

SUMMARY

The present disclosure encompasses a PD1 binder (*e.g.*, an immunoglobulin single variable domain (ISVD) or a Nanobody that binds to PD1 (*e.g.*, human PD1) comprising the amino acid sequence set forth in SEQ ID NO: 1 or 2; but which comprises a mutation at one or more of positions 1, 11, 14, 52a, 73, 74, 83, 89, 100a, 110 and 112 wherein said positions are numbered according to Kabat (*e.g.*, L11V, A14P, A74S, K83R, I89L and, optionally, E1D; or L11V,A14P,W52aV,N73P,A74S,K83R,I89L,W100a and, optionally, E1D), comprising CDR1 that comprises the amino acid sequence: IHAMG (SEQ ID NO: 3) or GSIASIHAMG (SEQ ID NO: 6); CDR2 that comprises the amino acid sequence: VITXSGGITYYADSVKG (SEQ ID NO: 4; wherein X is W or V) or VITXSGGITY (SEQ ID NO: 7; wherein X is W or V); and CDR3 that comprises the amino acid sequence: DKHQSSXYDY (SEQ ID NO: 5, wherein X is W or F) or DKHQSSXYDY (SEQ ID NO: 8, wherein X is W or F). In an embodiment, the PD1 binder (*e.g.*, an ISVD such as a Nanobody) comprises an amino acid residue at position 11 that is chosen from L or V; an amino acid residue at position 89 chosen from T, V or L; an amino acid residue at position 110 chosen from T, K or Q; and/or an amino acid residue at position 112 chosen from S, K or Q. In an embodiment, the PD1 binder (*e.g.*, an ISVD such as a Nanobody) comprises one or more mutations described is a member selected from the group consisting of: 89T; 89L in combination with 11V; 89L in combination with 110K or 110Q; 89L in combination with 112K or 112Q; 89L in combination with 11V and 110K or 110Q; 89L in combination with 11V and 112K or 112Q; 11V in combination with 110K or 110Q; 11V in combination with 112K or 112Q. In an embodiment, the amino acid at positions 11, 89, 110 and 112 are as any of those set forth in the Table B herein. In an embodiment, the PD1 binder (*e.g.*, an ISVD such as a Nanobody) comprises one or more mutations at a position selected from the group consisting of 1, 14, 41, 74, 83, 87 and 108 and/or one or more humanizing substitutions known per se (for which reference is made to the prior art cited herein,

Ala; (c) $n = 3$ and each $X = \text{Ala}$; (d) $n = 2$ and at least one $X = \text{Ala}$ and wherein the remaining amino acid residue(s) X are independently chosen from any naturally occurring amino acid; (e) $n = 3$ and at least one $X = \text{Ala}$ and wherein the remaining amino acid residue(s) X are independently chosen from any naturally occurring amino acid; (f) $n = 3$ and at least two $X = \text{Ala}$ and wherein the remaining amino acid residue(s) X are independently chosen from any naturally occurring amino acid; (g) $n = 1$ and $X = \text{Gly}$; (h) $n = 2$ and each $X = \text{Gly}$; (i) $n = 3$ and each $X = \text{Gly}$; (j) $n = 2$ and at least one $X = \text{Gly}$ wherein the remaining amino acid residue(s) X are independently chosen from any naturally occurring amino acid; (k) $n = 3$ and at least one $X = \text{Gly}$ wherein the remaining amino acid residue(s) X are independently chosen from any naturally occurring amino acid; (l) $n = 3$ and at least two $X = \text{Gly}$ wherein the remaining amino acid residue(s) X are independently chosen from any naturally occurring amino acid; (m) $n = 2$ and each $X = \text{Ala}$ or Gly ; (n) $n = 3$ and each $X = \text{Ala}$ or Gly ; (o) $n = 3$ and at least one $X = \text{Ala}$ or Gly wherein remaining amino acid residue(s) X are independently chosen from any naturally occurring amino acid; or (p) $n = 3$ and at least two $X = \text{Ala}$ or Gly wherein the remaining amino acid residue(s) X are independently chosen from any naturally occurring amino acid, *e.g.*, A, AA, AAA, G, GG, GGG, AG, GA, AAG, AGG, AGA, GGA, GAA or GAG. The present disclosure also provides a PD1 binder (*e.g.*, an ISVD such as a Nanobody) comprising one or mutations at position 11, 89, 110 and 112 and an amino acid sequence having at least 85% (*e.g.*, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 99.5, 99.9 or 100%) sequence identity with the amino acid sequence set forth in a member selected from the group consisting of SEQ ID NOs: 9-40 (in which any C-terminal extension that may be present as well as any CDRs are not taken into account for determining the degree of sequence identity).

In one aspect, there is provided a PD1 binder comprising an immunoglobulin single variable domain (ISVD) that binds to PD1, wherein the immunoglobulin single variable domain comprises the amino acid sequence:

DVQLVESGGG VVQPGGSLRL SCAASGSIAS IHAMGWFRQA PGKEREFVAV ITWSGGITYY
ADSVKGRFTI SRDNSKNTVY LQMNSLRPED TALYYCAGDK HQSSWYDYWG QGTLVTVSS

(SEQ ID NO: 57, or said sequence comprising a D1E mutation);

EVQLVESGGGVVQPGGSLRLSCAASGSIASIHAMGWFRQAPGKEREFVAVITWSGGITYYADSVKGRFTISRDN SKNT
VYLQMNSLRPEDTALYYCAGDKHQSSWYDYWGQGTLVTVSS

(SEQ ID NO: 99, or said sequence comprising a E1D mutation);

DVQLVESGGGVVQPGGSLRLSCAASGSIASIHAMGWFRQAPGKEREFVAVITVSGGITYYADSVKGRFTISRDN SKNT
VYLQMNSLRPEDTALYYCAGDKHQSSFYDYWGQGTLVTVSS

(SEQ ID NO: 103, or said sequence comprising a D1E and/or N73S mutation);

DVQLVESGGGVVQPGGSLRLSCAASGSIASIHAMGWFRQAPGKEREFVAVITVSGGITYYADSVKGRFTISRQSKNT
VYLQMNSLRPEDTALYYCAGDKHQSSFYDYWGQGTLLTVSS

(SEQ ID NO: 104, or said sequence comprising a D1E mutation); or

5 DVQLVESGGGVVQPGGSLRLSCAASGSIASIHAMGWFRQAPGKEREFVAVITVSGGITYYADSVKGRFTISRDPKNT
VYLQMNSLRPEDTALYYCAGDKHQSSFYDYWGQGTLLTVSS

(SEQ ID NO: 105, or said sequence comprising a D1E mutation), or

DVQLVESGGGVVQPGGSLRLSCAASGSIASIHAMGWFRQAPGKEREFVAVITVSGGITYYADSVKGRFTISRQSKNT
VYLQMNSLRPEDTALYYCAGDKHQSSFYDYWGQGTLLTVSS (amino acids 1-119 of SEQ ID NO: 117, or

10 said sequence comprising a D1E mutation); or

DVQLVESGGGVVQPGGSLRLSCAASGSIASIHAMGWFRQAPGKEREFVAVITVSGGITYYADSVKGRFTISRDSKNT
VYLQMNSLRPEDTALYYCAGDKHQSSFYDYWGQGTLLTVSS (amino acids 1-119 of SEQ ID NO: 121, or

said sequence comprising a D1E mutation);

and, optionally, comprising a half-life extender and/or a C-terminal extender.

15 The present disclosure also provides a multispecific immunoglobulin comprising a PD1
binder (*e.g.*, an ISVD such as a Nanobody) that binds to PD1 linked to one or more molecules
that bind to an epitope that is not the epitope to which the PD1 binder binds (*e.g.*, PD1, CTLA4,
LAG3, BTLA and/or CD27), *e.g.*, comprising a PD1 binding moiety and a CTLA4 binding
moiety; a PD1 binding moiety and a BTLA binding moiety; a PD1 binding moiety and a LAG3
20 binding moiety; or a PD1 binding moiety, a LAG3 binding moiety and a BTLA binding moiety,
optionally linked via one or more linkers, *e.g.*, peptide linkers. In one embodiment, the
multispecific immunoglobulin comprises a first PD1 Nanobody linked to one or more molecules
selected from the group consisting of an CTLA4 Nanobody, an LAG3 Nanobody, an BTLA
Nanobody, an

- a C-terminal extension such as an alanine;

or

- the PD1 binder 102C12 (E1D,L11V,A14P,A74S,K83R,I89L) or 1PD102C12 (E1D, L11V, A14P, W52aV, N73X (*e.g.*, N73P or N73Q or N73S), A74S, K83R, I89L, W100aF);

- a peptide linker such as 9GS, 20GS or 35 GS (*e.g.*, GGGGSGGGSGGGSGGGSGGGSGGGSGGGSGGGGS (SEQ ID NO: 70));

- the PD1 binder 102C12 (L11V,A14P,A74S,K83R,I89L) or 1PD102C12 (L11V, A14P, W52aV, N73X (*e.g.*, N73P or N73Q or N73S), A74S, K83R, I89L, W100aF);

- a peptide linker such as 9GS, 20GS or 35 GS (*e.g.*, GGGGSGGGSGGGSGGGSGGGSGGGSGGGSGGGGS (SEQ ID NO: 70));

- the LAG3 binder 11B09 (L11V,A14P,R41P,N43K,A62S,A74S,K83R,V89L);

- a peptide linker such as 9GS, 20GS or 35 GS (*e.g.*, GGGGSGGGSGGGSGGGSGGGSGGGSGGGSGGGGS (SEQ ID NO: 70));

- the LAG3 binder 11B09 (L11V,A14P,R41P,N43K,A62S,A74S,K83R,V89L);

- a peptide linker such as 9GS, 20GS or 35 GS (*e.g.*, GGGGSGGGSGGGSGGGSGGGSGGGSGGGSGGGGS (SEQ ID NO: 70));

- a half-life extender such as ALB11002; and, optionally,

- a C-terminal extension such as an alanine.

The half -life extender is, in an embodiment, a human serum albumin (HSA) binder such as ALB11002. In an embodiment, the HSA binder comprises CDR1 comprising the amino acid sequence GFTFSSFGMS (SEQ ID NO: 60); CDR2 comprising the amino acid sequence

SISGSGSDDL (SEQ ID NO: 61); and CDR3 comprising the amino acid sequence GGSLSR (SEQ ID NO: 62), *e.g.*, comprising the amino acid sequence EVQLVESGGG VVQPGNSLR

SCAASGFTFS SFGMSWVRQA PGKGLEWVSS ISGSGSDTLVADSVKGRFTI SRDNAKTTLY LQMNSLRPED TALYYCTIGG SLRSSQGTI VTVSSA (SEQ ID NO: 59). Such half-life extenders themselves are part of the present disclosure.

optionally administered in association with a further therapeutic agent or a therapeutic procedure *e.g.*, surgical tumorectomy.

In another aspect, there is provided use of a PD1 binder as disclosed herein in the manufacture of a medicament for the treatment of cancer or an infectious disease.

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DESCRIPTION OF THE FIGURES

Figure 1. A table listing some of the amino acid positions that will be specifically referred to herein and their numbering according to some alternative numbering systems (such as Aho and IMGT)

10 **Figure 2 (1-5).** PD1 binder sequences.

Figure 3 (A-B). Alignment of 102C12 sequence with that of the SEQ ID NOs: 9-40 (see WO 2008/071447, SEQ ID NO: 348).

15 **Figure 4.** Predominant N-linked glycans for monoclonal antibodies produced in Chinese hamster ovary cells (CHO N-linked glycans) and in engineered yeast cells (engineered yeast N-linked glycans): squares: N-acetylglucosamine (GlcNAc); circles: mannose (Man); diamonds: galactose (Gal); triangles: fucose (Fuc).

Figure 5. Monovalent binding of CHO-K1 expressing human PD1 by F023700275 (1PD102C12(A14P,A74S,K83R)-FLAG3-HIS6) or F023700706 (1PD102C12 (L11V,A14P,A74S,K83R,I89L)-FLAG3-HIS6). GAM=goat anti-mouse secondary antibody use
20 to detect mouse antibody that binds to FLAG epitope; α -FLAG + GAM=goat anti-mouse secondary antibody used plus mouse antibody that binds to FLAG epitope that is at C-terminus of the construct.

Figure 6. Monovalent binding of 3A9 cells expressing human LAG3 by F0237611B09-FLAG-His6 or F023700842 (F0237611B09(L11V,A14P,R41P,N43K,A62S,A74S,K83R,V89L)-
25 FLAG3-HIS6.

Figure 7 (A-B). Binding of (A) CHO expressing human PD-1 by F023700931, F023700924, F023700933 or F023700962 or (B) 3A9 expressing rhesus PD-1 by F023700931 (*Pichia* or CHO expressed), F023700924 (*Pichia* or CHO expressed), F023700933, F023700962, F023700678 (1PD102C12 (A14P,A74S,K83R)-35GS-ALB11002) or F023701127. ABH0074 +
30 GAM=goat anti-mouse secondary antibody used plus mouse antibody ABH0074 that binds to nanobody frameworks.

We claim:

1. A PD1 binder comprising an immunoglobulin single variable domain (ISVD) that binds to PD1, wherein the immunoglobulin single variable domain comprises the amino acid sequence:

DVQLVESGGG VVQPGGSLRL SCAASGSIAS IHAMGWFRQA PGKEREVAV ITWSGGITYY

5 ADSVKGRFTI SRDNSKNTVY LQMNSLRPED TALYYCAGDK HQSSWYDYWG QGTLVTVSS

(SEQ ID NO: 57, or said sequence comprising a D1E mutation);

EVQLVESGGGVVQPGGSLRLSCAASGSIASIHAMGWFRQAPGKEREVAVITWSGGITYYADSVKGRFTISRDNKNT
VYLQMNSLRPEDTALYYCAGDKHQSSWYDYWGQGTLVTVSS

(SEQ ID NO: 99, or said sequence comprising a E1D mutation);

10 DVQLVESGGGVVQPGGSLRLSCAASGSIASIHAMGWFRQAPGKEREVAVITVSGGITYYADSVKGRFTISRDNKNT
VYLQMNSLRPEDTALYYCAGDKHQSSFYDYWGQGTLVTVSS

(SEQ ID NO: 103, or said sequence comprising a D1E and/or N73S mutation);

DVQLVESGGGVVQPGGSLRLSCAASGSIASIHAMGWFRQAPGKEREVAVITVSGGITYYADSVKGRFTISRQSKNT
VYLQMNSLRPEDTALYYCAGDKHQSSFYDYWGQGTLVTVSS

15 (SEQ ID NO: 104, or said sequence comprising a D1E mutation); or

DVQLVESGGGVVQPGGSLRLSCAASGSIASIHAMGWFRQAPGKEREVAVITVSGGITYYADSVKGRFTISRDPKNT
VYLQMNSLRPEDTALYYCAGDKHQSSFYDYWGQGTLVTVSS

(SEQ ID NO: 105, or said sequence comprising a D1E mutation), or

DVQLVESGGGVVQPGGSLRLSCAASGSIASIHAMGWFRQAPGKEREVAVITVSGGITYYADSVKGRFTISRQSKNT

20 VYLQMNSLRPEDTALYYCAGDKHQSSFYDYWGQGTLVTVSS (amino acids 1-119 of SEQ ID NO: 117, or
said sequence comprising a D1E mutation); or

DVQLVESGGGVVQPGGSLRLSCAASGSIASIHAMGWFRQAPGKEREVAVITVSGGITYYADSVKGRFTISRDSKNT
VYLQMNSLRPEDTALYYCAGDKHQSSFYDYWGQGTLVTVSS (amino acids 1-119 of SEQ ID NO: 121, or

said sequence comprising a D1E mutation);

25 and, optionally, comprising a half-life extender and/or a C-terminal extender.

2. The PD1 binder of claim 1, wherein the PD1 binder is linked at the C-terminus to a half-life extender comprising the amino acid sequence of SEQ ID NO: 59.

30 3. The PD1 binder of claim 1 or 2 further comprising a C-terminal extender wherein the C-terminal extender is an alanine.

4. An injection device or vessel that comprises the binder of any one of claims 1 to 3.

35 5. A polynucleotide encoding the binder of any one of claims 1 to 3.

6. A vector comprising the polynucleotide of claim 5.
7. A host cell comprising the polynucleotide of claim 5 or the vector of claim 6.
- 5 8. A method for making a PD1 binder of any one of claims 1 to 3 comprising introducing a polynucleotide encoding the binder into a host cell and culturing the host cell in a medium under conditions favorable to expression of said binder from said polynucleotide and, optionally, purifying the binder from said host cell and/or said medium.
- 10 9. A PD1 binder produced by the method of claim 8.
10. A method for preventing PD1 from binding to PD-L1 and/or PD-L2 in vitro comprising contacting the PD1 with said binder of any one of claims 1 to 3 optionally in association with a further therapeutic agent.
- 15 11. A PD1 binder of any one of claims 1 to 3 for use in enhancing an immune response in a subject, optionally in association with a further therapeutic agent.
12. Use of a PD1 binder of any one of claims 1 to 3 in the manufacture of a medicament for
20 the treatment of cancer or an infectious disease.
13. The use of claim 12 wherein the cancer is metastatic cancer, a solid tumor, a hematologic cancer, leukemia, lymphoma, osteosarcoma, rhabdomyosarcoma, neuroblastoma, kidney cancer, leukemia, renal transitional cell cancer, bladder cancer, Wilm's cancer, ovarian cancer, pancreatic
25 cancer, breast cancer, prostate cancer, bone cancer, lung cancer, non-small cell lung cancer, gastric cancer, colorectal cancer, cervical cancer, synovial sarcoma, head and neck cancer, squamous cell carcinoma, multiple myeloma, renal cell cancer, retinoblastoma, hepatoblastoma, hepatocellular carcinoma, melanoma, rhabdoid tumor of the kidney, Ewing's sarcoma, chondrosarcoma, brain cancer, glioblastoma, meningioma, pituitary adenoma, vestibular
30 schwannoma, a primitive neuroectodermal tumor, medulloblastoma, astrocytoma, anaplastic astrocytoma, oligodendroglioma, ependymoma, choroid plexus papilloma, polycythemia vera, thrombocythemia, idiopathic myelofibrosis, soft tissue sarcoma, thyroid cancer, endometrial cancer, carcinoid cancer or liver cancer, breast cancer or gastric cancer.

14. The use of claim 12 wherein the infectious disease is a bacterial infection, a viral infection or a fungal infection.

- 5 15. The use of any one of claims 12-14 wherein the medicament is to be administered in association with other, further therapeutic agents and/or therapeutic procedures.