

ANTIBODIES SPECIFICALLY RECOGNIZING *PSEUDOMONAS* PCRV AND USES THEREOF

SUBMISSION OF SEQUENCE LISTING ON ASCII TEXT FILE

[0001] The content of the following submission on ASCII text file is incorporated herein
5 by reference in its entirety: a computer readable form (CRF) of the Sequence Listing (file
name: 710262000340SEQLIST.TXT, date recorded: March 31, 2019, size: 47 KB).

FIELD OF THE APPLICATION

[0002] This application pertains to antibodies that specifically recognize PcrV from
Pseudomonas aeruginosa, and methods of manufacture and uses thereof, including
10 methods of treating and preventing *Pseudomonas* infections.

BACKGROUND OF THE APPLICATION

[0003] *Pseudomonas aeruginosa* is an obligately aerobic gram negative *bacillus* being
widely existing in the natural world. Although its pathogenicity is usually low, it is a
pathogen that causes opportunistic infections, often occurring in patients suffering from
15 various pre-existing diseases such as cancer, diabetes, immunodeficiency diseases and
patients administered with pharmaceuticals exhibiting immune-inhibitory action. Patients
with breached skin mucous membrane are prone to *P. aeruginosa* infections while it also
poses considerable risk to patients with chronic structural lung diseases (such as COPD or
cystic fibrosis). *P. aeruginosa* may often cause pneumonia, urinary tract infection, sepsis
20 and the like, and often leading to severe results. Up to 10% of nosocomial infections are
attributed to *P. aeruginosa*, with mortality rates approaching 40% in patients with *P.*
aeruginosa bacteremia. In clinical fields, *P. aeruginosa* infection is considered as one
of the most difficult infections to be treated not only because *P. aeruginosa* has inherently
low sensitivity to existent antibiotics, but also because of its high tendency to acquire
25 resistance to various antibiotics. Thus the strategy of developing an arsenal of antibiotics
has limited merits in combating *P. aeruginosa* infections.

[0004] *Pseudomonas aeruginosa* is a major cause of hospital-acquired infections,
particularly in mechanically ventilated patients, and it is the leading cause of death in
cystic fibrosis patients. A key virulence factor associated with disease severity is the *P.*
30 *aeruginosa* type III secretion system (T3SS), which injects bacterial toxins directly into the
cytoplasm of host cells. High cytotoxicity of *Pseudomonas aeruginosa* is exerted by
injection of toxin into a eukaryotic cell via a type III exotoxin secretion system (T3SS).

PcrV is a protein of 294 residues (NCBI Accession No. AAC45935, SEQ NO: 71) constituting the type III exotoxin secretion system, and an operon sequence encoding the same is open to the public (US 6,551,795, Yahr, T. L. et al., *J. Bacteriol.*, 1997, vol. 179, p. 7165). The PcrV protein, located at the tip of the T3SS injectisome complex, is required for T3SS function and is a well-validated target in animal models of immunoprophylactic strategies targeting *P. aeruginosa*. The *P. aeruginosa* T3SS is a well-validated target for intervention in infections caused by this opportunistic pathogen. Both active vaccination with T3SS component proteins and passive immunotherapy targeting PcrV strongly attenuate *P. aeruginosa* disease in animal models. Since control for PcrV can possibly lead a therapeutic means in controlling *Pseudomonas aeruginosa* infection (T. Sawa et al., *Nature Medicine*, 1999, vol. 5, p. 392), polyclonal antibodies (Shime N et al., *J. Immunol.* 2001, vol. 167, p. 5880, Imamura Y et al., *Eur. Respir. J.*, 2007, Vol. 29, p. 965) and monoclonal antibodies (WO2002064161A2, Karine Faure et al., *J. Immune. Based. Therapies and Vaccines*, 2003, Vol. 1, Dara W. Frank et al., *J. Infect. Disease*, 2002, Vol. 186, p. 64) against PcrV having neutralizing activity are reported. However, polyclonal antibodies are difficult to be humanized and to be used as pharmaceutical compositions because of the difficulty in improving antigenicity. An antibody against PcrV, designated V2L2, is described in Warrener et al., 2014, *Antimicrob. Agents Chemother.*, 58, 4384–4391. A pegylated Fab fragment of an anti-PcrV Mab, based on the PcrV-specific mouse monoclonal antibody MAb166, is inactive for preventing *P. aeruginosa* respiratory infections in mechanically ventilated patients. While effective in blocking *P. aeruginosa* T3SS *in vitro*, MAb166 requires relatively high antibody doses for protection in animal models. The present application provides novel anti-PcrV mAbs that showed potent inhibition of PcrV *in vitro* and *in vivo*.

[0005] The disclosures of all publications, patents, patent applications and published patent applications referred to herein are hereby incorporated herein by reference in their entirety.

BRIEF SUMMARY OF THE APPLICATION

[0006] The present application provides an isolated anti-PcrV antibody that specifically binds to an epitope on *Pseudomonas* PcrV, and methods of use thereof for treating *Pseudomonas* infections.

65 [0007] In one aspect, the present application provides an isolated anti-PcrV antibody that specifically binds to an epitope on *Pseudomonas* PcrV, wherein the epitope comprises one, two, three, four, five, or six amino acid residues selected from the group consisting of Gln160, Asp165, Asp170, Asp173, Thr175, and Ser202 of *Pseudomonas* PcrV. In some embodiments, the anti- PcrV antibody binds to the *Pseudomonas* PcrV with a Kd from
70 about 0.1 pM to about 1 nM.

[0008] In one aspect, the present application provides an isolated anti- PcrV antibody, wherein the anti- PcrV antibody comprises a heavy chain variable domain (VH) comprising: a heavy chain complementarity determining region (HC-CDR) 1 comprising $X_1X_2X_3MS$ (SEQ ID NO: 39), or a variant thereof comprising up to about 3 amino acid
75 substitutions, wherein X_1 is D or S, X_2 is Y or N and X_3 is P, H, Y or S; an HC-CDR2 comprising $X_1ISESGGSTX_2X_3ADSVKG$ (SEQ ID NO: 40), or a variant thereof comprising up to about 3 amino acid substitutions, wherein X_1 is G or V; X_2 is N or Y; and X_3 is D or Y; and an HC-CDR3 comprising $GRFX_1X_2X_3X_4X_5X_6FX_7RAVYGMDV$ (SEQ ID NO: 41), or a variant thereof comprising up to about 3 amino acid substitutions,
80 wherein X_1 is S or C, X_2 is T, G, D, Y, Q or A, X_3 is S, D, N, E, L, A, or Y, X_4 is S, T, Y, or A, X_5 is S, H, Q, A, R, K, G, E, Y or D, X_6 is H or C, and X_7 is F or Y; and a light chain variable domain (VL) comprising: a light chain complementarity determining region (LC-CDR) 1 comprising $RASQGIX_1SYLA$ (SEQ ID NO: 42), or a variant thereof comprising up to about 3 amino acid substitutions, wherein X_1 is S or R; a LC-CDR2
85 comprising $AASTLQS$ (SEQ ID NO: 34), or a variant thereof comprising up to about 3 amino acid substitutions; and a LC-CDR3 comprising QLX_1SYPLX_2 (SEQ ID NO: 43), or a variant thereof comprising up to about 3 amino acid substitutions, wherein X_1 is S, N or K, and X_2 is S or T.

[0009] In some embodiments, there is provided an isolated anti- PcrV antibody,
90 wherein the anti- PcrV antibody comprises a heavy chain variable domain (VH) comprising: a heavy chain complementarity determining region (HC-CDR) 1 comprising DX_1X_2MS (SEQ ID NO: 44), or a variant thereof comprising up to about 3 amino acid substitutions, wherein X_1 is N or Y and X_2 is P, H, or Y; an HC-CDR2 comprising $X_1ISESGGSTNYADSVKG$ (SEQ ID NO: 45), or a variant thereof comprising up to about
95 3 amino acid substitutions, wherein X_1 is G or V; and an HC-CDR3 comprising $GRFSTX_1SX_2HFX_3RAVYGMDV$ (SEQ ID NO: 46), or a variant thereof comprising up to about 3 amino acid substitutions, wherein X_1 is N, S, D, or L, X_2 is S or A, and X_3 is F or

Y; and a light chain variable domain (VL) comprising: a light chain complementarity determining region (LC-CDR) 1 comprising RASQGISSYLA (SEQ ID NO: 32), or a
100 variant thereof comprising up to about 3 amino acid substitutions; a LC-CDR2 comprising AASTLQS (SEQ ID NO: 34), or a variant thereof comprising up to about 3 amino acid substitutions; and a LC-CDR3 comprising QQLSSYPLX₁ (SEQ ID NO: 47), or a variant thereof comprising up to about 3 amino acid substitutions, wherein X₁ is S or T. In some embodiments, there is provided an isolated anti- PcrV antibody, wherein the anti- PcrV
105 antibody comprises a VH comprising an HC-CDR1, an HC-CDR2, and an HC-CDR3 of the VH comprising the amino acid sequence of any one of SEQ ID NOs: 48-64; and a VL comprising a LC-CDR1, a LC-CDR2, and a LC-CDR3 of the VL comprising the amino acid sequence of any one of SEQ ID NOs: 65-70.

[0010] In some embodiments according to any one of the isolated anti- PcrV antibody
110 described above, the anti- PcrV antibody comprises: a VH comprising an HC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 1-8, or a variant thereof comprising up to about 3 amino acid substitutions; an HC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 9-14, or a variant thereof comprising up to about 3 amino acid substitutions; and an HC-CDR3 comprising the amino acid sequence of
115 any one of SEQ ID NOs: 15-31, or a variant thereof comprising up to about 3 amino acid substitutions; and a VL comprising a LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 32-33, or a variant thereof comprising up to about 3 amino acid substitutions; a LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 34, or a variant thereof comprising up to about 3 amino acid substitutions; and a LC-CDR3 comprising the amino
120 acid sequence of any one of SEQ ID NOs: 35-38, or a variant thereof comprising up to about 3 amino acid substitutions.

[0011] In some embodiments according to any one of the isolated anti- PcrV antibody described above, the anti- PcrV antibody comprises: (i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 1, an HC-CDR2 comprising the
125 amino acid sequence of SEQ ID NO: 9, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 15, or a variant thereof comprising up to about 5 amino acid substitutions in the HC-CDRs; and a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 32, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 34, and an LC-CDR3 comprising the amino acid sequence of
130 35, or a variant thereof comprising up to about 5 amino acid substitutions in the LC-CDRs;

(ii) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 2, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 10, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 16, or a variant thereof comprising up to about 5 amino acid substitutions in the HC-CDRs; and a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 32, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 34, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 35, or a variant thereof comprising up to about 5 amino acid substitutions in the LC-CDRs; (iii) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 1, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 9, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 17, or a variant thereof comprising up to about 5 amino acid substitutions in the HC-CDRs; and a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 32, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 34, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 36, or a variant thereof comprising up to about 5 amino acid substitutions in the LC-CDRs; (iv) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 3, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 10, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 18, or a variant thereof comprising up to about 5 amino acid substitutions in the HC-CDRs; and a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 32, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 34, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 37, or a variant thereof comprising up to about 5 amino acid substitutions in the LC-CDRs; (v) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 3, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 10, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 19, or a variant thereof comprising up to about 5 amino acid substitutions in the HC-CDRs; and a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 32, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 34, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 35, or a variant thereof comprising up to about 5 amino acid substitutions in the LC-CDRs; (vi) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 3, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 11, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 20, or a variant thereof comprising up to about 5 amino acid substitutions in

the HC-CDRs; and a V_L comprising an LC-CDR1 comprising the amino acid sequence of
165 SEQ ID NO: 32, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 34,
and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 35, or a variant
thereof comprising up to about 5 amino acid substitutions in the LC-CDRs; (vii) a V_H
comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 4, an
HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 10, and an HC-CDR3
170 comprising the amino acid sequence of SEQ ID NO: 21, or a variant thereof comprising up
to about 5 amino acid substitutions in the HC-CDRs; and a V_L comprising an LC-CDR1
comprising the amino acid sequence of SEQ ID NO: 32, an LC-CDR2 comprising the
amino acid sequence of SEQ ID NO: 34, and an LC-CDR3 comprising the amino acid
sequence of SEQ ID NO: 35, or a variant thereof comprising up to about 5 amino acid
175 substitutions in the LC-CDRs; (viii) a V_H comprising an HC-CDR1 comprising the amino
acid sequence of SEQ ID NO: 5, an HC-CDR2 comprising the amino acid sequence of
SEQ ID NO: 10, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO:
22, or a variant thereof comprising up to about 5 amino acid substitutions in the HC-CDRs;
and a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 32,
180 an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 34, and an LC-CDR3
comprising the amino acid sequence of SEQ ID NO: 37, or a variant thereof comprising
up to about 5 amino acid substitutions in the LC-CDRs; (ix) a V_H comprising an
HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 6, an HC-CDR2
comprising the amino acid sequence of SEQ ID NO: 10, and an HC-CDR3 comprising the
185 amino acid sequence of SEQ ID NO: 23, or a variant thereof comprising up to about 5
amino acid substitutions in the HC-CDRs; and a V_L comprising an LC-CDR1 comprising
the amino acid sequence of SEQ ID NO: 32, an LC-CDR2 comprising the amino acid
sequence of SEQ ID NO: 34, and an LC-CDR3 comprising the amino acid sequence of
SEQ ID NO: 37, or a variant thereof comprising up to about 5 amino acid substitutions in
190 the LC-CDRs; (x) a V_H comprising an HC-CDR1 comprising the amino acid sequence of
SEQ ID NO: 1, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 12, and
an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 24, or a variant thereof
comprising up to about 5 amino acid substitutions in the HC-CDRs; and a V_L comprising
an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 33, an LC-CDR2
195 comprising the amino acid sequence of SEQ ID NO: 34, and an LC-CDR3 comprising the
amino acid sequence of SEQ ID NO: 35, or a variant thereof comprising up to about 5

amino acid substitutions in the LC-CDRs; (xi) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 2, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 10, and an HC-CDR3 comprising the amino acid sequence of
200 SEQ ID NO: 25, or a variant thereof comprising up to about 5 amino acid substitutions in the HC-CDRs; and a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 32, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 34, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 37, or a variant thereof comprising up to about 5 amino acid substitutions in the LC-CDRs; (xii) a V_H
205 comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 1, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 9, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 26, or a variant thereof comprising up to about 5 amino acid substitutions in the HC-CDRs; and a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 32, an LC-CDR2 comprising the
210 amino acid sequence of SEQ ID NO: 34, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 35, or a variant thereof comprising up to about 5 amino acid substitutions in the LC-CDRs; (xiii) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 3, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 10, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO:
215 27, or a variant thereof comprising up to about 5 amino acid substitutions in the HC-CDRs; and a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 32, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 34, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 35, or a variant thereof comprising up to about 5 amino acid substitutions in the LC-CDRs; (xiv) a V_H comprising an HC-CDR1
220 comprising the amino acid sequence of SEQ ID NO: 1, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 10, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 28, or a variant thereof comprising up to about 5 amino acid substitutions in the HC-CDRs; and a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 32, an LC-CDR2 comprising the amino acid sequence of
225 SEQ ID NO: 34, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 35, or a variant thereof comprising up to about 5 amino acid substitutions in the LC-CDRs; (xv) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 7, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 13, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 29, or a variant thereof comprising up

230 to about 5 amino acid substitutions in the HC-CDRs; and a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 32, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 34, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 37, or a variant thereof comprising up to about 5 amino acid substitutions in the LC-CDRs; (xvi) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 1, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 10, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 30, or a variant thereof comprising up to about 5 amino acid substitutions in the HC-CDRs; and a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 32, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 34, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 35, or a variant thereof comprising up to about 5 amino acid substitutions in the LC-CDRs; (xvii) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 8, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 14, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 31, or a variant thereof comprising up to about 5 amino acid substitutions in the HC-CDRs; and a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 32, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 34, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 37, or a variant thereof comprising up to about 5 amino acid substitutions in the LC-CDRs; or (xviii) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 7, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 13, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 29, or a variant thereof comprising up to about 5 amino acid substitutions in the HC-CDRs; and a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 32, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 34, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 38, or a variant thereof comprising up to about 5 amino acid substitutions in the LC-CDRs.

[0012] In some embodiments according to any one of the isolated anti-PcrV antibodies described above, the anti-PcrV antibody comprises: (i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 3, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 10, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 18, or a variant thereof comprising up to about 5 amino acid substitutions in the HC-CDRs; and a V_L comprising an LC-CDR1 comprising the amino

acid sequence of SEQ ID NO: 32, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 34, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 37, or a variant thereof comprising up to about 5 amino acid substitutions in the LC-CDRs; (ii) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 3, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 10, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 19, or a variant thereof comprising up to about 5 amino acid substitutions in the HC-CDRs; and a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 32, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 34, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 35, or a variant thereof comprising up to about 5 amino acid substitutions in the LC-CDRs; (iii) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 1, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 10, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 28, or a variant thereof comprising up to about 5 amino acid substitutions in the HC-CDRs; and a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 32, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 34, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 35, or a variant thereof comprising up to about 5 amino acid substitutions in the LC-CDRs; or (iv) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 7, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 13, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 29, or a variant thereof comprising up to about 5 amino acid substitutions in the HC-CDRs; and a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 32, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 34, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 37, or a variant thereof comprising up to about 5 amino acid substitutions in the LC-CDRs.

[0013] In some embodiments according to any one of the isolated anti-PcrV antibodies described above, the isolated anti-PcrV antibody comprises: a V_H comprising the amino acid sequence of any one of SEQ ID NOs: 48-64, or a variant thereof having at least about 90% sequence identity to the amino acid sequence of any one of SEQ ID NOs: 48-64; and a V_L comprising the amino acid sequence of any one of SEQ ID NOs: 65-70, or a variant thereof having at least about 90% sequence identity to the amino acid sequence of any one of SEQ ID NOs: 65-70. In some embodiments, the isolated anti-PcrV antibody comprises: a V_H comprising the amino acid sequence of any one of SEQ ID NOs: 51-52

and 61-62, or a variant thereof having at least about 90% sequence identity to the amino acid sequence of any one of SEQ ID NOs: 51-52 and 61-62; and a V_L comprising the amino acid sequence of SEQ ID NO: 65 or 67, or a variant thereof having at least about 90% sequence identity to the amino acid sequence of SEQ ID NO: 65 or 67. In some

300 embodiments, the isolated anti- PcrV antibody comprises: (i) a V_H comprising the amino acid sequence of SEQ ID NO: 48 and a V_L comprising the amino acid sequence of SEQ ID NO: 65; (ii) a V_H comprising the amino acid sequence of SEQ ID NO: 49 and a V_L comprising the amino acid sequence of SEQ ID NO: 65; (iii) a V_H comprising the amino acid sequence of SEQ ID NO: 50 and a V_L comprising the amino acid sequence of SEQ ID

305 NO: 66; (iv) a V_H comprising the amino acid sequence of SEQ ID NO: 51 and a V_L comprising the amino acid sequence of SEQ ID NO: 67; (v) a V_H comprising the amino acid sequence of SEQ ID NO: 52 and a V_L comprising the amino acid sequence of SEQ ID NO: 65; a (vi) V_H comprising the amino acid sequence of SEQ ID NO: 53 and a V_L comprising the amino acid sequence of SEQ ID NO: 65; (vii) a V_H comprising the amino acid sequence of SEQ ID NO: 54 and a V_L comprising the amino acid sequence of SEQ ID

310 NO: 65; (viii) a V_H comprising the amino acid sequence of SEQ ID NO: 55 and a V_L comprising the amino acid sequence of SEQ ID NO: 67; (ix) a V_H comprising the amino acid sequence of SEQ ID NO: 56 and a V_L comprising the amino acid sequence of SEQ ID NO: 67; (x) a V_H comprising the amino acid sequence of SEQ ID NO: 57 and a V_L comprising the amino acid sequence of SEQ ID NO: 68; (xi) a V_H comprising the amino acid sequence of SEQ ID NO: 58 and a V_L comprising the amino acid sequence of SEQ ID

315 NO: 69; (xii) a V_H comprising the amino acid sequence of SEQ ID NO: 59 and a V_L comprising the amino acid sequence of SEQ ID NO: 65; (xiii) a V_H comprising the amino acid sequence of SEQ ID NO: 60 and a V_L comprising the amino acid sequence of SEQ ID NO: 65; (xiv) a V_H comprising the amino acid sequence of SEQ ID NO: 61 and a V_L comprising the amino acid sequence of SEQ ID NO: 65; (xv) a V_H comprising the amino acid sequence of SEQ ID NO: 62 and a V_L comprising the amino acid sequence of SEQ ID

320 NO: 67; (xvi) a V_H comprising the amino acid sequence of SEQ ID NO: 63 and a V_L comprising the amino acid sequence of SEQ ID NO: 65; (xvii) a V_H comprising the amino acid sequence of SEQ ID NO: 64 and a V_L comprising the amino acid sequence of SEQ ID NO: 67; or (xviii) a V_H comprising the amino acid sequence of SEQ ID NO: 62 and a V_L comprising the amino acid sequence of SEQ ID NO: 70.

325

[0014] In some embodiments, there is provided an isolated anti-PcrV antibody that specifically binds to PcrV competitively with the isolated anti-PcrV antibody of any one of
330 isolated anti-PcrV antibodies described above. In some embodiments, there is provided an isolated anti-PcrV antibody that specifically binds to the same epitope as any one of isolated anti-PcrV antibodies as described above.

[0015] In some embodiments according to any one of the isolated anti-PcrV antibodies described above, the anti- PcrV antibody comprises an Fc fragment. In some
335 embodiments, the anti-PcrV antibody is a full-length IgG antibody. In some embodiments, the anti- PcrV antibody is a full-length IgG1 or IgG4 antibody. In some embodiments, the anti- PcrV antibody is chimeric, human, or humanized. In some embodiments, the anti- PcrV antibody is an antigen binding fragment selected from the group consisting of a Fab, a Fab', a F(ab')₂, a Fab'-SH, a single-chain Fv (scFv), an Fv
340 fragment, a dAb, a Fd or a diabody.

[0016] In some embodiments, there is provided isolated nucleic acid molecule(s) that encodes any one of the anti-PcrV antibodies described above. In some embodiments, there is provided a vector comprising a nucleic acid molecule according to any one of the nucleic acid molecules described above. In some embodiments, there is provided a host
345 cell comprising any one of the anti-PcrV antibodies described above, any one of the nucleic acid molecules described above, or any one of the vectors described above. In some embodiments, there is provided a method of producing an anti-PcrV antibody, comprising: a) culturing any one of the host cells described above under conditions effective to express the anti-PcrV antibody; and b) obtaining the expressed anti-PcrV
350 antibody from the host cell.

[0017] In some embodiments, there is provided a method of preventing or treating a disease or condition in an individual in need thereof, comprising administering to the individual an effective amount of an anti-PcrV antibody according to any one of the anti-PcrV antibodies described above, or a pharmaceutical composition comprising an
355 anti-PcrV antibody according to any one of the pharmaceutical compositions described above. In some embodiments, use of the anti-PcrV antibody according to any one of the anti-PcrV antibodies described above, or a pharmaceutical composition comprising an anti-PcrV antibody according to any one of the pharmaceutical compositions described above in the manufacture of a medicament for treating a disease or condition. In some
360 embodiments, the disease or condition is a pathogenic infection. In some embodiments,

the infection is a gram-negative bacterial infection. In some embodiments, the bacterium is *Pseudomonas aeruginosa*. In some embodiments, the disease or condition comprises one or more symptoms caused by *Pseudomonas aeruginosa* infection. In some embodiments, the symptom comprises one or more of fever, chills, fatigues, muscle and joint pain, swelling of joints, headache, diarrhea, skin rashes, pus in wounds, bacteremia, acute pneumonia, or intraperitoneal infection.

[0018] In some embodiments according to any one of the methods of treatment described above, the method further comprises administering one or more therapeutic agents. In some embodiments, at least one of the therapeutic agents is an antibiotic. In some embodiments, the antibiotic is one or more of Imipenem, Tobramycin, Ciprofloxacin, Meropenem or Aztreonam .

[0019] Also provided are pharmaceutical compositions, kits and articles of manufacture comprising any one of the anti-PcrV antibodies, nucleic acids, vectors, isolated host cells described above.

BRIEF DESCRIPTION OF THE DRAWINGS

[0020] FIGS. 1A and 1B show the ability of lead optimized antibodies in inhibiting RBC lysis compared to parental 13-42 mAb and reference antibody V2L2-MD.

[0021] FIGS. 2A and 2B show the ability of lead optimized antibodies in inhibiting A549 cell lysis compared to parental 13-42 mAb.

[0022] FIG. 3 shows the cross-reactivity of lead optimized antibodies 7B1, 7C1, 8C1, and 6D10 to BV particles compared to reference antibodies Mab166 and V2L2-MD, as measured by ELISA.

[0023] FIG. 4A to 4E show the cross-reactivity of 13-42 mAb and optimized antibodies 7B1, 7C1, 8C1, and 6D10 to PcrV-negative A549 cells, as measured by FACS.

[0024] FIG. 5A, 5B and 5C show the ability of optimized antibodies 7B1, 7C1 and 8C1 in inhibiting RBC lysis caused by O1-52/66, O16-177/81 and O6-57/66 strain respectively, as compared to parental 13-42 mAb and V2L2-MD reference antibody.

[0025] FIG. 6A to 6D show the ability of optimized antibodies 7B1, 7C1 and 8C1 in inhibiting A549 cell lysis caused by O1-52/66, O6-57/66, O16-177/81 and O11-PA103 strain respectively, as compared to parental 13-42 mAb.

[0026] FIGS. 7A and 7B show the ability of lead optimized antibodies 7B1, 7C1 and 3G3 to improve survival prophylactically in a mouse acute pneumonia model, as compared to parental 13-42 mAb.

- [0027] FIG.8A and 8B show the ability of parental 13-42mAb, reference antibody
395 V2L2-MD and lead optimized antibodies 7B1, 7C1, 8C1 and 6D10 to prophylactically
reduce organ burden of *P. aeruginosa* in a mouse acute pneumonia model.
- [0028] FIG. 9 shows the ability of lead optimized antibodies 7B1, 7C1 and 8C1 to
improve survival prophylactically in a mouse intraperitoneal infection model, as compared
to parental 13-42 mAb.
- 400 [0029] FIG. 10 shows the ability of lead optimized antibodies 7B1, 7C1 and 8C1 to
improve survival prophylactically in an immunodeficiency mouse acute pneumonia model,
as compared to parental 13-42 mAb.
- [0030] FIG. 11A and 11B shows the ability of lead optimized antibodies 7B1, 7C1, 8C1
and 6D10 to improve survival therapeutically in a mouse acute pneumonia model, as
405 compared to reference antibody V2L2-MD.
- [0031] FIG. 12A and 12B show the ability of lead optimized antibodies 7B1 and 8C1 to
improve survival prophylactically in a mouse intraperitoneal infection model, alone or
when combined with antibiotics Meropenem (MEM), Tobramycin (TOB), or
Ciprofloxacin (CIP).
- 410 [0032] FIG. 13A and 13B shows the pharmacokinetic profiles in rat for optimized
antibody 7B1, 7C1, 8C1, 6D10 and reference antibody V2L2-MD, when administered
intravenously at 3 mg/kg and 30 mg/kg doses, respectively.
- [0033] FIGS. 14A to 14D show the binding affinity of optimized antibody 7B1 for wild
type PcrV and PcrV with mutations at exemplary amino acid residues, as measured by
415 ELISA.

DETAILED DESCRIPTION OF THE APPLICATION

- [0034] The present application in one aspect provides anti-PcrV antibodies. By using a
combination of selections on scFv phage libraries, affinity maturation and appropriately
420 designed biochemical and biological assays, we have identified highly potent antibody
molecules that bind to PcrV which inhibit the action of red blood cell and A549 cell lysis,
and which provide both therapeutic and prophylactic *in vivo* protection against
Pseudomonas aeruginosa. The results presented herein indicate that our antibodies bind a
different region or epitope of PcrV when compared with either of the known anti- PcrV
425 antibodies Mab166 or V2L2-MD, and surprisingly are even more potent than the known
anti- PcrV antibodies, as demonstrated in a variety of biological assays.

[0035] The anti-PcrV antibodies provided by the present application include, for example, full-length anti-PcrV antibodies, anti-PcrV scFvs, anti-PcrV Fc fusion proteins, multi-specific (such as bispecific) anti-PcrV antibodies, anti-PcrV immunoconjugates, and the like.

[0036] In one aspect, the present application provides an isolated anti-PcrV antibody that specifically binds to an epitope on *Pseudomonas* PcrV, wherein the epitope comprises one, two, three, four, five, or six amino acid residues selected from the group consisting of Gln160, Asp165, Asp170, Asp173, Thr175, and Ser202 of *Pseudomonas* PcrV.

[0037] Also provided are anti-PcrV antibodies having specific sequences and antibodies that compete with or binds to the same epitope as such antibodies.

[0038] Also provided are nucleic acids encoding the anti-PcrV antibodies, compositions comprising the anti-PcrV antibodies, and methods of making and using the anti-PcrV antibodies.

Definitions

[0039] As used herein, “treatment” or “treating” is an approach for obtaining beneficial or desired results, including clinical results. For purposes of this application, beneficial or desired clinical results include, but are not limited to, one or more of the following: alleviating one or more symptoms resulting from the disease, diminishing the extent of the disease, stabilizing the disease (*e.g.*, preventing or delaying the worsening of the disease), preventing or delaying the spread (*e.g.*, systemic spread of a pathogen) of the disease, preventing or delaying the recurrence of the disease, delay or slowing the progression of the disease, ameliorating the disease state, providing a remission (partial or total) of the disease, decreasing the dose of one or more other medications required to treat the disease, delaying the progression of the disease, increasing or improving the quality of life, increasing weight gain, and/or prolonging survival. Also encompassed by “treatment” is a reduction of pathological consequence of infection (such as, for example, host cell lysis or necrosis). The methods of the application contemplate any one or more of these aspects of treatment.

[0040] The term “prevent,” and similar words such as “prevented,” “preventing,” “prevention” etc., indicate an approach for preventing, inhibiting, or reducing the likelihood of the occurrence or recurrence of, a disease or condition, *e.g.*, a pathogenic infection. It also refers to delaying the occurrence or recurrence of a disease or condition,

or delaying the occurrence or recurrence of the symptoms of a disease or condition. As
460 used herein, “prevention” and similar words also includes reducing the intensity, effect,
symptoms and/or burden of a disease or condition prior to occurrence or recurrence of the
disease or condition. As used herein, “prevention” and similar words also includes
reducing the risk and susceptibility to occurrence or recurrence of the disease or condition,
e.g., a pathogenic infection.

465 **[0041]** The term “antibody” includes full-length antibodies and antigen-binding
fragments thereof. A full-length antibody comprises two heavy chains and two light chains.
The variable regions of the light and heavy chains are responsible for antigen binding. The
variable regions in both chains generally contain three highly variable loops called the
complementarity determining regions (CDRs) (light chain (LC) CDRs including LC-CDR1,
470 LC-CDR2, and LC-CDR3, heavy chain (HC) CDRs including HC-CDR1, HC-CDR2, and
HC-CDR3). CDR boundaries for the antibodies and antigen-binding fragments disclosed
herein may be defined or identified by the conventions of Kabat, Chothia, or Al-Lazikani
(Al-Lazikani 1997; Chothia 1985; Chothia 1987; Chothia 1989; Kabat 1987; Kabat 1991).
The three CDRs of the heavy or light chains are interposed between flanking stretches
475 known as framework regions (FRs), which are more highly conserved than the CDRs and
form a scaffold to support the hypervariable loops. The constant regions of the heavy and
light chains are not involved in antigen binding, but exhibit various effector functions.
Antibodies are assigned to classes based on the amino acid sequence of the constant region
of their heavy chain. The five major classes or isotypes of antibodies are IgA, IgD, IgE,
480 IgG, and IgM, which are characterized by the presence of α , δ , ϵ , γ , and μ heavy chains,
respectively. Several of the major antibody classes are divided into subclasses such as
IgG1 (γ 1 heavy chain), IgG2 (γ 2 heavy chain), IgG3 (γ 3 heavy chain), IgG4 (γ 4 heavy
chain), IgA1 (α 1 heavy chain), or IgA2 (α 2 heavy chain).

[0042] The term “antigen-binding fragment” as used herein refers to an antibody
485 fragment including, for example, a diabody, a Fab, a Fab', a F(ab')₂, an Fv fragment, a
disulfide stabilized Fv fragment (dsFv), a (dsFv)₂, a bispecific dsFv (dsFv-dsFv'), a
disulfide stabilized diabody (ds diabody), a single-chain Fv (scFv), an scFv dimer (bivalent
diabody), a multispecific antibody formed from a portion of an antibody comprising one or
more CDRs, a camelized single domain antibody, a nanobody, a domain antibody, a
490 bivalent domain antibody, or any other antibody fragment that binds to an antigen but does
not comprise a complete antibody structure. An antigen-binding fragment is capable of

binding to the same antigen to which the parent antibody or a parent antibody fragment (e.g., a parent scFv) binds. In some embodiments, an antigen-binding fragment may comprise one or more CDRs from a particular human antibody grafted to a framework region from one or more different human antibodies.

495 [0043] The term “epitope” as used herein refers to the specific group of atoms or amino acids on an antigen to which an antibody or antibody moiety binds. Two antibodies or antibody moieties may bind the same epitope within an antigen if they exhibit competitive binding for the antigen.

500 [0044] As used herein, a first antibody “competes” for binding to a target PcrV with a second antibody when the first antibody inhibits target PcrV binding of the second antibody by at least about 50% (such as at least about any of 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98% or 99%) in the presence of an equimolar concentration of the first antibody, or *vice versa*. A high throughput process for “binning” antibodies based upon their cross-competition is described in PCT Publication No. WO 03/48731.

505 [0045] As use herein, the term “specifically binds,” “specifically recognizing,” or “is specific for” refers to measurable and reproducible interactions, such as binding between a target and an antibody, that is determinative of the presence of the target in the presence of a heterogeneous population of molecules, including biological molecules. For example, an antibody that specifically recognizes a target (which can be an epitope) is an antibody that binds this target with greater affinity, avidity, more readily, and/or with greater duration than its bindings to other targets. In some embodiments, an antibody that specifically recognizes an antigen reacts with one or more antigenic determinants of the antigen with a binding affinity that is at least about 10 times its binding affinity for other targets.

515 [0046] An “isolated” anti-PcrV antibody as used herein refers to an anti-PcrV antibody that (1) is not associated with proteins found in nature, (2) is free of other proteins from the same source, (3) is expressed by a cell from a different species, or, (4) does not occur in nature.

520 [0047] The term “isolated nucleic acid” as used herein is intended to mean a nucleic acid of genomic, cDNA, or synthetic origin or some combination thereof, which by virtue of its origin the “isolated nucleic acid” (1) is not associated with all or a portion of a polynucleotide in which the “isolated nucleic acid” is found in nature, (2) is operably linked to a polynucleotide which it is not linked to in nature, or (3) does not occur in nature as part of a larger sequence.

525 [0048] As used herein, the term “CDR” or “complementarity determining region” is intended to mean the non-contiguous antigen combining sites found within the variable region of both heavy and light chain polypeptides. These particular regions have been described by Kabat *et al.*, J. Biol. Chem. 252:6609-6616 (1977); Kabat *et al.*, U.S. Dept. of Health and Human Services, “Sequences of proteins of immunological interest” (1991);

530 Chothia *et al.*, J. Mol. Biol. 196:901-917 (1987); Al-Lazikani B. *et al.*, J. Mol. Biol., 273: 927-948 (1997); MacCallum *et al.*, J. Mol. Biol. 262:732-745 (1996); Abhinandan and Martin, *Mol. Immunol.*, 45: 3832-3839 (2008); Lefranc M.P. *et al.*, *Dev. Comp. Immunol.*, 27: 55-77 (2003); and Honegger and Plückthun, *J. Mol. Biol.*, 309:657-670 (2001), where the definitions include overlapping or subsets of amino acid residues when compared

535 against each other. Nevertheless, application of either definition to refer to a CDR of an antibody or grafted antibodies or variants thereof is intended to be within the scope of the term as defined and used herein. The amino acid residues which encompass the CDRs as defined by each of the above cited references are set forth below in Table 1 as a comparison. CDR prediction algorithms and interfaces are known in the art, including, for

540 example, Abhinandan and Martin, *Mol. Immunol.*, 45: 3832-3839 (2008); Ehrenmann F. *et al.*, *Nucleic Acids Res.*, 38: D301-D307 (2010); and Adolf-Bryfogle J. *et al.*, *Nucleic Acids Res.*, 43: D432-D438 (2015). The contents of the references cited in this paragraph are incorporated herein by reference in their entireties for use in the present application and for possible inclusion in one or more claims herein.

545 TABLE 1: CDR DEFINITIONS

	Kabat ¹	Chothia ²	MacCallum ³	IMGT ⁴	AHo ⁵
V _H CDR1	31-35	26-32	30-35	27-38	25-40
V _H CDR2	50-65	53-55	47-58	56-65	58-77
V _H CDR3	95-102	96-101	93-101	105-117	109-137
V _L CDR1	24-34	26-32	30-36	27-38	25-40
V _L CDR2	50-56	50-52	46-55	56-65	58-77
V _L CDR3	89-97	91-96	89-96	105-117	109-137

¹Residue numbering follows the nomenclature of Kabat *et al.*, *supra*

²Residue numbering follows the nomenclature of Chothia *et al.*, *supra*

³Residue numbering follows the nomenclature of MacCallum *et al.*, *supra*

⁴Residue numbering follows the nomenclature of Lefranc *et al.*, *supra*

550 ⁵Residue numbering follows the nomenclature of Honegger and Plückthun, *supra*

[0049] The term “chimeric antibodies” refer to antibodies in which a portion of the heavy and/or light chain is identical with or homologous to corresponding sequences in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is identical with or homologous to
555 corresponding sequences in antibodies derived from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they exhibit a biological activity of this application (*see* U.S. Patent No. 4,816,567; and Morrison *et al.*, Proc. Natl. Acad. Sci. USA, 81:6851-6855 (1984)).

[0050] “Fv” is the minimum antibody fragment which contains a complete
560 antigen-recognition and -binding site. This fragment consists of a dimer of one heavy- and one light-chain variable region domain in tight, non-covalent association. From the folding of these two domains emanate six hypervariable loops (3 loops each from the heavy and light chain) that contribute the amino acid residues for antigen binding and confer antigen binding specificity to the antibody. However, even a single variable domain (or half of an
565 Fv comprising only three CDRs specific for an antigen) has the ability to recognize and bind antigen, although at a lower affinity than the entire binding site.

[0051] “Single-chain Fv,” also abbreviated as “sFv” or “scFv,” are antibody fragments that comprise the V_H and V_L antibody domains connected into a single polypeptide chain. In some embodiments, the scFv polypeptide further comprises a polypeptide linker
570 between the V_H and V_L domains which enables the scFv to form the desired structure for antigen binding. For a review of scFv, *see* Pluckthun in *The Pharmacology of Monoclonal Antibodies*, vol. 113, Rosenberg and Moore eds., Springer-Verlag, New York, pp. 269-315 (1994).

[0052] The term “diabodies” refers to small antibody fragments prepared by constructing
575 scFv fragments (see preceding paragraph) typically with short linkers (such as about 5 to about 10 residues) between the V_H and V_L domains such that inter-chain but not intra-chain pairing of the V domains is achieved, resulting in a bivalent fragment, *i.e.*, fragment having two antigen-binding sites. Bispecific diabodies are heterodimers of two “crossover” scFv fragments in which the V_H and V_L domains of the two antibodies are present on different
580 polypeptide chains. Diabodies are described more fully in, for example, EP 404,097; WO 93/11161; and Hollinger *et al.*, Proc. Natl. Acad. Sci. USA, 90:6444-6448 (1993).

[0053] “Humanized” forms of non-human (*e.g.*, rodent) antibodies are chimeric antibodies that contain minimal sequence derived from the non-human antibody. For the

most part, humanized antibodies are human immunoglobulins (recipient antibody) in
585 which residues from a hypervariable region (HVR) of the recipient are replaced by
residues from a hypervariable region of a non-human species (donor antibody) such as
mouse, rat, rabbit or non-human primate having the desired antibody specificity, affinity,
and capability. In some instances, framework region (FR) residues of the human
immunoglobulin are replaced by corresponding non-human residues. Furthermore,
590 humanized antibodies can comprise residues that are not found in the recipient antibody or
in the donor antibody. These modifications are made to further refine antibody
performance. In general, the humanized antibody will comprise substantially all of at least
one, and typically two, variable domains, in which all or substantially all of the
hypervariable loops correspond to those of a non-human immunoglobulin and all or
595 substantially all of the FRs are those of a human immunoglobulin sequence. The
humanized antibody optionally also will comprise at least a portion of an immunoglobulin
constant region (Fc), typically that of a human immunoglobulin. For further details, *see*
Jones *et al.*, *Nature* 321:522-525 (1986); Riechmann *et al.*, *Nature* 332:323-329 (1988);
and Presta, *Curr. Op. Struct. Biol.* 2:593-596 (1992).

600 **[0054]** “Percent (%) amino acid sequence identity” or “homology” with respect to the
polypeptide and antibody sequences identified herein is defined as the percentage of amino
acid residues in a candidate sequence that are identical with the amino acid residues in the
polypeptide being compared, after aligning the sequences considering any conservative
substitutions as part of the sequence identity. Alignment for purposes of determining
605 percent amino acid sequence identity can be achieved in various ways that are within the
skill in the art, for instance, using publicly available computer software such as BLAST,
BLAST-2, ALIGN, Megalign (DNASTAR), or MUSCLE software. Those skilled in the art
can determine appropriate parameters for measuring alignment, including any algorithms
needed to achieve maximal alignment over the full-length of the sequences being
610 compared. For purposes herein, however, % amino acid sequence identity values are
generated using the sequence comparison computer program MUSCLE (Edgar, R.C.,
Nucleic Acids Research 32(5):1792-1797, 2004; Edgar, R.C., *BMC Bioinformatics*
5(1):113, 2004).

[0055] The terms “Fc receptor” or “FcR” are used to describe a receptor that binds to the
615 Fc region of an antibody. In some embodiments, an FcR of this application is one that
binds an IgG antibody (a γ receptor) and includes receptors of the Fc γ RI, Fc γ RII, and

Fc γ RIII subclasses, including allelic variants and alternatively spliced forms of these receptors. Fc γ RII receptors include Fc γ RIIA (an “activating receptor”) and Fc γ RIIB (an “inhibiting receptor”), which have similar amino acid sequences that differ primarily in the cytoplasmic domains thereof. Activating receptor Fc γ RIIA contains an immunoreceptor tyrosine-based activation motif (ITAM) in its cytoplasmic domain. Inhibiting receptor Fc γ RIIB contains an immunoreceptor tyrosine-based inhibition motif (ITIM) in its cytoplasmic domain (*see review M. in Daëron, Annu. Rev. Immunol.* 15:203-234 (1997)). The term includes allotypes, such as Fc γ RIIA allotypes: Fc γ RIIA-Phe158, Fc γ RIIA-Val158, Fc γ RIIA-R131 and/or Fc γ RIIA-H131. FcRs are reviewed in Ravetch and Kinet, *Annu. Rev. Immunol* 9:457-92 (1991); Capel *et al.*, *Immunomethods* 4:25-34 (1994); and de Haas *et al.*, *J. Lab. Clin. Med.* 126:330-41 (1995). Other FcRs, including those to be identified in the future, are encompassed by the term “FcR” herein. The term also includes the neonatal receptor, FcRn, which is responsible for the transfer of maternal IgGs to the fetus (Guyer *et al.*, *J. Immunol.* 117:587 (1976) and Kim *et al.*, *J. Immunol.* 24:249 (1994)).

[0056] The term “FcRn” refers to the neonatal Fc receptor (FcRn). FcRn is structurally similar to major histocompatibility complex (MHC) and consists of an α -chain noncovalently bound to β 2-microglobulin. The multiple functions of the neonatal Fc receptor FcRn are reviewed in Ghetie and Ward (2000) *Annu. Rev. Immunol.* 18, 739-766. FcRn plays a role in the passive delivery of immunoglobulin IgGs from mother to young and the regulation of serum IgG levels. FcRn can act as a salvage receptor, binding and transporting pinocytosed IgGs in intact form both within and across cells, and rescuing them from a default degradative pathway.

[0057] The “CH1 domain” of a human IgG Fc region usually extends from about amino acid 118 to about amino acid 215 (EU numbering system).

[0058] “Hinge region” is generally defined as stretching from Glu216 to Pro230 of human IgG1 (Burton, *Molec. Immunol.* 22:161-206 (1985)). Hinge regions of other IgG isotypes may be aligned with the IgG1 sequence by placing the first and last cysteine residues forming inter-heavy chain S-S bonds in the same positions.

[0059] The “CH2 domain” of a human IgG Fc region usually extends from about amino acid 231 to about amino acid 340. The CH2 domain is unique in that it is not closely paired with another domain. Rather, two N-linked branched carbohydrate chains are interposed between the two CH2 domains of an intact native IgG molecule. It has been speculated that

650 the carbohydrate may provide a substitute for the domain-domain pairing and help stabilize the CH2 domain. Burton, *Molec Immunol.* 22:161-206 (1985).

[0060] The “CH3 domain” comprises the stretch of residues C-terminal to a CH2 domain in an Fc region (*i.e.* from about amino acid residue 341 to the C-terminal end of an antibody sequence, typically at amino acid residue 446 or 447 of an IgG).

655 [0061] A “functional Fc fragment” possesses an “effector function” of a native sequence Fc region. Exemplary “effector functions” include C1q binding; complement dependent cytotoxicity (CDC); Fc receptor binding; antibody-dependent cell-mediated cytotoxicity (ADCC); phagocytosis; down regulation of cell surface receptors (*e.g.* B cell receptor; BCR), etc. Such effector functions generally require the Fc region to be combined with a
660 binding domain (*e.g.* an antibody variable domain) and can be assessed using various assays known in the art.

[0062] An antibody with a variant IgG Fc with “altered” FcR binding affinity or ADCC activity is one which has either enhanced or diminished FcR binding activity (*e.g.*, Fc γ R or FcRn) and/or ADCC activity compared to a parent polypeptide or to a polypeptide
665 comprising a native sequence Fc region. The variant Fc which “exhibits increased binding” to an FcR binds at least one FcR with higher affinity (*e.g.*, lower apparent K_d or IC_{50} value) than the parent polypeptide or a native sequence IgG Fc. According to some embodiments, the improvement in binding compared to a parent polypeptide is about 3 fold, such as about any of 5, 10, 25, 50, 60, 100, 150, 200, or up to 500 fold, or about 25% to 1000%
670 improvement in binding. The polypeptide variant which “exhibits decreased binding” to an FcR, binds at least one FcR with lower affinity (*e.g.*, higher apparent K_d or higher IC_{50} value) than a parent polypeptide. The decrease in binding compared to a parent polypeptide may be about 40% or more decrease in binding.

[0063] “Antibody-dependent cell-mediated cytotoxicity” or “ADCC” refers to a form of
675 cytotoxicity in which secreted Ig bound to Fc receptors (FcRs) present on certain cytotoxic cells (*e.g.*, Natural Killer (NK) cells, neutrophils, and macrophages) enable these cytotoxic effector cells to bind specifically to an antigen-bearing target cell and subsequently kill the target cell with cytotoxins. The antibodies “arm” the cytotoxic cells and are required for such killing. The primary cells for mediating ADCC, NK cells, express Fc γ RIII only,
680 whereas monocytes express Fc γ RI, Fc γ RII and Fc γ RIII. FcR expression on hematopoietic cells is summarized in Table 3 on page 464 of Ravetch and Kinet, *Annu. Rev. Immunol* 9:457-92 (1991). To assess ADCC activity of a molecule of interest, an *in vitro* ADCC

assay, such as that described in US Patent No. 5,500,362 or 5,821,337 may be performed. Useful effector cells for such assays include peripheral blood mononuclear cells (PBMC) and Natural Killer (NK) cells. Alternatively, or additionally, ADCC activity of the molecule of interest may be assessed *in vivo*, *e.g.*, in an animal model such as that disclosed in Clynes *et al. PNAS (USA)* 95:652-656 (1998).

[0064] The polypeptide comprising a variant Fc region which “exhibits increased ADCC” or mediates ADCC in the presence of human effector cells more effectively than a polypeptide having wild type IgG Fc or a parent polypeptide is one which *in vitro* or *in vivo* is substantially more effective at mediating ADCC, when the amounts of polypeptide with variant Fc region and the polypeptide with wild type Fc region (or the parent polypeptide) in the assay are essentially the same. Generally, such variants will be identified using any *in vitro* ADCC assay known in the art, such as assays or methods for determining ADCC activity, *e.g.*, in an animal model *etc.* In some embodiments, the variant is from about 5 fold to about 100 fold, *e.g.* from about 25 to about 50 fold, more effective at mediating ADCC than the wild type Fc (or parent polypeptide) .

[0065] “Complement dependent cytotoxicity” or “CDC” refers to the lysis of a target cell in the presence of complement. Activation of the classical complement pathway is initiated by the binding of the first component of the complement system (C1q) to antibodies (of the appropriate subclass) which are bound to their cognate antigen. To assess complement activation, a CDC assay, *e.g.* as described in Gazzano-Santoro *et al., J. Immunol. Methods* 202:163 (1996), may be performed. Polypeptide variants with altered Fc region amino acid sequences and increased or decreased C1q binding capability are described in US patent No. 6,194,551B1 and WO99/51642. The contents of those patent publications are specifically incorporated herein by reference. *See also*, Idusogie *et al. J. Immunol.* 164: 4178-4184 (2000).

[0066] Unless otherwise specified, a “nucleotide sequence encoding an amino acid sequence” includes all nucleotide sequences that are degenerate versions of each other and that encode the same amino acid sequence. The phrase nucleotide sequence that encodes a protein or an RNA may also include introns to the extent that the nucleotide sequence encoding the protein may in some version contain an intron(s).

[0067] The term “operably linked” refers to functional linkage between a regulatory sequence and a heterologous nucleic acid sequence resulting in expression of the latter. For example, a first nucleic acid sequence is operably linked with a second nucleic acid

sequence when the first nucleic acid sequence is placed in a functional relationship with the second nucleic acid sequence. For instance, a promoter is operably linked to a coding sequence if the promoter affects the transcription or expression of the coding sequence. Generally, operably linked DNA sequences are contiguous and, where necessary to join two protein coding regions, in the same reading frame.

[0068] “Homologous” refers to the sequence similarity or sequence identity between two polypeptides or between two nucleic acid molecules. When a position in both of the two compared sequences is occupied by the same base or amino acid monomer subunit, *e.g.*, if a position in each of two DNA molecules is occupied by adenine, then the molecules are homologous at that position. The percent of homology between two sequences is a function of the number of matching or homologous positions shared by the two sequences divided by the number of positions compared times 100. For example, if 6 of 10 of the positions in two sequences are matched or homologous then the two sequences are 60% homologous. By way of example, the DNA sequences ATTGCC and TATGGC share 50% homology. Generally, a comparison is made when two sequences are aligned to give maximum homology.

[0069] An “effective amount” of an anti-PcrV antibody or composition as disclosed herein, is an amount sufficient to carry out a specifically stated purpose. An “effective amount” can be determined empirically and by known methods relating to the stated purpose.

[0070] The term “therapeutically effective amount” refers to an amount of an anti-PcrV antibody or composition as disclosed herein, effective to “treat” a disease or disorder in an individual. In the case of *P. aeruginosa* infection, the therapeutically effective amount of the anti-PcrV antibody or composition as disclosed herein can reduce the number of infected cells; inhibit (*i.e.*, slow to some extent and preferably stop) the spread of infection; and/or relieve to some extent one or more of the symptoms associated with the infection. To the extent the anti-PcrV antibody or composition as disclosed herein can prevent *P. aeruginosa* growth and/or kill *P. aeruginosa* in an infection, the anti-PcrV can be cytostatic and/or cytotoxic. In some embodiments, the therapeutically effective amount is an amount that inhibits infection in a patient. In some embodiments, the therapeutically effective amount is an amount that completely eradicates infection in a patient.

[0071] As used herein, by “pharmaceutically acceptable” or “pharmacologically compatible” is meant a material that is not biologically or otherwise undesirable, *e.g.*, the

material may be incorporated into a pharmaceutical composition administered to a patient
750 without causing any significant undesirable biological effects or interacting in a deleterious
manner with any of the other components of the composition in which it is contained.
Pharmaceutically acceptable carriers or excipients have preferably met the required
standards of toxicological and manufacturing testing and/or are included on the Inactive
Ingredient Guide prepared by the U.S. Food and Drug administration.

755 [0072] It is understood that embodiments of the application described herein include
“consisting” and/or “consisting essentially of” embodiments.

[0073] Reference to “about” a value or parameter herein includes (and describes)
variations that are directed to that value or parameter per se. For example, description
referring to “about X” includes description of “X”.

760 [0074] As used herein, reference to “not” a value or parameter generally means and
describes “other than” a value or parameter. For example, the method is not used to treat
infection of type X means the method is used to treat infection of types other than X.

[0075] As used herein and in the appended claims, the singular forms “a,” “or,” and “the”
include plural referents unless the context clearly dictates otherwise.

765

Anti-PcrV Antibodies

[0076] In one aspect, the present application provides anti-PcrV antibodies that
specifically bind to PcrV. Anti-PcrV antibodies include, but are not limited to, humanized
antibodies, chimeric antibodies, mouse antibodies, human antibodies, and antibodies
770 comprising the heavy chain and/or light chain CDRs discussed herein. In one aspect, the
application provides isolated antibodies that bind to PcrV. Contemplated anti-PcrV
antibodies include, for example, full-length anti-PcrV antibodies (*e.g.*, full-length IgG1,
IgG2 or IgG4), anti-PcrV scFvs, multi-specific (such as bispecific) anti-PcrV antibodies,
anti-PcrV immunoconjugates, and the like. In some embodiments, the anti-PcrV antibody
775 is a Fab, a Fab', a F(ab')₂, a Fab'-SH, a single-chain Fv (scFv), an Fv fragment, a dAb, a
Fd, or a diabody. In some embodiments, reference to an antibody that specifically binds to
PcrV means that the antibody binds to PcrV with an affinity that is at least about 10 times
(including for example at least about any of 10, 10², 10³, 10⁴, 10⁵, 10⁶, or 10⁷ times) its
binding affinity for non-target. In some embodiments, the non-target is an antigen that is
780 not PcrV. Binding affinity can be determined by methods known in the art, such as ELISA,

fluorescence activated cell sorting (FACS) analysis, or radioimmunoprecipitation assay (RIA). K_d can be determined by methods known in the art, such as surface plasmon resonance (SPR) assay or biolayer interferometry (BLI).

785 [0077] In certain aspects, the anti-PcrV antibody or antigen-binding fragment thereof that specifically binds to *Pseudomonas* PcrV (a) promotes, mediates, or enhances opsonophagocytic killing (OPK) of *P. aeruginosa*, and/or (b) disrupts the activity of the type III toxin secretion system.

790 [0078] Although anti-PcrV antibodies containing human sequences (*e.g.*, human heavy and light chain variable domain sequences comprising human CDR sequences) are extensively discussed herein, non-human anti-PcrV antibodies are also contemplated. In some embodiments, non-human anti-PcrV antibodies comprise human CDR sequences from an anti-PcrV antibody as described herein and non-human framework sequences. Non-human framework sequences include, in some embodiments, any sequence that can be used for generating synthetic heavy and/or light chain variable domains using one or
795 more human CDR sequences as described herein, including, *e.g.*, mammals, *e.g.*, mouse, rat, rabbit, pig, bovine (*e.g.*, cow, bull, buffalo), deer, sheep, goat, chicken, cat, dog, ferret, primate (*e.g.*, marmoset, rhesus monkey), etc. In some embodiments, a non-human anti-PcrV antibody includes an anti-PcrV antibody generated by grafting one or more human CDR sequences as described herein onto a non-human framework sequence (*e.g.*, a
800 mouse or chicken framework sequence).

[0079] The complete amino acid sequence of an exemplary PcrV protein comprises or consists of the amino acid sequence of SEQ ID NO: 71. In some embodiments, the anti-PcrV antibody described herein specifically recognizes an epitope within *Pseudomonas* PcrV. In some embodiments, the anti-PcrV antibody is specific for
805 *Pseudomonas* PcrV and does not exhibit species cross-reactivity or other types of non-*Pseudomonas* protein cross-reactivity.

[0080] In some embodiments, the anti-PcrV antibody described herein specifically binds to a linear epitope within *Pseudomonas* PcrV. In some embodiments, the anti-PcrV antibody described herein specifically binds to a nonlinear epitope within *Pseudomonas*
810 PcrV. In some embodiments, the anti-PcrV antibody described herein specifically binds to an epitope on *Pseudomonas* PcrV, where the epitope comprises at least any one of 1, 2, 3, 4, 5 or 6 amino acid residues selected from the group of Gln160, Asp165, Asp170, Asp173, Thr175, and Ser202 of *Pseudomonas* PcrV, according to SEQ ID NO: 71. In some

embodiments, the anti-PcrV antibody described herein specifically binds to an epitope on
815 *Pseudomonas* PcrV, where the epitope comprises at least 2 amino acid residues selected
from the group of Gln160, Asp165, Asp170, Asp173, Thr175, and Ser202 of
Pseudomonas PcrV, according to SEQ ID NO: 71. In some embodiments, the anti-PcrV
antibody described herein specifically binds to an epitope on *Pseudomonas* PcrV, where
the epitope comprises at least 3 amino acid residues selected from the group of Gln160,
820 Asp165, Asp170, Asp173, Thr175, and Ser202 of *Pseudomonas* PcrV, according to SEQ
ID NO: 71. In some embodiments, the anti-PcrV antibody described herein specifically
binds to an epitope on *Pseudomonas* PcrV, where the epitope comprises at least 4 amino
acid residues selected from the group of Gln160, Asp165, Asp170, Asp173, Thr175, and
Ser202 of *Pseudomonas* PcrV, according to SEQ ID NO: 71. In some embodiments, the
825 anti-PcrV antibody described herein specifically binds to an epitope on *Pseudomonas* PcrV,
where the epitope comprises at least 5 amino acid residues selected from the group of
Gln160, Asp165, Asp170, Asp173, Thr175, and Ser202 of *Pseudomonas* PcrV, according
to SEQ ID NO: 71. In some embodiments, the anti-PcrV antibody described herein
specifically binds to an epitope on *Pseudomonas* PcrV, where the epitope comprises
830 Gln160, Asp165, Asp170, Asp173, Thr175, and Ser202 of *Pseudomonas* PcrV, according
to SEQ ID NO: 71.

[0081] In some embodiments, the anti-PcrV antibody comprises an antibody heavy chain
constant region and an antibody light chain constant region. In some embodiments, the
anti-PcrV antibody comprises an IgG1 heavy chain constant region. In some embodiments,
835 the anti-PcrV antibody comprises an IgG2 heavy chain constant region. In some
embodiments, the anti-PcrV antibody comprises an IgG3 heavy chain constant region. In
some embodiments, the anti-PcrV antibody comprises an IgG4 heavy chain constant region.
In some embodiments, the IgG is a human IgG. In some embodiments, the heavy chain
constant region comprises or consists of the amino acid sequence of SEQ ID NO: 73. In
840 some embodiments, the heavy chain constant region comprises or consists of the amino
acid sequence of SEQ ID NO: 74. In some embodiments, the anti-PcrV comprises a
lambda light chain constant region. In some embodiments, the anti-PcrV antibody
comprises a kappa light chain constant region. In some embodiments, the light chain
constant region comprises or consists of the amino acid sequence of SEQ ID NO: 72. In
845 some embodiments, the anti-PcrV antibody comprises an antibody heavy chain variable
domain and an antibody light chain variable domain.

[0082] In one aspect, the present application provides an isolated anti- PcrV antibody, wherein the anti- PcrV antibody comprises a heavy chain variable domain (V_H) comprising: a heavy chain complementarity determining region (HC-CDR) 1 comprising X₁X₂X₃MS (SEQ ID NO: 39), or a variant thereof comprising up to about 3 amino acid substitutions, wherein X₁ is D or S, X₂ is Y or N and X₃ is P, H, Y or S; an HC-CDR2 comprising X₁ISESGGSTX₂X₃ADSVKG (SEQ ID NO: 40), or a variant thereof comprising up to about 3 amino acid substitutions, wherein X₁ is G or V; X₂ is N or Y; and X₃ is D or Y; and an HC-CDR3 comprising GRFX₁X₂X₃X₄X₅X₆FX₇RAVYGMDV (SEQ ID NO: 41), or a variant thereof comprising up to about 3 amino acid substitutions, wherein X₁ is S or C, X₂ is T, G, D, Y, Q or A, X₃ is S, D, N, E, L, A, or Y, X₄ is S, T, Y, or A, X₅ is S, H, Q, A, R, K, G, E, Y or D, X₆ is H or C, and X₇ is F or Y; and a light chain variable domain (V_L) comprising: a light chain complementarity determining region (LC-CDR) 1 comprising RASQGIX₁SYLA (SEQ ID NO: 42), or a variant thereof comprising up to about 3 amino acid substitutions, wherein X₁ is S or R; a LC-CDR2 comprising AASTLQS (SEQ ID NO: 34), or a variant thereof comprising up to about 3 amino acid substitutions; and a LC-CDR3 comprising QQLX₁SYPLX₂ (SEQ ID NO: 43), or a variant thereof comprising up to about 3 amino acid substitutions, wherein X₁ is S, N or K, and X₂ is S or T.

[0083] In some embodiments, the anti-PcrV antibody comprises a V_H comprising: an HC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 1-8, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions, an HC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 9-14, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions, and an HC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 15-31, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a V_L comprising: an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 32 or 33, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 34, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions, and an LC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 35-38, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions.

[0084] In some embodiments, the anti-PcrV antibody comprises a V_H comprising: an HC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 1-8, an

- 880 HC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 9-14, and an HC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 15-31; and a V_L comprising: an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 32 or 33 an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 34, and an LC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 35-38.
- 885 **[0085]** In some embodiments, the anti-PcrV antibody comprises a V_H comprising an HC-CDR1, an HC-CDR2 and an HC-CDR3 of the V_H comprising the amino acid sequence of any one of SEQ ID NOs: 48-64; and a V_L comprising a LC-CDR1, a LC-CDR2, and a LC-CDR3 of the V_L comprising the amino acid sequence of any one of SEQ ID NOs: 65-70.
- 890 **[0086]** In some embodiments, the anti-PcrV antibody comprises a V_H comprising the amino acid sequence of any one of SEQ ID NOs: 48-64, or a variant thereof having at least about 90% (for example at least about any of 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%) sequence identity, and a V_L comprising the amino acid sequence of any one of SEQ ID NOs: 65-70, or a variant thereof having at least about 90% sequence identity. In
- 895 some embodiments, the anti-PcrV antibody comprises a V_H comprising the amino acid sequence of any one of SEQ ID NOs: 48-64, and a V_L comprising the amino acid sequence of any one of SEQ ID NOs: 65-70.
- [0087]** In some embodiments, the anti-PcrV is a full-length antibody. In some embodiments, the anti-PcrV antibody comprises IgG1 constant domains. In some
- 900 embodiments, the IgG1 is human IgG1. In some embodiments, the anti-PcrV antibody comprises IgG4 constant domains. In some embodiments, the IgG4 is human IgG4. In some embodiments, the anti-PcrV heavy chain constant region comprises or consists of the amino acid sequence of SEQ ID NO: 73. In some embodiments, the anti-PcrV heavy chain constant region comprises or consists of the amino acid sequence of SEQ ID NO: 74. In
- 905 some embodiments, the anti-PcrV light chain constant region comprises or consists of the amino acid sequence of SEQ ID NO: 72.
- [0088]** In some embodiments, there is provided an isolated anti- PcrV antibody, wherein the anti- PcrV antibody comprises a heavy chain variable domain (V_H) comprising: a heavy chain complementarity determining region (HC-CDR) 1 comprising DX₁X₂MS (SEQ ID NO: 44), or a variant thereof comprising up to about 3 amino acid substitutions, wherein X₁ is N or Y and X₂ is P, H, or Y; an HC-CDR2 comprising X₁ISESGGSTNYADSVKG (SEQ ID NO: 45), or a variant thereof comprising up to about

3 amino acid substitutions, wherein X_1 is G or V; and an HC-CDR3 comprising GRFSTX₁SX₂HFX₃RAVYGMDV (SEQ ID NO: 46), or a variant thereof comprising up to about 3 amino acid substitutions, wherein X_1 is N, S, D, or L, X_2 is S or A, and X_3 is F or Y; and a light chain variable domain (V_L) comprising: a light chain complementarity determining region (LC-CDR) 1 comprising RASQGISSYLA (SEQ ID NO: 32), or a variant thereof comprising up to about 3 amino acid substitutions; a LC-CDR2 comprising AASTLQS (SEQ ID NO: 34), or a variant thereof comprising up to about 3 amino acid substitutions; and a LC-CDR3 comprising QQLSSYPLX₁ (SEQ ID NO: 47), or a variant thereof comprising up to about 3 amino acid substitutions, wherein X_1 is S or T.

[0089] In some embodiments, the anti- PcrV antibody comprises a V_H comprising HC-CDR1, HC-CDR2 and HC-CDR3 of the V_H of SEQ ID NO: 51, and a V_L comprising LC-CDR1, LC-CDR2 and LC-CDR3 of the V_L of SEQ ID NO: 67. In some embodiments, the anti- PcrV antibody comprises a V_H comprising HC-CDR1, HC-CDR2 and HC-CDR3 of the V_H of SEQ ID NO: 52, and a V_L comprising LC-CDR1, LC-CDR2 and LC-CDR3 of the V_L of SEQ ID NO: 65. In some embodiments, the anti- PcrV antibody comprises a V_H comprising HC-CDR1, HC-CDR2 and HC-CDR3 of the V_H of SEQ ID NO: 61, and a V_L comprising LC-CDR1, LC-CDR2 and LC-CDR3 of the V_L of SEQ ID NO: 65. In some embodiments, the anti- PcrV antibody comprises a V_H comprising HC-CDR1, HC-CDR2 and HC-CDR3 of the V_H of SEQ ID NO: 62, and a V_L comprising LC-CDR1, LC-CDR2 and LC-CDR3 of the V_L of SEQ ID NO: 67.

[0090] In some embodiments, the anti-PcrV antibody comprises a V_H comprising: an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 1, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 9, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 15, or a variant thereof comprising up to about 5 amino acid substitutions in the HC-CDRs; and a V_L comprising: an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 32, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 34, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 35, or a variant thereof comprising up to about 5 amino acid substitutions in the LC-CDRs.

[0091] In some embodiments, the anti-PcrV antibody comprises a V_H comprising: an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 1, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 9, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 15; and a V_L comprising: an LC-CDR1 comprising

the amino acid sequence of SEQ ID NO: 32, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 34, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 35. In some embodiments, there is provided an anti-PcrV antibody that specifically competes with an antibody comprising a V_H comprising: an HC-CDR1
950 comprising the amino acid sequence of SEQ ID NO: 1, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 9, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 15; and a V_L comprising: an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 32, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 34, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO:
955 35. In some embodiments, there is provided an anti-PcrV antibody that binds to the same epitope as an antibody comprising a V_H comprising: an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 1, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 9, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 15; and a V_L comprising: an LC-CDR1 comprising the amino acid sequence of SEQ ID NO:
960 32, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 34, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 35.

[0092] In some embodiments, the anti-PcrV antibody comprises a V_H comprising the amino acid sequence of SEQ ID NO: 48, or a variant thereof having at least about 90% (for example at least about any of 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%)
965 sequence identity, and a V_L comprising the amino acid sequence of SEQ ID NO: 65, or a variant thereof having at least about 90% sequence identity. In some embodiments, the anti-PcrV antibody comprises a V_H comprising the amino acid sequence of SEQ ID NO: 48 and a V_L comprising the amino acid sequence of SEQ ID NO: 65. In some embodiments, the anti-PcrV antibody comprises a V_H comprising an HC-CDR1, an
970 HC-CDR2 and an HC-CDR3 of the V_H comprising the amino acid sequence of SEQ ID NO: 48; and a V_L comprising a LC-CDR1, a LC-CDR2, and a LC-CDR3 of the V_L comprising the amino acid sequence of SEQ ID NOs: 65.

[0093] In some embodiments, the anti-PcrV antibody comprises a V_H comprising: an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 2, an HC-CDR2
975 comprising the amino acid sequence of SEQ ID NO: 10, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 16, or a variant thereof comprising up to about 5 amino acid substitutions in the HC-CDRs; and a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 32, an LC-CDR2 comprising the amino acid

sequence of SEQ ID NO: 34, and an LC-CDR3 comprising the amino acid sequence of
980 SEQ ID NO: 35, or a variant thereof comprising up to about 5 amino acid substitutions in
the LC-CDRs.

[0094] In some embodiments, the anti-PcrV antibody comprises a V_H comprising: an
HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 2, an HC-CDR2
comprising the amino acid sequence of SEQ ID NO: 10, and an HC-CDR3 comprising the
985 amino acid sequence of SEQ ID NO: 16; and a V_L comprising an LC-CDR1 comprising
the amino acid sequence of SEQ ID NO: 32, an LC-CDR2 comprising the amino acid
sequence of SEQ ID NO: 34, and an LC-CDR3 comprising the amino acid sequence of
SEQ ID NO: 35. In some embodiments, there is provided an anti-PcrV antibody that
specifically competes with an antibody comprising a V_H comprising: an HC-CDR1
990 comprising the amino acid sequence of SEQ ID NO: 2, an HC-CDR2 comprising the
amino acid sequence of SEQ ID NO: 10, and an HC-CDR3 comprising the amino acid
sequence of SEQ ID NO: 16; and a V_L comprising an LC-CDR1 comprising the amino
acid sequence of SEQ ID NO: 32, an LC-CDR2 comprising the amino acid sequence of
SEQ ID NO: 34, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO:
995 35. In some embodiments, there is provided an anti-PcrV antibody that binds to the same
epitope as an antibody comprising a V_H comprising: an HC-CDR1 comprising the amino
acid sequence of SEQ ID NO: 2, an HC-CDR2 comprising the amino acid sequence of
SEQ ID NO: 10, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO:
1000 16; and a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO:
32, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 34, and an
LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 35.

[0095] In some embodiments, the anti-PcrV antibody comprises a V_H comprising the
amino acid sequence of SEQ ID NO: 49, or a variant thereof having at least about 90% (for
example at least about any of 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%)
1005 sequence identity, and a V_L comprising the amino acid sequence of SEQ ID NO: 65, or a
variant thereof having at least about 90% sequence identity. In some embodiments, the
anti-PcrV antibody comprises a V_H comprising the amino acid sequence of SEQ ID NO:
49 and a V_L comprising the amino acid sequence of SEQ ID NO: 65. In some
embodiments, the anti-PcrV antibody comprises a V_H comprising an HC-CDR1, an
1010 HC-CDR2 and an HC-CDR3 of the V_H comprising the amino acid sequence of SEQ ID

NO: 49; and a V_L comprising a LC-CDR1, a LC-CDR2, and a LC-CDR3 of the V_L comprising the amino acid sequence of SEQ ID NOs: 65.

[0096] In some embodiments, the anti-PcrV antibody comprises a V_H comprising: an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 1, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 9, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 17, or a variant thereof comprising up to about 5 amino acid substitutions in the HC-CDRs; and a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 32, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 34, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 36, or a variant thereof comprising up to about 5 amino acid substitutions in the LC-CDRs.

[0097] In some embodiments, the anti-PcrV antibody comprises a V_H comprising: an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 1, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 9, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 17; and a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 32, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 34, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 36. In some embodiments, there is provided an anti-PcrV antibody that specifically competes with an antibody comprising a V_H comprising: an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 1, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 9, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 17; and a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 32, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 34, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 36. In some embodiments, there is provided an anti-PcrV antibody that binds to the same epitope as an antibody comprising a V_H comprising: an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 1, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 9, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 17; and a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 32, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 34, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 36.

[0098] In some embodiments, the anti-PcrV antibody comprises a V_H comprising the amino acid sequence of SEQ ID NO: 50, or a variant thereof having at least about 90% (for

example at least about any of 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%)
1045 sequence identity, and a V_L comprising the amino acid sequence of SEQ ID NO: 66, or a
variant thereof having at least about 90% sequence identity. In some embodiments, the
anti-PcrV antibody comprises a V_H comprising the amino acid sequence of SEQ ID NO:
50 and a V_L comprising the amino acid sequence of SEQ ID NO: 66. In some
embodiments, the anti-PcrV antibody comprises a V_H comprising an HC-CDR1, an
1050 HC-CDR2 and an HC-CDR3 of the V_H comprising the amino acid sequence of SEQ ID
NO: 50; and a V_L comprising a LC-CDR1, a LC-CDR2, and a LC-CDR3 of the V_L
comprising the amino acid sequence of SEQ ID NOs: 66.

[0099] In some embodiments, the anti-PcrV antibody comprises a V_H comprising: an
HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 3, an HC-CDR2
1055 comprising the amino acid sequence of SEQ ID NO: 10, and an HC-CDR3 comprising the
amino acid sequence of SEQ ID NO: 18, or a variant thereof comprising up to about 5
amino acid substitutions in the HC-CDRs; and a V_L comprising an LC-CDR1 comprising
the amino acid sequence of SEQ ID NO: 32, an LC-CDR2 comprising the amino acid
sequence of SEQ ID NO: 34, and an LC-CDR3 comprising the amino acid sequence of
1060 SEQ ID NO: 37, or a variant thereof comprising up to about 5 amino acid substitutions in
the LC-CDRs.

[00100] In some embodiments, the anti-PcrV antibody comprises a V_H comprising: an
HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 3, an HC-CDR2
comprising the amino acid sequence of SEQ ID NO: 10, and an HC-CDR3 comprising the
1065 amino acid sequence of SEQ ID NO: 18; and a V_L comprising an LC-CDR1 comprising
the amino acid sequence of SEQ ID NO: 32, an LC-CDR2 comprising the amino acid
sequence of SEQ ID NO: 34, and an LC-CDR3 comprising the amino acid sequence of
SEQ ID NO: 37. In some embodiments, there is provided an anti-PcrV antibody that
specifically competes with an antibody comprising a V_H comprising: an HC-CDR1
1070 comprising the amino acid sequence of SEQ ID NO: 3, an HC-CDR2 comprising the
amino acid sequence of SEQ ID NO: 10, and an HC-CDR3 comprising the amino acid
sequence of SEQ ID NO: 18; and a V_L comprising an LC-CDR1 comprising the amino
acid sequence of SEQ ID NO: 32, an LC-CDR2 comprising the amino acid sequence of
SEQ ID NO: 34, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO:
1075 37. In some embodiments, there is provided an anti-PcrV antibody that binds to the same
epitope as an antibody comprising a V_H comprising: an HC-CDR1 comprising the amino

acid sequence of SEQ ID NO: 3, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 10, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 18; and a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 32, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 34, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 37.

[00101] In some embodiments, the anti-PcrV antibody comprises a V_H comprising the amino acid sequence of SEQ ID NO: 51, or a variant thereof having at least about 90% (for example at least about any of 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%) sequence identity, and a V_L comprising the amino acid sequence of SEQ ID NO: 67, or a variant thereof having at least about 90% sequence identity. In some embodiments, the anti-PcrV antibody comprises a V_H comprising the amino acid sequence of SEQ ID NO: 51 and a V_L comprising the amino acid sequence of SEQ ID NO: 67. In some embodiments, the anti-PcrV antibody comprises a V_H comprising an HC-CDR1, an HC-CDR2 and an HC-CDR3 of the V_H comprising the amino acid sequence of SEQ ID NO: 51; and a V_L comprising a LC-CDR1, a LC-CDR2, and a LC-CDR3 of the V_L comprising the amino acid sequence of SEQ ID NO: 67.

[00102] In some embodiments, the anti-PcrV antibody comprises a V_H comprising: an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 3, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 10, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 19, or a variant thereof comprising up to about 5 amino acid substitutions in the HC-CDRs; and a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 32, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 34, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 35, or a variant thereof comprising up to about 5 amino acid substitutions in the LC-CDRs.

[00103] In some embodiments, the anti-PcrV antibody comprises a V_H comprising: an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 3, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 10, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 19; and a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 32, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 34, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 35. In some embodiments, there is provided an anti-PcrV antibody that specifically competes with an antibody comprising a V_H comprising: an HC-CDR1

- 1110 comprising the amino acid sequence of SEQ ID NO: 3, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 10, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 19; and a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 32, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 34, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 35.
- 1115 In some embodiments, there is provided an anti-PcrV antibody that binds to the same epitope as an antibody comprising a V_H comprising: an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 3, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 10, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 19; and a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 32, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 34, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 35.
- 1120 **[00104]** In some embodiments, the anti-PcrV antibody comprises a V_H comprising the amino acid sequence of SEQ ID NO: 52, or a variant thereof having at least about 90% (for example at least about any of 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%) sequence identity, and a V_L comprising the amino acid sequence of SEQ ID NO: 65, or a variant thereof having at least about 90% sequence identity. In some embodiments, the anti-PcrV antibody comprises a V_H comprising the amino acid sequence of SEQ ID NO: 52 and a V_L comprising the amino acid sequence of SEQ ID NO: 65. In some embodiments, the anti-PcrV antibody comprises a V_H comprising an HC-CDR1, an
- 1130 HC-CDR2 and an HC-CDR3 of the V_H comprising the amino acid sequence of SEQ ID NO: 52; and a V_L comprising a LC-CDR1, a LC-CDR2, and a LC-CDR3 of the V_L comprising the amino acid sequence of SEQ ID NOs: 65.
- [00105]** In some embodiments, the anti-PcrV antibody comprises a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 3, an HC-CDR2
- 1135 comprising the amino acid sequence of SEQ ID NO: 11, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 20, or a variant thereof comprising up to about 5 amino acid substitutions in the HC-CDRs; and a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 32, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 34, and an LC-CDR3 comprising the amino acid sequence of
- 1140 SEQ ID NO: 35, or a variant thereof comprising up to about 5 amino acid substitutions in the LC-CDRs.

[00106] In some embodiments, the anti-PcrV antibody comprises a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 3, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 11, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 20; and a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 32, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 34, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 35. In some embodiments, there is provided an anti-PcrV antibody that specifically competes with an antibody comprising a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 3, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 11, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 20; and a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 32, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 34, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 35.

In some embodiments, there is provided an anti-PcrV antibody that binds to the same epitope as an antibody comprising a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 3, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 11, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 20; and a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 32, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 34, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 35.

[00107] In some embodiments, the anti-PcrV antibody comprises a V_H comprising the amino acid sequence of SEQ ID NO: 53, or a variant thereof having at least about 90% (for example at least about any of 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%) sequence identity, and a V_L comprising the amino acid sequence of SEQ ID NO: 65, or a variant thereof having at least about 90% sequence identity. In some embodiments, the anti-PcrV antibody comprises a V_H comprising the amino acid sequence of SEQ ID NO: 53 and a V_L comprising the amino acid sequence of SEQ ID NO: 65. In some embodiments, the anti-PcrV antibody comprises a V_H comprising an HC-CDR1, an HC-CDR2 and an HC-CDR3 of the V_H comprising the amino acid sequence of SEQ ID NO: 53; and a V_L comprising a LC-CDR1, a LC-CDR2, and a LC-CDR3 of the V_L comprising the amino acid sequence of SEQ ID NOs: 65.

[00108] In some embodiments, the anti-PcrV antibody comprises a V_H comprising: an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 4, an HC-CDR2

1175 comprising the amino acid sequence of SEQ ID NO: 10, and an HC-CDR3 comprising the
amino acid sequence of SEQ ID NO: 21, or a variant thereof comprising up to about 5
amino acid substitutions in the HC-CDRs; and a V_L comprising an LC-CDR1 comprising
the amino acid sequence of SEQ ID NO: 32, an LC-CDR2 comprising the amino acid
sequence of SEQ ID NO: 34, and an LC-CDR3 comprising the amino acid sequence of
1180 SEQ ID NO: 35, or a variant thereof comprising up to about 5 amino acid substitutions in
the LC-CDRs.

[00109] In some embodiments, the anti-PcrV antibody comprises a V_H comprising: an
HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 4, an HC-CDR2
comprising the amino acid sequence of SEQ ID NO: 10, and an HC-CDR3 comprising the
1185 amino acid sequence of SEQ ID NO: 21; and a V_L comprising an LC-CDR1 comprising
the amino acid sequence of SEQ ID NO: 32, an LC-CDR2 comprising the amino acid
sequence of SEQ ID NO: 34, and an LC-CDR3 comprising the amino acid sequence of
SEQ ID NO: 35. In some embodiments, there is provided an anti-PcrV antibody that
specifically competes with an antibody comprising a V_H comprising: an HC-CDR1
1190 comprising the amino acid sequence of SEQ ID NO: 4, an HC-CDR2 comprising the
amino acid sequence of SEQ ID NO: 10, and an HC-CDR3 comprising the amino acid
sequence of SEQ ID NO: 21; and a V_L comprising an LC-CDR1 comprising the amino
acid sequence of SEQ ID NO: 32, an LC-CDR2 comprising the amino acid sequence of
SEQ ID NO: 34, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO:
1195 35. In some embodiments, there is provided an anti-PcrV antibody that binds to the same
epitope as an antibody comprising a V_H comprising: an HC-CDR1 comprising the amino
acid sequence of SEQ ID NO: 4, an HC-CDR2 comprising the amino acid sequence of
SEQ ID NO: 10, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO:
21; and a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO:
1200 32, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 34, and an
LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 35.

[00110] In some embodiments, the anti-PcrV antibody comprises a V_H comprising the
amino acid sequence of SEQ ID NO: 54, or a variant thereof having at least about 90% (for
example at least about any of 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%)
1205 sequence identity, and a V_L comprising the amino acid sequence of SEQ ID NO: 65, or a
variant thereof having at least about 90% sequence identity. In some embodiments, the
anti-PcrV antibody comprises a V_H comprising the amino acid sequence of SEQ ID NO:

54 and a V_L comprising the amino acid sequence of SEQ ID NO: 65. In some
embodiments, the anti-PcrV antibody comprises a V_H comprising an HC-CDR1, an
1210 HC-CDR2 and an HC-CDR3 of the V_H comprising the amino acid sequence of SEQ ID
NO: 54; and a V_L comprising a LC-CDR1, a LC-CDR2, and a LC-CDR3 of the V_L
comprising the amino acid sequence of SEQ ID NOs: 65.

[00111] In some embodiments, the anti-PcrV antibody comprises a V_H comprising: an
HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 5, an HC-CDR2
1215 comprising the amino acid sequence of SEQ ID NO: 10, and an HC-CDR3 comprising the
amino acid sequence of SEQ ID NO: 22, or a variant thereof comprising up to about 5
amino acid substitutions in the HC-CDRs; and a V_L comprising an LC-CDR1 comprising
the amino acid sequence of SEQ ID NO: 32, an LC-CDR2 comprising the amino acid
sequence of SEQ ID NO: 34, and an LC-CDR3 comprising the amino acid sequence of
1220 SEQ ID NO: 37, or a variant thereof comprising up to about 5 amino acid substitutions in
the LC-CDRs.

[00112] In some embodiments, the anti-PcrV antibody comprises a V_H comprising: an
HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 5, an HC-CDR2
comprising the amino acid sequence of SEQ ID NO: 10, and an HC-CDR3 comprising the
1225 amino acid sequence of SEQ ID NO: 22; and a V_L comprising an LC-CDR1 comprising
the amino acid sequence of SEQ ID NO: 32, an LC-CDR2 comprising the amino acid
sequence of SEQ ID NO: 34, and an LC-CDR3 comprising the amino acid sequence of
SEQ ID NO: 37. In some embodiments, there is provided an anti-PcrV antibody that
specifically competes with an antibody comprising a V_H comprising: an HC-CDR1
1230 comprising the amino acid sequence of SEQ ID NO: 5, an HC-CDR2 comprising the
amino acid sequence of SEQ ID NO: 10, and an HC-CDR3 comprising the amino acid
sequence of SEQ ID NO: 22; and a V_L comprising an LC-CDR1 comprising the amino
acid sequence of SEQ ID NO: 32, an LC-CDR2 comprising the amino acid sequence of
SEQ ID NO: 34, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO:
1235 37. In some embodiments, there is provided an anti-PcrV antibody that binds to the same
epitope as an antibody comprising a V_H comprising: an HC-CDR1 comprising the amino
acid sequence of SEQ ID NO: 5, an HC-CDR2 comprising the amino acid sequence of
SEQ ID NO: 10, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO:
22; and a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO:

- 1240 32, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 34, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 37.
- [00113] In some embodiments, the anti-PcrV antibody comprises a V_H comprising the amino acid sequence of SEQ ID NO: 55, or a variant thereof having at least about 90% (for example at least about any of 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%)
- 1245 sequence identity, and a V_L comprising the amino acid sequence of SEQ ID NO: 67, or a variant thereof having at least about 90% sequence identity. In some embodiments, the anti-PcrV antibody comprises a V_H comprising the amino acid sequence of SEQ ID NO: 55 and a V_L comprising the amino acid sequence of SEQ ID NO: 67. In some
- embodiments, the anti-PcrV antibody comprises a V_H comprising an HC-CDR1, an
- 1250 HC-CDR2 and an HC-CDR3 of the V_H comprising the amino acid sequence of SEQ ID NO: 55; and a V_L comprising a LC-CDR1, a LC-CDR2, and a LC-CDR3 of the V_L comprising the amino acid sequence of SEQ ID NOs: 67.
- [00114] In some embodiments, the anti-PcrV antibody comprises a V_H comprising: an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 6, an HC-CDR2
- 1255 comprising the amino acid sequence of SEQ ID NO: 10, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 23, or a variant thereof comprising up to about 5 amino acid substitutions in the HC-CDRs; and a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 32, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 34, and an LC-CDR3 comprising the amino acid sequence of
- 1260 SEQ ID NO: 37, or a variant thereof comprising up to about 5 amino acid substitutions in the LC-CDRs.
- [00115] In some embodiments, the anti-PcrV antibody comprises a V_H comprising: an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 6, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 10, and an HC-CDR3 comprising the
- 1265 amino acid sequence of SEQ ID NO: 23; and a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 32, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 34, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 37. In some embodiments, there is provided an anti-PcrV antibody that specifically competes with an antibody comprising a V_H comprising: an HC-CDR1
- 1270 comprising the amino acid sequence of SEQ ID NO: 6, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 10, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 23; and a V_L comprising an LC-CDR1 comprising the amino

acid sequence of SEQ ID NO: 32, an LC-CDR2 comprising the amino acid sequence of
SEQ ID NO: 34, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO:
1275 37. In some embodiments, there is provided an anti-PcrV antibody that binds to the same
epitope as an antibody comprising a V_H comprising: an HC-CDR1 comprising the amino
acid sequence of SEQ ID NO: 6, an HC-CDR2 comprising the amino acid sequence of
SEQ ID NO: 10, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO:
23; and a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO:
1280 32, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 34, and an
LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 37.

[00116] In some embodiments, the anti-PcrV antibody comprises a V_H comprising the
amino acid sequence of SEQ ID NO: 56, or a variant thereof having at least about 90% (for
example at least about any of 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%)
1285 sequence identity, and a V_L comprising the amino acid sequence of SEQ ID NO: 67, or a
variant thereof having at least about 90% sequence identity. In some embodiments, the
anti-PcrV antibody comprises a V_H comprising the amino acid sequence of SEQ ID NO:
56 and a V_L comprising the amino acid sequence of SEQ ID NO: 67. In some
embodiments, the anti-PcrV antibody comprises a V_H comprising an HC-CDR1, an
1290 HC-CDR2 and an HC-CDR3 of the V_H comprising the amino acid sequence of SEQ ID
NO: 56; and a V_L comprising a LC-CDR1, a LC-CDR2, and a LC-CDR3 of the V_L
comprising the amino acid sequence of SEQ ID NOs: 67.

[00117] In some embodiments, the anti-PcrV antibody comprises a V_H comprising: an
HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 1, an HC-CDR2
1295 comprising the amino acid sequence of SEQ ID NO: 12, and an HC-CDR3 comprising the
amino acid sequence of SEQ ID NO: 24, or a variant thereof comprising up to about 5
amino acid substitutions in the HC-CDRs; and a V_L comprising an LC-CDR1 comprising
the amino acid sequence of SEQ ID NO: 33, an LC-CDR2 comprising the amino acid
sequence of SEQ ID NO: 34, and an LC-CDR3 comprising the amino acid sequence of
1300 SEQ ID NO: 35, or a variant thereof comprising up to about 5 amino acid substitutions in
the LC-CDRs.

[00118] In some embodiments, the anti-PcrV antibody comprises a V_H comprising: an
HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 1, an HC-CDR2
comprising the amino acid sequence of SEQ ID NO: 12, and an HC-CDR3 comprising the
1305 amino acid sequence of SEQ ID NO: 24; and a V_L comprising an LC-CDR1 comprising

the amino acid sequence of SEQ ID NO: 33, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 34, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 35. In some embodiments, there is provided an anti-PcrV antibody that specifically competes with an antibody comprising a V_H comprising: an HC-CDR1

1310 comprising the amino acid sequence of SEQ ID NO: 1, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 12, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 24; and a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 33, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 34, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 35.

1315 In some embodiments, there is provided an anti-PcrV antibody that binds to the same epitope as an antibody comprising a V_H comprising: an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 1, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 12, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 24; and a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO:

1320 33, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 34, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 35.

[00119] In some embodiments, the anti-PcrV antibody comprises a V_H comprising the amino acid sequence of SEQ ID NO: 57, or a variant thereof having at least about 90% (for example at least about any of 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%)

1325 sequence identity, and a V_L comprising the amino acid sequence of SEQ ID NO: 68, or a variant thereof having at least about 90% sequence identity. In some embodiments, the anti-PcrV antibody comprises a V_H comprising the amino acid sequence of SEQ ID NO: 57 and a V_L comprising the amino acid sequence of SEQ ID NO: 68. In some

embodiments, the anti-PcrV antibody comprises a V_H comprising an HC-CDR1, an

1330 HC-CDR2 and an HC-CDR3 of the V_H comprising the amino acid sequence of SEQ ID NO: 57; and a V_L comprising a LC-CDR1, a LC-CDR2, and a LC-CDR3 of the V_L comprising the amino acid sequence of SEQ ID NOs: 68.

[00120] In some embodiments, the anti-PcrV antibody comprises a V_H comprising: an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 2, an HC-CDR2

1335 comprising the amino acid sequence of SEQ ID NO: 10, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 25, or a variant thereof comprising up to about 5 amino acid substitutions in the HC-CDRs; and a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 32, an LC-CDR2 comprising the amino acid

sequence of SEQ ID NO: 34, and an LC-CDR3 comprising the amino acid sequence of
1340 SEQ ID NO: 37, or a variant thereof comprising up to about 5 amino acid substitutions in
the LC-CDRs.

[00121] In some embodiments, the anti- PcrV antibody comprises a V_H comprising: an
HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 2, an HC-CDR2
comprising the amino acid sequence of SEQ ID NO: 10, and an HC-CDR3 comprising the
1345 amino acid sequence of SEQ ID NO: 25; and a V_L comprising an LC-CDR1 comprising
the amino acid sequence of SEQ ID NO: 32, an LC-CDR2 comprising the amino acid
sequence of SEQ ID NO: 34, and an LC-CDR3 comprising the amino acid sequence of
SEQ ID NO: 37. In some embodiments, there is provided an anti-PcrV antibody that
specifically competes with an antibody comprising a V_H comprising: an HC-CDR1
1350 comprising the amino acid sequence of SEQ ID NO: 2, an HC-CDR2 comprising the
amino acid sequence of SEQ ID NO: 10, and an HC-CDR3 comprising the amino acid
sequence of SEQ ID NO: 25; and a V_L comprising an LC-CDR1 comprising the amino
acid sequence of SEQ ID NO: 32, an LC-CDR2 comprising the amino acid sequence of
SEQ ID NO: 34, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO:
1355 37. In some embodiments, there is provided an anti-PcrV antibody that binds to the same
epitope as an antibody comprising a V_H comprising: an HC-CDR1 comprising the amino
acid sequence of SEQ ID NO: 2, an HC-CDR2 comprising the amino acid sequence of
SEQ ID NO: 10, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO:
25; and a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO:
1360 32, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 34, and an
LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 37.

[00122] In some embodiments, the anti-PcrV antibody comprises a V_H comprising the
amino acid sequence of SEQ ID NO: 58, or a variant thereof having at least about 90% (for
example at least about any of 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%)
1365 sequence identity, and a V_L comprising the amino acid sequence of SEQ ID NO: 69, or a
variant thereof having at least about 90% sequence identity. In some embodiments, the
anti-PcrV antibody comprises a V_H comprising the amino acid sequence of SEQ ID NO:
58 and a V_L comprising the amino acid sequence of SEQ ID NO: 69. In some
embodiments, the anti-PcrV antibody comprises a V_H comprising an HC-CDR1, an
1370 HC-CDR2 and an HC-CDR3 of the V_H comprising the amino acid sequence of SEQ ID

NO: 58; and a V_L comprising a LC-CDR1, a LC-CDR2, and a LC-CDR3 of the V_L comprising the amino acid sequence of SEQ ID NOs: 69.

[00123] In some embodiments, the anti- PcrV antibody comprises a V_H comprising: an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 1, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 9, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 26, or a variant thereof comprising up to about 5 amino acid substitutions in the HC-CDRs; and a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 32, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 34, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 35, or a variant thereof comprising up to about 5 amino acid substitutions in the LC-CDRs.

[00124] In some embodiments, the anti- PcrV antibody comprises a V_H comprising: an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 1, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 9, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 26; and a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 32, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 34, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 35. In some embodiments, there is provided an anti-PcrV antibody that specifically competes with an antibody comprising a V_H comprising: an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 1, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 9, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 26; and a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 32, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 34, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 35. In some embodiments, there is provided an anti-PcrV antibody that binds to the same epitope as an antibody comprising a V_H comprising: an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 1, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 9, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 26; and a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 32, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 34, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 35.

[00125] In some embodiments, the anti-PcrV antibody comprises a V_H comprising the amino acid sequence of SEQ ID NO: 59, or a variant thereof having at least about 90% (for

example at least about any of 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%)
1405 sequence identity, and a V_L comprising the amino acid sequence of SEQ ID NO: 65, or a
variant thereof having at least about 90% sequence identity. In some embodiments, the
anti-PcrV antibody comprises a V_H comprising the amino acid sequence of SEQ ID NO:
59 and a V_L comprising the amino acid sequence of SEQ ID NO: 65. In some
embodiments, the anti-PcrV antibody comprises a V_H comprising an HC-CDR1, an
1410 HC-CDR2 and an HC-CDR3 of the V_H comprising the amino acid sequence of SEQ ID
NO: 59; and a V_L comprising a LC-CDR1, a LC-CDR2, and a LC-CDR3 of the V_L
comprising the amino acid sequence of SEQ ID NOs: 65.

[00126] In some embodiments, the anti- PcrV antibody comprises a V_H comprising: an
HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 3, an HC-CDR2
1415 comprising the amino acid sequence of SEQ ID NO: 10, and an HC-CDR3 comprising the
amino acid sequence of SEQ ID NO: 27, or a variant thereof comprising up to about 5
amino acid substitutions in the HC-CDRs; and a V_L comprising an LC-CDR1 comprising
the amino acid sequence of SEQ ID NO: 32, an LC-CDR2 comprising the amino acid
sequence of SEQ ID NO: 34, and an LC-CDR3 comprising the amino acid sequence of
1420 SEQ ID NO: 35, or a variant thereof comprising up to about 5 amino acid substitutions in
the LC-CDRs.

[00127] In some embodiments, the anti- PcrV antibody comprises a V_H comprising: an
HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 3, an HC-CDR2
comprising the amino acid sequence of SEQ ID NO: 10, and an HC-CDR3 comprising the
1425 amino acid sequence of SEQ ID NO: 27; and a V_L comprising an LC-CDR1 comprising
the amino acid sequence of SEQ ID NO: 32, an LC-CDR2 comprising the amino acid
sequence of SEQ ID NO: 34, and an LC-CDR3 comprising the amino acid sequence of
SEQ ID NO: 35. In some embodiments, there is provided an anti-PcrV antibody that
specifically competes with an antibody comprising a V_H comprising: an HC-CDR1
1430 comprising the amino acid sequence of SEQ ID NO: 3, an HC-CDR2 comprising the
amino acid sequence of SEQ ID NO: 10, and an HC-CDR3 comprising the amino acid
sequence of SEQ ID NO: 27; and a V_L comprising an LC-CDR1 comprising the amino
acid sequence of SEQ ID NO: 32, an LC-CDR2 comprising the amino acid sequence of
SEQ ID NO: 34, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 35.

1435 In some embodiments, there is provided an anti-PcrV antibody that binds to the same
epitope as an antibody comprising a V_H comprising: an HC-CDR1 comprising the amino

acid sequence of SEQ ID NO: 3, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 10, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 27; and a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 32, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 34, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 35.

[00128] In some embodiments, the anti-PcrV antibody comprises a V_H comprising the amino acid sequence of SEQ ID NO: 60, or a variant thereof having at least about 90% (for example at least about any of 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%) sequence identity, and a V_L comprising the amino acid sequence of SEQ ID NO: 65, or a variant thereof having at least about 90% sequence identity. In some embodiments, the anti-PcrV antibody comprises a V_H comprising the amino acid sequence of SEQ ID NO: 60 and a V_L comprising the amino acid sequence of SEQ ID NO: 65. In some embodiments, the anti-PcrV antibody comprises a V_H comprising an HC-CDR1, an HC-CDR2 and an HC-CDR3 of the V_H comprising the amino acid sequence of SEQ ID NO: 60; and a V_L comprising a LC-CDR1, a LC-CDR2, and a LC-CDR3 of the V_L comprising the amino acid sequence of SEQ ID NOs: 65.

[00129] In some embodiments, the anti-PcrV antibody comprises a V_H comprising: an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 1, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 10, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 28, or a variant thereof comprising up to about 5 amino acid substitutions in the HC-CDRs; and a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 32, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 34, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 35, or a variant thereof comprising up to about 5 amino acid substitutions in the LC-CDRs.

[00130] In some embodiments, the anti-PcrV antibody comprises a V_H comprising: an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 1, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 10, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 28; and a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 32, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 34, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 35. In some embodiments, there is provided an anti-PcrV antibody that specifically competes with an antibody comprising a V_H comprising: an HC-CDR1

- 1470 comprising the amino acid sequence of SEQ ID NO: 1, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 10, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 28; and a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 32, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 34, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 35.
- 1475 In some embodiments, there is provided an anti-PcrV antibody that binds to the same epitope as an antibody comprising a V_H comprising: an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 1, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 10, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 28; and a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 32, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 34, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 35.
- 1480 **[00131]** In some embodiments, the anti-PcrV antibody comprises a V_H comprising the amino acid sequence of SEQ ID NO: 61, or a variant thereof having at least about 90% (for example at least about any of 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%) sequence identity, and a V_L comprising the amino acid sequence of SEQ ID NO: 65, or a variant thereof having at least about 90% sequence identity. In some embodiments, the anti-PcrV antibody comprises a V_H comprising the amino acid sequence of SEQ ID NO: 61 and a V_L comprising the amino acid sequence of SEQ ID NO: 65. In some embodiments, the anti-PcrV antibody comprises a V_H comprising an HC-CDR1, an HC-CDR2 and an HC-CDR3 of the V_H comprising the amino acid sequence of SEQ ID NO: 61; and a V_L comprising a LC-CDR1, a LC-CDR2, and a LC-CDR3 of the V_L comprising the amino acid sequence of SEQ ID NOs: 65.
- 1490 **[00132]** In some embodiments, the anti-PcrV antibody comprises a V_H comprising: an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 7, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 13, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 29, or a variant thereof comprising up to about 5 amino acid substitutions in the HC-CDRs; and a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 32, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 34, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 37, or a variant thereof comprising up to about 5 amino acid substitutions in the LC-CDRs.
- 1500

[00133] In some embodiments, the anti-PcrV antibody comprises a V_H comprising: an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 7, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 13, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 29; and a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 32, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 34, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 37. In some embodiments, there is provided an anti-PcrV antibody that specifically competes with an antibody comprising a V_H comprising: an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 7, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 13, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 29; and a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 32, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 34, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 37. In some embodiments, there is provided an anti-PcrV antibody that binds to the same epitope as an antibody comprising a V_H comprising: an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 7, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 13, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 29; and a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 32, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 34, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 37.

[00134] In some embodiments, the anti-PcrV antibody comprises a V_H comprising the amino acid sequence of SEQ ID NO: 62, or a variant thereof having at least about 90% (for example at least about any of 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%) sequence identity, and a V_L comprising the amino acid sequence of SEQ ID NO: 67, or a variant thereof having at least about 90% sequence identity. In some embodiments, the anti-PcrV antibody comprises a V_H comprising the amino acid sequence of SEQ ID NO: 62 and a V_L comprising the amino acid sequence of SEQ ID NO: 67. In some embodiments, the anti-PcrV antibody comprises a V_H comprising an HC-CDR1, an HC-CDR2 and an HC-CDR3 of the V_H comprising the amino acid sequence of SEQ ID NO: 62; and a V_L comprising a LC-CDR1, a LC-CDR2, and a LC-CDR3 of the V_L comprising the amino acid sequence of SEQ ID NOs: 67.

[00135] In some embodiments, the anti-PcrV antibody comprises a V_H comprising: an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 1, an HC-CDR2

1535 comprising the amino acid sequence of SEQ ID NO: 10, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 30, or a variant thereof comprising up to about 5 amino acid substitutions in the HC-CDRs; and a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 32, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 34, and an LC-CDR3 comprising the amino acid sequence of
 1540 SEQ ID NO: 35, or a variant thereof comprising up to about 5 amino acid substitutions in the LC-CDRs.

[00136] In some embodiments, the anti-PcrV antibody comprises a V_H comprising: an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 1, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 10, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 30; and a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 32, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 34, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 35. In some embodiments, there is provided an anti-PcrV antibody that specifically competes with an antibody comprising a V_H comprising: an HC-CDR1
 1545 comprising the amino acid sequence of SEQ ID NO: 1, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 10, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 30; and a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 32, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 34, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO:
 1550 35. In some embodiments, there is provided an anti-PcrV antibody that binds to the same epitope as an antibody comprising a V_H comprising: an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 1, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 10, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 30; and a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO:
 1560 32, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 34, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 35.

[00137] In some embodiments, the anti-PcrV antibody comprises a V_H comprising the amino acid sequence of SEQ ID NO: 63, or a variant thereof having at least about 90% (for example at least about any of 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%)
 1565 sequence identity, and a V_L comprising the amino acid sequence of SEQ ID NO: 65, or a variant thereof having at least about 90% sequence identity. In some embodiments, the anti-PcrV antibody comprises a V_H comprising the amino acid sequence of SEQ ID NO:

63 and a V_L comprising the amino acid sequence of SEQ ID NO: 65. In some
embodiments, the anti-PcrV antibody comprises a V_H comprising an HC-CDR1, an
1570 HC-CDR2 and an HC-CDR3 of the V_H comprising the amino acid sequence of SEQ ID
NO: 63; and a V_L comprising a LC-CDR1, a LC-CDR2, and a LC-CDR3 of the V_L
comprising the amino acid sequence of SEQ ID NOs: 65.

[00138] In some embodiments, the anti-PcrV antibody comprises a V_H comprising: an
HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 8, an HC-CDR2
1575 comprising the amino acid sequence of SEQ ID NO: 14, and an HC-CDR3 comprising the
amino acid sequence of SEQ ID NO: 31, or a variant thereof comprising up to about 5
amino acid substitutions in the HC-CDRs; and a V_L comprising an LC-CDR1 comprising
the amino acid sequence of SEQ ID NO: 32, an LC-CDR2 comprising the amino acid
sequence of SEQ ID NO: 34, and an LC-CDR3 comprising the amino acid sequence of
1580 SEQ ID NO: 37, or a variant thereof comprising up to about 5 amino acid substitutions in
the LC-CDRs.

[00139] In some embodiments, the anti-PcrV antibody comprises a V_H comprising: an
HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 8, an HC-CDR2
comprising the amino acid sequence of SEQ ID NO: 14, and an HC-CDR3 comprising the
1585 amino acid sequence of SEQ ID NO: 31; and a V_L comprising an LC-CDR1 comprising
the amino acid sequence of SEQ ID NO: 32, an LC-CDR2 comprising the amino acid
sequence of SEQ ID NO: 34, and an LC-CDR3 comprising the amino acid sequence of
SEQ ID NO: 37. In some embodiments, there is provided an anti-PcrV antibody that
specifically competes with an antibody comprising a V_H comprising: an HC-CDR1
1590 comprising the amino acid sequence of SEQ ID NO: 8, an HC-CDR2 comprising the
amino acid sequence of SEQ ID NO: 14, and an HC-CDR3 comprising the amino acid
sequence of SEQ ID NO: 31; and a V_L comprising an LC-CDR1 comprising the amino
acid sequence of SEQ ID NO: 32, an LC-CDR2 comprising the amino acid sequence of
SEQ ID NO: 34, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 37.
1595 In some embodiments, there is provided an anti-PcrV antibody that binds to the same
epitope as an antibody comprising a V_H comprising: an HC-CDR1 comprising the amino
acid sequence of SEQ ID NO: 8, an HC-CDR2 comprising the amino acid sequence of
SEQ ID NO: 14, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO:
31; and a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO:

1600 32, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 34, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 37.

[00140] In some embodiments, the anti-PcrV antibody comprises a V_H comprising the amino acid sequence of SEQ ID NO: 64, or a variant thereof having at least about 90% (for example at least about any of 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%)

1605 sequence identity, and a V_L comprising the amino acid sequence of SEQ ID NO: 67, or a variant thereof having at least about 90% sequence identity. In some embodiments, the anti-PcrV antibody comprises a V_H comprising the amino acid sequence of SEQ ID NO: 64 and a V_L comprising the amino acid sequence of SEQ ID NO: 67. In some

embodiments, the anti-PcrV antibody comprises a V_H comprising an HC-CDR1, an

1610 HC-CDR2 and an HC-CDR3 of the V_H comprising the amino acid sequence of SEQ ID NO: 64; and a V_L comprising a LC-CDR1, a LC-CDR2, and a LC-CDR3 of the V_L comprising the amino acid sequence of SEQ ID NOs: 67.

[00141] In some embodiments, the anti-PcrV antibody comprises a V_H comprising: an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 7, an HC-CDR2

1615 comprising the amino acid sequence of SEQ ID NO: 13, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 29, or a variant thereof comprising up to about 5 amino acid substitutions in the HC-CDRs; and a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 32, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 34, and an LC-CDR3 comprising the amino acid sequence of

1620 SEQ ID NO: 38, or a variant thereof comprising up to about 5 amino acid substitutions in the LC-CDRs.

[00142] In some embodiments, the anti-PcrV antibody comprises a V_H comprising: an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 7, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 13, and an HC-CDR3 comprising the

1625 amino acid sequence of SEQ ID NO: 29; and a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 32, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 34, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 38. In some embodiments, there is provided an anti-PcrV antibody that specifically competes with an antibody comprising a V_H comprising: an HC-CDR1

1630 comprising the amino acid sequence of SEQ ID NO: 7, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 13, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 29; and a V_L comprising an LC-CDR1 comprising the amino

acid sequence of SEQ ID NO: 32, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 34, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 38.

1635 In some embodiments, there is provided an anti-PcrV antibody that binds to the same epitope as an antibody comprising a V_H comprising: an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 7, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 13, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 29; and a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 32, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 34, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 38.

[00143] In some embodiments, the anti-PcrV antibody comprises a V_H comprising the amino acid sequence of SEQ ID NO: 62, or a variant thereof having at least about 90% (for example at least about any of 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%) sequence identity, and a V_L comprising the amino acid sequence of SEQ ID NO: 70, or a variant thereof having at least about 90% sequence identity. In some embodiments, the anti-PcrV antibody comprises a V_H comprising the amino acid sequence of SEQ ID NO: 62 and a V_L comprising the amino acid sequence of SEQ ID NO: 70. In some embodiments, the anti-PcrV antibody comprises a V_H comprising an HC-CDR1, an HC-CDR2 and an HC-CDR3 of the V_H comprising the amino acid sequence of SEQ ID NO: 62; and a V_L comprising a LC-CDR1, a LC-CDR2, and a LC-CDR3 of the V_L comprising the amino acid sequence of SEQ ID NOs: 70.

[00144] In some embodiments, competition assays may be used to identify a monoclonal antibody that competes with an anti-PcrV antibody described herein for binding to PcrV. Competition assays can be used to determine whether two antibodies bind the same epitope by recognizing identical or sterically overlapping epitopes or one antibody competitively inhibits binding of another antibody to the antigen. In certain embodiments, such a competing antibody binds to the same epitope that is bound by an antibody described herein. Exemplary competition assays include, but are not limited to, routine assays such as those provided in Harlow and Lane (1988) Antibodies: A Laboratory Manual ch.14 (Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.). Detailed exemplary methods for mapping an epitope to which an antibody binds are provided in Morris (1996) "Epitope Mapping Protocols," in Methods in Molecular Biology vol. 66 (Humana Press, Totowa, N.J.). In some embodiments, two antibodies are said to bind to the same epitope if each blocks binding of the other by 50% or more. In some embodiments, the antibody

that competes with an anti- PcrV antibody described herein is a chimeric, humanized or human antibody.

[00145] Exemplary anti- PcrV antibody sequences are shown in Tables 2 and 3. Those skilled in the art will recognize that many algorithms are known for prediction of CDR positions and for delimitation of antibody heavy chain and light chain variable regions. Anti- PcrV antibodies comprising CDRs, V_H and/or V_L sequences from antibodies described herein, but based on prediction algorithms other than those exemplified in the tables below, are within the scope of this invention.

Table 2. Exemplary anti-PcrV antibody CDR sequences.

Name	CDR H1	CDR H2	CDR H3
13-42	DYPMS (SEQ ID NO: 1)	GISEGGSTNDADSVKG (SEQ ID NO: 9)	GRFCTNSSCFRAVYGMDV (SEQ ID NO: 15)
2B1	SYPMs (SEQ ID NO: 2)	GISEGGSTNYADSVKG (SEQ ID NO: 10)	GRFSGESHFFRAVYGMDV (SEQ ID NO: 16)
3H10	DYPMS (SEQ ID NO: 1)	GISEGGSTNDADSVKG (SEQ ID NO: 9)	GRFSDYSQHFFRAVYGMDV (SEQ ID NO: 17)
8C1	DNHMS (SEQ ID NO: 3)	GISEGGSTNYADSVKG (SEQ ID NO: 10)	GRFSTNSAHFFRAVYGMDV (SEQ ID NO: 18)
7C1	DNHMS (SEQ ID NO: 3)	GISEGGSTNYADSVKG (SEQ ID NO: 10)	GRFSTSSSHFYRAVYGMDV (SEQ ID NO: 19)
2D3	DNHMS (SEQ ID NO: 3)	GISEGGSTYYADSVKG (SEQ ID NO: 11)	GRFSYDTRHFFRAVYGMDV (SEQ ID NO: 20)
3A9	DYSMS (SEQ ID NO: 4)	GISEGGSTNYADSVKG (SEQ ID NO: 10)	GRFSDSTKHFFRAVYGMDV (SEQ ID NO: 21)
3A11	SYHMS (SEQ ID NO: 5)	GISEGGSTNYADSVKG (SEQ ID NO: 10)	GRFSQSSSHFFRAVYGMDV (SEQ ID NO: 22)
3B12	SNHMS (SEQ ID NO: 6)	GISEGGSTNYADSVKG (SEQ ID NO: 10)	GRFSDEYGHFFRAVYGMDV (SEQ ID NO: 23)
3G3	DYPMS (SEQ ID NO: 1)	GISEGGSTYDADSVKG (SEQ ID NO: 12)	GRFSASAGHFFRAVYGMDV (SEQ ID NO: 24)
4A10	SYPMs (SEQ ID NO: 2)	GISEGGSTNYADSVKG (SEQ ID NO: 10)	GRFSDASEHFFRAVYGMDV (SEQ ID NO: 25)
5B3	DYPMS (SEQ ID NO: 1)	GISEGGSTNDADSVKG (SEQ ID NO: 9)	GRFSQDSYHFFRAVYGMDV (SEQ ID NO: 26)
6A10	DNHMS (SEQ ID NO: 3)	GISEGGSTNYADSVKG (SEQ ID NO: 10)	GRFSTSSSHFFRAVYGMDV (SEQ ID NO: 27)
6D10	DYPMS (SEQ ID NO: 1)	GISEGGSTNYADSVKG (SEQ ID NO: 10)	GRFSTDSSSHFYRAVYGMDV (SEQ ID NO: 28)
7B1	DNYMS	VISEGGSTNYADSVKG	GRFSTLSSHFFRAVYGMDV

	(SEQ ID NO: 7)	(SEQ ID NO: 13)	(SEQ ID NO: 29)
7B2	DYPMS (SEQ ID NO: 1)	GISESGGSTNYADSVKG (SEQ ID NO: 10)	GRFSTDSSHFFRAVYGMDV (SEQ ID NO: 30)
9B12	DNPMS (SEQ ID NO: 8)	VISESGGSTNDADSVKG (SEQ ID NO: 14)	GRFSTNSDHFYRAVYGMDV (SEQ ID NO: 31)
7B1-1F5	DNYMS (SEQ ID NO: 7)	VISESGGSTNYADSVKG (SEQ ID NO: 13)	GRFSTLSSHFFRAVYGMDV (SEQ ID NO: 29)
Name	CDR L1	CDR L2	CDR L3
13-42	RASQGISSYLA (SEQ ID NO: 32)	AATLQS (SEQ ID NO: 34)	QQLSSYPLS (SEQ ID NO: 35)
2B1	RASQGISSYLA (SEQ ID NO: 32)	AATLQS (SEQ ID NO: 34)	QQLSSYPLS (SEQ ID NO: 35)
3H10	RASQGISSYLA (SEQ ID NO: 32)	AATLQS (SEQ ID NO: 34)	QQLNSYPLT (SEQ ID NO: 36)
8C1	RASQGISSYLA (SEQ ID NO: 32)	AATLQS (SEQ ID NO: 34)	QQLSSYPLT (SEQ ID NO: 37)
7C1	RASQGISSYLA (SEQ ID NO: 32)	AATLQS (SEQ ID NO: 34)	QQLSSYPLS (SEQ ID NO: 35)
2D3	RASQGISSYLA (SEQ ID NO: 32)	AATLQS (SEQ ID NO: 34)	QQLSSYPLS (SEQ ID NO: 35)
3A9	RASQGISSYLA (SEQ ID NO: 32)	AATLQS (SEQ ID NO: 34)	QQLSSYPLS (SEQ ID NO: 35)
3A11	RASQGISSYLA (SEQ ID NO: 32)	AATLQS (SEQ ID NO: 34)	QQLSSYPLT (SEQ ID NO: 37)
3B12	RASQGISSYLA (SEQ ID NO: 32)	AATLQS (SEQ ID NO: 34)	QQLSSYPLT (SEQ ID NO: 37)
3G3	RASQGIRSYLA (SEQ ID NO: 33)	AATLQS (SEQ ID NO: 34)	QQLSSYPLS (SEQ ID NO: 35)
4A10	RASQGISSYLA (SEQ ID NO: 32)	AATLQS (SEQ ID NO: 34)	QQLSSYPLT (SEQ ID NO: 37)
5B3	RASQGISSYLA (SEQ ID NO: 32)	AATLQS (SEQ ID NO: 34)	QQLSSYPLS (SEQ ID NO: 35)
6A10	RASQGISSYLA (SEQ ID NO: 32)	AATLQS (SEQ ID NO: 34)	QQLSSYPLS (SEQ ID NO: 35)
6D10	RASQGISSYLA (SEQ ID NO: 32)	AATLQS (SEQ ID NO: 34)	QQLSSYPLS (SEQ ID NO: 35)
7B1	RASQGISSYLA (SEQ ID NO: 32)	AATLQS (SEQ ID NO: 34)	QQLSSYPLT (SEQ ID NO: 37)
7B2	RASQGISSYLA (SEQ ID NO: 32)	AATLQS (SEQ ID NO: 34)	QQLSSYPLS (SEQ ID NO: 35)
9B12	RASQGISSYLA	AATLQS	QQLSSYPLT

	(SEQ ID NO: 32)	(SEQ ID NO: 34)	(SEQ ID NO: 37)
7B1-1F5	RASQGISSYLA (SEQ ID NO: 32)	AATLQS (SEQ ID NO: 34)	QQLKSYPLT (SEQ ID NO: 38)

Table 3. Exemplary V_H and V_L sequences.

SEQ ID NO	Description	Sequence
48	13-42 V _H	EVQLLESGGGLVQPGGSLRLSCTASGFTFSDYPMSWVRQAPGK GLDWVSGISESGGSTNDADSVKGRFSTSRDNSKSTLYLDMNSLR AEDTAIYYCAKGRFCTNSSCFFRAVYGMDVWGQGTAVTVSS
49	2B1 V _H	EVQLLESGGGLVQPGGSLRLSCTASGFTFSSYPMSWVRQAPGK GLDWVSGISESGGSTNYADSVKGRFSTSRDNSKSTLYLDMNSLR AEDTAIYYCAKGRFSGESHFFRAVYGMDVWGQGTAVTVSS
50	3H10 V _H	EVQLLESGGGLVQPGGSLRLSCTASGFTFSDYPMSWVRQAPGK GLDWVSGISESGGSTNDADSVKGRFSTSRDNSKSTLYLDMNSLR AEDTAIYYCAKGRFSDYSQHFFRAVYGMDVWGQGTAVTVSS
51	8C1 V _H	EVQLLESGGGLVQPGGSLRLSCTASGFTFSDNHMSWVRQAPGK GLDWVSGISESGGSTNYADSVKGRFSTSRDNSKSTLYLDMNSLR AEDTAIYYCAKGRFSTNSAHFFRAVYGMDVWGQGTAVTVSS
52	7C1 V _H	EVQLLESGGGLVQPGGSLRLSCTASGFTFSDNHMSWVRQAPGK GLDWVSGISESGGSTNYADSVKGRFSTSRDNSKSTLYLDMNSLR AEDTAIYYCAKGRFSTSSSHFYRAVYGMDVWGQGTAVTVSS
53	2D3 V _H	EVQLLESGGGLVQPGGSLRLSCTASGFTFSDNHMSWVRQAPGK GLDWVSGISESGGSTYYADSVKGRFSTSRDNSKSTLYLDMNSLR AEDTAIYYCAKGRFSYDTRHFFRAVYGMDVWGQGTAVTVSS
54	3A9 V _H	EVQLLESGGGLVQPGGSLRLSCTASGFTFSDYMSWVRQAPGK GLDWVSGISESGGSTNYADSVKGRFSTSRDNSKSTLYLDMNSLR AEDTAIYYCAKGRFSDSTKHFFRAVYGMDVWGQGTAVTVSS
55	3A11 V _H	EVQLLESGGGLVQPGGSLRLSCTASGFTFSSYHMSWVRQAPGK GLDWVSGISESGGSTNYADSVKGRFSTSRDNSKSTLYLDMNSLR AEDTAIYYCAKGRFSQSSSHFFRAVYGMDVWGQGTAVTVSS
56	3B12 V _H	EVQLLESGGGLVQPGGSLRLSCTASGFTFSSNHMSWVRQAPGK GLDWVSGISESGGSTNYADSVKGRFSTSRDNSKSTLYLDMNSLR AEDTAIYYCAKGRFSDEYGHFFRAVYGMDVWGQGTAVTVSS
57	3G3 V _H	EVQLLESGGGLVQPGGSLRLSCTASGFTFSDYPMSWVRQAPGK GLDWVSGISESGGSTYDADSVKGRFSTSRDNSKSTLYLDMNSLR AEDTAIYYCAKGRFSASAGHFFRAVYGMDVWGQGTAVTVSS
58	4A10 V _H	EVQLLESGGGLVQPGGSLRLSCTASGFTFSSYPMSWVRQAPGK GLDWVSGISESGGSTNYADSVKGRFSTSRDNSKSTLYLDMNSLR

SEQ ID NO	Description	Sequence
		AEDTAIYYCAKGRFSDASEHFFRAVYGMDVWGQGTAVTVSS
59	5B3 V _H	EVQLLESGGGLVQPGGSLRLSCTASGFTFSDYPMSWVRQAPGK GLDWVSGISESGGSTNDADSVKGRFSTSRDNSKSTLYLDMNSLR AEDTAIYYCAKGRFSQDSYHFFRAVYGMDVWGQGTAVTVSS
60	6A10 V _H	EVQLLESGGGLVQPGGSLRLSCTASGFTFSDNHMSWVRQAPGK GLDWVSGISESGGSTNYADSVKGRFSTSRDNSKSTLYLDMNSLR AEDTAIYYCAKGRFSTSSSHFFRAVYGMDVWGQGTAVTVSS
61	6D10 V _H	EVQLLESGGGLVQPGGSLRLSCTASGFTFSDYPMSWVRQAPGK GLDWVSGISESGGSTNYADSVKGRFSTSRDNSKSTLYLDMNSLR AEDTAIYYCAKGRFSTDSSHFYRAVYGMDVWGQGTAVTVSS
62	7B1, 7B1-1F5 V _H	EVQLLESGGGLVQPGGSLRLSCTASGFTFSDNYMSWVRQAPGK GLDWVSVISESGGSTNYADSVKGRFSTSRDNSKSTLYLDMNSLR AEDTAIYYCAKGRFSTLSSHFFRAVYGMDVWGQGTAVTVSS
63	7B2 V _H	EVQLLESGGGLVQPGGSLRLSCTASGFTFSDYPMSWVRQAPGK GLDWVSGISESGGSTNYADSVKGRFSTSRDNSKSTLYLDMNSLR AEDTAIYYCAKGRFSTDSSHFRAVYGMDVWGQGTAVTVSS
64	9B12 V _H	EVQLLESGGGLVQPGGSLRLSCTASGFTFSDNPMSWVRQAPGK GLDWVSVISESGGSTNDADSVKGRFSTSRDNSKSTLYLDMNSLR AEDTAIYYCAKGRFSTNSDHFYRAVYGMDVWGQGTAVTVSS
65	13-42, 2B1, 7C1, 2D3, 3A9, 5B3, 6A10, 6D10, 7B2 V _L	DIQLTQSPSSLSASVGDRVTITCRASQGISSYLAWYQQKPGKAPK LLIYAASLTQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQL SSYPLSFGGGTKVEIK
66	3H10 V _L	DIQLTQSPSSLSASVGDRVTITCRASQGISSYLAWYQQKPGKAPK LLIYAASLTQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQL NSYPLTFGGGTKVEIK
67	8C1, 3A11, 3B12, 7B1, 9B12 V _L	DIQLTQSPSSLSASVGDRVTITCRASQGISSYLAWYQQKPGKAPK LLIYAASLTQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQL SSYPLTFGGGTKVEIK
68	3G3 V _L	DIQLTQSPSSLSASVGDRVTITCRASQGIRSYLAWYQQKPGKAP KLLIYAASLTQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQ QLSSYPLSFGGGTKVEIK
69	4A10 V _L	DIQLTQSPSSLSASVGDRVTITCRASQGISSYLAWYQQKPGKTPK LLIYAASLTQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQL SSYPLTFGGGTKVEIK
70	7B1-1F5 V _L	DIQLTQSPSSFSASTGDRVTITCRASQGISSYLAWYQQKPGKAPK VLIYAASLTQSGVPSRFSGSGSGTEFTLTISSLQPEDFATYYCQQL KSYPLTFGGGTKLEIK

Table 4: Exemplary Sequences		
SEQ ID NO:	Description	Sequence
71	Full-length PcrV	MEVRNLNAARELFLDELLAASAAPASAEQEELLALLRSERIVLAHAGQP LSEAQVLKALAWLLAANPSAPPGQGLEVLREVLQARRQPGAQWDLRE FLVSAYFSLHGRLDEDVIGVYKDV LQTQDGKRKALLDELKALTAELKVYSVIQSQINAALSAKQGIRIDAGGI DLVDPTLYGYAVGDPRWKDSPEYALLSNLDTFSGKLSIKDFLSGSPKQS GELKGLSDEYPFEDNNPVGNFATTVSDRSRPLNDKVNEKTTLLNDTSS RYNSAVEALNRFIQKYDSVLRDILSAI
72	Light chain constant region	RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQS GNSQESVTEQDSKDYSLSTLTLSKADYEKHKVYACEVTHQGLSSPV TKSFNRGEC
73	IgG1 heavy chain constant region	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSG VHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKV EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVD VSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKN QVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPVLDSGDSFFLYSKL TVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
74	IgG4 heavy chain constant region	ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSG VHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYTCNVDPKPSNTKVDKRV ESKYGPPCPSCAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVDSQ EDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLN GKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVS LTCLVKGFYPSDIAVEWESNGQPENNYKTTPVLDSGDSFFLYSRLTVD KSRWQEGNVFSCSVMHEALHNHYTQKSLSLSPGK

1680 Binding affinity

1685 **[00148]** Binding affinity can be indicated by K_d , K_{off} , K_{on} , or K_a . The term “ K_{off} ”, as used herein, is intended to refer to the off-rate constant for dissociation of an antibody from the antibody /antigen complex, as determined from a kinetic selection set up. The term “ K_{on} ”, as used herein, is intended to refer to the on-rate constant for association of an antibody to the antigen to form the antibody/antigen complex. The term equilibrium dissociation constant “ K_d ”, as used herein, refers to the dissociation constant of a particular antibody-antigen interaction, and describes the concentration of antigen

required to occupy one half of all of the antibody-binding domains present in a solution of antibody molecules at equilibrium, and is equal to $K_{\text{off}}/K_{\text{on}}$. The measurement of K_d presupposes that all binding agents are in solution. In the case where the antibody is tethered to a cell wall, *e.g.*, in a yeast expression system, the corresponding equilibrium rate constant is expressed as EC50, which gives a good approximation of K_d . The affinity constant, K_a , is the inverse of the dissociation constant, K_d .

[00149] The dissociation constant (K_d) is used as an indicator showing affinity of antibody moieties to antigens. For example, easy analysis is possible by the Scatchard method using antibodies marked with a variety of marker agents, as well as by using Biacore (made by Amersham Biosciences), analysis of biomolecular interactions by surface plasmon resonance, according to the user's manual and attached kit. The K_d value that can be derived using these methods is expressed in units of M (Mols). An antibody that specifically binds to a target may have a K_d of, for example, $\leq 10^{-7}$ M, $\leq 10^{-8}$ M, $\leq 10^{-9}$ M, $\leq 10^{-10}$ M, $\leq 10^{-11}$ M, $\leq 10^{-12}$ M, or $\leq 10^{-13}$ M.

[00150] Binding specificity of the antibody can be determined experimentally by methods known in the art. Such methods comprise, but are not limited to, Western blots, ELISA-, RIA-, ECL-, IRMA-, EIA-, BIAcore-tests and peptide scans.

[00151] In some embodiments, the anti-PcrV antibody specifically binds to a target PcrV with a K_d of about 10^{-7} M to about 10^{-13} M (such as about 10^{-7} M to about 10^{-13} M, about 10^{-8} M to about 10^{-13} M, about 10^{-9} M to about 10^{-13} M, or about 10^{-10} M to about 10^{-12} M). Thus in some embodiments, the K_d of the binding between the anti-PcrV antibody and PcrV, is about 10^{-7} M to about 10^{-13} M, about 1×10^{-7} M to about 5×10^{-13} M, about 10^{-7} M to about 10^{-12} M, about 10^{-7} M to about 10^{-11} M, about 10^{-7} M to about 10^{-10} M, about 10^{-7} M to about 10^{-9} M, about 10^{-8} M to about 10^{-13} M, about 1×10^{-8} M to about 5×10^{-13} M, about 10^{-8} M to about 10^{-12} M, about 10^{-8} M to about 10^{-11} M, about 10^{-8} M to about 10^{-10} M, about 10^{-8} M to about 10^{-9} M, about 5×10^{-9} M to about 1×10^{-13} M, about 5×10^{-9} M to about 1×10^{-12} M, about 5×10^{-9} M to about 1×10^{-11} M, about 5×10^{-9} M to about 1×10^{-10} M, about 10^{-9} M to about 10^{-13} M, about 10^{-9} M to about 10^{-12} M, about 10^{-9} M to about 10^{-11} M, about 10^{-9} M to about 10^{-10} M, about 5×10^{-10} M to about 1×10^{-13} M, about 5×10^{-10} M to about 1×10^{-12} M, about 5×10^{-10} M to about 1×10^{-11} M, about 10^{-10} M to about 10^{-13} M, about 1×10^{-10} M to about 5×10^{-13} M, about 1×10^{-10} M to about 1×10^{-12} M, about 1×10^{-10} M to about 5×10^{-12} M, about 1×10^{-10} M to about 1×10^{-11} M, about 10^{-11} M to about 10^{-13} M, about 1×10^{-11} M to about 5×10^{-13} M, about

10^{-11} M to about 10^{-12} M, or about 10^{-12} M to about 10^{-13} M. In some embodiments, the K_d of the binding between the anti-PcrV antibody and a PcrV is about 10^{-7} M to about 10^{-13} M.

[00152] In some embodiments, the K_d of the binding between the anti-PcrV antibody and a non-target is higher than the K_d of the binding between the anti-PcrV antibody and the target, and is herein referred to in some embodiments as the binding affinity of the anti-PcrV antibody to the target (*e.g.*, PcrV) is higher than that to a non-target. In some embodiments, the non-target is an antigen that is not PcrV. In some embodiments, the K_d of the binding between the anti-PcrV antibody (against PcrV) and a non-PcrV target can be at least about 10 times, such as about 10-100 times, about 100-1000 times, about 10^3 - 10^4 times, about 10^4 - 10^5 times, about 10^5 - 10^6 times, about 10^6 - 10^7 times, about 10^7 - 10^8 times, about 10^8 - 10^9 times, about 10^9 - 10^{10} times, about 10^{10} - 10^{11} times, or about 10^{11} - 10^{12} times of the K_d of the binding between the anti-PcrV antibody and a target PcrV.

Nucleic Acids

[00153] Nucleic acid molecules encoding the anti-PcrV antibodies are also contemplated. In some embodiments, there is provided a nucleic acid (or a set of nucleic acids) encoding a full-length anti-PcrV antibody, including any of the full-length anti-PcrV antibodies described herein. In some embodiments, the nucleic acid (or a set of nucleic acids) encoding the anti-PcrV antibody described herein may further comprises a nucleic acid sequence encoding a peptide tag (such as protein purification tag, *e.g.*, His-tag, HA tag).

[00154] Also contemplated here are isolated host cells comprising an anti-PcrV antibody, an isolated nucleic acid encoding the polypeptide components of the anti-PcrV antibody, or a vector comprising a nucleic acid encoding the polypeptide components of the anti-PcrV antibody described herein.

[00155] The present application also includes variants to these nucleic acid sequences. For example, the variants include nucleotide sequences that hybridize to the nucleic acid sequences encoding the anti-PcrV antibodies of the present application under at least moderately stringent hybridization conditions.

[00156] The present application also provides vectors in which a nucleic acid of the present application is inserted.

[00157] In brief summary, the expression of an anti-PcrV antibody (*e.g.*, full-length anti-PcrV antibody) by a natural or synthetic nucleic acid encoding the anti-PcrV antibody can be achieved by inserting the nucleic acid into an appropriate expression vector, such

that the nucleic acid is operably linked to 5' and 3' regulatory elements, including for example a promoter (*e.g.*, a lymphocyte-specific promoter) and a 3' untranslated region (UTR). The vectors can be suitable for replication and integration in eukaryotic host cells. Typical cloning and expression vectors contain transcription and translation terminators, initiation sequences, and promoters useful for regulation of the expression of the desired nucleic acid sequence.

[00158] The nucleic acids of the present application may also be used for nucleic acid immunization and gene therapy, using standard gene delivery protocols. Methods for gene delivery are known in the art. See, *e.g.*, U.S. Pat. Nos. 5,399,346; 5,580,859; 5,589,466, incorporated by reference herein in their entireties. In some embodiments, the application provides a gene therapy vector.

[00159] The nucleic acid can be cloned into a number of types of vectors. For example, the nucleic acid can be cloned into a vector including, but not limited to a plasmid, a phagemid, a phage derivative, an animal virus, and a cosmid. Vectors of particular interest include expression vectors, replication vectors, probe generation vectors, and sequencing vectors.

[00160] Further, the expression vector may be provided to a cell in the form of a viral vector. Viral vector technology is well known in the art and is described, for example, in Green and Sambrook (2013, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, New York), and in other virology and molecular biology manuals. Viruses which are useful as vectors include, but are not limited to, retroviruses, adenoviruses, adeno-associated viruses, herpes viruses, and lentiviruses. In general, a suitable vector contains an origin of replication functional in at least one organism, a promoter sequence, convenient restriction endonuclease sites, and one or more selectable markers (*see, e.g.*, WO 01/96584; WO 01/29058; and U.S. Pat. No. 6,326,193).

[00161] A number of viral based systems have been developed for gene transfer into mammalian cells. For example, retroviruses provide a convenient platform for gene delivery systems. A selected gene can be inserted into a vector and packaged in retroviral particles using techniques known in the art. The recombinant virus can then be isolated and delivered to cells of the subject either *in vivo* or *ex vivo*. A number of retroviral systems are known in the art. In some embodiments, adenovirus vectors are used. A number of adenovirus vectors are known in the art. In some embodiments, lentivirus vectors are used. Vectors derived from retroviruses such as the lentivirus are suitable tools to achieve

long-term gene transfer since they allow long-term, stable integration of a transgene and its propagation in daughter cells. Lentiviral vectors have the added advantage over vectors derived from onco-retroviruses such as murine leukemia viruses in that they can transduce non-proliferating cells, such as hepatocytes. They also have the added advantage of low immunogenicity.

[00162] Additional promoter elements, *e.g.*, enhancers, regulate the frequency of transcriptional initiation. Typically, these are located in the region 30-110 bp upstream of the start site, although a number of promoters have recently been shown to contain functional elements downstream of the start site as well. The spacing between promoter elements frequently is flexible, so that promoter function is preserved when elements are inverted or moved relative to one another. In the thymidine kinase (tk) promoter, the spacing between promoter elements can be increased to 50 bp apart before activity begins to decline.

[00163] One example of a suitable promoter is the immediate early cytomegalovirus (CMV) promoter sequence. This promoter sequence is a strong constitutive promoter sequence capable of driving high levels of expression of any polynucleotide sequence operatively linked thereto. Another example of a suitable promoter is Elongation Growth Factor-1 α (EF-1 α). However, other constitutive promoter sequences may also be used, including, but not limited to the simian virus 40 (SV40) early promoter, mouse mammary tumor virus (MMTV), human immunodeficiency virus (HIV) long terminal repeat (LTR) promoter, MoMuLV promoter, an avian leukemia virus promoter, an Epstein-Barr virus immediate early promoter, a Rous sarcoma virus promoter, as well as human gene promoters such as, but not limited to, the actin promoter, the myosin promoter, the hemoglobin promoter, and the creatine kinase promoter. Further, the application should not be limited to the use of constitutive promoters. Inducible promoters are also contemplated as part of the application. The use of an inducible promoter provides a molecular switch capable of turning on expression of the polynucleotide sequence which it is operatively linked when such expression is desired, or turning off the expression when expression is not desired. Examples of inducible promoters include, but are not limited to a metallothioneine promoter, a glucocorticoid promoter, a progesterone promoter, and a tetracycline promoter.

[00164] In some embodiments, the expression of the anti-PcrV antibody is inducible. In some embodiments, a nucleic acid sequence encoding the anti-PcrV antibody is operably linked to an inducible promoter, including any inducible promoter described herein.

Inducible promoters

[00165] The use of an inducible promoter provides a molecular switch capable of turning on expression of the polynucleotide sequence which it is operatively linked when such expression is desired, or turning off the expression when expression is not desired.

Exemplary inducible promoter systems for use in eukaryotic cells include, but are not limited to, hormone-regulated elements (e.g., see Mader, S. and White, J. H. (1993) Proc. Natl. Acad. Sci. USA 90:5603-5607), synthetic ligand-regulated elements (see, e.g., Spencer, D. M. et al 1993) Science 262: 1019-1024) and ionizing radiation-regulated elements (e.g., see Manome, Y. et al. (1993) Biochemistry 32: 10607-10613; Datta, R. et al. (1992) Proc. Natl. Acad. Sci. USA 89: 1014- 10153). Further exemplary inducible promoter systems for use in in vitro or in vivo mammalian systems are reviewed in Gingrich et al. (1998) Annual Rev. Neurosci 21:377-405. In some embodiments, the inducible promoter system for use to express the anti-PcrV antibody is the Tet system. In some embodiments, the inducible promoter system for use to express the anti-PcrV antibody is the lac repressor system from *E. coli*.

[00166] An exemplary inducible promoter system for use in the present application is the Tet system. Such systems are based on the Tet system described by Gossen et al. (1993). In an exemplary embodiment, a polynucleotide of interest is under the control of a promoter that comprises one or more Tet operator (TetO) sites. In the inactive state, Tet repressor (TetR) will bind to the TetO sites and repress transcription from the promoter. In the active state, e.g., in the presence of an inducing agent such as tetracycline (Tc), anhydrotetracycline, doxycycline (Dox), or an active analog thereof, the inducing agent causes release of TetR from TetO, thereby allowing transcription to take place.

Doxycycline is a member of the tetracycline family of antibiotics having the chemical name of

1-dimethylamino-2,4a,5,7,12-pentahydroxy-11-methyl-4,6-dioxo-1,4a,11,11a,12,12a-hexahydrotetracene-3-carboxamide.

[00167] In one embodiment, a TetR is codon-optimized for expression in mammalian cells, e.g., murine or human cells. Most amino acids are encoded by more than one codon due to the degeneracy of the genetic code, allowing for substantial variations in the

nucleotide sequence of a given nucleic acid without any alteration in the amino acid sequence encoded by the nucleic acid. However, many organisms display differences in codon usage, also known as “codon bias” (i.e., bias for use of a particular codon(s) for a given amino acid). Codon bias often correlates with the presence of a predominant species of tRNA for a particular codon, which in turn increases efficiency of mRNA translation. Accordingly, a coding sequence derived from a particular organism (*e.g.*, a prokaryote) may be tailored for improved expression in a different organism (*e.g.*, a eukaryote) through codon optimization.

[00168] Other specific variations of the Tet system include the following “Tet-Off” and “Tet-On” systems. In the Tet-Off system, transcription is inactive in the presence of Tc or Dox. In that system, a tetracycline-controlled transactivator protein (tTA), which is composed of TetR fused to the strong transactivating domain of VP16 from Herpes simplex virus, regulates expression of a target nucleic acid that is under transcriptional control of a tetracycline-responsive promoter element (TRE). The TRE is made up of TetO sequence concatamers fused to a promoter (commonly the minimal promoter sequence derived from the human cytomegalovirus (hCMV) immediate-early promoter). In the absence of Tc or Dox, tTA binds to the TRE and activates transcription of the target gene. In the presence of Tc or Dox, tTA cannot bind to the TRE, and expression from the target gene remains inactive.

[00169] Conversely, in the Tet-On system, transcription is active in the presence of Tc or Dox. The Tet-On system is based on a reverse tetracycline-controlled transactivator, rtTA. Like tTA, rtTA is a fusion protein comprised of the TetR repressor and the VP16 transactivation domain. However, a four amino acid change in the TetR DNA binding moiety alters rtTA's binding characteristics such that it can only recognize the tetO sequences in the TRE of the target transgene in the presence of Dox. Thus, in the Tet-On system, transcription of the TRE-regulated target gene is stimulated by rtTA only in the presence of Dox.

[00170] Another inducible promoter system is the lac repressor system from *E. coli* (See Brown et al., Cell 49:603-612 (1987)). The lac repressor system functions by regulating transcription of a polynucleotide of interest operably linked to a promoter comprising the lac operator (lacO). The lac repressor (lacR) binds to LacO, thus preventing transcription of the polynucleotide of interest. Expression of the polynucleotide of interest is induced by a suitable inducing agent, *e.g.*, isopropyl- β -D-thiogalactopyranoside (IPTG).

1885 [00171] In order to assess the expression of a polypeptide or portions thereof, the expression vector to be introduced into a cell can also contain either a selectable marker gene or a reporter gene or both to facilitate identification and selection of expressing cells from the population of cells sought to be transfected or infected through viral vectors. In other aspects, the selectable marker may be carried on a separate piece of DNA and used in a co-transfection procedure. Both selectable markers and reporter genes may be flanked
1890 with appropriate regulatory sequences to enable expression in the host cells. Useful selectable markers include, for example, antibiotic-resistance genes, such as neo and the like.

[00172] Reporter genes are used for identifying potentially transfected cells and for evaluating the functionality of regulatory sequences. In general, a reporter gene is a gene
1895 that is not present in or expressed by the recipient organism or tissue and that encodes a polypeptide whose expression is manifested by some easily detectable property, *e.g.*, enzymatic activity. Expression of the reporter gene is assayed at a suitable time after the DNA has been introduced into the recipient cells. Suitable reporter genes may include genes encoding luciferase, β -galactosidase, chloramphenicol acetyl transferase, secreted
1900 alkaline phosphatase, or the green fluorescent protein gene (*e.g.*, Ui-Tel *et al.*, 2000 *FEBS Letters* 479: 79-82). Suitable expression systems are well known and may be prepared using known techniques or obtained commercially. In general, the construct with the minimal 5' flanking region showing the highest level of expression of reporter gene is identified as the promoter. Such promoter regions may be linked to a reporter gene and
1905 used to evaluate agents for the ability to modulate promoter-driven transcription.

[00173] In some embodiments, there is provided nucleic acid encoding a full-length anti-PcrV antibody according to any of the full-length anti-PcrV antibodies described herein. In some embodiments, the nucleic acid comprises one or more nucleic acid sequences encoding the heavy and light chains of the full-length anti-PcrV antibody. In
1910 some embodiments, each of the one or more nucleic acid sequences is contained in separate vectors. In some embodiments, at least some of the nucleic acid sequences are contained in the same vector. In some embodiments, all of the nucleic acid sequences are contained in the same vector. Vectors may be selected, for example, from the group consisting of mammalian expression vectors and viral vectors (such as those derived from
1915 retroviruses, adenoviruses, adeno-associated viruses, herpes viruses, and lentiviruses).

1920 [00174] Methods of introducing and expressing genes into a cell are known in the art. In the context of an expression vector, the vector can be readily introduced into a host cell, *e.g.*, mammalian, bacterial, yeast, or insect cell by any method in the art. For example, the expression vector can be transferred into a host cell by physical, chemical, or biological means.

1925 [00175] Physical methods for introducing a polynucleotide into a host cell include calcium phosphate precipitation, lipofection, particle bombardment, microinjection, electroporation, and the like. Methods for producing cells comprising vectors and/or exogenous nucleic acids are well-known in the art. See, for example, Green and Sambrook (2013, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, New York). In some embodiments, the introduction of a polynucleotide into a host cell is carried out by calcium phosphate transfection.

1930 [00176] Biological methods for introducing a polynucleotide of interest into a host cell include the use of DNA and RNA vectors. Viral vectors, and especially retroviral vectors, have become the most widely used method of inserting genes into mammalian, *e.g.*, human cells. Other viral vectors can be derived from lentivirus, poxviruses, herpes simplex virus 1, adenoviruses and adeno-associated viruses, and the like. See, for example, U.S. Pat. Nos. 5,350,674 and 5,585,362.

1935 [00177] Chemical means for introducing a polynucleotide into a host cell include colloidal dispersion systems, such as macromolecule complexes, nanocapsules, microspheres, beads, and lipid-based systems including oil-in-water emulsions, micelles, mixed micelles, and liposomes. An exemplary colloidal system for use as a delivery vehicle *in vitro* and *in vivo* is a liposome (*e.g.*, an artificial membrane vesicle).

1940 [00178] In the case where a non-viral delivery system is utilized, an exemplary delivery vehicle is a liposome. The use of lipid formulations is contemplated for the introduction of the nucleic acids into a host cell (*in vitro*, *ex vivo* or *in vivo*). In another aspect, the nucleic acid may be associated with a lipid. The nucleic acid associated with a lipid may be encapsulated in the aqueous interior of a liposome, interspersed within the lipid bilayer of a liposome, attached to a liposome via a linking molecule that is associated with both the
1945 liposome and the oligonucleotide, entrapped in a liposome, complexed with a liposome, dispersed in a solution containing a lipid, mixed with a lipid, combined with a lipid, contained as a suspension in a lipid, contained or complexed with a micelle, or otherwise associated with a lipid. Lipid, lipid/DNA or lipid/expression vector associated

compositions are not limited to any particular structure in solution. For example, they may be present in a bilayer structure, as micelles, or with a “collapsed” structure. They may also simply be interspersed in a solution, possibly forming aggregates that are not uniform in size or shape. Lipids are fatty substances which may be naturally occurring or synthetic lipids. For example, lipids include the fatty droplets that naturally occur in the cytoplasm as well as the class of compounds which contain long-chain aliphatic hydrocarbons and their derivatives, such as fatty acids, alcohols, amines, amino alcohols, and aldehydes.

[00179] Regardless of the method used to introduce exogenous nucleic acids into a host cell or otherwise expose a cell to the inhibitor of the present application, in order to confirm the presence of the recombinant DNA sequence in the host cell, a variety of assays may be performed. Such assays include, for example, “molecular biological” assays well known to those of skill in the art, such as Southern and Northern blotting, RT-PCR and PCR; “biochemical” assays, such as detecting the presence or absence of a particular peptide, *e.g.*, by immunological means (ELISAs and Western blots) or by assays described herein to identify agents falling within the scope of the application.

Preparation of anti-PcrV antibodies

[00180] In some embodiments, the anti-PcrV antibody is a monoclonal antibody or derived from a monoclonal antibody. In some embodiments, the anti-PcrV antibody comprises V_H and V_L domains, or variants thereof, from the monoclonal antibody. In some embodiments, the anti-PcrV antibody further comprises C_H1 and C_L domains, or variants thereof, from the monoclonal antibody. Monoclonal antibodies can be prepared, *e.g.*, using known methods in the art, including hybridoma methods, yeast display, phage display methods, or using recombinant DNA methods. Additionally, exemplary yeast display and phage display methods are described herein and in the Examples below.

[00181] In a hybridoma method, a hamster, mouse, or other appropriate host animal is typically immunized with an immunizing agent to elicit lymphocytes that produce or are capable of producing antibodies that will specifically bind to the immunizing agent. Alternatively, the lymphocytes can be immunized *in vitro*. The immunizing agent can include a polypeptide or a fusion protein of the protein of interest. Generally, peripheral blood lymphocytes (“PBLs”) are used if cells of human origin are desired, or spleen cells or lymph node cells are used if non-human mammalian sources are desired. The lymphocytes are then fused with an immortalized cell line using a suitable fusing agent,

- such as polyethylene glycol, to form a hybridoma cell. Immortalized cell lines are usually transformed mammalian cells, particularly myeloma cells of rodent, bovine, and human origin. Usually, rat or mouse myeloma cell lines are employed. The hybridoma cells can be cultured in a suitable culture medium that preferably contains one or more substances that
- 1985 inhibit the growth or survival of the unfused, immortalized cells. For example, if the parental cells lack the enzyme hypoxanthine guanine phosphoribosyl transferase (HGPRT or HPRT), the culture medium for the hybridomas typically will include hypoxanthine, aminopterin, and thymidine ("HAT medium"), which prevents the growth of HGPRT-deficient cells.
- 1990 **[00182]** In some embodiments, the immortalized cell lines fuse efficiently, support stable high-level expression of antibody by the selected antibody-producing cells, and are sensitive to a medium such as HAT medium. In some embodiments, the immortalized cell lines are murine myeloma lines, which can be obtained, for instance, from the Salk
- 1995 Institute Cell Distribution Center, San Diego, California and the American Type Culture Collection, Manassas, Virginia. Human myeloma and mouse-human heteromyeloma cell lines also have been described for the production of human monoclonal antibodies.
- [00183]** The culture medium in which the hybridoma cells are cultured can then be assayed for the presence of monoclonal antibodies directed against the polypeptide. The binding specificity of monoclonal antibodies produced by the hybridoma cells can be
- 2000 determined by immunoprecipitation or by an *in vitro* binding assay, such as radioimmunoassay (RIA) or enzyme-linked immunoabsorbent assay (ELISA). Such techniques and assays are known in the art. The binding affinity of the monoclonal antibody can, for example, be determined by the Scatchard analysis of Munson and Pollard, *Anal. Biochem.*, 107:220 (1980).
- 2005 **[00184]** After the desired hybridoma cells are identified, the clones can be sub cloned by limiting dilution procedures and grown by standard methods. Goding, *supra*. Suitable culture media for this purpose include, for example, Dulbecco's Modified Eagle's Medium and RPMI-1640 medium. Alternatively, the hybridoma cells can be grown *in vivo* as ascites in a mammal.
- 2010 **[00185]** The monoclonal antibodies secreted by the sub clones can be isolated or purified from the culture medium or ascites fluid by conventional immunoglobulin purification procedures such as, for example, protein A-Sepharose, hydroxylapatite chromatography, gel electrophoresis, dialysis, or affinity chromatography.

[00186] In some embodiments, according to any of the anti-PcrV antibodies described herein, the anti-PcrV antibody comprises sequences from a clone selected from an antibody library (such as a phage library presenting scFv or Fab fragments). The clone may be identified by screening combinatorial libraries for antibody fragments with the desired activity or activities. For example, a variety of methods are known in the art for generating phage display libraries and screening such libraries for antibodies possessing the desired binding characteristics. Such methods are reviewed, *e.g.*, in Hoogenboom *et al.*, *Methods in Molecular Biology* 178:1-37 (O'Brien *et al.*, ed., Human Press, Totowa, N.J., 2001) and further described, *e.g.*, in McCafferty *et al.*, *Nature* 348:552-554; Clackson *et al.*, *Nature* 352: 624-628 (1991); Marks *et al.*, *J. Mol. Biol.* 222: 581-597 (1992); Marks and Bradbury, *Methods in Molecular Biology* 248:161-175 (Lo, ed., Human Press, Totowa, N.J., 2003); Sidhu *et al.*, *J. Mol. Biol.* 338(2): 299-310 (2004); Lee *et al.*, *J. Mol. Biol.* 340(5): 1073-1093 (2004); Fellouse, *Proc. Natl. Acad. Sci. USA* 101(34): 12467-12472 (2004); and Lee *et al.*, *J. Immunol. Methods* 284(1-2): 119-132(2004).

[00187] In certain phage display methods, repertoires of V_H and V_L genes are separately cloned by polymerase chain reaction (PCR) and recombined randomly in phage libraries, which can then be screened for antigen-binding phage as described in Winter *et al.*, *Ann. Rev. Immunol.*, 12: 433-455 (1994). Phages typically display antibody fragments, either as scFv fragments or as Fab fragments. Libraries from immunized sources provide high-affinity antibodies to the immunogen without the requirement of constructing hybridomas. Alternatively, the naive repertoire can be cloned (*e.g.*, from human) to provide a single source of antibodies to a wide range of non-self and also self-antigens without any immunization as described by Griffiths *et al.*, *EMBO J*, 12: 725-734 (1993). Finally, naive libraries can also be made synthetically by cloning unrearranged V-gene segments from stem cells, and using PCR primers containing random sequence to encode the highly variable CDR3 regions and to accomplish rearrangement *in vitro*, as described by Hoogenboom and Winter, *J. Mol. Biol.*, 227: 381-388 (1992). Patent publications describing human antibody phage libraries include, for example: U.S. Pat. No. 5,750,373, and US Patent Publication Nos. 2005/0079574, 2005/0119455, 2005/0266000, 2007/0117126, 2007/0160598, 2007/0237764, 2007/0292936, and 2009/0002360.

[00188] The anti-PcrV antibodies can be prepared using phage display to screen libraries for anti-PcrV antibody moieties specific to the target PcrV. The library can be a human scFv phage display library having a diversity of at least one $\times 10^9$ (such as at least about

any of 1×10^9 , 2.5×10^9 , 5×10^9 , 7.5×10^9 , 1×10^{10} , 2.5×10^{10} , 5×10^{10} , 7.5×10^{10} , or 1×10^{11}) unique human antibody fragments. In some embodiments, the library is a naïve human library constructed from DNA extracted from human PMBCs and spleens from healthy donors, encompassing all human heavy and light chain subfamilies. In some embodiments, the library is a naïve human library constructed from DNA extracted from PBMCs isolated from patients with various diseases, such as patients with autoimmune diseases, cancer patients, and patients with infectious diseases. In some embodiments, the library is a semi-synthetic human library, wherein heavy chain CDR3 is completely randomized, with all amino acids (with the exception of cysteine) equally likely to be present at any given position (*see, e.g.,* Hoet, R.M. *et al., Nat. Biotechnol.* 23(3):344-348, 2005). In some embodiments, the heavy chain CDR3 of the semi-synthetic human library has a length from about 5 to about 24 (such as about any of 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, or 24) amino acids. In some embodiments, the library is a fully-synthetic phage display library. In some embodiments, the library is a non-human phage display library.

[00189] Phage clones that bind to the target PcrV with high affinity can be selected by iterative binding of phage to the target PcrV, which is bound to a solid support (such as, for example, beads for solution panning or mammalian cells for cell panning), followed by removal of non-bound phage and by elution of specifically bound phage. The bound phage clones are then eluted and used to infect an appropriate host cell, such as *E. coli* XL1-Blue, for expression and purification. The panning can be performed for multiple (such as about any of 2, 3, 4, 5, 6 or more) rounds with solution panning, cell panning, or a combination of both, to enrich for phage clones binding specifically to the target PcrV. Enriched phage clones can be tested for specific binding to the target PcrV by any methods known in the art, including for example ELISA and FACS.

[00190] Monoclonal antibodies can also be made by recombinant DNA methods, such as those described in U.S. Patent No. 4,816,567. DNA encoding the monoclonal antibodies of the application can be readily isolated and sequenced using conventional procedures (*e.g.,* by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of murine antibodies). Hybridoma cells as described above or PcrV-specific phage clones of the application can serve as a source of such DNA. Once isolated, the DNA can be placed into expression vectors, which are then transfected into host cells such as simian COS cells, Chinese hamster ovary (CHO) cells, or myeloma cells

2080 that do not otherwise produce immunoglobulin protein, to obtain the synthesis of
monoclonal antibodies in the recombinant host cells. The DNA also can be modified, for
example, by substituting the coding sequence for human heavy- and light-chain constant
domains and/or framework regions in place of the homologous non-human sequences (U.S.
Patent No. 4,816,567; Morrison *et al.*, *supra*) or by covalently joining to the
2085 immunoglobulin coding sequence all or part of the coding sequence for a
non-immunoglobulin polypeptide. Such a non-immunoglobulin polypeptide can be
substituted for the constant domains of an antibody of the application, or can be substituted
for the variable domains of one antigen-combining site of an antibody of the application to
create a chimeric bivalent antibody.

2090 **[00191]** The antibodies can be monovalent antibodies. Methods for preparing monovalent
antibodies are known in the art. For example, one method involves recombinant expression
of immunoglobulin light chain and modified heavy chain. The heavy chain is truncated
generally at any point in the Fc region so as to prevent heavy-chain crosslinking.

Alternatively, the relevant cysteine residues are substituted with another amino acid
2095 residue or are deleted so as to prevent crosslinking.

[00192] *In vitro* methods are also suitable for preparing monovalent antibodies. Digestion
of antibodies to produce fragments thereof, particularly Fab fragments, can be
accomplished using any method known in the art.

[00193] Antibody variable domains with the desired binding specificities
2100 (antibody-antigen combining sites) can be fused to immunoglobulin constant-domain
sequences. The fusion preferably is with an immunoglobulin heavy-chain constant domain,
comprising at least part of the hinge, CH2, and CH3 regions. In some embodiments, the
first heavy-chain constant region (CH1) containing the site necessary for light-chain
binding is present in at least one of the fusions. DNAs encoding the immunoglobulin
2105 heavy-chain fusions and, if desired, the immunoglobulin light chain, are inserted into
separate expression vectors, and are co-transfected into a suitable host organism.

Human and Humanized Antibodies

[00194] The anti-PcrV antibodies (*e.g.*, full-length anti-PcrV antibodies) can be
humanized antibodies or human antibodies. Humanized forms of non-human (*e.g.*, murine)
2110 antibody moieties are chimeric immunoglobulins, immunoglobulin chains, or fragments
thereof (such as Fv, Fab, Fab', F(ab')₂, scFv, or other antigen-binding subsequences of
antibodies) that typically contain minimal sequence derived from non-human

immunoglobulin. Humanized antibody moieties include human immunoglobulins, immunoglobulin chains, or fragments thereof (recipient antibody) in which residues from a CDR of the recipient are replaced by residues from a CDR of a non-human species (donor antibody) such as mouse, rat, or rabbit having the desired specificity, affinity, and capacity. In some instances, Fv framework residues of the human immunoglobulin are replaced by corresponding non-human residues. Humanized antibody moieties can also comprise residues that are found neither in the recipient antibody nor in the imported CDR or framework sequences. In general, the humanized antibody can comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin, and all or substantially all of the FR regions are those of a human immunoglobulin consensus sequence.

[00195] Generally, a humanized antibody has one or more amino acid residues introduced into it from a source that is non-human. These non-human amino acid residues are often referred to as “import” residues, which are typically taken from an “import” variable domain. According to some embodiments, humanization can be essentially performed following the method of Winter and co-workers (Jones *et al.*, *Nature*, 321: 522-525 (1986); Riechmann *et al.*, *Nature*, 332: 323-327 (1988); Verhoeven *et al.*, *Science*, 239: 1534-1536 (1988)), by substituting rodent CDRs or CDR sequences for the corresponding sequences of a human antibody. Accordingly, such “humanized” antibody moieties are antibody moieties (U.S. Patent No. 4,816,567), wherein substantially less than an intact human variable domain has been substituted by the corresponding sequence from a non-human species. In practice, humanized antibody moieties are typically human antibody moieties in which some CDR residues and possibly some FR residues are substituted by residues from analogous sites in rodent antibodies.

[00196] As an alternative to humanization, human antibody moieties can be generated. For example, it is now possible to produce transgenic animals (*e.g.*, mice) that are capable, upon immunization, of producing a full repertoire of human antibodies in the absence of endogenous immunoglobulin production. For example, it has been described that the homozygous deletion of the antibody heavy-chain joining region (JH) gene in chimeric and germ-line mutant mice results in complete inhibition of endogenous antibody production. Transfer of the human germ-line immunoglobulin gene array into such germ-line mutant mice will result in the production of human antibodies upon antigen challenge. See, *e.g.*, Jakobovits *et al.*, *PNAS USA*, 90:2551 (1993); Jakobovits *et al.*, *Nature*, 362:255-258

(1993); Bruggemann *et al.*, *Year in Immunol.*, 7:33 (1993); U.S. Patent Nos. 5,545,806, 5,569,825, 5,591,669; 5,545,807; and WO 97/17852. Alternatively, human antibodies can be made by introducing human immunoglobulin loci into transgenic animals, *e.g.*, mice in which the endogenous immunoglobulin genes have been partially or completely

2150 inactivated. Upon challenge, human antibody production is observed that closely resembles that seen in humans in all respects, including gene rearrangement, assembly, and antibody repertoire. This approach is described, for example, in U.S. Patent Nos. 5,545,807; 5,545,806; 5,569,825; 5,625,126; 5,633,425; and 5,661,016, and Marks *et al.*, *Bio/Technology*, 10: 779-783 (1992); Lonberg *et al.*, *Nature*, 368: 856-859 (1994);

2155 Morrison, *Nature*, 368: 812-813 (1994); Fishwild *et al.*, *Nature Biotechnology*, 14: 845-851 (1996); Neuberger, *Nature Biotechnology*, 14: 826 (1996); Lonberg and Huszar, *Intern. Rev. Immunol.*, 13: 65-93 (1995).

[00197] Human antibodies may also be generated by *in vitro* activated B cells (see U.S. Patents 5,567,610 and 5,229,275) or by using various techniques known in the art,

2160 including phage display libraries. Hoogenboom and Winter, *J. Mol. Biol.*, 227:381 (1991); Marks *et al.*, *J. Mol. Biol.*, 222:581 (1991). The techniques of Cole *et al.* and Boerner *et al.* are also available for the preparation of human monoclonal antibodies. Cole *et al.*, *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, p. 77 (1985) and Boerner *et al.*, *J. Immunol.*, 147(1): 86-95 (1991).

2165 **Anti-PcrV antibody variants**

[00198] In some embodiments, amino acid sequence variants of the anti-PcrV antibodies (*e.g.*, full-length anti-PcrV antibody) provided herein are contemplated. For example, it may be desirable to improve the binding affinity and/or other biological properties of the antibody. Amino acid sequence variants of an antibody may be prepared by introducing

2170 appropriate modifications into the nucleotide sequence encoding the antibody, or by peptide synthesis. Such modifications include, for example, deletions from, and/or insertions into and/or substitutions of residues within the amino acid sequences of the antibody. Any combination of deletion, insertion, and substitution can be made to arrive at the final construct, provided that the final construct possesses the desired characteristics,

2175 *e.g.*, antigen-binding.

[00199] In some embodiments, anti-PcrV antibody variants having one or more amino acid substitutions are provided. Sites of interest for substitutional mutagenesis include the

2180 HVRs and FRs. Amino acid substitutions may be introduced into an antibody of interest and the products screened for a desired activity, *e.g.*, improved bioactivity, retained/improved antigen binding, decreased immunogenicity, or improved opsonophagocytic killing (OPK) of pathogens, such as *P. aeruginosa*.

[00200] Conservative substitutions are shown in Table 4 below.

TABLE 4: CONSERVATIVE SUBSTITUTIONS

Original Residue	Exemplary Substitutions	Preferred Substitutions
Ala (A)	Val; Leu; Ile	Val
Arg (R)	Lys; Gln; Asn	Lys
Asn (N)	Gln; His; Asp, Lys; Arg	Gln
Asp (D)	Glu; Asn	Glu
Cys (C)	Ser; Ala	Ser
Gln (Q)	Asn; Glu	Asn
Glu (E)	Asp; Gln	Asp
Gly (G)	Ala	Ala
His (H)	Asn; Gln; Lys; Arg	Arg
Ile (I)	Leu; Val; Met; Ala; Phe; Norleucine	Leu
Leu (L)	Norleucine; Ile; Val; Met; Ala; Phe	Ile
Lys (K)	Arg; Gln; Asn	Arg
Met (M)	Leu; Phe; Ile	Leu
Phe (F)	Trp; Leu; Val; Ile; Ala; Tyr	Tyr
Pro (P)	Ala	Ala
Ser (S)	Thr	Thr
Thr (T)	Val; Ser	Ser
Trp (W)	Tyr; Phe	Tyr
Tyr (Y)	Trp; Phe; Thr; Ser	Phe
Val (V)	Ile; Leu; Met; Phe; Ala; Norleucine	Leu

2185 [00201] Amino acids may be grouped into different classes according to common side-chain properties:

- a. hydrophobic: Norleucine, Met, Ala, Val, Leu, Ile;
- b. neutral hydrophilic: Cys, Ser, Thr, Asn, Gln;

c. acidic: Asp, Glu;

d. basic: His, Lys, Arg;

2190 e. residues that influence chain orientation: Gly, Pro;

f. aromatic: Trp, Tyr, Phe.

[00202] Non-conservative substitutions will entail exchanging a member of one of these classes for another class.

2195 [00203] An exemplary substitutional variant is an affinity matured antibody, which may be conveniently generated, *e.g.*, using phage display-based affinity maturation techniques. Briefly, one or more CDR residues are mutated and the variant antibody moieties displayed on phage and screened for a particular biological activity (*e.g.*, bioactivity based on RBC lysis inhibition assay or binding affinity). Alterations (*e.g.*, substitutions) may be made in HVRs, *e.g.*, to improve bioactivity based on RBC lysis inhibition assay or antibody affinity.

2200 Such alterations may be made in HVR “hotspots,” *i.e.*, residues encoded by codons that undergo mutation at high frequency during the somatic maturation process (*see, e.g.*, Chowdhury, *Methods Mol. Biol.* 207:179-196 (2008)), and/or specificity determining residues (SDRs), with the resulting variant V_H or V_L being tested for binding affinity. Affinity maturation by constructing and reselecting from secondary libraries has been

2205 described, *e.g.*, in Hoogenboom *et al.* in *Methods in Molecular Biology* 178:1-37 (O'Brien *et al.*, ed., Human Press, Totowa, NJ, (2001).)

[00204] In some embodiments of affinity maturation, diversity is introduced into the variable genes chosen for maturation by any of a variety of methods (*e.g.*, error-prone PCR, chain shuffling, or oligonucleotide-directed mutagenesis). A secondary library is then

2210 created. The library is then screened to identify any antibody variants with the desired affinity. Another method to introduce diversity involves HVR-directed approaches, in which several HVR residues (*e.g.*, 4-6 residues at a time) are randomized. HVR residues involved in antigen binding may be specifically identified, *e.g.*, using alanine scanning mutagenesis or modeling. CDR-H3 and CDR-L3 in particular are often targeted.

2215 [00205] In some embodiments, substitutions, insertions, or deletions may occur within one or more HVRs so long as such alterations do not substantially reduce the ability of the antibody to bind antigen. For example, conservative alterations (*e.g.*, conservative substitutions as provided herein) that do not substantially reduce binding affinity may be made in HVRs. Such alterations may be outside of HVR “hotspots” or SDRs. In some

2220 embodiments of the variant VH and VL sequences provided above, each HVR either is
unaltered, or contains no more than one, two or three amino acid substitutions.

[00206] A useful method for identification of residues or regions of an antibody that may
be targeted for mutagenesis is called “alanine scanning mutagenesis” as described by
Cunningham and Wells (1989) *Science*, 244:1081-1085. In this method, a residue or group
2225 of target residues (*e.g.*, charged residues such as arg, asp, his, lys, and glu) are identified
and replaced by a neutral or negatively charged amino acid (*e.g.*, alanine or polyalanine) to
determine whether the interaction of the antibody with antigen is affected. Further
substitutions may be introduced at the amino acid locations demonstrating functional
sensitivity to the initial substitutions. Alternatively, or additionally, a crystal structure of an
2230 antigen-antibody complex can be determined to identify contact points between the
antibody and antigen. Such contact residues and neighboring residues may be targeted or
eliminated as candidates for substitution. Variants may be screened to determine whether
they contain the desired properties.

[00207] Amino acid sequence insertions include amino- and/or carboxyl-terminal fusions
2235 ranging in length from one residue to polypeptides containing a hundred or more residues,
as well as intrasequence insertions of single or multiple amino acid residues. Examples of
terminal insertions include an antibody with an N-terminal methionyl residue. Other
insertional variants of the antibody molecule include the fusion to the N- or C-terminus of
the antibody to an enzyme (*e.g.* for ADEPT) or a polypeptide which increases the serum
2240 half-life of the antibody.

Fc Region Variants

[00208] In some embodiments, one or more amino acid modifications may be introduced
into the Fc region of an antibody (*e.g.*, a full-length anti-PcrV antibody or anti-PcrV Fc
2245 fusion protein) provided herein, thereby generating an Fc region variant. In some
embodiments, the Fc region variant has enhanced ADCC effector function, often related to
binding to Fc receptors (FcRs). In some embodiments, the Fc region variant has decreased
ADCC effector function. There are many examples of changes or mutations to Fc
sequences that can alter effector function. For example, WO 00/42072 and Shields *et al.* *J*
2250 *Biol. Chem.* 9(2): 6591-6604 (2001) describe antibody variants with improved or
diminished binding to FcRs. The contents of those publications are specifically
incorporated herein by reference.

[00209] Antibody-Dependent Cell-Mediated Cytotoxicity (ADCC) is a mechanism of action of therapeutic antibodies against tumor cells. ADCC is a cell-mediated immune defense whereby an effector cell of the immune system actively lyses a target cell (e.g., an infected cell), whose membrane-surface antigens have been bound by specific antibodies (e.g., an anti-PcrV antibody). The typical ADCC involves activation of NK cells by antibodies. An NK cell expresses CD16 which is an Fc receptor. This receptor recognizes, and binds to, the Fc portion of an antibody bound to the surface of a target cell. The most common Fc receptor on the surface of an NK cell is called CD16 or FcγRIII. Binding of the Fc receptor to the Fc region of an antibody results in NK cell activation, release of cytolytic granules and consequent target cell apoptosis.

[00210] In some embodiments, the application contemplates an anti-PcrV antibody variant (such as a full-length anti-PcrV antibody variant) comprising an Fc region that possesses some but not all effector functions, which makes it a desirable candidate for applications in which the half-life of the anti-PcrV antibody *in vivo* is important yet certain effector functions (such as CDC and ADCC) are unnecessary or deleterious. *In vitro* and/or *in vivo* cytotoxicity assays can be conducted to confirm the reduction/depletion of CDC and/or ADCC activities. For example, Fc receptor (FcR) binding assays can be conducted to ensure that the antibody lacks FcγR binding (hence likely lacking ADCC activity), but retains FcRn binding ability. The primary cells for mediating ADCC, NK cells, express FcγRIII only, whereas monocytes express FcγRI, FcγRII and FcγRIII. FcR expression on hematopoietic cells is summarized in Table 3 on page 464 of Ravetch and Kinet, *Annu. Rev. Immunol.* 9:457-492 (1991). Non-limiting examples of *in vitro* assays to assess ADCC activity of a molecule of interest is described in U.S. Pat. No. 5,500,362 (see, e.g. Hellstrom, I. *et al. Proc. Nat'l Acad. Sci. USA* 83:7059-7063 (1986)) and Hellstrom, I *et al., Proc. Nat'l Acad. Sci. USA* 82:1499-1502 (1985); U.S. Pat. No. 5,821,337 (see Bruggemann, M. *et al., J. Exp. Med.* 166:1351-1361 (1987)). Alternatively, non-radioactive assay methods may be employed (see, for example, ACTI™ non-radioactive cytotoxicity assay for flow cytometry (CellTechnology, Inc. Mountain View, Calif.; and CytoTox 96™ non-radioactive cytotoxicity assay (Promega, Madison, Wis.)). Useful effector cells for such assays include peripheral blood mononuclear cells (PBMC) and Natural Killer (NK) cells. Alternatively, or additionally, ADCC activity of the molecule of interest may be assessed *in vivo*, e.g., in an animal model such as that disclosed in Clynes *et al. Proc. Nat'l Acad. Sci. USA* 95:652-656 (1998). C1q binding

- assays may also be carried out to confirm that the antibody is unable to bind C1q and hence lacks CDC activity. See, *e.g.*, C1q and C3c binding ELISA in WO 2006/029879 and WO 2005/100402. To assess complement activation, a CDC assay may be performed (see, for example, Gazzano-Santoro *et al.*, *J. Immunol. Methods* 202:163 (1996); Cragg, M. S. *et al.*, *Blood* 101:1045-1052 (2003); and Cragg, M. S. and M. J. Glennie, *Blood* 103:2738-2743 (2004)). FcRn binding and *in vivo* clearance/half-life determinations can also be performed using methods known in the art (see, *e.g.*, Petkova, S. B. *et al.*, *Int'l. Immunol.* 18(12):1759-1769 (2006)).
- 2290 [00211] Antibodies with reduced effector function include those with substitution of one or more of Fc region residues 238, 265, 269, 270, 297, 327 and 329 (U.S. Pat. No. 6,737,056). Such Fc mutants include Fc mutants with substitutions at two or more of amino acid positions 265, 269, 270, 297 and 327, including the so-called "DANA" Fc mutant with substitution of residues 265 and 297 to alanine (U.S. Pat. No. 7,332,581).
- 2295 [00212] Certain antibody variants with improved or diminished binding to FcRs are described. (See, *e.g.*, U.S. Pat. No. 6,737,056; WO 2004/056312, and Shields *et al.*, *J. Biol. Chem.* 9(2): 6591-6604 (2001).)
- 2300 [00213] In some embodiments, alterations are made in the Fc region that result in altered (*i.e.*, either improved or diminished) opsonization, *e.g.* such as described in Moore *et al.*, *MAbs*. 2(2): 181-189 (2010) .
- 2305 [00214] In some embodiments, there is provided an anti-PcrV antibody (such as a full-length anti-PcrV antibody) variant comprising a variant Fc region comprising one or more amino acid substitutions which increase half-life and/or improve binding to the neonatal Fc receptor (FcRn). Antibodies with increased half-lives and improved binding to FcRn are described in US2005/0014934A1 (Hinton *et al.*). Those antibodies comprise an
- 2310 Fc region with one or more substitutions therein which improve binding of the Fc region to FcRn. Such Fc variants include those with substitutions at one or more of Fc region residues: 238, 256, 265, 272, 286, 303, 305, 307, 311, 312, 317, 340, 356, 360, 362, 376, 378, 380, 382, 413, 424 or 434, *e.g.*, substitution of Fc region residue 434 (U.S. Pat. No. 7,371,826).
- 2315 [00215] See also Duncan & Winter, *Nature* 322:738-40 (1988); U.S. Pat. No. 5,648,260; U.S. Pat. No. 5,624,821; and WO 94/29351 concerning other examples of Fc region variants.

[00216] Anti-PcrV antibodies (such as full-length anti-PcrV antibodies) comprising any of the Fc variants described herein, or combinations thereof, are contemplated.

2320 ***Glycosylation Variants***

[00217] In some embodiments, an anti-PcrV antibody (such as a full-length anti-PcrV antibody) provided herein is altered to increase or decrease the extent to which the anti-PcrV antibody is glycosylated. Addition or deletion of glycosylation sites to an anti-PcrV antibody may be conveniently accomplished by altering the amino acid sequence of the anti-PcrV antibody or polypeptide portion thereof such that one or more glycosylation sites is created or removed.

[00218] Where the anti-PcrV antibody comprises an Fc region, the carbohydrate attached thereto may be altered. Native antibodies produced by mammalian cells typically comprise a branched, biantennary oligosaccharide that is generally attached by an N-linkage to Asn297 of the CH2 domain of the Fc region. See, e.g., Wright *et al.*, *TIBTECH* 15:26-32 (1997). The oligosaccharide may include various carbohydrates, e.g., mannose, N-acetyl glucosamine (GlcNAc), galactose, and sialic acid, as well as a fucose attached to a GlcNAc in the “stem” of the biantennary oligosaccharide structure. In some embodiments, modifications of the oligosaccharide in an anti-PcrV antibody of the application may be made in order to create anti-PcrV antibody variants with certain improved properties.

[00219] The N-glycans attached to the CH2 domain of Fc is heterogeneous. Antibodies or Fc fusion proteins generated in CHO cells are fucosylated by fucosyltransferase activity. See Shoji-Hosaka *et al.*, *J. Biochem.* 2006, 140:777- 83. Normally, a small percentage of naturally occurring afucosylated IgGs may be detected in human serum. N-glycosylation of the Fc is important for binding to FcγR; and afucosylation of the N-glycan increases Fc's binding capacity to FcγRIIIa. Increased FcγRIIIa binding can enhance ADCC, which can be advantageous in certain antibody therapeutic applications in which cytotoxicity is desirable.

[00220] In some embodiments, an enhanced effector function can be detrimental when Fc-mediated cytotoxicity is undesirable. In some embodiments, the Fc fragment or CH2 domain is not glycosylated. In some embodiments, the N-glycosylation site in the CH2 domain is mutated to prevent from glycosylation.

[00221] In some embodiments, anti-PcrV antibody (such as a full-length anti-PcrV antibody) variants are provided comprising an Fc region wherein a carbohydrate structure attached to the Fc region has reduced fucose or lacks fucose, which may improve ADCC

function. Specifically, anti-PcrV antibodies are contemplated herein that have reduced fucose relative to the amount of fucose on the same anti-PcrV antibody produced in a wild-type CHO cell. That is, they are characterized by having a lower amount of fucose than they would otherwise have if produced by native CHO cells (*e.g.*, a CHO cell that
2355 produce a native glycosylation pattern, such as, a CHO cell containing a native FUT8 gene). In some embodiments, the anti-PcrV antibody is one wherein less than about 50%, 40%, 30%, 20%, 10%, or 5% of the N-linked glycans thereon comprise fucose. For example, the amount of fucose in such an anti-PcrV antibody may be from 1% to 80%, from 1% to 65%, from 5% to 65% or from 20% to 40%. In some embodiments, the
2360 anti-PcrV antibody is one wherein none of the N-linked glycans thereon comprise fucose, *i.e.*, wherein the anti-PcrV antibody is completely without fucose, or has no fucose or is afucosylated. The amount of fucose is determined by calculating the average amount of fucose within the sugar chain at Asn297, relative to the sum of all glycostructures attached to Asn 297 (*e. g.* complex, hybrid and high mannose structures) as measured by
2365 MALDI-TOF mass spectrometry, as described in WO 2008/077546, for example. Asn297 refers to the asparagine residue located at about position 297 in the Fc region (EU numbering of Fc region residues); however, Asn297 may also be located about ± 3 amino acids upstream or downstream of position 297, *i.e.*, between positions 294 and 300, due to minor sequence variations in antibodies. Such fucosylation variants may have improved
2370 ADCC function. See, *e.g.*, US Patent Publication Nos. US 2003/0157108 (Presta, L.); US 2004/0093621 (Kyowa Hakko Kogyo Co., Ltd). Examples of publications related to “defucosylated” or “fucose-deficient” antibody variants include: US 2003/0157108; WO 2000/61739; WO 2001/29246; US 2003/0115614; US 2002/0164328; US 2004/0093621; US 2004/0132140; US 2004/0110704; US 2004/0110282; US 2004/0109865; WO
2375 2003/085119; WO 2003/084570; WO 2005/035586; WO 2005/035778; WO2005/053742; WO2002/031140; Okazaki *et al. J. Mol. Biol.* 336:1239-1249 (2004); Yamane-Ohnuki *et al. Biotech. Bioeng.* 87: 614 (2004). Examples of cell lines capable of producing defucosylated antibodies include Lec13 CHO cells deficient in protein fucosylation (Ripka *et al. Arch. Biochem. Biophys.* 249:533-545 (1986); US Pat Appl No US 2003/0157108 A1,
2380 Presta, L; and WO 2004/056312 A1, Adams *et al.*, especially at Example 11), and knockout cell lines, such as α -1,6-fucosyltransferase gene, FUT8, knockout CHO cells (see, *e.g.*, Yamane-Ohnuki *et al. Biotech. Bioeng.* 87: 614 (2004); Kanda, Y. *et al., Biotechnol. Bioeng.*, 94(4):680-688 (2006); and WO2003/085107).

2385 [00222] Anti-PcrV antibody (such as a full-length anti-PcrV antibody) variants are further provided with bisected oligosaccharides, *e.g.*, in which a biantennary oligosaccharide attached to the Fc region of the anti-PcrV antibody is bisected by GlcNAc. Such anti-PcrV antibody (such as a full-length anti-PcrV antibody) variants may have reduced fucosylation and/or improved ADCC function. Examples of such antibody variants are described, *e.g.*, in WO 2003/011878 (Jean-Mairet *et al.*); U.S. Pat. No. 6,602,684 (Umana *et al.*); US 2390 2005/0123546 (Umana *et al.*), and Ferrara *et al.*, *Biotechnology and Bioengineering*, 93(5): 851-861 (2006). Anti-PcrV antibody (such as full-length anti-PcrV antibody) variants with at least one galactose residue in the oligosaccharide attached to the Fc region are also provided. Such anti-PcrV antibody variants may have improved CDC function. Such antibody variants are described, *e.g.*, in WO 1997/30087 (Patel *et al.*); WO 1998/58964 2395 (Raju, S.); and WO 1999/22764 (Raju, S.).

[00223] In some embodiments, the anti-PcrV antibody (such as a full-length anti-PcrV antibody) variants comprising an Fc region are capable of binding to an FcγRIII. In some embodiments, the anti-PcrV antibody (such as a full-length anti-PcrV antibody) variants comprising an Fc region have ADCC activity in the presence of human effector cells (*e.g.*, 2400 T cell) or have increased ADCC activity in the presence of human effector cells compared to the otherwise same anti-PcrV antibody (such as a full-length anti-PcrV antibody) comprising a human wild-type Fc region.

Cysteine Engineered Variants

[00224] In some embodiments, it may be desirable to create cysteine engineered 2405 anti-PcrV antibodies (such as a full-length anti-PcrV antibody) in which one or more amino acid residues are substituted with cysteine residues. In some embodiments, the substituted residues occur at accessible sites of the anti-PcrV antibody. By substituting those residues with cysteine, reactive thiol groups are thereby positioned at accessible sites of the anti-PcrV antibody and may be used to conjugate the anti-PcrV antibody to other 2410 moieties, such as drug moieties or linker-drug moieties, to create an anti-PcrV immunoconjugate, as described further herein. Cysteine engineered anti-PcrV antibodies (*e.g.*, full-length anti-PcrV antibodies) may be generated as described, *e.g.*, in U.S. Pat. No. 7,521,541.

Derivatives

2415 [00225] In some embodiments, an anti-PcrV antibody (such as a full-length anti-PcrV antibody) provided herein may be further modified to contain additional non-proteinaceous

moieties that are known in the art and readily available. The moieties suitable for derivatization of the anti-PcrV antibody include but are not limited to water soluble polymers. Non-limiting examples of water soluble polymers include, but are not limited to, polyethylene glycol (PEG), copolymers of ethylene glycol/propylene glycol, carboxymethylcellulose, dextran, polyvinyl alcohol, polyvinyl pyrrolidone, poly-1,3-dioxolane, poly-1,3,6-trioxane, ethylene/maleic anhydride copolymer, polyaminoacids (either homopolymers or random copolymers), and dextran or poly(n-vinyl pyrrolidone)polyethylene glycol, propylene glycol homopolymers, polypropylene oxide/ethylene oxide co-polymers, polyoxyethylated polyols (*e.g.*, glycerol), polyvinyl alcohol, and mixtures thereof. Polyethylene glycol propionaldehyde may have advantages in manufacturing due to its stability in water. The polymer may be of any molecular weight, and may be branched or unbranched. The number of polymers attached to the anti-PcrV antibody may vary, and if more than one polymer are attached, they can be the same or different molecules. In general, the number and/or type of polymers used for derivatization can be determined based on considerations including, but not limited to, the particular properties or functions of anti-PcrV antibody to be improved, whether the anti-PcrV antibody derivative will be used in a therapy under defined conditions, etc.

[00226] In some embodiments, conjugates of an anti-PcrV antibody (such as a full-length anti-PcrV antibody) and nonproteinaceous moiety that may be selectively heated by exposure to radiation are provided. In some embodiments, the nonproteinaceous moiety is a carbon nanotube (Kam *et al.*, *Proc. Natl. Acad. Sci. USA* 102: 11600-11605 (2005)). The radiation may be of any wavelength, and includes, but is not limited to, wavelengths that do not harm ordinary cells, but which heat the nonproteinaceous moiety to a temperature at which cells proximal to the anti-PcrV antibody-nonproteinaceous moiety are killed.

Pharmaceutical Compositions

[00227] Also provided herein are compositions (such as pharmaceutical compositions, also referred to herein as formulations) comprising any of the anti-PcrV antibodies (such as a full-length anti-PcrV antibody), nucleic acids encoding the antibodies, vectors comprising the nucleic acids encoding the antibodies, or host cells comprising the nucleic acids or vectors described herein. In some embodiments, there is provided a pharmaceutical composition comprising any one of the anti-PcrV antibodies described herein and a pharmaceutically acceptable carrier.

[00228] Suitable formulations of the anti-PcrV antibodies are obtained by mixing an anti-PcrV antibody having the desired degree of purity with optional pharmaceutically acceptable carriers, excipients or stabilizers (*Remington's Pharmaceutical Sciences* 16th edition, Osol, A. Ed. (1980)), in the form of lyophilized formulations or aqueous solutions. Acceptable carriers, excipients, or stabilizers are nontoxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride, benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propylparaben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrans; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (*e.g.* Zn-protein complexes); and/or non-ionic surfactants such as TWEEN™, PLURONICS™ or polyethylene glycol (PEG). Exemplary formulations are described in WO98/56418, expressly incorporated herein by reference. Lyophilized formulations adapted for subcutaneous administration are described in WO97/04801. Such lyophilized formulations may be reconstituted with a suitable diluent to a high protein concentration and the reconstituted formulation may be administered subcutaneously to the individual to be treated herein. Lipofectins or liposomes can be used to deliver the anti-PcrV antibodies of this application into cells.

[00229] The formulation herein may also contain one or more active compounds in addition to the anti-PcrV antibody (such as a full-length anti-PcrV antibody) as necessary for the particular indication being treated, preferably those with complementary activities that do not adversely affect each other. For example, it may be desirable to further provide an anti-neoplastic agent, a growth inhibitory agent, a cytotoxic agent, or a chemotherapeutic agent in addition to the anti-PcrV antibody. Such molecules are suitably present in combination in amounts that are effective for the purpose intended. The effective amount of such other agents depends on the amount of anti-PcrV antibody present in the formulation, the type of disease or disorder or treatment, and other factors discussed above.

These are generally used in the same dosages and with administration routes as described herein or about from 1 to 99% of the heretofore employed dosages.

- 2485 [00230] The anti-PcrV antibodies (*e.g.*, full-length anti-PcrV antibodies) may also be entrapped in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or gelatin-microcapsules and poly-(methylmethacrylate) microcapsules, respectively, in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles and nanocapsules) or in macroemulsions. Sustained-release preparations may be prepared.
- 2490 [00231] Sustained-release preparations of the anti-PcrV antibodies (*e.g.*, full-length anti-PcrV antibodies) can be prepared. Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing the antibody (or fragment thereof), which matrices are in the form of shaped articles, *e.g.*, films, or microcapsules. Examples of sustained-release matrices include polyesters, hydrogels (for example, poly(2-hydroxyethyl-methacrylate), or poly(vinylalcohol)), polylactides (U.S. Pat. No. 3,773,919), copolymers of L-glutamic acid and ethyl-L-glutamate, non-degradable ethylene-vinyl acetate, degradable lactic acid-glycolic acid copolymers such as the LUPRON DEPOTTM (injectable microspheres composed of lactic acid-glycolic acid copolymer and leuprolide acetate), and poly-D (-)-3-hydroxybutyric acid. While polymers
- 2495 such as ethylene-vinyl acetate and lactic acid-glycolic acid enable release of molecules for over 100 days, certain hydro gels release proteins for shorter time periods. When encapsulated antibody remain in the body for a long time, they can denature or aggregate as a result of exposure to moisture at 37 °C, resulting in a loss of biological activity and possible changes in immunogenicity. Rational strategies can be devised for stabilization of
- 2500 anti-PcrV antibodies depending on the mechanism involved. For example, if the aggregation mechanism is discovered to be intermolecular S-S bond formation through thio-disulfide interchange, stabilization can be achieved by modifying sulfhydryl residues, lyophilizing from acidic solutions, controlling moisture content, using appropriate additives, and developing specific polymer matrix compositions.
- 2505 [00232] In some embodiments, the anti-PcrV antibody (such as a full-length anti-PcrV antibody) is formulated in a buffer comprising a citrate, NaCl, acetate, succinate, glycine, polysorbate 80 (Tween 80), or any combination of the foregoing. In some embodiments, the anti-PcrV antibody is formulated in a buffer comprising about 100 mM to about 150 mM glycine. In some embodiments, the anti-PcrV antibody is formulated in a buffer

2515 comprising about 50mM to about 100 mM NaCl. In some embodiments, the anti-PcrV antibody is formulated in a buffer comprising about 10mM to about 50 mM acetate. In some embodiments, the anti-PcrV antibody is formulated in a buffer comprising about 10mM to about 50 mM succinate. In some embodiments, the anti-PcrV antibody is formulated in a buffer comprising about 0.005% to about 0.02% polysorbate 80. In some
2520 embodiments, the anti-PcrV antibody is formulated in a buffer having a pH between about 5.1 and 5.6. In some embodiments, the anti-PcrV antibody is formulated in a buffer comprising 10 mM citrate, 100 mM NaCl, 100mM glycine, and 0.01% polysorbate 80, wherein the formulation is at pH 5.5.

[00233] The formulations to be used for *in vivo* administration must be sterile. This is
2525 readily accomplished by, *e.g.*, filtration through sterile filtration membranes.

Methods of treatment or prevention using anti-PcrV antibodies

[00234] In certain aspects, there is provided a method of treating a *Pseudomonas* infection in an individual comprising administering to the individual an effective amount of a composition comprising any of the anti-PcrV antibodies described herein. In some
2530 embodiments, the method of treating a *Pseudomonas* infection further provides therapeutic or prophylactic effect on diseases and/or conditions associated with *Pseudomonas* infection. In some aspects, there is provided a method of preventing a *Pseudomonas* infection in an individual comprising administering to the individual an effective amount of a composition comprising any of the anti-PcrV antibodies described herein. In some embodiments, use of
2535 the anti-PcrV antibody according to any one of the anti-PcrV antibodies described above, or a pharmaceutical composition comprising an anti- PcrV antibody according to any one of the pharmaceutical compositions described above in the manufacture of a medicament for treating a disease or condition.

[00235] Diseases and/or conditions associated with *Pseudomonas* infection include, but
2540 are not limited to fever, chills, fatigues, muscle and joint pain, swelling of joints, headache, diarrhea, skin rashes, pus in wounds, bacteremia, acute pneumonia, intraperitoneal infection. Further exemplary diseases include, but are not limited to, respiratory tract infections, bacteremia, septic shock, suppurative arthritis, enteritis, skin and soft tissue infections (such as burn wound infections), urinary tract infections, intestinal infections,
2545 ulcerative keratitis, chronic suppurative otitis media, mastoiditis, sinusitis, and endocarditis.

In some embodiments, the method of treating or preventing a *Pseudomonas* infection reduces rate of mortality resulting from the *Pseudomonas* infection.

- [00236] In some embodiments, there is provided a method of treating or preventing a *Pseudomonas* infection in an individual comprising administering to the individual an effective amount of a composition comprising an anti-PcrV antibody. In some embodiments, the anti-PcrV antibody specifically binds to a linear epitope within *Pseudomonas* PcrV. In some embodiments, the anti-PcrV described herein specifically binds to a nonlinear epitope within *Pseudomonas* PcrV. In some embodiments, there is provided a method of treating or preventing a *Pseudomonas* infection in an individual comprising administering to the individual an effective amount of a composition comprising an anti-PcrV antibody, where the anti-PcrV antibody binds to an epitope comprising at least any one of 1, 2, 3, 4, 5, or 6 amino acid residues selected from the group of Gln160, Asp165, Asp170, Asp173, Thr175, and Ser202 of *Pseudomonas* PcrV, according to SEQ ID NO: 71.
- [00237] In some embodiments, there is provided a method of treating or preventing a *Pseudomonas* infection in an individual comprising administering to the individual an effective amount of a composition comprising an anti-PcrV antibody, wherein the anti-PcrV antibody comprises a V_H comprising an HC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 1-8, an HC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 9-14, and an HC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 15-31; and a V_L comprising an LC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 32-33, an LC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 34, and an LC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 35-38. In some embodiments, there is provided a method of treating or preventing a *Pseudomonas* infection in an individual comprising administering to the individual an effective amount of a composition comprising an anti-PcrV antibody, wherein the anti-PcrV antibody competes with an antibody comprising: a V_H comprising an HC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 1-8, an HC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 9-14, and an HC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 15-31; and a V_L comprising an LC-CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 32-33, an LC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 34, and an LC-CDR3

comprising the amino acid sequence of any one of SEQ ID NOs: 35-38. In some
2580 embodiments, there is provided a method of treating or preventing a *Pseudomonas*
infection in an individual comprising administering to the individual an effective amount
of a composition comprising an anti-PcrV antibody, wherein the anti-PcrV antibody binds
to the same epitope as an antibody comprising: a V_H comprising an HC-CDR1 comprising
the amino acid sequence of any one of SEQ ID NOs: 1-8, an HC-CDR2 comprising the
2585 amino acid sequence of any one of SEQ ID NOs: 9-14, and an HC-CDR3 comprising the
amino acid sequence of any one of SEQ ID NOs: 15-31; and a V_L comprising an
LC-CDR1 comprising the amino acid sequence of SEQ ID NOs: any one of 32-33, an
LC-CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 34, and an
LC-CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 35-38.

2590 **[00238]** In some embodiments, there is provided a method of treating or preventing a
Pseudomonas infection in an individual comprising administering to the individual an
effective amount of a composition comprising an anti-PcrV antibody, wherein the
anti-PcrV antibody comprises a V_H comprising the amino acid sequence of any one of SEQ
ID NOs: 48-64, and a V_L comprising the amino acid sequence of any one of SEQ ID NOs:
2595 65-70. In some embodiments, there is provided a method of treating or preventing a
Pseudomonas infection in an individual comprising administering to the individual an
effective amount of a composition comprising an anti-PcrV antibody, wherein the
anti-PcrV antibody competes with an antibody comprising: a V_H comprising the amino
acid sequence of any one of SEQ ID NOs: 48-64, and a V_L comprising the amino acid
2600 sequence of any one of SEQ ID NOs: 65-70. In some embodiments, there is provided a
method of treating or preventing a *Pseudomonas* infection in an individual comprising
administering to the individual an effective amount of a composition comprising an
anti-PcrV antibody, wherein the anti-PcrV antibody binds to the same epitope as an
antibody comprising: a V_H comprising the amino acid sequence of any one of SEQ ID NOs:
2605 48-64, and a V_L comprising the amino acid sequence of any one of SEQ ID NOs: 65-70.

[00239] In some embodiments, there is provided a method of treating or preventing a
Pseudomonas infection in an individual comprising administering to the individual an
effective amount of a composition comprising an anti-PcrV antibody, wherein the
anti-PcrV antibody competes with an antibody comprising: a V_H comprising an HC-CDR1
2610 comprising the amino acid sequence of SEQ ID NO: 1, an HC-CDR2 comprising the
amino acid sequence of SEQ ID NO: 9, and an HC-CDR3 comprising the amino acid

sequence of SEQ ID NO: 15; and a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 32, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 34, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 35.

2615 35. In some embodiments, there is provided a method of treating or preventing a *Pseudomonas* infection in an individual comprising administering to the individual an effective amount of a composition comprising an anti-PcrV antibody, wherein the anti-PcrV antibody binds to the same epitope as an antibody comprising: a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 1, an HC-CDR2

2620 comprising the amino acid sequence of SEQ ID NO: 9, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 15; and a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 32, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 34, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 35.

2625 [00240] In some embodiments, there is provided a method of treating or preventing a *Pseudomonas* infection in an individual comprising administering to the individual an effective amount of a composition comprising an anti-PcrV antibody, wherein the anti-PcrV antibody competes with an antibody comprising: a V_H comprising the amino acid sequence of SEQ ID NO: 48 and a V_L comprising the amino acid sequence of SEQ ID

2630 NO: 65. In some embodiments, there is provided a method of treating or preventing a *Pseudomonas* infection in an individual comprising administering to the individual an effective amount of a composition comprising an anti-PcrV antibody, wherein the anti-PcrV antibody binds to the same epitope as an antibody comprising: a V_H comprising the amino acid sequence of SEQ ID NO: 48 and a V_L comprising the amino acid sequence

2635 of SEQ ID NO: 65.

[00241] In some embodiments, there is provided a method of treating or preventing a *Pseudomonas* infection in an individual comprising administering to the individual an effective amount of a composition comprising an anti-PcrV antibody comprising: a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 1, an

2640 HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 9, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 15, or a variant thereof comprising up to 5 amino acid substitutions; and a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 32, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 34, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO:

2645 35, or a variant thereof comprising up to 5 amino acid substitutions. In some
embodiments, the anti-PcrV antibody comprises a V_H comprising the amino acid sequence
of SEQ ID NO: 48, or a variant thereof having at least 90% sequence identity to the amino
acid sequence of SEQ ID NO: 48; and a V_L comprising the amino acid sequence of SEQ
ID NO: 65, or a variant thereof having at least 90% sequence identity to the amino acid
2650 sequence of SEQ ID NO: 65.

[00242] In some embodiments, there is provided a method of treating or preventing a
Pseudomonas infection in an individual comprising administering to the individual an
effective amount of a composition comprising an anti-PcrV antibody, wherein the
anti-PcrV antibody competes with an antibody comprising: a V_H comprising an HC-CDR1
2655 comprising the amino acid sequence of SEQ ID NO: 2, an HC-CDR2 comprising the
amino acid sequence of SEQ ID NO: 10, and an HC-CDR3 comprising the amino acid
sequence of SEQ ID NO: 16; and a V_L comprising an LC-CDR1 comprising the amino
acid sequence of SEQ ID NO: 32, an LC-CDR2 comprising the amino acid sequence of
SEQ ID NO: 34, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO:

2660 35. In some embodiments, there is provided a method of treating or preventing a
Pseudomonas infection in an individual comprising administering to the individual an
effective amount of a composition comprising an anti-PcrV antibody, wherein the
anti-PcrV antibody binds to the same epitope as an antibody comprising: a V_H comprising
an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 2, an HC-CDR2
2665 comprising the amino acid sequence of SEQ ID NO: 10, and an HC-CDR3 comprising the
amino acid sequence of SEQ ID NO: 16; and a V_L comprising an LC-CDR1 comprising
the amino acid sequence of SEQ ID NO: 32, an LC-CDR2 comprising the amino acid
sequence of SEQ ID NO: 34, and an LC-CDR3 comprising the amino acid sequence of
SEQ ID NO: 35.

2670 [00243] In some embodiments, there is provided a method of treating or preventing a
Pseudomonas infection in an individual comprising administering to the individual an
effective amount of a composition comprising an anti-PcrV antibody, wherein the
anti-PcrV antibody competes with an antibody comprising: a V_H comprising the amino
acid sequence of SEQ ID NO: 49 and a V_L comprising the amino acid sequence of SEQ ID
2675 NO: 65. In some embodiments, there is provided a method of treating or preventing a
Pseudomonas infection in an individual comprising administering to the individual an
effective amount of a composition comprising an anti-PcrV antibody, wherein the

anti-PcrV antibody binds to the same epitope as an antibody comprising: a V_H comprising the amino acid sequence of SEQ ID NO: 49 and a V_L comprising the amino acid sequence of SEQ ID NO: 65.

[00244] In some embodiments, there is provided a method of treating or preventing a *Pseudomonas* infection in an individual comprising administering to the individual an effective amount of a composition comprising an anti-PcrV antibody comprising: a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 2, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 10, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 16, or a variant thereof comprising up to 5 amino acid substitutions; and a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 32, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 34, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 35, or a variant thereof comprising up to 5 amino acid substitutions. In some embodiments, the anti-PcrV antibody comprises a V_H comprising the amino acid sequence of SEQ ID NO: 49, or a variant thereof having at least 90% sequence identity to the amino acid sequence of SEQ ID NO: 49; and a V_L comprising the amino acid sequence of SEQ ID NO: 65, or a variant thereof having at least 90% sequence identity to the amino acid sequence of SEQ ID NO: 65.

[00245] In some embodiments, there is provided a method of treating or preventing a *Pseudomonas* infection in an individual comprising administering to the individual an effective amount of a composition comprising an anti-PcrV antibody, wherein the anti-PcrV antibody competes with an antibody comprising: a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 1, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 9, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 17; and a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 32, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 34, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 36.

In some embodiments, there is provided a method of treating or preventing a *Pseudomonas* infection in an individual comprising administering to the individual an effective amount of a composition comprising an anti-PcrV antibody, wherein the anti-PcrV antibody binds to the same epitope as an antibody comprising: a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 1, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 9, and an HC-CDR3 comprising the amino acid sequence of SEQ

ID NO: 17; and a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 32, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 34, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 36.

- [00246] In some embodiments, there is provided a method of treating or preventing a *Pseudomonas* infection in an individual comprising administering to the individual an effective amount of a composition comprising an anti-PcrV antibody, wherein the anti-PcrV antibody competes with an antibody comprising: a V_H comprising the amino acid sequence of SEQ ID NO: 50 and a V_L comprising the amino acid sequence of SEQ ID NO: 66. In some embodiments, there is provided a method of treating or preventing a *Pseudomonas* infection in an individual comprising administering to the individual an effective amount of a composition comprising an anti-PcrV antibody, wherein the anti-PcrV antibody binds to the same epitope as an antibody comprising: a V_H comprising the amino acid sequence of SEQ ID NO: 50 and a V_L comprising the amino acid sequence of SEQ ID NO: 66.
- [00247] In some embodiments, there is provided a method of treating or preventing a *Pseudomonas* infection in an individual comprising administering to the individual an effective amount of a composition comprising an anti-PcrV antibody comprising: a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 1, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 9, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 17, or a variant thereof comprising up to 5 amino acid substitutions; and a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 32, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 34, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 36, or a variant thereof comprising up to 5 amino acid substitutions. In some embodiments, the anti-PcrV antibody comprises a V_H comprising the amino acid sequence of SEQ ID NO: 50, or a variant thereof having at least 90% sequence identity to the amino acid sequence of SEQ ID NO: 50, and a V_L comprising the amino acid sequence of SEQ ID NO: 66, or a variant thereof having at least 90% sequence identity to the amino acid sequence of SEQ ID NO: 66.
- [00248] In some embodiments, there is provided a method of treating or preventing a *Pseudomonas* infection in an individual comprising administering to the individual an effective amount of a composition comprising an anti-PcrV antibody, wherein the anti-PcrV antibody competes with an antibody comprising: a V_H comprising an HC-CDR1

- comprising the amino acid sequence of SEQ ID NO: 3, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 10, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 18; and a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 32, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 34, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 37. In some embodiments, there is provided a method of treating or preventing a *Pseudomonas* infection in an individual comprising administering to the individual an effective amount of a composition comprising an anti-PcrV antibody, wherein the anti-PcrV antibody binds to the same epitope as an antibody comprising: a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 3, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 10, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 18; and a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 32, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 34, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 37.
- [00249]** In some embodiments, there is provided a method of treating or preventing a *Pseudomonas* infection in an individual comprising administering to the individual an effective amount of a composition comprising an anti-PcrV antibody, wherein the anti-PcrV antibody competes with an antibody comprising: a V_H comprising the amino acid sequence of SEQ ID NO: 51 and a V_L comprising the amino acid sequence of SEQ ID NO: 67. In some embodiments, there is provided a method of treating or preventing a *Pseudomonas* infection in an individual comprising administering to the individual an effective amount of a composition comprising an anti-PcrV antibody, wherein the anti-PcrV antibody binds to the same epitope as an antibody comprising: a V_H comprising the amino acid sequence of SEQ ID NO: 51 and a V_L comprising the amino acid sequence of SEQ ID NO: 67.
- [00250]** In some embodiments, there is provided a method of treating or preventing a *Pseudomonas* infection in an individual comprising administering to the individual an effective amount of a composition comprising an anti-PcrV antibody comprising: a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 3, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 10, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 18, or a variant thereof comprising up to 5 amino acid substitutions; and a V_L comprising an LC-CDR1 comprising the amino

acid sequence of SEQ ID NO: 32, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 34, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 37, or a variant thereof comprising up to 5 amino acid substitutions. In some
2780 embodiments, the anti-PcrV antibody comprises a V_H comprising the amino acid sequence of SEQ ID NO: 51, or a variant thereof having at least 90% sequence identity to the amino acid sequence of SEQ ID NO: 51; and a V_L comprising the amino acid sequence of SEQ ID NO: 67, or a variant thereof having at least 90% sequence identity to the amino acid sequence of SEQ ID NO: 67. In some embodiments, the method prevents a
2785 *Pseudomonas* infection in an individual.

[00251] In some embodiments, there is provided a method of treating or preventing a *Pseudomonas* infection in an individual comprising administering to the individual an effective amount of a composition comprising an anti-PcrV antibody, wherein the anti-PcrV antibody competes with an antibody comprising: a V_H comprising an HC-CDR1
2790 comprising the amino acid sequence of SEQ ID NO: 3, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 10, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 19; and a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 32, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 34, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 35.

2795 In some embodiments, there is provided a method of treating or preventing a *Pseudomonas* infection in an individual comprising administering to the individual an effective amount of a composition comprising an anti-PcrV antibody, wherein the anti-PcrV antibody binds to the same epitope as an antibody comprising: a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 3, an HC-CDR2 comprising the amino acid
2800 sequence of SEQ ID NO: 10, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 19; and a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 32, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 34, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 35.

[00252] In some embodiments, there is provided a method of treating or preventing a
2805 *Pseudomonas* infection in an individual comprising administering to the individual an effective amount of a composition comprising an anti-PcrV antibody, wherein the anti-PcrV antibody competes with an antibody comprising: a V_H comprising the amino acid sequence of SEQ ID NO: 52 and a V_L comprising the amino acid sequence of SEQ ID NO: 65. In some embodiments, there is provided a method of treating or preventing a

- 2810 *Pseudomonas* infection in an individual comprising administering to the individual an effective amount of a composition comprising an anti-PcrV antibody, wherein the anti-PcrV antibody binds to the same epitope as an antibody comprising: a V_H comprising the amino acid sequence of SEQ ID NO: 52 and a V_L comprising the amino acid sequence of SEQ ID NO: 65.
- 2815 [00253] In some embodiments, there is provided a method of treating or preventing a *Pseudomonas* infection in an individual comprising administering to the individual an effective amount of a composition comprising an anti-PcrV antibody comprising: a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 3, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 10, and an HC-CDR3
- 2820 comprising the amino acid sequence of SEQ ID NO: 19, or a variant thereof comprising up to 5 amino acid substitutions; and a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 32, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 34, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 35, or a variant thereof comprising up to 5 amino acid substitutions. In some embodiments,
- 2825 the anti-PcrV antibody comprises a V_H comprising the amino acid sequence of SEQ ID NO: 52, or a variant thereof having at least 90% sequence identity to the amino acid sequence of SEQ ID NO: 52; and a V_L comprising the amino acid sequence of SEQ ID NO: 65, or a variant thereof having at least 90% sequence identity to the amino acid sequence of SEQ ID NO: 65.
- 2830 [00254] In some embodiments, there is provided a method of treating or preventing a *Pseudomonas* infection in an individual comprising administering to the individual an effective amount of a composition comprising an anti-PcrV antibody, wherein the anti-PcrV antibody competes with an antibody comprising: a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 3, an HC-CDR2 comprising the
- 2835 amino acid sequence of SEQ ID NO: 11, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 20; and a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 32, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 34, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 35. In some embodiments, there is provided a method of treating or preventing a *Pseudomonas*
- 2840 infection in an individual comprising administering to the individual an effective amount of a composition comprising an anti-PcrV antibody, wherein the anti-PcrV antibody binds to the same epitope as an antibody comprising: a V_H comprising an HC-CDR1 comprising

the amino acid sequence of SEQ ID NO: 3, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 11, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 20; and a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 32, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 34, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 35.

[00255] In some embodiments, there is provided a method of treating or preventing a *Pseudomonas* infection in an individual comprising administering to the individual an effective amount of a composition comprising an anti-PcrV antibody, wherein the anti-PcrV antibody competes with an antibody comprising: a V_H comprising the amino acid sequence of SEQ ID NO: 53 and a V_L comprising the amino acid sequence of SEQ ID NO: 65. In some embodiments, there is provided a method of treating or preventing a *Pseudomonas* infection in an individual comprising administering to the individual an effective amount of a composition comprising an anti-PcrV antibody, wherein the anti-PcrV antibody binds to the same epitope as an antibody comprising: a V_H comprising the amino acid sequence of SEQ ID NO: 53 and a V_L comprising the amino acid sequence of SEQ ID NO: 65.

[00256] In some embodiments, there is provided a method of treating or preventing a *Pseudomonas* infection in an individual comprising administering to the individual an effective amount of a composition comprising an anti-PcrV antibody comprising: a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 3, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 11, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 20, or a variant thereof comprising up to 5 amino acid substitutions; and a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 32, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 34, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 35, or a variant thereof comprising up to 5 amino acid substitutions. In some embodiments, the anti-PcrV antibody comprises a V_H comprising the amino acid sequence of SEQ ID NO: 53, or a variant thereof having at least 90% sequence identity to the amino acid sequence of SEQ ID NO: 53; and a V_L comprising the amino acid sequence of SEQ ID NO: 65, or a variant thereof having at least 90% sequence identity to the amino acid sequence of SEQ ID NO: 65.

[00257] In some embodiments, there is provided a method of treating or preventing a *Pseudomonas* infection in an individual comprising administering to the individual an

effective amount of a composition comprising an anti-PcrV antibody, wherein the anti-PcrV antibody competes with an antibody comprising: a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 4, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 10, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 21; and a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 32, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 34, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 35. In some embodiments, there is provided a method of treating or preventing a *Pseudomonas* infection in an individual comprising administering to the individual an effective amount of a composition comprising an anti-PcrV antibody, wherein the anti-PcrV antibody binds to the same epitope as an antibody comprising: a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 4, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 10, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 21; and a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 32, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 34, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 35.

[00258] In some embodiments, there is provided a method of treating or preventing a *Pseudomonas* infection in an individual comprising administering to the individual an effective amount of a composition comprising an anti-PcrV antibody, wherein the anti-PcrV antibody competes with an antibody comprising: a V_H comprising the amino acid sequence of SEQ ID NO: 54 and a V_L comprising the amino acid sequence of SEQ ID NO: 65. In some embodiments, there is provided a method of treating or preventing a *Pseudomonas* infection in an individual comprising administering to the individual an effective amount of a composition comprising an anti-PcrV antibody, wherein the anti-PcrV antibody binds to the same epitope as an antibody comprising: a V_H comprising the amino acid sequence of SEQ ID NO: 54 and a V_L comprising the amino acid sequence of SEQ ID NO: 65.

[00259] In some embodiments, there is provided a method of treating or preventing a *Pseudomonas* infection in an individual comprising administering to the individual an effective amount of a composition comprising an anti-PcrV antibody comprising: a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 4, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 10, and an HC-CDR3

comprising the amino acid sequence of SEQ ID NO: 21, or a variant thereof comprising up to 5 amino acid substitutions; and a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 32, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 34, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 35, or a variant thereof comprising up to 5 amino acid substitutions. In some embodiments, the anti-PcrV antibody comprises a V_H comprising the amino acid sequence of SEQ ID NO: 54, or a variant thereof having at least 90% sequence identity to the amino acid sequence of SEQ ID NO: 54; and a V_L comprising the amino acid sequence of SEQ ID NO: 65, or a variant thereof having at least 90% sequence identity to the amino acid sequence of SEQ ID NO: 65.

[00260] In some embodiments, there is provided a method of treating or preventing a *Pseudomonas* infection in an individual comprising administering to the individual an effective amount of a composition comprising an anti-PcrV antibody, wherein the anti-PcrV antibody competes with an antibody comprising: a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 5, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 10, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 22; and a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 32, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 34, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 37. In some embodiments, there is provided a method of treating or preventing a *Pseudomonas* infection in an individual comprising administering to the individual an effective amount of a composition comprising an anti-PcrV antibody, wherein the anti-PcrV antibody binds to the same epitope as an antibody comprising: a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 5, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 10, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 22; and a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 32, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 34, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 37.

[00261] In some embodiments, there is provided a method of treating or preventing a *Pseudomonas* infection in an individual comprising administering to the individual an effective amount of a composition comprising an anti-PcrV antibody, wherein the anti-PcrV antibody competes with an antibody comprising: a V_H comprising the amino

acid sequence of SEQ ID NO: 55 and a V_L comprising the amino acid sequence of SEQ ID NO: 67. In some embodiments, there is provided a method of treating or preventing a *Pseudomonas* infection in an individual comprising administering to the individual an effective amount of a composition comprising an anti-PcrV antibody, wherein the anti-PcrV antibody binds to the same epitope as an antibody comprising: a V_H comprising the amino acid sequence of SEQ ID NO: 55 and a V_L comprising the amino acid sequence of SEQ ID NO: 67.

[00262] In some embodiments, there is provided a method of treating or preventing a *Pseudomonas* infection in an individual comprising administering to the individual an effective amount of a composition comprising an anti-PcrV antibody comprising: a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 5, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 10, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 22, or a variant thereof comprising up to 5 amino acid substitutions; and a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 32, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 34, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 37, or a variant thereof comprising up to 5 amino acid substitutions. In some embodiments, the anti-PcrV antibody comprises a V_H comprising the amino acid sequence of SEQ ID NO: 55, or a variant thereof having at least 90% sequence identity to the amino acid sequence of SEQ ID NO: 55; and a V_L comprising the amino acid sequence of SEQ ID NO: 67, or a variant thereof having at least 90% sequence identity to the amino acid sequence of SEQ ID NO: 67.

[00263] In some embodiments, there is provided a method of treating or preventing a *Pseudomonas* infection in an individual comprising administering to the individual an effective amount of a composition comprising an anti-PcrV antibody, wherein the anti-PcrV antibody competes with an antibody comprising: a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 6, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 10, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 23; and a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 32, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 34, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 37. In some embodiments, there is provided a method of treating or preventing a *Pseudomonas* infection in an individual comprising administering to the individual an

2975 effective amount of a composition comprising an anti-PcrV antibody, wherein the
 anti-PcrV antibody binds to the same epitope as an antibody comprising: a V_H comprising
 an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 6, an HC-CDR2
 comprising the amino acid sequence of SEQ ID NO: 10, and an HC-CDR3 comprising the
 amino acid sequence of SEQ ID NO: 23; and a V_L comprising an LC-CDR1 comprising
 2980 the amino acid sequence of SEQ ID NO: 32, an LC-CDR2 comprising the amino acid
 sequence of SEQ ID NO: 34, and an LC-CDR3 comprising the amino acid sequence of
 SEQ ID NO: 37.

[00264] In some embodiments, there is provided a method of treating or preventing a
Pseudomonas infection in an individual comprising administering to the individual an
 2985 effective amount of a composition comprising an anti-PcrV antibody, wherein the
 anti-PcrV antibody competes with an antibody comprising: a V_H comprising the amino
 acid sequence of SEQ ID NO: 56 and a V_L comprising the amino acid sequence of SEQ ID
 NO: 67. In some embodiments, there is provided a method of treating or preventing a
Pseudomonas infection in an individual comprising administering to the individual an
 2990 effective amount of a composition comprising an anti-PcrV antibody, wherein the
 anti-PcrV antibody binds to the same epitope as an antibody comprising: a V_H comprising
 the amino acid sequence of SEQ ID NO: 56 and a V_L comprising the amino acid sequence
 of SEQ ID NO: 67.

[00265] In some embodiments, there is provided a method of treating or preventing a
Pseudomonas infection in an individual comprising administering to the individual an
 2995 effective amount of a composition comprising an anti-PcrV antibody comprising: a V_H
 comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 6, an
 HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 10, and an HC-CDR3
 comprising the amino acid sequence of SEQ ID NO: 23, or a variant thereof comprising up
 3000 to 5 amino acid substitutions; and a V_L comprising an LC-CDR1 comprising the amino
 acid sequence of SEQ ID NO: 32, an LC-CDR2 comprising the amino acid sequence of
 SEQ ID NO: 34, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO:
 37, or a variant thereof comprising up to 5 amino acid substitutions. In some
 embodiments, the anti-PcrV antibody comprises a V_H comprising the amino acid sequence
 3005 of SEQ ID NO: 56, or a variant thereof having at least 90% sequence identity to the amino
 acid sequence of SEQ ID NO: 56; and a V_L comprising the amino acid sequence of SEQ

ID NO: 67, or a variant thereof having at least 90% sequence identity to the amino acid sequence of SEQ ID NO: 67.

[00266] In some embodiments, there is provided a method of treating or preventing a
3010 *Pseudomonas* infection in an individual comprising administering to the individual an
effective amount of a composition comprising an anti-PcrV antibody, wherein the
anti-PcrV antibody competes with an antibody comprising: a V_H comprising an HC-CDR1
comprising the amino acid sequence of SEQ ID NO: 1, an HC-CDR2 comprising the
amino acid sequence of SEQ ID NO: 12, and an HC-CDR3 comprising the amino acid
3015 sequence of SEQ ID NO: 24; and a V_L comprising an LC-CDR1 comprising the amino
acid sequence of SEQ ID NO: 33, an LC-CDR2 comprising the amino acid sequence of
SEQ ID NO: 34, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 35.
In some embodiments, there is provided a method of treating or preventing a *Pseudomonas*
infection in an individual comprising administering to the individual an effective amount
3020 of a composition comprising an anti-PcrV antibody, wherein the anti-PcrV antibody binds
to the same epitope as an antibody comprising: a V_H comprising an HC-CDR1 comprising
the amino acid sequence of SEQ ID NO: 1, an HC-CDR2 comprising the amino acid
sequence of SEQ ID NO: 12, and an HC-CDR3 comprising the amino acid sequence of
SEQ ID NO: 24; and a V_L comprising an LC-CDR1 comprising the amino acid sequence
3025 of SEQ ID NO: 33, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 34,
and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 35.

[00267] In some embodiments, there is provided a method of treating or preventing a
Pseudomonas infection in an individual comprising administering to the individual an
effective amount of a composition comprising an anti-PcrV antibody, wherein the
3030 anti-PcrV antibody competes with an antibody comprising: a V_H comprising the amino
acid sequence of SEQ ID NO: 57 and a V_L comprising the amino acid sequence of SEQ ID
NO: 68. In some embodiments, there is provided a method of treating or preventing a
Pseudomonas infection in an individual comprising administering to the individual an
effective amount of a composition comprising an anti-PcrV antibody, wherein the
3035 anti-PcrV antibody binds to the same epitope as an antibody comprising: a V_H comprising
the amino acid sequence of SEQ ID NO: 57 and a V_L comprising the amino acid sequence
of SEQ ID NO: 68.

[00268] In some embodiments, there is provided a method of treating or preventing a
Pseudomonas infection in an individual comprising administering to the individual an

3040 effective amount of a composition comprising an anti-PcrV antibody comprising: a V_H
comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 1, an
HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 12, and an HC-CDR3
comprising the amino acid sequence of SEQ ID NO: 24, or a variant thereof comprising up
to 5 amino acid substitutions; and a V_L comprising an LC-CDR1 comprising the amino
3045 acid sequence of SEQ ID NO: 33, an LC-CDR2 comprising the amino acid sequence of
SEQ ID NO: 34, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 35,
or a variant thereof comprising up to 5 amino acid substitutions. In some embodiments,
the anti-PcrV antibody comprises a V_H comprising the amino acid sequence of SEQ ID NO:
57, or a variant thereof having at least 90% sequence identity to the amino acid sequence
3050 of SEQ ID NO: 57; and a V_L comprising the amino acid sequence of SEQ ID NO: 68, or a
variant thereof having at least 90% sequence identity to the amino acid sequence of SEQ
ID NO: 68.

[00269] In some embodiments, there is provided a method of treating or preventing a
Pseudomonas infection in an individual comprising administering to the individual an
3055 effective amount of a composition comprising an anti-PcrV antibody, wherein the
anti-PcrV antibody competes with an antibody comprising: a V_H comprising an HC-CDR1
comprising the amino acid sequence of SEQ ID NO: 2, an HC-CDR2 comprising the
amino acid sequence of SEQ ID NO: 10, and an HC-CDR3 comprising the amino acid
sequence of SEQ ID NO: 25; and a V_L comprising an LC-CDR1 comprising the amino
3060 acid sequence of SEQ ID NO: 32, an LC-CDR2 comprising the amino acid sequence of
SEQ ID NO: 34, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO:
37. In some embodiments, there is provided a method of treating or preventing a
Pseudomonas infection in an individual comprising administering to the individual an
effective amount of a composition comprising an anti-PcrV antibody, wherein the
3065 anti-PcrV antibody binds to the same epitope as an antibody comprising: a V_H comprising
an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 2, an HC-CDR2
comprising the amino acid sequence of SEQ ID NO: 10, and an HC-CDR3 comprising the
amino acid sequence of SEQ ID NO: 25; and a V_L comprising an LC-CDR1 comprising
the amino acid sequence of SEQ ID NO: 32, an LC-CDR2 comprising the amino acid
3070 sequence of SEQ ID NO: 34, and an LC-CDR3 comprising the amino acid sequence of
SEQ ID NO: 37.

[00270] In some embodiments, there is provided a method of treating or preventing a *Pseudomonas* infection in an individual comprising administering to the individual an effective amount of a composition comprising an anti-PcrV antibody, wherein the
3075 anti-PcrV antibody competes with an antibody comprising: a V_H comprising the amino acid sequence of SEQ ID NO: 58 and a V_L comprising the amino acid sequence of SEQ ID NO: 69. In some embodiments, there is provided a method of treating or preventing a *Pseudomonas* infection in an individual comprising administering to the individual an effective amount of a composition comprising an anti-PcrV antibody, wherein the
3080 anti-PcrV antibody binds to the same epitope as an antibody comprising: a V_H comprising the amino acid sequence of SEQ ID NO: 58 and a V_L comprising the amino acid sequence of SEQ ID NO: 69.

[00271] In some embodiments, there is provided a method of treating or preventing a *Pseudomonas* infection in an individual comprising administering to the individual an effective amount of a composition comprising an anti-PcrV antibody comprising: a V_H
3085 comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 2, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 10, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 25, or a variant thereof comprising up to 5 amino acid substitutions; and a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 32, an LC-CDR2 comprising the amino acid sequence of
3090 SEQ ID NO: 34, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 37, or a variant thereof comprising up to 5 amino acid substitutions. In some embodiments, the anti-PcrV antibody comprises a V_H comprising the amino acid sequence of SEQ ID NO: 58, or a variant thereof having at least 90% sequence identity to the amino acid sequence of SEQ ID NO: 58; and a V_L comprising the amino acid sequence of SEQ
3095 ID NO: 69, or a variant thereof having at least 90% sequence identity to the amino acid sequence of SEQ ID NO: 69.

[00272] In some embodiments, there is provided a method of treating or preventing a *Pseudomonas* infection in an individual comprising administering to the individual an effective amount of a composition comprising an anti-PcrV antibody, wherein the
3100 anti-PcrV antibody competes with an antibody comprising: a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 1, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 9, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 26; and a V_L comprising an LC-CDR1 comprising the amino

3105 acid sequence of SEQ ID NO: 32, an LC-CDR2 comprising the amino acid sequence of
 SEQ ID NO: 34, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO:
 35. In some embodiments, there is provided a method of treating or preventing a
Pseudomonas infection in an individual comprising administering to the individual an
 effective amount of a composition comprising an anti-PcrV antibody, wherein the
 3110 anti-PcrV antibody binds to the same epitope as an antibody comprising: a V_H comprising
 an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 1, an HC-CDR2
 comprising the amino acid sequence of SEQ ID NO: 9, and an HC-CDR3 comprising the
 amino acid sequence of SEQ ID NO: 26; and a V_L comprising an LC-CDR1 comprising
 the amino acid sequence of SEQ ID NO: 32, an LC-CDR2 comprising the amino acid
 3115 sequence of SEQ ID NO: 34, and an LC-CDR3 comprising the amino acid sequence of
 SEQ ID NO: 35.

[00273] In some embodiments, there is provided a method of treating or preventing a
Pseudomonas infection in an individual comprising administering to the individual an
 effective amount of a composition comprising an anti-PcrV antibody, wherein the
 3120 anti-PcrV antibody competes with an antibody comprising: a V_H comprising the amino
 acid sequence of SEQ ID NO: 59 and a V_L comprising the amino acid sequence of SEQ ID
 NO: 65. In some embodiments, there is provided a method of treating or preventing a
Pseudomonas infection in an individual comprising administering to the individual an
 effective amount of a composition comprising an anti-PcrV antibody, wherein the
 3125 anti-PcrV antibody binds to the same epitope as an antibody comprising: a V_H comprising
 the amino acid sequence of SEQ ID NO: 59 and a V_L comprising the amino acid sequence
 of SEQ ID NO: 65.

[00274] In some embodiments, there is provided a method of treating or preventing a
Pseudomonas infection in an individual comprising administering to the individual an
 effective amount of a composition comprising an anti-PcrV antibody comprising: a V_H
 3130 comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 1, an
 HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 9, and an HC-CDR3
 comprising the amino acid sequence of SEQ ID NO: 26, or a variant thereof comprising up
 to 5 amino acid substitutions; and a V_L comprising an LC-CDR1 comprising the amino
 acid sequence of SEQ ID NO: 32, an LC-CDR2 comprising the amino acid sequence of
 3135 SEQ ID NO: 34, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO:
 35, or a variant thereof comprising up to 5 amino acid substitutions. In some

embodiments, the anti-PcrV antibody comprises a V_H comprising the amino acid sequence of SEQ ID NO: 59, or a variant thereof having at least 90% sequence identity to the amino acid sequence of SEQ ID NO: 59; and a V_L comprising the amino acid sequence of SEQ ID NO: 65, or a variant thereof having at least 90% sequence identity to the amino acid sequence of SEQ ID NO: 65.

[00275] In some embodiments, there is provided a method of treating or preventing a *Pseudomonas* infection in an individual comprising administering to the individual an effective amount of a composition comprising an anti-PcrV antibody, wherein the anti-PcrV antibody competes with an antibody comprising: a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 3, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 10, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 27; and a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 32, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 34, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 35. In some embodiments, there is provided a method of treating or preventing a *Pseudomonas* infection in an individual comprising administering to the individual an effective amount of a composition comprising an anti-PcrV antibody, wherein the anti-PcrV antibody binds to the same epitope as an antibody comprising: a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 3, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 10, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 27; and a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 32, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 34, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 35.

[00276] In some embodiments, there is provided a method of treating or preventing a *Pseudomonas* infection in an individual comprising administering to the individual an effective amount of a composition comprising an anti-PcrV antibody, wherein the anti-PcrV antibody competes with an antibody comprising: a V_H comprising the amino acid sequence of SEQ ID NO: 60 and a V_L comprising the amino acid sequence of SEQ ID NO: 65. In some embodiments, there is provided a method of treating or preventing a *Pseudomonas* infection in an individual comprising administering to the individual an effective amount of a composition comprising an anti-PcrV antibody, wherein the anti-PcrV antibody binds to the same epitope as an antibody comprising: a V_H comprising

3170 the amino acid sequence of SEQ ID NO: 60 and a V_L comprising the amino acid sequence of SEQ ID NO: 65.

[00277] In some embodiments, there is provided a method of treating or preventing a *Pseudomonas* infection in an individual comprising administering to the individual an effective amount of a composition comprising an anti-PcrV antibody comprising: a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 3, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 10, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 27, or a variant thereof comprising up to 5 amino acid substitutions; and a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 32, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 34, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 35, or a variant thereof comprising up to 5 amino acid substitutions. In some embodiments, the anti-PcrV antibody provided herein comprises a V_H comprising the amino acid sequence of SEQ ID NO: 60, or a variant thereof having at least 90% sequence identity to the amino acid sequence of SEQ ID NO: 60; and a V_L comprising the amino acid sequence of SEQ ID NO: 65, or a variant thereof having at least 90% sequence identity to the amino acid sequence of SEQ ID NO: 65.

[00278] In some embodiments, there is provided a method of treating or preventing a *Pseudomonas* infection in an individual comprising administering to the individual an effective amount of a composition comprising an anti-PcrV antibody, wherein the anti-PcrV antibody competes with an antibody comprising: a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 1, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 10, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 28; and a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 32, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 34, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 35. In some embodiments, there is provided a method of treating or preventing a *Pseudomonas* infection in an individual comprising administering to the individual an effective amount of a composition comprising an anti-PcrV antibody, wherein the anti-PcrV antibody binds to the same epitope as an antibody comprising: a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 1, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 10, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 28; and a V_L comprising an LC-CDR1 comprising the amino acid sequence

of SEQ ID NO: 32, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 34, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 35.

3205 [00279] In some embodiments, there is provided a method of treating or preventing a *Pseudomonas* infection in an individual comprising administering to the individual an effective amount of a composition comprising an anti-PcrV antibody, wherein the anti-PcrV antibody competes with an antibody comprising: a V_H comprising the amino acid sequence of SEQ ID NO: 61 and a V_L comprising the amino acid sequence of SEQ ID
3210 NO: 65. In some embodiments, there is provided a method of treating or preventing a *Pseudomonas* infection in an individual comprising administering to the individual an effective amount of a composition comprising an anti-PcrV antibody, wherein the anti-PcrV antibody binds to the same epitope as an antibody comprising: a V_H comprising the amino acid sequence of SEQ ID NO: 61 and a V_L comprising the amino acid sequence
3215 of SEQ ID NO: 65.

[00280] In some embodiments, there is provided a method of treating or preventing a *Pseudomonas* infection in an individual comprising administering to the individual an effective amount of a composition comprising an anti-PcrV antibody comprising: a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 1, an
3220 HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 10, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 28, or a variant thereof comprising up to 5 amino acid substitutions; and a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 32, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 34, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 35,
3225 or a variant thereof comprising up to 5 amino acid substitutions. In some embodiments, the anti-PcrV antibody comprises a V_H comprising the amino acid sequence of SEQ ID NO: 61, or a variant thereof having at least 90% sequence identity to the amino acid sequence of SEQ ID NO: 61; and a V_L comprising the amino acid sequence of SEQ ID NO: 65, or a variant thereof having at least 90% sequence identity to the amino acid sequence of SEQ
3230 ID NO: 65.

[00281] In some embodiments, there is provided a method of treating or preventing a *Pseudomonas* infection in an individual comprising administering to the individual an effective amount of a composition comprising an anti-PcrV antibody, wherein the anti-PcrV antibody competes with an antibody comprising: a V_H comprising an HC-CDR1
3235 comprising the amino acid sequence of SEQ ID NO: 7, an HC-CDR2 comprising the

amino acid sequence of SEQ ID NO: 13, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 29; and a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 32, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 34, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 37. In some embodiments, there is provided a method of treating or preventing a *Pseudomonas* infection in an individual comprising administering to the individual an effective amount of a composition comprising an anti-PcrV antibody, wherein the anti-PcrV antibody binds to the same epitope as an antibody comprising: a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 7, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 13, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 29; and a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 32, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 34, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 37.

[00282] In some embodiments, there is provided a method of treating or preventing a *Pseudomonas* infection in an individual comprising administering to the individual an effective amount of a composition comprising an anti-PcrV antibody, wherein the anti-PcrV antibody competes with an antibody comprising: a V_H comprising the amino acid sequence of SEQ ID NO: 62 and a V_L comprising the amino acid sequence of SEQ ID NO: 67. In some embodiments, there is provided a method of treating or preventing a *Pseudomonas* infection in an individual comprising administering to the individual an effective amount of a composition comprising an anti-PcrV antibody, wherein the anti-PcrV antibody binds to the same epitope as an antibody comprising: a V_H comprising the amino acid sequence of SEQ ID NO: 62 and a V_L comprising the amino acid sequence of SEQ ID NO: 67.

[00283] In some embodiments, there is provided a method of treating or preventing a *Pseudomonas* infection in an individual comprising administering to the individual an effective amount of a composition comprising an anti-PcrV antibody comprising: a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 7, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 13, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 29, or a variant thereof comprising up to 5 amino acid substitutions; and a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 32, an LC-CDR2 comprising the amino acid sequence of

SEQ ID NO: 34, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 37, or a variant thereof comprising up to 5 amino acid substitutions. In some embodiments, the anti-PcrV antibody comprises a V_H comprising the amino acid sequence of SEQ ID NO: 62, or a variant thereof having at least 90% sequence identity to the amino acid sequence of SEQ ID NO: 62; and a V_L comprising the amino acid sequence of SEQ ID NO: 67, or a variant thereof having at least 90% sequence identity to the amino acid sequence of SEQ ID NO: 67.

[00284] In some embodiments, there is provided a method of treating or preventing a *Pseudomonas* infection in an individual comprising administering to the individual an effective amount of a composition comprising an anti-PcrV antibody, wherein the anti-PcrV antibody competes with an antibody comprising: a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 1, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 10, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 30; and a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 32, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 34, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 35. In some embodiments, there is provided a method of treating or preventing a *Pseudomonas* infection in an individual comprising administering to the individual an effective amount of a composition comprising an anti-PcrV antibody, wherein the anti-PcrV antibody binds to the same epitope as an antibody comprising: a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 1, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 10, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 30; and a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 32, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 34, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 35.

[00285] In some embodiments, there is provided a method of treating or preventing a *Pseudomonas* infection in an individual comprising administering to the individual an effective amount of a composition comprising an anti-PcrV antibody, wherein the anti-PcrV antibody competes with an antibody comprising: a V_H comprising the amino acid sequence of SEQ ID NO: 63 and a V_L comprising the amino acid sequence of SEQ ID NO: 65. In some embodiments, there is provided a method of treating or preventing a *Pseudomonas* infection in an individual comprising administering to the individual an

effective amount of a composition comprising an anti-PcrV antibody, wherein the anti-PcrV antibody binds to the same epitope as an antibody comprising: a V_H comprising the amino acid sequence of SEQ ID NO: 63 and a V_L comprising the amino acid sequence of SEQ ID NO: 65.

[00286] In some embodiments, there is provided a method of treating or preventing a *Pseudomonas* infection in an individual comprising administering to the individual an effective amount of a composition comprising an anti-PcrV antibody comprising: a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 1, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 10, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 30, or a variant thereof comprising up to 5 amino acid substitutions; and a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 32, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 34, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 35, or a variant thereof comprising up to 5 amino acid substitutions. In some embodiments, the anti-PcrV antibody comprises a V_H comprising the amino acid sequence of SEQ ID NO: 63, or a variant thereof having at least 90% sequence identity to the amino acid sequence of SEQ ID NO: 63; and a V_L comprising the amino acid sequence of SEQ ID NO: 65, or a variant thereof having at least 90% sequence identity to the amino acid sequence of SEQ ID NO: 65.

[00287] In some embodiments, there is provided a method of treating or preventing a *Pseudomonas* infection in an individual comprising administering to the individual an effective amount of a composition comprising an anti-PcrV antibody, wherein the anti-PcrV antibody competes with an antibody comprising: a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 8, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 14, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 31; and a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 32, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 34, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 37.

In some embodiments, there is provided a method of treating or preventing a *Pseudomonas* infection in an individual comprising administering to the individual an effective amount of a composition comprising an anti-PcrV antibody, wherein the anti-PcrV antibody binds to the same epitope as an antibody comprising: a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 8, an HC-CDR2 comprising the amino acid

- 3335 sequence of SEQ ID NO: 14, and an HC-CDR3 comprising the amino acid sequence of
SEQ ID NO: 31; and a V_L comprising an LC-CDR1 comprising the amino acid sequence
of SEQ ID NO: 32, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 34,
and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 37.
- [00288] In some embodiments, there is provided a method of treating or preventing a
3340 *Pseudomonas* infection in an individual comprising administering to the individual an
effective amount of a composition comprising an anti-PcrV antibody, wherein the
anti-PcrV antibody competes with an antibody comprising: a V_H comprising the amino
acid sequence of SEQ ID NO: 64 and a V_L comprising the amino acid sequence of SEQ ID
NO: 67. In some embodiments, there is provided a method of treating or preventing a
3345 *Pseudomonas* infection in an individual comprising administering to the individual an
effective amount of a composition comprising an anti-PcrV antibody, wherein the
anti-PcrV antibody binds to the same epitope as an antibody comprising: a V_H comprising
the amino acid sequence of SEQ ID NO: 64 and a V_L comprising the amino acid sequence
of SEQ ID NO: 67.
- 3350 [00289] In some embodiments, there is provided a method of treating or preventing a
Pseudomonas infection in an individual comprising administering to the individual an
effective amount of a composition comprising an anti-PcrV antibody comprising: a V_H
comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 8, an
HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 14, and an HC-CDR3
3355 comprising the amino acid sequence of SEQ ID NO: 31, or a variant thereof comprising up
to 5 amino acid substitutions; and a V_L comprising an LC-CDR1 comprising the amino
acid sequence of SEQ ID NO: 32, an LC-CDR2 comprising the amino acid sequence of
SEQ ID NO: 34, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO:
37, or a variant thereof comprising up to 5 amino acid substitutions. In some
3360 embodiments, the anti-PcrV antibody comprises a V_H comprising the amino acid sequence
of SEQ ID NO: 64, or a variant thereof having at least 90% sequence identity to the amino
acid sequence of SEQ ID NO: 64; and a V_L comprising the amino acid sequence of SEQ
ID NO: 67, or a variant thereof having at least 90% sequence identity to the amino acid
sequence of SEQ ID NO: 67.
- 3365 [00290] In some embodiments, there is provided a method of treating or preventing a
Pseudomonas infection in an individual comprising administering to the individual an
effective amount of a composition comprising an anti-PcrV antibody, wherein the

anti-PcrV antibody competes with an antibody comprising: a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 7, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 13, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 29; and a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 32, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 34, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 38. In some embodiments, there is provided a method of treating or preventing a *Pseudomonas* infection in an individual comprising administering to the individual an effective amount of a composition comprising an anti-PcrV antibody, wherein the anti-PcrV antibody binds to the same epitope as an antibody comprising: a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 7, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 13, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 29; and a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 32, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 34, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 38.

[00291] In some embodiments, there is provided a method of treating or preventing a *Pseudomonas* infection in an individual comprising administering to the individual an effective amount of a composition comprising an anti-PcrV antibody, wherein the anti-PcrV antibody competes with an antibody comprising: a V_H comprising the amino acid sequence of SEQ ID NO: 62 and a V_L comprising the amino acid sequence of SEQ ID NO: 70. In some embodiments, there is provided a method of treating or preventing a *Pseudomonas* infection in an individual comprising administering to the individual an effective amount of a composition comprising an anti-PcrV antibody, wherein the anti-PcrV antibody binds to the same epitope as an antibody comprising: a V_H comprising the amino acid sequence of SEQ ID NO: 62 and a V_L comprising the amino acid sequence of SEQ ID NO: 70.

[00292] In some embodiments, there is provided a method of treating or preventing a *Pseudomonas* infection in an individual comprising administering to the individual an effective amount of a composition comprising an anti-PcrV antibody comprising: a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 7, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 13, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 29, or a variant thereof comprising up to 5 amino acid substitutions; and a V_L comprising an LC-CDR1 comprising the amino

acid sequence of SEQ ID NO: 32, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 34, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 38, or a variant thereof comprising up to 5 amino acid substitutions. In some embodiments, the anti-PcrV antibody comprises a V_H comprising the amino acid sequence of SEQ ID NO: 62, or a variant thereof having at least 90% sequence identity to the amino acid sequence of SEQ ID NO: 62; and a V_L comprising the amino acid sequence of SEQ ID NO: 70, or a variant thereof having at least 90% sequence identity to the amino acid sequence of SEQ ID NO: 70

[00293] In some embodiments, there is provided a method of treating or preventing a *Pseudomonas* infection in an individual comprising administering to the individual an effective amount of a composition comprising an anti-PcrV antibody, wherein the anti-PcrV antibody described herein specifically binds to an epitope on *Pseudomonas* PcrV, where the epitope comprises any one of 1, 2, 3, 4, 5 or 6 amino acid residues selected from the group of Gln160, Asp165, Asp170, Asp173, Thr175, and Ser202 of *Pseudomonas* PcrV, according to SEQ ID No: 71. In some embodiments, the anti-PcrV antibody in the composition competes with an antibody that binds to any one of 1, 2, 3, 4, 5, or 6 amino residues selected from the group of Gln160, Asp165, Asp170, Asp173, Thr175, and Ser202 of *Pseudomonas* PcrV, according to SEQ ID No: 71.

[00294] In some embodiments according to any of the methods of treatment or prevention described herein, the anti-PcrV antibody comprises an antibody heavy chain constant region and an antibody light chain constant region. In some embodiments, the anti-PcrV antibody comprises an IgG1 heavy chain constant region. In some embodiments, the anti-PcrV antibody comprises an IgG2 heavy chain constant region. In some embodiments, the anti-PcrV antibody comprises an IgG3 heavy chain constant region. In some embodiments, the anti-PcrV antibody comprises an IgG4 heavy chain constant region. In some embodiments, the IgG is a human IgG. In some embodiments, the heavy chain constant region comprises or consists of the amino acid sequence of SEQ ID NO: 73. In some embodiments, the heavy chain constant region comprises or consists of the amino acid sequence of SEQ ID NO: 74. In some embodiments, the anti-PcrV comprises a lambda light chain constant region. In some embodiments, the anti-PcrV antibody comprises a kappa light chain constant region. In some embodiments, the light chain constant region comprises or consists of the amino acid sequence of SEQ ID NO: 72. In

some embodiments, the anti-PcrV antibody comprises an antibody heavy chain variable domain and an antibody light chain variable domain.

3435 [00295] In some embodiments according to any of the methods of treatment or prevention described herein, the method further provides therapeutic or prophylactic effect on diseases and/or conditions associated with *Pseudomonas* infection. In some embodiments, the method prevents a *Pseudomonas* infection in an individual.

[00296] In some embodiments, the individual is a mammal (*e.g.*, human, non-human
3440 primate, rat, mouse, cow, horse, pig, sheep, goat, dog, cat, *etc.*). In some embodiments, the individual is a human. In some embodiments, the individual is a clinical patient, a clinical trial volunteer, an experimental animal, *etc.* In some embodiments, the individual is younger than about 60 years old (including for example younger than about any of 50, 40, 30, 25, 20, 15, or 10 years old). In some embodiments, the individual is older than about 60
3445 years old (including for example older than about any of 70, 80, 90, or 100 years old).

[00297] In some embodiments, the individual has one or more risk factors associated with *P. aeruginosa* infection. For example, in some embodiments, the individual has exposed or breached skin mucuous layer. In some embodiments, the individual has one or more burn wounds. In some embodiments, the individual has one or more surgery wounds. In
3450 some embodiments, the individual has skin disease. In some embodiments, the individual is inserted with a foreign body, such as, but not limited to a mechanical ventilator or catheter. In some embodiments, the individual is diagnosed with or genetically prone to immunodeficiency diseases, including but not limited to HIV infection, AIDS and/or neutrophil deficiency. In some embodiments, the individual has received one or more
3455 forms of chemotherapy. In some embodiments, the individual has received one or more forms of glucocorticoid treatment. In some embodiments, the individual has received one or more forms of chemotherapy. In some embodiments, the individual is diagnosed with or genetically prone to cancer, diabetes and/or chronic structural lung diseases (such as cystic fibrosis or COPD). In some embodiments, the individual is diagnosed with or
3460 genetically prone to flora imbalance in digestive system and/or in other organs. In some embodiments, the individual has one or more risk factors associated with one or more diseases or disorders described herein.

[00298] The present application in some embodiments provides a method of delivering an anti- PcrV antibody (such as any one of the anti- PcrV antibodies described herein, *e.g.*, an
3465 isolated anti- PcrV antibody) to a cell infected by a pathogen in an individual, the method

comprising administering to the individual a composition comprising the anti- PcrV antibody.

[00299] In some embodiments according to any one of the methods described herein, the method further comprises administering one or more additional therapeutic agents. In some
 3470 some embodiments, at least one of the therapeutic agents is an antibiotic. In some embodiments, the antibiotic is a penicillin, a cephalosporin, a carbapenem, a fluoroquinolone, an aminoglycoside, a monobactam, a polymyxin, an antibiotic combination containing β -lactamase inhibitor, or any combinations thereof. In some
 3475 embodiments, the antibiotic is Cefepim, Ceftazidime, Cefpirome, Imipenem, Meropenem, Ticarcillin, Piperacillin, Azlocillin, Carbenicillin, Mezlocillin, Aztreonam, Tobramycin, Gentamicin, Amikacin, Ciprofloxacin, Levofloxacin, Cefoperazon-Sulbactam, Piperacillin-Tazobactam, Fosfomycin, or any combinations thereof. In some
 3480 embodiments, the antibiotic is one or more of Imipenem, Tobramycin, Ciprofloxacin, Meropenem or Aztreonam. In some embodiments, the antibiotic is one or more of Gentamycin, Ampicillin or Kanamycin.

[00300] Many diagnostic methods for infectious agents exhibiting PcrV expression and the clinical delineation of those diseases are known in the art. Such methods include, but are not limited to, *e.g.*, immunohistochemistry, PCR, and fluorescent in situ hybridization (FISH).

3485 [00301] In some embodiments, the anti- PcrV antibodies (*e.g.*, full-length anti- PcrV antibodies) and/or compositions of the application are administered in combination with a second, third, or fourth agent (including, *e.g.*, an antibiotic) to treat or prevent diseases or disorders involving PcrV -expressing pathogens.

3490 ***Dosing and Method of Administering the anti-PCR V antibodies***

[00302] The dose of the anti-PCR V antibody (such as isolated anti-PCR V antibody) compositions administered to an individual (such as a human) may vary with the particular composition, the mode of administration, and the type of disease being treated. In some
 3495 embodiments, the amount of the composition (such as composition comprising isolated anti-PCR V antibody) is effective to result in an objective response (such as a partial response or a complete response) in the treatment or prevention of *Pseudomonas* infections. In some embodiments, the amount of the anti- PcrV antibody composition is sufficient to result in a complete response in the individual. In some embodiments, the amount of the

anti- PcrV antibody composition is sufficient to result in a partial response in the individual.

3500 In some embodiments, the amount of the anti- PcrV antibody composition administered (for example when administered alone) is sufficient to produce an overall response rate of more than about any of 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 64%, 65%, 70%, 75%, 80%, 85%, or 90% among a population of individuals treated with the anti-PCRv antibody composition. Responses of an individual to the treatment or
3505 prevention by the methods described herein can be determined, for example, based on detection of *Pseudomonas* by methods such as by Gram stains or other phenotypic tests.

[00303] In some embodiments, the amount of the composition (such as composition comprising isolated anti- PcrV antibody) is sufficient to prolong progress-free survival of the individual. In some embodiments, the amount of the composition is sufficient to
3510 prolong overall survival of the individual. In some embodiments, the amount of the composition (for example when administered along) is sufficient to produce clinical benefit of more than about any of 50%, 60%, 70%, or 77% among a population of individuals treated with the anti- PcrV antibody composition.

[00304] In some embodiments, the amount of the composition (such as composition comprising isolated anti- PcrV antibody), alone or in combination with a second, third, and/or fourth agent, is an amount sufficient to decrease the number of *Pseudomonas* organ
3515 burden by at least about any of 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95% or 100% compared to the corresponding organ burden in the same subject prior to treatment or compared to the corresponding activity in other subjects not receiving the treatment.

3520 Standard methods can be used to measure the magnitude of this effect, such as *in vitro* assays with purified enzyme, cell-based assays, animal models, or human testing.

[00305] In some embodiments, the amount of the anti- PcrV antibody (such as a full-length anti-PcrV antibody) in the composition is below the level that induces a toxicological effect (*i.e.*, an effect above a clinically acceptable level of toxicity) or is at a
3525 level where a potential side effect can be controlled or tolerated when the composition is administered to the individual.

[00306] In some embodiments, the amount of the composition is close to a maximum tolerated dose (MTD) of the composition following the same dosing regimen. In some
embodiments, the amount of the composition is more than about any of 80%, 90%, 95%,
3530 or 98% of the MTD.

[00307] In some embodiments, the amount of an anti-PcrV antibody (such as a full-length anti-PcrV antibody) in the composition is included in a range of about 0.001 μg to about 1000 μg .

[00308] In some embodiments, the composition or method further comprises one or more antibiotics. In some embodiments, the amount of an antibiotic (such as Imipenem, Tobramycin, Ciprofloxacin, Meropenem, Aztreonam, Ticarcillin, Piperacillin, Azlocillin, Carbenicillin, Mezlocillin, Gentamycin or Amikacin) in the composition is included in a range of about 0.001 μg to about 1000 μg .

[00309] In some embodiments of any of the above aspects, the effective amount of anti-PcrV antibody (such as a full-length anti-PcrV antibody) in the composition is in the range of about 0.1 $\mu\text{g}/\text{kg}$ to about 100 mg/kg of total body weight.

[00310] In some embodiments of any of the above aspects, the effective amount of antibiotic (such as Imipenem, Tobramycin, Ciprofloxacin, Meropenem, Aztreonam, Ticarcillin, Piperacillin, Azlocillin, Carbenicillin, Mezlocillin, Gentamycin or Amikacin) in the composition is in the range of about 0.1 $\mu\text{g}/\text{kg}$ to about 100 mg/kg of total body weight.

[00311] The anti-PcrV antibody compositions can be administered to an individual (such as human) via various routes, including, for example, intravenous, intra-arterial, intraperitoneal, intrapulmonary, oral, inhalation, intravesicular, intramuscular, intra-tracheal, subcutaneous, intraocular, intrathecal, transmucosal, and transdermal. In some embodiments, sustained continuous release formulation of the composition may be used. In some embodiments, the composition is administered intravenously. In some embodiments, the composition is administered intraportally. In some embodiments, the composition is administered intraarterially. In some embodiments, the composition is administered intraperitoneally. In some embodiments, the composition is administered intrahepatically. In some embodiments, the composition is administered by hepatic arterial infusion. In some embodiments, the administration is to an injection site distal to a first disease site.

Articles of Manufacture and Kits

[00312] In some embodiments of the application, there is provided an article of manufacture containing materials useful for treating or preventing a *Pseudomonas* infection in an individual, or for delivering an anti-PcrV antibody (such as a full-length

anti-PcrV antibody) to a cell attached by a pathogen expressing PcrV. The article of manufacture can comprise a container and a label or package insert on or associated with the container. Suitable containers include, for example, bottles, vials, syringes, etc. The containers may be formed from a variety of materials such as glass or plastic. Generally, the container holds a composition which is effective for treating a disease or disorder described herein, and may have a sterile access port (for example the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). At least one active agent in the composition is an anti-PcrV antibody of the application. The label or package insert indicates that the composition is used for treating the particular condition. The label or package insert will further comprise instructions for administering the anti-PcrV antibody composition to the patient. Articles of manufacture and kits comprising combinatorial therapies described herein are also contemplated.

[00313] Package insert refers to instructions customarily included in commercial packages of therapeutic products that contain information about the indications, usage, dosage, administration, contraindications and/or warnings concerning the use of such therapeutic products. In some embodiments, the package insert indicates that the composition is used for treating bacterial infections. In some embodiments, the package insert indicates that the composition is used for treating *Pseudomonas* infections.

[00314] Additionally, the article of manufacture may further comprise a second container comprising a pharmaceutically-acceptable buffer, such as bacteriostatic water for injection (BWFI), phosphate-buffered saline, Ringer's solution and dextrose solution. It may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, and syringes.

[00315] Kits are also provided that are useful for various purposes, *e.g.*, useful for treating or preventing a *Pseudomonas* infection in an individual, or for delivering an anti-PcrV antibody (such as a full-length anti-PcrV antibody) to a cell attached by a pathogen expressing PcrV, optionally in combination with the articles of manufacture. Kits of the application include one or more containers comprising anti-PcrV antibody composition (or unit dosage form and/or article of manufacture), and in some embodiments, further comprise another agent (such as the agents described herein) and/or instructions for use in accordance with any of the methods described herein. The kit may further comprise a description of selection of individuals suitable for treatment. Instructions supplied in the kits of the application are typically written instructions on a label or package insert (*e.g.*, a

paper sheet included in the kit), but machine-readable instructions (*e.g.*, instructions carried on a magnetic or optical storage disk) are also acceptable.

[00316] For example, in some embodiments, the kit comprises a composition comprising an anti-PcrV antibody (such as a full-length anti-PcrV antibody). In some embodiments, 3600 the kit comprises a) a composition comprising any one of the anti-PcrV antibodies described herein, and b) an effective amount of at least one other agent, wherein the other agent enhances the effect (*e.g.*, treatment effect, detecting effect) of the anti-PcrV antibody. In some embodiments, the kit comprises a) a composition comprising any one of the anti-PcrV antibodies described herein, and b) instructions for administering the anti-PcrV 3605 antibody composition to an individual for treating a *Pseudomonas* infection in an individual. In some embodiments, the kit comprises a) a composition comprising any one of the anti-PcrV antibodies described herein, b) an effective amount of at least one other agent, wherein the other agent enhances the effect (*e.g.*, treatment effect, detecting effect) of the anti-PcrV antibody, and c) instructions for administering the anti-PcrV 3610 composition and the other agent(s) to an individual for useful for treating a *Pseudomonas* infection in an individual. The anti-PcrV antibody and the other agent(s) can be present in separate containers or in a single container. For example, the kit may comprise one distinct composition or two or more compositions wherein one composition comprises an anti-PcrV antibody and another composition comprises another agent.

3615 [00317] In some embodiments, the kit comprises a nucleic acid (or set of nucleic acids) encoding an anti-PcrV antibody (such as a full-length anti-PcrV antibody). In some embodiments, the kit comprises a) a nucleic acid (or set of nucleic acids) encoding an anti-PcrV antibody, and b) a host cell for expressing the nucleic acid (or set of nucleic acids). In some embodiments, the kit comprises a) a nucleic acid (or set of nucleic acids) 3620 encoding an anti-PcrV antibody, and b) instructions for i) expressing the anti-PcrV antibody in a host cell, ii) preparing a composition comprising the anti-PcrV antibody, and iii) administering the composition comprising the anti-PcrV antibody to an individual for treating or preventing a *Pseudomonas* infection in an individual. In some embodiments, the kit comprises a) a nucleic acid (or set of nucleic acids) encoding an anti-PcrV antibody, b) 3625 a host cell for expressing the nucleic acid (or set of nucleic acids), and c) instructions for i) expressing the anti-PcrV antibody in the host cell, ii) preparing a composition comprising the anti-PcrV antibody, and iii) administering the composition comprising the anti-PcrV

antibody to an individual for treating or preventing a *Pseudomonas* infection in an individual.

3630 [00318] The kits of the application are in suitable packaging. Suitable packaging includes, but is not limited to, vials, bottles, jars, flexible packaging (*e.g.*, sealed Mylar or plastic bags), and the like. Kits may optionally provide additional components such as buffers and interpretative information. The present application thus also provides articles of manufacture, which include vials (such as sealed vials), bottles, jars, flexible packaging, 3635 and the like.

[00319] The instructions relating to the use of the anti-PcrV antibody compositions generally include information as to dosage, dosing schedule, and route of administration for the intended treatment. The containers may be unit doses, bulk packages (*e.g.*, multi-dose packages) or sub-unit doses. For example, kits may be provided that contain 3640 sufficient dosages of an anti-PcrV antibody (such as a full-length anti-PcrV antibody) as disclosed herein to provide effective treatment of an individual for an extended period, such as any of a week, 8 days, 9 days, 10 days, 11 days, 12 days, 13 days, 2 weeks, 3 weeks, 4 weeks, 6 weeks, 8 weeks, 3 months, 4 months, 5 months, 7 months, 8 months, 9 months, or more. Kits may also include multiple unit doses of the anti-PcrV antibody and 3645 pharmaceutical compositions and instructions for use and packaged in quantities sufficient for storage and use in pharmacies, for example, hospital pharmacies and compounding pharmacies.

[00320] Those skilled in the art will recognize that several embodiments are possible within the scope and spirit of this application. The application will now be described in 3650 greater detail by reference to the following non-limiting examples. The following examples further illustrate the application but, of course, should not be construed as in any way limiting its scope.

EXAMPLES

3655 **Example 1: Generation of recombinant *Pseudomonas* PcrV and selection of anti-PcrV scFv antibodies**

Generation of recombinant Pseudomonas PcrV

[00321] The full-length sequence of PcrV (PAO1) was synthesized (Generay, Shanghai) and subcloned into the expression vector pET using restriction enzyme recognition sites

NdeI and BamHI. His-tag or other conventionally used tags were used to tag PcrV.

3660 Expression vectors pET-6his -PcrV, pET-6his-Avi-PcrV were generated. In these constructs, “his” stands for His-tag, and “Avi” stands for Avidin tag. The expression and purification of recombinant PcrV including pET-6his -PcrV, pET-6His-Avi-PcrV were carried out according to manufacturer’s protocol. Briefly, *E. Coli* cells were transformed with the expression vectors, and the cells were induced with IPTG and cultured at 25°C, 3665 and 220rpm overnight. Subsequently, the *E. Coli* cells were sonicated, the cell debris were pelleted and removed, and the proteins were separated by centrifugation.

[00322] Proteins expressing his-tag were then purified using Ni Sepharose purification according to manufacturer’s protocol. Specifically, the Qiagen Ni-NTA superflow cartridges were used for immobilized metal affinity chromatography (IMAC) analysis. The 3670 cartridges were first equilibrated with buffer A1 (50mM Na₃PO₄, 0.15M NaCl, pH 7.2) with a flow rate of 150cm/h. The pH of the supernatant of the culture media was adjusted to 7.2 and flown through the cartridges at room temperature at 150cm/h. Next, buffer A1 (6 times the volume of that of the cartridges) was used to equilibrate the cartridges at 150cm/h. A 50mM PB solution (0.15M NaCl and 0.2M Imidazole, pH 7.2) with a volume 3675 that is 10 times that of the cartridges was used to wash the cartridges and the elution was collected.

Generation of biotinylated PcrV antigen

[00323] Biotinylation of 6His-Avi-PcrV using the biotin ligase B0101A (GENECOPOEIA™) was carried out according to the manufacturer’s protocol. Briefly, 3680 buffer A1B and Biotin Ligase were added to 6His-Avi-PcrV, followed by 1 hours of incubation at 30°C. The biotinylated PcrV is referred to as Bhavi-PcrV hereafter. The efficiency of biotinylation was measured using ELISA. Briefly, Bhavi-PcrV was removed by incubating with magnetic beads (Dynabeads™ MyOne™ Streptavidin T1), and the concentration of 6His-Avi-PcrV in supernatant was quantified by ELISA, using 3685 6His-Avi-PcrV with known concentration as a standard material. The biotinylation efficiency was determined to be 90%.

Selection of anti-PcrV scFv antibodies

[00324] *Generation of yeast scFv antibody display library*: RNA collected from 2000 human blood samples was reverse-transcribed into cDNA, and the VH and VK fragments 3690 were amplified using VH- and VK-specific primers. Upon gel extraction and purification, scFvs were generated by linking VH and VK via a linker. The scFvs were cloned into the

yeast display plasmid PYD1, which were then electroporated into yeast to generate the yeast scFv antibody display library.

Selection of anti-PcrV scFv antibodies as determined by PcrV binding:

3695 [00325] The scFvs which recognized PcrV were isolated from the yeast display library. Briefly, magnetic-activated cell sorting (MACS) was used to enrich for cells expressing anti-PcrV scFv antibodies. Bhavi-PcrV was mixed with magnetic beads (Dynabeads™ MyOne™ Streptavidin T1) overnight to coat the biotinylated PcrV onto magnetic beads, according to manufacturer's protocol. The scFv antibody yeast library was then mixed with
3700 the PcrV coated beads for enriching yeast that display PcrV-recognizing antibodies, whereas the non-binders are washed away in washing steps. Subsequently, the collected yeast cells were labeled with PcrV proteins, and sorted by FACS to select for yeast displaying antibodies with high affinity to PcrV. The FACS-mediated selection was repeated 2- 3 cycles. The selected yeast library cells were plated on agar, and single
3705 colonies were picked and assayed by further FACs analysis. From yeast clones that exhibited positive binding to PcrV, each scFV gene was subcloned into a prokaryotic expression vector and fused with a 6-his tag. The His-labeled scFv were then purified using Ni Sepharose purification as described above. A panel of positive scFv antibodies was obtained at the end of the selection process, and subjected to functional testing for the
3710 ability to inhibit RBC lysis caused by *P. aeruginosa*.

Analysis of anti-PcrV scFv candidates as determined by RBC lysis inhibition

[00326] Monoclonal scFv antibodies were selected and assessed for biological activity by RBC lysis assay. Briefly, red blood cells (RBCs) were prepared from fresh whole human or rabbit blood by centrifugation, supplemented with EDTA and rinsed with multiple
3715 phosphate-buffered saline (PBS) washes. Washed RBCs (2.5% [vol/vol] final) in Dulbecco's modified Eagle's medium (DMEM) plus 10% fetal bovine serum (FBS) (Gibco) and purified anti-PcrV antibodies diluted in PBS were combined into wells of a round-bottom 96-well plate. Strain 57/66(O6) *P. aeruginosa* was grown to mid-log phase in 2× YT medium (Oxford), harvested by centrifugation, and resuspended in DMEM-fetal
3720 bovine serum (FBS) at an optical density at 600 nm (OD600) of 0.15. Ten microliters of bacterial suspension was added to the RBC-antibody mixture, mixed by agitation, and incubated for 3 hours at 37°C and 5% CO₂. The plates were briefly centrifuged (1000 rpm, 1 min) to pellet the intact RBCs, the supernatants transferred to a flat-bottom 96-well plate,

and the OD405 was measured to detect any lysis, from which the relative amount of lysis inhibition was calculated and plotted. IC₅₀ values for the antibodies were also determined.

Example 2: Generation and characterization of full-length human anti-PcrV antibodies

Generation of full-length anti-PcrV antibodies

[00327] The most potent scFv antibodies were reformatted as human IgG1 antibody molecules with a human IgG1 heavy chain constant domain, and a human kappa light chain constant domain. VL and VH were amplified from the prokaryotic expression vector and introduced into eukaryotic expression vectors pTT5-L (containing kappa constant domain) and pTT5-H1 (containing IgG1 heavy chain constant domain). Plasmids expressing the light and heavy chains were extracted and used to transfect 293F cells. After the cells were cultured at 37°C, 8% CO₂ and 120rpm for 5 days, the antibodies in the culture media were purified using Protein A affinity chromatography.

[00328] Briefly, Protein A column was first equilibrated with a PBS buffer containing 50mM PBS and 0.15M NaCl (pH7.2), at a flow rate of 150cm/h and with a volume that is six times the volume of the column. The supernatant of the culture media (pH was adjusted to 7.2) was passed through the column at 150cm/h. Upon further equilibration, the column was washed off using 50mM sodium citrate (pH3.5) and the elution containing anti-PcrV antibodies was collected. The full length antibodies were functionally characterized for the ability to inhibit RBC lysis (see Example 1 for reference), A549/ U937 cell lysis, as well as survival improvement in an acute pneumonia mouse model, according to the methods in Example 1 and the methods described below.

Analysis of anti-PcrV candidates as determined by A549 or U937 lysis inhibition

[00329] To assay for ability to inhibit cytotoxicity and cell lysis caused by *P. aeruginosa*, the candidate anti-PcrV antibodies were added to human bronchoepithelial cell line A549 or human histiocytic lymphoma cell line U937 seeded in white 96-well plates (Nunc Nunclon Delta) in DMEM plus 10% fetal bovine serum. Log-phase strain PA103(O11) *P. aeruginosa* was added at a multiplicity of infection (MOI) of 10 and incubated for 2 h at 37°C and 5% CO₂, followed by 20 minutes of equilibration at room temperature. Lactate dehydrogenase (LDH) released from lysed cells was quantified using the CytoTox-ONE

3755 kit (Promega), to assay membrane integrity. The relative amount of lysis inhibition was plotted accordingly. IC₅₀ values for the antibodies were also determined.

Analysis of anti-PcrV candidates as determined in mouse acute pneumonia model

[00330] The candidate anti-PcrV antibodies were assayed for the ability to improve survival in a mouse acute pneumonia model. In a prophylactic model, 7-8 week old
3760 BALB/c mice (Vital River Laboratory) were administered the candidate antibodies or PBS intraperitoneally (i.p.) 24 hr before infection, at doses of 1, 5, 10 or 25 mg/kg mouse weight. To generate an acute pneumonia model, which was performed as described previously (DiGiandomenico et al., 2007, *Proc. Natl. Acad. Sci. U. S. A.*, 104:4624–4629), the BALB/c mice were intranasally inoculated with *P. aeruginosa* (PA103 strain)
3765 suspended in a 40µl inoculum at the lethal dose, or double the lethal dose (at 8×10^5 - 1.6×10^6 CFU; 1* or 2* LD₉₀). Mouse survival was recorded for up to 7 days post-infection.

13-42 mAb as lead antibody for further optimization

[00331] Out of the full-length antibodies that were generated, 13-42 monoclonal antibody
3770 was selected as the lead parent antibody, as determined by its ability to inhibit RBC lysis, A549 or U937 cell lysis, as well as the ability to improve survival in an acute pneumonia mouse model.

Example 3: Generation and characterization of optimized full-length anti-PcrV antibodies

3775 [00332] Out of the full-length antibodies that were generated, 13-42 was selected as the lead parental antibody for further optimization. In particular, 13-42 was optimized to remove free cysteine residues and glycosylation sites in CDR3 region of the heavy chain (HC-CDR3), as well as to improve homology to human germ line by selectively engineering 11 amino acid residues in HC-CDR1, HC-CDR2, and LC-CDR3. Two
3780 methodologies were employed to optimize 13-42 derived antibodies.

[00333] In the first method, the loci encoding the two cysteines in HC-CDR3 were first mutated to encode stop codons to generate an optimization template. Subsequently, the mutated loci encoding the stop codons (previously encoding the two cysteines) were randomly mutated to any of the 20 amino acids. At the same time, the glycosylation sites
3785 (*i.e.*, NSS) in HC-CDR3 were eliminated by mutating either Asn (N) or Ser (S) in any NXS sequences in HC-CDR3; and the locus for each of the remaining amino acid in

CDR-H3 was randomly mutated one at a time. Finally, the non-human amino acid sequences in CDR-L3, CDR-H1 and CDR-H2 were mutated to improve homology to human sequences.

3790 **[00334]** In an alternative scheme, the loci encoding the two cysteines in HC-CDR3 were first mutated to encode stop codons to generate an optimization template. Subsequently, the mutated loci encoding the stop codons (previously encoding the two cysteines) were randomly mutated to any of the 20 amino acids. At the same time, the loci for all amino acids previously flanked by the cysteines in CDR-H3 were randomly mutated

3795 simultaneously. Finally, the non-human amino acid sequences in CDR-L3, CDR-H1 and CDR-H2 were mutated to improve homology to human sequences.

[00335] Starting with the scFv of 13-42, a phage scFv display library containing mutations in the CDR regions was generated as described above. Variants that were able to bind PcrV with high affinity and with low dissociation rate were identified using ELISA or

3800 BLI, and their functions were tested for inhibition on RBC lysis and A549 cell lysis. The scFv antibodies optimized from 13-42 showing comparable or improved biological activity compared to the parental 13-42 scFv were used to generate full-length antibodies. A panel of optimized full-length antibodies was obtained at the end of the selection.

[00336] The optimized antibodies were tested for PcrV binding as assayed by ELISA.

3805 For the ELISA experiment, synthetic PcrV was used to coat the wells of a 96-well plate. On the following day, after washing with PBST, blocking with 200 μ L PBS-milk for an hour, and another wash with PBST, the antibodies were added and incubated for an hour at 37°C. The plate was washed with 0.1% TBST for 6 times before 100 μ L of Goat-anti-human Fc antibody-AP (1:3000 in PBS) was added to each well and incubated

3810 for an hour. After washing with 0.1% TBST for 6 times, 50 μ L of pNPP was added to each well and color was developed for 10-20 minutes at 37°C. The signals were read by a microplate reader at 410nm. The ELISA results (OD410) were analyzed and the EC50 values were calculated, as shown in Table 5.

Table 5: Binding affinity of Optimized anti-PcrV mAb clones to PcrV				
Optimized anti-PcrV mAb clones	ELISA assay EC50(ug/ml)		Optimized anti-PcrV mAb clones	ELISA assay EC50(ug/ml)
4A10	0.1133		2B1	0.1624
7B1	0.1252		7B2	0.09672

7C1	0.1337		3H10	0.1488
8C1	0.1107		3G3	0.09364
6A10	0.1043		3A11	0.09516
5B3	0.1094		3A9	0.1161
3B12	0.1178		13-42	0.1316

3815 [00337] The binding kinetics and affinity of optimized anti-PcrV mAb clones 7B1, 7C1, 8C1, 6A10, 5B3, 3B12, 4A10, 2B1, 7B2, 3H10, 3G3, 3A11, and 3A9 to PcrV were also tested by BLI and shown in Table 6.

Table 6: Binding affinity of Optimized anti-PcrV mAb clones to PcrV			
Optimized anti-PcrV mAb clones	kd(M)	Optimized anti-PcrV mAb clones	kd(M)
7B1	<1.0E-12	7B2	8.75E-11
7C1	1.01E-10	3H10	7.79E-10
8C1	3.30E-10	3G3	3.81E-10
6A10	2.04E-10	3A11	1.26E-10
5B3	4.55E-10	3A9	5.33E-10
3B12	1.67E-10	13-42	4.14E-10
4A10	6.06E-10		
2B1	1.94E-10		

3820 [00338] The optimized full length antibodies were then functionally characterized for the ability to inhibit RBC lysis and A549 cell lysis, according to the methods described in Examples 1 and 2.

Optimized anti-PcrV antibodies inhibit RBC and A549 cell lysis caused by P. aeruginosa
RBC lysis inhibition by optimized anti-PcrV antibodies

3825 [00339] The ability of optimized anti-PcrV mAb clones 7B1, 7C1, 8C1, 6D10, 6A10, 5B3, 3B12, 4A10, 2B1, 7B2, 3H10, 3G3, 9B12, 3A9, 2D3, 7B1-1F5, to inhibit red blood cell

lysis was compared to the parental 13-42 mAb and the reference antibody V2L2-MD. RBC lysis inhibition assay was performed as described in Example 1.

[00340] As shown in FIG. 1A and 1B and Table 7, all antibodies optimized from 13-42 exhibited better or comparable efficacies in inhibiting RBC lysis as parental 13-42 mAb and V2L2-MD.

Table 7: Potency of Optimized anti-PcrV mAb in inhibiting RBC lysis			
Optimized anti-PcrV mAb clones	RBC lysis assay IC ₅₀ (ug/ml)	Optimized anti-PcrV mAb clones	RBC lysis assay IC ₅₀ (ug/ml)
7B1	0.10	7B2	0.05
7C1	0.10	3H10	0.16
8C1	0.12	3G3	0.18
6D10	0.16	9B12	0.07
6A10	0.04	3A9	0.23
5B3	0.12	2D3	0.05
3B12	0.16	13-42	0.23
4A10	0.16	V2L2-MD	0.21
2B1	0.12	7B1-1F5	0.19

A549 cell lysis inhibition by optimized anti-PcrV antibodies

[00341] The ability of optimized anti-PcrV 7B1, 7C1, 8C1, 6D10, 6A10, 5B3, 3B12, 4A10, 2B1, 7B2, 3H10, 3G3, 3A11, 3A9 to inhibit A549 cell lysis was also evaluated, as compared to the parental 13-42 mAb. A549 cell lysis inhibition assay was performed as described in Example 2.

[00342] As shown in FIG. 2A and 2B and Table 8, all antibodies optimized from 13-42 exhibited better or comparable efficacies in inhibiting A549 lysis as parental 13-42 mAb.

Table 8: Potency of Optimized anti-PcrV mAb in inhibiting A549 lysis			
Optimized anti-PcrV mAb clones	A549 cytotoxicity assay IC ₅₀ (ug/ml)	Optimized anti-PcrV mAb clones	A549 cytotoxicity assay IC ₅₀ (ug/ml)
7B1	1.06	2B1	3.01
7C1	1.36	7B2	2.32
8C1	1.94	3H10	1.78

6D10	2.63	3G3	2.75
6A10	2.00	3A11	0.91
5B3	2.39	3A9	1.21
3B12	2.68	13-42	2.67
4A10	2.80		

3840 **Example 4: Characterizing the specificity and affinity of optimized anti-PcrV antibodies**

[00343] Of the optimized antibodies, 7C1, 7B1, 8C1 and 6D10 antibodies were further characterized for their affinity and specificity in PcrV binding.

Binding affinity of optimized antibodies to PcrV

3845 [00344] The association constant (k_a), dissociation constant (k_d) and the equilibrium dissociation constant (K_D) for binding of optimized anti-PcrV mAb clones 7B1, 7C1, 8C1, 6D10 and reference antibody V2L2-MD to PcrV were tested by BIACORE (GE company). The K_D values for optimized clones 7B1, 7C1, 8C1, 6D10 ($8.47E-11M$, $4.57E-11M$, $3.16E-11M$, $5.20E-11M$, respectively) were determined to be comparable to that for the

3850 V2L2-MD antibody ($4E-11M$). Furthermore, the optimized antibodies 7B1, 7C1, 8C1 and 6D10 displayed comparable association and dissociation kinetics in PcrV binding, as shown by comparable k_a and k_d values (data not shown).

Specificity of anti-PcrV antibodies

[00345] The specificity of the optimized anti-PcrV antibodies were characterized by

3855 measuring cross-reactivity to BV particles and to PcrV-negative A549 cells.

[00346] *Cross-reactivity to BV particles:* Using ELISA, optimized antibodies 7C1, 7B1, 8C1, 6D10, and reference antibodies V2L2-MD and Mab166 were tested for cross-reactivity to BV particles according to the method described previously (See Hötzel I, et al, 2012, mAbs 4:6, 753–760). As shown in FIG.3, Mab166 displayed a high level of

3860 non-specific binding to BV particles. In contrast, the antibodies 7C1, 7B1, 8C1, 6D10 displayed low levels of non-specific binding, similar to V2L2-MD and much lower than Mab166.

[00347] *Cross-reactivity to A549 cells:* Using FACS, parental antibody 13-42, optimized antibodies 7C1, 7B1, 8C1, 6D10 as well as V2L2-MD were tested for cross-reactivity to

3865 PcrV-negative A549 cells. As shown in FIGS. 4A-4E, 13-42 mAb as well as the optimized

antibodies 7C1, 7B1, 8C1, 6D10 all displayed similarly low levels of A549 binding as negative control (no antibody) and V2L2-MD, while a positive control antibody specific to GM-CSFR on A549 cells displayed a higher level of binding to A549 cells.

[00348] Taken together, these results indicated that optimized antibodies 7C1, 7B1, 8C1 and 6D10 displayed similarly low non-specific binding as parental antibody 13-42 mAb and reference antibody V2L2-MD, while displaying much lower non-specific binding than Mab166, another reference anti-PcrV antibody.

Example 5: Optimized full-length anti-PcrV antibodies broadly neutralize *P. aeruginosa* strains

[00349] To examine whether the optimized anti-PcrV antibodies can neutralize a broad spectrum of *P. aeruginosa* strains, the ability of optimized anti-PcrV antibodies to inhibit RBC and A549 cell lysis caused by multiple clinically relevant *P. aeruginosa* strains (O6, O11, O1, O16), which represent 70% of clinical cases of *P. aeruginosa* infections, was investigated.

RBC lysis inhibition by optimized anti-PcrV antibodies

[00350] The optimized anti-PcrV mAb clones 7C1, 8C1, and 7B1 were further analyzed for their abilities to inhibit red blood cell lysis by various strains of *P. aeruginosa*, namely strains O1-52/66, O16-177/81, and O6-57/66, as compared to the parental 13-42 mAb and the reference antibody V2L2-MD. RBC lysis inhibition assay was performed as described in Example 1.

[00351] As shown in FIG. 5A-5C and Table 9, the optimized mAb clones 7C1, 8C1, and 7B1 exhibited better efficacy in inhibiting RBC lysis by strains O1-52/66, O16-177/81, and O6-57/66 than either the reference antibody V2L2-MD or the parental 13-42 mAb.

Table 9: Efficacy of Optimized anti-PcrV in inhibiting RBC lysis caused by various <i>P. aeruginosa</i> strains				
	Strain of <i>P. aeruginosa</i>			
mAb clones	O1-52/66 IC50 (μg/mL)	O16-177/81 IC50 (μg/mL)	O6-57/66 IC50 (μg/mL)	Average IC50 (μg/mL)
7C1	0.3674	0.1409	0.0767	0.1950
8C1	0.4240	0.2522	0.1207	0.2656
7B1	0.3079	0.1974	0.1380	0.2144
13-42	0.4720	0.4635	0.1303	0.3553

V2L2-MD	0.6345	0.4769	0.1464	0.4193
---------	--------	--------	--------	--------

3890 *A549 cell lysis inhibition by optimized anti-PcrV antibodies*

[00352] The optimized anti-PcrV mAb clones 7C1, 8C1, and 7B1 were analyzed for their abilities to inhibit A549 cell lysis by various strains of *P. aeruginosa*, namely strains O6-57/66, O11-PA103, O1-52/66 and O16-177/81, as compared to the parental 13-42 mAb. A549 cell lysis inhibition assay was performed as described in Example 2.

3895 **[00353]** As shown in FIG. 6A-6D and Table 10, the optimized mAb clones 7C1, 8C1, and 7B1 exhibited comparable efficacy in inhibiting A549 lysis by strains O6-57/66, O11-PA103, O1-52/66 and O16-177/81 as compared to the parental 13-42 mAb.

Table 10: Efficacy in inhibiting A549 cell lysis caused by various <i>P. aeruginosa</i> strains					
	Strain of <i>P. aeruginosa</i>				
mAb clones	O6-57/66 IC50 (μg/mL)	O11-PA103 IC50 (μg/mL)	O1-52/66 IC50 (μg/mL)	O16-177/81 IC50 (μg/mL)	Average IC50 (μg/mL)
13-42	0.8909	1.207	2.339	1.078	1.3787
7C1	0.4962	1.117	1.998	1.095	1.1766
7B1	0.5031	2.607	4.558	0.8606	2.1322
8C1	0.7206	1.536	N/D	0.8811	1.0459

[00354] To further illustrate the optimized antibodies could broadly neutralize *P. aeruginosa* strains, the abilities of these antibodies to bind various forms of PcrV mutants were tested. Briefly, 100 PcrV sequences were randomly selected from Genbank database. In contrast to the wild type sequence as shown in SEQ ID No: 71, 13% of the selected sequences contained R, G, or K at position 225 (as compared of S in the wild type sequence SEQ No: 71), which was the main mutation around the epitope of 7B1.

3905 **[00355]** Optimized 7B1, 7C1, 8C1 and 6D10 antibodies were characterized for their affinity and specificity in binding to these PcrV mutants (with mutations at position 225), and were found to strongly bind these mutants as well (data not shown). These findings further illustrate that the optimized anti-PcrV antibodies could neutralize a broad spectrum of *P. aeruginosa* strains, which might express various forms of PcrV mutants.

3910

Example 6: Optimized anti-PcrV antibodies as prophylactic treatment for *P. aeruginosa* infection

[00356] The ability of the optimized anti-PcrV antibodies in protecting against *P. aeruginosa* infection prophylactically was demonstrated with survival improvement in mouse pneumonia model, intraperitoneal infection model and immunocompromised mouse pneumonia model.

Survival improvement in mouse pneumonia model by anti-PcrV antibodies

[00357] The ability of the optimized anti-PcrV antibodies 7C1, 7B1 and 3G3 to improve survival in mouse pneumonia model was evaluated in comparison to parental 13-42 mAb and HIV-10E8, a broadly neutralizing antibody against HIV described previously (See Huang J, et al, 2012, Nature 491:406), was used as a negative control.

[00358] In a prophylactic model, 7-8 week old BALB/c mice (Vital River Laboratory) were administered with antibodies intraperitoneally (i.p.) 24 h before infection, at doses of 1 or 10 mg/kg mouse weight. To induce acute pneumonia, the BALB/c mice were intranasally inoculated with *P. aeruginosa* (PA103 strain) suspended in a 40μl inoculum at the lethal dose (1*LD₉₀) or at double the lethal dose (2* LD₉₀) (8×10⁵~ 1.6×10⁶ CFU). Mouse survival was recorded for up to 7 days post-infection. The results were represented as Kaplan-Meier survival curves.

[00359] As shown by Kaplan-Meier survival curves in FIG. 7A and 7B, at both 1x and 2x the lethal doses (1*LD₉₀ or 2* LD₉₀; 8×10⁵~ 1.6×10⁶ CFU) of *P. aeruginosa* inoculation, all anti-PcrV antibodies exhibited significant survival improvement compared to negative control (P <0.001). Also, the optimized antibodies 7C1, 7B1 exhibited comparable or better survival improvements than parental 13-42 mAb (P <0.05). Differences in survival were calculated by the log rank test.

Organ burden in mouse pneumonia model administered with anti-PcrV antibodies

[00360] The ability of the parental anti-PcrV antibody 13-42 mAb in reducing organ burden for an acute pneumonia model was evaluated and compared to that of reference antibody V2L2-MD. The ability of the optimized anti-PcrV antibody clones 7B1, 7C1, 8C1, and 6D10 in reducing organ burden for an acute pneumonia model was further evaluated. HIV-10E8 was used as a negative control.

[00361] In a prophylactic model, 7-8 week old BALB/c mice (Vital River Laboratory) were administered with antibodies or PBS intraperitoneally (i.p.) 24 hr before infection, at

10 mg/kg mouse weight. To induce acute pneumonia, the BALB/c mice were intranasally inoculated with *P. aeruginosa* (PA103 strain) suspended in a 40µl inoculum at half the
 3945 lethal dose ($0.5 \times LD_{90}$; 3×10^5 CFU). 24 hours post-infection, mice were euthanized, where the lungs, spleens and kidneys were extracted, homogenized and diluted before plating on agar to measure the viable bacteria units (in CFU), which represented the *P. aeruginosa* burden in the respective organ sample.

[00362] As shown in FIG. 8A, 13-42 mAb exhibited better efficacy in reducing organ
 3950 burden in the lung, and similar efficacy in reducing organ burden in spleen and kidney in the mouse pneumonia model, as compared to V2L2-MD.

[00363] As shown in FIG. 8B, optimized anti-PcrV antibody clones 7B1, 7C1, 8C1, and 6D10 exhibited comparable efficacy in reducing organ burden in the lung, spleen and kidney as compared to V2L2-MD, and exhibited higher efficacy in reducing organ burden
 3955 in the lung, spleen and kidney in the mouse pneumonia model as compared to HIV-10E8.
Survival improvement in mouse intraperitoneal infection model using anti-PcrV antibodies

[00364] The ability of the optimized anti-PcrV antibodies 7C1, 7B1 and 8C1 to improve survival in a mouse intraperitoneal infection model was evaluated in comparison to parental 13-42 mAb. HIV-10E8 was used as negative control. The mouse
 3960 intraperitoneal infection model was generated as described previously (See Warrener et al., 2014, *Antimicrob. Agents Chemother.*, 58, 4384–4391).

[00365] In a prophylactic model, 7-8 week old BALB/c mice (Vital River Laboratory) were administered antibodies by intraperitoneal (i.p.) injection 24 h before infection, at doses of 5 or 25 mg/kg mouse weight. To induce intraperitoneal infection, the BALB/c
 3965 mice were intraperitoneally inoculated with *P. aeruginosa* (O6-57/66 strain) suspended in a 300µl inoculum at triple the lethal dose ($3 \times LD_{90} = 7 \times 10^5$ CFU). Mouse survival was recorded for up to 5 days post-infection.

[00366] As shown in FIG.9, at triple the lethal dose ($3 \times LD_{90}$) of *P. aeruginosa* inoculation, all anti-PcrV antibodies exhibited significant survival improvement compared
 3970 to negative control at the 25mg/kg antibody dose ($P < 0.0001$). Also, the optimized antibodies 7C1, 7B1 exhibited comparable or better survival improvement as parental 13-42 mAb, at the 5 mg/kg antibody dose ($P < 0.05$). Differences in survival were calculated by the log rank test.

Survival improvement in immunocompromised mouse pneumonia model by anti-PcrV antibodies
 3975

[00367] The ability of the optimized anti-PcrV antibodies 7C1, 7B1 and 8C1 to improve survival for acute pneumonia model in immunodeficient mice was evaluated in comparison to parental 13-42. HIV-10E8 was used as negative control. The acute pneumonia model in immunodeficient mice model was generated as described previously (DiGiandomenico et al., 2014, *Sci. Transl. Med.*, 6: 262ra155).

[00368] In a prophylactic model, to achieve immunosuppression, 7-8 week old BALB/c mice (Vital River Laboratory) were first administered 2 doses of 150 mg/kg of cyclophosphamide, at 4 days and 1 day before *P. aeruginosa* infection, to suppress white cell count. Antibodies were then administered intraperitoneally (i.p.) at 24 h before infection, at doses of 3.2 to 5 mg/kg mouse weight. To induce acute pneumonia, the BALB/c mice were intranasally inoculated with *P. aeruginosa* (O6-57/66 strain) suspended in a 40µl inoculum at the lethal dose ($1 \times LD_{90} = 10$ PFU). Mouse survival was recorded for up to 10 days post-infection.

[00369] As shown in FIG. 10, at the lethal dose ($1 \times LD_{90}$) of *P. aeruginosa* inoculation, all anti-PcrV antibodies exhibited significant survival improvement compared to negative control ($P < 0.05$). Also, the optimized antibody 7C1 exhibited better survival improvement than the parental 13-42 antibody ($P < 0.05$), while the optimized antibodies 7B1, 8C1 exhibited comparable or better protection than the parental 13-42 antibody ($P < 0.05$). Differences in survival were calculated by the log rank test.

[00370] Taken together, these results show that the anti-PcrV antibodies disclosed herein can be employed to neutralize *P. aeruginosa* infection prophylactically in an immunodeficient mice acute pneumonia model.

Example 7: Optimized anti-PcrV antibodies as therapeutic treatment for *P. aeruginosa* infection

[00371] The ability of the optimized anti-PcrV antibodies in neutralizing *P. aeruginosa* infection therapeutically was demonstrated with survival improvement in a mouse pneumonia model.

Survival improvement in mouse pneumonia model by optimized anti-PcrV antibodies

[00372] The ability of the optimized anti-PcrV antibodies 7C1, 7B1, 8C1 and 6D10 to improve survival for mouse acute pneumonia model was evaluated in comparison to positive control V2L2-MD. HIV-10E8 was used as negative control.

[00373] In a therapeutic model, to induce acute pneumonia, 7-8 week old BALB/c mice (Vital River Laboratory) were intranasally inoculated with *P. aeruginosa* (PA103 strain) suspended in a 40µl inoculum at a lethal dose ($1 \times LD_{90} = 8 \times 10^5$ CFU). Antibodies or PBS were administered intravenously (i.v.) at 1 h post-infection, at a dose of 1mg/kg or 10mg/kg mouse weight. Mouse survival was recorded for up to 6 days post-infection.

[00374] As shown in FIG. 11A-11B, at the lethal dose ($1 \times LD_{90}$) of *P. aeruginosa* inoculation, all anti-PcrV antibodies exhibited significant survival improvement compared to negative control ($p < 0.05$). Also, the optimized antibodies 7C1, 7B1, 8C1 and 6D10 displayed survival improvement comparable to that of V2L2-MD. Differences in survival were calculated by the log rank test. The results show that the anti-PcrV antibodies disclosed herein can be employed to neutralize *P. aeruginosa* infection therapeutically.

4020 **Example 8: Combination treatment of *P. aeruginosa* infection using optimized anti-PcrV antibodies and antibiotics**

Survival improvement in mouse intraperitoneal infection model by anti-PcrV antibodies in combination with antibiotics

[00375] The ability of the optimized anti-PcrV antibody 7B1 or 8C1 to improve survival in mouse intraperitoneal infection model was evaluated in comparison to antibiotics Meropenem, Tobramycin, Ciprofloxacin; and also in comparison to combination treatment of 7B1 or 8C1 with either antibiotic. HIV-10E8 was used as negative control.

[00376] In a prophylactic model for anti-PcrV testing, 7-8 week old BALB/c mice (Vital River Laboratory) were intraperitoneally injected with antibody 24h before infection or with antibiotic 1h post infection. Specifically, the mice were administered with either optimized anti-PcrV antibody clone 7B1 or 8C1 (at 5mg/kg), Meropenem (at 3mg/kg), Tobramycin (at 10mg/kg), Ciprofloxacin (at 30mg/kg), or a combination of 7B1 or 8C1 with one of the antibiotics. To induce intraperitoneal infection, the BALB/c mice were intraperitoneally inoculated with *P. aeruginosa* (PA103 strain) suspended in a 300µl inoculum at triple the lethal dose ($3 \times LD_{90} = 7 \times 10^5$ CFU). Mouse survival was recorded for up to 5 days post-infection.

[00377] As shown in FIG. 12A, at the lethal dose of $3 \times LD_{90}$ of *P. aeruginosa* (PA103 strain) inoculation, the optimized antibodies 7B1 (at 5mg/kg) exhibited better or comparable survival improvement as antibiotics Meropenem (at 3mg/kg). The combination of 7B1 (at 5mg/kg) with antibiotics Meropenem (at 3mg/kg), Tobramycin (at 10mg/kg) or

Ciprofloxacin (at 30mg/kg) exhibited enhanced survival improvement as compared to either the antibody treatment alone or the respective antibiotics treatment alone ($p < 0.05$). As shown in FIG. 12B, the combination of 8C1 (at 5mg/kg) with antibiotics Tobramycin (at 10mg/kg) or Ciprofloxacin (at 30mg/kg) also exhibited enhanced survival improvement as compared to either the antibody treatment alone or the respective antibiotics treatment ($p < 0.05$). These results here demonstrate the clinical potential of using the anti-PcrV antibodies disclosed herein combined with antibiotics in neutralizing *P. aeruginosa*.

Example 9: Pharmacokinetic profile of Optimized anti-PcrV antibodies

[00378] To investigate the *in vivo* pharmacokinetics of the parental 13-42 anti-PcrV antibody as well as the optimized clones, the plasma levels of 7C1, 7B1, 8C1 and 6D10 antibodies in rat were measured over time.

[00379] *Pharmacokinetic profile in rat:* 40 healthy adult rats (about 0.2kg by weight) were equally into two groups, where each group registered similar average weight. One group was injected intravenously with 30mg/kg of V2L2-MD, 7C1, 7B1, 8C1 or 6D10 and the other group was injected intravenously with 3 mg/kg of V2L2-MD, 7C1, 7B1, 8C1 or 6D10. Blood was collected at one hour after injection, and subsequently at 0 hour, 0.5 hour, 2 hour, 8 hour, 1 day, 3 days, 7 days, 11 days, 17 days, 23 days, 31 days, 41 days and 52 days after injection. After centrifugation, the plasma was used for analyzing antibody concentration using ELISA. For the ELISA experiment, synthetic PcrV was used to coat the wells of a 96-well plate. On the following day, after washing with PBST, blocking with 200 μ L PBS-milk for an hour, and another wash with PBST, the plasma was added and incubated for an hour at 37°C. The plate was washed with 0.1% TBST for 6 times before 100 μ L of Goat-anti-human Fc antibody-AP (1:3000 in PBS) was added to each well and incubated for an hour. After washing with 0.1% TBST for 6 times, 50 μ L of pNPP was added to each well and color was developed for 10-20 minutes at 37 °C. The signals were read by a microplate reader at 410nm.

[00380] As shown in FIGS. 13A and 13B, the half-lives of 7C1, 7B1, 8C1 or 6D10 at both high and low i.v. dosages (3 mg/kg or 30 mg/kg) were comparable to or longer than that of reference antibody V2L2-MD, indicating that the optimized antibodies displayed more stable pharmacokinetic profiles at various doses as compared to the reference antibody V2L2-MD.

Example 10: Epitope recognized by optimized antibodies

4075 [00381] To characterize the epitope bound by the anti-PcrV antibodies, mutations were introduced in the PcrV protein, and the ability of the optimized antibody 7B1 mAb to bind these PcrV mutants was measured by ELISA.

Epitope mapping of anti-PcrV antibodies

[00382] Using the discovery studio software, the predicted binding sites on PcrV for 7B1
4080 and the optimized antibodies were identified, and the amino acid residues within the binding sites and in proximity to the binding sites were selected and subjected to alanine scanning. Subsequently, PcrV proteins carrying these selected mutations were expressed. The binding affinity of 7B1-IgG1 for each mutated PcrV protein was analyzed using ELISA. FIGs. 14A-14D show the ELISA binding curves of the antibodies for mutated
4085 PcrV, where mutation positions were according to sequence of SEQ ID NO: 71. As used herein, PcrV represents a His-tagged wild-type *P. aeruginosa* PcrV (PcrV-6His). Mutations at various positions of the amino acid sequence of the wild type PcrV were generated using alanine scanning as described above. As shown in FIGS. 14A-14D, a mutation at position Q160 significantly affected binding by 7B1-IgG1 (FIG. 14C). In
4090 contrast, mutation of Q160 in PcrV does not affect binding by reference antibody V2L2-MD (data not shown). A mutation at position D165 also affected binding by 7B1-IgG1 (FIG. 14C). In addition, mutations at each of positions D170, D173, T175 and S202 affected binding by 7B1-IgG1 (FIG. 14D). Based on these results, exemplary epitopes of the 7B1-IgG1 antibodies were identified as comprising amino acid residues
4095 Gln160, Asp165, Asp170, Asp173, Thr175, and Ser202, according to SEQ ID NO: 71.