

**Anti-PcrV Antibodies that Bind PcrV, Compositions Comprising Anti-PcrV
Antibodies, and Methods of Use Thereof**

TECHNICAL FIELD

[0001] The present invention provides, in part, antibodies, bispecific antibodies, and antigen-binding fragments thereof, which specifically bind PcrV, as well as compositions and methods of treatment for *P. aeruginosa* infection.

SEQUENCE LISTING

[0002] An official copy of the sequence listing is submitted concurrently with the specification electronically via EFS-Web as an ASCII formatted sequence listing with a file name of "10494WO01_SEQ_LIST_ST25.txt", a creation date of June 10, 2020, and a size of about 76 KB. The sequence listing contained in this ASCII formatted document is part of the specification and is herein incorporated by reference in its entirety.

BACKGROUND

[0003] *Pseudomonas aeruginosa* (*P. aeruginosa*) is a Gram-negative bacillus present in a wide variety of environments. Requiring only simple nutrition, *P. aeruginosa* can grow in distilled water, and can grow well in acetate mediums and ammonium sulphate mediums. It can grow at temperatures as high as 42°C, and is resistant to high concentrations of salts, weak antiseptics, and many antibiotics.

[0004] An opportunistic pathogen, the bacteria are a major health concern, often drug resistant and causing community acquired and nosocomial infections. Such bacterial infections can be serious and life-threatening, with pneumonia being one of the most concerning manifestations. The bacterium rarely causes disease in healthy people and animals but is a significant problem for critically ill or immunocompromised individuals. For example, *P. aeruginosa* infection is a major problem in individuals who have cystic fibrosis (CF), resulting in progressive lung damage from recurrent and chronic respiratory tract infections with the bacterium. Others at risk include patients on mechanical ventilators, patients with tuberculosis, neutropenic cancer patients, and burn victims.

[0005] The bacterial type 3 secretion system (T3SS) is an important virulence factor of Gram-negative bacteria. T3SS is a complex multi-protein structure crossing the complete bacterial cell wall. Only two proteins are accessible to antibodies: the single barrel homopolymeric forming protein and the needle tip protein. *P. aeruginosa*'s V-tip

protein (PcrV) is an example of a V-tip protein common to many Gram negative bacterial T3SSs. The PcrV is located at the end of the T3SS protein, forming a pentameric ring-type structure on the tip of the needle.

[0006] The T3SS hollow needle-like molecular structure operates by translocating toxins (ExoS, ExoT, ExoU, and ExoY) into eukaryotic cells, causing cell death and lysis. However, the translocation pore itself is sufficient to cause the death of infected cells, either directly through pore-mediated increases in membrane permeability, or indirectly through the activation of broad cellular defense responses. By killing white blood cells and epithelial cells and triggering inflammation, the T3SS virulence mechanism enables *P. aeruginosa* to evade human immune defenses.

[0007] There remains a significant unmet medical need for improved antibiotic drugs that treat or prevent *P. aeruginosa* infection.

SUMMARY

[0008] Provided herein are antibodies and antigen-binding fragments thereof that bind *P. aeruginosa*'s V-tip protein (PcrV). Such antibodies are useful for inhibiting or neutralizing the activity of the bacterial type 3 secretion system (T3SS) in *P. aeruginosa*. In some embodiments, the antibodies are useful for blocking translocation of toxins from the bacteria to the host cell and/or for preventing death of the host cells. In some embodiments, the antibodies function by blocking pore-mediated membrane permeability in the host cell.

[0009] In certain embodiments, the antibodies are useful in preventing, treating or ameliorating at least one symptom of *P. aeruginosa* infection in a subject. In certain embodiments, the antibodies may be administered prophylactically or therapeutically to a patient having, or at risk of acquiring, a *P. aeruginosa* infection. In certain embodiments, compositions containing at least one antibody of the disclosure may be administered to a patient having a *P. aeruginosa* infection. In certain embodiments, compositions containing at least one antibody of the disclosure may be administered to a patient at risk of contracting a *P. aeruginosa* infection, for example, a patient with cystic fibrosis, with diabetes, on a mechanical ventilator, undergoing surgery, with tuberculosis, with HIV, with a compromised immune system, with neutropenia, with an indwelling catheter, after physical trauma, with burns, in an intensive care unit, who is bedridden, with malignancy, with chronic obstructive pulmonary disease, in a long-term care health facility, or who is an intravenous drug user.

[0010] The antibodies provided herein can be full-length (for example, an IgG1 or IgG4

antibody) or may comprise only an antigen-binding portion (for example, a Fab, F(ab')₂ or scFv fragment), and may be modified to affect functionality, e.g., to increase persistence in the host or to eliminate residual effector functions (Reddy *et al.*, 2000, J. Immunol. 164:1925-1933). In certain embodiments, the antibodies may be bispecific.

[0011] In some aspects, the present disclosure provides isolated recombinant monoclonal antibodies or antigen-binding fragments thereof that bind specifically to PcrV. Such antibodies are often functional antagonists of the T3SS, i.e., the antibodies bind to PcrV and inhibits the T3SS.

[0012] In one embodiment, the present disclosure provides an isolated recombinant antibody or antigen-binding fragment thereof that specifically binds to PcrV, wherein the antibody has one or more of the following characteristics:

(a) comprises three heavy chain complementarity determining regions (CDRs) (HCDR1, HCDR2 and HCDR3) contained within any one of the heavy chain variable region (HCVR) sequences selected from the group consisting of SEQ ID NOs: 2, 18, 34, and 50; and three light chain CDRs (LCDR1, LCDR2 and LCDR3) contained within any one of the light chain variable region (LCVR) sequences selected from the group consisting of SEQ ID NOs: 10, 26, 42, and 58;

(b) is a fully human monoclonal antibody;

(c) binds to full length PcrV with a dissociation constant (K_D) of less than 10^{-8} M, as measured in a surface plasmon resonance assay at 25°C;

(d) binds to full length PcrV with a dissociation constant (K_D) of less than 10^{-8} M, as measured in a surface plasmon resonance assay at 37°C;

(e) demonstrates neutralization of *P. aeruginosa* strain 6077 with an IC_{50} ranging from about 10^{-11} M to about 10^{-8} M in a cytotoxicity assay;

(f) demonstrates neutralization of *P. aeruginosa* strain ATCC 700888 with an IC_{50} ranging from about 10^{-9} M to about 10^{-7} M in a cytotoxicity assay;

(g) demonstrates neutralization of *P. aeruginosa* strain 6077 with an IC_{50} ranging from about 10^{-10} M to about 10^{-6} M in a hemolytic assay;

(h) demonstrates neutralization of *P. aeruginosa* strain ATCC 700888 with an IC_{50} ranging from about 10^{-10} M to about 10^{-7} M in a hemolytic assay;

(i) decreases mortality from *P. aeruginosa* strain 6206 or strain 6077 in mice treated prophylactically with 5 mg/kg relative to untreated mice in an acute pneumonia model;

(j) decreases mortality from *P. aeruginosa* strain 6206 or strain 6077 in mice treated prophylactically with 1.0, 0.2 or 0.04 mg/kg relative to untreated mice in an acute pneumonia model;

(k) decreases lung bacterial burden of *P. aeruginosa* strain 6206 in mice treated prophylactically at 0.1 mg/kg or 0.2 mg/kg relative to untreated mice in an acute pneumonia model;

(l) decreases lung bacterial burden of *P. aeruginosa* strain PA01 in mice treated prophylactically at 25 mg/kg relative to untreated mice in an acute pneumonia model; and/or

(m) cross-competes with a reference antibody, wherein the reference antibody comprises a heavy chain variable region (HCVR) and a light chain variable region (LCVR) amino acid sequence selected from the group consisting of any of the HCVR and LCVR amino acid sequences of Table 1.

[0013] In some aspects, the isolated antibody or antigen-binding fragment thereof further has one or more of the following characteristics:

(n) binds to full length PcrV (SEQ ID NO: 77) with an EC_{50} of less than about 10^{-8} M;

(o) binds to PcrV 136-233 (SEQ ID NO: 81) with an EC_{50} of less than about 10^{-8} M;

(p) interacts with at least one amino acid sequence selected from the group consisting of (i) amino acid residues ranging from about position 150 to about position 170 of SEQ ID NO: 78 and (ii) amino acid residues ranging from about position 155 to about 170 of SEQ ID NO: 78; and/or

(q) interacts with at least one amino acid sequence selected from the group consisting of SEQ ID NO: 85 and SEQ ID NO: 86.

[0014] Exemplary anti-PcrV antibodies provided herein are listed in Tables 1, 2, and 3 herein. Table 1 sets forth the amino acid sequence identifiers of the heavy chain variable regions (HCVRs), light chain variable regions (LCVRs), heavy chain complementarity determining regions (HCDR1, HCDR2 and HCDR3), and light chain complementarity determining regions (LCDR1, LCDR2 and LCDR3) of exemplary anti-PcrV antibodies. Table 2 sets forth the nucleic acid sequence identifiers of the HCVRs, LCVRs, HCDR1, HCDR2 HCDR3, LCDR1, LCDR2 and LCDR3 of the exemplary anti-PcrV antibodies. Table 3 provides the heavy chain and light chain nucleic acid and amino acid sequences of several exemplary antibodies.

[0015] Provided herein are antibodies, or antigen-binding fragments thereof,

comprising an HCVR comprising an amino acid sequence selected from any of the HCVR amino acid sequences listed in Table 1, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity thereto. In some aspects, the antibody or antigen-binding fragment thereof comprises an HCVR having an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 18, 34, and 50.

[0016] Also provided are antibodies, or antigen-binding fragments thereof, comprising an LCVR comprising an amino acid sequence selected from any of the LCVR amino acid sequences listed in Table 1, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity thereto. In some aspects, the antibody or antigen-binding fragment thereof comprises an LCVR having an amino acid sequence selected from the group consisting of SEQ ID NOs: 10, 26, 42, and 58.

[0017] Provided herein are antibodies, or antigen-binding fragments thereof, comprising an HCVR and an LCVR amino acid sequence pair (HCVR/LCVR) comprising any of the HCVR amino acid sequences listed in Table 1 paired with any of the LCVR amino acid sequences listed in Table 1. According to certain embodiments, the present disclosure provides antibodies, or antigen-binding fragments thereof, comprising an HCVR/LCVR amino acid sequence pair contained within any of the exemplary anti-PcrV antibodies listed in Table 1.

[0018] In one embodiment, the isolated antibody or antigen-binding fragment thereof comprises a HCVR/LCVR amino acid sequence pair selected from the group consisting of SEQ ID NOs: 2/10, 18/26, 34/42, and 50/58.

[0019] In one embodiment, the isolated antibody or antigen-binding fragment thereof comprises:

- (a) a HCDR1 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 4, 20, 36, and 52;
- (b) a HCDR2 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 6, 22, 38, and 54;
- (c) a HCDR3 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 8, 24, 40, and 56;
- (d) a LCDR1 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 12, 28, 44, and 60;
- (e) a LCDR2 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 14, 30, 46, and 62; and

(f) a LCDR3 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 16, 32, 48, and 64.

[0020] In certain embodiments, the HCVR/LCVR amino acid sequence pair is selected from the group consisting of SEQ ID NOs: 2/10 (H1H29329P), 18/26 (H1H29332P), 34/42 (H1H29336P), and 50/58 (H1H29339P).

[0021] Also provided herein are antibodies, or antigen-binding fragments thereof, comprising a heavy chain CDR1 (HCDR1) comprising an amino acid sequence selected from any of the HCDR1 amino acid sequences listed in Table 1 or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity.

[0022] Also provided herein are antibodies, or antigen-binding fragments thereof, comprising a heavy chain CDR2 (HCDR2) comprising an amino acid sequence selected from any of the HCDR2 amino acid sequences listed in Table 1 or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity.

[0023] Also provided herein are antibodies, or antigen-binding fragments thereof, comprising a heavy chain CDR3 (HCDR3) comprising an amino acid sequence selected from any of the HCDR3 amino acid sequences listed in Table 1 or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity.

[0024] Also provided herein are antibodies, or antigen-binding fragments thereof, comprising a light chain CDR1 (LCDR1) comprising an amino acid sequence selected from any of the LCDR1 amino acid sequences listed in Table 1 or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity.

[0025] Also provided herein are antibodies, or antigen-binding fragments thereof, comprising a light chain CDR2 (LCDR2) comprising an amino acid sequence selected from any of the LCDR2 amino acid sequences listed in Table 1 or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity.

[0026] Also provided herein are antibodies, or antigen-binding fragments thereof, comprising a light chain CDR3 (LCDR3) comprising an amino acid sequence selected from any of the LCDR3 amino acid sequences listed in Table 1 or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity.

[0027] Also provided herein are antibodies, or antigen-binding fragments thereof, comprising an HCDR3 and an LCDR3 amino acid sequence pair (HCDR3/LCDR3) comprising any of the HCDR3 amino acid sequences listed in Table 1 paired with any of the LCDR3 amino acid sequences listed in Table 1. According to certain embodiments, the present disclosure provides antibodies, or antigen-binding fragments thereof, comprising an HCDR3/LCDR3 amino acid sequence pair contained within any of the exemplary anti-PcrV antibodies listed in Table 1. In certain embodiments, the HCDR3/LCDR3 amino acid sequence pair is selected from the group consisting of SEQ ID NOs: 2/10 (H1H29329P), 18/26 (H1H29332P), 34/42 (H1H29336P), and 50/58 (H1H29339P).

[0028] Also provided herein are antibodies, or antigen-binding fragments thereof, comprising a set of six CDRs (*i.e.*, HCDR1-HCDR2-HCDR3-LCDR1-LCDR2-LCDR3) contained within any of the exemplary anti-PcrV antibodies listed in Table 1. In certain embodiments, the HCDR1-HCDR2-HCDR3-LCDR1-LCDR2-LCDR3 amino acid sequence set is selected from the group consisting of SEQ ID NOs: 4-6-8-12-14-16 (*e.g.*, H1H29329P), 20-22-24-28-30-32 (*e.g.*, H1H29332P); 36-38-40-44-46-48 (*e.g.*, H1H29336P); and 52-54-56-60-62-64 (*e.g.*, H1H29339P).

[0029] In a related embodiment, provided herein are antibodies, or antigen-binding fragments thereof, comprising a set of six CDRs (*i.e.*, HCDR1-HCDR2-HCDR3-LCDR1-LCDR2-LCDR3) contained within an HCVR/LCVR amino acid sequence pair as defined by any of the exemplary anti-PcrV antibodies listed in Table 1. For example, the present disclosure includes antibodies, or antigen-binding fragments thereof, comprising the HCDR1-HCDR2-HCDR3-LCDR1-LCDR2-LCDR3 amino acid sequences set contained within an HCVR/LCVR amino acid sequence pair selected from the group consisting of SEQ ID NOs: 2/10 (*e.g.*, H1H29329P), 18/26 (*e.g.*, H1H29332P), 34/42 (*e.g.*, H1H29336P), and 50/58 (*e.g.*, H1H29339P). Methods and techniques for identifying CDRs within HCVR and LCVR amino acid sequences are well known in the art and can be used to identify CDRs within the specified HCVR and/or LCVR amino acid sequences disclosed herein. Exemplary conventions that can be used to identify the boundaries of CDRs include, *e.g.*, the Kabat definition, the Chothia definition, and the AbM definition. In general terms, the Kabat definition is based on sequence variability, the Chothia definition is based on the location of the structural loop regions, and the AbM definition is a compromise between the Kabat and Chothia approaches. See, *e.g.*, Kabat, "Sequences of Proteins of Immunological Interest," National Institutes of Health, Bethesda, Md. (1991); Al-Lazikani *et al.*, *J. Mol. Biol.* 273:927-948 (1997); and Martin *et*

al., *Proc. Natl. Acad. Sci. USA* 86:9268-9272 (1989). Public databases are also available for identifying CDR sequences within an antibody.

[0030] Also provided herein are antibodies having any one of the heavy chain amino acid sequences provided in Table 3 and/or any one of the light chain amino acid sequences provided in Table 3.

[0031] Provided herein are antibodies comprising an HC comprising an amino acid sequence selected from any of the HC amino acid sequences listed in Table 3, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity thereto.

[0032] Also provided are antibodies comprising an LC comprising an amino acid sequence selected from any of the LC amino acid sequences listed in Table 3, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity thereto.

[0033] Provided herein are antibodies comprising an HC and an LC amino acid sequence pair (HC/LC) comprising any of the HC amino acid sequences listed in Table 3 paired with any of the LC amino acid sequences listed in Table 3. According to certain embodiments, the present disclosure provides antibodies comprising an HC/LC amino acid sequence pair contained within any of the exemplary anti-PcrV antibodies listed in Table 3.

[0034] In one embodiment, the isolated antibody thereof comprises a HC/LC amino acid sequence pair selected from the group consisting of SEQ ID NOs: 65/66, 67/68, 69/70, and 71/72.

[0035] The present disclosure includes anti-PcrV antibodies having a modified glycosylation pattern. In some embodiments, modification to remove undesirable glycosylation sites may be useful, or an antibody lacking a fucose moiety present on the oligosaccharide chain, for example, to increase antibody dependent cellular cytotoxicity (ADCC) function (see Shield et al. (2002) JBC 277:26733). In other applications, modification of galactosylation can be made in order to modify complement dependent cytotoxicity (CDC).

[0036] Also provided herein are antibodies and antigen-binding fragments thereof that compete for specific binding to *P. aeruginosa* PcrV with an antibody or antigen-binding fragment thereof comprising the CDRs of a HCVR and the CDRs of a LCVR, wherein the HCVR and LCVR each has an amino acid sequence selected from the HCVR and LCVR sequences listed in Table 1. In some aspects, the antibody or antigen-binding fragment thereof competes for binding to PcrV 136-233 (SEQ ID NO: 81) with the

reference antibody or antigen-binding fragment thereof.

[0037] Further provided are antibodies and antigen-binding fragments thereof that bind the same *P. aeruginosa* PcrV epitope as a reference antibody or antigen-binding fragment thereof comprising the CDRs of a HCVR and the CDRs of a LCVR, wherein the HCVR and LCVR each has an amino acid sequence selected from the HCVR and LCVR sequences listed in Table 1. In some aspects, the epitope comprises the residues of PcrV 136-233 (SEQ ID NO: 81) or the residues of PcrV 150-170 (SEQ ID NO: 86) or the residues of PcrV 155-170 (SEQ ID NO: 85).

[0038] Still further provided are isolated antibodies and antigen-binding fragments thereof that block *P. aeruginosa* PcrV translocation of toxins from the bacteria to a host cell. Still further provided are isolated antibodies and antigen-binding fragments thereof that block a T3SS pore-mediated increase in host cell membrane permeability.

[0039] In certain embodiments, the antibodies or antigen-binding fragments of the present disclosure are bispecific comprising a first binding specificity to a first epitope in the PcrV protein and a second binding specificity to a second epitope in the PcrV protein, wherein the first and second epitopes are distinct and non-overlapping. In certain embodiments the bispecific may comprise a first arm that binds to an epitope in the PcrV protein and a second arm that binds to a different *P. aeruginosa* antigen.

[0040] In another aspect, provided herein are nucleic acid molecules encoding anti-PcrV antibodies or portions thereof. For example, the present disclosure provides nucleic acid molecules encoding any of the HCVR amino acid sequences listed in Table 1; in certain embodiments the nucleic acid molecule comprises a polynucleotide sequence selected from any of the HCVR nucleic acid sequences listed in Table 2, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity thereto.

[0041] Also provided herein are nucleic acid molecules encoding any of the LCVR amino acid sequences listed in Table 1; in certain embodiments the nucleic acid molecule comprises a polynucleotide sequence selected from any of the LCVR nucleic acid sequences listed in Table 2, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity thereto.

[0042] Also provided herein are nucleic acid molecules encoding any of the HCDR1 amino acid sequences listed in Table 1; in certain embodiments the nucleic acid molecule comprises a polynucleotide sequence selected from any of the HCDR1 nucleic acid sequences listed in Table 2, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity thereto.

[0043] Also provided herein are nucleic acid molecules encoding any of the HCDR2 amino acid sequences listed in Table 1; in certain embodiments the nucleic acid molecule comprises a polynucleotide sequence selected from any of the HCDR2 nucleic acid sequences listed in Table 2, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity thereto.

[0044] Also provided herein are nucleic acid molecules encoding any of the HCDR3 amino acid sequences listed in Table 1; in certain embodiments the nucleic acid molecule comprises a polynucleotide sequence selected from any of the HCDR3 nucleic acid sequences listed in Table 2, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity thereto.

[0045] Also provided herein are nucleic acid molecules encoding any of the LCDR1 amino acid sequences listed in Table 1; in certain embodiments the nucleic acid molecule comprises a polynucleotide sequence selected from any of the LCDR1 nucleic acid sequences listed in Table 2, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity thereto.

[0046] Also provided herein are nucleic acid molecules encoding any of the LCDR2 amino acid sequences listed in Table 1; in certain embodiments the nucleic acid molecule comprises a polynucleotide sequence selected from any of the LCDR2 nucleic acid sequences listed in Table 2, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity thereto.

[0047] Also provided herein are nucleic acid molecules encoding any of the LCDR3 amino acid sequences listed in Table 1; in certain embodiments the nucleic acid molecule comprises a polynucleotide sequence selected from any of the LCDR3 nucleic acid sequences listed in Table 2, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity thereto.

[0048] Also provided herein are nucleic acid molecules encoding an HCVR, wherein the HCVR comprises a set of three CDRs (*i.e.*, HCDR1-HCDR2-HCDR3), wherein the HCDR1-HCDR2-HCDR3 amino acid sequence set is as defined by any of the exemplary anti-PcrV antibodies listed in Table 1.

[0049] Also provided herein are nucleic acid molecules encoding an LCVR, wherein the LCVR comprises a set of three CDRs (*i.e.*, LCDR1-LCDR2-LCDR3), wherein the LCDR1-LCDR2-LCDR3 amino acid sequence set is as defined by any of the exemplary anti-PcrV antibodies listed in Table 1.

[0050] Also provided herein are nucleic acid molecules encoding both an HCVR and an LCVR, wherein the HCVR comprises an amino acid sequence of any of the HCVR

amino acid sequences listed in Table 1, and wherein the LCVR comprises an amino acid sequence of any of the LCVR amino acid sequences listed in Table 1. In certain embodiments, the nucleic acid molecule comprises a polynucleotide sequence selected from any of the HCVR nucleic acid sequences listed in Table 2, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity thereto, and a polynucleotide sequence selected from any of the LCVR nucleic acid sequences listed in Table 2, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity thereto. In certain embodiments according to this aspect of the disclosure, the nucleic acid molecule encodes an HCVR and LCVR, wherein the HCVR and LCVR are both derived from the same PcrV antibody listed in Table 1.

[0051] Also provided herein are nucleic acid molecules encoding any of the heavy chain amino acid sequences listed in Table 1. The present disclosure also provides nucleic acid molecules encoding any of the light chain amino acid sequences listed in Table 1.

[0052] In a related aspect, provided herein are recombinant expression vectors capable of expressing a polypeptide comprising a heavy or light chain variable region of an anti-PcrV antibody. For example, the present disclosure includes recombinant expression vectors comprising any of the nucleic acid molecules mentioned above, *i.e.*, nucleic acid molecules encoding any of the HCVR, LCVR, and/or CDR sequences as set forth in Table 1. Also included within the scope of the present disclosure are host cells into which such vectors have been introduced, as well as methods of producing the antibodies or portions thereof by culturing the host cells under conditions permitting production of the antibodies or antibody fragments, and recovering the antibodies and antibody fragments so produced.

[0053] In another aspect, provided herein is a pharmaceutical composition comprising one or more isolated monoclonal antibodies or antigen-binding fragments thereof which specifically bind to PcrV as disclosed herein and a pharmaceutically acceptable carrier or diluent. The one or more isolated antibodies can comprise the CDRs within an HCVR/LCVR amino acid sequence pair selected from the group consisting of the HCVR and LCVR sequences listed in Table 1. The one or more isolated antibodies can comprise an HCVR/LCVR amino acid sequence pair comprising the HCVR and LCVR sequences listed in Table 1. In one embodiment, the HCVR/LCVR amino acid sequence pair is selected from the group consisting of SEQ ID NOs: 2/10, 18/26, 34/42, and 50/58. In one embodiment, the HCVR/LCVR amino acid sequence pair is selected from the

group consisting of SEQ ID NOs: 34/42 and 50/58.

[0054] In another related aspect, provided herein is a composition, which is a combination of an anti-PcrV antibody and one or more additional therapeutic agents.

[0055] In one embodiment, the additional therapeutic agent is any agent that is advantageously combined with an anti-PcrV antibody. Exemplary agents that may be advantageously combined with an anti-PcrV antibody include, without limitation, other agents that bind and/or inhibit *P. aeruginosa* activity (including other antibodies or antigen-binding fragments thereof, etc.) and/or agents which do not directly bind PcrV or another *P. aeruginosa* antigen but nonetheless inhibit bacterial activity including infectivity of host cells. In some aspects, the second therapeutic agent may be a therapeutic for treating infections associated with a different organism which may co-infect with *P. aeruginosa*, for example, an organism such as *S. aureus*. In some aspects, the additional therapeutic agent is selected from the group consisting of an antibiotic, an anti-inflammatory drug, a different antibody to *P. aeruginosa*, and a therapeutic useful for treating a co-infection. In some aspects, the additional therapeutic agent is useful for treating a *S. aureus* co-infection.

[0056] In a related aspect, provided herein is a method of neutralizing *P. aeruginosa*, the method comprising exposing a cell containing intracellular *P. aeruginosa* to a composition comprising one or more anti-PcrV antibodies or antigen-binding fragments thereof, wherein the exposing results in enhanced protection from cell death. In certain embodiments, the exposing may be *in vitro* or *in vivo*. In certain embodiments, the enhanced protection is observed when the antibody is used alone, or when it is used in combination with one or more additional therapeutic agents or antibodies against *P. aeruginosa*. In certain embodiments, the one or more additional therapeutic agents is selected from the group consisting of an antibiotic, an anti-inflammatory drug, a different antibody to *P. aeruginosa*, and a therapeutic useful for treating a co-infection. In some aspects, the one or more additional therapeutic agents is a therapeutic useful for treating a co-infection such as a *S. aureus* infection. In some aspects, the one or more additional therapeutic agents is a different anti-*P. aeruginosa* antibody.

[0057] In some embodiments, provided herein are methods of decreasing the risk of acquiring *P. aeruginosa* infection. In some aspects, the method comprises administering one or more anti-PcrV antibodies provided herein, or a pharmaceutical composition comprising one or more anti-PcrV antibodies. A patient at greater risk for *P. aeruginosa* infection can be a patient with cystic fibrosis, with diabetes, on a mechanical ventilator, undergoing surgery, with tuberculosis, with HIV, with a compromised immune system,

with neutropenia, with an indwelling catheter, after physical trauma, with burns, in an intensive care unit, who is bedridden, with malignancy, with chronic obstructive pulmonary disease, in a long-term care health facility, or who is an intravenous drug user.

[0058] In certain embodiments, provided herein are methods of decreasing bacterial load in a subject. In certain embodiments, decreasing bacterial load is apparent in a subject's lung. In some aspects, the method comprises administering to the subject a composition comprising one or more antibodies or antigen-binding fragments thereof that bind PcrV. In some aspects, the antibody or antigen-binding fragment thereof blocks *P. aeruginosa* delivery of toxins into the host cell. In some aspects, treatment with an anti-PcrV antibody provided herein decreases *P. aeruginosa* bacterial load. In some aspects, treatment with an anti-PcrV antibody provided herein decreases *P. aeruginosa* bacterial load and *S. aureus* bacterial load.

[0059] In some embodiments, provided herein are methods of increasing the survival, or the likelihood of survival, of a subject suffering from infection with *P. aeruginosa*, or a subject at risk for *P. aeruginosa* infection. In some aspects, the method comprises administering at least one anti-PcrV antibody or antigen-binding fragment thereof provided herein, or a pharmaceutical composition comprising at least one anti-PcrV antibody, to a subject in need thereof.

[0060] In some embodiments, provided herein are methods of increasing the survival, or the likelihood of survival, of a subject suffering from infection with *P. aeruginosa*, or a subject at risk for *P. aeruginosa* infection, wherein the subject suffers from cystic fibrosis. In some aspects, the method comprises administering at least one anti-PcrV antibody or antigen-binding fragment thereof provided herein, or a pharmaceutical composition comprising at least one anti-PcrV antibody, to the subject. In some aspects, the subject does not have pneumonia symptoms at the time of administration.

[0061] In some embodiments, provided herein are methods to ameliorate or reduce the severity, duration, or frequency of occurrence, of at least one symptom of a *P. aeruginosa* infection in a subject. In some aspects, the method comprises administering one or more anti-PcrV antibodies or antigen-binding fragments provided herein, or a pharmaceutical composition comprising at least one anti-PcrV antibody or antigen-binding fragment thereof, to a subject in need thereof. In some aspects, the at least one symptom is selected from the group consisting of fever, chills, headache, fatigue, joint pain, stiffness, myalgia, diarrhea, and vomiting; pain, itching, and liquid discharge in the ears; rashes, including pus-filled pimples on the skin; pain and redness in an eye;

pneumonia, coughing, and congestion; soft tissue discharge of green pus and a sweet, fruity smell; and urinary tract infection.

[0062] In some aspects, the subject has pneumonia, bacteremia, a bone infection, a joint infection, a skin infection, a burn infection, a wound infection, or any combination thereof, caused by *P. aeruginosa* infection.

[0063] In some aspects, the one or more anti-PcrV antibodies or antigen-binding fragments thereof provided herein, or the pharmaceutical composition comprising at least one anti-PcrV antibody or antigen-binding fragment thereof, is administered prophylactically or therapeutically to the subject in need thereof to treat or prevent the development of invasive infection with *P. aeruginosa*.

[0064] In one embodiment, the subject in need thereof is a subject with an active *P. aeruginosa* infection or a subject at risk for acquiring *P. aeruginosa* infection. In some aspects, the subject is selected from the group consisting of an immunocompromised individual, a hospitalized individual, an individual suffering from a major illness, an individual undergoing surgery, an individual undergoing an invasive procedure, a trauma patient, an intravenous drug user, an individual with severe burns, an individual using a breathing machine, an individual with a catheter, an individual receiving chemotherapy, an individual with diabetes, an individual with cystic fibrosis, an individual with HIV, an individual with tuberculosis, or an individual with any other medical condition that can compromise the immune system. In some aspects, the subject has a *P. aeruginosa* infection. In some aspects, the subject has a *P. aeruginosa* infection and a *S. aureus* infection. In some aspects, the subject has a *P. aeruginosa* infection and one or more other gram-negative or gram-positive co-infections. In some aspects, the subject has pneumonia, bacteremia, a bone infection, a joint infection, a skin infection, a burn infection, a wound infection, or any combination thereof, caused by *P. aeruginosa* infection. In some aspects, the *P. aeruginosa* is resistant or partially resistant to an antibiotic.

[0065] In one embodiment, the subject in need thereof may be administered at least one anti-PcrV antibody or an antigen-binding fragment thereof as provided herein, or a pharmaceutical composition comprising at least one antibody or antigen-binding fragment thereof, in combination with one or more additional therapeutic agents. The one or more additional therapeutic agents may be selected from the group consisting of an antibiotic, an anti-inflammatory drug (such as corticosteroids, and non-steroidal anti-inflammatory drugs), a different antibody to *P. aeruginosa*, a therapeutic useful for treating a co-infection such as a *S. aureus* infection, and any other drug or therapy

known in the art useful for ameliorating at least one symptom of a *P. aeruginosa* infection, or for reducing the bacterial load in a patient. In one embodiment, the one or more additional therapeutic agents comprise one or more anti-PcrV antibodies. In certain embodiments, the second therapeutic agent may be an agent that helps to counteract or reduce any possible side effect(s) associated with an antibody or antigen-binding fragment thereof of the disclosure, if such side effect(s) should occur.

[0066] In one embodiment, the pharmaceutical composition may be administered subcutaneously, intravenously, intradermally, intramuscularly, intranasally, or orally.

[0067] In certain embodiments, the one or more antibodies or antigen-binding fragments thereof may be administered prophylactically or therapeutically to a subject having, or at risk of having, or pre-disposed to developing a *P. aeruginosa* infection. The subjects at risk include, but are not limited to, an immunocompromised individual, a hospitalized individual, an individual suffering from a major illness, an individual undergoing surgery or another invasive procedure, a trauma patient, an intravenous drug user, an individual with severe burns, an individual using a breathing machine, an individual with a catheter, an individual receiving chemotherapy, an individual with tuberculosis, an individual with diabetes, an individual with cystic fibrosis, an individual with HIV, or an individual with any other medical condition that can compromise the immune system.

[0068] The present disclosure also includes an anti-PcrV antibody or antigen-binding fragment thereof as provided herein for use in treating a subject who has, or is at risk of acquiring, *P. aeruginosa* infection, or for use in the manufacture of a medicament for the treatment of a disease or disorder associated with a *P. aeruginosa* infection.

[0069] Provided herein are injection devices (*e.g.*, hypodermic needle and syringe, an autoinjector or a pre-filled syringe) or vessels (*e.g.*, a vial) that include an anti-PcrV antibody or antigen-binding fragment thereof as provided herein (*e.g.*, an antibody having an HCVR/LCVR amino acid sequence pair is selected from the group consisting of SEQ ID NOs: 2/10, 18/26, 34/42, and 50/58).

[0070] Further provided are methods for administering the composition to a subject (*e.g.*, a human) including the step of introducing the components of the composition into the body of the subject, *e.g.*, parenterally, for example, by injection using an injection device. In an embodiment of the disclosure, the subject suffers from a *P. aeruginosa* infection or is at risk of acquiring a *P. aeruginosa* infection.

[0071] Further provided are methods for making a composition comprising the anti-PcrV antibody (*e.g.*, a composition comprising an antibody having an HCVR/LCVR

amino acid sequence pair is selected from the group consisting of SEQ ID NOs: 2/10, 18/26, 34/42, and 50/58) and a pharmaceutically acceptable carrier.

[0072] Also provided are methods of making the device or vessel that comprises a composition disclosed herein comprising introducing the components of the combination into the vessel or device.

[0073] Other embodiments will become apparent from a review of the ensuing detailed description.

DETAILED DESCRIPTION

[0074] Before the present methods are described, it is to be understood that this invention is not limited to particular methods, and experimental conditions described, as such methods and conditions may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting, since the scope of the present invention will be limited only by the appended claims.

[0075] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, preferred methods and materials are now described.

Definitions

[0076] The term "antibody", as used herein, is intended to refer to immunoglobulin molecules comprised of four polypeptide chains, two heavy (H) chains and two light (L) chains inter-connected by disulfide bonds (*i.e.*, "full antibody molecules"), as well as multimers thereof (*e.g.* IgM) or antigen-binding fragments thereof. Each heavy chain is comprised of a heavy chain variable region ("HCVR" or " V_H ") and a heavy chain constant region (comprised of domains C_{H1} , C_{H2} and C_{H3}). Each light chain is comprised of a light chain variable region ("LCVR" or " V_L ") and a light chain constant region (C_L). The V_H and V_L regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDR), interspersed with regions that are more conserved, termed framework regions (FR). Each V_H and V_L is composed of three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. In certain embodiments of the disclosure, the FRs of the antibody (or antigen binding fragment thereof) may be identical to the

human germline sequences, or may be naturally or artificially modified. An amino acid consensus sequence may be defined based on a side-by-side analysis of two or more CDRs.

[0077] Substitution of one or more CDR residues or omission of one or more CDRs is also possible. Antibodies have been described in the scientific literature in which one or two CDRs can be dispensed with for binding. Padlan *et al.* (1995 FASEB J. 9:133-139) analyzed the contact regions between antibodies and their antigens, based on published crystal structures, and concluded that only about one fifth to one third of CDR residues actually contact the antigen. Padlan also found many antibodies in which one or two CDRs had no amino acids in contact with an antigen (see also, Vajdos *et al.* 2002 J Mol Biol 320:415-428).

[0078] CDR residues not contacting antigen can be identified based on previous studies (for example residues H60-H65 in CDRH2 are often not required), from regions of Kabat CDRs lying outside Chothia CDRs, by molecular modeling and/or empirically. If a CDR or residue(s) thereof is omitted, it is usually substituted with an amino acid occupying the corresponding position in another human antibody sequence or a consensus of such sequences. Positions for substitution within CDRs and amino acids to substitute can also be selected empirically. Empirical substitutions can be conservative or non-conservative substitutions.

[0079] The fully human anti-PcrV monoclonal antibodies disclosed herein may comprise one or more amino acid substitutions, insertions and/or deletions in the framework and/or CDR regions of the heavy and light chain variable domains as compared to the corresponding germline sequences. Such mutations can be readily ascertained by comparing the amino acid sequences disclosed herein to germline sequences available from, for example, public antibody sequence databases. The present disclosure includes antibodies, and antigen-binding fragments thereof, which are derived from any of the amino acid sequences disclosed herein, wherein one or more amino acids within one or more framework and/or CDR regions are mutated to the corresponding residue(s) of the germline sequence from which the antibody was derived, or to the corresponding residue(s) of another human germline sequence, or to a conservative amino acid substitution of the corresponding germline residue(s) (such sequence changes are referred to herein collectively as "germline mutations"). A person of ordinary skill in the art, starting with the heavy and light chain variable region sequences disclosed herein, can easily produce numerous antibodies and antigen-binding fragments which comprise one or more individual germline mutations or

combinations thereof. In certain embodiments, all of the framework and/or CDR residues within the V_H and/or V_L domains are mutated back to the residues found in the original germline sequence from which the antibody was derived. In other embodiments, only certain residues are mutated back to the original germline sequence, *e.g.*, only the mutated residues found within the first 8 amino acids of FR1 or within the last 8 amino acids of FR4, or only the mutated residues found within CDR1, CDR2 or CDR3. In other embodiments, one or more of the framework and/or CDR residue(s) are mutated to the corresponding residue(s) of a different germline sequence (*i.e.*, a germline sequence that is different from the germline sequence from which the antibody was originally derived). Furthermore, the antibodies disclosed herein may contain any combination of two or more germline mutations within the framework and/or CDR regions, *e.g.*, wherein certain individual residues are mutated to the corresponding residue of a particular germline sequence while certain other residues that differ from the original germline sequence are maintained or are mutated to the corresponding residue of a different germline sequence. Once obtained, antibodies and antigen-binding fragments that contain one or more germline mutations can be easily tested for one or more desired property such as, improved binding specificity, increased binding affinity, improved or enhanced antagonistic or agonistic biological properties (as the case may be), reduced immunogenicity, etc. Antibodies and antigen-binding fragments obtained in this general manner are encompassed within the present disclosure.

[0080] Also contemplated herein are fully human anti-PcrV monoclonal antibodies comprising variants of any of the HCVR, LCVR, and/or CDR amino acid sequences disclosed herein having one or more conservative substitutions. For example, the present disclosure includes anti-PcrV antibodies having HCVR, LCVR, and/or CDR amino acid sequences with, *e.g.*, 10 or fewer, 8 or fewer, 6 or fewer, 4 or fewer, etc. conservative amino acid substitutions relative to any of the HCVR, LCVR, and/or CDR amino acid sequences disclosed herein.

[0081] The term "human antibody", as used herein, is intended to include antibodies having variable and constant regions derived from human germline immunoglobulin sequences. The human mAbs of the disclosure may include amino acid residues not encoded by human germline immunoglobulin sequences (*e.g.*, mutations introduced by random or site-specific mutagenesis *in vitro* or by somatic mutation *in vivo*), for example in the CDRs and in particular CDR3. However, the term "human antibody", as used herein, is not intended to include mAbs in which CDR sequences derived from the germline of another mammalian species (*e.g.*, mouse), have been grafted onto human

FR sequences. The term includes antibodies recombinantly produced in a non-human mammal, or in cells of a non-human mammal. The term is not intended to include antibodies isolated from or generated in a human subject.

[0082] The term "recombinant", as used herein, refers to antibodies or antigen-binding fragments thereof of the disclosure created, expressed, isolated or obtained by technologies or methods known in the art as recombinant DNA technology which include, *e.g.*, DNA splicing and transgenic expression. The term refers to antibodies expressed in a non-human mammal (including transgenic non-human mammals, *e.g.*, transgenic mice), or a cell (*e.g.*, CHO cells) expression system or isolated from a recombinant combinatorial human antibody library.

[0083] The term "specifically binds," or "binds specifically to", or the like, means that an antibody or antigen-binding fragment thereof forms a complex with an antigen that is relatively stable under physiologic conditions. Specific binding can be characterized by an equilibrium dissociation constant of at least about 1×10^{-7} M or less (*e.g.*, a smaller K_D denotes a tighter binding). Methods for determining whether two molecules specifically bind are well known in the art and include, for example, equilibrium dialysis, surface plasmon resonance, and the like. As described herein, antibodies have been identified by surface plasmon resonance, *e.g.*, BIACORE™, which bind specifically to PcrV. Moreover, multi-specific antibodies that bind to *P. aeruginosa* PcrV and one or more additional *P. aeruginosa* antigens or a bi-specific that binds to two different regions of *P. aeruginosa* PcrV are nonetheless considered antibodies that "specifically bind", as used herein.

[0084] The term "high affinity" antibody refers to those mAbs having a binding affinity to PcrV, expressed as K_D , of at least 10^{-7} M; preferably 10^{-8} M; more preferably 10^{-9} M, even more preferably 10^{-10} M, even more preferably 10^{-11} M, even more preferably 10^{-12} M, as measured by surface plasmon resonance, *e.g.*, BIACORE™ or solution-affinity ELISA.

[0085] By the term "slow off rate", "Koff" or "kd" is meant an antibody that dissociates from PcrV with a rate constant of $1 \times 10^{-3} \text{ s}^{-1}$ or less, preferably $1 \times 10^{-4} \text{ s}^{-1}$ or less, as determined by surface plasmon resonance, *e.g.*, BIACORE™.

[0086] The terms "antigen-binding portion" of an antibody, "antigen-binding fragment" of an antibody, and the like, as used herein, include any naturally occurring, enzymatically obtainable, synthetic, or genetically engineered polypeptide or glycoprotein that specifically binds an antigen to form a complex. The terms "antigen-binding fragment" of an antibody, or "antibody fragment", as used herein, refers to one or

more fragments of an antibody that retain the ability to bind to PcrV.

[0087] In specific embodiments, antibody or antibody fragments of the disclosure may be conjugated to a moiety such a ligand or a therapeutic moiety ("immunoconjugate"), such as an antibiotic, a second anti-*P. aeruginosa* antibody, or any other therapeutic moiety useful for treating a *P. aeruginosa* infection.

[0088] An "isolated antibody", as used herein, is intended to refer to an antibody that is substantially free of other antibodies (Abs) having different antigenic specificities (e.g., an isolated antibody that specifically binds *P. aeruginosa* PcrV, or a fragment thereof, and is substantially free of Abs that specifically bind antigens other than PcrV).

[0089] A "blocking antibody" or a "neutralizing antibody", as used herein (or an "antibody that neutralizes *P. aeruginosa* activity" or "antagonist antibody"), is intended to refer to an antibody whose binding to PcrV results in inhibition of at least one biological activity of *P. aeruginosa*. For example, an antibody of the disclosure may prevent or block *P. aeruginosa* bacteria from translocating bacterial toxins into a host cell. In addition, a "neutralizing antibody" is one that can neutralize, i.e., prevent, inhibit, reduce, impede or interfere with, the ability of a pathogen to initiate and/or perpetuate an infection in a host. The terms "neutralizing antibody" and "an antibody that neutralizes" or "antibodies that neutralize" are used interchangeably herein. These antibodies can be used, alone or in combination, as prophylactic or therapeutic agents with other anti-bacterial agents upon appropriate formulation, or in association with active vaccination, or as a diagnostic tool.

[0090] "Antibody-dependent cell-mediated cytotoxicity" or "ADCC" is a mechanism of cell-mediated immune defense whereby an effector cell of the immune system actively lyses a target cell, whose membrane-surface antigens have been bound by specific antibodies, such as by those described herein. As such, it is one mechanism through which, for example, a bacteria specific antibody can act to limit the spread of infection. Classical ADCC is mediated by natural killer cells (NK cells), macrophages, neutrophils and in certain instances, eosinophils.

[0091] The term "surface plasmon resonance", as used herein, refers to an optical phenomenon that allows for the analysis of real-time biomolecular interactions by detection of alterations in protein concentrations within a biosensor matrix, for example using the BIACORE™ system (Pharmacia Biosensor AB, Uppsala, Sweden and Piscataway, N.J.).

[0092] The term " K_D ", as used herein, is intended to refer to the equilibrium dissociation constant of a particular antibody-antigen interaction.

[0093] The term “epitope” refers to an antigenic determinant that interacts with a specific antigen-binding site in the variable region of an antibody molecule known as a paratope. A single antigen may have more than one epitope. Thus, different antibodies may bind to different areas on an antigen and may have different biological effects. The term “epitope” also refers to a site on an antigen to which B and/or T cells respond. It also refers to a region of an antigen that is bound by an antibody. Epitopes may be defined as structural or functional. Functional epitopes are generally a subset of the structural epitopes and have those residues that directly contribute to the affinity of the interaction. Epitopes may also be conformational, that is, composed of non-linear amino acids. In certain embodiments, epitopes may include determinants that are chemically active surface groupings of molecules such as amino acids, sugar side chains, phosphoryl groups, or sulfonyl groups, and, in certain embodiments, may have specific three-dimensional structural characteristics, and/or specific charge characteristics.

[0094] The term “cross-competes”, as used herein, means an antibody or antigen-binding fragment thereof binds to an antigen and inhibits or blocks the binding of another antibody or antigen-binding fragment thereof. The term also includes competition between two antibodies in both orientations, *i.e.*, a first antibody that binds and blocks binding of second antibody and vice-versa. In certain embodiments, the first antibody and second antibody may bind to the same epitope. Alternatively, the first and second antibodies may bind to different, but overlapping epitopes such that binding of one inhibits or blocks the binding of the second antibody, *e.g.*, *via* steric hindrance. Cross-competition between antibodies may be measured by methods known in the art, for example, by a real-time, label-free bio-layer interferometry assay. To determine if a test antibody cross-competes with a reference anti-PcrV antibody of the disclosure, the reference antibody is allowed to bind to PcrV protein under saturating conditions. Next, the ability of a test antibody to bind to the PcrV protein is assessed. If the test antibody is able to bind to PcrV protein following saturation binding with the reference anti-PcrV antibody, it can be concluded that the test antibody binds to a different epitope than the reference anti-PcrV antibody. On the other hand, if the test antibody is not able to bind to the PcrV protein following saturation binding with the reference anti-PcrV antibody, then the test antibody may bind to the same epitope as the epitope bound by the reference anti-PcrV antibody.

[0095] Typically, an antibody or antigen-binding fragment provided herein which is modified in some way retains the ability to specifically bind to PcrV, *e.g.*, retains at least 10% of its PcrV binding activity (when compared to the parental antibody) when that

activity is expressed on a molar basis. In some aspects, an antibody or antigen-binding fragment of the disclosure retains at least 20%, 50%, 70%, 80%, 90%, 95% or 100% or more of the PcrV binding affinity as the parental antibody. It is also intended that an antibody or antigen-binding fragment of the present disclosure can include conservative or non-conservative amino acid substitutions (referred to as "conservative variants" or "function conserved variants" of the antibody) that do not substantially alter its biologic activity.

[0096] A "variant" of a polynucleotide refers to a polynucleotide comprising a nucleotide sequence that is at least about 70-99.9% (e.g., at least about 70, 72, 74, 75, 76, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 99.5, or 99.9%) identical to a referenced nucleotide sequence that is set forth herein (e.g., SEQ ID NO: 1, 9, 17, 25, 33, 41, 49, or 57); when the comparison is performed by a BLAST algorithm wherein the parameters of the algorithm are selected to give the largest match between the respective sequences over the entire length of the respective reference sequences (e.g., expect threshold: 10; word size: 28; max matches in a query range: 0; match/mismatch scores: 1, -2; gap costs: linear).

[0097] The term "substantial identity" or "substantially identical," when referring to a nucleic acid or fragment thereof, indicates that, when optimally aligned with appropriate nucleotide insertions or deletions with another nucleic acid (or its complementary strand), there is nucleotide sequence identity in at least about 90%, and more preferably at least about 95%, 96%, 97%, 98% or 99% of the nucleotide bases, as measured by any well-known algorithm of sequence identity, such as FASTA, BLAST or GAP, as discussed below. A nucleic acid molecule having substantial identity to a reference nucleic acid molecule may, in certain instances, encode a polypeptide having the same or substantially similar amino acid sequence as the polypeptide encoded by the reference nucleic acid molecule.

[0098] A "variant" of a polypeptide, such as an immunoglobulin chain (e.g., H1H29329P V_H, V_L, HC, or LC, H1H29332P V_H, V_L, HC, or LC, H1H29336P V_H, V_L, HC, or LC, or H1H29339P V_H, V_L, HC, or LC), refers to a polypeptide comprising an amino acid sequence that is at least about 70-99.9% (e.g., 70, 72, 74, 75, 76, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 99.5, 99.9%) identical or similar to a referenced amino acid sequence that is set forth herein (e.g., SEQ ID NO: 2, 10, 65, 66, 18, 26, 67, 68, 34, 42, 69, 70, 50, 58, 71, or 72); when the comparison is performed by a BLAST algorithm wherein the parameters of the algorithm are selected to give the largest match between the respective sequences over the entire length of the

respective reference sequences (e.g., expect threshold: 10; word size: 3; max matches in a query range: 0; BLOSUM 62 matrix; gap costs: existence 11, extension 1; conditional compositional score matrix adjustment).

[0099] As applied to polypeptides, the term "substantial similarity" or "substantially similar" means that two peptide sequences, when optimally aligned, such as by the programs GAP or BESTFIT using default gap weights, share at least 90% sequence identity, even more preferably at least 95%, 98% or 99% sequence identity. Preferably, residue positions, which are not identical, differ by conservative amino acid substitutions. A "conservatively modified variant" or a "conservative substitution" refers to a variant wherein there is one or more substitutions of amino acids in a polypeptide with other amino acids having similar characteristics (e.g. charge, side-chain size, hydrophobicity/hydrophilicity, backbone conformation and rigidity, etc.). Such changes can frequently be made without significantly disrupting the biological activity of the antibody or fragment. Those of skill in this art recognize that, in general, single amino acid substitutions in non-essential regions of a polypeptide do not substantially alter biological activity (see, e.g., Watson et al. (1987) *Molecular Biology of the Gene*, The Benjamin/Cummings Pub. Co., p. 224 (4th Ed.)). In addition, substitutions of structurally or functionally similar amino acids are less likely to significantly disrupt biological activity.

[00100] In cases where two or more amino acid sequences differ from each other by conservative substitutions, the percent or degree of similarity may be adjusted upwards to correct for the conservative nature of the substitution. Means for making this adjustment are well known to those of skill in the art. (See, e.g., Pearson (1994) *Methods Mol. Biol.* 24: 307-331). Examples of groups of amino acids that have side chains with similar chemical properties include 1) aliphatic side chains: glycine, alanine, valine, leucine and isoleucine; 2) aliphatic-hydroxyl side chains: serine and threonine; 3) amide-containing side chains: asparagine and glutamine; 4) aromatic side chains: phenylalanine, tyrosine, and tryptophan; 5) basic side chains: lysine, arginine, and histidine; 6) acidic side chains: aspartate and glutamate, and 7) sulfur-containing side chains: cysteine and methionine. Preferred conservative amino acids substitution groups are: valine-leucine-isoleucine, phenylalanine-tyrosine, lysine-arginine, alanine-valine, glutamate-aspartate, and asparagine-glutamine. Alternatively, a conservative replacement is any change having a positive value in the PAM250 log-likelihood matrix disclosed in Gonnet *et al.* (1992) *Science* 256: 1443-45. A "moderately conservative" replacement is any change having a nonnegative value in the PAM250 log-likelihood matrix.

[00101] Sequence similarity for polypeptides is typically measured using sequence analysis software. Protein analysis software matches similar sequences using measures of similarity assigned to various substitutions, deletions and other modifications, including conservative amino acid substitutions. For instance, GCG software contains programs such as GAP and BESTFIT which can be used with default parameters to determine sequence homology or sequence identity between closely related polypeptides, such as homologous polypeptides from different species of organisms or between a wild type protein and a mutein thereof. See, *e.g.*, GCG Version 6.1. Polypeptide sequences also can be compared using FASTA with default or recommended parameters; a program in GCG Version 6.1. FASTA (*e.g.*, FASTA2 and FASTA3) provides alignments and percent sequence identity of the regions of the best overlap between the query and search sequences (Pearson (2000) *supra*). Another preferred algorithm when comparing a sequence provided herein to a database containing a large number of sequences from different organisms is the computer program BLAST, especially BLASTP or TBLASTN, using default parameters. See, *e.g.*, Altschul *et al.* (1990) J. Mol. Biol. 215: 403-410 and (1997) Nucleic Acids Res. 25:3389-3402.

[00102] Anti-PcrV antigen-binding proteins, *e.g.*, antibodies and antigen-binding fragments thereof of the present disclosure, in one embodiment, include a heavy chain immunoglobulin variable region having at least 70% (*e.g.*, 80%, 85%, 90%, 95%, 99%) amino acid sequence identity to the amino acids set forth in SEQ ID NO: 2, 18, 34, or 50; and/or a light chain immunoglobulin variable region having at least 70% (*e.g.*, 80%, 85%, 90%, 95%, 99%) amino acid sequence identity to the amino acids set forth in SEQ ID NO: 10, 26, 42, or 58.

[00103] In addition, a variant anti-PcrV antigen-binding protein may include a polypeptide comprising an amino acid sequence that is set forth herein except for one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10) mutations such as, for example, missense mutations (*e.g.*, conservative substitutions), non-sense mutations, deletions, or insertions. For example, the present disclosure includes antigen-binding proteins which include an immunoglobulin heavy chain variant comprising the amino acid sequence set forth in SEQ ID NO: 2, 18, 34, or 50 but having one or more of such mutations and/or an immunoglobulin light chain variant comprising the amino acid sequence set forth in SEQ ID NO: 10, 26, 42, or 58 but having one or more of such mutations. In an embodiment of the disclosure, a variant anti-PcrV antigen-binding protein includes an immunoglobulin heavy chain variant comprising HCDR1, HCDR2, and HCDR3 wherein one or more

(*e.g.*, 1 or 2 or 3) of such CDRs has one or more of such mutations (*e.g.*, conservative substitutions) and/or an immunoglobulin light chain variant comprising LCDR1, LCDR2, and LCDR3 wherein one or more (*e.g.*, 1 or 2 or 3) of such CDRs has one or more of such mutations (*e.g.*, conservative substitutions).

[00104] The disclosure further provides variant anti-PcrV antigen-binding proteins, *e.g.*, antibodies or antigen-binding fragments thereof, comprising one or more variant CDRs (*e.g.*, any one or more of HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and/or LCDR3) that are set forth herein with at least 70%, 75%, 80%, 85%, 90%, 95%, 98%, 99% or 99.9% sequence identity or similarity to, *e.g.*, SEQ ID NO: 4, 6, 8, 12, 14, and/or 16; or 20, 22, 24, 28, 30, and/or 32; or 36, 38, 40, 44, 46, and/or 48; or 52, 54, 56, 60, 62, and/or 64.

[00105] Embodiments of the present disclosure also include variant antigen-binding proteins, *e.g.*, anti-PcrV antibodies and antigen-binding fragments thereof, that comprise immunoglobulin V_Hs and V_Ls, or HCs and LCs, which comprise an amino acid sequence having 70% or more (*e.g.*, 80%, 85%, 90%, 95%, 97% or 99%) overall amino acid sequence identity or similarity to the amino acid sequences of the corresponding V_Hs, V_Ls, HCs or LCs specifically set forth herein, but wherein the HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and/or LCDR3 of such immunoglobulins are not variants and comprise the amino acid sequence set forth in SEQ ID NO: 4, 6, 8, 12, 14, and/or 16; or 20, 22, 24, 28, 30, and/or 32; or 36, 38, 40, 44, 46, and/or 48; or 52, 54, 56, 60, 62, and/or 64, respectively. Thus, in such embodiments, the CDRs within variant antigen-binding proteins are not, themselves, variants.

[00106] Function-conservative variants of the anti-PcrV antibodies and antigen-binding fragments thereof are also part of the present invention. Any of the variants of the anti-PcrV antibodies and antigen-binding fragments thereof (as discussed herein) may be "function-conservative variants". Such function-conservative variants may, in some cases, also be characterized as conservatively modified variants. "Function-conservative variants," as used herein, refers to variants of the anti-PcrV antibodies or antigen-binding fragments thereof in which one or more amino acid residues have been changed without significantly altering one or more functional properties of the antibody or fragment. In an embodiment of the invention, a function-conservative variant of an anti-PcrV antibody or antigen-binding fragment thereof of the present disclosure comprises a variant amino acid sequence and exhibits one or more of the following functional properties:

- binds to full length PcrV with a dissociation constant (K_D) of less than 10^{-8} M, as measured in a surface plasmon resonance assay at 25°C;
- binds to full length PcrV with a dissociation constant (K_D) of less than 10^{-8} M, as measured in a surface plasmon resonance assay at 37°C;
- demonstrates neutralization of *P. aeruginosa* strain 6077 with an IC_{50} ranging from about 10^{-11} M to about 10^{-8} M in a cytotoxicity assay;
- demonstrates neutralization of *P. aeruginosa* strain ATCC 700888 with an IC_{50} ranging from about 10^{-9} M to about 10^{-7} M in a cytotoxicity assay;
- demonstrates neutralization of *P. aeruginosa* strain 6077 with an IC_{50} ranging from about 10^{-10} M to about 10^{-6} M in a hemolytic assay;
- demonstrates neutralization of *P. aeruginosa* strain ATCC 700888 with an IC_{50} ranging from about 10^{-10} M to about 10^{-7} M in a hemolytic assay;
- decreases mortality from *P. aeruginosa* strain 6206 or strain 6077 in mice treated prophylactically with 5 mg/kg relative to untreated mice in an acute pneumonia model;
- decreases mortality from *P. aeruginosa* strain 6206 or strain 6077 in mice treated prophylactically with 1.0, 0.2 or 0.04 mg/kg relative to untreated mice in an acute pneumonia model;
- decreases lung bacterial burden of *P. aeruginosa* strain 6206 in mice treated prophylactically at 0.1 mg/kg or 0.2 mg/kg relative to untreated mice in an acute pneumonia model;
- decreases lung bacterial burden of *P. aeruginosa* strain PA01 in mice treated prophylactically at 25 mg/kg relative to untreated mice in an acute pneumonia model; and/or
- cross-competes with a reference antibody, wherein the reference antibody comprises a heavy chain variable region (HCVR) and a light chain variable region (LCVR) amino acid sequence selected from the group consisting of any of the HCVR and LCVR amino acid sequences of Table 1;
- binds to full length PcrV (SEQ ID NO: 77) with an EC_{50} of less than about 10^{-8} M;
- binds to PcrV 136-233 (SEQ ID NO: 81) with an EC_{50} of less than about 10^{-8} M;
- interacts with amino acid residues ranging from about position 150 to about position 170 of SEQ ID NO: 78;

- interacts with amino acid residues ranging from about position 155 to about 170 of SEQ ID NO: 78; and/or
- interacts with at least one amino acid sequence selected from the group consisting of SEQ ID NO: 85 and SEQ ID NO: 86.

[00107] By the phrase “therapeutically effective amount” is meant an amount that produces the desired effect for which it is administered. The exact amount will depend on the purpose of the treatment, and will be ascertainable by one skilled in the art using known techniques (see, for example, Lloyd (1999) *The Art, Science and Technology of Pharmaceutical Compounding*).

[00108] As used herein, the term “subject” refers to an animal, preferably a mammal, more preferably a human, in need of amelioration, prevention and/or treatment of a disease or disorder such as *P. aeruginosa* infection. The subject may have a *P. aeruginosa* infection or is predisposed to developing a *P. aeruginosa* infection. Subjects “predisposed to developing a *P. aeruginosa* infection”, or subjects “who may be at elevated risk for contracting a *P. aeruginosa* infection”, are those subjects with a subject undergoing surgery, a subject being treated for a major illness, a trauma patient, an intravenous drug user, a subject having severe burns, a subject using a breathing machine, a subject with a catheter, a subject undergoing chemotherapy, a subject having diabetes, a subject with cystic fibrosis, a subject with tuberculosis, a subject with HIV, and a subject with a compromised immune system.

[00109] As used herein, the terms “treat”, “treating” or “treatment” refer to therapeutic treatment, wherein the object is to clear or reduce the bacterial burden of an infectious agent in a subject that has been clinically diagnosed with an infection, such as pneumonia, bacteremia, peritonitis, sepsis, and/or an abscess. The terms include inhibition of progression of disease or of worsening of infection. The terms also include positive prognosis of disease, i.e., the subject may be free of infection or may have reduced or no bacterial titers upon administration of a therapeutic agent such as an antibody disclosed herein. “Treatment” can also mean prolonging survival as compared to expected survival if not receiving treatment. Those in need of treatment include those already with the infection as well as those prone to acquire the *P. aeruginosa* infection, e.g., in burn patients or immunosuppressed patients susceptible to bacterial infection, e.g., *P. aeruginosa* infection. The therapeutic agent may be administered at a therapeutic dose to the subject.

[00110] As used herein, the terms “prevent” or “mitigate” refer to prophylactic or preventative measures, wherein the object is to prevent or slow down (lessen) a

P. aeruginosa infection, or symptom associated with the infection. Beneficial or desired clinical results include, but are not limited to, alleviation of symptoms, diminishment of extent of infection, stabilized (i.e., not worsening) state of disease, clearance or reduction of the infectious agent such as *P. aeruginosa* in a subject, a delay or slowing of disease progression, amelioration or palliation of the disease state, and remission (whether partial or total), whether detectable or undetectable.

[00111] As used herein, the terms "nosocomial disease" and "nosocomial infection" refer to a disease or infection originating in a hospital or other healthcare facility. Nosocomial infections can be caused by *P. aeruginosa*, e.g., *P. aeruginosa* resistant to antibiotics. In certain aspects, a nosocomial infection is not present or incubating prior to the subject being admitted to the hospital or healthcare facility, and is acquired or contracted after the subject's admittance to the hospital or healthcare facility.

General Description

[00112] As described above, the bacterial T3SS is an important virulence factor of Gram-negative bacteria, including *P. aeruginosa*. The PcrV protein, located at the end of the T3SS apparatus, forms a ring-type structure on the tip of the needle. The needle tip protein is accessible to antibodies as one of the two proteins present on the external surface of the bacteria.

[00113] As such, provided herein are antibodies, bispecific antigen binding molecules, and antigen-binding fragments thereof that bind *P. aeruginosa* PcrV. Antibodies that target PcrV can prevent injection of the bacterial toxins into an infected cell, leading to decreased inflammation, cell death, and dissemination of the bacteria. Antibodies that target PcrV can block pore-mediated membrane permeability in an infected host cell. These anti-PcrV antibodies, bispecific antigen-binding molecules, and antigen-binding fragments thereof are useful in treating and/or mitigating *P. aeruginosa* infection, treating and/or mitigating symptoms of *P. aeruginosa* infection, and preventing development of or progression of symptoms of *P. aeruginosa* infection. In some aspects, the anti-PcrV antibodies prevent development of or progression of *P. aeruginosa*-caused pneumonia.

[00114] Methods for treating patients with *P. aeruginosa* infection are provided herein. Methods for preventing development of or progression of symptoms of *P. aeruginosa* infection are provided herein. Methods for preventing signs of *P. aeruginosa*, such as a positive culture from blood, skin, urine, pus, or other body fluid samples or radiographic imaging suggestive of *P. aeruginosa* infection, or findings on physical exam suggestive of *P. aeruginosa* infection such as, but not limited to, skin or bone ulcers, or abnormal

vital signs, are provided herein. In some aspects, the patient may have cystic fibrosis. In some aspects, the patient may be on a mechanical ventilator. In some aspects the patient is a neutropenic cancer patient. In some aspects, the patient is a burn victim. In some aspects, the patient suffers from tuberculosis.

[00115] In some embodiments the patient has an antibiotic-resistant *P. aeruginosa* infection. In some embodiments the patient has a co-infection such as a *S. aureus* infection, for example, an antibiotic-resistant *S. aureus* infection. In some embodiments both the *S. aureus* and the *P. aeruginosa* infections are antibiotic-resistant. In some embodiments the patient has a co-infection with a Gram-negative bacteria or a Gram-positive bacteria.

General Methods

[00116] Standard methods in molecular biology are described Sambrook, Fritsch and Maniatis (1982 & 1989 2nd Edition, 2001 3rd Edition) Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.; Sambrook and Russell (2001) Molecular Cloning, 3rd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.; Wu (1993) Recombinant DNA, Vol. 217, Academic Press, San Diego, Calif.). Standard methods also appear in Ausbel, *et al.* (2001) Current Protocols in Molecular Biology, Vols. 1-4, John Wiley and Sons, Inc. New York, N.Y., which describes cloning in bacterial cells and DNA mutagenesis (Vol. 1), cloning in mammalian cells and yeast (Vol. 2), glycoconjugates and protein expression (Vol. 3), and bioinformatics (Vol. 4).

[00117] Methods for protein purification including immunoprecipitation, chromatography, electrophoresis, centrifugation, and crystallization are described (Coligan, *et al.* (2000) Current Protocols in Protein Science, Vol. 1, John Wiley and Sons, Inc., New York). Chemical analysis, chemical modification, post-translational modification, production of fusion proteins, glycosylation of proteins are described (see, *e.g.*, Coligan, *et al.* (2000) Current Protocols in Protein Science, Vol. 2, John Wiley and Sons, Inc., New York; Ausubel, *et al.* (2001) Current Protocols in Molecular Biology, Vol. 3, John Wiley and Sons, Inc., NY, NY, pp. 16.0.5-16.22.17; Sigma-Aldrich, Co. (2001) Products for Life Science Research, St. Louis, Mo.; pp. 45-89; Amersham Pharmacia Biotech (2001) BioDirectory, Piscataway, N.J., pp. 384-391). Production, purification, and fragmentation of polyclonal and monoclonal antibodies are described (Coligan, *et al.* (2001) Current Protocols in Immunology, Vol. 1, John Wiley and Sons, Inc., New York; Harlow and Lane (1999) Using Antibodies, Cold Spring Harbor Laboratory Press, Cold Spring Harbor,

N.Y.; Harlow and Lane, *supra*). Standard techniques for characterizing ligand/receptor interactions are available (see, *e.g.*, Coligan, *et al.* (2001) *Current Protocols in Immunology*, Vol. 4, John Wiley, Inc., New York).

[00118] Monoclonal, polyclonal, and humanized antibodies can be prepared (see, *e.g.*, Sheperd and Dean (eds.) (2000) *Monoclonal Antibodies*, Oxford Univ. Press, New York, N.Y.; Kontermann and Dubel (eds.) (2001) *Antibody Engineering*, Springer-Verlag, New York; Harlow and Lane (1988) *Antibodies A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., pp. 139-243; Carpenter, *et al.* (2000) *J. Immunol.* 165:6205; He, *et al.* (1998) *J. Immunol.* 160:1029; Tang *et al.* (1999) *J. Biol. Chem.* 274:27371-27378; Baca *et al.* (1997) *J. Biol. Chem.* 272:10678-10684; Chothia *et al.* (1989) *Nature* 342:877-883; Foote and Winter (1992) *J. Mol. Biol.* 224:487-499; U.S. Pat. No. 6,329,511).

[00119] An alternative to humanization is to use human antibody libraries displayed on phage or human antibody libraries in transgenic mice (Vaughan *et al.* (1996) *Nature Biotechnol.* 14:309-314; Barbas (1995) *Nature Medicine* 1:837-839; Mendez *et al.* (1997) *Nature Genetics* 15:146-156; Hoogenboom and Chames (2000) *Immunol. Today* 21:371-377; Barbas *et al.* (2001) *Phage Display: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.; Kay *et al.* (1996) *Phage Display of Peptides and Proteins: A Laboratory Manual*, Academic Press, San Diego, Calif.; de Bruin *et al.* (1999) *Nature Biotechnol.* 17:397-399). Single chain antibodies and diabodies are described (see, *e.g.*, Malecki *et al.* (2002) *Proc. Natl. Acad. Sci. USA* 99:213-218; Conrath *et al.* (2001) *J. Biol. Chem.* 276:7346-7350; Desmyter *et al.* (2001) *J. Biol. Chem.* 276:26285-26290; Hudson and Kortt (1999) *J. Immunol. Methods* 231:177-189; and U.S. Pat. No. 4,946,778). Bifunctional antibodies are provided (see, *e.g.*, Mack, *et al.* (1995) *Proc. Natl. Acad. Sci. USA* 92:7021-7025; Carter (2001) *J. Immunol. Methods* 248:7-15; Volkel, *et al.* (2001) *Protein Engineering* 14:815-823; Segal, *et al.* (2001) *J. Immunol. Methods* 248:1-6; Brennan, *et al.* (1985) *Science* 229:81-83; Raso, *et al.* (1997) *J. Biol. Chem.* 272:27623; Morrison (1985) *Science* 229:1202-1207; Traunecker, *et al.* (1991) *EMBO J.* 10:3655-3659; and U.S. Pat. Nos. 5,932,448, 5,532,210, and 6,129,914). Fully human antibodies may also be developed in genetically engineered mice such as the VelociMouse. See *e.g.*, DeChiara *et al.*, Producing fully ES cell-derived mice from eight-cell stage embryo injections, *Methods Enzymol.* 476:285-94 (2010); Dechiara *et al.*, VelociMouse: fully ES cell-derived F0-generation mice obtained from the injection of ES cells into eight-cell-stage embryos.

Methods Mol Biol, 530:311-24 (2009); U.S. patent nos. 7576259; 7659442; or 7294754, and US2008/0078000A1.

[00120] Purification of antigen is not typically necessary for the generation of antibodies. Animals can be immunized with cells bearing the antigen of interest. Splenocytes can then be isolated from the immunized animals, and the splenocytes can be fused with a myeloma cell line to produce a hybridoma (see, *e.g.*, Meyaard *et al.* (1997) *Immunity* 7:283-290; Wright *et al.* (2000) *Immunity* 13:233-242; Preston *et al.*, *supra*; Kaithamana *et al.* (1999) *J. Immunol.* 163:5157-5164).

[00121] Antibodies can be conjugated, *e.g.*, to small drug molecules, enzymes, liposomes, polyethylene glycol (PEG). Antibodies are useful for therapeutic, diagnostic, kit or other purposes, and include antibodies coupled, *e.g.*, to dyes, radioisotopes, enzymes, or metals, *e.g.*, colloidal gold (see, *e.g.*, Le Doussal *et al.* (1991) *J. Immunol.* 146:169-175; Gibellini *et al.* (1998) *J. Immunol.* 160:3891-3898; Hsing and Bishop (1999) *J. Immunol.* 162:2804-2811; Everts *et al.* (2002) *J. Immunol.* 168:883-889).

[00122] Methods for flow cytometry, including fluorescence activated cell sorting (FACS), are available (see, *e.g.*, Owens, *et al.* (1994) *Flow Cytometry Principles for Clinical Laboratory Practice*, John Wiley and Sons, Hoboken, N.J.; Givan (2001) *Flow Cytometry*, 2nd ed.; Wiley-Liss, Hoboken, N.J.; Shapiro (2003) *Practical Flow Cytometry*, John Wiley and Sons, Hoboken, N.J.). Fluorescent reagents suitable for modifying nucleic acids, including nucleic acid primers and probes, polypeptides, and antibodies, for use, *e.g.*, as diagnostic reagents, are available (Molecular Probes (2003) Catalogue, Molecular Probes, Inc., Eugene, Oreg.; Sigma-Aldrich (2003) Catalogue, St. Louis, Mo.).

[00123] Standard methods of histology of the immune system are described (see, *e.g.*, Muller-Harmelink (ed.) (1986) *Human Thymus: Histopathology and Pathology*, Springer Verlag, New York, N.Y.; Hiatt, *et al.* (2000) *Color Atlas of Histology*, Lippincott, Williams, and Wilkins, Phila, Pa.; Louis, *et al.* (2002) *Basic Histology: Text and Atlas*, McGraw-Hill, New York, N.Y.).

[00124] Software packages and databases for determining, *e.g.*, antigenic fragments, leader sequences, protein folding, functional domains, glycosylation sites, and sequence alignments, are available (see, *e.g.*, GenBank, Vector NTI® Suite (Informax, Inc, Bethesda, Md.); GCG Wisconsin Package (Accelrys, Inc., San Diego, Calif.); DeCypher® (TimeLogic Corp., Crystal Bay, Nev.); Menne, *et al.* (2000) *Bioinformatics* 16: 741-742; Menne, *et al.* (2000) *Bioinformatics Applications Note* 16:741-742; Wren, *et*

al. (2002) Comput. Methods Programs Biomed. 68:177-181; von Heijne (1983) Eur. J. Biochem. 133:17-21; von Heijne (1986) Nucleic Acids Res. 14:4683-4690).

Anti-PcrV Antibodies

[00125] Passive immunotherapy for prophylaxis or treatment of infectious diseases has been used for more than a century, usually in the form of convalescent human sera that contains high titers of neutralizing antibodies (Good *et al.*, 1991; Cancer 68: 1415-1421). Today, several purified monoclonal antibodies are currently in preclinical and clinical development for use as anti-microbials (Marasco *et al.* 2007; Nature Biotechnology 25: 1421-1434). Certain antibodies have been described that bind to *P. aeruginosa* PcrV (See *e.g.* François *et al.*, 2012; Crit. Care Med. 40: 2320-2326; and WO2009088032).

[00126] The inventors have described herein fully human antibodies and antigen-binding fragments thereof that specifically bind to *P. aeruginosa* PcrV and modulate the T3SS virulence mechanism. The anti-PcrV antibodies may bind to PcrV with high affinity. In certain embodiments, the antibodies may bind to PcrV and prevent or mitigate cell death. In certain embodiments, the antibodies may prevent the translocation of bacterial toxins into the host cell, and as such may inhibit or neutralize *P. aeruginosa* infection. In some embodiments, the antibodies may function by blocking T3SS pore-mediated membrane permeability in a host cell. In certain embodiments, the antibodies provided herein may mediate antibody dependent cell-mediated cytotoxicity (ADCC) and as such, may aid in destroying cells that harbor the bacteria. In certain embodiments, the antibodies may act in both fashions, *e.g.* they may neutralize bacterial infectivity and may mediate ADCC. In some aspects, the antibodies can decrease bacterial load, *e.g.* lung bacterial load, relative to similarly situated but untreated subjects or populations. In some aspects, the antibodies can increase survival or decrease mortality, relative to similarly situated but untreated subjects or populations. In some embodiments, the antibodies may be useful for treating a subject suffering from a *P. aeruginosa* infection. The antibodies when administered to a subject in need thereof may reduce the infection by *P. aeruginosa* in the subject. They may be used alone or as adjunct therapy with other therapeutic moieties or modalities known in the art for treating a bacterial infection. Furthermore, the identified antibodies can be used prophylactically (before infection) to protect a subject, *e.g.* a mammal, from infection, or can be used therapeutically (after infection is established) to ameliorate a previously established infection, or to ameliorate at least one symptom associated with the infection.

[00127] The full-length amino acid sequence of *P. aeruginosa* PcrV protein is shown in

GenBank as accession number 250397.1 and also in SEQ ID NO: 77. A truncated PcrV protein (PcrV_136-257) is shown in SEQ ID NO: 79. Both proteins can be labeled with a 6-histidine tag: SEQ ID NO: 78 for the full length PcrV and SEQ ID NO: 80 for the truncated PcrV.

[00128] In certain embodiments, the antibodies provided herein are obtained from mice immunized with a primary immunogen, such as a full-length PcrV, or with a truncated version of the protein. The immunogen may be any immunogenic fragment of the PcrV protein or DNA encoding the active fragment thereof. The peptides may be modified to include addition or substitution of certain residues for tagging or for purposes of conjugation to carrier molecules, such as, KLH. For example, a cysteine may be added at either the N terminal or C terminal end of a peptide, or a linker sequence may be added to prepare the peptide for conjugation to, for example, KLH for immunization.

[00129] Certain anti-PcrV antibodies disclosed herein are able to bind to and neutralize the activity of *P. aeruginosa*, as determined by *in vitro* or *in vivo* assays. The ability of the antibodies of this disclosure to bind to and neutralize the activity of *P. aeruginosa* may be measured using any standard method known to those skilled in the art, including binding assays, or activity assays, as described herein.

[00130] Non-limiting, exemplary *in vitro* assays for measuring binding activity are illustrated in Example 3, herein. In Example 3, the binding affinity and dissociation constants of anti-PcrV antibodies for full length PcrV (6his labeled, SEQ ID NO: 78) or truncated PcrV (6his labeled, SEQ ID NO: 80) were determined by Biacore. In Examples 6 and 7, neutralization assays were used to determine the ability of the antibodies to neutralize two different strains of *P. aeruginosa*.

[00131] The antibodies specific for PcrV may contain no additional labels or moieties, or they may contain an N-terminal or C-terminal label or moiety. In one embodiment, the label or moiety is biotin. In a binding assay, the location of a label (if any) may determine the orientation of the peptide relative to the surface upon which the peptide is bound. For example, if a surface is coated with avidin, a peptide containing an N-terminal biotin will be oriented such that the C-terminal portion of the peptide will be distal to the surface. In one embodiment, the label may be a radionuclide, a fluorescent dye or an MRI-detectable label. In certain embodiments, such labeled antibodies may be used in diagnostic assays including imaging assays.

Antigen-Binding Fragments of Antibodies

[00132] Unless specifically indicated otherwise, the term "antibody," as used herein,

shall be understood to encompass antibody molecules comprising two immunoglobulin heavy chains and two immunoglobulin light chains (*i.e.*, "full antibody molecules") as well as antigen-binding fragments thereof. The terms "antigen-binding portion" of an antibody, "antigen-binding fragment" of an antibody, and the like, as used herein, include any naturally occurring, enzymatically obtainable, synthetic, or genetically engineered polypeptide or glycoprotein that specifically binds an antigen to form a complex. The terms "antigen-binding fragment" of an antibody, or "antibody fragment", as used herein, refers to one or more fragments of an antibody that retain the ability to specifically bind to *P. aeruginosa* PcrV. An antibody fragment may include a Fab fragment, a F(ab')₂ fragment, a Fv fragment, a dAb fragment, a fragment containing a CDR, or an isolated CDR. In certain embodiments, the term "antigen-binding fragment" refers to a polypeptide fragment of a multi-specific antigen-binding molecule. Antigen-binding fragments of an antibody may be derived, *e.g.*, from full antibody molecules using any suitable standard techniques such as proteolytic digestion or recombinant genetic engineering techniques involving the manipulation and expression of DNA encoding antibody variable and (optionally) constant domains. Such DNA is known and/or is readily available from, *e.g.*, commercial sources, DNA libraries (including, *e.g.*, phage-antibody libraries), or can be synthesized. The DNA may be sequenced and manipulated chemically or by using molecular biology techniques, for example, to arrange one or more variable and/or constant domains into a suitable configuration, or to introduce codons, create cysteine residues, modify, add or delete amino acids, etc.

[00133] Non-limiting examples of antigen-binding fragments include: (i) Fab fragments; (ii) F(ab')₂ fragments; (iii) Fd fragments; (iv) Fv fragments; (v) single-chain Fv (scFv) molecules; (vi) dAb fragments; and (vii) minimal recognition units consisting of the amino acid residues that mimic the hypervariable region of an antibody (*e.g.*, an isolated complementarity determining region (CDR) such as a CDR3 peptide), or a constrained FR3-CDR3-FR4 peptide. Other engineered molecules, such as domain-specific antibodies, single domain antibodies, domain-deleted antibodies, chimeric antibodies, CDR-grafted antibodies, diabodies, triabodies, tetrabodies, minibodies, nanobodies (*e.g.* monovalent nanobodies, bivalent nanobodies, etc.), small modular immunopharmaceuticals (SMIPs), and shark variable IgNAR domains, are also encompassed within the expression "antigen-binding fragment," as used herein.

[00134] An antigen-binding fragment of an antibody will typically comprise at least one variable domain. The variable domain may be of any size or amino acid composition and will generally comprise at least one CDR, which is adjacent to or in frame with one or

more framework sequences. In antigen-binding fragments having a V_H domain associated with a V_L domain, the V_H and V_L domains may be situated relative to one another in any suitable arrangement. For example, the variable region may be dimeric and contain $V_H - V_H$, $V_H - V_L$ or $V_L - V_L$ dimers. Alternatively, the antigen-binding fragment of an antibody may contain a monomeric V_H or V_L domain.

[00135] In certain embodiments, an antigen-binding fragment of an antibody may contain at least one variable domain covalently linked to at least one constant domain. Non-limiting, exemplary configurations of variable and constant domains that may be found within an antigen-binding fragment of an antibody of the present disclosure include: (i) $V_H - C_H1$; (ii) $V_H - C_H2$; (iii) $V_H - C_H3$; (iv) $V_H - C_H1 - C_H2$; (v) $V_H - C_H1 - C_H2 - C_H3$; (vi) $V_H - C_H2 - C_H3$; (vii) $V_H - C_L$; (viii) $V_L - C_H1$; (ix) $V_L - C_H2$; (x) $V_L - C_H3$; (xi) $V_L - C_H1 - C_H2$; (xii) $V_L - C_H1 - C_H2 - C_H3$; (xiii) $V_L - C_H2 - C_H3$; and (xiv) $V_L - C_L$. In any configuration of variable and constant domains, including any of the exemplary configurations listed above, the variable and constant domains may be either directly linked to one another or may be linked by a full or partial hinge or linker region. A hinge region may consist of at least 2 (*e.g.*, 5, 10, 15, 20, 40, 60 or more) amino acids, which result in a flexible or semi-flexible linkage between adjacent variable and/or constant domains in a single polypeptide molecule. Moreover, an antigen-binding fragment of an antibody of the present disclosure may comprise a homo-dimer or hetero-dimer (or other multimer) of any of the variable and constant domain configurations listed above in non-covalent association with one another and/or with one or more monomeric V_H or V_L domain (*e.g.*, by disulfide bond(s)).

[00136] As with full antibody molecules, antigen-binding fragments may be mono-specific or multi-specific (*e.g.*, bi-specific). A multi-specific antigen-binding fragment of an antibody will typically comprise at least two different variable domains, wherein each variable domain is capable of specifically binding to a separate antigen or to a different epitope on the same antigen. Any multi-specific antibody format, including the exemplary bi-specific antibody formats disclosed herein, may be adapted for use in the context of an antigen-binding fragment of an antibody of the present disclosure using routine techniques available in the art.

Preparation of Human Antibodies

[00137] Methods for generating human antibodies in transgenic mice are known in the art. Any such known methods can be used in the context of the present disclosure to make human antibodies that specifically bind to *P. aeruginosa* PcrV. An immunogen

comprising any one of the following can be used to generate antibodies to PcrV. In certain embodiments, the antibodies of the disclosure are obtained from mice immunized with a full-length PcrV, for example, GenBank accession numbers NP_250397.1 (SEQ ID NO: 77) or a truncated PcrV protein, for example, PcrV_136-257 (SEQ ID NO: 79). Alternatively, the PcrV protein or a fragment thereof may be produced using standard biochemical techniques and modified and used as immunogen. In one embodiment, the immunogen is a recombinant PcrV protein or fragment thereof. In certain embodiments, the immunogen may be a commercially available PcrV protein. In certain embodiments, one or more booster injections may be administered. In certain embodiments, the booster injections may comprise one or more commercially available PcrV proteins. In certain embodiments, the immunogen may be a recombinant PcrV protein expressed in *E. coli* or in any other eukaryotic or mammalian cells such as Chinese hamster ovary (CHO) cells.

[00138] Using VELOCIMMUNE® technology (see, for example, US 6,596,541, Regeneron Pharmaceuticals, VELOCIMMUNE®) or any other known method for generating monoclonal antibodies, high affinity chimeric antibodies to PcrV are initially isolated having a human variable region and a mouse constant region. The VELOCIMMUNE® technology involves generation of a transgenic mouse having a genome comprising human heavy and light chain variable regions operably linked to endogenous mouse constant region loci such that the mouse produces an antibody comprising a human variable region and a mouse constant region in response to antigenic stimulation. The DNA encoding the variable regions of the heavy and light chains of the antibody are isolated and operably linked to DNA encoding the human heavy and light chain constant regions. The DNA is then expressed in a cell capable of expressing the fully human antibody.

[00139] Generally, a VELOCIMMUNE® mouse is challenged with the antigen of interest, and lymphatic cells (such as B-cells) are recovered from the mice that express antibodies. The lymphatic cells may be fused with a myeloma cell line to prepare immortal hybridoma cell lines, and such hybridoma cell lines are screened and selected to identify hybridoma cell lines that produce antibodies specific to the antigen of interest. DNA encoding the variable regions of the heavy chain and light chain may be isolated and linked to desirable isotypic constant regions of the heavy chain and light chain. Such an antibody protein may be produced in a cell, such as a CHO cell. Alternatively, DNA encoding the antigen-specific chimeric antibodies or the variable domains of the light and heavy chains may be isolated directly from antigen-specific lymphocytes.

[00140] Initially, high affinity chimeric antibodies are isolated having a human variable region and a mouse constant region. As in the experimental section below, the antibodies are characterized and selected for desirable characteristics, including affinity, selectivity, epitope, etc. The mouse constant regions are replaced with a desired human constant region to generate the fully human antibody, for example wild-type or modified IgG1 or IgG4. While the constant region selected may vary according to specific use, high affinity antigen-binding and target specificity characteristics reside in the variable region.

Bioequivalents

[00141] The anti-PcrV antibodies and antibody fragments disclosed herein encompass proteins having amino acid sequences that vary from those of the described antibodies, but that retain the ability to bind PcrV. Such variant antibodies and antibody fragments comprise one or more additions, deletions, or substitutions of amino acids when compared to parent sequence, but exhibit biological activity that is essentially equivalent to that of the described antibodies. Likewise, the antibody-encoding DNA sequences of the present disclosure encompass sequences that comprise one or more additions, deletions, or substitutions of nucleotides when compared to the disclosed sequence, but that encode an antibody or antibody fragment that is essentially bioequivalent to an antibody or antibody fragment disclosed herein.

[00142] Two antigen-binding proteins, or antibodies, are considered bioequivalent if, for example, they are pharmaceutical equivalents or pharmaceutical alternatives whose rate and extent of absorption do not show a significant difference when administered at the same molar dose under similar experimental conditions, either single dose or multiple doses. Some antibodies will be considered equivalents or pharmaceutical alternatives if they are equivalent in the extent of their absorption but not in their rate of absorption and yet may be considered bioequivalent because such differences in the rate of absorption are intentional and are reflected in the labeling, are not essential to the attainment of effective body drug concentrations on, *e.g.*, chronic use, and are considered medically insignificant for the particular drug product studied.

[00143] In one embodiment, two antigen-binding proteins are bioequivalent if there are no clinically meaningful differences in their safety, purity, or potency.

[00144] In one embodiment, two antigen-binding proteins are bioequivalent if a patient can be switched one or more times between the reference product and the biological product without an expected increase in the risk of adverse effects, including a clinically

significant change in immunogenicity, or diminished effectiveness, as compared to continued therapy without such switching.

[00145] In one embodiment, two antigen-binding proteins are bioequivalent if they both act by a common mechanism or mechanisms of action for the condition or conditions of use, to the extent that such mechanisms are known.

[00146] Bioequivalence may be demonstrated by *in vivo* and/or *in vitro* methods. Bioequivalence measures include, *e.g.*, (a) an *in vivo* test in humans or other mammals, in which the concentration of the antibody or its metabolites is measured in blood, plasma, serum, or other biological fluid as a function of time; (b) an *in vitro* test that has been correlated with and is reasonably predictive of human *in vivo* bioavailability data; (c) an *in vivo* test in humans or other mammals in which the appropriate acute pharmacological effect of the antibody (or its target) is measured as a function of time; and (d) in a well-controlled clinical trial that establishes safety, efficacy, or bioavailability or bioequivalence of an antibody.

[00147] Bioequivalent variants of the antibodies disclosed herein may be constructed by, for example, making various substitutions of residues or sequences or deleting terminal or internal residues or sequences not needed for biological activity. For example, cysteine residues not essential for biological activity can be deleted or replaced with other amino acids to prevent formation of unnecessary or incorrect intramolecular disulfide bridges upon renaturation. In other contexts, bioequivalent antibodies may include antibody variants comprising amino acid changes, which modify the glycosylation characteristics of the antibodies, *e.g.*, mutations that eliminate or remove glycosylation.

Anti-PcrV Antibodies Comprising Fc Variants

[00148] According to certain embodiments, anti-PcrV antibodies are provided comprising an Fc domain comprising one or more mutations that enhance or diminish antibody binding to the FcRn receptor, *e.g.*, at acidic pH as compared to neutral pH. For example, the present disclosure includes anti-PcrV antibodies comprising a mutation in the C_H2 or a C_H3 region of the Fc domain, wherein the mutation(s) increases the affinity of the Fc domain to FcRn in an acidic environment (*e.g.*, in an endosome where pH ranges from about 5.5 to about 6.0). Such mutations may result in an increase in serum half-life of the antibody when administered to an animal. Non-limiting examples of such Fc modifications include, *e.g.*, a modification at position 250 (*e.g.*, E or Q); 250 and 428 (*e.g.*, L or F); 252 (*e.g.*, L/Y/F/W or T), 254 (*e.g.*, S or T), and 256 (*e.g.*, S/R/Q/E/D or T);

or a modification at position 428 and/or 433 (e.g., H/L/R/S/P/Q or K) and/or 434 (e.g., A, W, H, F or Y [N434A, N434W, N434H, N434F or N434Y]); or a modification at position 250 and/or 428; or a modification at position 307 or 308 (e.g., 308F, V308F), and 434. In one embodiment, the modification comprises a 428L (e.g., M428L) and 434S (e.g., N434S) modification; a 428L, 259I (e.g., V259I), and 308F (e.g., V308F) modification; a 433K (e.g., H433K) and a 434 (e.g., 434Y) modification; a 252, 254, and 256 (e.g., 252Y, 254T, and 256E) modification; a 250Q and 428L modification (e.g., T250Q and M428L); and a 307 and/or 308 modification (e.g., 308F or 308P). In yet another embodiment, the modification comprises a 265A (e.g., D265A) and/or a 297A (e.g., N297A) modification.

[00149] For example, provided herein are anti-PcrV antibodies comprising an Fc domain comprising one or more pairs or groups of mutations selected from the group consisting of: 250Q and 248L (e.g., T250Q and M248L); 252Y, 254T and 256E (e.g., M252Y, S254T and T256E); 428L and 434S (e.g., M428L and N434S); 257I and 311I (e.g., P257I and Q311I); 257I and 434H (e.g., P257I and N434H); 376V and 434H (e.g., D376V and N434H); 307A, 380A and 434A (e.g., T307A, E380A and N434A); and 433K and 434F (e.g., H433K and N434F). All possible combinations of the foregoing Fc domain mutations and other mutations within the antibody variable domains disclosed herein are contemplated within the scope of the present disclosure.

[00150] The present disclosure also includes anti-PcrV antibodies comprising a chimeric heavy chain constant (C_H) region, wherein the chimeric C_H region comprises segments derived from the C_H regions of more than one immunoglobulin isotype. For example, the antibodies of the disclosure may comprise a chimeric C_H region comprising part or all of a C_{H2} domain derived from a human IgG1, human IgG2 or human IgG4 molecule, combined with part or all of a C_{H3} domain derived from a human IgG1, human IgG2 or human IgG4 molecule. According to certain embodiments, the antibodies provided herein comprise a chimeric C_H region having a chimeric hinge region. For example, a chimeric hinge may comprise an "upper hinge" amino acid sequence (amino acid residues from positions 216 to 227 according to EU numbering) derived from a human IgG1, a human IgG2 or a human IgG4 hinge region, combined with a "lower hinge" sequence (amino acid residues from positions 228 to 236 according to EU numbering) derived from a human IgG1, a human IgG2 or a human IgG4 hinge region. According to certain embodiments, the chimeric hinge region comprises amino acid residues derived from a human IgG1 or a human IgG4 upper hinge and amino acid residues derived from a human IgG2 lower hinge. An antibody comprising a chimeric C_H region as described

herein may, in certain embodiments, exhibit modified Fc effector functions without adversely affecting the therapeutic or pharmacokinetic properties of the antibody. (See, e.g., U.S. Provisional Appl. No. 61/759,578, filed February 1, 2013).

Biological Characteristics of the Antibodies

[00151] In general, the antibodies of the present disclosure function by binding to PcrV. For example, the present disclosure includes antibodies and antigen-binding fragments of antibodies that bind PcrV or PcrV₁₃₆₋₂₅₇ (e.g., at 25°C or at 37°C) with a K_D of less than 10^{-7} M, as measured by surface plasmon resonance, e.g., using the assay format as described herein. In certain embodiments, the antibodies or antigen-binding fragments thereof bind PcrV with a K_D of less than about 10nM, less than about 5nM, less than about 1nM, less than about 500pM, less than 250pM, or less than 100pM, as measured by surface plasmon resonance, e.g., using the assay format as described herein, or a substantially similar assay.

[00152] The present disclosure also includes antibodies and antigen-binding fragments thereof that bind PcrV with a dissociative half-life ($t_{1/2}$) of greater than about 10 minutes as measured by surface plasmon resonance at 25°C, or greater than about 1 minute as measured by surface plasmon resonance at 37°C e.g., using an assay format as defined herein, or a substantially similar assay. In certain embodiments, the antibodies or antigen-binding fragments of the present disclosure bind PcrV with a $t_{1/2}$ of greater than about 1 minute, of greater than about 10 minutes, of greater than about 30 minutes, of greater than about 60 minutes, of greater than about 100 minutes, of greater than about 200 minutes, greater than about 300 minutes, greater than about 400 minutes, greater than about 500 minutes, greater than about 600 minutes, greater than about 700 minutes, greater than about 800 minutes, greater than about 900 minutes, or greater than about 1000 minutes as measured by surface plasmon resonance at 25°C, or at 37°C e.g., using an assay format as defined herein (e.g., mAb-capture or antigen-capture format), or a substantially similar assay.

[00153] Also included herein are antibodies or antigen-binding fragments thereof that neutralize *P. aeruginosa* PcrV-mediated toxicity, for example, in A549 cells as shown in Example 6. In some embodiments, the antibodies exhibit a neutralization potency against *P. aeruginosa* strain 6077 with an IC_{50} ranging from about 10^{-11} M to about 10^{-7} M. In some embodiments, the antibodies exhibit a neutralization potency against *P. aeruginosa* strain ATCC 700888 with an IC_{50} ranging from about 10^{-9} M to about 10^{-7} M. The antibodies provided herein also neutralize *P. aeruginosa* PcrV-mediated RBC

hemolysis as shown in Example 7. In some embodiments, the antibodies exhibit a neutralization potency against strain 6077 with an IC_{50} ranging from about 10^{-10} M to about 10^{-7} M. In some embodiments, the antibodies exhibit a neutralization potency against strain ATCC 700888 with an IC_{50} ranging from about 10^{-10} M to about 10^{-8} M. Furthermore, the antibodies provided herein cross-compete with other antibodies that bind PcrV, as shown in Example 4.

[00154] Also included herein are antibodies that prevent individual mortality or decrease population mortality rates. In some embodiments, the antibodies can be administered prophylactically. In some embodiments, the antibodies can be administered therapeutically. In some embodiments, the antibodies, when dosed at 5 mg/kg, exhibit 100% improved survival in a mouse model of pneumonia, as shown in Example 8. In some embodiments, the antibodies exhibit improved survival even at lower dosages, for example, 1 mg/kg, or 0.2 mg/kg, or even 0.04 mg/kg, as shown in Example 9.

[00155] Also included herein are antibodies that decrease bacterial load, for example, lung bacterial load, in a subject or population. In some embodiments, the antibodies can be administered prophylactically. In some embodiments, the antibodies can be administered therapeutically. In some embodiments, the antibodies, when dosed at either 0.1 or 0.2 mg/kg, or at 25 mg/kg, demonstrate a decrease in bacterial load by 3 to 4 logs more than untreated subjects in a mouse model of pneumonia, as shown in Example 10 and Example 11.

[00156] In one embodiment, the antibodies provided herein can have one or more of the following characteristics: (a) comprise three heavy chain complementarity determining regions (CDRs) (HCDR1, HCDR2 and HCDR3) contained within any one of the heavy chain variable region (HCVR) sequences selected from the group consisting of SEQ ID NOs: 2, 18, 34, and 50; and three light chain CDRs (LCDR1, LCDR2 and LCDR3) contained within any one of the light chain variable region (LCVR) sequences selected from the group consisting of SEQ ID NOs: 10, 26, 42, and 58; (a) comprise three heavy chain complementarity determining regions (CDRs) (HCDR1, HCDR2 and HCDR3) contained within any one of the heavy chain variable region (HCVR) sequences selected from the group consisting of SEQ ID NOs: 2, 18, 34, and 50; and three light chain CDRs (LCDR1, LCDR2 and LCDR3) contained within any one of the light chain variable region (LCVR) sequences selected from the group consisting of SEQ ID NOs: 10, 26, 42, and 58; (b) are fully human monoclonal antibodies; (c) bind to full length PcrV with a dissociation constant (K_D) of less than 10^{-8} M, as measured in a surface plasmon resonance assay at 25°C; (d) bind to full length PcrV with a dissociation constant (K_D) of

less than 10^{-8} M, as measured in a surface plasmon resonance assay at 37°C ; (e) demonstrate neutralization of *P. aeruginosa* strain 6077 with an IC_{50} ranging from about 10^{-11} M to about 10^{-8} M in a cytotoxicity assay; (f) demonstrate neutralization of *P. aeruginosa* strain ATCC 700888 with an IC_{50} ranging from about 10^{-9} M to about 10^{-7} M in a cytotoxicity assay; (g) demonstrate neutralization of *P. aeruginosa* strain 6077 with an IC_{50} ranging from about 10^{-10} M to about 10^{-6} M in a hemolytic assay; (h) demonstrate neutralization of *P. aeruginosa* strain ATCC 700888 with an IC_{50} ranging from about 10^{-10} M to about 10^{-7} M in a hemolytic assay; (i) decrease mortality from *P. aeruginosa* strain 6206 or strain 6077 in mice treated prophylactically with 5 mg/kg relative to untreated mice in an acute pneumonia model; (j) decrease mortality from *P. aeruginosa* strain 6206 or strain 6077 in mice treated prophylactically with 1.0, 0.2 or 0.04 mg/kg relative to untreated mice in an acute pneumonia model; (k) decrease lung bacterial burden of *P. aeruginosa* strain 6206 in mice treated prophylactically at 0.1 mg/kg or 0.2 mg/kg relative to untreated mice in an acute pneumonia model; (l) decrease lung bacterial burden of *P. aeruginosa* strain PA01 in mice treated prophylactically at 25 mg/kg relative to untreated mice in an acute pneumonia model; and/or (m) cross-compete with a reference antibody, wherein the reference antibody comprises a heavy chain variable region (HCVR) and a light chain variable region (LCVR) amino acid sequence selected from the group consisting of any of the HCVR and LCVR amino acid sequences of Table 1.

[00157] In a related embodiment, the antibodies or antigen-binding fragments thereof as provided herein can have one or more of the following characteristics: (n) bind to full length PcrV (SEQ ID NO: 77) with an EC_{50} of less than about 10^{-8} M; (o) bind to PcrV 136-233 (SEQ ID NO: 81) with an EC_{50} of less than about 10^{-8} M; (p) interact with at least one amino acid sequence selected from the group consisting of (i) amino acid residues ranging from about position 150 to about position 170 of SEQ ID NO: 78 and (ii) amino acid residues ranging from about position 155 to about 170 of SEQ ID NO: 78; and/or (q) interact with at least one amino acid sequence selected from the group consisting of SEQ ID NO: 85 and SEQ ID NO: 86.

[00158] The antibodies provided herein may possess one or more of the aforementioned biological characteristics, or any combinations thereof. Certain of the properties of the antibodies are summarized below. Other biological characteristics of the antibodies will be evident to a person of ordinary skill in the art from a review of the present disclosure including the working Examples herein.

Epitope Mapping and Related Technologies

[00159] Provided herein are anti-PcrV antibodies that interact with one or more amino

acids found within the *P. aeruginosa* PcrV protein. The epitope to which the antibodies bind may consist of a single contiguous sequence of 3 or more (*e.g.*, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more) amino acids located within the PcrV protein (*e.g.* a linear epitope in a domain). Alternatively, the epitope may consist of a plurality of non-contiguous amino acids (or amino acid sequences) located within the PcrV protein (*e.g.* a conformational epitope). Illustratively, anti-PcrV antibodies or antigen-binding fragments thereof provided herein can bind to PcrV 136-233 (SEQ ID NO: 81); can interact with amino acid residues ranging from about position 150 to about position 170 of SEQ ID NO: 78; can interact with amino acid residues ranging from about position 155 to about 170 of SEQ ID NO: 78; and/or can interact with at least one amino acid sequence selected from the group consisting of SEQ ID NO: 85 and SEQ ID NO: 86.

[00160] Various techniques known to persons of ordinary skill in the art can be used to determine whether an antibody "interacts with one or more amino acids" within a polypeptide or protein. Exemplary techniques include, for example, routine cross-blocking assays, such as that described in Antibodies, Harlow and Lane (Cold Spring Harbor Press, Cold Spring Harbor, NY). Other methods include alanine scanning mutational analysis, peptide blot analysis (Reineke (2004) Methods Mol. Biol. 248: 443-63), peptide cleavage analysis crystallographic studies and NMR analysis. In addition, methods such as epitope excision, epitope extraction and chemical modification of antigens can be employed (Tomer (2000) Prot. Sci. 9: 487-496). Another method that can be used to identify the amino acids within a polypeptide with which an antibody interacts is hydrogen/deuterium exchange detected by mass spectrometry. In general terms, the hydrogen/deuterium exchange method involves deuterium-labeling the protein of interest, followed by binding the antibody to the deuterium-labeled protein. Next, the protein/antibody complex is transferred to water and exchangeable protons within amino acids that are protected by the antibody complex undergo deuterium-to-hydrogen back-exchange at a slower rate than exchangeable protons within amino acids that are not part of the interface. As a result, amino acids that form part of the protein/antibody interface may retain deuterium and therefore exhibit relatively higher mass compared to amino acids not included in the interface. After dissociation of the antibody, the target protein is subjected to protease cleavage and mass spectrometry analysis, thereby revealing the deuterium-labeled residues that correspond to the specific amino acids with which the antibody interacts. *See, e.g.*, Ehring (1999) Analytical Biochemistry 267: 252-259; Engen and Smith (2001) Anal. Chem. 73: 256A-

265A.

[00161] The term "epitope" refers to a site on an antigen to which B and/or T cells respond. B-cell epitopes can be formed both from contiguous amino acids and noncontiguous amino acids juxtaposed by tertiary folding of a protein. Epitopes formed from contiguous amino acids are typically retained on exposure to denaturing solvents, whereas epitopes formed by tertiary folding are typically lost on treatment with denaturing solvents. An epitope typically includes at least 3, and more usually, at least 5 or 8-10 amino acids in a unique spatial conformation.

[00162] Modification-Assisted Profiling (MAP), also known as Antigen Structure-based Antibody Profiling (ASAP) is a method that categorizes large numbers of monoclonal antibodies (mAbs) directed against the same antigen according to the similarities of the binding profile of each antibody to chemically or enzymatically modified antigen surfaces (see US 2004/0101920). Each category may reflect a unique epitope either distinctly different from or partially overlapping with epitope represented by another category. This technology allows rapid filtering of genetically identical antibodies, such that characterization can be focused on genetically distinct antibodies. When applied to hybridoma screening, MAP may facilitate identification of rare hybridoma clones that produce mAbs having the desired characteristics. MAP may be used to sort the antibodies into groups of antibodies binding different epitopes.

[00163] In certain embodiments, the anti-PcrV antibodies or antigen-binding fragments thereof bind an epitope within any one or more of the regions exemplified in PcrV protein, either in natural form, or recombinantly produced, or to a fragment thereof.

[00164] The present disclosure includes anti-PcrV antibodies that bind to the same epitope, or a portion of the epitope. Likewise, the present disclosure also includes anti-PcrV antibodies that compete for binding to the PcrV protein or a fragment thereof with any of the specific exemplary antibodies described herein. For example, the present disclosure includes anti-PcrV antibodies that cross-compete for binding to PcrV with one or more antibodies obtained from those antibodies described in Tables 1 and 2.

[00165] One can easily determine whether an antibody binds to the same epitope as, or competes for binding with, a reference anti-PcrV antibody by using routine methods known in the art. For example, to determine if a test antibody binds to the same epitope as a reference anti-PcrV antibody of the disclosure, the reference antibody is allowed to bind to the PcrV protein or peptide under saturating conditions. Next, the ability of a test antibody to bind to the PcrV protein is assessed. If the test antibody is able to bind to PcrV following saturation binding with the reference anti-PcrV antibody, it can be

concluded that the test antibody binds to a different epitope than the reference anti-PcrV antibody. On the other hand, if the test antibody is not able to bind to the PcrV protein following saturation binding with the reference anti-PcrV antibody, then the test antibody may bind to the same epitope as the epitope bound by the reference anti-PcrV antibody provided herein.

[00166] To determine if an antibody competes for binding with a reference anti- PcrV antibody, the above-described binding methodology is performed in two orientations: In a first orientation, the reference antibody is allowed to bind to a PcrV protein under saturating conditions followed by assessment of binding of the test antibody to the PcrV protein. In a second orientation, the test antibody is allowed to bind to a PcrV protein under saturating conditions followed by assessment of binding of the reference antibody to the PcrV protein. If, in both orientations, only the first (saturating) antibody is capable of binding to the PcrV protein, then it is concluded that the test antibody and the reference antibody compete for binding to PcrV. As will be appreciated by a person of ordinary skill in the art, an antibody that competes for binding with a reference antibody may not necessarily bind to the identical epitope as the reference antibody, but may sterically block binding of the reference antibody by binding an overlapping or adjacent epitope.

[00167] Two antibodies bind to the same or overlapping epitope if each competitively inhibits (blocks) binding of the other to the antigen. That is, a 1-, 5-, 10-, 20- or 100-fold excess of one antibody inhibits binding of the other by at least 50% but preferably 75%, 90% or even 99% as measured in a competitive binding assay (see, *e.g.*, Junghans *et al.*, Cancer Res. 1990 50:1495-1502). Alternatively, two antibodies have the same epitope if essentially all amino acid mutations in the antigen that reduce or eliminate binding of one antibody reduce or eliminate binding of the other. Two antibodies have overlapping epitopes if some amino acid mutations that reduce or eliminate binding of one antibody reduce or eliminate binding of the other.

[00168] Additional routine experimentation (*e.g.*, peptide mutation and binding analyses) can then be carried out to confirm whether the observed lack of binding of the test antibody is in fact due to binding to the same epitope as the reference antibody or if steric blocking (or another phenomenon) is responsible for the lack of observed binding. Experiments of this sort can be performed using ELISA, RIA, surface plasmon resonance, flow cytometry or any other quantitative or qualitative antibody-binding assay available in the art.

Immunoconjugates

[00169] The disclosure provides a human anti-PcrV monoclonal antibody conjugated to a therapeutic moiety ("immunoconjugate"), such as an antibiotic to treat *P. aeruginosa* infection. As used herein, the term "immunoconjugate" refers to an antibody, which is chemically or biologically linked to a radioactive agent, a cytokine, an interferon, a target or reporter moiety, an enzyme, a peptide or protein or a therapeutic agent. The antibody may be linked to the radioactive agent, cytokine, interferon, target or reporter moiety, enzyme, peptide or therapeutic agent at any location along the molecule so long as it is able to bind its target. Examples of immunoconjugates include antibody drug conjugates and antibody-toxin fusion proteins. In one embodiment, the agent may be a second different antibody to *P. aeruginosa*. In certain embodiments, the antibody may be conjugated to an agent specific for an infected cell. The type of therapeutic moiety that may be conjugated to the anti-PcrV antibody will take into account the condition to be treated and the desired therapeutic effect to be achieved. Examples of suitable agents for forming immunoconjugates are known in the art; see for example, WO 05/103081.

Multi-specific Antibodies

[00170] The antibodies provided herein may be mono-specific, bi-specific, or multi-specific. Multi-specific antibodies may be specific for different epitopes of one target polypeptide or may contain antigen-binding domains specific for more than one target polypeptide. See, e.g., Tutt et al., 1991, J. Immunol. 147:60-69; Kufer et al., 2004, Trends Biotechnol. 22:238-244.

[00171] Any of the multi-specific antigen-binding molecules provided herein, or variants thereof, may be constructed using standard molecular biological techniques (e.g., recombinant DNA and protein expression technology), as will be known to a person of ordinary skill in the art.

[00172] In some embodiments, *P. aeruginosa*-specific antibodies are generated in a bi-specific format (a "bi-specific") in which variable regions binding to distinct domains of *P. aeruginosa* PcrV are linked together to confer dual antigen specificity within a single binding molecule. Appropriately designed bi-specifics may enhance overall T3SS inhibitory efficacy through increasing both specificity and binding avidity. Variable regions with specificity for individual domains, or that can bind to different regions within one domain, are paired on a structural scaffold that allows each region to bind simultaneously to the separate epitopes, or to different regions within one domain. In one example for a bi-specific, heavy chain variable regions (V_H) from a binder with

specificity for one domain are recombined with light chain variable regions (V_L) from a series of binders with specificity for a second domain to identify non-cognate V_L partners that can be paired with an original V_H without disrupting the original specificity for that V_H . In this way, a single V_L segment (e.g., V_{L1}) can be combined with two different V_H domains (e.g., V_{H1} and V_{H2}) to generate a bi-specific comprised of two binding "arms" (V_{H1} - V_{L1} and V_{H2} - V_{L1}). Use of a single V_L segment reduces the complexity of the system and thereby simplifies and increases efficiency in cloning, expression, and purification processes used to generate the bi-specific (See, for example, USSN13/022759 and US2010/0331527).

[00173] Alternatively, antibodies that bind more than one domain and a second target, such as, but not limited to, for example, a second different anti-*P. aeruginosa* antibody, may be prepared in a bi-specific format using techniques described herein, or other techniques known to those skilled in the art. Antibody variable regions binding to distinct regions may be linked together with variable regions that bind to relevant sites on, for example, *P. aeruginosa*, to confer dual-antigen specificity within a single binding molecule. Appropriately designed bi-specifics of this nature serve a dual function. Variable regions with specificity for one *P. aeruginosa* antigen are combined with variable regions with specificity for PcrV and are paired on a structural scaffold that allows each variable region to bind to the separate antigens.

[00174] An exemplary bi-specific antibody format that can be used in the context of the present disclosure involves the use of a first immunoglobulin (Ig) C_H3 domain and a second Ig C_H3 domain, wherein the first and second Ig C_H3 domains differ from one another by at least one amino acid, and wherein at least one amino acid difference reduces binding of the bi-specific antibody to Protein A as compared to a bi-specific antibody lacking the amino acid difference. In one embodiment, the first Ig C_H3 domain binds Protein A and the second Ig C_H3 domain contains a mutation that reduces or abolishes Protein A binding such as an H95R modification (by IMGT exon numbering; H435R by EU numbering). The second C_H3 may further comprise a Y96F modification (by IMGT; Y436F by EU). Further modifications that may be found within the second C_H3 include: D16E, L18M, N44S, K52N, V57M, and V82I (by IMGT; D356E, L358M, N384S, K392N, V397M, and V422I by EU) in the case of IgG1 antibodies; N44S, K52N, and V82I (IMGT; N384S, K392N, and V422I by EU) in the case of IgG2 antibodies; and Q15R, N44S, K52N, V57M, R69K, E79Q, and V82I (by IMGT; Q355R, N384S, K392N, V397M, R409K, E419Q, and V422I by EU) in the case of IgG4 antibodies. Variations on the bi-specific antibody format described above are contemplated within the scope of the

present disclosure.

[00175] Other exemplary bispecific formats that can be used in the context of the present disclosure include, without limitation, *e.g.*, scFv-based or diabody bispecific formats, IgG-scFv fusions, dual variable domain (DVD)-Ig, Quadroma, knobs-into-holes, common light chain (*e.g.*, common light chain with knobs-into-holes, etc.), CrossMab, CrossFab, (SEED)body, leucine zipper, Duobody, IgG1/IgG2, dual acting Fab (DAF)-IgG, and Mab² bispecific formats (*see, e.g.*, Klein *et al.* 2012, mAbs 4:6, 1-11, and references cited therein, for a review of the foregoing formats). Bispecific antibodies can also be constructed using peptide/nucleic acid conjugation, *e.g.*, wherein unnatural amino acids with orthogonal chemical reactivity are used to generate site-specific antibody-oligonucleotide conjugates which then self-assemble into multimeric complexes with defined composition, valency and geometry. (*See, e.g.*, Kazane *et al.*, *J. Am. Chem. Soc.* [Epub: Dec. 4, 2012]).

Therapeutic Administration and Formulations

[00176] The disclosure provides therapeutic compositions comprising the anti-PcrV antibodies or antigen-binding fragments thereof as provided herein. Therapeutic compositions in accordance with the present disclosure will be administered with suitable carriers, excipients, and other agents that are incorporated into formulations to provide improved transfer, delivery, tolerance, and the like. A multitude of appropriate formulations can be found in the formulary known to all pharmaceutical chemists: Remington's Pharmaceutical Sciences, Mack Publishing Company, Easton, PA. These formulations include, for example, powders, pastes, ointments, jellies, waxes, oils, lipids, lipid (cationic or anionic) containing vesicles (such as LIPOFECTIN™), DNA conjugates, anhydrous absorption pastes, oil-in-water and water-in-oil emulsions, emulsions carbowax (polyethylene glycols of various molecular weights), semi-solid gels, and semi-solid mixtures containing carbowax. *See also* Powell *et al.* "Compendium of excipients for parenteral formulations" PDA (1998) J Pharm Sci Technol 52:238-311.

[00177] The dose of antibody may vary depending upon the age and the size of a subject to be administered, target disease, conditions, route of administration, and the like. When an antibody of the present disclosure is used for treating a disease or disorder in an adult patient, or for preventing such a disease, it is advantageous to administer the antibody normally at a single dose of about 0.01 to about 60 mg/kg body weight, for example, about 0.04 mg/kg, about 0.2 mg/kg, about 2.0 mg/kg, about 5 mg/kg, about 10 mg/kg, about 15 mg/kg, about 20 mg/kg, about 25 mg/kg, about 30

mg/kg, about 0.04 mg/kg to about 2.0 mg/kg, about 5 mg/kg to about 60 mg/kg, about 10 mg/kg to about 50 mg/kg, or about 20 mg/kg to about 50 mg/kg body weight. Depending on the severity of the condition, the frequency and the duration of the treatment can be adjusted. In certain embodiments, the antibody or antigen-binding fragment thereof can be administered as an initial dose of at least about 0.1 mg to about 800 mg, about 1 to about 500 mg, about 5 to about 300 mg, or about 10 to about 200 mg, to about 100 mg, or to about 50 mg. In certain embodiments, the initial dose may be followed by administration of a second or a plurality of subsequent doses of the antibody or antigen-binding fragment thereof in an amount that can be approximately the same or less than that of the initial dose, wherein the subsequent doses are separated by at least 6 hours to 24 hours, at least 1 day to 3 days; at least one week, at least 2 weeks; at least 3 weeks; at least 4 weeks; at least 5 weeks; at least 6 weeks; at least 7 weeks; at least 8 weeks; at least 9 weeks; at least 10 weeks; at least 12 weeks; or at least 14 weeks.

[00178] Various delivery systems are known and can be used to administer the pharmaceutical composition provided herein, *e.g.*, encapsulation in liposomes, microparticles, microcapsules, recombinant cells capable of expressing the mutant viruses, receptor mediated endocytosis (see, *e.g.*, Wu et al. (1987) J. Biol. Chem. 262:4429-4432). Methods of introduction include, but are not limited to, intradermal, transdermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural and oral routes. The composition may be administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (*e.g.*, oral mucosa, rectal and intestinal mucosa, etc.) and may be administered together with other biologically active agents. Administration can be systemic or local. The pharmaceutical composition can be also delivered in a vesicle, in particular a liposome (see, for example, Langer (1990) Science 249:1527-1533).

[00179] The use of nanoparticles to deliver the antibodies provided herein is also contemplated. Antibody-conjugated nanoparticles may be used both for therapeutic and diagnostic applications. Antibody-conjugated nanoparticles and methods of preparation and use are described in detail by Arruebo, M., et al. 2009 ("Antibody-conjugated nanoparticles for biomedical applications" in J. Nanomat. Volume 2009, Article ID 439389, 24 pages, doi: 10.1155/2009/439389). Nanoparticles may be developed and conjugated to antibodies contained in pharmaceutical compositions to target infected cells. Nanoparticles for drug delivery have also been described in, for example, US 8257740, or US 8246995.

[00180] In certain situations, the pharmaceutical composition can be delivered in a

controlled release system. In one embodiment, a pump may be used. In another embodiment, polymeric materials can be used. In yet another embodiment, a controlled release system can be placed in proximity of the composition's target, thus requiring only a fraction of the systemic dose.

[00181] The injectable preparations may include dosage forms for intravenous, subcutaneous, intracutaneous, intracranial, intraperitoneal and intramuscular injections, drip infusions, etc. These injectable preparations may be prepared by methods publicly known. For example, the injectable preparations may be prepared, *e.g.*, by dissolving, suspending or emulsifying the antibody or its salt described above in a sterile aqueous medium or an oily medium conventionally used for injections. As the aqueous medium for injections, there are, for example, physiological saline, an isotonic solution containing glucose and other auxiliary agents, etc., which may be used in combination with an appropriate solubilizing agent such as an alcohol (*e.g.*, ethanol), a polyalcohol (*e.g.*, propylene glycol, polyethylene glycol), a nonionic surfactant [*e.g.*, polysorbate 80, HCO-50 (polyoxyethylene (50 mol) adduct of hydrogenated castor oil)], etc. As the oily medium, there are employed, *e.g.*, sesame oil, soybean oil, etc., which may be used in combination with a solubilizing agent such as benzyl benzoate, benzyl alcohol, etc. The injection thus prepared is preferably filled in an appropriate ampoule.

[00182] A pharmaceutical composition can be delivered subcutaneously or intravenously with a standard needle and syringe. In addition, with respect to subcutaneous delivery, a pen delivery device readily has applications in delivering a pharmaceutical composition of the present disclosure. Such a pen delivery device can be reusable or disposable. A reusable pen delivery device generally utilizes a replaceable cartridge that contains a pharmaceutical composition. Once all of the pharmaceutical composition within the cartridge has been administered and the cartridge is empty, the empty cartridge can readily be discarded and replaced with a new cartridge that contains the pharmaceutical composition. The pen delivery device can then be reused. In a disposable pen delivery device, there is no replaceable cartridge. Rather, the disposable pen delivery device comes prefilled with the pharmaceutical composition held in a reservoir within the device. Once the reservoir is emptied of the pharmaceutical composition, the entire device is discarded.

[00183] Numerous reusable pen and autoinjector delivery devices have applications in the subcutaneous delivery of a pharmaceutical composition of the present disclosure. Examples include, but certainly are not limited to AUTOPEN™ (Owen Mumford, Inc., Woodstock, UK), DISETRONIC™ pen (Disetronic Medical Systems, Burghdorf,

Switzerland), HUMALOG MIX 75/25™ pen, HUMALOG™ pen, HUMALIN 70/30™ pen (Eli Lilly and Co., Indianapolis, IN), NOVOPEN™ I, II and III (Novo Nordisk, Copenhagen, Denmark), NOVOPEN JUNIOR™ (Novo Nordisk, Copenhagen, Denmark), BD™ pen (Becton Dickinson, Franklin Lakes, NJ), OPTIPEN™, OPTIPEN PRO™, OPTIPEN STARLET™, and OPTICLIK™ (Sanofi-Aventis, Frankfurt, Germany), to name only a few. Examples of disposable pen delivery devices having applications in subcutaneous delivery of a pharmaceutical composition of the present disclosure include, but certainly are not limited to the SOLOSTAR™ pen (Sanofi-Aventis), the FLEXPEN™ (Novo Nordisk), and the KWIKPEN™ (Eli Lilly), the SURECLICK™ Autoinjector (Amgen, Thousand Oaks, CA), the PENLET™ (Haselmeier, Stuttgart, Germany), the EPIPEN (Dey, L.P.) and the HUMIRA™ Pen (Abbott Labs, Abbott Park, IL), to name only a few.

[00184] Advantageously, the pharmaceutical compositions for oral or parenteral use described above are prepared into dosage forms in a unit dose suited to fit a dose of the active ingredients. Such dosage forms in a unit dose include, for example, tablets, pills, capsules, injections (ampoules), suppositories, etc. The amount of the antibody contained is generally about 1 to about 500 mg per dosage form in a unit dose; especially in the form of injection, it is preferred that the antibody is contained in about 1 mg to about 100 mg and in about 10 to about 250 mg for the other dosage forms.

Therapeutic Uses of the Antibodies

[00185] The antibodies provided herein are useful for the treatment, and/or prevention (e.g. prophylactic treatment) of a disease or disorder or condition associated with *P. aeruginosa* infection and/or for ameliorating at least one symptom associated with such disease, disorder or condition.

[00186] In certain embodiments, the antibodies disclosed herein are useful to treat subjects suffering from pneumonia, bacteremia, a bone infection, a joint infection, a skin infection, a burn infection, a wound infection, urinary tract infection, or any combination thereof, caused by *P. aeruginosa*. In one embodiment, an antibody or antigen-binding fragment thereof may be administered at a therapeutic dose to a patient with *P. aeruginosa* infection.

[00187] In some embodiments, provided herein are methods to ameliorate or reduce the severity, duration, or frequency of occurrence, of at least one symptom of a *P. aeruginosa* infection in a subject. For example, one or more anti-PcrV antibodies disclosed herein may be administered to relieve or prevent or decrease the severity of

one or more of the symptoms or conditions of the disease or disorder. The antibodies may be used to ameliorate or reduce the severity of at least one symptom of *P. aeruginosa* infection including, but not limited to fever, chills, headache, fatigue, joint pain, stiffness, myalgia, diarrhea, vomiting, pain, itching, liquid discharge in the ears, rashes, pus-filled pimples on the skin, eye pain, redness in an eye, pneumonia, coughing, congestion, soft tissue discharge of green pus, sweet, fruity smell, and urinary tract infection.

[00188] It is also contemplated herein to administer one or more anti-PcrV antibodies disclosed herein prophylactically to subjects at risk for developing infection such as a subject undergoing surgery, a subject being treated for a major illness, a subject having severe burns, a subject using a breathing machine, a subject with a catheter, a subject undergoing chemotherapy, a subject having diabetes, a subject with cystic fibrosis, a subject with tuberculosis, a subject with HIV, or a subject with a compromised immune system.

[00189] Other subjects at risk for acquiring a *P. aeruginosa* infection include, for example, a person who is immunocompromised because of autoimmune disease, or those persons receiving immunosuppressive therapy (for example, following organ transplant), certain forms of anemia that deplete or destroy white blood cells, those persons receiving radiation or chemotherapy, or those persons afflicted with an inflammatory disorder.

[00190] It is also contemplated herein to administer one or more anti-PcrV antibodies in order to neutralize a *P. aeruginosa* infection. Exposing an individual or cells results in enhanced protection from cell death. In certain embodiments, the exposing may be *in vitro* or *in vivo*. The enhanced protection can be observed when the antibody is used alone, or when it is used in combination with one or more additional therapeutic agents or antibodies against *P. aeruginosa*.

[00191] It is also contemplated herein to administer one or more anti-PcrV antibodies in order to decrease bacterial load in a subject. In some aspects, the one or more anti-PcrV antibodies or antigen-binding fragments thereof decrease bacterial load in a subject's lungs. The antibody or antigen-binding fragment thereof can block *P. aeruginosa* delivery of toxins into the host cell. In some aspects, treatment with an anti-PcrV antibody provided herein decreases *P. aeruginosa* bacterial load. In some aspects, treatment with an anti-PcrV antibody provided herein decreases bacterial load of *P. aeruginosa* and a co-infecting bacteria, for example, a Gram-negative or Gram-positive bacteria. In some aspects, treatment with an anti-PcrV antibody provided herein

decreases *P. aeruginosa* bacterial load and *S. aureus* bacterial load.

[00192] In some embodiments, provided herein are methods of increasing the survival, or the likelihood of survival, of a subject suffering from infection with *P. aeruginosa*, or a subject at risk for *P. aeruginosa* infection.

[00193] One or more anti-PcrV antibodies can be administered to increase the survival, or the likelihood of survival, of a subject suffering from cystic fibrosis. In some aspects, the subject does not have pneumonia symptoms at the time of administration.

[00194] In a further embodiment, the present anti-PcrV antibodies are used for the preparation of a pharmaceutical composition for treating patients at risk for or suffering from a *P. aeruginosa* infection. In another embodiment, the present antibodies are used as adjunct therapy with any other agent or any other therapy known to those skilled in the art useful for treating or ameliorating a *P. aeruginosa* infection.

Combination Therapies

[00195] Combination therapies may include an anti-PcrV antibody as disclosed herein and any additional therapeutic agent that may be advantageously combined with such an antibody or a biologically active fragment thereof. The antibodies may be combined synergistically with one or more drugs or agents used to treat *P. aeruginosa* infection.

[00196] Exemplary agents for treating a bacterial infection may include, *e.g.*, antibiotics, anti-inflammatory drugs (such as corticosteroids, and non-steroidal anti-inflammatory drugs), a different antibody to *P. aeruginosa*, or any other palliative therapy to treat a symptom of *P. aeruginosa* infection or for reducing the bacterial load in a patient. In certain embodiments, the second therapeutic agent may be an agent that helps to counteract or reduce any possible side effect(s) associated with an anti-PcrV antibody or antigen-binding fragment thereof, if such side effect(s) should occur.

[00197] Exemplary agents that may be advantageously combined with an anti-PcrV antibody include, without limitation, other agents that bind and/or inhibit *P. aeruginosa* activity (including other antibodies or antigen-binding fragments thereof, etc.) and/or agents which do not directly bind PcrV or another *P. aeruginosa* antigen but nonetheless inhibit bacterial activity including infectivity of host cells. In some aspects, the second therapeutic agent may be a therapeutic for treating infections associated with a different organism which may co-infect with *P. aeruginosa*, for example, a Gram-positive organism or a Gram-negative organism, *e.g.*, an organism such as *S. aureus*. In some aspects, the additional therapeutic agent is a therapeutic useful for treating a co-infection. In some aspects, the additional therapeutic agent is useful for treating a *S.*

aureus co-infection.

[00198] Exemplary antibiotics to combine with the anti-PcrV antibodies include: penicillins (piperacillin, piperacillin/tazobactam, mezlocillin, ticarcillin, ticarcillin/clavulanate), cephalosporins (ceftazidime, cefoperazone, cefepime), carbapenems (imipenem/cilastatin; meropenem), monobactams (aztreonam), aminoglycosides (tobramycin, gentamicin, amikacin), fluoroquinolones (ciprofloxacin, levofloxacin), and others (polymyxin B, colistin). Common treatment regimens include: for bacteremia: penicillin plus aminoglycoside; penicillin plus ciprofloxacin; cephalosporin, aztreonam or carbapenem plus aminoglycoside or ciprofloxacin; for CNS infection: ceftazidime, optionally plus an aminoglycoside; cefepime; ciprofloxacin; aztreonam; meropenem; for bone or joint infection: penicillin plus an aminoglycoside or ciprofloxacin; cephalosporin; aztreonam; fluoroquinolone; carbapenem; external otitis: cephalosporin; carbapenem; ciprofloxacin; cephalosporin plus aminoglycoside; keratitis/corneal ulcer (eye): tobramycin (topical), optionally with piperacillin or ticarcillin (topical); ciprofloxacin or ofloxacin (topical); and urinary tract infection: ciprofloxacin; aminoglycoside; penicillin; cephalosporin; carbapenem. (See, e.g., Kasper, D. L, et al, eds: Harrison's Principles of Internal Medicine, 16th Ed., McGraw-Hill, 2005).

[00199] In one embodiment, the one or more additional therapeutic agents comprise one or more anti-PcrV antibodies. In certain embodiments, the second therapeutic agent is another different antibody, for example, another *P. aeruginosa* antibody, wherein the different antibody or antibodies may or may not bind PcrV or bind to the same epitope on PcrV, or an overlapping epitope. In certain embodiments, the second therapeutic agent is an antibody to a different *P. aeruginosa* antigen. In certain embodiments, the second therapeutic agent is an antibody to a different infectious bacteria, e.g. *S. aureus*. In some embodiments, non-competing antibodies may be combined and administered to a subject in need thereof. The antibodies comprising the combination may block the activity of the T3SS mechanism and/or inhibit some other activity of the bacteria.

[00200] As used herein, the term "in combination with" means that additional therapeutically active component(s) may be administered prior to, concurrent with, or after the administration of at least one anti-PcrV antibody provided herein. The term "in combination with" also includes sequential or concomitant administration of an anti-PcrV antibody and a second therapeutic agent.

[00201] The additional therapeutically active component(s) may be administered to a subject prior to administration of an anti-PcrV antibody. For example, a first component may be deemed to be administered "prior to" a second component if the first component

is administered 1 week before, 72 hours before, 60 hours before, 48 hours before, 36 hours before, 24 hours before, 12 hours before, 6 hours before, 5 hours before, 4 hours before, 3 hours before, 2 hours before, 1 hour before, 30 minutes before, 15 minutes before, 10 minutes before, 5 minutes before, or less than 1 minute before administration of the second component. In other embodiments, the additional therapeutically active component(s) may be administered to a subject after administration of an anti-PcrV antibody. For example, a first component may be deemed to be administered "after" a second component if the first component is administered 1 minute after, 5 minutes after, 10 minutes after, 15 minutes after, 30 minutes after, 1 hour after, 2 hours after, 3 hours after, 4 hours after, 5 hours after, 6 hours after, 12 hours after, 24 hours after, 36 hours after, 48 hours after, 60 hours after, 72 hours after administration of the second component. In yet other embodiments, the additional therapeutically active component(s) may be administered to a subject concurrent with administration of an anti-PcrV antibody. "Concurrent" administration, for purposes of the present disclosure, includes, *e.g.*, administration of an anti-PcrV antibody and an additional therapeutically active component to a subject in a single dosage form, or in separate dosage forms administered to the subject within about 30 minutes or less of each other. If administered in separate dosage forms, each dosage form may be administered via the same route (*e.g.*, both the anti-PcrV antibody and the additional therapeutically active component may be administered intravenously, etc.); alternatively, each dosage form may be administered via a different route (*e.g.*, the anti-PcrV antibody may be administered intravenously, and the additional therapeutically active component may be administered orally). In any event, administering the components in a single dosage form, in separate dosage forms by the same route, or in separate dosage forms by different routes are all considered "concurrent administration," for purposes of the present disclosure. For purposes of the present disclosure, administration of an anti-PcrV antibody "prior to", "concurrent with," or "after" (as those terms are defined herein above) administration of an additional therapeutically active component is considered administration of an anti-PcrV antibody "in combination with" an additional therapeutically active component.

[00202] The present disclosure includes pharmaceutical compositions in which an anti-PcrV antibody described herein is co-formulated with one or more of the additional therapeutically active component(s) as described elsewhere herein.

Administration Regimens

[00203] According to certain embodiments, a single dose of an anti-PcrV antibody of the

disclosed herein (or a pharmaceutical composition comprising a combination of an anti-PcrV antibody and any of the additional therapeutically active agents mentioned herein) may be administered to a subject in need thereof. According to certain embodiments, multiple doses of an anti-PcrV antibody (or a pharmaceutical composition comprising a combination of an anti-PcrV antibody and any of the additional therapeutically active agents mentioned herein) may be administered to a subject over a defined time course. The methods according to this aspect of the disclosure comprise sequentially administering to a subject multiple doses of an anti-PcrV antibody described herein. As used herein, "sequentially administering" means that each dose of anti-PcrV antibody is administered to the subject at a different point in time, *e.g.*, on different days separated by a predetermined interval (*e.g.*, hours, days, weeks or months). The present disclosure includes methods which comprise sequentially administering to the patient a single initial dose of an anti-PcrV antibody, followed by one or more secondary doses of the anti-PcrV antibody, and optionally followed by one or more tertiary doses of the anti-PcrV antibody.

[00204] The terms "initial dose," "secondary doses," and "tertiary doses," refer to the temporal sequence of administration of the anti-PcrV antibody disclosed herein. Thus, the "initial dose" is the dose which is administered at the beginning of the treatment regimen (also referred to as the "baseline dose"); the "secondary doses" are the doses which are administered after the initial dose; and the "tertiary doses" are the doses which are administered after the secondary doses. The initial, secondary, and tertiary doses may all contain the same amount of anti-PcrV antibody, but generally may differ from one another in terms of frequency of administration. In certain embodiments, however, the amount of anti-PcrV antibody contained in the initial, secondary and/or tertiary doses varies from one another (*e.g.*, adjusted up or down as appropriate) during the course of treatment. In certain embodiments, two or more (*e.g.*, 2, 3, 4, or 5) doses are administered at the beginning of the treatment regimen as "loading doses" followed by subsequent doses that are administered on a less frequent basis (*e.g.*, "maintenance doses").

[00205] In certain exemplary embodiments, each secondary and/or tertiary dose is administered 1 to 48 hours (*e.g.*, 1, 1½, 2, 2½, 3, 3½, 4, 4½, 5, 5½, 6, 6½, 7, 7½, 8, 8½, 9, 9½, 10, 10½, 11, 11½, 12, 12½, 13, 13½, 14, 14½, 15, 15½, 16, 16½, 17, 17½, 18, 18½, 19, 19½, 20, 20½, 21, 21½, 22, 22½, 23, 23½, 24, 24½, 25, 25½, 26, 26½, or more) after the immediately preceding dose. The phrase "the immediately preceding dose," as used herein, means, in a sequence of multiple administrations, the dose of

anti-PcrV antibody, which is administered to a patient prior to the administration of the very next dose in the sequence with no intervening doses.

[00206] The methods may comprise administering to a patient any number of secondary and/or tertiary doses of an anti-PcrV antibody. For example, in certain embodiments, only a single secondary dose is administered to the patient. In other embodiments, two or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, or more) secondary doses are administered to the patient. Likewise, in certain embodiments, only a single tertiary dose is administered to the patient. In other embodiments, two or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, or more) tertiary doses are administered to the patient.

[00207] In certain embodiments, the frequency at which the secondary and/or tertiary doses are administered to a patient can vary over the course of the treatment regimen. The frequency of administration may also be adjusted during the course of treatment by a physician depending on the needs of the individual patient following clinical examination.

Pharmaceutical Compositions

[00208] Provided herein pharmaceutical formulations comprising one or more anti-PcrV antibodies according to Table 1, and including, for example, or one or more (*e.g.*, 1, 2, or 3) components thereof admixed with a pharmaceutically acceptable carrier or excipient. See, *e.g.*, Remington's Pharmaceutical Sciences and U.S. Pharmacopeia: National Formulary, Mack Publishing Company, Easton, Pa. (1984). Methods for making such a pharmaceutical formulation comprising admixing a pharmaceutically acceptable carrier or excipient with the component(s) forms part of the present disclosure as do the pharmaceutical compositions that are produced by such methods.

[00209] The scope of the present disclosure includes desiccated, *e.g.*, freeze-dried, anti-PcrV antibodies of the present disclosure, and a pharmaceutical composition thereof that includes a pharmaceutically acceptable carrier but substantially lacks water. In one embodiment, the pharmaceutical formulation is aqueous (includes water). In an embodiment of the disclosure, the pharmaceutical formulation is sterile.

[00210] Pharmaceutical formulations of therapeutic agents may be prepared by mixing with acceptable carriers, excipients, or stabilizers in the form of, *e.g.*, lyophilized powders, slurries, aqueous solutions or suspensions (see, *e.g.*, Hardman *et al.* (2001) Goodman and Gilman's The Pharmacological Basis of Therapeutics, McGraw-Hill, New York, N.Y.; Gennaro (2000) Remington: The Science and Practice of Pharmacy, Lippincott, Williams, and Wilkins, New York, N.Y.; Avis, *et al.* (eds.) (1993)

Pharmaceutical Dosage Forms: Parenteral Medications, Marcel Dekker, NY; Lieberman, *et al.* (eds.) (1990) Pharmaceutical Dosage Forms: Tablets, Marcel Dekker, NY; Lieberman, *et al.* (eds.) (1990) Pharmaceutical Dosage Forms: Disperse Systems, Marcel Dekker, NY; Weiner and Kotkoskie (2000) Excipient Toxicity and Safety, Marcel Dekker, Inc., New York, N.Y.).

[00211] The mode of administration of pharmaceutical compositions comprising the anti-PcrV antibodies can vary. Routes of administration include oral, rectal, transmucosal, intestinal, parenteral; intramuscular, subcutaneous, intradermal, intramedullary, intrathecal, direct intraventricular, intravenous, intraperitoneal, intranasal, intraocular, inhalation, insufflation, topical, cutaneous, transdermal, or intra-arterial.

[00212] The present disclosure provides methods for administering pharmaceutical formulations comprising an anti-PcrV antibody to a subject (*e.g.*, a human) comprising introducing the formulation into the body of the subject, *e.g.*, into a vein, the subcutis or the muscular tissue of the subject. For example, the method comprises piercing the body of the subject with a needle of a syringe and injecting the formulation into the body of the subject.

[00213] One or more vessels are provided herein (*e.g.*, a plastic or glass vial, *e.g.*, with a cap, or a chromatography column, hollow bore needle or a syringe cylinder) comprising an anti-PcrV antibody as disclosed herein or a pharmaceutical composition thereof comprising a pharmaceutically acceptable carrier. Methods for preparing one or more vessels comprising the composition are provided, the methods comprising introducing the components of the combination into one or more vessels, *e.g.*, a single vessel comprising a combination of components which are co-formulated. In an embodiment of the present disclosure, the vessel(s) is/are then introduced into a kit.

[00214] Also provided is a device, *e.g.*, an injection device, comprising an anti-PcrV antibody disclosed herein or a pharmaceutical composition thereof and methods of use thereof. An injection device is a device that introduces a substance into the body of a patient via a parenteral route, *e.g.*, intramuscular, subcutaneous or intravenous. For example, an injection device may be a syringe (*e.g.*, pre-filled with the pharmaceutical composition, such as an auto-injector, or filled at the point of use, *e.g.*, by the user or a clinician) which, for example, includes a cylinder or barrel for holding fluid to be injected (*e.g.*, comprising the antibody or fragment or a pharmaceutical composition thereof), a needle for piercing skin and/or blood vessels for injection of the fluid; and a plunger for pushing the fluid out of the cylinder and through the needle bore.

[00215] The pharmaceutical compositions disclosed herein may also be administered

with a needleless hypodermic injection device; such as the devices disclosed in U.S. patent nos. 6620135; 6096002; 5399163; 5383851; 5312335; 5064413; 4941880; 4790824 or 4596556. Such needleless devices and methods of use thereof comprising the pharmaceutical composition are also part of the present disclosure.

[00216] Provided herein are methods for preparing one or more injection devices (*e.g.*, pre-filled syringe or autoinjector) comprising an anti-PcrV antibody, the methods comprising introducing the components of the combination into one or more of such devices, *e.g.*, a single device comprising the anti-PcrV antibody. In one embodiment, the injection device(s) is/are then introduced into a kit.

[00217] Also provided are kits comprising an anti-PcrV antibody. In one embodiment, the kit comprises the antibody in a vessel or injection device (*e.g.*, pre-filled syringe or autoinjector). The kit can include a package insert including information concerning the pharmaceutical compositions and dosage forms in the kit. Generally, such information aids patients and physicians in using the enclosed pharmaceutical compositions effectively and safely. For example, any of the following information regarding antibodies provided herein may be supplied in the insert: pharmacokinetics, pharmacodynamics, clinical studies, efficacy parameters, indications and usage, contraindications, warnings, precautions, adverse reactions, overdosage, proper dosage and administration, how supplied, proper storage conditions, references, manufacturer/distributor information and patent information.

Diagnostic Uses of the Antibodies

[00218] The anti-PcrV antibodies provided herein may be used to detect and/or measure *P. aeruginosa* in a sample, *e.g.*, for diagnostic purposes. Some embodiments contemplate the use of one or more antibodies provided herein in assays to detect a disease or disorder such as *P. aeruginosa* infection. Exemplary diagnostic assays for *P. aeruginosa* may comprise, *e.g.*, contacting a sample, obtained from a patient, with an anti-PcrV antibody of the disclosure, wherein the anti-PcrV antibody is labeled with a detectable label or reporter molecule or used as a capture ligand to selectively isolate *P. aeruginosa* from patient samples. Alternatively, an unlabeled anti-PcrV antibody can be used in diagnostic applications in combination with a secondary antibody which is itself detectably labeled. The detectable label or reporter molecule can be a radioisotope, such as ³H, ¹⁴C, ³²P, ³⁵S, or ¹²⁵I; a fluorescent or chemiluminescent moiety such as fluorescein isothiocyanate, or rhodamine; or an enzyme such as alkaline phosphatase, β -galactosidase, horseradish peroxidase, or luciferase. Specific exemplary assays that

can be used to detect or measure *P. aeruginosa* in a sample include enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA), and fluorescence-activated cell sorting (FACS).

[00219] Samples that can be used in *P. aeruginosa* diagnostic assays according to the present disclosure include any tissue or fluid sample obtainable from a patient, which contains detectable quantities of either *P. aeruginosa*, or fragments thereof, under normal or pathological conditions. Generally, levels of *P. aeruginosa* in a particular sample obtained from a healthy patient (e.g., a patient not afflicted with a disease associated with *P. aeruginosa*) will be measured to initially establish a baseline, or standard, level of *P. aeruginosa*. This baseline level of *P. aeruginosa* can then be compared against the levels of *P. aeruginosa* measured in samples obtained from individuals suspected of having a *P. aeruginosa*-associated condition, or symptoms associated with such condition.

[00220] The antibodies specific for *P. aeruginosa* may contain no additional labels or moieties, or they may contain an N-terminal or C-terminal label or moiety. In one embodiment, the label or moiety is biotin. In a binding assay, the location of a label (if any) may determine the orientation of the peptide relative to the surface upon which the peptide is bound. For example, if a surface is coated with avidin, a peptide containing an N-terminal biotin will be oriented such that the C-terminal portion of the peptide will be distal to the surface.

EXAMPLES

[00221] The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to make and use the methods and compositions provided herein, and are not intended to limit the scope of what the inventors regard as their invention. Efforts have been made to ensure accuracy with respect to numbers used (e.g., amounts, temperature, etc.) but some experimental errors and deviations should be accounted for. Unless indicated otherwise, parts are parts by weight, molecular weight is average molecular weight, temperature is in degrees Centigrade, and pressure is at or near atmospheric.

Example 1. Generation of Anti-PcrV Antibodies

[00222] The DNA encoding full length PcrV or a truncated version of PcrV (PcrV 136-257) was cloned into target vectors for expression in *E. coli* BL21(DE3). The recombinant PcrV or truncated version of PcrV (PcrV 136-257) were purified from the supernatants of lysates

from transformed *E. coli* cells. Human antibodies to PcrV were generated using full length PcrV.6xHis (See also GenBank NP_250397.1; PAO1 strain; GenScript; See also SEQ ID NO: 77) or a truncated PcrV.6xHis protein (See PcrV_136-257; GenScript, See also SEQ ID NO: 79). The immunogen was administered directly, with an adjuvant to stimulate the immune response, to a VELOCIMMUNE® mouse (i.e., an engineered mouse comprising DNA encoding human Immunoglobulin heavy and kappa light chain variable regions), e.g., as described in U.S. Pat. No. 8,502,018. The antibody immune response was monitored by a PcrV-specific immunoassay. When a desired immune response was achieved, anti-PcrV antibodies were isolated directly from antigen-positive B cells without fusion to myeloma cells, as described in U.S. Pat. No. 7,582,298, herein specifically incorporated by reference in its entirety. Using this method, several fully human anti-PcrV antibodies (i.e., antibodies possessing human variable domains and human constant domains) were obtained.

[00223] Exemplary antibodies generated according to the foregoing methods were designated as follows: H1H29329P, H1H29332P, H1H29336P, and H1H29339P.

[00224] The biological properties of the exemplary antibodies generated in accordance with the methods of this Example are described in detail in the Examples set forth below.

Example 2: Heavy and Light Chain Variable Region Amino Acid and Nucleotide Sequences

[00225] Table 1 sets forth the amino acid sequence identifiers of the heavy and light chain variable regions and CDRs of selected exemplary anti-PcrV antibodies of the invention. The corresponding nucleic acid sequence identifiers are set forth in Table 2. Table 3 provides sequence identifiers for full length heavy and light chain amino acid sequences.

Table 1: Amino Acid Sequence Identifiers

Antibody Designation	SEQ ID NOs:							
	HCV	HCDR	HCDR	HCDR	LCV	LCDR	LCDR	LCDR
	R	1	2	3	R	1	2	3
H1H29329P	2	4	6	8	10	12	14	16
H1H29332P	18	20	22	24	26	28	30	32
H1H29336P	34	36	38	40	42	44	46	48
H1H29339P	50	52	54	56	58	60	62	64

Table 2: Nucleic Acid Sequence Identifiers

Antibody Designation	SEQ ID NOs:							
	HCV R	HCDR 1	HCDR 2	HCDR 3	LCV R	LCDR 1	LCDR 2	LCDR 3
H1H29329P	1	3	5	7	9	11	13	15
H1H29332P	17	19	21	23	25	27	29	31
H1H29336P	33	35	37	39	41	43	45	47
H1H29339P	49	51	53	55	57	59	61	63

Table 3: Sequence Identifiers for full length heavy and light chain sequences

Antibody Designation	SEQ ID NOs:	
	Full length Heavy Chain	Full length Light Chain
	Amino Acid	Amino Acid
H1H29329P	65	66
H1H29332P	67	68
H1H29336P	69	70
H1H29339P	71	72

[00226] Antibodies are typically referred to herein according to the following nomenclature: Fc prefix (*e.g.* "H4H"), followed by a numerical identifier (*e.g.* "13290," "13291," "13295," *etc.*), followed by a "P" suffix, as shown in Tables 1, 2, and 3. Thus, according to this nomenclature, an antibody may be referred to herein as, *e.g.*, "H1H29329P," "H1H29332P," "H1H29336P," *etc.* The prefix on the antibody designations used herein indicate the particular Fc region isotype of the antibody. In particular, an "H1H" antibody has a human IgG1 Fc (all variable regions are fully human as denoted by the first 'H' in the antibody designation). As will be appreciated by a person of ordinary skill in the art, an antibody having a particular Fc isotype can be converted to an antibody with a different Fc isotype (*e.g.*, an antibody with a mouse IgG1 Fc can be converted to an antibody with a human IgG4, *etc.*), but in any event, the variable domains (including the CDRs) – which are indicated by the numerical identifiers shown in Tables 1 - 3 – will remain the same, and the binding properties are expected to

be identical or substantially similar regardless of the nature of the Fc domain.

Antibody Comparators

[00227] A first comparator antibody, REGN3514 (Control I, HC/LC SEQ ID NOs: 73/74) is an anti-PcrV antibody having sequences reported in WO 2013/070615. A second comparator antibody, REGN3977 (Control III, HC/LC SEQ ID NOs: 75/76) is an anti-PcrV antibody having sequences first reported in US 7,494,653. A third comparator anti-PcrV antibody is REGN7070 (Control V, HC/LC SEQ ID NOs: 83/84). Isotype control antibodies REGN1932 and REGN684 (Controls II and IV, respectively) are used in the experiments below.

Example 3. Biacore Binding Affinities and Kinetic Constants of Human Monoclonal Anti-PcrV Antibodies

[00228] The equilibrium dissociation constant (K_D) for different PcrV reagents binding to purified anti-PcrV monoclonal antibodies were determined using a real-time surface plasmon resonance based Biacore T200 biosensor. All binding studies were performed in 10mM HEPES, 150mM NaCl, 3.4mM EDTA and 0.05% v/v Tween-20, pH 7.4 (HBS-EP) running buffer at 25°C and 37°C. The Biacore CM5 sensor chip surface was first derivatized by amine coupling with the anti-human Fc γ specific polyclonal antibody (Jackson ImmunoResearch Cat. #109-005-098) to capture anti-PcrV monoclonal antibodies. Binding studies were performed on different concentrations of full-length PcrV.6xhis (SEQ ID NO: 78) and PcrV (aa136-257).6xhis (SEQ ID NO: 80) (90nM – 3.33nM; 3-fold serial dilution) prepared in HBS-EP running buffer. Proteins were injected over the captured anti-PcrV monoclonal antibody surface for 4 minutes at a flow rate of 50 μ L/minute, while the dissociation of monoclonal antibody bound PcrV reagent was monitored for 10 minutes in HBS-EP running buffer.

[00229] Kinetic association (k_a) and dissociation (k_d) rate constants were determined by fitting the real-time sensorgrams to a 1:1 binding model using Scrubber 2.0c curve fitting software. Binding dissociation equilibrium constants (K_D) and dissociative half-lives ($t_{1/2}$) were calculated from the kinetic rate constants as:

$$K_D \text{ (M)} = \frac{k_d}{k_a}, \text{ and } t_{1/2} \text{ (min)} = \frac{\ln(2)}{60 \cdot k_d}$$

[00230] Binding kinetic parameters for full-length PcrV.6xhis and PcrV (aa136-257).6xhis binding to the anti-PcrV monoclonal antibodies at 25°C and 37°C are shown

in Tables 4 through 7.

Table 4: Binding Kinetics of Anti-PcrV mAbs to Full-length PcrV.6xhis at 25°C

mAb Captured	mAb Capture Level (RU)	90nM Ag Bound (RU)	k_a (1/Ms)	k_d (1/s)	K_D (M)	$t_{1/2}$ (min)
H1H29336P	283.9±2.2	102.1	4.90E+05	≤1.00E-05	2.04E-11	≥1155
H1H29339P	364.7±4.5	140.4	3.65E+05	≤1.00E-05	2.74E-11	≥1155
H1H29332P	504.4±2.8	151.9	2.37E+05	1.30E-05	5.48E-11	888.5
H1H29329P	438.4±2.3	81	1.84E+05	9.50E-04	5.16E-09	12.2
REGN3977 – Control III	498.9±2.5	146.4	2.07E+05	4.95E-04	2.39E-09	23.4
REGN3514 – Control I	618.6±4.9	169.4	2.67E+05	3.90E-05	1.46E-10	296.2
REGN1932 - Isotype Control II	262.2±0.4	-5.9	NB	NB	NB	NB

Table 5: Binding Kinetics of Anti-PcrV mAbs to Full-length PcrV.6xhis at 37°C

mAb Captured	mAb Capture Level (RU)	90nM Ag Bound (RU)	k_a (1/Ms)	k_d (1/s)	K_D (M)	$t_{1/2}$ (min)
H1H29336P	272.5±1.4	80.7	8.60E+05	≤1.00E-05	1.16E-11	≥1155
H1H29339P	370.8±4.4	129.6	5.08E+05	≤1.00E-05	1.97E-11	≥1155
H1H29332P	533.4±2.1	163.2	2.93E+05	2.68E-05	9.20E-11	431.5
H1H29329P	430.7±1.8	59.6	5.54E+05	5.05E-03	9.12E-09	2.3
REGN3977 – Control III	452.5±3.0	135.7	2.63E+05	9.17E-04	3.48E-09	12.6
REGN3514 – Control I	517.6±2.9	141.2	4.31E+05	1.96E-04	4.53E-10	59.1
REGN1932 – Isotype Control II	230.4±0.5	-22.5	NB	NB	NB	NB

Table 6: Binding Kinetics of Anti-PcrV mAbs to PcrV (aa136-257).6xhis at 25°C

mAb Captured	mAb Capture Level (RU)	90nM Ag Bound (RU)	k_a (1/Ms)	k_d (1/s)	K_D (M)	$t_{1/2}$ (min)
H1H29336P	287.6±1.5	48.2	3.67E+05	1.48E-05	4.08E-11	783.1
H1H29339P	370.9±3.3	62.9	2.93E+05	2.76E-05	9.55E-11	418
H1H29332P	505.4±1.2	68.5	1.33E+05	3.83E-05	2.85E-10	302
H1H29329P	431.6±1.1	27.5	1.53E+05	2.30E-03	1.50E-08	5

REGN3977 – Control III	497.7±2.6	66.6	2.23E+05	9.56E-04	4.28E-09	12.1
REGN3514 – Control I	614.9±3.9	75.2	1.47E+05	1.22E-04	8.30E-10	94.7
REGN1932 – Isotype Control II	262.4±0.3	0.4	NB	NB	NB	NB

Table 7: Binding Kinetics of Anti-PcrV mAbs to PcrV (aa136-257).6xhis at 37°C

mAb Captured	mAb Capture Level (RU)	90nM Ag Bound (RU)	k_a (1/Ms)	k_d (1/s)	K_D (M)	$t_{1/2}$ (min)
H1H29336P	270.2±0.7	40.1	5.44E+05	4.36E-05	8.09E-11	264.7
H1H29339P	364.9±4.7	55.3	4.35E+05	8.15E-05	1.86E-10	141.8
H1H29332P	530.4±1.9	69.4	1.78E+05	1.06E-04	5.95E-10	109.2
H1H29329P	421.7±3.6	16.4	1.03E+05	1.10E-02	1.07E-07	1
REGN3977 Control III	445.8±2.6	54.7	2.16E+05	2.45E-03	1.14E-08	4.7
REGN3514 - Control I	517.3±1.7	60.7	2.46E+05	5.96E-04	2.42E-09	19.4
REGN1932 - Isotype Control II	229.0±1.5	-3	NB	NB	NB	NB

[00231] At 25°C, anti-PcrV monoclonal antibodies bound to full-length PcrV.6xhis (SEQ ID NO: 78) with K_D values ranging from 20.4pM to 5.16nM, as shown in Table 4. At 37°C, anti-PcrV monoclonal antibodies bound to full-length PcrV.6xhis (SEQ ID NO: 78) with K_D values ranging from 11.6pM to 9.12nM, as shown in Table 5. The isotype control antibody REGN1932 (Control II) exhibited no binding.

[00232] At 25°C, anti-PcrV monoclonal antibodies bound to PcrV (aa136-257).6xhis (SEQ ID NO: 80) with K_D values ranging from 40.8pM to 15.0nM, as shown in Table 6. At 37°C, anti-PcrV monoclonal antibodies bound to PcrV (aa136-257).6xhis (SEQ ID NO: 80) with K_D values ranging from 80.9pM to 107nM, as shown in Table 7. The isotype control antibody REGN1932 (Control II) exhibited no binding.

Example 4: Octet Cross-Competition Between Anti-PcrV Monoclonal Antibodies

[00233] Binding competition between a panel of anti-PcrV monoclonal antibodies was determined using a real-time, label-free bio-layer interferometry assay on an Octet® HTX biosensor (ForteBio, A Division of Pall Life Sciences). The entire experiment was performed at 25°C in 10mM HEPES, 150mM NaCl, 3mM EDTA, 0.05% v/v Surfactant

Tween-20, and 1mg/mL BSA, pH7.4 (HBS-EBT) buffer with the plate shaking at 1000rpm. Octet biosensor tips (ForteBio Inc, # 18-5122) coated with anti-Penta-his antibody were submerged for 90 seconds in wells containing 20µg/mL solution of full-length PcrV with a C-terminal hexahistidine tag (PcrV.6xhis; SEQ ID: 78) to capture ~0.62-0.74nM PcrV.6xhis. The antigen captured biosensor tips were then saturated with anti-PcrV monoclonal antibody (subsequently referred to as mAb-1) by dipping into wells containing 50µg/mL solution of mAb-1 for 5 minutes. To assess whether 2 antibodies compete for binding to their respective epitopes, the biosensor tips were subsequently dipped into wells containing 50µg/mL solution of a second anti-PcrV monoclonal antibody (subsequently referred to as mAb-2) for 3 minutes. Biosensor tips were washed in HBS-EBT buffer between every step of the experiment. The real-time binding response was monitored over the entire course of the experiment and the binding response at the end of every step was recorded. The response of mAb-2 binding to full-length PcrV.6xhis pre-complexed with mAb-1 was compared and competitive/non-competitive behavior of different anti-PcrV monoclonal antibodies was determined as shown in Table 8.

Table 8: Cross-Competition of Anti-PcrV Antibodies for Binding to Full-Length PcrV.6xhis.

First mAb-1 Captured using AHC Octet Biosensors	mAb-2Antibodies Shown to Compete with mAb-1
H1H29339P	H1H29329P
	H1H29332P
	H1H29336P
	REGN3514
H1H29329P	H1H29339P
	H1H29332P
	H1H29336P
	REGN3514
H1H29332P	H1H29339P
	H1H29329P
	H1H29336P
	REGN3514
	H1H29339P

First mAb-1 Captured using AHC Octet Biosensors	mAb-2Antibodies Shown to Compete with mAb-1
H1H29336P	H1H29329P
	H1H29332P
	REGN3514
REGN3514 - Control I	H1H29339P
	H1H29329P
	H1H29332P
	H1H29336P

Example 5: Binding of Human Monoclonal Antibodies to *P. aeruginosa* PcrV Recombinant Proteins by ELISA

[00234] Anti-PcrV monoclonal antibodies (mAbs) were assessed by ELISA for their ability to bind to recombinant PcrV proteins. Nunc MicroSorp™ 96-well plates were coated with 0.2 µg per well of recombinant full-length *P. aeruginosa* PcrV (GenScript) (SEQ ID NO: 77) or a truncated form of the protein (encompassing amino acids 136 to 233 of the mature protein; GenScript) (SEQ ID NO: 81) and incubated overnight at 4°C. The following morning, plates were washed three times with wash buffer (Imidazole buffered saline with Tween-20) and blocked for 1.5 hours at 25°C with 200 µl of blocking buffer (3% BSA in PBS). Plates were washed once and titrations of antibodies and isotype-matched control antibody (ranging from 33 nM – 0.1 pM with 1:3 serial dilutions in 0.5% BSA/0.05% Tween-20/PBS) were added to the protein-containing wells and incubated for one hour at 25°C. Wells were washed three times and then incubated with 100 ng/ml anti-human HRP secondary antibody per well for one hour at 25°C. 100 µl of SuperSignal™ ELISA Pico Chemiluminescent Substrate was added to each well and signal was detected (Victor X3 plate reader, Perkin Elmer). Luminescence values were analyzed by a four-parameter logistic equation over a 10-point response curve (GraphPad Prism).

[00235] As shown in Table 9, all anti-PcrV antibodies showed sub-nanomolar EC₅₀ binding to *P. aeruginosa* full length PcrV and sub-nanomolar EC₅₀ binding to truncated PcrV protein. Sub-nanomolar EC₅₀ binding of the anti-PcrV comparator antibody (Control I – REGN3514) was observed to both full length PcrV protein and the truncated PcrV protein, while the isotype control mAb (Control IV – REGN684) did not bind to either protein.

Table 9: Binding of anti-PcrV mAbs to *P. aeruginosa* PcrV proteins

mAb	Binding (EC ₅₀) [M]	
	Full length PcrV	PcrV 136-233
H1H29329P	5.969E-10	7.438E-09
H1H29332P	2.119E-09	8.139E-10
H1H29336P	2.276E-09	1.027E-09
H1H29339P	1.961E-09	8.562E-10
Control I – REGN3514	6.957E-10	7.587E-09
Control IV – Isotype Control	no binding	no binding

Example 6: Ability of *P. aeruginosa* anti-PcrV Monoclonal Antibodies to Neutralize PcrV-Mediated Cytotoxicity

[00236] Anti-PcrV monoclonal antibodies were assessed for their ability to prevent PcrV-mediated lysis of A549 cells, a human lung epithelial cell line. A549 cells were seeded at a density of approximately 4.8×10^5 cells/ml in Ham's F-12K (supplemented with 10% heat-inactivated FBS and L-glutamine) into 96-well clear bottom-black tissue culture treated plates and incubated overnight at 37°C with 5% CO₂. The next day, media was removed from the cells and replaced with 100 µl assay medium (DMEM without phenol red, supplemented with 10% heat-inactivated FBS). Titrations of purified antibodies or isotype-matched control (ranging from 33.3 pM – 1.33 µM) were added in 50 µl and cells were incubated for 45 minutes at 37°C with 5% CO₂.

[00237] Meanwhile, log phase cultures of *P. aeruginosa* strains 6077 (Gerald Pier, Brigham and Women's Hospital, Harvard University) and ATCC 700888 (ATCC) were prepared as follows: overnight *P. aeruginosa* cultures were grown in LB, diluted 1:50 in fresh LB and grown to OD₆₀₀ = ~1 at 37°C with shaking. Cultures were washed once with PBS and diluted to OD₆₀₀ = 0.03 in PBS for both *P. aeruginosa* strains. Bacteria in 50 µl were added to the wells containing cells and antibody, incubated for two hours at 37°C with 5% CO₂. Cell death was determined using the CytoTox-Glo™ Assay kit (Promega). Luminescence was detected using a plate reader (Victor, Perkin Elmer) and luminescence values were analyzed by a four-parameter logistic equation (GraphPad Prism).

[00238] As shown in Table 10, anti-PcrV mAbs (H1H29329P, H1H29332P, H1H29336P and H1H29339P) showed efficacy in preventing A549 cell death. All four monoclonal antibodies protected against both *P. aeruginosa* strains. Control anti-PcrV mAb (Control

I – REGN3514) also demonstrated efficacy against both bacterial strains and the isotype control mAb (Control II) had no effect.

Table 10: Neutralization of *P. aeruginosa* PcrV-mediated Toxicity in an A549 Cytotoxicity Assay

mAb	A549 Cytotoxicity Assay (IC50) [M]	
	<i>P. aeruginosa</i> strain 6077	<i>P. aeruginosa</i> strain ATCC 700888
H1H29329P	1.079E-08	5.428E-08
H1H29332P	6.474E-09	3.288E-08
H1H29336P	8.400E-10	6.372E-09
H1H29339P	3.329E-11	7.818E-09
Control I – REGN3514	8.070E-10	1.784E-08
Control II – Isotype Control	no efficacy	no efficacy

Example 7: Ability of *P. aeruginosa* Anti-PcrV Monoclonal Antibodies to Neutralize PcrV-Mediated Cytotoxicity

[00239] Anti-PcrV monoclonal antibodies were assessed for their ability to prevent PcrV-mediated lysis of rabbit red blood cells (rRBCs; Colorado Serum Co.).

[00240] Overnight cultures of *P. aeruginosa* strain 6077 and strain ATCC 700888 were grown in LB, diluted 1:50 in fresh LB and grown to $OD_{600} = \sim 1$ at 37°C with shaking. The culture was washed once with PBS and diluted to $OD_{600} = 0.15$ in PBS for both *P. aeruginosa* strains. rRBCs were prepared by centrifuging a 50% rRBC suspension at 4°C for 10 minutes at 2000 xg, replacing the supernatant with PBS, gently mixing the rRBC and PBS, and diluting the rRBC to 5%. In 96 well round-bottom plates, 10 µl of *P. aeruginosa* strain 6077 or ATCC 700888 were mixed with titrations of purified antibodies or isotype-matched control (ranging from 33.3 pM – 1.33 µM) or Triton X-100 (lysis positive control) in 50 µl, followed by the addition of 50 µl 5% rRBC. Plates were incubated at 37°C for two hours, with shaking at 550rpm. At the end of the incubation period, plates were centrifuged at 25°C for one minute at 200 xg, 75 µl of the supernatant was transferred to a flat clear bottom plate and absorbance (A_{405}) was detected using a plate reader (Victor X3, Perkin Elmer), and absorbance values were analyzed by a four-parameter logistic equation (GraphPad Prism).

[00241] As shown in Table 11, all four anti-PcrV mAbs showed efficacy in preventing rRBC hemolysis and protected against both *P. aeruginosa* strains. Control anti-PcrV mAb (Control I – REGN3514) also demonstrated efficacy against both bacterial strains and the isotype control mAb (Control IV – REGN684) had no effect.

Table 11: Neutralization of *P. aeruginosa* PcrV in Rabbit RBC Hemolysis Assay

mAb	Rabbit RBC Hemolysis Assay (IC50) [M]	
	<i>P. aeruginosa</i> strain 6077	<i>P. aeruginosa</i> strain ATCC 700888
H1H29329P	5.640E-08	2.389E-09
H1H29332P	1.525E-08	4.062E-09
H1H29336P	2.051E-10	8.097E-10
H1H29339P	2.302E-09	5.586E-09
Control I - REGN3514	2.441E-09	1.011E-09
Control IV – Isotype Control	no efficacy	no efficacy

Example 8: Efficacy of Anti-PcrV Monoclonal Antibodies in an in vivo Model of Acute Pneumonia

[00242] Anti-PcrV monoclonal antibodies (mAbs) that prevented PcrV-mediated toxicity in either the rabbit red blood cell (RBC) hemolysis assay (Example 7) or the A549 cytotoxicity assay (Example 6) were assessed for their ability to prevent mortality in a murine acute pneumonia model. Female BALB/c-ELITE mice (Charles River; 7-8 weeks old; n=5 per group) were injected subcutaneously with a single dose of 5 mg/kg of the purified antibodies or isotype-matched control. Two days post-injection of the mAbs, mice were challenged intranasally with 20 μ l of either *P. aeruginosa* strain 6077 (at $\sim 4.2 \times 10^5$ CFU/mouse) or strain 6206 (at $\sim 1.2 \times 10^6$ CFU/mouse) that had been grown to log phase ($OD_{600} = 1$) in TSB at 37°C, washed once and resuspended in PBS. The mice were monitored for survival for a total of seven days post-infection.

[00243] As shown in Table 12, all four anti-PcrV mAbs, H1H29329P, H1H29332P, H1H29336P and H1H29339P, prevented death of mice in an acute pneumonia model when administered at 5 mg/kg prophylactically against both *P. aeruginosa* strains. Control anti-PcrV mAb (Control I – REGN3514) also demonstrated efficacy against both bacterial strains. The isotype control mAb (Control IV – REGN684) had no protective effect.

Table 12: Prophylactic Treatment with Anti-PcrV mAbs in an Acute Pneumonia Model

mAb	% Survival (Day 7 post-infection)	
	<i>P. aeruginosa</i> strain 6077	<i>P. aeruginosa</i> strain 6206
H1H29329P	100	100
H1H29332P	100	100
H1H29336P	100	100
H1H29339P	100	100
Control I – REGN3514	100	100
Control IV – REGN684	0	0

Example 9: In vivo Efficacy of anti-PcrV Monoclonal Antibodies in an Acute Pneumonia Model Using *P. aeruginosa* Strains 6077 and 6206

[00244] Anti-PcrV monoclonal antibodies (mAbs) that demonstrated efficacy in a murine acute pneumonia model when administered prophylactically at 5 mg/kg (H1H29329P, H1H29332P, H1H29336P, H1H29339P) were tested at lower doses to assess their ability to prevent mortality in a murine acute pneumonia model. Female BALB/c-ELITE mice (Charles River; 7-8 weeks old; n=5-10 per group) were injected subcutaneously with a single dose of either 1.0, 0.2 or 0.04 mg/kg of the purified antibodies or isotype-matched control. Two days post-injection of the mAbs, mice were challenged intranasally with 20 μ l of *P. aeruginosa* strain 6077 (at $\sim 4.5 \times 10^5$ CFU/mouse) or 6206 (at $\sim 9 \times 10^5$ CFU/mouse) that had been grown to log phase ($OD_{600} = 1$) in TSB at 37°C, washed once and resuspended in PBS. The mice were monitored for survival for a total of seven days post-infection.

[00245] As shown in Table 13, anti-PcrV mAbs H1H29336P and H1H29339P decreased mortality of mice when administered prophylactically at doses as low as 0.04 mg/kg against *P. aeruginosa* strain 6077 and as low as 0.2 mg/kg against *P. aeruginosa* strain 6206. In contrast, anti-PcrV mAbs H1H29329P and H1H29332P were unable to prevent mortality when tested at doses less than 1.0 mg/kg using the more cytotoxic strain 6206. Control anti-PcrV mAb (Control I – REGN3514) lost efficacy against *P. aeruginosa* strain 6077 and strain 6206 at doses less than 0.2 mg/kg and 1.0 mg/kg, respectively. Isotype control mAb (Control IV – REGN684) had no protective effect.

Table 13: Prophylactic Treatment with Anti-PcrV mAbs in an Acute Pneumonia Mouse Model

mAb	mAb dose (mg/kg)	% Survival (Day 7 post-infection)	
		<i>P. aeruginosa</i> strain 6077	<i>P. aeruginosa</i> strain 6206
H1H29329P	1	100	80
	0.2	n.d.	0
	0.04	n.d.	n.d.
H1H29332P	1	100	80
	0.2	n.d.	0
	0.04	n.d.	n.d.
H1H29336P	1	100	80
	0.2	100	80
	0.04	70	0
H1H29339P	1	100	80
	0.2	100	90
	0.04	60	0
REGN3514 – Control I	1	100	40
	0.2	80	0
	0.04	0	0
Control IV – Isotype Control	1	20	0
	0.2	0	0
	0.04	n.d.	n.d.

n.d.: no data (experiment not done)

Example 10: In vivo Efficacy of Prophylactic Treatment with Anti-PcrV Monoclonal Antibodies in an Acute Pneumonia Model Using *P. aeruginosa* Strain 6206

[00246] This Example demonstrated the ability of prophylactically administered anti-PcrV monoclonal antibody to decrease bacterial burden in the lungs in a murine acute pneumonia model using *P. aeruginosa* strain 6206.

[00247] The H1H29339P anti-PcrV antibody prevented mortality in a murine acute pneumonia model when administered prophylactically, as shown in Example 9. In this experiment, the antibody was tested at low doses for its ability to decrease the bacterial burden in the lungs of mice using the same acute pneumonia model. Female BALB/c-ELITE mice (Charles River; 7-8 weeks old; n=5 per group) were subcutaneously injected with a single dose of either 0.1 or 0.2 mg/kg of the purified antibody or 0.2 mg/kg of isotype-matched control. Two days post-injection of the antibody, mice were challenged intranasally with 20 µl of *P. aeruginosa* strain 6206 (at $\sim 1.2 \times 10^6$ CFU/mouse) that had been grown to log phase ($OD_{600} = 1$) in TSB at 37°C, washed once and resuspended in PBS. The mice were sacrificed 16 – 18 hours post-infection, lungs were harvested and

lung homogenates were plated for bacterial enumeration on LB agar plates.

[00248] As shown in Table 14, the anti-PcrV mAb H1H29339P decreased the bacterial burden in the lungs of mice infected with *P. aeruginosa* 6206 one log more than control anti-PcrV mAb (Control V – REGN7070) when administered at either 0.1 or 0.2 mg/kg and 3 - 4 logs more than the no antibody or isotype control mAb (Control IV) groups.

Table 14: Bacterial burden in the lungs of mice administered 0.1 or 0.2 mg/kg anti-PcrV mAb prophylactically in an acute pneumonia model using *P. aeruginosa* strain 6206

mAb	mAb dose (mg/kg)	<i>P. aeruginosa</i> 6206 in lungs (CFU/g lungs)
H1H29339P	0.1	3.23e6
	0.2	1.10e5
Control V – REGN7070	0.1	2.03e7
	0.2	2.22e6
Control IV – Isotype Control	0.2	1.21e9
No mAb	n.a.	1.33e9

n.a., not applicable

Example 11: In vivo Efficacy of Prophylactic Treatment with Anti-PcrV Monoclonal Antibodies in an Acute Pneumonia Model Using *P. aeruginosa* Strain PAO1

[00249] This Example demonstrated the ability of prophylactically administered anti-PcrV monoclonal antibody to decrease bacterial burden in the lungs in a murine acute pneumonia model using *P. aeruginosa* strain PAO1, the most commonly used strain for research and, relative to more recently isolated *P. aeruginosa* strains, less cytotoxic.

[00250] The H1H29336P anti-PcrV antibody demonstrated efficacy in a murine acute pneumonia model when administered prophylactically against *P. aeruginosa* cytotoxic strains 6077 and 6206, as shown in Examples 8 through 10. Here, the antibody was tested against a noncytotoxic strain, PAO1. Female BALB/c-ELITE mice (Charles River; 7-8 weeks old; n=10 per group) were subcutaneously injected with a single dose of 25 mg/kg of purified antibodies or isotype-matched control. Two days post-injection of the mAb, mice were challenged intranasally with 20 µl of *P. aeruginosa* strain PAO1 (at ~1x10⁸ CFU/mouse) that had been grown to log phase (OD₆₀₀ = 1) in TSB at 37°C, washed once and resuspended in PBS. The mice were sacrificed 16 – 18 hours post-infection, lungs were harvested and lung homogenates were plated for bacterial enumeration on LB agar plates.

[00251] As shown in Table 15, anti-PcrV mAb H1H29336P decreased the bacterial burden in the lungs of mice infected with noncytotoxic *P. aeruginosa* strain PAO1

approximately 2 logs more than control anti-PcrV mAb (Control V – REGN7070) and 4 logs more than the no antibody or isotype control mAb (Control IV) groups.

Table 15: Bacterial burden in the lungs of mice administered 25 mg/kg anti-PcrV mAb prophylactically in an acute pneumonia model using *P. aeruginosa* strain PAO1

mAb	mAb dose (mg/kg)	<i>P. aeruginosa</i> PAO1 in lungs (CFU/g lungs)
H1H29336P	25	5.11e7
Control V – REGN7070	25	1.24e9
Control IV – Isotype Control	25	9.34e11
No mAb	n.a.	2.34e11

n.a., not applicable

Example 12: Anti-PcrV Antibody Epitope Binding by HDX-MS

[00252] Hydrogen-Deuterium Exchange Mass Spectrometry (HDX-MS) was performed to determine the amino acid residues of the *Pseudomonas aeruginosa* PcrV (SEQ ID NO: 78) interacting with H1H29336P and H1H29339P antibodies. A general description of the HDX-MS method is provided in e.g., Ehring (1999) *Analytical Biochemistry* 267(2):252-259; and Engen and Smith (2001) *Anal. Chem.* 73:256A-265A.

[00253] The HDX-MS experiments were performed on an integrated HDX-MS platform, consisting of a Leaptec HDX PAL system for the deuterium labeling and quenching, a Waters Acquity M-Class (Auxiliary solvent manager) for the sample digestion and loading, a Waters Acquity M-Class (μ Binary solvent manager) for the analytical gradient, and a Thermo Q Exactive HF mass spectrometer for peptide mass measurement.

[00254] The labeling solution was prepared as PBS buffer in D₂O at pD 7.0 (10 mM phosphate buffer, 140 mM NaCl, and 3 mM KCl, equivalent to pH 7.4 at 25°C). For deuterium labeling, 10 μ l of PcrV (from GenScript, 57.3 μ M) or PcrV premixed with H1H29336P in 1:0.6 molar ratio (antigen to antibody complex) and 10 μ l of PcrV (from GenScript, 31.7 μ M) or PcrV premixed with H1H29339P in 1:0.6 molar ratio (antigen to antibody complex) were incubated at 20°C with 90 μ L of D₂O labeling solution for various time-points in duplicates (e.g., non-deuterated control = 0 second; deuterium-labeled for 5 minutes and 10 minutes). The deuteration reaction was quenched by adding 100 μ l of quench buffer (0.5 M TCEP-HCl, 8 M urea and 1% formic acid) to each sample for a 5-minute incubation at 20 °C. The quenched samples were then injected into a Waters HDX Manager for online pepsin/protease XIII digestion. The digested peptides were separated by a C8 column (1.0 mm x 50 mm, NovaBioassays) at -9.5 °C

with a 22-minute gradient from 0%-90% B (mobile phase A: 0.5% formic acid and 4.5% acetonitrile in water, mobile phase B: 0.5% formic acid in acetonitrile). The eluted peptides were analyzed by a Thermo Q Exactive HF mass spectrometry in LC-MS/MS or LC-MS mode.

[00255] The LC-MS/MS data of undeuterated PcrV sample were searched against a database including PcrV sequence and its reversed sequence using Byonic search engine (Protein Metrics). The search parameters were set as default using non-specific enzymatic digestion and human glycosylation as common variable modification. The list of identified peptides was then imported into the HDX Workbench software (version 3.3) to calculate the deuterium uptake of each peptide detected by LC-MS from all deuterated samples. For a given peptide, the centroid mass (intensity-weighted average mass) at each time point was used to calculate the deuterium uptake (D) and percentage of deuterium uptake (%D).

$$\text{Deuterium Uptake (D-uptake)} = \text{Average Mass (deuterated)} - \text{Average Mass (undeuterated)}$$

$$\text{Percentage of deuterium uptake (\%D)} = \frac{\text{D-uptake for peptide at each time point} \times 100\%}{\text{Maximum D-uptake of the peptide}}$$

[00256] A total of 127 peptides from PcrV were identified from both PcrV alone and PcrV in complex with H1H29336P samples, representing 95% sequence coverage of PcrV. Any peptide which exhibited a differential percent D-uptake value above 5% was defined as significantly protected. Peptides corresponding to amino acids 155-170 (ALSAKQGIRIDAGGID – SEQ ID NO: 85) on PcrV were significantly protected by H1H29336P (PcrV residues are numbered according to PcrV amino acid sequence of SEQ ID NO: 78). See Table 16.

[00257] A total of 133 peptides from PcrV were identified from both PcrV alone and PcrV in complex with H1H29339P samples, representing 98% sequence coverage of PcrV. Any peptide which exhibited a differential percent D-uptake value above 5% was defined as significantly protected. Peptides corresponding to amino acids 150-170 (SQINAALSAKQGIRIDAGGID – SEQ ID NO: 86) on PcrV were significantly protected by H1H29339P (PcrV residues are numbered according to PcrV amino acid sequence of SEQ ID NO: 78). See Table 17.

Table 16: PcrV peptides with significant protection upon formation of PcrV- H1H29336P complex compared to PcrV alone

PcrV Residues	Charge (+)	5 min			10 min			$\Delta\%D$
		PcrV- H1H29336P		PcrV	PcrV- H1H29336P		PcrV	
		Centroid MH ⁺	Centroid MH ⁺	ΔD	Centroid MH ⁺	Centroid MH ⁺	ΔD	
155-165	2	1173.10	1173.74	-0.64	1172.99	1173.80	-0.81	-9.0
155-170	2	1587.45	1588.32	-0.87	1587.37	1588.34	-0.97	-7.3
155-170	3	1587.59	1588.30	-0.70	1587.50	1588.29	-0.79	-5.9
157-165	2	988.46	988.91	-0.45	988.35	988.91	-0.55	-8.0
157-170	3	1402.70	1403.25	-0.55	1402.61	1403.22	-0.61	-5.4
157-170	2	1402.78	1403.33	-0.55	1402.68	1403.25	-0.57	-5.2
161-165	1	573.12	573.29	-0.17	573.08	573.28	-0.20	-6.8
161-170	2	987.44	987.80	-0.36	987.38	987.75	-0.37	-5.1

Table 17: PcrV peptides with significant protection upon formation of PcrV- H1H29339P complex compared to PcrV alone

PcrV Residues	Charge (+)	5 min			10 min			$\Delta\%D$
		PcrV- H1H29339P		PcrV	PcrV- H1H29339P		PcrV	
		Centroid MH ⁺	Centroid MH ⁺	ΔD	Centroid MH ⁺	Centroid MH ⁺	ΔD	
150-156	1	716.24	716.46	-0.22	716.23	716.63	-0.40	-6.8
155-165	2	1172.94	1173.78	-0.84	1172.84	1173.91	-1.07	-11.8
155-170	2	1587.22	1588.29	-1.07	1587.11	1588.49	-1.38	-9.7
157-165	2	988.35	988.85	-0.50	988.25	988.95	-0.70	-9.5
157-168	2	1173.96	1174.55	-0.59	1173.81	1174.68	-0.87	-8.1
157-170	2	1402.67	1403.30	-0.63	1402.46	1403.43	-0.97	-7.4
161-165	1	573.07	573.28	-0.21	573.06	573.30	-0.24	-8.3
161-170	2	987.31	987.71	-0.40	987.17	987.79	-0.62	-7.1

[00258] The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description and the accompanying figures. Such modifications are intended to fall within the scope of the appended claims.