

NEUTRALIZING HUMAN MONOCLONAL ANTIBODIES AGAINST P. AERUGINOSATechnical Field

The present invention relates to antibodies or antigen-binding fragments thereof against *Pseudomonas aeruginosa*, pharmaceutical compositions comprising such antibodies or antigen-binding fragments thereof, kits comprising such antibodies or antigen-binding fragments thereof, and the antibodies or antigen-binding fragments thereof, the pharmaceutical compositions and the kits for use as a medicament, and in the treatment or prevention of a disease caused by *Pseudomonas aeruginosa*. The present invention further relates to methods of treating, preventing or reducing the severity of an infection with *Pseudomonas aeruginosa*, and to nucleic acids encoding such antibodies or antigen-binding fragments thereof, expression vectors comprising such nucleic acids, host cells comprising such nucleic acids or expression vectors, and methods for the production of such antibodies or antigen-binding fragments thereof.

Technological Background

Antimicrobial resistance is an emerging global threat with increasing morbidity and mortality worldwide. This critical situation is aggravated by an innovation and discovery gap leading to a tremendous lack of substances with antibacterial activity. Alternative approaches such as antibody or bacteriophage-based therapies as well as anti-virulence or host directed drugs are required to meet the global needs of therapeutics active against drug-resistant bacteria.

In the last two decades, several studies demonstrated the therapeutic potential of neutralizing antibodies against viral infections. Here, broadly neutralizing antibodies (bNAbs) were mainly identified by performing a comprehensive assessment of antigen-reactive B cells derived from infected, convalescent, or vaccinated individuals. However, while numerous antibodies have been developed to target viral pathogens, antibody-mediated treatment approaches against bacterial pathogens were rarely successful and only a limited number of antibodies were identified to potentially neutralize bacterial pathogens.

Pseudomonas aeruginosa (hereinafter also referred to as *P. aeruginosa* or PA) is a Gram-negative pathogen which frequently causes severe nosocomial infections including pneumonia and sepsis. *Pseudomonas aeruginosa* has been classified as a serious threat to the public health by the Centers for Disease Control and Prevention (CDC) and the World Health Organization (WHO) due to

extensive intrinsic and extrinsic resistance mechanisms. Besides acute infections, *Pseudomonas aeruginosa* is also capable of causing chronic infections, for instance in patients with structural lung diseases such as chronic obstructive pulmonary disease (COPD) or cystic fibrosis (CF), a monogenetic disease determined by Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) mutations. Decreased mucociliary clearance of the bronchial system and production of a nutrient-rich, hyper-viscous airway mucus in this disease provide ideal growth conditions for opportunistic pathogens such as *Pseudomonas aeruginosa*.

A key virulence factor of *Pseudomonas aeruginosa* is the type III secretion system (T3SS), a syringe-like, multiprotein structure which injects effector toxins such as ExoS and ExoU into the cytosol of host cells leading to cell lysis and tissue damage. The T3SS has been linked to bacterial persistence, higher relapse rates and increased mortality in infected patients. PcrV, a pentameric structural protein forms the T3SS needle-tip complex which is required for appropriate assembly of the PopB/D translocon complex and its insertion into the host cell membrane. As immunogenicity of PcrV has been known for decades, several works have been focusing on antibody-mediated abrogation of PcrV function to inhibit virulence of *Pseudomonas aeruginosa*, for instance, US 2005/0063985 A1, US 2013/0108627 A1, and a study by DiGiandomenico (DiGiandomenico et al., 2014).

Safety, efficacy, and pharmacokinetics of the anti-PcrV monoclonal antibody of DiGiandomenico A *et al.* was evaluated in a clinical trial, where it was found that the anti-PcrV monoclonal antibody was unable to reduce the incidence of nosocomially-acquired pneumonia caused by *Pseudomonas aeruginosa* in mechanically ventilated patients infected with *Pseudomonas aeruginosa*. Previous works rely on immunization of mice to generate PcrV-specific antibody sequences, while in-depth investigations of the human B cell response to PcrV and subsequent exploitation of the B cell repertoire for the development of patient-derived highly neutralizing antibodies are lacking so far.

As *Pseudomonas aeruginosa* can reside over years in the airways of people with CF (pwCF), it is hypothesized that the repetitive antigen exposure in these patients fosters a highly affinity matured adaptive immune response, which results in the development of antibodies potentially inhibiting virulence of *Pseudomonas aeruginosa*.

Therefore, it is essential to develop fully human monoclonal antibodies which are able to strongly inhibit the cytotoxic effect of *Pseudomonas aeruginosa* in a human patient, achieve a comparable or improved effect in comparison to the antibiotic levofloxacin, and against which *Pseudomonas aeruginosa* has little to no natural resistance.

Thus, it is an object of the present invention to provide novel monoclonal antibodies against *Pseudomonas aeruginosa* which do not demonstrate autoreactivity and have excellent neutralization potency against circulating resistant *Pseudomonas aeruginosa* strains.

It is a further object of the present invention to provide novel monoclonal antibodies against *Pseudomonas aeruginosa* which can be used in treatment or prevention of a disease caused by *Pseudomonas aeruginosa* in human or animal subjects as well as in prevention of infection of a human or animal subject with *Pseudomonas aeruginosa*.

Summary of the invention

These objects have been solved by the present invention as specified hereinafter.

According to a first aspect of the present invention, an antibody or antigen-binding fragment thereof is provided which is directed against *Pseudomonas aeruginosa*, wherein the antibody or antigen-binding fragment thereof comprises the combination of the heavy chain CDR1 to CDR3 and the light chain CDR1 to CDR3 amino acid sequence of one antibody selected from the group comprising 30-D9 (having a CDR-H1 amino acid sequence of SEQ ID No. 21, a CDR-H2 amino acid sequence of SEQ ID No. 22, a CDR-H3 amino acid sequence of SEQ ID No. 23, a CDR-L1 amino acid sequence of SEQ ID No. 24, a CDR-L2 amino acid sequence of SEQ ID No. 25, a CDR-L3 amino acid sequence of SEQ ID No. 26), 30-B8 (having a CDR-H1 amino acid sequence of SEQ ID No. 27, a CDR-H2 amino acid sequence of SEQ ID No. 28, a CDR-H3 amino acid sequence of SEQ ID No. 29, a CDR-L1 amino acid sequence of SEQ ID No. 30, a CDR-L2 amino acid sequence of SEQ ID No. 31, a CDR-L3 amino acid sequence of SEQ ID No. 32), 30-D7 (having a CDR-H1 amino acid sequence of SEQ ID No. 33, a CDR-H2 amino acid sequence of SEQ ID No. 34, a CDR-H3 amino acid sequence of SEQ ID No. 35, a CDR-L1 amino acid sequence of SEQ ID No. 36, a CDR-L2 amino acid sequence of SEQ ID No. 37, a CDR-L3 amino acid sequence of SEQ ID No. 38), 11-A6 (having a CDR-H1 amino acid sequence of SEQ ID No. 39, a CDR-H2 amino acid sequence of SEQ ID No. 40, a CDR-H3 amino acid sequence of SEQ ID No. 41, a CDR-L1 amino acid sequence of SEQ ID No. 42, a CDR-L2 amino acid sequence of SEQ ID No. 43, a CDR-L3 amino acid sequence of SEQ ID No. 44), 23-A9 (having a CDR-H1 amino acid sequence of SEQ ID No. 45, a CDR-H2 amino acid sequence of SEQ ID No. 46, a CDR-H3 amino acid sequence of SEQ ID No. 47, a CDR-L1 amino acid sequence of SEQ ID No. 48, a CDR-L2 amino acid sequence of SEQ ID No. 49, a CDR-L3 amino acid sequence of SEQ ID No. 50), 11-C10 (having a CDR-H1 amino acid sequence of SEQ ID No. 51, a CDR-H2 amino acid sequence of SEQ ID No. 52, a CDR-H3 amino acid sequence of SEQ ID No. 53, a CDR-L1 amino acid sequence of SEQ ID No. 54, a CDR-L2 amino acid sequence of SEQ ID No. 55, a CDR-L3 amino acid sequence of SEQ ID No. 56), 23-F9 (having a CDR-H1 amino acid sequence of

SEQ ID No. 57, a CDR-H2 amino acid sequence of SEQ ID No. 58, a CDR-H3 amino acid sequence of SEQ ID No. 59, a CDR-L1 amino acid sequence of SEQ ID No. 60, a CDR-L2 amino acid sequence of SEQ ID No. 61, a CDR-L3 amino acid sequence of SEQ ID No. 62), 11-C4 (having a CDR-H1 amino acid sequence of SEQ ID No. 63, a CDR-H2 amino acid sequence of SEQ ID No. 64, a CDR-H3 amino acid sequence of SEQ ID No. 65, a CDR-L1 amino acid sequence of SEQ ID No. 66, a CDR-L2 amino acid sequence of SEQ ID No. 67, a CDR-L3 amino acid sequence of SEQ ID No. 68), 30-B9 (having a CDR-H1 amino acid sequence of SEQ ID No. 69, a CDR-H2 amino acid sequence of SEQ ID No. 70, a CDR-H3 amino acid sequence of SEQ ID No. 71, a CDR-L1 amino acid sequence of SEQ ID No. 72, a CDR-L2 amino acid sequence of SEQ ID No. 73, a CDR-L3 amino acid sequence of SEQ ID No. 74), and 30-C9 (having a CDR-H1 amino acid sequence of SEQ ID No. 75, a CDR-H2 amino acid sequence of SEQ ID No. 76, a CDR-H3 amino acid sequence of SEQ ID No. 77, a CDR-L1 amino acid sequence of SEQ ID No. 78, a CDR-L2 amino acid sequence of SEQ ID No. 79, a CDR-L3 amino acid sequence of SEQ ID No. 80).

In one embodiment of the first aspect of the invention, the antibody or antigen-binding fragment thereof comprises the combination of the variable region heavy chain amino acid sequence and of the variable region light chain amino acid sequence of one antibody selected from the group comprising 30-D9 (having the variable region heavy chain amino acid sequence of SEQ ID No. 1 and the variable region light chain amino acid sequence of SEQ ID No. 2), 30-B8 (having the variable region heavy chain amino acid sequence of SEQ ID No. 3 and the variable region light chain amino acid sequence of SEQ ID No. 4), 30-D7 (having the variable region heavy chain amino acid sequence of SEQ ID No. 5 and the variable region light chain amino acid sequence of SEQ ID No. 6), 11-A6 (having the variable region heavy chain amino acid sequence of SEQ ID No. 7 and the variable region light chain amino acid sequence of SEQ ID No. 8), 23-A9 (having the variable region heavy chain amino acid sequence of SEQ ID No. 9 and the variable region light chain amino acid sequence of SEQ ID No. 10), 11-C10 (having the variable region heavy chain amino acid sequence of SEQ ID No. 11 and the variable region light chain amino acid sequence of SEQ ID No. 12), 23-F9 (having the variable region heavy chain amino acid sequence of SEQ ID No. 13 and the variable region light chain amino acid sequence of SEQ ID No. 14), 11-C4 (having the variable region heavy chain amino acid sequence of SEQ ID No. 15 and the variable region light chain amino acid sequence of SEQ ID No. 16), 30-B9 (having the variable region heavy chain amino acid sequence of SEQ ID No. 17 and the variable region light chain amino acid sequence of SEQ ID No. 18), and 30-C9 (having the variable region heavy chain amino acid sequence of SEQ ID No. 19 and the variable region light chain amino acid sequence of SEQ ID No. 20).

In an embodiment of the first aspect of the invention, the amino acid sequences comprised are of one antibody selected from the group comprising 30-D9, 30-B8, 30-D7, 11-A6, 23-A9, and 11-C10, preferably of one antibody from the group comprising 30-D9, 30-B8, 30-D7, and 11-A6, more preferably of one antibody from the group comprising 30-D9 and 30-B8, particularly preferably of the antibody 30-B8.

In another embodiment of the first aspect of the invention, the antibody or antigen-binding fragment thereof is directed against a protein of the type III secretion system of *Pseudomonas aeruginosa*, preferably against the protein PcrV of *Pseudomonas aeruginosa* (UniProt accession number G3XD49).

In yet another embodiment of the first aspect of the invention, the amino acid sequences of the CDRs or of the variable regions comprised therein are from an antibody which is able to inhibit cytotoxicity of *Pseudomonas aeruginosa* wild type strain PAO1 as determined by a cytotoxicity assay as described in the description with an IC₅₀ of at most 2 µg/ml, preferably at most 1 µg/ml, more preferably at most 0.3 µg/ml, even more preferably at most 0.2 µg/ml, particularly preferably at most 0.1 µg/ml.

In one embodiment of the first aspect of the invention, the amino acid sequences of the CDRs or of the variable regions comprised therein are from an antibody which is able to inhibit *Pseudomonas aeruginosa* strain PAO1-induced cell death to obtain cell viability in comparison to uninfected controls in an assay as described in the description with at least 80% viability, preferably at least 84% viability, more preferably at least 90% viability, particularly preferably at least 95% viability.

In an embodiment of the first aspect of the invention, the antibody or antigen-binding fragment thereof does not display autoreactivity defined as detectable binding when tested against permeabilized HEp-2 cells using an antinuclear antibody (ANA) testing kit (NOVA-Lite HEp-2 ANA kit; Inova Diagnostics) at concentrations of 100 pg/ml of the antibody or binding fragment thereof.

According to a second aspect of the invention, a pharmaceutical composition is provided comprising an antibody or antigen-binding fragment thereof according to the first aspect of the invention and at least one pharmaceutically acceptable excipient.

According to a third aspect of the invention, a kit is provided comprising an antibody or antigen-binding fragment thereof according to the first aspect of the invention and a container.

According to a fourth aspect of the invention, the antibody or antigen-binding fragment thereof according to the first aspect of the invention, pharmaceutical composition according to the second aspect of the invention, or kit according to the third aspect of the invention for use as a medicament.

According to a fifth aspect of the invention, the antibody or antigen-binding fragment thereof according to the first aspect of the invention, pharmaceutical composition according to the second aspect of the invention, or kit according to the third aspect of the invention for use in the treatment or prevention of an infection with *Pseudomonas aeruginosa* in mammalian subjects, preferably in human subjects.

According to the sixth aspect of the invention, a nucleic acid is provided encoding an antibody or antigen-binding fragment thereof according to the first aspect of the invention.

According to the seventh aspect of the invention, an expression vector is provided comprising the nucleic acid according to the sixth aspect of the invention in functional association with an expression control sequence.

According to the eighth aspect of the invention, a host cell is provided comprising a nucleic acid according to the sixth aspect of the invention or the expression vector according to the seventh aspect of the invention.

According to the ninth aspect of the invention, a method of production of an antibody or antigen-binding fragment thereof according to the first aspect of the invention is provided, comprising (a) cultivating the host cell according to the eighth aspect of the invention under conditions allowing expression of the antibody or antigen-binding fragment thereof, and (b) recovering the antibody or antigen-binding fragment thereof.

Brief description of the drawings

The present disclosure will be more readily appreciated by reference to the following detailed description when being considered in connection with the accompanying drawings in which:

Figure 1 is a graph showing results of a cell line-based assay. A549 cells were infected with PAO1 for 150 min with a MOI of 0.5 in presence of monoclonal anti-PcrV antibodies (50 µg/mL). As control, cells were left uninfected or were infected in presence of a mock control, a humanized mouse anti-PcrV antibody (1F3) (50 µg/mL) or gentamicin (20 µg/mL). Relative fluorescence units (RFU) were measured after adding resazurin. Each data point represents the mean of technical replicates of an independent experiment. Box plots indicate the median, the upper and lower quartile and the

minimum and maximum values. Significance was calculated to infected cells treated with a mock control using a One-Way ANOVA with Tukey's multiple comparisons test.

Figure 2 are graphs showing results of a cell line-based assay. A549 cells were infected using different drug resistant *Pseudomonas aeruginosa* strains (A, B), both isolated from patients with blood-stream infections. Cells were treated with piperacillin/tazobactam (16 µg/mL), meropenem (8 µg/mL), ceftazidime (8 µg/mL), ciprofloxacin (1 µg/mL), gentamicin (4 µg/mL) as well as selected patient-derived monoclonal anti-PcrV antibodies (50 µg/mL). Significance was calculated in comparison to infected cells treated with a mock control using a One-Way ANOVA with Tukey's multiple comparisons test. Box plots are indicating the median, the 25th and 75th quartile, and minimum and maximum values of four independent experiments.

Figure 3 are graphs showing results of a hemolysis-based assay.

Figure 3A: CD-1 mice were treated with cyclophosphamide intraperitoneally at d -4 and d -1 to induce neutropenia. Subsequently, pulmonary infection was induced by nebulization of *Pseudomonas aeruginosa* (Boston 41501 strain). To confirm successful application of bacteria, an inoculum group was used. A vehicle control (PBS), levofloxacin (100 mg/kg), or mAbs (5 mg/kg) were administered 2 h later intraperitoneally. After 24 h experiments were terminated and lungs were homogenized followed by quantifications of CFUs.

Figure 3B: CD-1 mice were rendered neutropenic by administration of 150 mg/kg and 100 mg/kg cyclophosphamide intraperitoneally on day -4 and -1, respectively. 2 h prior to infection a control mAb (MCA1), or mAbs 30-B8 or 30-D9 (5 mg/kg) were administered intraperitoneally. Infection was initiated by intramuscular injection of 1.2×10^5 CFU/ml PA (Boston 41501) into each lateral thigh. As control levofloxacin (100 mg/kg) was given 2, 6 and 10 h post infection. To confirm bacterial infection after injection, six animals were used as inoculum control group. After 24 h animals were euthanized, muscles were homogenized and CFUs were determined. Box plots are indicating the median, the 25th and 75th quartile, and minimum and maximum values. Significance was calculated to animals treated with the control antibody using a One-Way ANOVA with Tukey's multiple comparisons test.

Figure 4 is a graph showing a titration curve of a cell line-based assay. A549 cells were infected in presence of the human PcrV mAb 30-D9, a bispecific PcrV-Psl antibody (MEDI3902), and 1F3 at a concentration ranging from 50 µg/mL to 24 ng/ml. RFUs were determined after adding resazurin. Box plots are indicating the median, the 25th and 75th quartile, and minimum and maximum values of two independent experiments.

Figure 5 are images showing results of an autoreactivity test. HepG2 cells were stained with 100 µg/mL anti-PcrV mAbs for 30 min at room temperature. After washing with PBS cells were labeled with a second FITC-conjugated anti-human IgG antibody for 30 min. A positive control was used according to the instructions of the kit used. As negative control, PBS was used instead a primary antibody. Stained slides were mounted and analyzed by microscopy with a 40-fold magnification.

Figure 6 is a graph showing results of a direct comparison of the viability of A549 cells upon infection with *Pseudomonas aeruginosa* wild type strain PAO1 in the presence of increased doses of reference antibody H1H29336P or of representative antibodies 30-B8 and 30-D9 of the present invention; higher RFU indicate increased viability, representing improved and superior neutralization strength of the antibody used.

Detailed description of preferred embodiments

In the following, the invention will be explained in more detail with reference to the accompanying figures. In the Figures, like elements are denoted by identical reference numerals and repeated description thereof may be omitted in order to avoid redundancies.

In order that the present description can be more readily understood, certain terms are first defined. Additional definitions are set forth throughout the detailed description.

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

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

It is understood that wherever aspects are described herein with the language "comprising," otherwise analogous aspects described in terms of "consisting of" and/or "consisting essentially of" are also provided.

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure is related. For example, the Concise Dictionary of Biomedicine and Molecular Biology, Juo, Pei-Show, 2nd ed., 2002, CRC Press; The Dictionary of Cell and Molecular Biology, 3rd ed., 1999, Academic Press; and the Oxford Dictionary Of Biochemistry And Molecular Biology, Revised, 2000, Oxford University Press, provide one of skill with a general dictionary of many of the terms used in this disclosure.

Units, prefixes, and symbols are denoted in their Système International d'Unités (SI) accepted form. Numeric ranges are inclusive of the numbers defining the range. Unless otherwise indicated, nucleotide sequences are written left to right in 5' to 3' orientation. Amino acid sequences are written left to right in amino to carboxy orientation. The headings provided herein are not limitations of the various aspects of the disclosure, which can be had by reference to the specification as a whole. Accordingly, the terms defined immediately below are more fully defined by reference to the specification in its entirety.

The term "about" is used herein to mean approximately, roughly, around, or in the regions of. When the term "about" is used in conjunction with a numerical range, it modifies that range by extending the boundaries above and below the numerical values set forth. In general, the term "about" can modify a numerical value above and below the stated value by a variance of, e.g., 10 percent, up or down (higher or lower).

The term "antibody" is used herein in the broadest sense to refer to molecules with an immunoglobulin-like domain (for example IgG, IgM, IgA, IgD or IgE) and includes monoclonal, recombinant, chimeric, human, humanized, multispecific antibodies, including bispecific antibodies, and heteroconjugate antibodies; a single variable domain (e.g., VH, VHH, VL, domain antibody), antigen binding antibody fragments, Fab, F(ab')₂, Fv, disulphide linked Fv, single chain Fv, disulphide-linked scFv, diabodies, etc. and modified versions of any of the foregoing.

The term "antibody" as used herein refers to a protein, which is capable of specifically binding to an antigen or an antigen-binding portion thereof. The term includes full length antibodies of any class or isotype and any single chain or fragment thereof. An antibody that specifically binds to an antigen, or antigen-binding portion thereof, may bind exclusively to that antigen, or portion thereof, or it may bind to a limited number of homologous antigens, or portions thereof. Full-length antibodies usually comprise at least four polypeptide chains: two heavy (H) chains and two light (L) chains that are interconnected by disulfide bonds.

One immunoglobulin sub-class of particular pharmaceutical interest is the IgG family. In humans, the IgG class may be sub-divided into 4 sub-classes: IgG1, IgG2, IgG3 and IgG4, based on the sequence of their heavy chain constant regions. The light chains can be divided into two types, kappa and lambda, based on differences in their sequence composition. IgG molecules are composed of two heavy chains, interlinked by two or more disulfide bonds, and two light chains, each attached to a heavy chain by a disulfide bond. A heavy chain may comprise a heavy chain variable region (VH) and up to three heavy chain constant (CH) regions: CH1, CH2 and CH3. A light chain may comprise a light chain variable region (VL) and a light chain constant region (CL).

VH and VL regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDRs), interspersed with regions that are more conserved, termed framework regions (FR). VH and VL regions are typically composed of three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. The hypervariable regions of the heavy and light chains form a binding domain that is capable of interacting with an antigen, while the constant region of an antibody may mediate binding of the immunoglobulin to host tissues or factors, including but not limited to various cells of the immune system (effector cells), Fc receptors and the first component (C1q) of the classical complement system. Antibodies of the current invention may be isolated.

The term "isolated antibody" refers to an antibody that has been separated and/or recovered from (an)other component(s) in the environment in which it was produced and/or that has been purified from a mixture of components present in the environment in which it was produced. Certain antigen-binding fragments of antibodies may be suitable in the context of the current invention, as it has been shown that the antigen-binding function of an antibody can be performed by fragments of a full-length antibody.

The term "binding fragment" or "antigen-binding fragment" of an antibody refers to one or more fragment(s) of an antibody that retain the ability to specifically bind to an antigen, such as a protein of the type III secretion system of *Pseudomonas aeruginosa*, as described herein.

Examples of antigen-binding fragments include Fab, Fab', F(ab)2, F(ab')2, F(ab)S, Fv (typically the VL and VH domains of a single arm of an antibody), single-chain Fv (scFv; see, e.g., Bird et al., 1988; Huston et al., 1988), dsFv, Fd (typically the VH and CH1 domain), and dAb (typically a VH domain) fragments; VH, VL, VHH, and V-NAR domains; monovalent molecules comprising a single VH and a single VL chain; minibodies, diabodies, triabodies, tetrabodies, and kappa bodies (see, e.g., Ill et al., 1997); camel IgG; IgNAR; as well as one or more isolated CDRs or a functional paratope, where the

isolated CDRs or antigen-binding residues or polypeptides can be associated or linked together so as to form a functional antibody fragment.

Various types of antibody fragments have been described or reviewed in, e.g., Holliger and Hudson, 2005; International Publ. No. WO 2005/040219, and U.S. Publ. Nos. 2005/0238646 and 2002/0161201. These antibody fragments may be obtained using conventional techniques known to those of skill in the art, and the fragments may be screened for utility in the same manner as intact antibodies.

A "human" antibody (HuMAb) refers to an antibody having variable regions in which both the framework and CDR regions are derived from human germline immunoglobulin sequences. Furthermore, if the antibody contains a constant region, the constant region also is derived from human germline immunoglobulin sequences. The antibodies described herein can include amino acid residues not encoded by human germline immunoglobulin sequences (e.g., mutations introduced by random or site-specific mutagenesis in vitro or by somatic mutation in vivo).

However, the term "human antibody", as used herein, is not intended to include antibodies in which CDR sequences derived from the germline of another mammalian species, such as a mouse, have been grafted onto human framework sequences. The terms "human" antibodies and "fully human" antibodies are used synonymously.

A "recombinant human antibody" refers to all human antibodies that are prepared, expressed, created or isolated by recombinant means, such as (a) antibodies isolated from an animal (e.g., a mouse) that is transgenic or transchromosomal for human immunoglobulin genes or a hybridoma prepared therefrom, (b) antibodies isolated from a host cell transformed to express the antibody, e.g., from a transfectoma, (c) antibodies isolated from a recombinant, combinatorial human antibody library, and (d) antibodies prepared, expressed, created or isolated by any other means that involve splicing of human immunoglobulin gene sequences to other DNA sequences.

Such recombinant human antibodies comprise variable and constant regions that utilize particular human germline immunoglobulin sequences are encoded by the germline genes, but include subsequent rearrangements and mutations which occur, for example, during antibody maturation. As known in the art (see, e.g., Lonberg, 2005), the variable region contains the antigen binding domain, which is encoded by various genes that rearrange to form an antibody specific for a foreign antigen. In addition to rearrangement, the variable region can be further modified by multiple single amino acid changes (referred to as somatic mutation or hypermutation) to increase the affinity of the antibody to

the foreign antigen. The constant region will change in further response to an antigen (i.e., isotype switch).

Therefore, the rearranged and somatically mutated nucleic acid molecules that encode the light chain and heavy chain immunoglobulin polypeptides in response to an antigen cannot have sequence identity with the original nucleic acid molecules, but instead will be substantially identical or similar (i.e., have at least 80% of identity).

A "chimeric antibody" refers to an antibody in which the variable regions are derived from one species and the constant regions are derived from another species, such as an antibody in which the variable regions are derived from a mouse antibody and the constant regions are derived from a human antibody.

Alternative antibody formats include alternative scaffolds in which the one or more CDRs of the antigen-binding portion can be arranged onto a suitable non-immunoglobulin protein scaffold or skeleton, such as an affibody, a SpA scaffold, an LDL receptor class A domain, an avimer or an EGF domain.

The term "domain" (interchangeably referred to as "region" herein) refers to a folded protein structure which retains its tertiary structure independent of the rest of the protein. Generally, domains are responsible for discrete functional properties of proteins and in many cases may be added, removed or transferred to other proteins without loss of function of the remainder of the protein and/or of the domain.

The term "variable domain" refers to a folded polypeptide domain comprising sequences characteristic of antibody variable domains. It, therefore, includes complete antibody variable domains such as VH, VHH and VL and modified antibody variable domains, for example, in which one or more loops have been replaced by sequences which are not characteristic of antibody variable domains, or antibody variable domains which have been truncated or comprise N- or C-terminal extensions, as well as folded fragments of variable domains which retain at least the binding activity and specificity of the full-length domain.

A single variable (V) domain is capable of binding an antigen or epitope independently of a different variable region or domain. A "domain antibody" or "dAbTM" may be considered the same as a "single variable domain". A single variable domain may be a human single variable domain, but also includes single variable domains from other species such as rodent nurse shark and Camelid VHH dAbsTM.

Camelid VHH are immunoglobulin single variable domain polypeptides that are derived from species including camel, llama, alpaca, dromedary, and guanaco, which produce heavy chain antibodies naturally devoid of light chains. Such VHH domains may be humanized according to standard techniques available in the art, and such domains are considered to be "single variable domains".

An antigen-binding fragment may be provided by means of arrangement of one or more CDRs on non-antibody protein scaffolds. "Protein Scaffold" may include an immunoglobulin (Ig) scaffold, for example an IgG scaffold, which may be a four chain or two chain antibody, or which may comprise only the Fc region of an antibody, or which may comprise one or more constant regions from an antibody, which constant regions may be of human or primate origin, or which may be an artificial chimera of human and primate constant regions.

Phrases like "an antibody recognizing an antigen" and "an antibody specific for an antigen" may be used interchangeably herein with the term "an antibody which binds specifically to an antigen."

By the terms "treat," "treating," or "treatment of" (or grammatically equivalent terms) it is meant that the severity of the subject's condition is reduced or at least partially improved or ameliorated and/or that some alleviation, mitigation or decrease in at least one clinical symptom is achieved and/or there is a delay in the progression of the condition.

As used herein, the terms "prevent," "prevents," or "prevention" and "inhibit," "inhibits," or "inhibition" (and grammatical equivalents thereof) are not meant to imply complete abolition of disease and encompasses any type of prophylactic treatment that reduces the incidence of the condition, delays the onset of the condition, and/or reduces the symptoms associated with the condition after onset.

An "effective," "prophylactically effective," or "therapeutically effective" amount as used herein is an amount that is sufficient to provide some improvement or benefit to the subject. Alternatively stated, an "effective," "prophylactically effective," or "therapeutically effective" amount is an amount that will provide some delay, alleviation, mitigation, or decrease in at least one clinical symptom in the subject. Those skilled in the art will appreciate that the effects need not be complete or curative, as long as some benefit is provided to the subject.

A "neutralizing antibody" may refer to any antibody or antigen-binding fragment thereof that binds to a pathogen and interferes with the ability of the pathogen to infect a cell and/or cause disease in a subject.

For polypeptides, the term "substantial homology" indicates that two polypeptides, or designated sequences thereof, when optimally aligned and compared, are identical, with appropriate amino acid insertions or deletions, in at least about 80% of the amino acids, at least about 90% to 95%, or at least about 98% to 99.5% of the amino acids.

The percent identity between two sequences is a function of the number of identical positions shared by the sequences (i.e., % homology = # of identical positions/total # of positions times 100), taking into account the number of gaps, and the length of each gap, which need to be introduced for optimal alignment of the two sequences. The comparison of sequences and determination of percent identity between two sequences can be accomplished using a mathematical algorithm, as described in the non-limiting examples below.

The nucleic acids can be present in whole cells, in a cell lysate, or in a partially purified or substantially pure form. A nucleic acid is "isolated" or "rendered substantially pure" when purified away from other cellular components or other contaminants, e.g., other cellular nucleic acids (e.g., the other parts of the chromosome) or proteins, by standard techniques, including alkaline/SDS treatment, CsCl banding, column chromatography, agarose gel electrophoresis and others well known in the art. See, Ausubel, 1987.

Nucleic acids, e.g., cDNA, can be mutated, in accordance with standard techniques to provide gene sequences. For coding sequences, these mutations, can affect amino acid sequence as desired. In particular, DNA sequences substantially homologous to or derived from native V, D, J, constant, switches and other such sequences described herein are contemplated (where "derived" indicates that a sequence is identical or modified from another sequence).

The term "vector," as used herein, is intended to refer to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. One type of vector is a "plasmid," which refers to a circular double stranded DNA loop into which additional DNA segments can be ligated. Another type of vector is a viral vector, wherein additional DNA or RNA segments can be ligated into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (e.g., bacterial vectors having a bacterial origin of replication and episomal mammalian vectors).

Other vectors (e.g., non-episomal mammalian vectors) can be integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. Moreover, certain vectors are capable of directing the expression of genes to which they are

operatively linked. Such vectors are referred to herein as "recombinant expression vectors" (or simply, "expression vectors"). In general, expression vectors of utility in recombinant DNA techniques are often in the form of plasmids. In the present specification, "plasmid" and "vector" can be used interchangeably as the plasmid is the most commonly used form of vector. However, also included are other forms of expression vectors, such as viral vectors (e.g., replication defective retroviruses, adenoviruses and adeno-associated viruses), which serve equivalent functions.

The term "recombinant host cell" (or simply "host cell"), as used herein, is intended to refer to a cell that comprises a nucleic acid that is not naturally present in the cell, and can be a cell into which a recombinant expression vector has been introduced. It should be understood that such terms are intended to refer not only to the particular subject cell but to the progeny of such a cell. Because certain modifications can occur in succeeding generations due to either mutation or environmental influences, such progeny cannot, in fact, be identical to the parent cell, but are still included within the scope of the term "host cell" as used herein.

As used herein, the term "linked" refers to the association of two or more molecules. The linkage can be covalent or non-covalent. The linkage also can be genetic (i.e., recombinantly fused). Such linkages can be achieved using a wide variety of art recognized techniques, such as chemical conjugation and recombinant protein production.

An "Fc receptor" or "FcR" is a receptor that binds to the Fc region of an immunoglobulin. FcRs that bind to an IgG antibody comprise receptors of the FcγR family, including allelic variants and alternatively spliced forms of these receptors. The FcγR family consists of three activating (FcγRI, FcγRIII, and FcγRIV in mice; FcγRIA, FcγRIIA, and FcγRIIIA in humans) and one inhibitory (FcγRIIB) receptor. Various properties of human FcγRs are known in the art. The majority of innate effector cell types coexpress one or more activating FcγR and the inhibitory FcγRIIB, whereas natural killer (NK) cells selectively express one activating Fc receptor (FcγRIII in mice and FcγRIIIA in humans) but not the inhibitory FcγRIIB in mice and humans. Human IgG1 binds to most human Fc receptors and is considered equivalent to murine IgG2a with respect to the types of activating Fc receptors that it binds to.

"Fc region" (fragment crystallizable region) or "Fc domain" or "Fc" refers to the C-terminal region of the heavy chain of an antibody that mediates the binding of the immunoglobulin to host tissues or factors, including binding to Fc receptors located on various cells of the immune system (e.g., effector cells) or to the first component (C1q) of the classical complement system. Thus, an Fc region

comprises the constant region of an antibody excluding the first constant region immunoglobulin domain (e.g., CH1 or CL).

The constant region may be modified to stabilize the antibody, e.g., to reduce the risk of a bivalent antibody separating into two monovalent VH-VL fragments. For example, in an IgG4 constant region, residue S228 (residue numbering according to the EU index) may be mutated to a proline (P) residue to stabilize inter heavy chain disulphide bridge formation at the hinge (see, e.g., Angal et al., 1993). Antibodies or fragments thereof can also be defined in terms of their complementarity-determining regions (CDRs).

The term "complementarity-determining region" or "hypervariable region", when used herein, refers to the regions of an antibody in which amino acid residues involved in antigen binding are situated. The region of hypervariability or CDRs can be identified as the regions with the highest variability in amino acid alignments of antibody variable domains. In general, databases can be used for CDR identification such as the Kabat database, the CDRs e.g., being defined as comprising amino acid residues 24-34 (CDR1), 50-59 (CDR2) and 89-97 (CDR3) of the light-chain variable region, and 31-35 (CDR1), 50-65 (CDR2) and 95-102 (CDR3) in the heavy-chain variable region; (Kabat et al. 1991). Alternatively, CDRs can generally be defined as those residues from a "hypervariable loop" (residues 26-33 (L1), 50-52 (L2) and 91-96 (L3) in the light-chain variable region and 26-32 (H1), 53-55 (H2) and 96-101 (H3) in the heavy-chain variable region (Chothia and Lesk, 1987).

The CDR regions of the antibody sequences described herein are preferably defined according to the numbering scheme of IMGT which is an adaptation of the numbering scheme of Chothia (ImMunoGeneTics information system®; Lefranc et al., 1999.; <http://imgt.org>).

As used herein, the terms "specific binding," "selective binding," "selectively binds," and "specifically binds," refer to antibody binding to an epitope on a predetermined antigen. Preferably, the antibody binds to the predetermined antigen with an affinity that is at least two-fold greater than its affinity for binding to a non-specific antigen (e.g., BSA, casein) other than the predetermined antigen or a closely-related antigen.

The term "binding affinity" herein refers to a measurement of the strength of a non-covalent interaction between two molecules, e.g. an antibody, or fragment thereof, and an antigen. The term "binding affinity" is used to describe monovalent interactions (intrinsic activity).

The binding affinity between two molecules, e.g. an antibody, or fragment thereof, and an antigen, through a monovalent interaction may be quantified by determination of the equilibrium dissociation constant (KD). In turn, KD can be determined by measurement of the kinetics of complex formation and dissociation, e.g. by the SPR method. The rate constants corresponding to the association and the dissociation of a monovalent complex are referred to as the association rate constant k_a (or k_{on}) and dissociation rate constant k_d (or k_{off}), respectively. KD is related to k_a and k_d through the equation $KD=k_d/k_a$. Following the above definition, binding affinities associated with different molecular interactions, such as comparison of the binding affinity of different antibodies for a given antigen, may be compared by comparison of the KD values for the individual antibody/antigen complexes.

The term "binding specificity" herein refers to the interaction of a molecule such as an antibody, or fragment thereof, with a single exclusive antigen, or with a limited number of highly homologous antigens (or epitopes). In contrast, antibodies that are capable of specifically binding to a protein of the type III secretion system of *Pseudomonas aeruginosa* are not capable of binding dissimilar molecules.

The specificity of an interaction and the value of an equilibrium binding constant can be determined directly by well-known methods. Standard assays to evaluate the ability of ligands (such as antibodies) to bind their targets are known in the art and include, for example, ELISAs, Western blots, RIAs, and flow cytometry analysis. The binding kinetics and binding affinity of the antibody also can be assessed by standard assays known in the art, such as SPR.

A "polypeptide" refers to a chain comprising at least two consecutively linked amino acid residues, with no upper limit on the length of the chain. One or more amino acid residues in the protein can contain a modification such as, but not limited to, glycosylation, phosphorylation or disulfide bond formation. A "protein" can comprise one or more polypeptides.

The term "nucleic acid" or "nucleic acid molecule," as used herein, is intended to include DNA molecules and RNA molecules. A nucleic acid molecule can be single-stranded or double-stranded, and can be cDNA.

The term "subject" includes human and other mammalian subjects that receive either prophylactic or therapeutic treatment. As used herein, the term "subject" includes any human or non-human animal. The term "non-human animal" includes all vertebrates, e.g., mammals and non-mammals, such as non-human primates, sheep, dog, cow, chickens, amphibians, reptiles, etc.

As used herein, the terms "ug" and "uM" are used interchangeably with "µg" and "µM," respectively.

As used herein, "administering" refers to the physical introduction of a composition comprising a therapeutic agent to a subject, using any of the various methods and delivery systems known to those skilled in the art. Different routes of administration for the antibodies described herein include intravenous, intraperitoneal, intramuscular, subcutaneous, spinal or other parenteral routes of administration, for example by injection or infusion.

The phrase "parenteral administration" as used herein means modes of administration other than enteral and topical administration, usually by injection, and includes, without limitation, intravenous, intraperitoneal, intramuscular, intraarterial, intrathecal, intralymphatic, intralesional, intracapsular, intraorbital, intracardiac, intradermal, transtracheal, subcutaneous, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal, epidural and intrasternal injection and infusion, as well as in vivo electroporation.

Alternatively, an antibody described herein can be administered via a non-parenteral route, such as a topical, epidermal or mucosal route of administration, for example, intranasally, orally, vaginally, rectally, sublingually or topically. Administering can also be performed, for example, once, a plurality of times, and/or over one or more extended periods.

As used herein "vaccination composition" means a pharmaceutical composition comprising at least one antibody or antigen-binding portion thereof of the present invention which is capable of providing active and/or passive immunity. "Active immunity" as used herein means inducing or enhancing a subject's immune response to an antigen. "Passive immunity" as used and preferred herein means supplementing a subject's immune response to an antigen or pathogen by providing antibodies and/or antigen-binding portions thereof which neutralize an antigen.

The present inventors have dedicated themselves to solving the problem of the present invention and were successful to find novel human monoclonal antibodies against *Pseudomonas aeruginosa* having superior neutralization potency against currently circulating resistant *Pseudomonas aeruginosa*.

Accordingly, the present invention provides antibodies or antigen-binding fragments thereof which are directed against *Pseudomonas aeruginosa*, wherein the antibody or antigen-binding fragment thereof comprises the combination of the heavy chain CDR1 to CDR3 and the light chain CDR1 to CDR3 amino acid sequence of one antibody selected from the group comprising 30-D9 (having a

CDR-H1 amino acid sequence of SEQ ID No. 21, a CDR-H2 amino acid sequence of SEQ ID No. 22, a CDR-H3 amino acid sequence of SEQ ID No. 23, a CDR-L1 amino acid sequence of SEQ ID No. 24, a CDR-L2 amino acid sequence of SEQ ID No. 25, a CDR-L3 amino acid sequence of SEQ ID No. 26), 30-B8 (having a CDR-H1 amino acid sequence of SEQ ID No. 27, a CDR-H2 amino acid sequence of SEQ ID No. 28, a CDR-H3 amino acid sequence of SEQ ID No. 29, a CDR-L1 amino acid sequence of SEQ ID No. 30, a CDR-L2 amino acid sequence of SEQ ID No. 31, a CDR-L3 amino acid sequence of SEQ ID No. 32), 30-D7 (having a CDR-H1 amino acid sequence of SEQ ID No. 33, a CDR-H2 amino acid sequence of SEQ ID No. 34, a CDR-H3 amino acid sequence of SEQ ID No. 35, a CDR-L1 amino acid sequence of SEQ ID No. 36, a CDR-L2 amino acid sequence of SEQ ID No. 37, a CDR-L3 amino acid sequence of SEQ ID No. 38), 11-A6 (having a CDR-H1 amino acid sequence of SEQ ID No. 39, a CDR-H2 amino acid sequence of SEQ ID No. 40, a CDR-H3 amino acid sequence of SEQ ID No. 41, a CDR-L1 amino acid sequence of SEQ ID No. 42, a CDR-L2 amino acid sequence of SEQ ID No. 43, a CDR-L3 amino acid sequence of SEQ ID No. 44), 23-A9 (having a CDR-H1 amino acid sequence of SEQ ID No. 45, a CDR-H2 amino acid sequence of SEQ ID No. 46, a CDR-H3 amino acid sequence of SEQ ID No. 47, a CDR-L1 amino acid sequence of SEQ ID No. 48, a CDR-L2 amino acid sequence of SEQ ID No. 49, a CDR-L3 amino acid sequence of SEQ ID No. 50), 11-C10 (having a CDR-H1 amino acid sequence of SEQ ID No. 51, a CDR-H2 amino acid sequence of SEQ ID No. 52, a CDR-H3 amino acid sequence of SEQ ID No. 53, a CDR-L1 amino acid sequence of SEQ ID No. 54, a CDR-L2 amino acid sequence of SEQ ID No. 55, a CDR-L3 amino acid sequence of SEQ ID No. 56), 23-F9 (having a CDR-H1 amino acid sequence of SEQ ID No. 57, a CDR-H2 amino acid sequence of SEQ ID No. 58, a CDR-H3 amino acid sequence of SEQ ID No. 59, a CDR-L1 amino acid sequence of SEQ ID No. 60, a CDR-L2 amino acid sequence of SEQ ID No. 61, a CDR-L3 amino acid sequence of SEQ ID No. 62), 11-C4 (having a CDR-H1 amino acid sequence of SEQ ID No. 63, a CDR-H2 amino acid sequence of SEQ ID No. 64, a CDR-H3 amino acid sequence of SEQ ID No. 65, a CDR-L1 amino acid sequence of SEQ ID No. 66, a CDR-L2 amino acid sequence of SEQ ID No. 67, a CDR-L3 amino acid sequence of SEQ ID No. 68), 30-B9 (having a CDR-H1 amino acid sequence of SEQ ID No. 69, a CDR-H2 amino acid sequence of SEQ ID No. 70, a CDR-H3 amino acid sequence of SEQ ID No. 71, a CDR-L1 amino acid sequence of SEQ ID No. 72, a CDR-L2 amino acid sequence of SEQ ID No. 73, a CDR-L3 amino acid sequence of SEQ ID No. 74), and 30-C9 (having a CDR-H1 amino acid sequence of SEQ ID No. 75, a CDR-H2 amino acid sequence of SEQ ID No. 76, a CDR-H3 amino acid sequence of SEQ ID No. 77, a CDR-L1 amino acid sequence of SEQ ID No. 78, a CDR-L2 amino acid sequence of SEQ ID No. 79, a CDR-L3 amino acid sequence of SEQ ID No. 80).

Within the context of the present invention, the antibodies, which have been generated and described herein, may be used and claimed as the complete monoclonal human antibody or as any functional

or antigen-binding fragment thereof. Preferably, the antibody or any kind of functional or antigen-binding fragment thereof should at least comprise the complementarity determining regions (CDR) 1 to 3 of the heavy chain and CDR 1 to 3 of the light chain of the antibody.

The CDR regions of the antibody sequences described herein are preferably defined according to the numbering scheme of IMGT which is an adaptation of the numbering scheme of Chothia (ImMunoGeneTics information system®; Lefranc et al., 1999; <http://imgt.org>).

Based on the common general knowledge and the information given herein on the heavy chain variable region amino acid sequences and the light chain variable region amino acid sequences of the antibodies of the invention, the CDRs can be easily and unambiguously determined by a skilled person.

According to one preferred embodiment of the present invention, the light and heavy chain variable region sequences of the preferred antibodies and antigen-binding fragments thereof described herein with the internal designations 30-D9, 30-B8, 30-D7, 11-A6, 23-A9, 11-C10, 23-F9, 11-C4, 30-B9, and 30-C9 are as follows:

SEQ ID NO	Source	Sequence
1	Heavy chain variable domain 30-D9	EVQLLESGGGLAQPGGSLRLSCAASGFTFSNNAMNWVRQAPGK RLEWVSGIIGNGTSTYYADSVKGRFTISRDN SKNTVSLQMSSLRA EDTAIYYCAKDRHPWRWLQLFDSWGQGTLTVSS
2	Light chain variable domain 30-D9	DIQMTQSPSTLSASLGDRVITICRASQSIGSWLAWYQQKPGKAP KLLIYKASNRESAVPSRFSGSESGTEFTLTISLQPD DFATYYCQY YNSYSQAAVTFGPGTKVDIK
3	Heavy chain variable domain 30-B8	EGQLLESGGGLAQPGGSLRLSCTASGFTFSKNAMNWVRQAPGK RLEWVAGIIGNGSDTYADSVKGRFTISRDN SKNTVSLQMNSLRA EDSAIYYCAKDRHPWRWLQLFDSWGQGTLTVSS
4	Light chain variable domain 30-B8	DIQLTQSPSTVSASLGDRVITICRASQDIGSWLAWYQQKPGKAPK LLIYKASNVESGVPSRFSGSESGTEFTLTISLQPD DVATYFCQYY NSYSHATVTFGPGTKVDIK
5	Heavy chain variable domain 30-D7	EVQLLESGGGLAQPGGSLRLSCAASGFTFSNNAMNWVRQAPGK RLEWVSGIIGNGTSTYYADSVKGRFTISRDN SKNTVSLQMNSLRA EDTAIYYCAKDRHPWRWLQLFDSWGQGTLTVSS

SEQ ID NO	Source	Sequence
6	Light chain variable domain 30-D7	DIQMTQSPSTLSASLGDRVITITCRASQSIGSWLAWYQQKAGKAP KLLIYKASNVESGVPSRFSGSESGTEFTLTISLQPDFFATYYCQY YNSYSQGAVTFGPGTKVDIK
7	Heavy chain variable domain 11-A6	QAQLVQSETEVKKPGASVKVSCKASGYTFTTHAIHWVRQAPGQS LEWMGWINGANGNTKYSQKFQDRVITITRDTSASTVYLEMSSLTS EDTAVYYCARLGWSYSTSPWAFDPWGQGTLIIVSS
8	Light chain variable domain 11-A6	QSGLTQPPSASGSPGQSVTISCTGTTDDIGSYDYVSWYQHHPGK APRLIIEVSKRPSGVPDRFSGSKSGDTASLTVSGLQAEDEADYIC SSHIGNNKVFVFGTGKVTVL
9	Heavy chain variable domain 23-A9	EVQLLESGGGLVQPGGSLRLSCVASGFTFKNFAMAWVRQAPGK GLEWVSAVSNSGGATYYADSVRGRLTISRDNSTRDTLYLQMNSLR AEDTAIYYCAKALANPHFYGSGTYDFYAMDVWGQGTTVTVTS
10	Light chain variable domain 23-A9	QSALTQPASVSASPGQSITISCSGTSSDIGGYKFSWYQQHPGKA PQLIIEVSNRPSGVSNRFGSGSKSANTASLTISGLQAEDEADYYCS SYARSGSLLFGSGTKVTVL
11	Heavy chain variable domain 11-C10	QAQLVQSETEVKKPGASVKLSCKASGYTFSTHAIHWVRQAPGQS LEWMGWINGANGNTKYSQRFQDRVITARDTAASTVYLEMTSLTS EDTAVYYCARLGWSYSSSPWAFDPWGQGTLIIVSS
12	Light chain variable domain 11-C10	QSGLTQPPSASGSPGQSVTLSTCTGTTDDIGSYDFVSWYQHHPG KAPRLIIEVKNRPSGVPDRFSGSKSGDTASLTVSGLQAEDEADYI CCSHVGNNKFVFGTGKIVIL
13	Heavy chain variable domain 23-F9	QVQLQESGPGLVKPSQTLSSLCTVSGGSIGSGSYWNWIRQPAG KGLEWIGRIYASGSTNYPNPSLKSRTMSLDTSKNQFYVNLTSVTA TDSAVYYCARGNGGQGFDFWGQGNLTVSS
14	Light chain variable domain 23-F9	SYVLTQPPSVSVAPGKTASISCGGNNLGRQSVQWYQQKPGQAP MRVIFYNSDRPSGIPERFSGSKSGNTATLTISRVEAGDEADYYCQ VWDNSSDQGVFGGGTKLTVL
15	Heavy chain variable domain 11-C4	EVQLVETGGGLVEPGGSLRLSCAASGITLTSHWMYVWRQIPGKG LMWVSRINSVSGGINYADSVKGRFTISRDNKNTLDLQMNSLRVD DSAVYYCATYSTTWKAFDYWGQGTLLTVSS
16	Light chain variable domain 11-C4	SYDLTQPMSVSVSAGQTASITCSGEKLGDRYASWYQQKPGQSP ALVIYQDDKRPSGIPERFSGSNSGDTATLTISGTQAIDEADYYCQA WDTKNLGFGGGTRLTVL

SEQ ID NO	Source	Sequence
17	Heavy chain variable domain 30-B9	QVQLQQWGAGLLKPSETLSLTCAVSGGSFNGYYWSWIRQPPGK GLEWIGEINDSGSTNYNPSLRSRVTSVDTSKNQVSVKLTSTVTA DTAVYYCARGTWGGGSGSWRNWFGPWGHGTLTVSS
18	Light chain variable domain 30-B9	DIQMTQSPSTLSASVGDRVSITCRANQTIYNWLAWYQQRPGKAP NLLIYKTSTLESGVPSRFSGSGSGTEFTLTISLQPDFAFYCQQ YSSYPRTFGQGKVEVK
19	Heavy chain variable domain 30-C9	EVQLVQSGAEVKKSGESLKISCEVSGYSFNRHWIGWVRQMPGK GLEWMGIIFPGSDTRYSPSFQGQITISADKSISTAYLQWSSLKAS DTAMYYCARHVRNYDEINGYYYLDYWGGGTPVTVSS
20	Light chain variable domain 30-C9	EIVMTQSPATLSVSPGDRASLSCRASQSVSSNLAWYQQKPGQAP RLLIYGASTRATGIPARFSGSGSGTEFTLTISLQSEDFAFYICQQ YDTWPPGMHTFGQGKLEIK

According to one embodiment of the present invention, the antibody or antigen-binding fragment thereof comprises a heavy chain variable region amino acid sequence of antibody 30-D9 (SEQ ID No. 1), or a heavy chain variable region amino acid sequence of antibody 30-B8 (SEQ ID No. 3), or a heavy chain variable region amino acid sequence of antibody 30-D7 (SEQ ID No. 5), or a heavy chain variable region amino acid sequence of antibody 11-A6 (SEQ ID No. 7), or a heavy chain variable region amino acid sequence of antibody 23-A9 (SEQ ID No. 9), or a heavy chain variable region amino acid sequence of antibody 11-C10 (SEQ ID No. 11), or a heavy chain variable region amino acid sequence of antibody 23-F9 (SEQ ID No. 13), or a heavy chain variable region amino acid sequence of antibody 11-C4 (SEQ ID No. 15), or a heavy chain variable region amino acid sequence of antibody 30-B9 (SEQ ID No. 17), or a heavy chain variable region amino acid sequence of antibody 30-C9 (SEQ ID No. 19).

According to an embodiment of the present invention, the antibody or antigen-binding fragment thereof comprises a light chain variable region amino acid sequence of antibody 30-D9 (SEQ ID No. 2), or a light chain variable region amino acid sequence of antibody 30-B8 (SEQ ID No. 4), or a light chain variable region amino acid sequence of antibody 30-D7 (SEQ ID No. 6), or a light chain variable region amino acid sequence of antibody 11-A6 (SEQ ID No. 8), or a light chain variable region amino acid sequence of antibody 23-A9 (SEQ ID No. 10), or a light chain variable region amino acid sequence of antibody 11-C10 (SEQ ID No. 12), or a light chain variable region amino acid sequence of antibody 23-F9 (SEQ ID No. 14), or a light chain variable region amino acid sequence of antibody

11-C4 (SEQ ID No. 16), or a light chain variable region amino acid sequence of antibody 30-B9 (SEQ ID No. 181), or a light chain variable region amino acid sequence of antibody 30-C9 (SEQ ID No. 20).

According to a preferred embodiment of the present invention, the antibody comprises a heavy chain variable region amino acid sequence of SEQ ID No. 1 and a light chain variable region amino acid sequence of SEQ ID No. 2, or the antibody comprises a heavy chain variable region amino acid sequence of SEQ ID No. 3 and a light chain variable region amino acid sequence of SEQ ID No. 4, or the antibody comprises a heavy chain variable region amino acid sequence of SEQ ID No. 5 and a light chain variable region amino acid sequence of SEQ ID No. 6, or the antibody comprises a heavy chain variable region amino acid sequence of SEQ ID No. 7 and a light chain variable region amino acid sequence of SEQ ID No. 8, or the antibody comprises a heavy chain variable region amino acid sequence of SEQ ID No. 9 and a light chain variable region amino acid sequence of SEQ ID No. 10, or the antibody comprises a heavy chain variable region amino acid sequence of SEQ ID No. 11 and a light chain variable region amino acid sequence of SEQ ID No. 12, or the antibody comprises a heavy chain variable region amino acid sequence of SEQ ID No. 13 and a light chain variable region amino acid sequence of SEQ ID No. 14, or the antibody comprises a heavy chain variable region amino acid sequence of SEQ ID No. 15 and a light chain variable region amino acid sequence of SEQ ID No. 16, or the antibody comprises a heavy chain variable region amino acid sequence of SEQ ID No. 17 and a light chain variable region amino acid sequence of SEQ ID No. 18, or the antibody comprises a heavy chain variable region amino acid sequence of SEQ ID No. 19 and a light chain variable region amino acid sequence of SEQ ID No. 20.

According to a specific embodiment of the present invention, the antibody consists of two heavy chains of sequence SEQ ID No. 1 and two light chains of sequence SEQ ID No. 2, or the antibody consists of two heavy chains of sequence SEQ ID No. 3 and two light chains of sequence SEQ ID No. 4, or the antibody consists of two heavy chains of sequence SEQ ID No. 5 and two light chains of sequence SEQ ID No. 6, or the antibody consists of two heavy chains of sequence SEQ ID No. 7 and two light chains of sequence SEQ ID No. 8, or the antibody consists of two heavy chains of sequence SEQ ID No. 9 and two light chains of sequence SEQ ID No. 10, or the antibody consists of two heavy chains of sequence SEQ ID No. 11 and two light chains of sequence SEQ ID No. 12, or the antibody consists of two heavy chains of sequence SEQ ID No. 13 and two light chains of sequence SEQ ID No. 14, or the antibody consists of two heavy chains of sequence SEQ ID No. 15 and two light chains of sequence SEQ ID No. 16, or the antibody consists of two heavy chains of sequence SEQ ID No. 17 and two light chains of sequence SEQ ID No. 18, or the antibody consists of two heavy chains of sequence SEQ ID No. 19 and two light chains of sequence SEQ ID No. 20.

According to one preferred embodiment of the present invention, the CDR sequences of the light and heavy chain variable region sequences of the antibodies and antigen-binding fragments thereof described herein are as follows:

Source	SEQ ID CDR1	CDR1	SEQ ID CDR2	CDR2	SEQ ID CDR3	CDR3
Heavy chain 30-D9	21	GFTFSNNA	22	IIGNGTST	23	AKDRHPWR WLQLFDS
Light chain 30-D9	24	QSIGSW	25	KAS	26	QYYNSYSQ AAVT
Heavy chain 30-B8	27	GFTFSKNA	28	IIGNGSDT	29	AKDRHPWR WLQLFDS
Light chain 30-B8	30	QDIGSW	31	KAS	32	QYYNSYSH ATVT
Heavy chain 30-D7	33	GFTFSSNA	34	IIGNGTST	35	AKDRHPWR WLQLFDS
Light chain 30-D7	36	QSIGSW	37	KAS	38	QYYNSYSQ GAVT
Heavy chain 11-A6	39	GYTFTTHA	40	INGANGNT	41	ARLGWSYS TSPWAFDP
Light chain 11-A6	42	TDDIGSYDY	43	EVS	44	SSHIGNNKF V
Heavy chain 23-A9	45	GFTFKNFA	46	VSNSGGAT	47	AKALANPH FYGSGTYD FYAMDV
Light chain 23-A9	48	SSDIGGYKF	49	EVS	50	SSYARSGS LL
Heavy chain 11-C10	51	GYTFSTHA	52	INGANGNT	53	ARLGWSYS SSPWAFDP
Light chain 11-C10	54	TDDIGSYDF	55	EVN	56	CSHVGNNK FV
Heavy chain 23-F9	57	GGSIGSGSY Y	58	IYASGST	59	ARGNGGQ GFDF

Source	SEQ ID CDR1	CDR1	SEQ ID CDR2	CDR2	SEQ ID CDR3	CDR3
Light chain 23-F9	60	NLGRQS	61	YNS	62	QVWDNSSD QGV
Heavy chain 11-C4	63	GITLTSHW	64	INSVGSGI	65	ATYSTTWK AFDY
Light chain 11-C4	66	KLGDYR	67	QDD	68	QAWDTKNL G
Heavy chain 30-B9	69	GGSFNGYY	70	INDSGST	71	ARGTWGG GSGSWRN WFGP
Light chain 30-B9	72	QTIYNW	73	KTS	74	QQYSSYP R
Heavy chain 30-C9	75	GYSFNRHW	76	IFPGSDT	77	ARHVRNYD EINGYYYLD Y
Light chain 30-C9	78	QSVSSN	79	GAS	80	QQYDTWPP GMHT

According to a preferred embodiment of the present invention, the antibody used as a source for sequences comprised in the antibody or antigen-binding fragment thereof according to the present invention is selected from the group comprising 30-D9, 30-B8, 30-D7, 11-A6, 23-A9, and 11-C10, preferably of one antibody from the group comprising 30-D9, 30-B8, 30-D7, and 11-A6, more preferably of one antibody from the group comprising 30-D9 and 30-B8, particularly preferably of the antibody 30-B8.

In another embodiment of the present invention, the antibody used as a source for sequences comprised in the antibody of the invention is 30-D9. In another embodiment of the present invention, the antibody used as a source for sequences comprised in the antibody of the invention is 30-B8. In one embodiment of the present invention, the antibody used as a source for sequences comprised in the antibody of the invention is 30-D7. In another embodiment of the present invention, the antibody used as a source for sequences comprised in the antibody of the invention is 11-A6.

According to another preferred embodiment of the present invention, the antibody or antigen-binding fragment thereof is directed against a protein of the type III secretion system of *Pseudomonas aeruginosa*, preferably against the protein PcrV of *Pseudomonas aeruginosa* (UniProt accession number G3XD49).

According to one embodiment of the present invention, the amino acid sequences of the CDRs or of the variable regions comprised therein are from an antibody which is able to inhibit cytotoxicity of *Pseudomonas aeruginosa* wild type strain PAO1 as determined by a cytotoxicity assay as described in the description with an IC₅₀ of at most 2 µg/ml, preferably at most 1 µg/ml, more preferably at most 0.3 µg/ml, even more preferably at most 0.2 µg/ml, particularly preferably at most 0.1 µg/ml.

According to the present invention, the neutralization assay for determining IC₅₀ values is to be carried out by serial dilution experiments as described in “*Neutralizing effects of human anti-PcrV antibodies against Pseudomonas aeruginosa in vitro*” in the examples below.

In general, the antibodies or antigen-binding fragments thereof as described herein further encompass antibody amino acid sequences being at least 80% identical to the sequences as defined above as long as they are still directed against a protein of the type III secretion system of *Pseudomonas aeruginosa*, preferably as long as they are still directed against the protein PcrV of *Pseudomonas aeruginosa*.

According to one other embodiment, the antibody or antigen-binding fragment thereof does not display autoreactivity defined as detectable binding when tested against permeabilized HEp-2 cells using an antinuclear antibody (ANA) testing kit (NOVA-Lite HEp-2 ANA kit; Inova Diagnostics) at concentrations of 100 pg/ml of the antibody or binding fragment thereof.

The sequence variations encompassed herein are meant to include sequences having trivial mutations, i.e., conservative mutations, of the antibody amino acid sequence which do not interfere with structural folds and the affinity of the antibody to a protein of the type III secretion system of *Pseudomonas aeruginosa*. Preferably, the deviations in the amino acid sequence leading to an at least 80%, 85%, 90% or 95% overall identity to the individualized sequences explicitly disclosed herein are present exclusively outside the CDR regions of the antibodies according to the invention, and are encompassed herein as forming part of the present invention. In particular, the present invention encompasses antibody amino acid sequences having 1, 2, 3, 4, 5, or 6 mutations within the constant regions of the antibody.

The antibodies according to the present invention are preferably of human origin. Thus, at least the sequences outside the CDRs, such as framework and constant regions of the antibody, are preferably of human origin or can be attributed to human origin. Furthermore, the antibodies of the present invention are preferably monoclonal.

In one preferred embodiment, the antibody is a monoclonal antibody or a fragment thereof that retains binding specificity and ability to neutralize infectious pathogen. In one preferred embodiment, the antibody is an IgG1, IgG2, IgG3, or IgG4 antibody. For example, the antibody may be an antibody comprising an Fc domain of any human IgG isotype (e.g. IgG1, IgG2, IgG3, or IgG4).

Optionally, the antigen-binding compound consists of or comprises a Fab, Fab', Fab'-SH, F(ab)2, Fv, a diabody, single-chain antibody fragment, or a multispecific antibody comprising multiple different antibody fragments.

Within the present invention, an antibody or antigen-binding fragment directed against *Pseudomonas aeruginosa* or PcrV of *Pseudomonas aeruginosa* means an antibody binding to PcrV of *Pseudomonas aeruginosa* with an at least 10-fold, more preferably at least 50-fold, particularly preferably at least 100-fold increased affinity compared to unrelated epitopes, proteins or protein regions.

It is a trivial task for a skilled person to determine if an antibody which exhibits a certain degree of identity is directed against PcrV of *Pseudomonas aeruginosa* based on the above or the common general knowledge.

The determination of percent identity between two sequences is accomplished according to the present invention by using the mathematical algorithm of Karlin and Altschul (Karlin and Altschul, 1993). Such an algorithm is the basis of the BLASTN and BLASTP programs of Altschul et al. (Altschul et al., 1990). BLAST nucleotide searches are performed with the BLASTN program. To obtain gapped alignments for comparative purposes, Gapped BLAST is utilized as described by Altschul et al. (Altschul et al., 1997). When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs are used.

According to a preferred embodiment of the present invention, antibody amino acid sequences form part of the invention which consist of or comprise a nucleic acid sequence being at least 85% identical to the sequences defined above and disclosed herein, more preferably at least 90% identical, even more preferred at least 95% identical.

In the description of the present application, antibody designations may be used. It is pointed out that the antibodies consist of heavy and light chains which also form part of the present description. If reference is made to an antibody by its designation or to a SEQ ID No., it should be understood that these ways of reference are interchangeable.

The present invention further relates to a pharmaceutical composition comprising an antibody or antigen-binding fragment thereof according to the invention as defined and further described herein and at least one pharmaceutically acceptable excipient. The pharmaceutical composition may be a vaccination composition for a human and/or animal subject.

The present invention also encompasses a kit comprising an antibody or antigen-binding fragment thereof according to the invention as defined and further described herein and a container.

In one aspect, the present invention is also directed to the antibody or antigen-binding fragment thereof according to the invention as defined and further described herein, the pharmaceutical composition as described herein, and the kit for use as a medicament.

In another aspect, the present invention is also directed to the antibody or antigen-binding fragment thereof according to the invention as defined and further described herein, the pharmaceutical composition as described herein, and the kit for use in the treatment of an infection with *Pseudomonas aeruginosa* in mammalian subjects, preferably in human subjects.

In one aspect, the present invention is also directed to the antibody or antigen-binding fragment thereof according to the invention as defined and further described herein, the pharmaceutical composition as described herein, and the kit for use in the prevention of an infection with *Pseudomonas aeruginosa* in mammalian subjects, preferably in human subjects.

An antibody and/or antigen-binding fragment thereof according to the invention may be administered to a patient in need thereof by intravenous injection or infusion, subcutaneous injection, intramuscular injection, or inhalative application, preferably by intravenous injection.

The dosage of an antibody or antigen-binding fragment thereof of the invention to be administered to a subject may vary depending on such things as the severity of the symptoms exhibited as well as the age, sex, and health of the subject.

An antibody according to the invention may be administered to a patient in need thereof by inhalative application. The antibody may be administered by inhalative application, wherein it is provided in a liquid pharmaceutical composition which is nebulized by a mesh nebulizer or a jet nebulizer prior to administration.

A pharmaceutical composition of the invention is formulated to be compatible with its intended route of administration. Examples of routes of administration include, but are not limited to, parenteral, e.g., intravenous, intradermal, subcutaneous, oral, intranasal (e.g., inhalation and inhaled through the mouth), transdermal (e.g., topical), transmucosal, and rectal administration.

In a specific embodiment, the composition is formulated in accordance with routine procedures as a pharmaceutical composition adapted for intravenous, subcutaneous, intramuscular, oral, intranasal, or topical administration to human beings. Typically, compositions for intravenous administration are solutions in sterile isotonic aqueous buffer. Where necessary, the composition may also include a solubilizing agent and a local anesthetic such as lignocaine to ease pain at the site of the injection.

The methods of the invention may comprise pulmonary administration, e.g., by use of an inhaler or nebulizer, of a composition formulated with an aerosolizing agent. See, e.g., U.S. Pat. Nos. 6,019,968, 5,985,320, 5,985,309, 5,934,272, 5,874,064, 5,855,913, 5,290,540, and 4,880,078; and PCT Publication Nos. WO 92/19244, WO 97/32572, WO 97/44013, WO 98/31346, and WO 99/66903, each of which is incorporated herein by reference their entireties.

The methods of the invention may also comprise administration of a composition formulated for parenteral administration by injection (e.g., by bolus injection or continuous infusion). The pharmaceutical formulation of the present invention may be provided in liquid form or may be provided in lyophilized form.

In one aspect, the present invention relates to a nucleic acid encoding an antibody or antigen-binding fragment thereof as described herein.

In another aspect, the present invention relates to an expression vector comprising the nucleic acid as described herein in functional association with an expression control sequence.

In yet another aspect, the present invention relates to a host cell comprising a nucleic acid as described herein.

In one aspect, the present invention relates to a host cell comprising an expression vector as described herein.

In another aspect, the present invention relates to a method of production of an antibody or antigen-binding fragment as described herein, comprising (a) cultivating a host cell as described herein under conditions allowing expression of the antibody or antigen-binding fragment thereof, and (b) recovering the antibody or antigen-binding fragment thereof.

In another aspect, the present invention is also directed to the use of the antibody or antigen-binding fragment thereof according to the invention or a pharmaceutical composition of the invention in the manufacture of a medicament for treatment of a disease caused by *Pseudomonas aeruginosa* in human or animal subjects.

All embodiments of the present invention as described and/or claimed herein are deemed to be combinable within the present invention in any combination, unless the skilled person considers such a combination to not make any technical sense or to be excluded by contradiction.

EXAMPLES

Neutralizing effects of human anti-PcrV antibodies against *Pseudomonas aeruginosa* *in vitro*

To determine neutralizing activity, antibodies 30-D9, 30-B8, 30-D7, 11-A6, 23-A9, 11-C10, 23-F9, 11-C4, 30-B9, and 30-C9 were screened at 5 µg/mL in a hemolysis assay. As control, hemolysis assays were performed with 5 µg/mL of PcrV-specific antibody 1F3 (US 2005/0063985 A1/US 8,501,179 B2), bispecific PcrV–Psl antibody MEDI3902 (DiGiandomenico et al), and polyclonal human antibodies (IVIG) and 20 µg/mL gentamicin.

The humanized mouse anti-PcrV antibody 1F3 used herein as a reference antibody for comparison of neutralization efficiency with the antibodies of the present invention is disclosed in the US patent US 8,501,179 B2 as best-in-class with a low IC₅₀ of 5,3 nM or 4,0 nM depending on the cell type used to test neutralization potency (cf. US 8,501,179 B2, column 15, line 28 and 33). The direct comparison between said reference antibody 1F3 and the antibodies of the present invention demonstrate the superiority of the present invention over the prior art (see Table below, Figure 1 and Figure 4).

The antibodies of the invention exhibited an inhibition of hemolysis of >50%, identifying them as highly neutralizing antibodies. The antibodies were further titrated to determine the half-maximal inhibitory concentration (IC₅₀).

Here, inhibitory effects of the antibodies were tested in the hemolysis assay with 12 different concentrations from 50 µg/ml to 24 ng/ml by using a 1:1 serial dilution. Percentage of hemolysis to the control (infected, untreated cells) were calculated for each concentration and plotted on a logarithmic scale x-axis (concentration) and linear scale y-axis (% hemolysis to control) to generate a dose-response curve. IC₅₀ values for each antibody were calculated using a variable slope model (four-parameter). The results are shown in the table below.

The neutralizing effects of the antibodies were further tested at a concentration of 50 µg/mL in an A549 cytotoxicity assay. T3SS dependent lysis of A549 cells was confirmed by incubating cells with the T3SS deficient PAO1FΔpscD strain which had no impact on cell viability. The antibodies inhibited bacteria-induced cell death with a cell viability of >50%, which was calculated in comparison to uninfected controls. The mouse-derived antibody 1F3 as well as IVIG failed to protect cells from *PA*-mediated cell death in this assay. The three antibodies 30-D9, 30-B8, and 30-D7 protected cells nearly completely (>90%), similar to treatment with the antibiotic gentamicin (30-B8: 90.45%, 30-D7: 91.83% and 30-D9: 96.42% vs gentamicin: 95.41% viability). The results are shown in Fig. 1 and the table below.

Serial dilution experiments were performed to determine IC₅₀ values for the antibodies of the invention in comparison to 1F3 and MEDI3902 (gremubamab), the latter of which showed good activity in pre-clinical experiments, but failed to prevent *Pseudomonas aeruginosa* associated pneumonia in early clinical trials. The human PcrV-antibodies of the present invention largely outperformed the mouse-derived antibodies. MEDI3902 protected A549 cells from *PA*-induced cytotoxicity with an IC₅₀ of 11.46 µg/mL whereas the most potent patient-derived antibody 30-D9 had a 144-fold improved IC₅₀ of 79.5 ng/mL.

IC₅₀ values were calculated by testing 12 different concentrations from 50 µg/ml to 24 ng/ml by using a 1:1 serial dilution. RFU values for each antibody at each concentration were plotted on a logarithmic scale x-axis (concentration) and linear scale y-axis (RFU) to generate a dose-response curve. IC₅₀ values for each antibody were calculated using a variable slope model (four-parameter). The results are shown in Fig. 4 and the table below.

Antibodies	Neutralization PcrV						
	Hemolysis assay			Cytotoxicity assay			
	Name	Hemolysis RBCs – PA14 ± SD (%)	P-Value (to negative control)	IC ₅₀ (µg/mL)	Cytotoxicity A549 - PAO1 ± SD (RFU)	P-Value (to infected cells)	Viability (%) IC ₅₀ (µg/mL)
	11-A6	20.56 ± 6.73	<0.0001	0.097	26310 ± 5258	<0.0001	88.81% -
	11-C4	25.65 ± 6.30	<0.0001	0.076	24182 ± 4013	<0.0001	79.10% 2.923
	11-C10	19.90 ± 1.30	<0.0001	0.115	25293 ± 4492	<0.0001	84.17% 0.161
	23-A9	17.94 ± 2.64	<0.0001	0.050	25470 ± 3057	<0.0001	84.98% 0.273
	23-F9	21.51 ± 5.70	<0.0001	0.087	24675 ± 2717	<0.0001	81.35% 0.230
	30-B8	38.04 ± 14.59	<0.0001	0.054	26971 ± 2085	<0.0001	91.83% 1.80
	30-B9	30.22 ± 4.86	<0.0001	0.340	22367 ± 3787	<0.0001	70.82% 0.99
	30-C9	41.69 ± 6.61	<0.0001	10.61	19294 ± 3434	<0.0001	56.80% -
	30-D7	18.98 ± 3,28	<0.0001	0.0576	26670 ± 2981	<0.0001	90.45% 0.11
	30-D9	18.16 ± 3.06	<0.0001	0.076	27978 ± 1851	<0.0001	96.42% 0.080
	1F3	29.98 ± 10.66	<0.0001	0.340	6971 ± 3601	0,9996	3.16% -
	MEDI3902	-	-	-	21680 ± 3391	<0.0001	75.10% 11.46
	IVIG	97.61 ± 5.17	-	-	5500 ± 2603	0.9994	0% -
	Gentamicin	23.40 ± 12.90	<0.0001	-	27689 ± 2734	<0.0001	95.41% -

None of the anti-PcrV antibodies of the invention showed autoreactivity in a HEp-2 cell-based autoreactivity assay, while moderate cytoplasmatic autoreactivity was observed for the mouse-derived PcrV mAb 1F3 (*cf.* Fig. 4).

Neutralizing effects of human anti-PcrV antibodies against drug resistant clinical *Pseudomonas aeruginosa* isolates of *in vitro*

The neutralizing activity of the anti-PcrV mAb 30-B8 against clinical strains isolates from patients with blood-stream infections were tested. Compared to the reference strain PAO1, the human mAb 30-B8 showed similar activity against clinical isolates, including those with high levels of resistance against commonly used antibiotics (*cf.* Fig. 2A and 2B). To exclude any natural resistance mechanisms by mutations and to estimate the effective spectrum of highly neutralizing patient-derived anti-PcrV mAbs in a clinical setting, the *pcrV* genes of 30 clinical isolates were sequenced and compared to the *pcrV* gene sequence of the reference strain PAO1. In most of the isolates, silent mutations were detected. In total, 5 different mutations were detected leading to amino-acid changes in PcrV. However, none of the observed mutations led to a loss of function of the tested human anti-PcrV mAb 30-B8.

Determination of *in vivo* potency of patient-derived anti-PcrV mAbs

To assess the *in vivo* function and efficacy of human B cell-derived mAbs, the therapeutic efficacy of the two highly neutralizing anti-PcrV mAbs 30-B8 and 30-D9 were tested in a pneumonia mouse model. CD-1 mice were first treated with cyclophosphamide four and one day before infection to induce neutropenia, which results in a higher susceptibility to *Pseudomonas aeruginosa* infection. Subsequently, pneumonia was induced by aerosolized delivery of *Pseudomonas aeruginosa* (Boston 41501 strain). Anti-PcrV mAbs (5 mg/kg), a vehicle control or the conventional antimicrobial levofloxacin (100 mg/kg) were administered 2 h later intraperitoneally. After 24 h, mice were sacrificed and the bacterial load was determined by counting colony forming units (CFUs) in lung tissue homogenates. The treatment of animals with patient-derived anti-PcrV mAbs 30-B8 and 30-D9 led to a pronounced reduction of the bacterial burden in mouse lungs (*cf.* Fig. 3A). For the mAb 30-B8, growth inhibitory effects were comparable to treatment with levofloxacin (mean CFUs: control 1.00×10^9 vs levofloxacin 1.15×10^5 , 30-B8 4.59×10^3 , and 30-D9 2.70×10^6). Moreover, mAb treatment strongly reduced the systemic inflammatory response in infected animals as shown by a significant decline of cytokine plasma levels (mean IL-6 / TNF plasma concentration: control 3362.54 / 27 ng/mL vs levofloxacin 54.88 / 3.15 ng/mL and 30-B8 15.92 / 0.84 ng/mL) (Fig. 6B and 6C). In line with these findings, a nearly complete prevention of hemorrhagic infiltrate formation in lung sections of infected and mAb treated animals was observed.

The mAbs were assessed for prophylactic activity in a neutropenic thigh infection model. Antibodies were applied intraperitoneally 2 h prior to intramuscular injection of *Pseudomonas aeruginosa* into the left and right lateral thigh followed by terminal analysis 24 h later. Treatment of mice with human anti-PcrV mAbs led to a significant reduction of the bacterial burden in tissue compared to a control antibody (MCA1; anti-MERS-CoV S glycoprotein antibody) (cf. Fig. 3B). Efficacy of anti-PcrV mAbs 30-B8 and 30-D9 was comparable to levofloxacin treatment (administered three times after infection vs single dose mAbs with 5 mg/kg before infection). In comparison to the lung infection model, the systemic inflammatory response observed in this experiment was lower. Nevertheless, it was found that PcrV mAb treatment and levofloxacin treatment reduced plasma IL-6 levels to the same extent.

Direct comparison to antibody H1H29336P of the prior art

The PcrV antibody H1H29336P of the prior art was reported as showing very low IC₅₀ values in A549 cytotoxicity assays with *P. aeruginosa* (against *P. aeruginosa* strains 6077 as well as ATCC 700888; cf. page 69, Table 10 of WO 2020/252029 A1).

In order to assess and compare the human monoclonal antibodies of the present invention to said reference antibody, serial dilution experiments were performed to determine the IC₅₀ values of H1H29336P in direct comparison to 30-B8 and 30-D9 (see Figure 6, and the results presented below).

For the infection experiments, the reference strain *P. aeruginosa* PAO1 was used, which is commonly utilized in *P. aeruginosa* infection studies (cf. Grace A, Sahu R, Owen DR, Dennis VA. *Pseudomonas aeruginosa* reference strains PAO1 and PA14: A genomic, phenotypic, and therapeutic review. *Front Microbiol.* 2022 Oct 13;13:1023523. doi: 10.3389/fmicb.2022.1023523. PMID: 36312971; PMCID: PMC9607943.).

Results obtained for a comparison between H1H29336P and the antibodies of the present invention are considered to allow an assessment of the efficacy and relative potency of the antibodies of the invention in comparison to H1H29336P as well as other reference antibodies disclosed in WO 2020/252029 A1.

In fact, H1H29336P showed clearly inferior efficacy in comparison to the representative antibodies of the present invention 30-B8 and 30-D9. In a comparative assay, **H1H29336P** exhibited a calculated IC₅₀ of **9.14 µg/ml**, wherein the representative antibodies of the present invention showed more than 30 times lower IC₅₀ values of **56.22 ng/ml** for **30-D9** (about 163x lower) and **195.9 ng/ml** for **30-B8** (about 47x lower). Results of this experiment are shown in Figure 6.

Additional comparative data has been raised for other antibodies of the present invention and is reported as **127.6 ng/ml** for **30-D7** (about 72x lower), **223.3 ng/ml** for **11-A6** (about 41x lower), **248,3 ng/ml** for **23-F9** (about 37x lower) and **262,7 ng/ml** for **23-A9** (about 35x lower than reference antibody H1H29336P).

It should be noted that in WO 2020/252029 A1, the competitor antibody REGN3514 (also known as MEDI3902) was used, as referenced in WO 2013/070615 (cf. paragraph [00227] on page 63 of WO 2020/252029 A1). A549 cytotoxicity experiments revealed IC₅₀ values for REGN3514 of 8.07x10⁻¹⁰ M for *P. aeruginosa* strain 6077 and 1.784x10⁻⁸ M for *P. aeruginosa* strain ATCC 700888 (cf. Table 10 in paragraph [00238] of WO 2020/252029 A1).

These values obtained in comparative experiments of Table 10 of WO 2020/252029 A1 were only surpassed by H1H29336P for both strains (24x lower for *P. aeruginosa* strain 6077 and 2.3x lower for *P. aeruginosa* strain ATCC 700888) and by H1H29336P for *P. aeruginosa* strain ATCC 700888 (2.8x lower, page 69, WO 2020/252029 A1).

Based on a comparison of the newly generated data with IC₅₀ values of MEDI3902 in *P. aeruginosa* strain PAO1 infection experiments (exemplarily shown for antibody 30-D9 in Fig. 4), representative antibodies of the present invention outperformed MEDI3902 in PAO1 infection experiments, with fold changes ranging from 25 to 204 (11-A6 = 51x higher, 11-C10 = 25x higher, 23-A9 = 44x higher, 23-F9 = 46x higher, 30-B8 = 59x higher, 30-D7 = 90x higher, and 30-D9 = 204x higher).

This data clearly demonstrates the unprecedented neutralization potency and breadth of the aforementioned antibodies of the invention, and allow the conclusion that all antibodies according to the present invention exhibit surprisingly improved properties over any and all antibodies of the prior art.

METHODS DETAILS

Isolation of serum and PBMCs from whole blood

Serum collection tubes (Sarstedt, Nuembrecht, Germany) were centrifuged at 3800 × g for 10 min at 4°C to separate serum from clotted blood. Serum was heat-inactivated at 56°C for 30 min and stored at -80°C. PBMCs were collected in Compoflex® CPDA-1 blood bags (Fresenius, Bad Homburg, Germany). PBMCs were purified by density gradient centrifugation using Cytiva Ficoll®-Paque (GE Healthcare, Chicago, USA) and Leucosep™ tubes (Greiner, Kremsmuenster, Austria). Subsequently, cells were stored in FBS (Thermo Fisher Scientific, Waltham, MA, USA) containing 10% (v/v) dimethyl sulfoxide (DMSO) (Merck, Darmstadt, Germany) at -150 °C.

Bacterial strains and culture of *Pseudomonas aeruginosa*

Pseudomonas aeruginosa strains PAO1, PAO1 Δ pscD, PA14 and clinical strains were used for *in vitro* experiments. All clinical strains were isolated from patients with blood stream infection. For infection experiments we inoculated bacteria (stored in glycerol stocks at -80°C) in 5 mL LB broth (LB) (Carl Roth, Karlsruhe, Germany) and incubated shaking at 37°C . The next day cultures were transferred into fresh LB and adjusted to an optical density (OD_{600}) of 0.2. Cultures were then incubated at $37^{\circ}\text{C}/200$ rpm until an exponential growth was achieved (OD_{600} 0.8 – 1.5). Cultures were washed twice in Dulbecco's phosphate buffered saline (DPBS) (Thermo Fisher Scientific) before infection.

Hemolysis assay

Human red blood cells from healthy donors were washed four times with Dulbecco's Phosphate Buffered Saline (DPBS) to remove residual serum components and were diluted to a final concentration of 2.5×10^6 cells/mL. 100 μL of the suspension were added to a 96-well plate and were infected with bacteria at a multiplicity of infection (MOI) of 1 (2.5×10^6 bacteria/mL in DPBS). Subsequently, the plate was centrifuged at $1000 \times g$ for 5 min and incubated for 2h at 37°C . After 2h cells were resuspended followed by a centrifugation step at $1500 \times g$ for 10 min. 100 μL of the supernatant of each well were transferred to a new plate and the OD at 540 nm was measured using a plate reader.

Recombinant expression and isolation of PcrV

Genomic DNA from strain PAO1 was isolated using a DNeasy® Blood & Tissue Kit (Qiagen, Hilden, Germany) according to the manufacturer's instruction. The *pcrV* gene was amplified using the Phusion high fidelity polymerase (Thermo Fisher Scientific) and the primer pair: *fwd* TCACCATCACGGATCCGAAGTCAGAAACCTTAATG and *rev* TCAGCTAATTAAG CTTCTA GATCGCGCTGAGAATG. After digestion of the expression vector (pQE80, Qiagen) with the restriction enzymes BamHI and HindIII (both NEB, Ipswich, MA, USA), the purified PCR product was cloned into the expression vector using the In-Fusion® HD EcoDry™ Cloning Kit with Stellar cells from Takara Bio (Kusatsu, Japan). Clones were selected on ampicillin supplemented agar plates (Sigma-Aldrich, St. Louis, MO, USA) and accurate insert of the *pcrV* gene into the expression vector was verified by sequencing. Colonies containing vectors were grown in liquid cultures, plasmids were isolated using a QIAprep® Spin Miniprep Kit (Qiagen) and transformed into competent BL21 *E. coli* by heat shock and selected on agar plates with ampicillin. Single clones were picked, incubated overnight in LB-media, and transferred into fresh LB-media. After reaching an OD_{600} of 0.5 300 μM IPTG (isopropyl- β -D-thiogalactopyranosid) (Sigma-Aldrich) were added, and bacteria were incubated for additional 3h with shaking at 30°C . Subsequently, bacteria were centrifuged at $4000 \times g$ for 5 min and lysed using the B-PER™ Bacterial Protein Extraction Reagent (Thermo Fisher Scientific)

according to the manufacturer's instruction. Recombinant PcrV was isolated using HisPur™ Ni-NTA Resin (Thermo Fisher Scientific) by gravity-flow column (Carl Roth). Briefly, the bacterial lysate was equilibrated with 15 mM imidazole (Sigma-Aldrich) and added on the Ni-NTA resin containing gravity columns. After washing multiple times with phosphate buffered saline (PBS) containing 25 mM imidazole, His-labelled PcrV was released using 250 mM imidazole and buffer was exchanged to PBS using 10 kDa centrifugal filters (Sigma-Aldrich). Purity of the recombinant PcrV was determined by SDS-PAGE using 4-12 % Bis-Tris protein gels (Thermo Fisher Scientific) and InstantBlue™ Protein Stain (Expedeon, Heidelberg, Germany).

Determination of anti-PcrV titers in serum

High-binding 96-well ELISA plates (Corning Inc., Corning, NY, USA) were coated with recombinant PcrV protein (2 µg/mL) in ELISA coating buffer (Biolegend, San Diego, CA, USA) at 4°C overnight, washed four times with PBS/0.05% Tween (Merck) (PBST) and blocked with PBS, containing 5 % BSA (Sigma-Aldrich) for 120 min at RT. Thereafter, serum was added in serial dilutions in PBS/5 % BSA for 60 min at RT. After washing with PBST, plates were incubated with horseradish peroxidase-conjugated goat anti-human IgG antibody (Jackson ImmunoResearch West Grove, PA, USA; 1:2500 in PBS/5 % BSA) for 60 min at RT. ELISAs were developed using 3,3',5,5'-tetramethylbenzidine (TMB) (Thermo Fisher Scientific). After 15 min sulfuric acid (Carl Roth) was added and absorbance was measured at 450 nm using a multiplate reader (Hidex, Turku, Finland).

Isolation of PcrV-specific B cells

PBMCs were enriched for CD19⁺ cells using CD19 micro beads (Miltenyi Biotec, Bergisch-Gladbach, Germany) according to the manufacturer's instruction. After a washing step with FACS buffer, cells were spun down and blocked for 30 min in 10% FCS. Subsequently, cells were resuspended in buffer with 4',6-diamidino-2-phenylindole (DAPI) (Thermo Fisher Scientific) (1:100), anti-human IgG-PE (clone: G18-145) (BD Biosciences) and anti-human CD20-Alexa Fluor 700 (clone: 2H7) (Biolegend), PcrV^{AF488} and PcrV^{AF647} (each 10 µg/mL) and incubated for 20 min at 4°C. Finally, cells were washed with 15 mL FACS buffer, spun down and resuspended in 500 µL FACS buffer. Cell suspensions were used for further sorting in a single cell manner into 96-well plates using a BD FACSAria™ III (BD Biosciences). All wells contained 4 µL lysis buffer, consisting of PBS, 0.5 U/µL RNAsin (Promega), 0.5 U/µL RNaseOUT™ (Thermo Fisher Scientific), and 10 mM DTT (Thermo Fisher Scientific). After sorting, plates were immediately stored at -80°C until further processing.

Ig heavy/light chain amplification and sequence analysis

Single-cell amplification of antibody heavy and light chains was performed as previously described (Gieselmann et al., 2021). Briefly, cDNA was generated by reverse transcription using Random Hexamer Primer and Superscript IV reverse transcriptase (both Thermo Fisher Scientific) in presence of RNaseOUT™ (Thermo Fisher Scientific) and RNasin® (Promega). Sequential semi-nested PCR using a Platinum™ Taq Hot Start polymerase (Thermo Fisher Scientific) and optimized V gene-specific primer mixes were used to amplify target sequences and sequenced subsequently (Kreer et al, 2020). Sequences were annotated with IgBLAST and trimmed to extract only the variable region from FWR1 to the end of the J gene (Ye et al., 2013). To identify clonally related sequences within a single subject, heavy chain sequences of that particular subject were grouped by identical VH and VJ genes and pairwise Levenshtein distances between CDRH3s within a VH/VJ group were determined. Sequences were assigned to the same clone if they shared the same VH/VJ gene combination and had a minimal CDRH3 amino acid identity of 75% (with respect to the shortest CDRH3). All clones were cross validated by the investigators taking shared mutations, IGHG isotype, and light chain information into account, where available.

Cloning and production of PcrV-specific mAbs

Antibody cloning from 1st PCR products was performed as previously described. Amplicons for cloning were produced from the 1st PCR using a Q5® Hot Start High Fidelity DNA Polymerase (New England Biolabs) and specific primer for overhangs for sequence and ligation independent cloning (SLIC). After purification (NucleoSpin® 96 PCR Clean-up, Macherey-Nagel, Dueren, Germany), target sequences were cloned into expression vectors by SLIC using T4 DNA polymerase (New England Biolabs) and chemical competent *E. coli* DH5α (Tiller et al., 2008). After verification of positive colonies by colony PCR and Sanger sequencing, plasmids were amplified and purified from midi cultures (Macherey-Nagel). HEK293-6E cells were co-transfected with human heavy chain (IgG1 isotype) and light chain antibody expression plasmids using polyethylenimine (PEI) (Sigma-Aldrich) and maintained in FreeStyle 293 Expression Medium (Thermo Fisher Scientific) with 0.2% penicillin/streptomycin (Thermo Fisher Scientific) at 37°C and 6% CO2 and kept under constant shaking at 90-120 rpm. To isolate monoclonal antibodies, supernatants were centrifuged seven days post transfection, filtrated using PES filters and incubated with Protein G-coupled Sepharose® beads (GE Life Sciences). Beads were centrifuged, washed with PBS and antibodies were eluted from the Protein G-coupled beads in chromatography columns using 0.1 M glycine (pH = 3) and buffered using 1 M Tris (pH = 8). Buffer exchange to PBS was performed using Amicon® 30kDa filter tubes (Millipore). Antibody concentrations were determined using UV spectrophotometry (Nanodrop, Thermo Fisher Scientific) and antibodies were stored at 4°C until further use.

ELISA analysis to determine antibody binding activity to PcrV

ELISA plates (Thermo Fisher Scientific) were coated with 2.5 µg/mL recombinant PcrV in PBS at 4°C overnight. ELISA plates were blocked with 2.5% BSA and 2.5% dry milk powder in PBS/0.05% Tween-20 (PBST) for 60 min at RT, incubated with primary antibody in 2.5% BSA and 2.5% dry milk powder in PBST for 120 min, followed by goat anti-human IgG-HRP (Southern Biotech) diluted 1:2000 in PBS for 60 min at RT. Between each step plates were washed three times with PBST. ELISA plates were developed with ABTS solution (Thermo Fisher Scientific) and absorbance was measured at 415 nm and 695 nm. Positive binding was defined by a minimal top OD ≥ 0.2 and an $EC_{50} \leq 30$ µg/mL.

A549 cytotoxicity assay

Human lung epithelial cells (A549) (ATCC, Manassas, VA, USA) were seeded in 100 µL RPMI-medium (Thermo Fisher Scientific) with 10% FBS at a density of 2×10^5 cells/mL in a 96-well plate (TPP, Trasadingen, Switzerland) and incubated at 37°C with 5% CO₂. After 24h, the supernatant was removed and cells were infected with bacteria resuspended in RPMI/10% FBS (100 µL/well). After 150 min, cells were washed with RPMI and 100 µL RPMI/10% FCS supplemented with 20 µg/mL gentamicin and 10 µg/mL moxifloxacin were added. After additional 18 h, 10 µL resazurin (Sigma-Aldrich) was added and the cells were incubated for 140 min. Fluorescence was measured at a wavelength of Ex_{560 nm}/Em_{590 nm}.

Sequencing of *pcrV* in clinical isolates

Bacterial genomic DNA was extracted with a DNeasy® Kit (Qiagen) according to the manufacturer's instruction. The *pcrV* gene region was amplified using a primer set fwd 5'-GCA GGG CGA GCA GGG TAC C-3' / 5'-GCC GAT GCG TGG CTT GTT G-3' and rev 5'-GCC TGT TGC TGG TCG GTG TC-3' / 5'-GCT GGT CGG TGT CGG AAG G-3' and sequenced (Microsynth Seqlab, Balgach, Switzerland).

***In vivo* infection experiments**

For the therapeutic pneumonia model, female CD-1 mice were rendered neutropenic by administration of 150 mg/kg and 100 mg/kg cyclophosphamide intraperitoneally (i.p.) on day -4 and -1, respectively. At day 0, mice were infected by nebulization of 20 µL 1.7×10^7 CFU/mL *PA* (Boston 41501). After 2 h, mice were treated with 5 mg/kg mAbs, 100 mg/kg levofloxacin or a vehicle control. To control bacterial burden after nebulization, two animals were used as inoculum control group. After 24 h p.i., mice were sacrificed for terminal analysis. After isolation of blood, lung and kidney were removed, weighed and homogenized in 3 mL 0.9% NaCl. For determination of CFUs, suspensions of homogenized organs were serially diluted, plated on agar plates and incubated overnight at 37°C. CFUs were determined by manual counting. TNF and IL-6 were determined in plasma using

commercial ELISA Kits (all Thermo Fisher Scientific) according to the manufacturer's instruction. Human IgG concentrations were determined as described above.

A prophylactic approach was tested with a neutropenic thigh infection model. Male CD-1 mice were rendered neutropenic by administration of 150 mg/kg and 100 mg/kg cyclophosphamide i.p. on day -4 and -1, respectively. 2 h prior infection antibodies were administered (5 mg/kg) intraperitoneally. Infection was initiated by intramuscular injection of 1.2×10^8 CFU/ml *PA* (Boston 41501) (in 30 μ l) into each lateral thigh. As control, levofloxacin (100 mg/kg) was given 2, 6 and 10 h after infection. To control the bacterial burden after injection, six animals were used as inoculum control group. For analgesia, all animals were treated with tramadol 20 mg/kg subcutaneously. After 24 h experiments were terminated and whole blood was collected into tubes coated with 0.5 M EDTA and immediately spun down at 13,000 rpm for 10 min at 4°C. The plasma was transferred into a new tube and analyzed as described above. Infected muscles were removed, homogenized and CFUs were determined.

Microscopic Analysis

To determine autoreactivity, HepG2 cells (NOVA Lite HEp-2 ANA Kit) (Inova Diagnostics, San Diego, CA, USA) were stained with 100 μ g/mL mAbs for 30 min at room temperature followed by washing with PBS and labeling with a second FITC-conjugated anti-human IgG antibody for 30 min. Stained slides were mounted and analyzed by microscopy. Each mAb was tested at least in duplicate.

For histological analyses mouse lungs were fixed for 24 h in formalin and stored in 70% ethanol. Fixed lungs were embedded in paraffin, cut by a microtome and stained with H&E. Slides were scanned using a S360 slide scanner (Hamamatsu Photonics, Hamamatsu, Japan) and images were generated using OMERO software (OME University of Dundee & Open Microscopy Environment). ImageJ software was used to quantify alveolar space by using a threshold of 205 and 246 respectively. Alveolar space was quantified relative to the total surface area.

Statistical analysis

Statistical analysis was performed with GraphPad Prism 8.0.2 software (GraphPad). *P*-values less than or equal to 0.05 were considered statistically significant. For comparison of multiple groups, we used one- or two-way ANOVA depending on the dataset (for all groups homogeneity variance was tested). T-test with Welch's corrections were used for comparison of two groups. Box plots indicate the median, the upper and lower quartile and the minimum and maximum values. Data points represent biological replicates.

Direct comparison to antibody H1H29336P

H1H29336P was expressed according to the sequence specified in the published international patent application WO 2020/252029 A1 (e.g. full length heavy chain amino acid sequence of SEQ ID NO: 69, and full length light chain amino acid sequence of SEQ ID NO: 70 as disclosed on page 62, Table 3 of WO 2020/252029 A1).

A549 cells were infected with *Pseudomonas aeruginosa* wild type strain PAO1 for 150 minutes with a MOI of 0.5 in the presence of H1H29336P in doses ranging from 5 µg/ml to 9.77 ng/ml. As controls, anti-PcrV mAbs 30-B8 and 30-D9 of the present invention, and human polyclonal IgGs (intravenous immunoglobulin (IVIG)), were tested in corresponding concentrations.

Metabolic activity, as an indicator of A549 cell viability, was determined using resazurin, a cell-permeable redox-sensitive fluorescent dye. Fluorescence was measured at a wavelength of Ex560 nm/Em590 nm, and values are indicated as relative fluorescence units (RFUs) in Figure 6.

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Claims

1. Antibody or antigen-binding fragment thereof directed against *Pseudomonas aeruginosa*, wherein the antibody or antigen-binding fragment thereof comprises the combination of the heavy chain CDR1 to CDR3 and the light chain CDR1 to CDR3 amino acid sequence of one antibody selected from the group comprising:

30-D9 (having a CDR-H1 amino acid sequence of SEQ ID No. 21, a CDR-H2 amino acid sequence of SEQ ID No. 22, a CDR-H3 amino acid sequence of SEQ ID No. 23, a CDR-L1 amino acid sequence of SEQ ID No. 24, a CDR-L2 amino acid sequence of SEQ ID No. 25, a CDR-L3 amino acid sequence of SEQ ID No. 26),

30-B8 (having a CDR-H1 amino acid sequence of SEQ ID No. 27, a CDR-H2 amino acid sequence of SEQ ID No. 28, a CDR-H3 amino acid sequence of SEQ ID No. 29, a CDR-L1 amino acid sequence of SEQ ID No. 30, a CDR-L2 amino acid sequence of SEQ ID No. 31, a CDR-L3 amino acid sequence of SEQ ID No. 32),

30-D7 (having a CDR-H1 amino acid sequence of SEQ ID No. 33, a CDR-H2 amino acid sequence of SEQ ID No. 34, a CDR-H3 amino acid sequence of SEQ ID No. 35, a CDR-L1 amino acid sequence of SEQ ID No. 36, a CDR-L2 amino acid sequence of SEQ ID No. 37, a CDR-L3 amino acid sequence of SEQ ID No. 38),

11-A6 (having a CDR-H1 amino acid sequence of SEQ ID No. 39, a CDR-H2 amino acid sequence of SEQ ID No. 40, a CDR-H3 amino acid sequence of SEQ ID No. 41, a CDR-L1 amino acid sequence of SEQ ID No. 42, a CDR-L2 amino acid sequence of SEQ ID No. 43, a CDR-L3 amino acid sequence of SEQ ID No. 44),

23-A9 (having a CDR-H1 amino acid sequence of SEQ ID No. 45, a CDR-H2 amino acid sequence of SEQ ID No. 46, a CDR-H3 amino acid sequence of SEQ ID No. 47, a CDR-L1 amino acid sequence of SEQ ID No. 48, a CDR-L2 amino acid sequence of SEQ ID No. 49, a CDR-L3 amino acid sequence of SEQ ID No. 50),

11-C10 (having a CDR-H1 amino acid sequence of SEQ ID No. 51, a CDR-H2 amino acid sequence of SEQ ID No. 52, a CDR-H3 amino acid sequence of SEQ ID No. 53, a CDR-L1 amino acid sequence of SEQ ID No. 54, a CDR-L2 amino acid sequence of SEQ ID No. 55, a CDR-L3 amino acid sequence of SEQ ID No. 56),

23-F9 (having a CDR-H1 amino acid sequence of SEQ ID No. 57, a CDR-H2 amino acid sequence of SEQ ID No. 58, a CDR-H3 amino acid sequence of SEQ ID No. 59, a CDR-L1 amino acid sequence of SEQ ID No. 60, a CDR-L2 amino acid sequence of SEQ ID No. 61, a CDR-L3 amino acid sequence of SEQ ID No. 62),

11-C4 (having a CDR-H1 amino acid sequence of SEQ ID No. 63, a CDR-H2 amino acid sequence of SEQ ID No. 64, a CDR-H3 amino acid sequence of SEQ ID No. 65, a CDR-L1 amino acid sequence of SEQ ID No. 66, a CDR-L2 amino acid sequence of SEQ ID No. 67, a CDR-L3 amino acid sequence of SEQ ID No. 68),

30-B9 (having a CDR-H1 amino acid sequence of SEQ ID No. 69, a CDR-H2 amino acid sequence of SEQ ID No. 70, a CDR-H3 amino acid sequence of SEQ ID No. 71, a CDR-L1 amino acid sequence of SEQ ID No. 72, a CDR-L2 amino acid sequence of SEQ ID No. 73, a CDR-L3 amino acid sequence of SEQ ID No. 74), and

30-C9 (having a CDR-H1 amino acid sequence of SEQ ID No. 75, a CDR-H2 amino acid sequence of SEQ ID No. 76, a CDR-H3 amino acid sequence of SEQ ID No. 77, a CDR-L1 amino acid sequence of SEQ ID No. 78, a CDR-L2 amino acid sequence of SEQ ID No. 79, a CDR-L3 amino acid sequence of SEQ ID No. 80).

2. Antibody or antigen-binding fragment thereof according to claim 1, wherein the antibody or antigen-binding fragment thereof comprises the combination of the variable region heavy chain amino acid sequence and of the variable region light chain amino acid sequence of one antibody selected from the group comprising:

30-D9 (having the variable region heavy chain amino acid sequence of SEQ ID No. 1 and the variable region light chain amino acid sequence of SEQ ID No. 2),

30-B8 (having the variable region heavy chain amino acid sequence of SEQ ID No. 3 and the variable region light chain amino acid sequence of SEQ ID No. 4),

30-D7 (having the variable region heavy chain amino acid sequence of SEQ ID No. 5 and the variable region light chain amino acid sequence of SEQ ID No. 6),

11-A6 (having the variable region heavy chain amino acid sequence of SEQ ID No. 7 and the variable region light chain amino acid sequence of SEQ ID No. 8),

23-A9 (having the variable region heavy chain amino acid sequence of SEQ ID No. 9 and the variable region light chain amino acid sequence of SEQ ID No. 10),

11-C10 (having the variable region heavy chain amino acid sequence of SEQ ID No. 11 and the variable region light chain amino acid sequence of SEQ ID No. 12),

23-F9 (having the variable region heavy chain amino acid sequence of SEQ ID No. 13 and the variable region light chain amino acid sequence of SEQ ID No. 14),

11-C4 (having the variable region heavy chain amino acid sequence of SEQ ID No. 15 and the variable region light chain amino acid sequence of SEQ ID No. 16),

30-B9 (having the variable region heavy chain amino acid sequence of SEQ ID No. 17 and the variable region light chain amino acid sequence of SEQ ID No. 18), and

30-C9 (having the variable region heavy chain amino acid sequence of SEQ ID No. 19 and the variable region light chain amino acid sequence of SEQ ID No. 20).

3. Antibody or antigen-binding fragment thereof according to claim 1 or 2, wherein the amino acid sequences comprised are of one antibody selected from the group comprising, 30-D9, 30-B8, 30-D7, 11-A6, 23-A9, and 11-C10, preferably of one antibody from the group comprising 30-D9, 30-B8, 30-D7, and 11-A6, more preferably of one antibody from the group comprising 30-D9 and 30-B8, particularly preferably of the antibody 30-B8.
4. Antibody or antigen-binding fragment thereof according to any one of claims 1 to 3, wherein the antibody or antigen-binding fragment thereof is directed against a protein of the type III secretion system of *Pseudomonas aeruginosa*, preferably against the protein PcrV of *Pseudomonas aeruginosa* (UniProt accession number G3XD49).
5. Antibody or antigen-binding fragment thereof according to any one of claims 1 to 4, wherein the amino acid sequences of the CDRs or of the variable regions comprised therein are from an antibody which is able to inhibit *Pseudomonas aeruginosa* wild type strain PAO1 in a cytotoxicity assay as described in the description with an IC₅₀ of at most 2 µg/ml, preferably at most 1 µg/ml, more preferably at most 0.3 µg/ml, even more preferably at most 0.2 µg/ml, particularly preferably at most 0.1 µg/ml.

6. Antibody or antigen-binding fragment thereof according to any one of claims 1 to 5, wherein the amino acid sequences of the CDRs or of the variable regions comprised therein are from an antibody which is able to inhibit *Pseudomonas aeruginosa* strain PAO1-induced cell death to obtain cell viability in comparison to uninfected controls in an assay as described in the description with at least 80% viability, preferably at least 84% viability, more preferably at least 90% viability, particularly preferably at least 95% viability.
7. Antibody or antigen-binding fragment thereof according to any one of claims 1 to 6, wherein the antibody or antigen-binding fragment thereof does not display autoreactivity defined as detectable binding when tested against permeabilized HEp-2 cells using an antinuclear antibody (ANA) testing kit (NOVA-Lite HEp-2 ANA kit; Inova Diagnostics) at concentrations of 100 pg/ml of the antibody or binding fragment thereof.
8. Pharmaceutical composition comprising an antibody or antigen-binding fragment thereof according to any one of claims 1 to 7 and at least one pharmaceutically acceptable excipient.
9. Kit comprising an antibody or antigen-binding fragment thereof according to any one of claims 1 to 7 and a container.
10. Antibody or antigen-binding fragment thereof according to any one of claims 1 to 7, pharmaceutical composition according to claim 8, or kit according to claim 9 for use as a medicament.
11. Antibody or antigen-binding fragment thereof according to any one of claims 1 to 7, pharmaceutical composition according to claim 8, or kit according to claim 9 for use in the treatment or prevention of an infection with *Pseudomonas aeruginosa* in mammalian subjects, preferably in human subjects.
12. Nucleic acid encoding an antibody or antigen-binding fragment thereof according to any of claims 1 to 7.
13. An expression vector comprising the nucleic acid of claim 12 in functional association with an expression control sequence.
14. Host cell comprising a nucleic acid according to claim 12 or the expression vector according to claim 13.

15. Method of production of an antibody or antigen-binding fragment thereof according to any one of claims 1 to 7, comprising
- (a) cultivating the host cell of claim 14 under conditions allowing expression of the antibody or antigen-binding fragment thereof, and
 - (b) recovering the antibody or antigen-binding fragment thereof.