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(54) Title: PD1 BINDING AGENTS

(57) Abstract: Provided herein are antibody molecules that bind specifically to Programmed cell death (PD1) and related nucleic acid molecules, vectors and host cells. Also provided herein are medical uses of such antibody molecules.

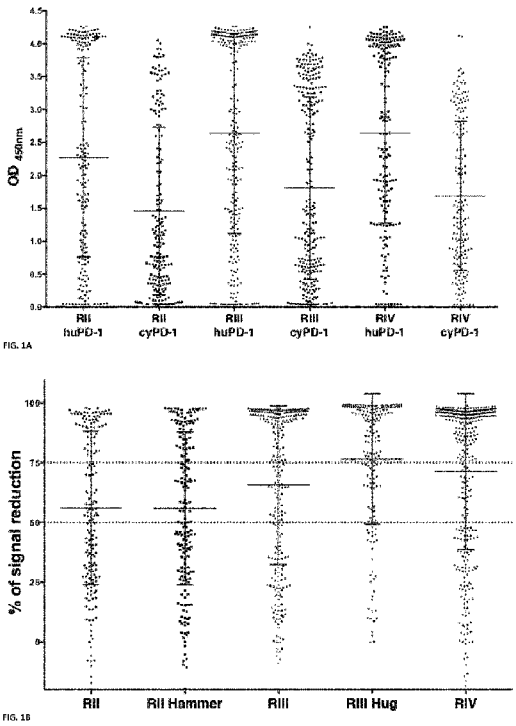

FIG. 1A

FIG. 1B

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PD1 BINDING AGENTS

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of GB Patent Application No. 1813405.6, filed on August 16, 2018, and GB Patent Application No. 1803746.5, 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: ULNL_001_00WO_SeqList_ST25.txt, date recorded: March 7, 2019, file size 153,562 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 clinical evidence suggests that blocking PD1/PD-L1 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. Antibodies such as PD1 inhibitors that target receptors on immune cells, and whose pharmacological function is to stimulate immune responses, are at heightened risk of provoking anti-drug antibody responses. These anti-drug antibody responses in the patient can reduce drug half-life, potency and safety during clinical use. 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 subjected 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 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 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 can have dramatic negative effects on all of these desired molecular properties.

WO2015/035606 A1 describes an antagonistic murine anti-PD1 IgG molecule termed "Mu317", and also the preparation of humanized forms of Mu317 (designated hu317). Those humanized forms of Mu317 were produced using classical humanization techniques, i.e. by grafting 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 Mu317 murine residues. For reasons noted above, such humanized forms of Mu317 described in WO2015/035606 A1 are not ideal.

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 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 or a conservative substitution of T (for example S)-F or a conservative substitution of F (for example L)-S or a

conservative substitution of S (for example T)-S-Y-G or any amino acid (for example W)-M or a conservative substitution of M (for example V)-S or any amino acid (for example H) (SEQ ID NO:20);

an HCDR2 having amino acids in sequence in the following order: V or a conservative substitution of V (for example L)-A or a conservative substitution of A (for example G)-V/N-I-W/K-Q/A-D/G-G-S-T/E-N/K/S-Y-N/V-D-S-V-K-G (SEQ ID NO:21); and

an HCDR3 having amino acids in sequence in the following order: A or any amino acid (for example D/E/G/H/I/K/L/H/Q/S/V/W)-Y or a conservative substitution of Y (for example F/W)-G-N or any amino acid (for example A/E/F/G/H/I/Q/R/S/T/V/W/Y)-Y or a conservative substitution of Y (for example V/W)-W or any amino acid (for example F/H/M/P/Q/S/V)-Y-I or any amino acid (for example L/M/S/T/V/Y)-D or a conservative substitution of D (for example E)-V or any amino acid (for example A/E/F/G/H/I/K/L/M/R/S/T/W/Y) (SEQ ID NO:22).

In aspects of the invention, the HCDR1 of the antibody molecule or antigen-binding portion may exclude the sequences GFSLTSYGVH (SEQ ID NO:23; Mu317 murine/humanized antibody HCDR1 disclosed in WO2015/035606 A1; US2015/079109A1), VIWAGGSTNYNSALMS (SEQ ID NO:24; Mu317 murine/humanized antibody HCDR2 disclosed in WO2015/035606 A1; US2015/079109A1), and/or the HCDR3 of the antibody molecule or antigen-binding portion may exclude the sequence AYGNYWYIDV (SEQ ID NO:25; Mu317 murine/humanized antibody HCDR3 disclosed in WO2015/035606 A1; US2015/079109A1).

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: K-S-S-Q/E-S-V-S or a conservative substitution of S (such as G/T)-N-D-L or a conservative substitution of L (such as V)-A (SEQ ID NO:26);

an LCDR2 having amino acids in sequence in the following order: Y-A-F or any amino acid (for example S)-H or any amino acid (for example P/T)-R-F-S or a conservative substitution of S (such as A/T) (SEQ ID NO:27); and

an LCDR3 having amino acids in sequence in the following order: Q or any amino acid (for example H)-Q-A or any amino acid (for example S)-Y-S-T or any amino acid (for example, N/S)-P-Y or any amino acid (for example W)-T (SEQ ID NO:28).

In aspects of the invention, the LCDR1 of the antibody molecule or antigen-binding portion may exclude the sequence KASQSVSNDVA / KSSQEVSNDVA (SEQ ID NO:29 / SEQ ID NO:30; Mu317 murine/humanized antibody LCDR1 disclosed in WO2015/035606 A1;

US2015/079109A1), and/or the LCDR2 of the antibody molecule or antigen-binding portion may exclude the sequence YAFHRFT (SEQ ID NO:31; Mu317 murine/humanized antibody LCDR2 disclosed in WO2015/035606 A1; US2015/079109A1) and/or the LCDR3 of the antibody molecule or antigen-binding portion may exclude the sequence HQAYSSPYT (SEQ ID NO:32; Mu317 murine/humanized antibody LCDR3 disclosed in WO2015/035606 A1; US2015/079109A1).

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-X_1-X_2-X_3-S-Y-X_4-X_5-X_6, wherein X_1 is T or a conservative substitution of T, X_2 is F or a conservative substitution of F, X_3 is S or a conservative substitution of S, X_4 is G or any other amino acid, X_5 is M or a conservative substitution of M, and X_6 is S or any other amino acid (SEQ ID NO:20);

(b) the HCDR2 comprises the amino acid sequence X_1-X_2-X_3-I-X_4-X_5-X_6-G-S-X_7-X_8-Y-X_9-D-S-V-K-G, wherein X_1 is V or a conservative substitution of V, X_2 is A or a conservative substitution of A, X_3 is V or N, X_4 is W or K, X_5 is Q or A, X_6 is D or G, X_7 is T or E, X_8 is N, K, or S, and X_9 is N or V (SEQ ID NO:21);

(c) the HCDR3 comprises the amino acid sequence X_1-X_2-G-X_3-X_4-X_5-Y-X_6-X_7-X_8, wherein X_1 is A or any other amino acid, X_2 is Y or a conservative substitution of Y, X_3 is N or any other amino acid, X_4 is Y or a conservative substitution of Y, X_5 is W or any other amino acid, X_6 is I or any other amino acid, X_7 is D or a conservative substitution of D, and X_8 is V or any other amino acid (SEQ ID NO:22);

(d) the LCDR1 comprises the amino acid sequence K-S-S-X_1-S-V-X_2-N-D-X_3-A, wherein X_1 is Q or E, X_2 is S or a conservative substitution of S, and X_3 is L or a conservative substitution of L(SEQ ID NO:26);

(e) the LCDR2 comprises the amino acid sequence Y-A-X_1-X_2-R-F-X_3, wherein X_1 is F or any other amino acid, X_2 is H or any other amino acid, and X_3 is S or a conservative substitution of S (SEQ ID NO:27); and

(f) the LCDR3 comprises the amino acid sequence X_1-Q-X_2-Y-S-X_3-P-X_4-T, wherein X_1 is Q or any other amino acid, X_2 is A or any other amino acid, X_3 is T or any other amino acid, and X_4 is Y or any other amino acid (SEQ ID NO:28).

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 GFTFSSYGMS (SEQ ID NO:33), HCDR2 of VANIWQDGSTKYVDSVKG (SEQ ID NO:34), and HCDR3 of AYGNYWYIDR (SEQ ID NO:35); and the VL region amino acid sequence comprises LCDR1 of KSSQSVTNDVA (SEQ ID NO:36), LCDR2 of YAYHRFT (SEQ ID NO:37), and LCDR3 of HQAYSSPYT (SEQ ID NO:38);

(b) the VH region amino acid sequence comprises HCDR1 of GFSLSSYGMS (SEQ ID NO:39), HCDR2 of VAVIWQDGSTNYVDSVKG (SEQ ID NO:40), and HCDR3 of AYGNYWYIDR (SEQ ID NO:35); and the VL region amino acid sequence comprises LCDR1 of KSSQSVTNDVA (SEQ ID NO:36), LCDR2 of YAYHRFT (SEQ ID NO:37), and LCDR3 of HQAYSSPYT (SEQ ID NO:38);

(c) the VH region amino acid sequence comprises HCDR1 of GFTFSSYGMS (SEQ ID NO:33), HCDR2 of VANIWQDGSEKYVDSVKG (SEQ ID NO:41), and HCDR3 of AYGNYWYIDR (SEQ ID NO:35); and the VL region amino acid sequence comprises LCDR1 of KSSQSVTNDVA (SEQ ID NO:36), LCDR2 of YAYHRFT (SEQ ID NO:37), and LCDR3 of HQAYSSPYT (SEQ ID NO:38);

(d) the VH region amino acid sequence comprises HCDR1 of GFSFSSYGMH (SEQ ID NO:42), HCDR2 of VANIWQDGSTNYVDSVKG (SEQ ID NO:43), and HCDR3 of AYGNYWYIDR (SEQ ID NO:35); and the VL region amino acid sequence comprises LCDR1 of KSSQSVSNDVA (SEQ ID NO:44), LCDR2 of YASHRFT (SEQ ID NO:45), and LCDR3 of HQAYSTPYT (SEQ ID NO:46);

(e) the VH region amino acid sequence comprises HCDR1 of GFSFTSYGMS (SEQ ID NO:47), HCDR2 of LANIWQDGSTNYVDSVKG (SEQ ID NO:48), and HCDR3 of AYGNYWYIDR (SEQ ID NO:35); and the VL region amino acid sequence comprises LCDR1 of KSSQSVSNDVA (SEQ ID NO:44), LCDR2 of YASHRFT (SEQ ID NO:45), and LCDR3 of HQAYSTPYT (SEQ ID NO:46);

(f) the VH region amino acid sequence comprises HCDR1 of GFTFSSYGVH (SEQ ID NO:49), HCDR2 of VGVIWQDGSENYVDSVKG (SEQ ID NO:50), and HCDR3 of AYGNYWYIDR (SEQ ID NO:35); and the VL region amino acid sequence comprises LCDR1 of KSSQSVSNDLA (SEQ ID NO:51), LCDR2 of YASHRFT (SEQ ID NO:45), and LCDR3 of QQAYSTPYT (SEQ ID NO:52);

(g) the VH region amino acid sequence comprises HCDR1 of GFSFSSYGMH (SEQ ID NO:42), HCDR2 of VANIWQDGSTNYVDSVKG (SEQ ID NO:43), and HCDR3 of AYGNYWYIDR (SEQ ID NO:35); and the VL region amino acid sequence comprises LCDR1 of KSSQSVSNDVA (SEQ ID NO:44), LCDR2 of YASHRFT (SEQ ID NO:45), and LCDR3 of HQAYSTPYT (SEQ ID NO:46);

(h) the VH region amino acid sequence comprises HCDR1 of GFSFSSYGMH (SEQ ID NO:42), HCDR2 of LANIWQDGSENYVDSVKG (SEQ ID NO:53), and HCDR3 of

AYGNYWYIDR (SEQ ID NO:35); and the VL region amino acid sequence comprises LCDR1 of KSSQSVSNDVA (SEQ ID NO:44), LCDR2 of YASHRFT (SEQ ID NO:45), and LCDR3 of QQAYSTPYT (SEQ ID NO:52);

(i) the VH region amino acid sequence comprises HCDR1 of GFTLSSYGMH (SEQ ID NO: 54), HCDR2 of LAVIWADGSTNYVDSVKG (SEQ ID NO:55), and HCDR3 of AYGNYMYIDV (SEQ ID NO:56); and the VL region amino acid sequence comprises LCDR1 of KSSQSVSNDVA (SEQ ID NO:44), LCDR2 of YASHRFT (SEQ ID NO:45), and LCDR3 of HQAYSTPYT (SEQ ID NO:46);

(j) the VH region amino acid sequence comprises HCDR1 of GFTFSSYGMH (SEQ ID NO:57), HCDR2 of VGVIKQDGSENYVDSVKG (SEQ ID NO:58), and HCDR3 of AYGNYWYIDR (SEQ ID NO:35); and the VL region amino acid sequence comprises LCDR1 of KSSQSVSNDVA (SEQ ID NO:44), LCDR2 of YASHRFT (SEQ ID NO:45), and LCDR3 of HQAYSTPYT (SEQ ID NO:46); or

(k) the VH region amino acid sequence comprises HCDR1 of GFSFTSYGMH (SEQ ID NO:59), HCDR2 of VGVIWQDGSTNYVDSVKG (SEQ ID NO:60), and HCDR3 of GYGNYWYIDV (SEQ ID NO:61); and the VL region amino acid sequence comprises LCDR1 of KSSQSVSNDLA (SEQ ID NO:51), LCDR2 of YASHRFT (SEQ ID NO:45), and LCDR3 of QQAYSTPYT (SEQ ID NO:52).

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: 33, SEQ ID NO: 39, SEQ ID NO: 42, SEQ ID NO: 47, SEQ ID NO: 49, SEQ ID NO: 54, SEQ ID NO: 57 or SEQ ID NO: 59;

(b) HCDR2 of SEQ ID NO: 34, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 43, SEQ ID NO: 48, SEQ ID NO: 50, SEQ ID NO: 53, SEQ ID NO: 55, SEQ ID NO: 58 or SEQ ID NO: 60; and

(c) HCDR3 of SEQ ID NO: 35, SEQ ID NO: 56 or SEQ ID NO: 61; and

the VL region amino acid sequence comprises:

(a') LCDR1 of SEQ ID NO: 36, SEQ ID NO: 44 or SEQ ID NO: 51;

(b') LCDR2 of SEQ ID NO: 37 or SEQ ID NO: 45; and

(c') LCDR3 of SEQ ID NO: 38, SEQ ID NO: 46 or SEQ ID NO: 52.

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 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;

(c) the VH region amino acid sequence comprises SEQ ID NO:5 and the VL region amino acid sequence comprises SEQ ID NO:6; or

(d) the VH region amino acid sequence comprises SEQ ID NO:7 and the VL region amino acid sequence comprises SEQ ID NO:8.

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.

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.

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

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.

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.

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.

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 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 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 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 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.

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 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.

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. 1A – FIG. 1B. Direct binding ELISA of library-derived anti-PD1 Fabs against human and cyno PD1-Fc proteins and HTRF competition assay. 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 RI-RIV. After each round of selection, library-derived clones were screened as periplasmically-expressed Fab proteins in ELISA (FIG. 1A) against both human (huPD1) and cyno (cyPD1). Fab samples were also tested in parallel for their ability to compete for the binding epitope of mu317 on human PD1 by HTRF (FIG. 1B). Mean ± SD values in each round are represented in grey bars.

FIG. 2A – FIG. 2B. 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 712 unique scFv clones is shown for V_L (SEQ ID NOs: 409-411) (FIG. 2A) and V_H (SEQ ID NOs: 412-414)(FIG. 2B) 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 (IGKV4-1 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. 3A – FIG. 3B. Direct titration ELISA for library-derived IgG1null clones binding to human and cyno PD1-Fc proteins. Mu317 and library-derived IgGs were titrated (in nM) in a direct binding ELISA against human (FIG. 3A) and cyno (FIG. 3B) PD1-Fc proteins.

FIG. 4A – FIG. 4B. Flow cytometric binding to human and cyno PD1+ CHO cells. Mu317, lead library-derived and designer IgGs were examined for specific binding on human (FIG. 4A) and cyno (FIG. 4B) CHO cells expressing human PD1. Concentration-dependent binding was observed against human PD1 for all clones, with no binding being observed for Isotype control IgG1. No binding signal was observed for any clone against untransfected CHO cells.

FIG. 5. Cell-based PD1/PD-L1 antagonism assay. Analyses of antagonism of human PD1 function at the cell surface, for multiple lead clones in human IgG1null format, showed that all novel clones exhibited strong, concentration-dependent, antagonistic activity.

FIG. 6A – FIG 6C. Cell-based PD1/PD-L1 antagonism assay for maximally humanized lead clones MH4, MH8 AND MH12 versus mu317 and hu317. Analyses of antagonism of

human PD1 function, for multiple lead clones in human IgG1null format. These analyses showed that clones MH4 (FIG. 6A), MH8 (FIG. 6B) and MH12 (FIG. 6C) exhibited antagonistic potency overlapping with both mu317 and hu317. Values expressed as Mean ± SD fold induction.

FIG. 7A – FIG. 7H. T cell epitope peptide content in lead antibody v-domains. The v-domains of Hu317 and multiple lead antibodies were examined for the presence of Germline (GE), High Affinity Foreign (HAF), Low Affinity Foreign (LAF) and TCED+ T cell receptor epitopes. Both the VH and VL domains of Hu317 were found to contain multiple high-risk human T cell epitopes and few germline epitopes. In all lead clones, the high-risk epitope content was significantly reduced and germline epitope content significantly improved.

FIG. 8A – FIG. 8C. MLR cell-based PD1/PD-L1 antagonism assay. Effect of anti-PD1 IgGs on IFN-γ Production in an Allogeneic MLR assay. Control analyses (FIG. 8A), 3-point analyses for lead clones 09C06, MH4, MH8, MH12 (FIG. 8B), and multi-point analyses for clones MH4, MH8 and Hu317 (FIG. 8C) are shown. In each case, Isotype controls were used (human IgG4, or IgG1).

FIG. 9. Charge variant profiles of purified IgGs. (1) 09C06, (2) MH4, (3) MH8, (4) MH12 and (5) Hu317 are shown. Y axis signals measured in Fluorescence Units.

FIG. 10. DSC Thermograms of purified IgGs. (1) 09C06, (2) MH4, (3) MH8, (4) MH12 and (5) Hu317 are shown.

FIG. 11. Flow cytometric binding of Hu317, lead and control IgGs to HEK293 cells transfected with PD1 or potential off-target binding proteins. Analyses of binding specificity were performed on HEK293 cells transiently transfected with plasmids encoding either human PD1, human BTN2A1, human LSAMP, human CD20 or ZS green marker only. Each antibody was used in staining at 2 μg/ml concentration and histograms are indicated per antibody in this figure. In each panel of this figure, where target binding events were observed (indicated by a movement of all, or the major portion of the requisite histogram towards higher AF647 signal), the peak of interest is named (PD1, CD20, LSAMP). No binding signals were observed for any IgG against BTN2A1.

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 or a conservative substitution of T (for example S)-F or a conservative substitution of F (for example L)-S or a conservative substitution of S (for example T)-S-Y-G or any amino acid (for example W)-M

or a conservative substitution of M (for example V)-S or any amino acid (for example H) (SEQ ID NO:20);

an HCDR2 having amino acids in sequence in the following order: V or a conservative substitution of V (for example L)-A or a conservative substitution of A (for example G)-V/N-I-W/K-Q/A-D/G-G-S-T/E-N/K/S-Y-N/V-D-S-V-K-G (SEQ ID NO:21); and

an HCDR3 having amino acids in sequence in the following order: A or any amino acid (for example D/E/G/H/I/K/L/H/Q/S/V/W)-Y or a conservative substitution of Y (for example F/W)-G-N or any amino acid (for example A/E/F/G/H/I/Q/R/S/T/V/W/Y)-Y or a conservative substitution of Y (for example V/W)-W or any amino acid (for example F/H/M/P/Q/S/V)-Y-I or any amino acid (for example L/M/S/T/V/Y)-D or a conservative substitution of D (for example E)-V or any amino acid (for example A/E/F/G/H/I/K/L/M/R/S/T/W/Y) (SEQ ID NO:22).

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:16 or SEQ ID NO:17. 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:16 or SEQ ID NO:17.

In aspects of the invention, the HCDR1 of the antibody molecule or antigen-binding portion may exclude the sequences GFSLTSYGVH (SEQ ID NO:23; Mu317 murine/humanized antibody HCDR1 disclosed in WO2015/035606 A1; US2015/079109A1), VIWAGGSTNYNSALMS (SEQ ID NO:24; Mu317 murine/humanized antibody HCDR2 disclosed in WO2015/035606 A1; US2015/079109A1), and/or the HCDR3 of the antibody molecule or antigen-binding portion may exclude the sequence AYGNYWYIDV (SEQ ID NO:25; Mu317 murine/humanized antibody HCDR3 disclosed in WO2015/035606 A1; US2015/079109A1).

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: K-S-S-Q/E-S-V-S or a conservative substitution of S (such as G/T)-N-D-L or a conservative substitution of L (such as V)-A (SEQ ID NO:26);

an LCDR2 having amino acids in sequence in the following order: Y-A-F or any amino acid (for example S/Y)-H or any amino acid (for example P/T)-R-F-S or a conservative substitution of S (such as A/T) (SEQ ID NO:27); and

an LCDR3 having amino acids in sequence in the following order: Q or any amino acid (for example H)-Q-A or any amino acid (for example S)-Y-S-T or any amino acid (for example, N/S)-P-Y or any amino acid (for example W)-T (SEQ ID NO:28).

In aspects of the invention, the LCDR1 of the antibody molecule or antigen-binding portion may exclude the sequence KASQSVSNDVA / KSSQEVSNDVA (SEQ ID NO:29 / SEQ ID NO:30; Mu317 murine/humanized antibody LCDR1 disclosed in WO2015/035606 A1; US2015/079109A1), and/or the LCDR2 of the antibody molecule or antigen-binding portion may exclude the sequence YAFHRFT (SEQ ID NO:31; Mu317 murine/humanized antibody LCDR2 disclosed in WO2015/035606 A1; US2015/079109A1) and/or the LCDR3 of the antibody molecule or antigen-binding portion may exclude the sequence HQAYSSPYT (SEQ ID NO:32; Mu317 murine/humanized antibody LCDR3 disclosed in WO2015/035606 A1; US2015/079109A1).

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-X_1-X_2-X_3-S-Y-X_4-X_5-X_6, wherein X_1 is T or a conservative substitution of T (for example, S), X_2 is F or a conservative substitution of F (for example, L), X_3 is S or a conservative substitution of S (for example, T), X_4 is G or any other amino acid (for example, W), X_5 is M or a conservative substitution of M (for example, V), and X_6 is S or any other amino acid (for example, H) (SEQ ID NO:20);

(b) the HCDR2 comprises the amino acid sequence X_1-X_2-X_3-I-X_4-X_5-X_6-G-S-X_7-X_8-Y-X_9-D-S-V-K-G, wherein X_1 is V or a conservative substitution of V (for example, L), X_2 is A or a conservative substitution of A (for example, G), X_3 is V or N, X_4 is W or K, X_5 is Q or A, X_6 is D or G, X_7 is T or E, X_8 is N, K, or S, and X_9 is N or V (SEQ ID NO:21);

(c) the HCDR3 comprises the amino acid sequence X_1-X_2-G-X_3-X_4-X_5-Y-X_6-X_7-X_8, wherein X_1 is A or any other amino acid (for example, D, E, G, H, I, K, L, H, Q, S, V, or W), X_2 is Y or a conservative substitution of Y (for example, F or W), X_3 is N or any other amino acid (for example, A, E, F, G, H, I, Q, R, S, T, V, W, or Y), X_4 is Y or a conservative substitution of Y (for example, V or W), X_5 is W or any other amino acid (for example, F, H, M, P, Q, S, or V), X_6 is I or any other amino acid (for example, L, M, S, T, V, or Y), X_7 is D or a conservative substitution of D (for example, E), and X_8 is V or any other amino acid (for example, A, E, F, G, H, I, K, L, M, R, S, T, W, or Y) (SEQ ID NO:22);

(d) the LCDR1 comprises the amino acid sequence K-S-S-X_1-S-V-X_2-N-D-X_3-A, wherein X_1 is Q or E, X_2 is S or a conservative substitution of S (for example, G or T), and X_3 is L or a conservative substitution of L (for example, V) (SEQ ID NO:26);

(e) the LCDR2 comprises the amino acid sequence Y-A-X_1-X_2-R-F-X_3, wherein X_1 is F or any other amino acid (for example, S), X_2 is H or any other amino acid (for example, P or T), and X_3 is S or a conservative substitution of S (for example, A or T) (SEQ ID NO:27); and

(f) the LCDR3 comprises the amino acid sequence X_1-Q-X_2-Y-S-X_3-P-X_4-T, wherein X_1 is Q or any other amino acid (for example, H), X_2 is A or any other amino acid (for example, S), X_3 is T or any other amino acid (for example, N or S), and X_4 is Y or any other amino acid (for example, W) (SEQ ID NO:28).

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 order, FR1-LCDR1-FR2-LCDR2-FR3-LCDR3-FR4, wherein the HCDR1 is SEQ ID NO:20, the HCDR2 is SEQ ID NO:32, the HCDR3 is SEQ ID NO:33, the LCDR1 is SEQ ID NO:26, the LCDR2 is SEQ ID NO:27 and the LCDR3 is SEQ ID NO:28, 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: 116 (see Table 2) and wherein the light chain FR1, FR2, FR3 and FR4 amino acid sequences are the light chain FR1, FR2, FR3 and FR4 amino acid sequences in SEQ ID NO: 119 (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 murine anti-PD1 antibody Mu317 disclosed in WO2015/035606 A1; US2015/079109A1. Refining of the optimized antibody molecules as described herein has provided improved variable domain stability, high expression yields, and/or reduced immunogenicity potential without compromising binding specificity to both human PD1 as well as cynomolgus monkey PD1 (to facilitate maximally accurate primate toxicology and pk studies) and pharmacological potency in PD1 antagonism assays.

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

are not necessary “maximally optimized” in terms of anti-PD1 binding characteristics and/or other desirable features.

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 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 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 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.

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-G-M/V-S/H (SEQ ID NO:62); the HCDR2 may have the amino acid sequence: V-A-V/N-I-W/K-Q-D-G-S-E/T-N/K-Y-V/N-D-S-V-K-G (SEQ ID NO:63); and the HCDR3 may have the amino acid sequence: A/D/E/G/H/I/K/L/H/Q/S/V/W-Y/F/W-G-N/A/E/F/G/H/I/Q/R/S/T/V/W/Y-Y/V/W-W/F/H/M/P/Q/S/V-Y-I/L/M/S/T/V/Y-D/E-V/A/E/F/G/H/I/Q/R/S/T/W/Y (SEQ ID NO:64).

For example, the HCDR1 may have the amino acid sequence: G-F-T-F-S-S-Y-G-M-S/H (SEQ ID NO:65); the HCDR2 may have the amino acid sequence: V-A-V/N-I-W-Q-D-G-S-

E/T-N/K-Y-V-D-S-V-K-G (SEQ ID NO:66); and the HCDR3 may have the amino acid sequence: A/G-Y-G-N-Y-W/M-Y-I-D-R/V (SEQ ID NO:67).

In the antibody molecule or antigen-binding portion thereof, the LCDR1 may have the amino acid sequence: K-S-S-Q/E-S-V-S/G/T-N-D-L/V-A (SEQ ID NO:68); the LCDR2 may have the amino acid sequence Y-A-F/S/Y-H/P/T-R-F-S/T (SEQ ID NO:69); and the LCDR3 may have the amino acid sequence: Q/H-Q-A/S-Y-S-T/N/S-P-Y-T (SEQ ID NO:70).

For example, the LCDR1 may have the amino acid sequence: K-S-S-Q-S-V-S/G/T-N-D-L/V-A (SEQ ID NO:71); the LCDR2 may have the amino acid sequence Y-A-S/Y-H-R-F-S/T (SEQ ID NO:72); and the LCDR3 may have the amino acid sequence: Q/H-Q-A-Y-S-T/S-P-Y-T (SEQ ID NO:73).

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

(a) the amino acid sequences
GFSFTSYGMS (SEQ ID NO:47; HCDR1), LANIWQDGSTNYVDSVKG (SEQ ID NO:48; HCDR2), AYGNYWYIDR (SEQ ID NO:35; HCDR3), KSSQSVSNDVA (SEQ ID NO:44; LCDR1), YASHRFT (SEQ ID NO:45; LCDR2) and HQAYSTPYT (SEQ ID NO:46; LCDR3) [Clone 20C07]; or

(b) the amino acid sequences
GFTFSSYGVH (SEQ ID NO:49; HCDR1), VGVIWQDGSENYVDSVKG (SEQ ID NO:50; HCDR2), AYGNYWYIDR (SEQ ID NO:35; HCDR3), KSSQSVSNDLA (SEQ ID NO:51; LCDR1), YASHRFT (SEQ ID NO:45; LCDR2) and QQAYSTPYT (SEQ ID NO:52; LCDR3) [Clone 11C08];

(c) the amino acid sequences
GFSFSSYGMH (SEQ ID NO:42; HCDR1), VANIWQDGSTNYVDSVKG (SEQ ID NO:43; HCDR2), AYGNYWYIDR (SEQ ID NO:35; HCDR3), KSSQSVSNDVA (SEQ ID NO:44; LCDR1), YASHRFT (SEQ ID NO:45; LCDR2) and HQAYSTPYT (SEQ ID NO:46; LCDR3) [Clone 09C06];

(d) the amino acid sequences
GFSFSSYGMH (SEQ ID NO:42; HCDR1), LANIWQDGSENYVDSVKG (SEQ ID NO:53; HCDR2), AYGNYWYIDR (SEQ ID NO:35; HCDR3), KSSQSVSNDVA (SEQ ID NO:44; LCDR1), YASHRFT (SEQ ID NO:45; LCDR2) and QQAYSTPYT (SEQ ID NO:52; LCDR3) [Clone 17A12];

(e) the amino acid sequences

GFTLSSYGMH (SEQ ID NO:54; HCDR1), LAVIWADGSTNYVDSVKG (SEQ ID NO:55; HCDR2), AYGNYMYIDV (SEQ ID NO:56; HCDR3), KSSQSVSNDVA (SEQ ID NO:44; LCDR1), YASHRFT (SEQ ID NO:45; LCDR2) and HQAYSTPYT (SEQ ID NO:46; LCDR3) [Clone 17D08];

(f) the amino acid sequences
GFTFSSYGMH (SEQ ID NO:57; HCDR1), VGVIKQDGSENYVDSVKG (SEQ ID NO:58; HCDR2), AYGNYWYIDR (SEQ ID NO:35; HCDR3), KSSQSVSNDVA (SEQ ID NO:44; LCDR1), YASHRFT (SEQ ID NO:45; LCDR2) and HQAYSTPYT (SEQ ID NO:46; LCDR3) [Clone 17G08];

(g) the amino acid sequences
GFSFTSYGMH (SEQ ID NO:59; HCDR1), VGVIWQDGSTNYVDSVKG (SEQ ID NO:60; HCDR2), GYGNYWYIDV (SEQ ID NO:61; HCDR3), KSSQSVSNDLA (SEQ ID NO:51; LCDR1), YASHRFT (SEQ ID NO:45; LCDR2) and QQAYSTPYT (SEQ ID NO:52; LCDR3) [Clone 15D07];

(h) the amino acid sequences
GFSLSSYGMS (SEQ ID NO:39; HCDR1), VAVIWQDGSTNYVDSVKG (SEQ ID NO:40; HCDR2), AYGNYWYIDR (SEQ ID NO:35; HCDR3), KSSQSVGNDVA (SEQ ID NO:74; LCDR1), YASHRFT (SEQ ID NO:45; LCDR2) and QQAYSSPYT (SEQ ID NO:75; LCDR3) [Clone MH1];

(i) the amino acid sequences
GFSLSSYGMS (SEQ ID NO:39; HCDR1), VAVIWQDGSTNYVDSVKG (SEQ ID NO:40; HCDR2), AYGNYWYIDR (SEQ ID NO:35; HCDR3), KSSQSVTNDLA (SEQ ID NO:76; LCDR1), YASHRFT (SEQ ID NO:45; LCDR2) and QQAYSSPYT (SEQ ID NO:75; LCDR3) [Clone MH2];

(j) the amino acid sequences
GFSLSSYGMS (SEQ ID NO:39; HCDR1), VAVIWQDGSTNYVDSVKG (SEQ ID NO:40; HCDR2), AYGNYWYIDR (SEQ ID NO:35; HCDR3), KSSQSVGNDLA (SEQ ID NO:77; LCDR1), YAYHRFS (SEQ ID NO:78; LCDR2) and QQAYSSPYT (SEQ ID NO:75; LCDR3) [Clone MH3];

(k) the amino acid sequences
GFSLSSYGMS (SEQ ID NO:39; HCDR1), VAVIWQDGSTNYVDSVKG (SEQ ID NO:40; HCDR2), AYGNYWYIDR (SEQ ID NO:35; HCDR3), KSSQSVTNDVA (SEQ ID NO:36; LCDR1), YAYHRFT (SEQ ID NO:37; LCDR2) and HQAYSSPYT (SEQ ID NO:38; LCDR3) [Clone MH4];

(l) the amino acid sequences
GFTFSSYGMS (SEQ ID NO:33; HCDR1), VANIWQDGSTKYVDSVKG (SEQ ID NO:34; HCDR2), AYGNYWYIDR (SEQ ID NO:35; HCDR3), KSSQSVGNDVA (SEQ ID NO:74;

LCDR1), YASHRFT (SEQ ID NO:45; LCDR2) and QQAYSSPYT (SEQ ID NO:75; LCDR3) [Clone MH5];

(m) the amino acid sequences GFTFSSYGMS (SEQ ID NO:33; HCDR1), VANIWQDGSTKYVDSVKG (SEQ ID NO:34; HCDR2), AYGNYWYIDR (SEQ ID NO:35; HCDR3), KSSQSVTNDLA (SEQ ID NO:76; LCDR1), YASHRFT (SEQ ID NO:45; LCDR2) and QQAYSSPYT (SEQ ID NO:75; LCDR3) [Clone MH6];

(n) the amino acid sequences GFTFSSYGMS (SEQ ID NO:33; HCDR1), VANIWQDGSTKYVDSVKG (SEQ ID NO:34; HCDR2), AYGNYWYIDR (SEQ ID NO:35; HCDR3), KSSQSVGNDLA (SEQ ID NO:77; LCDR1), YAYHRFS (SEQ ID NO:78; LCDR2) and QQAYSSPYT (SEQ ID NO:75; LCDR3) [Clone MH7];

(o) the amino acid sequences GFTFSSYGMS (SEQ ID NO:33; HCDR1), VANIWQDGSTKYVDSVKG (SEQ ID NO:34; HCDR2), AYGNYWYIDR (SEQ ID NO:35; HCDR3), KSSQSVTNDVA (SEQ ID NO:36; LCDR1), YAYHRFT (SEQ ID NO:37; LCDR2) and HQAYSSPYT (SEQ ID NO:38; LCDR3) [Clone MH8];

(p) the amino acid sequences GFTFSSYGMS (SEQ ID NO:33; HCDR1), VANIWQDGSEKYVDSVKG (SEQ ID NO:41; HCDR2), AYGNYWYIDR (SEQ ID NO:35; HCDR3), KSSQSVGNDVA (SEQ ID NO:74; LCDR1), YASHRFT (SEQ ID NO:45; LCDR2) and QQAYSSPYT (SEQ ID NO:75; LCDR3) [Clone MH9];

(q) the amino acid sequences GFTFSSYGMS (SEQ ID NO:33; HCDR1), VANIWQDGSEKYVDSVKG (SEQ ID NO:41; HCDR2), AYGNYWYIDR (SEQ ID NO:35; HCDR3), KSSQSVTNDLA (SEQ ID NO:76; LCDR1), YASHRFT (SEQ ID NO:45; LCDR2) and QQAYSSPYT (SEQ ID NO:75; LCDR3) [Clone MH10];

(r) the amino acid sequences GFTFSSYGMS (SEQ ID NO:33; HCDR1), VANIWQDGSEKYVDSVKG (SEQ ID NO:41; HCDR2), AYGNYWYIDR (SEQ ID NO:35; HCDR3), KSSQSVGNDLA (SEQ ID NO:77; LCDR1), YAYHRFS (SEQ ID NO:78; LCDR2) and QQAYSSPYT (SEQ ID NO:75; LCDR3) [Clone MH11];

(s) the amino acid sequences GFTFSSYGMS (SEQ ID NO:33; HCDR1), VANIWQDGSEKYVDSVKG (SEQ ID NO:41; HCDR2), AYGNYWYIDR (SEQ ID NO:35; HCDR3), KSSQSVTNDVA (SEQ ID NO:36; LCDR1), YAYHRFT (SEQ ID NO:37; LCDR2) and HQAYSSPYT (SEQ ID NO:38; LCDR3) [Clone MH12];

(t) the amino acid sequences
GFTFSSYGMS (SEQ ID NO:33; HCDR1), VANIWQDGSTKYVDSVKG (SEQ ID NO:34; HCDR2), AYGNYWYIDR (SEQ ID NO:35; HCDR3), KSSQSVTNDLA (SEQ ID NO:76; LCDR1), YAYHRFT (SEQ ID NO:37; LCDR2) and HQAYSSPYT (SEQ ID NO:38; LCDR3) [Clone MH13];
(u) the amino acid sequences
GFTFSSYGMS (SEQ ID NO:33; HCDR1), VANIWQDGSTKYVDSVKG (SEQ ID NO:34; HCDR2), AYGNYWYIDR (SEQ ID NO:35; HCDR3), KSSQSVTNDLA (SEQ ID NO:76; LCDR1), YAYHRFS (SEQ ID NO:78; LCDR2) and HQAYSSPYT (SEQ ID NO:38; LCDR3) [Clone MH14];
(v) the amino acid sequences
GFTFSSYGMS (SEQ ID NO:33; HCDR1), VANIWQDGSTKYVDSVKG (SEQ ID NO:34; HCDR2), AYGNYWYIDR (SEQ ID NO:35; HCDR3), KSSQSVTNDLA (SEQ ID NO:76; LCDR1), YASHRFS (SEQ ID NO:79; LCDR2) and HQAYSSPYT (SEQ ID NO:38; LCDR3) [Clone MH15];
(w) the amino acid sequences
GFTFSSYGMS (SEQ ID NO:33; HCDR1), VANIWQDGSEKYVDSVKG (SEQ ID NO:41; HCDR2), AYGNYWYIDR (SEQ ID NO:35; HCDR3), KSSQSVTNDLA (SEQ ID NO:76; LCDR1), YAYHRFT (SEQ ID NO:37; LCDR2) and HQAYSSPYT (SEQ ID NO:38; LCDR3) [Clone MH16];
(x) the amino acid sequences
GFTFSSYGMS (SEQ ID NO:33; HCDR1), VANIWQDGSEKYVDSVKG (SEQ ID NO:41; HCDR2), AYGNYWYIDR (SEQ ID NO:35; HCDR3), KSSQSVTNDLA (SEQ ID NO:76; LCDR1), YAYHRFS (SEQ ID NO:78; LCDR2) and HQAYSSPYT (SEQ ID NO:38; LCDR3) [Clone MH17];
(y) the amino acid sequences
GFTFSSYGMS (SEQ ID NO:33; HCDR1), VANIWQDGSEKYVDSVKG (SEQ ID NO:41; HCDR2), AYGNYWYIDR (SEQ ID NO:35; HCDR3), KSSQSVTNDLA (SEQ ID NO:76; LCDR1), YASHRFS (SEQ ID NO:79; LCDR2) and HQAYSSPYT (SEQ ID NO:38; LCDR3) [Clone MH18];

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 GFTFSSYGMS (SEQ ID NO:33), HCDR2 of VANIWQDGSTKYVDSVKG (SEQ ID NO:34), and HCDR3 of AYGNYWYIDR (SEQ ID NO:35); and the VL region amino acid sequence comprises LCDR1

of KSSQSVTNDVA (SEQ ID NO:36), LCDR2 of YAYHRFT (SEQ ID NO:37), and LCDR3 of HQAYSSPYT (SEQ ID NO:38);

(b) the VH region amino acid sequence comprises HCDR1 of GFSLSSYGMS (SEQ ID NO:39), HCDR2 of VAVIWQDGSTNYVDSVKG (SEQ ID NO:40), and HCDR3 of AYGNYWYIDR (SEQ ID NO:35); and the VL region amino acid sequence comprises LCDR1 of KSSQSVTNDVA (SEQ ID NO:36), LCDR2 of YAYHRFT (SEQ ID NO:37), and LCDR3 of HQAYSSPYT (SEQ ID NO:38);

(c) the VH region amino acid sequence comprises HCDR1 of GFTFSSYGMS (SEQ ID NO:33), HCDR2 of VANIWQDGSEKYVDSVKG (SEQ ID NO:41), and HCDR3 of AYGNYWYIDR (SEQ ID NO:35); and the VL region amino acid sequence comprises LCDR1 of KSSQSVTNDVA (SEQ ID NO:36), LCDR2 of YAYHRFT (SEQ ID NO:37), and LCDR3 of HQAYSSPYT (SEQ ID NO:38);

(d) the VH region amino acid sequence comprises HCDR1 of GFSFSSYGMH (SEQ ID NO:42), HCDR2 of VANIWQDGSTNYVDSVKG (SEQ ID NO:43), and HCDR3 of AYGNYWYIDR (SEQ ID NO:35); and the VL region amino acid sequence comprises LCDR1 of KSSQSVSNDVA (SEQ ID NO:44), LCDR2 of YASHRFT (SEQ ID NO:45), and LCDR3 of HQAYSTPYT (SEQ ID NO:46);

(e) the VH region amino acid sequence comprises HCDR1 of GFSFTSYGMS (SEQ ID NO:47), HCDR2 of LANIWQDGSTNYVDSVKG (SEQ ID NO:48), and HCDR3 of AYGNYWYIDR (SEQ ID NO:35); and the VL region amino acid sequence comprises LCDR1 of KSSQSVSNDVA (SEQ ID NO:44), LCDR2 of YASHRFT (SEQ ID NO:45), and LCDR3 of HQAYSTPYT (SEQ ID NO:46);

(f) the VH region amino acid sequence comprises HCDR1 of GFTFSSYGVH (SEQ ID NO:49), HCDR2 of VGVIWQDGSENYVDSVKG (SEQ ID NO:50), and HCDR3 of AYGNYWYIDR (SEQ ID NO:35); and the VL region amino acid sequence comprises LCDR1 of KSSQSVSNDLA (SEQ ID NO:51), LCDR2 of YASHRFT (SEQ ID NO:45), and LCDR3 of QQAYSTPYT (SEQ ID NO:52);

(g) the VH region amino acid sequence comprises HCDR1 of GFSFSSYGMH (SEQ ID NO:42), HCDR2 of VANIWQDGSTNYVDSVKG (SEQ ID NO:43), and HCDR3 of AYGNYWYIDR (SEQ ID NO:35); and the VL region amino acid sequence comprises LCDR1 of KSSQSVSNDVA (SEQ ID NO:44), LCDR2 of YASHRFT (SEQ ID NO:45), and LCDR3 of HQAYSTPYT (SEQ ID NO:46);

(h) the VH region amino acid sequence comprises HCDR1 of GFSFSSYGMH (SEQ ID NO:42), HCDR2 of LANIWQDGSENYVDSVKG (SEQ ID NO:53), and HCDR3 of AYGNYWYIDR (SEQ ID NO:35); and the VL region amino acid sequence comprises LCDR1 of KSSQSVSNDVA (SEQ ID NO:44), LCDR2 of YASHRFT (SEQ ID NO:45), and LCDR3 of QQAYSTPYT (SEQ ID NO:52);

(i) the VH region amino acid sequence comprises HCDR1 of GFTLSSYGMH (SEQ ID NO:54), HCDR2 of LAVIWADGSTNYVDSVKG (SEQ ID NO:55), and HCDR3 of AYGNYMYIDV (SEQ ID NO:56); and the VL region amino acid sequence comprises LCDR1 of KSSQSVSNDVA (SEQ ID NO:44), LCDR2 of YASHRFT (SEQ ID NO:45), and LCDR3 of HQAYSTPYT (SEQ ID NO:46);

(j) the VH region amino acid sequence comprises HCDR1 of GFTFSSYGMH (SEQ ID NO:57), HCDR2 of VGVIKQDGSENYVDSVKG (SEQ ID NO:58), and HCDR3 of AYGNYWYIDR (SEQ ID NO:35); and the VL region amino acid sequence comprises LCDR1 of KSSQSVSNDVA (SEQ ID NO:44), LCDR2 of YASHRFT (SEQ ID NO:45), and LCDR3 of HQAYSTPYT (SEQ ID NO:46); or

(k) the VH region amino acid sequence comprises HCDR1 of GFSFTSYGMH (SEQ ID NO:59), HCDR2 of VGVIWQDGSTNYVDSVKG (SEQ ID NO:60), and HCDR3 of GYGNYWYIDV (SEQ ID NO:61); and the VL region amino acid sequence comprises LCDR1 of KSSQSVSNDLA (SEQ ID NO:51), LCDR2 of YASHRFT (SEQ ID NO:45), and LCDR3 of QQAYSTPYT (SEQ ID NO:52).

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 9 and the VL region comprises any one of the VL region amino acid sequences in Table 9.

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 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;

(c) the VH region amino acid sequence comprises SEQ ID NO:5 and the VL region amino acid sequence comprises SEQ ID NO:6; or

(d) the VH region amino acid sequence comprises SEQ ID NO:7 and the VL region amino acid sequence comprises SEQ ID NO:8.

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;

(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;

(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; or

(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%, 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. 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.

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 CDRs disclosed herein. In some embodiments, the invention provides an 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; and/or (b) binds specifically to human PD1 and cynomolgus PD1; and/or (c) does not comprise an oxidation site in the LCDR2; and/or (d) comprises a human germline peptide sequence with high MHC class II binding affinity (e.g., VKGRFTISR (SEQ ID NO:81)) in the HCDR2/framework 3 region of the VH domain; and/or (e) does not comprise a human T cell epitope sequence in the HCDR1/framework 2 region of the VH domain; and/or (f) does not comprise a human T cell epitope sequence in the HCDR3/framework 4 region of the VH domain; and/or (g) exhibits reduced charge species heterogeneity compared to antibody Hu317, when in IgG1 effector null antibody format. In some embodiments, a KD value of an antibody or antigen-binding portion may be determined by 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).

The terms "cross-compete", "cross-competition", "cross-block", "cross-blocked" and "cross-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 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 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 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 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.

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 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 Mu317 murine LCDR2 (as defined herein, i.e. the amino acid sequence YAFHRFT (SEQ ID NO:31)) has been identified to have a putative oxidation site at residue 3 (F). Removal of this site at equivalent positions in an LCDR2 of the invention, for example by conservative or non-conservative substitution (such as to S or Y), is envisaged (as for example in clones 09C06 and other clones in Tables 3 and 4).

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

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.

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.

The antibody molecule or antigen-binding portion thereof may comprise an IGKV4-1 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 IGKV4-1 human germline scaffold amino acid sequence into which a set of corresponding LCDR1, LCDR2 and LCDR3 amino acid sequences have been inserted.

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 IGKV4-1 human germline scaffold into which the corresponding LCDR 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 and a VL region that comprises an IGKV4-1 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 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).

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 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 10. The Fc region sequences in Table 10 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 RDELT (SEQ ID NO:18) motif or an REEM (SEQ ID NO:19) motif (underlined in Table 18). The REEM (SEQ ID NO:19) allotype is found in a smaller human population than the RDELT (SEQ ID NO:18) allotype. In some aspects, an anti-PD1 antibody may comprise an immunoglobulin constant region comprising any one of SEQ ID NOS:9-15. 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 10. 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 10 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 GFTFSSYGMS (SEQ ID NO:33), HCDR2 of VANIWQDGSTKYVDSVKG (SEQ ID NO:34), and HCDR3 of AYGNYWYIDR (SEQ ID NO:35); the VL region amino acid sequence comprises LCDR1 of KSSQSVTNDVA (SEQ ID NO:36), LCDR2 of YAYHRFT (SEQ ID NO:37), and LCDR3 of HQAYSSPYT (SEQ ID NO:38); and the heavy chain constant region comprises any one of SEQ ID NOS: 9-15;

(b) the VH region amino acid sequence comprises HCDR1 of GFSLSSYGMS (SEQ ID NO:39), HCDR2 of VAVIWQDGSTNYVDSVKG (SEQ ID NO:40), and HCDR3 of AYGNYWYIDR (SEQ ID NO:35); the VL region amino acid sequence comprises LCDR1 of KSSQSVTNDVA (SEQ ID NO:36), LCDR2 of YAYHRFT (SEQ ID NO:37), and LCDR3 of HQAYSSPYT (SEQ ID NO:38); and the heavy chain constant region comprises any one of SEQ ID NOS: 9-15;

(c) the VH region amino acid sequence comprises HCDR1 of GFTFSSYGMS (SEQ ID NO:33), HCDR2 of VANIWQDGSEKYVDSVKG (SEQ ID NO:41), and HCDR3 of AYGNYWYIDR (SEQ ID NO:35); the VL region amino acid sequence comprises LCDR1 of KSSQSVTNDVA (SEQ ID NO:36), LCDR2 of YAYHRFT (SEQ ID NO:37), and LCDR3 of HQAYSSPYT (SEQ ID NO:38); and the heavy chain constant region comprises any one of SEQ ID NOS: 9-15;

(d) the VH region amino acid sequence comprises HCDR1 of GFSFSSYGMH (SEQ ID NO:42), HCDR2 of VANIWQDGSTNYVDSVKG (SEQ ID NO:43), and HCDR3 of AYGNYWYIDR (SEQ ID NO:35); the VL region amino acid sequence comprises LCDR1 of KSSQSVSNDVA (SEQ ID NO:44), LCDR2 of YASHRFT (SEQ ID NO:45), and LCDR3 of HQAYSTPYT (SEQ ID NO:46); and the heavy chain constant region comprises any one of SEQ ID NOS: 9-15;

(e) the VH region amino acid sequence comprises HCDR1 of GFSFTSYGMS (SEQ ID NO:47), HCDR2 of LANIWQDGSTNYVDSVKG (SEQ ID NO:48), and HCDR3 of AYGNYWYIDR (SEQ ID NO:35); the VL region amino acid sequence comprises LCDR1 of KSSQSVSNDVA (SEQ ID NO:44), LCDR2 of YASHRFT (SEQ ID NO:45), and LCDR3 of HQAYSTPYT (SEQ ID NO:46); and the heavy chain constant region comprises any one of SEQ ID NOS: 9-15;

(f) the VH region amino acid sequence comprises HCDR1 of GFTFSSYGVH (SEQ ID NO:49), HCDR2 of VGVIWQDGSENYVDSVKG (SEQ ID NO:50), and HCDR3 of AYGNYWYIDR (SEQ ID NO:35); the VL region amino acid sequence comprises LCDR1 of KSSQSVSNDLA (SEQ ID NO:51), LCDR2 of YASHRFT (SEQ ID NO:45), and LCDR3 of QQAYSTPYT (SEQ ID NO:52); and the heavy chain constant region comprises any one of SEQ ID NOS: 9-15;

(g) the VH region amino acid sequence comprises HCDR1 of GFSFSSYGMH (SEQ ID NO:42), HCDR2 of VANIWQDGSTNYVDSVKG (SEQ ID NO:43), and HCDR3 of AYGNYWYIDR (SEQ ID NO:35); the VL region amino acid sequence comprises LCDR1 of KSSQSVSNDVA (SEQ ID NO:44), LCDR2 of YASHRFT (SEQ ID NO:45), and LCDR3 of HQAYSTPYT (SEQ ID NO:46); and the heavy chain constant region comprises any one of SEQ ID NOS: 9-15;

(h) the VH region amino acid sequence comprises HCDR1 of GFSFSSYGMH (SEQ ID NO:42), HCDR2 of LANIWQDGSENYVDSVKG (SEQ ID NO:53), and HCDR3 of AYGNYWYIDR (SEQ ID NO:35); the VL region amino acid sequence comprises LCDR1 of KSSQSVSNDVA (SEQ ID NO:44), LCDR2 of YASHRFT (SEQ ID NO:45), and LCDR3 of QQAYSTPYT (SEQ ID NO:52); and the heavy chain constant region comprises any one of SEQ ID NOS: 9-15;

(i) the VH region amino acid sequence comprises HCDR1 of GFTLSSYGMH (SEQ ID NO:54), HCDR2 of LAVIWADGSTNYVDSVKG (SEQ ID NO:55), and HCDR3 of AYGNYMYIDV (SEQ ID NO:56); the VL region amino acid sequence comprises LCDR1 of KSSQSVSNDVA (SEQ ID NO:44), LCDR2 of YASHRFT (SEQ ID NO:45), and LCDR3 of HQAYSTPYT (SEQ ID NO:46); and the heavy chain constant region comprises any one of SEQ ID NOS: 9-15;

(j) the VH region amino acid sequence comprises HCDR1 of GFTFSSYGMH (SEQ ID NO:57), HCDR2 of VGVIKQDGSENYVDSVKG (SEQ ID NO:58), and HCDR3 of AYGNYWYIDR (SEQ ID NO:35); the VL region amino acid sequence comprises LCDR1 of KSSQSVSNDVA (SEQ ID NO:44), LCDR2 of YASHRFT (SEQ ID NO:45), and LCDR3 of HQAYSTPYT (SEQ ID NO:46); and the heavy chain constant region comprises any one of SEQ ID NOS: 9-15; or

(k) the VH region amino acid sequence comprises HCDR1 of GFSFTSYGMH (SEQ ID NO:59), HCDR2 of VGVIWQDGSTNYVDSVKG (SEQ ID NO:60), and HCDR3 of GYGNYWYIDV (SEQ ID NO:61); the VL region amino acid sequence comprises LCDR1 of KSSQSVSNDLA (SEQ ID NO:51), LCDR2 of YASHRFT (SEQ ID NO:45), and LCDR3 of QQAYSTPYT (SEQ ID NO:52); and the heavy chain constant region comprises any one of SEQ ID NOS: 9-15.

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, 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 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;or

(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 comprising the amino acid substitutions L234A, L235A and G237A.

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: 9-15;

(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: 9-15;

(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: 9-15; or

(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: 9-15.

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 FcγRI, FcγRIIa, FcγRIIIa and FcγRIIIb receptors but maintain binding to human FcγRIIb receptor and optionally maintain binding to human FcRn receptor. FcγRI, FcγRIIa, FcγRIIIa and FcγRIIIb are examples of activating receptors. FcγRIIb 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 10) 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 10) 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).

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.

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 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 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 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, idarubicin, aclarubicin, zorubicin, mitoxantrone, epirubicin, carubicin, nogalamycin, menogaril, pitarubicin, valrubicin, cytarabine, gemcitabine, trifluridine, ancitabine, enocitabine, azacitidine, doxifluhdine, pentostatin, broxuhdine, capecitabine, cladhbine, decitabine, floxuhdine, fludarabine, gougerotin, puromycin, tegafur, tiazofuhn, adhamycin, cisplatin, carboplatin, cyclophosphamide, dacarbazine, vinblastine, vincristine, mitoxantrone, 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.

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.

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.

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.

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.

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.

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 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 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 example, the cancer may be pancreatic cancer, melanoma, breast cancer, lung cancer, bronchial cancer, colorectal cancer, prostate cancer, stomach cancer, ovarian cancer, 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.

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 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 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 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 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.

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.

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.

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; 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, disaccharides and other carbohydrates including glucose, mannose or dextrins; chelating agents, such as EDTA; sugars, such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions, such as sodium; metal complexes (e.g. Zn-protein complexes); and/or non-ionic surfactants, such as TWEEN™, PLURONICS™ or polyethylene glycol (PEG).

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.

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.

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 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 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, 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 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, weekly or monthly intervals, at the discretion of the physician. The treatment schedule for an individual may be dependent on the pharmocokinetic 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 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 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 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 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:

(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).

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

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 amino acid sequences are provided in Table 11. 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 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.

PD1 “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).

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.

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. 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.

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.

Table 1 shows the amino acid sequences of the Mu317 murine anti-PD1 antibody CDRs as defined herein (a "Unified" scheme), in comparison to well-known alternative systems for defining the same CDRs.

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):

	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
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
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
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
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, 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) 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.

"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.

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.

"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.

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.

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 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

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_{off} / k_{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.

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.

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 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 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 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 “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

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. 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 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.

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

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.

Materials and methods

PD1 library generation and selection

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

Production of soluble Fabs in individual *E. coli* clones was performed. *E. coli* TG1 cells in logarhythmic 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 were collected after shaking at room temperature and centrifugation.

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 Mu317 and hu317 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 FcγR interactions) and human Cκ domains, respectively. Co-transfection of heavy and light chain containing vector in mammalian expression system was

performed, followed by protein A-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 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 µg/ml. The Fab samples were applied at 1:5 dilution in 1% Skimmed Milk /1x PBS; 1h at RT; 100 µl/well. IgGs 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 IgG1null 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.

Flow cytometry of IgGs

Purified IgGs were tested in FACs for binding to CHO-K1 stable cell lines expressing human (Genscript, Cat. nr. M00529), and cyno (Genscript, Cat. nr. M00572) PD1, 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 (AttuneTM NxT Acoustic

Focusing Cytometer, Invitrogen/ ThermoFisher Scientific). The EC50 values were calculated using the MFI values in GraphPad Prism software (GraphPad Software, La Jolla, CA) and 4 parameters.

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), mu317 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.

Allogeneic mixed leucocyte reaction (MLR) assay

Human PBMCs from healthy donors were prepared from buffy coats. Immature monocyte-derived dendritic cells (Mo-DCs) were prepared by isolating CD14+ cells using magnetic-activated cell sorting (MACs, Stemcell) and cultured with differentiation medium (Miltenyi) for 7 days. At the end of this 7-day period the Mo-DCs were classed as 'immature' and are ready to use in an immature MLR. Responder T cells (using cryopreserved PBMCs) were prepared on the day required, from a different donor to that used for the Mo-DCs, using a T cell isolation kit (Stemcell). Under each experimental condition (immature and MoDCs) 6 separate MLRs were performed to provide biological variation, all conditions were prepared in technical triplicate. Cells were co-cultured: $1x10^4$ Mo-DC and $1x10^5$ CD4+ T cell per well +/- test IgG antibodies for 4 days. Single cell type (T cell/Mo-DC) controls were included in each assay. Supernatants were collected at the end of culture period and assessed for IFN-γ by ELISA.

Charge variant Analyses

Charge variant profiling of test articles was determined by Protein Charge Variant Assay on a LabChip GXII Touch HT (PerkinElmer, Beaconsfield, UK), according to the manufacturer's protocol.

DSC Analyses

The Tm 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 Tm of the protein was determined from the heating scan data.

Forced Oxidation Analyses

For forced oxidation analysis, test articles in PBS were treated with 0.5 % H2O2 at room temperature for 2 hours and then stored at - 80 °C prior to SEC, HIC and RP analysis (intact antibodies and subunits) on a Dionex Ultimate 3000RS HPLC system (ThermoFisher Scientific, Hemel Hempstead, UK), as detailed below. 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.

RP analysis of intact antibodies and subunits - Chromatographic separation was performed using a PLRP-S 1000, 5 µm, 2.1mm x 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 75% buffer A (0.075% TFA, 7.5% acetonitrile in H2O) to 50% buffer B (0.075% TFA, 7.5% H2O in acetonitrile) for mAbs 1-4, or 90% buffer B for mAb 5, 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.

SEC Analysis - Chromatographic separation was performed using an Acquity UPLC Protein BEH SEC Column, 200Å, 1.7 µm, 4.6 mm X 150 mm (Waters, Elstree, UK) and an Acquity UPLC Protein BEH SEC guard column 30 x 4.6 mm, 1.7 µm, 200 Å (Waters, Elstree, UK) connected to a Dionex Ultimate 3000RS HPLC system (ThermoFisher Scientific, Hemel Hempstead, UK). The method consisted of an isocratic elution over 15 minutes and the mobile phase was 0.2 M potassium phosphate pH 6.8, 0.2 M potassium chloride. The flow rate was 0.25 mL/minute. Detection was carried out by UV absorption at 280 nm.

HIC Analysis - 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.

Antibody v-domain specificity testing: human receptor array analyses

Human cell membrane receptor proteome arrays were performed at Retrogenix Ltd. Primary screens: 2 µg/ml of each antibody was screened for binding against fixed HEK293 cells/slides expressing 5528 human plasma membrane and tethered secreted proteins individually. 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. Confirmation/specificity screens: Expression vectors encoding ZsGreen1 only, or ZsGreen1 and CD20, PD1, BTN2A1, or LSAMP were transfected into human HEK293 cells. Each live transfectant was incubated with 2 µg/ml of each of the test antibodies, the positive control antibody Rituximab, or no primary 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.

Results and Discussion

CDR grafting onto preferred human germline v-genes

The CDRs of an agonistic murine anti-PD1 IgG Mu317 (WO2015/035606 A1 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 IGKV4-1, 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 hu317 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/IGKV4-1 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/IGKV4-1 v-domain sequences were combined into a Fab phage display format and a mutagenesis library cassette was generated by oligo synthesis and assembly. Two separate Fab libraries were initially constructed that sampled mutational diversity in either the heavy or light chain v-domain sequences in combination with the cognate paired grafted v-domain. Both VH and VL libraries were separately ligated into a phage display vector and transformed into *E. coli* via electroporation to generate 3.15×10^9 and 2.27×10^9 independent clones, respectively. Library build quality was verified by sequencing 96 clones per library. 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% and that positions sampled with non-binary amino acid content sampled the position in question with the intended amino acids. Libraries were rescued using helper phage M13 and selections performed on biotinylated human and cynomolgus monkey PD1-Fc proteins. After an initial round of selection, the two pools of mutated VH and VL domains were recombined into a secondary library and three further rounds of standard selection, plus two 'hammer-hug' rounds performed.

Post-selection screening (Fig. 1A, 1B) and DNA sequencing revealed the presence of 712 Fab clones that exhibited strong binding to human and cyno PD1 in ELISA and >50% inhibition of Mu317 IgG1 binding to human and cyno PD1 in Alphascreen assay. Amongst these 712 clones, germlining mutations were 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 7 top clones from this ranking were then sub-cloned into IgG expression vectors for further testing as below (Table 4).

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 712 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. 2A, 2B). 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 5 of 15 murine residues in the CDR-H1 and H2 exhibited retention frequency above 75% (Fig. 2B). In the V_L domain, only 6 of 12 murine CDR residues derived from the Mu317 sequence were retained with frequencies >75% (Fig. 2A). This analysis strongly suggested that the LCDR1 and LCDR3 sequences could be rendered close to germline identity to IGKV4-1.

Designs containing combination of those murine residues with RF > 75% were given the prefix "MH" (MH = Maximally Humanized). In total 12 initial designer V_H and V_L designer domain combinations were generated: MH1-MH12 (Table 4). The MH clones were generated by gene synthesis and (along with the 7 library-derived clones outlined above and positive controls Mu317 and hu317), cloned into human expression vectors for production in IgG1null format. All IgGs were readily expressed and purified from transient transfections of mammalian cells.

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 all library-derived clones and a select subset of designer clones (MH4, MH8, MH12) had human and cyno PD1 binding profiles similar to, or improved over, Mu317 (Fig. 3A, 3B). The majority of designer clones (MH1, MH2, MH3, MH5, MH6, MH7, MH9, MH19, MH11) exhibited reduced binding to one or both orthologs of PD1, demonstrating that the mutations found in these clones are disruptive to binding when used in combination. This finding highlighted the inability to predict precisely which combinations of mutations will lead to an optimal outcome, even in the possession of the mutational map outlined in Figs 2A and 2B.

An Alphascreen assay was established to allow the testing of designer IgGs for PD1 binding affinity and epitope competition with mu317 IgG binding to biotinylated monomeric human PD1. In this assay, while all 3 clones exhibited full epitope competition with Mu317, clone MH8 exhibited an IC50 value closest to mu317 (mu317 – 0.37 nM, MH8 – 0.39 nM, MH12 0.624 nM, MH4, 0.630 nM).

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

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 and designer clones exhibit concentration-dependent binding to membrane-presented human or cyno PD1 (Fig.

4A, 4B) with potencies similar to, or improved over, the hu317. No binding signals were observed 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. Lead clones MH4, MH8, MH12, 09C06, 17A12 and 20C07 demonstrated fold induction curves and maximal signal values highly similar to mu317 (Fig. 5). Maximally humanized clones MH4, MH8 and MH12 (Fig 6A, 6B, 6C, respectively) were then re-examined in this assay, being run alongside both mu317 and hu317 IgG1. These analyses showed that these three clones had fully maintained the potency of both mu317 and hu317, with concentration-dependent fold induction curves that are overlapping.

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 Hu317 and lead antibody v-domains. Analysis of the v-domain sequences was performed with overlapping 9mer peptides (with each overlapping the last 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 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 that are found 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 Figure 7 and Table 5, key lead v-domains exhibited significant beneficial changes in peptide epitope content in comparison to Hu317. As the v-domain engineering process undertaken here had successfully selected for antibodies that maintained anti-PD1 potency while humanizing a significant number of the murine residues included in the CDRs of Hu317 (Table 2), multiple HAF and LAF epitopes found in the v-domains of Hu317 (Fig. 7A) were absent in library-derived and designer leads (Table 5). Unexpectedly, GE epitope content was found to be significantly increased (from 5 to ≥ 14 in all leads), particularly in the VH regions of lead clones (Fig. 7B-H), and TCED+ epitopes were reduced in all leads (Table 5). The marked improvements in immunogenicity risk in the VH domain were mediated specifically by adapting the CDRs of Hu317 to fit the IGHV3-7 germline, which we have now shown (for the first time) contains more GE epitopes in its frameworks than are found in the IGHV4-4 VH grafted Hu317 frameworks. For example, the germlining of the last 7 residues of the HCDR2 sequence to match IGHV3-7 (YVDSVKG (SEQ ID NO:80) in all lead clones, Table 4) allowed a GE to be restored that spans the c-terminal HCDR2 and n-terminal framework 3 regions (VKGRFTISR (SEQ ID NO:81)). In addition, mutation of the HCDR1 sequence from 'GFSLTSYGVH' (SEQ ID NO:82) to the sequence 'GFTFSSYGMH' (SEQ ID NO:57) (found in clones 09C06, 17A12, 17D08, 17G08 and 15D07) not only ablated the LAF sequence 'LTSYGVHWI' (SEQ ID NO:83) which spans the HCDR1 and framework 2 regions of Hu317, but also rendered this CDR fully germline identity to the human v-genes IGHV3-33 and IGHV3-30-5. The HCDR1 sequence 'GFTFSSYGMS' (SEQ ID NO:33) (found in clones 20C07 and all designer clones MH1-MH18, Table 4) also ablated the LAF sequence 'LTSYGVHWI' (SEQ ID NO:83). Both humanized CDR1 sequences encoded a GE peptide sequence 'YGM(H/S)WVRQA' (SEQ ID NO:84) that was not found in either Hu317 or the library-derived clone 11C08, due to the presence of the residue valine in their HCDR1s at position 9 (e.g. GFSLTSYGVH (SEQ ID NO:82)).

Importantly, multiple foreign epitopes were also eliminated by germlining mutations found in the other CDRs of lead clones. For example, a TCED+ and LAF peptide 'YWYIDVWGQ' (SEQ ID NO:85) which spans the HCDR3 and framework 4 of Hu317 was eliminated in the

majority of lead clones by the mutation V>R at position 6 (Table 4). Similarly, in clones MH1-MH18 and 09C06, the HAF peptide 'LEWIGVIYA' (SEQ ID NO:86) and LAF peptide 'IGVIYADGS' (SEQ ID NO:87) which are both found in Hu317 were simultaneously ablated by mutation of the residues 'IGV' to 'VAN' (Table 4, Table 5). A HAF peptide 'LEWVGVIWQ' (SEQ ID NO:88) was found in the VH domain of clone 11C08, which overlaps with the 'LEWIGVIYA' (SEQ ID NO:86) HAF from Hu317, while the LAF peptide 'IGVIYADGS' (SEQ ID NO:87) was ablated. In clone 17A12, the 'LEWIGVIYA' (SEQ ID NO:86) HAF from Hu317 was partially ablated, by mutation to the LAF peptide 'LEWLANIWQ'.

In the VL region of Hu317 and all leads, the total balance of GE versus foreign epitopes was not increased (FIG. 7, TABLE 5), despite the introduction of the mutations outlined above that ablated the oxidation risk in the LCDR2 by mutating the F at position 3 of the LCDR2 (YAFHRFT (SEQ ID NO:31)). In total, the immunogenicity findings outlined above demonstrated that through extensive, yet unpredictable germlining of multiple CDR positions in the VH domain (specifically into the IGHV3-7 framework), the lead antibodies achieved the removal of several significant high-risk human t cell epitope peptide sequences and acquired several GEs with the potential to reduce immunogenicity, greatly improving the ratio of GE to foreign epitopes.

Lead IgG potency in MLR assay

The aim of this study was to characterise the potency of lead PD-1 targeted antibodies in an human allogeneic MLR, by measurement of IFN-γ in the culture supernatants. In control conditions (Fig. 8A) the cytokine levels demonstrated that a Nivolumab analog significantly increased IFN-γ levels compared to isotype control human IgG4. Isotype showed no difference compared to cells only, and the single cell conditions showed little to no measurable cytokine.

As all controls had demonstrated the expected signals, clones 09C06, MH4, MH8 and MH12 were examined for activity in the assay. The four test antibodies were used at three concentrations (0.1, 1 and 10µg/mL) and compared to the appropriate isotype control, human-IgG1. All four test antibodies significantly increased IFN-γ production within the cell culture supernatants in a dose- dependent manner (Fig. 8B). The data shows that all four test antibodies provided could enhance IFN-γ production in an allogeneic MLR when compared to hIgG1. Finally, clones Hu317, MH4 and MH8 were tested in the assay across a wide concentration range (Fig. 8C). This analysis demonstrated that both clones MH4 and

MH8 retained fully overlapping concentration-dependent activity with Hu317 in a relevant human *ex-vivo* potency assay.

Charge variant Analyses

Charge heterogeneity analysis is used widely in the characterisation of monoclonal antibodies because it provides important information about product quality and uniformity. Heterogeneity can be caused by enzymatic post-translational modifications such as glycosylation, lysine truncation, oxidation or deamidation. Heterogeneity in IgGs is a manufacturing risk and can cause increased complexity in bioprocessing protocols.

The Protein Charge Variant Assay allows identification of basic and acidic protein variants relative to the dominant product peak. The microfluidics chip technology resolves protein charge variants after fluorescent labelling. The charge variant profiles for 09C06, MH4, MH8 MH12 and hu317 IgGs are depicted in Figure 9. All of mAbs 09C06, MH4, MH8 and MH12 display a highly homogeneous profile, with the main isoform comprising 77.4-82.8% of the total protein (Table 6). In contrast, however, Hu317 showed an unexpectedly more complex profile with 4 distinct peaks, a higher percentage of basic isoforms (23.1%) and with the main isoform representing < 69% of total. These significant improvements in charge homogeneity for clones 09C06, MH4, MH8 and MH12 over Hu317 were not immediately explained by the removal of potential post-translational modification sites in the CDRs of the lead IgGs, as any potential o-link glycosylation, deamidation or isomerisation sites in the CDRs were maintained and no n-link glycosylation sites had been introduced (Tables 2 and 4). As such improved homogeneity could also be caused by improvements in thermal stability, or improved resistance to oxidation, we investigated those characteristics.

Thermal Stability Evaluation Using DSC

A protein's DSC profile provides information about its total structural stability, via its thermal stability, but can also represent a structural 'fingerprint' for assessing structural modifications. The thermogram of an intact antibody could present two or three peaks, with the largest peak containing the contribution from the Fab fragment. Usually the first transition covers the unfolding of the CH2 domain, while the last one would include the unfolding of the CH3 domain. The Fab unfolding could occur at a distinct temperature compared to the unfolding of CH2 and CH3 domains, but it is also possible that the Fab fragment unfolding 'buries' the unfolding of either CH2 or CH3 domains, resulting in peak overlap. As the constant domains are virtually identical for antibodies of the same subclass, the CH2 and CH3 domains should have comparable Tm, while the Fab's Tm will vary from antibody to antibody.

All five IgGs tested (09C06, MH4, MH8, MH12 and Hu317) were fully compatible with DSC analysis and gave good resolution data (Fig. 10), presenting comparable sample homogeneity and cooperativity. In Figure 10A (09C06 IgG), the first transition represents CH2 domain unfolding, the second transition represents the unfolding of the Fab fragment and the third transition represents CH3 domain unfolding. For clones MH4, MH8 and MH12, the major Fab transition is lower, overlapping with the CH2 domain (Fig. 10B-D). For clone Hu317, the Fab domain transition is actually higher and overlapping with the CH3 (Fig. 10E). Overall, the Fab unfolding Tm values demonstrated that the v-domains of lead clones 09C06, MH4, MH8 and MH12, while still relatively stable, were actually reduced in overall stability in comparison to those of Hu317 (Table 7).

Forced Oxidation Analyses

Oxidation of exposed amino acid residues, such as tryptophan and methionine is a common degradation pathway for mAbs, altering their structural integrity with potential impact on their biological activity and heterogeneity. In this study, forced oxidation using 0.5% H2O2 for 2 hours at room temperature was applied to IgGs 09C06, MH4, MH8, MH12 and Hu317. As oxidation can alter overall hydrophobicity of an antibody, either by increasing the polarity of the oxidised form or through conformational changes, potential changes induced by forced oxidation were analysed by Size Exclusion (SEC), Reverse Phase (RP) and Hydrophobic Interaction (HIC) Chromatographies.

SEC analysis - No significant changes in SEC profile, induced by forced oxidation, were observed for any of the five IgGs, with higher molecular weight isoforms remaining below 0.6% of total protein (Table 8).

RP analysis - No significant changes in the retention time of intact mAbs (non-reduced and reduced) were observed upon H2O2 treatment, implying minimal oxidation of exposed amino acids (Table 8).

HIC analysis - H2O2 treatment induced only a minor (0.4-0.6) minutes decrease in the retention on HIC for all 5 test articles, implying limited oxidation of exposed amino acids (Table 8).

Together, these data confirmed that the lead IgGs 09C06, MH4, MH8 and MH12 did not exhibit improved stability or reduced oxidative degradation potential in comparison to Hu317 and that neither stability changes nor changes in post-translational CDR modification motifs were responsible for the observed improvements in charge homogeneity.

Antibody binding specificity analyses

To examine the proteomic selectivity of Hu317 and clones MH4, MH8 and MH12, *in vitro* technologies (Retrogenix, Ltd.), which are based on using high-density arrays of cells expressing 5528 unique human membrane receptors and tethered secreted proteins, were used to screen for off-target binding specificities in purified IgG form. This receptor array binding screen identified that Hu317 exhibited strong binding to membrane-expressed PD1 exclusively, with no measurable binding to any other proteins (Table 12). In contrast, clones MH4, MH8 and MH12 also exhibited very limited signals, with strongest signal for PD1, but also had 2 potential new binding specificities: BNT2A1 (accession number NM_007049) and LSAMP (BC033803). MH12 was the only clone to show any binding signal to BNT2A1. To examine these findings with an orthogonal, high-sensitivity assay, the plasmids encoding for CD20, PD1, BTN2A1, LSAMP, and ZS green only (negative control) were used to perform transient transfection of the human cell line HEK293. Transfected cells were then stained using Hu317, MH4, MH8, MH12 and Rituximab IgGs. In staining of PD1-transfected cells at 2 μg/ml, all tested antibodies other than the Rituximab and secondary only controls showed the expected strong, specific staining of PD1-transfected cells but not ZS green-transfected (Fig. 11). Hu317 IgG showed no binding to any cells other than PD1-transfected cells, confirming the findings of the chip analyses, where this IgG was fully PD1 selective. Rituximab positive control exhibited strong, specific staining of CD20-transfected cells and the negative control (secondary labelled antibody only) exhibited no binding to any cells (Fig. 11). In contrast, only clones MH8 and MH12 exhibited clear binding signal on both PD1 and LSAMP, but no binding of BTN2A1, both in histogram analysis (Fig. 11) and in median fold-change of mean fluorescence measurements over ZSgreen-only transfected cells (Table 13). This unexpected, newly-acquired, reactivity to a single new protein in the human receptor proteome for clones MH8 and MH12 may be of potential therapeutic benefit, as LSAMP is a cell surface receptor whose expression has been shown to be upregulated in specific tumour types such as Acute Myeloid Leukaemia (Coccaro, N. *et al.* Cancer Genetics 2015, 208(10):517-522) and may therefore be of value in co-targeting along with PD1.

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 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.

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, that they constitute valid prior art or form part of the common general knowledge in any country in the world.

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

Scheme	HCDR1	HCDR2	HCDR3	LCDR1	LCDR2	LCDR3
Unified	GFSLTSYGVH (SEQ ID NO:89)	LGVIWAGGSTNYNSALMS (SEQ ID NO:94)	AYGNYWYIDV (SEQ ID NO:101)	KASQSVSNDVA (SEQ ID NO:105)	YAFHRFT (SEQ ID NO:109)	HQAYSSPYT (SEQ ID NO:111)
Kabat	SYGVH (SEQ ID NO:90)	VIWAGGSTNYNSALMS (SEQ ID NO:95)	AYGNYWYIDV (SEQ ID NO:101)	KASQSVSNDVA (SEQ ID NO:105)	YAFHRFT (SEQ ID NO:109)	HQAYSSPYT (SEQ ID NO:111)
Chotia	GFSLTSY (SEQ ID NO:91)	WAGGST (SEQ ID NO:96)	AYGNYWYIDV (SEQ ID NO:101)	KASQSVSNDVA (SEQ ID NO:105)	YAFHRFT (SEQ ID NO:109)	HQAYSSPYT (SEQ ID NO:111)
IMGT	GFSLTSYG (SEQ ID NO:92)	IWAGGSTN (SEQ ID NO:97)	ARAYGNYWYIDV (SEQ ID NO:102)	QSVSND (SEQ ID NO:106)	YAF	HQAYSSPYT (SEQ ID NO:111)
AHo	GFSLTSYGVH (SEQ ID NO:89)	IWAGGSTNYNSALMS (SEQ ID NO:98)	AYGNYWYID (SEQ ID NO:103)	ASQSVSND (SEQ ID NO:107)	YAFHRFT (SEQ ID NO:109)	AYSSPY (SEQ ID NO:112)
AbM	GFSLTSYGVH (SEQ ID NO:89)	VIWAGGSTNY (SEQ ID NO:99)	AYGNYWYIDV (SEQ ID NO:101)	KASQSVSNDVA (SEQ ID NO:105)	YAFHRFT (SEQ ID NO:109)	HQAYSSPYT (SEQ ID NO:111)
Contact	TSYGVH (SEQ ID NO:93)	LGVIWAGGSTNY (SEQ ID NO:100)	ARAYGNYWYID (SEQ ID NO:104)	SNDVAWY (SEQ ID NO:108)	LLIYYAFHRF (SEQ ID NO:110)	HQAYSSPY (SEQ ID NO:113)

Table 2. Amino acid sequence of MU317 murine anti-PD1 v-domains (mVH/mVL) and human germline CDR grafts (VH1/VL1).

V DOMAIN	Human germline[1]	Amino acid sequence[2]
Mu317 VH	n/a	QVQLKESGPGLVAPSKNLSITCTVS**GFSLTSYGVH**WIRQPPGKGLEW**LGVIWAGGSTNYNSALMS**RLSISKDNSRSQVFLRMNSLQTDDTAMYYCAR**AYGNYWYIDV**WGAGTTVTVSS
		(SEQ ID NO:114)
Hu317 VH	IGHV4-4	QVQLQESGPGLVKPSETLSLTCTVS**GFSLTSYGVH**WIRQPPGKGLEW**IGVIYADGSTNYNPSLKS**RVTISKDTSKNQVSLKLSSVTAADTAVYYCAR**AYGNYWYIDV**WGQGTTVTVSS
		(SEQ ID NO:115)
VH GRAFT	IGHV3-7[3]	EVQLVESGGGLVQPGGSLRLSCAAS**GFSLTSYGVH**WVRQAPGKGLEW**LGVIWAGGSTNYNDSVKG**RFTISRDNAKNSLYLQMNSLRAEDTAVYYCAR**AYGNYWYIDV**WGQGTTVTVSS
		(SEQ ID NO:116)
Mu317 VL	n/a	DIVMTQTPKFLLVSAGDRVTITC**KASQSVSNDVA**WYQQKPGQSPKLLIN**YAFHRFT**GVPDRFTGSGYGTDFIFTISTVQAEDLAVYFC**HQAYSSPYT**FGGGTKLEMK
		(SEQ ID NO:117)
Hu317 VL	IGKV4-1	DIVMTQSPDSLAVSLGERATINC**KSSESVSNDVA**WYQQKPGQPPKLLIN**YAFHRFT**GVPDRFSGSGYGTDFTLTISSLQAEDVAVYYC**HQAYSSPYT**FGQGTKLEIK
		(SEQ ID NO:118)
VL GRAFT	IGKV4-1[3]	DIVMTQSPDSLAVSLGERATINC**KSSQSVSNDVA**WYQQKPGQPPKLLIN**YAFHRFT**GVPDRFSGSGYGTDFTLTISSLQAEDVAVYYC**HQAYSSPYT**FGQGTKLEIK
		(SEQ ID NO:119)

[1]Human germline definitions used for grafting, based on IMGT system. [2]CDR 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. [3]Grafts used for library construction.

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

HCDR1	HCDR2	HCDR2 contd	HCDR2 contd	HCDR3	LCDR1	LCDR2	LCDR3
GFNFSSYGMH (SEQ ID NO:120)	LANIKADGSEKYVDSVKG (SEQ ID NO:147)	LGNIKQGGSENYNDSVKG (SEQ ID NO:210)	VANIWQDGSTNYNDSVKG (SEQ ID NO:233)	AFGNYWYIDV (SEQ ID NO:337)	KSSESVGNDLA (SEQ ID NO:397)	YAFHRES (SEQ ID NO:400)	HQAYSNPYT (SEQ ID NO:407)
GFSFSSYGMH (SEQ ID NO:42)	LANIKADGSENYNDSVKG (SEQ ID NO:148)	LGNIKQGGSTKYVDSVKG (SEQ ID NO:211)	VANIWQGGSEKYNDSVKG (SEQ ID NO:275)	AWGNYWYIDV (SEQ ID NO:338)	KSSESVGNDVA (SEQ ID NO:398)	YAFHRET (SEQ ID NO:401)	HQAYSTPYT (SEQ ID NO:46)
GFSFSSYGMS (SEQ ID NO:121)	LANIKADGSTKYVDSVKG (SEQ ID NO:149)	LGNIKQGGSTNYNDSVKG (SEQ ID NO:212)	VANIWQGGSTKYNDSVKG (SEQ ID NO:276)	AYGAYWYIDV (SEQ ID NO:339)	KSSESVSNDLA (SEQ ID NO:399)	YAFHRFA (SEQ ID NO:402)	QQAYSSPYT (SEQ ID NO:75)
GFSFSSYGVH (SEQ ID NO:122)	LANIKADGSTNYVDSVKG (SEQ ID NO:150)	LGNIWADGSEKYNDSVKG (SEQ ID NO:213)	VAVIKADGSTNYNDSVKG (SEQ ID NO:277)	AYGEYWYIDV (SEQ ID NO:340)	KSSQSVGNDLA (SEQ ID NO:77)	YAFHRFS (SEQ ID NO:403)	QQAYSTPYT (SEQ ID NO:52)
GFSFSSYGVS (SEQ ID NO:123)	LANIKAGGSEKYNDSVKG (SEQ ID NO:151)	LGNIWADGSEKYVDSVKG (SEQ ID NO:214)	VAVIKQDGSENYVDSVKG (SEQ ID NO:278)	AYGFYWYIDV (SEQ ID NO:341)	KSSQSVGNDVA (SEQ ID NO:74)	YAFPRFT (SEQ ID NO:404)	QQSYSTPYT (SEQ ID NO:408)
GFSFSSYWMH (SEQ ID NO:124)	LANIKAGGSEKYVDSVKG (SEQ ID NO:152)	LGNIWADGSENYNDSVKG (SEQ ID NO:215)	VAVIKQDGSTKYNDSVKG (SEQ ID NO:279)	AYGGYWYIDV (SEQ ID NO:342)	KSSQSVSNDLA (SEQ ID NO:51)	YAFTRFS (SEQ ID NO:405)	
GFSFTSYGMH (SEQ ID NO:59)	LANIKAGGSENYNDSVKG (SEQ ID NO:153)	LGNIWADGSENYVDSVKG (SEQ ID NO:216)	VAVIKQDGSTNYNDSVKG (SEQ ID NO:280)	AYGHYWYIDV (SEQ ID NO:343)	KSSQSVSNDVA (SEQ ID NO:44)	YAFTRFT (SEQ ID NO:406)	
GFSFTSYGMS (SEQ ID NO:47)	LANIKQDGSEKYVDSVKG (SEQ ID NO:154)	LGNIWADGSTKYNDSVKG (SEQ ID NO:217)	VAVIKQDGSTNYVDSVKG (SEQ ID NO:281)	AYGIYWYIDN (SEQ ID NO:344)	KSSQSVTNDVA (SEQ ID NO:36)	YASHRFS (SEQ ID NO:79)	
GFSFTSYGVH (SEQ ID NO:125)	LANIKQDGSENYVDSVKG (SEQ ID NO:155)	LGNIWADGSTKYVDSVKG (SEQ ID NO:218)	VAVIWADGSEKYVDSVKG (SEQ ID NO:282)	AYGNVWYIDV (SEQ ID NO:396)		YASHRFT (SEQ ID NO:45)	
GFSFTSYGVS (SEQ ID NO:126)	LANIKQDGSTKYNDSVKG (SEQ ID NO:156)	LGNIWADGSTNYNDSVKG (SEQ ID NO:219)	VAVIWADGSENYNDSVKG (SEQ ID NO:283)	AYGNWWYIDV (SEQ ID NO:345)		YAYHRFT (SEQ ID NO:37)	
GFSFTSYWMH (SEQ ID NO:127)	LANIKQDGSTKYVDSVKG (SEQ ID NO:157)	LGNIWADGSTNYVDSVKG (SEQ ID NO:220)	VAVIWADGSTKYNDSVKG (SEQ ID NO:284)	AYGNYFYIDV (SEQ ID NO:346)		YAYHRFS (SEQ ID NO:78)	
GFSLSSYGIS (SEQ ID NO:128)	LANIWADGSEKYNDSVKG (SEQ ID NO:158)	LGNIWAGGSENYVDSVKG (SEQ ID NO:221)	VAVIWADGSTKYVDSVKG (SEQ ID NO:285)	AYGNYFYIDW (SEQ ID NO:347)			
GFSLSSYGMH (SEQ ID NO:129)	LANIWADGSEKYVDSVKG (SEQ ID NO:159)	LGNIWQDGSEKYNDSVKG (SEQ ID NO:222)	VAVIWADGSTNYNDSVKG (SEQ ID NO:286)	AYGNYHYIDV (SEQ ID NO:348)			

GFSLSSYGMS (SEQ ID NO:39)	LANIWADGSENYNDSVKG (SEQ ID NO:160)	LGNIWQDGSEKYVDSVKG (SEQ ID NO:223)	VAVIWQDGSEKYNDSVKG (SEQ ID NO:287)	AYGNYMYIDV (SEQ ID NO:56)			
GFSLSSYGVH (SEQ ID NO:130)	LANIWADGSENYVDSVKG (SEQ ID NO:161)	LGNIWQDGSENYNDSVKG (SEQ ID NO:224)	VAVIWQDGSENYNDSVKG (SEQ ID NO:288)	AYGNYPYIDV (SEQ ID NO:349)			
GFSLSSYGVS (SEQ ID NO:131)	LANIWADGSTKYNDSVKG (SEQ ID NO:162)	LGNIWQDGSENYVDSVKG (SEQ ID NO:225)	VAVIWQDGSENYVDSVKG (SEQ ID NO:289)	AYGNYQYIDV (SEQ ID NO:350)			
GFSLSSYWMH (SEQ ID NO:132)	LANIWADGSTKYVDSVKG (SEQ ID NO:163)	LGNIWQDGSTKYNDSVKG (SEQ ID NO:226)	VAVIWQDGSTKYNDSVKG (SEQ ID NO:290)	AYGNYSYIDV (SEQ ID NO:351)			
GFSLTSYWMH (SEQ ID NO:133)	LANIWADGSTNYNDSVKG (SEQ ID NO:164)	LGNIWQDGSTKYVDSVKG (SEQ ID NO:227)	VAVIWQDGSTKYVDSVKG (SEQ ID NO:291)	AYGNYVYIDV (SEQ ID NO:352)			
GFTFSSYGMH (SEQ ID NO:57)	LANIWADGSTNYVDSVKG (SEQ ID NO:165)	LGNIWQDGSTNYNDSVKG (SEQ ID NO:228)	VAVIWQDGSTNYNDSVKG (SEQ ID NO:292)	AYGNYWYADV (SEQ ID NO:353)			
GFTFSSYGMS (SEQ ID NO:33)	LANIWAGGSTNYNDSVKG (SEQ ID NO:166)	LGNIWQDGSTNYVDSVKG (SEQ ID NO:229)	VAVIWQDGSTNYVDSVKG (SEQ ID NO:40)	AYGNYWYGDV (SEQ ID NO:354)			
GFTFSSYGVH (SEQ ID NO:49)	LANIWQDGSEKYNDSVKG (SEQ ID NO:167)	LGNIWQGGSTNYNDSVKG (SEQ ID NO:230)	VAVIWQGGSTKYNDSVKG (SEQ ID NO:293)	AYGNYWYIDA (SEQ ID NO:355)			
GFTFSSYGVS (SEQ ID NO:134)	LANIWQDGSEKYVDSVKG (SEQ ID NO:168)	LGVIKADGSENYNDSVKG (SEQ ID NO:231)	VGNIKADGSENYNDSVKG (SEQ ID NO:294)	AYGNYWYIDE (SEQ ID NO:356)			
GFTFTSYGMH (SEQ ID NO:135)	LANIWQDGSENYNDSVKG (SEQ ID NO:169)	LGVIKADGSTKYNDSVKG (SEQ ID NO:232)	VGNIKADGSTKYNDSVKG (SEQ ID NO:295)	AYGNYWYIDF (SEQ ID NO:357)			
GFTFTSYGMS (SEQ ID NO:136)	LANIWQDGSENYVDSVKG (SEQ ID NO:53)	LGVIKADGSTNYNDSVKG (SEQ ID NO:234)	VGNIKADGSTNYNDSVKG (SEQ ID NO:296)	AYGNYWYIDG (SEQ ID NO:358)			
GFTFTSYGVH (SEQ ID NO:137)	LANIWQDGSTKYNDSVKG (SEQ ID NO:170)	LGVIKQDGSENYNDSVKG (SEQ ID NO:235)	VGNIKAGGSTNYVDSVKG (SEQ ID NO:297)	AYGNYWYIDH (SEQ ID NO:359)			
GFTFTSYGVS (SEQ ID NO:138)	LANIWQDGSTKYVDSVKG (SEQ ID NO:171)	LGVIKQDGSTKYNDSVKG (SEQ ID NO:236)	VGNIKQDGSENYNDSVKG (SEQ ID NO:298)	AYGNYWYIDI (SEQ ID NO:360)			
GFTLSSYGMH (SEQ ID NO:54)	LANIWQDGSTNYNDSVKG (SEQ ID NO:172)	LGVIKQDGSTKYVDSVKG (SEQ ID NO:237)	VGNIKQDGSENYVDSVKG (SEQ ID NO:299)	AYGNYWYIDK (SEQ ID NO:361)			
GFTLSSYGMS (SEQ ID NO:139)	LANIWQDGSTNYVDSVKG (SEQ ID NO:48)	LGVIKQDGSTNYNDSVKG (SEQ ID NO:238)	VGNIKQDGSTKYNDSVKG (SEQ ID NO:300)	AYGNYWYIDL (SEQ ID NO:362)			

GFTLSSYGVH (SEQ ID NO:140)	LANIWQGGSENYVDSVKG (SEQ ID NO:173)	LGVIKQGGSENYNDSVKG (SEQ ID NO:239)	VGNIKQDGSTNYNDSVKG (SEQ ID NO:301)	AYGNYWYIDM (SEQ ID NO:363)		
GFTLSSYGVS (SEQ ID NO:141)	LAVIKADGSEKYNDSVKG (SEQ ID NO:174)	LGVIWADGSEKYNDSVKG (SEQ ID NO:240)	VGNIKQDGSTNYVDSVKG (SEQ ID NO:302)	AYGNYWYIDR (SEQ ID NO:35)		
GFTLSSYWMH (SEQ ID NO:142)	LAVIKADGSENYNDSVKG (SEQ ID NO:175)	LGVIWADGSENYNDSVKG (SEQ ID NO:241)	VGNIKQGGSTKYNDSVKG (SEQ ID NO:303)	AYGNYWYIDS (SEQ ID NO:364)		
GFTLTSYGMH (SEQ ID NO:143)	LAVIKADGSTKYVDSVKG (SEQ ID NO:176)	LGVIWADGSTKYNDSVKG (SEQ ID NO:242)	VGNIWADGSENYNDSVKG (SEQ ID NO:304)	AYGNYWYIDT (SEQ ID NO:365)		
GFTLTSYGMS (SEQ ID NO:144)	LAVIKADGSTNYNDSVKG (SEQ ID NO:177)	LGVIWADGSTKYVDSVKG (SEQ ID NO:243)	VGNIWADGSTKYVDSVKG (SEQ ID NO:305)	AYGNYWYIDW (SEQ ID NO:366)		
GFTLTSYGVH (SEQ ID NO:145)	LAVIKAGGSTNYVDSVKG (SEQ ID NO:178)	LGVIWADGSTNYNDSVKG (SEQ ID NO:244)	VGNIWADGSTNYNDSVKG (SEQ ID NO:306)	AYGNYWYIDY (SEQ ID NO:367)		
GFTLTSYGVS (SEQ ID NO:146)	LAVIKQDGSEKYNDSVKG (SEQ ID NO:179)	LGVIWADGSTNYVDSVKG (SEQ ID NO:245)	VGNIWADGSTNYVDSVKG (SEQ ID NO:307)	AYGNYWYIEV (SEQ ID NO:368)		
	LAVIKQDGSTNYNDSVKG (SEQ ID NO:180)	LGVIWADGSTSYNDSVKG (SEQ ID NO:246)	VGNIWQDGSEKYNDSVKG (SEQ ID NO:308)	AYGNYWYLDV (SEQ ID NO:369)		
	LAVIKQDGSTNYVDSVKG (SEQ ID NO:181)	LGVIWAGGSTNYNDSVKG (SEQ ID NO:247)	VGNIWQDGSEKYVDSVKG (SEQ ID NO:309)	AYGNYWYMDV (SEQ ID NO:370)		
	LAVIWADGSEKYNDSVKG (SEQ ID NO:182)	LGVIWPDGSTNYNDSVKG (SEQ ID NO:248)	VGNIWQDGSENYVDSVKG (SEQ ID NO:310)	AYGNYWYSDV (SEQ ID NO:371)		
	LAVIWADGSENYNDSVKG (SEQ ID NO:183)	LGVIWQDGSEKYNDSVKG (SEQ ID NO:249)	VGNIWQDGSTKYNDSVKG (SEQ ID NO:311)	AYGNYWYTDV (SEQ ID NO:372)		
	LAVIWADGSENYVDSVKG (SEQ ID NO:184)	LGVIWQDGSEKYVDSVKG (SEQ ID NO:250)	VGNIWQDGSTKYVDSVKG (SEQ ID NO:312)	AYGNYWYVDV (SEQ ID NO:373)		
	LAVIWADGSTKYNDSVKG (SEQ ID NO:185)	LGVIWQDGSENYNDSVKG (SEQ ID NO:251)	VGNIWQDGSTNYNDSVKG (SEQ ID NO:313)	AYGNYWYYDV (SEQ ID NO:374)		
	LAVIWADGSTKYVDSVKG (SEQ ID NO:186)	LGVIWQDGSENYVDSVKG (SEQ ID NO:252)	VGNIWQDGSTNYVDSVKG (SEQ ID NO:314)	AYGQYWYIDV (SEQ ID NO:375)		
	LAVIWADGSTNYNDSVKG (SEQ ID NO:187)	LGVIWQDGSTKYNDSVKG (SEQ ID NO:253)	VGNIWQGGSEKYNDSVKG (SEQ ID NO:315)	AYGRYWYIDV (SEQ ID NO:376)		

LAVIWADGSTNYVDSVKG (SEQ ID NO:55)	LGVIWQDGSTKYVDSVKG (SEQ ID NO:254)	VGVIKADGSTKYNDSVKG (SEQ ID NO:316)	AYGSYWYIDV (SEQ ID NO:377)			
LAVIWAGGSEKYNDSVKG (SEQ ID NO:188)	LGVIWQDGSTNYNDSVKG (SEQ ID NO:255)	VGVIKADGSTNYNDSVKG (SEQ ID NO:317)	AYGTYWYIDV (SEQ ID NO:378)			
LAVIWAGGSENYNDSVKG (SEQ ID NO:189)	LGVIWQDGSTNYVDSVKG (SEQ ID NO:256)	VGVIKADGSTNYVDSVKG (SEQ ID NO:318)	AYGVYWYIDR (SEQ ID NO:379)			
LAVIWAGGSTKYNDSVKG (SEQ ID NO:190)	LGVIWQGGSTKYNDSVKG (SEQ ID NO:257)	VGVIKQDGSENYNDSVKG (SEQ ID NO:319)	AYGVYWYIDV (SEQ ID NO:380)			
LAVIWQDGSEKYNDSVKG (SEQ ID NO:191)	LGVIWQGGSTNYNDSVKG (SEQ ID NO:258)	VGVIKQDGSTKYNDSVKG (SEQ ID NO:320)	AYGWYWYIDV (SEQ ID NO:381)			
LAVIWQDGSEKYVDSVKG (SEQ ID NO:192)	VANIKADGSEKYVDSVKG (SEQ ID NO:259)	VGVIKQDGSTNYNDSVKG (SEQ ID NO:321)	AYGYYWYIDV (SEQ ID NO:382)			
LAVIWQDGSENYNDSVKG (SEQ ID NO:193)	VANIKADGSENYVDSVKG (SEQ ID NO:260)	VGVIWADGSEKYNDSVKG (SEQ ID NO:322)	DYGNYWYIDV (SEQ ID NO:383)			
LAVIWQDGSENYVDSVKG (SEQ ID NO:194)	VANIKADGSTKYVDSVKG (SEQ ID NO:261)	VGVIWADGSENYNDSVKG (SEQ ID NO:323)	EYGNYWYIDR (SEQ ID NO:384)			
LAVIWQDGSTKYNDSVKG (SEQ ID NO:195)	VANIKQDGSEKYNDSVKG (SEQ ID NO:262)	VGVIWADGSENYVDSVKG (SEQ ID NO:324)	EYGNYWYIDV (SEQ ID NO:385)			
LAVIWQDGSTKYVDSVKG (SEQ ID NO:196)	VANIKQDGSEKYVDSVKG (SEQ ID NO:263)	VGVIWADGSTKYNDSVKG (SEQ ID NO:325)	GYGNYWYIDV (SEQ ID NO:61)			
LAVIWQDGSTNYNDSVKG (SEQ ID NO:197)	VANIKQDGSTKYNDSVKG (SEQ ID NO:264)	VGVIWADGSTKYVDSVKG (SEQ ID NO:326)	HYGNYWYIDV (SEQ ID NO:386)			
LAVIWQDGSTNYVDSVKG (SEQ ID NO:198)	VANIKQDGSTNYNDSVKG (SEQ ID NO:265)	VGVIWADGSTNYNDSVKG (SEQ ID NO:327)	IYGNYWYIDR (SEQ ID NO:387)			
LAVIWQGGSEKYNDSVKG (SEQ ID NO:199)	VANIWADGSEKYNDSVKG (SEQ ID NO:266)	VGVIWADGSTNYVDSVKG (SEQ ID NO:328)	IYGNYWYIDV (SEQ ID NO:388)			
LAVIWQGGSENYNDSVKG (SEQ ID NO:200)	VANIWADGSTKYNDSVKG (SEQ ID NO:267)	VGVIWQDGSEKYNDSVKG (SEQ ID NO:329)	LYGNYWYIDV (SEQ ID NO:389)			
LGNIKADGSENYNDSVKG (SEQ ID NO:201)	VANIWADGSTNYNDSVKG (SEQ ID NO:268)	VGVIWQDGSEKYVDSVKG (SEQ ID NO:330)	MYGNYWYIDV (SEQ ID NO:390)			

LGNIKADGSTKYNDSVKG (SEQ ID NO:202)	VANIWADGSTNYVDSVKG (SEQ ID NO:269)	VGVIWQDGSENYNDSVKG (SEQ ID NO:331)	QYGNYWYIDR (SEQ ID NO:391)			
LGNIKADGSTKYVDSVKG (SEQ ID NO:203)	VANIWAGGSENYNDSVKG (SEQ ID NO:270)	VGVIWQDGSENYVDSVKG (SEQ ID NO:50)	QYGNYWYIDV (SEQ ID NO:392)			
LGNIKADGSTNYNDSVKG (SEQ ID NO:204)	VANIWQDGSEKYNDSVKG (SEQ ID NO:271)	VGVIWQDGSTKYNDSVKG (SEQ ID NO:332)	SYGNYWYIDV (SEQ ID NO:393)			
LGNIKAGGSTNYNDSVKG (SEQ ID NO:205)	VANIWQDGSEKYVDSVKG (SEQ ID NO:41)	VGVIWQDGSTKYVDSVKG (SEQ ID NO:333)	VYGNYWYIDV (SEQ ID NO:394)			
LGNIKAGGSTNYVDSVKG (SEQ ID NO:206)	VANIWQDGSENYNDSVKG (SEQ ID NO:272)	VGVIWQDGSTNYNDSVKG (SEQ ID NO:334)	WYGNYWYIDV (SEQ ID NO:395)			
LGNIKQDGSENYNDSVKG (SEQ ID NO:207)	VANIWQDGSENYVDSVKG (SEQ ID NO:273)	VGVIWQDGSTNYVDSVKG (SEQ ID NO:60)				
LGNIKQDGSTKYNDSVKG (SEQ ID NO:208)	VANIWQDGSTKYNDSVKG (SEQ ID NO:274)	VGVIWQGGSEKYVDSVKG (SEQ ID NO:335)				
LGNIKQDGSTNYNDSVKG (SEQ ID NO:209)		VGVIWQGGSTNYNDSVKG (SEQ ID NO:336)				

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

Clone name	LCDR1	LCDR2	LCDR3	HCDR1	HCDR2	HCDR3
20C07	KSSQSVSNDVA (SEQ ID NO:44)	YASHRFT (SEQ ID NO:45)	HQAYSTPYT (SEQ ID NO:46)	GFSFTSYGMS (SEQ ID NO:47)	LANIWQDGSTNYVDSVKG (SEQ ID NO:48)	AYGNYWYIDR (SEQ ID NO:35)
11C08	KSSQSVSNDLA (SEQ ID NO:51)	YASHRFT (SEQ ID NO:45)	QQAYSTPYT (SEQ ID NO:52)	GFTFSSYGVH (SEQ ID NO:49)	VGVIWQDGSENYVDSVKG (SEQ ID NO:50)	AYGNYWYIDR (SEQ ID NO:35)
09C06	KSSQSVSNDVA (SEQ ID NO:44)	YASHRFT (SEQ ID NO:45)	HQAYSTPYT (SEQ ID NO:46)	GFSFSSYGMH (SEQ ID NO:42)	VANIWQDGSTNYVDSVKG (SEQ ID NO:43)	AYGNYWYIDR (SEQ ID NO:35)
17A12	KSSQSVSNDVA (SEQ ID NO:44)	YASHRFT (SEQ ID NO:45)	QQAYSTPYT (SEQ ID NO:52)	GFSFSSYGMH (SEQ ID NO:42)	LANIWQDGSENYVDSVKG (SEQ ID NO:53)	AYGNYWYIDR (SEQ ID NO:35)
17D08	KSSQSVSNDVA (SEQ ID NO:44)	YASHRFT (SEQ ID NO:45)	HQAYSTPYT (SEQ ID NO:46)	GFTLSSYGMH (SEQ ID NO:54)	LAVIWADGSTNYVDSVKG (SEQ ID NO:55)	AYGNYMYIDV (SEQ ID NO:56)
17G08	KSSQSVSNDVA (SEQ ID NO:44)	YASHRFT (SEQ ID NO:45)	HQAYSTPYT (SEQ ID NO:46)	GFTFSSYGMH (SEQ ID NO:57)	VGVIKQDGSENYVDSVKG (SEQ ID NO:58)	AYGNYWYIDR (SEQ ID NO:35)
15D07	KSSQSVSNDLA (SEQ ID NO:51)	YASHRFT (SEQ ID NO:45)	QQAYSTPYT (SEQ ID NO:52)	GFSFTSYGMH (SEQ ID NO:59)	VGVIWQDGSTNYVDSVKG (SEQ ID NO:60)	GYGNYWYIDV (SEQ ID NO:61)
MH1	KSSQSVGNDVA (SEQ ID NO:74)	YASHRFT (SEQ ID NO:45)	QQAYSSPYT (SEQ ID NO:75)	GFSLSSYGMS (SEQ ID NO:39)	VAVIWQDGSTNYVDSVKG (SEQ ID NO:40)	AYGNYWYIDR (SEQ ID NO:35)
MH2	KSSQSVTNDLA (SEQ ID NO:76)	YASHRFT (SEQ ID NO:45)	QQAYSTPYT (SEQ ID NO:52)	GFSLSSYGMS (SEQ ID NO:39)	VAVIWQDGSTNYVDSVKG (SEQ ID NO:40)	AYGNYWYIDR (SEQ ID NO:35)
MH3	KSSQSVGNDLA (SEQ ID NO:77)	YAYHRFS (SEQ ID NO:78)	QQAYSTPYT (SEQ ID NO:52)	GFSLSSYGMS (SEQ ID NO:39)	VAVIWQDGSTNYVDSVKG (SEQ ID NO:40)	AYGNYWYIDR (SEQ ID NO:35)
MH4	KSSQSVTNDVA (SEQ ID NO:36)	YAYHRFT (SEQ ID NO:37)	HQAYSTPYT (SEQ ID NO:46)	GFSLSSYGMS (SEQ ID NO:39)	VAVIWQDGSTNYVDSVKG (SEQ ID NO:40)	AYGNYWYIDR (SEQ ID NO:35)
MH5	KSSQSVGNDVA (SEQ ID NO:74)	YASHRFT (SEQ ID NO:45)	QQAYSSPYT (SEQ ID NO:75)	GFTFSSYGMS (SEQ ID NO:33)	VANIWQDGSTKYVDSVKG (SEQ ID NO:34)	AYGNYWYIDR (SEQ ID NO:35)
MH6	KSSQSVTNDLA (SEQ ID NO:76)	YASHRFT (SEQ ID NO:45)	QQAYSTPYT (SEQ ID NO:52)	GFTFSSYGMS (SEQ ID NO:33)	VANIWQDGSTKYVDSVKG (SEQ ID NO:34)	AYGNYWYIDR (SEQ ID NO:35)
MH7	KSSQSVGNDLA (SEQ ID NO:77)	YAYHRFS (SEQ ID NO:78)	QQAYSTPYT (SEQ ID NO:52)	GFTFSSYGMS (SEQ ID NO:33)	VANIWQDGSTKYVDSVKG (SEQ ID NO:34)	AYGNYWYIDR (SEQ ID NO:35)
MH8	KSSQSVTNDVA (SEQ ID NO:36)	YAYHRFT (SEQ ID NO:37)	HQAYSTPYT (SEQ ID NO:46)	GFTFSSYGMS (SEQ ID NO:33)	VANIWQDGSTKYVDSVKG (SEQ ID NO:34)	AYGNYWYIDR (SEQ ID NO:35)
MH9	KSSQSVGNDVA (SEQ ID NO:74)	YASHRFT (SEQ ID NO:45)	QQAYSSPYT (SEQ ID NO:75)	GFTFSSYGMS (SEQ ID NO:33)	VANIWQDGSEKYVDSVKG (SEQ ID NO:41)	AYGNYWYIDR (SEQ ID NO:35)
MH10	KSSQSVTNDLA (SEQ ID NO:76)	YASHRFT (SEQ ID NO:45)	QQAYSTPYT (SEQ ID NO:52)	GFTFSSYGMS (SEQ ID NO:33)	VANIWQDGSEKYVDSVKG (SEQ ID NO:41)	AYGNYWYIDR (SEQ ID NO:35)
MH11	KSSQSVGNDLA (SEQ ID NO:77)	YAYHRFS (SEQ ID NO:78)	QQAYSTPYT (SEQ ID NO:52)	GFTFSSYGMS (SEQ ID NO:33)	VANIWQDGSEKYVDSVKG (SEQ ID NO:41)	AYGNYWYIDR (SEQ ID NO:35)

MH12	KSSQSVTNDVA (SEQ ID NO:36)	YAYHRFT (SEQ ID NO:37)	HQAYSTPYT (SEQ ID NO:46)	GFTFSSYGMS (SEQ ID NO:33)	VANIWQDGSEKYVDSVKG (SEQ ID NO:41)	AYGNYWYIDR (SEQ ID NO:35)
MH13	KSSQSVTNDLA (SEQ ID NO:76)	YAYHRFT (SEQ ID NO:37)	HQAYSTPYT (SEQ ID NO:46)	GFTFSSYGMS (SEQ ID NO:33)	VANIWQDGSTKYVDSVKG (SEQ ID NO:34)	AYGNYWYIDR (SEQ ID NO:35)
MH14	KSSQSVTNDLA (SEQ ID NO:76)	YAYHRFS (SEQ ID NO:78)	HQAYSTPYT (SEQ ID NO:46)	GFTFSSYGMS (SEQ ID NO:33)	VANIWQDGSTKYVDSVKG (SEQ ID NO:34)	AYGNYWYIDR (SEQ ID NO:35)
MH15	KSSQSVTNDLA (SEQ ID NO:76)	YASHRFS (SEQ ID NO:79)	HQAYSTPYT (SEQ ID NO:46)	GFTFSSYGMS (SEQ ID NO:33)	VANIWQDGSTKYVDSVKG (SEQ ID NO:34)	AYGNYWYIDR (SEQ ID NO:35)
MH16	KSSQSVTNDLA (SEQ ID NO:76)	YAYHRFT (SEQ ID NO:37)	HQAYSTPYT (SEQ ID NO:46)	GFTFSSYGMS (SEQ ID NO:33)	VANIWQDGSEKYVDSVKG (SEQ ID NO:41)	AYGNYWYIDR (SEQ ID NO:35)
MH17	KSSQSVTNDLA (SEQ ID NO:76)	YAYHRFS (SEQ ID NO:78)	HQAYSTPYT (SEQ ID NO:46)	GFTFSSYGMS (SEQ ID NO:33)	VANIWQDGSEKYVDSVKG (SEQ ID NO:41)	AYGNYWYIDR (SEQ ID NO:35)
MH18	KSSQSVTNDLA (SEQ ID NO:76)	YASHRFS (SEQ ID NO:79)	HQAYSTPYT (SEQ ID NO:46)	GFTFSSYGMS (SEQ ID NO:33)	VANIWQDGSEKYVDSVKG (SEQ ID NO:41)	AYGNYWYIDR (SEQ ID NO:35)

Table 5. Predicted t cell epitope content scores for Hu317 and example lead clones.

Clone Name	GE	HAF	LAF	TCED+
Hu317	9	4	7	2
09C06	15	4	2	1
20C07	15	4	3	1
11C08	14	4	5	1
17A12	15	4	3	1
MH4	15	4	3	1
MH8	15	3	3	1
MH12	15	3	3	1

Table 6. Charge isoform distributions for Hu317 and example lead clones.

Clone Name	% Basic Isoforms	% Main Peak	% Acidic Isoforms
Hu317	23.1	68.9	8
09C06	3.5	82.8	13.7
MH4	7	81.6	11.4
MH8	6	77.4	16.6
MH12	7.4	80.4	12.2

Table 7. Thermal transition midpoints for Hu317 and example lead clones.

Clone	Tm1 °C	Tm2 °C	Tm3 °C
Hu317	72.3	79.4*	86.1
09C06		76.6*	86.5
MH4		75.3*	86.2
MH8		74.2*	86.2
MH12	71.9		86.5*

* Fab Tm

Table 8. IgGs show high integrity before and after forced oxidation with 0.5% H2O2, measured by Size Exclusion (SEC), Reverse Phase (RP) and Hydrophobic Interaction (HIC) Chromatographies

	% HMW by SEC		Retention RP (mins)		RP Heavy (mins)		RP Light (mins)		Retention HIC (Mins)	
	0 mins	120 min	0 mins	120 min	0 mins	120 min	0 mins	120 min	0 mins	120 min
Clone	H202	H202	H202	H202	H202	H202	H202	H202	H202	H202
Hu317	0.13	0.17	8.3	8.2	7.7	7.7	6.5	6.5	7.2	6.9
09C06	0.27	0.27	12.8	12.7	11.7	11.6	8.6	8.6	6.3	5.7
MH4	0.28	0.41	13.7	13.7	12.8	12.5	8.9	8.8	7.2	6.8
MH8	0.3	0.25	13.2	13.1	12	11.9	8.8	8.8	6.9	6.5
MH12	0.52	0.31	13.2	13.2	12	12	8.9	8.8	7	6.9

Table 9. Examples of antibody variable region amino acid sequences.

Antibody MH8 heavy chain variable (VH) region
EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYGMSWVRQAPGKGLEWVANIWQDGSTK
YVDSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCARAYGNYWYIDRWGQGTTVTVS
S (SEQ ID NO: 1)

Antibody MH8 light chain variable (VL) region
DIVMTQSPDSLAVSLGERATINCKSSQSVTNDVAWYQQKPGQPPKLLINYAYHRFTGVPD
RFSGSGYGTDFTLTISSLQAEDVAVYYCHQAYSTPYTFGQGTKLEIK (SEQ ID NO: 2)

Antibody MH4 heavy chain variable (VH) region
EVQLVESGGGLVQPGGSLRLSCAASGFSLSSYGMSWVRQAPGKGLEWVAVIWQDGSTN
YVDSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCARAYGNYWYIDRWGQGTTVTVS
S (SEQ ID NO: 3)

Antibody MH4 light chain variable (VL) region
DIVMTQSPDSLAVSLGERATINCKSSQSVTNDVAWYQQKPGQPPKLLINYAYHRFTGVPD
RFSGSGYGTDFTLTISSLQAEDVAVYYCHQAYSTPYTFGQGTKLEIK (SEQ ID NO: 4)

Antibody MH12 heavy chain variable (VH) region
EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYGMSWVRQAPGKGLEWVANIWQDGSE
KYVDSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCARAYGNYWYIDRWGQGTTVTV
SS (SEQ ID NO: 5)

Antibody MH12 light chain variable (VL) region
DIVMTQSPDSLAVSLGERATINCKSSQSVTNDVAWYQQKPGQPPKLLINYAYHRFTGVPD
RFSGSGYGTDFTLTISSLQAEDVAVYYCHQAYSTPYTFGQGTKLEIK (SEQ ID NO: 6)

Antibody 09C06 heavy chain variable (VH) region
EVQLVESGGGLVQPGGSLRLSCAASGFSFSSYGMHWVRQAPGKGLEWVANIWQDGST
NYVDSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCARAYGNYWYIDRWGQGTTVTV
SS (SEQ ID NO: 7)

Antibody 09C06 light chain variable (VL) region
DIVMTQSPDSLAVSLGERATINCKSSQSVSNDVAWYQQKPGQPPKLLINYASHRFTGVPD
RFSGSGYGTDFTLTISSLQAEDVAVYYCHQAYSTPYTFGQGTKLEIK (SEQ ID NO: 8)

Table 10. Examples of antibody Fc region amino acid sequences.

Human IgG4 wild type
ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS
GLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRVESKYGPPCPSCPAPEFLGGPSV
FLFPPKPKDTLMISRTPEVTCVVVDVSQED PEVQFNWYVDGVEVHNAKTKPREEQFNST
YRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMT
KNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQ
EGNVFSCSVMHEALHNHYTQKS LSLSLGK (SEQ ID NO: 9)

Human IgG4(S228P)
ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS
GLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEFLGGPSV
FLFPPKPKDTLMISRTPEVTCVVVDVSQED PEVQFNWYVDGVEVHNAKTKPREEQFNST
YRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMT

KNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQ
EGNVFSCSVMHEALHNHYTQKS LSLSLGK (SEQ ID NO: 10)

Human IgG1 wild type
ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS
GLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGG
PSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYN
STYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDEL
TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRW
QQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 11)

Human IgG1-3M
ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS
GLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGA
PSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYN
STYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDEL
TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRW
QQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 12)

Human IgG2 wild type
ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS
GLYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKCCVECPPCPAPPVAGPSV
FLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTF
RVVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTK
NQVSLTCLVKGFYPSDISVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQ
GNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 13)

Human IgG1 wild type “REEM” allotype
ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS
GLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGG
PSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYN
STYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREE
MTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR
WQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 14)

Human IgG1-3M “REEM” allotype
ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS
GLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGA
PSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYN
STYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREE
MKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRW
QQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 15)

Table 11. Examples of target protein amino acid sequences.

Human PD1 sequence
MQIPQAPWPVVWAVLQLGWRPGWFLDSPDRPWNPPTFSPALLVVTEGDNATFTCSFSN
TSESFVLNWYRMSPSNQTDKLAAFPEDRSQPGQDCRFRVTQLPNGRDFHMSVVRARRN
DSGTYLCGAISLAPKAQIKESLRAELRVTERRAEVPTAHPSPSPRPAGQFQTLVVGVVGG
LLGSLVLLVWVLAVICSRAARGTIGARRTGQPLKEDPSAVPVFSVDYGELDFQWREKTPE
PPVPCVPEQTEYATIVFPSGMGTSSPARRGSADGPRSAQPLRPEDGHCSWPL (SEQ ID
NO: 16)

Cynomolgus monkey PD1 sequence
MQIPQAPWPVVWAVLQLGWRPGWFLESPDRPWNAPTFSPALLLVTEGDNATFTCSFSN
ASESFVLNWYRMSPSNQTDKLAAFPEDRSQPGQDCRFRVTRLPNGRDFHMSVVRARRN
DSGTYLCGAISLAPKAQIKESLRAELRVTERRAEVPTAHPSPSPRPAGQFQALVVGVVGG
LLGSLVLLVWVLAVICSRAAQGTIEARRTGQPLKEDPSAVPVFSVDYGELDFQWREKTPE
PPAPCVPEQTEYATIVFPSGLGTSSPARRGSADGPRSPRPLRPEDGHCSWPL (SEQ ID NO: 17)

Human LSAMP sequence
MVRRVQPDRKQLPLVLLRLLCLLPTGLPVRSVDFNRGTDNITVRQGDTAILRCVVEDKNSK
VAWLNRSGIIFAGHDKWSLDPRVELEKRHSLEYSLRIQKVDVYDEGSYTCSVQTQHEPKT
SQVYLIVQVPPKISNISSDVTVNEGSNVTLVCMANGRPEPVITWRHLTPTGREFEGEEEYL
EILGITREQSGKYECKAANEVSSADVKQVKVTVNYPPTITESKSNEATTGRQASLKCEASA
VPAPDFEWYRDDTRINSANGLEIKSTEGQSSLTVTNVTEEHYGNYTCVAANKLGVTNASL
VLFRPGSVRGINGSISLAVPLWLLAASLLCLLSKC (SEQ ID NO: 415)

Human BTN2A1 sequence
MESAAALHFSRPASLLLLLLSLCALVSAQFIVVGPTDPILATVGENTTLRCHLSPEKNAEDM
EVRWFRSQFSPAVFVYKGGRERTEEQMEEYRGRTTFVSKDISRGSVALVIHNITAQENGT
YRCYFQEGRSYDEAILHLVVAGLGSKPLISMRGHEDGGIRLECISRGWYPKPLTVWRDPY
GGVAPALKEVSMPDADGLFMVTTAVIIRDKSVRNMSCSINNTLLGQKKESVIFIPESFMPSV
SPCAVALPIIVVILMIPIAVCIYWINKLQKEKKILSGEKEFERETREIALKELEKERVQKEEELQ
VKEKLQEELRWRRTFLHAVDVVLDPDTAHPDLFLSEDRRSVRRCPFRHLGESVPDNPER
FDSQPCVLGRESFASGKHYWEVEVENVIEWTVGVCRDSVERKGEVLLIPQNGFWTLEMH
KGQYRAVSSPDRILPLKESLCRVGVFLDYEAGDVSFYNMRDRSHIYTCPRSAFSVPVRPF
FRLGCEDSPIFICPALTGANGVTVPEEGLTLHRVGTHQSL (SEQ ID NO: 416)

Table 12. Target proteins identified via IgG screening of proteome arrays

IgG clone	Number of hits in chip screen	PD1 (PDCD1)	BTN2A1	LSAMP
		NM_005018	NM_007049	BC033803
MH4 (2µg/ml)	2	strong		weak
MH8 (2ug/ml)	2	strong		weak
MH12 (2ug/ml)	3	strong	weak/med	weak
Ilu317 (2ug/ml)	1	strong		
Anti-fluorescein (4-4-20e) Human IgG4 kappa. Ab00102-13.0 negative control (2ug/ml)	0			
PBS (secondary only)	0			

Table 13. Fold fluorescence changes in IgG binding on non-target protein-transfected cells versus mock (ZSgreen only) cells

IgG clone	BTN2A1	LSAMP	CD20
Hu317	1.2	1.5	1.4
MH4	1.2	1.9	1.1
MH8	1.1	7.1	0.9
MH12	1.3	4.1	1.1
Secondary only	1.1	1.5	0.9

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CLAIMS

1. 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 GFTFSSYGMS (SEQ ID NO:33), HCDR2 of VANIWQDGSTKYVDSVKG (SEQ ID NO:34), and HCDR3 of AYGNYWYIDR (SEQ ID NO:35); and the VL region amino acid sequence comprises LCDR1 of KSSQSVTNDVA (SEQ ID NO:36), LCDR2 of YAYHRFT (SEQ ID NO:37), and LCDR3 of HQAYSSPYT (SEQ ID NO:38);

(b) the VH region amino acid sequence comprises HCDR1 of GFSLSSYGMS (SEQ ID NO:39), HCDR2 of VAVIWQDGSTNYVDSVKG (SEQ ID NO:40), and HCDR3 of AYGNYWYIDR (SEQ ID NO:35); and the VL region amino acid sequence comprises LCDR1 of KSSQSVTNDVA (SEQ ID NO:36), LCDR2 of YAYHRFT (SEQ ID NO:37), and LCDR3 of HQAYSSPYT (SEQ ID NO:38);

(c) the VH region amino acid sequence comprises HCDR1 of GFTFSSYGMS (SEQ ID NO:33), HCDR2 of VANIWQDGSEKYVDSVKG (SEQ ID NO:41), and HCDR3 of AYGNYWYIDR (SEQ ID NO:35); and the VL region amino acid sequence comprises LCDR1 of KSSQSVTNDVA (SEQ ID NO:36), LCDR2 of YAYHRFT (SEQ ID NO:37), and LCDR3 of HQAYSSPYT (SEQ ID NO:38);

(d) the VH region amino acid sequence comprises HCDR1 of GFSFSSYGMH (SEQ ID NO:42), HCDR2 of VANIWQDGSTNYVDSVKG (SEQ ID NO:43), and HCDR3 of AYGNYWYIDR (SEQ ID NO:35); and the VL region amino acid sequence comprises LCDR1 of KSSQSVSNDVA (SEQ ID NO:44), LCDR2 of YASHRFT (SEQ ID NO:45), and LCDR3 of HQAYSTPYT (SEQ ID NO:46);

(e) the VH region amino acid sequence comprises HCDR1 of GFSFTSYGMS (SEQ ID NO:47), HCDR2 of LANIWQDGSTNYVDSVKG (SEQ ID NO:48), and HCDR3 of AYGNYWYIDR (SEQ ID NO:35); and the VL region amino acid sequence comprises LCDR1 of KSSQSVSNDVA (SEQ ID NO:44), LCDR2 of YASHRFT (SEQ ID NO:45), and LCDR3 of HQAYSTPYT (SEQ ID NO:46);

(f) the VH region amino acid sequence comprises HCDR1 of GFTFSSYGVH (SEQ ID NO:49), HCDR2 of VGVIWQDGSENYVDSVKG (SEQ ID NO:50), and HCDR3 of AYGNYWYIDR (SEQ ID NO:35); and the VL region amino acid sequence comprises LCDR1 of KSSQSVSNDLA (SEQ ID NO:51), LCDR2 of YASHRFT (SEQ ID NO:45), and LCDR3 of QQAYSTPYT (SEQ ID NO:52);

(g) the VH region amino acid sequence comprises HCDR1 of GFSFSSYGMH (SEQ ID NO:42), HCDR2 of VANIWQDGSTNYVDSVKG (SEQ ID NO:43), and HCDR3 of AYGNYWYIDR (SEQ ID NO:35); and the VL region amino acid sequence comprises LCDR1

of KSSQSVSNDVA (SEQ ID NO:44), LCDR2 of YASHRFT (SEQ ID NO:45), and LCDR3 of HQAYSTPYT (SEQ ID NO:46);

(h) the VH region amino acid sequence comprises HCDR1 of GFSFSSYGMH (SEQ ID NO:42), HCDR2 of LANIWQDGSENYVDSVKG (SEQ ID NO:53), and HCDR3 of AYGNYWYIDR (SEQ ID NO:35); and the VL region amino acid sequence comprises LCDR1 of KSSQSVSNDVA (SEQ ID NO:44), LCDR2 of YASHRFT (SEQ ID NO:45), and LCDR3 of QQAYSTPYT (SEQ ID NO:52);

(i) the VH region amino acid sequence comprises HCDR1 of GFTLSSYGMH (SEQ ID NO: 54), HCDR2 of LAVIWADGSTNYVDSVKG (SEQ ID NO:55), and HCDR3 of AYGNYMYIDV (SEQ ID NO:56); and the VL region amino acid sequence comprises LCDR1 of KSSQSVSNDVA (SEQ ID NO:44), LCDR2 of YASHRFT (SEQ ID NO:45), and LCDR3 of HQAYSTPYT (SEQ ID NO:46);

(j) the VH region amino acid sequence comprises HCDR1 of GFTFSSYGMH (SEQ ID NO:57), HCDR2 of VGVIKQDGSENYVDSVKG (SEQ ID NO:58), and HCDR3 of AYGNYWYIDR (SEQ ID NO:35); and the VL region amino acid sequence comprises LCDR1 of KSSQSVSNDVA (SEQ ID NO:44), LCDR2 of YASHRFT (SEQ ID NO:45), and LCDR3 of HQAYSTPYT (SEQ ID NO:46); or

(k) the VH region amino acid sequence comprises HCDR1 of GFSFTSYGMH (SEQ ID NO:59), HCDR2 of VGVIWQDGSTNYVDSVKG (SEQ ID NO:60), and HCDR3 of GYGNYWYIDV (SEQ ID NO:61); and the VL region amino acid sequence comprises LCDR1 of KSSQSVSNDLA (SEQ ID NO:51), LCDR2 of YASHRFT (SEQ ID NO:45), and LCDR3 of QQAYSTPYT (SEQ ID NO:52).

2. The antibody or antigen-binding portion of claim 1, 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;

(c) the VH region amino acid sequence comprises SEQ ID NO:5 and the VL region amino acid sequence comprises SEQ ID NO:6; or

(d) the VH region amino acid sequence comprises SEQ ID NO:7 and the VL region amino acid sequence comprises SEQ ID NO:8.

3. 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-X_1-X_2-X_3-S-Y-X_4-X_5-X_6, wherein X_1 is T or a conservative substitution of T, X_2 is F or a conservative substitution of F, X_3 is S or a conservative substitution of S, X_4 is G or any other amino acid, X_5 is M or a conservative substitution of M, and X_6 is S or any other amino acid (SEQ ID NO:20);

(b) the HCDR2 comprises the amino acid sequence X_1-X_2-X_3-I-X_4-X_5-X_6-G-S-X_7-X_8-Y-X_9-D-S-V-K-G, wherein X_1 is V or a conservative substitution of V, X_2 is A or a conservative substitution of A, X_3 is V or N, X_4 is W or K, X_5 is Q or A, X_6 is D or G, X_7 is T or E, X_8 is N, K, or S, and X_9 is N or V (SEQ ID NO:21);

(c) the HCDR3 comprises the amino acid sequence X_1-X_2-G-X_3-X_4-X_5-Y-X_6-X_7-X_8, wherein X_1 is A or any other amino acid, X_2 is Y or a conservative substitution of Y, X_3 is N or any other amino acid, X_4 is Y or a conservative substitution of Y, X_5 is W or any other amino acid, X_6 is I or any other amino acid, X_7 is D or a conservative substitution of D, and X_8 is V or any other amino acid (SEQ ID NO:22);

(d) the LCDR1 comprises the amino acid sequence K-S-S-X_1-S-V-X_2-N-D-X_3-A, wherein X_1 is Q or E, X_2 is S or a conservative substitution of S, and X_3 is L or a conservative substitution of L (SEQ ID NO:26);

(e) the LCDR2 comprises the amino acid sequence Y-A-X_1-X_2-R-F-X_3, wherein X_1 is F or any other amino acid, X_2 is H or any other amino acid, and X_3 is S or a conservative substitution of S (SEQ ID NO:27); and

(f) the LCDR3 comprises the amino acid sequence X_1-Q-X_2-Y-S-X_3-P-X_4-T, wherein X_1 is Q or any other amino acid, X_2 is A or any other amino acid, X_3 is T or any other amino acid, and X_4 is Y or any other amino acid (SEQ ID NO:28).

4. An 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 of any one of claims 1-3; and

(a) comprises fully germline human framework amino acid sequences; and/or

(b) binds specifically to human PD1 and cynomolgus PD1; and/or

(c) does not comprise an oxidation site in the LCDR2; and/or

(d) comprises a human germline peptide sequence with high MHC class II binding affinity in the HCDR2/framework 3 region of the VH domain; and/or

(e) does not comprise a human T cell epitope sequence in the HCDR1/framework 2 region of the VH domain; and/or

(f) does not comprise a human T cell epitope sequence in the HCDR3/framework 4 region of the VH domain; and/or

(g) exhibits reduced charge species heterogeneity compared to antibody Hu317, when in IgG1 effector null antibody format.

5. The antibody or antigen-binding portion of any one of claims 1-4, wherein the antibody is human, humanized or chimeric.

6. The antibody or antigen-binding portion of any one of claims 1-5, wherein the VH region, the VL region, or both the VH and the VL region comprise one or more human framework region amino acid sequences.

7. The antibody or antigen-binding portion of any one of claims 1-6, wherein the VH region, the VL region, or both the VH and the VL region comprise a human variable region framework scaffold amino acid sequence into which the CDRs have been inserted.

8. The antibody or antigen-binding portion of claim 1 or 3, wherein the VH region comprises an IGHV3-7 human germline scaffold amino acid sequence into which the HCDR1, HCDR2 and HCDR3 amino acid sequences have been inserted.

9. The antibody or antigen-binding portion of any one of claims 1, 3 and 8, wherein the VL region comprises an IGKV4-1 human germline scaffold amino acid sequence into which the LCDR1, LCDR2 and LCDR3 amino acid sequences have been inserted.

10. The antibody or antigen-binding portion of any one of claims 1-9, wherein the antibody comprises an immunoglobulin constant region.

11. The antibody or antigen-binding portion of claim 10, wherein the immunoglobulin constant region is IgG, IgE, IgM, IgD, IgA or IgY.

12. The antibody or antigen-binding portion of claim 11, wherein the immunoglobulin constant region is IgG1, IgG2, IgG3, IgG4, IgA1 or IgA2.

13. The antibody or antigen-binding portion of claim 10, wherein the immunoglobulin constant region is immunologically inert.

14. The antibody or antigen-binding portion of claim 10, wherein the immunoglobulin constant region is a wild-type human IgG4 constant region, a human IgG4 constant region comprising the amino acid substitution S228P, a wild-type human IgG1 constant region, a human IgG1 constant region comprising the amino acid substitutions L234A, L235A and G237A or a wild-type human IgG2 constant region.

15. The antibody or antigen-binding portion of claim 13, wherein the immunoglobulin constant region comprises any one of SEQ ID NOS:9-15.

16. The antigen-binding portion of any one of claims 1-15, wherein the antibody or antigen-binding portion is an Fab, an Fab', an $F(ab')_2$, an Fd, an Fv, an scFv, a single domain antibody (dAb), a maxibody, a minibody, an intrabody, a diabody, a triabody, a tetrabody, a v-NAR or a bis-scFv.

17. The antibody or antigen-binding portion of any one of claims 1-16, wherein the antibody is monoclonal.

18. The antibody or antigen-binding portion of any one of claims 1-17, wherein the antibody is a tetrameric antibody, a tetravalent antibody or a multispecific antibody.

19. The antibody or antigen-binding portion of any one of claims 1-18, wherein the antibody is 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.

20. The antibody or antigen-binding portion of any one of claims 1-19, wherein the antibody or antigen-binding portion binds specifically to (a) human PD1 or (b) human PD1 and cynomolgus PD1.

21. An immunoconjugate comprising the antibody or antigen-binding portion of any one of claims 1-20 linked to a therapeutic agent.

22. The immunoconjugate of claim 21, wherein the therapeutic agent is a cytotoxin, a radioisotope, a chemotherapeutic agent, an immunomodulatory agent, an anti-angiogenic agent, an antiproliferative agent, a pro-apoptotic agent, a cytostatic enzyme, a cytolytic enzymes, a therapeutic nucleic acid, an anti-angiogenic agent, an anti-proliferative agent, or a pro-apoptotic agent.

23. A pharmaceutical composition comprising the antibody or antigen-binding portion of any one of claims 1-20 or the immunoconjugate of claim 21 or 22, and a pharmaceutically acceptable carrier, diluent or excipient.

24. An nucleic acid molecule encoding

(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 the antibody or antigen-binding portion of any one of claims 1-20.

25. An expression vector comprising the nucleic acid molecule of claim 24.

26. A recombinant host cell comprising the nucleic acid molecule of claim 24 or the expression vector of claim 25.

27. A method of producing an anti-PD1 antibody or an antigen-binding portion thereof, the method comprising:

culturing a recombinant host cell comprising the expression vector of claim 25 under conditions whereby the nucleic acid molecule is expressed, thereby producing the antibody or antigen-binding portion; and

isolating the antibody or antigen-binding portion from the host cell or culture.

28. A method for enhancing an immune response in a subject, comprising administering to the subject a therapeutically effective amount of the antibody or antigen-binding portion of any one of claims 1-20, the immunoconjugate of claim 21 or 22 or the pharmaceutical composition of claim 23.

29. A method of treating or preventing cancer, an infectious disease, or an immune disease in a subject, comprising administering to the subject a therapeutically effective amount of the antibody or antigen-binding portion of any one of claims 1-20, the immunoconjugate of claim 21 or 22 or the pharmaceutical composition of claim 23.

30. The method of claim 29, wherein the cancer is pancreatic cancer, melanoma, breast cancer, lung cancer, bronchial cancer, colorectal cancer, prostate cancer, stomach cancer, ovarian cancer, 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.

31. The method of claim 29, wherein the infectious disease is viral, bacterial, fungal, or parasitic.

32. The method of claim 29, the infectious disease is human immunodeficiency virus (HIV) infection.

33. The antibody or antigen-binding portion of any one of claims 1-20, the immunoconjugate of claim 21 or 22 or the pharmaceutical composition of claim 23 for use in the treatment of cancer, an infectious disease, or an immune disease.

34. The antibody or antigen-binding portion, the immunoconjugate or the pharmaceutical composition for use according to claim 33, wherein the cancer is pancreatic cancer, melanoma, breast cancer, lung cancer, bronchial cancer, colorectal cancer, prostate cancer, stomach cancer, ovarian cancer, 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.

35. The antibody or antigen-binding portion, the immunoconjugate or the pharmaceutical composition for use according to claim 33, wherein the infectious disease is viral, bacterial, fungal, or parasitic.

36. The antibody or antigen-binding portion, the immunoconjugate or the pharmaceutical composition for use according to claim 33, wherein the infectious disease is human immunodeficiency virus (HIV) infection.

37. The antibody or antigen-binding portion of any one of claims 1-20, the immunoconjugate of claim 21 or 22 or the pharmaceutical composition of claim 23, for use as a medicament.

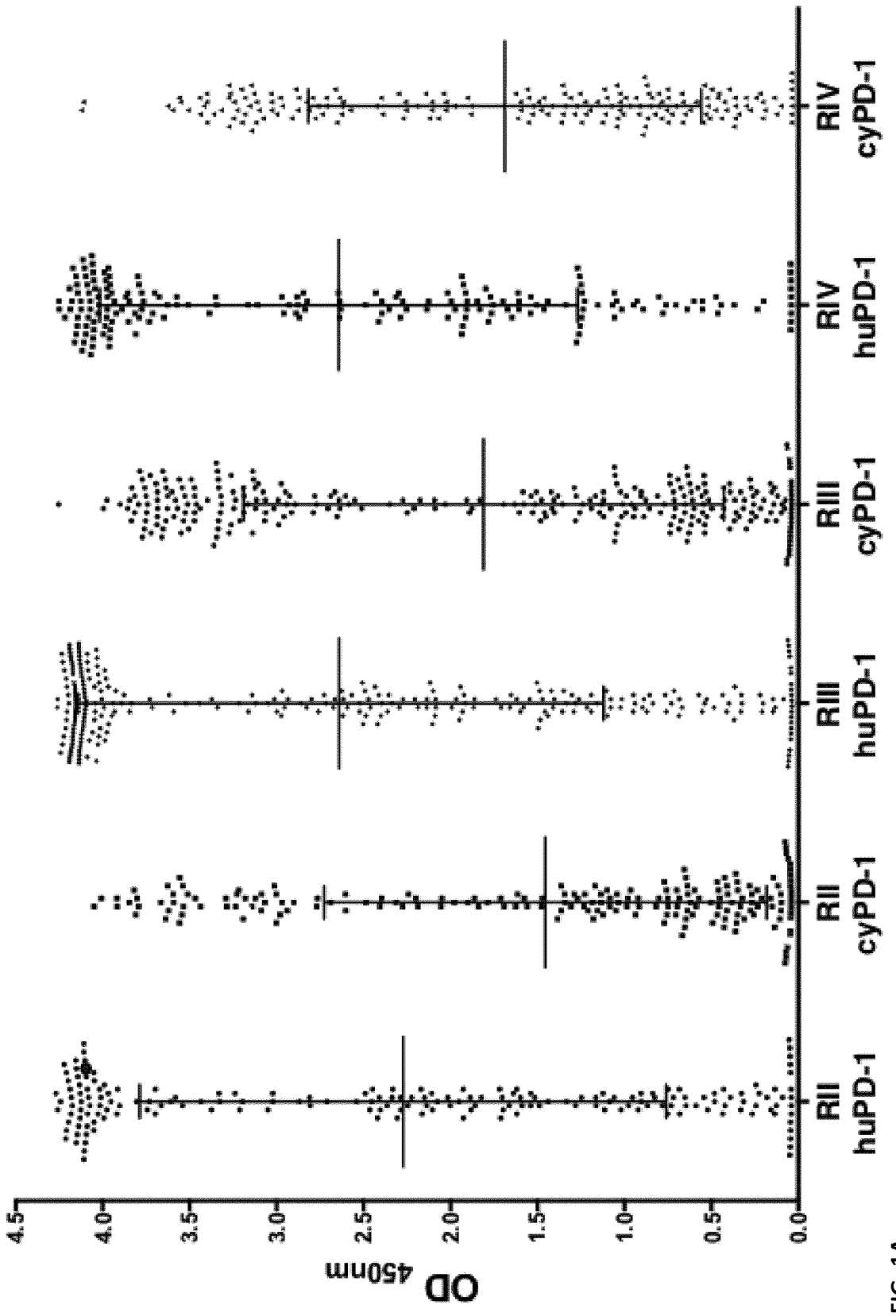

OD 450nm
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
RII huPD-1
RII cyPD-1
RIII huPD-1
RIII cyPD-1
RIV huPD-1
RIV cyPD-1

FIG. 1A

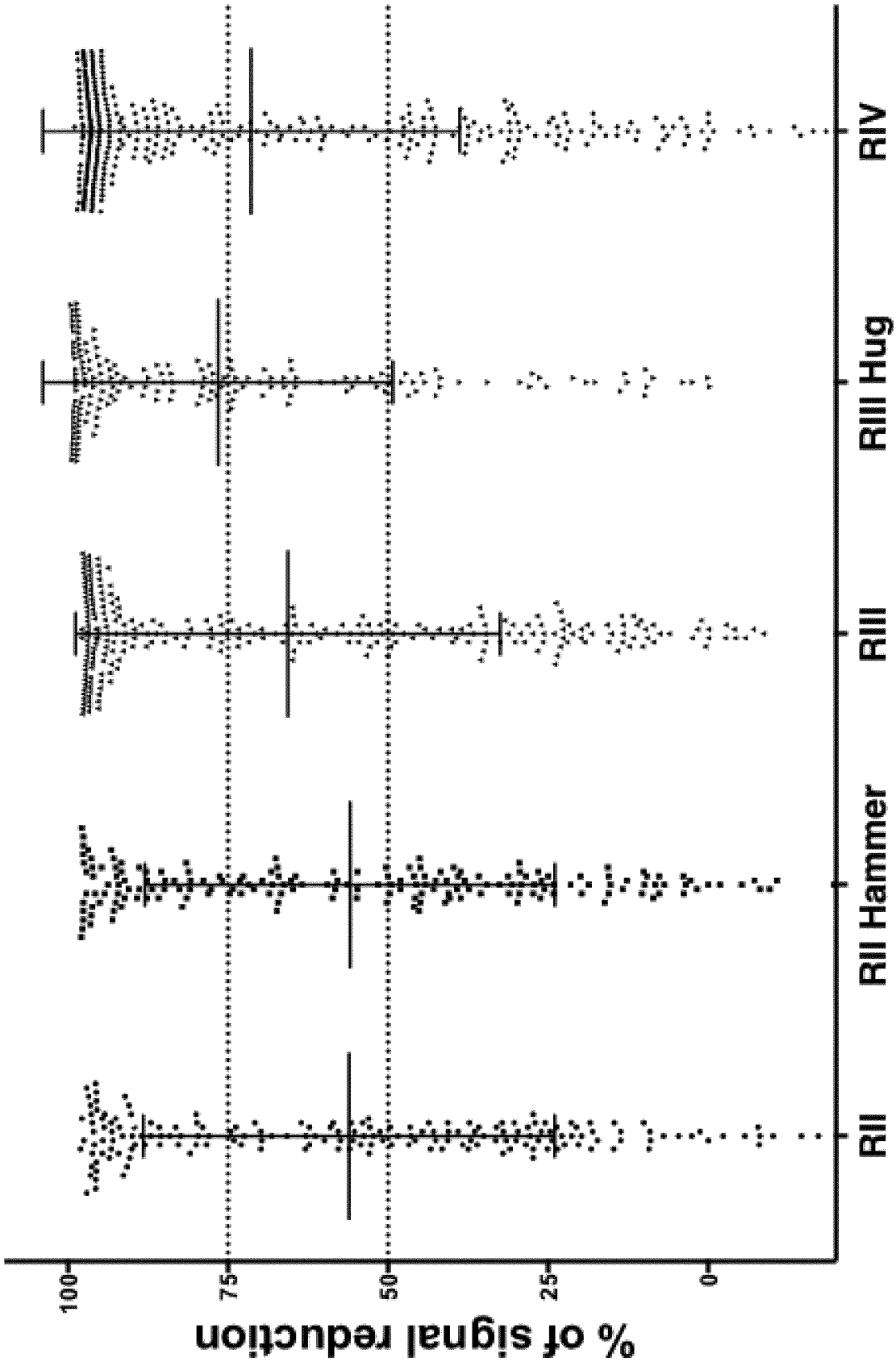

% of signal reduction
100
75
50
25
0
RII
RII Hammer
RIII
RIII Hug
RIV

FIG. 1B

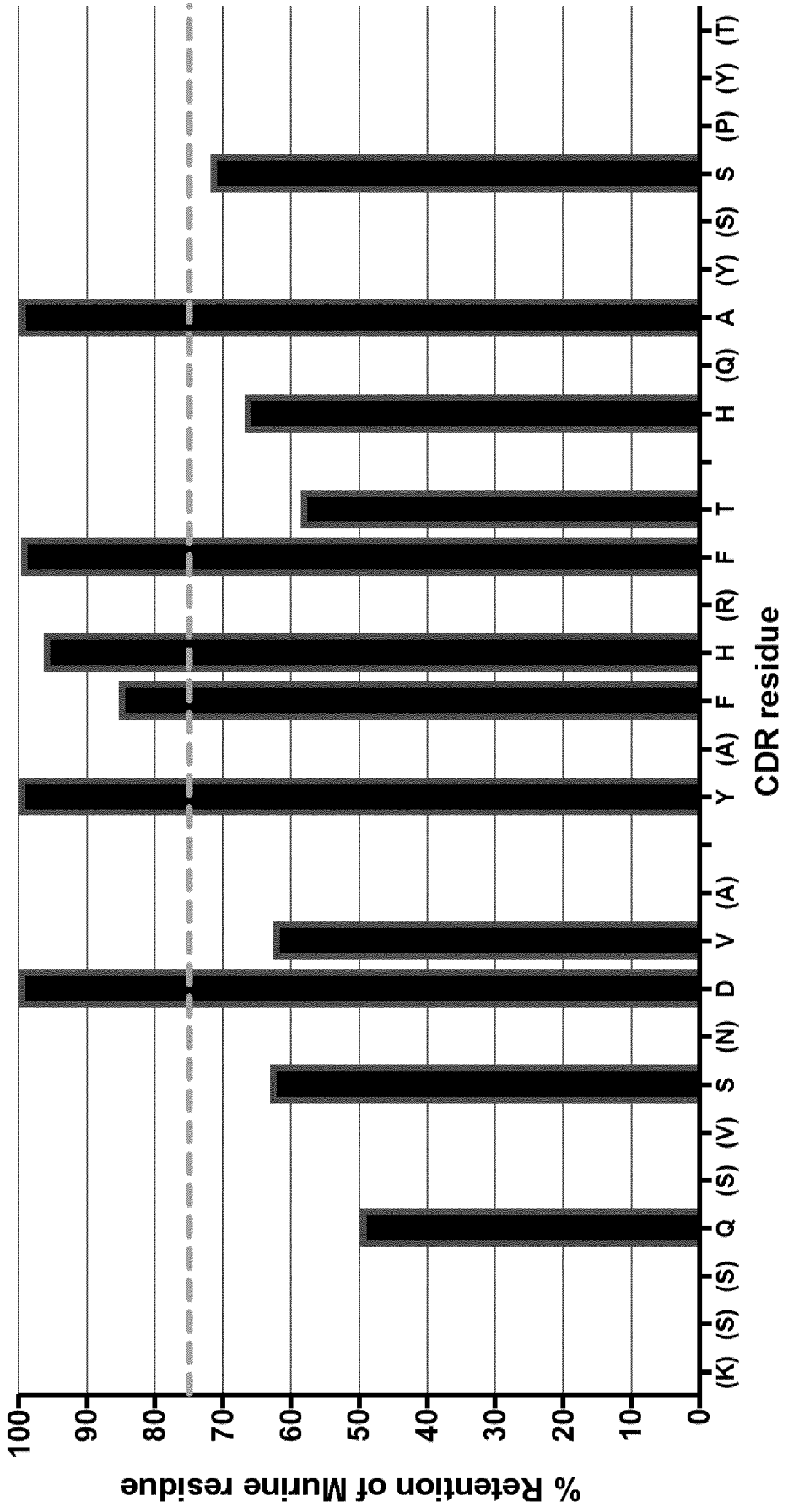

% Retention of Murine residue
100
90
80
70
60
50
40
30
20
10
0
(K) (S) (S) Q (S) (V) S (N) D V (A) Y (A) F H (R) F T H (Q) A (Y) (S) S (P) (Y) (T)
CDR residue

FIG. 2A

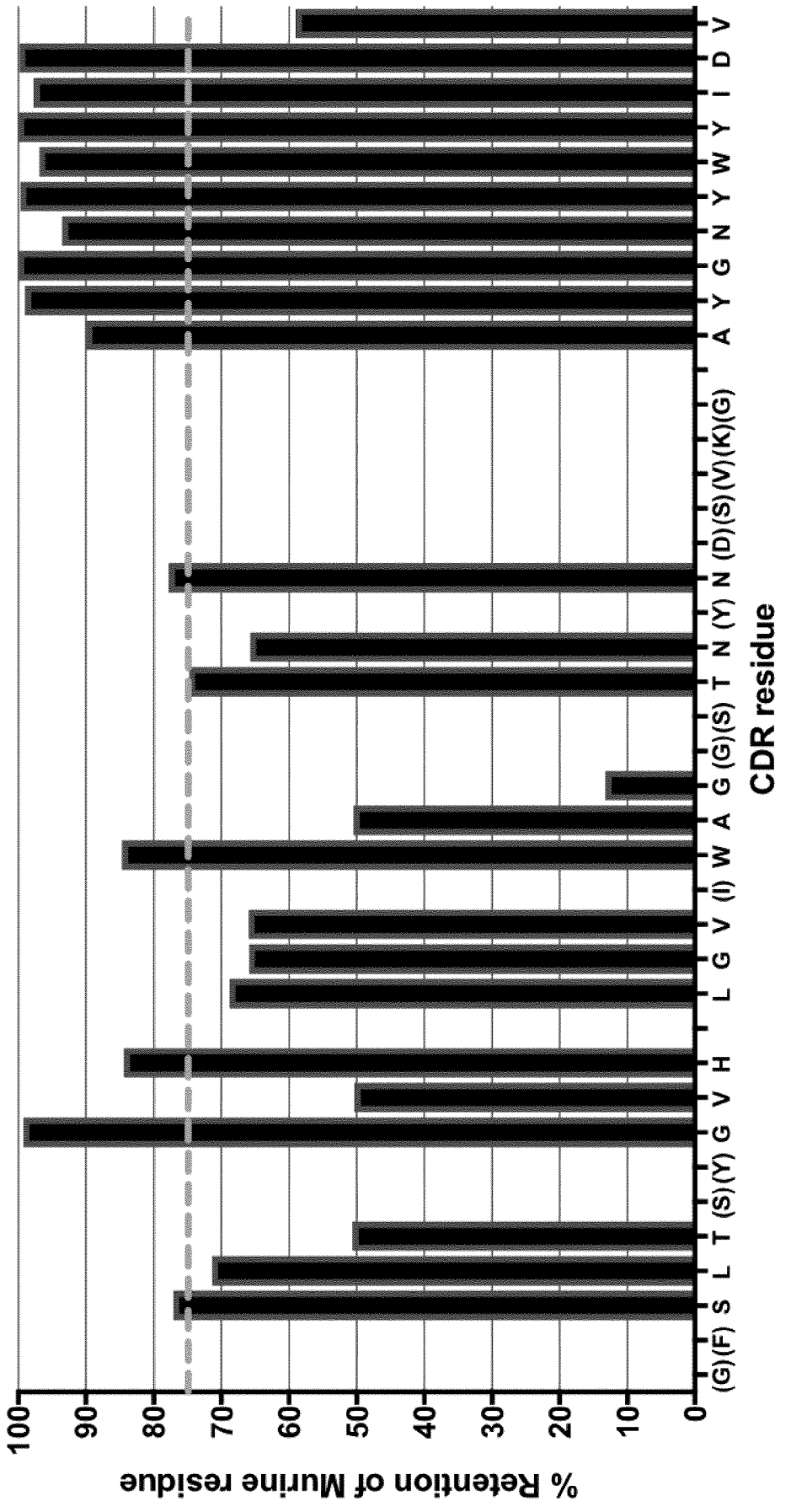

% Retention of Murine residue
100
90
80
70
60
50
40
30
20
10
0
(G)(F) S L T (S)(Y) G V H L G V (I) W A G (G)(S) T N (Y) N (D)(S)(V)(K)(G) A Y G N Y W Y I D V
CDR residue

FIG. 2B

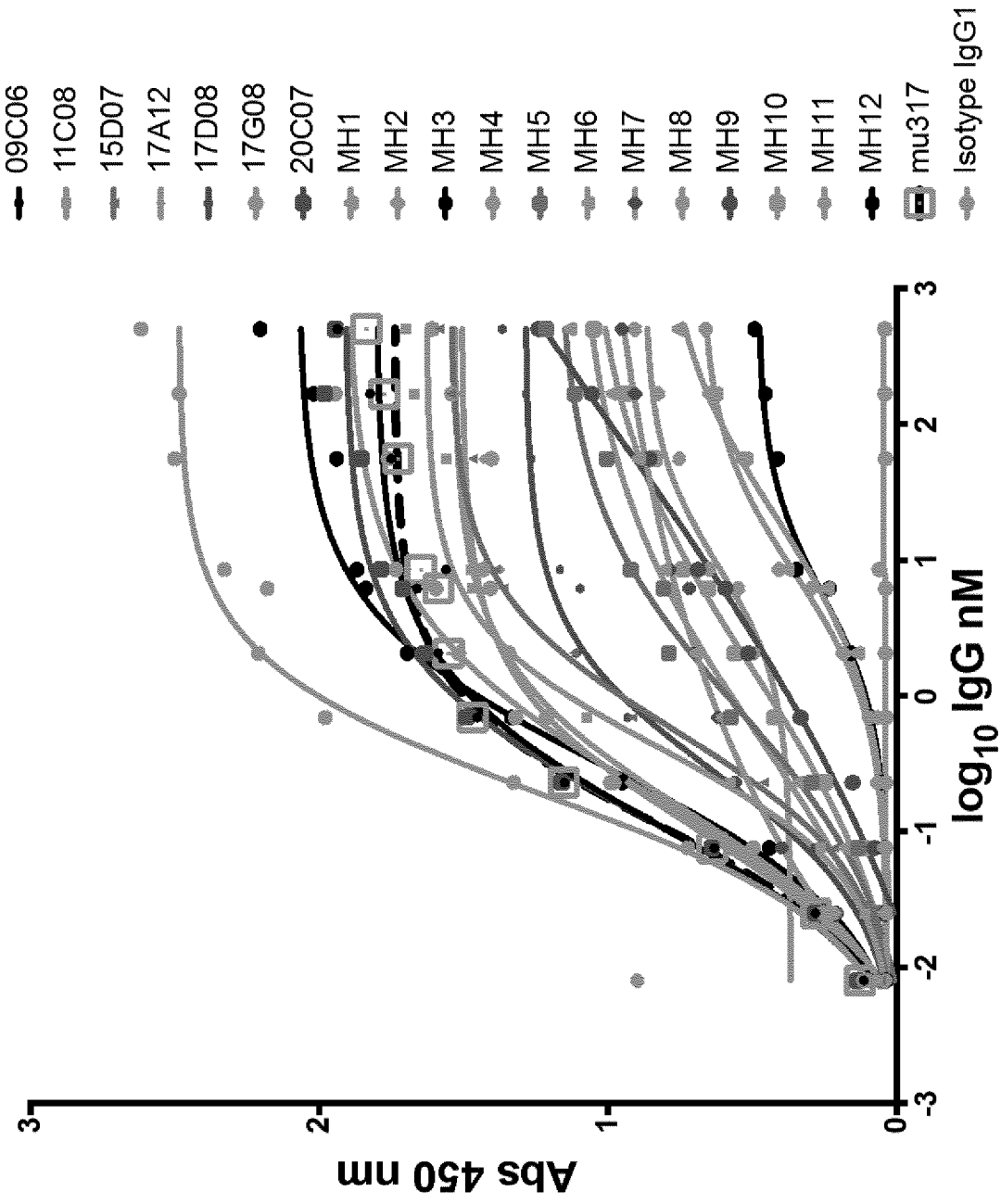

09C06
11C08
15D07
17A12
17D08
17G08
20C07
MH1
MH2
MH3
MH4
MH5
MH6
MH7
MH8
MH9
MH10
MH11
MH12
mu317
Isotype IgG1
Abs 450 nm
log10 IgG nM
3
2
1
0
-3
-2
-1
0
1
2
3

FIG. 3A

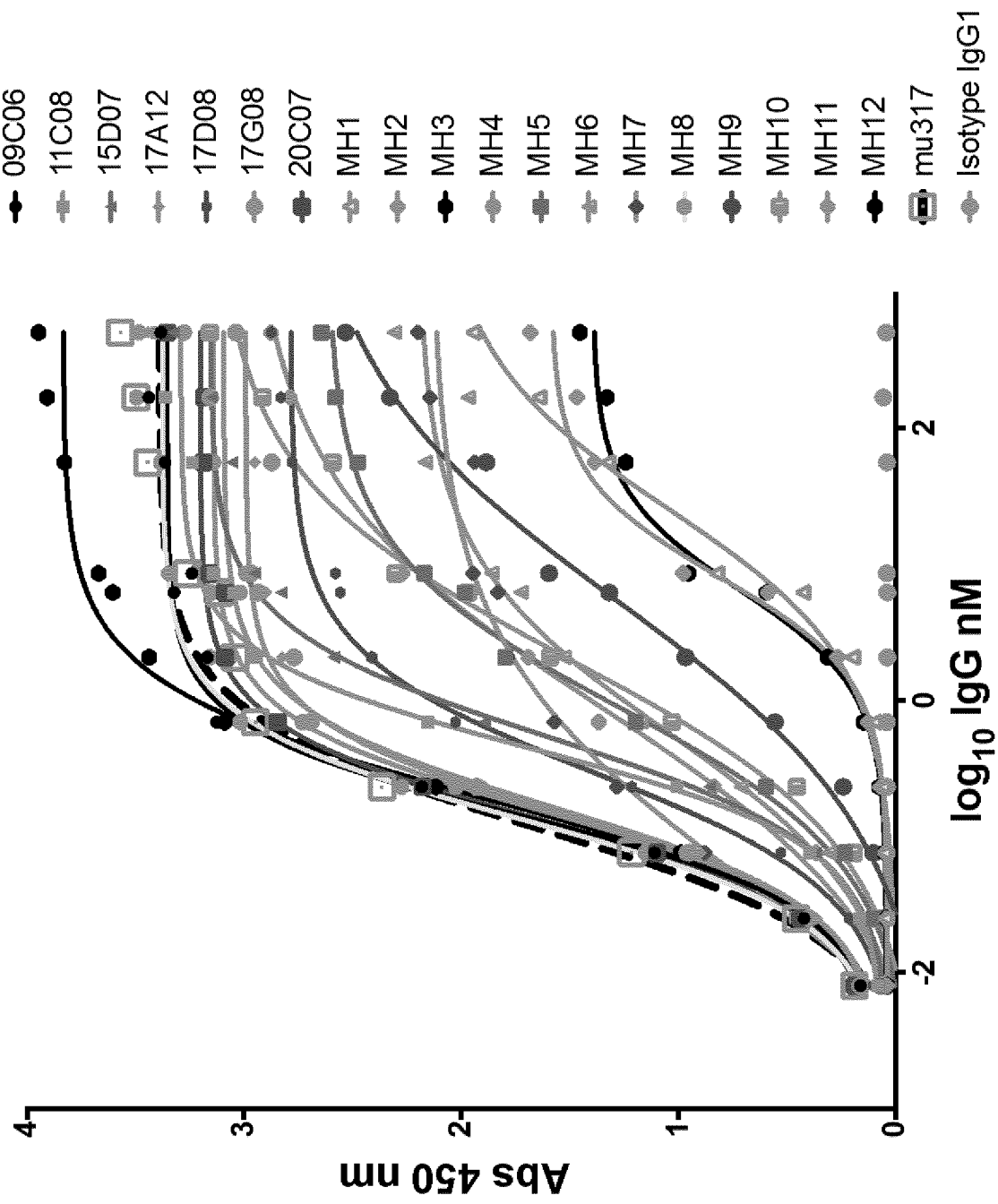
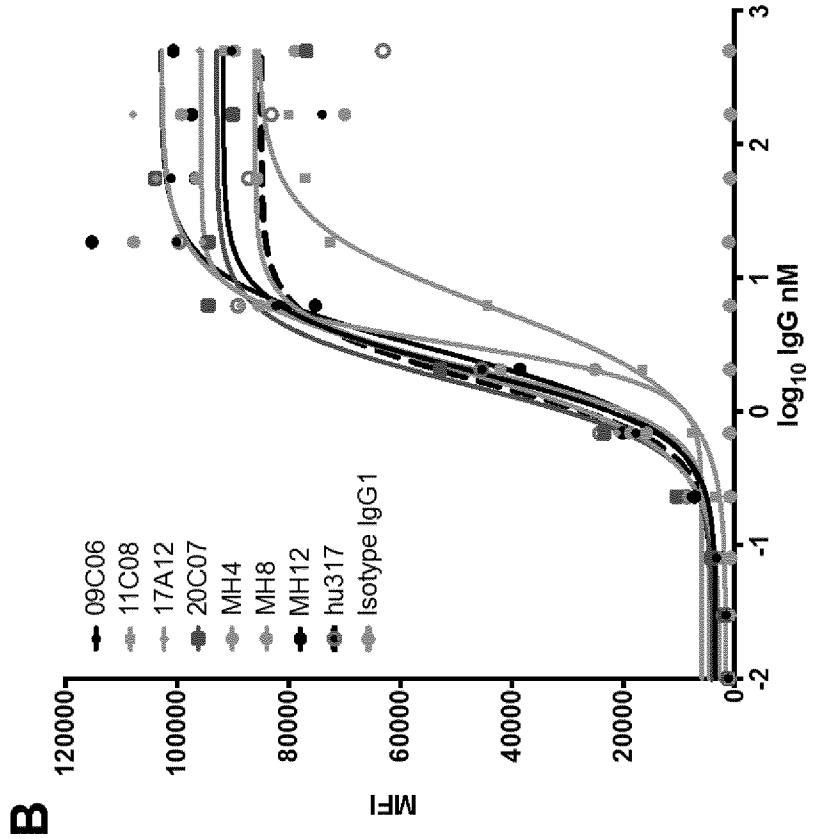

09C06
11C08
15D07
17A12
17D08
17G08
20C07
MH1
MH2
MH3
MH4
MH5
MH6
MH7
MH8
MH9
MH10
MH11
MH12
mu317
Isotype IgG1
Abs 450 nm
0
1
2
3
4
-2
0
2
log10 IgG nM

FIG. 3B


B
MFI
120000
100000
80000
60000
40000
20000
0
-2
-1
0
1
2
3
log10 IgG nM
09C06
11C08
17A12
20C07
MH4
MH8
MH12
hu317
Isotype IgG1

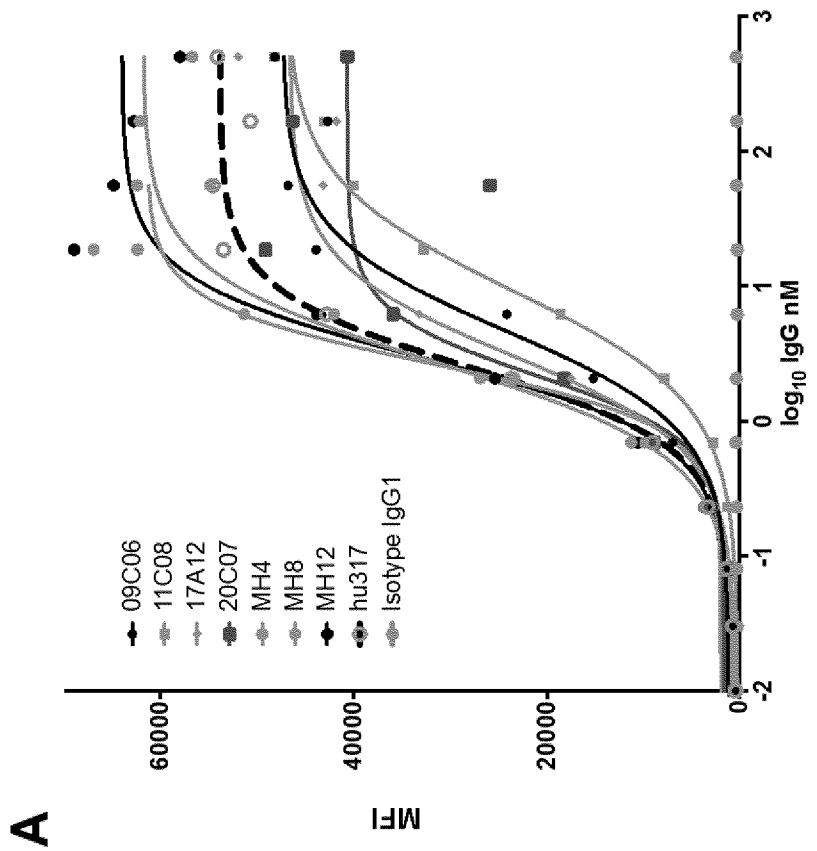

A
MFI
60000
40000
20000
0
-2
-1
0
1
2
3
log10 IgG nM
09C06
11C08
17A12
20C07
MH4
MH8
MH12
hu317
Isotype IgG1

FIG. 4

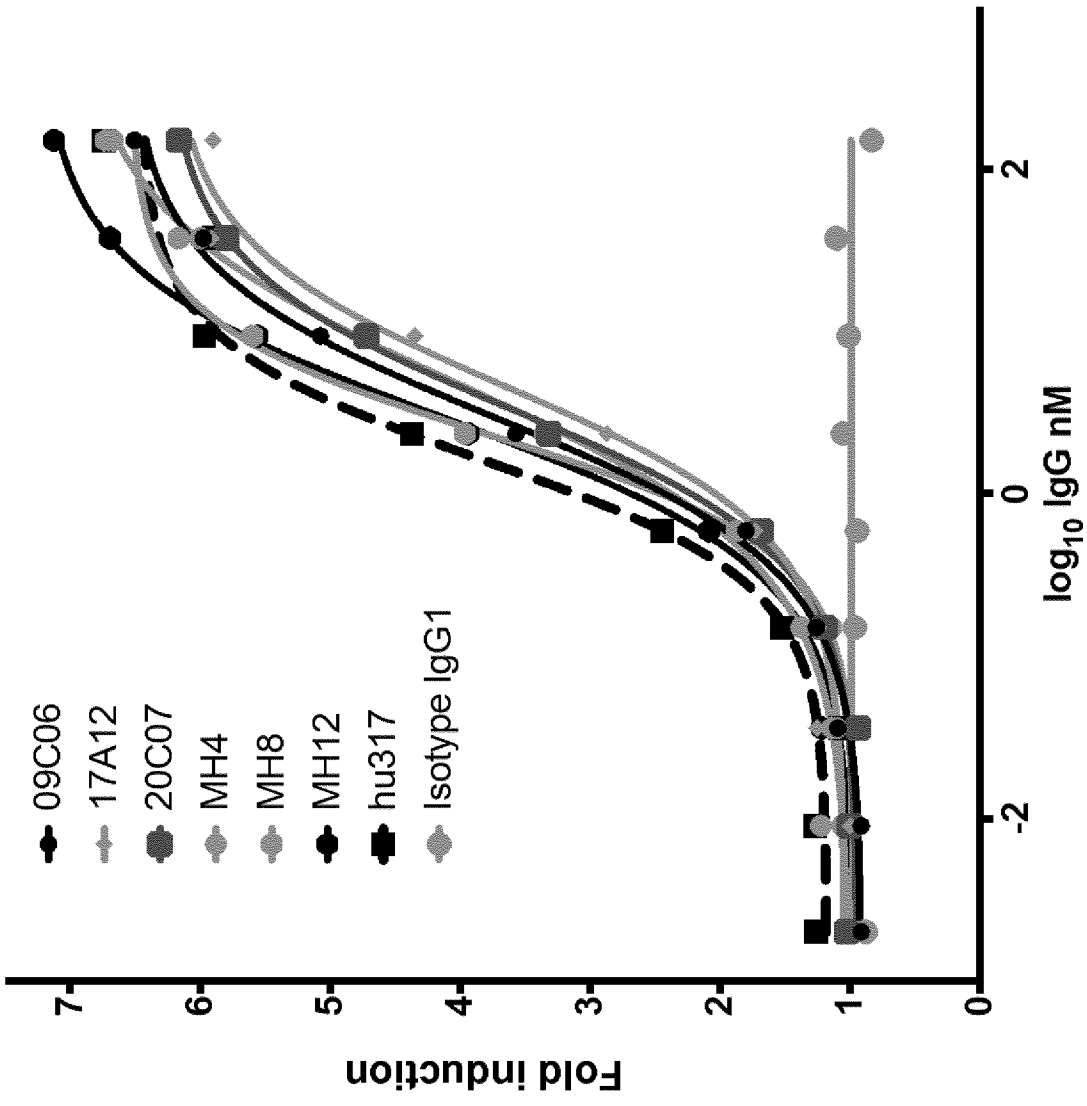

Fold induction
0
1
2
3
4
5
6
7
-2
0
2
log10 IgG nM
09C06
17A12
20C07
MH4
MH8
MH12
hu317
Isotype IgG1

FIG. 5

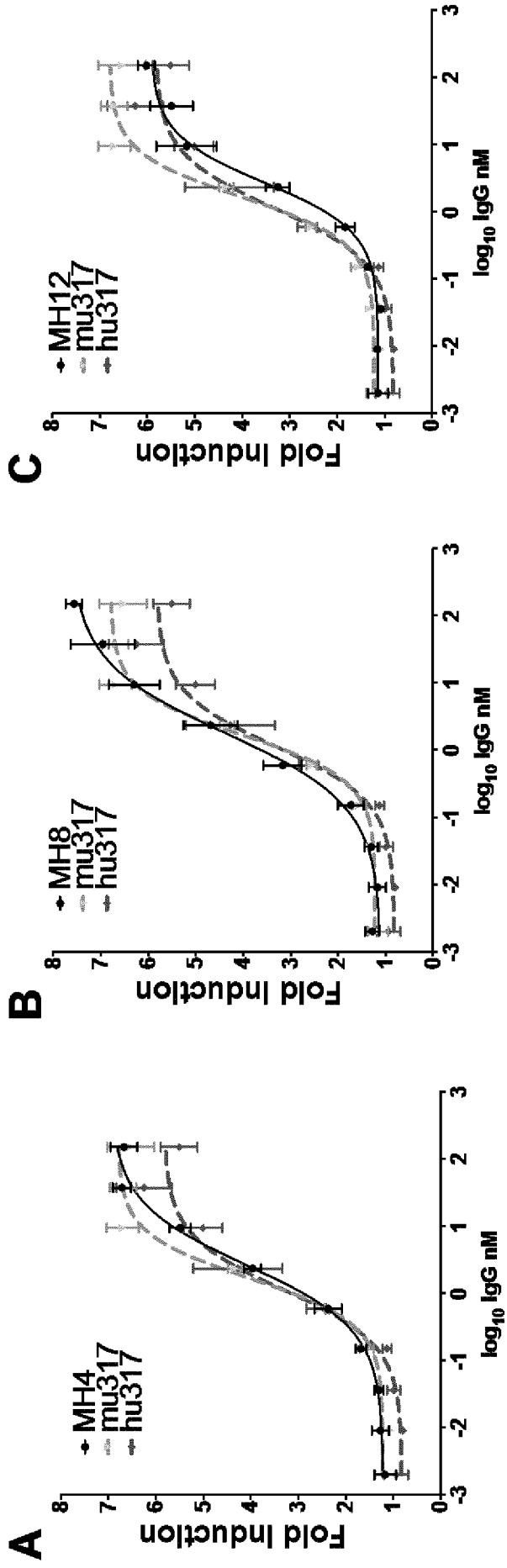


FIG. 6

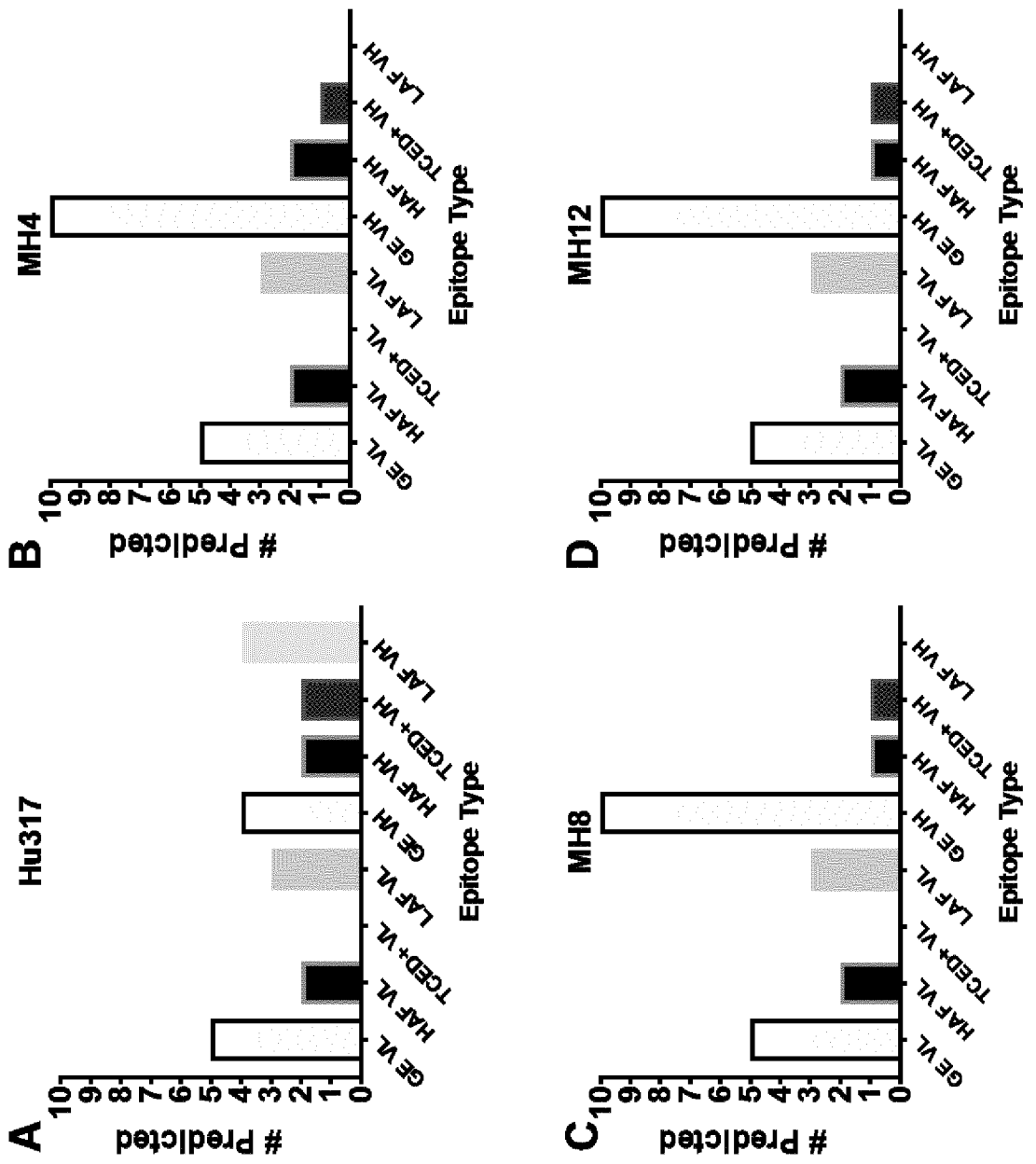

A
Hu317
B
MH4
C
MH8
D
MH12
Predicted
Epitope Type
GE VL
HAF VL
TCED+ VL
LAF VL
GE VH
HAF VH
TCED+ VH
LAF VH

FIG. 7A-7D

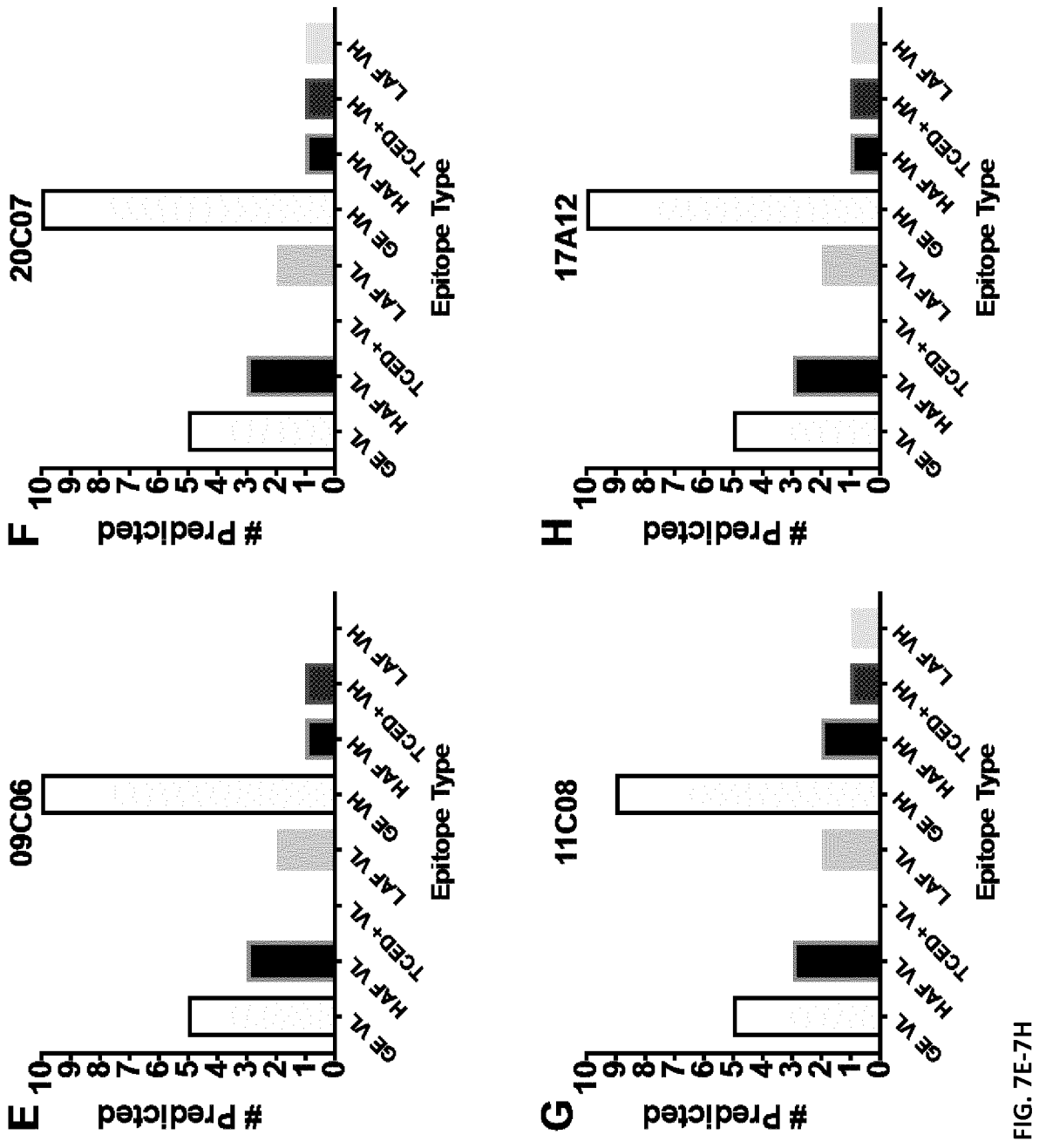

E
09C06
Predicted
Epitope Type
GE VL
HAF VL
TCED+ VL
LAF VL
GE VH
HAF VH
TCED+ VH
LAF VH
F
20C07
G
11C08
H
17A12

FIG. 7E-7H

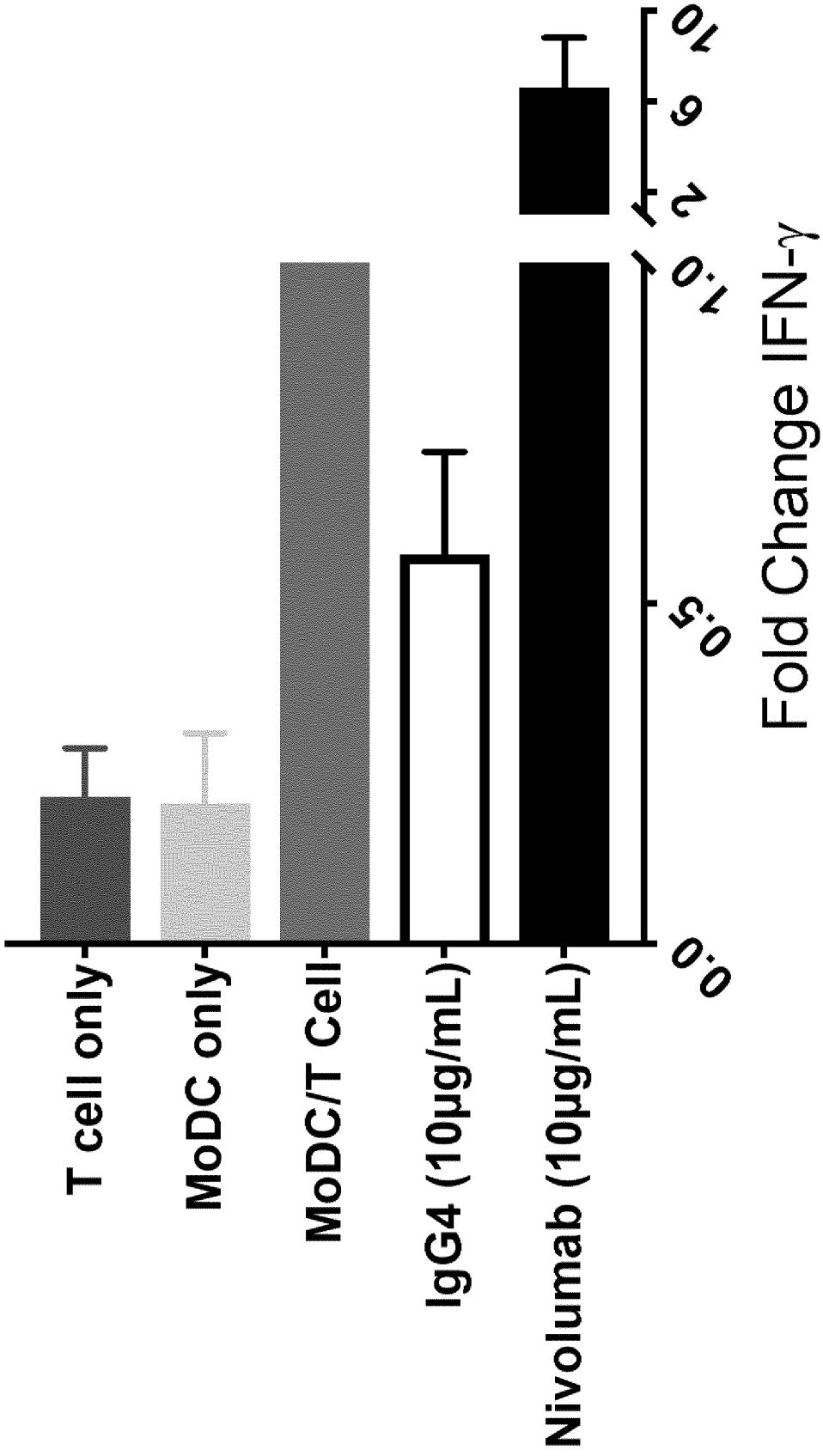

T cell only
MoDC only
MoDC/T Cell
IgG4 (10µg/mL)
Nivolumab (10µg/mL)
0.0
0.5
1.0
2
6
10
Fold Change IFN-γ

FIG. 8A

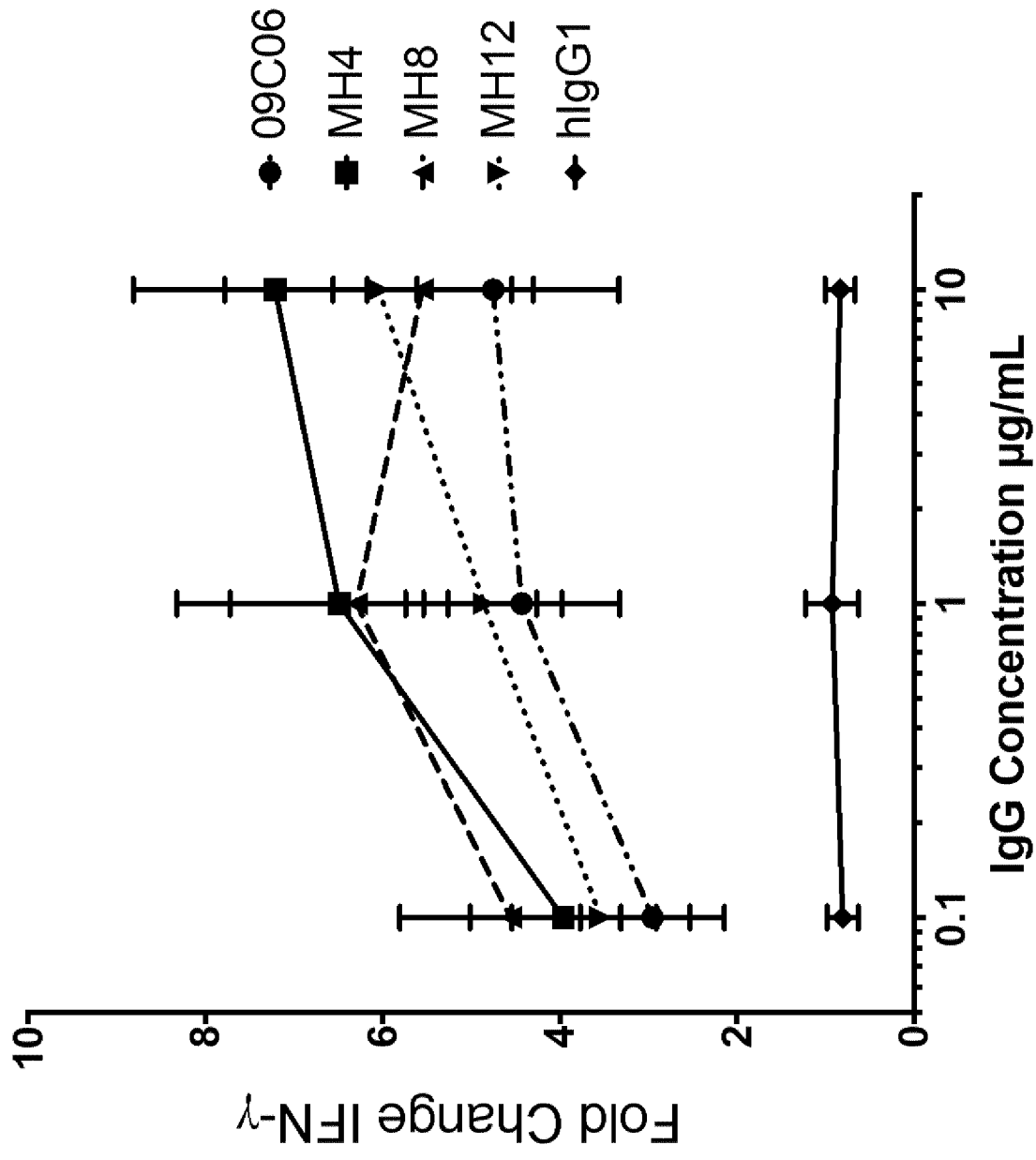

09C06
MH4
MH8
MH12
hIgG1
Fold Change IFN-γ
0
2
4
6
8
10
0.1
1
10
IgG Concentration µg/mL

FIG. 8B

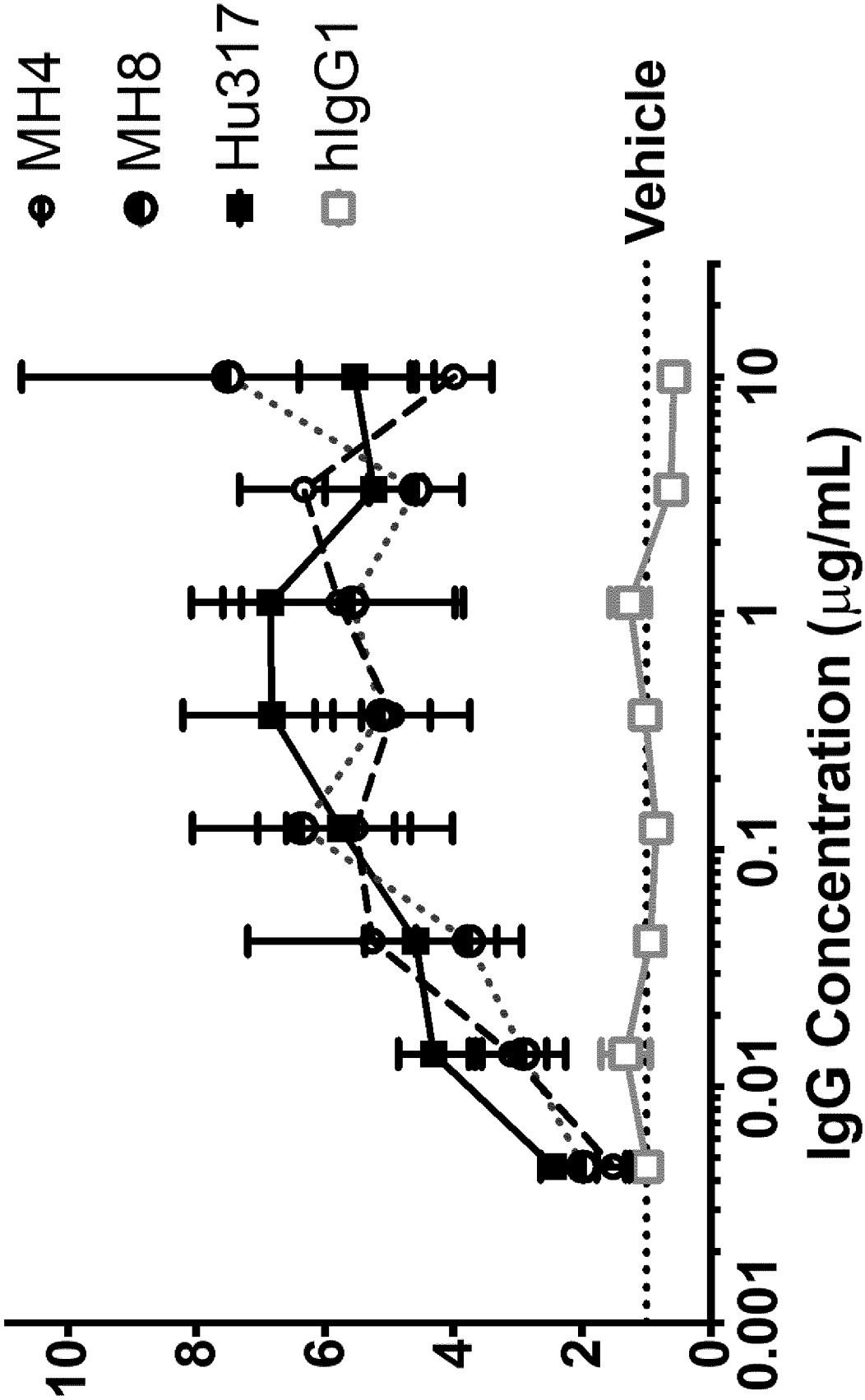
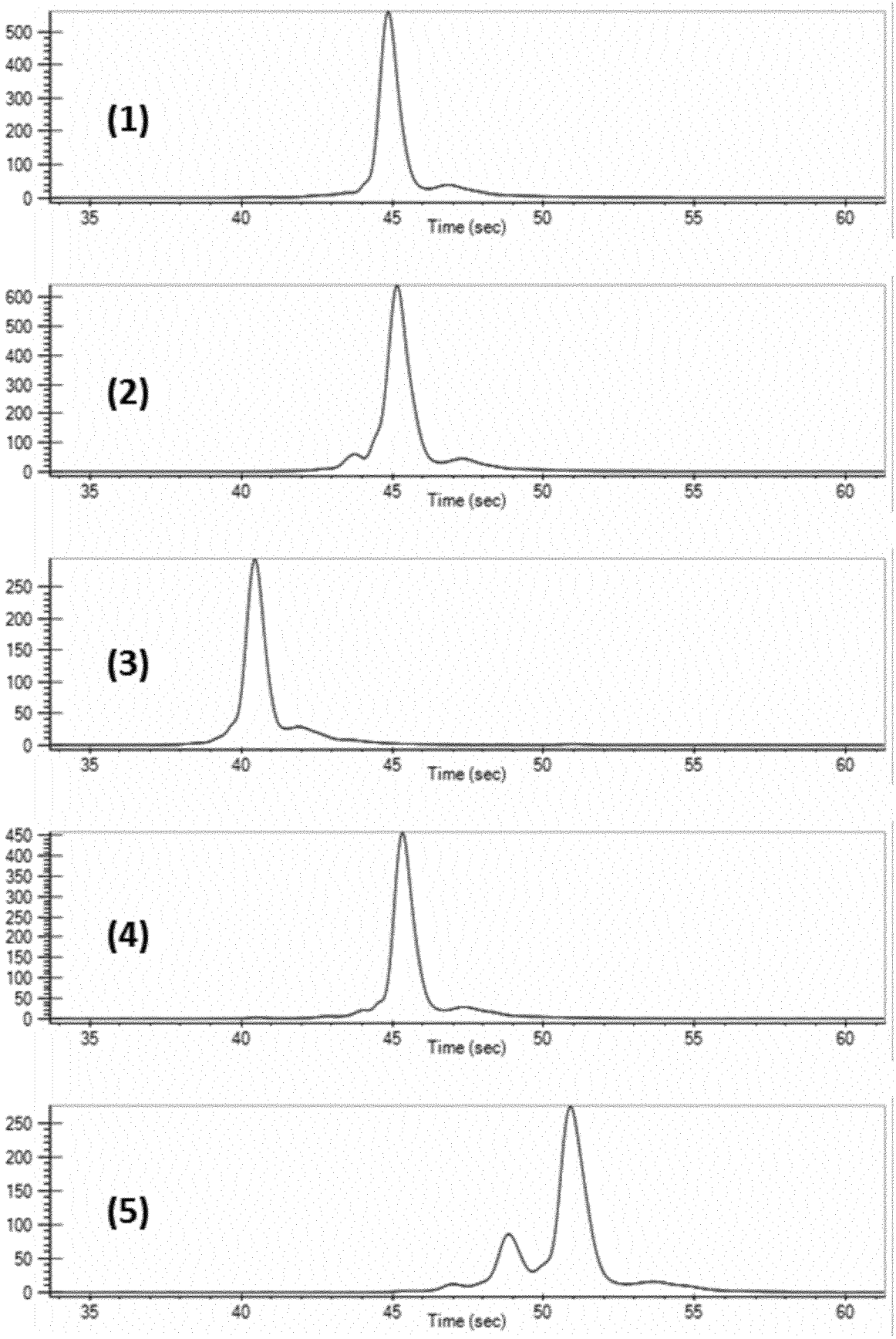

MH4
MH8
Hu317
hIgG1
Vehicle
Fold Change IFN-γ
IgG Concentration (μg/mL)
0
2
4
6
8
10
0.001
0.01
0.1
1
10

FIG. 8C

FIG. 9



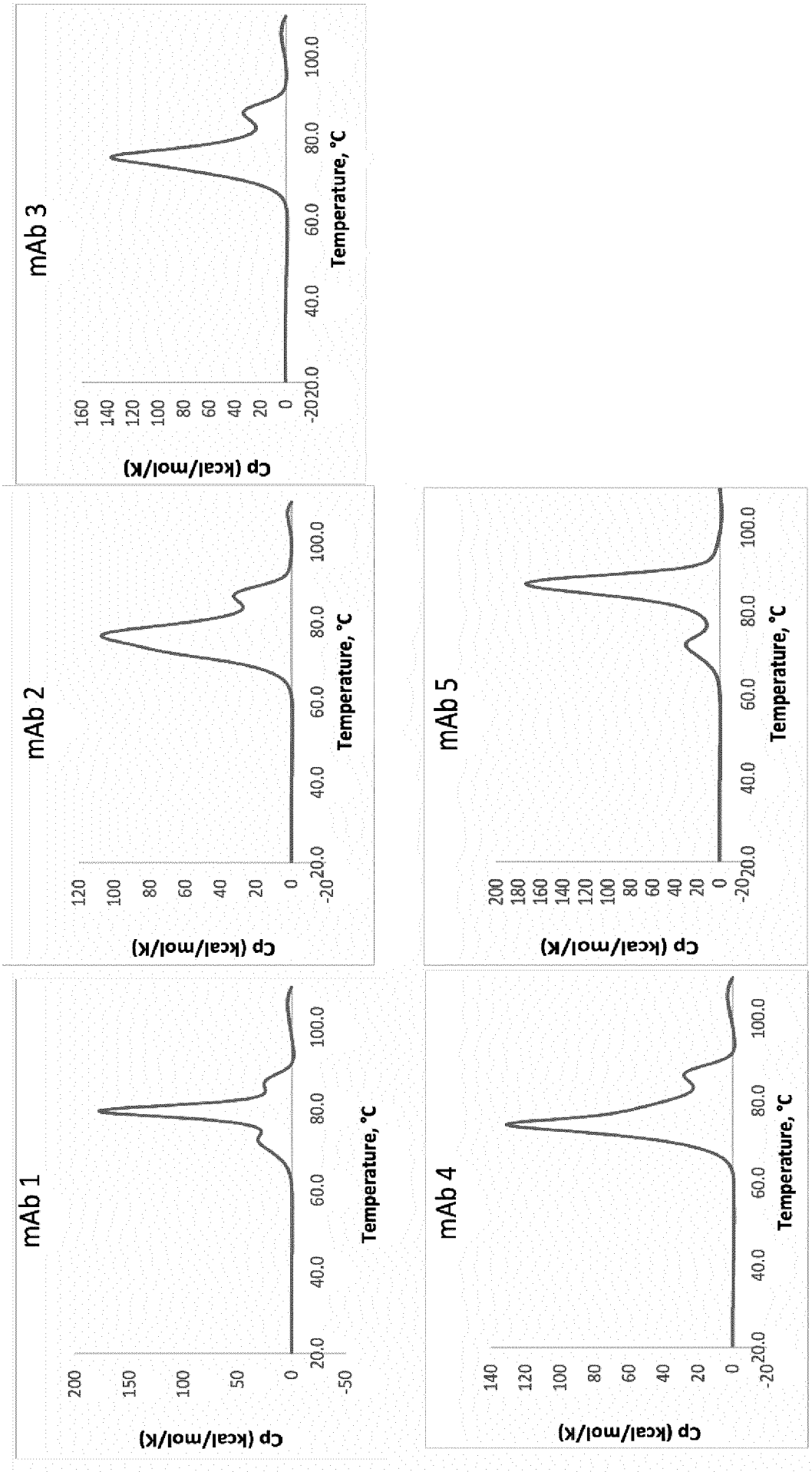

mAb 1
Cp (kcal/mol/K)
Temperature, °C
mAb 2
Cp (kcal/mol/K)
Temperature, °C
mAb 3
Cp (kcal/mol/K)
Temperature, °C
mAb 4
Cp (kcal/mol/K)
Temperature, °C
mAb 5
Cp (kcal/mol/K)
Temperature, °C

FIG. 10

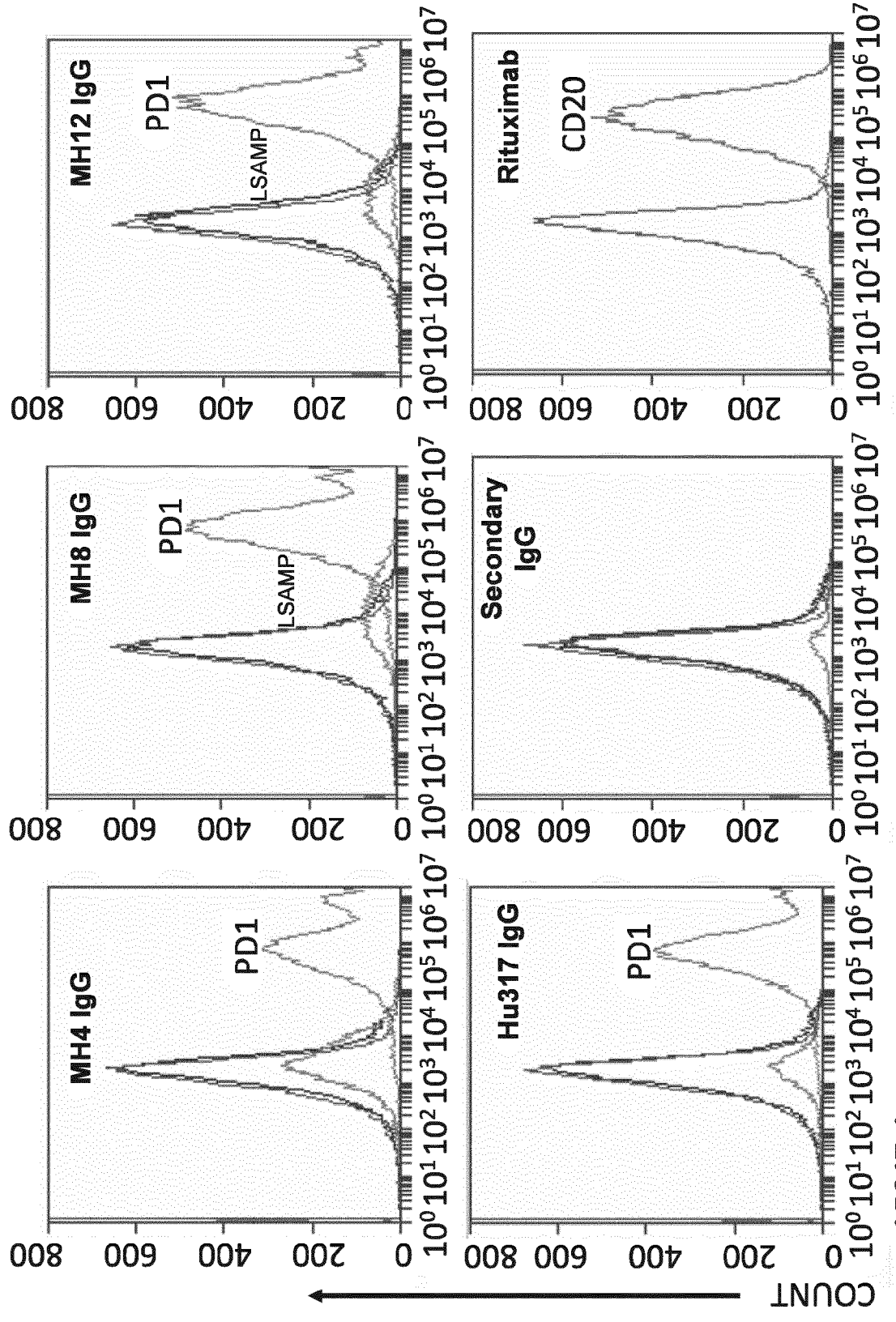

MH4 IgG
PD1
MH8 IgG
PD1
LSAMP
MH12 IgG
PD1
LSAMP
Hu317 IgG
PD1
Secondary IgG
Rituximab
CD20
COUNT
AF647-A
0
200
400
600
800
10^0 10^1 10^2 10^3 10^4 10^5 10^6 10^7

FIG. 11

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2019/055927

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K16/28 A61P35/00
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07K A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 8 735 553 B1 (LI K; LIU Q; PENG A; PENG H; SONG J; XU L; ZHANG T; ZHNG T) 27 May 2014 (2014-05-27) claims 1-35; examples 1-12	1,3-37
X	WO 2006/121168 A1 (ONO PHARMACEUTICAL CO [JP]; MEDAREX INC [US] ET AL.) 16 November 2006 (2006-11-16) claims 1-105; examples 1-25	1,3-37
A	EP 3 081 576 A1 (SHANGHAI HENGRUI PHARM CO LTD [CN]; JIANGSU HENGRUI MEDICINE CO [CN]) 19 October 2016 (2016-10-19) claims 1-27; examples 1-11	1-37

-/--

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
24 May 2019	11/06/2019

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Le Flao, Katell

1

Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2017/055404 A1 (F HOFFMANN-LA ROCHE AG [CH]; HOFFMANN-LA ROCHE INC [US]) 6 April 2017 (2017-04-06) page 1, line 27 - page 3, line 29; claims 1-30	1-37

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