binding sites for complement and Fc receptors, including the FcRn receptor (see below). The Fc region contains the entire second constant domain CH2 (residues 231-340 of human IgG1, according to the Kabat numbering system) and the third constant domain CH3 (residues 341-447). Amino acid residues of the IgG constant and variable domains referred to herein are numbered according to the EU numbering index of Kabat *et al.* (*Sequences of Proteins of Immunological Interest*, 5th ed., 1991 NIH Pub. No. 91-3242, which is incorporated by reference herein in its entirety), and include corresponding residues in other IgG constant domains as determined by sequence alignment.

[0076] The terms “hinge-Fc region,” “Fc-hinge region,” “hinge-Fc domain,” or “Fc-hinge domain,” as used herein are used interchangeably and refer to a region of an IgG molecule consisting of the Fc region (residues 231-447) and a hinge region (residues 216-230) extending from the N-terminus of the Fc region.

[0077] Antibody fragments including single-chain antibodies can comprise the variable region(s) alone or in combination with the entirety or a portion of the following: hinge region, CH1, CH2, and CH3 domains.

- [0078] Also included are antigen-binding fragments also comprising any combination of variable region(s) with a hinge region, CH1, CH2, and CH3 domains.
- [0079] Antibodies, or antigen-binding fragments thereof disclosed herein can be from any animal origin including birds and mammals. The antibodies can be human, murine, donkey, rabbit, goat, guinea pig, camel, llama, horse, or chicken antibodies.
- [0080] Light chains are classified as either kappa or lambda (κ , λ). Each heavy chain class can be bound with either a kappa or lambda light chain. In general, the light and heavy chains are covalently bonded to each other, and the "tail" portions of the two heavy chains are bonded to each other by covalent disulfide linkages or non-covalent linkages when the immunoglobulins are generated either by hybridomas, B cells or genetically engineered host cells. In the heavy chain, the amino acid sequences run from an N-terminus at the forked ends of the Y configuration to the C-terminus at the bottom of each chain.
- [0081] Both the light and heavy chains are divided into regions of structural and functional homology. The terms "constant" and "variable" are used functionally. The term "constant domain" refers to the portion of an immunoglobulin molecule having a more conserved amino acid sequence relative to the other portion of the immunoglobulin, the variable domain, which contains the antigen binding site. The heavy chain constant domain contains the CH1, CH2 and CH3 domains and the light chain constant domain contains the CL domain. The variable domains of both the light (VL) and heavy (VH) chain portions determine antigen recognition and specificity. Conversely, the constant domains of the light chain (CL) and the heavy chain (CH1, CH2 or CH3) confer important biological properties such as secretion, transplacental mobility, Fc receptor binding, complement binding, and the like. By convention the numbering of the constant region domains increases as they become more distal from the antigen binding site or amino-terminus of the antibody. The N-terminal portion is a variable region and at the C-terminal portion is a constant region; the CH3 and CL domains comprise the carboxy-terminus of the heavy and light chain, respectively.
- [0082] As indicated above, the variable region allows the binding molecule to selectively recognize and specifically bind epitopes on antigens. That is, the VL domain and VH domain, or subset of the complementarity determining regions (CDRs), of a binding molecule, *e.g.*, an antibody combine to form the variable region that defines a three dimensional antigen binding site. This quaternary binding molecule structure forms the

antigen binding site present at the end of each arm of the Y. More specifically, the antigen binding site is defined by three CDRs on each of the VH and VL chains.

[0083] In naturally occurring antibodies, the six “complementarity determining regions” or “CDRs” present in each antigen binding domain are short, non-contiguous sequences of amino acids that are specifically positioned to form the antigen binding domain as the antibody assumes its three dimensional configuration in an aqueous environment. The remainder of the amino acids in the antigen binding domains, referred to as “framework” regions, show less inter-molecular variability. The framework regions largely adopt a β -sheet conformation and the CDRs form loops that connect, and in some cases form part of, the β -sheet structure. Thus, framework regions act to form a scaffold that provides for positioning the CDRs in correct orientation by inter-chain, non-covalent interactions. The antigen binding domain formed by the positioned CDRs defines a surface complementary to the epitope on the immunoreactive antigen. This complementary surface promotes the non-covalent binding of the antibody to its cognate epitope. The amino acids comprising the CDRs and the framework regions, respectively, can be readily identified for any given heavy or light chain variable region by one of ordinary skill in the art, since they have been precisely defined (*see*, “Sequences of Proteins of Immunological Interest,” Kabat, E., *et al.*, U.S. Department of Health and Human Services, (1983); and Chothia and Lesk, *J. Mol. Biol.*, 196:901-917 (1987), which are incorporated herein by reference in their entireties).

[0084] The terms “Kabat numbering”, “EU numbering index of Kabat” and like terms are recognized in the art and refer to a system of numbering amino acid residues in the heavy and light chain variable regions of an antibody or an antigen-binding fragment thereof. In certain aspects, CDRs can be determined according to the Kabat numbering system (*see*, *e.g.*, Kabat EA & Wu TT (1971) *Ann NY Acad Sci* 190: 382-391 and Kabat EA *et al.*, (1991) *Sequences of Proteins of Immunological Interest*, Fifth Edition, U.S. Department of Health and Human Services, NIH Publication No. 91-3242). Using the Kabat numbering system, CDRs within an antibody heavy chain molecule are typically present at amino acid positions 31 to 35, which optionally can include one or two additional amino acids, following 35 (referred to in the Kabat numbering scheme as 35A and 35B) (CDR1), amino acid positions 50 to 65 (CDR2), and amino acid positions 95 to 102 (CDR3). Using the Kabat numbering system, CDRs within an antibody light chain molecule are typically present at amino acid positions 24 to 34 (CDR1), amino acid positions 50 to 56 (CDR2),

and amino acid positions 89 to 97 (CDR3). In some aspects, the CDRs of the antibodies described herein have been determined according to the Kabat numbering scheme.

[0085] Chothia refers instead to the location of the structural loops (Chothia and Lesk, J. Mol. Biol. 196:901-917 (1987)). The end of the Chothia CDR-H1 loop when numbered using the Kabat numbering convention varies between H32 and H34 depending on the length of the loop (this is because the Kabat numbering scheme places the insertions at H35A and H35B; if neither 35A nor 35B is present, the loop ends at 32; if only 35A is present, the loop ends at 33; if both 35A and 35B are present, the loop ends at 34). The AbM hypervariable regions represent a compromise between the Kabat CDRs and Chothia structural loops, and are used by Oxford Molecular's AbM antibody modeling software.

Loop	Kabat	AbM	Chothia
L1	L24-L34	L24-L34	L24-L34
L2	L50-L56	L50-L56	L50-L56
L3	L89-L97	L89-L97	L89-L97
H1	H31-H35B	H26-H35B (Kabat Numbering)	H26-H32..34
H1	H31-H35	H26-H35 (Chothia Numbering)	H26-H32
H2	H50-H65	H50-H58	H52-H56
H3	H95-H102	H95-H102	H95-H102

[0086] Single chain Fvs (scFv) molecules are known in the art and are described, *e.g.*, in US patent 5,892,019. Immunoglobulin or antibody molecules encompassed by this disclosure can be of any type (*e.g.*, IgG, IgE, IgM, IgD, IgA, and IgY), class (*e.g.*, IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2) or subclass of immunoglobulin molecule.

[0087] A "monoclonal" antibody or antigen-binding fragment thereof refers to a homogeneous antibody or antigen-binding fragment population involved in the highly specific recognition and binding of a single antigenic determinant, or epitope. This is in contrast to polyclonal antibodies that typically include different antibodies directed against different antigenic determinants. The term "monoclonal" antibody or antigen-binding fragment thereof encompasses both intact and full-length monoclonal antibodies as well as antibody fragments (such as Fab, Fab', F(ab')₂, Fv), single chain (scFv) mutants, fusion proteins comprising an antibody portion, and any other modified immunoglobulin molecule

comprising an antigen recognition site. Furthermore, a "monoclonal" antibody or antigen-binding fragment thereof refers to such antibodies and antigen-binding fragments thereof made in any number of manners including but not limited to by hybridoma, phage selection, recombinant expression, and transgenic animals.

[0088] As used herein, "human" antibodies include antibodies having the amino acid sequence of a human immunoglobulin and include antibodies isolated from human immunoglobulin libraries or from animals transgenic for one or more human immunoglobulins and that do not express endogenous immunoglobulins, as described *infra* and, for example in, U.S. Pat. No. 5,939,598 by Kucherlapati *et al.*

[0089] By "specifically binds," it is generally meant that a binding molecule, *e.g.*, a bispecific antibody or fragment, variant, or derivative thereof binds to an epitope via an antigen binding domain, and that the binding entails some complementarity between an antigen binding domain and the epitope. A binding molecule as provided herein can contain one, two, three, four, or more binding domains that can be the same or different, and that can bind to the same epitope, or to two or more different epitopes. According to this definition, a binding molecule is said to "specifically bind" to an epitope when it binds to that epitope, via its antigen binding domain more readily than it would bind to a random, unrelated epitope. The term "specificity" is used herein to qualify the relative affinity by which a certain binding molecule binds to a certain epitope. For example, binding molecule "A" may be deemed to have a higher specificity for a given epitope than binding molecule "B," or binding molecule "A" may be said to bind to epitope "C" with a higher specificity than it has for related epitope "D."

[0090] An antibody that "binds to the same epitope" as a reference antibody refers to an antibody that contacts the same amino acid and/or sugar residues as the reference antibody. The ability of an antibody to bind to the same epitope as a reference antibody can be determined using peptide scanning mutagenesis or high throughput alanine scanning mutagenesis.

[0091] An antibody is said to "competitively inhibit" binding of a reference antibody to a given epitope if it preferentially binds to that epitope or an overlapping epitope such that it blocks, to some degree, binding of the reference antibody to the epitope. Competitive inhibition may be determined by any method known in the art, for example, competition ELISA assays. An antibody may be said to competitively inhibit binding of the reference

antibody to a given epitope by at least 90%, at least 80%, at least 70%, at least 60%, or at least 50%.

[0092] The term "bispecific antibody" as used herein refer to an antibody that has binding domains specific for two different antigens or epitopes within a single antibody molecule. It will be appreciated that other molecules in addition to the canonical antibody structure can be constructed with two binding specificities. It will further be appreciated that antigen binding by bispecific antibodies can be simultaneous or sequential. Triomas and hybrid hybridomas are two examples of cell lines that can secrete bispecific antibodies. Bispecific antibodies can also be constructed by recombinant means. (Ströhlein and Heiss, *Future Oncol.* 6:1387-94 (2010); Mabry and Snavely, *IDrugs.* 13:543-9 (2010)).

[0093] As used herein, the term "MEDI3902" or "gremubamab" refers to a bispecific antibody having a heavy chain with the amino acid sequence of SEQ ID NO:19 and a light chain with the amino acid sequence of SEQ ID NO:20. MEDI3902 is also known as Gremubamab.

[0094] As used herein, the term "AZD0292" refers to a bispecific antibody having a heavy chain with the amino acid sequence of SEQ ID NO:31 and a light chain with the amino acid sequence of SEQ ID NO:20.

[0095] The term "FcRn receptor" or "FcRn" as used herein refers to an Fc receptor ("n" indicates neonatal) which is known to be involved in transfer of maternal IgGs to a fetus through the human or primate placenta, or yolk sac (rabbits) and to a neonate from the colostrum through the small intestine. It is also known that FcRn is involved in the maintenance of constant serum IgG levels by binding the IgG molecules and recycling them into the serum. The binding of FcRn to naturally occurring IgG1, IgG2, and IgG4 molecules is strictly pH-dependent with optimum binding at pH 6. IgG3 has a known variation at position 435 (i.e., human IgG has R435 instead of H435 found in human IgG1, IgG2 and IgG4), which may result in reduced binding at pH 6. FcRn comprises a heterodimer of two polypeptides, whose molecular weights are approximately 50 kD and 15 kD, respectively. The extracellular domains of the 50 kD polypeptide are related to major histocompatibility complex (MHC) class I α -chains and the 15 kD polypeptide was shown to be the non-polymorphic β_2 -microglobulin (β_2 -m). In addition to placenta and neonatal intestine, FcRn is also expressed in various tissues across species as well as various types of endothelial cell lines. It is also expressed in human adult vascular endothelium, muscle vasculature and

hepatic sinusoids and it is suggested that the endothelial cells may be most responsible for the maintenance of serum IgG levels in humans and mice. FcRn receptors include, *e.g.*, human and murine FcRn proteins as well as homologs thereof having FcRn activity.

[0096] An "FcRn-binding fragment" of an IgG constant domain, as that term is used herein, refers to a fragment of an IgG constant domain that binds to the FcRn receptor. An FcRn-binding fragment of an IgG constant domain can include the Fc region, or the hinge-Fc region; thus it can include portions of the heavy chain CH2-CH3 region or the hinge-CH2-CH3 region that are involved in binding to FcRn (see Roopenian *et al.*, Nature Rev. Immunol. 7:715-725 (2007)).

[0097] "KD" as that term is used herein (sometimes also referred to as K_d , K_D or K_d) is the equilibrium dissociation constant a binding interaction between two molecules, such as an IgG and FcRn. KD can be calculated from observed rate constants for association (k_{on}) and dissociation (k_{off}), such that KD is equal to the ratio of the k_{off}/k_{on} .

[0098] The term "*in vivo* half-life" as used herein refers to a biological half-life of a particular type of IgG molecule or its fragments containing FcRn-binding sites in the circulation of a given animal and is represented by a time required for half the quantity administered in the animal to be cleared from the circulation and/or other tissues in the animal. When a clearance curve of a given IgG is constructed as a function of time, the curve is usually biphasic with a rapid α -phase which represents an equilibration of the injected IgG molecules between the intra- and extra-vascular space and which is, in part, determined by the size of molecules, and a longer β -phase which represents the catabolism of the IgG molecules in the intravascular space. The term "*in vivo* half-life" practically corresponds to the half-life of the IgG molecules in the β -phase.

[0099] As used herein, the term "engineered antibody" refers to an antibody in which the variable domain in either the heavy and light chain or both is altered by at least partial replacement of one or more CDRs from an antibody of known specificity and, if necessary, by partial framework region replacement and sequence changing. Although the CDRs can be derived from an antibody of the same class or even subclass as the antibody from which the framework regions are derived, it is envisaged that the CDRs will be derived from an antibody of different class and preferably from an antibody from a different species. An engineered antibody in which one or more "donor" CDRs from a non-human antibody of known specificity is grafted into a human heavy or light chain framework region is referred

to herein as a "humanized antibody." It may not be necessary to replace all of the CDRs with the complete CDRs from the donor variable region to transfer the antigen binding capacity of one variable domain to another. Rather, it may only be necessary to transfer those residues that are necessary to maintain the activity of the target binding site. Given the explanations set forth in, *e.g.*, U. S. Pat. Nos. 5,585,089, 5,693,761, 5,693,762, and 6,180,370, it will be well within the competence of those skilled in the art, either by carrying out routine experimentation or by trial and error testing to obtain a functional engineered or humanized antibody.

[0100] A polypeptide, antibody, polynucleotide, vector, cell, or composition which is "isolated" is a polypeptide, antibody, polynucleotide, vector, cell, or composition which is in a form not found in nature. Isolated polypeptides, antibodies, polynucleotides, vectors, cells or compositions include those which have been purified to a degree that they are no longer in a form in which they are found in nature. In some aspects, an antibody, polynucleotide, vector, cell, or composition which is isolated is substantially pure. As used herein, "substantially pure" refers to material which is at least 50% pure (*i.e.*, free from contaminants), at least 90% pure, at least 95% pure, at least 98% pure, or at least 99% pure.

[0101] The terms "polypeptide," "peptide," and "protein" are used interchangeably herein to refer to polymers of amino acids of any length. The polymer can be linear or branched, it can comprise modified amino acids, and it can be interrupted by non-amino acids. The terms also encompass an amino acid polymer that has been modified naturally or by intervention; for example, disulfide bond formation, glycosylation, lipidation, acetylation, phosphorylation, or any other manipulation or modification, such as conjugation with a labeling component. Also included within the definition are, for example, polypeptides containing one or more analogs of an amino acid (including, for example, unnatural amino acids, *etc.*), as well as other modifications known in the art. It is understood that, because the polypeptides of this disclosure are based upon antibodies, in some aspects, the polypeptides can occur as single chains or associated chains.

[0102] Administration "in combination with" one or more further therapeutic agents (*e.g.*, an antibiotic) includes simultaneous (concurrent) or consecutive administration in any order.

[0103] Terms such as "treating" or "treatment" or "to treat" or "alleviating" or "to alleviate" refer to therapeutic measures that cure, slow down, lessen symptoms of, and/or halt

progression of a diagnosed pathologic condition or disorder. Thus, those in need of treatment include those already diagnosed with or suspected of having the disorder.

- [0104] The term “airway neutrophilia” refers to an accumulation of neutrophils in the airspace of the lungs.
- [0105] The term “sputum neutrophilia” refers to the presence of neutrophils in the sputum of a subject. In some aspects, the neutrophils in the sputum of a subject in need of treatment, *e.g.*, a subject with bronchiectasis, are increased relative to neutrophils in the sputum of healthy controls.
- [0106] By "subject" or "individual" or "animal" or "patient" or “mammal,” is meant any subject, *e.g.*, a mammalian subject, for whom diagnosis, prognosis, or therapy is desired. Mammalian subjects include humans, domestic animals, farm animals, and zoo, sports, or pet animals such as dogs, cats, guinea pigs, rabbits, rats, mice, horses, cattle, cows, bears, and so on.

II. Bispecific Anti-*Pseudomonas* Antibodies with Modified Fc Regions

- [0107] Provided herein are bispecific antibodies that specifically bind to *Pseudomonas aeruginosa* Psl and PcrV and that comprise modified Fc regions. As provided herein, it was surprisingly discovered that modifications to the Fc regions of such bispecific antibodies can result in increased half-life, but also in decreased manufacturing-associated aggregation without decreasing potency against *Pseudomonas aeruginosa*.
- [0108] Exemplary sequences that can be present in a bispecific antibody that specifically binds to *Pseudomonas aeruginosa* Psl and PcrV are presented in Table 1, defined according to Kabat nomenclature/numbering.

Table 1: Bispecific Antibody Sequences

SEQ ID NO:	Description	Sequence
1	Anti-PcrV HCDR1	SYAMN
2	Anti-PcrV HCDR2	AITMSGITAYYTDDVKG
3	Anti-PcrV HCDR3	EEFLPGTHYYYGMDV

SEQ ID NO:	Description	Sequence
4	Anti-PcrV LCDR1	RASQGIRNDLG
5	Anti-PcrV LCDR2	SASTLQS
6	Anti-PcrV LCDR3	LQDYNYPWT
7	Anti-Psl HCDR1	PYYWT
8	Anti-Psl HCDR2	YIHSSGYTDYNPSLKS
9	Anti-Psl HCDR3	ADWDRLRALDI
10	Anti-Psl LCDR1	RASQSIRSHLN
11	Anti-Psl LCDR2	GASNLQS
12	Anti-Psl LCDR3	QQSTGAWN
13	Anti-PcrV VH	EVQLLES G GGLVQPGGSLRLSCAASGFTFSSYAMNWVRQAPG KGLEWVSAITMSGITAYYTD DVKGRFTISRDN SKNTLYLQMNS LRAEDTAVYYCAKEEFLPGTHYYYGMDVWGQGTTVTVSS
14	Anti-PcrV VL	AIQMTQSPSSLSASVGDRVITITCRASQGIRNDLGWYQQKPGKA PKLLIYSASTLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCL QDYNYPWTFGQGTKVEIK
15	Anti-Psl VH	QVQLQESGPGLVKPSSETLSLTCTVSGGSISPYWWTWIRQPPGKC LELIGYIHSSGYTDYNPSLKS RVTISGDTSKKQFSLKLSSVTAAD TAVYYCARADWDRLRALDIWGQGTMTVTVSS
16	Anti-Psl VL	DIQLTQSPSSLSASVGDRVITITCRASQSIRSHLNWYQQKPGKAP KLLIYGASNLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQ QSTGAWNWF GCGTKVEIK

SEQ ID NO:	Description	Sequence
17	Anti-Psl scFv	QVQLQESGPGLVKPSSETLSLTCTVSGGSISPYWWTWIRQPPGKC LELIGYIHSSGYTDYNPSLKSRVTISGDTSKKQFSLKLSSVTAAD TAVYYCARADWDRRLRALDIWGQGMVTVSSGGGSGGGGSG GGGSGGGGSDIQLTQSPSSLSASVGDRVITICRASQSIRSHLNW YQQKPGKAPKLLIYGASNLQSGVPSRFSGSGSGTDFTLTISLQPE DFATYYCQQSTGAWNWFSGCGTKVEIK
18	Anti-Psl scFv linker	GGGSGGGGSGGGGSGGGGS
19	MEDI3902 Heavy	EVQLLESQGLVQPGSLRLSCAASGFTSSYAMNWRQAPG KGLEWVSAITMSGITAYTDDVKGRFTISRDNKNTLYLQMNS LRAEDTAVYYCAKEEFLPGTHYYYGMDVWGQGTITVTVSSAS TKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGA LTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNHKP SNTKVDKRVPEPKSCGGGSGGGGSGVQLQESGPGLVKPSSETLS LTCTVSGGSISPYWWTWIRQPPGKCLELIGYIHSSGYTDYNPSL KSRVTISGDTSKKQFSLKLSSVTAADTAVYYCARADWDRRLRA LDIWGQGMVTVSSGGGSGGGGSGGGGSGGGGSDIQLTQSP SSLSASVGDRVITICRASQSIRSHLNWYQQKPGKAPKLLIYGAS NLQSGVPSRFSGSGSGTDFTLTISLQPEDFATYYCQQSTGAWN WFGCGTKVEIKGGGSGGGGSGDKTHTCPPCPAPELLGGPSVFL FPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEV HNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSN KALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLV KGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTV DKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
20	MEDI3902 and AZD0292 Light	AIQMTQSPSSLSASVGDRVITICRASQGIRNDLGWYQQKPGKA PKLLIYSASTLQSGVPSRFSGSGSGTDFTLTISLQPEDFATYYCL QDYNYPWTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASV VCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKSTYS LSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

SEQ ID NO:	Description	Sequence
21	CH1	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNS GALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNH KPSNTKVDKRV
22	H1	EPKSC
23	H2	DKTHTCPPCP
24	CL	RTVAAPSVFIFPPSDEQLKSGTASVVCLLNFFYPREAKVQWKV DNALQSGNSQESVTEQDSKDYSLSTLTLSKADYEEKHKVYA CEVTHQGLSSPVTKSFNRGEC
25	MEDI3902 CH2-CH3	APELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVK FNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLN GKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMT KNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSGD SFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSP GK
26	Linker	[GGGGS]n, where n is 0, 1, 2, 3, 4, or 5
27	Linker	[GGGG]n, where n is 0, 1, 2, 3, 4, or 5
28	L1	[GGGGS]n, where n is 2
29	L2	[GGGGS]n, where n is 4
30	CH2-CH3 (N3Y)	APELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVK FNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLN GKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMT KNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSGD SFFLYSKLTVDKSRWQQGNVFSCSVMHEACSYHLCQKSLSLSP GK
31	AZD0292 Heavy chain	EVQLLESQGLVQPGGSLRLSCAASGFTSSYAMNWVRQAPG KGLEWVSAITMSGITAYTDDVKGRFTISRDNKNTLYLQMNS LRAEDTAVYYCAKEEFLPGTHYYYGMDVWGQGTITVTVSSAS TKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGA LTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPS NTKVDKRVKPSCKGGGSGGGGSQVQLQESGPGLVKPSETLS

SEQ ID NO:	Description	Sequence
		LTCTVSGGSISPYWWTWIRQPPGKCLELIGYIHSSGYTDYNPSL KSRVTISGDTSKKQFSLKLSSVTAADTAVYYCARADWDRLRA LDIWGQGTMTVTSSGGGSGGGGSGGGGSGGGGSDIQLTQSP SSLSASVGDRVITICRASQSIRSHLNWYQQKPGKAPKLLIYGAS NLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSTGAWN WFGCGTKVEIKGGGSGGGGSDKTHTCPPCPAPELLGGPSVFL FPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEV HNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSN KALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLV KGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTV DKSRWQQGNVFSCSVMHEACSYHLCQKSLSLSPGK
32	Wild type Fc sub- sequence	MHEALHNHYTQKSLSLS
33	N3Y- containing Fc sub- sequence	MHEAC <u>SYHLC</u> QKSLSLS
34	WT His435 loop of IgG1, IgG2, IgG4	LHNHYT
35	WT His435 loop of IgG3	LHNRFT
36	Modified His435 loop region variant	CXRHXC

SEQ ID NO:	Description	Sequence
37	Modified His435 loop region variant	CRRHXC
38	Modified His435 loop region variant	CXRHRC
39	Modified His435 loop region	CSWHLC
40	Modified His435 loop region	CSWHLE
41	Modified His435 loop region variant	CXXXXC, wherein each X indicates any amino acid
42	Modified His435 loop region variant	CXXXXCE, wherein each X indicates any amino acid
43	Gremubam-ab YTE variant	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMNWVRQAPG KGLEWVSAITMSGITAYYTDVKGRTISRDN SKNTLYLQMNS LRAEDTAVYYCAKEEFLPGTHYYYGMDVWGQGTTVTVSSAS TKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGA LTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKP SNTKVDKRVEPKSCGGGSGGGGSQVQLQESGPGLVKPSETLS LTCTVSGGSISPYWWTWIRQPPGKCLELIGYIHSSGYTDYNPSL KSRVTISGDTSKKQFSLKLSSVTAADTAVYYCARADWDRRLRA

SEQ ID NO:	Description	Sequence
		LDIWGQGTMTVTSSGGGSGGGGSGGGGSGGGGSDIQLTQSP SSLSASVGDRVTITCRASQSIRSHLNWYQQKPGKAPKLLIYGAS NLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSTGAWN WFGCGTKVEIKGGGSGGGGSDKTHTCPPCPAPPELLGGPSVFL FPPKPKDTLYITREPEVTCVVVDVSHEDPEVKFNWYVDGVEVH NAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK ALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVK GFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVD KSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
44	Modified His435 loop region ("N3Y")	CSYHLC
45	AZD0292 Heavy chain - cDNA	tcagatctca ccgtccttga cacgaagctt actgagcaca caggacctgc agggcccgcca ccatgggctg gtcctgcac atcctgttgc tgggtggccac agctacaggt gtacactccg aggtgcagct gttggagct gggggaggt tggtagacc tgggggggcc ctgagactct cctgtgcagc ctctggattc accttagca gctatgcat gaactgggtc cgccaggctc caggggaagg gctggagtgg gtctcagcta ttactatgag tgggtatacc gcatactaca ccgacgacgt gaaggggccg ttaccatct ccagagacaa ttccaagaac acgtatata tgcaaatgaa cagcctgagg gccgaggaca cgcccgata ttactgtgcg aaggaagaat ttttacctg aacgcactac tactacggtg tggacgtctg gggccaagg accacggta ccgtctctc agcgtcgacc aaggcccat ccgtctccc cctggcacc tctccaaga gcacctctg gggcacagcg gcctgggct gcctggtaa ggactactc ccgaaccgg tgacgggtgc ctggaacta ggcgctctga ccagcggcgt gcacacctc ccggctgcc tacgtctc aggactctac tccctcagca cgtgtgtgac cgtgccctcc agcagctgg gcacccagac ctacatctgc aacgtgaac acaagcccag caacaccaag gtggacaaga gagttgagcc caaatctgt ggcggagggg gctctggcgg agggggatcc caggtgcagc tgcaggatc tggcctggc ctctgaagc cctccgagac actgtctctg acctgcaccg tgcggcgg ctccatctc cttactact ggacctgag cagacagccc cctggcaagt gcctggaact gatcggtac atccactct ccggctacac cgactacaac ccagcctga agtccagagt gacctctc ggcgacacct ccaagaagca gttctccctg aagctgtct ccgtgaccgc cgctgatacc gccgtgtact actgcgccag agccgactgg gacagactga gagccctgga catctggggc cagggcacaa tggtcaccgt gtctagcggg ggcggaggat ctgtgtgtgg tggatctggc ggcggaggaa gtgtgtggcg aggtctgat atccagctga cccagtcctc ctccagcctg tctgtctctg tggcgaccg cgtgaccat acctgtagag cctccagtc catccgtcc cacctgaact ggtatcagca gaagccggc agggcccca agctgtgat ctacggcgcc

SEQ ID NO:	Description	Sequence
		tccaatctgc agtccggcgt gccctctaga ttctccggat ctggctccgg caccgacttt accctgacca tcagctccct gcagcccag gacttcgcca cctactactg ccagcagtct accggcgctt ggaattgggt cggctcgggc accaaggtgg aatacaaggg cggaggtggc tctggcggag ggggatccga caaaactcac acatgcccac cgtgccagc acctgaactc ctggggggac cgtcagtttt cctctcccc ccaaaacca aggacacct catgatctcc cggaccctg aggtcacatg cgtggtgggt gacgtgagcc acgaagacc tgaggtcaag ttcaactggt acgtggacgg cgtggaggtg cataatgcca agacaaagcc cggggaggag cagtacaaca gcacgtaccg tgggtcagc gtcttcaccg tctgcacca ggactggctg aatggcaagg agtacaagtg caaggtctcc aacaaagccc tccagcccc catcgagaaa accatctcca aagccaaagg gcagcccga gaaccacagg tctacacct gccccatcc cgggaggaga tgaccaagaa ccaggtcagc ctgacctgcc tggcaaaagg ctctatccc agcgacatcg ccgtggagtg ggagagcaat gggcagccgg agaacaacta caagaccacg cctcccgtgc tggactccga cggctccttc ttctctata gcaagctcac cgtggacaag agcaggtggc agcaggggaa cgtcttctca tgcctcgtga tgcattgggc ttgtcatat cacctctgcc agaagagctt aagcctgtct ccgggtaaat gagcggccgc gaattcacgt gccagccatc tgtgtttgc cctccccgc tgcctctt gacctggaa ggtgccactc ccactgtct ttctataaa aatgaggaag ttgcatcgca ttgtctgagt aggtgtcatt ctattctggg ggggtgggtg gggcaggaca gcaaggggga ggattgggaa gacaatagca ggcatgatca attt
46	AZD0292 Light chain - cDNA	tcagatctca ccgtcttga cacgaagcta ttgcaactac agttactgag cacacaggac ctgctaccgc catctacca tggacatgag ggtgcccccc cagctgctgg gcctgctgt gctgtggctg cccggggcca agtgcgtat tcagatgacc cagtcacctt ccagcctgtc cgcctctgt ggagacagag tgaccatcac ctgtcggggc tccaggggca tcagaaacga cctgggctgg tatcagcaga agcccgccaa gggcccaag ctgtgatct actctgctc caccctgag tccggcgtgc cctccagatt ttccggctct ggctctggca ccgactttac cctgaccatc agtccctgc agcccgagga ctccgccacc tactactgtc tgaagacta caactacccc tggaccttcg gccagggcac caaggtggaa atcaagcgga ccgtggccgc tctctcgtg ttcatcttc caccctccga cgagcagctg aagtcgggca ccgctcctgt ggtctgctg ctgaacaact tctaccccc cgaggccaag gtgcagtga aggtggacaa cgcctcgtg tccggcaact ccaggaatc cgtcaccgag caggactcca aggacagcac ctactcctg tccagcacc tgacctgtc caagccgac tacgagaagc acaagtgta cgcctgcga gtgaccacc agggcctgtc cagccctgtg accaagtct tcaaccgggg cgagtgtga tgaacgtgcc agccatctgt tgtttgcccc tccccgtc ctctctgac cctggaaggt gccactcca ctgtccttc ctaataaaat gaggaattg catcgattg tctgagtagg tgtatttcta ttctggggg tgggtgggg caggacagca agggggagga ttgggaagac aatagcaggc a

- a. Antigen-binding domains of bispecific anti-*Pseudomonas* antibodies with modified Fc regions

[0109] Provided herein are bispecific antibodies that specifically bind to *Pseudomonas aeruginosa* Psl exopolysaccharide (Psl) and the type 3 secretion protein, PcrV.

Accordingly, in some aspects, the bispecific antibodies comprise a Psl-binding domain and a PcrV-binding domain.

[0110] A bispecific antibody provided herein can comprise an antigen-binding domain that specifically binds to *P. aeruginosa* PcrV and competitively inhibits binding to PcrV of an antibody comprising a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:13 and a light chain variable region comprising the amino acid sequence of SEQ ID NO:14. In some aspects, a bispecific antibody provided herein comprises an antigen-binding domain that specifically binds to *P. aeruginosa* PcrV and binds to the same epitope of PcrV as an antibody comprising a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:13 and a light chain variable region comprising the amino acid sequence of SEQ ID NO:14.

[0111] In some aspects, a bispecific antibody provided herein comprises an antigen-binding domain that specifically binds to *P. aeruginosa* PcrV and comprises (i) a heavy chain CDR1, CDR2, and CDR3 comprising the amino acid sequences of the heavy chain CDR1, CDR2, and CDR3 sequences in SEQ ID NO:13 (*e.g.*, the Kabat-defined, AbM-defined, or Chothia-defined CDRs) and (ii) a light chain CDR1, CDR2, and CDR3 comprising the amino acid sequences of the heavy chain CDR1, CDR2, and CDR3 sequences in SEQ ID NO:14 (*e.g.*, the Kabat-defined, AbM-defined, or Chothia-defined CDRs).

[0112] In some aspects, a bispecific antibody provided herein comprises an antigen-binding domain that specifically binds to *P. aeruginosa* PcrV and comprises a heavy chain CDR1 comprising the amino acid sequence of SEQ ID NO:1, a heavy chain CDR2 comprising the amino acid sequence of SEQ ID NO:2, a heavy chain CDR3 comprising the amino acid sequence of SEQ ID NO:3, a light chain CDR1 comprising the amino acid sequence of SEQ ID NO:4, a light chain CDR2 comprising the amino acid sequence of SEQ ID NO:5, and a light chain CDR3 comprising the amino acid sequence of SEQ ID NO:6. In some aspects, a bispecific antibody provided herein comprises an antigen-binding domain that specifically binds to *P. aeruginosa* PcrV and comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:13. In some aspects, a bispecific antibody provided herein comprises an antigen-binding domain that specifically binds to *P. aeruginosa* PcrV and comprises a light chain variable region comprising the amino acid sequence of SEQ ID NO:14. In some aspects, a bispecific antibody provided herein

comprises an antigen-binding domain that specifically binds to *P. aeruginosa* PcrV and comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:13 and a light chain variable region comprising the amino acid sequence of SEQ ID NO:14.

[0113] In some aspects, a bispecific antibody provided herein comprises a PcrV-binding domain with a heavy chain variable region and a light chain variable region on separate polypeptides.

[0114] In some aspects, a bispecific antibody provided herein comprises a PcrV-binding domain with a heavy chain variable region and a light chain variable region on the same polypeptide. In some aspects a PcrV-binding domain with a heavy chain variable region and a light chain variable region on the same polypeptide comprises a linker. The linker can be, for example, between the heavy chain variable region and the light chain variable region. The linker can be, for example, a glycine-rich linker or a glycine-serine linker. In some aspects, the linker comprises the amino acid sequence of SEQ ID NO:18.

[0115] In some aspects, a bispecific antibody provided herein comprises a PcrV-binding domain that is an scFv. In some aspects, the PcrV-binding scFv is in the orientation VH-VL, e.g., VH-linker-VL. In some aspects, the PcrV-binding scFv is in the orientation VL-VH, e.g., VL-linker-VH.

[0116] In some aspects, a bispecific antibody provided herein comprises an antigen binding domain that specifically binds to *P. aeruginosa* Psl and competitively inhibits binding to Psl of an antibody comprising a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:15 and a light chain variable region comprising the amino acid sequence of SEQ ID NO:16. In some aspects, a bispecific antibody provided herein comprises an antigen-binding domain that specifically binds to *P. aeruginosa* Psl and binds to the same epitope of Psl as an antibody comprising a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:15 and a light chain variable region comprising the amino acid sequence of SEQ ID NO:16.

[0117] In some aspects, a bispecific antibody provided herein comprises an antigen-binding domain that specifically binds to *P. aeruginosa* Psl and comprises (i) a heavy chain CDR1, CDR2, and CDR3 comprising the amino acid sequences of the heavy chain CDR1, CDR2, and CDR3 sequences in SEQ ID NO:15 (e.g., the Kabat-defined, AbM-defined, or Chothia-defined CDRs) and (ii) a light chain CDR1, CDR2, and CDR3 comprising the

amino acid sequences of the heavy chain CDR1, CDR2, and CDR3 sequences in SEQ ID NO:16 (e.g., the Kabat-defined, AbM-defined, or Chothia-defined CDRs).

[0118] In some aspects, a bispecific antibody provided herein comprises an antigen-binding domain that specifically binds to *P. aeruginosa* Psl and comprises a heavy chain CDR1 comprising the amino acid sequence of SEQ ID NO:7, a heavy chain CDR2 comprising the amino acid sequence of SEQ ID NO:8, a heavy chain CDR3 comprising the amino acid sequence of SEQ ID NO:9, a light chain CDR1 comprising the amino acid sequence of SEQ ID NO:10, a light chain CDR2 comprising the amino acid sequence of SEQ ID NO:11, and a light chain CDR3 comprising the amino acid sequence of SEQ ID NO:12. In some aspects, a bispecific antibody provided herein comprises an antigen-binding domain that specifically binds to *P. aeruginosa* Psl and comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:15. In some aspects, a bispecific antibody provided herein comprises an antigen-binding domain that specifically binds to *P. aeruginosa* Psl and comprises a light chain variable region comprising the amino acid sequence of SEQ ID NO:16. In some aspects, a bispecific antibody provided herein comprises an antigen-binding domain that specifically binds to *P. aeruginosa* Psl and comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:15 and a light chain variable region comprising the amino acid sequence of SEQ ID NO:16.

[0119] In some aspects, a bispecific antibody provided herein comprises an antigen-binding domain that specifically binds to *P. aeruginosa* Psl and comprises a heavy chain variable region and a light chain variable region on the same polypeptide. In some aspects a PcrV-binding domain with a heavy chain variable region and a light chain variable region on the same polypeptide comprises a linker. The linker can be, for example, between the heavy chain variable region and the light chain variable region. The linker can be, for example, a glycine-rich linker or a glycine-serine linker. In some aspects, the linker comprises the amino acid sequence of SEQ ID NO:18.

[0120] In some aspects, the bispecific antibody comprises a Psl-binding domain that is an scFv. The scFv can comprise a linker. The linker can be, for example, a glycine-rich linker or a glycine-serine linker. In some aspects, the linker comprises the amino acid sequence of SEQ ID NO:18. In some aspects, the scFv is in the orientation VH-VL, e.g.,

VH-linker-VL. In some aspects, the scFv is in the orientation VL-VH, e.g., VL-linker-VH. In some aspects, the scFv comprises the amino acid sequence of SEQ ID NO:17.

[0121] In some aspects, a bispecific antibody provided herein comprises a Psl-binding domain with a heavy chain variable region and a light chain variable region on separate polypeptides.

b. Structure of bispecific anti-*Pseudomonas* antibodies with modified Fc regions

[0122] In some aspects, a bispecific antibody provided herein is an IgG antibody. The IgG antibody can be, for example, an IgG1 antibody. In some aspects, an IgG1 antibody is a human IgG1 antibody. In some aspects, an IgG1 antibody is a humanized IgG1 antibody.

[0123] The IgG antibody can be, for example, an IgG2 antibody. In some aspects, an IgG2 antibody is a human IgG2 antibody. In some aspects, an IgG2 antibody is a humanized IgG1 antibody.

[0124] The IgG antibody can be, for example, an IgG3 antibody. In some aspects, an IgG3 antibody is a human IgG3 antibody. In some aspects, an IgG3 antibody is a humanized IgG3 antibody.

[0125] The IgG antibody can be, for example, an IgG4 antibody. In some aspects, an IgG4 antibody is a human IgG4 antibody. In some aspects, an IgG4 antibody is a humanized IgG4 antibody.

[0126] In some aspects, a bispecific antibody as disclosed herein has the structure of BS1, BS2, BS3, or BS4, all as shown in FIG. 17 of WO 2013/070615, which is incorporated by reference herein in its entirety.

[0127] In some aspects, a bispecific antibody as disclosed herein has the BS4 structure, disclosed in detail in WO 2013/070615, which is incorporated herein by reference in its entirety. For example, this disclosure provides a bispecific antibody in which an anti-Psl scFv molecule is inserted into the hinge region of each heavy chain of an anti-PcrV antibody or fragment thereof.

[0128] In some aspects, a bispecific antibody provided herein comprises (i) a heavy chain of the formula VH-CH1-H1-L1-S-L2-H2-CH2-CH3, wherein VH is an anti-*P. aeruginosa* PcrV heavy chain variable domain; CH1 is a heavy chain constant region domain 1; H1 is a first heavy chain hinge region fragment; L1 is a first linker; S is an anti-*P. aeruginosa* Psl ScFv molecule; L2 is a second linker; H2 is a second heavy chain hinge region fragment;

CH2 is a heavy chain constant region domain-2; and CH3 is a heavy chain constant region domain-3; and (ii) a light chain of the formula VL-CL, wherein VL is an anti-*P. aeruginosa* PcrV light chain variable domain, and CL is an antibody light chain kappa constant region or an antibody light chain lambda region. In some aspects, CL is an antibody light chain kappa constant region. In some aspects, the VH comprises the amino acid sequence of SEQ ID NO:13, and the VL comprises the amino acid sequence of SEQ ID NO:14. In some aspects, the scFv comprises the amino acid sequence of SEQ ID NO:15 and the amino acid sequence of SEQ ID NO:16. In some aspects, the VH comprises the amino acid sequence of SEQ ID NO:13, the VL comprises the amino acid sequence of SEQ ID NO:14, the scFv comprises the amino acid sequence of SEQ ID NO:15 and the amino acid sequence of SEQ ID NO:16. In some aspects, the scFv comprises the amino acid sequence of SEQ ID NO:17. In some aspects, the VH comprises the amino acid sequence of SEQ ID NO:13, the VL comprises the amino acid sequence of SEQ ID NO:14, the scFv comprises the amino acid sequence of SEQ ID NO:17. In some aspects, CH1 comprises the amino acid sequence of SEQ ID NO:21. In some aspects, L1 and L2 can be the same or different, and can independently comprise (a) [GGGGS]_n, where n is 0, 1, 2, 3, 4, or 5 (SEQ ID NO:26), (b) [GGGG]_n, where n is 0, 1, 2, 3, 4, or 5 (SEQ ID NO:27), or a combination of (a) and (b). In some aspects, H1 comprises the amino acid sequence EPKSC (SEQ ID NO:22). In some aspects, L1 comprises [GGGGS]_n, where n is 2 (SEQ ID NO:28). In some aspects, L2 comprises [GGGGS]_n, where n is 2 (SEQ ID NO:28). In some aspects, H2 comprises the amino acid sequence DKTHTCPPCP (SEQ ID NO:23). In some aspects CH2-CH3 comprises the amino acid sequence of SEQ ID NO:30. In some aspects, CL comprises the amino acid sequence of SEQ ID NO:24.

[0129] In some aspects, a bispecific antibody provided herein comprises a polypeptide comprising the amino acid sequence of SEQ ID NO:31. In some aspects, a bispecific antibody provided herein comprises a polypeptide comprising the amino acid sequence of SEQ ID NO:20. In some aspects, a bispecific antibody provided herein comprises a polypeptide comprising the amino acid sequence of SEQ ID NO:31 and a polypeptide comprising the amino acid sequence of SEQ ID NO:20.

[0130] In some aspects, the bispecific antibodies provided herein can be a tandem single chain (sc) Fv fragment, which contain two different scFv fragments covalently tethered together by a linker (*e.g.*, a polypeptide linker). (Ren-Heidenreich *et al. Cancer* 100:1095-

1103 (2004); Korn *et al. J Gene Med* 6:642-651 (2004)). In some aspects, the linker can contain, or be, all or part of a heavy chain polypeptide constant region such as a CH1 domain. In some aspects, the two antibody fragments can be covalently tethered together by way of a polyglycine-serine or polyserine-glycine linker as described in, *e.g.*, U.S. Pat. Nos. 7,112,324 and 5,525,491, respectively. Methods for generating bispecific tandem scFv antibodies are described in, *e.g.*, Maletz *et al. Int J Cancer* 93:409-416 (2001); and Honemann *et al. Leukemia* 18:636-644 (2004). Alternatively, the antibodies can be "linear antibodies" as described in, *e.g.*, Zapata *et al. Protein Eng.* 8:1057-1062 (1995). Briefly, these antibodies comprise a pair of tandem Fd segments (VH-CH1-VH-CH1) that form a pair of antigen binding regions.

[0131] The disclosure also embraces variant forms of bispecific antibodies such as the tetravalent dual variable domain immunoglobulin (DVD-Ig) molecules described in Wu *et al.* (2007) *Nat Biotechnol* 25(11):1290-1297. The DVD-Ig molecules are designed such that two different light chain variable domains (VL) from two different parent antibodies are linked in tandem directly or via a short linker by recombinant DNA techniques, followed by the light chain constant domain. For example, the DVD-Ig light chain polypeptide can contain in tandem: (a) the VL from a PcrV-binding domain; and (b) the VL from a Psl-binding domain. Similarly, the heavy chain comprises the two different heavy chain variable domains (VH) linked in tandem, followed by the constant domain CH1 and Fc region. For example, the DVD-Ig heavy chain polypeptide can contain in tandem: (a) the VH from a PcrV-binding domain; and (b) the VH from a Psl-binding domain. In this case, expression of the two chains in a cell results in a heterotetramer containing four antigen combining sites, two that specifically bind to PcrV and two that specifically bind to Psl. Methods for generating DVD-Ig molecules from two parent antibodies are further described in, *e.g.*, PCT Publication Nos. WO 2008/024188 and WO 2007/024715, each of which are incorporated by reference in its entirety.

[0132] In some aspects, a bispecific antibody provided herein does not comprise a modification that enhances hexamer formation. In some aspects, a bispecific antibody provided herein is not a HexaBody. In some aspects, a bispecific antibody provided herein is not an IgG-HexaBody. HexaBody technology is discussed, for example, in de Jong RN, *et al.*, *PLoS Biol.* 2016 Jan 6;14(1):e1002344. doi: 10.1371/journal.pbio.1002344. PMID: 26736041; PMCID: PMC4703389, which is herein incorporated by reference in its entirety.

[0133] In some aspects, a bispecific antibody provided herein does not comprise an E345R substitution. In some aspects, a bispecific antibody provided herein does not comprise an E430G substitution. In some aspects, a bispecific antibody provided herein does not comprise an S440Y substitution. In some aspects, a bispecific antibody provided herein does not comprise an E345R, E430G, or S440Y substitution.

c. Modified Fc regions in bispecific anti-*Pseudomonas* antibodies

[0134] As provided herein, a bispecific antibody with a modified Fc region can have one or more altered properties as compared to a “corresponding” antibody without the modified Fc region (*e.g.*, with a wild-type Fc region). A “corresponding” antibody to a reference antibody with a modified Fc region is an antibody that contains the same amino acid sequence as the reference sequence except for the specified modifications in the Fc region. Thus, for example, for AZD0292, gremubamab (MEDI3902) is a “corresponding” antibody because gremubamab contains the same amino acid sequence as AZD0292 except for the N3Y modification in the Fc region of AZD0292.

[0135] Anti-*Pseudomonas aeruginosa* Psl and PcrV bispecific antibodies provided herein can exploit FcRn-mediated recycling to achieve serum half-lives that can be similar to or different from that of endogenous IgG, depending on the desired properties. By further endowing biological therapeutic and diagnostic agents with improved pharmacokinetic properties, the present disclosure provides opportunities for more desirable dosages, reduced frequency of administration, or improved clearance, while maintaining efficacy.

[0136] The present disclosure provides Anti-*Pseudomonas aeruginosa* Psl and PcrV bispecific antibodies whose *in vivo* half-lives are altered (increased or decreased) by the presence of an IgG constant domain, or FcRn binding fragment thereof (*e.g.*, an Fc region or hinge-Fc region) (*e.g.*, from a human IgG, *e.g.*, human IgG1), that have modifications of one or more of amino acid residues in at least the CH3 domain. The modifications can include amino acid substitutions, insertions, deletions, or any combination thereof. It should be understood that all references to amino acid residues of the IgG constant and variable domains that appear herein are numbered according to the EU numbering index of Kabat *et al.* (Sequences of Proteins of Immunological Interest, 5th ed., 1991 NIH Pub. No. 91-3242, which is incorporated by reference herein in its entirety), and include corresponding residues in other IgG constant domains as determined by sequence alignment.

[0137] More particularly, the disclosure provides anti-*Pseudomonas aeruginosa* Psl and PcrV bispecific antibodies whose *in vivo* half-lives are altered (increased or decreased) by the presence of an IgG constant domain, or FcRn binding fragment thereof (*e.g.*, an Fc region or hinge-Fc region (*e.g.*, from a human IgG, *e.g.*, human IgG1)), that have modifications of one or more of amino acid residues 432, 433, 434, 435, 436, or 437, and/or that have a single amino acid insertion between amino acids 437 and 438, which insertion is referred to herein as 437*, in the His435 loop region of the CH3 domain, which amino acid substitutions and/or insertions alter (increase or decrease) the binding affinity of the IgG constant domain or FcRn-binding fragment thereof for FcRn at a particular pH (*e.g.* pH 6.0 or pH 7.4). Such modifications, including insertions between residues 437 and 438, will be referred to generally as modifications within the His435 loop region, *i.e.*, at amino acid residues 432-437. In certain aspects, these modifications can exclude residue 435, such that the modified IgG constant domain, or FcRn-binding portion thereof (*e.g.*, an Fc region or hinge-Fc region), contains His435 which is found in wild-type human IgG1, IgG2, and IgG4. In certain aspects, for example modification of the analogous His435 loop region in human IgG3 which in the wild-type molecule includes the arginine at position (R435) instead of the histidine (H435) found in IgG1, IgG2 and IgG4 and further, is a site of known allelic variation, these modifications include the substitution of a wild-type non-histidine residue 435 with a histidine, to yield H435. In one aspect, the modified IgG constant domain, or FcRn-binding portion thereof (*e.g.*, an Fc region or hinge-Fc region), is a human or humanized IgG constant domain or FcRn-binding portion thereof, although it may be murine. The human or humanized IgG constant domain can be a constant domain from an IgG1, IgG2, IgG3 or IgG4 domain, or any subtype thereof.

[0138] As provided herein, amino acid modifications in the His435 loop region of the CH3 domain of the Fc fragment of human IgG, can affect the binding affinity of the bispecific antibody to FcRn at one or more pHs. These modifications can result in an alteration in the pH dependence of binding of the bispecific antibody to FcRn. In some aspects, the amino acid modifications in the His435 loop region can result in a higher binding affinity of the bispecific antibody for FcRn at pH 6, at pH 7.4, or at both pH 6.0 and 7.4, than exhibited by a corresponding antibody with a wild-type IgG constant domain. Additionally or alternatively, the modifications may affect the *in vivo* half-life of the molecule.

[0139] The His435 loop region includes amino acid residues 432, 433, 434, 435, 436 and 437. The wild type amino acid sequence of the His435 loop region (residues 432 to 437) of the CH3 domain of the Fc fragment of human IgG1, IgG2 and IgG4 is Leu-His-Asn-His-Tyr-Thr (SEQ ID NO:34) and of human IgG3 is Leu-His-Asn-Arg-Phe-Thr (SEQ ID NO:35). In some aspects, one or more amino acid modifications in an Fc region are made in or near one or more of residues 432, 433, 434, 435, 436 and 437, *e.g.*, in a human IgG constant domain, or FcRn-binding domain thereof (*e.g.*, an Fc region or hinge-Fc region), or analogous residues thereof, as determined by amino acid sequence alignment, in other IgGs. Such mutations include amino acid substitutions as well as deletions and insertions. An illustrative site for an amino acid insertion is between residues 437 and 438, which added position is referred to herein as 437*.

[0140] In one aspect of the modified IgG constant domain, or FcRn-binding fragment thereof (*e.g.*, an Fc region or hinge-Fc region), residue 435 is maintained as a histidine (His435) (such as in wild-type human IgG1, IgG2 and IgG4) or mutated to a histidine (as in IgG3, which natively contains R435, and is thus mutated to R435H), while at least one of residues 432, 433, 434, 436, or 437 is substituted, and/or an insertion is made at position 437*. In one aspect, neither residue 435 (His435) nor residue 433 (His 433) is mutated (except that, for human IgG3, residue 435 has the R435H mutation so that it is His435), while at least one of residues 432, 434, 436, or 437 is substituted, and/or an insertion is made at position 437*. In one aspect, the FcRn binding domain has a substitution at 1, 2, 3, 4, or all 5 of residues 432, 433, 434, 436, 437, and/or has an insertion at position 437* in the His435 loop region. In another aspect, the FcRn binding domain has a substitution at three or more of positions 432, 433, 434, 435, 436 or 437. In another aspect, the FcRn binding domain has a substitution at four or more of positions 432, 433, 434, 435, 436 or 437.

[0141] In one aspect, at least one of positions 432 and 437 is substituted with cysteine, and residues 433, 434, 435, and 436 are each independently either substituted or not substituted. In certain aspects, residues 432 and 437 are both substituted with cysteines, and residues 433, 434, 435, and 436 are each independently either substituted or not substituted.

[0142] In one aspect, at least one of positions 432 and 437 is substituted with an amino acid selected from the group consisting of glutamine, glutamic acid, aspartic acid, lysine, arginine, and histidine, and residues 433, 434, 435, and 436 are each independently either

substituted or not substituted. In certain aspects, both of positions 432 and 437 are substituted with an amino acid independently selected from the group consisting of glutamine, glutamic acid, aspartic acid, lysine, arginine, and histidine, and residues 433, 434, 435, and 436 are each independently either substituted or not substituted.

[0143] In one aspect, a modified IgG constant domain, or FcRn-binding fragment thereof (*e.g.*, an Fc region or hinge-Fc region), contains at least three mutations in the His435 loop region, and has a histidine at position 435 (the histidine at position 435 may be a wild type residues or a mutation). Any of the various permutations of three or more mutations (not including a mutation at 435, if present) is encompassed by the disclosure, including without limitation mutations at the following sites:

Positions: 432, 433, and any one of 434, 436, 437, and 437*

Positions: 432, 434, and any one of 436, 437, and 437*

Positions: 432, 436, and any one of 437 and 437*

Positions: 432, 437, and 437*

Positions: 433, 434, and any one of 436, 437, and 437*

Positions: 433, 436, and any one of 437 and 437*

Positions: 433, 437, and 437*

Positions: 434, 436, and any one of 437 and 437*

Positions: 434, 437, and 437*

Positions: 436, 437, and 437*

Positions: 432, 433, 434, and any one of 436, 437, and 437*

Positions: 432, 433, 436, and any one of 437 and 437*

Positions: 432, 433, 437, and 437*

Positions: 432, 434, 436, and any one of 437 and 437*

Positions: 432, 434, 437, and 437*

Positions: 432, 436, 437, and 437*

Positions: 433, 434, 436, and any one of 437 and 437*

Positions: 433, 434, 437, and 437*

Positions: 434, 436, 437, and 437*

Positions: 432, 433, 434, 436, and any one of 437 and 437*

Positions: 432, 433, 434, 437, and 437*

Positions: 432, 433, 436, 437, and 437*

Positions: 432, 434, 436, 437, and 437*

Positions: 433, 434, 436, 437, and 437*

Positions: 432, 433, 434, 436, 437, and 437*

[0144] In some aspects, the modified IgG constant domain, or FcRn-binding fragment thereof (*e.g.*, an Fc region or hinge-Fc region), contains a mutation in the His435 loop region at positions 432, 433, 434, 436 and 437, and has a histidine at position 435 (the histidine at position 435 may be a wild type residue or a mutation). In one aspect, a modified IgG Fc region comprises the amino acid sequence of SEQ ID NO:44. In one aspect, a modified IgG1 Fc region comprises the amino acid sequence of SEQ ID NO:44. In one aspect, a modified human IgG Fc region comprises the amino acid sequence of SEQ ID NO:44. In one aspect, a modified human IgG1 Fc region comprises the amino acid sequence of SEQ ID NO:44. The amino acid sequence of SEQ ID NO:44 may be referred to as the “N3Y” modification, “N3Y” mutations and the like.

[0145] In one aspect, a modified IgG Fc region comprises the amino acid sequence of SEQ ID NO:33. In one aspect, a modified IgG1 Fc region comprises the amino acid sequence of SEQ ID NO:33. In one aspect, a modified human IgG Fc region comprises the amino acid sequence of SEQ ID NO:33. In one aspect, a modified human IgG1 Fc region comprises the amino acid sequence of SEQ ID NO:33.

[0146] In one aspect of the modified IgG constant domain, or FcRn-binding fragment thereof (*e.g.*, an Fc region or hinge-Fc region), the His435 loop region of the CH3 domain of the Fc fragment has the amino acid sequence CXXXXC (residues 432-437; SEQ ID NO:41) or CXXXXCE (residues 432 through 437* wherein 437* is an insertion; SEQ ID NO:42). In one aspect, exemplary His435 loop amino acid sequences for various HB20.3 IgG mutants can be generated from a CXXXXCE (SEQ ID NO:42).

[0147] Without intending to be bound by theory, the two cysteine residues may exert a stabilizing effect on the loop region, possibly by forming a disulfide cystine. The predicted distance between the two cysteines is about 6.7Å, which is within the range (4.6-7Å) compatible with the formation of a cystine. In certain aspects based on the His435 loop motif CXXXXC (SEQ ID NO:41), the amino acid modifications for a modified IgG constant domain, or FcRn-binding fragment thereof (*e.g.*, an Fc region or hinge-Fc region), are substitutions at one or more of positions 432-437, with both of positions 432 and 437 being substituted with cysteine. Position 435 is histidine. Position 433 can be substituted

with arginine, proline, threonine, lysine, serine, alanine, methionine, or asparagine; in one aspect, position 433 is serine. Position 434 can be substituted with arginine, tryptophan, histidine, phenylalanine, tyrosine, serine, methionine or threonine; in one aspect, position 434 is tyrosine. Position 436 can be substituted with leucine, arginine, isoleucine, lysine, methionine, valine, histidine, serine, or threonine; in one aspect, position 436 is leucine. In some aspects, the mutated His435 loop region contains a glutamic acid insertion at position 437*.

[0148] In one aspect, positions 432 and 437 are cysteine; position 433 is arginine, proline, threonine, lysine, serine, alanine, methionine, asparagine, or histidine; position 434 is arginine, tryptophan, histidine, phenylalanine, tyrosine, serine, methionine, threonine, or asparagine; position 435 is histidine; and position 436 is leucine, arginine, isoleucine, lysine, methionine, valine, histidine, serine, threonine, tyrosine, or phenylalanine. In one aspect, the His435 loop region is CXRHXC (SEQ ID NO:36), wherein position 433 is histidine or is substituted with arginine, proline, serine, or asparagine, and wherein position 436 is substituted with leucine, arginine, isoleucine, methionine, or serine. In one aspect, the His435 loop region is CRRHXC (SEQ ID NO:37) wherein position 436 is substituted with leucine, arginine, isoleucine, lysine, methionine, valine, histidine, serine, or threonine. In one aspect, the His435 loop region is CXRHRC (SEQ ID NO:38) wherein position 433 is arginine, proline, threonine, lysine, serine, alanine, methionine, or asparagine. In one aspect, the His435 loop region is CSWHLC (SEQ ID NO:39) or CSWHLE (SEQ ID NO:40). In some aspects, in any of these aspects, the mutated His435 loop region contains a glutamic acid insertion at position 437*.

[0149] Amino acid modifications can be made by any method known in the art and many such methods are well known and routine for the skilled artisan. For example, but not by way of limitation, amino acid substitutions, deletions and insertions may be accomplished using any well-known PCR-based technique. Amino acid substitutions may be made by site-directed mutagenesis (see, for example, Zoller and Smith, Nucl. Acids Res. 10:6487-6500, 1982; Kunkel, Proc. Natl. Acad. Sci USA 82:488, 1985, which are hereby incorporated by reference in their entireties). Mutants that result in increased affinity for FcRn and increased *in vivo* half-life may readily be screened using well-known and routine assays, such as those described herein. Amino acid substitutions can be introduced at one or more residues in the IgG constant domain or FcRn-binding fragment thereof (*e.g.*, an Fc

region or hinge-Fc region), and the mutated constant domains or fragments can be expressed on the surfaces of bacteriophage which are then screened for increased FcRn binding affinity.

[0150] Once generated, a mutated IgG constant domain, or fragment thereof (*e.g.*, an Fc region or hinge-Fc region), may be used in the construction of a bispecific antibody (*e.g.*, by fusing to the variable portions of a bispecific antibody of interest) or an Fc fusion molecule (*e.g.*, by fusing/conjugating a heterologous moiety). A modified IgG or Fc fusion molecule of the disclosure may be generated by methods well known to one skilled in the art. Briefly, such methods include but are not limited to, combining a variable region with the desired specificity (*e.g.*, a variable region isolated from a phage display or expression library or derived from a human or non-human antibody) with a modified IgG constant region, or an FcRn-binding fragment thereof (*e.g.*, an Fc region or hinge-Fc region) having an altered half-life as provided herein. Alternatively, one skilled in the art may generate a modified IgG or Fc fusion molecule of the disclosure by substituting at least one amino acid residue in the Fc region of an antibody or Fc fusion molecule.

[0151] In addition to affecting half-life, the amino acid modifications described herein may alter (*i.e.*, increase or decrease) the bioavailability (*e.g.*, transport to mucosal surfaces, or other target tissues) of the molecules, in particular, alters (*i.e.*, increases or decreases) transport (or concentration or half-life) of the molecule to mucosal surfaces (*e.g.*, of the lungs) or other portions of a target tissue. In some aspects, the amino acid modifications alter (*e.g.*, increase or decrease) transport or concentration or half-life of the molecule to the lungs. In some aspects, the amino acid modifications alter (*e.g.*, increase or decrease) transport (or concentration or half-life) of the molecule to the heart, pancreas, liver, kidney, bladder, stomach, large or small intestine, respiratory tract, lymph nodes, nervous tissue (central and/or peripheral nervous tissue), muscle, epidermis, bone, cartilage, joints, blood vessels, bone marrow, prostate, ovary, uterine, tumor or cancer tissue, etc.

[0152] In some aspects, the amino acid modifications do not abolish, or do not alter, one or more other immune effector or receptor binding functions of the constant domain, for example, but not limited to complement fixation, antibody-dependent cellular cytotoxicity (ADCC), complement dependent cytotoxicity (CDC), antibody dependent cellular phagocytosis (ADCP) and/or binding to one or more Fc gamma receptors such as Fc γ RI,

FcγRII, and FcγRIII. Modified IgGs and other molecules of the disclosure can be evaluated for effector function using methods well-known and routine in the art.

[0153] Moreover, the disclosure provides modified IgGs well-suited for a variety of diagnostic and therapeutic purposes. The disclosure provides modified IgGs exhibiting varying levels of pH dependence of their binding to FcRn. Different levels of pH dependence may result in or correlate with different pharmacokinetic properties, which in turn yield modified IgGs that are better suited for some purposes than for others.

[0154] For example, some of the modified IgGs described herein exhibit high affinity binding to FcRn at pH 6.0 along with a high level of pH dependence in their binding to FcRn, and an observed increase in in vivo half-life. Modified IgGs of this aspect of the disclosure have utility, for example, when employed as therapeutic agents in applications wherein a longer in vivo half-life is desirable. Optionally the modified IgG exhibits other improved pharmacokinetic properties as well, such as retained or enhanced ability to interact with Fc-ligands such as Fcγ receptors and C1q, robust opsonophagocytic killing (OPK) activity, and the ability to mediate Fc effector functions (*e.g.*, CDC, ADCC).

[0155] In contrast, some of the modified IgGs described herein exhibit high affinity binding to FcRn at pH 6.0 along with a lower level of pH dependence in their binding to FcRn (typically as a result of enhanced affinity for FcRn at pH 7.4), and an observed decrease in in vivo half-life. Modified IgGs of this aspect of the disclosure have utility, for example, when employed as therapeutic agents in applications wherein a shorter in vivo half-life is desirable, such as in treating certain autoimmune conditions. They may also be well-suited to diagnostic applications, such as when used as a biological imaging agent, where quick clearance from bodily fluids or tissue is desired.

[0156] Additionally, the disclosure relates to amino acid modifications (*e.g.*, substitutions, insertions or deletions) in the IgG constant domain, or FcRn binding fragment thereof (*e.g.*, an Fc region or hinge-Fc region), that have been discovered to increase the affinity of the IgG constant domain, or fragment thereof, for FcRn at pH 6, and that optionally alter the affinity of the IgG or fragment thereof for FcRn at pH 7.4, thereby altering the pH dependence of binding affinity of the IgG constant domain, or fragment thereof (*e.g.*, an Fc region or hinge-Fc region) for FcRn. Further, these modifications may either increase or decrease the in vivo half-life of the molecule.

[0157] In one aspect, the disclosure addresses the pharmaceutical importance of increasing the *in vivo* half-lives of anti-*Pseudomonas aeruginosa* Psl and PcrV bispecific antibodies. To this end, the disclosure provides anti-*Pseudomonas aeruginosa* Psl and PcrV bispecific antibodies containing a modified IgG constant domain or FcRn-binding fragment thereof (*e.g.*, an Fc region or hinge-Fc region (*e.g.*, from a human IgG, *e.g.*, human IgG1)) that confer increased *in vivo* half-life on immunoglobulins and other bioactive molecules. In this aspect, the present disclosure relates to anti-*Pseudomonas aeruginosa* Psl and PcrV bispecific antibodies that have an increased *in vivo* half-life by virtue of the presence of a modified IgG constant domain, or FcRn-binding fragment thereof (*e.g.*, an Fc region or hinge-Fc region (*e.g.*, from a human IgG, *e.g.*, human IgG1)) wherein the IgG constant domain, or fragment thereof, is modified (*e.g.*, by amino acid substitution, deletion or insertion) to change (increase or decrease) the binding affinity of the IgG constant domain or FcRn-binding fragment for FcRn at a particular pH (*e.g.* pH 6.0 or pH 7.4). In one aspect, the IgG constant domain, or FcRn-binding fragment thereof, is modified to increase the binding affinity for FcRn at pH 6.0 relative to the binding affinity for FcRn at pH 7.4. The *in vivo* half-lives of the modified IgGs of the disclosure can be conveniently evaluated in a human transgenic mouse model or a cynomolgus monkey primate model, as described, *e.g.*, in Example 2 below.

[0158] Most modified antibodies of the disclosure, whether they exhibit increased or decreased *in vivo* half-lives compared to each other or their unmodified or wild-type counterparts, contain an IgG constant domain, or FcRn-binding fragment thereof, that exhibits higher binding affinity toward FcRn at pH 6.0 than wild-type IgG constant domain.

[0159] More generally, one skilled in the art will understand that the Fc variants of the disclosure, whether they exhibit increased or decreased *in vivo* half-lives compared to each other or their unmodified or wild-type counterparts, may have altered FcRn binding properties. Examples of binding properties include but are not limited to, binding specificity, equilibrium dissociation constant (KD), dissociation and association rates (k_{on} , and k_{off} respectively), binding affinity and/or avidity. It is well known in the art that the equilibrium dissociation constant (KD) is defined as k_{off}/k_{on} . It is understood that a higher affinity interaction will have a lower KD and conversely that a lower affinity interaction will have a higher KD. However, in some instances the value k_{on} or k_{off} may be more relevant than the value of the KD.

[0160] While the relationships among IgG binding affinity for FcRn, the pH dependence of such binding affinity, and *in vivo* half-life are complex, for those IgG constant domains that exhibit high affinity binding to FcRn at pH 6.0 (*e.g.*, KDs of less than about 500 nM), as the binding affinity for FcRn at pH 7.4 increases (generally reflecting reduced pH dependence of FcRn binding), for example, if the KD at pH 7.4 falls below about 1 μ M into the nanomolar range, the result can in some instances be a shorter *in vivo* half-life for the modified IgG. In contrast, reduced binding affinity for FcRn at pH 7.4 (*e.g.*, KDs at pH 7.4 above about 1 μ M) coupled with high binding affinity at pH 6.0 (*e.g.*, KDs of less than about 500 nM), generally reflecting a greater pH dependence of FcRn binding, can in some instances result in a longer *in vivo* half-life.

[0161] In some aspects, modified IgGs and other molecules of the disclosure contain a modified IgG constant domain or FcRn-binding fragment thereof (*e.g.*, an Fc region or hinge-Fc region), that exhibits a KD for binding to FcRn at pH 6.0 of less than 100 nM, less than 200 nM, less than 300 nM, less than 400 nM, less than 500 nM or less than 1000 nM. Modified IgGs of the disclosure can, for example, be characterized by KD values for FcRn binding at pH 6.0 of 10 nM to 500 nM, 50 nM to 500 nM. In some aspects, the modified IgG of the disclosure exhibits at least a 10-fold enhancement, at least a 20-fold enhancement, or at least a 50-fold enhancement of binding affinity for FcRn at pH 6.0 compared to a wild-type IgG constant domain, or FcRn-binding fragment thereof.

[0162] Additionally or alternatively, a modified IgG can exhibit a binding affinity for FcRn at pH 7.4 that is between 10 nM and 50 μ M. Without intending to be bound by theory, it is observed that a threshold may exist: at pH 7.4, a KD of about 1 μ M or higher (*e.g.*, a KD of 1 μ M, 5 μ M, 10 μ M, 20 μ M, 30 μ M, 40 μ M, 50 μ M, or higher; that is, binding affinities in the micromolar or millimolar range evidencing lower binding affinity for FcRn) may be associated with a modified IgG or other molecule having longer half-life (slower clearance), while a KD of less than 1 μ M at pH 7.4 (*e.g.*, a KD of 50 nM, 100 nM, 200 nM, 500 nM, 800 nM up to about 1000 nM; that is, binding affinities in the nanomolar range, evidencing higher binding affinity for FcRn) may be associated with a modified IgG or other molecule having a shortened half-life (faster clearance). An increased half-life for the modified IgG or other molecule is generally, but not always, associated with pH-dependent binding to FcRn characterized by a KD of 50 nM to 400 nM or 500 nM for binding at pH 6, and a KD of more than 1 μ M at pH 7.4.

- [0163] The structure of the IgG constant domain (or FcRn-binding fragment thereof, *e.g.*, an Fc region or hinge-Fc region) outside the His435 loop region may be referred to herein as the molecule's IgG "base structure" or "background" and these two terms are used interchangeably. The disclosure thus contemplates modified IgGs with mutations in the His435 loop region incorporated into either a wild-type IgG base structure or a mutant IgG base structure. Any mutant IgG base structure can be utilized; exemplary but nonlimiting mutant IgG base structures are described herein. In certain aspects, the IgG base structure has a sequence according to SEQ ID NO: 25 or 19.
- [0164] The modified immunoglobulin molecules of the disclosure include IgG molecules that naturally contain an FcRn binding domain, as well as other non-IgG immunoglobulins (*e.g.*, IgE, IgM, IgD, IgA and IgY) or fragments of immunoglobulins that have been engineered to contain an FcRn-binding fragment (*i.e.*, fusion proteins comprising non-IgG immunoglobulin or a fragment thereof and an FcRn binding domain, such as an Fc region or Fc hinge region). In both cases the FcRn-binding domain has one or more amino acid modifications that increase the affinity of the constant domain fragment for FcRn at pH 6.0 and, optionally, affect (either increase or decrease) the pH dependence of binding to FcRn.
- [0165] The modified immunoglobulins include any immunoglobulin molecule that binds (preferably, immunospecifically, *i.e.*, competes off non-specific binding), as determined by immunoassays well known in the art for assaying specific antigen-antibody binding) an antigen and contains an FcRn-binding fragment. Such antibodies include, but are not limited to, polyclonal, monoclonal, bi-specific, multi-specific, human, humanized, and chimeric antibodies, single chain antibodies.
- [0166] The immunoglobulins (and other proteins used herein) may be from any animal origin including birds and mammals. The antibodies can be, for example, human, rodent (*e.g.*, mouse and rat), donkey, sheep, rabbit, goat, guinea pig, camel, horse, or chicken. As used herein, "human" antibodies include antibodies having the amino acid sequence of a human immunoglobulin and include antibodies isolated from human immunoglobulin libraries or from animals transgenic for one or more human immunoglobulin and that do not express endogenous immunoglobulins, as described *infra* and, for example, in U.S. Patent No. 5,939,598 by Kucherlapati *et al.*

- d. Additional properties of bispecific anti-*Pseudomonas* antibodies with modified Fc regions

[0167] In some aspects, a bispecific antibody provided herein (*e.g.*, AZD0292) mediates cytotoxic activity against *Pseudomonas* (*e.g.*, *Pseudomonas aeruginosa*). In some aspects, a bispecific antibody provided herein (*e.g.*, AZD0292) targets *Pseudomonas* (*e.g.*, *Pseudomonas aeruginosa*) for opsonophagocytic killing (OPK). Methods of assessing cytotoxic activity and/or OPK are known in the art and provided herein, *e.g.*, in Example 1.

[0168] In some aspects, a bispecific antibody provided herein (*e.g.*, AZD0292) has similar cytotoxic activity against *Pseudomonas* (*e.g.*, *Pseudomonas aeruginosa*) as gremubamab (MEDI3902). In some aspects, a bispecific antibody provided herein (*e.g.*, AZD0292) has similar OPK activity against *Pseudomonas* (*e.g.*, *Pseudomonas aeruginosa*) as gremubamab. In some aspects, a bispecific antibody provided herein (*e.g.*, AZD0292) has similar cytotoxic activity and OPK activity against *Pseudomonas* (*e.g.*, *Pseudomonas aeruginosa*) as gremubamab.

[0169] In some aspects, a bispecific antibody provided herein (*e.g.*, AZD0292) prevents cell attachment, *e.g.*, prevents attachment of *Pseudomonas* (*e.g.*, *Pseudomonas aeruginosa*), to host cells. Methods of assessing prevention of attachment are known in the art and provided, *e.g.*, in WO 2013/070615, which is herein incorporated by reference in its entirety. In some aspects, a bispecific antibody provided herein (*e.g.*, AZD0292) disrupts biofilm formation. In some aspects, a bispecific antibody provided herein (*e.g.*, AZD0292) inhibits primary colony formation.

[0170] In some aspects, a bispecific antibody provided herein (*e.g.*, AZD0292) exhibits less aggregation in solution than gremubamab. In some aspects, a bispecific antibody provided herein (*e.g.*, AZD0292) exhibits less aggregation in a shake plate overgrow screen than gremubamab (see *e.g.*, Example 4).

III. Uses of Bispecific Anti-*Pseudomonas* Antibodies with Modified Fc Regions

[0171] Also provided herein are methods of preparing and administering anti-*Pseudomonas* Psl and/or PcrV binding molecules, *e.g.*, an antibody or fragment, variant or derivative thereof, as disclosed herein to a subject in need thereof. The route of administration of the anti-*Pseudomonas* Psl and/or PcrV binding molecules, *e.g.*, antibody or fragment, variant or derivative thereof, can be, for example, parenteral. The term

parenteral as used herein includes, *e.g.*, intravenous, intraarterial, intraperitoneal, intramuscular, or subcutaneous administration. A suitable form for administration would be a solution for injection, in particular for intravenous or intraarterial injection or drip. However, in other methods compatible with the teachings herein, an anti-*Pseudomonas* Psl and/or PcrV binding molecules, *e.g.*, antibody or fragment, variant or derivative thereof, as disclosed herein can be delivered directly to the site of the adverse cellular population *e.g.*, infection, thereby increasing the exposure of the diseased tissue to the therapeutic agent. For example, an anti-*Pseudomonas* Psl and/or PcrV binding molecule can be directly administered to ocular tissue, burn injury, or lung tissue.

[0172] Anti-*Pseudomonas* Psl and/or PcrV binding molecules, *e.g.*, antibodies or fragments, variants or derivatives thereof, as disclosed herein can be administered in a pharmaceutically effective amount for the *in vivo* treatment of a *Pseudomonas* infection. In this regard, it will be appreciated that the disclosed bispecific antibodies will be formulated so as to facilitate administration and promote stability of the active agent. For the purposes of the instant application, a pharmaceutically effective amount refers to an amount sufficient to achieve effective binding to a target and to achieve a benefit, *e.g.*, treat, ameliorate, lessen, clear, or prevent *Pseudomonas* infection.

[0173] Some aspects are directed to a method of preventing or treating a *Pseudomonas* infection in a subject in need thereof, comprising administering to the subject an effective amount of the binding molecule or fragment thereof, the antibody or fragment thereof. In further aspects, the *Pseudomonas* infection is a *P. aeruginosa* infection. In some aspects, the subject is a human. In certain aspects, the infection is an ocular infection, a lung infection, a burn infection, a wound infection, a skin infection, a blood infection, a bone infection, or a combination of two or more of said infections. In further aspects, the subject suffers from acute pneumonia, burn injury, corneal infection, cystic fibrosis, or a combination thereof.

[0174] Certain aspects are directed to a method of blocking or preventing attachment of *P. aeruginosa* to epithelial cells comprising contacting a mixture of epithelial cells and *P. aeruginosa* with the binding molecule or fragment thereof, the antibody or fragment thereof described herein.

[0175] Also disclosed is a method of enhancing OPK of *P. aeruginosa* comprising contacting a mixture of phagocytic cells and *P. aeruginosa* with the binding molecule or

fragment thereof, the antibody or fragment thereof, the composition, the polynucleotide, the vector, or the host cell described herein. In further aspects, the phagocytic cells are differentiated HL-60 cells or human polymorphonuclear leukocytes (PMNs).

a. Methods and Uses of Bispecific Anti-*Pseudomonas* Antibodies with Modified Fc Regions in the Treatment of Bronchiectasis

[0176] As demonstrated herein, bispecific antibodies that specifically bind to *Pseudomonas aeruginosa* Psl and PcrV and have modified Fc regions (*e.g.*, AZD0292) are useful in treating subjects with bronchiectasis. Accordingly, provided herein are methods of treatment using such bispecific antibodies, uses of such bispecific antibodies in the preparation of medicaments, and bispecific antibodies for use in treatments.

[0177] In some aspects, provided herein are methods of treating bronchiectasis (*e.g.*, non-cystic fibrosis bronchiectasis). The methods can comprise administering to the subject a bispecific antibody that specifically binds to *Pseudomonas aeruginosa* PcrV protein and Psl exopolysaccharide and that comprises a modified Fc region (*e.g.*, AZD0292). The subject can be, *e.g.*, a subject that is colonized with *Pseudomonas aeruginosa*.

[0178] In some aspects, provided herein are methods of improving pre-bronchodilator forced expiratory volume 1 (FEV₁) in a subject with bronchiectasis (*e.g.*, non-cystic fibrosis bronchiectasis). The methods can comprise administering to the subject a bispecific antibody that specifically binds to *Pseudomonas aeruginosa* PcrV protein and Psl exopolysaccharide and that comprises a modified Fc region (*e.g.*, AZD0292). The subject can be, *e.g.*, a subject that is colonized with *Pseudomonas aeruginosa*.

[0179] In some aspects, provided herein are methods of reducing *Pseudomonas aeruginosa* load in a subject with bronchiectasis (*e.g.*, non-cystic fibrosis bronchiectasis). The methods can comprise administering to the subject a bispecific antibody that specifically binds to *Pseudomonas aeruginosa* PcrV protein and Psl exopolysaccharide and that comprises a modified Fc region (*e.g.*, AZD0292). The subject can be, *e.g.*, a subject that is colonized with *Pseudomonas aeruginosa*.

[0180] In some aspects, provided herein are methods of reducing bronchiectasis exacerbations in a subject with bronchiectasis (*e.g.*, non-cystic fibrosis bronchiectasis). The methods can, for example, reduce bronchiectasis exacerbations requiring hospitalization and/or reduce bronchiectasis exacerbations requiring antibiotics. The methods can comprise administering to the subject a bispecific antibody that specifically

binds to *Pseudomonas aeruginosa* PcrV protein and Psl exopolysaccharide and that comprises a modified Fc region (e.g., AZD0292). The subject can be, e.g., a subject that is colonized with *Pseudomonas aeruginosa*.

[0181] In some aspects, provided herein are methods of reducing the need for intravenous antibiotics in a subject with bronchiectasis (e.g., non-cystic fibrosis bronchiectasis). The methods can comprise administering to the subject a bispecific antibody that specifically binds to *Pseudomonas aeruginosa* PcrV protein and Psl exopolysaccharide and that comprises a modified Fc region (e.g., AZD0292). The subject can be, e.g., a subject that is colonized with *Pseudomonas aeruginosa*.

[0182] In some aspects, provided herein are methods of stabilizing lung function in a subject with bronchiectasis (e.g., non-cystic fibrosis bronchiectasis). The methods can comprise administering to the subject a bispecific antibody that specifically binds to *Pseudomonas aeruginosa* PcrV protein and Psl exopolysaccharide and that comprises a modified Fc region (e.g., AZD0292). The subject can be, e.g., a subject that is colonized with *Pseudomonas aeruginosa*.

[0183] In some aspects, provided herein are methods of improving cough frequency and/or intensity in a subject with bronchiectasis (e.g., non-cystic fibrosis bronchiectasis). The methods can comprise administering to the subject a bispecific antibody that specifically binds to *Pseudomonas aeruginosa* PcrV protein and Psl exopolysaccharide and that comprises a modified Fc region (e.g., AZD0292). The subject can be, e.g., a subject that is colonized with *Pseudomonas aeruginosa*.

[0184] In some aspects, provided herein are methods of decreasing bronchiectasis symptoms in a subject with bronchiectasis (e.g., non-cystic fibrosis bronchiectasis). The methods can comprise administering to the subject a bispecific antibody that specifically binds to *Pseudomonas aeruginosa* PcrV protein and Psl exopolysaccharide and that comprises a modified Fc region (e.g., AZD0292). The subject can be, e.g., a subject that is colonized with *Pseudomonas aeruginosa*.

[0185] In some aspects, provided herein are methods of improving the quality of life in a subject with bronchiectasis (e.g., non-cystic fibrosis bronchiectasis). The methods can comprise administering to the subject a bispecific antibody that specifically binds to *Pseudomonas aeruginosa* PcrV protein and Psl exopolysaccharide and that comprises a

modified Fc region (e.g., AZD0292). The subject can be, e.g., a subject that is colonized with *Pseudomonas aeruginosa*.

[0186] In some aspects, provided herein are methods of eradicating *Pseudomonas aeruginosa* in a subject with bronchiectasis (e.g., non-cystic fibrosis bronchiectasis). The methods can comprise administering to the subject a bispecific antibody that specifically binds to *Pseudomonas aeruginosa* PcrV protein and Psl exopolysaccharide and that comprises a modified Fc region (e.g., AZD0292). The subject can be, e.g., a subject that is colonized with *Pseudomonas aeruginosa*.

[0187] In some aspects, provided herein are methods of inducing sustained *Pseudomonas aeruginosa* suppression in a subject with bronchiectasis (e.g., non-cystic fibrosis bronchiectasis). The methods can comprise administering to the subject a bispecific antibody that specifically binds to *Pseudomonas aeruginosa* PcrV protein and Psl exopolysaccharide and that comprises a modified Fc region (e.g., AZD0292). The subject can be, e.g., a subject that is colonized with *Pseudomonas aeruginosa*.

[0188] In some aspects, provided herein are methods of reducing the risk of bronchiectasis progression related to *Pseudomonas aeruginosa* in a subject with bronchiectasis (e.g., non-cystic fibrosis bronchiectasis). The methods can comprise administering to the subject a bispecific antibody that specifically binds to *Pseudomonas aeruginosa* PcrV protein and Psl exopolysaccharide and that comprises a modified Fc region (e.g., AZD0292). The subject can be, e.g., a subject that is colonized with *Pseudomonas aeruginosa*.

[0189] A bronchiectasis suitable for treatment in accordance with the methods and uses provided herein can be non-cystic fibrosis bronchiectasis. In some aspects, the non-cystic fibrosis bronchiectasis was confirmed by chest computed tomography (CT) demonstrating bronchiectasis affecting 1 or more lobes in a subject.

[0190] A subject with bronchiectasis (e.g., non-cystic fibrosis bronchiectasis) suitable for treatment in accordance with the methods and uses provided herein can be a subject that is colonized with *Pseudomonas aeruginosa*. Subjects with *Pseudomonas aeruginosa* colonization can be identified, e.g., using routine sputum culture. In some aspects, the subject is colonized with a *Pseudomonas aeruginosa* strain comprising a genome comprising a Psl-operon. In some aspects, the subject is colonized with a *Pseudomonas aeruginosa* strain comprising a genome comprising a PcrV-encoding loci.

- [0191] In some aspects, the subject is chronically infected with *Pseudomonas aeruginosa*. As used herein, a subject who is “chronically infected” refers to a subject colonized with at least two isolates of *Pseudomonas aeruginosa*, concurrently or sequentially, while clinically stable over a year.
- [0192] A subject with bronchiectasis (e.g., non-cystic fibrosis bronchiectasis) suitable for treatment in accordance with the methods and uses provided herein can be a subject with airway neutrophilia and/or sputum neutrophilia.
- [0193] A subject with bronchiectasis (e.g., non-cystic fibrosis bronchiectasis) suitable for treatment in accordance with the methods and uses provided herein can be a subject with a history of at least two moderate to severe bronchiectasis exacerbations per year requiring antibiotics.
- [0194] A subject with bronchiectasis (e.g., non-cystic fibrosis bronchiectasis) suitable for treatment in accordance with the methods and uses provided herein can be a subject with a history of at least one exacerbation requiring hospital care.
- [0195] A subject with bronchiectasis (e.g., non-cystic fibrosis bronchiectasis) suitable for treatment in accordance with the methods and uses provided herein can be a subject on long term nebulized antibiotics.
- [0196] A subject with bronchiectasis (e.g., non-cystic fibrosis bronchiectasis) suitable for treatment in accordance with the methods and uses provided herein can be a subject with chronic obstructive pulmonary disease (COPD).
- [0197] A subject with bronchiectasis (e.g., non-cystic fibrosis bronchiectasis) suitable for treatment in accordance with the methods and uses provided herein can be a human subject.
- [0198] As provided herein, bronchiectasis (e.g., non-cystic fibrosis bronchiectasis) of any cause can be treated in accordance with the methods and uses provided herein. For example, in some aspects, the bronchiectasis (e.g., non-cystic fibrosis bronchiectasis) was caused by hypogammaglobulinemia. In some aspects, the bronchiectasis (e.g., non-cystic fibrosis bronchiectasis) was caused by common variable immunodeficiency. In some aspects, the bronchiectasis (e.g., non-cystic fibrosis bronchiectasis) was caused by alpha-1-antitrypsin deficiency.
- [0199] The methods and uses provided herein can comprise administering a bispecific antibody that specifically binds to *Pseudomonas aeruginosa* PcrV protein and Psl exopolysaccharide and that comprises a modified Fc region (e.g., AZD0292) to a subject.

The administration can be intravenous administration. The administration can be subcutaneous administration.

[0200] The methods and uses provided herein are effective in treating bronchiectasis (*e.g.*, non-cystic fibrosis bronchiectasis). In some aspects of the methods and uses provided herein, administration of a bispecific antibody that specifically binds to *Pseudomonas aeruginosa* PcrV protein and Psl exopolysaccharide and that comprises a modified Fc region (*e.g.*, AZD0292) reduces *Pseudomonas aeruginosa* in sputum cultures obtained from the subject, *e.g.*, as compared to the *Pseudomonas aeruginosa* in sputum cultures obtained from the subject prior to the administration. The reduction can occur, *e.g.*, within 12 weeks of the first administration of the bispecific antibody, within 8 weeks of the first administration of the bispecific antibody, or within 4 weeks of the first administration of the bispecific antibody. In some aspects of the methods and uses provided herein, administration of a bispecific antibody that specifically binds to *Pseudomonas aeruginosa* PcrV protein and Psl exopolysaccharide and that comprises a modified Fc region (*e.g.*, AZD0292) decreases antibiotic usage by the subject, *e.g.*, as compared to the subject's usage prior to the administration. In some aspects of the methods and uses provided herein, administration of a bispecific antibody that specifically binds to *Pseudomonas aeruginosa* PcrV protein and Psl exopolysaccharide (*e.g.*, AZD0292) eradicates *Pseudomonas aeruginosa* in the subject.

[0201] The methods and uses provided herein can comprise administering a bispecific antibody that specifically binds to *Pseudomonas aeruginosa* PcrV protein and Psl exopolysaccharide and that comprises a modified Fc region (*e.g.*, AZD0292) to a subject in combination with an antibiotic. In some aspects, the methods and uses provided herein comprise administering a bispecific antibody that specifically binds to *Pseudomonas aeruginosa* PcrV protein and Psl exopolysaccharide and that comprises a modified Fc region (*e.g.*, AZD0292) to a subject in combination with an aminoglycoside, ticarcillin, a ureidopenicillin, ciprofloxacin, cefepime, gentamicin, amikacin, tobramycin, ceftazidime, aztreonam, cefotaxime, meropenem, polymyxin b, or any combination thereof.

- b. Methods and Uses of Bispecific Anti-Pseudomonas Antibodies with Modified Fc Regions in the Prevention or Treatment of Nosocomial Infections

[0202] This disclosure provides a method of preventing nosocomial infection in a susceptible human subject, where the method includes administering to the subject a bispecific antibody that specifically binds to *Pseudomonas aeruginosa* PcrV protein and Psl exopolysaccharide and that comprises a modified Fc region (*e.g.*, AZD0292). A susceptible human subject is a person who is at risk of contracting a nosocomial infection but at the time of administration does not have an infection or shows no symptoms of an infection; or a person who has contracted a nosocomial infection that requires intervention or mitigation. The method further includes monitoring the subject for symptoms following administration of the bispecific antibody for, *e.g.*, through 1 day, 3 days, 5 days, 7 days, 10 days, 15 days, 21 days, 28 days, or 30 days. In an aspect, the method includes monitoring the subject for symptoms through at least about 21 days or more from the day of administration. “Nosocomial infections” are defined elsewhere herein and include, *e.g.*, pneumonia, bacteremia, bone infection, joint infection, skin infection, burn infection, wound infection, peritonitis, sepsis, and/or an abscess. Symptoms associated with nosocomial infections, *e.g.*, pneumonia, are known in the art. In certain aspects the nosocomial infection is caused by, or is exacerbated by *P. aeruginosa*. According to this method, the human subject is successfully treated if, *e.g.*, at 1 day, 3 days, 5 days, 8 days, 10 days, 15 days, 21 days, 28 days, or 30 days post-administration, the subject remains symptom-free (if the subject was symptom free at the time of administration) or displays less severe symptoms than would be expected if not treated with a bispecific anti-*Pseudomonas* antibody that comprises a modified Fc region (*e.g.*, AZD0292). In an aspect, the human subject is successfully treated if at 21 days post-administration, the subject remains symptom-free (if the subject was symptom free at the time of administration) or displays less severe symptoms than would be expected if not treated with a bispecific anti-*Pseudomonas* antibody that comprises a modified Fc region (*e.g.*, AZD0292). In another aspect, the human subject is successfully treated if at 28 or 30 days post-administration, the subject remains symptom-free (if the subject was symptom free at the time of administration) or displays less severe symptoms than would be expected if not treated with a bispecific anti-*Pseudomonas* antibody that comprises a modified Fc region (*e.g.*, AZD0292).

[0203] In another aspect this disclosure provides a method of preventing or treating pneumonia, *e.g.*, hospital acquired or not hospital acquired pneumonia in a susceptible human subject, where the method includes administering to the subject a bispecific antibody that specifically binds to *Pseudomonas aeruginosa* PcrV protein and Psl exopolysaccharide and that comprises a modified Fc region (*e.g.*, AZD0292). In certain aspects the pneumonia is nosocomial or iatrogenic. A susceptible human subject is a person who is at risk of contracting pneumonia but at the time of administration does not have pneumonia symptoms; or a person who has contracted pneumonia that requires intervention or mitigation. The method further includes monitoring the subject for pneumonia symptoms following administration of the bispecific antibody for, *e.g.*, through 1 day, 3 days, 5 days, 7 days, 10 days, 15 days, 21 days, 28 days, or 30 days. In an aspect, the method includes monitoring the subject for symptoms at least about 21 days from the day of administration. Symptoms associated with pneumonia are known in the art. In certain aspects the pneumonia is caused by, or is exacerbated by, *P. aeruginosa*. According to this method, the human subject is successfully treated if, *e.g.*, at 1 day, 3 days, 5 days, 8 days, 10 days, 15 days, 21 days, 28 days, or 30 days post-administration, the subject remains symptom-free (if the subject was symptom free at the time of administration) or displays less severe symptoms than would be expected if not treated with a bispecific anti-*Pseudomonas* antibody that comprises a modified Fc region (*e.g.*, AZD0292). In an aspect, the human subject is successfully treated if at 7 days post-administration, the subject remains symptom-free (if the subject was symptom-free at the time of administration) or displays less severe symptoms than would be expected if not treated with a bispecific anti-*Pseudomonas* antibody that comprises a modified Fc region (*e.g.*, AZD0292). In an aspect, the human subject is successfully treated if at 21 days post-administration, the subject remains symptom-free (if the subject was symptom-free at the time of administration) or displays less severe symptoms than would be expected if not treated with a bispecific anti-*Pseudomonas* antibody that comprises a modified Fc region (*e.g.*, AZD0292). In another aspect, the human subject is successfully treated if at 28 or 30 days post-administration, the subject remains symptom-free (if the subject was symptom free at the time of administration) or displays less severe symptoms than would be expected if not treated with a bispecific anti-*Pseudomonas* antibody that comprises a modified Fc region (*e.g.*, AZD0292).

[0204] In another aspect this disclosure provides a method of preventing or treating a disease caused by *Pseudomonas aeruginosa*, e.g., pneumonia, tracheobronchitis, bacteremia, endocarditis, meningitis, otitis media, bacterial keratitis, endophthalmitis, osteomyelitis, gastrointestinal disease, skin infection, septicemia, or any combination thereof, in a susceptible human subject. In certain aspects the disease caused by *Pseudomonas aeruginosa* is nosocomial or iatrogenic. A susceptible human subject is a person who is at risk of contracting a disease treatable or preventable by the methods provided herein but at the time of administration does not have disease symptoms; or a person who has contracted disease caused by *Pseudomonas aeruginosa* that requires treatment, intervention or mitigation.

[0205] The methods provided herein are suitable for use with susceptible human subjects as described elsewhere herein. Examples include subjects who are about to be hospitalized, are currently hospitalized, were recently hospitalized, are about to be, currently, or recently on a mechanical ventilator, or a combination thereof. Hospitalization, in some instances, can be in an intensive care unit (ICU). Mechanical ventilation, if required, can be through intubation, e.g., through an endotracheal or nasotracheal tube, or through a tracheostomy. Patients who are about to be, are currently, or were recently on mechanical ventilation can have a heightened risk of contracting a respiratory infection, e.g., pneumonia, e.g., *Pseudomonas aeruginosa* pneumonia. In those situations where mechanical ventilation is indicated, administration of a bispecific antibody as provided by the disclosed methods can reduce the risk of contracting pneumonia, for example, while currently on mechanical ventilation, after mechanical ventilation is no longer required, or a combination thereof.

[0206] In certain aspects the subject is colonized with *Pseudomonas aeruginosa* in the respiratory tract, e.g., the lower respiratory tract, at the time of administration of the bispecific antibody. In certain aspects the subject's respiratory tract is colonized with *P. aeruginosa* one, two, three, or four days prior to administration of the bispecific antibody. Colonization can be measured, e.g., by detection of *P. aeruginosa* in a tracheal aspirate within 6 hours, 12 hours, 24 hours, 48 hours, 72 hours, or 96 hours prior to administration of the bispecific antibody. In certain aspects of the methods provided herein, the subject has not received antibiotics considered active against the *P. aeruginosa* strain with which the subject is colonized prior to administration of the bispecific antibody. In certain aspects, the subject's respiratory tract can be additionally colonized by *Staphylococcus aureus* at

the time of administration of the bispecific anti-*Pseudomonas* antibody that comprises a modified Fc region (*e.g.*, AZD0292).

[0207] In certain aspects the subject does not have pneumonia symptoms at the time of administration of the bispecific antibody. Symptoms can be measured according to the Clinical Pulmonary Infection Score (CPIS). A lack of symptoms can be inferred *e.g.*, if at 24 hours prior to the administration of the bispecific antibody the subject has a CPIS of less than 6.

[0208] The methods provided herein include monitoring a subject for disease symptoms, *e.g.*, pneumonia symptoms, following administration of the bispecific antibody. In certain aspects, the subject can be monitored for pneumonia by chest x-ray, observation of respiratory signs or symptoms of pneumonia, microbiologic confirmation of pneumonia, or any combination thereof. A subject can be determined to have pneumonia, *e.g.*, when a new or worsening infiltrate consistent with pneumonia is observed on a chest x-ray, when the subject displays at least two minor or at least one major respiratory sign or symptoms of pneumonia, when a specimen obtained from the subject is positive for *P. aeruginosa* by culture, or any combination thereof. In certain aspects the specimen is a respiratory secretion of the subject. A respiratory secretion can be obtained from expectorated sputum, by endotracheal aspiration, by bronchoscopy with bronchoalveolar lavage, by use of a protected-specimen brush sampling in an intubated subject, or any combination thereof.

[0209] Minor respiratory signs or symptoms of pneumonia include, without limitation, a body temperature of greater than about 38°C, a core body temperature of less than about 35°C, a white blood cell count of greater than about 10,000 cells per cubic millimeter (mm³), a white blood cell count of less than about 4,500 cells per mm³, a band neutrophil count of greater than about 15%, production of new purulent endotracheal secretions or sputum, new auscultatory findings, dullness to percussion, a new onset of cough, dyspnea, tachypnea, hypoxemia, or any combination thereof. Major respiratory signs or symptoms of pneumonia can include, without limitation, an acute change made in the ventilatory support system to enhance oxygenation comprising a PaO₂/FiO₂ ratio less than about 240 mm Hg maintained for at least four hours, a decrease in the PaO₂/FiO₂ ratio of greater than about 50 mm Hg maintained for at least four hours, the necessity to initiate or reinstitute mechanical ventilation in a non-mechanically ventilated subject, or any combination thereof. Microbiologic confirmation of pneumonia can include, without limitation, a

respiratory specimen positive for *P. aeruginosa* by culture, a blood culture positive for *P. aeruginosa*, a pleural fluid aspirate or lung tissue culture positive for *P. aeruginosa*, or any combination thereof.

[0210] In certain aspects, the methods provided by this disclosure can further include administering an antibiotic to the subject prior to, concurrently with, and/or following administration of the bispecific antibody. Suitable antibiotics can include, without limitation, aminoglycosides, ticarcillin, ureidopenicillins, ciprofloxacin, cefepime, gentamicin, amikacin, tobramycin, ceftazidime, aztreonam, cefotaxime, or any combination thereof. Suitable dosages and length of treatment can be readily determined by a healthcare provider. In certain aspects, the *P. aeruginosa* strain with which the subject is colonized is sensitive to the antibiotic chosen for administration. In other aspects, however, the *P. aeruginosa* strain with which the subject is colonized is resistant or partially resistant to one or more of the available antibiotics chosen for administration.

IV. Methods for producing bispecific anti-Pseudomonas antibodies

[0211] A bispecific anti-*Pseudomonas* antibody that comprises a modified Fc region (e.g., AZD0292) of the disclosure can be produced by any method known in the art for the synthesis of antibodies, in particular, by chemical synthesis or by recombinant expression techniques.

[0212] The present disclosure also provides polynucleotides comprising a nucleic acid sequence encoding an anti-*Pseudomonas aeruginosa* Psl and PcrV bispecific antibody comprising a modified IgG constant domain of the disclosure or an FcRn-binding fragment thereof (e.g., an Fc region or hinge-Fc region), as well as vectors comprising said polynucleotides. In particular aspects, the present disclosure provides an isolated polynucleotide comprising a nucleic acid molecule encoding the heavy chain of the bispecific antibody described herein. In some aspects, the isolated polynucleotide further comprises a nucleic acid molecule encoding the light chain of the bispecific antibody described herein.

[0213] The nucleotide sequence of the anti-*Pseudomonas aeruginosa* Psl and PcrV bispecific antibodies comprising modified IgG constant domain and the polynucleotides encoding the same may be obtained by any methods known in the art, including general DNA sequencing method, such as dideoxy chain termination method (Sanger sequencing), and oligonucleotide priming in combination with PCR, respectively.

- [0214] The nucleotide sequence encoding an antibody may be obtained from any information available to those of skill in the art (i.e., from Genbank, the literature, or by routine cloning). If a clone containing a nucleic acid encoding a particular antibody or an epitope-binding fragment thereof is not available, but the sequence of the antibody molecule or epitope-binding fragment thereof is known, a nucleic acid encoding the immunoglobulin may be chemically synthesized or obtained from a suitable source (*e.g.*, an antibody cDNA library, or a cDNA library generated from, or nucleic acid, for example poly A⁺ RNA, isolated from any tissue or cells expressing the antibody, such as hybridoma cells selected to express an antibody) by PCR amplification using synthetic primers hybridizable to the 3' and 5' ends of the sequence or by cloning using an oligonucleotide probe specific for the particular gene sequence to identify, *e.g.*, a cDNA clone from a cDNA library that encodes the antibody. Amplified nucleic acids generated by PCR may then be cloned into replicable cloning vectors using any method well known in the art.
- [0215] Once the nucleotide sequence of the antibody is determined, the nucleotide sequence of the antibody may be manipulated using methods well known in the art for the manipulation of nucleotide sequences, *e.g.*, recombinant DNA techniques, site directed mutagenesis, PCR, etc. (see, for example, the techniques described in Sambrook *et al.*, 1990, Molecular Cloning, A Laboratory Manual, 2d Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY; and Ausubel *et al.*, eds., 1998, Current Protocols in Molecular Biology, John Wiley & Sons, NY, which are both incorporated by reference herein in their entireties), to generate antibodies having a different amino acid sequence by, for example, introducing amino acid substitutions, deletions, and/or insertions into the epitope-binding domain regions of the antibodies, for example, into the hinge-Fc regions of the antibodies which are involved in the interaction with FcRn. Antibodies having one or more modifications in amino acid residues 432-437 or other locations can be generated.
- [0216] In particular embodiments, the nucleic acid molecule encoding the heavy chain of the bispecific antibody has a sequence according to SEQ ID NO: 45. In particular embodiments, the nucleic acid molecule encoding the light chain of the bispecific antibody has a sequence according to SEQ ID NO: 46.
- [0217] Recombinant expression of an antibody requires construction of an expression vector containing a nucleotide sequence that encodes the antibody. Once a nucleotide sequence encoding an antibody molecule or a heavy or light chain of an antibody, or portion

thereof (optionally, but not necessarily, containing the heavy or light chain variable region) has been obtained, the vector for the production of the antibody molecule may be produced by recombinant DNA technology using techniques well known in the art. Thus, methods for preparing a protein by expressing a polynucleotide containing an antibody encoding nucleotide sequence are described herein. Methods which are well known to those skilled in the art can be used to construct expression vectors containing antibody coding sequences and appropriate transcriptional and translational control signals. These methods include, for example, in vitro recombinant DNA techniques, synthetic techniques, and in vivo genetic recombination. The disclosure, thus, provides replicable vectors comprising a nucleotide sequence encoding the constant region of the antibody molecule with one or more modifications in the amino acid residues involved in the interaction with FcRn (see, *e.g.*, PCT Publication WO 86/05807; PCT Publication WO 89/01036; and U.S. Patent No. 5,122,464). The nucleotide sequence encoding the heavy-chain variable region, light-chain variable region, both the heavy-chain and light-chain variable regions, an epitope-binding fragment of the heavy- and/or light-chain variable region, or one or more complementarity determining regions (CDRs) of an antibody may be cloned into such a vector for expression.

[0218] Thus, in some aspects provided herein, the present disclosure relates to a vector comprising (i) a nucleic acid molecule encoding the heavy chain of the bispecific antibody, or (ii) a nucleic acid molecule encoding the heavy chain of the bispecific antibody described herein and a nucleic acid molecule encoding the light chain of the bispecific antibody described herein. In some aspects, the present disclosure relates to a pair of vectors, wherein the first vector of the pair of vectors comprises a nucleic acid molecule encoding the heavy chain of the bispecific antibody described herein, and the second vector of the pair of vectors comprises a nucleic acid molecule encoding the light chain of the bispecific antibody described herein. In particular embodiments, the nucleic acid molecule encoding the heavy chain of the bispecific antibody has a sequence according to SEQ ID NO: 45. In particular embodiments, the nucleic acid molecule encoding the light chain of the bispecific antibody has a sequence according to SEQ ID NO: 46.

[0219] The expression vector is transferred to a host cell by conventional techniques and the transfected cells are then cultured by conventional techniques to produce an antibody having an increased affinity for FcRn and an increased in vivo half-life. Thus, the disclosure

includes host cells containing a polynucleotide encoding an antibody, a constant domain or a FcRn binding fragment thereof having one or more modifications in amino acid residues 432-437 or other locations, optionally operably linked to a heterologous promoter.

[0220] A variety of host-expression vector systems may be utilized to express the antibody molecules of the disclosure. Such host-expression systems represent vehicles by which the coding sequences of interest may be produced and subsequently purified, but also represent cells which may, when transformed or transfected with the appropriate nucleotide coding sequences, express an antibody molecule of the disclosure *in situ*. These include, but are not limited to, microorganisms such as bacteria (*e.g.*, *E. coli* and *B. subtilis*) transformed with recombinant bacteriophage DNA, plasmid DNA or cosmid DNA expression vectors containing antibody coding sequences; yeast (*e.g.*, *Saccharomyces* and *Pichia*) transformed with recombinant yeast expression vectors containing antibody coding sequences; insect cell systems infected with recombinant virus expression vectors (*e.g.*, baculovirus) containing antibody coding sequences; plant cell systems infected with recombinant virus expression vectors (*e.g.*, cauliflower mosaic virus, CaMV; and tobacco mosaic virus, TMV) or transformed with recombinant plasmid expression vectors (*e.g.*, Ti plasmid) containing antibody coding sequences; and mammalian cell systems (*e.g.*, COS, CHO, BHK, 293, 3T3 and NS0 cells) harboring recombinant expression constructs containing promoters derived from the genome of mammalian cells (*e.g.*, metallothionein promoter) or from mammalian viruses (*e.g.*, the adenovirus late promoter; the vaccinia virus 7.5K promoter). Bacterial cells such as *Escherichia coli*, and eukaryotic cells, which are well-suited for the expression of whole recombinant antibody molecule, are used for the expression of a recombinant antibody molecule. For example, mammalian cells such as Chinese hamster ovary cells (CHO), in conjunction with a vector such as the major intermediate early gene promoter element from human cytomegalovirus is an effective expression system for antibodies (Foecking *et al.*, *Gene*, 45:101, 1986, and Cockett *et al.*, *Bio/Technology*, 8:2, 1990).

[0221] In bacterial systems, a number of expression vectors may be advantageously selected depending upon the use intended for the antibody molecule being expressed. For example, when a large quantity of such a protein is to be produced, for the generation of pharmaceutical compositions of an antibody molecule, vectors which direct the expression of high levels of fusion protein products that are readily purified may be desirable. Such vectors include, but are not limited to, the *E. coli* expression vector pUR278 (Ruther *et al.*,

EMBO, 12:1791, 1983), in which the antibody coding sequence may be ligated individually into the vector in frame with the lacZ coding region so that a fusion protein is produced; and pIN vectors (Inouye & Inouye, Nucleic Acids Res., 13:3101-3109, 1985 and Van Heeke & Schuster, J. Biol. Chem., 24:5503-5509, 1989).

[0222] In an insect system, Autographa californica nuclear polyhedrosis virus (AcNPV) is used as a vector to express foreign genes. The virus grows in Spodoptera frugiperda cells. The antibody coding sequence may be cloned individually into non-essential regions (for example the polyhedrin gene) of the virus and placed under control of an AcNPV promoter (for example the polyhedrin promoter).

[0223] In mammalian host cells, a number of viral-based expression systems may be utilized to express an antibody molecule of the disclosure. In cases where an adenovirus is used as an expression vector, the antibody coding sequence of interest may be ligated to an adenovirus transcription/translation control complex, *e.g.*, the late promoter and tripartite leader sequence. This chimeric gene may then be inserted in the adenovirus genome by *in vitro* or *in vivo* recombination. Insertion in a non-essential region of the viral genome (*e.g.*, region EI or E3) will result in a recombinant virus that is viable and capable of expressing the antibody molecule in infected hosts (*e.g.*, see Logan & Shenk, Proc. Natl. Acad. Sci. USA, 81:355-359, 1984). Specific initiation signals may also be required for efficient translation of inserted antibody coding sequences. These signals include the ATG initiation codon and adjacent sequences. Furthermore, the initiation codon must be in phase with the reading frame of the desired coding sequence to ensure translation of the entire insert. These exogenous translational control signals and initiation codons can be of a variety of origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of appropriate transcription enhancer elements, transcription terminators, etc. (see, *e.g.*, Bitter *et al.*, Methods in Enzymol., 153:516-544, 1987).

[0224] In addition, a host cell strain may be chosen which modulates the expression of the antibody sequences, or modifies and processes the antibody in the specific fashion desired. Such modifications (*e.g.*, glycosylation) and processing (*e.g.*, cleavage) of protein products may be important for the function of the antibody. Different host cells have characteristic and specific mechanisms for the post-translational processing and modification of proteins and gene products. Appropriate cell lines or host systems can be chosen to ensure the correct modification and processing of the antibody expressed. To this end, eukaryotic host

cells which possess the cellular machinery for proper processing of the primary transcript, glycosylation, and phosphorylation of the gene product may be used. Such mammalian host cells include but are not limited to CHO, VERY, BHK, HeLa, COS, MDCK, 293, 3T3, W138, and in particular, myeloma cells such as NS0 cells, and related cell lines, see, for example, Morrison *et al.*, U.S. Patent No. 5,807,715, which is hereby incorporated by reference in its entirety.

[0225] For long-term, high-yield production of recombinant antibodies, stable expression is preferred. For example, cell lines which stably express the antibody molecule may be engineered. Rather than using expression vectors which contain viral origins of replication, host cells can be transformed with DNA controlled by appropriate expression control elements (*e.g.*, promoter, enhancer, sequences, transcription terminators, polyadenylation sites, etc.), and a selectable marker. Following the introduction of the foreign DNA, engineered cells may be allowed to grow for 1-2 days in an enriched media, and then are switched to a selective media. The selectable marker in the recombinant plasmid confers resistance to the selection and allows cells to stably integrate the plasmid into their chromosomes and grow to form foci which in turn can be cloned and expanded into cell lines. This method may advantageously be used to engineer cell lines which express the antibody molecule. Such engineered cell lines may be particularly useful in screening and evaluation of compositions that interact directly or indirectly with the antibody molecule.

[0226] A number of selection systems may be used, including but not limited to, the herpes simplex virus thymidine kinase (Wigler *et al.*, Cell, 11:223, 1977), hypoxanthineguanine phosphoribosyltransferase (Szybalska & Szybalski, Proc. Natl. Acad. Sci. USA, 48:202, 1992), and adenine phosphoribosyltransferase (Lowy *et al.*, Cell, 22:8-17, 1980) genes can be employed in tk-, hgprt- or aprt- cells, respectively. Also, antimetabolite resistance can be used as the basis of selection for the following genes: dhfr, which confers resistance to methotrexate (Wigler *et al.*, Natl. Acad. Sci. USA, 77:357, 1980 and O'Hare *et al.*, Proc. Natl. Acad. Sci. USA, 78:1527, 1981); gpt, which confers resistance to mycophenolic acid (Mulligan & Berg, Proc. Natl. Acad. Sci. USA, 78:2072, 1981); neo, which confers resistance to the aminoglycoside G-418 (Wu and Wu, Biotherapy, 3:87-95, 1991; Tolstoshev, Ann. Rev. Pharmacol. Toxicol., 32:573-596, 1993; Mulligan, Science, 260:926-932, 1993; and Morgan and Anderson, Ann. Rev. Biochem., 62: 191-217, 1993; and May, TIB TECH, 11(5):155-215, 1993); and hygromycin, which confers resistance to

hygromycin (Santerre *et al.*, Gene, 30:147, 1984). Methods commonly known in the art of recombinant DNA technology may be routinely applied to select the desired recombinant clone, and such methods are described, for example, in Ausubel *et al.* (eds.), 1993, Current Protocols in Molecular Biology, John Wiley & Sons, NY; Kriegler, 1990, Gene Transfer and Expression, A Laboratory Manual, Stockton Press, NY; in Chapters 12 and 13, Dracopoli *et al.* (eds), 1994, Current Protocols in Human Genetics, John Wiley & Sons, NY; and Colberre-Garapin *et al.*, J. Mol. Biol., 150:1, 1981, which are incorporated by reference herein in their entireties.

[0227] The expression levels of an antibody molecule can be increased by vector amplification (for a review, see Bebbington and Hentschel, 1987, The use of vectors based on gene amplification for the expression of cloned genes in mammalian cells in DNA cloning, Vol.3. Academic Press, New York). When a marker in the vector system expressing antibody is amplifiable, increase in the level of inhibitor present in culture of host cell will increase the number of copies of the marker gene. Since the amplified region is associated with the antibody gene, production of the antibody will also increase (Crouse *et al.*, Mol., Cell. Biol., 3:257, 1983).

[0228] The host cell may be co-transfected with two expression vectors of the disclosure, the first vector encoding a heavy chain derived polypeptide and the second vector encoding a light chain derived polypeptide. The two vectors may contain identical selectable markers which enable equal expression of heavy and light chain polypeptides or different selectable markers to ensure maintenance of both plasmids. Alternatively, a single vector may be used which encodes, and is capable of expressing, both heavy and light chain polypeptides. In such situations, the light chain should be placed before the heavy chain to avoid an excess of toxic free heavy chain (Proudfoot, Nature, 322:52, 1986; and Kohler, Proc. Natl. Acad. Sci. USA, 77:2 197, 1980). The coding sequences for the heavy and light chains may comprise cDNA or genomic DNA.

[0229] Once a bispecific anti-*Pseudomonas* antibody that comprises a modified Fc region (*e.g.*, AZD0292) of the disclosure has been produced by recombinant expression, it may be purified by any method known in the art for purification of an immunoglobulin molecule, for example, by chromatography (*e.g.*, ion exchange, affinity, particularly by affinity for the specific antigen after Protein A purification, and sizing column chromatography), centrifugation, differential solubility, or by any other standard techniques for the

purification of proteins. Further, the antibodies of the present disclosure or fragments thereof may be fused to heterologous polypeptide sequences described herein or otherwise known in the art to facilitate purification.

V. Pharmaceutical Compositions

- [0230] Pharmaceutical compositions used in this disclosure can comprise anti-*Pseudomonas aeruginosa* Psl and PcrV bispecific antibodies that comprise modified Fc regions (e.g., AZD0292) and pharmaceutically acceptable carriers well known to those of ordinary skill in the art. Preparations for parenteral administration include sterile aqueous or non-aqueous solutions, suspensions, and emulsions.
- [0231] The route of administration of an anti-*Pseudomonas aeruginosa* Psl and PcrV bispecific antibody with a modified Fc region can be, for example, parenteral. The term parenteral as used herein includes, e.g., intravenous and subcutaneous administration. Accordingly, a pharmaceutical composition comprising an anti-*Pseudomonas aeruginosa* Psl and PcrV bispecific antibody that comprises a modified Fc region (e.g., AZD0292) can be formulated for intravenous administration. In some aspects, a pharmaceutical composition comprising an anti-*Pseudomonas aeruginosa* Psl and PcrV bispecific antibody that comprises a modified Fc region (e.g., AZD0292) can be formulated for subcutaneous administration. A suitable form for administration would be a solution for injection.
- [0232] Pharmaceutical compositions comprising anti-*Pseudomonas aeruginosa* Psl and PcrV bispecific antibodies that comprise modified Fc regions (e.g., AZD0292) can be administered and/or formulated, e.g., for the treatment of a disease of the disclosure.
- [0233] Anti-*Pseudomonas aeruginosa* Psl and PcrV bispecific antibodies that comprise a modified Fc region (e.g., AZD0292) can be administered in a pharmaceutically effective amount for the *in vivo* treatment of or prevention of *Pseudomonas aeruginosa* infection.
- [0234] As provided herein, pharmaceutical compositions comprising anti-*Pseudomonas aeruginosa* Psl and PcrV bispecific antibodies that comprise a modified Fc region (e.g., AZD0292) can be formulated for administration in combination with an antibiotic. In some aspects, the bispecific antibodies are formulated for administration in combination with an aminoglycoside, ticarcillin, a ureidopenicillin, ciprofloxacin, cefepime, gentamicin, amikacin, tobramycin, ceftazidime, aztreonam, cefotaxime, meropenem, polymyxin b, or any combination thereof.

VI. Kits Comprising Bispecific Anti-*Pseudomonas* Antibodies with Modified Fc Regions

[0235] The disclosure also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the pharmaceutical compositions of the disclosure. Optionally associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration.

[0236] The present disclosure provides kits that can be used in the above methods. In one aspect, a kit comprises an anti-*Pseudomonas aeruginosa* Psl and PcrV bispecific antibody that comprises a modified Fc region of the disclosure (e.g., AZD0292), optionally in a purified form, in one or more containers. In a specific aspect, the kits of the present disclosure contain a substantially isolated antigen or combination of antigens (e.g., PcrV and Psl) as a control. Optionally, the kits of the present disclosure further comprise a control antibody, fusion protein, or conjugated molecule which does not react with the antigen included in the kit. In another specific aspect, the kits of the present disclosure contain a means for detecting the binding of an anti-*Pseudomonas aeruginosa* Psl and PcrV bispecific antibody that comprises a modified Fc region of the disclosure (e.g., AZD0292) to an antigen (e.g., the anti-*Pseudomonas aeruginosa* Psl and PcrV bispecific antibody that comprises a modified Fc region of the disclosure (e.g., AZD0292), may be conjugated to a detectable substrate such as a fluorescent compound, an enzymatic substrate, a radioactive compound or a luminescent compound, or a second antibody which recognizes the first antibody may be conjugated to a detectable substrate). In specific aspects, the kit may include a recombinantly produced or chemically synthesized antigen or combination of antigens. The antigen(s) provided in the kit may also be attached to a solid support. In a more specific aspect the detecting means of the above-described kit includes a solid support to which an antigen or a combination of antigens is attached. Such a kit may also include a non-attached reporter-labeled anti-human antibody. In this aspect, binding of the antibody to the antigen can be detected by binding of the said reporter-labeled antibody.

VII. Immunoassays

[0237] Anti-*Pseudomonas* Psl and PcrV bispecific antibodies that comprise a modified Fc region of the disclosure (e.g., AZD0292) can be assayed for immunospecific binding by

any method known in the art. The immunoassays which can be used include but are not limited to competitive and non-competitive assay systems using techniques such as western blots, radioimmunoassays, ELISA (enzyme linked immunosorbent assay), "sandwich" immunoassays, immunoprecipitation assays, precipitin reactions, gel diffusion precipitin reactions, immunodiffusion assays, agglutination assays, complement-fixation assays, immunoradiometric assays, fluorescent immunoassays, protein A immunoassays, to name but a few. Such assays are routine and well known in the art (*see, e.g., Ausubel et al., eds, Current Protocols in Molecular Biology, John Wiley & Sons, Inc., New York, Vol. 1 (1994), which is incorporated by reference herein in its entirety*). Exemplary immunoassays are described briefly below (but are not intended by way of limitation).

[0238] There are a variety of methods available for measuring the affinity of an antibody-antigen interaction, but relatively few for determining rate constants. Most of the methods rely on either labeling antibody or antigen, which inevitably complicates routine measurements and introduces uncertainties in the measured quantities. Antibody affinity can be measured by a number of methods, including OCTET[®], BIACORE[®], ELISA, and FACS.

[0239] The OCTET[®] system uses biosensors in a 96-well plate format to report kinetic analysis. Protein binding and dissociation events can be monitored by measuring the binding of one protein in solution to a second protein immobilized on the FortéBio biosensor. In the case of measuring binding of anti-*Pseudomonas* Psl and PcrV bispecific antibodies with Fc region modifications by immobilizing onto OCTET[®] tips followed by analysis of binding of the antibody, which is in solution. Association and disassociation of antibody to immobilized anti-*Pseudomonas* Psl and PcrV bispecific antibodies with Fc region modifications is then detected by the instrument sensor. The data is then collected and exported to GraphPad Prism for affinity curve fitting.

[0240] Surface plasmon resonance (SPR) as performed on BIACORE[®] offers a number of advantages over conventional methods of measuring the affinity of antibody-antigen interactions: (i) no requirement to label either antibody or antigen; (ii) antibodies do not need to be purified in advance, cell culture supernatant can be used directly; (iii) real-time measurements, allowing rapid semi-quantitative comparison of different monoclonal antibody interactions, are enabled and are sufficient for many evaluation purposes; (iv) biospecific surface can be regenerated so that a series of different monoclonal antibodies

can easily be compared under identical conditions; (v) analytical procedures are fully automated, and extensive series of measurements can be performed without user intervention. BIAapplications Handbook, version AB (reprinted 1998), BIACORE[®] code No. BR-1001-86; BIAtechnology Handbook, version AB (reprinted 1998), BIACORE[®] code No. BR-1001-84.

[0241] SPR based binding studies require that one member of a binding pair be immobilized on a sensor surface. The binding partner immobilized is referred to as the ligand. The binding partner in solution is referred to as the analyte. In some cases, the ligand is attached indirectly to the surface through binding to another immobilized molecule, which is referred to as the capturing molecule. SPR response reflects a change in mass concentration at the detector surface as analytes bind or dissociate.

[0242] Based on SPR, real-time BIACORE[®] measurements monitor interactions directly as they happen. The technique is well suited to determination of kinetic parameters. Comparative affinity ranking is extremely simple to perform, and both kinetic and affinity constants can be derived from the sensorgram data.

[0243] When analyte is injected in a discrete pulse across a ligand surface, the resulting sensorgram can be divided into three essential phases: (i) Association of analyte with ligand during sample injection; (ii) Equilibrium or steady state during sample injection, where the rate of analyte binding is balanced by dissociation from the complex; (iii) Dissociation of analyte from the surface during buffer flow.

[0244] The association and dissociation phases provide information on the kinetics of analyte-ligand interaction (k_a and k_d , the rates of complex formation and dissociation, $k_d/k_a = K_D$). The equilibrium phase provides information on the affinity of the analyte-ligand interaction (K_D).

[0245] BIAevaluation software provides comprehensive facilities for curve fitting using both numerical integration and global fitting algorithms. With suitable analysis of the data, separate rate and affinity constants for interaction can be obtained from simple BIACORE[®] investigations. The range of affinities measurable by this technique is very broad ranging from mM to pM.

[0246] Epitope specificity is an important characteristic of a monoclonal antibody. Epitope mapping with BIACORE[®], in contrast to conventional techniques using radioimmunoassay, ELISA or other surface adsorption methods, does not require labeling

or purified antibodies, and allows multi-site specificity tests using a sequence of several monoclonal antibodies. Additionally, large numbers of analyses can be processed automatically.

[0247] Pair-wise binding experiments test the ability of two MAb to bind simultaneously to the same antigen. MAbs directed against separate epitopes will bind independently, whereas MAbs directed against identical or closely related epitopes will interfere with each other's binding. These binding experiments with BIACORE® are straightforward to carry out.

[0248] For example, one can use a capture molecule to bind the first Mab, followed by addition of antigen and second Mab sequentially. The sensorgrams will reveal: 1. how much of the antigen binds to first Mab, 2. to what extent the second MAb binds to the surface-attached antigen, 3. if the second MAb does not bind, whether reversing the order of the pair-wise test alters the results.

[0249] Peptide inhibition is another technique used for epitope mapping. This method can complement pair-wise antibody binding studies, and can relate functional epitopes to structural features when the primary sequence of the antigen is known. Peptides or antigen fragments are tested for inhibition of binding of different MAbs to immobilized antigen. Peptides which interfere with binding of a given MAb are assumed to be structurally related to the epitope defined by that MAb.

VIII. Diagnostic Uses

[0250] In some aspects provided herein, the anti-*Pseudomonas aeruginosa* Psl and PcrV bispecific antibodies with modified Fc regions are useful for detecting the presence of *Pseudomonas aeruginosa* Psl and/or PcrV in a sample or an individual. The term "detecting" as used herein encompasses quantitative or qualitative detection. Provided herein are methods of using the antibodies of this disclosure for diagnostic purposes, such as the detection of *Pseudomonas aeruginosa* Psl and/or PcrV in an individual or in tissue samples derived from an individual. In some aspects, the individual is a human. The detection method can involve quantification of the antigen-bound antibody. Antibody detection in biological samples may occur with any method known in the art, including immunofluorescence microscopy, immunocytochemistry, immunohistochemistry, ELISA, FACS analysis, immunoprecipitation, or micro-positron emission tomography. In certain aspects, the antibody is radiolabeled, for example with ¹⁸F and subsequently detected

utilizing micro-positron emission tomography analysis. Antibody-binding may also be quantified in a patient by non-invasive techniques such as positron emission tomography (PET), X-ray computed tomography, single-photon emission computed tomography (SPECT), computed tomography (CT), and computed axial tomography (CAT).

[0251] The practice of the disclosure will employ, unless otherwise indicated, conventional techniques of cell biology, cell culture, molecular biology, transgenic biology, microbiology, recombinant DNA, and immunology, which are within the skill of the art. Such techniques are explained fully in the literature. See, for example, *Molecular Cloning A Laboratory Manual*, 2nd Ed., Sambrook *et al.*, ed., Cold Spring Harbor Laboratory Press: (1989); *Molecular Cloning: A Laboratory Manual*, Sambrook *et al.*, ed., Cold Springs Harbor Laboratory, New York (1992), *DNA Cloning*, D. N. Glover ed., Volumes I and II (1985); *Oligonucleotide Synthesis*, M. J. Gait ed., (1984); Mullis *et al.* U.S. Pat. No: 4,683,195; *Nucleic Acid Hybridization*, B. D. Hames & S. J. Higgins eds. (1984); *Transcription And Translation*, B. D. Hames & S. J. Higgins eds. (1984); *Culture Of Animal Cells*, R. I. Freshney, Alan R. Liss, Inc., (1987); *Immobilized Cells And Enzymes*, IRL Press, (1986); B. Perbal, *A Practical Guide To Molecular Cloning* (1984); the treatise, *Methods In Enzymology*, Academic Press, Inc., N.Y.; *Gene Transfer Vectors For Mammalian Cells*, J. H. Miller and M. P. Calos eds., Cold Spring Harbor Laboratory (1987); *Methods In Enzymology*, Vols. 154 and 155 (Wu *et al.* eds.); *Immunochemical Methods In Cell And Molecular Biology*, Mayer and Walker, eds., Academic Press, London (1987); *Handbook Of Experimental Immunology*, Volumes I-IV, D. M. Weir and C. C. Blackwell, eds., (1986); *Manipulating the Mouse Embryo*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., (1986); and in Ausubel *et al.*, *Current Protocols in Molecular Biology*, John Wiley and Sons, Baltimore, Maryland (1989).

[0252] General principles of antibody engineering are set forth in *Antibody Engineering*, 2nd edition, C.A.K. Borrebaeck, Ed., Oxford Univ. Press (1995). General principles of protein engineering are set forth in *Protein Engineering, A Practical Approach*, Rickwood, D., *et al.*, Eds., IRL Press at Oxford Univ. Press, Oxford, Eng. (1995). General principles of antibodies and antibody-hapten binding are set forth in: Nisonoff, A., *Molecular Immunology*, 2nd ed., Sinauer Associates, Sunderland, MA (1984); and Steward, M.W., *Antibodies, Their Structure and Function*, Chapman and Hall, New York, NY (1984).

Additionally, standard methods in immunology known in the art and not specifically described are generally followed as in *Current Protocols in Immunology*, John Wiley & Sons, New York; Stites *et al.* (eds), *Basic and Clinical Immunology* (8th ed.), Appleton & Lange, Norwalk, CT (1994) and Mishell and Shiigi (eds), *Selected Methods in Cellular Immunology*, W.H. Freeman and Co., New York (1980).

[0253] Standard reference works setting forth general principles of immunology include *Current Protocols in Immunology*, John Wiley & Sons, New York; Klein, J., *Immunology: The Science of Self-Nonself Discrimination*, John Wiley & Sons, New York (1982); Kennett, R., *et al.*, eds., *Monoclonal Antibodies, Hybridoma: A New Dimension in Biological Analyses*, Plenum Press, New York (1980); Campbell, A., "Monoclonal Antibody Technology" in Burden, R., *et al.*, eds., *Laboratory Techniques in Biochemistry and Molecular Biology*, Vol. 13, Elsevier, Amsterdam (1984), *Kuby Immunology* 4th ed. Ed. Richard A. Goldsby, Thomas J. Kindt and Barbara A. Osborne, H. Freeman & Co. (2000); Roitt, I., Brostoff, J. and Male D., *Immunology* 6th ed. London: Mosby (2001); Abbas A., Abul, A. and Lichtman, A., *Cellular and Molecular Immunology* Ed. 5, Elsevier Health Sciences Division (2005); Kontermann and Dubel, *Antibody Engineering*, Springer Verlag (2001); Sambrook and Russell, *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor Press (2001); Lewin, *Genes VIII*, Prentice Hall (2003); Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Press (1988); Dieffenbach and Dveksler, *PCR Primer* Cold Spring Harbor Press (2003).

EXAMPLES

[0254] The present disclosure is illustrated by the following examples. It is to be understood that the particular examples, materials, amounts, and procedures are to be interpreted broadly in accordance with the scope and spirit of the disclosure as set forth herein.

Example 1: AZD0292 construction and opsonophagocytic killing and anti-cytotoxicity activity

[0255] The gremubamab antibody (also known as MEDI3902) is a bispecific antibody containing an anti-Psl antigen-binding domain and an anti-PcrV antigen-binding domain. Gremubamab antibody sequences are provided above in Table 1. A new antibody, called

AZD0292 was constructed using the same anti-Psl and anti-PcrV antigen-binding domains, but containing an N3Y half-life extension modification within the CH3 domain. The AZD0292 antibody was constructed as described in DiGiandomenico et al., 2014 (Sci. Trans. Med.) except that the wild-type sequence MHEALHNHYTQKSLSLS (SEQ ID NO: 32) was replaced with that of a modified Fc sub-sequence comprising the N3Y mutations (underlined): MHEACSYHLCQKSLSLS (SEQ ID NO: 33).

[0256] Monoclonal antibodies targeting Psl and PcrV are known to mediate opsonophagocytic killing (OPK) and anti-cytotoxic activity, respectively, against *P. aeruginosa*. (See DiGiandomenico et al., 2014 Sci. Trans. Med.). Therefore, AZD0292 was evaluated for its ability to mediate complement-dependent opsonophagocytic killing activity of a luminescent *P. aeruginosa* strain (DiGiandomenico et al., 2014 Sci. Trans. Med) in comparison to gremubamab, anti-Psl monoclonal antibody (mAb) Psl0096, anti-PcrV mAb V2L2-MD, and a control IgG. This assay was performed in 96-well plate using 0.025 mL of each component; luminescent *P. aeruginosa* strain PAO1, (DiGiandomenico et al., 2012 J. Exp. Med), diluted baby rabbit serum (Cedar Lane), differentiated HL-60 cells, and monoclonal antibody. Data was acquired utilizing a Tecan Spark Multimode Microplate Reader (Tecan), and then subsequently plotted as percent killed, in comparison to a control lacking antibody at various temperatures. The results are shown in **FIG. 1A**. AZD0292, gremubamab and anti-Psl mAb Psl0096 mediated similar OPK activity against *P. aeruginosa*. As expected, no opsonophagocytic killing activity was observed with anti-PcrV mAb V2L2-MD or the control IgG antibodies.

[0257] AZD0292 was also evaluated for its anti-cytotoxic activity. This assay was performed as described in DiGiandomenico et al., 2014., Sci. Trans. Med. The tested antibodies were first added to A549 cells (2×10^4 cells/well), which were seeded in white 96-well plates (provided by Nunc Nunclon Delta) in Dulbecco's modified Eagle's medium plus 10% fetal bovine serum. Log-phase *P. aeruginosa* strain 6077, which is capable of expressing Exoenzyme U, was then added at a desired multiplicity of infection (MOI) of 10, and incubated for 2 hours at 37°C with 5% CO₂. Afterwards, release of lactate dehydrogenase from lysed cells was measured. Data was acquired utilizing a Tecan Spark Multimode Microplate Reader (Tecan). The results are shown in **FIG. 1B**. In this assay, AZD0292, gremubamab, and anti-PcrV mAb V2L2-MD prevented cell death at similar

efficacies, whereas a control IgG and anti-Psl mAb Psl0096 had no protective activity as expected.

[0258] These results indicate that AZD0292 and gremubamab function equivalently in both anti-Psl and anti-PcrV functional activity assays.

[0259] Next, to test the impact of different half-life extension technologies, AZD0292 was evaluated for its ability to mediate complement-dependent opsonophagocytic killing activity of a luminescent *P. aeruginosa* strain (DiGiandomenico *et al.*, 2014 Sci. Trans. Med) in comparison to gremubamab, afucosylated (Afuc) versions of gremubamab withYTE half-life extension (SEQ ID NO:43) and without theYTE half-life extension (M252Y/S254T/T256E substitutions in the Fc region, numbering according to Kabat) and a control IgG. A Chinese Hamster Ovary (CHO) transient expression system was used to generate afucosylated IgG. There are several methods now established to generate recombinant afucosylated proteins. For example titration of fucose into cell culture media (Louie *et al* Biotechnol Bioeng. 2017 Mar;114(3):632-644), co-expression of GDP-6-deoxy-D-lyxo-4-hexulose reductase (von Horsten *et al* Glycobiology. 2010 Dec;20(12):1607-18), co-expression of an anti-FUT8 intrabody (Joubert *et al* Biotechnol Bioeng. 2022 Aug;119(8):2206-2220), FUT8 knockout cell line (Yamane-Ohnuki *et al* Biotechnol Bioeng. 2004 Sep 5;87(5):614-22; Malphettes *et al* Biotechnol Bioeng. 2010 Aug 1;106(5):774-83) and other strategies (Pereira *et al* MAbs. 2018 Jul;10(5):693-711).

[0260] This assay was performed in 96-well plate using 0.025 mL of each component; luminescent *P. aeruginosa* strain PAO1, (DiGiandomenico *et al.*, 2012 J. Exp. Med), diluted baby rabbit serum (Cedar Lane), differentiated HL-60 cells, and monoclonal antibody. Data was acquired utilizing a Tecan Spark Multimode Microplate Reader (Tecan), and then subsequently plotted as percent killed, in comparison to a control lacking antibody at various temperatures. The results are shown in **FIG. 2**. AZD0292, gremubamab and gremubamab-Afuc mediated similar OPK activity against *P. aeruginosa*. Whereas, Gremubamab-Afuc-YTE exhibited reduced activity compared to AZD0292, gremubamab and gremubamab-Afuc. No opsonophagocytic killing activity was observed with control IgG antibody.

[0261] These results surprisingly indicate that different half-life extension technologies modifying the Fc region can impact the opsonophagocytic killing activity of the antibody. Whilst theYTE half-life extension modification caused a significant decrease in activity

(noting that afucosylated gremubamab activity was similar to gremubamab), the N3Y half-life extension modification did not affect opsonophagocytic killing activity.

Example 2: AZD0292 has increased serum exposure compared to gremubamab

[0262] The N3Y modification of AZD0292 enables increased binding affinity to the neonatal Fc receptor (FcRn), which functions as a recycling receptor that is responsible for maintaining IgG in the circulation. To confirm that AZD0292 exhibited increased exposure versus gremubamab, the pharmacokinetics of each molecule was compared in a Tg32 human FcRn transgenic mouse model.

[0263] In the pharmacokinetic analysis, AZD0292 and gremubamab (10 mg/kg) were both intravenously delivered to 7-week old mice, followed by blood sampling at 1, 4, 12, 54, 72, and 102 hours, as well as 7, 9, 11, 14, 17, 21, 24, 28, 35, and 42 days, post-antibody administration. Blood was collected in BD microcontainer blood collection tubes, and the serum was subsequently processed by centrifugation at 500x g for approximately 10 minutes. Processed serum was stored at -80°C until antibody quantification.

[0264] Quantification of gremubamab and AZD0292 in mouse sera was performed using antigen-specific ELISA. Nunc MaxiSorp plates (Thermo Fisher Scientific) were coated overnight at 4°C with an anti-idiotypic antibody which targeted the anti-PcrV portion of gremubamab and AZD0292. Following a PBS wash, which contained 0.1% Tween 20 (wash buffer), the plates were blocked for 1 hour at room temperature (RT) with PBS + 5% BSA. Following three washes with wash buffer, plates were incubated with mouse sera diluted in PBS. AZD0292 or gremubamab were then utilized as standards.

[0265] Following a 1.5 hour incubation, while shaking (200 rpm) at room temperature (RT), plates were washed and incubated for 30 min with 0.05 mL of an anti-idiotypic antibody targeting the anti-Psl arm of Gremubamab/MEDI3902 and AZD0292. Following three washes with wash buffer, 0.05 mL of horseradish peroxidase (HRP)-conjugated goat anti-human IgG (1:10,000; Jackson Laboratories) was added and incubated for 30 minutes at RT. After washing, 0.05 mL of 3,3',5,5'-tetramethylbenzidine (TMB) substrate (KPL) was added and the reaction was stopped after approximately 10 minutes with 0.05 mL of 0.2 M H₂SO₄. The optical density at 450 nm (OD₄₅₀) was measured with a spectrophotometer (Molecular Devices).

[0266] The results are shown in **FIG. 3**. Following administration of 10 mg/kg IV of AZD0292 and gremubamab, a 50% lower clearance was estimated for AZD0292 compared to gremubamab.

Example 3: Photo- and heat-stressed AZD0292 retain anti-Psl and anti-PcrV functional activity

[0267] To determine whether AZD0292 retained functional activity following exposure to photo- and heat- stressing conditions, its activity was evaluated in comparison to similarly treated gremubamab in OPK and anti-cytotoxicity assays.

[0268] Accelerated photo- and heat- stress stability assays were performed as described (Dippel et al., 2023 MABS). Briefly, in heat-stress assays, antibodies were diluted to 1 mg/ml in PBS (pH 7.2), and incubated for 2 weeks at either 4°C or 45°C. In photo-stress assays, antibodies were formulated at 2.5 mg/mL in PBS (pH 7.2), filled into 1 cc Schott glass vials, stoppered/sealed, and placed into an ICH-compliant photo-stability chamber (Caron Model 6545-2). Samples were exposed to cool white light at 3000 lux over the course of 1 week, for a total exposure of approximately 500000 lux hours.

[0269] In the OPK and anti-cytotoxicity assays, photo- and heat-stressed AZD0292 and gremubamab antibodies performed similarly to non-stressed AZD0292 and gremubamab. See **FIGs. 4A** and **4B**. The percent change in monomer, aggregate, and fragment formation compared to non-stressed material is presented in Table 2.

Table 2.

Antibody	Photostability			Accelerated heat stability		
	Change in monomer (%)	Change in aggregate (%)	Change in fragment (%)	Change in monomer (%)	Change in aggregate (%)	Change in fragment (%)
Gremubamab	1.27	1.27	0.00	2.61	0.00	2.61
AZD0292	1.49	1.49	0.00	2.70	0.00	2.70

[0270] This data indicated no difference in the functional activity of stressed and non-stressed AZD0292 or gremubamab.

Example 4: AZD0292 surprisingly demonstrates reduced aggregation

- [0271] Studies were performed to evaluate whether there was any product quality difference between clones expressing AZD0292 and MEDI3902 (specifically evaluating aggregate levels, monomer levels, protein concentration, and expressed titer).
- [0272] In order to assess the effect of the N3Y mutation in AZD0292 on affinity product aggregate levels a shake plate overgrow screen (SPOG) was performed. In the study, 384 clones were screened, and the top 96 were selected based on their expressed titers. Aggregation analysis was performed on the top 96 clones using small scale Protein A capture followed by high throughput size exclusion chromatography (HTSEC).
- [0273] For the shake plate overgrow screen, in brief, upon clonal cell line expansion, cell culture from each stable clone was seeded at 0.7×10^5 cells/ml in a total volume of 350 μ l in a 96 deep-well plate and underwent a 10-day fed-batch process using cell-growth medium and bolus additions of nutrient feeds and glucose. On day 10 of this fed-batch experiment, cell culture from each stable clone assessed was then harvested. The viable cell density and cell viability of each clone was measured. The cell culture medium was then clarified by centrifugation, and supernatant samples were sent for rProtein titre analysis (quantified by using a protein A high-performance liquid chromatography (HPLC) on an Agilent HP1100 or HP1200 (Agilent Technologies, Santa Clara, CA) by comparing peak size from each sample with a calibration curve). HTP-aggregation analysis is described in more detail below.
- [0274] Protein purification from 96-deep well plate cell culture was performed using PhyTip 200 μ L volume columns, containing 20 μ L of ProPlus (MabSelect SuRe™) affinity resin (Biotage GB Limited, Hengoed, United Kingdom) operated on a Tecan Freedom EVO® 200 robotic liquid handling platform (TECAN Group Ltd.) with Freedom EVOware®, Version 2.7 (TECAN Group Ltd.).
- [0275] Phosphate buffer saline was used as column equilibration and first wash step (wash 1) buffer whilst the second wash step (wash 2) buffer was composed of 25 mM sodium acetate, 120 mM sodium chloride, pH 5.5. Elution was carried out by using 100 mM glycine buffer pH 2.6 or 25 mM sodium acetate buffer, pH 3.6. After purification, the samples were neutralised by using 1M Tris buffer pH 7.5.
- [0276] Aggregation analysis was performed by high-throughput size exclusion chromatography (HTSEC) monitoring UV absorbance at 280 nm. Equipment and software packages for chromatography analysis were all purchased from Agilent Technologies Inc.

and involved Agilent 1260 Infinity II UHPLC system including a degasser, quaternary pump, thermostatted multi-sampler, and diode array detector (DAD) in conjunction with a multi-column compartment with a column selection valve. All UHPLC parts were joined by 1.6 mm OD, 0.12 μ m ID stainless steel capillary tubing with stainless steel fittings. System control and data analysis were accomplished with Agilent OpenLAB CDS ChemStation Edition, version C.01.07. HTSEC analysis was performed using the Acquity UPLC Protein BEH SEC 200 Å, 1.7 μ m (Waters) column with 2.1 $\times$ 150 mm (ID $\times$ length) and mobile phase composed of 50 mM sodium phosphate, 450 mM arginine, pH7.0. Data visualization and statistical analysis were performed using the software package JMP Pro16 (SAS).

[0277] **FIGs. 5A and 5B** show bar graphs representation of the data obtained from the study for percent monomer (**FIG. 5A**) and percent higher molecular weight aggregate species (**FIG. 5B**) for AZD0292 and MEDI3902. “Percent monomer” refers to the expected monoclonal antibody (with 2 heavy chains and 2 light chains). Data from A5, A6, A9, B9, and E6 are missing due to insufficient sample availability for analysis. The data obtained from this study show that, surprisingly, AZD0292 had significantly less higher molecular weight aggregate species than MEDI3902. This demonstration across multiple expressing clones indicates that the observation is not simply an artifact of a single clone, but rather a fundamental aspect of the N3Y mutations of AZD0292 as compared with MEDI3902.

[0278] Another representation of this data from **FIG. 5B** is shown in **FIG. 6**. **FIG. 6** shows % aggregation (percent high molecular weight species) for each clone. The light (less aggregates) to dark (more aggregates) coloring is proportional to the % aggregates measured in each sample by HTSEC. This visual representation clearly demonstrates the surprising decrease in % aggregation of AZD0292 as compared against MEDI3902.

[0279] The data was plotted in **FIG. 7** to evaluate the presence of any correlation between the percent high molecular weight species (e.g., aggregates), the mean concentration of the PhyTip Protein A purified sample, and the titer on the final day from the fed batch 96-well plate bioreactor. However, no correlation was observed in the scatter plot matrix (**FIG. 7**). These data indicate that aggregation is not induced by the sample concentration levels over the range evaluated and that aggregation is not induced by the fed batch day final titer.

[0280] To further compare the levels of aggregates between clones expressing MEDI3902 and AZD0292, the data were tested by aligning with the normality assumption required for

using the student's t-test statistic. Both Anderson-Darling and Shapiro-Wilk tests (**FIGs. 8A** and **8B** for the AZD0292 samples and MEDI3902 samples, respectively) confirmed that the data is normally distributed (p-value > 0.05). The t-test approach used assumed equal variances based on a two-sided F-test assessment giving a p-value > 0.05. T-test results showed that the average aggregation levels observed in clones expressing MEDI3902 versus AZD0292 are different with statistical significance (p-value < 0.05 based on the 95% confidence interval (**FIG. 8C**)). On average the difference observed was 2.99% less aggregates for the AZD0292 samples as compared to the MEDI3902 samples (**FIG. 8C**).

[0281] **FIG. 9A** shows the % aggregation and **FIG. 9B** shows antibody titer levels for the clones expressing AZD0292 and MEDI3902 measured in all samples and ordered from low to high aggregate levels (left to right in **FIG. 9A** with the corresponding fed batch day final titer level below in **FIG. 9B** for the same clone). **FIG. 9A** shows reduced aggregation of AZD0292 samples as compared to MEDI3902 samples. The data indicates that the decrease in aggregation for AZD0292 is not correlated with reductions in titer level.

[0282] **FIG. 10** shows the percent aggregate data for AZD0292 and MEDI3902 over three months at 40°C. The increase in percent aggregate for AZD0292 and MEDI3902 over time were comparable, indicating that both molecules (MEDI3902 and AZD0292) had similar degradation rates.

[0283] Overall, these results demonstrate that clones expressing AZD0292 had significantly less aggregates than those expressing MEDI3902. Although the mean expression titer of MEDI3902 expressing clones is slightly higher than those expressing AZD0292, the difference is not significant. The data shows that the relative aggregate levels in AZD0292 ranged from 3.9% to 18.0%, and in MEDI3902 the aggregate levels from 7.0% to 22.2% (**FIG. 9A** and **FIG. 9B**). However, clones expressing AZD0292 reported 3% less aggregates than those expressing MEDI3902. This percent aggregate level difference is greater than the analytical method error (0.1% area difference) and is therefore a meaningful difference. Therefore, clones expressing AZD0292 reported significantly less aggregates than those expressing MEDI3902. The level of aggregation observed showed no correlation with expression titer, confluence, viable cell density, or cell viability. These data surprisingly indicate that the N3Y mutation in AZD0292 reduces aggregate levels relative to MEDI3902.

[0284] The complete disclosures of all patents, patent applications including provisional patent applications, publications including patent publications and nonpatent publications, and electronically available material (including, for example, nucleotide sequence submissions in, e.g., GenBank and RefSeq, and amino acid sequence submissions in, e.g., SwissProt, PIR, PRF, PDB, and translations from annotated coding regions in GenBank and RefSeq) cited herein are incorporated by reference. The foregoing detailed description and examples have been given for clarity of understanding only. No unnecessary limitations are to be understood therefrom. The invention is not limited to the exact details shown and described, for variations obvious to one skilled in the art will be included within the invention defined by the claims.

WHAT IS CLAIMED:

1. A bispecific antibody that specifically binds to *Pseudomonas aeruginosa* PcrV protein and Psl exopolysaccharide, wherein the antibody comprises a modified IgG Fc region, the modified IgG Fc region comprising amino acid substitutions at two or more of positions 432 to 437, numbered according to the EU numbering index of Kabat, relative to a wild-type IgG Fc region; wherein
 - (i) positions 432 and 437 are each substituted with cysteine;
 - (ii) position 433 is histidine or is substituted with arginine, proline, threonine, lysine, serine, alanine, methionine, or asparagine;
 - (iii) position 434 is asparagine or is substituted with arginine, tryptophan, histidine, phenylalanine, tyrosine, serine, methionine or threonine;
 - (iv) position 435 is histidine; and
 - (v) position 436 is tyrosine or phenylalanine or is substituted with leucine, arginine, isoleucine, lysine, methionine, valine, histidine, serine, or threonine; andwherein the antibody has an increased half-life compared to the half-life of a corresponding antibody having the wild-type IgG Fc region.
2. The bispecific antibody of claim 1, wherein the modified IgG Fc region is a modified IgG1 Fc region.
3. The bispecific antibody of claim 1 or 2, wherein the modified IgG Fc region is a modified human IgG Fc region.
4. The bispecific antibody of any one of claims 1-3, wherein the bispecific antibody exhibits less aggregation in solution than an antibody comprising a heavy chain comprising the amino acid sequence of SEQ ID NO:19 and a light chain comprising the amino acid sequence of SEQ ID NO:20.
5. The bispecific antibody of any one of claims 1-4, wherein the bispecific antibody promotes opsonophagocytic killing activity of *P. aeruginosa*, optionally wherein the bispecific antibody mediates similar *in vitro* opsonophagocytic killing activity of *P. aeruginosa* as an antibody comprising a heavy chain comprising the amino acid sequence

of SEQ ID NO:19 and a light chain comprising the amino acid sequence of SEQ ID NO:20.

6. The bispecific antibody of any one of claims 1-5, further comprising an amino acid insertion after position 437, optionally wherein the amino acid insertion is glutamic acid.
7. The bispecific antibody of any one of claims 1-6, wherein the binding affinity of the bispecific antibody for FcRn at pH 6.0 is higher than the binding affinity for FcRn of a corresponding antibody having the wild-type human IgG1 Fc region at pH 6.
8. The bispecific antibody of any one of claims 1-7, wherein the binding affinity of the bispecific antibody for FcRn at pH 7.4 is higher than the binding affinity for FcRn of a corresponding antibody having the wild-type human IgG1 Fc region at pH 7.4.
9. The bispecific antibody of any one of claims 1-8, wherein the modified human IgG1 Fc region exhibits increased pH dependence on binding affinity for FcRn compared to a corresponding antibody having the wild-type human IgG1 Fc region.
10. The bispecific antibody of any one of claims 1-9, wherein the modified human IgG1 Fc region has amino acid substitutions at three of positions 432, 433, 434, 435, 436, and 437.
11. The bispecific antibody of any one of claims 1-9, wherein the modified human IgG1 Fc region has amino acid substitutions at four of positions 432, 433, 434, 435, 436, and 437.
12. The bispecific antibody of any one of claims 1-9, wherein the modified human IgG1 Fc region has amino acid substitutions at five of positions 432, 433, 434, 435, 436, and 437.
13. The bispecific antibody of any one of claims 1-9, wherein the modified human IgG1 Fc region has amino acid substitutions at six of positions 432, 433, 434, 435, 436, and 437.
14. The bispecific antibody of any one of claims 1-9, wherein the modified human IgG1 Fc region comprises the amino acid sequence of SEQ ID NO:44 or the amino acid sequence of SEQ ID NO:33.
15. The bispecific antibody of any one of claims 1-14, wherein the bispecific antibody is not a HexaBody.

16. The bispecific antibody of any one of claims 1-15, wherein the bispecific antibody competitively inhibits binding to PcrV of an antibody comprising a heavy chain variable region (VH) comprising the amino acid sequence of SEQ ID NO:13 and a light chain variable region (VL) comprising the amino acid sequence of SEQ ID NO:14.
17. The bispecific antibody of any one of claims 1-16, wherein the bispecific antibody binds to the same epitope of PcrV as an antibody comprising a VH comprising the amino acid sequence of SEQ ID NO:13 and a VL comprising the amino acid sequence of SEQ ID NO:14.
18. The bispecific antibody of any one of claims 1-17, wherein the bispecific antibody comprises an antigen-binding domain that binds to *Pseudomonas aeruginosa* PcrV protein and comprises a VH-CDR1 comprising the amino acid sequence of SEQ ID NO:1, a VH-CDR2 comprising the amino acid sequence of SEQ ID NO:2, a VH-CDR3 comprising the amino acid sequence of SEQ ID NO:3, a VL-CDR1 comprising the amino acid sequence of SEQ ID NO:4, a VL-CDR2 comprising the amino acid sequence of SEQ ID NO:5, and a VL-CDR3 comprising the amino acid sequence of SEQ ID NO:6.
19. The bispecific antibody of claim 18, wherein the antigen-binding domain that binds to *Pseudomonas aeruginosa* PcrV protein comprises a VH comprising the amino acid sequence of SEQ ID NO:13 and/or a VL comprising the amino acid sequence of SEQ ID NO:14.
20. The bispecific antibody of claim 18 or claim 19, wherein the antigen-binding domain that binds to *Pseudomonas aeruginosa* PcrV protein comprises a heavy chain variable region and a light chain variable region on separate polypeptides.
21. The bispecific antibody of any one of claims 1-20, wherein the bispecific antibody competitively inhibits binding to Psl of an antibody comprising a VH comprising the amino acid sequence of SEQ ID NO:15 and a VL comprising the amino acid sequence of SEQ ID NO:16.
22. The bispecific antibody of any one of claims 1-21, wherein the bispecific antibody binds to the same epitope of Psl as an antibody comprising a VH comprising the amino acid

- sequence of SEQ ID NO:15 and a VL comprising the amino acid sequence of SEQ ID NO:16.
23. The bispecific antibody of any one of claims 1-22, wherein the antibody comprises an antigen-binding domain that binds to *Pseudomonas aeruginosa* Psl exopolysaccharide and comprises a heavy chain variable region VH-CDR1 comprising the amino acid sequence of SEQ ID NO:7, a VH-CDR2 comprising the amino acid sequence of SEQ ID NO:8, a VH-CDR3 comprising the amino acid sequence of SEQ ID NO:9, a light chain variable region VL-CDR1 comprising the amino acid sequence of SEQ ID NO:10, a VL-CDR2 comprising the amino acid sequence of SEQ ID NO:11, and a VL-CDR3 comprising the amino acid sequence of SEQ ID NO:12.
24. The bispecific antibody of claim 23, wherein the antigen-binding domain that binds to *Pseudomonas aeruginosa* Psl exopolysaccharide comprises a VH comprising the amino acid sequence of SEQ ID NO:15 and/or a VL comprising the amino acid sequence of SEQ ID NO:16.
25. The bispecific antibody of claim 23 or 24, wherein the antigen-binding domain that binds to *Pseudomonas aeruginosa* Psl exopolysaccharide comprises a VH and a VL on the same polypeptide.
26. The bispecific antibody of any one of claims 23-25, wherein the antigen-binding domain that binds to *Pseudomonas aeruginosa* Psl exopolysaccharide comprises a linker between the VH and the VL, optionally wherein the linker comprises the amino acid sequence of SEQ ID NO:18.
27. The bispecific antibody of any one of claims 23-26, wherein the antigen-binding domain that binds to *Pseudomonas aeruginosa* Psl exopolysaccharide comprises an scFv.
28. The bispecific antibody of claim 27, wherein the scFv comprises a linker, optionally wherein the linker comprises the amino acid sequence of SEQ ID NO:18.
29. The bispecific antibody of claim 27 or claim 28, wherein the scFv is in the orientation VH-linker-VL.

30. The bispecific antibody of any one of claims 27-29, wherein the scFv comprises the amino acid sequence of SEQ ID NO:17.
31. The bispecific antibody of any one of claims 27-30, wherein the scFv is on the same polypeptide chain as the VH of the antigen-binding domain that binds to *Pseudomonas aeruginosa* PcrV protein.
32. The bispecific antibody of any one of claims 27-31, wherein the scFv is C-terminal to the VH of the antigen-binding domain that binds to *Pseudomonas aeruginosa* PcrV protein.
33. The bispecific antibody of any one of claims 1-32, wherein the bispecific antibody comprises (i) a heavy chain of the formula VH-CH1-H1-L1-S-L2-H2-CH2-CH3, wherein VH is an anti-*Pseudomonas aeruginosa* PcrV heavy chain variable domain; CH1 is a heavy chain constant region domain 1; H1 is a first heavy chain hinge region fragment; L1 is a first linker; S is an anti-*Pseudomonas aeruginosa* Psl scFv molecule; L2 is a second linker; H2 is a second heavy chain hinge region fragment; CH2 is a heavy chain constant region domain-2; and CH3 is a heavy chain constant region domain-3; and (ii) a light chain of the formula VL-CL, wherein VL is an anti-*Pseudomonas aeruginosa* PcrV light chain variable domain, and CL is an antibody light chain kappa constant region or an antibody light chain lambda constant region.
34. The bispecific antibody of claim 33, wherein CH1 comprises the amino acid sequence of SEQ ID NO:21.
35. The bispecific antibody of claim 33 or claim 34, wherein H1 comprises the amino acid sequence of SEQ ID NO:22.
36. The bispecific antibody of any one of claims 33-35, wherein L1 comprises the amino acid sequence of SEQ ID NO:28.
37. The bispecific antibody of any one of claims 33-36, wherein L2 comprises the amino acid sequence of SEQ ID NO:28.
38. The bispecific antibody of any one of claims 33-37, wherein H2 comprises the amino acid sequence of SEQ ID NO:23.

39. The bispecific antibody of any one of claims 33-38, wherein CH2-CH3 comprises the amino acid sequence of SEQ ID NO:30.
40. The bispecific antibody of any one of claims 33-39, wherein CL is an antibody light chain kappa constant region.
41. The bispecific antibody of any one of claims 33-40, wherein CL comprises the amino acid sequence of SEQ ID NO:24.
42. The bispecific antibody of any one of claims 1-41, comprising a heavy chain comprising the amino acid sequence of SEQ ID NO:31 and/or a light chain comprising the amino acid sequence of SEQ ID NO:20.
43. An isolated polynucleotide comprising a nucleic acid molecule encoding the heavy chain of the bispecific antibody of any one of claims 1-42.
44. The isolated polynucleotide of claim 43, further comprising a nucleic acid molecule encoding the light chain of the bispecific antibody of any one of claims 1-42.
45. A vector comprising the polynucleotide of claim 43 or 44.
46. A host cell comprising (i) the polynucleotide of claim 43 or 44, or (ii) the vector of claim 45.
47. A method of producing a bispecific antibody, the method comprising culturing the host cell of claim 46, and optionally isolating the bispecific antibody.
48. A bispecific antibody produced by the method of claim 47.
49. A composition comprising the bispecific antibody of any one of claims 1-42 or 48, and a pharmaceutically acceptable carrier.
50. A method of treating or preventing a *Pseudomonas* infection in a subject in need thereof, the method comprising administering the bispecific antibody of any one of claims 1-42 or 48, or the composition of claim 49 to the subject.

51. The method of claim 50, wherein the infection is a lung infection, a respiratory tract infection, pneumonia, bacteremia, a bone infection, a joint infection, a skin infection, a burn infection, a wound infection, or any combination thereof.
52. A method of treating bronchiectasis in a subject in need thereof, the method comprising administering the bispecific antibody of any one of claims 1-42 or 48, or the composition of claim 49 to the subject.
53. A method of improving pre-bronchodilator forced expiratory volume 1 (FEV₁) in a subject with bronchiectasis, the method comprising administering the bispecific antibody of any one of claims 1-42 or 48, or the composition of claim 49 to the subject.
54. A method of reducing *Pseudomonas aeruginosa* load in a subject with bronchiectasis, the method comprising administering the bispecific antibody of any one of claims 1-42 or 48, or the composition of claim 49 to the subject.
55. A method of reducing bronchiectasis exacerbations in a subject in need thereof, the method comprising administering the bispecific antibody of any one of claims 1-42 or 48, or the composition of claim 49 to the subject.
56. A method of reducing the need for intravenous antibiotics in a subject with bronchiectasis, the method comprising administering the bispecific antibody of any one of claims 1-42 or 48, or the composition of claim 49 to the subject.
57. A method of stabilizing lung function in a subject with bronchiectasis, the method comprising administering the bispecific antibody of any one of claims 1-42 or 48, or the composition of claim 49 to the subject.
58. The method of any one of claims 52-57, wherein the bronchiectasis is non-cystic fibrosis bronchiectasis.
59. The method of any one of claims 50-58, further comprising administering an antibiotic.
60. The method of any one of claims 50-59, wherein the subject is colonized with *Pseudomonas aeruginosa*, optionally wherein the respiratory tract of the subject is colonized with *Pseudomonas aeruginosa*.

61. Use of the bispecific antibody of any one of claims 1-42 or 48, or the composition of claim 49 in the preparation of a medicament for use in the method of any one of claims 50-60.
62. The bispecific antibody of any one of claims 1-42 or 48, or the composition of claim 49 for use in the method of any one of claims 50-60.