COMBINATION THERAPIES USING ANTI- *PSEUDOMONAS* PSL AND PCRV BINDING MOLECULES

REFERENCE TO SEQUENCE LISTING SUBMITTED ELECTRONICALLY

[0001] The content of the electronically submitted sequence listing in ASCII text file entitled PSEUD-101WO1.txt created on November 5, 2013, and having a size of 382 kilobytes filed with the application is incorporated herein by reference in its entirety.

BACKGROUND

Field of the Disclosure

[0002] This disclosure relates to combination therapies using anti-*Pseudomonas* Psl and PcrV binding domains for use in the prevention and treatment of *Pseudomonas* infection. Furthermore, the disclosure provides compositions useful in such therapies.

Background of the Disclosure

[0003] *Pseudomonas aeruginosa* (*P. aeruginosa*) is a gram-negative opportunistic pathogen that causes both acute and chronic infections in compromised individuals (Ma *et al., Journal of Bacteriology 189(22)*:8353-8356 (2007)). This is partly due to the high innate resistance of the bacterium to clinically used antibiotics, and partly due to the formation of highly antibiotic-resistant biofilms (Drenkard E., *Microbes Infect 5:*1213-1219 (2003); Hancokc & Speert, *Drug Resist Update 3*:247-255 (2000)).

[0004] *P. aeruginosa* is a common cause of hospital-acquired infections in the Western world. It is a frequent causative agent of bacteremia in burn victims and immune compromised individuals (Lyczak *et al., Microbes Infect 2:*1051-1060 (2000)). It is also the most common cause of nosocomial gram-negative pneumonia (Craven *et al., Semin Respir Infect 11*:32-53 (1996)), especially in mechanically ventilated patients, and is the most prevalent pathogen in the lungs of individuals with cystic fibrosis (Pier *et al., ASM News 6*:339-347 (1998)).

[0005] *Pseudomonas* Psl exopolysaccharide is reported to be anchored to the surface of *P. aeruginosa* and is thought to be important in facilitating colonization of host tissues and in establishing/maintaining biofilm formation (Jackson, K.D., *et al., J Bacteriol 186,*

4466-4475 (2004)). Its structure comprises mannose-rich repeating pentasaccharide (Byrd, M.S., *et al., Mol Microbiol 73*, 622-638 (2009)).

[0006] PcrV is a relatively conserved component of the type III secretion system. PcrV appears to be an integral component of the translocation apparatus of the type III secretion system mediating the delivery of the type III secretory toxins into target eukaryotic cells (Sawa T., *et al. Nat. Med. 5*, 392-398 (1999)). Active and passive immunization against PcrV improved acute lung injury and mortality of mice infected with cytotoxic *P. aeruginosa* (Sawa *et al.* 2009). The major effect of immunization against PcrV was due to the blockade of translocation of the type III secretory toxins into eukaryotic cells.

[0007] Due to increasing multidrug resistance, there remains a need in the art for the development of novel strategies for the identification of new *Pseudomonas*-specific prophylactic and therapeutic agents.

BRIEF SUMMARY

[0008] This disclosure provides an isolated binding molecule which specifically binds to *Pseudomonas* PcrV, where the binding molecule includes an immunoglobulin VH and an immunoglobulin VL, and where the VH has the amino acid sequence of SEQ ID NO:255 or SEQ ID NO:257, and where the VL has the amino acid sequence of SEQ ID NO:256.

[0009] In a related embodiment, the disclosure provides an isolated binding molecule which specifically binds to *Pseudomonas* PcrV, where the binding molecule includes an immunoglobulin VH and an immunoglobulin VL, each of which include three complementarity determining regions: a CDR1, CDR2, and CDR3. The VH CDR1 can be SEQ ID NO:311 or a variant thereof with 1, 2, 3, or 4 conservative amino acid substitutions, the VH CDR2 can be SEQ ID NO:312, or a variant thereof with 1, 2, 3, or 4 conservative amino acid substitutions, and the VHCDR3 can be SEQ ID NO: 313, or a variant thereof with 1, 2, 3, or 4 conservative amino acid substitutions. Additionally or alternatively, the VL CDR1 can be SEQ ID NO:314, or a variant thereof with 1, 2, 3, or 4 conservative amino acid substitutions, the VL CDR2 can be SEQ ID NO:315, or a variant thereof with 1, 2, 3, or 4 conservative amino acid substitutions, and the VL CDR3 can be SEQ ID NO:316, or a variant thereof with 1, 2, 3, or 4 conservative amino acid substitutions. According to these embodiments, the VH and VL CDRs are according to the Kabat numbering system.

[0010] In another embodiment, the disclosure provides an isolated binding molecule which specifically binds to *Pseudomonas* PcrV, where the binding molecule includes an immunoglobulin VH and an immunoglobulin VL, where the VH has an amino acid sequence at least 90% identical, or fully identical, to SEQ ID NO:317, and additionally or alternatively, the VL has an amino acid sequence at least 90% identical, or fully identical, to SEQ ID NO:318.

[0011] In any of these embodiments, the binding molecule can be an anti-PcrV antibody or antigen-binding fragment thereof. For example the binding molecule can be an antibody or fragment thereof in which the VH is part of an antibody heavy chain which can have one or more heavy chain constant regions, and in which the VL is part of an antibody light chain which can have a light chain constant region. In certain embodiments, the antibody or fragment thereof has two antibody heavy chains, which can be identical, and two antibody light chains, which can be identical.

[0012] In certain embodiments, the antibody or fragment thereof is a bispecific antibody. For example a bispecific antibody that specifically binds to *Pseudomonas* PcrV and *Pseudomonas* Psl. In certain aspects the binding domain of the bispecific antibody which specifically binds to *Pseudomonas* Psl can be an anti-Psl ScFv molecule. In certain aspects the anti-Psl ScFv molecule can include an amino acid sequence of SEQ ID NO:240 – SEQ ID NO:254, or any combination of two or more amino acid sequences of SEQ ID NO:240 – SEQ ID NO:254. In certain embodiments an anti-Psl ScFv molecule is inserted into the hinge region of each heavy chain of an anti-PcrV antibody or fragment thereof as described above.

[0013] In certain bispecific antibodies disclosed herein each antibody heavy chain includes the formula VH-CH1-H1-L1-S-L2-H2-CH2-CH3, where CH1 is a heavy chain constant region domain-1, H1 is a first heavy chain hinge region fragment, L1 is a first linker, S is an anti-PcrV ScFv molecule, L2 is a second linker, H2 is a second heavy chain hinge region fragment, CH2 is a heavy chain constant region domain-2, and CH3 is a heavy chain constant region domain-3. For example, the binding molecule can include the VH amino acid sequence of SEQ ID NO:255, SEQ ID NO:257, or SEQ ID NO:317, and the CH1 can include SEQ ID NO:319. In certain aspects L1 and L2 can b the same or different, and each can include the amino acid sequence (a) [GGGGS]n, wherein n is 0, 1, 2, 3, 4, or 5, (b) [GGGG]n, wherein n is 0, 1, 2, 3, 4, or 5, or a combination of the

amino acid sequences (a) and (b). In certain aspects H1 can be EPKSC (SEQ ID NO:320), and H2 can be DKTHTCPPCP (SEQ ID NO:321). In certain embodiments S can include one or more of the amino acid sequences SEQ ID NO:240 to SEQ ID NO:254. As a further aspect, CH2-CH3 can be APELLGGPSVFLFPPKPKDT L**X1**I**X2**R**X3**PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYR VVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRE EMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPSLDSDGSFFLYSKLT VDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:322), where X1 is M or Y, X2 is S or T, and X3 is T or E. In certain bispecific antibodies disclosed herein each antibody light chain can include the formula VL-CL, CL can be an antibody light chain kappa constant region or an antibody light chain lambda constant region, and VL can be the amino acid sequence of SEQ ID NO:256 or SEQ ID NO:318.

[0014] Certain bispecific antibodies provided herein include two identical heavy chains and two identical light chains, where each antibody heavy chain includes SEQ ID NO:264, and each antibody light chain includes SEQ ID NO: 263. Specifically, this disclosure provides a bispecific antibody which specifically binds to *Pseudomonas* Psl and *Pseudomonas* PcrV, where the antibody includes an immunoglobulin heavy chain and an immunoglobulin light chain, where the heavy chain includes the amino acid sequence of SEQ ID NO:264, and the light chain includes the amino acid sequence of SEQ ID NO:263.

[0015] In certain embodiments, the disclosure provides an isolated binding molecule which specifically binds to *Pseudomonas* Psl, where the binding molecule can have an immunoglobulin VH and an immunoglobulin VL, each including three complementarity determining regions CDR1, CDR2, and CDR3. According to these embodiments, the VH CDR1 can be SEQ ID NO:47, the VH CDR2 can be SEQ ID NO: 48, the VH CDR3 can be SEQ ID NO:258, SEQ ID NO:267, SEQ ID NO:268, SEQ ID NO:269, SEQ ID NO:270, SEQ ID NO:271, SEQ ID NO:272, SEQ ID NO:273, SEQ ID NO:274, SEQ ID NO:275, SEQ ID NO:276, SEQ ID NO:277, SEQ ID NO:278, or SEQ ID NO:279, the VL CDR1 can be SEQ ID NO:50, the VL CDR2 can be SEQ ID NO:51, and the VL CDR3 can be SEQ ID NO:280, SEQ ID NO:281, SEQ ID NO:282, SEQ ID NO:52, SEQ ID NO:283, SEQ ID NO:284, SEQ ID NO:285, SEQ ID NO:286, or SEQ ID NO:287. The VH and VL CDRs are are according to the Kabat numbering system.

[0016] In certain of these anti-Psl binding molecules, the VH CDR3 is SEQ ID NO:258, and the VL CDR3 is SEQ ID NO:280. More specifically the VH includes SEQ ID NO:288, and the VL includes SEQ ID NO:289.

[0017] In certain of these anti-Psl binding molecules, the VH CDR3 is SEQ ID NO:267, and the VL CDR3 is SEQ ID NO:281. More specifically the VH includes SEQ ID NO:290, and the VL includes SEQ ID NO:291.

[0018] In certain of these anti-Psl binding molecules, the VH CDR3 is SEQ ID NO:268, and the VL CDR3 is SEQ ID NO:282. More specifically the VH includes SEQ ID NO:292, and the VL includes SEQ ID NO:293.

[0019] In certain of these anti-Psl binding molecules, the VH CDR3 is SEQ ID NO:269, and the VL CDR3 SEQ ID NO:52. More specifically the VH includes SEQ ID NO:294, and the VL includes SEQ ID NO:11.

[0020] In certain of these anti-Psl binding molecules, the VH CDR3 is SEQ ID NO:270, and the VL CDR3 is SEQ ID NO:283. More specifically the VH includes SEQ ID NO:295, and the VL includes SEQ ID NO:296.

[0021] In certain of these anti-Psl binding molecules, the VH CDR3 is SEQ ID NO:271, and the VL CDR3 is SEQ ID NO:284. More specifically the VH includes SEQ ID NO:297, and the VL includes SEQ ID NO:298.

[0022] In certain of these anti-Psl binding molecules, the VH CDR3 is SEQ ID NO:272, and the VL CDR3 is SEQ ID NO:285. More specifically the VH includes SEQ ID NO:299, and the VL includes SEQ ID NO:300.

[0023] In certain of these anti-Psl binding molecules, the VH CDR3 is SEQ ID NO:273, and the VL CDR3 is SEQ ID NO:286. More specifically the VH includes SEQ ID NO:301, and the VL includes SEQ ID NO:302.

[0024] In certain of these anti-Psl binding molecules, the VH CDR3 is SEQ ID NO:274, and the VL CDR3 is SEQ ID NO:52. More specifically the VH includes SEQ ID NO:303, and the VL includes SEQ ID NO:11.

[0025] In certain of these anti-Psl binding molecules, the VH CDR3 is SEQ ID NO:275, and the VL CDR3 is SEQ ID NO:52. More specifically the VH includes SEQ ID NO:304, and the VL includes SEQ ID NO:11.

[0026] In certain of these anti-Psl binding molecules, the VH CDR3 is SEQ ID NO:276, and the VL CDR3 is SEQ ID NO:52. More specifically the VH includes SEQ ID NO:305, and the VL includes SEQ ID NO:11.

[0027] In certain of these anti-Psl binding molecules, the VH CDR3 is SEQ ID NO:277, and the VL CDR3 is SEQ ID NO:52. More specifically the VH includes SEQ ID NO:306, and the VL includes SEQ ID NO:11.

[0028] In certain of these anti-Psl binding molecules, the VH CDR3 is SEQ ID NO:278, and the VL CDR3 is SEQ ID NO:52. More specifically the VH includes SEQ ID NO:307, and the VL includes SEQ ID NO:11.

[0029] In certain of these anti-Psl binding molecules, the VH CDR3 is SEQ ID NO:279, and the VL CDR3 is SEQ ID NO:287. More specifically the VH includes SEQ ID NO:308, and the VL includes SEQ ID NO:325.

[0030] In certain of these anti-Psl binding molecules, the VH includes the amino acid sequence of SEQ ID NO:309, and the VL includes the amino acid sequence of SEQ ID NO:310.

[0031] Each of the anti-Psl binding molecules described above can be an antibody or antigen-binding fragment thereof, for example, a single-chain Fv (ScFv) antibody molecule. In some aspects the ScFv includes the formula: VH-L-VL, and wherein L is a linker, on other aspects the ScFv includes the formula: VL-L-VH, and wherein L is a linker. In each of these aspects, L can be the amino acid sequence (a) [GGGGS]n, wherein n is 0, 1, 2, 3, 4, or 5, (b) [GGGG]n, wherein n is 0, 1, 2, 3, 4, or 5, or L can be a combination of (a) and (b). In further aspects, L can further include the amino acids ala-leu at the C-terminus of the linker.

[0032] Certain anti-Psl ScFvs include the amino acid sequence SEQ ID NO:240. Certain anti-Psl ScFvs include the amino acid sequence SEQ ID NO:262. Other anti-Psl ScFvs provided in this disclosure include one or more of the amino acid sequences SEQ ID NO:241, SEQ ID NO:242, SEQ ID NO:243, SEQ ID NO:244, SEQ ID NO:245, SEQ ID NO:246, SEQ ID NO:247, SEQ ID NO:248, SEQ ID NO:249, SEQ ID NO:250, SEQ ID NO:251, SEQ ID NO:252, SEQ ID NO:253, or SEQ ID NO:254.

[0033] Further provided herein is an isolated polypeptide which includes any one or more of the amino acid sequences described above, for example, SEQ ID NO:240, SEQ ID NO:241, SEQ ID NO:242, SEQ ID NO:243, SEQ ID NO:244, SEQ ID NO:245, SEQ ID

NO:246, SEQ ID NO:247, SEQ ID NO:248, SEQ ID NO:249, SEQ ID NO:250, SEQ ID NO:251, SEQ ID NO:252, SEQ ID NO:253, SEQ ID NO:254, SEQ ID NO:262, SEQ ID NO:255, SEQ ID NO:256, SEQ ID NO:257, SEQ ID NO:263, SEQ ID NO:264, SEQ ID NO:258, SEQ ID NO:263, SEQ ID NO:264, SEQ ID NO:267, SEQ ID NO:268, SEQ ID NO:269, SEQ ID NO:270, SEQ ID NO:271, SEQ ID NO:272, SEQ ID NO:273, SEQ ID NO:274, SEQ ID NO:275, SEQ ID NO:276, SEQ ID NO:277 SEQ ID NO:278 SEQ ID NO:279, SEQ ID NO:280, SEQ ID NO:281, SEQ ID NO:282, SEQ ID NO:283, SEQ ID NO:284, SEQ ID NO:285, SEQ ID NO:286, SEQ ID NO:287, SEQ ID NO:288, SEQ ID NO:289, SEQ ID NO:290, SEQ ID NO:291, SEQ ID NO:292, SEQ ID NO:293, SEQ ID NO:294, SEQ ID NO:295, SEQ ID NO:296, SEQ ID NO:298, SEQ ID NO:299,300, SEQ ID NO:301, SEQ ID NO:302, SEQ ID NO:303, SEQ ID NO:304, SEQ ID NO:305, SEQ ID NO:306, SEQ ID NO:307, SEQ ID NO:308, SEQ ID NO:309, SEQ ID NO:310, SEQ ID NO:311, SEQ ID NO:312, SEQ ID NO:313, SEQ ID NO:315, SEQ ID NO:316, SEQ ID NO:317, SEQ ID NO:318, or SEQ ID NO:325.

[0034] Further provided is a cell which incorporates or can produce one or more of any binding molecule described above, or a subunit thereof, any bispecific antibody described above, or a subunit thereof, or any polypeptide described above.

[0035] This disclosure also provides an isolated polynucleotide which includes a nucleic acid encoding one or more of any binding molecule described above, or a subunit thereof, any bispecific antibody described above, or a subunit thereof, or any polypeptide described above. Further provided is a vector which includes such a polynucleotide, and a cell which includes such a polynucleotide or vector.

[0036] Also provided is a composition which includes one or more of any binding molecule described above, or a subunit thereof, any bispecific antibody described above, or a subunit thereof, or any polypeptide described above, and a pharmaceutically acceptable carrier. In certain aspects a component of such a composition binds to at least 80%, at least 85%, at least 90% or at least 95% of *P. aeruginosa* strains isolated from infected patients, where the *P. aeruginosa* strain can be isolated from one or more of lung, sputum, eye, pus, feces, urine, sinus, a wound, skin, blood, bone, or knee fluid. In certain compositions described herein, the binding molecule or a subunit thereof, or the bispecific antibody or a subunit thereof, can be conjugated to one or more agents such as an antimicrobial agent, a therapeutic agent, a prodrug, a peptide, a protein, an enzyme, a

lipid, a biological response modifier, pharmaceutical agent, a lymphokine, a heterologous antibody or fragment thereof, a detectable label, or polyethylene glycol (PEG). The detectable label can be one or more of an enzyme, a fluorescent label, a chemiluminescent label, a bioluminescent label, or a radioactive label. Any composition provided herein can further include one or more antibiotics, including, but not limited to Ciprofloxacin or Meropenem.

[0037] This disclosure further provides a method of preventing or treating a *Pseudomonas* infection in a subject in need thereof, where the method includes administering to a subject an effective amount of any composition described herein.

[0038] Further provided is a method of preventing or treating a *Pseudomonas* infection in a subject in need thereof, where the method includes administering to a subject an effective amount of any bispecific antibody described herein or any composition described herein which includes a bispecific antibody. In certain embodiments, such an administration provides a synergistic therapeutic effect in the prevention or treatment of the *Pseudomonas* infection in the subject, and wherein the synergistic effect is greater than the sum of the individual effects of administration of equimolar quantities of monospecific binding molecules with the same *Pseudomonas* Psl and *Pseudomonas* PcrV binding specificities as the bispecific antibody. In certain aspects, the synergistic therapeutic effect results in greater percent survival than the additive percent survival of subjects to which only one of the binding domains has been administered.

[0039] Also provided is a method of preventing or treating a *Pseudomonas* infection in a subject in need thereof, where the method includes administering to a subject an effective amount of ny composition described herein where the composition includes an antibiotic, where the administration provides a synergistic therapeutic effect in the prevention or treatment of the *Pseudomonas* infection in the subject, where the synergistic effect is greater than the sum of the individual effects of administration of equimolar quantities of one or more of (a) just the bispecific antibody, (b), the monospecific binding molecules with the same *Pseudomonas* Psl and *Pseudomonas* PcrV binding specificities as the bispecific antibody and (c) the antibiotic.

[0040] According to any of the methods provided herein, a composition or a bispecific antibody can be administered for two or more prevention/treatment cycles, the *Pseudomonas* infection can be a *P. aeruginosa* infection, and the subject can be a human.

In certain aspects the infection is one or more of, but is not limited to, an ocular infection, a lung infection, a burn infection, a wound infection, a skin infection, a blood infection, or a bone infection. In certain aspects the subject suffers from one or more of acute pneumonia, burn injury, corneal infection, or cystic fibrosis.

[0041] This disclosure further provides a kit comprising any of the compositions described herein.

BRIEF DESCRIPTION OF THE DRAWINGS/FIGURES

[0042] Figure 1 (A-F): Phenotypic whole cell screening with human antibody phage libraries identified *P. aeruginosa* functionally active specific antibodies. (A) Overview of complete antibody selection strategy. (B) Flow diagram describing the process to isolate antibody variable region genes from patients recently exposed to *P. aeruginosa*. (C) Characteristics of the scFv phage display libraries, indicating the size and diversity of the cloned antibody repertoire. (D) Comparison of the phage display selection efficiency using either the patient antibody library or a naïve antibody library, when selected on *P. aeruginosa* 3064 Δ WapR (^{1}) or *P. aeruginosa* PAO1 MexAB OprM Δ WapR (^{2}) in suspension. Bars indicate the output titers (in CFU) at each round of selection, and circles indicate the proportion of duplicated VH CDR3 sequences, an indication of clonal enrichment. (E) ELISA screen of scFv from phage display to test binding to multiple strains of *P. aeruginosa*. ELISA data (absorbance at 450 nm) are shown for eight individual phage-scFvs from selections and one irrelevant phage-scFv. (F) FACS binding of *P. aeruginosa* specific antibodies with representative strains from unique *P. aeruginosa* serotypes. For each antibody tested a human IgG negative control antibody is shown as a shaded peak.

[0043] Figure 2 (A-B): Evaluation of mAbs promoting OPK of *P. aeruginosa* (A) Opsonophagocytosis assay with luminescent *P. aeruginosa* serogroup O5 strain (PAO1.lux), with dilutions of purified monoclonal antibodies derived from phage panning. (B) Opsonophagocytosis assay with luminescent *P. aeruginosa* serogroup O11 strain (9882-80.lux), with dilutions of purified WapR-004 and Cam-003 monoclonal antibodies derived from phage panning. In both A and B, R347, an isotype matched human monoclonal antibody that does not bind *P. aeruginosa* antigens, was used as a negative control.

[0044] Figure 3 (A-I): Identification of the *P. aeruginosa* Psl exopolysaccharide target of antibodies derived from phenotypic screening. Reactivity of antibodies was determined by indirect ELISA on plates coated with indicated *P. aeruginosa* strains: (A) wild type PAO1, PAO1Δ*wbpL,* PAO1Δ*rmlC* and PAO1Δ*galU*. (B) PAO1Δ*pslA*. The Genway antibody is specific to a *P. aeruginosa* outer membrane protein and was used as a positive control. (C) FACS binding analysis of Cam-003 to PAO1 and PAO1Δ*pslA*. Cam-003 is indicated by a solid black line and clear peak; an isotype matched non-*P. aeruginosa*-specific human IgG1 antibody was used as a negative control and is indicated by a gray line and shaded peak. (D) LPS purified from PAO1 and PAO1Δ*pslA* was resolved by SDS-PAGE and immunobloted with antisera derived from mice vaccinated with PAO1Δ*wapR*Δ*algD*, a mutant strain deficient in O-antigen transport to the outer membrane and alginate production. (E) Cam-003 ELISA binding data with isogenic mutants of PAO1. Cam-003 is only capable of binding to strains expressing Psl. pPW145 is a pUCP expression vector containing *pslA*. (F and G) Opsonophagocytosis assays indicating that Cam-003 only mediates killing of strains capable of producing Psl (wild type PAO1 and PAO1Δ*pslA* complemented *in trans* with the *pslA* gene). (H and I) ELISA data indicating reactivity of anti-Psl antibodies WapR-001, WapR-004, and WapR-016 with PAO1 Δ*wbpL*Δ*algD* and PAO1 Δ*wbpL*Δ*algD*Δ*pslA*. R347 was used as a negative control in all experiments.

[0045] Figure 4: Anti-Psl mAbs inhibit cell attachment of luminescent *P. aeruginosa* strain PAO1.lux to A549 cells. Log-phase PAO1.lux were added to a confluent monolayer of A549 cells at an MOI of 10 followed by analysis of RLU after repeated washing to remove unbound *P. aeruginosa*. Results are representative of three independent experiments performed in duplicate for each antibody concentration.

[0046] Figure 5 (A-C): *In vivo* passaged *P. aeruginosa* strains maintain/increase expression of Psl**.** The Cam-003 antibody is shown by a solid black line and a clear peak; the human IgG negative control antibody is shown as a gray line and a shaded peak. (A) For the positive control, Cam-003 was assayed for binding to strains grown to log phase from an overnight culture (~5 x 10^8/ml). (B) The inocula for each strain were prepared to 5 x 10^8 CFU/ml from an overnight TSA plate grown to lawn and tested for reactivity to Cam-003 by flow cytometry. (C) Four hours post intraperitoneal challenge, bacteria was

harvested from mice by peritoneal lavage and assayed for the presence of Psl with Cam-003 by flow cytometry.

[0047] Figure 6 (A-F): Survival rates for animals treated with anti-Psl monoclonal antibodies Cam-003 or WapR-004 in a *P. aeruginosa* acute pneumonia model. (A-D) Animals were treated with Cam-003 at 45, 15, and 5mg/kg and R347 at 45mg/kg or PBS 24 hours prior to intranasal infection with (A) PAO1 (1.6×10^7 CFU), (B) 33356 (3×10^7 CFU), (C) 6294 (7×10^6 CFU), (D) 6077 (1×10^6 CFU). (E-F) Animals were treated with WapR-004 at 5 and 1mg/kg as indicated followed by infection with 6077 at (E) (8×10^5 CFU), or (F) (6×10^5 CFU). Animals were carefully monitored for survival up to 72 hours (A-D) or for 120 hours (E-F). In all experiments, PBS and R347 served as negative controls. Results are represented as Kaplan-Meier survival curves; differences in survival were calculated by the Log-rank test for Cam-003 vs. R347. (A) Cam-003 (45mg/kg – $P<0.0001$; 15mg/kg – $P=0.0003$; 5mg/kg – $P=0.0033$). (B) Cam-003 (45mg/kg – $P=0.0012$; 15mg/kg – $P=0.0012$; 5mg/kg – $P=0.0373$). (C) Cam-003 (45mg/kg – $P=0.0007$; 15mg/kg – $P=0.0019$; 5mg/kg – $P=0.0212$). (D) Cam-003 (45mg/kg – $P<0.0001$; 15mg/kg – $P<0.0001$; 5mg/kg – $P=0.0001$). Results are representative of at least two independent experiments. (E) [Cam-003 (5mg/kg) vs. R347 (5mg/kg): $P=0.02$; Cam-003 (1mg/kg) vs. R347 (5mg/kg): $P=0.4848$; WapR-004 (5mg/kg) vs. R347 (5mg/kg): $P<0.0001$; WapR-004 (1mg/kg) vs. R347 (5mg/kg): $P=0.0886$; WapR-004 (5mg/kg) vs. Cam-003 (5mg/kg): $P=0.0017$; WapR-004 (1mg/kg) vs. Cam-003 (1mg/kg): $P=0.2468$; R347 (5mg/kg) vs. PBS: P=0.6676] (F) [Cam-003 (5mg/kg) vs. R347 (5mg/kg): $P=0.0004$; Cam-003 (1mg/kg) vs. R347 (5mg/kg): $P<0.0001$; WapR-004 (5mg/kg) vs. R347 (5mg/kg): $P<0.0001$; WapR-004 (1mg/kg) vs. R347 (5mg/kg): $P<0.0001$; WapR-004 (5mg/kg) vs. Cam-003 (5mg/kg): $P=0.0002$; WapR-004 (1mg/kg) vs. Cam-003 (1mg/kg): $P=0.2628$; R347 (5mg/kg) vs. PBS: P=0.6676]. Results are representative of five independent experiments.

[0048] Figure 7 (A-F): Anti-Psl monoclonal antibodies, Cam-003 and WapR-004, reduce organ burden after induction of acute pneumonia. Mice were treated with Cam-003 antibody 24 hours prior to infection with (A) PAO1 (1.1×10^7 CFU), (B) 33356 (1×10^7 CFU), (C) 6294 (6.25×10^6 CFU) (D) 6077 (1×10^6 CFU), and WapR-004 antibody 24 hours prior to infection with (E) 6294 (~1×10^7 CFU), and (F) 6206 (~1×10^6 CFU). 24 hours post-infection, animals were euthanized followed by harvesting or organs for

identification of viable CFU. Differences in viable CFU were determined by the Mann-Whitney U-test for Cam-003 or WapR-004 vs. R347. (A) Lung: Cam-003 (45mg/kg – P=0.0015; 15mg/kg – P=0.0021; 5mg/kg – P=0.0015); Spleen: Cam-003 (45mg/kg – P=0.0120; 15mg/kg – P=0.0367); Kidneys: Cam-003 (45mg/kg – P=0.0092; 15mg/kg – P=0.0056); (B) Lung: Cam-003 (45mg/kg – P=0.0010; 15mg/kg – P<0.0001; 5mg/kg – P=0.0045); (C) Lung: Cam-003 (45mg/kg – P=0.0003; 15mg/kg – P=0.0039; 5mg/kg – P=0.0068); Spleen: Cam-003 (45mg/kg – P=0.0057; 15mg/kg – P=0.0230; 5mg/kg – P=0.0012); (D) Lung: Cam-003 (45mg/kg – P=0.0005; 15mg/kg – P=0.0003; 5mg/kg – P=0.0007); Spleen: Cam-003 (45mg/kg – P=0.0015; 15mg/kg – P=0.0089; 5mg/kg – P=0.0089); Kidneys: Cam-003 (45mg/kg – P=0.0191; 15mg/kg – P=0.0355; 5mg/kg – P=0.0021). (E) Lung: WapR-004 (15mg/kg – P=0.0011; 5mg/kg – P=0.0004; 1mg/kg – P=0.0002); Spleen: WapR-004 (15mg/kg – P<0.0001; 5mg/kg – P=0.0014; 1mg/kg – P<0.0001); F) Lung: WapR-004 (15mg/kg – P<0.0001; 5mg/kg – P=0.0006; 1mg/kg – P=0.0079); Spleen: WapR-004 (15mg/kg – P=0.0059; 5mg/kg – P=0.0261; 1mg/kg – P=0.0047); Kidney: WapR-004 (15mg/kg – P=0.0208; 5mg/kg – P=0.0268.

[0049] Figure 8 (A-G): Anti-Psl monoclonal antibodies Cam-003 and WapR-004 are active in a *P. aeruginosa* keratitis model and thermal injury model. Mice were treated with a control IgG1 antibody or Cam-003 at 45mg/kg (A, B) or 15mg/kg (C, D) or PBS or a control IgG1 antibody or Cam-003 at 45mg/kg or WapR-004 at 45mg/kg or 15mg/kg or 5mg/kg (F, G) 24 hours prior to infection with 6077 (O11-cytotoxic – $2x10^6$ CFU). Immediately before infection, three 1 mm scratches were made on the left cornea of each animal followed by topical application of *P. aeruginosa* in a 5 µl inoculum. 24 hours after infection, the corneal pathology scores were calculated followed by removal of the eye for determination of viable CFU. Differences in pathology scores and viable CFU were determined by the Mann-Whitney U-test. (A) P=0.0001, (B) P<0.0001, (C) P=0.0003, (D) P=0.0015. (F) and (G) Cam-003 (45mg/kg) vs. WapR-004 (45mg/kg): P=0.018; Cam-003 (45mg/kg) vs. WapR-004 (15mg/kg): P=0.0025; WapR-004 (45mg/kg) vs. WapR-004 (15mg/kg): P=0.1331; WapR-004 (5mg/kg) vs. Ctrl: P<0.0001. Results are representative of five independent experiments. (E) Survival analysis from Cam-003 and R347 treated CF-1 mice in a *P. aeruginosa* thermal injury model after 6077 infection (2×10^5 CFU) (log-rank: R347 vs. Cam-003 15mg/kg, P=0.0094; R347 vs. Cam-003 5mg/kg, P=0.0017). Results are representative of at least three independent

experiments. (n) refers to number of animals in each group. Figure 8 (H): Anti-Psl and anti-PcrV monoclonal antibodies are active in a *P. aeruginosa* mouse ocular keratitis model. Mice were injected intraperitoneally (IP) with PBS or a control IgG1 antibody (R347) at 45mg/kg or WapR-004 (α-Psl) at 5mg/kg or V2L2 (α-PcrV) at 5mg/kg, 16 hours prior to infection with 6077 (O11-cytotoxic – $1x10^6$ CFU). Immediately before infection, mice were anesthetized followed by initiation of three 1 mm scratches on the cornea and superficial stroma of one eye of each mouse using a 27-gauge needle under a dissection microscope, followed by topical application of *P. aeruginosa* 6077 strain in a 5 µl inoculum.

[0050] Figure 9 (A-C): A Cam-003 Fc mutant antibody, Cam-003-TM, has diminished OPK and *in vivo* efficacy but maintains anti-cell attachment activity. (A) PAO1.lux OPK assay with Cam-003 and Cam-003-TM, which harbors mutations in the Fc domain that prevents Fc interactions with Fcγ receptors (Oganesyan, V., et al., *Acta Crystallogr D Biol Crystallogr 64*, 700-704 (2008)). R347 was used as a negative control. (B) PAO1 cell attachment assay with Cam-003 and Cam-003-TM. (C) Acute pneumonia model comparing efficacy of Cam-003 vs. Cam-003-TM.

[0051] Figure 10 (A-C): A: Epitope mapping and identification of the relative binding affinity for anti-Psl monoclonal antibodies. Epitope mapping was performed by competition ELISA and confirmed using an OCTET® flow system with Psl derived from the supernatant of an overnight culture of *P. aeruginosa* strain PAO1. Relative binding affinities were measured on a FORTEBIO® OCTET® 384 instrument. Also shown are antibody concentrations where cell attachment was maximally inhibited and OPK EC50 values for each antibody. B, C. Relative binding affinities of various WapR-004 mutants as measured on a FORTEBIO® OCTET® 384 instrument. Also shown are OPK EC50 values for the various mutants.

[0052] Figure 11 (A-M): Evaluation of WapR-004 (W4) mutants clones in the *P. aeruginosa* opsonophagocytic killing (OPK) assay (A-M) OPK assay with luminescent *P. aeruginosa* serogroup O5 strain (PAO1.lux), with dilutions of different W4 mutant clones in scFv-Fc format. In some instances, W4 IgG1 was included in the assay and is indicated as W4-IgG1. W4-RAD-Cam and W4-RAD-GB represent the same WapR-004RAD sequence described herein. "W4-RAD" is a shorthand name for WapR-004RAD, and W4-RAD-Cam and W4-RAD-GB designations in panels D through M

represent two different preparations of WapR-004RAD. (N-Q): Evaluation of the optimized anti-Psl mAbs derived from lead (WapR-004) optimization in the *P. aeruginosa* OPK assay. (N-O) OPK assay with luminescent PAO1.lux using dilutions of purified lead optimized monoclonal antibodies. (P-Q) Repeat OPK assay with PAO1.lux with dilutions of purified mAbs to confirm results. (N-Q): W4-RAD was used as a comparative positive control. In all experiments, R347, a human IgG1 monoclonal antibody that does not bind *P. aeruginosa* antigens, was used as a negative control.

[0053] Figure 12 (A-H): (A) The PcrV epitope diversity. . (B) Percent inhibition of cytotoxicity analysis for the parental V2L2 mAb, mAb166 (positive control) and R347 (negative control). (C) Evaluation of the V2L2 mAb, mAb166 (positive control) and R347 (negative control) ability to prevent lysis of RBCs. (D) Evaluation of the V2L2-germlined mAb (V2L2-GL) and optimized V2L2-GL mAbs (V2L2-P4M, V2L2-MFS, V2L2-MD and V2L2-MR) to prevent lysis of RBCs. (E) Evaluation of mAbs 1E6, 1F3, 11A6, 29D2, PCRV02 and V2L7 to prevent lysis of RBCs (F) Evaluation of mAbs V2L2 and 29D2 to prevent lysis of RBCs. (G-H) Relative binding affinities of V2L2-GL and V2L2-MD antibodies.

[0054] Figure 13 (A-I): *In vivo* survival study of anti-PcrV antibody treated mice. (A) Mice were treated 24 hours prior to infection with: 1.03 x 10^6 CFU 6077 ($exoU^+$) with 45 mg/kg R347 (negative control), 45 mg/kg, 15.0 mg/kg, 5.0 mg/kg, or 1.0 mg/kg mAb166 (positive control), or 15 mg/kg, 5.0 mg/kg, 1.0 mg/kg, or 0.2 mg/kg V2L2. Survival was monitored for 96 hours. (B) Mice were treated 24 hours prior to infection with: 2.1 x 10^7 CFU 6294 ($exoS^+$) with 15 mg/kg R347 (negative control), 15.0 mg/kg, 5.0 mg/kg, or 1.0 mg/kg mAb166 (positive control), or 15 mg/kg, 5.0 mg/kg, or 1.0 mg/kg V2L2. Survival was monitored for 168 hours. Mice were treated 24 hours prior to infection with: (C) 6294 (O6) or (D) PA103A with R347 (negative control), 5mg/kg of the PcrV antibody PcrV-02, or 5mg/kg, 1.0mg/kg, 0.2mg/kg, or 0.04mg/kg V2L2. Mice were treated 24 hours prior to infection with strain 6077 with R347 (negative control), 5mg/kg of the PcrV antibody PcrV-02, V2L7 (5mg/kg or 1mg/kg), 3G5 (5mg/kg or 1mg/kg), or 11A6 (5mg/kg or 1mg/kg) (E), or 25mg/kg of the V2L7, 1E6, 1F3, 29D2, R347 or 1mg/kg of the PcrV antibody PcrV-01 (F), or 25mg/kg of the 21F1, V2L2, 2H3, 4A8, SH3, LE10, R347 or 1mg/kg of the PcrV-02 (G), or the 29D2 (1 mg/kg, 3mg/kg or 10 mg/kg), the V2L2 (1 mg/kg, 3mg/kg or 10 mg/kg) R347 or 1mg/kg of the PcrV-02 (H). Mice were

treated 24 hours prior to infection with: 6294 (O6) or PA103A with the V2L2 (0.04mg/kg, 0.2mg/kg, 1 mg/kg or 5 mg/kg), R347 or 5mg/kg of the PcrV-02. Percent survival was assayed in an acute pneumonia model.

[0055] Figure 14: Organ burden analysis of V2L2 treated mice. Mice were treated 24 hours prior to infection with 6206 with (A) R347 (negative control), 1 mg/kg, 0.2 mg/kg, or 0.07 mg/kg V2L2 and (B) 15 mg/kg R347 (negative control); 15.0 mg/kg, 5.0 mg/kg, or 1.0 mg/kg mAb166 (positive control); or 5.0 mg/kg, 1.0 mg/kg, or 0.2 mg/kg V2L2. Colony forming units were identified per gram of tissue in lung, spleen, and kidney.

[0056] Figure 15: Organ burden analysis of V2L2 and WapR-004 (W4) treated mice. Mice were treated 24 hours prior to infection with 6206 (O11-ExoU+) with R347 (negative control), V2L2 alone, or V2L2 (0.1mg/kg) in combination with increasing concentrations of W4 (0.1, 0.5, 1.0, or 2.0 mg/kg). Colony forming units were identified per gram of tissue in lung, spleen, and kidney. .

[0057] Figure 16 (A-G): Survival rates for animals treated with anti-PcrV monoclonal antibody V2L2 in a *P. aeruginosa* acute pneumonia model. V2L2-GL, V2L2-MD, V2L2-PM4, V2L2-A and V2L2-MFS designations in panels A through G represent different preparations of V2L2. (A-C) Animals were treated with V2L2 at 1mg/kg, 0.5mg/kg or R347 at 0.5mg/kg prior to intranasal infection with (A) 6077 (9.75×10^5 CFU), (B, C) 6077 (9.5×10^5 CFU). (D-F) Animals were treated with V2L2 at 0.5mg/kg, 0.1mg/kg or R347 at 0.5mg/kg followed by infection with 6077 (D) (1×10^6 CFU), (E) (9.5×10^5 CFU) or F (1.026×10^6 CFU). (G) Animals were treated with V2L2-MD at (0.04mg/kg, 0.2mg/kg, 1mg/kg or 5mg/kg), mAb166 (positive control) at (0.2mg/kg, 1mg/kg, 5mg/kg or 15mg/kg), or R347 at 0.5mg/kg followed by infection with 6206 ($2 \times 10^{7+}$ CFU).

[0058] Figure 17 (A-B): Schematic representation of (A) Bs1-TNFα/W4, Bs2-TNFα/W4, Bs3-TNFα/W4 and (B) Bs2-V2L2/W4-RAD, Bs3-V2L2/W4-RAD, and Bs4-V2L2-W4-RAD Psl/PcrV bispecific antibodies. (A) For Bs1-TNFα/W4, the W4 scFv is fused to the amino-terminus of TNFα VL through a (G4S)2 linker. For Bs2-TNFα/W4, the W4 scFv is fused to the amino-terminus of TNFα VH through a (G4S)2 linker. For Bs3-TNFα/W4, the W4 scFv is fused to the carboxy-terminus of CH3 through a (G4S)2 linker. (B) For Bs2-V2L2-2C, the W4-RAD scFv is fused to the amino-terminus of V2L2 VH through a (G4S)2 linker. For Bs2-W4-RAD-2C, the V2L2 scFv is fused to the

amino-terminus of W4-RAD VH through a (G4S)2 linker. For Bs3-V2L2-2C, the W4-RAD scFv is fused to the carboxy-terminus of CH3 through a (G4S)2 linker. For Bs4-V2L2-2C, the W4-RAD scFv is inserted in the hinge region, linked by (G4S)2 linker on the N-terminal and C-terminal of the scFv.

[0059] Figure 18: Evaluation of WapR-004 (W4) scFv activity in a bispecific constructs depicted in Figure 17A. The W4 scFv was ligated onto two different bispecific constructs (in alternating N- or C-terminal orientations) having a TNFα binding arm. Each W4-TNFα bispecific construct (Bs1-TNFα/W4, Bs2-TNFα/W4 and Bs3-TNFα/W4) retained the ability to inhibit cell attachment similarly as W4 using the PAO1.lux (O5) assay indicating that the W4 scFv retains its activity in a bispecific format. R347 was used as a negative control.

[0060] Figure 19 (A-C): Anti-Psl and anti-PcrV binding domains were combined in the bispecific format by replacing the TNFα antibody of Figure 17B with V2L2. These constructs are identical to those depicted in Figure 17B with the exception of using the non-stabilized W4-scFv in place of the stabilized W4-RAD scFv. Both W4 and W4-RAD target identical epitopes and have identical functional activities. Percent inhibition of cytotoxicity was analysed for both BS2-V2L2 and BS3-V2L2 using both (A) 6206 and (B) 6206Δ*pslA* treated A549 cells. (C) BS2-V2L2, BS3-V2L2, and BS4-V2L2 were evaluated for their ability to prevent lysis of RBCs compared to the parental control. All bi-specific constructs retained anti-cytotoxicity activity similar to the parental V2L2 antibody using 6206 and 6206Δ*pslA* infected cells and prevented lysis of RBCs similar to the parental control (V2L2). R347 was used as a negative control in all experiments.

[0061] Figure 20 (A-C): Evaluation of anti-Psl/anti-PcrV bispecific constructs for promoting OPK of *P. aeruginosa*. Opsonophagocytosis assay is shown with luminescent *P. aeruginosa* serogroup O5 strain (PAO1.lux), with dilutions of purified Psl/TNFα bispecific antibodies (Bs2-TNFα and Bs3-TNFα); the W4-RAD or V2L2-IgG1 parental antibodies; the Psl/PcrV bispecific antibodies Bs2- V2L2 or Bs3-V2L2, or the Bs2-V2L2-2C, Bs3-V2L2-2C, Bs4-V2L2-2C or the Bs4-V2L2-2C antibody harboring a YTE mutation (Bs4-V2L2-2C-YTE). (A) While the Bs2-V2L2 antibody showed similar killing compared to the parental W4-RAD antibody, the killing for the Bs3-V2L2 antibody was decreased. (B) While the Bs2-V2L2-2C and Bs4-V2L2-2C antibodies showed similar killing compared to the parental W4-RAD antibody, the killing for the Bs3-V2L2-2C

antibody was decreased. (C) W4-RAD and W4-RAD-YTE designations represent different preparations of W4-RAD. Bs4-V2L2-2C (old lot) and Bs4-V2L2-2C (new lot), designations represent different preparations of Bs4-V2L2-2C. The YTE modification in Bs4-V2L2-2C-YTE is a modification made to antibodies that increases the half-life of antibodies. Different preparations of Bs4 antibodies (old lot vs. new lot) showed similar killing compared to the parental W4-RAD antibody, however the Bs4-V2L2-2C-YTE antibodies had a 3-fold drop in OPK activity when compared to Bs4-V2L2-2C (See EC50 table). R347 was used as a negative control in all experiments.

[0062] Figure 21 (A-I): *In vivo* survival study of anti-Psl/anti-PcrV bispecific antibodies Bs2-V2L2, Bs3-V2L2, Bs4-V2L2-2C and Bs4-V2L2-2C-YTE-treated mice in a 6206 acute pneumonia model system. Mice (n=10) were treated with (A): R347 (negative control, 0.2 mg/kg), Bs2-V2L2 (0.28 mg/kg), Bs3-V2L2 (0.28 mg/kg), V2L2 (0.2 mg/kg) or W4-RAD (0.2 mg/kg); (B-C): R347 (negative control, 1 mg/kg), Bs2-V2L2 (0.5 mg/kg or 1 mg/kg), or Bs4-V2L2-2C (0.5 mg/kg or 1 mg/kg); (D): R347 (negative control, 1 mg/kg), Bs3-V2L2 (0.5 mg/kg or 1 mg/kg), or Bs4-V2L2-2C (0.5 mg/kg or 1 mg/kg); (E): R347 (negative control, 2 mg/kg), a combination of the individual W4 and V2L2 antibodies (0.5 mg/kg or 1 mg/kg each) or Bs4-V2L2-2C (1 mg/kg or 2 mg/kg); (F): R347 (negative control, 1 mg/kg), a mixture of the individual W4 and V2L2 antibodies (0.5 mg/kg or 1 mg/kg each) or Bs4-V2L2-2C (1 mg/kg or 0.5 mg/kg). Twenty-four hours post-treatment, all mice were infected with ~ ($6.25x10^5$-$1x10^6$ CFU/animal) 6206 (O11-ExoU+). All mice were monitored for 120 hours. (A): All of the control mice succumbed to infection by approximately 30 hours post-infection. All of the Bs3-V2L2 animals survived, along with those which received the V2L2 control. Approximately 90% of the W4-RAD immunized animals survived. In contrast, approximately 50% of the Bs2-V2L2 animals succumbed to infection by 120 hours. (B-F): All of the control mice succumbed to infection by approximately 48 hours post-infection. (B): Bs4-V2L2-2C had greater activity in comparison to Bs2-V2L2 at both 1.0 and 0.5 mg/kg. (C): Bs4-V2L2-2C appeared to have greater activity in comparison to Bs2-V2L2 at 1.0 mg/kg (results are not statistically significant). (D): Bs4-V2L2-2C had greater activity in comparison to Bs3-V2L2 at 0.5 mg/kg. (E): Bs4-V2L2-2C at both 2 mg/kg and 1 mg/kg had greater activity in comparison to the antibody mixture at both 1.0 and 0.5 mg/kg. (F): Bs4-V2L2 (1 mg/kg) has similar activity at both 1.0 and 0.5 mg/kg. (G-H): Both Bs4-

V2L2-2C and Bs4-V2L2-2C-YTE had similar activity at both 1.0 and 0.5 mg/kg. Results are represented as Kaplan-Meier survival curves; differences in survival were calculated by the Log-rank test for (B) Bs4-V2L2-2C vs. Bs2-V2L2 (1 mg/kg – P=0.034; 0.5mg/kg – P=0.0002); (D) Bs4-V2L2-2C vs. Bs3-V2L2 (0.5 mg/kg – P<0.0001); (E): Bs4-V2L2-2C (2 mg/kg) vs. antibody mixture (1 mg/kg each)-P=0.0012; Bs4-V2L2-2C (1 mg/kg) vs. antibody mixture (0.5 mg/kg each)-P=0.0002. (G-H): Mice (n=8) were treated with: R347 (negative control, 1 mg/kg), Bs4-V2L2-2C (1 and 0.5 mg/kg), and Bs4-V2L2-2C-YTE (1 and 0.5 mg/kg) and 6206 (9e5 CFU). No difference in survival between Bs4-V2L2-2C and Bs4-V2L2-2C-YTE at either dose were observed by Log-Rank. (I): To analyze the efficacy of each antibody construct, mice were treated with 0.1 mg/kg, 0.2 mg/kg, 0.5 mg/kg, 1 mg/kg, 2 mg/kg, 5 mg/kg, 10 mg/kg or 15 mg/kg and analyzed for survival in a 6206 lethal pneumonia model. The percent survival is indicated in the table with the number of animals for each comparison indicated in parentheses.

[0063] Figure 22: Organ burden analysis of anti-Psl/PcrV bispecific antibody-treated animals using the 6206 acute pneumonia model. Mice were treated 24 hours prior to infection with 6206 (O11-ExoU+) with R347 (negative control), V2L2 or W4-RAD alone (0.2 mg/kg), Bs2-V2L2 (0.28 mg/kg), or BS3-V2L2 (0.28 mg/kg). Colony forming units were identified per gram of tissue in lung, spleen, and kidney. At the concentration tested, both Bs2-V2L2 and Bs3-V2L2 significantly decreased organ burden in lung. However, neither of the bispecific constructs was able to significantly affect organ burden in spleen or kidney compared to the parental antibodies.

[0064] Figure 23 (A-B): Organ burden analysis of anti-Psl/PcrV bispecific antibody-treated animals using a 6294 model system. Mice were treated 24 hours prior to infection with 6294 with R347 (negative control), V2L2 or W4-RAD alone (0.5 mg/kg), Bs2-V2L2 (0.7 mg/kg), or Bs3-V2L2 (0.7 mg/kg) (A), or V2L2 or W4-RAD alone (0.2 mg/kg), Bs2-V2L2 (0.2 mg/kg), Bs3-V2L2 (0.2 mg/kg) or a combination of the individual W4-RAD and V2L2 antibodies (0.1 mg/kg each) (B). Twenty-four hours post-administration of antibody, all mice were infected with an inoculum containing 2.5 x 10^7 CFU 6294 (A) or 1.72 x 10^7 CFU 6294 (B). Colony forming units were identified per gram of tissue in lung, spleen, and kidney. Using the 6294 model system, (A) both the BS2-V2L2 and BS3-V2L2 significantly decreased organ burden in all of the tissues to a level comparable to that of the V2L2 parental antibody. The W4-RAD parental antibody had no effect on

decreasing organ burden. (B) Bs2-V2L2, Bs3-V2L2, and W4-RAD+V2L2 combination significantly decreased organ burden in all of the tissues to a level comparable to that of the V2L2 parental antibody.

[0065] Figure 24: *In vivo* survival study of Bs2-W4/V2L2 and Bs3-W4/V2L2-treated mice in a 6294 model system. Mice were treated with R347 (negative control, 0.2 mg/kg), Bs2-V2L2 (0.28 mg/kg), Bs3-V2L2 (0.28 mg/kg), V2L2 (0.2 mg/kg) or W4-RAD (0.2 mg/kg). Twenty-four hours post-treatment, all mice were infected with 6294. All mice were monitored for 120 hours. All of the control mice succumbed to infection by approximately 75 hours post-infection. Sixty percent of the Bs3-V2L2 and 50% of the Bs2-V2L2 animals survived after 120 hours post-inoculation. As was seen in the organ burden studies, W4-RAD immunization did not affect survival with all mice succumbing to infection at approximately the same time as the controls.

[0066] Figure 25 (A-D): Organ burden analysis of anti-Psl/PcrV bispecific antibody or W4 + V2L2 combination therapy in the 6206 model system. Suboptimal concentrations of antibody were used (A-C) to enable the ability to decipher antibody activity. (D) High concentrations of Bs4 were used. Mice were treated 24 hours prior to infection with 6206 with R347 (negative control), V2L2 or W4-RAD alone (0.2 mg/kg), Bs2-V2L2 (0.2 mg/kg), Bs3-V2L2 (0.2 mg/kg), Bs4 (15.0, 5.0 and 1.0 mg/kg) or a combination of the individual W4 and V2L2 antibodies (0.1 mg/kg each). Twenty-four hours post-administration of antibody, all mice were infected with an inoculum containing (A), (B) 4.75×10^5 CFU 6206 (O11-ExoU+), or (C) 7.75×10^5 CFU 6206 (O11-ExoU+) or (D) 9.5×10^5 CFU 6206 (O11-ExoU+). Colony forming units were identified per gram of tissue in lung, spleen, and kidney. Using the 6206 model system, both the BS2-V2L2 and BS3-V2L2 decreased organ burden in the lung, spleen and kidneys to a level comparable to that of the W4 + V2L2 combination. In the lung, the combination significantly reduced bacterial CFUs Bs2- and Bs3-V2L2 and V2L2 using the Kruskal-Wallis with Dunn's post test. Significant differences in bacterial burden in the spleen and kidney were not observed, although a trend towards reduction was noted. (D) When optimal concentrations of Bs4-V2L2-2C were used (15.0, 5.0, and 1.0), rapid and efficient bacterial clearance was observed from the lung. In addition, bacterial dissemination to the spleen and kidneys were also ablated. Asterisks indicate statistical significance when compared to the R347 control using the Kruskal-Wallis with Dunn's post test.

[0067] Figure 26 (A-J): Therapeutic adjunctive therapy: Bs4-V2L2-2C + antibiotic. (A)-(B) Mice were treated 24 hours prior to infection with 1 x 10^6 CFU 6206 with 0.5 mg/kg R347 (negative control) or Bs4-V2L2-2C (0.2 mg/kg or 0.5 mg/kg) or Ciprofloxacin (CIP) (20 mg/kg or 6.7 mg/kg) 1 hour post infection, or a combination of the Bs4-V2L2-2C 24 hours prior to infection and CIP 1 hour post infection (0.5 mg/kg + 20 mg/kg or 0.5 mg/kg + 6.7 mg/kg or 0.2 mg/kg + 20 mg/kg or 0.2 mg/kg + 6.7 mg/kg, respectively). (C) Mice were treated 1 hour post infection with 9.5 x 10^5 CFU 6206 with 5 mg/kg R347 or CIP (20 mg/kg or 6.7 mg/kg) or Bs4-V2L2-2C (1 mg/kg or 5 mg/kg), or a combination of the Bs4-V2L2-2C and CIP (5 mg/kg + 20 mg/kg or 5 mg/kg + 6.7 mg/kg or 1 mg/kg + 20 mg/kg or 1 mg/kg + 6.7 mg/kg, respectively). (D) Mice were treated 2 hours post infection with 9.5 x 10^5 CFU 6206 with 5 mg/kg R347 or CIP (20 mg/kg or 6.7 mg/kg) or Bs4-V2L2-2C (1 mg/kg or 5 mg/kg), or a combination of the Bs4-V2L2-2C and Cipro (5 mg/kg + 20 mg/kg or 5 mg/kg + 6.7 mg/kg or 1 mg/kg + 20 mg/kg or 1 mg/kg + 6.7 mg/kg, respectively). (E) Mice were treated 2 hours post infection with 9.75 x 10^5 CFU 6206 with 5 mg/kg R347or Bs4-V2L2-2C (1 mg/kg or 5 mg/kg) or CIP (20 mg/kg or 6.7 mg/kg) 1 hour post infection, or a combination of the Bs4-V2L2-2C 2 hours post infection and CIP 1 hour post infection (5 mg/kg + 20 mg/kg or 5 mg/kg + 6.7 mg/kg or 1 mg/kg + 20 mg/kg or 1 mg/kg + 6.7 mg/kg, respectively). (F) Mice were treated 1 hour post infection with 9.5 x 10^5 CFU 6206 with 5 mg/kg R347 or Meropenem (MEM) (0.75 mg/kg or 2.3 mg/kg) or Bs4-V2L2-2C (1 mg/kg or 5 mg/kg), or a combination of the Bs4-V2L2-2C and MEM (5 mg/kg + 2.3 mg/kg or 5 mg/kg + 0.75 mg/kg or 1 mg/kg + 2.3 mg/kg or 1 mg/kg + 0.75 mg/kg, respectively). (G) Mice were treated 2 hours post infection with 9.75 x 10^5 CFU 6206 with 5 mg/kg R347 or Bs4-V2L2-2C (1 mg/kg or 5 mg/kg) or MEM (0.75 mg/kg or 2.3 mg/kg) 1 hour post infection, or a combination of the Bs4-V2L2-2C 2 hours post infection and MEM 1 hour post infection (5 mg/kg + 2.3 mg/kg or 5 mg/kg + 0.75 mg/kg or 1 mg/kg + 2.3 mg/kg or 1 mg/kg + 0.75 mg/kg, respectively). (H) Mice were treated 2 hours post infection with 1 x 10^6 CFU 6206 with 5 mg/kg R347 or Bs4-V2L2-2C (1 mg/kg or 5 mg/kg) or MEM (0.75 mg/kg or 2.3 mg/kg), or a combination of the Bs4-V2L2-2C 2 and MEM (5 mg/kg + 2.3 mg/kg or 5 mg/kg + 0.75 mg/kg or 1 mg/kg + 2.3 mg/kg or 1 mg/kg + 0.75 mg/kg, respectively). (I) Mice were treated 4 hour post infection with 9.25 x 10^5 CFU 6206 with 5 mg/kg R347 or CIP (6.7 mg/kg) or Bs4-V2L2-2C (1 mg/kg or 5 mg/kg) or a combination of the Bs4-V2L2-

2C and CIP (5 mg/kg + 6.7 mg/kg or 1 mg/kg + 6.7 mg/kg, respectively) , (J) Mice were treated 4 hour post infection with 1.2 x 10^6 CFU 6206 with 5 mg/kg R347 + CIP (6.7 mg/kg), CIP (6.7 mg/kg), or Bs4-V2L2-2C (1 mg/kg or 5 mg/kg) or a combination of the Bs4-V2L2-2C and CIP (5 mg/kg + 6.7 mg/kg or 1 mg/kg + 6.7 mg/kg, respectively). (A-J) Bs4 antibody combined with either CIP or MEM increases efficacy of antibiotic therapy, indicating synergistic protection when the molecules are combined. In addition, although antibiotic delivered by itself or in combination with a *P. aeruginosa* non-specific antibody can reduce or control bacterial CFU in the lung, antibiotic alone does not protect mice from lethality in this setting. Optimal protection in this setting requires including Bs4-V2L2-2C in combination with antibiotic.

[0068] Figure 27 (A-C): Difference in functional activity of bi-specific antibodies BS4-WT, BS4-GL and BS4-GLO: opsonophagocytic killing assay (A), anti-cell attachment assay (B), and a RBC lysis anti-cytotoxicity assay (C).

[0069] Figure 28 (A-B): Percent protection against lethal pneumonia in mice challenged in prophylactic (A) or therapeutic (B) settings with *P. aeruginosa* strains. The percent survival is indicated in the table with the number of animals for each comparison indicated in parentheses. The dashes indicate not tested.

[0070] Figure 29 (A-B): Survival rates for animals treated with bispecific antibody Bs4-GLO in a *P. aeruginosa* lethal bacteremia model. (A) Animals were treated with Bs4-GLO at 15mg/kg, 5mg/kg, 1mg/kg or R347 at 15mg/kg 24 hours prior to intraperitoneal infection with 6294 (O6) (5.58 x 10^7 CFU). (B) Animals were treated with Bs4-GLO at 5 mg/kg, 1 mg/kg, 0.2 mg/kg or R347 at 5mg/kg 24 hours prior to intraperitoneal infection with 6206 (O11-ExoU$^+$) (6.48 x 10^6 CFU). Results are represented as Kaplan-Meier survival curves; differences in survival were calculated by the Log-rank test for BS4-GLO at each concentration vs. R347. (A) Bs4-GLO at all concentrations vs. R347 $P<0.0001$. (B) Bs4-GLO at all concentrations vs. R347 $P=0.0003$. Results are representative of three independent experiments.

[0071] Figure 30 (A-C): Survival rates for animals prophylactically treated (prevention) with Bs4-GLO in a *P. aeruginosa* thermal injury model. (A) Animals were treated with Bs4-GLO at 15mg/kg, 5mg/kg or R347 at 15mg/kg 24 hours prior to induction of thermal injury and subcutaneous infection with *P. aeruginosa* strain 6077 (O11-ExoU$^+$) with 1.4 x 10^5 CFU directly under the wound. (B) Animals were treated with Bs4-GLO at 15mg/kg

or R347 at 15mg/kg 24 hours prior to induction of thermal injury and subcutaneous infection with *P. aeruginosa* strain 6206 (O11-ExoU$^+$) with 4.15 x 10^4 CFU directly under the wound. (C) Animals were treated with Bs4-GLO at 15mg/kg, 5mg/kg or R347 at 15mg/kg 24 hours prior to induction of thermal injury and subcutaneous infection with *P. aeruginosa* strain 6294 (O6) with 7.5 x 10^1 CFU directly under the wound. Results are represented as Kaplan-Meier survival curves; differences in survival were calculated by the Log-rank test for Bs4-GLO at each concentration vs. R347. (A-C) Bs4-GLO at all concentrations vs. R347 - $P<0.0001$. Results are representative of two independent experiments for each *P. aeruginosa* strain.

[0072] Figure 31 (A-B): Survival rates for animals therapeutically treated (treatment)) with Bs4-GLO in a *P. aeruginosa* thermal injury model. (A) Animals were treated with Bs4-GLO at 42.6mg/kg, 15 mg/kg or R347 at 45mg/kg 4h hours after induction of thermal injury and subcutaneous infection with *P. aeruginosa* strain 6077 (O11-ExoU$^+$) with 1.6 x 10^5 CFU directly under the wound. (B) Animals were treated with Bs4-GLO at 15mg/kg, 5 mg/kg or R347 at 15mg/kg 12h hours after induction of thermal injury and subcutaneous infection with *P. aeruginosa* strain 6077 (O11-ExoU$^+$) with 1.0 x 10^5 CFU directly under the wound. Results are represented as Kaplan-Meier survival curves; differences in survival were calculated by the Log-rank test for BS4-GLO at each concentration vs. R347. (A) Bs4-GLO at both concentrations vs. R347 – $P=0.0004$. (B) Bs4-GLO at 5mg/kg vs. R347 - $P=0.048$. Results are representative of two independent experiments.

[0073] Figure 32 (A-B): Therapeutic adjunctive therapy: Bs4GLO + ciprofloxacin (CIP): (A) Mice were treated 4 hour post infection with 9.5 x 10^5 CFU 6206 with 5 mg/kg R347 + CIP (6.7 mg/kg) or Bs4-WT (1 mg/kg or 5 mg/kg) or a combination of the Bs4-WT and CIP (5 mg/kg + 6.7 mg/kg or 1 mg/kg + 6.7 mg/kg, respectively). (B) Mice were treated 4 hour post infection with 9.5 x 10^5 CFU 6206 with 5 mg/kg R347 + CIP (6.7 mg/kg) or Bs4-GLO (1 mg/kg or 5 mg/kg) or a combination of the Bs4-GLO and CIP (5 mg/kg + 6.7 mg/kg or 1 mg/kg + 6.7 mg/kg, respectively

[0074] Figure 33 (A-B): Therapeutic adjunctive therapy: Bs4-GLO + meropenem (MEM): (A) Mice were treated 4 hour post infection with 9.5 x 10^5 CFU 6206 with 5 mg/kg R347 + MEM (0.75 mg/kg) or Bs4-WT (1 mg/kg or 5 mg/kg) or a combination of the Bs4-WT and MEM (5 mg/kg + 0.75 mg/kg or 1 mg/kg + 0.75 mg/kg, respectively).

(B) Mice were treated 4 hour post infection with 9.5 x 10^5 CFU 6206 with 5 mg/kg R347 + MEM (0.75 mg/kg) or Bs4-GLO (1 mg/kg or 5 mg/kg) or a combination of the Bs4-GLO and MEM (5 mg/kg + 0.75 mg/kg or 1 mg/kg + 0.75 mg/kg, respectively).

[0075] Figure 34 (A-C): Therapeutic adjunctive therapy: Bs4-GLO + antibiotic in a lethal bacteremia model. Mice were treated 24 hours prior to intraperitoneal infection with *P. aeruginosa* strain 6294 (O6) 9.3 x 10^7 with Bs4-GLO at (0.25mg/kg or 0.5mg/kg) or R347 (negative control). One hour post infection, mice were treated subcutaneously with (A) 1mg/kg CIP, (B) 2.5mg/kg MEM or (C) 2.5mg/kg TOB. Results are represented as Kaplan-Meier survival curves; differences in survival were calculated by the Log-rank test for Bs4-GLO at each concentration vs. R347.

[0076] Figure 35 (A-B) Schematic representation of alternative formats for Bs4 constructs (A) anti-PcrV variable regions are present separately on the heavy and light chains while the anti-Psl variable regions are present as an scFv within the hinge region of the heavy chain and (B) anti-Psl variable regions are present separately on the heavy and light chains while the anti-PcrV variable regions are present as an scFv within the hinge region of the heavy chain.

DETAILED DESCRIPTION

I. DEFINITIONS

[0077] It is to be noted that the term "a" or "an" entity refers to one or more of that entity; for example, "a binding molecule which specifically binds to *Pseudomonas* Psl and/or PcrV," is understood to represent one or more binding molecules which specifically bind to *Pseudomonas* Psl and/or PcrV. As such, the terms "a" (or "an"), "one or more," and "at least one" can be used interchangeably herein.

[0078] As used herein, the term “polypeptide” is intended to encompass a singular “polypeptide” as well as plural “polypeptides,” and refers to a molecule composed of monomers (amino acids) linearly linked by amide bonds (also known as peptide bonds). The term "polypeptide" refers to any chain or chains of two or more amino acids, and does not refer to a specific length of the product. Thus, peptides, dipeptides, tripeptides, oligopeptides, “protein,” “amino acid chain,” or any other term used to refer to a chain or chains of two or more amino acids are included within the definition of "polypeptide,”

and the term “polypeptide” can be used instead of, or interchangeably with any of these terms. The term "polypeptide" is also intended to refer to the products of post-expression modifications of the polypeptide, including without limitation glycosylation, acetylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, or modification by non-naturally occurring amino acids. A polypeptide can be derived from a natural biological source or produced by recombinant technology, but is not necessarily translated from a designated nucleic acid sequence. It can be generated in any manner, including by chemical synthesis.

[0079] A polypeptide as disclosed herein can be of a size of about 3 or more, 5 or more, 10 or more, 20 or more, 25 or more, 50 or more, 75 or more, 100 or more, 200 or more, 500 or more, 1,000 or more, or 2,000 or more amino acids. Polypeptides can have a defined three-dimensional structure, although they do not necessarily have such structure. Polypeptides with a defined three-dimensional structure are referred to as folded, and polypeptides which do not possess a defined three-dimensional structure, but rather can adopt a large number of different conformations, and are referred to as unfolded. As used herein, the term glycoprotein refers to a *protein* coupled to at least one carbohydrate moiety that is attached to the protein via an oxygen-containing or a nitrogen-containing side chain of an amino acid residue, *e.g.*, a serine residue or an asparagine residue.

[0080] By an "isolated" polypeptide or a fragment, variant, or derivative thereof is intended a polypeptide that is not in its natural milieu. No particular level of purification is required. For example, an isolated polypeptide can be removed from its native or natural environment. Recombinantly produced polypeptides and proteins expressed in host cells are considered isolated as disclosed herein, as are native or recombinant polypeptides which have been separated, fractionated, or partially or substantially purified by any suitable technique.

[0081] Other polypeptides disclosed herein are fragments, derivatives, analogs, or variants of the foregoing polypeptides, and any combination thereof. The terms "fragment," "variant," "derivative" and "analog" when referring to a binding molecule such as an antibody which specifically binds to *Pseudomonas* Psl and/or PcrV as disclosed herein include any polypeptides which retain at least some of the antigen-binding properties of the corresponding native antibody or polypeptide. Fragments of polypeptides include, for example, proteolytic fragments, as well as deletion fragments, in

addition to specific antibody fragments discussed elsewhere herein. Variants of a binding molecule, *e.g.*, an antibody which specifically binds to *Pseudomonas* Psl and/or PcrV as disclosed herein include fragments as described above, and also polypeptides with altered amino acid sequences due to amino acid substitutions, deletions, or insertions. Variants can occur naturally or be non-naturally occurring. Non-naturally occurring variants can be produced using art-known mutagenesis techniques. Variant polypeptides can comprise conservative or non-conservative amino acid substitutions, deletions or additions. Derivatives of a binding molecule, *e.g.*, an antibody which specifically binds to *Pseudomonas* Psl and/or PcrV as disclosed herein are polypeptides which have been altered so as to exhibit additional features not found on the native polypeptide. Examples include fusion proteins. Variant polypeptides can also be referred to herein as "polypeptide analogs." As used herein a "derivative" of a binding molecule, *e.g.*, an antibody which specifically binds to *Pseudomonas* Psl and/or PcrV refers to a subject polypeptide having one or more residues chemically derivatized by reaction of a functional side group. Also included as "derivatives" are those peptides which contain one or more naturally occurring amino acid derivatives of the twenty standard amino acids. For example, 4-hydroxyproline can be substituted for proline; 5-hydroxylysine can be substituted for lysine; 3-methylhistidine can be substituted for histidine; homoserine can be substituted for serine; and ornithine can be substituted for lysine.

[0082] The term "polynucleotide" is intended to encompass a singular nucleic acid as well as plural nucleic acids, and refers to an isolated nucleic acid molecule or construct, *e.g.*, messenger RNA (mRNA) or plasmid DNA (pDNA). A polynucleotide can comprise a conventional phosphodiester bond or a non-conventional bond (*e.g.*, an amide bond, such as found in peptide nucleic acids (PNA)). The term "nucleic acid" refers to any one or more nucleic acid segments, *e.g.*, DNA or RNA fragments, present in a polynucleotide. By "isolated" nucleic acid or polynucleotide is intended a nucleic acid molecule, DNA or RNA, which has been removed from its native environment. For example, a recombinant polynucleotide encoding a binding molecule, *e.g.*, an antibody which specifically binds to *Pseudomonas* Psl and/or PcrV contained in a vector is considered isolated as disclosed herein. Further examples of an isolated polynucleotide include recombinant polynucleotides maintained in heterologous host cells or purified (partially or substantially) polynucleotides in solution. Isolated RNA molecules include *in vivo* or *in*

vitro RNA transcripts of polynucleotides. Isolated polynucleotides or nucleic acids further include such molecules produced synthetically. In addition, polynucleotide or a nucleic acid can be or can include a regulatory element such as a promoter, ribosome binding site, or a transcription terminator.

[0083] As used herein, a "coding region" is a portion of nucleic acid which consists of codons translated into amino acids. Although a "stop codon" (TAG, TGA, or TAA) is not translated into an amino acid, it can be considered to be part of a coding region, but any flanking sequences, for example promoters, ribosome binding sites, transcriptional terminators, introns, and the like, are not part of a coding region. Two or more coding regions can be present in a single polynucleotide construct, *e.g.*, on a single vector, or in separate polynucleotide constructs, *e.g.*, on separate (different) vectors. Furthermore, any vector can contain a single coding region, or can comprise two or more coding regions, *e.g.*, a single vector can separately encode an immunoglobulin heavy chain variable region and an immunoglobulin light chain variable region. In addition, a vector, polynucleotide, or nucleic acid can encode heterologous coding regions, either fused or unfused to a nucleic acid encoding an a binding molecule which specifically binds to *Pseudomonas* Psl and/or PcrV, *e.g.*, an antibody, or antigen-binding fragment, variant, or derivative thereof. Heterologous coding regions include without limitation specialized elements or motifs, such as a secretory signal peptide or a heterologous functional domain.

[0084] In certain embodiments, the polynucleotide or nucleic acid is DNA. In the case of DNA, a polynucleotide comprising a nucleic acid which encodes a polypeptide normally can include a promoter and/or other transcription or translation control elements operably associated with one or more coding regions. An operable association is when a coding region for a gene product, *e.g.*, a polypeptide, is associated with one or more regulatory sequences in such a way as to place expression of the gene product under the influence or control of the regulatory sequence(s). Two DNA fragments (such as a polypeptide coding region and a promoter associated therewith) are "operably associated" if induction of promoter function results in the transcription of mRNA encoding the desired gene product and if the nature of the linkage between the two DNA fragments does not interfere with the ability of the expression regulatory sequences to direct the expression of the gene product or interfere with the ability of the DNA template to be transcribed. Thus, a

promoter region would be operably associated with a nucleic acid encoding a polypeptide if the promoter was capable of effecting transcription of that nucleic acid. The promoter can be a cell-specific promoter that directs substantial transcription of the DNA only in predetermined cells. Other transcription control elements, besides a promoter, for example enhancers, operators, repressors, and transcription termination signals, can be operably associated with the polynucleotide to direct cell-specific transcription. Suitable promoters and other transcription control regions are disclosed herein.

[0085] A variety of transcription control regions are known to those skilled in the art. These include, without limitation, transcription control regions which function in vertebrate cells, such as, but not limited to, promoter and enhancer segments from cytomegaloviruses (the immediate early promoter, in conjunction with intron-A), simian virus 40 (the early promoter), and retroviruses (such as Rous sarcoma virus). Other transcription control regions include those derived from vertebrate genes such as actin, heat shock protein, bovine growth hormone and rabbit ß-globin, as well as other sequences capable of controlling gene expression in eukaryotic cells. Additional suitable transcription control regions include tissue-specific promoters and enhancers as well as lymphokine-inducible promoters (*e.g.*, promoters inducible by interferons or interleukins).

[0086] Similarly, a variety of translation control elements are known to those of ordinary skill in the art. These include, but are not limited to ribosome binding sites, translation initiation and termination codons, and elements derived from picornaviruses (particularly an internal ribosome entry site, or IRES, also referred to as a CITE sequence).

[0087] In other embodiments, a polynucleotide can be RNA, for example, in the form of messenger RNA (mRNA).

[0088] Polynucleotide and nucleic acid coding regions can be associated with additional coding regions which encode secretory or signal peptides, which direct the secretion of a polypeptide encoded by a polynucleotide as disclosed herein, *e.g.*, a polynucleotide encoding a binding molecule which specifically binds to *Pseudomonas* Psl and/or PcrV, *e.g.*, an antibody, or antigen-binding fragment, variant, or derivative thereof. According to the signal hypothesis, proteins secreted by mammalian cells have a signal peptide or secretory leader sequence which is cleaved from the mature protein once export of the growing protein chain across the rough endoplasmic reticulum has been initiated. Those

of ordinary skill in the art are aware that polypeptides secreted by vertebrate cells generally have a signal peptide fused to the N-terminus of the polypeptide, which is cleaved from the complete or "full length" polypeptide to produce a secreted or "mature" form of the polypeptide. In certain embodiments, the native signal peptide, *e.g.*, an immunoglobulin heavy chain or light chain signal peptide is used, or a functional derivative of that sequence that retains the ability to direct the secretion of the polypeptide that is operably associated with it. Alternatively, a heterologous mammalian signal peptide, or a functional derivative thereof, can be used. For example, the wild-type leader sequence can be substituted with the leader sequence of human tissue plasminogen activator (TPA) or mouse ß-glucuronidase.

[0089] Disclosed herein are certain binding molecules, or antigen-binding fragments, variants, or derivatives thereof. Unless specifically referring to full-sized antibodies such as naturally-occurring antibodies, the term "binding molecule" encompasses full-sized antibodies as well as antigen-binding fragments, variants, analogs, or derivatives of such antibodies, *e.g.*, naturally occurring antibody or immunoglobulin molecules or engineered antibody molecules or fragments that bind antigen in a manner similar to antibody molecules.

[0090] As used herein, the term “binding molecule” refers in its broadest sense to a molecule that specifically binds an antigenic determinant. As described further herein, a binding molecule can comprise one of more of the binding domains described herein. As used herein, a "binding domain" includes a site that specifically binds the antigenic determinant. A non-limiting example of an antigen binding molecule is an antibody or fragment thereof that retains antigen-specific binding.

[0091] The terms "antibody" and "immunoglobulin" can be used interchangeably herein. An antibody (or a fragment, variant, or derivative thereof as disclosed herein comprises at least the variable domain of a heavy chain and at least the variable domains of a heavy chain and a light chain. Basic immunoglobulin structures in vertebrate systems are relatively well understood. *See*, *e.g.*, Harlow *et al.*, Antibodies: A Laboratory Manual, (Cold Spring Harbor Laboratory Press, 2nd ed. 1988).

[0092] As will be discussed in more detail below, the term “immunoglobulin” comprises various broad classes of polypeptides that can be distinguished biochemically. Those skilled in the art will appreciate that heavy chains are classified as gamma, mu, alpha,

delta, or epsilon, (γ, μ, α, δ, ε) with some subclasses among them (*e.g.*, γ1-γ4). It is the nature of this chain that determines the "class" of the antibody as IgG, IgM, IgA IgG, or IgE, respectively. The immunoglobulin subclasses (isotypes) *e.g.*, IgG_1, IgG_2, IgG_3, IgG_4, IgA_1, etc. are well characterized and are known to confer functional specialization. Modified versions of each of these classes and isotypes are readily discernible to the skilled artisan in view of the instant disclosure and, accordingly, are within the scope of this disclosure.

[0093] Light chains are classified as either kappa or lambda (κ, λ). Each heavy chain class can be bound with either a kappa or lambda light chain. In general, the light and heavy chains are covalently bonded to each other, and the "tail" portions of the two heavy chains are bonded to each other by covalent disulfide linkages or non-covalent linkages when the immunoglobulins are generated either by hybridomas, B cells or genetically engineered host cells. In the heavy chain, the amino acid sequences run from an N-terminus at the forked ends of the Y configuration to the C-terminus at the bottom of each chain.

[0094] Both the light and heavy chains are divided into regions of structural and functional homology. The terms "constant" and "variable" are used functionally. In this regard, it will be appreciated that the variable domains of both the light (VL) and heavy (VH) chain portions determine antigen recognition and specificity. Conversely, the constant domains of the light chain (CL) and the heavy chain (CH1, CH2 or CH3) confer important biological properties such as secretion, transplacental mobility, Fc receptor binding, complement binding, and the like. By convention the numbering of the constant region domains increases as they become more distal from the antigen binding site or amino-terminus of the antibody. The N-terminal portion is a variable region and at the C-terminal portion is a constant region; the CH3 and CL domains actually comprise the carboxy-terminus of the heavy and light chain, respectively.

[0095] As indicated above, the variable region allows the binding molecule to selectively recognize and specifically bind epitopes on antigens. That is, the VL domain and VH domain, or subset of the complementarity determining regions (CDRs), of a binding molecule, *e.g.*, an antibody combine to form the variable region that defines a three dimensional antigen binding site. This quaternary binding molecule structure forms the

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

[0096] In naturally occurring antibodies, the six "complementarity determining regions" or "CDRs" present in each antigen binding domain are short, non-contiguous sequences of amino acids that are specifically positioned to form the antigen binding domain as the antibody assumes its three dimensional configuration in an aqueous environment. The remainder of the amino acids in the antigen binding domains, referred to as "framework" regions, show less inter-molecular variability. The framework regions largely adopt a β-sheet conformation and the CDRs form loops which connect, and in some cases form part of, the β-sheet structure. Thus, framework regions act to form a scaffold that provides for positioning the CDRs in correct orientation by inter-chain, non-covalent interactions. The antigen binding domain formed by the positioned CDRs defines a surface complementary to the epitope on the immunoreactive antigen. This complementary surface promotes the non-covalent binding of the antibody to its cognate epitope. The amino acids comprising the CDRs and the framework regions, respectively, can be readily identified for any given heavy or light chain variable region by one of ordinary skill in the art, since they have been precisely defined (*see*, "Sequences of Proteins of Immunological Interest," Kabat, E., *et al.*, U.S. Department of Health and Human Services, (1983); and Chothia and Lesk, *J. Mol. Biol.*, *196*:901-917 (1987), which are incorporated herein by reference in their entireties).

[0097] In the case where there are two or more definitions of a term which is used and/or accepted within the art, the definition of the term as used herein is intended to include all such meanings unless explicitly stated to the contrary. A specific example is the use of the term "complementarity determining region" ("CDR") to describe the non-contiguous antigen combining sites found within the variable region of both heavy and light chain polypeptides. This particular region has been described by Kabat *et al.*, U.S. Dept. of Health and Human Services, "Sequences of Proteins of Immunological Interest" (1983) and by Chothia *et al.*, *J. Mol. Biol.* 196:901-917 (1987), which are incorporated herein by reference, where the definitions include overlapping or subsets of amino acid residues when compared against each other. Nevertheless, application of either definition to refer to a CDR of an antibody or variants thereof is intended to be within the scope of the term as defined and used herein. The appropriate amino acid residues which encompass the

CDRs as defined by each of the above cited references are set forth below in Table I as a comparison. The exact residue numbers which encompass a particular CDR will vary depending on the sequence and size of the CDR. Those skilled in the art can routinely determine which residues comprise a particular CDR given the variable region amino acid sequence of the antibody.

TABLE 1: CDR Definitions[1]

	Kabat	Chothia
VH CDR1	31-35	26-32
VH CDR2	50-65	52-58
VH CDR3	95-102	95-102
VL CDR1	24-34	26-32
VL CDR2	50-56	50-52
VL CDR3	89-97	91-96

[1]Numbering of all CDR definitions in Table 1 is according to the numbering conventions set forth by Kabat *et al.* (see below).

[0098] Kabat *et al.* also defined a numbering system for variable domain sequences that is applicable to any antibody. One of ordinary skill in the art can unambiguously assign this system of "Kabat numbering" to any variable domain sequence, without reliance on any experimental data beyond the sequence itself. As used herein, "Kabat numbering" refers to the numbering system set forth by Kabat *et al.*, U.S. Dept. of Health and Human Services, "Sequence of Proteins of Immunological Interest" (1983). Unless otherwise specified, references to the numbering of specific amino acid residue positions in a binding molecule which specifically binds to *Pseudomonas* Psl and/or PcrV, *e.g*, an antibody, or antigen-binding fragment, variant, or derivative thereof as disclosed herein are according to the Kabat numbering system.

[0099] Binding molecules, *e.g.*, antibodies or antigen-binding fragments, variants, or derivatives thereof include, but are not limited to, polyclonal, monoclonal, human, humanized, or chimeric antibodies, single chain antibodies, epitope-binding fragments, *e.g.*, Fab, Fab' and $F(ab')_2$, Fd, Fvs, single-chain Fvs (scFv), single-chain antibodies, disulfide-linked Fvs (sdFv), fragments comprising either a VL or VH domain, fragments produced by a Fab expression library. ScFv molecules are known in the art and are described, *e.g.*, in US patent 5,892,019. Immunoglobulin or antibody molecules encompassed by this disclosure can be of any type (*e.g.*, IgG, IgE, IgM, IgD, IgA, and

IgY), class (*e.g.*, IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2) or subclass of immunoglobulin molecule.

[0100] By "specifically binds," it is generally meant that a binding molecule, *e.g.*, an antibody or fragment, variant, or derivative thereof binds to an epitope via its antigen binding domain, and that the binding entails some complementarity between the antigen binding domain and the epitope. According to this definition, a binding molecule is said to "specifically bind" to an epitope when it binds to that epitope, via its antigen binding domain more readily than it would bind to a random, unrelated epitope. The term "specificity" is used herein to qualify the relative affinity by which a certain binding molecule binds to a certain epitope. For example, binding molecule "A" may be deemed to have a higher specificity for a given epitope than binding molecule "B," or binding molecule "A" may be said to bind to epitope "C" with a higher specificity than it has for related epitope "D."

[0101] By "preferentially binds," it is meant that the antibody specifically binds to an epitope more readily than it would bind to a related, similar, homologous, or analogous epitope. Thus, an antibody which "preferentially binds" to a given epitope would more likely bind to that epitope than to a related epitope, even though such an antibody can cross-react with the related epitope.

[0102] By way of non-limiting example, a binding molecule, *e.g.*, an antibody can be considered to bind a first epitope preferentially if it binds said first epitope with a dissociation constant (K_D) that is less than the antibody's K_D for the second epitope. In another non-limiting example, a binding molecule such as an antibody can be considered to bind a first antigen preferentially if it binds the first epitope with an affinity that is at least one order of magnitudeless than the antibody's K_D for the second epitope. In another non-limiting example, a binding molecule can be considered to bind a first epitope preferentially if it binds the first epitope with an affinity that is at least two orders of magnitude less than the antibody's K_D for the second epitope.

[0103] In another non-limiting example, a binding molecule, *e.g.*, an antibody or fragment, variant, or derivative thereof can be considered to bind a first epitope preferentially if it binds the first epitope with an off rate (k(off)) that is less than the antibody's k(off) for the second epitope. In another non-limiting example, a binding molecule can be considered to bind a first epitope preferentially if it binds the first

epitope with an affinity that is at least one order of magnitude less than the antibody's k(off) for the second epitope. In another non-limiting example, a binding molecule can be considered to bind a first epitope preferentially if it binds the first epitope with an affinity that is at least two orders of magnitude less than the antibody's k(off) for the second epitope.

[0104] A binding molecule, *e.g.*, an antibody or fragment, variant, or derivative thereof disclosed herein can be said to bind a target antigen, *e.g.*, a polysaccharide disclosed herein or a fragment or variant thereof with an off rate (k(off)) of less than or equal to 5 X 10^{-2} sec^{-1}, 10^{-2} sec^{-1}, 5 X 10^{-3} sec^{-1} or 10^{-3} sec^{-1}. A binding molecule as disclosed herein can be said to bind a target antigen, *e.g.*, a polysaccharide with an off rate (k(off)) less than or equal to 5 X 10^{-4} sec^{-1}, 10^{-4} sec^{-1}, 5 X 10^{-5} sec^{-1}, or 10^{-5} sec^{-1} 5 X 10^{-6} sec^{-1}, 10^{-6} sec^{-1}, 5 X 10^{-7} sec^{-1} or 10^{-7} sec^{-1}.

[0105] A binding molecule, *e.g.*, an antibody or antigen-binding fragment, variant, or derivative disclosed herein can be said to bind a target antigen, *e.g.*, a polysaccharide with an on rate (k(on)) of greater than or equal to 10^{3} M^{-1} sec^{-1}, 5 X 10^{3} M^{-1} sec^{-1}, 10^{4} M^{-1} sec^{-1} or 5 X 10^{4} M^{-1} sec^{-1}. A binding molecule as disclosed herein can be said to bind a target antigen, *e.g.*, a polysaccharide with an on rate (k(on)) greater than or equal to 10^{5} M^{-1} sec^{-1}, 5 X 10^{5} M^{-1} sec^{-1}, 10^{6} M^{-1} sec^{-1}, or 5 X 10^{6} M^{-1} sec^{-1} or 10^{7} M^{-1} sec^{-1}.

[0106] A binding molecule, *e.g.*, an antibody or fragment, variant, or derivative thereof is said to competitively inhibit binding of a reference antibody or antigen binding fragment to a given epitope if it preferentially binds to that epitope to the extent that it blocks, to some degree, binding of the reference antibody or antigen binding fragment to the epitope. Competitive inhibition can be determined by any method known in the art, for example, competition ELISA assays. A binding molecule can be said to competitively inhibit binding of the reference antibody or antigen binding fragment to a given epitope by at least 90%, at least 80%, at least 70%, at least 60%, or at least 50%.

[0107] As used herein, the term "affinity" refers to a measure of the strength of the binding of an individual epitope with the CDR of an immunoglobulin molecule. *See*, *e.g.*, Harlow *et al.*, Antibodies: A Laboratory Manual, (Cold Spring Harbor Laboratory Press, 2nd ed. 1988) at pages 27-28. As used herein, the term "avidity" refers to the overall stability of the complex between a population of immunoglobulins and an antigen, that is, the functional combining strength of an immunoglobulin mixture with the antigen. *See*,

e.g. , Harlow at pages 29-34. Avidity is related to both the affinity of individual immunoglobulin molecules in the population with specific epitopes, and also the valencies of the immunoglobulins and the antigen. For example, the interaction between a bivalent monoclonal antibody and an antigen with a highly repeating epitope structure, such as a polymer, would be one of high avidity.

[0108] Binding molecules or antigen-binding fragments, variants or derivatives thereof as disclosed herein can also be described or specified in terms of their cross-reactivity. As used herein, the term "cross-reactivity" refers to the ability of a binding molecule, *e.g.*, an antibody or fragment, variant, or derivative thereof, specific for one antigen, to react with a second antigen; a measure of relatedness between two different antigenic substances. Thus, a binding molecule is cross reactive if it binds to an epitope other than the one that induced its formation. The cross reactive epitope generally contains many of the same complementary structural features as the inducing epitope, and in some cases, can actually fit better than the original.

[0109] A binding molecule, *e.g.*, an antibody or fragment, variant, or derivative thereof can also be described or specified in terms of their binding affinity to an antigen. For example, a binding molecule can bind to an antigen with a dissociation constant or K_D no greater than 5×10^{-2} M, 10^{-2} M, 5×10^{-3} M, 10^{-3} M, 5×10^{-4} M, 10^{-4} M, 5×10^{-5} M, 10^{-5} M, 5×10^{-6} M, 10^{-6} M, 5×10^{-7} M, 10^{-7} M, 5×10^{-8} M, 10^{-8} M, 5×10^{-9} M, 10^{-9} M, 5×10^{-10} M, 10^{-10} M, 5×10^{-11} M, 10^{-11} M, 5×10^{-12} M, 10^{-12} M, 5×10^{-13} M, 10^{-13} M, 5×10^{-14} M, 10^{-14} M, 5×10^{-15} M, or 10^{-15} M.

[0110] Antibody fragments including single-chain antibodies can comprise the variable region(s) alone or in combination with the entirety or a portion of the following: hinge region, CH1, CH2, and CH3 domains. Also included are antigen-binding fragments also comprising any combination of variable region(s) with a hinge region, CH1, CH2, and CH3 domains. Binding molecules, *e.g.*, antibodies, or antigen-binding fragments thereof disclosed herein can be from any animal origin including birds and mammals. The antibodies can be human, murine, donkey, rabbit, goat, guinea pig, camel, llama, horse, or chicken antibodies. In another embodiment, the variable region can be condricthoid in origin (*e.g.*, from sharks). As used herein, "human" antibodies include antibodies having the amino acid sequence of a human immunoglobulin and include antibodies isolated from human immunoglobulin libraries or from animals transgenic for one or more human

immunoglobulins and that do not express endogenous immunoglobulins, as described *infra* and, for example in, U.S. Pat. No. 5,939,598 by Kucherlapati *et al.*

[0111] As used herein, the term "heavy chain portion" includes amino acid sequences derived from an immunoglobulin heavy chain. a binding molecule, *e.g.*, an antibody comprising a heavy chain portion comprises at least one of: a CH1 domain, a hinge (*e.g.*, upper, middle, and/or lower hinge region) domain, a CH2 domain, a CH3 domain, or a variant or fragment thereof. For example, a binding molecule, *e.g.*, an antibody or fragment, variant, or derivative thereof can comprise a polypeptide chain comprising a CH1 domain; a polypeptide chain comprising a CH1 domain, at least a portion of a hinge domain, and a CH2 domain; a polypeptide chain comprising a CH1 domain and a CH3 domain; a polypeptide chain comprising a CH1 domain, at least a portion of a hinge domain, and a CH3 domain, or a polypeptide chain comprising a CH1 domain, at least a portion of a hinge domain, a CH2 domain, and a CH3 domain. In another embodiment, a binding molecule, *e.g.*, an antibody or fragment, variant, or derivative thereof comprises a polypeptide chain comprising a CH3 domain. Further, a binding molecule for use in the disclosure can lack at least a portion of a CH2 domain (*e.g.*, all or part of a CH2 domain). As set forth above, it will be understood by one of ordinary skill in the art that these domains (*e.g.*, the heavy chain portions) can be modified such that they vary in amino acid sequence from the naturally occurring immunoglobulin molecule.

[0112] The heavy chain portions of a binding molecule, *e.g.*, an antibody as disclosed herein can be derived from different immunoglobulin molecules. For example, a heavy chain portion of a polypeptide can comprise a CH1 domain derived from an IgG1 molecule and a hinge region derived from an IgG3 molecule. In another example, a heavy chain portion can comprise a hinge region derived, in part, from an IgG1 molecule and, in part, from an IgG3 molecule. In another example, a heavy chain portion can comprise a chimeric hinge derived, in part, from an IgG1 molecule and, in part, from an IgG4 molecule.

[0113] As used herein, the term "light chain portion" includes amino acid sequences derived from an immunoglobulin light chain. The light chain portion comprises at least one of a VL or CL domain.

[0114] Binding molecules, *e.g.*, antibodies or antigen-binding fragments, variants, or derivatives thereof disclosed herein can be described or specified in terms of the

epitope(s) or portion(s) of an antigen, *e.g.*, a target polysaccharide that they recognize or specifically bind. The portion of a target polysaccharide which specifically interacts with the antigen binding domain of an antibody is an "epitope," or an "antigenic determinant." A target antigen, *e.g.*, a polysaccharide can comprise a single epitope, but typically comprises at least two epitopes, and can include any number of epitopes, depending on the size, conformation, and type of antigen.

[0115] As previously indicated, the subunit structures and three dimensional configuration of the constant regions of the various immunoglobulin classes are well known. As used herein, the *term* "VH domain" includes the amino terminal variable domain of an immunoglobulin heavy chain and the term "CH1 domain" includes the first (most amino terminal) constant region domain of an immunoglobulin heavy chain. The CH1 domain is adjacent to the VH domain and is amino terminal to the hinge region of an immunoglobulin heavy chain molecule.

[0116] As used herein the term "CH2 domain" includes the portion of a heavy chain molecule that *extends*, *e.g.*, from about residue 244 to residue 360 of an antibody using conventional numbering schemes (residues 244 to 360, Kabat numbering system; and residues 231-340, EU numbering system; see Kabat EA *et al. op. cit.* 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 is also well documented that the CH3 domain extends from the CH2 domain to the C-terminal of the IgG molecule and comprises approximately 108 residues.

[0117] As used herein, the term "hinge region" includes the portion of a heavy chain molecule that joins the CH1 domain to the CH2 domain. This hinge region comprises approximately 25 residues and is flexible, thus allowing the two N-terminal antigen binding regions to move independently. Hinge regions can be subdivided into three distinct domains: upper, middle, and lower hinge domains (Roux *et al.*, *J. Immunol.* *161*:4083 (1998)).

[0118] As used herein the term "disulfide bond" includes the covalent bond formed between two sulfur atoms. The amino acid cysteine comprises a thiol group that can form a disulfide bond or bridge with a second thiol group. In most naturally occurring IgG molecules, the CH1 and CL regions are linked by a disulfide bond and the two heavy

chains are linked by two disulfide bonds at positions corresponding to 239 and 242 using the Kabat numbering system (position 226 or 229, EU numbering system).

[0119] As used herein, the term "chimeric antibody" will be held to mean any antibody wherein the immunoreactive region or site is obtained or derived from a first species and the constant region (which can be intact, partial or modified) is obtained from a second species. In some embodiments the target binding region or site will be from a non-human source (*e.g.* mouse or primate) and the constant region is human.

[0120] The term "bispecific antibody" as used herein refers to an antibody that has binding sites for two different antigens within a single antibody molecule. It will be appreciated that other molecules in addition to the canonical antibody structure can be constructed with two binding specificities. It will further be appreciated that antigen binding by bispecific antibodies can be simultaneous or sequential. Triomas and hybrid hybridomas are two examples of cell lines that can secrete bispecific antibodies. Bispecific antibodies can also be constructed by recombinant means. (Ströhlein and Heiss, *Future Oncol. 6*:1387-94 (2010); Mabry and Snavely, *IDrugs. 13*:543-9 (2010)).

[0121] As used herein, the term "engineered antibody" refers to an antibody in which the variable domain in either the heavy and light chain or both is altered by at least partial replacement of one or more CDRs from an antibody of known specificity and, if necessary, by partial framework region replacement and sequence changing. Although the CDRs can be derived from an antibody of the same class or even subclass as the antibody from which the framework regions are derived, it is envisaged that the CDRs will be derived from an antibody of different class and preferably from an antibody from a different species. An engineered antibody in which one or more "donor" CDRs from a non-human antibody of known specificity is grafted into a human heavy or light chain framework region is referred to herein as a "humanized antibody." It may not be necessary to replace all of the CDRs with the complete CDRs from the donor variable region to transfer the antigen binding capacity of one variable domain to another. Rather, it may only be necessary to transfer those residues that are necessary to maintain the activity of the target binding site. Given the explanations set forth in, *e.g.*, U. S. Pat. Nos. 5,585,089, 5,693,761, 5,693,762, and 6,180,370, it will be well within the competence of those skilled in the art, either by carrying out routine experimentation or by trial and error testing to obtain a functional engineered or humanized antibody.

[0122] As used herein the term “properly folded polypeptide” includes polypeptides (*e.g.*, anti-*Pseudomonas* Psl and PcrV antibodies) in which all of the functional domains comprising the polypeptide are distinctly active. As used herein, the term “improperly folded polypeptide” includes polypeptides in which at least one of the functional domains of the polypeptide is not active. In one embodiment, a properly folded polypeptide comprises polypeptide chains linked by at least one disulfide bond and, conversely, an improperly folded polypeptide comprises polypeptide chains not linked by at least one disulfide bond.

[0123] As used herein the term “engineered” includes manipulation of nucleic acid or polypeptide molecules by synthetic means (*e.g.* by recombinant techniques, *in vitro* peptide synthesis, by enzymatic or chemical coupling of peptides or some combination of these techniques).

[0124] As used herein, the terms "linked," "fused" or "fusion" are used interchangeably. These terms refer to the joining together of two more elements or components, by whatever means including chemical conjugation or recombinant means. An "in-frame fusion" refers to the joining of two or more polynucleotide open reading frames (ORFs) to form a continuous longer ORF, in a manner that maintains the correct translational reading frame of the original ORFs. Thus, a recombinant fusion protein is a single protein containing two or more segments that correspond to polypeptides encoded by the original ORFs (which segments are not normally so joined in nature.) Although the reading frame is thus made continuous throughout the fused segments, the segments can be physically or spatially separated by, for example, in-frame linker sequence. For example, polynucleotides encoding the CDRs of an immunoglobulin variable region can be fused, in-frame, but be separated by a polynucleotide encoding at least one immunoglobulin framework region or additional CDR regions, as long as the "fused" CDRs are co-translated as part of a continuous polypeptide.

[0125] In the context of polypeptides, a "linear sequence" or a "sequence" is an order of amino acids in a polypeptide in an amino to carboxyl terminal direction in which residues that neighbor each other in the sequence are contiguous in the primary structure of the polypeptide.

[0126] The term “expression” as used herein refers to a process by which a gene produces a biochemical, for example, a polypeptide. The process includes any manifestation of the

functional presence of the gene within the cell including, without limitation, gene knockdown as well as both transient expression and stable expression. It includes without limitation transcription of the gene into messenger RNA (mRNA), and the translation of such mRNA into polypeptide(s). If the final desired product is a biochemical, expression includes the creation of that biochemical and any precursors. Expression of a gene produces a "gene product." As used herein, a gene product can be either a nucleic acid, *e.g.*, a messenger RNA produced by transcription of a gene, or a polypeptide which is translated from a transcript. Gene products described herein further include nucleic acids with post transcriptional modifications, *e.g.*, polyadenylation, or polypeptides with post translational modifications, *e.g.*, methylation, glycosylation, the addition of lipids, association with other protein subunits, proteolytic cleavage, and the like.

[0127] As used herein, the terms "treat" or "treatment" refer to both therapeutic treatment and prophylactic or preventative measures, wherein the object is to prevent or slow down (lessen) an undesired physiological change, infection, or disorder. Beneficial or desired clinical results include, but are not limited to, alleviation of symptoms, diminishment of extent of disease, stabilized (*i.e.*, not worsening) state of disease, clearance or reduction of an infectious agent such as *P. aeruginosa* in a subject, a delay or slowing of disease progression, amelioration or palliation of the disease state, and remission (whether partial or total), whether detectable or undetectable. "Treatment" can also mean prolonging survival as compared to expected survival if not receiving treatment. Those in need of treatment include those already with the infection, condition, or disorder as well as those prone to have the condition or disorder or those in which the condition or disorder is to be prevented, *e.g.*, in burn patients or immunosuppressed patients susceptible to *P. aeruginosa* infection.

[0128] By "subject" or "individual" or "animal" or "patient" or “mammal,” is meant any subject, particularly a mammalian subject, for whom diagnosis, prognosis, or therapy is desired. Mammalian subjects include humans, domestic animals, farm animals, and zoo, sports, or pet animals such as dogs, cats, guinea pigs, rabbits, rats, mice, horses, cattle, cows, bears, and so on.

[0129] As used herein, phrases such as “a subject that would benefit from administration of anti-*Pseudomonas* Psl and PcrV binding domains or binding molecules” and “an animal in need of treatment” includes subjects, such as mammalian subjects, that would

benefit from administration of anti-*Pseudomonas* Psl and PcrV binding domains or a binding molecule, such as an antibody, comprising one or more of the binding domains. Such binding domains, or binding molecules can be used, *e.g.*, for detection of *Pseudomonas* Psl or PcrV (*e.g.*, for a diagnostic procedure) and/or for treatment, *i.e.*, palliation or prevention of a disease, with anti-*Pseudomonas* Psl and PcrV binding molecules. As described in more detail herein, the anti-*Pseudomonas* Psl and PcrV binding molecules can be used in unconjugated form or can be conjugated, *e.g.*, to a drug, prodrug, or an isotope.

[0130] The term "synergistic effect", as used herein, refers to a greater-than-additive therapeutic effect produced by a combination of compounds wherein the therapeutic effect obtained with the combination exceeds the additive effects that would otherwise result from individual administration the compounds alone. Certain embodiments include methods of producing a synergistic effect in the treatment of *Pseudomonas* infections, wherein said effect is at least 5%, at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 100%, at least 200%, at least 500%, or at least 1000% greater than the corresponding additive effect.

[0131] "Co-administration" refers to the administration of different compounds, such as an anti-Psl and an anti-PcrV binding domain, or binding molecule comprising one or both an anti-Ps1 and anti-PcrV binding domain, such that the compounds elicit a synergistic effect on anti-*Pseudomonas* immunity. The compounds can be administered in the same or different compositions which if separate are administered proximate to one another, generally within 24 hours of each other and more typically within about 1-8 hours of one another, and even more typically within 1-4 hours of each other or close to simultaneous administration. The relative amounts are dosages that achieve the desired synergism.

II. BINDING DOMAINS AND BINDING MOLECULES

[0132] Antibodies that bind Psl and formats for using these antibodies have been described in the art. See, for example, International Application Nos. PCT/US2012/041538, filed June 8, 2012, and PCT/US2012/63639, filed November 6, 2012 (attorney docket no. AEMS-115WO1, entitled "MULTISPECIFIC AND MULTIVALENT BINDING PROTEINS AND USES THEREOF"), which are herein incorporated in their entireties by reference.

[0133] One embodiment is directed to binding domains that specifically bind to *Pseudomonas* PcrV, wherein binding can disrupt the activity of the type III toxin secretion system. In certain embodiments, the binding domains have the same *Pseudomonas* binding specificity as the antibody V2L2.

[0134] Another embodiment is directed to binding domains that specifically bind to *Pseudomonas* Psl or PcrV, wherein administration of both binding domains results in synergistic effects against *Pseudomonas* infections by (a) inhibiting attachment of *Pseudomonas aeruginosa* to epithelial cells, (b) promoting, mediating, or enhancing opsonophagocytic killing (OPK) of *P. aeruginosa*, (c) inhibiting attachment of *P. aeruginosa* to epithelial cells, or (d) disrupting the activity of the type III toxin secretion system. In certain embodiments, the binding domains have the same *Pseudomonas* binding specificity as the antibodies Cam-003, WapR-004, V2L2, or 29D2.

[0135] Other embodiments are directed to an isolated binding molecule(s) comprising one or both binding domains that specifically bind to *Pseudomonas* Psl and/or PcrV, wherein administration of the binding molecule results in synergistic effects against *Pseudomonas* infections. In certain embodiments, the binding molecule can comprise a binding domain from the antibodies or fragments thereof that include, but are not limited to Cam-003,WapR-004, V2L2, or 29D22.

[0136] As used herein, the terms "binding domain" or "antigen binding domain" includes a site that specifically binds an epitope on an antigen (*e.g.*, an epitope of *Pseudomonas* Psl or PcrV). The antigen binding domain of an antibody typically includes at least a portion of an immunoglobulin heavy chain variable region and at least a portion of an immunoglobulin light chain variable region. The binding site formed by these variable regions determines the specificity of the antibody.

[0137] The disclosure is more specifically directed to a composition comprising at least two anti-*Pseudomonas* binding domains, wherein one binding domain specifically binds Psl and the other binding domain specifically binds PcrV. In one embodiment, the composition comprises one binding domain that specifically binds to the same *Pseudomonas* Psl epitope as an antibody or antigen-binding fragment thereof comprising the heavy chain variable region (VH) and light chain variable region (VL) region of WapR-004, Cam-003, Cam-004, Cam-005, WapR-001, WapR-002, WapR-003, or WapR-016. In certain embodiments, the second binding domain specifically binds to the

same *Pseudomonas* PcrV epitope as an antibody or antigen binding fragment thereof comprising the heavy chain variable region (VH) and light chain variable region (VL) of V2L2 or 29D2.

[0138] In one embodiment, the composition comprises one binding domain that specifically binds to *Pseudomonas* Psl and/or competitively inhibits *Pseudomonas* Psl binding by an antibody or antigen-binding fragment thereof comprising the VH and VL of WapR-004, Cam-003, Cam-004, Cam-005, WapR-001, WapR-002, WapR-003, or WapR-016. In certain embodiments, the second binding domain specifically binds to the same *Pseudomonas* PcrV epitope and/or competitively inhibits *Pseudomonas* PcrV binding by an antibody or antigen binding fragment thereof comprising the heavy chain variable region (VH) and light chain variable region (VL) of V2L2 or 29D2.

[0139] Another embodiment is directed to an isolated binding molecule, *e.g.*, an antibody or antigen-binding fragment thereof which specifically binds to the same *Pseudomonas* PcrV epitope as an antibody or antigen-binding fragment thereof comprising the VH and VL region of V2L2 or 29D2.

[0140] Also included is an isolated binding molecule, *e.g.*, an antibody or fragment thereof which specifically binds to *Pseudomonas* PcrV and competitively inhibits *Pseudomonas* PcrV binding by an antibody or antigen-binding fragment thereof comprising the VH and VL of V2L2 or 29D2.

[0141] One embodiment is directed to an isolated binding molecule, *e.g.*, an antibody or antigen-binding fragment thereof which specifically binds to the same *Pseudomonas* Psl epitope as an antibody or antigen-binding fragment thereof comprising the VH and VL region of WapR-001, WapR-002, or WapR-003.

[0142] Also included is an isolated binding molecule, *e.g.*, an antibody or fragment thereof which specifically binds to *Pseudomonas* Psl and competitively inhibits *Pseudomonas* Psl binding by an antibody or antigen-binding fragment thereof comprising the VH and VL of WapR-001, WapR-002, or WapR-003.

[0143] Further included is an isolated binding molecule, *e.g.*, an antibody or fragment thereof which specifically binds to the same *Pseudomonas* Psl epitope as an antibody or antigen-binding fragment thereof comprising the VH and VL of WapR-016.

[0144] Also included is an isolated binding molecule, *e.g.*, an antibody or fragment thereof which specifically binds to *Pseudomonas* Psl and competitively inhibits

Pseudomonas Psl binding by an antibody or antigen-binding fragment thereof comprising the VH and VL of WapR-016.

[0145] Methods of making antibodies are well known in the art and described herein. Once antibodies to various fragments of, or to the full-length *Pseudomonas* Psl or PcrV without the signal sequence, have been produced, determining which amino acids, or epitope, of *Pseudomonas* Psl or PcrV to which the antibody or antigen binding fragment binds can be determined by epitope mapping protocols as described herein as well as methods known in the art (*e.g.* double antibody-sandwich ELISA as described in "Chapter 11 - Immunology," *Current Protocols in Molecular Biology*, Ed. Ausubel *et al.*, v.2, John Wiley & Sons, Inc. (1996)). Additional epitope mapping protocols can be found in Morris, G. *Epitope Mapping Protocols,* New Jersey: Humana Press (1996), which are both incorporated herein by reference in their entireties. Epitope mapping can also be performed by commercially available means (*i.e.* ProtoPROBE, Inc. (Milwaukee, Wisconsin)).

[0146] In certain aspects, the disclosure is directed to a binding molecule, *e.g.*, an antibody or fragment, variant, or derivative thereof which specifically binds to *Pseudomonas* Psl and/or PcrV with an affinity characterized by a dissociation constant (K_D) which is less than the K_D for said reference monoclonal antibody.

[0147] In certain embodiments an anti-*Pseudomonas* Psl and/or PcrV binding molecule, *e.g.*, an antibody or antigen-binding fragment, variant or derivative thereof as disclosed herein binds specifically to at least one epitope of Psl or PcrV, *i.e.*, binds to such an epitope more readily than it would bind to an unrelated, or random epitope; binds preferentially to at least one epitope of Psl or PcrV, *i.e.*, binds to such an epitope more readily than it would bind to a related, similar, homologous, or analogous epitope; competitively inhibits binding of a reference antibody which itself binds specifically or preferentially to a certain epitope of Psl or PcrV; or binds to at least one epitope of Psl or PcrV with an affinity characterized by a dissociation constant K_D of less than about 5 x 10^{-2} M, about 10^{-2} M, about 5 x 10^{-3} M, about 10^{-3} M, about 5 x 10^{-4} M, about 10^{-4} M, about 5 x 10^{-5} M, about 10^{-5} M, about 5 x 10^{-6} M, about 10^{-6} M, about 5 x 10^{-7} M, about 10^{-7} M, about 5 x 10^{-8} M, about 10^{-8} M, about 5 x 10^{-9} M, about 10^{-9} M, about 5 x 10^{-10} M, about 10^{-10} M, about 5 x 10^{-11} M, about 10^{-11} M, about 5 x 10^{-12} M, about 10^{-12} M, about 5

x 10^{-13} M, about 10^{-13} M, about 5 x 10^{-14} M, about 10^{-14} M, about 5 x 10^{-15} M, or about 10^{-15} M.

[0148] As used in the context of binding dissociation constants, the term "about" allows for the degree of variation inherent in the methods utilized for measuring antibody affinity. For example, depending on the level of precision of the instrumentation used, standard error based on the number of samples measured, and rounding error, the term "about 10^{-2} M" might include, for example, from 0.05 M to 0.005 M.

[0149] In specific embodiments a binding molecule, *e.g.*, an antibody, or antigen-binding fragment, variant, or derivative thereof binds *Pseudomonas* Psl and/or PcrV with an off rate (k(off)) of less than or equal to 5 X 10^{-2} sec^{-1}, 10^{-2} sec^{-1}, 5 X $l0^{-3}$ sec^{-1} or $l0^{-3}$ sec^{-1}. Alternatively, an antibody, or antigen-binding fragment, variant, or derivative thereof binds *Pseudomonas* Psl and/or PcrV with an off rate (k(off)) of less than or equal to 5 X 10^{-4} sec^{-1}, 10^{-4} sec^{-1}, 5 X 10^{-5} sec^{-1}, or 10^{-5} sec^{-1} 5 X 10^{-6} sec^{-1}, 10^{-6} sec^{-1}, 5 X 10^{-7} sec^{-1} or 10^{-7} sec^{-1}.

[0150] In other embodiments, a binding molecule, *e.g.*, an antibody, or antigen-binding fragment, variant, or derivative thereof as disclosed herein binds *Pseudomonas* Psl and/or PcrV with an on rate (k(on)) of greater than or equal to 10^{3} M^{-1} sec^{-1}, 5 X 10^{3} M^{-1} sec^{-1}, 10^{4} M^{-1} sec^{-1} or 5 X 10^{4} M^{-1} sec^{-1}. Alternatively, a binding molecule, *e.g.*, an antibody, or antigen-binding fragment, variant, or derivative thereof as disclosed herein binds *Pseudomonas* Psl and/or PcrV with an on rate (k(on)) greater than or equal to 10^{5} M^{-1} sec^{-1}, 5 X 10^{5} M^{-1} sec^{-1}, 10^{6} M^{-1} sec^{-1}, or 5 X 106 M^{-1} sec^{-1} or 10^{7} M^{-1} sec^{-1}.

[0151] In various embodiments, an anti-*Pseudomonas* Psl and/or PcrV binding molecule, *e.g.*, an antibody, or antigen-binding fragment, variant, or derivative thereof as described herein promotes opsonophagocytic killing of *Pseudomonas*, or inhibits *Pseudomonas* binding to epithelial cells. In certain embodiments described herein, the *Pseudomonas* Psl or PcrV target is *Pseudomonas aeruginosa* Psl or PcrV. In other embodiments, certain binding molecules described herein can bind to structurally related polysaccharide molecules regardless of their source. Such Psl-like molecules would be expected to be identical to or have sufficient structural relatedness to *P. aeruginosa* Psl to permit specific recognition by one or more of the binding molecules disclosed. In other embodiments, certain binding molecules described herein can bind to structurally related polypeptide molecules regardless of their source. Such PcrV-like molecules would be expected to be

identical to or have sufficient structural relatedness to *P. aeruginosa* PcrV to permit specific recognition by one or more of the binding molecules disclosed. Therefore, for example, certain binding molecules described herein can bind to Psl-like and/or PcrV-like molecules produced by other bacterial species, for example, Psl-like or PcrV-like molecules produced by other *Pseudomonas* species, *e.g.*, *Pseudomonas fluorescens, Pseudomonas putida, or Pseudomonas alcaligenes.* Alternatively, certain binding molecules as described herein can bind to Psl-like and/or PcrV-like molecules produced synthetically or by host cells genetically modified to produce Psl-like or PcrV-like molecules.

[0152] Unless it is specifically noted, as used herein a "fragment thereof" in reference to a binding molecule, *e.g.*, an antibody refers to an antigen-binding fragment, *i.e.*, a portion of the antibody which specifically binds to the antigen.

[0153] Anti-*Pseudomonas* Psl and/or PcrV binding molecules, *e.g.*, antibodies or antigen-binding fragments, variants, or derivatives thereof can comprise a constant region which mediates one or more effector functions. For example, binding of the C1 component of complement to an antibody constant region can activate the complement system. Activation of complement is important in the opsonization and lysis of pathogens. The activation of complement also stimulates the inflammatory response and can also be involved in autoimmune hypersensitivity. Further, antibodies bind to receptors on various cells via the Fc region, with a Fc receptor binding site on the antibody Fc region binding to a Fc receptor (FcR) on a cell. There are a number of Fc receptors which are specific for different classes of antibody, including IgG (gamma receptors), IgE (epsilon receptors), IgA (alpha receptors) and IgM (mu receptors). Binding of antibody to Fc receptors on cell surfaces triggers a number of important and diverse biological responses including engulfment and destruction of antibody-coated particles, clearance of immune complexes, lysis of antibody-coated target cells by killer cells (called antibody-dependent cell-mediated cytotoxicity, or ADCC), release of inflammatory mediators, placental transfer and control of immunoglobulin production.

[0154] Accordingly, certain embodiments disclosed herein include an anti-*Pseudomonas* Psl and/or PcrV binding molecule, *e.g.*, an antibody, or antigen-binding fragment, variant, or derivative thereof, in which at least a fraction of one or more of the constant region domains has been deleted or otherwise altered so as to provide desired biochemical

characteristics such as reduced effector functions, the ability to non-covalently dimerize, increased ability to localize at the site of a tumor, reduced serum half-life, or increased serum half-life when compared with a whole, unaltered antibody of approximately the same immunogenicity. For example, certain binding molecules described herein are domain deleted antibodies which comprise a polypeptide chain similar to an immunoglobulin heavy chain, but which lack at least a portion of one or more heavy chain domains. For instance, in certain antibodies, one entire domain of the constant region of the modified antibody will be deleted, for example, all or part of the CH2 domain will be deleted.

[0155] Modified forms of anti-*Pseudomonas* Psl and/or PcrV binding molecules, *e.g.*, antibodies or antigen-binding fragments, variants, or derivatives thereof can be made from whole precursor or parent antibodies using techniques known in the art. Exemplary techniques are discussed elsewhere herein.

[0156] In certain embodiments both the variable and constant regions of anti-*Pseudomonas* Psl and/or PcrV binding molecules, *e.g.*, antibodies or antigen-binding fragments are fully human. Fully human antibodies can be made using techniques that are known in the art and as described herein. For example, fully human antibodies against a specific antigen can be prepared by administering the antigen to a transgenic animal which has been modified to produce such antibodies in response to antigenic challenge, but whose endogenous loci have been disabled. Exemplary techniques that can be used to make such antibodies are described in US patents: 6,150,584; 6,458,592; 6,420,140. Other techniques are known in the art. Fully human anti bodies can likewise be produced by various display technologies, *e.g.*, phage display or other viral display systems, as described in more detail elsewhere herein.

[0157] Anti-*Pseudomonas* Psl and/or PcrV binding molecules, *e.g.*, antibodies or antigen-binding fragments, variants, or derivatives thereof as disclosed herein can be made or manufactured using techniques that are known in the art. In certain embodiments, binding molecules or fragments thereof are "recombinantly produced," *i.e.*, are produced using recombinant DNA technology. Exemplary techniques for making antibody molecules or fragments thereof are discussed in more detail elsewhere herein.

[0158] In certain anti-*Pseudomonas* Psl and/or PcrV binding molecules, *e.g.*, antibodies or antigen-binding fragments, variants, or derivatives thereof described herein, the Fc

portion can be mutated to decrease effector function using techniques known in the art. For example, the deletion or inactivation (through point mutations or other means) of a constant region domain can reduce Fc receptor binding of the circulating modified antibody thereby increasing tumor localization. In other cases it can be that constant region modifications moderate complement binding and thus reduce the serum half-life and nonspecific association of a conjugated cytotoxin. Yet other modifications of the constant region can be used to modify disulfide linkages or oligosaccharide moieties that allow for enhanced localization due to increased antigen specificity or antibody flexibility. The resulting physiological profile, bioavailability and other biochemical effects of the modifications, such as localization, biodistribution and serum half-life, can easily be measured and quantified using well known immunological techniques without undue experimentation.

[0159] In certain embodiments, anti-*Pseudomonas* Psl and/or PcrV binding molecules, *e.g.*, antibodies or antigen-binding fragments, variants, or derivatives thereof will not elicit a deleterious immune response in the animal to be treated, *e.g.*, in a human. In one embodiment, anti-*Pseudomonas* Psl and/or PcrV binding molecules, *e.g.*, antibodies or antigen-binding fragments, variants, or derivatives thereof are modified to reduce their immunogenicity using art-recognized techniques. For example, antibodies can be humanized, de-immunized, or chimeric antibodies can be made. These types of antibodies are derived from a non-human antibody, typically a murine or primate antibody, that retains or substantially retains the antigen-binding properties of the parent antibody, but which is less immunogenic in humans. This can be achieved by various methods, including (a) grafting the entire non-human variable domains onto human constant regions to generate chimeric antibodies; (b) grafting at least a part of one or more of the non-human complementarity determining regions (CDRs) into a human framework and constant regions with or without retention of critical framework residues; or (c) transplanting the entire non-human variable domains, but "cloaking" them with a human-like section by replacement of surface residues. Such methods are disclosed in Morrison *et al.*, *Proc. Natl. Acad. Sci. 81*:6851-6855 (1984); Morrison *et al.*, *Adv. Immunol. 44*:65-92 (1988); Verhoeyen *et al.*, *Science 239*:1534-1536 (1988); Padlan, *Molec. Immun. 28*:489-498 (1991); Padlan, *Molec. Immun. 31*:169-217 (1994), and U.S.

Pat. Nos. 5,585,089, 5,693,761, 5,693,762, and 6,190,370, all of which are hereby incorporated by reference in their entirety.

[0160] De-immunization can also be used to decrease the immunogenicity of an antibody. As used herein, the term "de-immunization" includes alteration of an antibody to modify T cell epitopes (see, *e.g.*, WO9852976A1, WO0034317A2). For example, VH and VL sequences from the starting antibody are analyzed and a human T cell epitope "map" from each V region showing the location of epitopes in relation to complementarity-determining regions (CDRs) and other key residues within the sequence. Individual T cell epitopes from the T cell epitope map are analyzed in order to identify alternative amino acid substitutions with a low risk of altering activity of the final antibody. A range of alternative VH and VL sequences are designed comprising combinations of amino acid substitutions and these sequences are subsequently incorporated into a range of binding polypeptides, *e.g.*, *Pseudomonas* Psl- and/or PcrV-specific antibodies or antigen-binding fragments thereof disclosed herein, which are then tested for function. Complete heavy and light chain genes comprising modified V and human C regions are then cloned into expression vectors and the subsequent plasmids introduced into cell lines for the production of whole antibody. The antibodies are then compared in appropriate biochemical and biological assays, and the optimal variant is identified.

[0161] Anti-*Pseudomonas* Psl and/or PcrV binding molecules, *e.g.*, antibodies or antigen-binding fragments, variants, or derivatives thereof can be generated by any suitable method known in the art. Polyclonal antibodies to an antigen of interest can be produced by various procedures well known in the art. For example, an anti-*Pseudomonas* Psl and/or PcrV antibody or antigen-binding fragment thereof can be administered to various host animals including, but not limited to, rabbits, mice, rats, chickens, hamsters, goats, donkeys, etc., to induce the production of sera containing polyclonal antibodies specific for the antigen. Various adjuvants can be used to increase the immunological response, depending on the host species, and include but are not limited to, Freund's (complete and incomplete), mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanins, dinitrophenol, and potentially useful human adjuvants such as BCG (bacille Calmette-Guerin) and *Corynebacterium parvum*. Such adjuvants are also well known in the art.

[0162] Monoclonal antibodies can be prepared using a wide variety of techniques known in the art including the use of hybridoma, recombinant, and phage display technologies, or a combination thereof. For example, monoclonal antibodies can be produced using hybridoma techniques including those known in the art and taught, for example, in Harlow *et al.,* Antibodies*: A Laboratory Manual,* Cold Spring Harbor Laboratory Press, 2nd ed. (1988)

[0163] DNA encoding antibodies or antibody fragments (*e.g.*, antigen binding sites) can also be derived from antibody libraries, such as phage display libraries. In a particular, such phage can be utilized to display antigen-binding domains expressed from a repertoire or combinatorial antibody library (*e.g.*, human or murine). Phage expressing an antigen binding domain that binds the antigen of interest can be selected or identified with antigen, *e.g.*, using labeled antigen or antigen bound or captured to a solid surface or bead. Phage used in these methods are typically filamentous phage including fd and M13 binding domains expressed from phage with scFv, Fab, Fv OE DAB (individual Fv region from light or heavy chains) or disulfide stabilized Fv antibody domains recombinantly fused to either the phage gene III or gene VIII protein. Exemplary methods are set forth, for example, in EP 368 684 B1; U.S. patent. 5,969,108, Hoogenboom, H.R. and Chames, *Immunol. Today 21*:371 (2000); Nagy *et al. Nat. Med. 8*:801 (2002); Huie *et al., Proc. Natl. Acad. Sci. USA 98*:2682 (2001); Lui *et al., J. Mol. Biol. 315*:1063 (2002), each of which is incorporated herein by reference. Several publications (*e.g.*, Marks *et al., Bio/Technology 10*:779-783 (1992)) have described the production of high affinity human antibodies by chain shuffling, as well as combinatorial infection and *in vivo* recombination as a strategy for constructing large phage libraries. In another embodiment, Ribosomal display can be used to replace bacteriophage as the display platform (*see, e.g.*, Hanes *et al., Nat. Biotechnol. 18*:1287 (2000); Wilson *et al., Proc. Natl. Acad. Sci. USA 98*:3750 (2001); or Irving *et al., J. Immunol. Methods 248*:31 (2001)). In yet another embodiment, cell surface libraries can be screened for antibodies (Boder *et al., Proc. Natl. Acad. Sci. USA 97*:10701 (2000); Daugherty *et al., J. Immunol. Methods 243*:211 (2000)). Such procedures provide alternatives to traditional hybridoma techniques for the isolation and subsequent cloning of monoclonal antibodies.

[0164] In phage display methods, functional antibody domains are displayed on the surface of phage particles which carry the polynucleotide sequences encoding them. For

example, DNA sequences encoding VH and VL regions are amplified from animal cDNA libraries (*e.g.*, human or murine cDNA libraries of lymphoid tissues) or synthetic cDNA libraries. In certain embodiments, the DNA encoding the VH and VL regions are joined together by an scFv linker by PCR and cloned into a phagemid vector (*e.g.*, p CANTAB 6 or pComb 3 HSS). The vector is electroporated in *E. coli* and the *E. coli* is infected with helper phage. Phage used in these methods are typically filamentous phage including fd and M13 and the VH or VL regions are usually recombinantly fused to either the phage gene III or gene VIII. Phage expressing an antigen binding domain that binds to an antigen of interest (*i.e.*, *Pseudomonas* Psl or PcrV) can be selected or identified with antigen, *e.g.*, using labeled antigen or antigen bound or captured to a solid surface or bead.

[0165] Additional examples of phage display methods that can be used to make the antibodies include those disclosed in Brinkman *et al.*, *J. Immunol. Methods 182*:41-50 (1995); Ames *et al.*, *J. Immunol. Methods 184*:177-186 (1995); Kettleborough *et al.*, *Eur. J. Immunol. 24*:952-958 (1994); Persic *et al.*, *Gene 187*:9-18 (1997); Burton *et al.*, *Advances in* Immunology *57*:191-280 (1994); PCT Application No. PCT/GB91/01134; PCT publications WO 90/02809; WO 91/10737; WO 92/01047; WO 92/18619; WO 93/11236; WO 95/15982; WO 95/20401; and U.S. Pat. Nos. 5,698,426; 5,223,409; 5,403,484; 5,580,717; 5,427,908; 5,750,753; 5,821,047; 5,571,698; 5,427,908; 5,516,637; 5,780,225; 5,658,727; 5,733,743 and 5,969,108; each of which is incorporated herein by reference in its entirety.

[0166] As described in the above references and in the examples below, after phage selection, the antibody coding regions from the phage can be isolated and used to generate whole antibodies, including human antibodies, or any other desired antigen binding fragment, and expressed in any desired host, including mammalian cells, insect cells, plant cells, yeast, and bacteria. For example, techniques to recombinantly produce Fab, Fab' and $F(ab')_2$ fragments can also be employed using methods known in the art such as those disclosed in PCT publication WO 92/22324; Mullinax *et al.*, *BioTechniques 12(6)*:864-869 (1992); and Sawai *et al.*, *AJRI 34*:26-34 (1995); and Better *et al.*, *Science 240*:1041-1043 (1988) (said references incorporated by reference in their entireties).

[0167] Examples of techniques which can be used to produce single-chain Fvs and antibodies include those described in U.S. Pat. Nos. 4,946,778 and 5,258,498; Huston *et*

al., *Methods in Enzymology 203*:46-88 (1991); Shu *et al.*, *PNAS 90*:7995-7999 (1993); and Skerra *et al.*, *Science 240*:1038-1040 (1988). In certain embodiments such as therapeutic administration, chimeric, humanized, or human antibodies can be used. A chimeric antibody is a molecule in which different portions of the antibody are derived from different animal species, such as antibodies having a variable region derived from a murine monoclonal antibody and a human immunoglobulin constant region. Methods for producing chimeric antibodies are known in the art. *See, e.g.*, Morrison, *Science 229*:1202 (1985); Oi *et al.*, *BioTechniques 4*:214 (1986); Gillies *et al.*, *J. Immunol. Methods 125*:191-202 (1989); U.S. Pat. Nos. 5,807,715; 4,816,567; and 4,816397, which are incorporated herein by reference in their entireties. Humanized antibodies are antibody molecules from non-human species antibody that binds the desired antigen having one or more complementarity determining regions (CDRs) from the non-human species and framework regions from a human immunoglobulin molecule. Often, framework residues in the human framework regions will be substituted with the corresponding residue from the CDR donor antibody to alter, preferably improve, antigen binding. These framework substitutions are identified by methods well known in the art, *e.g.*, by modeling of the interactions of the CDR and framework residues to identify framework residues important for antigen binding and sequence comparison to identify unusual framework residues at particular positions. (See, *e.g.*, Queen *et al.*, U.S. Pat. No. 5,585,089; Riechmann *et al.*, *Nature 332*:323 (1988), which are incorporated herein by reference in their entireties.) Antibodies can be humanized using a variety of techniques known in the art including, for example, CDR-grafting (EP 239,400; PCT publication WO 91/09967; U.S. Pat. Nos. 5,225,539; 5,530,101; and 5,585,089), veneering or resurfacing (EP 592,106; EP 519,596; Padlan, *Molecular Immunology 28(4/5)*:489-498 (1991); Studnicka *et al.*, *Protein Engineering 7(6)*:805-814 (1994); Roguska. *et al.*, *PNAS 91*:969-973 (1994)), and chain shuffling (U.S. Pat. No. 5,565,332).

[0168] Fully human antibodies are particularly desirable for therapeutic treatment of human patients. Human antibodies can be made by a variety of methods known in the art including phage display methods described above using antibody libraries derived from human immunoglobulin sequences. See also, U.S. Pat. Nos. 4,444,887 and 4,716,111; and PCT publications WO 98/46645, WO 98/50433, WO 98/24893, WO 98/16654, WO

96/34096, WO 96/33735, and WO 91/10741; each of which is incorporated herein by reference in its entirety.

[0169] Human antibodies can also be produced using transgenic mice which are incapable of expressing functional endogenous immunoglobulins, but which can express human immunoglobulin genes. For example, the human heavy and light chain immunoglobulin gene complexes can be introduced randomly or by homologous recombination into mouse embryonic stem cells. In addition, various companies can be engaged to provide human antibodies produced in transgenic mice directed against a selected antigen using technology similar to that described above.

[0170] Fully human antibodies which recognize a selected epitope can be generated using a technique referred to as "guided selection." In this approach a selected non-human monoclonal antibody, *e.g.*, a mouse antibody, is used to guide the selection of a completely human antibody recognizing the same epitope. (Jespers *et al.*, *Bio/Technology 12*:899-903 (1988). *See also*, U.S. Patent No. 5,565,332.)

[0171] In another embodiment, DNA encoding desired monoclonal antibodies 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). Isolated and subcloned hybridoma cells or isolated phage, for example, can serve as a source of such DNA. Once isolated, the DNA can be placed into expression vectors, which are then transfected into prokaryotic or eukaryotic host cells such as *E. coli* cells, simian COS cells, Chinese Hamster Ovary (CHO) cells or myeloma cells that do not otherwise produce immunoglobulins. More particularly, the isolated DNA (which can be synthetic as described herein) can be used to clone constant and variable region sequences for the manufacture antibodies as described in Newman *et al.*, U.S. Pat. No. 5,658,570, filed January 25, 1995, which is incorporated by reference herein. Transformed cells expressing the desired antibody can be grown up in relatively large quantities to provide clinical and commercial supplies of the immunoglobulin.

[0172] In one embodiment, an isolated binding molecule, *e.g.*, an antibody comprises at least one heavy or light chain CDR of an antibody molecule. In another embodiment, an isolated binding molecule comprises at least two CDRs from one or more antibody molecules. In another embodiment, an isolated binding molecule comprises at least three

CDRs from one or more antibody molecules. In another embodiment, an isolated binding molecule comprises at least four CDRs from one or more antibody molecules. In another embodiment, an isolated binding molecule comprises at least five CDRs from one or more antibody molecules. In another embodiment, an isolated binding molecule of the description comprises at least six CDRs from one or more antibody molecules.

[0173] In a specific embodiment, the amino acid sequence of the heavy and/or light chain variable domains can be inspected to identify the sequences of the complementarity determining regions (CDRs) by methods that are well-known in the art, *e.g.*, by comparison to known amino acid sequences of other heavy and light chain variable regions to determine the regions of sequence hypervariability. Using routine recombinant DNA techniques, one or more of the CDRs can be inserted within framework regions, *e.g.*, into human framework regions to humanize a non-human antibody. The framework regions can be naturally occurring or consensus framework regions, and preferably human framework regions (see, *e.g.*, Chothia *et al.*, *J. Mol. Biol. 278*:457-479 (1998) for a listing of human framework regions). The polynucleotide generated by the combination of the framework regions and CDRs encodes an antibody that specifically binds to at least one epitope of a desired antigen, *e.g.,* Psl or PcrV. One or more amino acid substitutions can be made within the framework regions, and, the amino acid substitutions improve binding of the antibody to its antigen. Additionally, such methods can be used to make amino acid substitutions or deletions of one or more variable region cysteine residues participating in an intrachain disulfide bond to generate antibody molecules lacking one or more intrachain disulfide bonds. Other alterations to the polynucleotide are encompassed by the present disclosure and are within the capabilities of a person of skill of the art.

[0174] Also provided are binding molecules that comprise, consist essentially of, or consist of, variants (including derivatives) of antibody molecules (*e.g.*, the VH regions and/or VL regions) described herein, which binding molecules or fragments thereof specifically bind to *Pseudomonas* Psl or PcrV. Standard techniques known to those of skill in the art can be used to introduce mutations in the nucleotide sequence encoding a binding molecule or fragment thereof which specifically binds to *Pseudomonas* Psl and/or PcrV, including, but not limited to, site-directed mutagenesis and PCR-mediated mutagenesis which result in amino acid substitutions. The variants (including

derivatives) encode polypeptides comprising less than 50 amino acid substitutions, less than 40 amino acid substitutions, less than 30 amino acid substitutions, less than 25 amino acid substitutions, less than 20 amino acid substitutions, less than 15 amino acid substitutions, less than 10 amino acid substitutions, less than 5 amino acid substitutions, less than 4 amino acid substitutions, less than 3 amino acid substitutions, or less than 2 amino acid substitutions relative to the reference VH region, VHCDR1, VHCDR2, VHCDR3, VL region, VLCDR1, VLCDR2, or VLCDR3. A "conservative amino acid substitution" is one in which the amino acid residue is replaced with an amino acid residue having a side chain with a similar charge. Families of amino acid residues having side chains with similar charges have been defined in the art. These families include amino acids with basic side chains (*e.g.*, lysine, arginine, histidine), acidic side chains (*e.g.*, aspartic acid, glutamic acid), uncharged polar side chains (*e.g.*, glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (*e.g.*, alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (*e.g.*, threonine, valine, isoleucine) and aromatic side chains (*e.g.*, tyrosine, phenylalanine, tryptophan, histidine). Alternatively, mutations can be introduced randomly along all or part of the coding sequence, such as by saturation mutagenesis, and the resultant mutants can be screened for biological activity to identify mutants that retain activity (*e.g.*, the ability to bind an *Pseudomonas* Psl or PcrV).

[0175] For example, it is possible to introduce mutations only in framework regions or only in CDR regions of an antibody molecule. Introduced mutations can be silent or neutral missense mutations, *i.e.*, have no, or little, effect on an antibody's ability to bind antigen. These types of mutations can be useful to optimize codon usage, or improve a hybridoma's antibody production. Alternatively, non-neutral missense mutations can alter an antibody's ability to bind antigen. The location of most silent and neutral missense mutations is likely to be in the framework regions, while the location of most non-neutral missense mutations is likely to be in CDR, though this is not an absolute requirement. One of skill in the art would be able to design and test mutant molecules with desired properties such as no alteration in antigen binding activity or alteration in binding activity (*e.g.*, improvements in antigen binding activity or change in antibody specificity). Following mutagenesis, the encoded protein can routinely be expressed and the functional and/or biological activity of the encoded protein, (*e.g.*, ability to bind at least one epitope

of *Pseudomonas* Psl or PcrV) can be determined using techniques described herein or by routinely modifying techniques known in the art.

[0176] One embodiment provides a bispecific antibody comprising an anti-*Pseudomonas* Psl and PcrV binding domain disclosed herein. In certain embodiments, the bispecific antibody contains a first Psl binding domain, and the second PcrV binding domain. Bispecific antibodies with more than two valencies are contemplated. For example, trispecific antibodies can also be prepared using the methods described herein. (Tutt et al., J. Immunol., 147:60 (1991)).

[0177] One embodiment provides a method of producing a bispecific antibody, that utilizes a single light chain that can pair with both heavy chain variable domains present in the bispecific molecule. To identify this light chain, various strategies can be employed. In one embodiment, a series of monoclonal antibodies are identified to each antigen that can be targeted with the bispecific antibody, followed by a determination of which of the light chains utilized in these antibodies is able to function when paired with the heavy chain of any of the antibodies identified to the second target. In this manner a light chain that can function with two heavy chains to enable binding to both antigens can be identified. In another embodiment, display techniques, such as phage display, can enable the identification of a light chain that can function with two or more heavy chains. In one embodiment, a phage library is constructed which comprises a diverse repertoire of heavy chain variable domains and a single light chain variable domain. This library can further be utilized to identify antibodies that bind to various antigens of interest. Thus, in certain embodiments, the antibodies identified will share a common light chain.

[0178] In certain embodiments, the bispecific antibody comprises at least one single chain Fv (scFv). In certain embodiments the bispecific antibody comprises two scFvs. For example, a scFv can be fused to one or both of a CH3 domain-containing polypeptide contained within an antibody. Some methods comprise producing a bispecific molecule wherein one or both of the heavy chain constant regions comprising at least a CH3 domain is utilized in conjunction with a single chain Fv domain to provide antigen binding.

III. ANTIBODY POLYPEPTIDES

[0179] The disclosure is further directed to isolated polypeptides which make up binding molecules, *e.g.*, antibodies or antigen-binding fragments thereof, which specifically bind to *Pseudomonas* Psl and/or PcrV and polynucleotides encoding such polypeptides. Binding molecules, *e.g.*, antibodies or fragments thereof as disclosed herein, comprise polypeptides, *e.g.*, amino acid sequences encoding, for example, Psl-specific and/or PcrV-specific antigen binding regions derived from immunoglobulin molecules. A polypeptide or amino acid sequence "derived from" a designated protein refers to the origin of the polypeptide. In certain cases, the polypeptide or amino acid sequence which is derived from a particular starting polypeptide or amino acid sequence has an amino acid sequence that is essentially identical to that of the starting sequence, or a portion thereof, wherein the portion consists of at least 10-20 amino acids, at least 20-30 amino acids, at least 30-50 amino acids, or which is otherwise identifiable to one of ordinary skill in the art as having its origin in the starting sequence.

[0180] Also disclosed is an isolated binding molecule, *e.g.*, an antibody or antigen-binding fragment thereof which specifically binds to *Pseudomonas* Psl comprising an immunoglobulin heavy chain variable region (VH) amino acid sequence at least 80%, 85%, 90% 95% or 100% identical to one or more of: SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, or SEQ ID NO: 74 as shown in Table 2.

[0181] Further disclosed is an isolated binding molecule, *e.g.*, an antibody or antigen-binding fragment thereof which specifically binds to *Pseudomonas* Psl comprising a VH amino acid sequence identical to, or identical except for one, two, three, four, five, or more amino acid substitutions to one or more of: SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, or SEQ ID NO: 74 as shown in Table 2.

[0182] Some embodiments include an isolated binding molecule, *e.g.*, an antibody or antigen-binding fragment thereof which specifically binds to *Pseudomonas* Psl comprising a VH, where one or more of the VHCDR1, VHCDR2 or VHCDR3 regions of the VH are at least 80%, 85%, 90%, 95% or 100% identical to one or more reference heavy chain VHCDR1, VHCDR2 or VHCDR3 amino acid sequences of one or more of: SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID

NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, or SEQ ID NO: 74 as shown in Table 2.

[0183] Further disclosed is an isolated binding molecule, *e.g.*, an antibody or antigen-binding fragment thereof which specifically binds to *Pseudomonas* Psl comprising a VH, where one or more of the VHCDR1, VHCDR2 or VHCDR3 regions of the VH are identical to, or identical except for four, three, two, or one amino acid substitutions, to one or more reference heavy chain VHCDR1, VHCDR2 and/or VHCDR3 amino acid sequences of one or more of: SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, or SEQ ID NO: 74 as shown in Table 2. Thus, according to this embodiment the VH comprises one or more of a VHCDR1, VHCDR2, or VHCDR3 identical to or identical except for four, three, two, or one amino acid substitutions, to one or more of the VHCDR1, VHCDR2, or VHCDR3 amino acid sequences shown in Table 3.

[0184] Also disclosed is an isolated binding molecule, *e.g.*, an antibody or antigen-binding fragment thereof which specifically binds to *Pseudomonas* Psl comprising an immunoglobulin light chain variable region (VL) amino acid sequence at least 80%, 85%, 90% 95% or 100% identical to one or more of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, or SEQ ID NO: 16 as shown in Table 2.

[0185] Some embodiments disclose an isolated binding molecule, *e.g.*, an antibody or antigen-binding fragment thereof which specifically binds to *Pseudomonas* Psl comprising a VL amino acid sequence identical to, or identical except for one, two, three, four, five, or more amino acid substitutions, to one or more of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, or SEQ ID NO: 16 as shown in Table 2.

[0186] Also provided is an isolated binding molecule, *e.g.*, an antibody or antigen-binding fragment thereof which specifically binds to *Pseudomonas* Psl comprising a VL, where one or more of the VLCDR1, VLCDR2 or VLCDR3 regions of the VL are at least 80%, 85%, 90%, 95% or 100% identical to one or more reference light chain VLCDR1, VLCDR2 or VLCDR3 amino acid sequences of one or more of: SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, or SEQ ID NO: 16 as shown in Table 2.

[0187] Further provided is an isolated binding molecule, *e.g.*, an antibody or antigen-binding fragment thereof which specifically binds to *Pseudomonas* Psl comprising a VL, where one or more of the VLCDR1, VLCDR2 or VLCDR3 regions of the VL are identical to, or identical except for four, three, two, or one amino acid substitutions, to one or more reference heavy chain VLCDR1, VLCDR2 and/or VLCDR3 amino acid sequences of one or more of: SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, or SEQ ID NO: 16 as shown in Table 2. Thus, according to this embodiment the VL comprises one or more of a VLCDR1, VLCDR2, or VLCDR3 identical to or identical except for four, three, two, or one amino acid substitutions, to one or more of the VLCDR1, VLCDR2, or VLCDR3 amino acid sequences shown in Table 3.

[0188] In other embodiments, an isolated antibody or antigen-binding fragment thereof which specifically binds to *Pseudomonas* Psl, comprises, consists essentially of, or consists of VH and VL amino acid sequences at least 80%, 85%, 90% 95% or 100% identical to:

(a) SEQ ID NO: 1 and SEQ ID NO: 2, respectively,(b) SEQ ID NO: 3 and SEQ ID NO:2, respectively,(c) SEQ ID NO: 4 and SEQ ID NO: 2 , respectively, (d) SEQ ID NO: 5 and SEQ ID NO: 6, respectively,(e) SEQ ID NO: 7 and SEQ ID NO: 8, respectively,(f) SEQ ID NO: 9 and SEQ ID NO: 10, respectively,(g) SEQ ID NO: 11 and SEQ ID NO: 12, respectively,(h) SEQ ID NO: 13 and SEQ ID NO: 14, respectively; (i) SEQ ID NO: 15 and SEQ ID NO: 16, respectively; or (j) SEQ ID NO: 74 and SEQ ID NO: 12, respectively. In certain embodiments, the above-described antibody or antigen-binding fragment thereof comprises a VH with the amino acid sequence SEQ ID NO: 11 and a VL with the amino acid sequence of SEQ ID NO: 12. In some embodiments, the above-described antibody or antigen-binding fragment thereof comprises a VH with the amino acid sequence SEQ ID NO: 1 and a VL with the amino acid sequence of SEQ ID NO: 2. In other embodiments, the above-described antibody or antigen-binding fragment thereof comprises a VH with the amino acid sequence SEQ ID NO: 11 and a VL with the amino acid sequence of SEQ ID NO: 12.

[0189] Certain embodiments provide an isolated binding molecule, e.g, an antibody, or antigen-binding fragment thereof which specifically binds to *Pseudomonas* Psl,

comprising an immunoglobulin VH and an immunoglobulin VL, each comprising a complementarity determining region 1 (CDR1), CDR2, and CDR3, wherein the VH CDR1 is PYYWT (SEQ ID NO:47), the VH CDR2 is YIHSSGYTDYNPSLKS (SEQ ID NO: 48), the VH CDR3 is selected from the group consisting of ADWDRLRALDI (Psl0096, SEQ ID NO:258), AMDIEPHALDI (Psl0225, SEQ ID NO:267), ADDPFPGYLDI (Psl0588, SEQ ID NO:268), ADWNEGRKLDI (Psl0567, SEQ ID NO:269), ADWDHKHALDI (Psl0337, SEQ ID NO:270), ATDEADHALDI (Psl0170, SEQ ID NO:271), ADWSGTRALDI (Psl0304, SEQ ID NO:272), GLPEKPHALDI (Psl0348, SEQ ID NO:273), SLFTDDHALDI (Psl0573, SEQ ID NO:274), ASPGVVHALDI (Psl0574, SEQ ID NO:275), AHIESHHALDI (Psl0582, SEQ ID NO:276), ATQAPAHALDI (Psl0584, SEQ ID NO:277), SQHDLEHALDI (Psl0585, SEQ ID NO:278), and AMPDMPHALDI (Psl0589, SEQ ID NO:279), the VL CDR1 is RASQSIRSHLN (SEQ ID NO:50), the VL CDR2 is GASNLQS (SEQ ID NO:51), and the VL CDR3 is selected from the group consisting of QQSTGAWNW (Psl0096, SEQ ID NO:280), QQDFFHGPN (Psl0225, SEQ ID NO:281), QQSDTFPLK (Psl0588, SEQ ID NO:282), QQSYSFPLT (WapR0004, Psl0567, Psl0573, Psl00574, Psl0582, Psl0584, Psl0585, SEQ ID NO:52), QDSSSWPLT (Psl0337, SEQ ID NO:283), SQSDTFPLT (Psl0170, SEQ ID NO:284), GQSDAFPLT (Psl0304, SEQ ID NO:285), LQGDLWPLT (Psl0348, SEQ ID NO:286), and QQSLEFPLT (Psl0589, SEQ ID NO:287), wherein the VH and VL CDRs are according to the Kabat numbering system.

[0190] Certain embodiments provide an isolated binding molecule, e.g, an antibody, or antigen-binding fragment thereof which specifically binds to *Pseudomonas* Psl, comprising an immunoglobulin VH and an immunoglobulin VL, each comprising a complementarity determining region 1 (CDR1), CDR2, and CDR3, wherein the VH CDR1 is PYYWT (SEQ ID NO:47), the VH CDR2 is YIHSSGYTDYNPSLKS (SEQ ID NO: 48), the VL CDR1 is RASQSIRSHLN (SEQ ID NO:50), the VL CDR2 is GASNLQS (SEQ ID NO:51), and the VH CDR3 and the VL CDR3 comprise, respectively, ADWDRLRALDI (Psl0096, SEQ ID NO:258) and QQSTGAWNW (Psl0096, SEQ ID NO:280); AMDIEPHALDI (Psl0225, SEQ ID NO:267) and QQDFFHGPN (Psl0225, SEQ ID NO:281); ADDPFPGYLDI (Psl0588, SEQ ID NO:268) and QQSDTFPLK (Psl0588, SEQ ID NO:282); ADWNEGRKLDI (Psl0567, SEQ ID NO:269) and the VL CDR3 is QQSYSFPLT (WapR0004, Psl0567, Psl0573, Psl00574,

Psl0582, Psl0584, Psl0585, SEQ ID NO:52); ADWDHKHALDI (Psl0337, SEQ ID NO:270) and QDSSSWPLT (Psl0337, SEQ ID NO:283); ATDEADHALDI (Psl0170, SEQ ID NO:271) and SQSDTFPLT (Psl0170, SEQ ID NO:284); ADWSGTRALDI (Psl0304, SEQ ID NO:272) and GQSDAFPLT (Psl0304, SEQ ID NO:285); GLPEKPHALDI (Psl0348, SEQ ID NO:273) and (Psl0348, SEQ ID NO:286); SLFTDDHALDI (Psl0573, SEQ ID NO:274) and SEQ ID NO:52; ASPGVVHALDI (Psl0574, SEQ ID NO:275) and SEQ ID NO:52; AHIESHHALDI (Psl0582, SEQ ID NO:276) and SEQ ID NO:52; ATQAPAHALDI (Psl0584, SEQ ID NO:277) and SEQ ID NO:52; SQHDLEHALDI (Psl0585, SEQ ID NO:278) and SEQ ID NO:52; or AMPDMPHALDI (Psl0589, SEQ ID NO:279) and QQSLEFPLT (Psl0589, SEQ ID NO:287).

[0191] Certain embodiments provide an isolated binding molecule, e.g., an antibody or antigen-binding fragment thereof which specifically binds to *Pseudomonas* Psl, comprising an immunoglobulin VH and an immunoglobulin VL, wherein the VH comprises QVQLQESGPGLVKPSETLSLTCTVSGGSISPYYWTWIRQPPGK**X1**LELIGYIHSSGY TDYNPSLKSRVTISGDTSKKQFSLKLSSVTAADTAVYYCARADWDRLRALDIWG QGTMVTVSS, wherein X1 is G or C (Psl0096, SEQ ID NO:288), and the VL comprises DIQLTQSPSSLSASVGDRVTITCRASQSIRSHLNWYQQKPGKAPKLLIYGASNLQS GVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSTGAWNWFG**X2**GTKVEIK, wherein X2 is G or C (Psl0096, SEQ ID NO:289); wherein the VH comprises QVQLQESGPGLVKPSETLSLTCTVSGGSISPYYWTWIRQPPGKGLELIGYIHSSGY TDYNPSLKSRVTISGDTSKKQFSLKLSSVTAADTAVYYCARAMDIEPHALDIWGQ GTMVTVSS (Psl0225, SEQ ID NO:290), and the VL comprises DIQLTQSPSSLSASVGDRVTITCRASQSIRSHLNWYQQKPGKAPKLLIYGASNLQS GVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSDDGFPNFGGGTKVEIK (Psl0225, SEQ ID NO:291); wherein the VH comprises QVQLQESGPGLVKPSETLSLTCTVSGGSISPYYWTWIRQPPGKGLELIGYIHSSGY TDYNPSLKSRVTISGDTSKKQFSLKLSSVTAADTAVYYCARADDPFPGYLDIWGQ GTMVTVSS (Psl0588, SEQ ID NO:292), and the VL comprises DIQLTQSPSSLSASVGDRVTITCRASQSIRSHLNWYQQKPGKAPKLLIYGASNLQS GVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSDTFPLKFGGGTKVEIK

(Psl0588, SEQ ID NO:293); wherein the VH comprises QVQLQESGPGLVKPSETLSLTCTVSGGSISPYYWTWIRQPPGKGLELIGYIHSSGY TDYNPSLKSRVTISGDTSKKQFSLKLSSVTAADTAVYYCARADWNEGRKLDIWG QGTMVTVSS (Psl0567, SEQ ID NO:294), and the VL comprises SEQ ID NO:11; herein the VH comprises QVQLQESGPGLVKPSETLSLTCTVSGGSISPYYWTWIRQPPGKGLELIGYIHSSGY TDYNPSLKSRVTISGDTSKKQFSLKLSSVTAADTAVYYCARADWDHKHALDIWG QGTMVTVSS (Psl0337, SEQ ID NO:295), and the VL comprises DIQLTQSPSSLSASVGDRVTITCRASQSIRSHLNWYQQKPGKAPKLLIYGASNLQS GVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQDSSSWPLTFGGGTKVEIK (Psl0337, SEQ ID NO:296); wherein the VH comprises EVQLLESGPGLVKPSETLSLTCNVAGGSISPYYWTWIRQPPGKGLELIGYIHSSGY TDYNPSLKSRVTISGDTSKKQFSLHVSSVTAADTAVYFCARATDEADHALDIWG QGTLVTVSS (Psl0170, SEQ ID NO:297), and the VL comprises EIVLTQSPSSLSTSVGDRVTITCRASQSIRSHLNWYQQKPGKAPKLLIYGASNLQS GVPSRFSGSGSGTDFTLTISSLQPEDFATYYCSQSDTFPLTFGGGTKLEIK (Psl0170, SEQ ID NO:298); wherein the VH comprises EVQLLESGPGLVKPSETLSLTCNVAGGSISPYYWTWIRQPPGKGLELIGYIHSSGY TDYNPSLKSRVTISGDTSKKQFSLHVSSVTAADTAVYFCARADWSGTRALDIWG QGTLVTVSS (Psl0304, SEQ ID NO:299), and the VL comprises EIVLTQSPSSLSTSVGDRVTITCWASQSIRSHLNWYQQKPGKAPKLLIYGASNLQS GVPSRFSGSGSGTDFTLTISSLQPEDFATYYCGQSDAFPLTFGGGTKLEIK (Psl0304, SEQ ID NO:300); wherein the VH comprises EVQLLESGPGLVKPSETLSLTCNVAGGSISPYYWTWIRQPPGKGLELIGYIHSSGY TDYNPSLKSRVTISGDTSKKQFSLHVSSVTAADTAVYFCARGLPEKPHALDIWGQ GTLVTVSS (Psl0348, SEQ ID NO:301), and the VL comprises EIVLTQSPSSLSTSVGDRVTITCRASQSIRSHLNWYQQKPGKAPKLLIYGASNLQS GVPSRFSGSGSGTDFTLTISSLQPEDFATYYCLQGDLWPLTFGGGTKLEIK (Psl0348, SEQ ID NO:302); wherein the VH comprises EVQLLESGPGLVKPSETLSLTCNVAGGSISPYYWTWIRQPPGKGLELIGYIHSSGY TDYNPSLKSRVTISGDTSKKQFSLHVSSVTAADTAVYFCARSLFTDDHALDIWGQ GTLVTVSS (Psl0573, SEQ ID NO:303), and the VL comprises SEQ ID NO:11; wherein

the VH comprises EVQLLESGPGLVKPSETLSLTCNVAGGSISPYYWTWIRQPPGKGLELIGYIHSSGY TDYNPSLKSRVTISGDTSKKQFSLHVSSVTAADTAVYFCARASPGVVHALDIWGQ GTLVTVSS (Psl0574, SEQ ID NO:304), and the VL comprises SEQ ID NO:11; wherein the VH comprises EVQLLESGPGLVKPSETLSLTCNVAGGSISPYYWTWIRQPPGKGLELIGYIHSSGY TDYNPSLKSRVTISGDTSKKQFSLHVSSVTAADTAVYFCARAHIESHHALDIWGQ GTLVTVSS (Psl0582, SEQ ID NO:305), and the VL comprises SEQ ID NO:11; wherein the VH comprises EVQLLESGPGLVKPSETLSLTCNVAGGSISPYYWTWIRQPPGKGLELIGYIHSSGY TDYNPSLKSRVTISGDTSKKQFSLHVSSVTAADTAVYFCARATQAPAHALDIWG QGTLVTVSS (Psl0584, SEQ ID NO:306), and the VL comprises SEQ ID NO:11; wherein the VH comprises EVQLLESGPGLVKPSETLSLTCNVAGGSISPYYWTWIRQPPGKGLELIGYIHSSGY TDYNPSLKSRVTISGDTSKKQFSLHVSSVTAADTAVYFCARSQHDLEHALDIWGQ GTLVTVSS (Psl0585, SEQ ID NO:307), and the VL comprises SEQ ID NO:11; or wherein the VH comprises EVQLLESGPGLVKPSETLSLTCNVAGGSISPYYWTWIRQPPGKGLELIGYIHSSGY TDYNPSLKSRVTISGDTSKKQFSLHVSSVTAADTAVYFCARAMPDMPHALDIWG QGTLVTVSS (Psl0589, SEQ ID NO:308), and the VL comprises EIVLTQSPSSLSTSVGDRVTITCRASQSIRSHLNWYQQKPGKAPKLLIYGASNLQS GVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSLEFPLTFGGGTKLEIK (Psl0589, SEQ ID NO:325).

[0192] Also disclosed is an isolated antibody single chain Fv (ScFv) fragment which specifically binds to *Pseudomonas* Psl (an "anti-Psl ScFv"), comprising the formula VH-L-VL or alternatively VL-L-VH, where L is a linker sequence. In certain aspects the linker can comprise (a) [GGGGS]n, wherein n is 0, 1, 2, 3, 4, or 5, (b) [GGGG]n, wherein n is 0, 1, 2, 3, 4, or 5, or a combination of (a) and (b). For example, an exemplary linker comprises: GGGGSGGGGSGGGGSGGGGSGGGGS (SEQ ID NO:326). In certain embodiments the linker further comprises the amino acids ala-leu at the C-terminus of the linker. In certain embodiments the anti-Psl ScFv comprises the amino acid sequence of SEQ ID NO:240, SEQ ID NO:241, SEQ ID NO:242, SEQ ID NO:243, SEQ ID NO:244,

SEQ ID NO:245, SEQ ID NO:246, SEQ ID NO:247, SEQ ID NO:248, SEQ ID NO:249, SEQ ID NO:250, SEQ ID NO:251, SEQ ID NO:252, SEQ ID NO:253, SEQ ID NO:254, or SEQ ID NO:262.

[0193] Also disclosed is an isolated antibody single chain Fv (ScFv) fragment which specifically binds to *Pseudomonas* PcrV (an "anti-PcrV ScFv"), comprising the formula VH-L-VL or alternatively VL-L-VH, where L is a linker sequence. In certain aspects the linker can comprise (a) [GGGGS]n, wherein n is 0, 1, 2, 3, 4, or 5, (b) [GGGG]n, wherein n is 0, 1, 2, 3, 4, or 5, or a combination of (a) and (b). For example, an exemplary linker comprises: GGGGSGGGGSGGGGSGGGGSGGGGS (SEQ ID NO:326). In certain embodiments the linker further comprises the amino acids ala-leu at the C-terminus of the linker.

[0194] Also disclosed is an isolated binding molecule, *e.g.*, an antibody or antigen-binding fragment thereof which specifically binds to *Pseudomonas* PcrV comprising an immunoglobulin heavy chain variable region (VH) and/or light chain variable region (VL) amino acid sequence at least 80%, 85%, 90% 95% or 100% identical to SEQ ID NO: 216 or SEQ ID NO: 217.

[0195] Further disclosed is an isolated binding molecule, *e.g.*, an antibody or antigen-binding fragment thereof which specifically binds to *Pseudomonas* PcrV comprising a VH, where one or more of the VHCDR1, VHCDR2 or VHCDR3 regions of the VH are identical to, or identical except for four, three, two, or one amino acid substitutions, to one or more reference heavy chain VHCDR1, VHCDR2 and/or VHCDR3 amino acid sequences of one or more of: SEQ ID NOs: 218-220 as shown in Table 3. Thus, according to this embodiment the VH comprises one or more of a VHCDR1, VHCDR2, or VHCDR3 identical to or identical except for four, three, two, or one amino acid substitutions, to one or more of the VHCDR1, VHCDR2, or VHCDR3 amino acid sequences shown in Table 3.

[0196] Further provided is an isolated binding molecule, *e.g.*, an antibody or antigen-binding fragment thereof which specifically binds to *Pseudomonas* PcrV comprising a VL, where one or more of the VLCDR1, VLCDR2 or VLCDR3 regions of the VL are identical to, or identical except for four, three, two, or one amino acid substitutions, to one or more reference heavy chain VLCDR1, VLCDR2 and/or VLCDR3 amino acid sequences of one or more of: SEQ ID NOs: 221-223 as shown in Table 3. Thus,

according to this embodiment the VL comprises one or more of a VLCDR1, VLCDR2, or VLCDR3 identical to or identical except for four, three, two, or one amino acid substitutions, to one or more of the VLCDR1, VLCDR2, or VLCDR3 amino acid sequences shown in Table 3.

[0197] Also provided is an isolated binding molecule, *e.g.*, an antibody or antigen-binding fragment thereof which specifically binds to *Pseudomonas* PcrV comprising a VH and a VL, wherein the VH comprises an amino acid sequence selected from the group consisting of SEQ ID NO:255 and SEQ ID NO:257, and wherein the VL comprises the amino acid sequence of SEQ ID NO:256.

[0198] Further provided is an isolated binding molecule, *e.g.*, an antibody or antigen-binding fragment thereof which specifically binds to *Pseudomonas* PcrV comprising a VH and a VL, each comprising a CDR1, CDR2, and CDR3, wherein the VH CDR1 is (a) SYAMS (SEQ ID NO:311), or a variant thereof comprising 1, 2, 3, or 4 conservative amino acid substitutions, the VH CDR2 is AISGSGYSTYYADSVKG (SEQ ID NO: 312), or a variant thereof comprising 1, 2, 3, or 4 conservative amino acid substitutions, and the VHCDR3 is EYSISSNYYYGMDV (SEQ ID NO: 313), or a variant thereof comprising 1, 2, 3, or 4 conservative amino acid substitutions; or (b) wherein the VL CDR1 is WASQGISSYLA (SEQ ID NO:314), or a variant thereof comprising 1, 2, 3, or 4 conservative amino acid substitutions, the VL CDR2 is AASTLQS (SEQ ID NO:315), or a variant thereof comprising 1, 2, 3, or 4 conservative amino acid substitutions, and the VL CDR3 is QQLNSSPLT (SEQ ID NO:316), or a variant thereof comprising 1, 2, 3, or 4 conservative amino acid substitutions; or (c) a combination of (a) and (b); wherein the VH and VL CDRs are according to the Kabat numbering system. In certain aspects of this embodiment, (a) the VH comprises an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98% 99%, or 100% identical to SEQ ID NO:317, (b) the VL comprises an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98% 99%, or 100% identical to SEQ ID NO:318; or (c) a combination of (a) and (b).

[0199] Also disclosed is an isolated bispecific binding molecule, *e.g.*, a bispecific antibody or antigen-binding fragment thereof which specifically binds to both *Pseudomonas* Psl and *Pseudomonas* PcrV comprising an immunoglobulin heavy chain variable region (VH) and/or light chain variable region (VL) amino acid sequence at least

80%, 85%, 90% 95% or 100% identical to SEQ ID NO: 228, SEQ ID NO:229, or SEQ ID NO: 235.

[0200] In certain embodiments, a bispecific antibody as disclosed herein has the structure of BS1, BS2, BS3, or BS4, all as shown in FIG. 17. In certain bispecific antibodies disclosed herein the binding domain which specifically binds to *Pseudomonas* Psl comprises an anti-Psl ScFv molecule. In other aspects the binding domain which specifically binds to *Pseudomonas* Psl comprises a conventional heavy chain and light chain. Similarly in certain bispecific antibodies disclosed herein the binding domain which specifically binds to *Pseudomonas* PcrV comprises an anti-PcrV ScFv molecule. In other aspects the binding domain which specifically binds to *Pseudomonas* PcrV comprises a conventional heavy chain and light chain.

[0201] In certain aspects a bispecific antibody as disclosed herein had the BS4 structure, disclosed in detail in U.S. Provisional Appl. No. 61/624,651filed on April 16, 2012 and International Application No: PCT/US2012/ 63639, filed November 6, 2012 (attorney docket no. AEMS-115WO1, entitled "MULTISPECIFIC AND MULTIVALENT BINDING PROTEINS AND USES THEREOF"), which is incorporated herein by reference in its entirety. For example, this disclosure provides a bispecific antibody in which an anti-Psl ScFv molecule is inserted into the hinge region of each heavy chain of an anti-PcrV antibody or fragment thereof.

[0202] This disclosure provides an isolated binding molecule, e.g., a bispecfic antibody comprising an antibody heavy chain and an antibody light chain, where the antibody heavy chain comprises the formula VH-CH1-H1-L1-S-L2-H2-CH2-CH3, wherein CH1 is a heavy chain constant region domain-1, H1 is a first heavy chain hinge region fragment, L1 is a first linker, S is an anti-PcrV ScFv molecule, L2 is a second linker, H2 is a second heavy chain hinge region fragment, CH2 is a heavy chain constant region domain-2, and CH3 is a heavy chain constant region domain-3. In certain aspects the VH comprises the amino acid sequence of SEQ ID NO:255, SEQ ID NO:257, or SEQ ID NO:317. In certain aspects L1 and L2 are the same or different, and independently comprise (a) [GGGGS]n, wherein n is 0, 1, 2, 3, 4, or 5, (b) [GGGG]n, wherein n is 0, 1, 2, 3, 4, or 5, or a combination of (a) and (b). In certain embodiments H1 comprises EPKSC (SEQ ID NO:320), and H2 comprises DKTHTCPPCP (SEQ ID NO:321).

[0203] In certain aspects, S comprises an anti-Psl ScFv molecule having the amino acid sequence of SEQ ID NO:240, SEQ ID NO:241, SEQ ID NO:242, SEQ ID NO:243, SEQ ID NO:244, SEQ ID NO:245, SEQ ID NO:246, SEQ ID NO:247, SEQ ID NO:248, SEQ ID NO:249, SEQ ID NO:250, SEQ ID NO:251, SEQ ID NO:252, SEQ ID NO:253, SEQ ID NO:254, or SEQ ID NO:262, or any combination of two or more of these amino acid sequences.

[0204] In further aspects, CH2-CH3 comprises (SEQ ID NO:322), wherein X1 is M or Y, X2 is S or T, and X3 is T or E. In further aspects the antibody light chain comprises VL-CL, wherein CL is an antibody light chain kappa constant region or am an antibody light chain lambda constant region. In further aspects VL comprises the amino acid sequence of SEQ ID NO:256 or SEQ ID NO:318. CL can comprise, *e.g.*, the amino acid sequence of SEQ ID NO:323

[0205] Further provided is an isolated binding molecule, *e.g.*, a bispecific antibody which specifically binds to both *Pseudomonas* Psl and *Pseudomonas* PcrV comprising a VH comprising the amino acid sequence SEQ ID NO:264, and a VL comprising the amino acid sequence SEQ ID NO:263.

[0206] In some embodiments, the bispecific antibodies of the invention can be a tandem single chain (sc) Fv fragment, which contain two different scFv fragments (i.e., V2L2 and W4) covalently tethered together by a linker (e.g., a polypeptide linker). (Ren-Heidenreich *et al. Cancer 100*:1095-1103 (2004); Korn *et al. J Gene Med 6*:642-651 (2004)). In some embodiments, the linker can contain, or be, all or part of a heavy chain polypeptide constant region such as a CH1 domain. In some embodiments, the two antibody fragments can be covalently tethered together by way of a polyglycine-serine or polyserine-glycine linker as described in, *e.g.*, U.S. Pat. Nos. 7,112,324 and 5,525,491, respectively. Methods for generating bispecific tandem scFv antibodies are described in, *e.g.*, Maletz *et al. Int J Cancer 93*:409-416 (2001); and Honemann *et al. Leukemia 18*:636-644 (2004). Alternatively, the antibodies can be "linear antibodies" as described in, *e.g.*, *Zapata et al. Protein Eng. 8*:1057-1062 (1995). Briefly, these antibodies comprise a pair of tandem Fd segments (VH-CH1-VH-CH1) that form a pair of antigen binding regions.

[0207] The disclosure also embraces variant forms of bispecific antibodies such as the tetravalent dual variable domain immunoglobulin (DVD-Ig) molecules described in Wu

et al. (2007) Nat Biotechnol 25(11):1290-1297. The DVD-Ig molecules are designed such that two different light chain variable domains (VL) from two different parent antibodies are linked in tandem directly or via a short linker by recombinant DNA techniques, followed by the light chain constant domain. For example, the DVD-Ig light chain polypeptide can contain in tandem: (a) the VL from V2L2; and (b) the VL from WapR-004. Similarly, the heavy chain comprises the two different heavy chain variable domains (VH) linked in tandem, followed by the constant domain CH1 and Fc region. For example, the DVD-Ig heavy chain polypeptide can contain in tandem: (a) the VH from V2L2; and (b) the VH from WapR-004. In this case, expression of the two chains in a cell results in a heterotetramer containing four antigen combining sites, two that specifically bind to V2L2 and two that specifically bind to Psl. Methods for generating DVD-Ig molecules from two parent antibodies are further described in, *e.g.*, PCT Publication Nos. WO 2008/024188 and WO 2007/024715.

[0208] In certain embodiments, an isolated binding molecule, *e.g.*, an antibody or antigen-binding fragment thereof as described herein specifically binds to *Pseudomonas* Psl and/or PcrV with an affinity characterized by a dissociation constant (K_D) no greater than 5×10^{-2} M, 10^{-2} M, 5×10^{-3} M, 10^{-3} M, 5×10^{-4} M, 10^{-4} M, 5×10^{-5} M, 10^{-5} M, 5×10^{-6} M, 10^{-6} M, 5×10^{-7} M, 10^{-7} M, 5×10^{-8} M, 10^{-8} M, 5×10^{-9} M, 10^{-9} M, 5×10^{-10} M, 10^{-10} M, 5×10^{-11} M, 10^{-11} M, 5×10^{-12} M, 10^{-12} M, 5×10^{-13} M, 10^{-13} M, 5×10^{-14} M, 10^{-14} M, 5×10^{-15} M, or 10^{-15} M.

[0209] In specific embodiments, an isolated binding molecule, *e.g.*, an antibody or antigen-binding fragment thereof as described herein specifically binds to *Pseudomonas* Psl and/or PcrV, with an affinity characterized by a dissociation constant (K_D) in a range of about 1×10^{-10} to about 1×10^{-6} M. In one embodiment, an isolated binding molecule, *e.g.*, an antibody or antigen-binding fragment thereof as described herein specifically binds to *Pseudomonas* Psl and/or PcrV, with an affinity characterized by a K_D of about 1.18×10^{-7} M, as determined by the OCTET® binding assay described herein. In another embodiment, an isolated binding molecule, *e.g.*, an antibody or antigen-binding fragment thereof as described herein specifically binds to *Pseudomonas* Psl and/or PcrV, with an affinity characterized by a K_D of about 1.44×10^{-7} M, as determined by the OCTET® binding assay described herein.

[0210] Some embodiments include the isolated binding molecules *e.g.*, an antibody or fragment thereof as described above, which (a) can inhibit attachment of *Pseudomonas aeruginosa* to epithelial cells, (b) can promote OPK of *P. aeruginosa*, or (c) can inhibit attachment of *P. aeruginosa* to epithelial cells and can promote OPK of *P. aeruginosa*.

[0211] In some embodiments the isolated binding molecule *e.g.*, an antibody or fragment thereof as described above, where maximum inhibition of *P. aeruginosa* attachment to epithelial cells is achieved at an antibody concentration of about 50 μg/ml or less, 5.0 μg/ml or less, or about 0.5 μg/ml or less, or at an antibody concentration ranging from about 30 μg/ml to about 0.3 μg/ml, or at an antibody concentration of about 1 μg/ml, or at an antibody concentration of about 0.3 μg/ml.

[0212] Certain embodiments include the isolated binding molecule *e.g.*, an antibody or fragment thereof as described above, where the OPK EC50 is less than about 0.5 μg/ml, less than about 0.05 μg/ml, or less than about 0.005 μg/ml, or where the OPK EC50 ranges from about 0.001 μg/ml to about 0.5 μg/ml, or where the OPK EC50 ranges from about 0.02 μg/ml to about 0.08 μg/ml, or where the OPK EC50 ranges from about 0.002 μg/ml to about 0.01 μg/ml or where the OPK EC50 is less than about 0.2 μg/ml, or wherein the OPK EC50 is less than about 0.02 μg/ml. In certain embodiments, an anti-*Pseudomonas* Psl binding molecule, *e.g.*, antibody or fragment, variant or derivative thereof described herein specifically binds to the same Ps1 epitope as monoclonal antibody WapR-004, WapR-004RAD, Cam-003, Cam-004, or Cam-005, or will competitively inhibit such a monoclonal antibody from binding to *Pseudomonas* Psl. WapR-004RAD is identical to WapR-004 except for an amino acid substitution G98A of the VH amino acid sequence of SEQ ID NO:11.

[0213] Some embodiments include WapR-004 (W4) mutants comprising an scFv-Fc molecule amino acid sequence identical to, or identical except for one, two, three, four, five, or more amino acid substitutions to one or more of: SEQ ID NO: 78, SEQ ID NO: 79, SEQ ID NO: 80, SEQ ID NO: 81, SEQ ID NO: 82, SEQ ID NO: 83, SEQ ID NO: 84, SEQ ID NO: 85, SEQ ID NO: 86, SEQ ID NO: 87, SEQ ID NO: 88, SEQ ID NO: 89, SEQ ID NO: 90, SEQ ID NO: 91, SEQ ID NO: 92, SEQ ID NO: 93, SEQ ID NO: 94, SEQ ID NO: 95, SEQ ID NO: 96, SEQ ID NO: 97, SEQ ID NO: 98, SEQ ID NO: 99, SEQ ID NO: 100, SEQ ID NO: 101, SEQ ID NO: 102, SEQ ID NO: 103, SEQ ID NO: 104, SEQ ID NO: 105, SEQ ID NO: 106, SEQ ID NO: 107, SEQ ID NO: 108, SEQ ID

NO: 109, SEQ ID NO: 110, SEQ ID NO: 111, SEQ ID NO: 112, SEQ ID NO: 113, SEQ ID NO: 114, SEQ ID NO: 115, SEQ ID NO: 116, SEQ ID NO: 117, SEQ ID NO: 118, SEQ ID NO: 119, SEQ ID NO: 120, SEQ ID NO: 121, SEQ ID NO: 122, SEQ ID NO: 123, SEQ ID NO: 124, SEQ ID NO: 125, SEQ ID NO: 126, SEQ ID NO: 127, SEQ ID NO: 128, SEQ ID NO: 129, SEQ ID NO: 130, SEQ ID NO: 131, SEQ ID NO: 132, SEQ ID NO: 133, SEQ ID NO: 134, SEQ ID NO: 135, SEQ ID NO: 136, SEQ ID NO: 137, SEQ ID NO: 138, SEQ ID NO: 139, SEQ ID NO: 140, SEQ ID NO: 141, SEQ ID NO: 142, SEQ ID NO: 143, SEQ ID NO: 144, SEQ ID NO: 145; or SEQ ID NO: 146.

[0214] Other embodiments include WapR-004 (W4) mutants comprising an scFv-Fc molecule amino acid sequence at least 80%, 85%, 90% 95% or 100% identical to one or more of: SEQ ID NO: 78, SEQ ID NO: 79, SEQ ID NO: 80, SEQ ID NO: 81, SEQ ID NO: 82, SEQ ID NO: 83, SEQ ID NO: 84, SEQ ID NO: 85, SEQ ID NO: 86, SEQ ID NO: 87, SEQ ID NO: 88, SEQ ID NO: 89, SEQ ID NO: 90, SEQ ID NO: 91, SEQ ID NO: 92, SEQ ID NO: 93, SEQ ID NO: 94, SEQ ID NO: 95, SEQ ID NO: 96, SEQ ID NO: 97, SEQ ID NO: 98, SEQ ID NO: 99, SEQ ID NO: 100, SEQ ID NO: 101, SEQ ID NO: 102, SEQ ID NO: 103, SEQ ID NO: 104, SEQ ID NO: 105, SEQ ID NO: 106, SEQ ID NO: 107, SEQ ID NO: 108, SEQ ID NO: 109, SEQ ID NO: 110, SEQ ID NO: 111, SEQ ID NO: 112, SEQ ID NO: 113, SEQ ID NO: 114, SEQ ID NO: 115, SEQ ID NO: 116, SEQ ID NO: 117, SEQ ID NO: 118, SEQ ID NO: 119, SEQ ID NO: 120, SEQ ID NO: 121, SEQ ID NO: 122, SEQ ID NO: 123, SEQ ID NO: 124, SEQ ID NO: 125, SEQ ID NO: 126, SEQ ID NO: 127, SEQ ID NO: 128, SEQ ID NO: 129, SEQ ID NO: 130, SEQ ID NO: 131, SEQ ID NO: 132, SEQ ID NO: 133, SEQ ID NO: 134, SEQ ID NO: 135, SEQ ID NO: 136, SEQ ID NO: 137, SEQ ID NO: 138, SEQ ID NO: 139, SEQ ID NO: 140, SEQ ID NO: 141, SEQ ID NO: 142, SEQ ID NO: 143, SEQ ID NO: 144, SEQ ID NO: 145; or SEQ ID NO: 146.

[0215] In some embodiments, an anti-*Pseudomonas* Psl binding molecule, *e.g.*, antibody or fragment, variant or derivative thereof described herein specifically binds to the same epitope as monoclonal antibody WapR-001, WapR-002, or WapR-003, or will competitively inhibit such a monoclonal antibody from binding to *Pseudomonas* Psl.

[0216] In certain embodiments, an anti-*Pseudomonas* Psl binding molecule, *e.g.*, antibody or fragment, variant or derivative thereof described herein specifically binds to

the same epitope as monoclonal antibody WapR-016, or will competitively inhibit such a monoclonal antibody from binding to *Pseudomonas* Psl.

TABLE 2: Reference VH and VL amino acid sequences*

Antibody Name	VH	VL
Cam-003	QVRLQQSGPGLVKPSETLSLTCTVSGGSTS**PYFWS**WLRQPPGKGLEWIG**YIHSNGGTNYNPSLKS**RLTISGDTSKNQFSLNLSFVTAADTALYYCAR**TDYDVYGPAFDI**WGQGTMVTV SEQ ID NO:1	SSELTQDPAVSVALGQTVRITC**QGDSLRSYYAS**WYQQKPGQAPVLVIY**GKNNRPS**GIPDRFSGSSSGNTASLTITGAQAEDEADYYC**NSRDSSGNHVV**FGGGTKLTVL SEQ ID NO:2
Cam-004	QVQLQQSGPGRVKPSETLSLTCTVSGYSVS**SGYYWG**WIRQSPGTGLEWIG**SISHSGSTYYNPSLKS**RVTISGDASKNQFFLRLTSVTAADTAVYYCAR**SEATANFDS**WGRGTLVTVSS SEQ ID NO:3	SSELTQDPAVSVALGQTVRITC**QGDSLRSYYAS**WYQQKPGQAPVLVIY**GKNNRPS**GIPDRFSGSSSGNTASLTITGAQAEDEADYYC**NSRDSSGNHVV**FGGGTKLTVL SEQ ID NO:2

Antibody Name	VH	VL
Cam-005	QVQLQQSGPGLVKPSETLSLTCTVSGGSVS**SSGYYWT**WIRQPPGKGLEWIG**SIYSSGSTYYSPSLKS**RVTISGDTSKNQFSLKLSSVTAADTAVYYCAR**LNWGTVSAFDI**WGRGTLVTV SEQ ID NO:4	SSELTQDPAVSVALGQTVRITC**QGDSLRSYYAS**WYQQKPGQAPVLVIY**GKNNRPS**GIPDRFSGSSSGNTASLTITGAQAEDEADYYC**NSRDSSGNHVV**FGGGTKLTVL SEQ ID NO:2
WapR-001	EVQLLESGGGLVQPGGSLRLSCSASGFTFS**RYPMH**WVRQAPGKGLEYVS**DIGTNGGSTNYADSVKG**RFTISRDNSKNTVYLQMSSLRAEDTAVYHCVA**GIAAAYGFDV**WGQGTMVTVSS SEQ ID NO:5	QAGLTQPASVSGSPGQSITISC**TGTSSDIATYNYVS**WYQQHPGKAPKLMIY**EGTKRPS**GVSNRFSGSKSGNTASLTISGLQAEDEADYYC**SSYARSYTYV**FGTGTELTVL SEQ ID NO:6
WapR-002	QVQLVQSGGGLVQPGGSLRLSCSASGFTFS**SYPMH**WVRQAPGKGLDYVS**DISPNGGSTNYADSVKG**RFTISRDNSKNTLFLQMSSLRAEDTAVYYCVM**GLVPYGFDI**WGQGTLVTVSS SEQ ID NO:7	QTVVTQPASVSGSPGQSITISC**TGTSSDVGGYNYVS**WYQQHPGKAPKLMIY**EVSNRPS**GVSNHFSGSKSGNTASLTISGLQAEDEADYYC**SSYTTSSTYV**FGTGTKVTVL SEQ ID NO:8

Antibody Name	VH	VL
WapR-003	QMQLVQSGGGLVQPGG SLRLSCSASGFTFS**SYP MH**WVRQAPGKGLDYV S**DISPNGGATNYADSV KG**RFTISRDNSKNTVYL QMSSLRAEDTAVYYCV M**GLVPYGFDN**WGQGT MVTVSS SEQ ID NO:9	QTVVTQPASVSASPGQSITISC**AGTSG DVGNYNFVS**WYQQHPGKAPKLLIY**E GSQRPS**GVSNRFSGSRSGNTASLTIS GLQAEDEADYYC**SSYARSYTYV**FGT GTKLTVL SEQ ID NO:10
WapR-004	EVQLLESGPGLVKPSET LSLTCNVAGGSIS**PYYW T**WIRQPPGKGLELIG**YI HSSGYTDYNPSLKS**RV TISGDTSKKQFSLHVSS VTAADTAVYFCAR**GD WDLLHALDI**WGQGTL VTVSS SEQ ID NO:11	EIVLTQSPSSLSTSVGDRVTITC**RASQ SIRSHLN**WYQQKPGKAPKLLIY**GAS NLQS**GVPSRFSGSGSGTDFTLTISSLQ PEDFATYYC**QQSYSFPLT**FGGGTKLE IK SEQ ID NO:12
WapR-007	EVQLVQSGADVKKPGA SVRVTCKASGYTFT**GH NIH**WVRQAPGQGLEW MG**WINPDSGATSYAQ KFQG**RVTMTRDTSITT AYMDLSRLRSDDTAVY YCAT**DTLLSNH**WGQGT LVTVSS SEQ ID NO:13	SSELTQDPAVSVALGQTVRITC**QGDS LRSYYTN**WFQQKPGQAPLLVVY**AK NKRPP**GIPDRFSGSSSGNTASLTITGA QAEDEADYYC**HSRDSSGNHVV**FGG GTKLTVL SEQ ID NO:14

Antibody Name	VH	VL
WapR-016	EVQLVESGGGLVQPGGSL RLSCAASGYTFS**SYATS**WV RQAPGKGLEWVA**GISGSG DTTDYVDSVKG**RFTVSRD NSKNTLYLQMNSLRADDT AVYYCAS**RGGLGGYYRG GFDF**WGQGTMVTVSS SEQ ID NO:15	QSVLTQPASVSGSPGQSITISC**TGTSSDVG GYNYVS**WYQQHPGKAPKLMIY**EVSNRPS** GVSNRFSGSKSGNTASLTISGLQAEDEAD YC**SSYSSGTVV**FGGGTELTVL SEQ ID NO:16
WapR-004RAD	EVQLLESGPGLVKPSET LSLTCNVAGGSIS**PYYW T**WIRQPPGKGLELIG**YI HSSGYTDYNPSLKS**RV TISGDTSKKQFSLHVSS VTAADTAVYFCAR**AD WDLLHALDI**WGQGTL VTVSS SEQ ID NO:74	EIVLTQSPSSLSTSVGDRVTITC**RASQ SIRSHLN**WYQQKPGKAPKLLIY**GAS NLQS**GVPSRFSGSGSGTDFTLTISSLQ PEDFATYYC**QQSYSFPLT**FGGGTKLE IK SEQ ID NO:12
V2L2	EMQLLESGGGLVQPGG SLRLSCAASGFTFS**SYA MN**WVRQAPGEGLEWV S**AITISGITAYYTDSVK G**RFTISRDNSKNTLYLQ MNSLRAGDTAVYYCA K**EEFLPGTHYYYGMD V**WGQGTTVTVSS SEQ ID NO:216	AIQMTQSPSSLSASVGDRVTITC**RAS QGIRNDLG**WYQQKPGKAPKLVIY**SA STLQS**GVPSRFSGSGSGTDFTLSISSL QPDDFATYYC**LQDYNYPWT**FGQGT KVEIK SEQ ID NO:217

*VH and VL CDR1, CDR2, and CDR3 amino acid sequences are underlined

TABLE 3: Reference VH and VL CDR1, CDR2, and CDR3 amino acid sequences

Antibody Name	VHCDR1	VHCDR2	VHCDR3	VLCDR1	VLCDR2	VLCDR3
Cam-003	PYFWS SEQ ID NO:17	YIHSNGG TNYNPSL KS SEQ ID NO:18	TDYDVY GPAFDI SEQ ID NO:19	QGDSLRSY YAS SEQ ID NO:20	GKNNRPS SEQ ID NO:21	NSRDSSGNH VV SEQ ID NO:22
Cam-004	SGYYW G SEQ ID NO:23	SISHSGST YYNPSLK S SEQ ID NO:24	SEATAN FDS SEQ ID NO:25	QGDSLRSY YAS SEQ ID NO:20	GKNNRPS SEQ ID NO:21	NSRDSSGNH VV SEQ ID NO:22
Cam-005	SSGYYW T SEQ ID NO:26	SIYSSGST YYSPSLKS SEQ ID NO:27	LNWGTV SAFDI SEQ ID NO:28	QGDSLRSY YAS SEQ ID NO:20	GKNNRPS SEQ ID NO:21	NSRDSSGNH VV SEQ ID NO:22
WapR-001	RYPMH SEQ ID NO:29	DIGTNG GSTNYA DSVKG SEQ ID NO:30	GIAAAY GFDV SEQ ID NO:31	TGTSSDIAT YNYVS SEQ ID NO:32	EGTKRPS SEQ ID NO:33	SSYARSYT YV SEQ ID NO:34
WapR-002	SYPMH SEQ ID NO:35	DISPNGG STNYAD SVKG SEQ ID NO:36	GLVPY GFDI SEQ ID NO:37	TGTSSDV GGYNYVS SEQ ID NO:38	EVSNRPS SEQ ID NO:39	SSYTTSSTY V SEQ ID NO:40

Antibody Name	VHCDR1	VHCDR2	VHCDR3	VLCDR1	VLCDR2	VLCDR3
WapR-003	SYPMH SEQ ID NO:41	DISPNGGATNYADSVKG SEQ ID NO:42	GLVPYGFDN SEQ ID NO:43	AGTSGDVGNYNFVS SEQ ID NO:44	EGSQRPS SEQ ID NO:45	SSYARSYTYV SEQ ID NO:46
WapR-004	PYYWT SEQ ID NO:47	YIHSSGYTDYNPSLKS SEQ ID NO:48	GDWDLLHALDI SEQ ID NO:49	RASQSIRSHLN SEQ ID NO:50	GASNLQS SEQ ID NO:51	QQSYSFPLT SEQ ID NO:52
WapR-007	GHNIH SEQ ID NO:53	WINPDSGATSYAQKFQG SEQ ID NO:54	DTLLSNH SEQ ID NO:55	QGDSLRSYYTN SEQ ID NO:56	AKNKRPP SEQ ID NO:57	HSRDSSGNHVV SEQ ID NO:58
WapR-016	SYATS SEQ ID NO:59	GISGSGDTTDYVDSVKG SEQ ID NO:60	RGGLGGYYRGGFDF SEQ ID NO:61	TGTSSDVGGYNYVS SEQ ID NO:62	EVSNRPS SEQ ID NO:63	SSYSSGTVV SEQ ID NO:64
WapR-004RAD	PYYWT SEQ ID NO:47	YIHSSGYTDYNPSLKS SEQ ID NO:48	ADWDLLHALDI SEQ ID NO:75	RASQSIRSHLN SEQ ID NO:50	GASNLQS SEQ ID NO:51	QQSYSFPLT SEQ ID NO:52

Antibody Name	VHCDR1	VHCDR2	VHCDR3	VLCDR1	VLCDR2	VLCDR3
V2L2	SYAMN SEQ ID NO:218	AITISGIT AYYTDS VKG SEQ ID NO:219	EEFLPG THYYY GMDV SEQ ID NO:220	RASQGIRN DLG SEQ ID NO:221	SASTLQS SEQ ID NO:222	LQDYNYP WT SEQ ID NO:223

[0217] In certain embodiments, an anti-*Pseudomonas* PcrV binding molecule, *e.g.*, antibody or fragment, variant or derivative thereof described herein specifically binds to the same PcrV epitope as monoclonal antibody V2L2, and/or will competitively inhibit such a monoclonal antibody from binding to *Pseudomonas* PcrV.

[0218] For example, in certain aspects the anti-*Pseudomonas* PcrV binding molecule, *e.g.*, antibody or fragment, variant or derivative thereof comprises V2L2-GL and/or V2L2-MD.

[0219] In certain embodiments, an anti-*Pseudomonas* PcrV binding molecule, *e.g.*, antibody or fragment, variant or derivative thereof described herein specifically binds to the same PcrV epitope as monoclonal antibody 29D2, and/or will competitively inhibit such a monoclonal antibody from binding to *Pseudomonas* PcrV.

[0220] Any anti-*Pseudomonas* Psl and/or PcrV binding molecules, *e.g.*, antibodies or fragments, variants or derivatives thereof described herein can further include additional polypeptides, *e.g.*, a signal peptide to direct secretion of the encoded polypeptide, antibody constant regions as described herein, or other heterologous polypeptides as described herein. Additionally, binding molecules or fragments thereof of the description include polypeptide fragments as described elsewhere. Additionally anti-*Pseudomonas* Psl and/or PcrV binding molecules, *e.g.*, antibodies or fragments, variants or derivatives thereof described herein can be fusion polypeptides, Fab fragments, scFvs, or other derivatives, as described herein.

[0221] Also, as described in more detail elsewhere herein, the disclosure includes compositions comprising anti-*Pseudomonas* Psl and/or PcrV binding molecules, *e.g.*, antibodies or fragments, variants or derivatives thereof described herein.

[0222] It will also be understood by one of ordinary skill in the art that anti-*Pseudomonas* Psl and/or PcrV binding molecules, *e.g.*, antibodies or fragments, variants or derivatives thereof described herein can be modified such that they vary in amino acid sequence from the naturally occurring binding polypeptide from which they were derived. For example, a polypeptide or amino acid sequence derived from a designated protein can be similar, *e.g.*, have a certain percent identity to the starting sequence, *e.g.*, it can be 60%, 70%, 75%, 80%, 85%, 90%, or 95% identical to the starting sequence.

[0223] As known in the art, "sequence identity" between two polypeptides is determined by comparing the amino acid sequence of one polypeptide to the sequence of a second polypeptide. When discussed herein, whether any particular polypeptide is at least about 70%, 75%, 80%, 85%, 90% or 95% identical to another polypeptide can be determined using methods and computer programs/software known in the art such as, but not limited to, the BESTFIT program (Wisconsin Sequence Analysis Package, Version 8 for Unix, Genetics Computer Group, University Research Park, 575 Science Drive, Madison, WI 53711). BESTFIT uses the local homology algorithm of Smith and Waterman, Advances in Applied Mathematics 2:482-489 (1981), to find the best segment of homology between two sequences. When using BESTFIT or any other sequence alignment program to determine whether a particular sequence is, for example, 95% identical to a reference sequence, the parameters are set, of course, such that the percentage of identity is calculated over the full length of the reference polypeptide sequence and that gaps in homology of up to 5% of the total number of amino acids in the reference sequence are allowed.

[0224] Percentage of “sequence identity” can also be determined by comparing two optimally aligned sequences over a comparison window. In order to optimally align sequences for comparison, the portion of a polynucleotide or polypeptide sequence in the comparison window can comprise additions or deletions termed gaps while the reference sequence is kept constant. An optimal alignment is that alignment which, even with gaps, produces the greatest possible number of “identical” positions between the reference and comparator sequences. Percentage “sequence identity” between two sequences can be determined using the version of the program “BLAST 2 Sequences” which was available from the National Center for Biotechnology Information as of September 1, 2004, which program incorporates the programs BLASTN (for nucleotide sequence comparison) and

BLASTP (for polypeptide sequence comparison), which programs are based on the algorithm of Karlin and Altschul (*Proc. Natl. Acad. Sci. USA* 90(12):5873-5877, 1993). When utilizing "BLAST 2 Sequences," parameters that were default parameters as of September 1, 2004, can be used for word size (3), open gap penalty (11), extension gap penalty (1), gap drop-off (50), expect value (10) and any other required parameter including but not limited to matrix option.

[0225] Furthermore, nucleotide or amino acid substitutions, deletions, or insertions leading to conservative substitutions or changes at "non-essential" amino acid regions can be made. For example, a polypeptide or amino acid sequence derived from a designated protein can be identical to the starting sequence except for one or more individual amino acid substitutions, insertions, or deletions, *e.g.*, one, two, three, four, five, six, seven, eight, nine, ten, fifteen, twenty or more individual amino acid substitutions, insertions, or deletions. In certain embodiments, a polypeptide or amino acid sequence derived from a designated protein has one to five, one to ten, one to fifteen, or one to twenty individual amino acid substitutions, insertions, or deletions relative to the starting sequence.

[0226] An anti-*Pseudomonas* Psl and/or PcrV binding molecule, *e.g.*, an antibody or fragment, variant or derivative thereof described herein can comprise, consist essentially of, or consist of a fusion protein. Fusion proteins are chimeric molecules which comprise, for example, an immunoglobulin antigen-binding domain with at least one target binding site, and at least one heterologous portion, *i.e.*, a portion with which it is not naturally linked in nature. The amino acid sequences can normally exist in separate proteins that are brought together in the fusion polypeptide or they can normally exist in the same protein but are placed in a new arrangement in the fusion polypeptide. Fusion proteins can be created, for example, by chemical synthesis, or by creating and translating a polynucleotide in which the peptide regions are encoded in the desired relationship.

[0227] The term "heterologous" as applied to a polynucleotide, polypeptide, or other moiety means that the polynucleotide, polypeptide, or other moiety is derived from a distinct entity from that of the rest of the entity to which it is being compared. In a non-limiting example, a "heterologous polypeptide" to be fused to a binding molecule, *e.g.*, an antibody or an antigen-binding fragment, variant, or derivative thereof is derived from a non-immunoglobulin polypeptide of the same species, or an immunoglobulin or non-immunoglobulin polypeptide of a different species.

IV. FUSION PROTEINS AND ANTIBODY CONJUGATES

[0228] In some embodiments, the anti-*Pseudomonas* Psl and/or PcrV binding molecules, *e.g.*, antibodies or fragments, variants or derivatives thereof can be administered multiple times in conjugated form. In still another embodiment, the anti-*Pseudomonas* Psl and/or PcrV binding molecules, *e.g.*, antibodies or fragments, variants or derivatives thereof can be administered in unconjugated form, then in conjugated form, or vice versa.

[0229] In specific embodiments, the anti-*Pseudomonas* Psl and/or PcrV binding molecules, *e.g.*, antibodies or fragments, variants or derivatives thereof can be conjugated to one or more antimicrobial agents, for example, Polymyxin B (PMB). PMB is a small lipopeptide antibiotic approved for treatment of multidrug-resistant Gram-negative infections. In addition to its bactericidal activity, PMB binds lipopolysaccharide (LPS) and neutralizes its proinflammatory effects. (Dixon, R.A. & Chopra, I. *J Antimicrob Chemother 18*, 557-563 (1986)). LPS is thought to significantly contribute to inflammation and the onset of Gram-negative sepsis. (Guidet, B., et al., *Chest 106*, 1194-1201 (1994)). Conjugates of PMB to carrier molecules have been shown to neutralize LPS and mediate protection in animal models of endotoxemia and infection. (Drabick, J.J., *et al. Antimicrob Agents Chemother 42*, 583-588 (1998)). Also disclosed is a method for attaching one or more PMB molecules to cysteine residues introduced into the Fc region of monoclonal antibodies (mAb) of the disclosure. For example, the Cam-003-PMB conjugates retained specific, mAb-mediated binding to *P. aeruginosa* and also retained OPK activity. Furthermore, mAb-PMB conjugates bound and neutralized LPS *in vitro*. In specific embodiments, the anti-*Pseudomonas* Psl and/or PcrV binding molecules, *e.g.*, antibodies or fragments, variants or derivatives thereof can be combined with antibiotics (*e.g.*, Ciprofloxacin, Meropenem, Tobramycin, Aztreonam).

[0230] In certain embodiments, an anti-*Pseudomonas* Psl and/or PcrV binding molecule, *e.g.*, an antibody or fragment, variant or derivative thereof described herein can comprise a heterologous amino acid sequence or one or more other moieties not normally associated with an antibody (*e.g.*, an antimicrobial agent, a therapeutic agent, a prodrug, a peptide, a protein, an enzyme, a lipid, a biological response modifier, pharmaceutical agent, a lymphokine, a heterologous antibody or fragment thereof, a detectable label, polyethylene glycol (PEG), and a combination of two or more of any said agents). In further embodiments, an anti-*Pseudomonas* Psl and/or PcrV binding molecule, *e.g.*, an

antibody or fragment, variant or derivative thereof can comprise a detectable label selected from the group consisting of an enzyme, a fluorescent label, a chemiluminescent label, a bioluminescent label, a radioactive label, or a combination of two or more of any said detectable labels.

V. POLYNUCLEOTIDES ENCODING BINDING MOLECULES

[0231] Also provided herein are nucleic acid molecules encoding the anti-*Pseudomonas* Psl and/or PcrV binding molecules, *e.g.*, antibodies or fragments, variants or derivatives thereof described herein. .

[0232] One embodiment provides an isolated polynucleotide comprising, consisting essentially of, or consisting of a nucleic acid encoding an immunoglobulin heavy chain variable region (VH) amino acid sequence at least 80%, 85%, 90% 95% or 100% identical to one or more of: SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, SEQ IS NO: 74, or SEQ ID NO:216 as shown in Table 2.

[0233] One embodiment provides an isolated polynucleotide comprising, consisting essentially of, or consisting of a nucleic acid encoding an immunoglobulin heavy chain variable region (VH) amino acid sequence of SEQ ID NO:257 or SEQ ID NO:259. For example the nucleic acid sequences of SEQ ID NO:261, and SEQ ID NO:: 259, respectively.

[0234] Another embodiment provides an isolated polynucleotide comprising, consisting essentially of, or consisting of a nucleic acid encoding a VH amino acid sequence identical to, or identical except for one, two, three, four, five, or more amino acid substitutions to one or more of: SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 74, or SEQ ID NO:216 as shown in Table 2.

[0235] Further embodiment provides an isolated polynucleotide comprising, consisting essentially of, or consisting of a nucleic acid encoding a VH, where one or more of the VHCDR1, VHCDR2 or VHCDR3 regions of the VH are identical to, or identical except for four, three, two, or one amino acid substitutions, to one or more reference heavy chain VHCDR1, VHCDR2 and/or VHCDR3 amino acid sequences of one or more of: SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9,

SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 74, or SEQ ID NO:216 as shown in Table 2.

[0236] Another embodiment provides an isolated polynucleotide comprising, consisting essentially of, or consisting of a nucleic acid encoding an isolated binding molecule, *e.g.*, an antibody or antigen-binding fragment thereof which specifically binds to *Pseudomonas* Psl comprising a VH, where one or more of the VHCDR1, VHCDR2 or VHCDR3 regions of the VH are identical to, or identical except for four, three, two, or one amino acid substitutions, to one or more reference heavy chain VHCDR1, VHCDR2 and/or VHCDR3 amino acid sequences of one or more of: SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, or SEQ ID NO: 74 as shown in Table 2.

[0237] A further embodiment provides an isolated binding molecule *e.g.*, an antibody or antigen-binding fragment comprising the VH encoded by the polynucleotide specifically or preferentially binds to *Pseudomonas* Psl and/or PcrV.

[0238] Another embodiment provides an isolated polynucleotide comprising, consisting essentially of, or consisting of a nucleic acid encoding an immunoglobulin light chain variable region (VL) amino acid sequence at least 80%, 85%, 90% 95% or 100% identical to one or more of: SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16, or SEQ ID NO:217 as shown in Table 2.

[0239] Another embodiment provides an isolated polynucleotide comprising, consisting essentially of, or consisting of a nucleic acid encoding the immunoglobulin light chain variable region (VL) amino acid sequence of SEQ ID NO:256, e.g., the nucleic acid sequence SEQ ID NO:260..

[0240] A further embodiment provides an isolated polynucleotide comprising, consisting essentially of, or consisting of a nucleic acid encoding a VL amino acid sequence identical to, or identical except for one, two, three, four, five, or more amino acid substitutions to one or more of: SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16, or SEQ ID NO:217 as shown in Table 2.

[0241] Another embodiment provides an isolated polynucleotide comprising, consisting essentially of, or consisting of a nucleic acid encoding a VL, where one or more of the

VLCDR1, VLCDR2 or VLCDR3 regions of the VL are at least 80%, 85%, 90%, 95% or 100% identical to one or more reference light chain VLCDR1, VLCDR2 or VLCDR3 amino acid sequences of one or more of: SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, or SEQ ID NO: 16, or SEQ ID NO:217 as shown in Table 2.

[0242] A further embodiment provides an isolated polynucleotide comprising, consisting essentially of, or consisting of a nucleic acid encoding an isolated binding molecule, *e.g.*, an antibody or antigen-binding fragment thereof which specifically binds to *Pseudomonas* Psl comprising an VL, where one or more of the VLCDR1, VLCDR2 or VLCDR3 regions of the VL are identical to, or identical except for four, three, two, or one amino acid substitutions, to one or more reference heavy chain VLCDR1, VLCDR2 and/or VLCDR3 amino acid sequences of one or more of: SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16, or SEQ ID NO:217 as shown in Table 2.

[0243] In another embodiment, isolated binding molecules *e.g.*, an antibody or antigen-binding fragment comprising the VL encoded by the polynucleotide specifically or preferentially bind to *Pseudomonas* Psl and/or PcrV.

[0244] One embodiment provides an isolated polynucleotide comprising, consisting essentially of, or consisting of a nucleic acid which encodes an scFv molecule including a VH and a VL, where the scFv is at least 80%, 85%, 90% 95% or 100% identical to one or more of SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:69, or SEQ ID NO:70 as shown in Table 4.

TABLE 4: Reference scFv nucleic acid sequences

Antibody Name	scFv nucleotide sequences
Cam-003	CAGCCGGCCATGGCCCAGGTACAGCTGCAGCAGTCAGGCCCAGG ACTGGTGAAGCCTTCGGAGACCCTGTCCCTCACCTGCACTGTCTCT GGTGGCTCCACCAGTCCTTACTTCTGGAGCTGGCTCCGGCAGCCC CCAGGGAAGGGACTGGAGTGGATTGGTTATATCCATTCCAATGGG GGCACCAACTACAACCCCTCCCTCAAGAGTCGACTCACCATATCA GGAGACACGTCCAAGAACCAATTCTCCCTGAATCTGAGTTTTGTG ACCGCTGCGGACACGGCCCTCTATTACTGTGCGAGAACGGACTAC GATGTCTACGGCCCCCGCTTTTGATATCTGGGGCCAGGGGACAATG GTCACCGTCTCGAGTGGTGGAGGCGGTTCAGGCGGAGGTGGCAG CGGCGGTGGCGGATCGTCTGAGCTGACTCAGGACCCTGCTGTGTC TGTGGCCTTGGGACAGACAGTCAGGATCACATGCCAAGGAGACA GCCTCAGAAGCTATTATGCAAGCTGGTACCAGCAGAAGCCAGGA CAGGCCCCTGTACTTGTCATCTATGGTAAAAACAACCGGCCCTCA GGGATCCCAGACCGATTCTCTGGCTCCAGCTCAGGAAACACAGCT TCCTTGACCATCACTGGGGCTCAGGCGGAAGATGAGGCTGACTAT TACTGTAACTCCCGGGACAGCAGTGGTAACCATGTGGTATTCGGC GGAGGGACCAAGCTGACCGTCCTAGGTGCGGCCGCA SEQ ID NO:65

Antibody Name	scFv nucleotide sequences
Cam-004	CAGCCGGCCATGGCCCAGGTACAGCTGCAGCAGTCAGGCCCAGG ACGGGTGAAGCCTTCGGAGACGCTGTCCCTCACCTGCACTGTCTC TGGTTACTCCGTCAGTAGTGGTTACTACTGGGGCTGGATCCGGCA GTCCCCAGGGACGGGGCTGGAGTGGATTGGGAGTATCTCTCATAG TGGGAGCACCTACTACAACCCGTCCCTCAAGAGTCGAGTCACCAT ATCAGGAGACGCATCCAAGAACCAGTTTTTCCTGAGGCTGACTTC TGTGACCGCCGCGGACACGGCCGTTTATTACTGTGCGAGATCTGA GGCTACCGCCAACTTTGATTCTTGGGGCAGGGGCACCCTGGTCAC CGTCTCTTCAGGTGGAGGCGGTTCAGGCGGAGGTGGCAGCGGCG GTGGCGGATCGTCTGAGCTGACTCAGGACCCTGCCGTGTCTGTGG CCTTGGGACAGACAGTCAGGATCACATGCCAAGGAGACAGCCTC AGAAGCTATTATGCAAGCTGGTACCAGCAGAAGCCAGGACAGGC CCCTGTACTTGTCATCTATGGTAAAAACAACCGGCCCTCAGGGAT CCCAGACCGATTCTCTGGCTCCAGCTCAGGAAACACAGCTTCCTT GACCATCACTGGGGCTCAGGCGGAAGATGAGGCTGACTATTACTG TAACTCCCGGGACAGCAGTGGTAACCATGTGGTATTCGGCGGAGG GACCAAGCTGACCGTCCTAGGTGCGGCCGCA SEQ ID NO:66

Antibody Name	scFv nucleotide sequences
Cam-005	CAGCCGGCCATGGCCCAGGTACAGCTGCAGCAGTCAGGCCCAGG ACTGGTGAAGCCTTCGGAGACCCTGTCCCTCACCTGCACTGTCTCT GGTGGCTCCGTCAGCAGTAGTGGTTATTACTGGACCTGGATCCGC CAGCCCCCAGGGAAGGGGCTGGAGTGGATTGGGAGTATCTATTCT AGTGGGAGCACATATTACAGCCCGTCCCTCAAGAGTCGAGTCACC ATATCCGGAGACACGTCCAAGAACCAGTTCTCCCTCAAGCTGAGC TCTGTGACCGCCGCAGACACAGCCGTGTATTACTGTGCGAGACTT AACTGGGGCACTGTGTCTGCCTTTGATATCTGGGGCAGAGGCACC CTGGTCACCGTCTCGAGTGGTGGAGGCGGTTCAGGCGGAGGTGGC AGCGGCGGTGGCGGATCGTCTGAGCTGACTCAGGACCCTGCTGTG TCTGTGGCCTTGGGACAGACAGTCAGGATCACATGCCAAGGAGAC AGCCTCAGAAGCTATTATGCAAGCTGGTACCAGCAGAAGCCAGG ACAGGCCCCTGTACTTGTCATCTATGGTAAAAACAACCGGCCCTC AGGGATCCCAGACCGATTCTCTGGCTCCAGCTCAGGAAACACAGC TTCCTTGACCATCACTGGGGCTCAGGCGGAAGATGAGGCTGACTA TTACTGTAACTCCCGGGACAGCAGTGGTAACCATGTGGTATTCGG CGGAGGGACCAAGCTGACCGTCCTAGGTGCGGCCGCA SEQ ID NO:67

Antibody Name	scFv nucleotide sequences
WapR-001	TCTATGCGGCCCAGCCGGCCATGGCCGAGGTGCAGCTGTTGGAGT CTGGGGGAGGTTTGGTCCAGCCTGGGGGGTCCCTGAGACTCTCCT GTTCAGCCTCTGGGTTCACCTTCAGTCGGTATCCTATGCATTGGGT CCGCCAGGCTCCAGGGAAGGGACTGGAATATGTTTCAGATATTGG TACTAATGGGGGTAGTACAAACTACGCAGACTCCGTGAAGGGCA GATTCACCATCTCCAGAGACAATTCCAAGAACACGGTGTATCTTC AAATGAGCAGTCTGAGAGCTGAGGACACGGCTGTGTATCATTGTG TGGCGGGTATAGCAGCCGCCTATGGTTTTGATGTCTGGGGCCAAG GGACAATGGTCACCGTCTCGAGTGGAGGCGGCGGTTCAGGCGGA GGTGGCTCTGGCGGTGGCGGAAGTGCACAGGCAGGGCTGACTCA GCCTGCCTCCGTGTCTGGGTCTCCTGGACAGTCGATCACCATCTCC TGCACTGGAACCAGCAGTGACATTGCTACTTATAACTATGTCTCCT GGTACCAACAGCACCCAGGCAAAGCCCCCAAACTCATGATTTATG AGGGCACTAAGCGGCCCTCAGGGGTTTCTAATCGCTTCTCTGGCT CCAAGTCTGGCAACACGGCCTCCCTGACAATCTCTGGGCTCCAGG CTGAGGACGAGGCTGATTATTACTGTTCCTCATATGCACGTAGTT ACACTTATGTCTTCGGAACTGGGACCGAGCTGACCGTCCTAGCGG CCGC SEQ ID NO:68

Antibody Name	scFv nucleotide sequences
WapR-002	CTATGCGGCCCAGCCGGCCATGGCCCAGGTGCAGCTGGTGCAGTC TGGGGGAGGCTTGGTCCAGCCTGGGGGGTCCCTGAGACTCTCCTG TTCAGCCTCTGGATTCACCTTCAGTAGCTATCCTATGCACTGGGTC CGCCAGGCTCCAGGGAAGGGACTGGATTATGTTTCAGACATCAGT CCAAATGGGGGTTCCACAAACTACGCAGACTCCGTGAAGGGCAG ATTCACCATCTCCAGAGACAATTCCAAGAACACACTGTTTCTTCA AATGAGCAGTCTGAGAGCTGAGGACACGGCTGTGTATTATTGTGT GATGGGGTTAGTACCCTATGGTTTTGATATCTGGGGCCAAGGCAC CCTGGTCACCGTCTCGAGTGGAGGCGGCGGTTCAGGCGGAGGTGG CTCTGGCGGTGGCGGAAGTGCACAGACTGTGGTGACCCAGCCTGC CTCCGTGTCTGGGTCTCCTGGACAGTCGATCACCATCTCCTGCACT GGAACCAGCAGTGACGTTGGTGGTTATAACTATGTCTCCTGGTAC CAACAGCACCCAGGCAAAGCCCCCAAACTCATGATTTATGAGGTC AGTAATCGGCCCTCAGGGGTTTCTAATCACTTCTCTGGCTCCAAGT CTGGCAACACGGCCTCCCTGACCATCTCTGGGCTCCAGGCTGAGG ACGAGGCTGATTATTACTGCAGCTCATATACAACCAGCAGCACTT ATGTCTTCGGAACTGGGACCAAGGTCACCGTCCTAGCGGCCG SEQ ID NO:69

Antibody Name	scFv nucleotide sequences
WapR-003	CGGCCCAGCCGGCCATGGCCCAGATGCAGCTGGTGCAGTCGGGG GGAGGCTTGGTCCAGCCTGGGGGGTCCCTGAGACTCTCCTGTTCA GCCTCTGGATTCACCTTCAGTAGCTATCCTATGCACTGGGTCCGCC AGGCTCCAGGGAAGGGACTGGATTATGTTTCAGACATCAGTCCAA ATGGGGGTGCCACAAACTACGCAGACTCCGTGAAGGGCAGATTC ACCATCTCCAGAGACAATTCCAAGAACACGGTGTATCTTCAAATG AGCAGTCTGAGAGCTGAAGACACGGCTGTCTATTATTGTGTGATG GGGTTAGTGCCCTATGGTTTTGATAACTGGGGCCAGGGGACAATG GTCACCGTCTCGAGTGGAGGCGGCGGTTCAGGCGGAGGTGGCTCT GGCGGTGGCGGAAGTGCACAGACTGTGGTGACCCAGCCTGCCTCC GTGTCTGCATCTCCTGGACAGTCGATCACCATCTCCTGCGCTGGA ACCAGCGGTGATGTTGGGAATTATAATTTTGTCTCCTGGTACCAA CAACACCCAGGCAAAGCCCCCAAACTCCTGATTTATGAGGGCAGT CAGCGGCCCTCAGGGGTTTCTAATCGCTTCTCTGGCTCCAGGTCTG GCAACACGGCCTCCCTGACAATCTCTGGGCTCCAGGCTGAGGACG AGGCTGATTATTACTGTTCCTCATATGCACGTAGTTACACTTATGT CTTCGGAACTGGGACCAAGCTGACCGTCCTAGCGGCCGCA SEQ ID NO:70

Antibody Name	scFv nucleotide sequences
WapR-004	TATGCGGCCCAGCCGGCCATGGCCGAGGTGCAGCTGTTGGAGTCG GGCCCAGGACTGGTGAAGCCTTCGGAGACCCTGTCCCTCACCTGC AATGTCGCTGGTGGCTCCATCAGTCCTTACTACTGGACCTGGATCC GGCAGCCCCCAGGGAAGGGCCTGGAGTTGATTGGTTATATCCACT CCAGTGGGTACACCGACTACAACCCCTCCCTCAAGAGTCGAGTCA CCATATCAGGAGACACGTCCAAGAAGCAGTTCTCCCTGCACGTGA GCTCTGTGACCGCTGCGGACACGGCCGTGTACTTCTGTGCGAGAG GCGATTGGGACCTGCTTCATGCTCTTGATATCTGGGGCCAAGGGA CCCTGGTCACCGTCTCGAGTGGAGGCGGCGGTTCAGGCGGAGGTG GCTCTGGCGGTGGCGGAAGTGCACTCGAAATTGTGTTGACACAGT CTCCATCCTCCCTGTCTACATCTGTAGGAGACAGAGTCACCATCA CTTGCCGGGCAAGTCAGAGCATTAGGAGCCATTTAAATTGGTATC AGCAGAAACCAGGGAAAGCCCCTAAACTCCTGATCTATGGTGCAT CCAATTTGCAAAGTGGGGTCCCATCAAGGTTCAGTGGCAGTGGAT CTGGGACAGATTTCACTCTCACCATTAGTAGTCTGCAACCTGAAG ATTTTGCAACTTACTACTGTCAACAGAGTTACAGTTTCCCCCTCAC TTTCGGCGGAGGGACCAAGCTGGAGATCAAAGCGGCCGC SEQ ID NO:71

Antibody Name	scFv nucleotide sequences
WapR-007	GCGGCCCAGCCGGCCATGGCCGAAGTGCAGCTGGTGCAGTCTGG GGCTGACGTAAAGAAGCCTGGGGCCTCAGTGAGGGTCACCTGCA AGGCTTCTGGATACACCTTCACCGGCCACAACATACACTGGGTGC GACAGGCCCCTGGACAAGGGCTTGAATGGATGGGATGGATCAAC CCTGACAGTGGTGCCACAAGCTATGCACAGAAGTTTCAGGGCAGG GTCACCATGACCAGGGACACGTCCATCACCACAGCCTACATGGAC CTGAGCAGGCTGAGATCTGACGACACGGCCGTATATTACTGTGCG ACCGATACATTACTGTCTAATCACTGGGGCCAAGGAACCCTGGTC ACCGTCTCGAGTGGTGGAGGCGGTTCAGGCGGAGGTGGCAGCGG CGGTGGCGGATCGTCTGAGCTGACTCAGGACCCTGCTGTGTCTGT GGCCTTGGGACAGACAGTCAGGATCACTTGCCAAGGAGACAGTCT CAGAAGCTATTACACAAACTGGTTCCAGCAGAAGCCAGGACAGG CCCCTCTACTTGTCGTCTATGCTAAAAATAAGCGGCCCCCAGGGA TCCCAGACCGATTCTCTGGCTCCAGCTCAGGAAACACAGCTTCCT TGACCATCACTGGGGCTCAGGCGGAAGATGAGGCTGACTATTACT GTCATTCCCGGGACAGCAGTGGTAACCATGTGGTATTCGGCGGAG GGACCAAGCTGACCGTCCTAGGTGCGGCCGCA SEQ ID NO:72

Antibody Name	scFv nucleotide sequences
WapR-016	CAGCCGGCCATGGCCGAGGTGCAGCTGGTGGAGTCTGGGGGAGG CTTGGTACAGCCTGGGGGGTCCCTGAGACTCTCCTG TGCAGCCTCTGGATACACCTTTAGCAGCTATGCCACGAGCTGGGT CCGCCAGGCTCCAGGGAAGGGGCTGGAGTGGGTCG CAGGTATTAGTGGTAGTGGTGATACCACAGACTACGTAGACTCCG TGAAGGGCCGGTTCACCGTCTCCAGAGACAATTCC AAGAACACCCTATATCTGCAAATGAACAGCCTGAGAGCCGACGA CACGGCCGTGTATTACTGTGCGTCGAGAGGAGGTTT AGGGGGTTATTACCGGGGCGGCTTTGACTTCTGGGGCCAGGGGAC AATGGTCACCGTCTCGAGTGGAGGCGGCGGTTCAG GCGGAGGTGGCTCTGGCGGTGGCGGAAGTGCACAGTCTGTGCTGA CGCAGCCTGCCTCCGTGTCTGGGTCTCCTGGACAG TCGATCACCATCTCCTGCACTGGAACCAGCAGTGACGTTGGTGGT TATAACTATGTCTCCTGGTACCAACAGCACCCAGG CAAAGCCCCCAAACTCATGATTTATGAGGTCAGTAATCGGCCCTC AGGGGTTTCTAATCGCTTCTCTGGCTCCAAGTCTG GCAACACGGCCTCCCTGACCATCTCTGGGCTCCAGGCTGAGGACG AGGCTGATTATTACTGCAGCTCATATACAAGCAGC GGCACTGTGGTATTCGGCGGAGGGACCGAGCTGACCGTCCTAGCG GCCGCA SEQ ID NO:73

Antibody Name	
V2L2 – VH	GAGATGCAGCTGTTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGG GGGTCCCTGAGACTCTCCTGTGCAGCCTCTGGATTCACCTTTAGCA GCTATGCCATGAACTGGGTCCGCCAGGCTCCAGGGGAGGGGCTGG AGTGGGTCTCAGCTATTACTATTAGTGGTATTACCGCATACTACAC CGACTCCGTGAAGGGCCGGTTCACCATCTCCAGAGACAATTCCAA GAACACGCTATATCTGCAAATGAACAGCCTGAGGGCCGGGGACAC GGCCGTATATTACTGTGCGAAGGAAGAATTTTTACCTGGAACGCA CTACTACTACGGTATGGACGTCTGGGGCCAAGGGACCACGGTCAC CGTCTCCTCA SEQ ID NO: 238

V2L2 – VL	GCCATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAG GAGACAGAGTCACCATCACTTGCCGGGCAAGTCAGGGCATTAGAA ATGATTTAGGCTGGTATCAACAGAAGCCAGGGAAAGCCCCTAAAC TCGTGATCTATTCTGCATCCACTTTACAAAGTGGGGTCCCATCAAG GTTCAGCGGCAGTGGATCTGGCACAGATTTCACTCTCTCCATCAGC AGCCTGCAGCCTGACGATTTTGCAACTTATTACTGTCTACAAGATT ACAATTACCCGTGGACGTTCGGCCAAGGGACCAAGGTTGAAATCA AA SEQ ID NO: 239

[0245] In some embodiments, an isolated antibody or antigen-binding fragment thereof encoded by one or more of the polynucleotides described above, which specifically binds to *Pseudomonas* Psl and/or PcrV, comprises, consists essentially of, or consists of VH and VL amino acid sequences at least 80%, 85%, 90%, 95% or 100% identical to:

(a) SEQ ID NO: 1 and SEQ ID NO: 2, respectively, (b) SEQ ID NO: 3 and SEQ ID NO: 2, respectively, (c) SEQ ID NO: 4 and SEQ ID NO: 2 , respectively, (d) SEQ ID NO: 5 and SEQ ID NO: 6 , respectively, (e) SEQ ID NO: 7 and SEQ ID NO: 8, respectively, (f) SEQ ID NO: 9 and SEQ ID NO: 10, respectively, (g) SEQ ID NO: 11 and SEQ ID NO: 12 , respectively, (h) SEQ ID NO: 13 and SEQ ID NO: 14, respectively; (i) SEQ ID NO: 15 and SEQ ID NO: 16, respectively; or (j) SEQ ID NO: 74 and SEQ ID NO: 12 , respectively.

[0246] In certain embodiments, an isolated binding molecule, *e.g.*, an antibody or antigen-binding fragment thereof encoded by one or more of the polynucleotides described above, specifically binds to *Pseudomonas* Psl and/or PcrV with an affinity characterized by a dissociation constant (K_D) no greater than 5×10^{-2} M, 10^{-2} M, 5×10^{-3} M, 10^{-3} M, 5×10^{-4} M, 10^{-4} M, 5×10^{-5} M, 10^{-5} M, 5×10^{-6} M, 10^{-6} M, 5×10^{-7} M, 10^{-7} M, 5×10^{-8} M, 10^{-8} M, 5×10^{-9} M, 10^{-9} M, 5×10^{-10} M, 10^{-10} M, 5×10^{-11} M, 10^{-11} M, 5×10^{-12} M, 10^{-12} M, 5×10^{-13} M, 10^{-13} M, 5×10^{-14} M, 10^{-14} M, 5×10^{-15} M, or 10^{-15} M.

[0247] In specific embodiments, an isolated binding molecule, *e.g.*, an antibody or antigen-binding fragment thereof encoded by one or more of the polynucleotides described above, specifically binds to *Pseudomonas* Psl and/or PcrV, with an affinity characterized by a dissociation constant (K_D) in a range of about 1×10^{-10} to about 1×10^{-6} M. In one embodiment, an isolated binding molecule, *e.g.*, an antibody or antigen-binding fragment thereof encoded by one or more of the polynucleotides described above, specifically binds to *Pseudomonas* Psl and/or PcrV, with an affinity characterized by a K_D of about 1.18×10^{-7} M, as determined by the OCTET® binding assay described herein. In

another embodiment, an isolated binding molecule, *e.g.*, an antibody or antigen-binding fragment thereof encoded by one or more of the polynucleotides described above, specifically binds to *Pseudomonas* Psl and/or PcrV, with an affinity characterized by a K_D of about 1.44 x 10^{-7} M, as determined by the OCTET® binding assay described herein.

[0248] In certain embodiments, an anti-*Pseudomonas* Psl and/or PcrV binding molecule, *e.g.*, antibody or fragment, variant or derivative thereof encoded by one or more of the polynucleotides described above, specifically binds to the same Ps1 epitope as monoclonal antibody WapR-004, WapR-004RAD, Cam-003, Cam-004, or Cam-005, or will competitively inhibit such a monoclonal antibody from binding to *Pseudomonas* Psl; and/or specifically binds to the same PcrV epitope as monoclonal antibody V2L2, or will competitively inhibit such a monoclonal antibody from binding to *Pseudomonas* PcrV. WapR-004RAD is identical to WapR-004 except for a nucleic acid substitution G293C of the VH nucleic acid sequence encoding the VH amino acid sequence of SEQ ID NO:11 (a substitution of the nucleotide in the VH-encoding portion of SEQ ID NO:71 at position 317). The nucleic acid sequence encoding the WapR-004RAD VH is presented as SEQ ID NO 76.

[0249] Some embodiments provide an isolated polynucleotide comprising, consisting essentially of, or consisting of a nucleic acid encoding a W4 mutant scFv-Fc molecule amino acid sequence identical to, or identical except for one, two, three, four, five, or more amino acid substitutions to one or more of: SEQ ID NO: 78, SEQ ID NO: 79, SEQ ID NO: 80, SEQ ID NO: 81, SEQ ID NO: 82, SEQ ID NO: 83, SEQ ID NO: 84, SEQ ID NO: 85, SEQ ID NO: 86, SEQ ID NO: 87, SEQ ID NO: 88, SEQ ID NO: 89, SEQ ID NO: 90, SEQ ID NO: 91, SEQ ID NO: 92, SEQ ID NO: 93, SEQ ID NO: 94, SEQ ID NO: 95, SEQ ID NO: 96, SEQ ID NO: 97, SEQ ID NO: 98, SEQ ID NO: 99, SEQ ID NO: 100, SEQ ID NO: 101, SEQ ID NO: 102, SEQ ID NO: 103, SEQ ID NO: 104, SEQ ID NO: 105, SEQ ID NO: 106, SEQ ID NO: 107, SEQ ID NO: 108, SEQ ID NO: 109, SEQ ID NO: 110, SEQ ID NO: 111, SEQ ID NO: 112, SEQ ID NO: 113, SEQ ID NO: 114, SEQ ID NO: 115, SEQ ID NO: 116, SEQ ID NO: 117, SEQ ID NO: 118, SEQ ID NO: 119, SEQ ID NO: 120, SEQ ID NO: 121, SEQ ID NO: 122, SEQ ID NO: 123, SEQ ID NO: 124, SEQ ID NO: 125, SEQ ID NO: 126, SEQ ID NO: 127, SEQ ID NO: 128, SEQ ID NO: 129, SEQ ID NO: 130, SEQ ID NO: 131, SEQ ID NO: 132, SEQ ID NO: 133, SEQ ID NO: 134, SEQ ID NO: 135, SEQ ID NO: 136, SEQ ID NO: 137, SEQ ID

NO: 138, SEQ ID NO: 139, SEQ ID NO: 140, SEQ ID NO: 141, SEQ ID NO: 142, SEQ ID NO: 143, SEQ ID NO: 144, SEQ ID NO: 145; or SEQ ID NO: 146.

[0250] Other embodiments provide an isolated polynucleotide comprising, consisting essentially of, or consisting of a nucleic acid encoding a W4 mutant scFv-Fc molecule amino acid sequence at least 80%, 85%, 90% 95% or 100% identical to one or more of: SEQ ID NO: 78, SEQ ID NO: 79, SEQ ID NO: 80, SEQ ID NO: 81, SEQ ID NO: 82, SEQ ID NO: 83, SEQ ID NO: 84, SEQ ID NO: 85, SEQ ID NO: 86, SEQ ID NO: 87, SEQ ID NO: 88, SEQ ID NO: 89, SEQ ID NO: 90, SEQ ID NO: 91, SEQ ID NO: 92, SEQ ID NO: 93, SEQ ID NO: 94, SEQ ID NO: 95, SEQ ID NO: 96, SEQ ID NO: 97, SEQ ID NO: 98, SEQ ID NO: 99, SEQ ID NO: 100, SEQ ID NO: 101, SEQ ID NO: 102, SEQ ID NO: 103, SEQ ID NO: 104, SEQ ID NO: 105, SEQ ID NO: 106, SEQ ID NO: 107, SEQ ID NO: 108, SEQ ID NO: 109, SEQ ID NO: 110, SEQ ID NO: 111, SEQ ID NO: 112, SEQ ID NO: 113, SEQ ID NO: 114, SEQ ID NO: 115, SEQ ID NO: 116, SEQ ID NO: 117, SEQ ID NO: 118, SEQ ID NO: 119, SEQ ID NO: 120, SEQ ID NO: 121, SEQ ID NO: 122, SEQ ID NO: 123, SEQ ID NO: 124, SEQ ID NO: 125, SEQ ID NO: 126, SEQ ID NO: 127, SEQ ID NO: 128, SEQ ID NO: 129, SEQ ID NO: 130, SEQ ID NO: 131, SEQ ID NO: 132, SEQ ID NO: 133, SEQ ID NO: 134, SEQ ID NO: 135, SEQ ID NO: 136, SEQ ID NO: 137, SEQ ID NO: 138, SEQ ID NO: 139, SEQ ID NO: 140, SEQ ID NO: 141, SEQ ID NO: 142, SEQ ID NO: 143, SEQ ID NO: 144, SEQ ID NO: 145; or SEQ ID NO: 146.

[0251] One embodiment provides an isolated polynucleotide comprising, consisting essentially of, or consisting of a nucleic acid which encodes a W4 mutant scFv-Fc molecule, where the nucleic acid is at least 80%, 85%, 90% 95% or 100% identical to one or more of SEQ ID NO: 147, SEQ ID NO: 148, SEQ ID NO: 149, SEQ ID NO: 150, SEQ ID NO: 151, or SEQ ID NO: 152, SEQ IS NO: 153, SEQ ID NO: 154, SEQ ID NO: 155, SEQ ID NO: 156, SEQ ID NO: 157, SEQ ID NO: 158, SEQ ID NO: 159, SEQ ID NO: 160, SEQ ID NO: 161, SEQ ID NO: 162, SEQ ID NO: 163, SEQ ID NO: 164, SEQ ID NO: 165, SEQ ID NO: 166, SEQ ID NO: 167, SEQ ID NO: 168, SEQ ID NO: 169, SEQ ID NO: 170, SEQ ID NO: 171, SEQ ID NO: 172, SEQ ID NO: 173, SEQ ID NO: 174, SEQ ID NO: 175, SEQ ID NO: 176, SEQ ID NO: 177, SEQ ID NO: 178, SEQ ID NO: 179, SEQ ID NO: 180, SEQ ID NO: 181, SEQ ID NO: 182, SEQ ID NO: 183, SEQ ID NO: 184, SEQ ID NO: 185, SEQ ID NO: 186, SEQ ID NO: 187, SEQ ID NO: 188, SEQ

ID NO: 189, SEQ ID NO: 190, SEQ ID NO: 191, SEQ ID NO: 192, SEQ ID NO: 193, SEQ ID NO: 194, SEQ ID NO: 195, SEQ ID NO: 196, SEQ ID NO: 197, SEQ ID NO: 198, SEQ ID NO: 199, SEQ ID NO: 200, SEQ ID NO: 201, SEQ ID NO: 202, SEQ ID NO: 203, SEQ ID NO: 204, SEQ ID NO: 205, SEQ ID NO: 206, SEQ ID NO: 207, SEQ ID NO: 208, SEQ ID NO: 209, SEQ ID NO: 210, SEQ ID NO: 211, SEQ ID NO: 212, SEQ ID NO: 213, SEQ ID NO: 214; or SEQ ID NO: 215.

[0252] One embodiment provides an isolated polynucleotide comprising, consisting essentially of, or consisting of a nucleic acid which encodes a V2L2 polypeptide, where the nucleic acid is at least 80%, 85%, 90% 95% or 100% identical to one or more of SEQ ID NO: 238 or SEQ ID NO: 239.

[0253] In other embodiments, an anti-*Pseudomonas* Psl and/or PcrV binding molecule, *e.g.*, antibody or fragment, variant or derivative thereof encoded by one or more of the polynucleotides described above, specifically binds to the same epitope as monoclonal antibody WapR-001, WapR-002, or WapR-003, or will competitively inhibit such a monoclonal antibody from binding to *Pseudomonas* Psl.

[0254] In certain embodiments, an anti-*Pseudomonas* Psl and/or PcrV binding molecule, *e.g.*, antibody or fragment, variant or derivative thereof encoded by one or more of the polynucleotides described above, specifically binds to the same epitope as monoclonal antibody WapR-016, or will competitively inhibit such a monoclonal antibody from binding to *Pseudomonas* Psl.

[0255] The disclosure also includes fragments of the polynucleotides as described elsewhere herein. Additionally polynucleotides which encode fusion polynucleotides, Fab fragments, and other derivatives, as described herein, are also provided.

[0256] The polynucleotides can be produced or manufactured by any method known in the art. For example, if the nucleotide sequence of the antibody is known, a polynucleotide encoding the antibody can be assembled from chemically synthesized oligonucleotides (*e.g.*, as described in Kutmeier *et al.*, BioTechniques *17*:242 (1994)), which, briefly, involves the synthesis of overlapping oligonucleotides containing portions of the sequence encoding the antibody, annealing and ligating of those oligonucleotides, and then amplification of the ligated oligonucleotides by PCR.

[0257] Alternatively, a polynucleotide encoding an anti-*Pseudomonas* Psl and/or PcrV binding molecule, *e.g.*, antibody or fragment, variant or derivative thereof can be

generated from nucleic acid from a suitable source. If a clone containing a nucleic acid encoding a particular antibody is not available, but the sequence of the antibody molecule is known, a nucleic acid encoding the antibody can be chemically synthesized or obtained from a suitable source (*e.g.*, an antibody cDNA library, or a cDNA library generated from, or nucleic acid, preferably poly A+RNA, isolated from, any tissue or cells expressing the antibody or such as hybridoma cells selected to express an antibody) by PCR amplification using synthetic primers hybridizable to the 3' and 5' ends of the sequence or by cloning using an oligonucleotide probe specific for the particular gene sequence to identify, *e.g.*, a cDNA clone from a cDNA library that encodes the antibody. Amplified nucleic acids generated by PCR can then be cloned into replicable cloning vectors using any method well known in the art.

[0258] Once the nucleotide sequence and corresponding amino acid sequence of an anti-*Pseudomonas* Psl and/or PcrV binding molecule, *e.g.*, antibody or fragment, variant or derivative thereof is determined, its nucleotide sequence can be manipulated using methods well known in the art for the manipulation of nucleotide sequences, *e.g.*, recombinant DNA techniques, site directed mutagenesis, PCR, etc. (see, for example, the techniques described in Sambrook *et* al., *Molecular Cloning, A Laboratory Manual*, 2d Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y. (1990) and Ausubel *et al.*, eds., *Current Protocols in Molecular Biology*, John Wiley & Sons, NY (1998), which are both incorporated by reference herein in their entireties), to generate antibodies having a different amino acid sequence, for example to create amino acid substitutions, deletions, and/or insertions.

[0259] A polynucleotide encoding an anti-*Pseudomonas* Psl and/or PcrV binding molecule, *e.g.*, antibody or fragment, variant or derivative thereof can be composed of any polyribonucleotide or polydeoxribonucleotide, which can be unmodified RNA or DNA or modified RNA or DNA. For example, a polynucleotide encoding an anti-*Pseudomonas* Psl and/or PcrV binding molecule, *e.g.*, antibody or fragment, variant or derivative thereof can be composed of single- and double-stranded DNA, DNA that is a mixture of single- and double-stranded regions, single- and double-stranded RNA, and RNA that is mixture of single- and double-stranded regions, hybrid molecules comprising DNA and RNA that can be single-stranded or, more typically, double-stranded or a mixture of single- and double-stranded regions. In addition, a polynucleotide encoding an

anti-*Pseudomonas* Psl and/or PcrV binding molecule, *e.g.*, antibody or fragment, variant or derivative thereof can be composed of triple-stranded regions comprising RNA or DNA or both RNA and DNA. A polynucleotide encoding an anti-*Pseudomonas* Psl and/or PcrV binding molecule, *e.g.*, antibody or fragment, variant or derivative thereof can also contain one or more modified bases or DNA or RNA backbones modified for stability or for other reasons. "Modified" bases include, for example, tritylated bases and unusual bases such as inosine. A variety of modifications can be made to DNA and RNA; thus, "polynucleotide" embraces chemically, enzymatically, or metabolically modified forms.

[0260] An isolated polynucleotide encoding a non-natural variant of a polypeptide derived from an immunoglobulin (*e.g.*, an immunoglobulin heavy chain portion or light chain portion) can be created by introducing one or more nucleotide substitutions, additions or deletions into the nucleotide sequence of the immunoglobulin such that one or more amino acid substitutions, additions or deletions are introduced into the encoded protein. Mutations can be introduced by standard techniques, such as site-directed mutagenesis and PCR-mediated mutagenesis. Conservative amino acid substitutions are made at one or more non-essential amino acid residues.

VI. EXPRESSION OF ANTIBODY POLYPEPTIDES

[0261] As is well known, RNA can be isolated from the original hybridoma cells or from other transformed cells by standard techniques, such as guanidinium isothiocyanate extraction and precipitation followed by centrifugation or chromatography. Where desirable, mRNA can be isolated from total RNA by standard techniques such as chromatography on oligo dT cellulose. Suitable techniques are familiar in the art.

[0262] In one embodiment, cDNAs that encode the light and the heavy chains of the anti-*Pseudomonas* Psl and/or PcrV binding molecule, *e.g.*, antibody or fragment, variant or derivative thereof can be made, either simultaneously or separately, using reverse transcriptase and DNA polymerase in accordance with well-known methods. PCR can be initiated by consensus constant region primers or by more specific primers based on the published heavy and light chain DNA and amino acid sequences. As discussed above, PCR also can be used to isolate DNA clones encoding the antibody light and heavy

chains. In this case the libraries can be screened by consensus primers or larger homologous probes, such as mouse constant region probes.

[0263] DNA, typically plasmid DNA, can be isolated from the cells using techniques known in the art, restriction mapped and sequenced in accordance with standard, well known techniques set forth in detail, *e.g.*, in the foregoing references relating to recombinant DNA techniques. Of course, the DNA can be synthetic according to the present disclosure at any point during the isolation process or subsequent analysis.

[0264] Following manipulation of the isolated genetic material to provide an anti-*Pseudomonas* Psl and/or PcrV binding molecule, *e.g.*, antibody or fragment, variant or derivative thereof of the disclosure, the polynucleotides encoding anti-*Pseudomonas* Psl and/or PcrV binding molecules, are typically inserted in an expression vector for introduction into host cells that can be used to produce the desired quantity of anti-*Pseudomonas* Psl and/or PcrV binding molecules.

[0265] Recombinant expression of an antibody, or fragment, derivative or analog thereof, *e.g.*, a heavy or light chain of an antibody which binds to a target molecule described herein, *e.g.*, Psl and/or PcrV, requires construction of an expression vector containing a polynucleotide that encodes the antibody. Once a polynucleotide encoding an antibody molecule or a heavy or light chain of an antibody, or portion thereof (containing the heavy or light chain variable domain), of the disclosure has been obtained, the vector for the production of the antibody molecule can be produced by recombinant DNA technology using techniques well known in the art. Thus, methods for preparing a protein by expressing a polynucleotide containing an antibody encoding nucleotide sequence are described herein. Methods which are well known to those skilled in the art can be used to construct expression vectors containing antibody coding sequences and appropriate transcriptional and translational control signals. These methods include, for example, *in vitro* recombinant DNA techniques, synthetic techniques, and *in vivo* genetic recombination. The disclosure, thus, provides replicable vectors comprising a nucleotide sequence encoding an antibody molecule of the disclosure, or a heavy or light chain thereof, or a heavy or light chain variable domain, operably linked to a promoter. Such vectors can include the nucleotide sequence encoding the constant region of the antibody molecule (see, *e.g.*, PCT Publication WO 86/05807; PCT Publication WO 89/01036; and

U.S. Pat. No. 5,122,464) and the variable domain of the antibody can be cloned into such a vector for expression of the entire heavy or light chain.

[0266] The term "vector" or "expression vector" is used herein to mean vectors used in accordance with the present disclosure as a vehicle for introducing into and expressing a desired gene in a host cell. As known to those skilled in the art, such vectors can easily be selected from the group consisting of plasmids, phages, viruses and retroviruses. In general, vectors compatible with the instant disclosure will comprise a selection marker, appropriate restriction sites to facilitate cloning of the desired gene and the ability to enter and/or replicate in eukaryotic or prokaryotic cells.

[0267] For the purposes of this disclosure, numerous expression vector systems can be employed. For example, one class of vector utilizes DNA elements which are derived from animal viruses such as bovine papilloma virus, polyoma virus, adenovirus, vaccinia virus, baculovirus, retroviruses (RSV, MMTV or MOMLV) or SV40 virus. Others involve the use of polycistronic systems with internal ribosome binding sites. Additionally, cells which have integrated the DNA into their chromosomes can be selected by introducing one or more markers which allow selection of transfected host cells. The marker can provide for prototrophy to an auxotrophic host, biocide resistance (*e.g.*, antibiotics) or resistance to heavy metals such as copper. The selectable marker gene can either be directly linked to the DNA sequences to be expressed, or introduced into the same cell by cotransformation. Additional elements can also be needed for optimal synthesis of mRNA. These elements can include signal sequences, splice signals, as well as transcriptional promoters, enhancers, and termination signals.

[0268] In some embodiments the cloned variable region genes are inserted into an expression vector along with the heavy and light chain constant region genes (*e.g.*, human) synthetic as discussed above. Of course, any expression vector which is capable of eliciting expression in eukaryotic cells can be used in the present disclosure. Examples of suitable vectors include, but are not limited to plasmids pcDNA3, pHCMV/Zeo, pCR3.1, pEF1/His, pIND/GS, pRc/HCMV2, pSV40/Zeo2, pTRACER-HCMV, pUB6/V5-His, pVAX1, and pZeoSV2 (available from Invitrogen, San Diego, CA), and plasmid pCI (available from Promega, Madison, WI). In general, screening large numbers of transformed cells for those which express suitably high levels if

immunoglobulin heavy and light chains is routine experimentation which can be carried out, for example, by robotic systems.

[0269] More generally, once the vector or DNA sequence encoding a monomeric subunit of the anti-*Pseudomonas* Psl and/or PcrV binding molecule, *e.g.*, antibody or fragment, variant or derivative thereof of the disclosure has been prepared, the expression vector can be introduced into an appropriate host cell. Introduction of the plasmid into the host cell can be accomplished by various techniques well known to those of skill in the art. These include, but are not limited to, transfection (including electrophoresis and electroporation), protoplast fusion, calcium phosphate precipitation, cell fusion with enveloped DNA, microinjection, and infection with intact virus. *See*, Ridgway, A. A. G. "*Mammalian Expression Vectors*" Vectors, Rodriguez and Denhardt, Eds., Butterworths, Boston, Mass., Chapter 24.2, pp. 470-472 (1988). Typically, plasmid introduction into the host is via electroporation. The host cells harboring the expression construct are grown under conditions appropriate to the production of the light chains and heavy chains, and assayed for heavy and/or light chain protein synthesis. Exemplary assay techniques include enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA), or fluorescence-activated cell sorter analysis (FACS), immunohistochemistry and the like.

[0270] The expression vector is transferred to a host cell by conventional techniques and the transfected cells are then cultured by conventional techniques to produce an antibody for use in the methods described herein. Thus, the disclosure includes host cells containing a polynucleotide encoding an anti-*Pseudomonas* Psl and/or PcrV binding molecule, *e.g.*, antibody or fragment, variant or derivative thereof, or a heavy or light chain thereof, operably linked to a heterologous promoter. In some embodiments for the expression of double-chained antibodies, vectors encoding both the heavy and light chains can be co-expressed in the host cell for expression of the entire immunoglobulin molecule, as detailed below.

[0271] Certain embodiments include an isolated polynucleotide comprising a nucleic acid which encodes the above-described VH and VL, wherein a binding molecule or antigen-binding fragment thereof expressed by the polynucleotide specifically binds *Pseudomonas* Psl and/or PcrV. In some embodiments the polynucleotide as described encodes an scFv molecule including VH and VL, at least 80%, 85%, 90% 95% or 100%

identical to one or more of SEQ ID NO: 65, SEQ ID NO: 66, SEQ ID NO: 67, SEQ ID NO: 68, SEQ ID NO: 69, or SEQ ID NO: 70 as shown in Table 4.

[0272] Some embodiments include vectors comprising the above-described polynucleotides. In further embodiments, the polynucleotides are operably associated with a promoter. In additional embodiments, the disclosure provides host cells comprising such vectors. In further embodiments, the disclosure provides vectors where the polynucleotide is operably associated with a promoter, wherein vectors can express a binding molecule which specifically binds *Pseudomonas* Psl and/or PcrV in a suitable host cell.

[0273] Also provided is a method of producing a binding molecule or fragment thereof which specifically binds *Pseudomonas* Psl and/or PcrV, comprising culturing a host cell containing a vector comprising the above-described polynucleotides, and recovering said antibody, or fragment thereof. In further embodiments, the disclosure provides an isolated binding molecule or fragment thereof produced by the above-described method.

[0274] As used herein, "host cells" refers to cells which harbor vectors constructed using recombinant DNA techniques and encoding at least one heterologous gene. In descriptions of processes for isolation of antibodies from recombinant hosts, the terms "cell" and "cell culture" are used interchangeably to denote the source of antibody unless it is clearly specified otherwise. In other words, recovery of polypeptide from the "cells" can mean either from spun down whole cells, or from the cell culture containing both the medium and the suspended cells.

[0275] A variety of host-expression vector systems can be utilized to express antibody molecules for use in the methods described herein. Such host-expression systems represent vehicles by which the coding sequences of interest can be produced and subsequently purified, but also represent cells which can, when transformed or transfected with the appropriate nucleotide coding sequences, express an antibody molecule of the disclosure *in situ*. These include but are not limited to microorganisms such as bacteria (*e.g.*, *E. coli*, *B. subtilis*) transformed with recombinant bacteriophage DNA, plasmid DNA or cosmid DNA expression vectors containing antibody coding sequences; yeast (*e.g.*, *Saccharomyces*, *Pichia*) transformed with recombinant yeast expression vectors containing antibody coding sequences; insect cell systems infected with recombinant virus expression vectors (*e.g.*, baculovirus) containing antibody coding sequences; plant

cell systems infected with recombinant virus expression vectors (*e.g.*, cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or transformed with recombinant plasmid expression vectors (*e.g.*, Ti plasmid) containing antibody coding sequences; or mammalian cell systems (*e.g.*, COS, CHO, BLK, 293, 3T3 cells) harboring recombinant expression constructs containing promoters derived from the genome of mammalian cells (*e.g.*, metallothionein promoter) or from mammalian viruses (*e.g.*, the adenovirus late promoter; the vaccinia virus 7.5K promoter). Bacterial cells such as *Escherichia coli*, or eukaryotic cells, especially for the expression of whole recombinant antibody molecule, are used for the expression of a recombinant antibody molecule. For example, mammalian cells such as Chinese hamster ovary cells (CHO), in conjunction with a vector such as the major intermediate early gene promoter element from human cytomegalovirus is an effective expression system for antibodies (Foecking *et al.*, *Gene 45*:101 (1986); Cockett *et al.*, *Bio/Technology 8*:2 (1990)).

[0276] The host cell line used for protein expression is often of mammalian origin; those skilled in the art are credited with ability to determine particular host cell lines which are best suited for the desired gene product to be expressed therein. Exemplary host cell lines include, but are not limited to, CHO (Chinese Hamster Ovary), DG44 and DUXB11 (Chinese Hamster Ovary lines, DHFR minus), HELA (human cervical carcinoma), CVI (monkey kidney line), COS (a derivative of CVI with SV40 T antigen), VERY, BHK (baby hamster kidney), MDCK, 293, WI38, R1610 (Chinese hamster fibroblast) BALBC/3T3 (mouse fibroblast), HAK (hamster kidney line), SP2/O (mouse myeloma), P3x63-Ag3.653 (mouse myeloma), BFA-1c1BPT (bovine endothelial cells), RAJI (human lymphocyte) and 293 (human kidney). Host cell lines are typically available from commercial services, the American Tissue Culture Collection or from published literature.

[0277] In addition, a host cell strain can be chosen which modulates the expression of the inserted sequences, or modifies and processes the gene product in the specific fashion desired. Such modifications (*e.g.*, glycosylation) and processing (*e.g.*, cleavage) of protein products can be important for the function of the protein. Different host cells have characteristic and specific mechanisms for the post-translational processing and modification of proteins and gene products. Appropriate cell lines or host systems can be chosen to ensure the correct modification and processing of the foreign protein expressed.

To this end, eukaryotic host cells which possess the cellular machinery for proper processing of the primary transcript, glycosylation, and phosphorylation of the gene product can be used.

[0278] For long-term, high-yield production of recombinant proteins, stable expression is preferred. For example, cell lines which stably express the antibody molecule can be engineered. Rather than using expression vectors which contain viral origins of replication, host cells can be transformed with DNA controlled by appropriate expression control elements (*e.g.*, promoter, enhancer, sequences, transcription terminators, polyadenylation sites, etc.), and a selectable marker. Following the introduction of the foreign DNA, engineered cells can be allowed to grow for 1-2 days in an enriched media, and then are switched to a selective media. The selectable marker in the recombinant plasmid confers resistance to the selection and allows cells to stably integrate the plasmid into their chromosomes and grow to form foci which in turn can be cloned and expanded into cell lines. This method can advantageously be used to engineer cell lines which stably express the antibody molecule.

[0279] A number of selection systems can be used, including but not limited to the herpes simplex virus thymidine kinase (Wigler *et al.*, *Cell 11*:223 (1977)), hypoxanthine-guanine phosphoribosyltransferase (Szybalska & Szybalski, *Proc. Natl. Acad. Sci. USA 48*:202 (1992)), and adenine phosphoribosyltransferase (Lowy *et al.*, *Cell 22*:817 1980) genes can be employed in tk-, hgprt- or aprt-cells, respectively. Also, antimetabolite resistance can be used as the basis of selection for the following genes: dhfr, which confers resistance to methotrexate (Wigler *et al.*, *Natl. Acad. Sci. USA 77*:357 (1980); O'Hare *et al.*, *Proc. Natl. Acad. Sci. USA 78*:1527 (1981)); gpt, which confers resistance to mycophenolic acid (Mulligan & Berg, *Proc. Natl. Acad. Sci. USA 78*:2072 (1981)); neo, which confers resistance to the aminoglycoside G-418 *Clinical Pharmacy 12*:488-505; Wu and Wu, *Biotherapy 3*:87-95 (1991); Tolstoshev, *Ann. Rev. Pharmacol. Toxicol. 32*:573-596 (1993); Mulligan, *Science 260*:926-932 (1993); and Morgan and Anderson, *Ann. Rev. Biochem. 62*:191-217 (1993);, *TIB TECH 11(5)*:155-215 (May, 1993); and hygro, which confers resistance to hygromycin (Santerre *et al.*, *Gene 30*:147 (1984). Methods commonly known in the art of recombinant DNA technology which can be used are described in Ausubel *et al.* (eds.), *Current Protocols in Molecular Biology*, John Wiley & Sons, NY (1993); Kriegler, *Gene Transfer and Expression*, A Laboratory

Manual, Stockton Press, NY (1990); and in Chapters 12 and 13, Dracopoli *et al.* (eds), *Current Protocols in Human Genetics*, John Wiley & Sons, NY (1994); Colberre-Garapin *et al.*, *J. Mol. Biol. 150*:1 (1981), which are incorporated by reference herein in their entireties.

[0280] The expression levels of an antibody molecule can be increased by vector amplification (for a review, *see* Bebbington and Hentschel, *The use of* vectors *based on gene amplification for the expression of cloned genes in mammalian cells in DNA cloning*, Academic Press, New York, Vol. 3. (1987)). When a marker in the vector system expressing antibody is amplifiable, increase in the level of inhibitor present in culture of host cell will increase the number of copies of the marker gene. Since the amplified region is associated with the antibody gene, production of the antibody will also increase (Crouse *et al.*, *Mol. Cell. Biol. 3*:257 (1983)).

[0281] *In vitro* production allows scale-up to give large amounts of the desired polypeptides. Techniques for mammalian cell cultivation under tissue culture conditions are known in the art and include homogeneous suspension culture, *e.g.* in an airlift reactor or in a continuous stirrer reactor, or immobilized or entrapped cell culture, *e.g.* in hollow fibers, microcapsules, on agarose microbeads or ceramic cartridges. If necessary and/or desired, the solutions of polypeptides can be purified by the customary chromatography methods, for example gel filtration, ion-exchange chromatography, chromatography over DEAE-cellulose or (immuno-)affinity chromatography, *e.g.*, after preferential biosynthesis of a synthetic hinge region polypeptide or prior to or subsequent to the HIC chromatography step described herein.

[0282] Constructs encoding anti-*Pseudomonas* Psl and/or PcrV binding molecules, *e.g.*, antibodies or fragments, variants or derivatives thereof, as disclosed herein can also be expressed non-mammalian cells such as bacteria or yeast or plant cells. Bacteria which readily take up nucleic acids include members of the enterobacteriaceae, such as strains of *Escherichia coli* or *Salmonella*; Bacillaceae, such as *Bacillus subtilis*; *Pneumococcus*; *Streptococcus*, and *Haemophilus influenzae*. It will further be appreciated that, when expressed in bacteria, the heterologous polypeptides typically become part of inclusion bodies. The heterologouspolypeptides must be isolated, purified and then assembled into functional molecules. Where tetravalent forms of antibodies are desired, the subunits will then self-assemble into tetravalent antibodies (WO02/096948A2).

[0283] In bacterial systems, a number of expression vectors can be advantageously selected depending upon the use intended for the antibody molecule being expressed. For example, when a large quantity of such a protein is to be produced, for the generation of pharmaceutical compositions of an antibody molecule, vectors which direct the expression of high levels of fusion protein products that are readily purified can be desirable. Such vectors include, but are not limited, to the *E. coli* expression vector pUR278 (Ruther *et al.*, *EMBO J. 2*:1791 (1983)), in which the antibody coding sequence can be ligated individually into the vector in frame with the lacZ coding region so that a fusion protein is produced; pIN vectors (Inouye & Inouye, *Nucleic Acids Res. 13*:3101-3109 (1985); Van Heeke & Schuster, *J. Biol. Chem. 24*:5503-5509 (1989)); and the like. pGEX vectors can also be used to express foreign polypeptides as fusion proteins with glutathione S-transferase (GST). In general, such fusion proteins are soluble and can easily be purified from lysed cells by adsorption and binding to a matrix glutathione-agarose beads followed by elution in the presence of free glutathione. The pGEX vectors are designed to include thrombin or factor Xa protease cleavage sites so that the cloned target gene product can be released from the GST moiety.

[0284] In addition to prokaryotes, eukaryotic microbes can also be used. *Saccharomyces cerevisiae*, or common baker's yeast, is the most commonly used among eukaryotic microorganisms although a number of other strains are commonly available, *e.g.*, *Pichia pastoris*.

[0285] For expression in *Saccharomyces*, the plasmid YRp7, for example, (Stinchcomb *et al.*, Nature *282*:39 (1979); Kingsman *et al.*, *Gene 7*:141 (1979); Tschemper *et al.*, *Gene 10*:157 (1980)) is commonly used. This plasmid already contains the TRP1 gene which provides a selection marker for a mutant strain of yeast lacking the ability to grow in tryptophan, for example ATCC No. 44076 or PEP4-1 (Jones, *Genetics 85*:12 (1977)). The presence of the trpl lesion as a characteristic of the yeast host cell genome then provides an effective environment for detecting transformation by growth in the absence of tryptophan.

[0286] In an insect system, *Autographa californica* nuclear polyhedrosis virus (AcNPV) is typically used as a vector to express foreign genes. The virus grows in *Spodoptera frugiperda* cells. The antibody coding sequence can be cloned individually into non-

essential regions (for example the polyhedrin gene) of the virus and placed under control of an AcNPV promoter (for example the polyhedrin promoter).

[0287] Once the anti-*Pseudomonas* Psl and/or PcrV binding molecule, *e.g.*, antibody or fragment, variant or derivative thereof, as disclosed herein has been recombinantly expressed, it can be purified by any method known in the art for purification of an immunoglobulin molecule, for example, by chromatography (*e.g.*, ion exchange, affinity, particularly by affinity for the specific antigen after Protein A, and sizing column chromatography), centrifugation, differential solubility, or by any other standard technique for the purification of proteins. Another method for increasing the affinity of antibodies of the disclosure is disclosed in US 2002 0123057 A1.

VII. IDENTIFICATION OF SEROTYPE-INDIFFERENT BINDING MOLECULES

[0288] The disclosure encompasses a target indifferent whole-cell approach to identify serotype independent therapeutic binding molecules *e.g.*, antibodies or fragments thereof with superior or desired therapeutic activities. The method can be utilized to identify binding molecules which can antagonize, neutralize, clear, or block an undesired activity of an infectious agent, *e.g.*, a bacterial pathogen. As is known in the art, many infectious agents exhibit significant variation in their dominant surface antigens, allowing them to evade immune surveillance. The identification method described herein can identify binding molecules which target antigens which are shared among many different *Pseudomonas* species or other Gram-negative pathogens, thus providing a therapeutic agent which can target multiple pathogens from multiple species. For example, the method was utilized to identify a series of binding molecules which bind to the surface of *P. aeruginosa* in a serotype-independent manner, and when bound to bacterial pathogens, mediate, promote, or enhance opsonophagocytic (OPK) activity against bacterial cells such as bacterial pathogens, *e.g.* opportunistic *Pseudomonas* species (*e.g.*, *Pseudomonas aeruginosa*, *Pseudomonas fluorescens, Pseudomonas putida, and Pseudomonas alcaligenes*) and/or inhibit the attachment of such bacterial cells to epithelial cells.

[0289] Certain embodiments disclose a method of identifying serotype-indifferent binding molecules comprising: (a) preparing naïve and/or convalescent antibody libraries in phage, (b) removing serotype-specific antibodies from the library by depletion panning, (c) screening the library for antibodies that specifically bind to whole cells

independent of serotype, and (d) screening of the resulting antibodies for desired functional properties.

[0290] Certain embodiments provide a whole-cell phenotypic screening approach as disclosed herein with antibody phage libraries derived from either naive or *P. aeruginosa* infected convalescing patients. Using a panning strategy that initially selected against serotype-specific reactivity, different clones that bound *P. aeruginosa* whole cells were isolated. Selected clones were converted to human IgG1 antibodies and were confirmed to react with *P. aeruginosa* clinical isolates regardless of serotype classification or site of tissue isolation (See Examples). Functional activity screens described herein indicated that the antibodies were effective in preventing *P. aeruginosa* attachment to mammalian cells and mediated opsonophagocytic (OPK) killing in a concentration-dependent and serotype-independent manner.

[0291] In further embodiments, the above-described binding molecules or fragments thereof, antibodies or fragments thereof, or compositions, bind to two or more, three or more, four or more, or five or more different *P. aeruginosa* serotypes, or to at least 80%, at least 85%, at least 90% or at least 95% of *P. aeruginosa* strains isolated from infected patients. In further embodiments, the *P. aeruginosa* strains are isolated from one or more of lung, sputum, eye, pus, feces, urine, sinus, a wound, skin, blood, bone, or knee fluid.

VIII. PHARMACEUTICAL COMPOSITIONS COMPRISING ANTI- *PSEUDOMONAS* PSL AND/OR PCRV BINDING MOLECULES

[0292] The pharmaceutical compositions used in this disclosure comprise pharmaceutically acceptable carriers well known to those of ordinary skill in the art. Preparations for parenteral administration include sterile aqueous or non-aqueous solutions, suspensions, and emulsions. Certain pharmaceutical compositions as disclosed herein can be orally administered in an acceptable dosage form including, *e.g.*, capsules, tablets, aqueous suspensions or solutions. Certain pharmaceutical compositions also can be administered by nasal aerosol or inhalation. Preservatives and other additives can also be present such as for example, antimicrobials, antioxidants, chelating agents, and inert gases and the like. Suitable formulations for use in the therapeutic methods disclosed herein are described in Remington's Pharmaceutical Sciences, Mack Publishing Co., 16th ed. (1980).

[0293] The amount of an anti-*Pseudomonas* Psl and/or PcrV binding molecule, *e.g.*, antibody or fragment, variant or derivative thereof, that can be combined with the carrier materials to produce a single dosage form will vary depending upon the host treated and the particular mode of administration. Dosage regimens also can be adjusted to provide the optimum desired response (*e.g.*, a therapeutic or prophylactic response). The compositions can also comprise the anti-*Pseudomonas* Psl and/or PcrV binding molecules, *e.g.*, antibodies or fragments, variants or derivatives thereof dispersed in a biocompatible carrier material that functions as a suitable delivery or support system for the compounds.

IX. TREATMENT METHODS USING THERAPEUTIC BINDING MOLECULES

[0294] Methods of preparing and administering anti-*Pseudomonas* Psl and/or PcrV binding molecules, *e.g.*, an antibody or fragment, variant or derivative thereof, as disclosed herein to a subject in need thereof are well known to or are readily determined by those skilled in the art. The route of administration of the anti-*Pseudomonas* Psl and/or PcrV binding molecules, *e.g.*, antibody or fragment, variant or derivative thereof, can be, for example, oral, parenteral, by inhalation or topical. The term parenteral as used herein includes, *e.g.*, intravenous, intraarterial, intraperitoneal, intramuscular, or subcutaneous administration. A suitable form for administration would be a solution for injection, in particular for intravenous or intraarterial injection or drip. However, in other methods compatible with the teachings herein, an anti-*Pseudomonas* Psl and/or PcrV binding molecules, *e.g.*, antibody or fragment, variant or derivative thereof, as disclosed herein can be delivered directly to the site of the adverse cellular population *e.g.*, infection thereby increasing the exposure of the diseased tissue to the therapeutic agent. For example, an anti-*Pseudomonas* Psl and/or PcrV binding molecule can be directly administered to ocular tissue, burn injury, or lung tissue.

[0295] Anti-*Pseudomonas* Psl and/or PcrV binding molecules, *e.g.*, antibodies or fragments, variants or derivatives thereof, as disclosed herein can be administered in a pharmaceutically effective amount for the *in vivo* treatment of *Pseudomonas* infection. In this regard, it will be appreciated that the disclosed binding molecules will be formulated so as to facilitate administration and promote stability of the active agent. For the purposes of the instant application, a pharmaceutically effective amount shall be held to

mean an amount sufficient to achieve effective binding to a target and to achieve a benefit, *e.g.*, treat, ameliorate, lessen, clear, or prevent *Pseudomonas* infection.

[0296] Some embodiments are directed to a method of preventing or treating a *Pseudomonas* infection in a subject in need thereof, comprising administering to the subject an effective amount of the binding molecule or fragment thereof, the antibody or fragment thereof, the composition, the polynucleotide, the vector, or the host cell described herein. In further embodiments, the *Pseudomonas* infection is a *P. aeruginosa* infection. In some embodiments, the subject is a human. In certain embodiments, the infection is an ocular infection, a lung infection, a burn infection, a wound infection, a skin infection, a blood infection, a bone infection, or a combination of two or more of said infections. In further embodiments, the subject suffers from acute pneumonia, burn injury, corneal infection, cystic fibrosis, or a combination thereof.

[0297] Certain embodiments are directed to a method of blocking or preventing attachment of *P. aeruginosa* to epithelial cells comprising contacting a mixture of epithelial cells and *P. aeruginosa* with the binding molecule or fragment thereof, the antibody or fragment thereof, the composition, the polynucleotide, the vector, or the host cell described herein.

[0298] Also disclosed is a method of enhancing OPK of *P. aeruginosa* comprising contacting a mixture of phagocytic cells and *P. aeruginosa* with the binding molecule or fragment thereof, the antibody or fragment thereof, the composition, the polynucleotide, the vector, or the host cell described herein. In further embodiments, the phagocytic cells are differentiated HL-60 cells or human polymorphonuclear leukocytes (PMNs).

[0299] In keeping with the scope of the disclosure, anti-*Pseudomonas* Psl and/or PcrV binding molecules, *e.g.*, antibodies or fragments, variants or derivatives thereof, can be administered to a human or other animal in accordance with the aforementioned methods of treatment in an amount sufficient to produce a therapeutic effect. The anti-*Pseudomonas* Psl and/or PcrV binding molecules, *e.g.*, antibodies or fragments, variants or derivatives thereof, disclosed herein can be administered to such human or other animal in a conventional dosage form prepared by combining the antibody of the disclosure with a conventional pharmaceutically acceptable carrier or diluent according to known techniques.

[0300] Effective doses of the compositions of the present disclosure, for treatment of *Pseudomonas* infection vary depending upon many different factors, including means of administration, target site, physiological state of the patient, whether the patient is human or an animal, other medications administered, and whether treatment is prophylactic or therapeutic. Usually, the patient is a human but non-human mammals including transgenic mammals can also be treated. Treatment dosages can be titrated using routine methods known to those of skill in the art to optimize safety and efficacy.

[0301] Anti-*Pseudomonas* Psl and/or PcrV binding molecules, *e.g.*, antibodies or fragments, variants or derivatives thereof can be administered multiple occasions at various frequencies depending on various factors known to those of skill in the art.. Alternatively, anti-*Pseudomonas* Psl and/or PcrV binding molecules, *e.g.*, antibodies or fragments, variants or derivatives thereof can be administered as a sustained release formulation, in which case less frequent administration is required. Dosage and frequency vary depending on the half-life of the antibody in the patient.

[0302] The compositions of the disclosure can be administered by any suitable method, *e.g.*, parenterally, intraventricularly, orally, by inhalation spray, topically, rectally, nasally, buccally, vaginally or via an implanted reservoir. The term "parenteral" as used herein includes subcutaneous, intravenous, intramuscular, intra-articular, intra-synovial, intrasternal, intrathecal, intrahepatic, intralesional and intracranial injection or infusion techniques.

X. SYNERGY

[0303] Chou and Talalay (*Adv. Enzyme Regul., 22*:27-55 (1984)) developed a mathematical method to describe the experimental findings of combined drug effects in a qualitative and quantitative manner. For mutually exclusive drugs, they showed that the generalized isobol equation applies for any degree of effect (see page 52 in Chou and Talalay). An isobol or isobologram is the graphic representation of all dose combinations of two drugs that have the same degree of effect. In isobolograms, a straight line indicates additive effects, a concave curve (curve below the straight line) represents synergistic effects, and a convex curve (curve above the straight line) represents antagonistic effects. These curves also show that a combination of two mutually exclusive drugs will show the same type of effect over the whole concentration range,

either the combination is additive, synergistic, or antagonistic. Most drug combinations show an additive effect. In some instances however, the combinations show less or more than an additive effect. These combinations are called antagonistic or synergistic, respectively. A combination manifests therapeutic synergy if it is therapeutically superior to one or other of the constituents used at its optimum dose. See, T. H. Corbett et al., Cancer Treatment Reports, 66, 1187 (1982). Tallarida RJ (J Pharmacol Exp Ther. 2001 Sep; 298 (3):865-72) also notes "Two drugs that produce overtly similar effects will sometimes produce exaggerated or diminished effects when used concurrently. A quantitative assessment is necessary to distinguish these cases from simply additive action."

[0304] A synergistic effect can be measured using the combination index (CI) method of Chou and Talalay (see Chang et al., Cancer Res. 45: 2434-2439, (1985)) which is based on the median-effect principle. This method calculates the degree of synergy, additivity, or antagonism between two drugs at various levels of cytotoxicity. Where the CI value is less than 1, there is synergy between the two drugs. Where the CI value is 1, there is an additive effect, but no synergistic effect. CI values greater than 1 indicate antagonism. The smaller the CI value, the greater the synergistic effect. In another embodiment, a synergistic effect is determined by using the fractional inhibitory concentration (FIC). This fractional value is determined by expressing the IC50 of a drug acting in combination, as a function of the IC50 of the drug acting alone. For two interacting drugs, the sum of the FIC value for each drug represents the measure of synergistic interaction. Where the FIC is less than 1, there is synergy between the two drugs. An FIC value of 1 indicates an additive effect. The smaller the FIC value, the greater the synergistic interaction.

[0305] In some embodiments, a synergistic effect is obtained in *Pseudomonas* treatment wherein one or more of the binding agents are administered in a "low dose" (*i.e.*, using a dose or doses which would be considered non-therapeutic if administered alone), wherein the administration of the low dose binding agent in combination with other binding agents (administered at either a low or therapeutic dose) results in a synergistic effect which exceeds the additive effects that would otherwise result from individual administration of the binding agent alone. In some embodiments, the synergistic effect is achieved via

administration of one or more of the binding agents administered in a "low dose" wherein the low dose is provided to reduce or avoid toxicity or other undesirable side effects.

XI. IMMUNOASSAYS

[0306] Anti-*Pseudomonas* Psl and/or PcrV binding molecules, *e.g.*, antibodies or fragments, variants or derivatives thereof can be assayed for immunospecific binding by any method known in the art. The immunoassays which can be used include but are not limited to competitive and non-competitive assay systems using techniques such as western blots, radioimmunoassays, ELISA (enzyme linked immunosorbent assay), "sandwich" immunoassays, immunoprecipitation assays, precipitin reactions, gel diffusion precipitin reactions, immunodiffusion assays, agglutination assays, complement-fixation assays, immunoradiometric assays, fluorescent immunoassays, protein A immunoassays, to name but a few. Such assays are routine and well known in the art (*see, e.g.*, Ausubel *et al.*, eds, *Current Protocols in Molecular Biology*, John Wiley & Sons, Inc., New York, Vol. 1 (1994), which is incorporated by reference herein in its entirety). Exemplary immunoassays are described briefly below (but are not intended by way of limitation).

[0307] There are a variety of methods available for measuring the affinity of an antibody-antigen interaction, but relatively few for determining rate constants. Most of the methods rely on either labeling antibody or antigen, which inevitably complicates routine measurements and introduces uncertainties in the measured quantities. Antibody affinity can be measured by a number of methods, including OCTET®, BIACORE®, ELISA, and FACS.

[0308] The OCTET® system uses biosensors in a 96-well plate format to report kinetic analysis. Protein binding and dissociation events can be monitored by measuring the binding of one protein in solution to a second protein immobilized on the FortéBio biosensor. In the case of measuring binding of anti-Psl or PcrV antibodies to Psl or PcrV, the Psl or PcrV is immobilized onto OCTET® tips followed by analysis of binding of the antibody, which is in solution. Association and disassociation of antibody to immobilized Psl or PcrV is then detected by the instrument sensor. The data is then collected and exported to GraphPad Prism for affinity curve fitting.

[0309] Surface plasmon resonance (SPR) as performed on BIACORE® offers a number of advantages over conventional methods of measuring the affinity of antibody-antigen interactions: (i) no requirement to label either antibody or antigen; (ii) antibodies do not need to be purified in advance, cell culture supernatant can be used directly; (iii) real-time measurements, allowing rapid semi-quantitative comparison of different monoclonal antibody interactions, are enabled and are sufficient for many evaluation purposes; (iv) biospecific surface can be regenerated so that a series of different monoclonal antibodies can easily be compared under identical conditions; (v) analytical procedures are fully automated, and extensive series of measurements can be performed without user intervention. BIAapplications Handbook, version AB (reprinted 1998), BIACORE® code No. BR-1001-86; BIAtechnology Handbook, version AB (reprinted 1998), BIACORE® code No. BR-1001-84.

[0310] SPR based binding studies require that one member of a binding pair be immobilized on a sensor surface. The binding partner immobilized is referred to as the ligand. The binding partner in solution is referred to as the analyte. In some cases, the ligand is attached indirectly to the surface through binding to another immobilized molecule, which is referred as the capturing molecule. SPR response reflects a change in mass concentration at the detector surface as analytes bind or dissociate.

[0311] Based on SPR, real-time BIACORE® measurements monitor interactions directly as they happen. The technique is well suited to determination of kinetic parameters. Comparative affinity ranking is extremely simple to perform, and both kinetic and affinity constants can be derived from the sensorgram data.

[0312] When analyte is injected in a discrete pulse across a ligand surface, the resulting sensorgram can be divided into three essential phases: (i) Association of analyte with ligand during sample injection; (ii) Equilibrium or steady state during sample injection, where the rate of analyte binding is balanced by dissociation from the complex; (iii) Dissociation of analyte from the surface during buffer flow.

[0313] The association and dissociation phases provide information on the kinetics of analyte-ligand interaction (k_a and k_d, the rates of complex formation and dissociation, $k_d/k_a = K_D$). The equilibrium phase provides information on the affinity of the analyte-ligand interaction (K_D).

[0314] BIAevaluation software provides comprehensive facilities for curve fitting using both numerical integration and global fitting algorithms. With suitable analysis of the data, separate rate and affinity constants for interaction can be obtained from simple BIACORE® investigations. The range of affinities measurable by this technique is very broad ranging from mM to pM.

[0315] Epitope specificity is an important characteristic of a monoclonal antibody. Epitope mapping with BIACORE®, in contrast to conventional techniques using radioimmunoassay, ELISA or other surface adsorption methods, does not require labeling or purified antibodies, and allows multi-site specificity tests using a sequence of several monoclonal antibodies. Additionally, large numbers of analyses can be processed automatically.

[0316] Pair-wise binding experiments test the ability of two MAbs to bind simultaneously to the same antigen. MAbs directed against separate epitopes will bind independently, whereas MAbs directed against identical or closely related epitopes will interfere with each other's binding. These binding experiments with BIACORE® are straightforward to carry out.

[0317] For example, one can use a capture molecule to bind the first Mab, followed by addition of antigen and second MAb sequentially. The sensorgrams will reveal: 1. how much of the antigen binds to first Mab, 2. to what extent the second MAb binds to the surface-attached antigen, 3. if the second MAb does not bind, whether reversing the order of the pair-wise test alters the results.

[0318] Peptide inhibition is another technique used for epitope mapping. This method can complement pair-wise antibody binding studies, and can relate functional epitopes to structural features when the primary sequence of the antigen is known. Peptides or antigen fragments are tested for inhibition of binding of different MAbs to immobilized antigen. Peptides which interfere with binding of a given MAb are assumed to be structurally related to the epitope defined by that MAb.

XII. ADMINISTRATION

[0319] A composition comprising either an anti-Psl binding domain or anti-PcrV binding domain, or a composition comprising both an anti -Psl and anti-PcrV binding domain are administered in such a way that they provide a synergistic effect in the treatment of

Pseudomonas in a patient. Administration can be by any suitable means provided that the administration provides the desired therapeutic effect, i.e., synergism. In certain embodiments, the antibodies are administered during the same cycle of therapy, e.g., during one cycle of therapy during a prescribed time period, both of the antibodies are administered to the subject. In some embodiments, administration of the antibodies can be during sequential administration in separate therapy cycles, e.g., the first therapy cycle involving administration of an anti-Psl antibody and the second therapy cycle involving administration of an anti-PcrV antibody. The dosage of the binding domains administered to a patient will also depend on frequency of administration and can be readily determined by one of ordinary skill in the art.

[0320] In other embodiments the binding domains are administered more than once during a treatment cycle. For example, in some embodiments, the binding domains are administered weekly for three consecutive weeks in a three or four week treatment cycle.

[0321] Administration of the composition comprising one or more of the binding domains can be on the same or different days provided that administration provides the desired therapeutic effect.

[0322] It will be readily apparent to those skilled in the art that other doses or frequencies of administration that provide the desired therapeutic effect are suitable for use in the present invention.

XII. KITS

[0323] In yet other embodiments, the present invention provides kits that can be used to perform the methods described herein. In certain embodiments, a kit comprises a binding molecule disclosed herein in one or more containers. One skilled in the art will readily recognize that the disclosed binding domains, polypeptides and antibodies of the present invention can be readily incorporated into one of the established kit formats which are well known in the art.

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[0324] The practice of the disclosure will employ, unless otherwise indicated, conventional techniques of cell biology, cell culture, molecular biology, transgenic biology, microbiology, recombinant DNA, and immunology, which are within the skill of the art. Such techniques are explained fully in the literature. See, for example, Molecular Cloning A Laboratory Manual, 2nd Ed., Sambrook *et al.*, ed., Cold Spring Harbor

Laboratory Press: (1989); *Molecular Cloning: A Laboratory Manual,* Sambrook *et al.*, ed., Cold Springs Harbor Laboratory, New York (1992), *DNA Cloning*, D. N. Glover ed., Volumes I and II (1985); *Oligonucleotide Synthesis*, M. J. Gait ed., (1984); Mullis *et al.* U.S. Pat. No: 4,683,195; *Nucleic Acid Hybridization*, B. D. Hames & S. J. Higgins eds. (1984); *Transcription And Translation*, B. D. Hames & S. J. Higgins eds. (1984); *Culture Of Animal Cells*, R. I. Freshney, Alan R. Liss, Inc., (1987); *Immobilized Cells And Enzymes*, IRL Press, (1986); B. Perbal, *A Practical Guide To Molecular Cloning* (1984); the treatise, *Methods In Enzymology*, Academic Press, Inc., N.Y.; *Gene Transfer Vectors For Mammalian Cells*, J. H. Miller and M. P. Calos eds., Cold Spring Harbor Laboratory (1987); *Methods In Enzymology*, Vols. 154 and 155 (Wu *et al.* eds.); *Immunochemical Methods In Cell And Molecular Biology*, Mayer and Walker, eds., Academic Press, London (1987); *Handbook Of Experimental Immunology*, Volumes I-IV, D. M. Weir and C. C. Blackwell, eds., (1986); *Manipulating the Mouse Embryo*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., (1986); and in Ausubel *et al.*, *Current Protocols in Molecular Biology,* John Wiley and Sons, Baltimore, Maryland (1989).

[0325] General principles of antibody engineering are set forth in *Antibody Engineering,* 2nd edition, C.A.K. Borrebaeck, Ed., Oxford Univ. Press (1995). General principles of protein engineering are set forth in *Protein Engineering, A Practical Approach,* Rickwood, D., *et al.*, Eds., IRL Press at Oxford Univ. Press, Oxford, Eng. (1995). General principles of antibodies and antibody-hapten binding are set forth in: Nisonoff, A., *Molecular Immunology,* 2nd ed., Sinauer Associates, Sunderland, MA (1984); and Steward, M.W., *Antibodies, Their Structure and Function,* Chapman and Hall, New York, NY (1984). Additionally, standard methods in immunology known in the art and not specifically described are generally followed as in *Current Protocols in Immunology*, John Wiley & Sons, New York; Stites *et al.* (eds) , *Basic and Clinical -Immunology* (8th ed.), Appleton & Lange, Norwalk, CT (1994) and Mishell and Shiigi (eds), *Selected Methods in Cellular Immunology,* W.H. Freeman and Co., New York (1980).

[0326] Standard reference works setting forth general principles of immunology include *Current Protocols in Immunology*, John Wiley & Sons, New York; Klein, J., Immunology*: The Science of Self-Nonself Discrimination*, John Wiley & Sons, New York (1982); Kennett, R., *et al.*, eds., *Monoclonal Antibodies, Hybridoma: A New Dimension in Biological Analyses*, Plenum Press, New York (1980); Campbell, A., "Monoclonal

Antibody Technology" in Burden, R., *et al.*, eds., *Laboratory Techniques in Biochemistry and Molecular Biology*, Vol. 13, Elsevere, Amsterdam (1984), *Kuby Immunnology* 4th ed. Ed. Richard A. Goldsby, Thomas J. Kindt and Barbara A. Osborne, H. Freemand & Co. (2000); Roitt, I., Brostoff, J. and Male D., *Immunology* 6th ed. London: Mosby (2001); Abbas A., Abul, A. and Lichtman, A., *Cellular and Molecular Immunology* Ed. 5, Elsevier Health Sciences Division (2005); Kontermann and Dubel, *Antibody Engineering*, Springer Verlan (2001); Sambrook and Russell, *Molecular Cloning: A Laboratory Manual.* Cold Spring Harbor Press (2001); Lewin, *Genes VIII*, Prentice Hall (2003); Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Press (1988); Dieffenbach and Dveksler, *PCR Primer* Cold Spring Harbor Press (2003).

EXAMPLES

Example 1: Construction and screening of human antibody phage display libraries

[0327] This example describes a target indifferent whole cell panning approach with human antibody phage libraries derived from both naive and *P. aeruginosa* infected convalescing patients to identify novel protective antigens against *Pseudomonas* infection (Figure 1A). Assays included in the *in vitro* functional screens included opsonophagocytosis (OPK) killing assays and cell attachment assays using the epithelial cell line A549. The lead candidates, based on superior *in vitro* activity, were tested in *P. aeruginosa* acute pneumonia, keratitis, and burn infection models.

[0328] Figure 1B shows construction of patient antibody phage display library. Whole blood was pooled from 6 recovering patients 7-10 days post diagnosis followed by RNA extraction and phage library construction as previously described (Vaughan, T.J., *et al., Nat Biotechnol 14*, 309-314 (1996); Wrammert, J., *et al., Nature* **453**, 667-671 (2008)). Figure 1C shows that the final cloned scFv library contained 5.4 x 10^8 transformants and sequencing revealed that 79% of scFv genes were full-length and in frame. The VH CDR3 loops, often important for determining epitope specificity, were 84% diverse at the amino acid level prior to library selection.

[0329] In addition to the patient library, a naïve human scFv phage display library containing up to 1x10^{11} binding members (Lloyd, C., *et al., Protein Eng Des Sel 22*, 159-168 (2009)) was used for antibody isolation (Vaughan, T.J., *et al., Nat Biotechnol 14*, 309-314 (1996)). Heat killed *P.aeruginosa* (1x10^9) was immobilized in IMMUNO™

Tubes (Nunc; MAXISORP™) followed for phage display selections as described (Vaughan, T.J., *et al., Nat Biotechnol 14*, 309-314 (1996)) with the exception of triethanolamine (100nM) being used as the elution buffer. For selection on *P. aeruginosa* in suspension, heat killed cells were blocked followed by addition of blocked phage to cells. After washing, eluted phage was used to infect *E. coli* cells as described (Vaughan, 1996). Rescue of phage from *E. coli* and binding to heat-killed *P. aeruginosa* by ELISA was performed as described (Vaughan, 1996).

[0330] Following development and validation of the whole-cell affinity selection methodology, both the new convalescing patient library and a previously constructed naive library (Vaughan, T.J., *et al., Nat Biotechnol 14*, 309-314 (1996)) underwent affinity selection on suspensions of *P. aeruginosa* strain 3064 possessing a complete O-antigen as well as an isogenic *wapR* mutant strain which lacked surface expression of O-antigen. Figure 1D shows that output titers from successive patient library selections were found to increase at a greater rate for the patient library than for the naïve library ($1x10^7$ vs $3x10^5$ at round 3, respectively). In addition, duplication of VH CDR3 loop sequences in the libraries (a measure of clonal enrichment during selection), was also found to be higher in the patient library, reaching 88-92%, compared to 15-25% in the naïve library at round 3 (Figure 1D). Individual scFv phage from affinity selections were next screened by ELISA for reactivity to *P. aeruginosa* heterologous serotype strains (Figure 1E). ELISA plates (Nunc; MAXISORP™) were coated with *P. aeruginosa* strains from overnight cultures as described (DiGiandomenico, A., et al., *Infect Immun 72*, 7012-7021 (2004)). Diluted antibodies were added to blocked plates for 1 hour, washed, and treated with HRP-conjugated anti-human secondary antibodies for 1 hour followed by development and analysis as described (Ulbrandt, N.D., *et al., J Virol 80*, 7799-7806 (2006)). The dominant species of phage obtained from whole cell selections with both libraries yielded serotype specific reactivity (data not shown). Clones exhibiting serotype independent binding in the absence of nonspecific binding to *E. coli* or bovine serum albumin were selected for further evaluation.

[0331] For IgG expression, the VH and VL chains of selected antibodies were cloned into human IgG1 expression vectors, co-expressed in HEK293 cells, and purified by protein A affinity chromatography as described (Persic, L., *et al., Gene 187*, 9-18 (1997)). Human IgG1 antibodies made with the variable regions from these selected serotype independent

phage were confirmed for *P. aeruginosa* specificity and prioritized for subsequent analysis by whole cell binding to dominant clinically relevant serotypes by FACS analysis (Figure 1F), since this method is more stringent than ELISA. For the flow cytometry based binding assays mid-log phase *P. aeruginosa* strains were concentrated in PBS to an OD_{650} of 2.0. After incubation of antibody (10 μg/mL) and bacteria (~1 x 10^7 cells) for 1 hr at 4°C with shaking, washed cells were incubated with an ALEXA FLUOR 647® goat anti- human IgG antibody (Invitrogen, Carlsbad, CA) for 0.5 hr at 4°C. Washed cells were stained with BACLIGHT™ green bacterial stain as recommended (Invitrogen, Carlsbad, CA). Samples were run on a LSR II flow cytometer (BD Biosciences) and analyzed using BD FacsDiva (v. 6.1.3) and FlowJo (v. 9.2; TreeStar). Antibodies exhibiting binding by FACS were further prioritized for functional activity testing in an opsonophagocytosis killing (OPK) assay.

Example 2: Evaluation of mAbs promoting OPK of *P. aeruginosa*

[0332] This example describes the evaluation of prioritized human IgG1 antibodies to promote OPK of *P. aeruginosa*. Figure 2A shows that with the exception of WapR-007 and the negative control antibody R347, all antibodies mediated concentration dependent killing of luminescent *P. aeruginosa* serogroup O5 strain (PAO1.lux). WapR-004 and Cam-003 exhibited superior OPK activity. OPK assays were performed as described in (DiGiandomenico, A., *et al.*, *Infect Immun 72*, 7012-7021 (2004)), with modifications. Briefly, assays were performed in 96-well plates using 0.025 ml of each OPK component; *P. aeruginosa* strains; diluted baby rabbit serum; differentiated HL-60 cells; and monoclonal antibody. In some OPK assays, luminescent *P. aeruginosa* strains, which were constructed as described (Choi, K.H., *et al., Nat Methods 2*, 443-448 (2005))., were used. Luminescent OPK assays were performed as described above but with determination of relative luciferase units (RLUs) using a Perkin Elmer ENVISION Multilabel plate reader (Perkin Elmer).

[0333] The ability of the WapR-004 and Cam-003 antibodies to mediate OPK activity against another clinically relevant O-antigen serotype strain, 9882-80.lux, was evaluated. Figure 2B shows that enhanced WapR-004 and Cam-003 OPK activity extends to strain 9882-80 (O11).

[0334] In addition, this example describes the evaluation of WapR-004 (W4) mutants in scFv-Fc format to promote OPK of *P. aeruginosa*. One mutant, Wap-004RAD (W4-RAD), was specifically created through site-directed mutagenesis to remove an RGD motif in VH. Other W4 mutants were prepared as follows. Nested PCR was performed as described (Roux, K.H., *PCR Methods Appl* **4**, S185-194 (1995)), to amplify W4 variants (derived from somatic hypermutation) from the scFv library derived from the convalescing P. aeruginosa infected patients , for analysis. This is the library from which WapR-004 was derived. W4 variant fragments were subcloned and sequenced using standard procedures known in the art. W4 mutant light chains (LC) were recombined with the WapR-004 heavy chain (HC) to produce W4 mutants in scFv-Fc format. In addition WapR-004 RAD heavy chain (HC) mutants were recombined with parent LCs of M7 and M8 in the scFv-Fc format. Constructs were prepared using standard procedures known in the art. Figures 11 (A-M) show that with the exception of the negative control antibody R347, all WapR-004 (W4) mutants mediated concentration dependent killing of luminescent *P. aeruginosa* serogroup O5 strain (PAO1.lux).

[0335] The WapR-004-RAD variable region was germ-lined to reduce potential immunogenicity, producing WapR-004-germline ("WapR-004-GL"), and was lead optimized via site-directed mutagenesis. Clones with improved affinity for Psl were selected in competition-based screens. Top clones were ranked by affinity improvement and analyzed in an *in vitro* functional assay. The 14 lead optimized clones are: Psl0096, Psl0170, Psl0225, Psl0304, Psl0337, Psl348, Psl0567, Psl0573, Psl0574, Psl0582, Psl0584, Psl0585, Psl0588 and Psl0589.

Example 3: Serotype independent anti-*P.aeruginosa* antibodies target the Psl exopolysaccharide

[0336] This example describes identification of the target of anti-*P. aeruginosa* antibodies derived from phenotypic screening. Target analysis was performed to test whether the serotype independent antibodies targeted protein or carbohydrate antigens. No loss of binding was observed in ELISA toPAO1 whole cell extracts exhaustively digested with proteinase K, suggesting that reactivity targeted surface accessible carbohydrate residues (data not shown). Isogenic mutants were constructed in genes responsible for O-antigen, alginate, and LPS core biosynthesis; *wbpL* (O-antigen-deficient); *wbpL/algD* (O-antigen and alginate deficient); *rmlC* (O-antigen-deficient and

truncated outer core); and *galU* (O-antigen-deficient and truncated inner core). *P. aeruginosa* mutants were constructed based on the allele replacement strategy described by Schweizer (Schweizer, H.P., *Mol Microbiol 6*, 1195-1204 (1992); Schweizer, H.D., *Biotechniques* **15**, 831-834 (1993)). Vectors were mobilized from *E. coli* strain S17.1 into *P. aeruginosa* strain PAO1; recombinants were isolated as described (Hoang, T.T., *et al.*, *Gene 212*, 77-86 (1998)). Gene deletion was confirmed by PCR. *P. aeruginosa* mutants were complemented with pUCP30T-based constructs harboring wild type genes. Reactivity of antibodies was determined by indirect ELISA on plates coated with above indicated *P. aeruginosa* strains: Figure 3A shows that Cam-003 binding to the *wbpL* or the *wbpL/algD* double mutant was unaffected, however binding to the *rmlC* and *galU* mutants were abolished. While these results were consistent with binding to LPS core, reactivity to LPS purified from PAO1 was not observed. The *rmlC* and *galU* genes were recently shown to be required for biosynthesis of the Psl exopolysaccharide, a repeating pentasaccharide polymer consisting of D-mannose, L-rhamnose, and D-glucose. Cam-003 binding to an isogenic *pslA* knockout PAO1Δ*pslA*, was tested, as *pslA* is required for Psl biosynthesis (Byrd, M.S., *et al., Mol Microbiol 73*, 622-638 (2009)). Binding of Cam-003 to PAO1Δ*pslA* was abolished when tested by ELISA (Figure 3B) and FACS (Figure 3C), while the LPS molecule in this mutant was unaffected (Figure 3D). Binding of Cam-003 was restored in a PAO1Δ*wbpL/algD/pslA* triple mutant complemented with *pslA* (Figure 3E) as was the ability of Cam-003 to mediate opsonic killing to complemented PAO1Δ*pslA* in contrast to the mutant (Figure 3F and 3G). Binding of Cam-003 antibody to a Pel exopolysaccharide mutant was also unaffected further confirming Psl as our antibody target (Figure 3E). Binding assays confirmed that the remaining antibodies also bound Psl (Figure 3H and 3I).

Example 4: Anti-Psl mAbs block attachment of *P. aeruginosa* to cultured epithelial cells.

[0337] This example shows that anti-Psl antibodies blocked *P. aeruginosa* association with epithelial cells. Anti-Psl antibodies were added to a confluent monolayer of A549 cells (an adenocarcinoma human alveolar basal epithelial cell line) grown in opaque 96-well plates (Nunc Nunclon Delta). Log-phase luminescent *P. aeruginosa* PAO1 strain (PAO1.lux) was added at an MOI of 10. After incubation of PAO1.lux with A549 cells at 37°C for 1 hour, the A549 cells were washed, followed by addition of LB+0.5% glucose. Bacteria were quantified following a brief incubation at 37°C as performed in the OPK

assay described in Example 2. Measurements from wells without A549 cells were used to correct for non-specific binding. Figure 4 shows that with the exception of Cam-005 and WapR-007, all antibodies reduced association of PAO1.lux to A549 cells in a dose-dependent manner. The mAbs which performed best in OPK assays, WapR-004 and Cam-003 (see Figures 2A-B, and Example 2), were also most active at inhibiting *P. aeruginosa* cell attachment to A549 lung epithelial cells, providing up to ~80% reduction compared to the negative control. WapR-016 was the third most active antibody, showing similar inhibitory activity as WapR-004 and Cam-003 but at 10-fold higher antibody concentration.

Example 5: *In vivo* passaged *P. aeruginosa* strains maintain/increase expression of Psl

[0338] To test if Psl expression *in vivo* is maintained, mice were injected intraperitoneally with *P. aeruginosa* isolates followed by harvesting of bacteria by peritoneal lavage four hours post-infection. The presence of Psl was analyzed with a control antibody and Cam-003 by flow cytometry as conditions for antibody binding are more stringent and allow for quantification of cells that are positive or negative for Psl expression. For *ex vivo* binding, bacterial inocula (0.1ml) was prepared from an overnight TSA plate and delivered intraperitoneally to BALB/c mice. At 4 hr. following challenge, bacteria were harvested, RBCs lysed, sonicated and resuspended in PBS supplemented with 0.1% Tween-20 and 1% BSA. Samples were stained and analyzed as previously described in Example 1. Figure 5 shows that bacteria harvested after peritoneal lavage with three wild type *P. aeruginosa* strains showed strong Cam-003 staining, which was comparable to log phase cultured bacteria (compare Figures 5A and 5C). *In vivo* passaged wild type bacteria exhibited enhanced staining when compared to the inoculum (compare Figures 5B and 5C). Within the inocula, Psl was not detected for strain 6077 and was minimally detected for strains PAO1 (O5) and 6206 (O11-cytotoxic). The binding of Cam-003 to bacteria increased in relation to the inocula indicating that Psl expression is maintained or increased *in vivo*. Wild type strains 6077, PAO1, and 6206 express Psl after *in vivo* passage, however strain PAO1 harboring a deletion of *pslA* (PAO1Δ*pslA*) is unable to react with Cam-003. These results further emphasize Psl as the target of the monoclonal antibodies.

Example 6: Survival rates for animals treated with anti-Psl monoclonal antibodies Cam-003 and WapR-004 in a *P. aeruginosa* acute pneumonia model

[0339] Antibodies or PBS were administered 24 hours before infection in each model. *P. aeruginosa* acute pneumonia, keratitis, and thermal injury infection models were performed as described (DiGiandomenico, A., *et al., Proc Natl Acad Sci U S A 104*, 4624-4629 (2007)), with modifications. In the acute pneumonia model, BALB/c mice (The Jackson Laboratory) were infected with *P. aeruginosa* strains suspended in a 0.05 ml inoculum. In the thermal injury model, CF-1 mice (Charles River) received a 10% total body surface area burn with a metal brand heated to 92°C for 10 seconds. Animals were infected subcutaneously with *P. aeruginosa* strain 6077 at the indicated dose. For organ burden experiments, acute pneumonia was induced in mice followed by harvesting of lungs, spleens, and kidneys 24 hours post-infection for determination of CFU.

[0340] Monoclonal antibodies Cam-003 and WapR-004 were evaluated in an acute lethal pneumonia model against *P. aeruginosa* strains representing the most frequent serotypes associated with clinical disease. Figures 6A and 6C show significant concentration-dependent survival in Cam-003-treated mice infected with strains PAO1 and 6294 when compared to controls. Figures 6B and 6D show that complete protection from challenge with 33356 and cytotoxic strain 6077 was afforded by Cam-003 at 45 and 15 mg/kg while 80 and 90% survival was observed at 5mg/kg for 33356 and 6077, respectively. Figures 6E and 6F show significant concentration-dependent survival in WapR-004-treated mice in the acute pneumonia model with strain 6077 (O11) (8×10^5 CFU) (Figure 6E), or 6077 (011) (6×10^5 CFU) (Figure 6F).

[0341] Cam-003 and WapR-004 were next examined for their ability to reduce *P. aeruginosa* organ burden in the lung and spread to distal organs, and later the animals were treated with various concentrations of WapR-004, Cam-003, or control antibodies at several different concentrations. Cam-003 was effective at reducing *P. aeruginosa* lung burden against all four strains tested. Cam-003 was most effective against the highly pathogenic cytotoxic strain, 6077, where the low dose was as effective as the higher dose (Figures 7D). Cam-003 also had a marked effect in reducing dissemination to the spleen and kidneys in mice infected with PAO1 (Figure 7A), 6294 (Figure 7C), and 6077 (Figure 7D), while dissemination to these organs was not observed in 33356 infected mice (Figure 7B). Figures 7E and 7F show that similarly, WapR-004 reduced organ burden

after induction of acute pneumonia with 6294 (O6) and 6206 (O11). Specifically, WapR-004 was effective at reducing *P. aeruginosa* dissemination to the spleen and kidneys in mice infected.

Example 7: Construction of anti-PcrV monoclonal antibody V2L2

[0342] VelocImmune® mice (Regeneron Pharmaceuticals) were immunized by Ultra-Short immunization method with r-PcrV and serum titers were followed for binding to PcrV and neutralizing the hemolytic activity of live *P.aeruginosa.* Mice showing anti-hemolytic activity in the serum were sacrificed and the spleen and lymph nodes (axial, inguinal and popliteal) were harvested. The cell populations from these organs were panned with biotinylated r-Pcrv to select for anti-PcrV specific B-cells. The selected cells were then fused with mouse myeloma partner P3X63-Ag8 and seeded at 25Kcells/well in hybridoma selection medium. After 10 days the medium from the hybridoma wells were completely changed with fresh medium and after another 3-4 days the hybridoma supernatants were assayed for anti-hemolytic activity. Colonies showing anti-hemolytic activity were limited dilution cloned at 0.2 cells/well of 96-well plates and the anti-hemolytic activity assay was repeated. Clones showing anti-hemolytic activity were adapted to Ultra-low IgG containing hybridoma culture medium. The IgG from the conditioned media were purified and assayed for in vitro anti-hemolytic activity and in vivo for protection against infection by *P.aeruginosa.* The antibodies were also categorized by competition assay into different groups. The variable (V) domains from the antibodies of interest were subcloned from the cDNA derived from their different respective clones. The subcloned V-segments were fused in frame with the cDNA for the corresponding constant domain in a mammalian expression plasmid. Recombinant IgG were expressed and purified from HEK293 cells. In instances where more than one cDNA V- sequence was obtained from a particular clone, all combinations of variable heavy and light chains were expressed and characterized to identify the functional IgG.

Example 8: Survival rates for animals treated with anti-Psl monoclonal antibodies Cam-003, WapR-004 and anti-PcrV monoclonal antibody V2L2 in a *P. aeruginosa* corneal infection model

[0343] Cam-003 and WapR-004 efficacy was next evaluated in a *P. aeruginosa* corneal infection model which emphasizes the pathogens ability to attach and colonize damaged tissue. Figures 8 A-D and 8 F-G show that mice receiving Cam-003 and WapR-004 had

significantly less pathology and reduced bacterial counts in total eye homogenates than was observed in negative control-treated animals. Figure 8E shows that Cam-003 was also effective when tested in a thermal injury model, providing significant protection at 15 and 5mg/kg when compared to the antibody-treated control. Figure 8 (H): The activity of anti-Psl and anti-PcrV monoclonal antibodies V2L2 was tested in a *P. aeruginosa* mouse ocular keratitis model. C3H/HeN mice were injected intraperitoneally (IP) with PBS or a control IgG1 antibody (R347) at 45mg/kg or WapR-004 (α-Psl) at 5mg/kg or V2L2 (α-PcrV) at 5mg/kg, 16 hours prior to infection with 6077 (O11-cytotoxic – $1x10^6$ CFU). Immediately before infection, mice were anesthetized followed by initiation of three 1 mm scratches on the cornea and superficial stroma of one eye of each mouse using a 27-gauge needle under a dissection microscope, followed by topical application of *P. aeruginosa* 6077 strain in a 5 µl inoculum. Eyes were photographed at 48 hours post infection followed by corneal grading by visualization of eyes under a dissection microscope. Grading of corneal infection was performed as previously described by Preston *et al.* (Preston, MJ., 1995, Infect. Immun. 63:3497). Briefly, infected eyes were graded 48 h after infection with strain 6077 by an investigator who was unaware of the animal treatments. The following grading scheme was used: grade 0, eye macroscopically identical to an uninfected eye; grade 1, faint opacity partially covering the pupil; grade 2, dense opacity covering the pupil; grade 3, dense opacity covering the entire pupil; grade 4, perforation of the cornea (shrinkage of the eyeball). Mice receiving systemically dosed (IP) Cam-003 or WapR-004RAD showed significantly less pathology and reduced bacterial colony forming units (CFU) in total eye homogenates than was observed in the R347 control mAb-treated animals. Similar results were observed in V2L2-treated animals when compared to R347-treated controls.

Example 9: A Cam-003 Fc mutant antibody, Cam-003-TM, has diminished OPK and *in vivo* efficacy but maintains anti-cell attachment activity.

[0344] Given the potential for dual mechanisms of action, a Cam-003 Fc mutant, Cam-003-TM, was created which harbors mutations in the Fc domain that reduces its interaction with Fcγ receptors (Oganesyan, V., *et al.*, *Acta Crystallogr D Biol Crystallogr 64*, 700-704 (2008)), to identify if protection was more correlative to anti-cell attachment or OPK activity. *P. aeruginosa* mutants were constructed based on the allele replacement strategy described by Schweizer (Schweizer, H.P., *Mol Microbiol* **6**, 1195-1204 (1992);

Schweizer, H.D., *Biotechniques 15*, 831-834 (1993)). Vectors were mobilized from *E. coli* strain S17.1 into *P. aeruginosa* strain PAO1; recombinants were isolated as described (Hoang, T.T., *et al.*, *Gene 212*, 77-86 (1998)). Gene deletion was confirmed by PCR. *P. aeruginosa* mutants were complemented with pUCP30T-based constructs harboring wild type genes. Figures 9A shows that Cam-003-TM exhibited a 4-fold drop in OPK activity compared to Cam-003 (EC_{50} of 0.24 and 0.06, respectively) but was as effective in the cell attachment assay (Figure 9B). Figure 9C shows that Cam-003-TM was also less effective against pneumonia suggesting that optimal OPK activity is necessary for optimal protection. OPK and cell attachment assays were performed as previously described in Examples 2 and 4, respectively.

Example 10: Epitope mapping and relative affinity for anti-Psl antibodies

[0345] Epitope mapping was performed by competition ELISA and confirmed using an OCTET® flow system with Psl derived from the supernatant of an overnight culture of *P. aeruginosa* strain PAO1. For competition ELISA, antibodies were biotinylated using the EZ-Link Sulfo-NHS-Biotin and Biotinylation Kit (Thermo Scientific). Antigen coated plates were treated with the EC_{50} of biotinylated antibodies coincubated with unlabeled antibodies. After incubation with HRP-conjugated streptavidin (Thermo Scientific), plates were developed as described above. Competition experiments between anti-Psl mAbs determined that antibodies targeted at least three unique epitopes, referred to as class 1, 2, and 3 antibodies (Figure 10A). Class 1 and 2 antibodies do not compete for binding, however the class 3 antibody, WapR-016, partially inhibits binding of the Class 1 and 2 antibodies.

[0346] Antibody affinity was determined by the OCTET® binding assays using Psl derived from the supernatant of overnight PAO1 cultures. Antibody K_D was determined by averaging the binding kinetics of seven concentrations for each antibody. Affinity measurements were taken with a FORTEBIO® OCTET® 384 instrument using 384 slanted well plates. The supernatant from overnight PAO1 cultures ± the *pslA* gene were used as the Psl source. Samples were loaded onto OCTET® AminoPropylSilane (hydrated in PBS) sensors and blocked, followed by measurement of anti-Psl mAb binding at several concentrations, and disassociation into PBS + 1% BSA. All procedures were performed as described (Wang, X., *et al.*, *J Immunol Methods 362*, 151-160). Association and disassociation raw ΔnM data were curve-fitted with GraphPad Prism.

Figure 10A shows the relative binding affinities of anti-Psl antibodies characterized above. Class 2 antibodies had the highest affinities of all the anti-Psl antibodies. Figure 10A also shows a summary of cell attachment and OPK data experiments. Figure 10B shows the relative binding affinities and OPK EC50 values of the Wap-004RAD (W4RAD) mutant as well as other W4 mutants lead optimized via site-directed mutagenesis as described in Example 2. Figure 10C shows the relative binding affinities of the Wap-004RAD (W4RAD), Wap-004RAD-Germline (W4RAD-GL) as well as lead optimized anti-Psl monoclonal antibodies (Psl0096, Psl0170, Psl0225, Psl0304, Psl0337, Psl348, Psl0567, Psl0573, Psl0574, Psl0582, Psl0584, Psl0585, Psl0588 and Psl0589). Highlighted clones Psl0096, Psl0225, Psl0337, Psl0567 and Psl0588 were selected based on their enhanced OPK activity, as shown in Example 10 below.

Example 11: Evaluation of lead optimized WapR-004 (W4) mutant clones and lead optimized anti-Psl monoclonal antibodies in the *P. aeruginosa* opsonophagocytic killing (OPK) assay

[0347] This example describes the evaluation of lead optimized WapR-004 (W4) mutant clones and lead optimized anti-Psl monoclonal antibodies to promote OPK of *P. aeruginosa* using the method described in Example 2. Figures 11A-Q show that with the exception of the negative control antibody R347, all antibodies mediated concentration dependent killing of luminescent *P. aeruginosa* serogroup O5 strain (PAO1.lux).

Example 12: Anti-PcrV monoclonal antibody V2L2 reduces lethality from acute pneumonia from multiple strains

[0348] The PcrV epitope diversity was analyzed using three approaches: bead based flow cytometry method, competition ELISA and western blotting of fragmented rPcrV. Competition experiments between anti-PcrV mAbs determined that antibodies targeted at least six unique epitopes, referred to as class 1, 2, 3, 4, 5 and 6 antibodies (Figure 12A). Class 2 and 3 antibodies partially compete for binding. mAbs representing additional epitope classes: class 1 (V2L7, 3G5, 4C3 and 11A6), class 2 (1E6 and 1F3), class 3 (29D2, 4A8 and 2H3), class 4 (V2L2) and class 5 (21F1, LE10 and SH3) were tested for *in vivo* protection as below described.

[0349] Novel anti-PcrV mAbs were isolated using hybridoma technology and the most potent T3SS inhibitors were selected using a rabbit red blood cell lysis inhibition assay. Percent inhibition of cytotoxicity analysis was analysed for the parental V2L2 mAb,

mAb166 (positive control) and R347 (negative control), where the antibodies were administered to cultured broncho-epithelial cell line A549 combined with log-phase *P. aeruginosa* strain 6077 (exoU+) at a MOI of approximately 10. A549 lysis was assayed by measuring released lactate dehydrogenase (LDH) activity and lysis in the presence of mAbs was compared to wells without mAb to determine percent inhibition. The V2L2 mAb, mAb166 (positive control) and R347 (negative control) were evaluated for their ability to prevent lysis of RBCs, where the antibodies were mixed with log-phase *P. aeruginosa* 6077 ($exoU^+$) and washed rabbit red blood cells (RBCs) and incubated for 2 hours at 37°. Intact RBCs were pelleted and the extent of lysis determined by measuring the OD_{405} of the cell-free supernatant. Lysis in the presence of anti-PcrV mAbs was compared to wells without mAb to determine percent inhibition. The positive control antibody, mAb166, is a previously characterized anti-PcrV antibody (*J Infect Dis.* 186: 64-73 (2002), *Crit Care Med.* 40: 2320-2326 (2012)).(B) The parental V2L2 mAb demonstrated inhibition of cytotoxicity with an IC50 of 0.10 μg/ml and exhibited an IC50 concentration 28-fold lower than mAb166 (IC50 of 2.8 μg/ml). (C) V2L2 also demonstrated prevention of RBC lysis with an IC50 of 0.37 μg/ml and exhibited an IC50 concentration 10-fold lower than mAb166 (IC50 of 3.7 μg/ml).

[0350] The V2L2 variable region was fully germlined to reduce potential immunogenicity. V2L2 was affinity matured using the parsimonious mutagenesis approach to randomize each position with 20 amino acids for all six CDRs, identifying affinity-improved single mutations. A combinatorial library was then used, encoding all possible combinations of affinity-improved single mutations. Clones with improved affinity to PcrV were selected using binding ELISA in IgG format. Top clones were ranked by affinity improvement and analyzed in an in vitro functional assay.V2L2 CDRs were systematically mutagenized and clones with improved affinity to PcrV were selected in competition-based screens. Clones were ranked by increases in affinity and analyzed in a functional assay. As shown in Figure 12D, RBC lysis was analyzed for V2L2-germlined MAb (V2L2-GL), V2L2-GL optimized mAbs (V2L2-P4M, V2L2-MFS, V2L2-MD and V2L2-MR), and a negative control antibody R347 using *Pseudomonas* strain 6077 infected A549 cells. V2L2-GL, V2L2-P4M, V2L2-MFS, V2L2-MD and V2L2-MR demonstrated prevention of RBC lysis.. As shown in Figure 12E, mAbs 1E6, 1F3, 11A6,

29D2, PCRV02 and V2L7 demonstrated prevention of RBC lysis. As shown in Figure 12F, V2L2 was more potent in prevention of RBC lysis than the 29D2.

[0351] Binding kinetics of V2L2-GL and V2L2-MD were measured using a Bio-Rad ProteOn™ XPR36 instrument. Antibodies were captured on a GLC bisensor chip using anti-human IgG reagents. rPcrV protein was injected at multiple concentrations and the dissociation phase followed for 600 seconds. Data was captured and analyzed using ProteOn Manager software. Figure 12 (G-H) shows the relative binding affinities of (G) V2L2-GL and (H) V2L2-MD antibodies. The clone V2L2-MD had increased Kd by 2-3 folds over V2L2-GL.

[0352] The *in vivo* effect of administration of an anti-PcrV antibodies was studied in mice using an acute pneumonia model. Groups of mice were treated with either increasing concentrations of the V2L2 antibody, a positive control anti-PcrV antibody (mAb166), or a negative control (R347), as shown in Figure 13 (A-B). Groups of mice were also treated with either increasing concentrations of the V2L2 antibody, the PcrV antibody PcrV-02, or a negative control (R347), as shown in Figure 13 (C-D). Twenty-four hours after treatment, all mice were infected with 5 x 10^7 CFU (C) *Pseudomonas aeruginosa* 6294 (O6) or (D) PA103A (O11). As shown in Figure 13, nearly all control treated animals succumbed to infection by 48 hours post infection. However, V2L2 showed a dose-dependent effect on improved survival even out to 168 hours post-infection. Further, V2L2 provided significantly more potent protection than mAb166 at similar doses ($P=0.025$, 5 mg/kg for strain 6077; $P < 0.0001$, 1 mg/kg for strain 6294).

[0353] Groups of mice were treated with either increasing concentrations of the 11A6, 3G5 or V2L7, the same concentrations of 29D2, 1F3, 1E6, V2L2, LE10, SH3, 4A8, 2H3, or 21F1, increasing concentrations of the 29D2, increasing concentrations of the V2L2, the PcrV antibody PcrV-02, or a negative control (R347), as shown in Figure 13 (E-H). Mice were injected intraperitoneally (IP) with mAbs 24 hours prior to to intranasal infection with *Pseudomonas* strain 6077 (1 x 10^6 CFU/animal). As shown in Figure 13E mAbs 11A6, 3G5 and V2L7 did not provide protection *in vivo*. As shown in Figure 13F, mAb 29D2 provides protection *in vivo*. As shown in Figure 13G, mAb V2L2 also provides protection *in vivo*. Figure 13H shows *in vivo* comparison of 29D2 and V2L2. Figure 13I shows that mAb V2L2 protects against additional *Pseudomonas* strains (*i.e.*,6294 and PA103A).

[0354] Organ burden of *Pseudomonas*-infected mice was also studied in response to administration of V2L2. Figure 14 (A) Mice were treated with either 1 mg/kg R347 (control), or 1 mg/kg, 0.2 mg/kg, or 0.07 mg/kg of V2L2 and then were infected intranasally with 1.2 x 10^6 cfu of *Pseudomonas* 6206. Figure 14 (B) Mice were also treated with either 15 mg/kg R347 (negative control); 15.0 mg/kg, 5.0 mg/kg, or 1.0 mg/kg mAb166 (positive control); or 5.0 mg/kg, 1.0 mg/kg, or 0.2 mg/kg V2L2 and then were infected intranasally with 5.5 x 10^6 cfu of *Pseudomonas* 6206. As shown in Figure 14 (A-B), while V2L2 had little effect on clearance in the kidney, it greatly reduced dissemination to both the lung and spleen in a dose-dependent manner. In addition, V2L2 provided significantly greater reduction in organ CFU than mAb166 at similar doses ($P < 0.0001$, 1 mg/kg, lung).

Example 13: *In vivo* activity of combination therapy using WapR-004 (anti-Psl) and V2L2 (anti-PcrV) antibodies

[0355] The *in vivo* effect of combination administration of anti-Psl and anti-PcrV binding domains was further studied in mice using the antibodies V2L2 and WapR-004 (RAD). Groups of mice were treated with R347 (2.1 mg/kg - negative control), V2L2 (0.1mg/kg), W4-RAD (0.5 mg/kg), or V2L2/W4 combination (either 0.1, 0.5, 1.0 or 2.0 mg/kg each). Twenty-four hours post-administration of antibody, all mice were infected with an inoculum containing 5.25 x 10^5 cfu 6206 (O11-ExoU+). Twenty-four hours post infection, lungs, spleens, and kidneys were harvested, homogenized, and plated for colony forming unit (CFU) identification per gram of tissue. As shown in Figure 15, at the concentrations tested, both V2L2 and W4 were effective in lowering organ burden, the V2L2/W4 combination showed an additive effect in tissue clearance. Histological examination of lung tissue revealed less hemorrhaging, less edema, and less inflammatory infiltrate compared to mice receiving V2L2 or WapR-004 alone (Table 5).

[0356] Similarly immunized animals were also assessed for survival from acute pneumonia infections.

Table 5

Group (n)	Treatment	Overall Impression (Involved Lung Surface Area)	Hemorrhage	Edema	Inflammatory Infiltrate	Gram Stain
1(2)	R347 (2.1 mg/kg)	Broncho interstitial pneumonia (75%) fibrinoid necrosis and marked congestion	3+	3+	PMN 3+	
6(3)	V2L2 (0.1 mg/kg)	Broncho interstitial pneumonia (55%) broncho epithelial injury and marked congestion	3+	3+	PMN 3+	
7(3)	WapR-004 (0.5 mg/kg)	Broncho interstitial pneumonia (50%) broncho epithelial injury and marked congestion	2-3+	3+	PMN 2-3+	
2(3)	V2L2 + WapR-004 (0.1 mg/kg + 0.1 mg/kg)	Broncho interstitial pneumonia (15%) Mild broncho epithelial injury	1+	3+	PMN 1+	
3(3)	V2L2 + WapR-004 (0.1 mg/kg + 0.5 mg/kg)	Broncho interstitial pneumonia (40%), Moderate congestion	2+	3+	PMN 2+	
4(3)	V2L2 + WapR-004 (0.1 mg/kg + 1.0 mg/kg)	Primarily Broncho pneumonia; Broncho interstitial pneumonia (20%)	1-3+	3+	PMN1-2+	
5(3)	V2L2 + WapR-004 (0.1 mg/kg + 2.0 mg/kg)	Mild Broncho pneumonia (20%)	1+	3+	PMN1-2+	

Example 14: Survival rates for animals treated with anti-PcrV monoclonal antibody V2L2 in a *P. aeruginosa* acute pneumonia model

[0357] Monoclonal antibodies V2L2-GL, V2L2-MD, V2L2-A, V2L2-C, V2L2-PM4 and V2L2-MFS were evaluated in an acute lethal pneumonia model against *P. aeruginosa* 6077 strain as previously described in Example 11. Figures 16 (A-F) show survival in all V2L2 treated mice infected with strain 6077 when compared to control. However, no significant difference in survival is observed between V2L2 antibodies at either dose: 0.5mg/kg and 1mg/kg (A-C) or 0.5mg/kg and 0.1mg/kg (D-F). Figures 16 (G-I) show survival in all V2L2 treated mice infected with strain 6077 when compared to control. No significant difference in survival is observed between V2L2 antibodies at either dose: 0.5mg/kg and 1mg/kg (G-I). (A-H)

[0358] All of the control mice succumbed to infection by approximately 48 hours post-infection.

Example 15: Construction of WapR-004/V2L2 bispecific antibodies

[0359] Figure 17A shows TNFα bispecific model constructs. For Bs1-TNFα/W4, the W4 scFv is fused to the amino-terminus of TNFα VL through a (G4S)2 linker. For Bs2-TNFα/W4, the W4 scFv is fused to the amino-terminus of TNFα VH through a (G4S)2 linker. For Bs3-TNFα/W4, the W4 scFv is fused to the carboxy-terminus of CH3 through a (G4S)2 linker.

[0360] Since the combination of WapR-004 + V2L2 provide protection against *Pseudomonas* challenge, bispecific constructs were generated comprising a WapR-004 scFv (W4-RAD) and V2L2 IgG (Figure 17B). To generate Bs2-V2L2-2C, the W4-RAD scFv is fused to N-terminal of V2L2 VH through (G4S)2 linker. To generate Bs3-V2L2-2C, W4-RAD scFv was fused to C-terminal of CH3 through (G4S)2 linker. To generate Bs4-V2L2-2C, the W4-RAD scFv was inserted in hinge region, linked by (G4S)2 linker on N-terminal and C-terminal of scFv. To generate Bs2-W4-RAD-2C, the V2L2 scFv was fused to the amino-terminus of W4-RAD VH through a (G4S)2 linker.

[0361] To generate the W4-RAD scFv for the Bs3 construct, the W4-RAD VH and VL were amplified by PCR. The primers used to amplify the W4-RAD VH were: W4-RAD VH forward primer: includes (G4S)2 linker and 22bp of VH N-terminal sequence

(GTAAAGGCGGAGGGGGATCCGGCGGAGGGGGCTCTGAGGTGCAGCTGTTGG AGTCGG (SEQ ID NO:224)); and W4-RAD VH reverse primer: includes part of (G4S)4 linker and 22 bp of VH C-terminal sequence (GATCCTCCGCCGCCGCTGCCCCCTCCCCCAGAGCCCCCTCCGCCACTCGAGA CGGTGACCAGGGTC (SEQ ID NO:225). Similarly, the W4-RAD VL was amplified by PCR using the primers: W4-RAD VL forward primer: includes part of (G4S)2 linker and 22 bp of VL N-terminal sequence (AGGGGGCAGCGGCGGCGGAGGATCTGGGGGAGGGGGCAGCGAAATTGTGTT GACACAGTCTC (SEQ ID NO:226)); and W4-RAD VL reverse primer: includes part of vector sequence and 22 bp of VL C-terminal sequence (CAATGAATTCGCGGCCGCTCATTTGATCTCCAGCTTGGTCCCAC SEQ ID NO:227)). The overlapping fragments were then fused together to form the W4-RAD scFv.

W4-RAD scFv sequence in Bs3 vector: underlined sequences are G4S linker

GGGGSGGGGSEVQLLESGPGLVKPSETLSLTCNVAGGSISPYYWTWIRQPPGKCLELIGY IHSSGYTDYNPSLKSRVTISGDTSKKQFSLHVSSVTAADTAVYFCARADWDLLHALDIW GQGTLVTVSSGGGGSGGGGSGGGGSGGGGSEIVLTQSPSSLSTSVGDRVTITCRASQSIRS HLNWYQQKPGKAPKLLIYGASNLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQS YSFPLTFGCGTKLEIK (SEQ ID NO:228)

[0362] After the W4-RAD scFv fragment was amplified, it was then gel purified and ligated into the Bs3 vector which had been digested with BamHI/NotI. The ligation was done using the In-Fusion system, followed by transformation in Stellar competent cells. Colonies were sequenced to confirm the correct W4-RAD scFv insert.

[0363] To generate the Bs3-V2L2-2C, the IgG portion in the Bs3 vector was replaced with V2L2 IgG. Briefly, the Bs3 vector which contains W4-RAD scFv was digested with BssHII / SalI and the resultant vector band was gel purified. Similarly, the vector containing V2L2 vector was digested with BssHII / SalI and the V2L2 insert was gel purified. The V2L2 insert was then ligated with the Bs3-W4-RAD scFv vector and colonies were sequenced to confirm the correct V2L2 IgG insert.

[0364] A similar approach was used to generate Bs2-V2L2-2C.

W4-RAD scFv-V2L2 VH sequences in Bs2 vector: underlined sequences are G4S linker

EVQLLESGPGLVKPSETLSLTCNVAGGSISPYYWTWIRQPPGKCLELIGYIHSSGYTDYNP
SLKSRVTISGDTSKKQFSLHVSSVTAADTAVYFCARADWDLLHALDIWGQGTLVTVSSG
GGGSGGGGSGGGGSGGGGSEIVLTQSPSSLSTSVGDRVTITCRASQSIRSHLNWYQQKPG
KAPKLLIYGASNLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYSFPLTFGCGT
KLEIKGGGGSGGGGSEMQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMNWVRQAPGE
GLEWVSAITISGITAYYTDSVKGRFTISRDNSKNTLYLQMNSLRAGDTAVYYCAKEEFLP
GTHYYYGMDVWGQGTTVTVSS (SEQ ID NO:229)

[0365] The following primers were used to amplify W4-RAD scFv. VH (forward primer) and VL (reverse primer): W4-RAD VH forward primer for Bs2 vector which includes some intron, 3' signal peptide and 22bp of W4-RAD VH N-terminal sequence (TTCTCTCCACAGGTGTACACTCCGAGGTGCAGCTGTTGGAGTCGG (SEQ ID NO:230)) and W4-RAD VL reverse primer for Bs2 vector: include (G4S)2 linker and 32 bp of VL C-terminal sequence (CCCCCTCCGCCGGATCCCCCTCCGCCTTTGATCTCCAGCTTGGTCCCACAGCC GAAAG (SEQ ID NO:231))

[0366] To amplify the V2L2 VH region the following primers were used: V2L2 VH forward primer: includes (G4S)2 linker and 22 bp of V2L2 VH N-terminal sequence (GGCGGAGGGGGATCCGGCGGAGGGGGCTCTGAGATGCAGCTGTTGGAGTCT GG (SEQ ID NO:232)), and V2L2 VH reverse primer: includes some of CH1 N-terminal sequence and 22 bp of V2L2 VH C-terminal sequence (ATGGGCCCTTGGTCGACGCTGAGGAGACGGTGACCGTGGTC (SEQ ID NO: 233)).

[0367] These primers were then used to amplify V2L2 VH, which was then joined by overlap with W4-RAD scFv and V2L2 VH to get W4-RAD scFv-V2L2-VH. The W4-RAD scFv-V2L2 VH was then ligated into Bs2 vector by gel purifying W4-RAD scFv – V2L2 VH (from overlap PCR); digesting Bs2 vector with BsrGI/SalI, and gel purifying vector band. The W4-RAD scFv-V2L2-VH was then ligated with Bs2 vector by In-Fusion system and transformed into Stellar competent cells and the colonies were confirmed for the correct W4-RAD scFv-V2L2 VH insert. To replace VL in Bs2 vector with V2L2 VL, the Bs2 vector which contains W4-RAD scFv-V2L2-VH was digested with BssHII / BsiWI and the vector band was gel purified. The pOE-V2L2 vector was then digested with BssHII / BsiWI and the V2L2 VL insert was gel purified. The V2L2 VL insert was then ligated with Bs2-W4-RAD scFv-V2L2-VH vector and the colonies were sequenced for correct V2L2 IgG insert.

[0368] Finally, a similar PCR-based approach was used to generate the Bs4-V2L2-2C construct. The hinge region with linker sequence is shown below:

Hinge region with linker sequence:

KVDKRVEPKSC*GGGGSGGGGS – N-terminus of scFv* **(SEQ ID NO:329)**
CH1 hinge linker

***C-terminus of scFv – GGGGSGGGGS*DKTHTCPPCPAPELL** **(SEQ ID NO:330)**
linker hinge CH2

W4-RAD scFv sequences in BS4 vector: W4-RAD scFv is in *bolded italics* with the G4S linkers *underlined in bolded italics*; hinge regions are doubled underlined

KVDKRVEPKSC***GGGGSGGGGSEVQLLESGPGLVKPSETLSLTCNVAGGSISPYYWTWIR QPPGKCLELIGYIHSSGYTDYNPSLKSRVTISGDTSKKQFSLHVSSVTAADTAVYFCARAD WDLLHALDIWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSEIVLTQSPSSLSTSVGDRV TITCRASQSIRSHLNWYQQKPGKAPKLLIYGASNLQSGVPSRFSGSGSGTDFTLTISSLQP EDFATYYCQQSYSFPLTFGCGTKLEIKGGGGSGGGGSDKTHTCPPCP***APELL**(SEQ ID NO:324)**

W4-RAD scFv is presented in *bolded italics* with the G4S linkers *underlined in bolded italics*

EVQLLESGPGLVKPSETLSLTCNVAGGSISPYYWTWIRQPPGKCLELIGYIHSSGYTDYNP SLKSRVTISGDTSKKQFSLHVSSVTAADTAVYFCARADWDLLHALDIWGQGTLVTVSSGG GGSGGGGSGGGGSGGGGSEIVLTQSPSSLSTSVGDRVTITCRASQSIRSHLNWYQQKPGK APKLLIYGASNLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYSFPLTFGCGTK LEIK

[0369] W4-RAD scFv was generated using PCR and the following primers: W4-RAD VH forward primer for Bs4 vector: includes some of linker sequences and 24 bp of W4-RAD VH N-terminal sequence (GAGGTGCAGCTGTTGGAGTCGGGC (SEQ ID NO:236)); and W4-RAD VL reverse primer for Bs4 vector: includes some hinge sequence, linker and 21 bp of W4-RAD VL C-terminal sequence (GTGTGAGTTTTGTCggatccCCCTCCGCCAGAGCCACCTCCGCCTTTGATCTCCA GCTTGGTCCC (SEQ ID NO: 237)).

[0370] W4-RAD scFv was then ligated into Bs4 vector to get Bs4-V2L2-2C by gel purifying W4-RAD scFv (from PCR); the Bs4-V2L2 vector was digested with BamHI and the vector band was gel purified. The W4-RAD scFv was ligated with Bs4 vector by In-Fusion system and the vector transform Stellar competent cells. Colonies were sequenced for the correct W4-RAD scFv insert.

[0371] The sequences for the light chain and heavy chain of the Bs4-V2L2-2C construct are provided in SEQ ID NOS: 327 and 328, respectively.

Example 16: A Psl/PcrV bispecific antibody promotes survival in pneumonia models

[0372] As an initial matter, the Bs2 and Bs3 bispecific antibodies were tested to examine whether they retained their W4 or V2L2 activity in a bispecific format. For the parental W4 scFv, a bispecific antibody was generated having W4 and a TNF-alpha binding arm. A cell attachment assay was performed as described above using the luminescent *P. aeruginosa* strain PAO1.lux. As shown in Figure 18, all bispecific constructs performed similarly to the parent W4-IgG1 construct.

[0373] As shown in Figure 19 (A-C), percent inhibition of cytotoxicity was analyzed for both Bs2-V2L2 and Bs3-V2L2 using both (A) 6206 and (B) 6206Δ*pslA* infected cells, and (C) percent inhibition of RBC lysis was analyzed for Bs2-V2L2-2C, Bs3-V2L2-2C and Bs4-V2L2-2C using 6206 infected cells. As shown in Figure 19 (A-C), all bispecific antibodies retained anti-cytotoxicity activity and inhibited RBC lysis at levels similar to the parental V2L2 antibody using 6206 and 6206Δ*pslA* infected cells.

[0374] The ability of the Bs2 and Bs3 bispecific antibodies to mediate OPK of *P. aeruginosa* was assessed using the method described in Example 2. While the Bs2-V2L2 antibody showed similar killing compared to the parental W4-RAD antibody, the killing for the Bs3-V2L2 antibody was decreased (Figure 20A). While the Bs2-V2L2-2C and Bs4-V2L2-2C antibodies showed similar killing compared to the parental W4-RAD antibody, the killing for the Bs3-V2L2-2C antibody was decreased (Figure 20B). Figure 20C shows that different preparations of Bs4 antibodies (old lot vs. new lot) showed similar killing compared to the parental W4-RAD antibody, however the Bs4-V2L2-2C-YTE antibodies had a 3-fold drop in OPK activity when compared to Bs4-V2L2-2C. A YTE mutant comprises a combination of three "YTE mutations": M252Y, S254T, and T256E, wherein the numbering is according to the EU index as set forth in Kabat, introduced into the heavy chain of an IgG. *See* U.S. Patent No. 7,658,921, which is incorporated by reference herein. The YTE mutant has been shown to increase the serum half-life of antibodies approximately four-times as compared to wild-type versions of the same antibody. *See, e.g.,* Dall'Acqua *et al.*, J. Biol. Chem. 281:23514-24 (2006) and U.S. Patent No. 7,083,784, which are hereby incorporated by reference in their entireties.

[0375] Following confirmation that both W4 and V2L2 retained activity in a bispecific format, the Bs2-V2L2, Bs3-V2L2 and Bs4-V2L2 constructs were assessed for survival from acute pneumonia infections. As shown in Figure 21A, all of the control mice succumbed to infection by approximately 30 hours post-infection. All of the Bs3-V2L2 animals survived, along with those which received the V2L2 control. Approximately 90% of the W4-RAD immunized animals survived. In contrast, Figures B-F show that approximately 50% of the Bs2-V2L2 animals succumbed to infection by 120 hours. All of the control mice succumbed to infection by approximately 48 hours post-infection. Figures G-H do not show difference in survival between Bs4-V2L2-2C and Bs4-V2L2-2C-YTE treated mice at either dose. These results suggest that both antibodies function equivalently in the 6206 acute pneumonia model. Figure 21 I shows that Bs2-V2L2, Bs4-V2L2-2C, and W4-RAD + V2L2 antibody mixture are the most effective in protection against lethal pneumonia in mice challenged with *P. aeruginosa* strain 6206 (ExoU+).

[0376] Organ burden was also assessed for similar immunized mice as described above. Following immunization as above, mice were challenged with 2.75×10^5 CFU 6206. As shown in Figure 22, at the concentration tested, both Bs2-V2L2 and Bs3-V2L2 significantly decreased organ burden in lung. However, neither of the bispecific constructs was able to significantly affect organ burden in spleen or kidney compared to the parental antibodies due to the use of suboptimal concentrations of the bispecific constructs. Suboptimal concentrations were used to enable the ability to decipher antibody activity.

[0377] Survival and organ burden effects of the bispecific antibodies were also addressed using the 6294 strain. Using the 6294 model system, both the BS2-V2L2 and BS3-V2L2 significantly decreased organ burden in all of the tissues to a level comparable to that of the V2L2 parental antibody. The W4-RAD parental antibody had no effect on decreasing organ burden (Figure 23A). As shown in Figure 23B, Bs2-V2L2, Bs3-V2L2, and W4-RAD+V2L2 combination significantly decreased organ burden in all of the tissues to a level comparable to that of the V2L2 parental antibody.

[0378] The survival data for immunized mice was similar in the 6294 challenged mice as before. As shown in Figure 24, BS3-V2L2 showed similar survival activity to V2L2 alone-treated mice, while BS2-V2L2 treated mice showed a slightly lower level of protection from challenge.

[0379] Organ burden was also assessed in bispecific antibodies treated in comparison with combination-treated animals as described above. As shown in Figures 25 (A-C), both the BS2-V2L2 and BS3-V2L2 decreased organ burden in the lung, spleen and kidneys to a level comparable to that of the W4 + V2L2 combination. In the lung, the combination significantly reduced bacterial CFUs Bs2- and Bs3-V2L2 and V2L2 using the Kruskal-Wallis with Dunn's post test. Significant differences in bacterial burden in the spleen and kidney were not observed, although a trend towards reduction was noted. An organ burden study was also performed with Bs4-GLO using 6206 in the pneumonia model. As shown in Figure 25 (D), when higher concentrations of antibody are used in prophylaxis of mice, a significant (Kruskal-Wallis with Dunn's post test) level of reduction in bacterial burden from the lung was observed. Significant reductions in bacterial dissemination to the spleen and kidneys were also observed when using higher concentrations of Bs4-GLO in this model.

[0380] These results were confirmed by histological examination of lung tissue of immunized BALB/c mice challenged with $1.33x10^7$ CFU using *P. aeruginosa* strain 6294 (Table 6A), $1.7x10^7$ CFU using *P. aeruginosa* strain 6294 (Table 6B) and 9.25×10^5 CFU using *P. aeruginosa* strain 6206 (Table 7).

Example 17: Therapeutic adjunctive therapy: Bs4-V2L2-2C + antibiotic

[0381] Survival effect of the Bs4 bispecific antibody and antibiotic adjunctive therapy was evaluated in an acute lethal pneumonia model against *P. aeruginosa* 6206 strain as previously described in Example 6 (Figure 26 (A-J)). (A-B) Mice were treated 24 hours prior to infection with 6206 with R347 (negative control) or Bs4-V2L2-2C or Ciprofloxacin (CIP) 1 hour post infection, or a combination of the Bs4-V2L2-2C 24 hours prior to infection and Cipro 1 hour post infection. (C) Mice were treated 1 hour post infection with 6206 with R347 or CIP or Bs4-V2L2-2C, or a combination of the Bs4-V2L2-2C and CIP. (D) Mice were treated 2 hours post infection with 6206 with R347 or CIP or Bs4-V2L2-2C, or a combination of the Bs4-V2L2-2C and CIP. (E) Mice were treated 2 hours post infection with 6206 with R347or Bs4-V2L2-2C or CIP 1 hour post infection, or a combination of the Bs4-V2L2-2C 2 hours post infection and CIP 1 hour post infection. (F) Mice were treated 1 hour post infection with 6206 with R347 or Meropenem (MEM) or Bs4-V2L2-2C, or a combination of the Bs4-V2L2-2C and MEM.

(G) Mice were treated 2 hours post infection with 6206 with R347 or Bs4-V2L2-2C or MEM 1 hour post infection, or a combination of the Bs4-V2L2-2C 2 hours post infection and MEM 1 hour post infection. (H) Mice were treated 2 hours post infection with 6206 with R347 or Bs4-V2L2-2C or MEM, or a combination of the Bs4-V2L2-2C 2 and MEM. (I) Mice were treated 4 hour post infection with 6206 with R347 or Cipro or Bs4-V2L2-2C or a combination of the Bs4-V2L2-2C and Cipro. All of the control mice succumbed to infection by approximately 24 hours post-infection. As shown in Figures 26 (A-I) Bs4 antibody combined with either CIP or MEM increases efficacy of antibiotic therapy, indicating synergistic protection when the molecules are combined. Further studies focused on the level of bacterial burden in mice treated with Bs4 or CIP alone or in combination (Bs4+CIP). As shown in Figure 26 (J), the level of bacterial burden in all organs (lung, spleen and kidneys) were similar in R347+CIP and Bs4+CIP, however only mice where Bs4 was included in the combination with CIP survive the infection (Figures 26 (A-E, I)). Altogether, these data indicate the antibiotics are important for reducing the bacterial burden in this animal model setting, however the specific antibody is required to reduce bacterial pathogenicity, thus protecting normal host immunity.

[0382] Survival effect of the Bs4 bispecific antibody and Tobramycin antibiotic adjunctive therapy will be evaluated in an acute lethal pneumonia model against *P. aeruginosa* 6206 strain as previously described in Example 6. Mice will be treated 24 hours prior to infection with 6206 with R347 (negative control) or Bs4-V2L2-2C or Tobramycin 1 hour post infection, or a combination of the Bs4-V2L2-2C 24 hours prior to infection and Tobramycin 1 hour post infection. Mice will also be treated 1 hour post infection with 6206 with R347 or Tobramycin or Bs4-V2L2-2C, or a combination of the Bs4-V2L2-2C and Tobramycin. In addition, mice will be treated 2 hours post infection with 6206 with R347 or Tobramycin or Bs4-V2L2-2C, or a combination of the Bs4-V2L2-2C and Tobramycin. Furthermore, mice will be treated 2 hours post infection with 6206 with R347 or Bs4-V2L2-2C or Tobramycin 1 hour post infection, or a combination of the Bs4-V2L2-2C 2 hours post infection and Tobramycin 1 hour post infection. Mice will be treated 4 hour post infection with 6206 with R347 or Tobramycin or Bs4-V2L2-2C or a combination of the Bs4-V2L2-2C and Tobramycin.

[0383] Survival effect of the Bs4 bispecific antibody and Aztreonam antibiotic adjunctive therapy will be evaluated in an acute lethal pneumonia model against *P. aeruginosa* 6206

strain as previously described in Example 6. Mice will be treated 24 hours prior to infection with 6206 with R347 (negative control) or Bs4-V2L2-2C or Aztreonam 1 hour post infection, or a combination of the Bs4-V2L2-2C 24 hours prior to infection and Aztreonam 1 hour post infection. Mice will also be treated 1 hour post infection with 6206 with R347 or Aztreonam or Bs4-V2L2-2C, or a combination of the Bs4-V2L2-2C and Aztreonam. In addition, mice will be treated 2 hours post infection with 6206 with R347 or Aztreonam or Bs4-V2L2-2C, or a combination of the Bs4-V2L2-2C and Aztreonam. Furthermore, mice will be treated 2 hours post infection with 6206 with R347 or Bs4-V2L2-2C or Aztreonam 1 hour post infection, or a combination of the Bs4-V2L2-2C 2 hours post infection and Aztreonam 1 hour post infection. Mice will be treated 4 hour post infection with 6206 with R347 or Aztreonam or Bs4-V2L2-2C or a combination of the Bs4-V2L2-2C and Aztreonam.

Example 18: Construction of the BS4-GLO bispecific antibody

[0384] The BS4-GLO (Germlined Lead Optimized) bispecific construct was generated comprising anti-Psl scFv (Psl0096 scfv) and V2L2-MD (VH+VL) as shown in Figure 35A. The BS4-GLO light chain comprises germilined lead optimized anti-PcrV antibody light chain variable region (*i.e.*, V2L2-MD). The BS4-GLO heavy chain comprises the formula VH-CH1-H1-L1-S-L2-H2-CH2-CH3, wherein CH1 is a heavy chain constant region domain-1, H1 is a first heavy chain hinge region fragment, L1 is a first linker, S is an anti-PcrV ScFv molecule, L2 is a second linker, H2 is a second heavy chain hinge region fragment, CH2 is a heavy chain constant region domain-2, and CH3 is a heavy chain constant region domain-3.

Bs4-GLO light chain:

AIQMTQSPSSLSASVGDRVTITCRASQGIRNDLGWYQQKPGKAPKLLIYSASTLQS GVPSRFSGSGSGTDFTLTISSLQPEDFATYYCLQDYNYPWTFGQGTKVEIKRTVA APSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQ DSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO:…)

GLO (germlined lead optimized) V2L2 (*i.e.*, V2L2-MD) light chain variable region is underlined

Bs4-GLO heavy chain:

EMQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMNWVRQAPGEGLEWVSAITIS GITAYYTDSVKGRFTISRDNSKNTLYLQMNSLRAGDTAVYYCAKEEFLPGTHYY YGMDVWGQGTTVTVSS[ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVT VSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTK VDKRV]EPKSC***GGGGSGGGGSEVQLLESGPGLVKPSETLSLTCNVAGGSISPYYW TWIRQPPGKCLELIGYIHSSGYTDYNPSLKSRVTISGDTSKKQFSLHVSSVTAADTA VYFCARADWDLLHALDIWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSEIVLTQ SPSSLSTSVGDRVTITCRASQSIRSHLNWYQQKPGKAPKLLIYGASNLQSGVPSRFS GSGSGTDFTLTISSLQPEDFATYYCQQSYSFPLTFGCGTKLEIKGGGGSGGGGS***DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNW YVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP APIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNG QPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQ KSLSLSPGK GLO (germlined -lead optimized) V2L2 (*i.e.*, V2L2-MD) heavy chain variable region is underlined; CH1 is bracketed **[]**; GLO (germlined-lead optimized) W4-RAD (*i.e.*, Psl0096) scFv is in ***bolded italics*** with the G4S linkers ***underlined in bolded italics***; hinge regions are doubled underlined.

[0385] An alternative Bs4-GLO bispecific construct comprising an anti-PcrV ScFv and an anti-Psl (VH+VL) is shown in Figure 35B, and is generated similarly.

Example 19: Evaluation of the functional activity and efficacy of the Bs4-GLO bispecific antibody

[0386] Bispecific antibodies Bs4-WT (also referred to herein as Bs4-V2L2-2C), Bs4-GL (comprising germlined anti-PcrV and anti-Psl variable regions) and Bs4-GLO produced as described in Example 18 were tested for differences in functional activity in an opsonophagocytic killing assay (Figure 27A), as previously described in Example 2, anti-cell attachment assay (Figure 27B), as previously described in Example 4 and a RBC lysis anti-cytotoxicity assay (Figure 27C), as previously described in Example 12. No *in vitro* difference in functional activities between the antibodies was observed.

[0387] *In vivo* efficacy of Bs4-GLO was examined as follows. For prophylactic evaluation, mice were prophylactically treated with several concentrations of the Bs4-GLO (*i.e.*, 0.007mg/kg, 0.02mg/kg, 0.07mg/kg, 0.2mg/kg, 0.5mg/kg, 1mg/kg, 3mg/kg,

5mg/kg, 10mg/kg or 15mg/kg) (Figure 28A), 24 hours before infection with the following *P. aeruginosa* strains (6206 (1.0 x 10^6), 6077 (1.0 x 10^6), 6294 (2.0 x 10^7) or PA103 (1.0 x 10^6)). For therapeutic evaluation, mice were therapeutically treated with several concentrations of the Bs4-GLO (*i.e.*, 0.03mg/kg, 0.3mg/kg, 0.5mg/kg, 1mg/kg, 2mg/kg, 5mg/kg, 10mg/kg, 15mg/kg, or 45mg/kg) (Figure 28B), at one hour after infection with the following *P. aeruginosa* strains (6206 (1.0 x 10^6), 6077 (1.0 x 10^6), 6294 (2.0 x 10^7) or PA103 (1.0 x 10^6)).

[0388] Survival effect of the Bs4-GLO bispecific antibody was evaluated in an acute lethal pneumonia model against different *P. aeruginosa* strains as previously described in Example 6. Figure 29 shows survival rates for animals treated with the Bs4-GLO in a *P. aeruginosa* lethal bacteremia model. Aspects of the bacteremia model are disclosed in detail in U.S. Provisional Appl. No. 61/723,128, filed November 6, 2012 (attorney docket no. ATOX-500P1, entitled "METHODS OF TREATING *S. AUREUS* ASSOCIATED DISEASES"), which is incorporated herein by reference in its entirety.

[0389] Animals were treated with Bs4-GLO or R347, 24 hours prior to intraperitoneal infection with (A) 6294 (O6) or (B) 6206. The BS4-GLO is effective at all tested concentrations in protection against lethal pneumonia in mice challenged with *P. aeruginosa* strains (A) 6294 and (B) 6206.

[0390] Survival effect of the Bs4-GLO bispecific antibody was evaluated in a *P. aeruginosa* thermal injury model against different *P. aeruginosa* strains. Figure 30 shows survival rates for animals prophylactically treated with the Bs4-GLO in a *P. aeruginosa* thermal injury model. Animals were treated with Bs4-GLO or R347 hours prior to induction of thermal injury and subcutaneous infection with *P. aeruginosa* strain (A) 6077 (O11-ExoU$^+$) or (B) 6206 (O11-ExoU$^+$) or (C) 6294 (O6) directly under the wound. The BS4-GLO is effective at all tested concentrations in prevention in a *P. aeruginosa* thermal injury model in mice challenged with *P. aeruginosa* strains (A) 6077, (B) 6206 and (C) 6294.

[0391] Figure 31 shows survival rates for animals therapeutically treated with bispecific antibody Bs4-GLO in a *P. aeruginosa* thermal injury model. (A) Animals were treated with Bs4-GLO or R347 (A) 4h hours or (B) 12 hours after induction of thermal injury and subcutaneous infection with *P. aeruginosa* strain 6077 (O11-ExoU$^+$) directly under the wound. The Bs4-GLO is effective at all tested concentrations in treatment in a *P.*

aeruginosa thermal injury model in mice treated with Bs4-GLO (B) 4h hours or (B) 12 hours after induction of thermal injury and subcutaneous infection with *P. aeruginosa* strain 6077.

Example 20: Therapeutic adjunctive therapy: Bs4-GLO + antibiotic

[0392] Survival effect of the Bs4-GLO bispecific antibody and antibiotic adjunctive therapy was evaluated in an acute lethal pneumonia model against *P. aeruginosa* 6206 strain as previously described in Example 6.

[0393] Figure 32 shows therapeutic adjunctive therapy with ciprofloxacin (CIP). (A) Mice were treated 4 hour post infection with *P. aeruginosa* strain 6206 with R347 + CIP or Bs4-WT or a combination of the Bs4-WT and CIP. (B) Mice were treated 4 hour post infection with *P. aeruginosa* strain 6206 with R347 + CIP or Bs4-GLO or a combination of the Bs4-GLO and CIP. (A-B) Bs4-WT or BS4-GLO antibody combined with CIP increased efficacy of antibiotic therapy.

[0394] Figure 33 shows therapeutic adjunctive therapy with meropenem (MEM): (A) Mice were treated 4 hour post infection with *P. aeruginosa* strain 6206 with R347 + MEM or Bs4-WT or a combination of the BS4-WT and MEM. (B) Mice were treated 4 hour post infection with *P. aeruginosa* strain 6206 with R347 + MEM or BS4 or a combination of the Bs4-GLO and MEM. (A-B) Bs4-WT or Bs4-GLO antibody combined with MEM increases efficacy of antibiotic therapy.

[0395] Figure 34 shows therapeutic adjunctive therapy: Bs4-GLO plus antibiotic in a lethal bacteremia model. Mice were treated 24 hours prior to intraperitoneal infection with *P. aeruginosa* strain 6294 with Bs4-GLO at the indicated concentrations, which were previously determine to be sub-therapeutic protective doses in this model and R347 (negative control). One hour post infection, mice were treated subcutaneously with antibiotics at the indicated concentrations, which were previously determined to be sub-therapeutic protective doses (A) Ciprofloxacin (CIP), (B) Meropenem (MEM) or (C) Tobramycin (TOB). Animals were carefully monitored for survival up to 72 hours post-infection. Bs4-GLO antibody combined with either CIP, MEM or TOB, at sub-protective doses, increases efficacy of antibiotic therapy.

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[0396] The disclosure is not to be limited in scope by the specific embodiments described which are intended as single illustrations of individual aspects of the disclosure, and any compositions or methods which are functionally equivalent are within the scope of this disclosure. Indeed, various modifications of the disclosure in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description and accompanying drawings. Such modifications are intended to fall within the scope of the appended claims.

[0397] All publications and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference. In addition, U.S. Provisional Application Nos.: 61/556,645 filed November 7, 2011; 61/624,651 filed April 16, 2012; 61/625,299 filed April 17, 2012; 61/697,585 filed September 6, 2012 and International Application No: PCT/US2012/63639, filed November 6, 2012 (attorney docket no. AEMS-115WO1, entitled "MULTISPECIFIC AND MULTIVALENT BINDING PROTEINS AND USES THEREOF") are incorporated by reference in their entirety for all purposes.

Table 6A

Group(n)	Treatment	Overall Impression (Involved Lung Surface Area)	Hemorrhage	Edema	Inflammatory Infiltrate	Bacteria
4(3)	R347 (0.2mg/kg)	Broncho interstitial pneumonia (57%), epithelial injury, marked congestion	3+ Extensive	3+	PMN 3+	2+
1(3)	V2L2 (0.2mg/kg)	Broncho interstitial pneumonia (57%), mild epithelial injury, moderate congestion	3+ Extensive	3+	PMN 3+	Neg-1+
6(3)	WapR-004 (0.2mg/kg)	Broncho interstitial pneumonia (57%), mod epithelial injury, marked congestion	3+ Extensive	3+	PMN 3+	2+
2(3)	BS2-V2L2 (0.2mg/kg)	Broncho interstitial pneumonia (27%), mild epithelial injury, mild to moderate congestion	3+ Moderate	2+-3+	PMN 2+	±
3(3)	BS3-V2L2 (0.2mg/kg)	Broncho interstitial pneumonia (20%), mild epithelial injury, mild to moderate congestion	3+ Mild	2+	PMN 1+-2+	±
5(2)	WapR-4 + V2L2 (0.1mg/kg ea)	Primarily Broncho pneumonia (20%) mild epithelial injury, mild congestion	3+ Mild	2+	PMN 1+-2+	Neg- ±

Table 6B

Group(n)	Treatment	Overall Impression (Involved Lung Surface Area)	Hemorrhage	Edema	Inflammatory Infiltrate	Bacteria
4(3)	R347 (0.2mg/kg)	Broncho interstitial pneumonia (40%), mild epithelial injury, moderate congestion	3+	3+	PMN 2+	2+
1(3)	V2L2 (0.2mg/kg)	Broncho interstitial pneumonia (30%), mild epithelial injury, mild congestion	2+	3+	PMN 2+	Neg
6(3)	WapR-004 (0.2mg/kg)	Broncho interstitial pneumonia (40%), mod epithelial injury, moderate congestion	3+	3+	PMN 2+	Neg-2+
2(3)	BS2-V2L2 (0.2mg/kg)	Broncho interstitial pneumonia (20%), mild epithelial injury, mild congestion	2+	2+	PMN 1+	Neg
3(3)	BS3-V2L2 (0.2mg/kg)	Broncho pneumonia mild epithelial injury	1+	±	±	Neg
5(2)	WapR-4 + V2L2 (0.1mg/kg ea)	Primarily Broncho pneumonia mild epithelial injury,	1+	±	±	Neg

Table 7

Group (n)	Treatment	Overall Impression (Involved Lung Surface Area)	Hemorrhage	Edema	Inflammatory Infiltrate	Bacteria
4(3)	R347 (0.2 mg/kg)	Broncho interstitial pneumonia (57%), epithelial injury, marked congestion	3+	3+	PMN 3+	1+
1(3)	V2L2 (0.2 mg/kg)	Broncho interstitial pneumonia (40%), mild epithelial injury, moderate congestion	3+	3+	PMN 2-3+	±
6(3)	WapR-004 (0.2 mg/kg)	Broncho interstitial pneumonia (36%), mild epithelial injury, marked congestion	3+	3+	PMN 2+	Neg-1+
2(3)	BS2-V2L2 (0.2 mg/kg)	Broncho interstitial pneumonia (22%), mild to moderate congestion	1+-2+	1+-2+	PMN 1-2+	Neg
3(3)	BS3-V2L2 (0.2 mg/kg)	Broncho interstitial pneumonia (20%), mild to moderate congestion	1+	1+	PMN 1+	Neg
5(3)	WapR-4 + V2L2 (0.1mg/kg ea)	Primarily Broncho pneumonia (<10%) mild congestion	1+	2+	±	Neg

WHAT IS CLAIMED IS:

1. An isolated binding molecule which specifically binds to *Pseudomonas* PcrV, comprising an immunoglobulin VH and an immunoglobulin VL, wherein the VH comprises an amino acid sequence selected from the group consisting of SEQ ID NO:255 and SEQ ID NO:257, and wherein the VL comprises the amino acid sequence of SEQ ID NO:256.

2. The binding molecule of claim 1, wherein the VH comprises SEQ ID NO:255.

3. The binding molecule of claim 1, wherein the VH comprises SEQ ID NO:257.

4. An isolated binding molecule which specifically binds to *Pseudomonas* PcrV, comprising an immunoglobulin VH and an immunoglobulin VL, each comprising a CDR1, CDR2, and CDR3,

(a) wherein the VH CDR1 is SEQ ID NO:311) or a variant thereof comprising 1, 2, 3, or 4 conservative amino acid substitutions, the VH CDR2 is SEQ ID NO:312, or a variant thereof comprising 1, 2, 3, or 4 conservative amino acid substitutions, and the VHCDR3 is SEQ ID NO: 313, or a variant thereof comprising 1, 2, 3, or 4 conservative amino acid substitutions; or

(b) wherein the VL CDR1 is SEQ ID NO:314, or a variant thereof comprising 1, 2, 3, or 4 conservative amino acid substitutions, the VL CDR2 is SEQ ID NO:315, or a variant thereof comprising 1, 2, 3, or 4 conservative amino acid substitutions, and the VL CDR3 is SEQ ID NO:316, or a variant thereof comprising 1, 2, 3, or 4 conservative amino acid substitutions; or

(c) a combination of (a) and (b);

wherein the VH and VL CDRs are according to the Kabat numbering system.

5. The binding molecule of claim 4,

(a) wherein the VH CDR1 is SEQ ID NO:311) the VH CDR2 is SEQ ID NO:312, and the VHCDR3 is SEQ ID NO:313; or

(b) wherein the VL CDR1 is SEQ ID NO:314, the VL CDR2 is SEQ ID NO:315, and the VL CDR3 is SEQ ID NO:316; or

(c) a combination of (a) and (b).

6. An isolated binding molecule which specifically binds to *Pseudomonas* PcrV, comprising an immunoglobulin VH and an immunoglobulin VL,

(a) wherein the VH comprises an amino acid sequence at least 90% identical to SEQ ID NO:317; or

(b) wherein the VL comprises an amino acid sequence at least 90% identical to SEQ ID NO:318; or

(c) a combination of (a) and (b).

7. The binding molecule of claim 6, wherein the VH comprises SEQ ID NO:317 and the VL comprises SEQ ID NO:318.

8. The binding molecule of any one of claims 1 to 7, which comprises an anti-PcrV antibody or antigen-binding fragment thereof.

9. The binding molecule of claim 8, wherein the VH is part of an antibody heavy chain which further comprises one or more heavy chain constant regions, and wherein the VL is part of an antibody light chain which further comprises a light chain constant region.

10. The binding molecule of claim 9, comprising two antibody heavy chains and two antibody light chains.

11. The binding molecule of any one of claims 8 to 10, which comprises a bispecific antibody.

12. The binding molecule of claim 11, further comprising a binding domain which specifically binds to *Pseudomonas* Psl.

13. The binding molecule of claim 12, wherein the binding domain which specifically binds to *Pseudomonas* Psl comprises an anti-Psl ScFv molecule.

14. The binding molecule of claim 13, wherein the anti-Psl ScFv molecule comprises an amino acid sequence of SEQ ID NO:240 – SEQ ID NO:254, or any combination of two or more amino acid sequences of SEQ ID NO:240 – SEQ ID NO:254.

15. The binding molecule of claim 13 or claim 14, wherein an anti-Psl ScFv molecule is inserted into the hinge region of each heavy chain of the anti-PcrV antibody or fragment thereof.

16. The binding molecule of claim 15, wherein each antibody heavy chain comprises the formula VH-CH1-H1-L1-S-L2-H2-CH2-CH3, wherein CH1 is a heavy chain constant region domain-1, H1 is a first heavy chain hinge region fragment, L1 is a first linker, S is an anti-PcrV ScFv molecule, L2 is a second linker, H2 is a second heavy chain hinge region fragment, CH2 is a heavy chain constant region domain-2, and CH3 is a heavy chain constant region domain-3.

17. The binding molecule of claim 16, wherein VH comprises the amino acid sequence of SEQ ID NO:255, SEQ ID NO:257, or SEQ ID NO:317.

18. The binding molecule of claim 17, wherein CH1 comprises SEQ ID NO:319.

19. The binding molecule of any one of claims 16 to 18, wherein L1 and L2 are the same or different, and independently comprise (a) [GGGGS]n, wherein n is 0, 1, 2, 3, 4, or 5, (b) [GGGG]n, wherein n is 0, 1, 2, 3, 4, or 5, or a combination of (a) and (b).

20. The binding molecule of any one of claims 16 to 19, wherein H1 comprises EPKSC (SEQ ID NO:320).

21. The binding molecule of any one of claims 16 to 20, wherein H2 comprises DKTHTCPPCP (SEQ ID NO:321).

22. The binding molecule of any one of claims 16 to 21, wherein S comprises an amino acid sequence selected from the group consisting of SEQ ID NO:240 to SEQ ID NO:254, and any combination thereof.

23. The binding molecule of any one of claims 16 to 22, wherein CH2-CH3 comprises APELLGGPSVFLFPPKPKDTL**X1**I**X2**R**X3**PEVTCVVVDVSHEDPEVKFNWY VDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTI

SKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTP PSLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:322), wherein X1 is M or Y, X2 is S or T, and X3 is T or E.

24. The binding molecule of any one of claims 16 to 23, wherein each antibody light chain comprises VL-CL, wherein CL is an antibody light chain kappa constant region or an antibody light chain lambda constant region.

25. The binding molecule of claim 24, wherein VL comprises the amino acid sequence of SEQ ID NO:256 or SEQ ID NO:318.

26. The binding molecule of claim 24, wherein CL comprises the amino acid sequence of SEQ ID NO:323.

27. The binding molecule of any one of claims 15 to 26, comprising two identical heavy chains and two identical light chains, wherein each antibody heavy chain comprises SEQ ID NO:264, and wherein each antibody light chain comprises SEQ ID NO: 263.

28. A bispecific antibody which specifically binds to *Pseudomonas* Psl and *Pseudomonas* PcrV, comprising an immunoglobulin heavy chain and an immunoglobulin light chain, wherein the heavy chain comprises the amino acid sequence of SEQ ID NO:264, and the light chain comprises the amino acid sequence of SEQ ID NO:263.

29. An isolated binding molecule which specifically binds to *Pseudomonas* Psl, comprising an immunoglobulin VH and an immunoglobulin VL, each comprising a complementarity determining region 1 (CDR1), CDR2, and CDR3, wherein the VH CDR1 is SEQ ID NO:47, the VH CDR2 is SEQ ID NO: 48, the VH CDR3 is selected from the group consisting of SEQ ID NO:258, SEQ ID NO:267, SEQ ID NO:268, SEQ ID NO:269, SEQ ID NO:270, SEQ ID NO:271, SEQ ID NO:272, SEQ ID NO:273, SEQ ID NO:274, SEQ ID NO:275, SEQ ID NO:276, SEQ ID NO:277, SEQ ID NO:278, and SEQ ID NO:279, the VL CDR1 is SEQ ID NO:50, the VL CDR2 is SEQ ID NO:51, and the VL CDR3 is selected from the group consisting of SEQ ID NO:280, SEQ ID NO:281, SEQ ID NO:282, SEQ ID NO:52, SEQ ID NO:283, SEQ ID NO:284, SEQ ID NO:285, SEQ ID NO:286, and SEQ ID NO:287, wherein the VH and VL CDRs are according to the Kabat numbering system.

30. The binding molecule of claim 29, wherein the VH CDR3 is SEQ ID NO:258, and the VL CDR3 is SEQ ID NO:280.

31. The binding molecule of claim 30, wherein the VH comprises SEQ ID NO:288, and the VL comprises SEQ ID NO:289.

32. The binding molecule of claim 29, wherein the VH CDR3 is SEQ ID NO:267, and the VL CDR3 is SEQ ID NO:281.

33. The binding molecule of claim 32, wherein the VH comprises SEQ ID NO:290, and the VL comprises SEQ ID NO:291.

34. The binding molecule of claim 29, wherein the VH CDR3 is SEQ ID NO:268, and the VL CDR3 is SEQ ID NO:282.

35. The binding molecule of claim 34, wherein the VH comprises SEQ ID NO:292, and the VL comprises SEQ ID NO:293.

36. The binding molecule of claim 29, wherein the VH CDR3 is SEQ ID NO:269, and the VL CDR3 SEQ ID NO:52.

37. The binding molecule of claim 36, wherein the VH comprises SEQ ID NO:294, and the VL comprises SEQ ID NO:11.

38. The binding molecule of claim 29, wherein the VH CDR3 is SEQ ID NO:270, and the VL CDR3 is SEQ ID NO:283.

39. The binding molecule of claim 38, wherein the VH comprises SEQ ID NO:295, and the VL comprises SEQ ID NO:296.

40. The binding molecule of claim 29, wherein the VH CDR3 is SEQ ID NO:271, and the VL CDR3 is SEQ ID NO:284.

41. The binding molecule of claim 40, wherein the VH comprises SEQ ID NO:297, and the VL comprises SEQ ID NO:298.

42. The binding molecule of claim 29, wherein the VH CDR3 is SEQ ID NO:272, and the VL CDR3 is SEQ ID NO:285.

43. The binding molecule of claim 42, wherein the VH comprises SEQ ID NO:299, and the VL comprises SEQ ID NO:300.

44. The binding molecule of claim 29, wherein the VH CDR3 is SEQ ID NO:273, and the VL CDR3 is SEQ ID NO:286.

45. The binding molecule of claim 44, wherein the VH comprises SEQ ID NO:301, and the VL comprises SEQ ID NO:302.

46. The binding molecule of claim 29, wherein the VH CDR3 is SEQ ID NO:274, and the VL CDR3 is SEQ ID NO:52.

47. The binding molecule of claim 46, wherein the VH comprises SEQ ID NO:303, and the VL comprises SEQ ID NO:11.

48. The binding molecule of claim 29, wherein the VH CDR3 is SEQ ID NO:275, and the VL CDR3 is SEQ ID NO:52.

49. The binding molecule of claim 48, wherein the VH comprises SEQ ID NO:304, and the VL comprises SEQ ID NO:11.

50. The binding molecule of claim 29, wherein the VH CDR3 is SEQ ID NO:276, and the VL CDR3 is SEQ ID NO:52.

51. The binding molecule of claim 50, wherein the VH comprises SEQ ID NO:305, and the VL comprises SEQ ID NO:11.

52. The binding molecule of claim 29, wherein the VH CDR3 is SEQ ID NO:277, and the VL CDR3 is SEQ ID NO:52.

53. The binding molecule of claim , wherein the VH comprises SEQ ID NO:306, and the VL comprises SEQ ID NO:11.

54. The binding molecule of claim 29, wherein the VH CDR3 is SEQ ID NO:278, and the VL CDR3 is SEQ ID NO:52.

55. The binding molecule of claim 54, wherein the VH comprises SEQ ID NO:307, and the VL comprises SEQ ID NO:11.

56. The binding molecule of claim 1, wherein the VH CDR3 is SEQ ID NO:279, and the VL CDR3 is SEQ ID NO:287.

57. The binding molecule of claim 56, wherein the VH comprises SEQ ID NO:308, and the VL comprises SEQ ID NO:325.

58. An isolated binding molecule which specifically binds to *Pseudomonas* Psl, comprising an immunoglobulin VH and an immunoglobulin VL, wherein the VH comprises the amino acid sequence of SEQ ID NO:309, and wherein the VL comprises the amino acid sequence of SEQ ID NO:310.

59. The binding molecule of any one of claims 29 to 58, which comprises an anti-Psl antibody or antigen-binding fragment thereof.

60. The binding molecule of claim 59, which comprises a single-chain Fv (ScFv) antibody molecule.

61. The binding molecule of claim 60, wherein the ScFv comprises the formula: VH-L-VL, and wherein L is a linker.

62. The binding molecule of claim 61, wherein the ScFv comprises the formula: VL-L-VH, and wherein L is a linker.

63. The binding molecule of claim 61 or claim 62, wherein L comprises (a) [GGGGS]n, wherein n is 0, 1, 2, 3, 4, or 5, (b) [GGGG]n, wherein n is 0, 1, 2, 3, 4, or 5, or a combination of (a) and (b).

64. The binding molecule of claim 63, wherein the linker further comprises ala-leu at the C-terminus of the linker.

65. The binding molecule of any one of claims 29, 30, 31, 59 to 61, 63, or 64, comprising the amino acid sequence of SEQ ID NO:240.

66. The binding molecule of any one of claims 29, 30, 31, 59 to 61, 63, or 64, comprising the amino acid sequence of SEQ ID NO:262.

67. The binding molecule of any one of claims 29, 32 to 61, 63, or 64, comprising an amino acid sequence selected from the group consisting of SEQ ID NO:241, SEQ ID NO:242, SEQ ID NO:243, SEQ ID NO:244, SEQ ID NO:245, SEQ ID NO:246, SEQ ID NO:247, SEQ ID NO:248, SEQ ID NO:249, SEQ ID NO:250, SEQ ID NO:251, SEQ ID NO:252, SEQ ID NO:253, SEQ ID NO:254, and any combination thereof.

68. An isolated polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO:240, SEQ ID NO:241, SEQ ID NO:242, SEQ ID NO:243, SEQ ID NO:244, SEQ ID NO:245, SEQ ID NO:246, SEQ ID NO:247, SEQ ID NO:248, SEQ ID NO:249, SEQ ID NO:250, SEQ ID NO:251, SEQ ID NO:252, SEQ ID NO:253, SEQ ID NO:254, SEQ ID NO:262, SEQ ID NO:255, SEQ ID NO:256, SEQ ID NO:257, SEQ ID NO:263, SEQ ID NO:264, SEQ ID NO:258, SEQ ID NO:263, SEQ ID NO:264, SEQ ID NO:267, SEQ ID NO:268, SEQ ID NO:269, SEQ ID NO:270, SEQ ID NO:271, SEQ ID NO:272, SEQ ID NO:273, SEQ ID NO:274, SEQ ID NO:275, SEQ ID NO:276, SEQ ID NO:277 SEQ ID NO:278 SEQ ID NO:279, SEQ ID NO:280, SEQ ID NO:281, SEQ ID NO:282, SEQ ID NO:283, SEQ ID NO:284, SEQ ID NO:285, SEQ ID NO:286, SEQ ID NO:287, SEQ ID NO:288, SEQ ID NO:289, SEQ ID NO:290, SEQ ID NO:291, SEQ ID NO:292, SEQ ID NO:293, SEQ ID NO:294, SEQ ID NO:295, SEQ ID NO:296, SEQ ID NO:298, SEQ ID NO:299,300, SEQ ID NO:301, SEQ ID NO:302, SEQ ID NO:303, SEQ ID NO:304, SEQ ID NO:305, SEQ ID NO:306, SEQ ID NO:307, SEQ ID NO:308, SEQ ID NO:309, SEQ ID NO:310, SEQ ID NO:311, SEQ ID NO:312, SEQ ID NO:313, SEQ ID NO:315, SEQ ID NO:316, SEQ ID NO:317, SEQ ID NO:318, SEQ ID NO:325, and any combination thereof.

69. A cell comprising or producing the binding molecule of any one of claims 1-27, or 29-67, or a subunit thereof, the bispecific antibody of claim 28 or a subunit thereof, the polypeptide of claim 68, or any combination thereof.

70. An isolated polynucleotide comprising a nucleic acid which encodes the binding molecule of any one of claims 1-27, or 29-67, or a subunit thereof, the bispecific antibody of claim 28 or a subunit thereof, the polypeptide of claim 68, or any combination thereof.

71. A vector comprising the polynucleotide of claim 70.

72. A cell comprising the polynucleotide of claim 69 or the vector of claim 70.

73. A composition comprising the binding molecule of any one of claims 1-27, or 29-67, or an antigen-binding subunit thereof, and a pharmaceutically acceptable carrier.

74. A composition comprising the bispecific antibody of claim 28 or an antigen-binding subunit thereof, and a pharmaceutically acceptable carrier.

75. The composition of claim 73 or claim 74, which binds to at least 80%, at least 85%, at least 90% or at least 95% of *P. aeruginosa* strains isolated from infected patients.

76. The composition of claim 75, wherein the *P. aeruginosa* strains are isolated from one or more of lung, sputum, eye, pus, feces, urine, sinus, a wound, skin, blood, bone, or knee fluid.

77. The composition of any of claims 73 to 76, wherein the binding molecule or a subunit thereof, or the bispecific antibody or a subunit thereof, is conjugated to an agent selected from the group consisting of antimicrobial agent, a therapeutic agent, a prodrug, a peptide, a protein, an enzyme, a lipid, a biological response modifier, pharmaceutical agent, a lymphokine, a heterologous antibody or fragment thereof, a detectable label, polyethylene glycol (PEG), and a combination of two or more of any said agents.

78. The composition of claim 77, wherein the detectable label is selected from the group consisting of an enzyme, a fluorescent label, a chemiluminescent label, a bioluminescent label, a radioactive label, or a combination of two or more of any said detectable labels.

79. The composition of any of claims 73 to 78, further comprising an antibiotic.

80. The composition of claim 79, wherein the antibiotic is selected from the group consisting of Ciprofloxacin, Meropenem, and a combination thereof.

81. A method of preventing or treating a *Pseudomonas* infection in a subject in need thereof, comprising administering to a subject an effective amount of the composition of any one of claims 73-80.

82. A method of preventing or treating a *Pseudomonas* infection in a subject in need thereof, comprising administering to a subject an effective amount of the bispecific antibody of claim 28 or the composition of any one of claims 74-80 wherein the composition comprises a bispecific antibody.

83. The method of claim 82, wherein the administration provides a synergistic therapeutic effect in the prevention or treatment of the *Pseudomonas* infection in the subject, and wherein the synergistic effect is greater than the sum of the individual effects of administration of equimolar quantities of monospecific binding molecules with the same *Pseudomonas* Psl and *Pseudomonas* PcrV binding specificities as the bispecific antibody.

84. The method of claim 83, wherein the synergistic therapeutic effect results in greater percent survival than the additive percent survival of subjects to which only one of the binding domains has been administered.

85. The method of any one of claims 81 to 84, wherein said composition is administered for two or more prevention/treatment cycles.

86. A method of preventing or treating a *Pseudomonas* infection in a subject in need thereof, comprising administering to a subject an effective amount of the composition of any one of claims 79 or 80, wherein said administration provides a synergistic therapeutic effect in the prevention or treatment of the *Pseudomonas* infection in the subject, and wherein said synergistic effect is greater than the sum of the individual effects of administration of equimolar quantities of one or more of (a) just the bispecific antibody, (b), the monospecific binding molecules with the same *Pseudomonas* Psl and *Pseudomonas* PcrV binding specificities as the bispecific antibody and (c) the antibiotic.

87. The method of claim 86, wherein the composition is administered for two or more prevention/treatment cycles.

88. The method of any of claims 81 to 87, wherein the *Pseudomonas* infection is a *P. aeruginosa* infection.

89. The method of any of claims 81 to 88, wherein the subject is a human

90. The method of any one of claims 81 to 89, wherein the infection is an ocular infection, a lung infection, a burn infection, a wound infection, a skin infection, a blood infection, a bone infection, or a combination of two or more of said infections.

91. The method of any one of claims 81 to 90, wherein the subject has acute pneumonia, burn injury, corneal infection, cystic fibrosis, or a combination thereof.

92. A kit comprising the composition of any one of claims 73 to 80.