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 one aspect, there is provided a PD1 binder comprising an immunoglobulin single variable domain (ISVD) that binds to PD1 comprising the amino acid sequence set forth in SEQ ID NO: 1 or 2 and further comprising a substitution of the amino acids at positions 11 and 89 with amino acids V and L, respectively wherein said residue numbers are Kabat residue numbers; and, optionally, a half-life extender and/or a C-terminal extender. 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,

such as WO 08/020079 or WO 09/138519. In an embodiment, the PD1 binder (*e.g.*, an ISVD such as a Nanobody) comprises a C-terminal extension of 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 amino acids. For example, in an embodiment, the PD1 binder (*e.g.*, an ISVD such as a Nanobody) comprises a C-terminal extension according to the formula -X(n), wherein X and n are as follows: (a) n = 1 and X = Ala; (b) n = 2 and each X =

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 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 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) or an infectious disease (bacterial infection, a viral infection or a fungal infection) in the body of a subject comprising administering an effective amount of PD1 binder (*e.g.*, immunoglobulin single variable domain (ISVD) or multispecific ISVD such as a Nanobody) optionally in association with a further therapeutic agent to the subject. In an embodiment, the subject is also administered a further therapeutic agent or a therapeutic procedure in association with the PD1 binder.

The present disclosure provides PD1 binders (*e.g.*, ISVDs such as Nanobodies) that bind to PD1 comprising: CDR1 comprising the amino acid sequence IHAMG (SEQ ID NO: 3) or GSIASIHAMG (SEQ ID NO: 6); CDR2 comprising 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 comprising the amino acid sequence DKHQSSXYDY (SEQ ID NO: 5, wherein X is W or F), which comprise one or more mutations at position 11 (*e.g.*, L11V) and 89 (*e.g.*, I89L) or one or more mutations selected from

The present disclosure also provides LAG3 binders that bind to LAG3 comprising CDR1 comprising the amino acid sequence GRTFSDYVMG (SEQ ID NO: 65); CDR2 comprising the amino acid sequence AISESGGRTH (SEQ ID NO: 66); and CDR3 comprising the amino acid sequence TLLWWTSEYAPIKANDYDY (SEQ ID NO: 67), *e.g.*, comprising the amino acid sequence: EVQLVE SGGGVVQPGG SLRLSCAASG RTFSDYVMGW FRQAPGKERE FVAAISESGG RTHYADSVKG RFTISRDNSK NTLYLQMNSL RPEDTALYYC ATTLLWWTSE YAPIKANDYD YWGQGTLVTV SS (SEQ ID NO: 64).

The PD1 and LAG3 binders may be in a single molecule such as a PD1/LAG3 binder, which is part of the present disclosure, that binds to PD1 and LAG3 that comprises: a PD1 binder comprising: CDR1 comprising the amino acid sequence IHAMG (SEQ ID NO: 3) or GSIASIHAMG (SEQ ID NO: 6); CDR2 comprising 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 comprising the amino acid sequence DKHQSSXYDY (SEQ ID NO: 5, wherein X is W or F); and a LAG3 binder comprising: CDR1 comprising the amino acid

- 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.*, GGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGS (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.*, GGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGS (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.*, GGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGS (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.*, GGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGS (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 SISGSGSDTL (SEQ ID NO: 61); and CDR3 comprising the amino acid sequence GGSLSR (SEQ ID NO: 62), *e.g.*, comprising the amino acid sequence EVQLVESGGG VVQPGNSLRL SCAASGFTFS SFGMSWVRQA PGKGLEWVSS ISGSGSDTLYADSVKGRFTI SRDNAKTTLY LQMNSLRPED TALYYCTIGG SLSRSSQGTL VTVSSA (SEQ ID NO: 59). Such half-life extenders themselves are part of the present disclosure.

In another aspect, there is provided a binder that cross-blocks a binder as disclosed herein from binding to PD1.

In an embodiment, the PD1 binder, LAG3 binder, HSA binder and/or PD1/LAG3 binder is in an injection device or vessel optionally in association with a further therapeutic agent. Such a device or vessel is part of the present disclosure. In another aspect, there is provided an injection device or vessel that comprises the binder as disclosed herein.

The present disclosure also provides a polynucleotide encoding any of the binders set forth herein as well as any vector (*e.g.*, plasmid) comprising the polynucleotide as well as any host cell (*e.g.*, CHO or fungal cell such as *Pichia*, *e.g.*, *P. pastoris*) comprising the polynucleotide or vector, *e.g.*, ectopic or integrated into one or more host cell chromosomes. In another aspect, there is provided a polynucleotide encoding the binder as disclosed herein. In another aspect, there is provided a vector comprising the polynucleotide as disclosed herein. In another aspect, there is provided a host cell comprising the polynucleotide or vector as disclosed herein.

The present disclosure, in another aspect, provides a method for making any of the binders set forth herein comprising introducing a polynucleotide encoding the binder into a host cell (*e.g.*, CHO or fungal cell such as *Pichia*, *e.g.*, *P. pastoris*) 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. Optionally, the vector or polynucleotide is integrated into one or more host cell chromosomes. In another aspect, there is provided a PD1 binder produced by such a method.

The present disclosure also provides a method for preventing PD1 from binding to PD-L1 and/or PD-L2 or for inhibiting any PD1 activity, *e.g.*, as discussed herein, comprising contacting said PD1 with a PD1 binder of the present disclosure (*e.g.*, a PD1/LAG3 binder) optionally in association with a further therapeutic agent. In another aspect, there is provided a method for preventing PD1 from binding to PD-L1 and/or PD-L2 in vitro comprising contacting the PD1 with said binder as disclosed herein optionally in association with a further therapeutic agent. The present disclosure also provides a method for preventing LAG3 from binding to MHC class II or for inhibiting any PD1 activity, *e.g.*, as discussed herein comprising contacting said LAG3 with a LAG3 binder of the present disclosure (*e.g.*, a PD1/LAG3 binder) optionally in association with a further therapeutic agent.

The present disclosure also provides a method for enhancing an immune response in the body of a subject (*e.g.*, a human) comprising administering an effective amount of binder of the present disclosure (*e.g.*, PD1/LAG3 binder) to the subject optionally in association with a further therapeutic agent. In another aspect, there is provided a PD1 binder as disclosed herein for use in enhancing an immune response in a subject, optionally in association with a further therapeutic agent. The present disclosure also provides a method for treating or preventing cancer (*e.g.*, metastatic cancer, a solid tumor or a hematologic cancer) or an infectious disease (*e.g.*, bacterial infection, a viral infection or a fungal infection) in the body of a subject (*e.g.*, a human) comprising administering an effective amount of binder of the present disclosure (*e.g.*, a PD1/LAG3 binder) optionally in association with a further therapeutic agent to the subject. When administered to a subject, a PD1 and/or LAG3 binder of the present disclosure (*e.g.*, a PD1/LAG3 binder) is

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 or prevention of cancer or an infectious disease.

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)

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).

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 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)-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 + 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 comprising the amino acid sequence set forth in SEQ ID NO: 1 or 2 and further comprising a substitution of the amino acids at positions 11 and 89 with amino acids V and L, respectively wherein said residue numbers are Kabat residue numbers;
and, optionally, a half-life extender and/or a C-terminal extender.

2. The PD1 binder of claim 1 further comprising:
a CDR1 comprising the amino acid sequence IHAMG (SEQ ID NO: 3) or GSIASIHAMG (SEQ ID NO: 6);
a CDR2 comprising 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
a CDR3 comprising the amino acid sequence DKHQSSXYDY (SEQ ID NO: 5, wherein X is W or F).

3. The PD1 binder of claim 2 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);
EVQLVESGGGVVQPGGSLRLSCAASGSIASIHAMGWFRQAPGKEREFVAVITWSGGITYYADSVKGRFTISRDNSKNT VYLQMNSLRPEDTALYYCAGDKHQSSWYDYWGQGTLVTVSS
(SEQ ID NO: 99, or said sequence comprising a E1D mutation);
DVQLVESGGGVVQPGGSLRLSCAASGSIASIHAMGWFRQAPGKEREFVAVITVSGGITYYADSVKGRFTISRDNSKNT VYLQMNSLRPEDTALYYCAGDKHQSSFYDYWGQGTLVTVSS
(SEQ ID NO: 103, or said sequence comprising a D1E and/or N73S mutation);
DVQLVESGGGVVQPGGSLRLSCAASGSIASIHAMGWFRQAPGKEREFVAVITVSGGITYYADSVKGRFTISRDQSKNT VYLQMNSLRPEDTALYYCAGDKHQSSFYDYWGQGTLVTVSS
(SEQ ID NO: 104, or said sequence comprising a D1E mutation); or
DVQLVESGGGVVQPGGSLRLSCAASGSIASIHAMGWFRQAPGKEREFVAVITVSGGITYYADSVKGRFTISRDPSKNT VYLQMNSLRPEDTALYYCAGDKHQSSFYDYWGQGTLVTVSS
(SEQ ID NO: 105, or said sequence comprising a D1E mutation), or
DVQLVESGGGVVQPGGSLRLSCAASGSIASIHAMGWFRQAPGKEREFVAVITVSGGITYYADSVKGRFTISRDQSKNT VYLQMNSLRPEDTALYYCAGDKHQSSFYDYWGQGTLVTVSS (amino acids 1-119 of SEQ ID NO: 117, or said sequence comprising a D1E mutation); or

DVQLVESGGGVVQPGGSLRLSCAASGSIASIHAMGWFRQAPGKEREFVAVITVSGGITYYADSVKGRFTISRDSSKNT
VYLQMNSLRPEDTALYYCAGDKHQSSFYDYWGQGTLVTVSS (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.

4. The PD1 binder of calim 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.

5. The PD1 binder of claim 1 or 4 further comprising a C-terminal extender wherein the C-terminal extender is an alanine.

6. A binder that cross-blocks a binder of claim 1 from binding to PD1.

7. An injection device or vessel that comprises the binder of claim 1.

8. A polynucleotide encoding the binder of claim 1.

9. A vector comprising the polynucleotide of claim 8.

10. A host cell comprising the polynucleotide of claim 8 or the vector of claim 9.

11. A method for making a PD1 binder of claim 1 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.

12. A PD1 binder produced by the method of claim 11.

13. 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 6 optionally in association with a further therapeutic agent.

14. A PD1 binder of claim 1 for use in enhancing an immune response in a subject, optionally in association with a further therapeutic agent.

15. Use of a PD1 binder of claim 1 in the manufacture of a medicament for the treatment or prevention of cancer or an infectious disease.

16. The use of claim 15 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 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 schwannoma, a primitive neuroectodermal tumor, medulloblastoma, astrocytoma, anaplastic astrocytoma, oligodendroglioma, ependymoma, choroid plexus papilloma, polycythemia vera, thrombocythemia, idiopathic myelfibrosis, soft tissue sarcoma, thyroid cancer, endometrial cancer, carcinoid cancer or liver cancer, breast cancer or gastric cancer.

17. The use of claim 15 wherein the infectious disease is a bacterial infection, a viral infection or a fungal infection.

18. The use of any one of claims 15-17 wherein the medicament is to be administered in association with other, further therapeutic agents and/or therapeutic procedures.