

PD1 BINDING AGENTS

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of GB Patent Application No. 1817652.9, filed on October 29, 2018, GB Patent Application No. 1816372.5, filed on October 8, 2018, GB Patent Application No. 1811302.7, filed on July 10, 2018, GB Patent Application No. 1807176.1, filed on May 1, 2018, and GB Patent Application No. 1803745.7, filed on March 8, 2018, the disclosure of each of which is hereby incorporated by reference in its entirety.

DESCRIPTION OF THE TEXT FILE SUBMITTED ELECTRONICALLY

The contents of the text file submitted electronically herewith are incorporated herein by reference in their entirety: A computer readable format copy of the Sequence Listing (filename: UHEL_001_01WO_SeqList_ST25.txt, date recorded: March 7, 2019, file size 113,139 bytes).

FIELD OF THE INVENTION

The invention relates to antibody molecules binding specifically to PD1 (also known as Programmed cell death 1, PDCD1, CD279, PD-1, SLEB2, PD-I, SLE1) and medical uses thereof.

BACKGROUND OF THE INVENTION

PD1 is a cell surface receptor that is a member of the immunoglobulin superfamily and is principally expressed on T cells, but has also been observed on pro-B cells. PD1 binds two known ligands, PD-L1 and PD-L2. The interaction of these ligands with PD1 on T cells down-regulates T cell inflammatory activity, which promotes immune self-tolerance. PD1 is, therefore, described as being an immune "checkpoint". This checkpoint activity has been demonstrated to minimise autoimmunity risk by promoting apoptosis (programmed cell death) in lymph node-resident T cells that are reactive to self-antigens. Importantly, PD1 ligation also promotes the survival of regulatory (anti-inflammatory) T cells.

While the role of PD1/PD-L1(2) binding in non-disease states is important for self-tolerance, it is also a key mechanism by which tumour cells escape identification as "foreign" during immune surveillance. Indeed, PD-L1 has been shown to be highly expressed in several cancers. Monoclonal antibodies that target PD1 and antagonise its function can therefore be of significant potential value in treating cancer, by boosting the immune response against malignant cells with high PD-L1 expression and/or which exhibit high levels of mutation. A significant body of preclinical and more recently, clinical, evidence suggests that blocking

PD1/PD-L1(2) binding can enhance the anti-tumour activity of T cells and inhibit the growth of both haematological and solid malignancies. As PD1/PD-L1 activity can also be induced in chronic diseases such as HIV, anti-PD1 antagonistic antibodies may also have therapeutic value in infectious disease settings. Hence, antagonistic anti-PD1 mAbs have the potential to act as immunotherapeutic agents in cancer, immune and infectious disease settings, and to amplify the effectiveness of currently established therapies.

The majority of currently approved antibody therapeutics are derived from immunized rodents. Many of those antibodies have undergone a process known as "humanization", via the "grafting" of murine CDRs into human v-gene framework sequences (see Nelson *et al.*, 2010, Nat Rev Drug Discov 9: 767-774). This process is often inaccurate and leads to a reduction in target binding affinity of the resulting antibody. To return the binding affinity of the original antibody, murine residues are usually introduced at key positions in the variable domain frameworks of the grafted v-domains (also known as "back-mutations").

While antibodies humanized via CDR grafting and back mutations have been shown to induce lower immune response rates in the clinic in comparison to those with fully murine v-domains, antibodies humanized using this basic grafting method still carry significant clinical development risks due to the potential physical instability and immunogenicity motifs still housed in the grafted CDR loops. As animal testing of protein immunogenicity is often non-predictive of immune responses in man, antibody engineering for therapeutic use focuses on minimizing predicted human T-cell epitope content, non-human germline amino acid content and aggregation potential in the purified protein.

The ideal humanized agonistic anti-PD1 antibody would therefore have as many identical residues as possible in the v-domains to those found in both the frameworks and CDRs of well-characterized human germline sequences. Townsend *et al.* (2015; PNAS 112: 15354-15359) describe a method for generating antibodies in which CDRs derived from rat, rabbit and mouse antibodies were grafted into preferred human frameworks and then subject to a human germ-lining approach termed "Augmented Binary Substitution". Although the approach demonstrated a fundamental plasticity in the original antibody paratopes, even when an investigator is in possession of highly accurate antibody-antigen co-crystal structural data, it is still not possible to reliably predict which individual residues in the CDR loops of any given antibody can be converted to human germline, and in what combination. Additionally, the Townsend *et al.* study did not address the addition of mutagenesis beyond the residues found in the human germline at positions where the removal of development risk motifs might be beneficial. This is a technological limitation which renders the process

inherently inefficient, requiring an extra stage of modification of the starting antibody sequence. In addition, it cannot currently be accurately predicted what modifications in distal positions of the protein sequence of an individual v-domain, or even on the partner v-domain, might facilitate the removal of risk motifs while maintaining antigen binding affinity and
5 specificity.

CDR germ-lining and development quality optimisation is thus a complex, multifactorial problem, as multiple functional properties of the molecule should preferably be maintained or improved, including in this instance: target binding specificity, PD1/PD-L1 signalling
10 antagonism, affinity to PD1 from both human and animal test species (e.g. cynomolgus monkey, also known as the crab-eating macaque, i.e. *Macaca fascicularis*) should be as similar as possible to facilitate highly accurate preclinical safety testing, v-domain biophysical stability and/or IgG expression yield should be optimal for manufacturing purposes. Antibody engineering studies have shown that mutation of even single residue positions in key CDRs
15 can have dramatic negative effects on all of these desired molecular properties.

WO2015/085847A1 describes an antagonistic murine anti-PD1 IgG molecule termed "MAb005", and also the preparation of humanized forms of MAb005. Those humanized forms of MAb005 were produced using classical humanization techniques, i.e. by grafting
20 of Kabat-defined murine CDRs into human heavy and light chain framework sequences, with some of the human framework residues being potentially back-mutated to the correspondingly positioned MAb005 murine residues. For reasons noted above, such humanized forms of MAb005 described in WO2015/085847A1 are not ideal.

25 SUMMARY OF THE INVENTION

The present invention provides a number of anti-PD1 antibodies and medical uses thereof.

According to one aspect of the invention, there is provided an antibody molecule which specifically binds to human PD1, and optionally also to cynomolgus monkey PD1, or an
30 antigen-binding portion thereof, wherein the antibody molecule or antigen-binding portion comprises a heavy chain variable region with:

an HCDR1 having amino acids in sequence in the following order: G-F-T-F-S-S-Y-L or any amino acid (for example A/D/E/F/G/H/I/N/P/Q/S/T/V/W/Y)-M-S (SEQ ID NO: 26);

an HCDR2 having amino acids in sequence in the following order: V or a conservative
35 substitution of V-A-T/N-I-S-G-G-G-A/S-E/N-T/K-Y-Y-P/V-D-S-V-K-G (SEQ ID NO: 27); and

an HCDR3 having amino acids in sequence in the following order: Q or any amino acid (for example T/L/M/N)-L or any amino acid (for example G/K/M/Q/S/V)-Y or a conservative

substitution of Y (for example H)-Y or any amino acid (for example A/D/F/G/M-A/D/E/F/I/K/M/S/W)-F or any amino acid (for example A/D/E/I/K/M/S/W)-D-Y (SEQ ID NO: 28).

- 5 In aspects of the invention, the HCDR1 of the antibody molecule or antigen-binding portion may exclude the sequences GFTFSSYMMS (SEQ ID NO: 29; MAb005 murine/humanized antibody HCDR1 disclosed in WO2015/085847A1; US2016/376367A1), TISGGGANTYYPDSVKG (SEQ ID NO: 30; MAb005 murine/humanized antibody HCDR2 disclosed in WO2015/085847A1; US2016/376367A1), and/or the HCDR3 of the antibody molecule or antigen-binding portion may exclude the sequence QLYYFDY (SEQ ID NO: 31; MAb005 murine/humanized antibody HCDR3 disclosed in WO2015/085847A1; US2016/376367A1).

15 The antibody molecule or antigen-binding portion may further comprise a light chain variable region with:

- an LCDR1 having amino acids in sequence in the following order: R-A-S-Q-S-I-S or a conservative substitution of S-S or a conservative substitution of S -Y or a conservative substitution of Y-L or a conservative substitution of L-N/T or a conservative substitution of N or T (SEQ ID NO: 32);
- 20 an LCDR2 having amino acids in sequence in the following order: A/T or a conservative substitution of A or T-A-S or a conservative substitution of S-S-L-Q or any amino acid (for example, A/H)-S or any amino acid (for example, D, Y) (SEQ ID NO: 33); and
- an LCDR3 having amino acids in sequence in the following order: Q-Q-S or any amino acid (for example, V)-Y-S-T or any amino acid (for example, I)-P-W or any amino acid (for example, L)-T (SEQ ID NO: 34).
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In aspects of the invention, the LCDR1 of the antibody molecule or antigen-binding portion may exclude the sequence LASQTIGTWLT (SEQ ID NO: 35; MAb005 murine/humanized antibody LCDR1 disclosed in WO2015/085847A1; US2016/376367A1), and/or the LCDR2 of the antibody molecule or antigen-binding portion may exclude the sequence TATSLAD (SEQ ID NO: 36; MAb005 murine/humanized antibody LCDR2 disclosed in WO2015/085847A1; US2016/376367A1) and/or the LCDR3 of the antibody molecule or antigen-binding portion may exclude the sequence QQVYSIPWT (SEQ ID NO: 37; MAb005 murine/humanized antibody LCDR3 disclosed in WO2015/085847A1; US2016/376367A1).

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In some aspects, disclosed herein is an anti-PD1 antibody or an antigen-binding portion thereof, wherein the antibody comprises a heavy chain variable (VH) region comprising

HCDR1, HCDR2, and HCDR3 and a light chain variable (VL) region comprising LCDR1, LCDR2, and LCDR3, wherein

- (a) the HCDR1 comprises the amino acid sequence G-F-T-F-S-S-Y-X₁-M-S, wherein X₁ is L or any other amino acid (SEQ ID NO: 26);
- 5 (b) the HCDR2 comprises the amino acid sequence X₁-A-X₂-I-S-G-G-G-X₃-X₄-X₅-Y-Y-X₆-D-S-V-K-G, wherein X₁ is V or a conservative substitution of V, X₂ is T or N, X₃ is A or S, X₄ is E or N, X₅ is T or K, and X₆ is P or V (SEQ ID NO: 27);
- (c) the HCDR3 comprises the amino acid sequence X₁-X₂-X₃-X₄-X₅-D-Y, wherein X₁ is Q or any other amino acid (for example, T, L, M, or N), X₂ is L or any other amino acid (for example, G, K, M, Q, S, or V), X₃ is Y or a conservative substitution of Y (for example, H),
- 10 X₄ is Y or any other amino acid (for example, A, D, F, G, M, E, I, K, S, or W) and X₅ is F or any other amino acid (for example, A, D, E, I, K, M, S, or W) (SEQ ID NO: 28);
- (d) the LCDR1 comprises the amino acid sequence R-A-S-Q-S-I-X₁-X₂-X₃-X₄-X₅, wherein X₁ is S or a conservative substitution of S, X₂ is S or a conservative substitution of S, X₃ is Y or a conservative substitution of Y, X₄ is L or a conservative substitution of L, and
- 15 X₅ is N or T or a conservative substitution of N or T (SEQ ID NO: 32);
- (e) the LCDR2 comprises the amino acid sequence X₁-A-X₂-S-L-X₃-X₄, wherein X₁ is A or T or a conservative substitution of A or T, X₂ is S or a conservative substitution of S, X₃ is Q or any other amino acid, and X₄ is S or any other amino acid (SEQ ID NO: 33); and
- 20 (f) the LCDR3 comprises the amino acid sequence Q-Q-X₁-Y-S-X₂-P-X₃-T, wherein X₁ is S or any other amino acid, X₂ is T or any other amino acid, and X₃ is W or any other amino acid (SEQ ID NO: 34).

In some aspects, the invention provides an anti-PD1 antibody or an antigen-binding portion thereof, wherein the antibody comprises a heavy chain variable (VH) region and a light chain variable (VL) region, wherein

- (a) the VH region amino acid sequence comprises HCDR1 of GFTFSSYLMS (SEQ ID NO: 38), HCDR2 of VATISGGGSEKYYVDSVKG (SEQ ID NO: 39) and HCDR3 of QLYGFDY (SEQ ID NO: 40); and the VL region amino acid sequence comprises LCDR1 of
- 30 RASQSISSWLN (SEQ ID NO: 41), LCDR2 of AASSLQS (SEQ ID NO: 42) and LCDR3 of QQSYSTPWT (SEQ ID NO: 43);
- (b) the VH region amino acid sequence comprises HCDR1 of GFTFSSYLMS (SEQ ID NO: 38), HCDR2 of VATISGGGSEKYYVDSVKG (SEQ ID NO: 39) and HCDR3 of QVYYFDY (SEQ ID NO: 44); and the VL region amino acid sequence comprises LCDR1 of
- 35 RASQSISSWLN (SEQ ID NO: 41), LCDR2 of AASSLQS (SEQ ID NO: 42) and LCDR3 of QQSYSTPWT (SEQ ID NO: 43);

(c) the VH region amino acid sequence comprises HCDR1 of GFTFSSYLMS (SEQ ID NO: 38), HCDR2 of VATISGGGSEKYYVDSVKG (SEQ ID NO: 39) and HCDR3 of QLYFFDY (SEQ ID NO: 45); and the VL region amino acid sequence comprises LCDR1 of RASQSISSWLN (SEQ ID NO: 41), LCDR2 of AASSLQS (SEQ ID NO: 42) and LCDR3 of QQSYSTPWT (SEQ ID NO: 43);

(d) the VH region amino acid sequence comprises HCDR1 of GFTFSSYLMS (SEQ ID NO: 38), HCDR2 of VATISGGGSEKYYVDSVKG (SEQ ID NO: 39) and HCDR3 of QLYAFDY (SEQ ID NO: 46); and the VL region amino acid sequence comprises LCDR1 of RASQSISSWLN (SEQ ID NO: 41), LCDR2 of AASSLQS (SEQ ID NO: 42) and LCDR3 of QQSYSTPWT (SEQ ID NO: 43);

(e) the VH region amino acid sequence comprises HCDR1 of GFTFSSYLMS (SEQ ID NO: 38), HCDR2 of VATISGGGSETYYVDSVKG (SEQ ID NO: 48) and HCDR3 of QLYGFDY (SEQ ID NO: 40); and the VL region amino acid sequence comprises LCDR1 of RASQSISSWLN (SEQ ID NO: 41), LCDR2 of AASSLQS (SEQ ID NO: 42) and LCDR3 of QQSYSIPWT (SEQ ID NO: 47);

(f) the VH region amino acid sequence comprises HCDR1 of GFTFSSYLMS (SEQ ID NO: 38), HCDR2 of VATISGGGAEKYYVDSVKG (SEQ ID NO: 49) and HCDR3 of QLYGFDY (SEQ ID NO: 40); and the VL region amino acid sequence comprises LCDR1 of RASQSISSWLN (SEQ ID NO: 41), LCDR2 of AASSLQD (SEQ ID NO: 50) and LCDR3 of QQSYSTPWT (SEQ ID NO: 43);

(g) the VH region amino acid sequence comprises HCDR1 of GFTFSSYLMS (SEQ ID NO: 38), HCDR2 of VATISGGGSETYYVDSVKG (SEQ ID NO: 48), and HCDR3 of QLYGFDY (SEQ ID NO: 40); and the VL region amino acid sequence comprises LCDR1 of RASQSISSWLN (SEQ ID NO: 41), LCDR2 of AASSLQS (SEQ ID NO: 42), and LCDR3 of QQSYSIPWT (SEQ ID NO: 47);

(h) the VH region amino acid sequence comprises HCDR1 of GFTFSSYLMS (SEQ ID NO: 38), HCDR2 of VATISGGGAEKYYVDSVKG (SEQ ID NO: 49), and HCDR3 of QLYGFDY (SEQ ID NO: 40); and the VL region amino acid sequence comprises LCDR1 of RASQSISSWLN (SEQ ID NO: 41), LCDR2 of AASSLQD (SEQ ID NO: 50), and LCDR3 of QQSYSTPWT (SEQ ID NO: 43);

(i) the VH region amino acid sequence comprises HCDR1 of GFTFSSYLMS (SEQ ID NO: 38), HCDR2 of VATISGGGSNTYYVDSVKG (SEQ ID NO: 51), and HCDR3 of QLYGFDY (SEQ ID NO: 40); and the VL region amino acid sequence comprises LCDR1 of RASQSISTWLN (SEQ ID NO: 52), LCDR2 of AASSLAS (SEQ ID NO: 53), and LCDR3 of QQSYSTPWT (SEQ ID NO: 43);

(j) the VH region amino acid sequence comprises HCDR1 of GFTFSSYLMS (SEQ ID NO: 38), HCDR2 of VATISGGGSEKYYVDSVKG (SEQ ID NO: 39), and HCDR3 of

- QVYYFDY (SEQ ID NO: 44); and the VL region amino acid sequence comprises LCDR1 of RASQSISSWLN (SEQ ID NO: 41), LCDR2 of AASSLHS (SEQ ID NO: 54), and LCDR3 of QQSYSTPWT (SEQ ID NO: 43); or
- (k) the VH region amino acid sequence comprises HCDR1 of GFTFSSYLMS (SEQ ID NO: 38), HCDR2 of VATISGGGSEKYYVDSVKG (SEQ ID NO: 39), and HCDR3 of QVYYFDY (SEQ ID NO: 44); and the VL region amino acid sequence comprises LCDR1 of RASQSISSWLN (SEQ ID NO: 41), LCDR2 of AASSLQS (SEQ ID NO: 42), and LCDR3 of QQSYSTPWT (SEQ ID NO: 43).
- 10 In some aspects, disclosed herein is an anti-PD1 antibody or an antigen-binding portion thereof, wherein the antibody comprises a heavy chain variable (VH) region and a light chain variable (VL) region, wherein
- the VH region amino acid sequence comprises:
- (a) HCDR1 of SEQ ID NO: 38;
- 15 (b) HCDR2 of SEQ ID NO: 39, SEQ ID NO: 48, SEQ ID NO: 49 or SEQ ID NO: 51;
- and
- (c) HCDR3 of SEQ ID NO: 40, SEQ ID NO: 44, SEQ ID NO: 45 or SEQ ID NO: 46;
- and
- the VL region amino acid sequence comprises:
- 20 (a') LCDR1 of SEQ ID NO: 41 or SEQ ID NO: 52;
- (b') LCDR2 of SEQ ID NO: 42, SEQ ID NO: 50, SEQ ID NO: 53 or SEQ ID NO: 54;
- and
- (c') LCDR3 of SEQ ID NO: 43 or SEQ ID NO: 47.
- 25 In some aspects, disclosed herein is an anti-PD1 antibody or an antigen-binding portion thereof, wherein the antibody comprises a heavy chain variable (VH) region and a light chain variable (VL) region, wherein
- (a) the VH region amino acid sequence comprises SEQ ID NO:1 and the VL region amino acid sequence comprises SEQ ID NO:2;
- 30 (b) the VH region amino acid sequence comprises SEQ ID NO:3 and the VL region amino acid sequence comprises SEQ ID NO:4;
- (c) the VH region amino acid sequence comprises SEQ ID NO:5 and the VL region amino acid sequence comprises SEQ ID NO:6;
- (d) the VH region amino acid sequence comprises SEQ ID NO:7 and the VL region amino acid sequence comprises SEQ ID NO:8;
- 35 (e) the VH region amino acid sequence comprises SEQ ID NO:9 and the VL region amino acid sequence comprises SEQ ID NO:10; or

(f) the VH region amino acid sequence comprises SEQ ID NO:11 and the VL region amino acid sequence comprises SEQ ID NO:12.

5 Also provided according to the invention is an immunoconjugate comprising the antibody molecule or antigen-binding portion thereof as defined herein linked, fused or conjugated to a therapeutic agent.

10 In another aspect the invention provides a nucleic acid molecule encoding the antibody molecule or antigen-binding portion thereof as defined herein.

Further provided is a vector comprising the nucleic acid molecule of the invention.

15 Also provided is a host cell comprising the nucleic acid molecule or the vector of the invention as defined herein.

20 In a further aspect there is provided a method of producing an anti-PD1 antibody and/or an antigen-binding portion thereof, comprising culturing the host cell of the invention under conditions that result in expression and/or production of the antibody and/or the antigen-binding portion thereof, and isolating the antibody and/or the antigen-binding portion thereof from the host cell or culture.

25 In another aspect of the invention there is provided a pharmaceutical composition comprising the antibody molecule or antigen-binding portion thereof of the invention as defined herein, or the nucleic acid molecule of the invention as defined herein, or the vector of the invention as defined herein.

30 Further provided is a method for enhancing an immune response in a subject, comprising administering an effective amount of the antibody molecule or antigen-binding portion thereof of the invention as defined herein, or the immunoconjugate of the invention as defined herein, or the nucleic acid molecule of the invention as defined herein, or the vector of the invention as defined herein, or the pharmaceutical composition of the invention as defined herein.

35 In a further aspect there is provided a method for treating or preventing cancer in a subject, comprising administering an effective amount of the antibody molecule or antigen-binding portion thereof of the invention as defined herein, or the immunoconjugate of the invention as defined herein, or the nucleic acid molecule of the invention as defined herein, or the

vector of the invention as defined herein, or the pharmaceutical composition of the invention as defined herein.

Further provided herein is an antibody molecule or antigen-binding portion thereof as defined
5 herein, or the immunoconjugate as defined herein, or the nucleic acid molecule as defined
herein, or the vector as defined herein, or the pharmaceutical composition as defined herein,
for use as a medicament. The invention also provides an antibody molecule or antigen-
binding portion thereof of the invention as defined herein, or the immunoconjugate of the
10 invention as defined herein, or the nucleic acid molecule of the invention as defined herein,
or the vector of the invention as defined herein, or the pharmaceutical composition of the
invention as defined herein, for use in the treatment of cancer.

In another aspect the invention provides the antibody molecule, or antigen-binding portion
thereof, or the immunoconjugate, or the nucleic acid molecule, or the vector for use, or the
15 method of treatment of the invention as defined herein, for separate, sequential or
simultaneous use in a combination combined with a second therapeutic agent, for example
an anti-cancer agent.

In a further aspect there is provided the use of an antibody molecule or antigen-binding
20 portion thereof of the invention as defined herein, or an immunoconjugate of the invention
as defined herein, or a nucleic acid molecule of the invention as defined herein, or a vector
of the invention as defined herein, or a pharmaceutical composition of the invention as
defined herein, in the manufacture of a medicament for the treatment of cancer.

25 The invention also provides a method for treating or preventing an infectious disease in a
subject, comprising administering an effective amount of the antibody molecule or antigen-
binding portion thereof as defined herein, or the immunoconjugate as defined here, or the
nucleic acid molecule as defined herein, or the vector as defined herein, or the
pharmaceutical composition as defined herein.

30 The infectious disease may be selected in all aspects from the group consisting of: viral,
bacterial, fungal or parasitic. In one embodiment, the infectious disease is human
immunodeficiency virus (HIV) infection.

35 Also provided is an antibody molecule or antigen-binding portion thereof as defined herein,
or the immunoconjugate as defined herein, or the nucleic acid molecule as defined herein,

or the vector as defined herein, or the pharmaceutical composition as defined herein, for use in the treatment of an infectious disease.

Further provided is the use of an antibody molecule or antigen-binding portion thereof as defined herein, or an immunoconjugate as defined herein, or a nucleic acid molecule as defined herein, or a vector as defined herein, or a pharmaceutical composition as defined herein, in the manufacture of a medicament for the treatment of an infectious disease.

The invention also provides a method for treating or preventing an infectious disease in a subject, comprising administering an effective amount of the antibody molecule or antigen-binding portion thereof as defined herein, or the immunoconjugate as defined here, or the nucleic acid molecule as defined herein, or the vector as defined herein, or the pharmaceutical composition as defined herein.

The invention also provides a method of producing an antibody molecule which specifically binds to human PD1 and optionally also to cynomolgus monkey PD1, or an antigen-binding portion thereof, comprising the steps of:

- (1) grafting anti-PD1 CDRs from a non-human source into a human v-domain framework to produce a humanized anti-PD1 antibody molecule or antigen-binding portion thereof;
- (2) generating a library of clones of the humanized anti-PD1 antibody molecule or antigen-binding portion thereof comprising one or more mutations in the CDRs;
- (3) screening the library for binding to human PD1 and optionally also to cynomolgus monkey PD1;
- (4) selecting clones from the screening step (3) having binding specificity to human PD1 and optionally also to cynomolgus monkey PD1; and
- (5) producing an antibody molecule which specifically binds to human PD1 and optionally also to cynomolgus monkey PD1, or an antigen-binding portion thereof from clones selected from step (4).

The method may comprise a further step of producing additional clones based on the clones selected in step (4), for example based on further exploratory mutagenesis at specific positions in the CDRs of the clones selected in step (4), to enhance humanization and/or minimise human T cell epitope content and/or improve manufacturing properties in the antibody molecule or antigen-binding portion thereof produced in step (5).

BRIEF DESCRIPTION OF THE FIGURES

FIG. 1. Direct binding ELISA of library-derived anti-PD1 Fabs against human and cyno

PD1-Fc proteins. Clones were derived from multiple phage selection branches where phage populations were selected on biotinylated human, or cynomolgus monkey PD1 proteins in each round. Standard selections are numbered R2-R4. 'Hammer-Hug' rounds are numbered R2-H and R3-H. After each round of selection, library-derived clones (black circles) were screened as periplasmically-expressed Fab proteins, against both human (h) and cyno (c) PD1. Mean $\pm$ SD values in each round are represented in grey bars. mAb005 v-domains expressed as human IgG1 Fab were used as positive controls on each plate (grey circles).

FIG. 2A – FIG. 2B. Direct titration ELISA for purified monomeric Fab binding to human and cyno PD1-Fc proteins. Mab005 and library-derived clones in human Fab format were titrated (in nM) in a direct binding ELISA against human (FIG. 2A) and cyno (FIG. 2B) PD1-Fc proteins.

FIG. 3A – FIG. 3B. Analysis of CDR residue tolerance for mutation to germline. A plot of murine amino acid retention frequencies in the CDRs of the ELISA-positive population of 64 unique scFv clones is shown for V_L (FIG. 3A; SEQ ID NOs: 110, 114 and 116) and V_H (FIG. 3B; SEQ ID NOs: 95, 92 and 106) domains, respectively. Only those residues targeted for human/murine residue mutagenesis are plotted, other than in the HCDR3. CDR residues noted in parentheses on the X-axes were identical to those found in the human germlines used for grafting (IGKV1-39 and IGHV3-7). In both plots the dashed line in grey at 75% represents the cut-off for tolerance of murine residue replacement by human germline.

FIG. 4A – FIG. 4B. Direct titration ELISA for library-derived IgG1null clones binding to human and cyno PD1-Fc proteins. Mab005 and library-derived clones in human IgG1null format were titrated (in nM) in a direct binding ELISA against human (FIG. 4A) and cyno (FIG. 4B) PD1-Fc proteins.

FIG. 5A – FIG. 5D. Direct titration ELISA for designer IgG1null clones IgG1-01 to IgG1-15 binding to human and cyno PD1-Fc proteins. Mab005 and designer clones in human IgG1null format were titrated (in nM) in a direct binding ELISA against human (FIG. 5A, FIG. 5B) and cyno (FIG. 5C, FIG. 5D) PD1-Fc proteins.

FIG. 6A – FIG. 6B. Epitope competition analysis of IgG1null proteins in Alphascreen Anti-PD1 IgG1null clones were applied in an epitope competition assay using Alphascreen technology. In this assay, library-derived (FIG. 6A) and designer (FIG. 6B) IgGs were analysed for their relative affinities and retention of the parental Mab005 epitope by competing for Mab005 IgG1null binding to human PD1 protein, in solution. All clones analysed showed strong, concentration-dependent neutralisation of Mab-005 binding to PD1.

FIG. 7A - FIG. 7C. Flow cytometric binding to human PD1+ CHO cells. IgG1-Mab005 (humanized), lead library-derived (FIG. 7A) and designer (FIG. 7B) IgGs were examined for specific binding on CHO-K1 cells expressing human PD1. Concentration-dependent binding

was observed against human PD1 for almost all clones, with weaker binding being observed for IgG1-Mab005 (humanized). Clone IgG1-15 was a single exception, showing weaker binding than IgG1-Mab005 (humanized) (FIG. 7C).

FIG. 8A – FIG. 8B. Flow cytometric testing of binding to cyno PD1+ CHO cells. IgG1-Mab005 (humanized), lead library-derived (FIG. 8A) and designer (FIG. 8B) IgGs were examined for specific binding on CHO-K1 cells expressing cyno PD1. Concentration-dependent binding was observed against cyno PD1 for all clones, with significantly stronger binding than that observed for IgG1-Mab005 (humanized).

FIG. 9. Cell-based PD1/PD-L1 antagonism assay. Analyses of antagonism of human PD1 function at the cell surface, for lead clones IgG1-11, IgG1-14, IgG1-06D02 and IgG1-16H10 in human IgG1null format, showed that all novel clones exhibited concentration-dependent antagonistic activity, with higher relative potency in comparison to both IgG1-Mab005 (humanized) and IgG4 Nivolumab analog.

FIG. 10A – FIG. 10E. Off-target binding analysis chip array assay. After performing an array-based binding screen on 4975 human receptors for IgG1-Mab005 (humanized), confirmatory analyses of binding specificity were performed on chips in which plasmids encoding PD1 and putative Mab005 off-target binding proteins were arrayed and used to transfect HEK293 cells. Effective transfection of all plasmids was confirmed by screening for the co-encoded marker ZS green (FIG. 10A). Separate chips were then probed in duplicate using IgG1-Mab005 (humanized) (FIG. 10B), Isotype IgG1 (FIG. 10C), Rituximab (FIG. 10D), and no primary antibody (FIG. 10E). These analyses confirmed that only IgG1-Mab005 (humanized) exhibited binding to PD1, but IgG1-Mab005 (humanized) also exhibited unexpected off-target binding to KDR, FZD5 and ULBP2 proteins.

FIG. 11A – FIG. 11T. Off-target binding analysis re-array assay. Analyses of binding specificity were performed on chips in which plasmids encoding relevant targets were arrayed and used to transfect HEK293 cells. Transfection of all plasmids was confirmed by screening for the co-encoded marker ZS green (FIG. 11A). Separate chips were then probed in duplicate using Pembrolizumab analog (FIG. 11B), Rituximab (FIG. 11C), Isotype IgG1 (FIG. 11D), mVH/mVL Mab005-IgG1 (FIG. 11E), IgG1-Mab005 (humanized) (FIG. 11F) and library-derived and designer lead IgGs (FIG. 11G - FIG. 11T). These analyses confirmed that mVH/mVL Mab005 IgG1 (original murine hybridoma v-domains) and IgG1-Mab005 (humanized) (humanized v-domains) both exhibited binding to PD1, but also exhibited unexpected off-target binding to KDR, FZD5 and ULBP2 proteins. In contrast, none of the lead antibodies (FIG. 11G - FIG. 11T) exhibited measurable signal (i.e., binding) on any protein other than PD1.

FIG. 12A – FIG. 12P. PD1 binding analysis by flow cytometry using transiently-transfected HEK293 cells. Analyses of binding specificity were performed on HEK293 cells

transiently transfected with plasmids encoding either human PD1 (grey line) or ZS green marker (black line). Transfected cells were then stained using mVH/mVL Mab005-IgG1, IgG1-Mab005 (humanized), Pembrolizumab analog, isotype IgG1 and a subset of lead antibodies (IgG1-06D02, IgG1-12HO4, IgG1-05, IgG1-08). Each antibody was used in repeat staining at high (5 µg/ml) and moderate (1 µg/ml) concentration. These analyses confirmed that all antibodies (other than the isotype control IgG1) exhibited binding to PD1, but no antibody exhibited measurable signal on ZS-green transfected cells.

FIG. 13A – FIG. 13P. FZD5 binding analysis by flow cytometry using transiently-transfected HEK293 cells. Analyses of binding specificity were performed on HEK293 cells transiently transfected with plasmids encoding either human FZD5 (grey line) or ZS green marker (black line). Transfected cells were then stained using mVH/mVL Mab005-IgG1, IgG1-Mab005 (humanized), Pembrolizumab analog, isotype IgG1 and a subset of lead antibodies (IgG1-06D02, IgG1-12HO4, IgG1-05, IgG1-08). Each antibody was used in repeat staining at high (5 µg/ml) and moderate (1 µg/ml) concentration. These analyses confirmed that the only antibodies which exhibited binding to FZD5-expressing cells were mVH/mVL Mab005-IgG1 and IgG1-Mab005 (humanized).

FIG. 14A – FIG. 14P. KDR binding analysis by flow cytometry using transiently-transfected HEK293 cells. Analyses of binding specificity were performed on HEK293 cells transiently transfected with plasmids encoding either human KDR (grey line) or ZS green marker (black line). Transfected cells were then stained using mVH/mVL Mab005-IgG1, IgG1-Mab005 (humanized), Pembrolizumab analog, isotype IgG1 and a subset of lead antibodies (IgG1-06D02, IgG1-12HO4, IgG1-05, IgG1-08). Each antibody was used in repeat staining at high (5 µg/ml) and moderate (1 µg/ml) concentration. These analyses confirmed that the only antibodies which exhibited binding to KDR-expressing cells were mVH/mVL Mab005-IgG1 and IgG1-Mab005 (humanized).

FIG. 15A – FIG. 15P. ULBP2 binding analysis by flow cytometry using transiently-transfected HEK293 cells. Analyses of binding specificity were performed on HEK293 cells transiently transfected with plasmids encoding either human ULBP2 (grey line) or ZS green marker (black line). Transfected cells were then stained using mVH/mVL Mab005-IgG1, IgG1-Mab005 (humanized), Pembrolizumab analog, isotype IgG1 and a subset of lead antibodies (IgG1-06D02, IgG1-12HO4, IgG1-05, IgG1-08). Each antibody was used in repeat staining at high (5 µg/ml) and moderate (1 µg/ml) concentration. These analyses confirmed that the only antibodies which exhibited binding to ULBP2-expressing cells were mVH/mVL Mab005-IgG1 and IgG1-Mab005 (humanized).

FIG. 16A – FIG. 16F. Off-target binding analysis ELISA assay. IgG1-Mab005 (humanized), mVH/mVL Mab005-IgG1, library-derived clones and designer clones in human

IgG1null format were titrated (in $\mu\text{g/ml}$) in a direct binding ELISA against human PD1 (FIG. 16A) and cyno PD1 (FIG. 16B), human VEGFR2 (FIG. 16C) and rhesus VEGFR2 (FIG. 16D), human FZD5 (FIG. 16E) and BSA (FIG. 16F) proteins. These analyses confirmed that all anti-PD1 antibodies exhibited binding to PD1, but only IgG1-Mab005 (humanized) and mVH/mVL Mab005-IgG1 exhibited measurable off-target binding to VEGFR2 and FZD5 proteins.

FIG. 17. VEGFR2 binding for mVH/mVL Mab005-IgG1 and IgG1-Mab005 (humanized) by Biacore. Human and rhesus monomeric VEGFR2-his proteins were titrated (in nM) in a direct binding ELISA against mVH/mVL Mab005-IgG1 and IgG1-Mab005 (humanized), captured by anti-human IgG1-Fc antibody. These analyses confirmed that both anti-PD1 antibodies exhibited binding to VEGFR2 recombinant protein, but binding was only observed at high concentrations of soluble analyte. As a result, reliable KD values could not be generated.

FIG. 18. Cell-based VEGFR2 agonism assay. mVH/mVL Mab005-IgG1, IgG1-Mab005 (humanized), library-derived clones and designer clones in human IgG1null format were titrated (in nM) in a human VEGFR2 signalling assay. mVH/mVL Mab005-IgG1, IgG1-Mab005 (humanized) and the positive control protein (human VEGF-165) all induced strong, concentration-dependent VEGFR2 agonism. Lead clones IgG1-04, IgG1-08, IgG1-06D02, and IgG1-12H04 showed no measurable agonism, even at concentrations as high as 1 μM .

FIG. 19. Differential Scanning Calorimetry (DSC) of IgGs. DSC assay data for the following antibodies in IgG1null form: (mAb-1) IgG1-05, (mAb-2) IgG1-08, (mAb-3) IgG1-Mab005 (humanized), (mAb-4) IgG1-06D02, and (mAb-5) IgG1-12H04.

FIG. 20A – FIG. 20B. Reverse Phase Chromatography analyses of tryptic peptides from IgGs before and after forced oxidation. Reverse phase chromatograms showed that for antibodies in IgG1null form: IgG1-Mab005 (humanized) (FIG. 20A) demonstrated up to 6 peptide changes post-oxidation, whereas lead clones such as IgG1-06D02 (FIG. 20B) showed greater resistance to oxidation with only 2 modifications.

DETAILED DESCRIPTION OF THE INVENTION

According to a first aspect of the invention, there is provided an antibody molecule which specifically binds to human PD1 and also to cynomolgus monkey PD1, or an antigen-binding portion thereof, wherein the antibody molecule or antigen-binding portion comprises a heavy chain variable region with:

- an HCDR1 having amino acids in sequence in the following order: G-F-T-F-S-S-Y-L or any amino acid (for example A/D/E/F/G/H/I/N/P/Q/S/T/V/W/Y)-M-S (SEQ ID NO: 26);
- an HCDR2 having amino acids in sequence in the following order: V or a conservative substitution of V-A-T/N-I-S-G-G-G-A/S-E/N-T/K-Y-Y-P/V-D-S-V-K-G (SEQ ID NO: 27); and

an HCDR3 having amino acids in sequence in the following order: Q or any amino acid (for example T/L/M/N)-L or any amino acid (for example G/K/M/Q/S/V)-Y or a conservative substitution of Y (for example H)-Y or any amino acid (for example A/D/F/G/M-A/D/E/F/I/K/M/S/W)-F or any amino acid (for example A/D/E/I/K/M/S/W)-D-Y (SEQ ID NO: 28).

In some aspects an anti-PD1 antibody or antigen-binding portion provided herein specifically binds to a PD1 protein comprising or consisting of SEQ ID NO:20 or SEQ ID NO:21. In some aspects an anti-PD1 antibody or antigen-binding portion provided herein specifically binds to a PD1 protein having an amino acid sequence that is at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98% or at least about 99% identical to SEQ ID NO:20 or SEQ ID NO:21.

In aspects of the invention, the HCDR1 of the antibody molecule or antigen-binding portion may exclude the sequence GFTFSSYMMS (SEQ ID NO: 29; MAb005 murine/humanized antibody HCDR1 disclosed in WO2015/085847A1; US2016/376367A1), and/or the HCDR2 of the antibody molecule or antigen-binding portion may exclude the sequence TISGGGANTYYPDSVKG (SEQ ID NO: 30; MAb005 murine/humanized antibody HCDR2 disclosed in WO2015/085847A1; US2016/376367A1), and/or the HCDR3 of the antibody molecule or antigen-binding portion may exclude the sequence QLYYFDY (SEQ ID NO: 31; MAb005 murine/humanized antibody HCDR3 disclosed in WO2015/085847A1; US2016/376367A1).

The antibody molecule or antigen-binding portion thereof according to the invention may further comprise a light chain variable region with:

an LCDR1 having amino acids in sequence in the following order: R-A-S-Q-S-I-S or a conservative substitution of S-S or a conservative substitution of S -Y or a conservative substitution of Y-L or a conservative substitution of L-N/T or a conservative substitution of N or T (SEQ ID NO: 32);

an LCDR2 having amino acids in sequence in the following order: A/T or a conservative substitution of A or T-A-S or a conservative substitution of S-S-L-Q or any amino acid (for example, A/H)-S or any amino acid (for example, D, Y) (SEQ ID NO: 33); and

an LCDR3 having amino acids in sequence in the following order: Q-Q-S or any amino acid (for example, V)-Y-S-T or any amino acid (for example, I)-P-W or any amino acid (for example, L)-T (SEQ ID NO: 34).

- In aspects of the invention, the LCDR1 of the antibody molecule or antigen-binding portion may exclude the sequence LASQTIGTWLT (SEQ ID NO: 35; MAb005 murine/humanized antibody LCDR1 disclosed in WO2015/085847A1; US2016/376367A1), and/or the LCDR2 of the antibody molecule or antigen-binding portion may exclude the sequence TATSLAD (SEQ ID NO: 36; MAb005 murine/humanized antibody LCDR2 disclosed in WO2015/085847A1; US2016/376367A1) and/or the LCDR3 of the antibody molecule or antigen-binding portion may exclude the sequence QQVYSIPWT (SEQ ID NO: 37; MAb005 murine/humanized antibody LCDR3 disclosed in WO2015/085847A1; US2016/376367A1).
- 10 In some aspects, disclosed herein is an anti-PD1 antibody or an antigen-binding portion thereof, wherein the antibody comprises a heavy chain variable (VH) region comprising HCDR1, HCDR2, and HCDR3 and a light chain variable (VL) region comprising LCDR1, LCDR2, and LCDR3, wherein
- (a) the HCDR1 comprises the amino acid sequence G-F-T-F-S-S-Y-X₁-M-S, wherein
- 15 X₁ is L or any other amino acid (for example, A, D, E, F, G, H, I, N, P, Q, S, T, V, W, or Y) (SEQ ID NO: 26);
- (b) the HCDR2 comprises the amino acid sequence X₁-A-X₂-I-S-G-G-X₃-X₄-X₅-Y-X₆-D-S-V-K-G, wherein X₁ is V or a conservative substitution of V, X₂ is T or N, X₃ is A or S, X₄ is E or N, X₅ is T or K, and X₆ is P or V (SEQ ID NO: 27);
- 20 (c) the HCDR3 comprises the amino acid sequence X₁-X₂-X₃-X₄-X₅-D-Y, wherein X₁ is Q or any other amino acid (for example, T, L, M, or N), X₂ is L or any other amino acid (for example, G, K, M, Q, S, or V), X₃ is Y or a conservative substitution of Y (for example, H), X₄ is Y or any other amino acid (for example, A, D, F, G, M, E, I, K, S, or W) and X₅ is F or any other amino acid (for example A, D, E, I, K, M, S, or W) (SEQ ID NO: 28);
- 25 (d) the LCDR1 comprises the amino acid sequence R-A-S-Q-S-I-X₁-X₂-X₃-X₄-X₅, wherein X₁ is S or a conservative substitution of S, X₂ is S or a conservative substitution of S, X₃ is Y or a conservative substitution of Y, X₄ is L or a conservative substitution of L, and X₅ is N or T or a conservative substitution of N or T (SEQ ID NO: 32);
- (e) the LCDR2 comprises the amino acid sequence X₁-A-X₂-S-L-X₃-X₄, wherein X₁
- 30 is A or T or a conservative substitution of A or T, X₂ is S or a conservative substitution of S, X₃ is Q or any other amino acid (for example, A or H), and X₄ is S or any other amino acid (for example, D or Y) (SEQ ID NO: 33); and
- (f) the LCDR3 comprises the amino acid sequence Q-Q-X₁-Y-S-X₂-P-X₃-T, wherein X₁ is S or any other amino acid (for example, V), X₂ is T or any other amino acid (for example, I), and X₃ is W or any other amino acid (for example, L) (SEQ ID NO: 34).
- 35

In some aspects, disclosed herein is an anti-PD1 antibody or an antigen-binding portion thereof, wherein the antibody comprises a heavy chain variable (VH) region comprising, in amino-terminal to carboxyl-terminal order, FR1-HCDR1-FR2-HCDR2-FR3-HCDR3-FR4 and a light chain variable (VL) region comprising, in amino-terminal to carboxyl-terminal
5 order, FR1-LCDR1-FR2-LCDR2-FR3-LCDR3-FR4, wherein the HCDR1 is SEQ ID NO:26, the HCDR2 is SEQ ID NO:27, the HCDR3 is SEQ ID NO:28, the LCDR1 is SEQ ID NO:32, the LCDR2 is SEQ ID NO:33 and the LCDR3 is SEQ ID NO:34, wherein the heavy chain FR1, FR2, FR3 and FR4 amino acid sequences are the heavy chain FR1, FR2, FR3 and FR4 amino acid sequences in SEQ ID NO: 120 (see Table 2) and wherein the light chain
10 FR1, FR2, FR3 and FR4 amino acid sequences are the light chain FR1, FR2, FR3 and FR4 amino acid sequences in SEQ ID NO: 122 (see Table 2).

As elaborated herein, the present inventors have succeeded for the first time in generating a number of optimized anti-PD1 antibody molecules using CDR sequences derived from the
15 murine anti-PD1 antibody MAb005 disclosed in WO2015/085847A1; US2016/376367A1. In embodiments of the present invention, these antibody molecules have been selected to have equivalency in binding specificity and affinity to both human PD1 as well as cynomolgus monkey PD1 (to facilitate maximally accurate primate toxicology and pk studies). Further refining of the optimized antibody molecules as described herein has provided improved
20 binding to the cynomolgus monkey orthologues of PD1, improved potency in neutralisation of PD1/PD-L1 signalling, improved variable domain stability, high expression yields, and/or reduced immunogenicity potential. Critically, these antibodies also dramatically improved the specificity of PD1 binding in comparison to MAb005, by ablating off-target binding to the human receptors KDR (also known as VEGFR2), FZD5 and ULBP2.

25 In some aspects, optimized anti-PD1 antibody molecules of the present invention do not necessarily have the maximum number of human germline substitutions at corresponding murine CDR or other (such as framework) amino acid positions. As elaborated in the experimental section below, we have found that "maximally humanized" antibody molecules
30 are not necessary "maximally optimized" in terms of anti-PD1 binding characteristics and/or other desirable features.

In some embodiments, optimized anti-PD1 antibody molecules of the present invention are improved over the Mab005 antibody (WO2015/085847A1; US2016/376367A1) in their
35 binding of a functionally identical epitope on cyno PD1. As elaborated in the experimental section below, we have found that some optimized clones exhibit Biacore KD values lower than 32nM for binding to monomeric cyno PD1 protein (e.g. between 2.7 and 15 nM) and

EC50 values lower than 6.5 nM (e.g. 0.86-2.17 nM) in flow cytometric staining of cyno PD1-expressing CHO cells.

5 The present invention encompasses modifications to the amino acid sequence of the antibody molecule or antigen-binding portion thereof as defined herein. For example, the invention includes antibody molecules and corresponding antigen-binding portions thereof comprising functionally equivalent variable regions and CDRs which do not significantly affect their properties as well as variants which have enhanced or decreased activity and/or affinity. For example, the amino acid sequence may be mutated to obtain an antibody with
10 the desired binding affinity to PD1. Insertions which include amino- and/or carboxyl-terminal fusions ranging in length from one residue to polypeptides containing a hundred or more residues, as well as intrasequence insertions of single or multiple amino acid residues, are envisaged. Examples of terminal insertions include an antibody molecule with an N-terminal methionyl residue or the antibody molecule fused to an epitope tag. Other insertional variants
15 of the antibody molecule include the fusion to the N- or C-terminus of the antibody of an enzyme or a polypeptide which increases the half-life of the antibody in the blood circulation.

The antibody molecule or antigen-binding portion of the invention may include glycosylated and nonglycosylated polypeptides, as well as polypeptides with other post-translational
20 modifications, such as, for example, glycosylation with different sugars, acetylation, and phosphorylation. The antibody molecule or antigen-binding portion of the invention may be mutated to alter such post-translational modifications, for example by adding, removing or replacing one or more amino acid residues to form or remove a glycosylation site.

25 The antibody molecule or antigen-binding portion of the invention may be modified for example by amino acid substitution to remove potential proteolytic sites in the antibody.

In the antibody molecule or antigen-binding portion thereof, the HCDR1 may have the amino acid sequence: G-F-T-F-S-S-Y-A/D/E/F/H/I/L/N/P/Q/S/T/V/W/Y-M-S (SEQ ID NO: 55); the
30 HCDR2 may have the amino acid sequence: V-A-T/N-I-S-G-G-G-S/A-E/N-T/K-Y-Y-V/P-D-S-V-K-G (SEQ ID NO: 56); and the HCDR3 may have the amino acid sequence: Q/L/M/N/T-L/G/K/Q/M/S/V-Y/H-Y/A/D/G/F/M-F/A/D/E/I/K/M/S/W/Y-D-Y (SEQ ID NO: 57).

For example, the HCDR1 may have the amino acid sequence: G-F-T-F-S-S-Y-L/A/S-M-S
35 (SEQ ID NO: 58); the HCDR2 may have the amino acid sequence: V-A-T-I-S-G-G-G-S/A-

E/N-T/K-Y-Y-V-D-S-V-K-G (SEQ ID NO: 59); and the HCDR3 may have the amino acid sequence: Q-L/V-Y-Y/G/F/A-F/Y/A-D-Y (SEQ ID NO: 60).

In the antibody molecule or antigen-binding portion thereof, the LCDR1 may have the amino acid sequence: R-A-S-Q-T/S-I-G/S-T/S-W/Y-L-T/N (SEQ ID NO: 61); the LCDR2 may have the amino acid sequence T/A-A-T/S-S-L-A/Q/H-D/S (SEQ ID NO: 62); and the LCDR3 may have the amino acid sequence: Q-Q-V/S-Y-S-I/T-P-W/L-T (SEQ ID NO: 63).

For example, the LCDR1 may have the amino acid sequence: R-A-S-Q-S-I-G/S-T/S-W/Y-L-N (SEQ ID NO: 64); the LCDR2 may have the amino acid sequence A-A-S-S-L-Q/H-S (SEQ ID NO: 65); and the LCDR3 may have the amino acid sequence: Q-Q-S-Y-S-I/T-P-W-T (SEQ ID NO: 66).

In specific embodiments of the invention, the antibody molecule or antigen-binding portion may comprise:

(a) the amino acid sequences

GFTFSSYLMS (SEQ ID NO: 38; HCDR1), VATISGGGSETYYVDSVKG (SEQ ID NO: 48; HCDR2), QLYGFDY (SEQ ID NO: 40; HCDR3), RASQSISSWLN (SEQ ID NO: 41; LCDR1), AASSLQS (SEQ ID NO: 42; LCDR2) and QQSYSIPWT (SEQ ID NO: 47; LCDR3) [Clone 06D02]; or

(b) the amino acid sequences

GFTFSSYAMS (SEQ ID NO: 67; HCDR1), VATISGGGSNKYYVDSVKG (SEQ ID NO: 68; HCDR2), QLYGFDY (SEQ ID NO: 40; HCDR3), RASQSISTWLN (SEQ ID NO: 52; LCDR1), AATSLAS (SEQ ID NO: 69; LCDR2) and QQSYSIPWT (SEQ ID NO: 47; LCDR3) [Clone 08F04];

(c) the amino acid sequences

GFTFSSYLMS (SEQ ID NO: 38; HCDR1), VATISGGGAETYYVDSVKG (SEQ ID NO: 70; HCDR2), QLYFFDY (SEQ ID NO: 45; HCDR3), RASQSISSWLN (SEQ ID NO: 41; LCDR1), TASSLQD (SEQ ID NO: 71; LCDR2) and QQSYSTPWT (SEQ ID NO: 43; LCDR3) [Clone 11G05];

(d) the amino acid sequences

GFTFSSYAMS (SEQ ID NO: 67; HCDR1), VATISGGGSEKYYVDSVKG (SEQ ID NO: 39; HCDR2), QLYYADY (SEQ ID NO: 72; HCDR3), RASQSISSWLN (SEQ ID NO: 41; LCDR1), TASSLAD (SEQ ID NO: 73; LCDR2) and QQSYSIPWT (SEQ ID NO: 47; LCDR3) [Clone 12E02];

(e) the amino acid sequences

- GFTFSSYLMS (SEQ ID NO: 38; HCDR1), VATISGGGAEKYYVDSVKG (SEQ ID NO: 49; HCDR2), QLYGFDY (SEQ ID NO: 40; HCDR3), RASQSISSWLN (SEQ ID NO: 41; LCDR1), AASSLQD (SEQ ID NO: 50; LCDR2) and QQSYSTPWT (SEQ ID NO: 43; LCDR3) [Clone 12H04];
- 5 (f) the amino acid sequences
GFTFSSYLMS (SEQ ID NO: 38; HCDR1), VATISGGGSNTYYVDSVKG (SEQ ID NO: 51; HCDR2), QLYGFDY (SEQ ID NO: 40; HCDR3), RASQSISTWLN (SEQ ID NO: 52; LCDR1), AASSLAS (SEQ ID NO: 53; LCDR2) and QQSYSTPWT (SEQ ID NO: 43; LCDR3) [Clone 12B07];
- 10 (g) the amino acid sequences
GFTFSSYLMS (SEQ ID NO: 38; HCDR1), VATISGGGAEKYYVDSVKG (SEQ ID NO: 49; HCDR2), QLYGFDY (SEQ ID NO: 40; HCDR3), RASQSISSYLN (SEQ ID NO: 74; LCDR1), AASSLQS (SEQ ID NO: 42; LCDR2) and QQSYSTPWT (SEQ ID NO: 43; LCDR3) [Clone 13G02];
- 15 (h) the amino acid sequences
GFTFSSYSMS (SEQ ID NO: 75; HCDR1), VATISGGGAETYYVDSVKG (SEQ ID NO: 70; HCDR2), QLYGFDY (SEQ ID NO: 40; HCDR3), RASQSISSWLN (SEQ ID NO: 41; LCDR1), AASSLQS (SEQ ID NO: 42; LCDR2) and QQSYSIPWT (SEQ ID NO: 47; LCDR3) [Clone 14C07];
- 20 (i) the amino acid sequences
GFTFSSYLMS (SEQ ID NO: 38; HCDR1), VATISGGGSNKYYVDSVKG (SEQ ID NO: 68; HCDR2), QLYGFDY (SEQ ID NO: 40; HCDR3), RASQSIGTYLN (SEQ ID NO: 76; LCDR1), AATSLQS (SEQ ID NO: 77; LCDR2) and QQSYSIPWT (SEQ ID NO: 47; LCDR3) [Clone 15C10];
- 25 (j) the amino acid sequences
GFTFSSYLMS (SEQ ID NO: 38; HCDR1), VATISGGGAEKYYVDSVKG (SEQ ID NO: 49; HCDR2), QLYAFDY (SEQ ID NO: 46; HCDR3), RASQSIGSYLN (SEQ ID NO: 78; LCDR1), TASSLQS (SEQ ID NO: 79; LCDR2) and QQSYSIPWT (SEQ ID NO: 47; LCDR3) [Clone 16C07];
- 30 (k) the amino acid sequences
GFTFSSYLMS (SEQ ID NO: 38; HCDR1), VATISGGGSEKYYVDSVKG (SEQ ID NO: 39; HCDR2), QVYYFDY (SEQ ID NO: 44; HCDR3), RASQSISSWLN (SEQ ID NO: 41; LCDR1), AASSLHS (SEQ ID NO: 54; LCDR2) and QQSYSTPWT (SEQ ID NO: 43; LCDR3) [Clone 16H10];
- 35 (l) the amino acid sequences
GFTFSSYPMS (SEQ ID NO: 80; HCDR1), VATISGGGSETYYVDSVKG (SEQ ID NO: 48; HCDR2), QLYYYDY (SEQ ID NO: 81; HCDR3), RASQSISTWLN (SEQ ID NO: 52; LCDR1),

AASSLQY (SEQ ID NO: 82; LCDR2) and QQSYSTPWT (SEQ ID NO: 43; LCDR3) [Clone 17B11];

(m) the amino acid sequences

GFTFSSYAMS (SEQ ID NO: 67; HCDR1), VATISGGGSEKYYVDSVKG (SEQ ID NO: 39; HCDR2), QLYYADY (SEQ ID NO: 72; HCDR3), RASQSISSWLN (SEQ ID NO: 41; LCDR1), AASSLQS (SEQ ID NO: 42; LCDR2) and QQSYSTPWT (SEQ ID NO: 43; LCDR3) [Clone IgG1-01 (VL domain contains JK4 sequence)];

(n) the amino acid sequences

GFTFSSYAMS (SEQ ID NO: 67; HCDR1), VATISGGGSEKYYVDSVKG (SEQ ID NO: 39; HCDR2), QLYYADY (SEQ ID NO: 72; HCDR3), RASQSISSWLN (SEQ ID NO: 41; LCDR1), AASSLQS (SEQ ID NO: 42; LCDR2) and QQSYSTPWT (SEQ ID NO: 43; LCDR3) [Clone IgG1-02 (VL domain contains JK1 sequence)];

(o) the amino acid sequences

GFTFSSYAMS (SEQ ID NO: 67; HCDR1), VATISGGGSEKYYVDSVKG (SEQ ID NO: 39; HCDR2), QLYYADY (SEQ ID NO: 72; HCDR3), RASQSISSYLN (SEQ ID NO: 74; LCDR1), AASSLQS (SEQ ID NO: 42; LCDR2) and QQSYSTPWT (SEQ ID NO: 43; LCDR3) [Clone IgG1-03 (VL domain contains JK1 sequence)];

(p) the amino acid sequences

GFTFSSYLMS (SEQ ID NO: 38; HCDR1), VATISGGGSEKYYVDSVKG (SEQ ID NO: 39; HCDR2), QVYYFDY (SEQ ID NO: 44; HCDR3), RASQSISSWLN (SEQ ID NO: 41; LCDR1), AASSLQS (SEQ ID NO: 42; LCDR2) and QQSYSTPWT (SEQ ID NO: 43; LCDR3) [Clone IgG1-04 (VL domain contains JK4 sequence)];

(q) the amino acid sequences

GFTFSSYLMS (SEQ ID NO: 38; HCDR1), VATISGGGSEKYYVDSVKG (SEQ ID NO: 39; HCDR2), QVYYFDY (SEQ ID NO: 44; HCDR3), RASQSISSWLN (SEQ ID NO: 41; LCDR1), AASSLQS (SEQ ID NO: 42; LCDR2) and QQSYSTPWT (SEQ ID NO: 43; LCDR3) [Clone IgG1-05 (VL domain contains JK1 sequence)];

(r) the amino acid sequences

GFTFSSYLMS (SEQ ID NO: 38; HCDR1), VATISGGGSEKYYVDSVKG (SEQ ID NO: 39; HCDR2), QVYYFDY (SEQ ID NO: 44; HCDR3), RASQSISSYLN (SEQ ID NO: 74; LCDR1), AASSLQS (SEQ ID NO: 42; LCDR2) and QQSYSTPWT (SEQ ID NO: 43; LCDR3) [Clone IgG1-06 (VL domain contains JK1 sequence)];

(s) the amino acid sequences

GFTFSSYLMS (SEQ ID NO: 38; HCDR1), VATISGGGSEKYYVDSVKG (SEQ ID NO: 39; HCDR2), QLYGFDY (SEQ ID NO: 40; HCDR3), RASQSISSWLN (SEQ ID NO: 41; LCDR1), AASSLQS (SEQ ID NO: 42; LCDR2) and QQSYSTPWT (SEQ ID NO: 43; LCDR3) [Clone IgG1-07 (VL domain contains JK4 sequence)];

- (t) the amino acid sequences
 GFTFSSYLMS (SEQ ID NO: 38; HCDR1), VATISGGGSEKYYVDSVKG (SEQ ID NO: 39; HCDR2), QLYGFDY (SEQ ID NO: 40; HCDR3), RASQSISSWLN (SEQ ID NO: 41; LCDR1), AASSLQS (SEQ ID NO: 42; LCDR2) and QQSYSTPWT (SEQ ID NO: 43; LCDR3) [Clone
 5 IgG1-08 (VL domain contains JK1 sequence)];
- (u) the amino acid sequences
 GFTFSSYLMS (SEQ ID NO: 38; HCDR1), VATISGGGSEKYYVDSVKG (SEQ ID NO: 39; HCDR2), QLYGFDY (SEQ ID NO: 40; HCDR3), RASQSISSYLN (SEQ ID NO: 74; LCDR1), AASSLQS (SEQ ID NO: 42; LCDR2) and QQSYSTPWT (SEQ ID NO: 43; LCDR3) [Clone
 10 IgG1-09 (VL domain contains JK1 sequence)];
- (v) the amino acid sequences
 GFTFSSYLMS (SEQ ID NO: 38; HCDR1), VATISGGGSEKYYVDSVKG (SEQ ID NO: 39; HCDR2), QLYFFDY (SEQ ID NO: 45; HCDR3), RASQSISSWLN (SEQ ID NO: 41; LCDR1), AASSLQS (SEQ ID NO: 42; LCDR2) and QQSYSTPWT (SEQ ID NO: 43; LCDR3) [Clone
 15 IgG1-10 (VL domain contains JK4 sequence)];
- (w) the amino acid sequences
 GFTFSSYLMS (SEQ ID NO: 38; HCDR1), VATISGGGSEKYYVDSVKG (SEQ ID NO: 39; HCDR2), QLYFFDY (SEQ ID NO: 45; HCDR3), RASQSISSWLN (SEQ ID NO: 41; LCDR1), AASSLQS (SEQ ID NO: 42; LCDR2) and QQSYSTPWT (SEQ ID NO: 43; LCDR3) [Clone
 20 IgG1-11 (VL domain contains JK1 sequence)];
- (x) the amino acid sequences
 GFTFSSYLMS (SEQ ID NO: 38; HCDR1), VATISGGGSEKYYVDSVKG (SEQ ID NO: 39; HCDR2), QLYFFDY (SEQ ID NO: 45; HCDR3), RASQSISSYLN (SEQ ID NO: 74; LCDR1), AASSLQS (SEQ ID NO: 42; LCDR2) and QQSYSTPWT (SEQ ID NO: 43; LCDR3) [Clone
 25 IgG1-12 (VL domain contains JK1 sequence)];
- (y) the amino acid sequences
 GFTFSSYLMS (SEQ ID NO: 38; HCDR1), VATISGGGSEKYYVDSVKG (SEQ ID NO: 39; HCDR2), QLYAFDY (SEQ ID NO: 46; HCDR3), RASQSISSWLN (SEQ ID NO: 41; LCDR1), AASSLQS (SEQ ID NO: 42; LCDR2) and QQSYSTPWT (SEQ ID NO: 43; LCDR3) [Clone
 30 IgG1-13 (VL domain contains JK4 sequence)];
- (z) the amino acid sequences
 GFTFSSYLMS (SEQ ID NO: 38; HCDR1), VATISGGGSEKYYVDSVKG (SEQ ID NO: 39; HCDR2), QLYAFDY (SEQ ID NO: 46; HCDR3), RASQSISSWLN (SEQ ID NO: 41; LCDR1), AASSLQS (SEQ ID NO: 42; LCDR2) and QQSYSTPWT (SEQ ID NO: 43; LCDR3) [Clone
 35 IgG1-14 (VL domain contains JK1 sequence)];
- (z1) the amino acid sequences

GFTFSSYLMS (SEQ ID NO: 38; HCDR1), VATISGGGSEKYYVDSVKG (SEQ ID NO: 39; HCDR2), QLYAFDY (SEQ ID NO: 46; HCDR3), RASQSISSYLN (SEQ ID NO: 74; LCDR1), AASSLQS (SEQ ID NO: 42; LCDR2) and QQSYSTPWT (SEQ ID NO: 43; LCDR3) [Clone IgG1-15 (VL domain contains JK1 sequence)].

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In some aspects, the invention provides an anti-PD1 antibody or an antigen-binding portion thereof, wherein the antibody comprises a heavy chain variable (VH) region and a light chain variable (VL) region, wherein

(a) the VH region amino acid sequence comprises HCDR1 of GFTFSSYLMS (SEQ ID NO: 38), HCDR2 of VATISGGGSEKYYVDSVKG (SEQ ID NO: 39) and HCDR3 of QLYGFDY (SEQ ID NO: 40); and the VL region amino acid sequence comprises LCDR1 of RASQSISSWLN (SEQ ID NO: 41), LCDR2 of AASSLQS (SEQ ID NO: 42) and LCDR3 of QQSYSTPWT (SEQ ID NO: 43);

(b) the VH region amino acid sequence comprises HCDR1 of GFTFSSYLMS (SEQ ID NO: 38), HCDR2 of VATISGGGSEKYYVDSVKG (SEQ ID NO: 39) and HCDR3 of QVYYFDY (SEQ ID NO: 44); and the VL region amino acid sequence comprises LCDR1 of RASQSISSWLN (SEQ ID NO: 41), LCDR2 of AASSLQS (SEQ ID NO: 42) and LCDR3 of QQSYSTPWT (SEQ ID NO: 43);

(c) the VH region amino acid sequence comprises HCDR1 of GFTFSSYLMS (SEQ ID NO: 38), HCDR2 of VATISGGGSEKYYVDSVKG (SEQ ID NO: 39) and HCDR3 of QLYFFDY (SEQ ID NO: 45); and the VL region amino acid sequence comprises LCDR1 of RASQSISSWLN (SEQ ID NO: 41), LCDR2 of AASSLQS (SEQ ID NO: 42) and LCDR3 of QQSYSTPWT (SEQ ID NO: 43);

(d) the VH region amino acid sequence comprises HCDR1 of GFTFSSYLMS (SEQ ID NO: 38), HCDR2 of VATISGGGSEKYYVDSVKG (SEQ ID NO: 39) and HCDR3 of QLYAFDY (SEQ ID NO: 46); and the VL region amino acid sequence comprises LCDR1 of RASQSISSWLN (SEQ ID NO: 41), LCDR2 of AASSLQS (SEQ ID NO: 42) and LCDR3 of QQSYSTPWT (SEQ ID NO: 43);

(e) the VH region amino acid sequence comprises HCDR1 of GFTFSSYLMS (SEQ ID NO: 38), HCDR2 of VATISGGGSEKYYVDSVKG (SEQ ID NO: 39) and HCDR3 of QLYGFDY (SEQ ID NO: 40); and the VL region amino acid sequence comprises LCDR1 of RASQSISSWLN (SEQ ID NO: 41), LCDR2 of AASSLQS (SEQ ID NO: 42) and LCDR3 of QQSYSIPWT (SEQ ID NO: 47);

(f) the VH region amino acid sequence comprises HCDR1 of GFTFSSYLMS (SEQ ID NO: 38), HCDR2 of VATISGGGAEEKYYVDSVKG (SEQ ID NO: 49) and HCDR3 of QLYGFDY (SEQ ID NO: 40); and the VL region amino acid sequence comprises LCDR1 of

RASQSISSWLN (SEQ ID NO: 41), LCDR2 of AASSLQD (SEQ ID NO: 50) and LCDR3 of QQSYSTPWT (SEQ ID NO: 43);

(g) the VH region amino acid sequence comprises HCDR1 of GFTFSSYLMS (SEQ ID NO: 38), HCDR2 of VATISGGGSETYYVDSVKG (SEQ ID NO: 48), and HCDR3 of QLYGFDY (SEQ ID NO: 40); and the VL region amino acid sequence comprises LCDR1 of RASQSISSWLN (SEQ ID NO: 41), LCDR2 of AASSLQS (SEQ ID NO: 42), and LCDR3 of QQSYSIPWT (SEQ ID NO: 47);

(h) the VH region amino acid sequence comprises HCDR1 of GFTFSSYLMS (SEQ ID NO: 38), HCDR2 of VATISGGGAEKYYVDSVKG (SEQ ID NO: 49), and HCDR3 of QLYGFDY (SEQ ID NO: 40); and the VL region amino acid sequence comprises LCDR1 of RASQSISSWLN (SEQ ID NO: 41), LCDR2 of AASSLQD (SEQ ID NO: 50), and LCDR3 of QQSYSTPWT (SEQ ID NO: 43);

(i) the VH region amino acid sequence comprises HCDR1 of GFTFSSYLMS (SEQ ID NO: 38), HCDR2 of VATISGGGSNTYYVDSVKG (SEQ ID NO: 51), and HCDR3 of QLYGFDY (SEQ ID NO: 40); and the VL region amino acid sequence comprises LCDR1 of RASQSISTWLN (SEQ ID NO: 52), LCDR2 of AASSLAS (SEQ ID NO: 53), and LCDR3 of QQSYSTPWT (SEQ ID NO: 43);

(j) the VH region amino acid sequence comprises HCDR1 of GFTFSSYLMS (SEQ ID NO: 38), HCDR2 of VATISGGGSEKYYVDSVKG (SEQ ID NO: 39), and HCDR3 of QVYYFDY (SEQ ID NO: 44); and the VL region amino acid sequence comprises LCDR1 of RASQSISSWLN (SEQ ID NO: 41), LCDR2 of AASSLHS (SEQ ID NO: 54), and LCDR3 of QQSYSTPWT (SEQ ID NO: 43); or

(k) the VH region amino acid sequence comprises HCDR1 of GFTFSSYLMS (SEQ ID NO: 38), HCDR2 of VATISGGGSEKYYVDSVKG (SEQ ID NO: 39), and HCDR3 of QVYYFDY (SEQ ID NO: 44); and the VL region amino acid sequence comprises LCDR1 of RASQSISSWLN (SEQ ID NO: 41), LCDR2 of AASSLQS (SEQ ID NO: 42), and LCDR3 of QQSYSTPWT (SEQ ID NO: 43).

In some aspects, disclosed herein is anti-PD1 antibody or an antigen-binding portion thereof, wherein the antibody comprises a heavy chain variable (VH) region and a light chain variable (VL) region, wherein the VH region comprises any one of the VH region amino acid sequences in Table 17 and the VL region comprises any one of the VL region amino acid sequences in Table 17.

In some aspects, disclosed herein is an anti-PD1 antibody or an antigen-binding portion thereof, wherein the antibody comprises a heavy chain variable (VH) region and a light chain variable (VL) region, wherein

- (a) the VH region amino acid sequence comprises SEQ ID NO:1 and the VL region amino acid sequence comprises SEQ ID NO:2;
- (b) the VH region amino acid sequence comprises SEQ ID NO:3 and the VL region amino acid sequence comprises SEQ ID NO:4;
- 5 (c) the VH region amino acid sequence comprises SEQ ID NO:5 and the VL region amino acid sequence comprises SEQ ID NO:6;
- (d) the VH region amino acid sequence comprises SEQ ID NO:7 and the VL region amino acid sequence comprises SEQ ID NO:8;
- (e) the VH region amino acid sequence comprises SEQ ID NO:9 and the VL region amino acid sequence comprises SEQ ID NO:10; or
- 10 (f) the VH region amino acid sequence comprises SEQ ID NO:11 and the VL region amino acid sequence comprises SEQ ID NO:12.

15 In some aspects, disclosed herein is an anti-PD1 antibody or an antigen-binding portion thereof, wherein the antibody comprises a heavy chain variable (VH) region and a light chain variable (VL) region, wherein

- (a) the VH region amino acid sequence is at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98% or at least about 99% identical to SEQ ID NO:1 and the VL region amino acid sequence is at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98% or at least about 99% identical to SEQ ID NO:2;
- 20 (b) the VH region amino acid sequence is at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98% or at least about 99% identical to SEQ ID NO:3 and the VL region amino acid sequence is at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98% or at least about 99% identical to SEQ ID NO:4;
- 25 (c) the VH region amino acid sequence is at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98% or at least about 99% identical to SEQ ID NO:5 and the VL region amino acid sequence is at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98% or at least about 99% identical to SEQ ID NO:6;
- 30

(d) the VH region amino acid sequence is at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98% or at least about 99% identical to SEQ ID NO:7 and the VL region amino acid sequence is at least about 90%, at least about 91%,
5 at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98% or at least about 99% identical to SEQ ID NO:8;

(e) the VH region amino acid sequence is at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least
10 about 96%, at least about 97%, at least about 98% or at least about 99% identical to SEQ ID NO:9 and the VL region amino acid sequence is at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98% or at least about 99% identical to SEQ ID NO:10; or

(f) the VH region amino acid sequence is at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98% or at least about 99% identical to SEQ ID
15 NO:11 and the VL region amino acid sequence is at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98% or at least about 99% identical to SEQ
20 ID NO:12. In some aspects, the CDR amino acid sequences of an anti-PD1 antibody are 100% identical to the CDR amino acid sequences in the recited sequences while the FR amino acid sequences are less than 100% identical to the FR amino acid sequences in the recited sequences.

25 In some aspects, the antibody or antigen-binding portion as defined herein may be isolated.

The antibody molecule or antigen-binding portion as defined herein may cross-compete for binding to PD1 with an antibody or antigen-binding portion thereof comprising the sets of
30 CDRs disclosed herein. In some embodiments, the invention provides an isolated anti-PD1 antibody or an antigen-binding portion thereof, wherein the antibody or antigen-binding portion cross-competes for binding to PD1 with the antibody or antigen-binding portion comprising the sets of CDRs disclosed herein; and (a) comprises fully germline human framework amino acid sequences; (b) binds specifically to human PD1 and
35 cynomolgus PD1; (c) antagonizes binding of human PD1 to human PD-L1 with an EC50 lower than 260 ng/ml; (d) binds to monomeric cynomolgus PD1 with a KD lower than 32 nM; (e) binds to cyno PD1-expressing cells with an EC50 lower than 6.5 nM; (f) binds to a

- functionally identical epitope on cynomolgus PD1 and human PD1; (g) comprises a human germline peptide sequence with high MHC class II binding affinity in LCDR2; (h) comprises a reduced number of immunogenic peptides compared to an anti-PD1 antibody comprising the variable domain sequences of antibody Mab005; (i) exhibits reduced immunogenicity
- 5 compared to an anti-PD1 antibody comprising the variable domain sequences of antibody Mab005; (j) comprises a deimmunized VL region (e.g., a fully deimmunized VL region); (k) exhibits no binding to one or more of KDR, FZD5, ULBP2 and EPHB6; and/or (l) does not agonise or minimally agonises one or more of KDR, FZD5, ULBP2 and EPHB6. In some embodiments, a KD value of an antibody or antigen-binding portion may be determined by
- 10 Biacore analysis. In some embodiments, an EC50 value of an antibody or antigen-binding portion may be determined by flow cytometric staining of PD-1 expressing cells (e.g., CHO cells). In some embodiments, binding to KDR, FZD5, ULBP2 or EPHB6 may be determined by flow cytometry analysis.
- 15 In some embodiments, an anti-PD1 antibody or antigen-binding portion has low immunogenicity. In certain cases, an antibody or antigen-binding portion exhibits reduced immunogenicity compared to an anti-PD1 antibody comprising HCDR1 of GFTFSSYMMS (SEQ ID NO: 29), HCDR2 of TISGGGANTYYPDSVKG (SEQ ID NO: 30), HCDR3 of QLYYFDY (SEQ ID NO: 31), LCDR1 of LASQTIGTWLT (SEQ ID NO: 35), LCDR2 of
- 20 TATSLAD (SEQ ID NO: 36), and LCDR3 of QQVYSIPWT (SEQ ID NO: 37). In some examples, immunogenicity risk of an antibody or antigen-binding portion may be determined *in silico* by identifying the location of T cell epitopes in the antibody or portion (e.g., in the variable regions of the antibody or portion).
- 25 For example, T cell epitopes in an antibody or antigen-binding portion may be identified by using iTope™. iTope™ can be used to analyse VL and VH region sequences for peptides with promiscuous high affinity binding to human MHC class II. Promiscuous high affinity MHC class II binding peptides are thought to correlate with the presence of T cell epitopes that are high risk indicators for clinical immunogenicity of drug proteins. The iTope™ software
- 30 predicts favourable interactions between amino acid side chains of a peptide and specific binding pockets (in particular pocket positions; p1, p4, p6, p7 and p9) within the open-ended binding grooves of 34 human MHC class II alleles. These alleles represent the most common HLA-DR alleles found world-wide with no weighting attributed to those found most prevalently in any particular ethnic population. Twenty of the alleles contain the 'open' p1
- 35 configuration and 14 contain the 'closed' configuration where glycine at position 83 is replaced by a valine. The location of key binding residues is achieved by the *in silico* generation of 9mer peptides that overlap by eight amino acids spanning the test protein

sequence. This process successfully discriminates with high accuracy between peptides that either bind or do not bind MHC class II molecules.

5 T cell epitopes in an antibody or antigen-binding portion may be identified by analysing VL and VH region sequences using TCED™ (T Cell Epitope Database™) to search for matches to T cell epitopes previously identified by *in vitro* human T cell epitope mapping analyses of other protein sequences. The TCED™ is used to search any test sequence against a large (>10,000 peptides) database of peptides derived from unrelated protein and antibody sequences.

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In some embodiments, an anti-PD1 antibody or antigen-binding portion may exhibit a low immunogenicity because the antibody or portion has a low number of one or more of the following peptides in its sequences: High Affinity Foreign ('HAF' – high immunogenicity risk), Low Affinity Foreign ('LAF' – lower immunogenicity risk), and/or TCED+ (previously identified epitope in TCED™ database).

15

In some embodiments, an anti-PD1 antibody or antigen-binding portion may have high Germline Epitope (GE) content in its sequence. In some examples, an anti-PD1 antibody or antigen-binding portion has 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 (or greater than 20) germline epitopes in its sequence (e.g., in the VL and/or VH region sequence). Germline Epitope may be defined as a human germline peptide sequence with high MHC Class II binding affinity. Germline Epitope 9mer peptides are unlikely to have immunogenic potential due to T cell tolerance, as validated by previous studies with a wide range of germline peptides. Importantly, such germline v-domain epitopes (aided further by similar sequences in the human antibody constant regions) also compete for MHC Class II occupancy at the membrane of antigen presenting cells, reducing the risk of foreign peptide presentation being sufficient to achieve the 'activation threshold' required for T cell stimulation. High GE content is therefore a beneficial quality in clinical development of an antibody therapeutic and can provide low immunogenicity. In some examples, an anti-PD1 antibody or antigen-binding portion comprises a human germline peptide sequence with high MHC class II binding affinity (e.g., germline epitope) in the LCDR2.

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In certain embodiments, an anti-PD1 antibody or antigen-binding portion may have a reduced number of HAF, LAF and/or TCED+ epitopes found in the frameworks of both the heavy and light chain variable regions compared to an anti-PD1 antibody comprising the variable domain sequences of antibody Mab005 (Table 2). In some embodiments, HAF,

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LAF and/or TCED+ epitopes are not present in the VL and/or VH region sequences of an anti-PD1 antibody or antigen-binding portion.

For example, a TCED+ and HAF peptide 'VTITCLASQ' (SEQ ID NO: 83) found in the
 5 LCDR-1 of Mab005 may be eliminated by the mutation L>R at position 6, converting this sequence to the light chain GE 'VTITCRASQ' (SEQ ID NO: 84) in an anti-PD1 antibody or antigen-binding portion.

In some embodiments, an LAF peptide 'IGTWLTWYQ' (SEQ ID NO: 85) found in the
 10 LCDR-1 of Mab005 may be eliminated by retaining only a single murine residue W at position 4, converting this sequence to 'ISSWLNWYQ' (SEQ ID NO: 86) in an anti-PD1 antibody or antigen-binding portion (Table 12).

In some embodiments, the Mab005 HAF peptides 'LLIYTATSL' (SEQ ID NO: 87) and
 15 'LIYTATSLA' (SEQ ID NO: 88), and LAF peptide 'IYTATSLAD' (SEQ ID NO: 89) may be eliminated and converted to GE sequences by mutation of the LCDR2 from the murine sequence 'TATSLAD' (SEQ ID NO: 36) to the fully germline sequence 'AASSLQS' (SEQ ID NO: 42) in an anti-PD1 antibody or antigen-binding portion.

20 The Mab005 FW3/LCDR3 region also encodes a LAF peptide 'YYCQQVYSI' (SEQ ID NO: 90). In some embodiments, this epitope may be eliminated by the germlining mutation V>S at position 6 in an anti-PD1 antibody or antigen-binding portion.

In the VH region of Mab005, the peptide sequence 'LYYFDYWGQ' (SEQ ID NO: 91)
 25 (spanning the HCDR3 and FW4) was found to be a LAF. In some embodiments, the mutation Y>A at position 3 in this peptide allows the elimination of this epitope in an anti-PD1 antibody or antigen-binding portion (Tables 4, 12).

The terms "cross-compete", "cross-competition", "cross-block", "cross-blocked" and "cross-
 30 blocking" are used interchangeably herein to mean the ability of an antibody or portion thereof to interfere with the binding directly or indirectly through allosteric modulation of the anti-PD1 antibodies of the invention to the target PD1 (e.g., human PD1). The extent to which an antibody or portion thereof is able to interfere with the binding of another to the target, and therefore whether it can be said to cross-block or cross-compete according to
 35 the invention, can be determined using competition binding assays. One example of a binding competition assay is Homogeneous Time Resolved Fluorescence (HTRF). One particularly suitable quantitative cross-competition assay uses a FACS- or an AlphaScreen-

based approach to measure competition between the labelled (e.g. His tagged, biotinylated or radioactive labelled) antibody or portion thereof and the other antibody or portion thereof in terms of their binding to the target. In general, a cross-competing antibody or portion thereof is, for example, one which will bind to the target in the cross-competition assay
5 such that, during the assay and in the presence of a second antibody or portion thereof, the recorded displacement of the immunoglobulin single variable domain or polypeptide according to the invention is up to 100% (e.g. in a FACS based competition assay) of the maximum theoretical displacement (e.g. displacement by cold (e.g. unlabeled) antibody or fragment thereof that needs to be cross-blocked) by the potentially cross-blocking antibody
10 or fragment thereof that is present in a given amount. Preferably, cross-competing antibodies or portions thereof have a recorded displacement that is between 10% and 100%, or between 50% and 100%.

The anti-PD1 antibody molecule or antigen-binding portion as defined herein may be
15 thermally stable. In some cases, an antibody molecule or antigen-binding portion may have substantially the same thermal stability as murine anti-PD1 antibody Mab005 or Mab005-IgG1 (humanized). In some cases, an antibody molecule or antigen-binding portion may be more thermally stable than murine anti-PD1 antibody Mab005 or Mab005-IgG1 (humanized). In some examples, an antibody molecule or antigen-binding portion
20 may have a melting temperature (T_m) from about 90°C to about 93°C and may be in an IgG1 format. In some aspects, an antibody molecule or antigen-binding portion may have a T_m from about 90.8°C to about 92.2°C and may be in an IgG1 format. In some aspects, the antigen-binding portion may be in an Fab format. The melting temperature of an antibody molecule or antigen-binding portion thereof may be analysed by a differential
25 scanning calorimetry (DSC) assay.

The anti-PD1 antibody molecule or antigen-binding portion as defined herein may be resistant to oxidation (e.g., resistant to oxidation of exposed amino acid residues). In some cases, an antibody molecule or antigen-binding portion may undergo reduced oxidation of
30 exposed amino acid residues compared to murine anti-PD1 antibody Mab005 or Mab005-IgG1 (humanized), or an anti-PD1 antibody comprising the variable domain sequences of antibody Mab005-IgG1 (humanized). Oxidation resistance of an antibody molecule or antigen-binding portion thereof may be analysed by adding an oxidative reagent (e.g., H_2O_2) to the antibody molecule or antigen-binding portion and analysing changes induced
35 by oxidation by Reverse Phase (RP) and Hydrophobic Interaction Chromatography (HIC) methods.

In certain embodiments, an antibody molecule or antigen-binding portion thereof binds specifically to PD1 and does not bind (or does not bind specifically) to one or more of the membrane receptors KDR (also known as VEGFR2), FZD5 (frizzled class receptor 5), ULBP2 (UL16 binding protein 2) and EPHB6. In some aspects, KDR, FZD5, ULBP2 and/or

5 EPHB6 are human proteins. In some embodiments, KDR is a rhesus protein. In some embodiments, the human KDR protein comprises or consists of the amino acid sequence of SEQ ID NO:22 or an amino acid sequence that is at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98% or at least about 99% identical to

10 SEQ ID NO:22. In some embodiments, the rhesus KDR protein comprises or consists of the amino acid sequence of SEQ ID NO:23 or an amino acid sequence that is at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98% or at least about 99% identical to SEQ ID NO:23. In some embodiments, the human FZD5

15 protein comprises or consists of the amino acid sequence of SEQ ID NO:24 or an amino acid sequence that is at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98% or at least about 99% identical to SEQ ID NO:24. In some embodiments, the human ULBP2 protein comprises or consists of the amino acid

20 sequence of SEQ ID NO:25 or an amino acid sequence that is at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98% or at least about 99% identical to SEQ ID NO:25. In one embodiment, an antibody molecule or antigen-binding portion thereof does not bind to any of KDR, FZD5, ULBP2 and EPHB6. In some

25 embodiments, an antibody molecule or antigen-binding portion thereof exhibits reduced binding to one or more of KDR, FZD5, ULBP2 and EPHB6 compared to the binding exhibited by antibody Mab005 or IgG1-Mab005 (humanized) to said membrane receptors. In some cases, binding of an antibody or antigen-binding portion thereof to PD1, KDR, FZD5, ULBP2 or EPHB6 may be determined by flow cytometry analysis.

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In some embodiments, an antibody molecule or antigen-binding portion thereof disclosed herein binds specifically to PD1 and does not agonise or minimally agonises one or more of KDR, FZD5, ULBP2 and EPHB6 (*e.g.*, human KDR, human FZD5, human ULBP2 or human EPHB6). In some embodiments, an antibody molecule or antigen-binding portion

35 thereof disclosed herein binds specifically to PD1 and exhibits reduced agonism of one or more of KDR, FZD5, ULBP2 and EPHB6 (*e.g.*, human KDR, human FZD5, human ULBP2 or human EPHB6) compared to the agonism of KDR, FZD5, ULBP2 or EPHB6 exhibited by

antibody Mab005 or IgG1-Mab005 (humanized). In some cases, agonism of KDR, FZD5, ULBP2 or EPHB6 by an antibody molecule or antigen-binding portion thereof may be determined by a KDR (or VEGFR2), FZD5, ULBP2 or EPHB6 signaling reporter assay.

- 5 The antibody molecule or antigen-binding portion as defined herein may comprise one or more substitutions, deletions and/or insertions which remove a post-translational modification (PTM) site, for example a glycosylation site (N-linked or O-linked), a deamination site, a phosphorylation site or an isomerisation/fragmentation site.
- 10 More than 350 types of PTM are known. Key forms of PTM include phosphorylation, glycosylation (*N*- and *O*-linked), sumoylation, palmitoylation, acetylation, sulfation, myristoylation, prenylation and methylation (of K and R residues). Statistical methods to identify putative amino acid sites responsible for specific PTMs are well known in the art (see Zhou *et al.*, 2016, Nature Protocols 1: 1318-1321). Removal of such a site for example by
- 15 substitution, deletion and/or insertion and then optionally testing (experimentally and/or theoretically) for (a) binding activity and/or (b) loss of the PTM is contemplated.

- For example, the MAb005 murine HCDR2 (as defined herein, i.e. the amino acid sequence VATISGGGANTYYPDSVKG (SEQ ID NO: 92)) has been identified to have a putative
- 20 deamidation site at residue 10 (N). Removal of this site at equivalent positions in an HCDR2 of the invention, for example by conservative or non-conservative substitution (such as to E, Q, S or D), is envisaged (as for example in clone 06D02 and other clones in Tables 3 and 4).

- 25 Similarly, the MAb005 murine HCDR1 (as defined herein, i.e. the amino acid sequence GFTFSSYMMS (SEQ ID NO: 29)) has been identified to have a putative oxidation site at residue 8 (M). Removal of this site at an equivalent position in an HCDR1 of the invention, for example by conservative or non-conservative substitution (such as to A/D/E/F/H/I/L/N/P/Q/S/T/V/W/Y), is envisaged (as exemplified in clone 06D02 and the further
- 30 sequences found in Tables 3 and 4).

The antibody molecule or antigen-binding portion thereof may be human, humanized or chimeric.

- 35 The antibody molecule or antigen-binding portion thereof may comprise one or more human variable domain framework scaffolds into which the CDRs have been inserted. For example,

the VH region, the VL region, or both the VH and the VL region may comprise one or more human framework region amino acid sequences.

5 The antibody molecule or antigen-binding portion thereof may comprise an IGHV3-7 human germline scaffold into which the corresponding HCDR sequences have been inserted. The antibody molecule or antigen-binding portion thereof may comprise a VH region that comprises an IGHV3-7 human germline scaffold amino acid sequence into which a set of corresponding HCDR1, HCDR2 and HCDR3 amino acid sequences have been inserted.

10 The antibody molecule or antigen-binding portion thereof may comprise an IGKV1-39 human germline scaffold into which the corresponding LCDR sequences have been inserted. The antibody molecule or antigen-binding portion thereof may comprise a VL region that comprises an IGKV1-39 human germline scaffold amino acid sequence into which a set of corresponding LCDR1, LCDR2 and LCDR3 amino acid sequences have been inserted.

15 The antibody molecule or antigen-binding portion thereof may comprise an IGHV3-7 human germline scaffold into which the corresponding HCDR sequences have been inserted and an IGKV1-39 human germline scaffold into which the corresponding LCDR sequences have been inserted. The antibody molecule or antigen-binding portion thereof may comprise a VH
20 region that comprises an IGHV3-7 human germline scaffold amino acid sequence into which a set of corresponding HCDR1, HCDR2 and HCDR3 amino acid sequences have been inserted and a VL region that comprises an IGKV1-39 human germline scaffold amino acid sequence into which a set of corresponding LCDR1, LCDR2 and LCDR3 amino acid sequences have been inserted. The HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3
25 amino acid sequences may be the HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3 amino acid sequences of any one of the clones in Table 4 (with all six CDR sequences being from the same clone).

30 In some aspects, the antibody molecule or antigen-binding portion thereof may comprise an immunoglobulin constant region. In some embodiments, the immunoglobulin constant region is IgG1, IgG2, IgG3, IgG4, IgA1 or IgA2. In additional embodiments, the immunoglobulin constant region is IgG1, IgG2, IgG3, IgG4, IgA1 or IgA2. The antibody molecule or antigen-binding portion thereof may comprise an immunologically inert constant region. In some aspects, an anti-PD1 antibody or antigen-binding portion thereof may
35 comprise an immunoglobulin constant region comprising a wild-type human IgG1 constant region, a human IgG1 constant region comprising the amino acid substitutions L234A, L235A and G237A or a human IgG1 constant region comprising the amino acid substitutions L234A,

L235A, G237A and P331S. In some aspects, an anti-PD1 antibody or antigen-binding portion thereof may comprise an immunoglobulin constant region comprising a wild-type human IgG2 constant region or a wild-type human IgG4 constant region. In some aspects, an anti-PD1 antibody may comprise an immunoglobulin constant region comprising any one of the amino acid sequences in Table 18. The Fc region sequences in Table 18 begin at the CH1 domain. In some aspects, an anti-PD1 antibody may comprise an immunoglobulin constant region comprising an amino acid sequence of an Fc region of human IgG4, human IgG4(S228P), human IgG2, human IgG1, human IgG1-3M or human IgG1-4M. For example, the human IgG4(S228P) Fc region comprises the following substitution compared to the wild-type human IgG4 Fc region: S228P. For example, the human IgG1-3M Fc region comprises the following substitutions compared to the wild-type human IgG1 Fc region: L234A, L235A and G237A, while the human IgG1-4M Fc region comprises the following substitutions compared to the wild-type human IgG1 Fc region: L234A, L235A, G237A and P331S. In some aspects, a position of an amino acid residue in a constant region of an immunoglobulin molecule is numbered according to EU nomenclature (Ward *et al.*, 1995 *Therap. Immunol.* 2:77-94). In some aspects, an immunoglobulin constant region may comprise an RDEL (SEQ ID NO: 93) motif or an REEM (SEQ ID NO: 94) motif (underlined in Table 18). The REEM (SEQ ID NO: 94) allotype is found in a smaller human population than the RDEL (SEQ ID NO: 93) allotype. In some aspects, an anti-PD1 antibody may comprise an immunoglobulin constant region comprising any one of SEQ ID NOS:13-19. In some aspects, an anti-PD1 antibody may comprise the six CDR amino acid sequences of any one of the clones in Table 4 and any one of the Fc region amino acid sequences in Table 18. In some aspects, an anti-PD1 antibody may comprise an immunoglobulin heavy chain constant region comprising any one of the Fc region amino acid sequences in Table 18 and an immunoglobulin light chain constant region that is a kappa light chain constant region or a lambda light chain constant region.

In some aspects, disclosed herein is an anti-PD1 antibody or an antigen-binding portion thereof, wherein the antibody comprises a heavy chain variable (VH) region, a light chain variable (VL) region and a heavy chain constant region, wherein

(a) the VH region amino acid sequence comprises HCDR1 of GFTFSSYLMS (SEQ ID NO: 38), HCDR2 of VATISGGGSEKYYVDSVKG (SEQ ID NO: 39) and HCDR3 of QLYGFDY (SEQ ID NO: 40); the VL region amino acid sequence comprises LCDR1 of RASQSISSWLN (SEQ ID NO: 41), LCDR2 of AASSLQS (SEQ ID NO: 42) and LCDR3 of QQSYSTPWT (SEQ ID NO: 43); and the heavy chain constant region comprises any one of SEQ ID NOS: 13-19;

(b) the VH region amino acid sequence comprises HCDR1 of GFTFSSYLMS (SEQ ID NO: 38), HCDR2 of VATISGGGSEKYYVDSVKG (SEQ ID NO: 39) and HCDR3 of QVYYFDY (SEQ ID NO: 44); the VL region amino acid sequence comprises LCDR1 of RASQSISSWLN (SEQ ID NO: 41), LCDR2 of AASSLQS (SEQ ID NO: 42) and LCDR3 of QQSYSTPWT (SEQ ID NO: 43); and the heavy chain constant region comprises any one of SEQ ID NOS: 13-19;

(c) the VH region amino acid sequence comprises HCDR1 of GFTFSSYLMS (SEQ ID NO: 38), HCDR2 of VATISGGGSEKYYVDSVKG (SEQ ID NO: 39) and HCDR3 of QLYFFDY (SEQ ID NO: 45); the VL region amino acid sequence comprises LCDR1 of RASQSISSWLN (SEQ ID NO: 41), LCDR2 of AASSLQS (SEQ ID NO: 42) and LCDR3 of QQSYSTPWT (SEQ ID NO: 43); and the heavy chain constant region comprises any one of SEQ ID NOS: 13-19;

(d) the VH region amino acid sequence comprises HCDR1 of GFTFSSYLMS (SEQ ID NO: 38), HCDR2 of VATISGGGSEKYYVDSVKG (SEQ ID NO: 39) and HCDR3 of QLYAFDY (SEQ ID NO: 46); the VL region amino acid sequence comprises LCDR1 of RASQSISSWLN (SEQ ID NO: 41), LCDR2 of AASSLQS (SEQ ID NO: 42) and LCDR3 of QQSYSTPWT (SEQ ID NO: 43); and the heavy chain constant region comprises any one of SEQ ID NOS: 13-19;

(e) the VH region amino acid sequence comprises HCDR1 of GFTFSSYLMS (SEQ ID NO: 38), HCDR2 of VATISGGGSEYTYVDSVKG (SEQ ID NO: 48) and HCDR3 of QLYGFDY (SEQ ID NO: 40); the VL region amino acid sequence comprises LCDR1 of RASQSISSWLN (SEQ ID NO: 41), LCDR2 of AASSLQS (SEQ ID NO: 42) and LCDR3 of QQSYSIPWT (SEQ ID NO: 47); and the heavy chain constant region comprises any one of SEQ ID NOS: 13-19;

(f) the VH region amino acid sequence comprises HCDR1 of GFTFSSYLMS (SEQ ID NO: 38), HCDR2 of VATISGGGAEKYYVDSVKG (SEQ ID NO: 49) and HCDR3 of QLYGFDY (SEQ ID NO: 40); the VL region amino acid sequence comprises LCDR1 of RASQSISSWLN (SEQ ID NO: 41), LCDR2 of AASSLQD (SEQ ID NO: 50) and LCDR3 of QQSYSTPWT (SEQ ID NO: 43); and the heavy chain constant region comprises any one of SEQ ID NOS: 13-19;

(g) the VH region amino acid sequence comprises HCDR1 of GFTFSSYLMS (SEQ ID NO: 38), HCDR2 of VATISGGGSEYTYVDSVKG (SEQ ID NO: 48), and HCDR3 of QLYGFDY (SEQ ID NO: 40); the VL region amino acid sequence comprises LCDR1 of RASQSISSWLN (SEQ ID NO: 41), LCDR2 of AASSLQS (SEQ ID NO: 42), and LCDR3 of QQSYSIPWT (SEQ ID NO: 47); and the heavy chain constant region comprises any one of SEQ ID NOS: 13-19;

(h) the VH region amino acid sequence comprises HCDR1 of GFTFSSYLMS (SEQ ID NO: 38), HCDR2 of VATISGGGAEKYYVDSVKG (SEQ ID NO: 49), and HCDR3 of QLYGFDY (SEQ ID NO: 40); the VL region amino acid sequence comprises LCDR1 of RASQSISSWLN (SEQ ID NO: 41), LCDR2 of AASSLQD (SEQ ID NO: 50), and LCDR3 of QQSYSTPWT (SEQ ID NO: 43); and the heavy chain constant region comprises any one of SEQ ID NOS: 13-19;

(i) the VH region amino acid sequence comprises HCDR1 of GFTFSSYLMS (SEQ ID NO: 38), HCDR2 of VATISGGGSNTYYVDSVKG (SEQ ID NO: 51), and HCDR3 of QLYGFDY (SEQ ID NO: 40); the VL region amino acid sequence comprises LCDR1 of RASQSISTWLN (SEQ ID NO: 52), LCDR2 of AASSLAS (SEQ ID NO: 53), and LCDR3 of QQSYSTPWT (SEQ ID NO: 43); and the heavy chain constant region comprises any one of SEQ ID NOS: 13-19;

(j) the VH region amino acid sequence comprises HCDR1 of GFTFSSYLMS (SEQ ID NO: 38), HCDR2 of VATISGGGSEKYYVDSVKG (SEQ ID NO: 39), and HCDR3 of QVYYFDY (SEQ ID NO: 44); the VL region amino acid sequence comprises LCDR1 of RASQSISSWLN (SEQ ID NO: 41), LCDR2 of AASSLHS (SEQ ID NO: 54), and LCDR3 of QQSYSTPWT (SEQ ID NO: 43); and the heavy chain constant region comprises any one of SEQ ID NOS: 13-19; or

(k) the VH region amino acid sequence comprises HCDR1 of GFTFSSYLMS (SEQ ID NO: 38), HCDR2 of VATISGGGSEKYYVDSVKG (SEQ ID NO: 39), and HCDR3 of QVYYFDY (SEQ ID NO: 44); the VL region amino acid sequence comprises LCDR1 of RASQSISSWLN (SEQ ID NO: 41), LCDR2 of AASSLQS (SEQ ID NO: 42), and LCDR3 of QQSYSTPWT (SEQ ID NO: 43); and the heavy chain constant region comprises any one of SEQ ID NOS: 13-19.

25

In some aspects, disclosed herein is an anti-PD1 antibody or an antigen-binding portion thereof, wherein the antibody comprises a heavy chain variable (VH) region, a light chain variable (VL) region and a heavy chain constant region, wherein

a) the VH region amino acid sequence comprises or consists of SEQ ID NO:1; the VL region amino acid sequence comprises or consists of SEQ ID NO:2; and the heavy chain constant region comprises a wild-type human IgG4 constant region, a human IgG4 constant region comprising the amino acid substitution S228P, a wild-type human IgG2 constant region; a wild-type human IgG1 constant region or a human IgG1 constant region comprising the amino acid substitutions L234A, L235A and G237A;

b) the VH region amino acid sequence comprises or consists of SEQ ID NO:3; the VL region amino acid sequence comprises or consists of SEQ ID NO:4; and the heavy chain constant region comprises a wild-type human IgG4 constant region, a human IgG4

constant region comprising the amino acid substitution S228P, a wild-type human IgG2 constant region; a wild-type human IgG1 constant region or a human IgG1 constant region comprising the amino acid substitutions L234A, L235A and G237A;

(c) the VH region amino acid sequence comprises or consists of SEQ ID NO:5; the
5 VL region amino acid sequence comprises or consists of SEQ ID NO:6; and the heavy chain constant region comprises a wild-type human IgG4 constant region, a human IgG4 constant region comprising the amino acid substitution S228P, a wild-type human IgG2 constant region; a wild-type human IgG1 constant region or a human IgG1 constant region comprising the amino acid substitutions L234A, L235A and G237A;

10 (d) the VH region amino acid sequence comprises or consists of SEQ ID NO:7; the VL region amino acid sequence comprises or consists of SEQ ID NO:8; and the heavy chain constant region comprises a wild-type human IgG4 constant region, a human IgG4 constant region comprising the amino acid substitution S228P, a wild-type human IgG2 constant region; a wild-type human IgG1 constant region or a human IgG1 constant region
15 comprising the amino acid substitutions L234A, L235A and G237A;

(e) the VH region amino acid sequence comprises or consists of SEQ ID NO:9; the VL region amino acid sequence comprises or consists of SEQ ID NO:10; and the heavy chain constant region comprises a wild-type human IgG4 constant region, a human IgG4 constant region comprising the amino acid substitution S228P, a wild-type human IgG2
20 constant region; a wild-type human IgG1 constant region or a human IgG1 constant region comprising the amino acid substitutions L234A, L235A and G237A; or

(f) the VH region amino acid sequence comprises or consists of SEQ ID NO:11; the VL region amino acid sequence comprises or consists of SEQ ID NO:12; and the heavy chain constant region comprises a wild-type human IgG4 constant region, a human IgG4
25 constant region comprising the amino acid substitution S228P, a wild-type human IgG2 constant region; a wild-type human IgG1 constant region or a human IgG1 constant region comprising the amino acid substitutions L234A, L235A and G237A.

30 In some aspects, disclosed herein is an anti-PD1 antibody or an antigen-binding portion thereof, wherein the antibody comprises a heavy chain variable (VH) region, a light chain variable (VL) region and a heavy chain constant region, wherein

a) the VH region amino acid sequence comprises or consists of SEQ ID NO:1; the VL region amino acid sequence comprises or consists of SEQ ID NO:2; and the heavy chain constant region comprises any one of SEQ ID NOS: 13-19;

35 (b) the VH region amino acid sequence comprises or consists of SEQ ID NO:3; the VL region amino acid sequence comprises or consists of SEQ ID NO:4; and the heavy chain constant region comprises any one of SEQ ID NOS: 13-19;

- (c) the VH region amino acid sequence comprises or consists of SEQ ID NO:5; the VL region amino acid sequence comprises or consists of SEQ ID NO:6; and the heavy chain constant region comprises any one of SEQ ID NOS: 13-19;
- (d) the VH region amino acid sequence comprises or consists of SEQ ID NO:7; the VL region amino acid sequence comprises or consists of SEQ ID NO:8; and the heavy chain constant region comprises any one of SEQ ID NOS: 13-19;
- (e) the VH region amino acid sequence comprises or consists of SEQ ID NO:9; the VL region amino acid sequence comprises or consists of SEQ ID NO:10; and the heavy chain constant region comprises any one of SEQ ID NOS: 13-19; or
- (f) the VH region amino acid sequence comprises or consists of SEQ ID NO:11; the VL region amino acid sequence comprises or consists of SEQ ID NO:12; and the heavy chain constant region comprises any one of SEQ ID NOS: 13-19.

In some aspects, an anti-PD1 antibody may be immune effector null. In some aspects, an anti-PD1 antibody or an antigen-binding portion thereof does not induce immune effector function and, optionally, suppresses immune effector function. In some aspects, an anti-PD1 antibody may lack measurable binding to human Fc γ RI, Fc γ RIIIa, Fc γ RIIIa and Fc γ RIIIb receptors but maintain binding to human Fc γ RIIIb receptor and optionally maintain binding to human FcRn receptor. Fc γ RI, Fc γ RIIIa, Fc γ RIIIa and Fc γ RIIIb are examples of activating receptors. Fc γ RIIIb is an example of an inhibitory receptor. FcRn is an example of a recycling receptor. In some aspects, binding affinity of an anti-PD1 antibody or an antigen-binding portion thereof for human Fc receptors may be measured by BIACORE[®] analysis. In some aspects, Homogeneous Time Resolved Fluorescence (HTRF) can be used to study binding of an anti-PD1 antibody to human Fc receptors. In one example of HTRF, human IgG1 (wild type) is labelled, as is the full suite of Fc gamma receptors and then antibodies with engineered Fc fragments are used in titration competition. In some aspects, PD1-positive cells may be mixed with human white blood cells and anti-PD1 antibodies, and cell killing by CDC, ADCC and/or ADCP may be measured. In some aspects, an anti-PD1 antibody comprising an amino acid sequence of an Fc region of human IgG1-3M (see Table 18) is effector null. In some aspects, an anti-PD1 antibody comprising an amino acid sequence of an Fc region of human IgG1-3M (see Table 18) is not effector null.

The antibody molecule or antigen-binding portion thereof may be a Fab fragment, a F(ab)₂ fragment, an Fv fragment, a tetrameric antibody, a tetravalent antibody, a multispecific antibody (for example, a bivalent antibody), a domain-specific antibody, a single domain antibody, a monoclonal antibody or a fusion protein. In one embodiment, an antibody may be a bispecific antibody that binds specifically to a first antigen and a second antigen, wherein

the first antigen is PD1 and the second antigen is not PD1. Antibody molecules and methods for their construction and use are described, in for example Holliger & Hudson (2005, Nature Biotechnol. 23(9): 1126-1136).

- 5 In another aspect of the invention, there is provided an immunoconjugate comprising the antibody molecule or antigen-binding portion thereof of the invention as defined herein linked a therapeutic agent.

10 Examples of suitable therapeutic agents include cytotoxins, radioisotopes, chemotherapeutic agents, immunomodulatory agents, anti-angiogenic agents, antiproliferative agents, pro-apoptotic agents, and cytostatic and cytolytic enzymes (for example RNases). Further therapeutic agents include a therapeutic nucleic acid, such as a gene encoding an immunomodulatory agent, an anti-angiogenic agent, an anti-proliferative agent, or a pro-apoptotic agent. These drug descriptors are not mutually exclusive, and thus a therapeutic
15 agent may be described using one or more of the above terms.

Examples of suitable therapeutic agents for use in immunoconjugates include the taxanes, maytansines, CC-1065 and the duocarmycins, the calicheamicins and other enediynes, and the auristatins. Other examples include the anti-folates, vinca alkaloids, and the
20 anthracyclines. Plant toxins, other bioactive proteins, enzymes (i.e., ADEPT), radioisotopes, photosensitizers may also be used in immunoconjugates. In addition, conjugates can be made using secondary carriers as the cytotoxic agent, such as liposomes or polymers. Suitable cytotoxins include an agent that inhibits or prevents the function of cells and/or results in destruction of cells. Representative cytotoxins include antibiotics, inhibitors of
25 tubulin polymerization, alkylating agents that bind to and disrupt DNA, and agents that disrupt protein synthesis or the function of essential cellular proteins such as protein kinases, phosphatases, topoisomerases, enzymes, and cyclins.

Representative cytotoxins include, but are not limited to, doxorubicin, daunorubicin,
30 idarubicin, aclarubicin, zorubicin, mitoxantrone, epirubicin, carubicin, nogalamycin, menogaril, pitarubicin, valrubicin, cytarabine, gemcitabine, trifluridine, ancitabine, enocitabine, azacitidine, doxifludine, pentostatin, broxuhdine, capecitabine, cladhbine, decitabine, floxuhdine, fludarabine, gougerotin, puromycin, tegafur, tiazofuhn, adhamycin, cisplatin, carboplatin, cyclophosphamide, dacarbazine, vinblastine, vincristine, mitoxantrone,
35 bleomycin, mechlorethamine, prednisone, procarbazine, methotrexate, flurouracils, etoposide, taxol, taxol analogs, platins such as cis-platin and carbo-platin, mitomycin, thiotepa, taxanes, vincristine, daunorubicin, epirubicin, actinomycin, authramycin,

azaserines, bleomycins, tamoxifen, idarubicin, dolastatins/auristatins, hemiasterlins, esperamicins and maytansinoids.

5 Suitable immunomodulatory agents include anti-hormones that block hormone action on tumors and immunosuppressive agents that suppress cytokine production, down-regulate self-antigen expression, or mask MHC antigens.

10 Also provided is a nucleic acid molecule encoding the antibody molecule or antigen-binding portion thereof of the invention as defined herein. A nucleic acid molecule may encode (a) the VH region amino acid sequence; (b) the VL region amino acid sequence; or (c) both the VH and the VL region amino acid sequences of an anti-PD1 antibody or an antigen-binding portion thereof described herein. In some aspects, the nucleic acid molecule as defined herein may be isolated.

15 Further provided is a vector comprising the nucleic acid molecule of the invention as defined herein. The vector may be an expression vector.

Also provided is a host cell comprising the nucleic acid molecule or the vector of the invention as defined herein. The host cell may be a recombinant host cell.

20 In a further aspect there is provided a method of producing an anti-PD1 antibody and/or an antigen-binding portion thereof, comprising culturing the host cell of the invention under conditions that result in expression and/or production of the antibody and/or the antigen-binding portion thereof, and isolating the antibody and/or the antigen-binding portion thereof
25 from the host cell or culture.

In another aspect of the invention there is provided a pharmaceutical composition comprising the antibody molecule or antigen-binding portion thereof of the invention as defined herein, or the nucleic acid molecule of the invention as defined herein, or the vector of the invention
30 as defined herein.

Further provided is a method for enhancing an immune response in a subject, comprising administering to the subject an effective amount of the antibody molecule or antigen-binding portion thereof of the invention as defined herein, or the immunoconjugate of the invention
35 as defined herein, or the nucleic acid molecule of the invention as defined herein, or the vector of the invention as defined herein, or the pharmaceutical composition of the invention as defined herein.

In a further aspect there is provided a method for treating or preventing cancer in a subject, comprising administering to the subject an effective amount of the antibody molecule or antigen-binding portion thereof of the invention as defined herein, or the immunoconjugate
5 of the invention as defined herein, or the nucleic acid molecule of the invention as defined herein, or the vector of the invention as defined herein, or the pharmaceutical composition of the invention as defined herein. In some cases, the administration to a subject of the antibody molecule or antigen-binding portion thereof of the invention as defined herein, or the immunoconjugate of the invention as defined herein, or the nucleic acid molecule of the
10 invention as defined herein, or the vector of the invention as defined herein does not induce or induces minimal hemangioma in the subject.

For example, the cancer may be pancreatic cancer, melanoma, breast cancer, lung cancer, bronchial cancer, colorectal cancer, prostate cancer, stomach cancer, ovarian cancer,
15 urinary bladder cancer, brain or central nervous system cancer, peripheral nervous system cancer, esophageal cancer, cervical cancer, uterine or endometrial cancer, cancer of the oral cavity or pharynx, liver cancer, kidney cancer, testicular cancer, biliary tract cancer, small bowel or appendix cancer, salivary gland cancer, thyroid gland cancer, adrenal gland cancer, osteosarcoma, chondrosarcoma, or cancer of hematological tissues.

20 The invention also provides an antibody molecule or antigen-binding portion thereof of the invention as defined herein, or the immunoconjugate of the invention as defined herein, or the nucleic acid molecule of the invention as defined herein, or the vector of the invention as defined herein, or the pharmaceutical composition of the invention as defined herein, for use
25 in the treatment of cancer.

In another aspect the invention provides the antibody molecule, or antigen-binding portion thereof, or the immunoconjugate, or the nucleic acid molecule, or the vector for use, or the method of treatment of the invention as defined herein, for separate, sequential or
30 simultaneous use in a combination combined with a second therapeutic agent, for example an anti-cancer agent.

In a further aspect there is provided the use of an antibody molecule or antigen-binding portion thereof of the invention as defined herein, or an immunoconjugate of the invention as defined herein, or a nucleic acid molecule of the invention as defined herein, or a vector
35 of the invention as defined herein, or a pharmaceutical composition of the invention as defined herein, in the manufacture of a medicament for the treatment of cancer.

The invention also provides a method for treating or preventing an infectious or immune disease in a subject, comprising administering to the subject an effective amount of the antibody molecule or antigen-binding portion thereof as defined herein, or the immun

5 conjugate as defined here, or the nucleic acid molecule as defined herein, or the vector as defined herein, or the pharmaceutical composition as defined herein.

In one embodiment, the invention provides an anti-PD1 antibody or an antigen-binding portion thereof comprising the amino acid sequences disclosed herein for use in therapy.

10

The pharmaceutical composition of the invention may comprise a pharmaceutically acceptable excipient, carrier, or diluent. A pharmaceutically acceptable excipient may be a compound or a combination of compounds entering into a pharmaceutical composition which does not provoke secondary reactions and which allows, for example, facilitation of the administration of the anti-PD1 antibody molecule, an increase in its lifespan and/or in its efficacy in the body or an increase in its solubility in solution. These pharmaceutically acceptable vehicles are well known and will be adapted by the person skilled in the art as a function of the mode of administration of the anti-PD1 antibody molecule.

20 In some embodiments, the anti-PD1 antibody molecule may be provided in a lyophilised form for reconstitution prior to administration. For example, lyophilised antibody molecules may be re-constituted in sterile water and mixed with saline prior to administration to an individual.

The anti-PD1 antibody molecules will usually be administered in the form of a pharmaceutical composition, which may comprise at least one component in addition to the antibody molecule. Thus pharmaceutical compositions may comprise, in addition to the anti-PD1 antibody molecule, a pharmaceutically acceptable excipient, carrier, buffer, stabilizer or other materials well known to those skilled in the art. Such materials should be non-toxic and should not interfere with the efficacy of the anti-PD1 antibody molecule. The precise nature of the carrier or other material will depend on the route of administration, which may be by bolus, infusion, injection or any other suitable route, as discussed below.

For parenteral, for example sub-cutaneous or intra-venous administration, e.g. by injection, the pharmaceutical composition comprising the anti-PD1 antibody molecule may be in the form of a parenterally acceptable aqueous solution which is pyrogen-free and has suitable pH, isotonicity and stability. Those of relevant skill in the art are well able to prepare suitable solutions using, for example, isotonic vehicles, such as Sodium Chloride Injection, Ringe's

Injection, Lactated Ringer's Injection. Preservatives, stabilizers, buffers, antioxidants and/or other additives may be employed as required including buffers such as phosphate, citrate and other organic acids; antioxidants, such as ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; 5 benzalkonium chloride; benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens, such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3'-pentanol; and m-cresol); low molecular weight polypeptides; proteins, such as serum albumin, gelatin or immunoglobulins; hydrophilic polymers, such as polyvinylpyrrolidone; amino acids, such as glycine, glutamine, asparagines, histidine, arginine, or lysine; monosaccharides, 10 disaccharides and other carbohydrates including glucose, mannose or dextrans; chelating agents, such as EDTA; sugars, such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions, such as sodium; metal complexes (e.g. Zn-protein complexes); and/or non-ionic surfactants, such as TWEENTM, PLURONICSTM or polyethylene glycol (PEG).

15 A pharmaceutical composition comprising an anti-PD1 antibody molecule may be administered alone or in combination with other treatments, either simultaneously or sequentially dependent upon the condition to be treated.

20 An anti-PD1 antibody molecule as described herein may be used in a method of treatment of the human or animal body, including prophylactic or preventative treatment (e.g. treatment before the onset of a condition in an individual to reduce the risk of the condition occurring in the individual; delay its onset; or reduce its severity after onset). The method of treatment may comprise administering the anti-PD1 antibody molecule to an individual in need thereof.

25 Administration is normally in a "therapeutically effective amount", this being sufficient to show benefit to a patient. Such benefit may be at least amelioration of at least one symptom. The actual amount administered, and rate and time-course of administration, will depend on the nature and severity of what is being treated, the particular mammal being treated, the clinical condition of the individual patient, the cause of the disorder, the site of delivery of the composition, the method of administration, the scheduling of administration and other factors 30 known to medical practitioners. Prescription of treatment, e.g. decisions on dosage etc., is within the responsibility of general practitioners and other medical doctors and may depend on the severity of the symptoms and/or progression of a disease being treated. Appropriate doses of antibody molecules are well known in the art (Ledermann J.A. *et al.*, 1991, *Int. J. Cancer* 47: 659-664; Bagshawe K.D. *et al.*, 1991, *Antibody, Immunoconjugates and Radiopharmaceuticals* 4: 915-922). Specific dosages may be indicated herein or in the Physician's Desk Reference (2003) as appropriate for the type of medicament being 35

administered may be used. A therapeutically effective amount or suitable dose of an antibody molecule may be determined by comparing its *in vitro* activity and *in vivo* activity in an animal model. Methods for extrapolation of effective dosages in mice and other test animals to humans are known. The precise dose will depend upon a number of factors,
5 including whether the antibody is for prevention or for treatment, the size and location of the area to be treated, the precise nature of the antibody (e.g. whole antibody, fragment) and the nature of any detectable label or other molecule attached to the antibody.

A typical antibody dose will be in the range 100 µg to 1 g for systemic applications, and 1 µg
10 to 1 mg for topical applications. An initial higher loading dose, followed by one or more lower doses, may be administered. Typically, the antibody will be a whole antibody, e.g. the IgG1 or IgG4 isotype. This is a dose for a single treatment of an adult patient, which may be proportionally adjusted for children and infants, and also adjusted for other antibody formats in proportion to molecular weight. Treatments may be repeated at daily, twice-weekly,
15 weekly or monthly intervals, at the discretion of the physician. The treatment schedule for an individual may be dependent on the pharmacokinetic and pharmacodynamic properties of the antibody composition, the route of administration and the nature of the condition being treated.

Treatment may be periodic, and the period between administrations may be about two weeks
20 or more, e.g. about three weeks or more, about four weeks or more, about once a month or more, about five weeks or more, or about six weeks or more. For example, treatment may be every two to four weeks or every four to eight weeks. Treatment may be given before, and/or after surgery, and/or may be administered or applied directly at the anatomical site of
25 surgical treatment or invasive procedure. Suitable formulations and routes of administration are described above.

In some embodiments, anti-PD1 antibody molecules as described herein may be
30 administered as sub-cutaneous injections. Sub-cutaneous injections may be administered using an auto-injector, for example for long term prophylaxis/treatment.

In some embodiments, the therapeutic effect of the anti-PD1 antibody molecule may persist for several half-lives, depending on the dose. For example, the therapeutic effect of a single
35 dose of the anti-PD1 antibody molecule may persist in an individual for 1 month or more, 2 months or more, 3 months or more, 4 months or more, 5 months or more, or 6 months or more.

The invention also provides a method of producing an antibody molecule which specifically binds to human PD1 and optionally also to cynomolgus monkey PD1, or an antigen-binding portion thereof, comprising the steps of:

- 5 (1) grafting anti-PD1 CDRs from a non-human source into a human v-domain framework to produce a humanized anti-PD1 antibody molecule or antigen-binding portion thereof;
- (2) generating a library of clones of the humanized anti-PD1 antibody molecule or antigen-binding portion thereof comprising one or more mutations in the CDRs;
- (3) screening the library for binding to human PD1 and optionally also to cynomolgus monkey PD1;
- 10 (4) selecting clones from the screening step (3) having binding specificity to human PD1 and optionally also to cynomolgus monkey PD1; and
- (5) producing an antibody molecule which specifically binds to human PD1 and optionally also to cynomolgus monkey PD1, or an antigen-binding portion thereof from clones selected from step (4).

- 15 The method may comprise a further step of producing additional clones based on the clones selected in step (4), for example based on further exploratory mutagenesis at specific positions in the CDRs of the clones selected in step (4), to enhance humanization and/or minimise human T cell epitope content and/or improve manufacturing properties in the
- 20 antibody molecule or antigen-binding portion thereof produced in step (5).

Refinements applicable to the above method are as described in Example 1 below.

- 25 As used herein, the term "PD1" refers to Programmed Cell Death Protein 1 and variants thereof that retain at least part of the biological activity of PD1. As used herein, PD1 includes all species of native sequence PD1, including human, rat, mouse and chicken. The term "PD1" is used to include variants, isoforms and species homologs of human PD1. Antibodies of the invention may cross-react with PD1 from species other than human, in particular PD1 from cynomolgus monkey (*Macaca fascicularis*). Examples of human and cynomolgus PD1
- 30 amino acid sequences are provided in Table 19. In certain embodiments, the antibodies may be completely specific for human PD1 and may not exhibit non-human cross-reactivity.

- As used herein, an "antagonist" as used in the context of the antibody of the invention or an "anti-PD1 antagonist antibody" (interchangeably termed "anti-PD1 antibody") refers to an
- 35 antibody which is able to bind to PD1 and inhibit PD1 biological activity and/or downstream pathway(s) mediated by PD1 signalling. An anti-PD1 antagonist antibody encompasses antibodies that can block, antagonize, suppress or reduce (including significantly) PD1

biological activity, including downstream pathways mediated by PD1 signalling, such as receptor binding and/or elicitation of a cellular response to PD1. For the purposes of the present invention, it will be explicitly understood that the term "anti- PD1 antagonist antibody" encompass all the terms, titles, and functional states and characteristics whereby PD1 itself, and PD1 biological activity (including but not limited to its ability to suppress the activation of anti-tumour cell activity of T cells), or the consequences of the activity or biological activity, are substantially nullified, decreased, or neutralized in any meaningful degree.

The antibody "specifically binds" "specifically interacts", "preferentially binds", "binds" or "interacts" with PD1 if it binds with greater affinity, avidity, more readily and/or with greater duration than it binds to other receptors.

An "antibody molecule" is an immunoglobulin molecule capable of specific binding to a target, such as a carbohydrate, polynucleotide, lipid, polypeptide, etc., through at least one antigen recognition site, located in the variable region of the immunoglobulin molecule. As used herein, the term "antibody molecule" encompasses not only intact polyclonal or monoclonal antibodies, but also any antigen binding fragment (for example, an "antigen-binding portion") or single chain thereof, fusion proteins comprising an antibody, and any other modified configuration of the immunoglobulin molecule that comprises an antigen recognition site including, for example without limitation, scFv, single domain antibodies (for example, shark and camelid antibodies), maxibodies, minibodies, intrabodies, diabodies, triabodies, tetrabodies, v-NAR and bis-scFv.

An "antibody molecule" encompasses an antibody of any class, such as IgG, IgA, or IgM (or sub-class thereof), and the antibody need not be of any particular class. Depending on the antibody amino acid sequence of the constant region of its heavy chains, immunoglobulins can be assigned to different classes. There are five major classes of immunoglobulins: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into subclasses (isotypes), for example IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2. The heavy-chain constant regions that correspond to the different classes of immunoglobulins are called alpha, delta, epsilon, gamma, and mu, respectively. The subunit structures and three-dimensional configurations of different classes of immunoglobulins are well known.

The term "antigen binding portion" of an antibody molecule, as used herein, refers to one or more fragments of an intact antibody that retain the ability to specifically bind to PD1. Antigen binding functions of an antibody molecule can be performed by fragments of an intact antibody. Examples of binding fragments encompassed within the term "antigen binding

portion" of an antibody molecule include Fab; Fab'; F(ab')₂; an Fd fragment consisting of the VH and CH1 domains; an Fv fragment consisting of the VL and VH domains of a single arm of an antibody; a single domain antibody (dAb) fragment, and an isolated complementarity determining region (CDR).

5

The term "Fc region" is used to define a C-terminal region of an immunoglobulin heavy chain. The "Fc region" may be a native sequence Fc region or a variant Fc region. Although the boundaries of the Fc region of an immunoglobulin heavy chain might vary, the human IgG heavy chain Fc region is usually defined to stretch from an amino acid residue at position Cys226, or from Pro230, to the carboxyl-terminus thereof. The numbering of the residues in the Fc region is that of the EU index as in Kabat. The Fc region of an immunoglobulin generally comprises two constant domains, CH2 and CH3. As is known in the art, an Fc region can be present in dimer or monomeric form.

10

15 A "variable region" of an antibody refers to the variable region of the antibody light chain or the variable region of the antibody heavy chain, either alone or in combination. As known in the art, the variable regions of the heavy and light chain each consist of four framework regions (FRs) connected by three complementarity determining regions (CDRs) also known as hypervariable regions, contribute to the formation of the antigen binding site of antibodies.

20 When choosing FR to flank CDRs, for example when humanizing or optimizing an antibody, FRs from antibodies which contain CDR sequences in the same canonical class are preferred.

25

The CDR definitions used in the present application combine the domains used in the many disparate, often conflicting schemes that have been created in the field, which are based on the combination of immunoglobulin repertoire analyses and structural analyses of antibodies in isolation and in their co-crystals with antigens (see review by Swindells *et al.*, 2016, abYsis: Integrated Antibody Sequence and Structure-Management, Analysis, and Prediction. J Mol Biol. [PMID: 27561707; Epub 22 August 2016]). The CDR definition used herein (a "Unified" definition) incorporates the lessons of all such prior insights and includes all appropriate loop positions required to sample the full residue landscape that potentially mediates target-binding complementarity.

30

Table 1 shows the amino acid sequences of the MAb005 murine anti-PD1 antibody CDRs as defined herein (a "Unified" scheme), in comparison to well-known alternative systems for defining the same CDRs.

35

The term “Mab005-IgG1 (humanized)” refers to an anti-PD1 antibody comprising the variable heavy region sequence labelled PD1-VH1 and the variable light region sequence labelled PD1-VL1 in Table 2 and a human IgG1 constant region.

- 5 As used herein the term “conservative substitution” refers to replacement of an amino acid with another amino acid which does not significantly deleteriously change the functional activity. A preferred example of a “conservative substitution” is the replacement of one amino acid with another amino acid which has a value ≥ 0 in the following BLOSUM 62 substitution matrix (see Henikoff & Henikoff, 1992, PNAS 89: 10915-10919):

10

	A	R	N	D	C	Q	E	G	H	I	L	K	M	F	P	S	T	W	Y	V
A	4	-1	-2	-2	0	-1	-1	0	-2	-1	-1	-1	-1	-2	-1	1	0	-3	-2	0
R	-1	5	0	-2	-3	1	0	-2	0	-3	-2	2	-1	-3	-2	-1	-1	-3	-2	-3
N	-2	0	6	1	-3	0	0	0	1	-3	-3	0	-2	-3	-2	1	0	-4	-2	-3
15 D	-2	-2	1	6	-3	0	2	-1	-1	-3	-4	-1	-3	-3	-1	0	-1	-4	-3	-3
C	0	-3	-3	-3	9	-3	-4	-3	-3	-1	-1	-3	-1	-2	-3	-1	-1	-2	-2	-1
Q	-1	1	0	0	-3	5	2	-2	0	-3	-2	1	0	-3	-1	0	-1	-2	-1	-2
E	-1	0	0	2	-4	2	5	-2	0	-3	-3	1	-2	-3	-1	0	-1	-3	-2	-2
G	0	-2	0	-1	-3	-2	-2	6	-2	-4	-4	-2	-3	-3	-2	0	-2	-2	-3	-3
20 H	-2	0	1	-1	-3	0	0	-2	8	-3	-3	-1	-2	-1	-2	-1	-2	-2	2	-3
I	-1	-3	-3	-3	-1	-3	-3	-4	-3	4	2	-3	1	0	-3	-2	-1	-3	-1	3
L	-1	-2	-3	-4	-1	-2	-3	-4	-3	2	4	-2	2	0	-3	-2	-1	-2	-1	1
K	-1	2	0	-1	-3	1	1	-2	-1	-3	-2	5	-1	-3	-1	0	-1	-3	-2	-2
M	-1	-1	-2	-3	-1	0	-2	-3	-2	1	2	-1	5	0	-2	-1	-1	-1	-1	1
25 F	-2	-3	-3	-3	-2	-3	-3	-3	-1	0	0	-3	0	6	-4	-2	-2	1	3	-1
P	-1	-2	-2	-1	-3	-1	-1	-2	-2	-3	-3	-1	-2	-4	7	-1	-1	-4	-3	-2
S	1	-1	1	0	-1	0	0	0	-1	-2	-2	0	-1	-2	-1	4	1	-3	-2	-2
T	0	-1	0	-1	-1	-1	-1	-2	-2	-1	-1	-1	-1	-2	-1	1	5	-2	-2	0
W	-3	-3	-4	-4	-2	-2	-3	-2	-2	-3	-2	-3	-1	1	-4	-3	-2	11	2	-3
30 Y	-2	-2	-2	-3	-2	-1	-2	-3	2	-1	-1	-2	-1	3	-3	-2	-2	2	7	-1
V	0	-3	-3	-3	-1	-2	-2	-3	-3	3	1	-2	1	-1	-2	-2	0	-3	-1	4.

- The term “monoclonal antibody” (Mab) refers to an antibody, or antigen-binding portion thereof, that is derived from a single copy or clone, including for example any eukaryotic, 35 prokaryotic, or phage clone, and not the method by which it is produced. Preferably, a monoclonal antibody of the invention exists in a homogeneous or substantially homogeneous population.

- A “humanized” antibody molecule refers to a form of non-human (for example, murine) 40 antibody molecules, or antigen-binding portion thereof, that are chimeric immunoglobulins, immunoglobulin chains, or fragments thereof (such as Fv, Fab, Fab', F(ab')₂ or other antigen-binding subsequences of antibodies) that contain minimal sequence derived from non-human immunoglobulin. Humanized antibodies may be human immunoglobulins (recipient antibody) in which residues from a CDR of the recipient are replaced by residues from a CDR

of a non-human species (donor antibody) such as mouse, rat, or rabbit having the desired specificity, affinity, and capacity.

5 “Human antibody or fully human antibody” refers to an antibody molecule, or antigen-binding portion thereof, derived from transgenic mice carrying human antibody genes or from human cells.

10 The term “chimeric antibody” is intended to refer to an antibody molecule, or antigen-binding portion thereof, in which the variable region sequences are derived from one species and the constant region sequences are derived from another species, such as an antibody molecule in which the variable region sequences are derived from a mouse antibody and the constant region sequences are derived from a human antibody.

15 “Antibody-drug conjugate” and “immunoconjugate” refer to an antibody molecule, or antigen-binding portion thereof, including antibody derivatives that binds to PD1 and is conjugated to cytotoxic, cytostatic and/or therapeutic agents.

20 Antibody molecules of the invention, or antigen-binding portion thereof, can be produced using techniques well known in the art, for example recombinant technologies, phage display technologies, synthetic technologies or combinations of such technologies or other technologies readily known in the art.

25 The term “isolated molecule” (where the molecule is, for example, a polypeptide, a polynucleotide, or an antibody) is a molecule that by virtue of its origin or source of derivation (1) is not associated with naturally associated components that accompany it in its native state, (2) is substantially free of other molecules from the same species (3) is expressed by a cell from a different species, or (4) does not occur in nature. Thus, a molecule that is chemically synthesized, or expressed in a cellular system different from the cell from which it naturally originates, will be “isolated” from its naturally associated components. A molecule
30 also may be rendered substantially free of naturally associated components by isolation, using purification techniques well known in the art. Molecule purity or homogeneity may be assayed by a number of means well known in the art. For example, the purity of a polypeptide sample may be assayed using polyacrylamide gel electrophoresis and staining of the gel to visualize the polypeptide using techniques well known in the art. For certain purposes, higher
35 resolution may be provided by using HPLC or other means well known in the art for purification.

The term "epitope" refers to that portion of a molecule capable of being recognized by and bound by an antibody molecule, or antigen-binding portion thereof, at one or more of the antibody molecule's antigen-binding regions. Epitopes can consist of defined regions of primary secondary or tertiary protein structure and includes combinations of secondary structural units or structural domains of the target recognised by the antigen binding regions of the antibody, or antigen-binding portion thereof. Epitopes can likewise consist of a defined chemically active surface grouping of molecules such as amino acids or sugar side chains and have specific three-dimensional structural characteristics as well as specific charge characteristics. The term "antigenic epitope" as used herein, is defined as a portion of a polypeptide to which an antibody molecule can specifically bind as determined by any method well known in the art, for example, by conventional immunoassays, antibody competitive binding assays or by x-ray crystallography or related structural determination methods (for example NMR).

The term "binding affinity" or "KD" refers to the dissociation rate of a particular antigen-antibody interaction. The KD is the ratio of the rate of dissociation, also called the "off-rate (k_{off})", to the association rate, or "on-rate (k_{on})". Thus, K_D equals $k_{\text{off}} / k_{\text{on}}$ and is expressed as a molar concentration (M). It follows that the smaller the K_D , the stronger the affinity of binding. Therefore, a K_D of 1 μM indicates weak binding affinity compared to a K_D of 1 nM. KD values for antibodies can be determined using methods well established in the art. One method for determining the KD of an antibody is by using surface plasmon resonance (SPR), typically using a biosensor system such as a Biacore® system.

The term "potency" is a measurement of biological activity and may be designated as IC_{50} , or effective concentration of an antibody or antibody drug conjugate to the antigen PD1 to inhibit 50% of activity measured in a PD1 activity assay as described herein.

The phrase "effective amount" or "therapeutically effective amount" as used herein refers to an amount necessary (at dosages and for periods of time and for the means of administration) to achieve the desired therapeutic result. An effective amount is at least the minimal amount, but less than a toxic amount, of an active agent which is necessary to impart therapeutic benefit to a subject.

The term "inhibit" or "neutralize" as used herein with respect to bioactivity of an antibody molecule of the invention means the ability of the antibody to substantially antagonize, prohibit, prevent, restrain, slow, disrupt, eliminate, stop, reduce or reverse for example

progression or severity of that which is being inhibited including, but not limited to, a biological activity or binding interaction of the antibody molecule to PD1.

5 A "host cell" includes an individual cell or cell culture that can be or has been a recipient for vector(s) for incorporation of polynucleotide inserts. Host cells include progeny of a single host cell, and the progeny may not necessarily be completely identical (in morphology or in genomic DNA complement) to the original parent cell due to natural, accidental, or deliberate mutation. A host cell includes cells transfected in vivo with a polynucleotide(s) of this invention.

10

As used herein, "vector" means a construct, which is capable of delivering, and, preferably, expressing, one or more gene(s) or sequence(s) of interest in a host cell. Examples of vectors include, but are not limited to, viral vectors, naked DNA or RNA expression vectors, plasmid, cosmid or phage vectors, DNA or RNA expression vectors associated with cationic
15 condensing agents, DNA or RNA expression vectors encapsulated in liposomes, and certain eukaryotic cells, such as producer cells.

The term "treating", as used herein, unless otherwise indicated, means reversing, alleviating, inhibiting the progress of, delaying the progression of, delaying the onset of, or preventing
20 the disorder or condition to which such term applies, or one or more symptoms of such disorder or condition. The term "treatment", as used herein, unless otherwise indicated, refers to the act of treating as defined above. The term "treating" also includes adjuvant and neoadjuvant treatment of a subject. For the avoidance of doubt, reference herein to "treatment" includes reference to curative, palliative and prophylactic treatment. For the
25 avoidance of doubt, references herein to "treatment" also include references to curative, palliative and prophylactic treatment.

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

Where aspects or embodiments of the invention are described in terms of a Markush group or other grouping of alternatives, the present invention encompasses not only the entire group listed as a whole, but each member of the group individually and all possible
35 subgroups of the main group, but also the main group absent one or more of the group members. The present invention also envisages the explicit exclusion of one or more of any of the group members in the claimed invention.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. In case of conflict, the present specification, including definitions, will control.

5 Throughout this specification and claims, the word "comprise," or variations such as "comprises" or "comprising" will be understood to imply the inclusion of a stated integer or group of integers but not the exclusion of any other integer or group of integers. Unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular. Any example(s) following the term "e.g." or "for example" is not meant

10 to be exhaustive or limiting.

The practice of the present invention will employ, unless otherwise indicated, conventional techniques of molecular biology (including recombinant techniques), microbiology, cell biology, biochemistry and immunology, which are within the skill of the art.

15 Particular non-limiting embodiments of the present invention will now be described with reference to accompanying drawings.

EXAMPLE 1. Generation of optimized anti-PD1 therapeutic antibodies

20 Introduction

In this example, we successfully generate a panel of agonistic, optimized anti-PD1 antibodies. These anti-PD1 antibodies are well expressed, biophysically stable, highly soluble and of maximized identity to preferred human germlines.

25 Materials and methods

PD1 library generation and selection

The PD1 Fab repertoire was assembled by mass oligo synthesis and PCR. The amplified Fab repertoire was then cloned via restriction-ligation into a phagemid vector, transformed

30 into *E.coli* TG-1 cells, and the phage repertoire rescued essentially as previously described in detail (Finlay *et al.*, 2011, Methods Mol Biol 681: 383-401).

Phage selections were performed by coating streptavidin magnetic microbeads with biotinylated PD1 target protein (either human or cyno), washing the beads thrice with PBS

35 and resuspending in PBS pH7.4 plus 5% skim milk protein. These beads were coated at 100 nM target protein in round 1 of selection, followed by reduced antigen concentrations in three

successive rounds. In each round, phage were eluted using trypsin before re-infection into TG1 cells.

Periplasmic extracts production (small-scale)

- 5 Production of soluble Fabs in individual *E. coli* clones was performed. *E. coli* TG1 cells in logarithmic growth phase were induced with isopropyl 1-thio- β -D-galactopyranoside. Periplasmic extracts containing soluble Fab were generated by a freeze/thaw cycle: Bacterial cell pellets were frozen at -20 °C for overnight and then thawed at room temperature and resuspended in PBS pH 7.4. The supernatants containing the soluble Fab
10 were collected after shaking at room temperature and centrifugation.

Fab Production and Purification

- A panel of anti-PD1 Fabs were selected for larger-scale production. *E. coli* TG1 cultures (500 ml) were prepared and soluble Fab expressions induced, as above. Periplasmic extracts
15 containing soluble Fab were extracted by a freeze/thaw cycle as above. The supernatants containing the soluble Fabs were collected after 1 hour with head-over-head rotation at room temperature, centrifugation and filtration through a 0.22 μ m membrane. Soluble Fabs were purified by two step purification procedure using His-Trap HP Affinity column (for purification via the associated His tag) followed by purification on modified protein A or CaptureSelect™
20 IgG-CH1 Affinity Matrix columns.

IgG expression and purification

- Mammalian codon-optimized synthetic genes encoding the heavy and light chain variable domains of the lead panel anti-PD1 antibodies plus the Mab005 variants and a
25 Pembrolizumab analog were cloned into mammalian expression vectors comprising effector function null human IgG1 ('IgG1null'; human IgG1 containing L234A, L235A, G237A mutations in the lower hinge that abrogate normal immunoglobulin ADCC, ADCP and CDC functions) and human C κ domains, respectively. Co-transfection of heavy and light chain containing vector in mammalian expression system was performed, followed by protein A-
30 based purification of the IgG, quantification and QC on denaturing and non-denaturing SDS-PAGE.

Direct binding ELISA for Fab and IgG

- Binding and cross-reactivity of the lead panel to the recombinant proteins was initially
35 assessed by binding ELISA. The human PD1 human Fc tagged recombinant protein and the cynomolgus monkey PD1 human Fc tagged recombinant protein were coated to the surface of MaxiSorp™ flat-bottom 96 well plate at 1 μ g/ml. The purified Fab or IgG samples were

titrated in two fold serial dilutions starting from 500 nM to 0.98 nM and allowed to bind to the coated antigens. The Fabs were detected using mouse anti-c-myc antibody followed by donkey anti-mouse IgG conjugated to horseradish peroxidase. The IgGs were detected using the mouse anti-human IgG conjugated to horseradish peroxidase. Binding signals were visualized with 3,3',5,5'-Tetramethylbenzidine Substrate Solution (TMB) and the absorbance measured at 450 nm.

Alphascreen epitope competition assay for IgG1 null antibodies

The AlphaScreen assay (Perkin Elmer) was performed in a 25 µl final volume in 384-well white microtiter plates (Greiner). The reaction buffer contained 1xPBS pH 7.3 (Oxoid, Cat. nr. BR0014G) and 0.05 % (v/v) Tween® 20 (Sigma, Cat. nr. P9416). Purified IgG samples were titrated in three fold serial dilutions starting at 500 nM final concentration and incubated with biotinylated human PD1-His/AviTag at 0.6 nM final concentration for 20 minutes at room temperature. The parental IgG at 0.3 nM and the anti-human IgG1 Acceptor beads at 20 µg/ml (final concentrations) were added and the mix was incubated for 1 hour at room temperature. Followed by addition of the Streptavidin Donor beads at 20 µg/ml (final concentration) and incubation for 30 minutes at room temperature. The emission of light was measured in the EnVision multilabel plate reader (Perkin Elmer) and analysed using the EnVision manager software. Values were reported as Counts Per Second (CPS) and corrected for crosstalk. The EC50 values were calculated using the MFI values in GraphPad Prism software (GraphPad Software, La Jolla, CA) and 4 parameters.

Biacore analyses of IgG affinity for monomeric human and cyno PD1 in solution

Affinity (KD) of purified IgGs was determined via SPR with antigen in-solution on a Biacore 3000 (GE). A mouse anti-human antibody (CH1 specific) was immobilized on a CM5 Sensor Chip to a level of 2000 RU in acetate buffer at pH 4.5 using amine coupling following the Wizard instructions for two channels. One channel was used for background signal correction. The standard running buffer HBS-EP pH 7.4 was used. Regeneration was performed with a single injection of 10 µl of 10 mM Glycine at pH 1.5 at 20 µl/minute. IgG samples were injected for 2 minutes at 50 nM at 30 µl/min followed by an off-rate of 60 seconds. The monomeric antigen (human PD1 His tagged or cynomolgus monkey PD1 His tag) was injected in two fold serial dilutions from 100 nM down to 3.1 nM, for 2 minutes at 30 µl/min followed by an off-rate of 300 seconds. The obtained sensorgrams were analysed using the Biacore 3000 evaluation (BIAevaluation) software. The KD was calculated by simultaneous fitting of the association and dissociation phases to a 1:1 Langmuir binding model.

Flow cytometry of IgGs

Purified IgGs were tested in FACs for binding to human and cyno PD1 expressed on CHO-K1 stable cell lines and CHO-K1 wild-type cells. The IgG samples were titrated in three-fold serial dilutions starting at 500 nM to 0.98 nM. Binding of IgGs was detected with a mouse anti-human IgG conjugated to FITC. Results were analyzed by examining the Mean Fluorescence Intensity (MFI) of 10000 cells per sample in the BL-1 channel detector of a flow cytometer (Attune™ NxT Acoustic Focusing Cytometer, Invitrogen/ ThermoFisher Scientific).

PD1/PD-L1 cell-based antagonism assay

The PD1/PD-L1 blockade cell-based bioassay (Promega), was used to measure the potency of antibodies in blocking the PD1/PD-L1 interaction. On the day before the assay, PD-L1 aAPC/CHO-K1 cells were thawed and transferred into cell recovery medium (90% Ham's F12/10% FBS). The cell suspension was dispensed to each of the inner 60 wells of two 96-well, white, flat-bottom assay plates, at 100 µl per well. Cell recovery medium was added to each of the outside wells and the assay plates and incubated overnight at 37 °C/5% CO₂. On the day of the assay the sample IgGs were diluted 4-fold in assay buffer (99% RPMI 1640/1% FBS) from 300 nM to 0,04 nM and 40 µl per dilution added to the assay plates containing the PD-L1 aAPC/CHO-K1 cells. Positive inhibition controls included the human PD1 Antibody AF1086 (R&D systems), mAb005 in IgG1null form and a pembrolizumab mAb analogue in IgG1null form. As a negative inhibition control, an irrelevant IgG was included. PD1 Effector Cells were then thawed in assay buffer (99% RPMI 1640/1% FBS) and the cell suspension added to the wells of the assay plates containing the PD-L1 aAPC/CHO-K1 cells and the IgG titration samples. The assay plates were incubated for six hours in a 37 °C/5 % CO₂ incubator, allowed to equilibrate to ambient temperature for 5–10 minutes, then 80 µl of Bio-Glo™ Reagent (Promega) was added. Assay plates were incubated at ambient temperature for a further 5–30 minutes and luminescence signals subsequently measured at 10, 20 and 30 minutes.

Antibody v-domain T cell epitope content: *in silico* analyses

In silico technologies (Abzena, Ltd.), which are based on identifying the location of T cell epitopes in therapeutic antibodies and proteins, were used for assessing potential immunogenicity in antibody v-domains. iTope™ was used to analyse the VL and a VH sequences of key leads for peptides with promiscuous high affinity binding to human MHC class II. Promiscuous high affinity MHC class II binding peptides are thought to correlate with the presence of T cell epitopes that are high risk indicators for clinical immunogenicity of drug proteins. The iTope™ software predicts favourable interactions between amino acid

side chains of a peptide and specific binding pockets (in particular pocket positions; p1, p4, p6, p7 and p9) within the open-ended binding grooves of 34 human MHC class II alleles. These alleles represent the most common HLA-DR alleles found world-wide with no weighting attributed to those found most prevalently in any particular ethnic population.

5 Twenty of the alleles contain the 'open' p1 configuration and 14 contain the 'closed' configuration where glycine at position 83 is replaced by a valine. The location of key binding residues is achieved by the *in silico* generation of 9mer peptides that overlap by eight amino acids spanning the test protein sequence. This process successfully discriminates with high accuracy between peptides that either bind or do not bind MHC class II molecules.

10

In addition, the sequences were analysed using TCED™ (T Cell Epitope Database™) search for matches to T cell epitopes previously identified by *in vitro* human T cell epitope mapping analyses of other protein sequences. The TCED™ is used to search any test sequence against a large (>10,000 peptides) database of peptides derived from unrelated protein and antibody sequences.

15

Antibody v-domain specificity testing: human receptor array analyses

Human cell membrane receptor proteome arrays were performed at Retrogenix Ltd. Primary screens: 5 µg/ml of IgG1-Mab005 (humanized) antibody was screened for binding against

20 fixed HEK293 cells/slides expressing 4975 human plasma membrane proteins individually (14 slide sets, n=2 slides per slide set). All transfection efficiencies exceeded the minimum threshold. Antibody binding was detected using AF647 fluorescent secondary anti-human IgG1 antibody. Primary hits (duplicate spots) were identified by analysing fluorescence (AF647 and ZsGreen1) on ImageQuant. Vectors encoding all hits were sequenced to confirm

25 their correct identities. Confirmation/specificity screens: Vectors encoding all hits, plus control vectors encoding MS4A1 (CD20) and EGFR, were spotted in duplicate on new slides, and used to reverse transfect human HEK293 cells as before. All transfection efficiencies exceeded the minimum threshold. Identical fixed slides were treated with 5 µg/ml of each test antibody, 5 µg/ml of the negative control antibody, 1 µg/ml Rituximab biosimilar (positive control), Isotype IgG1 (Ab00102 human IgG1 anti-Fluorescein) or no test molecule

30 (secondary only; negative control) (n=2 slides per treatment). Slides were analysed as above. Flow cytometry confirmation screen: Expression vectors encoding ZsGreen1 only, or ZsGreen1 and PD1, FZD5, KDR or ULBP2, were transfected into human HEK293 cells. Each live transfectant was incubated with 1 and 5 mg/ml of each of the test antibodies and the

35 Isotype control antibody. Cells were washed, and incubated with the same AF647 anti-human IgG Fc detection antibody as used in the cell microarray screens. Cells were again washed, and analysed by flow cytometry using an Accuri flow cytometer (BD). A 7AAD

live/dead dye was used to exclude dead cells, and ZsGreen1-positive cells (i.e. transfected cells) were selected for analyses.

DSC Analysis

- 5 The T_m of test articles was analysed using a MicroCal PEAQ-DSC (Malvern Instruments, Malvern, UK) running version 1.22 software. The samples were heated at a rate of 200 °C/hour over a range of 20-110 °C. Thermal data was normalised based on protein concentration. The T_m of the protein was determined from the heating scan data.

10 Forced Oxidation Analyses

- For forced oxidation analysis of intact IgGs: IgG1null samples in PBS buffer were treated with 0.5% H₂O₂ at room temperature for 2 hours and then stored at -80 °C prior to RP analysis (intact antibodies and subunits, tryptic peptides) on a Dionex Ultimate 3000RS HPLC system (ThermoFisher Scientific, Hemel Hempstead, UK). For intact antibody reduction, DTT was added to a final concentration of 0.33 M and samples were incubated for 1 hour at room temperature and immediately analysed by RP. Chromatographic separation was performed using a PLRP-S 1000, 5 µm, 2.1 mm × 50 mm column (Agilent Technologies, Stockport, UK) connected to a Dionex Ultimate 3000RS HPLC system (ThermoFisher Scientific, Hemel Hempstead, UK). The method consisted of a linear gradient from 80% buffer A (0.02% TFA, 7.5% acetonitrile in H₂O) to 50% buffer B (0.02% TFA, 7.5% H₂O in acetonitrile) over 24 minutes. The flow rate was 0.5 mL/minute and the temperature was maintained at 70 °C throughout the analysis. Detection was carried out by UV absorption at 280 nm.

- 25 For forced oxidation analysis of digested IgGs: Native and oxidised IgG1null samples were digested with trypsin using the SMART Digest™ kit (ThermoFisher Scientific, Hemel Hempstead, UK) by following the manufacturer's protocol. The resulting tryptic peptides were immediately analysed by RP. Chromatographic separation was performed using an Acquity UPLC CSH C18 Column, 130 Å, 1.7 µm, 2.1 mm × 150 mm (Waters, Elstree, UK) connected to a Dionex Ultimate 3000RS HPLC system (ThermoFisher Scientific, Hemel Hempstead, UK). The method consisted of a linear gradient from 95% buffer A (0.1% FA in H₂O) to 15% buffer B (0.085% FA in 75% acetonitrile) over 4 minutes, followed by a linear gradient from 15% buffer B to 60% buffer B over 22 minutes. The flow rate was 0.2 mL/minute and the temperature was maintained at 40 °C throughout the analysis. Detection was carried out by UV absorption at 280 nm.

For HIC analyses, chromatographic separation was performed using a TSKgel Butyl-NPR 4.6 mm × 35 mm HIC column (TOSOH Bioscience Ltd., Reading, UK) connected to a Dionex Ultimate 3000RS HPLC system (ThermoFisher Scientific, Hemel Hempstead, UK). The method consisted of a linear gradient from 60% Buffer A (100 mM sodium phosphate pH 7.0, 2 M ammonium sulphate) to 90% Buffer B (100 mM sodium phosphate pH 7.0) over 9 minutes. The flow rate was 1.2 mL/minute. Detection was carried out by UV absorption at 280 nm.

Results and Discussion

CDR grafting onto preferred human germline v-genes

The CDRs of an agonistic murine anti-PD1 IgG MAb005 (mVH/mVL; see WO2015/085847A1 and Table 2) were initially introduced to human germline immunoglobulin v-domain framework sequence scaffolds using CDR grafting. To bias our engineering efforts towards final lead therapeutic IgG compounds with optimal drug-like properties, we chose to graft the CDRs of the parental antibody onto “preferred” germline scaffolds IGHV3-7 and IGKV1-39, which are known to have good solubility and drug development qualities, and are used at high frequency in the expressed human antibody repertoire.

Those scaffolds and grafted CDR definitions are outlined in Table 2. The heavy and light chain sequences for murine anti-PD1 antibody are also shown in Table 2. While this process of CDR grafting is well known, it is still problematic to predict whether a given set of human v-domain sequences will act as suitable acceptor frameworks for non-human CDR grafting. The use of unsuitable frameworks can lead to the loss of target binding function, protein stability issues or even impaired expression of the final IgG. The IGHV3-7/IGKV1-39 graft was therefore taken forward as the template for CDR mutagenesis and selection of improved clones.

Library generation and screening

The CDR-grafted IGHV3-7/IGKV1-39 v-domain sequences were combined into a Fab phage display format and a mutagenesis library cassette was generated by oligo synthesis and assembly. The final Fab library was ligated into a phage display vector and transformed into *E. coli* via electroporation to generate 1.3×10^9 independent clones. Library build quality was verified by sequencing 96 clones. This sequencing data showed that the positions encoding either the murine or human germline residue at each position of variance had been effectively sampled at a frequency of approximately 50%. Libraries were rescued using helper phage

M13 and selections performed on biotinylated human and cynomolgus monkey PD1-Fc proteins in multiple separate branches.

Post-selection screening (Fig. 1) and DNA sequencing revealed the presence of 64 unique, human and cyno PD1-binding Fab clones that exhibited strong binding to human and cyno PD1 in ELISA and >50% inhibition of IgG1-Mab005 (humanized) binding to human and cyno PD1 in Alphascreen assay. Amongst these 64 clones, the framework sequences remained fully germline while mutations were also observed in all CDRs (Table 3). Lead clones were ranked based on level of CDR germ-lining versus ELISA signals for binding to both human and cyno PD1-Fc. The v-domains of the 12 top clones from this ranking were then sub-cloned into IgG expression vectors for further testing as below (Table 4). The top 12 clones were also expressed as Fab protein from *E. coli* expression, purified to homogeneity, quantified, and applied in direct ELISA binding analyses on human PD1-Fc and cyno PD1-Fc (Fig. 2A&B, Table 5). Unexpectedly, the titration of the Fab proteins on cyno PD1 protein demonstrated significantly improved binding EC50 values for several library-derived clones over that of the Mab005 (humanized) Fab (Table 5). This led to EC50 values for all library derived clones that were approximately equivalent to those observed for human PD1 binding, while the cyno binding of the Mab005 (humanized) Fab was significantly lower than for human. Indeed, binding of the Mab005 (humanized) Fab was sufficiently weak on cyno PD1 that it did not achieve binding signal saturation, even at the highest concentration applied (500nM), meaning that a cyno PD1 EC50 value could not be calculated for this protein (Table 5). As these values were generated with proven monomeric Fab proteins, they reflected 1:1 binding improvements for cyno protein in several clones.

While germ-lining mutations were observed in all CDRs for the lead clones derived directly from library selections, it remained possible that sequence analyses might allow further clones to be designed to have maximal humanization. The 64 sequence-unique hits with binding signals against human and cyno protein were therefore used to analyse the retention frequency for murine amino acids in the CDRs of this functionally characterized population. Positional amino acid retention frequency was expressed as a percentage found in the V_L and V_H domains (Fig. 3A&B, respectively). Murine residues with RF < 75% were regarded as positions that are possibly not essential to the target-binding paratope and are likely to be open to germ-lining, in a series of combinatorial designs (Table 4). In the V_H domain (excluding the CDR-H3), only 4 of 9 murine residues in the CDR-H1 and H2 exhibited retention frequency above 75% and the sole murine residue (M) found in the CDR-H1 was not found in any functional clones, giving it a frequency value of 0% (Fig. 3B). In the V_L domain, only 2 of 13 murine CDR residues derived from the Mab005 sequence were retained

with frequencies >75% (Fig. 3A). This analysis strongly suggested that the entire CDR-L2 sequence could be rendered germline identity to IGKV1-39. Importantly, the Tryptophan (W) residue found in the CDR-L3 of Mab005 and retained at almost 100% (Fig. 3A) is in a position donated by the J segment during light chain splicing, and was only regarded as non-human due to the use of the human JK4 sequence in the starting library. This observation allowed a redesign of the light chain in a series of designer clones to include the human JK1 sequence instead of JK4, as human JK1 naturally contains a W residue at that position, rendering the resulting light chain sequence fully germline in CDR-L2 and L3 (Table 4).

Designs containing only those murine residues with RF > 75% were given the prefix "MH" (MH = Maximally Humanized). Another designer v-domain set ('TTP' = Total Theoretically Possible) was also created that combined the most humanized CDRs observed in the population, for both V_L and V_H domains. In total 5 designer V_H and 3 designer V_L domains were generated: V_H domains MH-AA-VH, MH-LV-VH, MH-LG-VH, MH-LF-VH, MH-LA-VH sampled variant sequences in the CDR-H1 and H3, while MH-VL, MH-JK1-VL, TTP-JK1-VL sampled variants in the CDR-L1 and also the JK4>JK1 swap. Indeed, the W>Y and JK4>JK1 mutations in TTP-JK1-VL rendered this sequence fully human germline across the entire v-domain. These constructs were co-transfected in a matrixed fashion to create 15 final designer IgGs in total (Table 4). The MH and TTP clones were generated by gene synthesis and (along with the 12 library-derived clones outlined above and positive control IgG1-Mab005 (humanized)), cloned into human expression vectors for production in IgG1null format. All IgGs were readily expressed and purified from transient transfections of mammalian cells.

25

Lead IgG specificity and potency characteristics

The purified IgGs described above were then tested for binding to human and cyno PD1-Fc in direct titration ELISA format. This analysis demonstrated that several library-derived clones had human and cyno PD1 binding profiles and EC50 values similar to (within 2-fold), or improved over, IgG1-Mab005 (humanized) (Fig. 4A&B, Table 6). One notable exception was clone IgG1-14C07 which exhibited poor binding to both orthologs of PD1, making it impossible to determine an EC50 value for this clone. Similarly, the majority of designer IgGs had EC50 values similar to (within 3-fold), or improved over, IgG1-Mab005 (humanized) (Fig. 5A-D, Table 7). Clones IgG1-01, IgG1-02 and IgG1-03 exhibited poor binding to one or both orthologs of PD1, however, demonstrating that the CDR-H1 and H3 mutations found in these clones are disruptive to binding when used in combination. As the ELISA EC50 values for directly binding IgGs are strongly influenced by avidity, rather than true 1:1 binding affinity,

we then proceeded to perform higher-sensitivity, solution-phase epitope competition and Biacore binding affinity determinations, as outlined below.

5 An Alphascreen assay was established to allow the testing of IgGs for epitope competition with IgG1-Mab005 (humanized) binding to biotinylated monomeric human PD1. In this assay, the top-performing library-derived and designer IgGs were more effectively differentiated via IC50 values (Table 8). While many clones exhibited equivalent or improved competition for the Mab005 epitope over IgG1-Mab005 (humanized) (Fig. 6A&6B), several exhibited impaired epitope competition (>2-fold lower than Mab005) including: IgG1-15C10, IgG1-13G02, IgG1-08F04, IgG1-16C07, IgG1-12H11, IgG1-12E02, IgG1-14C07, IgG1-01, IgG1-02, IgG1-03, IgG1-06, IgG1-09, IgG1-12 and IgG1-15 (Table 8). Notably, the lowest-performing designer IgGs in this assay included those previously shown to have poor cyno ortholog cross-reactivity in the ELISA assay and also all clones that contained the CDR-L1 germlining mutation W>Y.

15 Biacore analyses of binding affinity was performed for all IgGs to solution-phase, monomeric human and cyno PD1 proteins. In all cases, accurate 1:1 binding affinities with low Chi² values were obtained (Table 9). These analyses showed that library-derived clones which consistently gave the highest EC50 and IC50 values in Fab and IgG ELISA and Alphascreen assays also showed highest affinity binding to human and cyno PD1. Importantly, library-derived clones IgG1-06D02, IgG1-12B07, IgG1-12H04 and IgG1-16H10 and designer clones IgG1-04, IgG1-05, IgG1-08, IgG1-10, IgG1-11, IgG1-13 and IgG1-14 all exhibited improved binding affinities for both human and cyno PD1 in comparison to IgG1-Mab005 (humanized). Importantly, these improvements in affinity unexpectedly normalised the human/cyno affinities for these clones to within 3-fold (all KD values < 4.9 nM), as opposed to IgG1-Mab005 (humanized), which exhibited an 8-fold differential (human KD – 4.0 nM, cyno KD – 32.0 nM). Affinity differentials of less than 3-fold between human and cyno target orthologs are highly beneficial in pre-clinical drug development analyses as they allow significantly better design and interpretation of e.g. monkey safety, PK and PD modelling experiments. This relative normalisation of the binding to both PD1 orthologs rendered these lead clones highly similar in binding to the IgG1-Pembrolizumab analog, which exhibited an affinity differential of 2.7. In addition, comparison of the affinities of clones IgG1-14 and IgG1-15 confirmed the influence of the single 'murine' W residue in the CDR-L1 of IgG1-14 (Table 4), as mutation of this single residue to Y in IgG1-15 resulted in approximately 10-fold loss of KD against both human and cyno PD1 (Table 9). This finding highlighted the difficulty in deciding '*a priori*' which CDR residues may or may not be converted to human germline

identity, as the W>Y mutation would normally be assumed to be a relatively conservative substitution.

Flow cytometric analyses of lead IgG binding specificity at the cell membrane

- 5 Antibodies to PD1 were analysed for concentration-dependent binding at the cell surface via flow cytometry. Initial analyses were performed on CHO cells stably-transfected with human or cyno PD1. These analyses showed that lead library-derived (Fig. 7A) and designer clones (Fig. 7B, 7C) exhibit concentration-dependent binding to membrane-presented human PD1 with potencies equivalent to, or improved over, the IgG1-Mab005 (humanized) (Table 10). Importantly, the influence of the CDR-L1 'W' residue was again
10 observed as IgG1-14 exhibited stronger binding than IgG1-15 for human PD1 (Fig. 7C). Analyses performed on cyno PD1 CHO cells further confirmed that several library-derived (Fig. 8A) and designer leads (Fig. 8B) exhibited significantly improved binding for cyno PD1 in comparison to IgG1-Mab005 (humanized) (Table 10). No binding signals were observed
15 for any clone, even at the highest concentrations, on untransfected CHO cells.

Lead IgG analyses in PD1-PDL1 blockade assay

- In a cell-based PD1/PD-L1 blockade reporter assay, all clones tested exhibited concentration-dependent antagonism of PD1. Importantly, multiple clones (including IgG1-
20 06D02, IgG1-12H04, IgG1-16H10, IgG1-05, IgG1-08, IgG1-11 and IgG1-14) exhibited improved potency in PD1 blockade in comparison to IgG1-Mab005 (humanized) (Table 11). In addition, clones IgG1-06D02 and IgG1-16H10 exhibited increased maximal signal in the assay, over IgG1-Mab005 (humanized) and an IgG4 Nivolumab analogue (Figure 9).

Antibody v-domain T cell epitope analyses

- In silico* technologies (Abzena, Ltd.), which are based on identifying the location of T cell epitopes in therapeutic antibodies and proteins, were used for assessing the immunogenicity of both the Mab005 and lead antibody v-domains. Analysis of the v-domain sequences was performed with overlapping 9mer peptides (with each overlapping the last
30 peptide by 8 residues) which were tested against each of the 34 MHC class II allotypes. Each 9mer was scored based on the potential 'fit' and interactions with the MHC class II molecules. The peptide scores calculated by the software lie between 0 and 1. Peptides that produced a high mean binding score (>0.55 in the iTope™ scoring function) were highlighted and, if >50% of the MHC class II binding peptides (i.e. 17 out of 34 alleles) had
35 a high binding affinity (score >0.6), such peptides were defined as 'high affinity' MHC class II binding peptides which are considered a high risk for containing CD4+ T cell epitopes. Low affinity MHC class II binding peptides bind a high number of alleles (>50%) with a

binding score >0.55 (but without a majority >0.6). Further analysis of the sequences was performed using the TCED™. The sequences were used to interrogate the TCED™ by BLAST search in order to identify any high sequence homology between peptides (T cell epitopes) from unrelated proteins/antibodies that stimulated T cell responses in previous *in vitro* T cell epitope mapping studies performed at Abzena Ltd.

Peptides were grouped into four classes: High Affinity Foreign ('HAF' – high immunogenicity risk), Low Affinity Foreign ('LAF' – lower immunogenicity risk), TCED+ (previously identified epitope in TCED database), and Germline Epitope ('GE' - human germline peptide sequence with high MHC Class II binding affinity). Germline Epitope 9mer peptides are unlikely to have immunogenic potential due to T cell tolerance, as validated by previous studies with a wide range of germline peptides. Importantly, such germline v-domain epitopes (aided further by similar sequences in the human antibody constant regions) also compete for MHC Class II occupancy at the membrane of antigen presenting cells, reducing the risk of foreign peptide presentation being sufficient to achieve the 'activation threshold' required for T cell stimulation. High GE content is therefore a beneficial quality in clinical development of an antibody therapeutic.

As shown in Table 12, key lead v-domains exhibited significant beneficial changes in peptide epitope content in comparison to IgG1-Mab005 (humanized) (Table 12). As the v-domain framework regions (i.e. outside the CDR sequences) of IgG1-Mab005 (humanized) and all leads were germline in sequence (Table 2), all improvements in predicted immunogenicity came about as a result of the germlining of CDR residues (Tables 4, 12). Indeed, in several clones, the VL domains were found to be fully deimmunized. GE epitope content was also found to be significantly increased in the VL regions of lead clones (from 1 to ≥ 3 in all leads), and TCED+ epitopes were eliminated from the VL domains in all leads (Table 12). Importantly, multiple HAF and LAF epitopes were eliminated by germlining mutations found in the VL CDRs of lead clones. For example, a TCED+ and HAF peptide 'VTITCLASQ' (SEQ ID NO: 83) found in the LCDR-1 of IgG1-Mab005 (humanized) was eliminated in all lead clones by the mutation L>R at position 6, converting this sequence to the light chain GE 'VTITCRASQ' (SEQ ID NO: 84). Similarly, an LAF peptide 'IGTWLTWYQ' (SEQ ID NO: 85) found in the LCDR-1 of IgG1-Mab005 (humanized) was eliminated in all lead clones by retaining only a single murine residue W at position 4, converting this sequence to 'ISSWLNWYQ' (SEQ ID NO: 86) (Table 12). In clones 06D02, IgG1-05, IgG1-08, IgG1-11 and IgG1-14, the IgG1-Mab005 (humanized) HAF peptides 'LLIYTATSL' (SEQ ID NO: 87) and 'LIYTATSLA' (SEQ ID NO: 88), and LAF peptide 'IYTATSLAD' (SEQ ID NO: 89) were eliminated and converted to GE sequences by

mutation of the LCDR-2 from the murine sequence 'TATSLAD' (SEQ ID NO: 36), to the fully germline sequence 'AASSLQS' (SEQ ID NO: 42). The IgG1-Mab005 (humanized) FW3/LCDR3 region also encoded for a LAF peptide 'YYCQQVYSI' (SEQ ID NO: 90). This epitope was eliminated in all leads by the germlining mutation V>S at position 6. In the VH region of IgG1-Mab005 (humanized), the peptide sequence 'LYYFDYWGQ' (SEQ ID NO:91) (spanning the HCDR3 and FW4) was found to be a LAF. The mutation Y>A at position 3 in this peptide allowed the elimination of this epitope in clones IgG1-14 (Tables 4, 12).

10 **Antibody binding specificity analyses**

In early clinical trials, a humanized form of Mab005 has been reported to induce unusual toxicities in human patients, such as hemangiomas, which have not been seen with other anti-PD1 or anti-PD-L1 therapeutics. These reports suggested that humanized Mab005 might have unique, 'off-target' binding characteristics that are not found in other anti-PD1 antibodies. Hemangioma is a benign tumor formed by a collection of excess blood vessels, often developing in the skin, but they can also develop in the liver and other organs. We hypothesised that Mab005 might bind to unidentified and unpredictable receptors associated with vascular development or tissue differentiation. To examine this possibility, *in vitro* technologies (Retrogenix, Ltd.), which are based on using high-density arrays of cells expressing 4975 unique human membrane receptors, were used to screen for off-target binding specificities in IgG1-Mab005 (humanized). This receptor array binding screen identified that IgG1-Mab005 (humanized) exhibited strong binding to membrane-expressed PD1, but also had 4 potential off-target binding specificities: FZD5 (frizzled class receptor 5), ULBP2 (UL16 binding protein 2), EphB6 and KDR (also known as VEGFR2). Hemangiomas are known to develop spontaneously in patients with mutations in vascular biology-associated receptors such as VEGFR2 and are a reported side effect in the use of vascular-targeting antibodies for cancer therapy, such as ramucirumab (Anti-VEGFR2). In addition, FZD5 is a Wnt pathway signalling receptor associated with vascular development, so modulating the function of either of these receptors is likely to modulate vascular biology. In addition, ULBP2 is a known ligand for the natural killer cell activating receptor NKG2D, so binding to this protein during cancer therapy with an anti-PD1 is of unknown consequence.

To confirm these off-target binding events, the plasmids encoding for these receptors and controls were submitted for DNA sequencing. These analyses confirmed that the encoded proteins were indeed the correct sequences. The plasmid samples for control and potential target receptors were then re-arrayed onto new chips for repeat analyses in duplicate. The

effective induction of expression from all re-arrayed plasmids was confirmed via scanning the chips for ZS green, which is co-encoded on all expression plasmids as an internal control marker. This analysis showed clearly detectable ZS expression in all positions where plasmids were spotted (Fig. 10A). Further, identically-spotted slides were then used to re-probe transfected cells with IgG1-Mab005 (humanized) (Fig. 10B), isotype IgG1 (negative control, Fig. 10C), Rituximab (IgG1 positive control, Fig. 10D), and a chip where no primary antibody probe was applied (Fig. 10E). These analyses showed that IgG1-Mab005 (humanized) again demonstrated measurable binding over background (on both chips) on cells transfected with PD1, FZD5, ULBP2 and KDR, but no binding to Fc γ R1a (due to IgG1 null isotype) or any other spots, including EphB6 (Fig 10B). Rituximab demonstrated binding to CD20 and Fc γ R1a (due to IgG1 isotype) as expected, with no observable binding to PD1, FZD5, ULBP2, EphB6 and KDR (Fig. 10D). In the chips probed with isotype control antibody (Fig. 10C) and no primary antibody (Fig. 10E), only the expected control proteins showed any signal. This clean performance of the control chips confirmed that IgG1-Mab005 (humanized) binding signals on PD1, FZD5, ULBP2 and KDR were specific.

To investigate the origin of this off-target binding activity and whether or not it was retained in library-derived and designer IgGs generated in this study, further chip binding analyses were performed (Fig. 11A-T). As above, the re-arrayed plasmids were again checked for transfection quality and the induction of ZS green expression was confirmed (Fig. 11A). Additional control antibodies; Pembrolizumab analog (Fig. 11B) and Rituximab (Fig. 11C) both bound only their respective cognate targets and the isotype control IgG1 showed no binding (Fig. 11D). Primary analyses using mVH/mVL Mab005-IgG1 and IgG1-Mab005 (humanized) (Figs 11E and F, respectively), showed that the off-target binding signals previously only observed with IgG1-Mab005 (humanized) (Fig 10B) were also present in chips probed with the mVH/mVL Mab005-IgG1. This finding confirmed that the off-target binding reactivity of IgG1-Mab005 (humanized) derived directly from the original murine hybridoma and was not a result of polyreactivity being induced during the humanization process. The off-target binding activity of IgG1-Mab005 (humanized) was therefore housed directly in the CDRs, as it was retained when the murine CDRs were grafted onto human germline frameworks.

Lead library-derived and designer antibodies were then also used to probe this same chip set. Surprisingly, none of the antibodies IgG1-06D02 (Fig. 11G), IgG1-11G05 (Fig. 11H), IgG1-12H04 (Fig. 11I), IgG1-16H10 (Fig. 11J), IgG1-04 (Fig. 11K), IgG1-05 (Fig. 11L), IgG1-06 (Fig. 11M), IgG1-08 (Fig. 11N), IgG1-11 (Fig. 11O), IgG1-13 (Fig. 11P), IgG1-14

(Fig. 11Q), IgG1-15 (Fig. 11R), IgG1-12B07 (Fig. 11S) and IgG1-10 (Fig. 11T) showed any measurable binding to any other target than PD1, on any of the duplicate analyses. This finding demonstrated that the lead antibodies retained highly specific and potent binding to PD1 only, and that the off-target reactivity observed for IgG1-Mab005 (humanized) had
5 been ablated.

To finally confirm these findings with an orthogonal, high-sensitivity assay, the sequence-verified plasmids for KDR, ULBP2, FZD5, and ZS green only (negative control) were used to perform transient transfection of the human cell line HEK293. Transfected cells were
10 then stained using mVH/mVL Mab005-IgG1, IgG1-Mab005 (humanized), Pembrolizumab analog, isotype IgG1 and a subset of lead antibodies (IgG1-06D02, IgG1-12HO4, IgG1-05, IgG1-08). Each antibody was used in repeat staining at high (5 µg/ml) and moderate (1 µg/ml) concentration against both receptor-transfected and ZS green-transfected cells (to measure background binding). In staining of PD1-transfected cells at both 5 and 1 µg/ml
15 (Figs. 12A-12P), all tested antibodies other than the isotype control showed the expected strong, specific staining of PD1-transfected cells but not ZS green-transfected. In contrast, staining of FZ5 (Figs. 13A-13P), KDR (Figs. 14A-14P) and ULBP2-transfected cells (Figs. 15A-15P) fully correlated with the chip data from Figure 11, with only mVH/mVL Mab005-IgG1 and IgG1-Mab005 (humanized) exhibiting strong binding signal on all 3 targets at
20 both 5 and 1 µg/ml. None of the antibodies IgG1-06D02, IgG1-12HO4, IgG1-05, IgG1-08, Pembrolizumab analog, nor isotype IgG1 exhibited any measurable binding above background, at either concentration, to any of FZ5, KDR or ULBP2-transfected cells. In a further, high-sensitivity, ELISA assay, these antibodies were also found to bind human and cyno PD1 (Fig. 16 A,B) but not to bind to recombinant ectodomains of human or rhesus
25 VEGFR2 (Fig. 16 C, D), human FZD5 (Fig. 16E), or to BSA protein (Fig. 16F). These findings fully confirmed that the CDR sequences derived in the mutagenesis and reselection process had unexpectedly ablated the off-target binding of receptors KDR, FZD5 and ULBP2 that may be primary drivers of the clinical toxicities associated with IgG1-Mab005 (humanized).

30 Finally, an attempt was made to estimate the affinity of mVH/mVL Mab005-IgG1 and IgG1-Mab005 (humanized) for VEGFR2 via Biacore, using the conditions described above for PD1 affinity evaluation. Human and rhesus monomeric VEGFR2-his proteins were titrated against mVH/mVL Mab005-IgG1 and IgG1-Mab005 (humanized). Both antibodies again exhibited
35 binding to VEGFR2 recombinant protein, but binding signal was only observed at very high concentrations of soluble analyte (Fig. 17). As a result, complex curves with poor fit values ($\text{Chi}^2 > 6.6$) were all that could be generated and reliable KD values could not be accurately

derived. This indicated that the affinity of both antibodies for either human or rhesus VEGFR2 was low, likely in the μM range.

VEGFR2 activation analyses

- 5 To investigate whether the VEGFR2 reactivity in IgG1-Mab005 (humanized) was capable of activating the receptor, a human VEGFR2 reporter assay was used to examine induction of luciferase expression under control of the natural VEGF response element NFAT (Promega). In this assay, both IgG1-Mab005 (humanized) and mVH/mVL Mab005-IgG1 exhibited strong, concentration-dependent activation of VEGFR2 signalling (in the nM
- 10 range), with IgG1-Mab005 (humanized) being more potent than mVH/mVL Mab005-IgG1 (Fig. 18). The activation potency of IgG1-Mab005 (humanized) was, however, significantly lower than that of recombinant human VEGF-163 (Fig. 18). Importantly, each of clones MAB04, MAB08, 06D02 and 12H04 were also analysed in this assay and demonstrated no observable activation signal, even at concentrations as high as 1 μM . Indeed, the signals at
- 15 maximum concentration for the lead IgG clones were lower than the signals observed for the isotype control IgG1 (Fig. 18).

IgG stability analyses

- Differential Scanning Calorimetry (DSC) is used to measure thermal stability of proteins, as
- 20 an indicator of overall molecular structural stability. All IgG1 null proteins tested were fully compatible with DSC analysis, presenting comparable sample homogeneity and cooperativity (Fig. 19). The measured thermal transition midpoints (T_m) for each antibody are indicated in Table 13. All five IgGs demonstrated similar CH2 domain T_{m1} values from 72.2 $^{\circ}\text{C}$ to 72.4 $^{\circ}\text{C}$, indicating that all samples had high integrity. Unexpectedly, however,
- 25 all lead IgGs (IgG1-05, IgG1-08, IgG1-06D02 and IgG1-12H04) demonstrated extremely high stability in their Fab domains, with T_{m2} values ranging from 90.8 $^{\circ}\text{C}$ to 92.2 $^{\circ}\text{C}$ (Table 13). Mab005-IgG1 (humanized), in contrast, had a significantly lower stability Fab T_m value of 83.8 $^{\circ}\text{C}$ (Table 13). The significant increases in Fab stability for the lead antibodies over Mab005-IgG1 (humanized) were unexpected as the variable domain framework regions
- 30 and antibody constant regions of all antibodies were identical in sequence and all improvements were therefore mediated solely by differences in CDR sequences.

- Oxidation of exposed amino acid residues, such as tryptophan and methionine is a common degradation pathway for mAbs. Importantly, oxidation of critical side chains in the
- 35 CDRs of antibodies can also potentially impact on their biological activity, by causing a reduction in target binding affinity. Oxidation is a process that usually happens over time in the storage of proteins, so the standard laboratory method of analysing oxidative risk in

real time is to add an oxidative reagent to the protein. In this study, forced oxidation was applied to the IgGs by treating with 0.5 % H₂O₂ in PBS, for 2 hours at room temperature. As oxidation can alter overall hydrophobicity of an antibody, for example by increasing the polarity of the oxidised form, potential changes induced by forced oxidation were analysed
5 by Reverse Phase (RP) and Hydrophobic Interaction Chromatography (HIC) methods.

In RP analyses, no changes in retention times were observed for any of clones IgG1-05, IgG1-08, IgG1-06D02 and IgG1-12H04, in either intact (Table 14) or reduced (Table 15) IgG forms, suggesting that no significant oxidation of side chains had occurred. In contrast,
10 upon H₂O₂ treatment of Mab005-IgG1 (humanized), a decrease of approximately 0.6 min in the column retention time of intact IgG (Table 14) and 0.8 min for the reduced light chain (Table 15) was observed, suggesting oxidation of exposed amino acids was specifically occurring in the antibody light chain. RP analysis of tryptic peptide fingerprints before and after H₂O₂ treatment also showed that side chain oxidation changes were minimal for all
15 IgGs apart from for Mab005-IgG1 (humanized). For Mab005-IgG1 (humanized), the loss or reduction of 5 peptides after forced oxidation, was followed by appearance of 6 additional peptides (Fig. 20A). In contrast, clones IgG1-05, IgG1-08, IgG1-06D02 and IgG1-12H04 showed the concomitant loss of significantly fewer peptides. For example, IgG1-06D02 exhibited loss of just two tryptic peptides and appearance of two additional peaks after
20 H₂O₂ treatment (Fig. 20B).

H₂O₂ treatment also induced a decrease in the retention time on HIC for all 5 test articles, suggesting minor oxidation of exposed amino acids (Table 16). Again, the highest decrease in the retention on HIC (1.1 min) was observed for Mab005-IgG1 (humanized),
25 while for clones IgG1-05, IgG1-08, IgG1-06D02 and IgG1-12H04 the decrease is only 0.2-0.3 min. In total, these findings suggested that lead clones IgG1-05, IgG1-08, IgG1-06D02 and IgG1-12H04 had unexpectedly improved physical stability and resistance to oxidative challenge in comparison to Mab005-IgG1 (humanized).

30 The combined analyses outlined herein demonstrated that, surprisingly, deep sampling of both germline and non-germline amino acids in the CDRs of these antibodies allowed the simultaneous optimisation of target binding specificity, immunogenicity risk, potency, biophysical stability and chemical stability risks in multiple final molecules.

35 All documents, or portions of documents, cited herein, including but not limited to patents, patent applications, articles, books, and treatises, are hereby expressly incorporated by reference in their entirety for any purpose. In the event that one or more of the incorporated

documents or portions of documents define a term that contradicts that term's definition in the application, the definition that appears in this application controls. However, mention of any reference, article, publication, patent, patent publication, and patent application cited herein is not, and should not be taken as an acknowledgment, or any form of suggestion,
5 that they constitute valid prior art or form part of the common general knowledge in any country in the world.

Although the present invention has been described with reference to preferred or exemplary embodiments, those skilled in the art will recognize that various modifications and variations
10 to the same can be accomplished without departing from the spirit and scope of the present invention and that such modifications are clearly contemplated herein. No limitation with respect to the specific embodiments disclosed herein and set forth in the appended claims is intended nor should any be inferred.

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Table 1. Amino acid sequences murine anti-PD1 CDRs as defined here ("Unified" scheme) in comparison to alternative definitions.

Scheme	<u>HCDR1</u>	<u>HCDR2</u>	<u>HCDR3</u>	<u>LCDR1</u>	<u>LCDR2</u>	<u>LCDR3</u>
Unified	GFTFSSYMS (SEQ ID NO: 95)	VATISGGGANTYYPDSVKG (SEQ ID NO: 92)	QLYYFDY (SEQ ID NO: 106)	LASQTIGTWLT (SEQ ID NO: 110)	TATSLAD (SEQ ID NO: 114)	QQVYSIPWT (SEQ ID NO: 116)
Kabat	SYNMS (SEQ ID NO: 96)	TISGGGANTYYPDSVKG (SEQ ID NO: 100)	QLYYFDY (SEQ ID NO: 106)	LASQTIGTWLT (SEQ ID NO: 110)	TATSLAD (SEQ ID NO: 114)	QQVYSIPWT (SEQ ID NO: 116)
Chotia	GFTFSSY (SEQ ID NO: 97)	SGGGAN (SEQ ID NO: 101)	QLYYFDY (SEQ ID NO: 106)	LASQTIGTWLT (SEQ ID NO: 110)	TATSLAD (SEQ ID NO: 114)	QQVYSIPWT (SEQ ID NO: 116)
IMGT	GFTFSSYM (SEQ ID NO: 98)	ISGGGANT (SEQ ID NO: 102)	ARQLYYFDY (SEQ ID NO: 107)	QTIGTW (SEQ ID NO: 111)	TAT	QQVYSIPWT (SEQ ID NO: 116)
AHo	GFTFSSYMS (SEQ ID NO: 95)	ISGGGANTYYPDSVKG (SEQ ID NO: 103)	QLYYFD (SEQ ID NO: 108)	ASQTIGTW (SEQ ID NO: 112)	TATSLAD (SEQ ID NO: 114)	VYSIPW (SEQ ID NO: 117)
Abm	GFTFSSYMS (SEQ ID NO: 95)	TISGGGANTY (SEQ ID NO: 104)	QLYYFDY (SEQ ID NO: 106)	LASQTIGTWLT (SEQ ID NO: 110)	TATSLAD (SEQ ID NO: 114)	QQVYSIPWT (SEQ ID NO: 116)
Contact	SSYMS (SEQ ID NO: 99)	VATISGGGANTY (SEQ ID NO: 105)	ARQLYYFD (SEQ ID NO: 109)	GTWLTWY (SEQ ID NO: 113)	LLIYTATSLA (SEQ ID NO: 115)	QQVYSIPW (SEQ ID NO: 118)

Table 2. Amino acid sequence of MAB005 murine anti-PD1 v-domains (mVH/mVL) and human germline CDR grafts (VH1/VL1).

<u>V DOMAIN</u>	<u>Human germline¹</u>	<u>Amino acid sequence²</u>
PD1-mVH	n/a	EVMLVESGGGLVKPGGSLKLSCAAS GFTESSYMM SWVRQTPPEKRLIEW VATISGGGANTYYPDSVKG RFTISRDNAKNLIYLQMSLSRSED ~~TALYYCAK~~ QLYYFDY WGQGTTITLVSS (SEQ ID NO: 119)
PD1-VH1	IGHV3-7	EVQLVESGGGLVQPGGSLRLSCAAS GFTESSYMM SWVRQAPGKGLIEW VATISGGGANTYYPDSVKG RFTISRDNAKNSLYLQMSLSRAEDTAVYYCAK QLYYFDY WGQGTTITVSS (SEQ ID NO: 120)
PD1-mVL	n/a	DIQMTQSPASASLGEGVTTITC LASQITGTWL TWYQOKPGKSPOLLII TATSLAD GVPSRFSGSGGTFSEKISSIQAEDFVITYY QOVYSIPWT FGGGTKLEIK (SEQ ID NO: 121)
PD1-VL1	IGKV1-39	DIQMTQSPSSLASAVGDRVTITC LASQITGTWL TWYQOKPGKAPKLLII TATSLAD GVPSRFSGSGGTDFTLTISLQPEDFATYY QOVYSIPWT FGGGTKVEIK (SEQ ID NO: 122)

¹Human germline definitions used for grafting, based on IMGT system. ²CDR residues are in bold and underlined. As noted above, the “Unified” CDR definitions used in this manuscript are an expanded definition in comparison to the classical Kabat definition. Each sequence above shows the framework regions (FRs) and the CDRs in the following order: FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4.

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Table 3. Unique CDRs from Fab clones shown to bind human and cyno PD1 proteins.

LCDR1	LCDR2	LCDR3	HCDR1	HCDR2	HCDR3
RASQSIGSWLT (SEQ ID NO: 123)	AASLAD (SEQ ID NO: 128)	QOYSIPLT (SEQ ID NO: 132)	GTFSSYAMS (SEQ ID NO: 67)	VANISGGAEKYYPDVSKG (SEQ ID NO: 147)	LLYYEDY (SEQ ID NO: 165)
RASQSIGSYLN (SEQ ID NO: 78)	AASLAS (SEQ ID NO: 53)	QOYSIPWT (SEQ ID NO: 47)	GTFSSYDMS (SEQ ID NO: 135)	VANISGGAEKYYPDVSKG (SEQ ID NO: 147)	MLYYDDY (SEQ ID NO: 166)
RASQSIGTWLN (SEQ ID NO: 124)	AASLHS (SEQ ID NO: 54)	QOYSTPWT (SEQ ID NO: 43)	GTFSSYEMS (SEQ ID NO: 136)	VANISGGAEKYYVDVSKG (SEQ ID NO: 148)	NLYYFDY (SEQ ID NO: 167)
RASQSIGTYLN (SEQ ID NO: 76)	AASLQD (SEQ ID NO: 50)	QOVSIPLT (SEQ ID NO: 133)	GTFSSYFMS (SEQ ID NO: 137)	VANISGGAEKYYVDVSKG (SEQ ID NO: 148)	QGYFFDY (SEQ ID NO: 168)
RASQSIGSWLN (SEQ ID NO: 41)	AASLQS (SEQ ID NO: 42)	QOYSTPWT (SEQ ID NO: 134)	GTFSSYGMS (SEQ ID NO: 138)	VANISGGAEKYYPDVSKG (SEQ ID NO: 149)	QKYFFDY (SEQ ID NO: 169)
RASQSIGSWLT (SEQ ID NO: 125)	AATSLAS (SEQ ID NO: 69)		GTFSSYHMS (SEQ ID NO: 139)	VANISGGAEKYYVDVSKG (SEQ ID NO: 150)	QLHYFDY (SEQ ID NO: 170)
RASQSIGSYLN (SEQ ID NO: 74)	AATSLQD (SEQ ID NO: 129)		GTFSSYIMS (SEQ ID NO: 140)	VANISGGANKYYPDVSKG (SEQ ID NO: 151)	QLYAFDY (SEQ ID NO: 46)
RASQSIGTWLN (SEQ ID NO: 52)	AATSLQS (SEQ ID NO: 77)		GTFSSYIMS (SEQ ID NO: 38)	VANISGGANKYYVDVSKG (SEQ ID NO: 152)	QLYDFDY (SEQ ID NO: 171)
RASQSIGTWLT (SEQ ID NO: 126)	TASSLAD (SEQ ID NO: 73)		GTFSSYIMS (SEQ ID NO: 141)	VANISGGSEKYYPDVSKG (SEQ ID NO: 153)	QLYFFDY (SEQ ID NO: 45)
RASQSIGTYLN (SEQ ID NO: 127)	TASSLQD (SEQ ID NO: 71)		GTFSSYDMS (SEQ ID NO: 80)	VANISGGSEKYYVDVSKG (SEQ ID NO: 154)	QLYGFYD (SEQ ID NO: 40)
	TASSLQS (SEQ ID NO: 79)		GTFSSYQMS (SEQ ID NO: 142)	VANISGGSETYYPDVSKG (SEQ ID NO: 155)	QLYMFYD (SEQ ID NO: 172)
	TATSLAS (SEQ ID NO: 130)		GTFSSYDMS (SEQ ID NO: 75)	VANISGGSETYYVDVSKG (SEQ ID NO: 156)	QLYYADY (SEQ ID NO: 72)
	TATSLQD (SEQ ID NO: 131)		GTFSSYIMS (SEQ ID NO: 143)	VANISGGSNKYYPDVSKG (SEQ ID NO: 157)	QLYYEDY (SEQ ID NO: 173)
			GTFSSYDMS (SEQ ID NO: 144)	VANISGGSNKYYVDVSKG (SEQ ID NO: 158)	QLYYIDY (SEQ ID NO: 174)
			GTFSSYDMS (SEQ ID NO: 145)	VATISGGAEKYYPDVSKG (SEQ ID NO: 159)	QLYYKDY (SEQ ID NO: 175)
			GTFSSYDMS (SEQ ID NO: 146)	VATISGGAEKYYPDVSKG (SEQ ID NO: 159)	QLYYMDY (SEQ ID NO: 176)

				VATISGGGAETYYVDSVKG (SEQ ID NO: 49)	QLYYSDY (SEQ ID NO: 177)
				VATISGGGAETYYVDSVKG (SEQ ID NO: 49)	QLYYWDY (SEQ ID NO: 178)
				VATISGGGAETYYVDSVKG (SEQ ID NO: 160)	QMYFDY (SEQ ID NO: 179)
				VATISGGGAETYYVDSVKG (SEQ ID NO: 70)	QQYFDY (SEQ ID NO: 180)
				VATISGGGANKYYVDSVKG (SEQ ID NO: 164)	QSYFDY (SEQ ID NO: 181)
				VATISGGGANKYYVDSVKG (SEQ ID NO: 161)	QVYFDY (SEQ ID NO: 44)
				VATISGGGSEKYYVDSVKG (SEQ ID NO: 162)	TLYYFDY (SEQ ID NO: 182)
				VATISGGGSEKYYVDSVKG (SEQ ID NO: 39)	
				VATISGGGSETYYVDSVKG (SEQ ID NO: 162)	
				VATISGGGSETYYVDSVKG (SEQ ID NO: 48)	
				VATISGGGSNKYYVDSVKG (SEQ ID NO: 163)	
				VATISGGGSNKYYVDSVKG (SEQ ID NO: 68)	

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Table 4. CDR sequences of unique, library-derived and designer, PD1 antagonistic IgGs.

Clone name	LCDR1	LCDR2	LCDR3	HCDR1	HCDR2	HCDR3
06D02	RASQSISSWLN (SEQ ID NO: 41)	AASLQS (SEQ ID NO: 42)	QQSYSTPWT (SEQ ID NO: 47)	GFTFSSYLMS (SEQ ID NO: 38)	VATISGGSEIYYVDSVKG (SEQ ID NO: 48)	QLYGFDY (SEQ ID NO: 40)
08F04	RASQSISSWLN (SEQ ID NO: 52)	AATSLAS (SEQ ID NO: 69)	QQSYSTPWT (SEQ ID NO: 47)	GFTFSSYAMS (SEQ ID NO: 67)	VATISGGSNKYVDSVKG (SEQ ID NO: 68)	QLYGFDY (SEQ ID NO: 40)
11G05	RASQSISSWLN (SEQ ID NO: 41)	TASSLQD (SEQ ID NO: 71)	QQSYSTPWT (SEQ ID NO: 43)	GFTFSSYLMS (SEQ ID NO: 38)	VATISGGGAETYYVDSVKG (SEQ ID NO: 70)	QLYFFDY (SEQ ID NO: 45)
12E02	RASQSISSWLN (SEQ ID NO: 41)	TASSLAD (SEQ ID NO: 73)	QQSYSTPWT (SEQ ID NO: 47)	GFTFSSYAMS (SEQ ID NO: 67)	VATISGGSEKYVDSVKG (SEQ ID NO: 39)	QLYYADY (SEQ ID NO: 72)
12H04	RASQSISSWLN (SEQ ID NO: 41)	AASLQD (SEQ ID NO: 50)	QQSYSTPWT (SEQ ID NO: 43)	GFTFSSYLMS (SEQ ID NO: 38)	VATISGGGAETYYVDSVKG (SEQ ID NO: 49)	QLYGFDY (SEQ ID NO: 40)
12B07	RASQSISSWLN (SEQ ID NO: 52)	AASLAS (SEQ ID NO: 53)	QQSYSTPWT (SEQ ID NO: 43)	GFTFSSYLMS (SEQ ID NO: 38)	VATISGGSNKYVDSVKG (SEQ ID NO: 51)	QLYGFDY (SEQ ID NO: 40)
13G02	RASQSISSWLN (SEQ ID NO: 74)	AASLQS (SEQ ID NO: 42)	QQSYSTPWT (SEQ ID NO: 43)	GFTFSSYLMS (SEQ ID NO: 38)	VATISGGGAETYYVDSVKG (SEQ ID NO: 49)	QLYGFDY (SEQ ID NO: 40)
14C07	RASQSISSWLN (SEQ ID NO: 41)	AASLQS (SEQ ID NO: 42)	QQSYSTPWT (SEQ ID NO: 47)	GFTFSSYAMS (SEQ ID NO: 75)	VANISGGGAETYYVDSVKG (SEQ ID NO: 150)	QLYGFDY (SEQ ID NO: 40)
15C10	RASQSISSWLN (SEQ ID NO: 76)	AATSLQS (SEQ ID NO: 77)	QQSYSTPWT (SEQ ID NO: 47)	GFTFSSYLMS (SEQ ID NO: 38)	VATISGGSNKYVDSVKG (SEQ ID NO: 68)	QLYGFDY (SEQ ID NO: 40)
16C07	RASQSISSWLN (SEQ ID NO: 78)	TASSLQS (SEQ ID NO: 79)	QQSYSTPWT (SEQ ID NO: 47)	GFTFSSYLMS (SEQ ID NO: 38)	VATISGGGAETYYVDSVKG (SEQ ID NO: 49)	QLYAFDY (SEQ ID NO: 46)
16H10	RASQSISSWLN (SEQ ID NO: 41)	AASLHS (SEQ ID NO: 54)	QQSYSTPWT (SEQ ID NO: 43)	GFTFSSYLMS (SEQ ID NO: 38)	VATISGGSEKYVDSVKG (SEQ ID NO: 39)	QVYVFDY (SEQ ID NO: 44)
12H11	RASQSISSWLN (SEQ ID NO: 52)	AASLQY (SEQ ID NO: 82)	QQSYSTPWT (SEQ ID NO: 43)	GFTFSSYPMS (SEQ ID NO: 80)	VATISGGSEIYYVDSVKG (SEQ ID NO: 162)	QLYYYDY (SEQ ID NO: 81)
MH-AA-VH				GFTFSSYAMS (SEQ ID NO: 67)	VATISGGSEKYVDSVKG (SEQ ID NO: 39)	QLYYADY (SEQ ID NO: 72)
MH-LV-VH				GFTFSSYLMS (SEQ ID NO: 38)	VATISGGSEKYVDSVKG (SEQ ID NO: 39)	QVYVFDY (SEQ ID NO: 44)
MH-LG-VH				GFTFSSYLMS (SEQ ID NO: 38)	VATISGGSEKYVDSVKG (SEQ ID NO: 39)	QLYGFDY (SEQ ID NO: 40)
MH-LF-VH				GFTFSSYLMS (SEQ ID NO: 38)	VATISGGSEKYVDSVKG (SEQ ID NO: 39)	QLYFFDY (SEQ ID NO: 45)

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Table 5. ELISA EC50 values of unique, library-derived anti-PD1 Fabs.

<u>Clone name</u>	<u>huPD1-Fc EC50 (nM)</u>	<u>cyPD1-Fc EC50 (nM)</u>
Fab12B07	0.2374	3.116
Fab12H04	0.7078	1.402
Fab06D02	0.7434	1.013
Fab-Mab005	1.245	ND
Fab14C07	1.704	2.551
Fab16H10	2.055	1.105
Fab12H11	2.804	4.269
Fab16C07	3.703	4.874
Fab11G05	4.27	6.379
Fab13G02	4.626	5.445
Fab15C10	4.838	6.408
Fab08F04	6.861	14.2
Fab12E02	ND	ND

5 N.D. = Not Determined

Table 6. ELISA EC50 values of unique, library-derived anti-PD1 IgG1null sequences.

<u>Clone name</u>	<u>huPD-1-Fc EC50 (nM)</u>	<u>cyPD-1-Fc EC50 (nM)</u>
IgG1-06D02	0.065	0.1036
IgG1-08F04	0.096	0.126
IgG1-12H04	0.1116	0.1204
IgG1-Mab005	0.1141	0.1322
IgG1-12B07	0.117	0.0932
IgG1-15C10	0.1393	0.1826
IgG1-16H10	0.1543	0.2337
IgG1-13G02	0.1595	0.21
IgG1-11G05	0.1671	0.2451
IgG1-12E02	0.1741	0.1943
IgG1-12H11	0.1795	0.2088
IgG1-16C07	0.2445	0.2372
IgG1-14C07	N.D.	N.D.

N.D. = Not Determined.

Table 7. ELISA EC50 values of unique, designer anti-PD1 IgG1null sequences.

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<u>Clone name</u>	<u>huPD-1-Fc EC50 (nM)</u>	<u>cyPD-1-Fc EC50 (nM)</u>
IgG1-11	0.04921	0.04975
IgG1-07	0.08801	0.06449
IgG1-13	0.107	0.09845
IgG1-04	0.1083	0.138
IgG1-05	0.1213	0.1349
IgG1-14	0.1246	0.09586
IgG1-10	0.128	0.1692
IgG1-08	0.1339	0.07714
IgG1-Mab005	0.1368	0.1525
IgG1-09	0.1497	0.1253
IgG1-15	0.172	0.1695
IgG1-06	0.1752	0.1482
IgG1-01	0.1781	N.D.
IgG1-02	0.1832	N.D.
IgG1-12	0.2718	0.2704
IgG1-03	N.D.	N.D.

N.D. = Not Determined.

Table 8. Alphascreen IC50 values of anti-PD1 IgG1null sequences.

<u>Clone ID</u>	<u>CPS IC50 nM</u>
IgG1-11	1.413
IgG1-05	1.66
IgG1-Mab005	2.142
IgG1-08	2.361
IgG1-16H10	2.498
IgG1-12B07	2.557
IgG1-07	2.615
IgG1-06D02	2.626
IgG1-14	3.004
IgG1-04	3.24
IgG1-10	3.416
IgG1-13	3.615
IgG1-12H04	4.013
IgG1-11G05	4.225
IgG1-15C10	5.868
IgG1-13G02	7.169
IgG1-08F04	7.744
IgG1-16C07	9.267
IgG1-12H11	15.47
IgG1-15	ND
IgG1-12E02	ND
IgG1-14C07	ND
IgG1-01	ND
IgG1-02	ND
IgG1-03	ND
IgG1-06	ND
IgG1-09	ND
IgG1-12	ND

ND = not determined

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Table 9. Biacore affinity values for IgG1null binding to human and cyno monomeric PD1.

Clone name	Human PD1				Cyno PD1			
	ka (1/Ms)	kd (1/s)	Chi2	KD (nM)	ka (1/Ms)	kd (1/s)	Chi2	KD (nM)
IgG1-Pembro*	4.9E+05	2.4E-03	1.3	4.8	3.9E+05	6.8E-04	1.7	1.8
IgG1-Mab005	1.2E+05	4.7E-04	1.3	4.0	8.8E+04	2.8E-03	0.4	32.0
IgG1-16H10	1.6E+05	3.4E-04	1.2	2.1	9.3E+04	4.2E-04	0.3	4.5
IgG1-12H04	2.0E+05	3.2E-04	1.1	1.6	1.4E+05	6.4E-04	0.8	4.7
IgG1-06D02	2.0E+05	2.5E-04	3.0	1.3	1.3E+05	6.2E-04	0.4	4.9
IgG1-12B07	1.7E+05	2.5E-04	2.2	1.5	1.1E+05	1.6E-03	0.5	14.2
IgG1-11G05	1.2E+05	5.8E-04	0.4	4.7	5.5E+04	1.3E-03	0.3	24.0
IgG1-13G02	1.5E+05	2.0E-03	0.4	13.4	9.7E+04	6.4E-03	0.4	65.5
IgG1-16C07	1.5E+05	1.8E-03	0.3	12.0	9.6E+04	7.5E-03	0.4	77.7
IgG1-12H11	1.5E+05	2.8E-03	0.3	18.1	9.9E+04	1.0E-02	0.4	103.0
IgG1-15C10	1.4E+05	1.8E-03	1.3	12.3	9.6E+04	1.4E-02	0.4	144.0
IgG1-08F04	1.3E+05	2.5E-03	1.1	19.5	1.5E+05	2.6E-02	0.7	174.0
IgG1-08	1.9E+05	2.8E-04	3.4	1.47	1.5E+05	5.2E-04	1.6	3.4
IgG1-06	1.8E+05	2.9E-04	2.9	1.59	1.5E+05	5.2E-04	1.6	3.4
IgG1-04	1.5E+05	2.7E-04	1.6	1.87	1.2E+05	3.2E-04	0.8	2.7
IgG1-05	1.5E+05	2.9E-04	1.1	1.91	1.2E+05	3.3E-04	1.3	2.8
IgG1-13	1.8E+05	4.4E-04	1.9	2.45	1.4E+05	9.8E-04	1.2	6.8
IgG1-14	1.8E+05	4.6E-04	2.2	2.57	1.4E+05	9.7E-04	1.2	6.9
IgG1-10	1.5E+05	4.0E-04	1.2	2.7	1.2E+05	7.1E-04	1.4	6.1
IgG1-11	1.3E+05	4.0E-04	1.4	3.01	1.0E+05	7.3E-04	0.9	7.1
IgG1-015	1.6E+05	3.5E-03	1.6	21.5	1.2E+05	8.6E-03	0.9	69.9

*Pembrolizumab analog

Table 10. EC50 values for IgG1null binding to human and cyno PD1-CHO cells.

<u>Clone name</u>	<u>huPD-1-Fc EC50 (nM)</u>	<u>cynoPD-1-Fc EC50 (nM)</u>
IgG1-Mab005	2.319	6.532
IgG1-06D02	1.779	1.561
IgG1-12H04	1.952	2.172
IgG1-16H10	1.681	1.221
IgG1-05	1.656	2.521
IgG1-08	1.551	2.031
IgG1-11	0.978	0.861
IgG1-14	1.866	1.326
IgG1-15	2.133	ND

ND = Not Determined

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Table 11. EC50 values for IgG1null blockade of human PD1/PD-L1.

<u>Clone name</u>	<u>EC50 (ng/ml)</u>
IgG1-Mab005	285
IgG1-16H10	252
IgG1-12H04	188
IgG1-06D02	224
IgG1-08	127
IgG1-05	156
IgG1-14	212
IgG1-11	172

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Table 12. Human T cell epitope content in v-domains predicted by iTOPE™ and TCED™.

Clone Name	Germline epitopes	Low Affinity Foreign	High Affinity Foreign	TCED+
Mab005 VL	1	4	2	1
Mab005 VH	9	3	1	2
06D02 VL	5	0	0	0
06D02 VH	9	3	1	2
12H04 VL	4	1	0	0
12H04 VH	9	3	1	2
16H10 VL	3	0	2	0
16H10 VH	9	3	1	2
IgG1-05 VL	5	0	0	0
IgG1-05 VH	9	3	1	2
IgG1-08 VL	5	0	0	0
IgG1-08 VH	9	3	1	2
IgG1-11 VL	5	0	0	0
IgG1-11 VH	9	3	1	2
IgG1-14 VL	5	0	0	0
IgG1-14 VH	9	2	1	2

Table 13. IgG thermal stability analysis by DSC – TM values in Degrees C.

<u>IgG</u>	<u>TM1 (CH2)</u>	<u>TM2 (Fab)</u>
Mab005-IgG1 (hum)	72.2	83.8
IgG1-05	72.4	90.8
IgG1-08	72.4	91.1
IgG1-06D02	72.4	92.2
IgG1-12H04	72.4	91.5

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Table 14. Reverse Phase Chromatography retention time for intact IgGs - before (0 mins) and after 120 mins of forced oxidation with 0.5% H₂O₂

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<u>IgG</u>	<u>0 mins</u>	<u>120 mins</u>
Mab005-IgG1 (hum)	16.4	15.8
IgG1-05	15.5	15.5
IgG1-08	15.5	15.5
IgG1-06D02	15.8	15.8
IgG1-12H04	15.7	15.7

Table 15. Reverse Phase Chromatography retention time for light and heavy chains of reduced IgGs - before (0 mins) and after 120 mins of forced oxidation with 0.5% H₂O₂

IgG	<u>Light Chain</u>		<u>Heavy Chain</u>	
	<u>0 mins</u>	<u>120 mins</u>	<u>0 mins</u>	<u>120 mins</u>
Mab005-IgG1 (hum)	13.8	13	14.2	14.4
IgG1-05	11.8	11.9	14.3	14.3
IgG1-08	11.9	11.9	14.4	14.4
IgG1-06D02	12.2	12.2	14.5	14.5
IgG1-12H04	11.9	11.9	14.5	14.5

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Table 16. Hydrophobic Interaction Chromatography retention time for IgGs - before (0 mins) and after 120 mins of forced oxidation with 0.5% H₂O₂

<u>IgG</u>	<u>0 mins</u>	<u>120 mins</u>
Mab005-IgG1 (hum)	7.7	6.6
IgG1-05	7	6.7
IgG1-08	7.3	7
IgG1-06D02	7.8	7.6
IgG1-12H04	7.4	7.1

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Table 17. Examples of antibody variable region amino acid sequences.

- 15 Antibody IgG1-08 heavy chain variable (VH) region
EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYLSWVRQAPGKGLEWVATISGGGSEK
YYVDSVKGRFTISRDNKNSLYLQMNSLRAEDTAVYYCARQLYGFQDYWGQGTLTVSS
(SEQ ID NO: 1)
- 20 Antibody IgG1-08 light chain variable (VL) region
DIQMTQSPSSLSASVGDRVTITCRASQSISSWLNWYQQKPGKAPKLLIYAASSLQSGVPS
RFGSGSGTDFTLTISLQPEDFATYYCQQSYSTPWTFGQGTKVEIK (SEQ ID NO: 2)
- 25 Antibody IgG1-05 heavy chain variable (VH) region
EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYLSWVRQAPGKGLEWVATISGGGSEK
YYVDSVKGRFTISRDNKNSLYLQMNSLRAEDTAVYYCARQVYYFDYWQGTLTVSS
(SEQ ID NO: 3)
- 30 Antibody IgG1-05 light chain variable (VL) region
DIQMTQSPSSLSASVGDRVTITCRASQSISSWLNWYQQKPGKAPKLLIYAASSLQSGVPS
RFGSGSGTDFTLTISLQPEDFATYYCQQSYSTPWTFGQGTKVEIK (SEQ ID NO: 4)

- Antibody IgG1-11 heavy chain variable (VH) region
 EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYLMWVRQAPGKGLEWVATISGGGSEK
 YYVDSVKGRFTISRDNANKNSLYLQMNSLRAEDTAVYYCARQLYFFDYWGQGTLLTVSS
 (SEQ ID NO: 5)
- 5 Antibody IgG1-11 light chain variable (VL) region
 DIQMTQSPSSLSASVGDRVTITCRASQSISSWLNWYQQKPGKAPKLLIYAASSLQSGVPS
 RFGSGSGTDFTLTISLQPEDFATYYCQQSYSTPWTFGQGTKVEIK (SEQ ID NO: 6)
- 10 Antibody IgG1-14 heavy chain variable (VH) region
 EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYLMWVRQAPGKGLEWVATISGGGSEK
 YYVDSVKGRFTISRDNANKNSLYLQMNSLRAEDTAVYYCARQLYAFDYWGQGTLLTVSS
 (SEQ ID NO: 7)
- 15 Antibody IgG1-14 light chain variable (VL) region
 DIQMTQSPSSLSASVGDRVTITCRASQSISSWLNWYQQKPGKAPKLLIYAASSLQSGVPS
 RFGSGSGTDFTLTISLQPEDFATYYCQQSYSTPWTFGQGTKVEIK (SEQ ID NO: 8)
- 20 Antibody 06D02 heavy chain variable (VH) region
 EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYLMWVRQAPGKGLEWVATISGGGSET
 YYVDSVKGRFTISRDNANKNSLYLQMNSLRAEDTAVYYCARQLYGFYWGQGTLLTVSS
 (SEQ ID NO: 9)
- 25 Antibody 06D02 light chain variable (VL) region
 DIQMTQSPSSLSASVGDRVTITCRASQSISSWLNWYQQKPGKAPKLLIYAASSLQSGVPS
 RFGSGSGTDFTLTISLQPEDFATYYCQQSYSTPWTFGGGTKVEIK (SEQ ID NO: 10)
- 30 Antibody 12H04 heavy chain variable (VH) region
 EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYLMWVRQAPGKGLEWVATISGGGAEK
 YYVDSVKGRFTISRDNANKNSLYLQMNSLRAEDTAVYYCARQLYGFYWGQGTLLTVSS
 (SEQ ID NO: 11)
- 35 Antibody 12H04 light chain variable (VL) region
 DIQMTQSPSSLSASVGDRVTITCRASQSISSWLNWYQQKPGKAPKLLIYAASSLQDGVPS
 RFGSGSGTDFTLTISLQPEDFATYYCQQSYSTPWTFGGGTKVEIK (SEQ ID NO: 12)

Table 18. Examples of antibody Fc region amino acid sequences.

- 40 Human IgG4 wild type
 ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTWNSGALTSKVHTFPFAVLQSS
 GLYSLSSVTVPSSSLGKTYTCNVDHKPSNTKVDKRVESEKYGPPCPSCPAPEFLGGPSV
 FLFPPKPKDTLMISRTPEVTCVVVDVSQED PEVQFNWYVDGVEVHNAKTKPREEQFNST
 YRVVSVLTVHLQDNLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMT
 45 KNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSRLTVDKSRWQ
 EGNVFSCSVMHEALHNHYTQKS LSLSLGK (SEQ ID NO: 13)
- Human IgG4(S228P)
 ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTWNSGALTSKVHTFPFAVLQSS
 50 GLYSLSSVTVPSSSLGKTYTCNVDHKPSNTKVDKRVESEKYGPPCPSCPAPEFLGGPSV
 FLFPPKPKDTLMISRTPEVTCVVVDVSQED PEVQFNWYVDGVEVHNAKTKPREEQFNST
 YRVVSVLTVHLQDNLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMT
 KNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSRLTVDKSRWQ
 55 EGNVFSCSVMHEALHNHYTQKS LSLSLGK (SEQ ID NO: 14)

- Human IgG1 wild type
 ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS
 GLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGG
 PSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYN
 5 STYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDEL
 TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRW
 QQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 15)
- Human IgG1-3M
 10 ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS
 GLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGA
 PSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYN
 STYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDEL
 15 TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRW
 QQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 16)
- Human IgG2 wild type
 ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS
 GLYSLSSVTVPSNFGTQTYTCNVDHKPSNTKVDKTVKCCVECPPCPAPPVAGPSV
 20 FLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTF
 RVVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTK
 NQVSLTCLVKGFYPSDISVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQ
 GNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 17)
- Human IgG1 wild type "REEM" allotype
 25 ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS
 GLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGG
 PSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYN
 STYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREE
 30 MTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR
 WQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 18)
- Human IgG1-3M "REEM" allotype
 35 ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS
 GLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGA
 PSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYN
 STYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREE
 MKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRW
 40 QQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 19)
- Table 19.** Examples of membrane protein amino acid sequences.
- Human PD1 sequence
 45 MQIPQAPWPVVWAVLQLGWRPGWFLDSPDRPWNPTFSPALLVTEGDNATFTCSFSN
 TSESVLNWYRMSPSNQTDKLAAPEDRSQPGQDCRFRVTQLPNGRDFHMSVVRARRN
 DSGTYLCAISLAPKAQIKESLRAELRVTERRAEVPTAHPSPSPRPAGQFQTLVGVVGG
 LLGSLVLLVWVLAVICSRAARGTIGARRTGQPLKEDPSAVPVFSVDYGELDFQWREKTPE
 PPVPCVPEQTEYATIVFPSGMGTSSPARRGSADGPRSAQPLRPEDGHCSWPL (SEQ ID
 NO: 20)
- Cynomolgus monkey PD1 sequence
 50 MQIPQAPWPVVWAVLQLGWRPGWFLESPDRPWNAPTFSALLVTEGDNATFTCSFSN
 ASESFLNWYRMSPSNQTDKLAAPEDRSQPGQDCRFRVTRLNNGRDFHMSVVRARRN
 DSGTYLCAISLAPKAQIKESLRAELRVTERRAEVPTAHPSPSPRPAGQFQALVGVVGG
 55 LLGSLVLLVWVLAVICSRAAQGTIEARRTGQPLKEDPSAVPVFSVDYGELDFQWREKTPE

PPAPCVPEQTEYATIVFPSGLGTSSPARRGSADGPRSPRPLRPEDGHCSWPL (SEQ ID NO: 21)

Human KDR (VEGFR2) sequence

5 MQSKVLLAVALWLCVETRAASVGLPSVSLDLPRLSIQKDILTIKANTTLQITCRGQRDLDWL
 WPNNQSGSEQRVEVTECSDGLFCKTLTIKPVIGNDTGAYKCFYRETDLASVIYVYVQDYR
 SPFIASVSDQHGVVYITENKNKTVIPCLGSISNLNVSLCARYPEKRFVPDGNRISWDSKK
 GFTIPSYMISYAGMVFCEAKINDESYQSIMYIVVVVGYRIYDVVLSPSHGIELSVGEKLVNLC
 TARTELVNGIDFNWEYPSSKHQHKLVNRDLKTQSGSEMKKFLSTLTIDGVTRSDQGLYT
 10 CAASSGLMTKKNSTFVRVHEKPFVAFSGMESLVEATVGERVRIPAKYLGYPPEIKWYK
 NGIPLESNHTIKAGHVLTIMEVSEKDTGNYTVILTNPISKEKQSHVVSLVVYVPPQIGESLI
 SPVDSYQYGTQTTLCTVYAIPPPHHHWYVWQLEEECANEPSQAVSVTNYPYCEEWRVS
 EDFQGGNKIEVNKNQFALIEGKNKTVSTLVIQAANVSALYKCEAVNKVGRGERVISFHVTR
 GPEITLQPDMLQPTTEQESVSLWCTADRSTFENLTWYKLGQPLPIHVGELPTPVCKNLDL
 15 WKLNATMFSNSTNDILIMELKNASLQDQGDYVCLAQDRKTKKRHCVRQLTVLERVAPMIT
 GNLENQTTSIGESIEVSCTASGNPPQIMWFKDNETLVEDSGIVLKDGNRNLTIIRVRKED
 EGLYTCQACSVLGCACVEAFFIIEGAQEKTNLEIILVGTAVIAMFFWLLLVIILRTVKRANGG
 ELKTGYLSIVMDPDELPLDEHCERLPYDASKWEFPRDRLKLGKPLGRGAFGQVIEADAFGI
 DKTATCRTVAVKMLKEGATHSEHRALMSELKILIHIGHHLNVVNLGACTKPGGPLMVIVEF
 20 CKFGNLSTYLRSKRNEFVPYKTKGARFRQGKDYVGAIPVDLKRRLDSITSSQSSASSGFV
 EEKSLSDVEEEEAPEDLYKDFTLLEHLICYSFQVAKGMEFLASRKCIHRDLAARNILLSEKN
 VVKICDFGLARDIYKDPDYVRKGDARLPLKWMAPETIFDRVYTIQSDVWSFGVLLWEIFSL
 GASPYPGVKIDEEFCRRLKEGTRMRAPDYTTPEMYQTMDCWHGEPSSQRPFTFSELVEHL
 GNLLQANAQQDGKDYIVLPISETLSMEEDSGLSLPTSPVSCMEEEEVCDPKFHYDNTAGIS
 25 QYLQNSKRKSRPVSVKTFEDIPLEEPEVKVIPDDNQDTSGMVLASEELKTLEDRTKLSPSF
 GGMVPSKSRESVASEGSNQTSQYQSGYHSDDTDTTVYSSEEAECLKIEIGVQTGSTAQI
 LQPDSGTTLSSPPV (SEQ ID NO: 22)

Rhesus KDR (VEGFR2) sequence

30 MASKVLLAVALWLCVETRAASVGLPSVSLDLPRLSIQKDILTIKANTTLQITCRGQRDLDWL
 WPNNQSGSEQRVEVTECSDGLFCKTLTIKPVIGNDTGAYKCFYRETDLASVIYVYVQDYR
 SPFIASVSDQHGVVYITENKNKTVIPCLGSISNLNVSLCARYPEKRFVPDGNRISWDSKK
 GFTIPSYMISYAGMVFCEAKINDESYQSIMYIVVVVGYRIYDVVLSPSHGVELSVGEKLVNLC
 CTARTELVNGIDFNWEYPSSKHQHKLVNRDLKTQSGSEMKKFLSTLTIDGVTRSDQGLYT
 35 TCAASSGLMTKKNSTFVRVHEKPFVAFSGMESLVEATVGERVRIPVKYLGYPPEIKWYK
 KNGIPLESNHTVKGHVLTIMEVSEKDTGNYTVILTNPISKEKQSHVVSLVVYVPPQIGESK
 LISPVDSYQYGTQTTLCTVYAIPPPHHHWYVWQLEEECPNEPSQAVSVTNYPYCEEWRVS
 VEDFQGGNKIEVNKNQFALIEGKNKTVSTLVIQAANVSALYKCEAVNKVGRGERVISFHVTR
 RGPEITLQPDMLQPTTEQESVSLWCTADKSTFENLTWYKLGQPLPVHVGELPTPVCKNLDT
 40 LWKLNATMFSNSTNDILIMELKNASLQDQGDYVCLAQDRKTKKRHCVRQLTVLERVAPMI
 TGNLENQTTSIGETIEVSCTASGNPPQIMWFKDNETLVEDSGIVLKDGNRNLTIIRVRKE
 DEGLYTCQACSVLGCACVEAFFIIEGAQEKTNLEIILVGTAVIAMFFWLLLVIILRTVKRANG
 GELKTGYLSIVMDPDELPLDEHCERLPYDASKWEFPRDRLKLGKPLGRGAFGQVIEADAF
 GIDKTATCRTVAVKMLKEGATHSEHRALMSELKILIHIGHHLNVVNLGACTKPGGPLMVIV
 45 EFCKFGNLSTYLRSKRNEFVPYKTKGARFRQGKDYVGAIPVDLKRRLDSITSSQSSASSG
 FVEEKSLSDVEEEEAPEDLYKDFTLLEHLICYSFQVAKGMEFLASRKCIHRDLAARNILLSE
 KNVVKICDFGLARDIYKDPDYVRKGDARLPLKWMAPETIFDRVYTIQSDVWSFGVLLWEIF
 SLGASPYPGVKIDEEFCRRLKEGTRMRAPDYTTPEMYQTMDCWHGEPSSQRPFTFSELVE
 HLGNLQANAQQDGKDYIVLPISETLSMEEDSGLSLPTSPVSCMEEEEVCDPKFHYDNTA
 50 GISQYLQNSKRKSRPVSVKTFEDIPLEEPEVKVIPDDNQDTSGMVLASEELKTLEDRTKLA
 PSFGMVSSKSRESVASEGSNQTSQYQSGYHSDDTDTTVYSSEEAECLKIEIGVQTGST
 AQILQPDSGTTLSSPPV (SEQ ID NO: 23)

55

Human FZD5 sequence

MARPDPSAPPSLLLLLLAQLVGRAAAASKAPVCQEITVPMCRGIGYNLTHMPNQFNHDTQ
DEAGLEVHQFWPLVEIQCSPLRFFLCSTYPICLPDYHKPLPPCRSV CERAKAGCSPLM
RQYGFAWPERMSCDRLPVLGRDAEVL CMDYNRSEATTAPPRPFPKPTLPGPPGAPAS
5 GGECFAGGPFVCKCREPFVPILKESHPLYNKVRTGQVPNCAVPCYQPSFSADERTFATF
WIGLWSVLCFISTSTTVATFLIDMERFRYPERPIIFLSACYLCVSLGFLVRLVVGHASVACSR
EHNHIIHYETTGPALCTIVFLLVYFFGMASSIWWVILSLTWFLAAGMKWGNEAIAQYQYFH
LAWLIPSVKSITALALSSVDGDPVAGICYVGNQNLNSLRGFVLGPLVLYLLVGTFLLAGF
VSLFRIRSVIKQGGTKTDKLEKLMIRIGIFTLLYTPASIVVACYLYEQHYRESWEAALTCAC
10 PGHDTGQPRAKPEYWVLMKYFMCLVVGITSGVWIWSGKTVESWRRFTSRCCCRPRRG
HKSGGAMAAGDYPEASAALTGRTGPPGPAATYHKQVSLSHV (SEQ ID NO: 24)

Human ULBP2 sequence

MAAAAATKILLCLPLLLLLSGWSRAGRADPHSLCYDITVIPKFRPGPRWCAVQGGQVDEKTF
15 LHYDCGNKTVTPVSPLGKKLVTTAWKAQNPVLREVVDILTEQLRDIQLENYTPKEPLTLQ
ARMSCEQKAEGHSSGSWQFSFDGQIFLLFDSEKRMWTTVHPGARKMKEKWENDKVVA
MSFHYFSMGDCIGWLEDFLMGMDSTLEPSAGAPLAMSSGTTQLRATATTLILCCLLILPC
FILPGI (SEQ ID NO: 25)