

CTLA-4 is upregulated and competes with CD28 for CD80 and CD86 binding on APCs but with significantly higher affinity, therefore downregulating—or deactivating—the T cell (Fig. 1). CTLA-4, therefore, downregulates T-cell responses and APC function, resulting in a decreased immune response to tumor-associated antigens and immune tolerance.

The mechanisms whereby CTLA4 and PD1 exert their inhibitory effects on T-cell activation are multifaceted. CTLA4 functions primarily to limit T-cell activation and clonal expansion, whereas PD1 functions primarily to limit effector T-cell function in the peripheral tissues. Their distinct molecular structures, regulation, signaling pathways, ligand distribution, and function on Tregs and other immune cells suggest that combined therapeutic blockade of CTLA4 and PD1 could synergize to mediate anti-tumor immunity. Intlekofer & Thompson, J. Leuko. Biol. 94(1): 25-39 (2013); Hurwitz *et al.* Proc. Natl. Acad. Sci. USA 95: 10067–10071 (1998); Parry *et al.* Mol. Cell. Biol. 25(21): 9543-9553 (2005); Callahan *et al.* Front. Oncol. Vol 4, Art. 385 (2015).

One method by which to inhibit CTLA4-mediated downregulation is by interfering with its interaction with its ligands by binding it with a Nanobody. The possibility exists that Nanobodies, originating in llamas, could cause an unwanted anti-drug immune response, *e.g.*, by binding of the Nanobodies by pre-existing antibodies in the patient's serum. Thus, novel methods by which to humanize Nanobodies so as to decrease or eliminate such a response are particularly valuable as are Nanobodies that are created by such methods.

SUMMARY

The present disclosure provides a multispecific immunoglobulin single variable domain (ISVD) such as a Nanobody that binds to human CTLA4 by contacting human CTLA4 at one or more of the following residues VRVTVL (amino acids 33 – 38 of SEQ ID NO: 110), ADSQVTEVC (amino acids 41-49 of SEQ ID NO: 110) and CKVELMYPPPYLG (amino acids 93-106 of SEQ ID NO: 110), *e.g.*, all all three sites. In an aspect, there is provided a CTLA4 binder comprising an immunoglobulin single variable domain (ISVD) that binds to human CTLA4 at one or more of the following residues: VRVTVL (amino acids 33 – 38 of SEQ ID NO: 110), ADSQVTEVC (amino acids 41-49 of SEQ ID NO: 110) and CKVELMYPPPYLG (amino acids 93-106 of SEQ ID NO: 110); wherein the ISVD comprises a mutation at residues 11 and 89 wherein said residue numbers are Kabat residue numbers. For example, the binder protects the residues from hydrogen-deuterium exchange in the presence of a deuterium source such as D₂O. In an embodiment, the

ISVD binds to human CTLA4 and generates a binding heat map (*e.g.*, as generated in a hydrogen-deuterium exchange assay) essentially as set forth in Figure 13.

The present disclosure also provides a CTLA4 binder comprising one or more (*e.g.*, 2) immunoglobulin single variable domains (ISVDs) that bind to human CTLA4 comprising:

5 CDR1

that comprises the amino acid sequence FYGMG (amino acids 6-10 of SEQ ID NO: 2) or GGTF SFYGMG (SEQ ID NO: 2); CDR2 that comprises the amino acid sequence DIRTSAGRTYYADSVKG (SEQ ID NO: 3) or DIRTSAGRTY (amino acids 1-10 of SEQ ID NO: 3); CDR3 that comprises the amino acid sequence EXSGISGWDY (SEQ ID NO: 4); optionally, wherein the ISVD comprises a mutation at residues 11 and 89 (*e.g.*, L11V and/or V89L, for example (E1D, L11V, A14P, Q45R, A74S, K83R, V89L, M96P, Q108L) wherein said residue numbers are Kabat residue numbers. In another aspect, there is provided a CTLA4 binder comprising one or more immunoglobulin single variable domains (ISVDs) that bind to human CTLA4 comprising: CDR1 that comprises the amino acid sequence FYGMG (amino acids 6-10 of SEQ ID NO: 2) or GGTF SFYGMG (SEQ ID NO: 2); CDR2 that comprises the amino acid sequence DIRTSAGRTYYADSVKG (SEQ ID NO: 3) or DIRTSAGRTY (amino acids 1-10 of SEQ ID NO: 3); CDR3 that comprises the amino acid sequence EXSGISGWDY (SEQ ID NO: 4), wherein X is M or P; wherein the ISVD comprises a mutation at residues 11 and 89 wherein said residue numbers are Kabat residue numbers.

The present disclosure provides a CTLA4 binder (*e.g.*, an ISVD, *e.g.*, a Nanobody) comprising CDR1, CDR2 and CDR3 of an immunoglobulin comprising amino acid sequence set forth in SEQ ID NO: 1 wherein said CTLA4 binder comprises at least one mutation with respect to the amino acid sequence set forth in SEQ ID NO: 1 wherein said at least one mutation is at a position selected from the group consisting of 11, 89, 110 and 112, wherein said positions are numbered according to Kabat; and optionally including any number of additional mutations that are set forth herein or otherwise, *e.g.* up to 10 (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10) additional mutations (*e.g.*, point mutations, substitutions, deletions, insertions). For example, in an embodiment, CDR1 comprises the amino acid sequence: FYGMG (SEQ ID NO: 2) or GGTF SFYGMG (SEQ ID NO: 5); CDR2 comprises the amino acid sequence: DIRTSAGRTYYADSVKG (SEQ ID NO: 3) or DIRTSAGRTY (SEQ ID NO: 6); and CDR3 comprises the amino acid sequence: EMSGISGWDY (SEQ ID NO: 4). For example, in an embodiment, the CTLA4 binder has a mutation relative to SEQ ID NO: 1 wherein the amino acid residue at position 11 is chosen from L or V; the amino acid residue at position 89 is suitably chosen from T, V or L; the amino acid residue at position 110 is suitably chosen from T, K or Q; and/or the amino acid residue at position 112 is suitably chosen from S, K or Q; *e.g.*, wherein the mutation is 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; and/or 11V in combination with 112K or 112Q. In

an embodiment, the mutation at positions 11, 89, 110 and/or 112 is as set forth in Table B. In an embodiment, the CTLA4 binder further comprises one or more mutations, relative to the SEQ ID NO: 1, at positions 1, 14, 45, 74, 83 and/or 108. In an embodiment, the CTLA4 binder has a C-terminal extension of 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 amino acids. For example, in

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embodiment, the C-terminal extension has the formula -X(n), wherein X and n are as follows:

(a) n = 1 and X = Ala; (b) n = 2 and each X = Ala; (c) n = 3 and each X = Ala; (d) n = 2 and at least one X = Ala (with the remaining amino acid residue(s) X being independently chosen from any naturally occurring amino acid); (e) n = 3 and at least one X = Ala (with the remaining amino acid residue(s) X being independently chosen from any naturally occurring amino acid); (f) n = 3 and at least two X = Ala (with the remaining amino acid residue(s) X being independently chosen from any naturally occurring amino acid); (g) n = 1 and X = Gly; (h) n = 2 and each X = Gly; (i) n = 3 and each X = Gly; (j) n = 2 and at least one X = Gly (with the remaining amino acid residue(s) X being independently chosen from any naturally occurring amino acid); (k) n = 3 and at least one X = Gly (with the remaining amino acid residue(s) X being independently chosen from any naturally occurring amino acid); (l) n = 3 and at least two X = Gly (with the remaining amino acid residue(s) X being independently chosen from any naturally occurring amino acid); (m) n = 2 and each X = Ala or Gly; (n) n = 3 and each X = Ala or Gly; (o) n = 3 and at least one X = Ala or Gly (with the remaining amino acid residue(s) X being independently chosen from any naturally occurring amino acid); or (p) n = 3 and at least two X = Ala or Gly (with the remaining amino acid residue(s) X being independently chosen from any naturally occurring amino acid). For example, in an embodiment, the C-terminal extension is A, AA, AAA, G, GG, GGG, AG, GA, AAG, AGG, AGA, GGA, GAA or GAG. The present disclosure includes a CTLA4 binder (*e.g.*, an ISVD such as a Nanobody) which comprises 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: 8-43 wherein the CTLA4 binder or ISVD comprises CDR1, CDR2 and CDR3 of an immunoglobulin comprising an amino acid sequence set forth in SEQ ID NO: 1 wherein said CTLA4 binder or ISVD comprises at least one mutation with respect to the amino acid sequence set forth in SEQ ID NO: 1 wherein said at least one mutation is at a position selected from the group consisting of 11, 89, 110 and 112, wherein said positions are numbered according to Kabat. The present disclosure also provides a CTLA4 binder, ISVD, Nanobody or polypeptide comprising the amino acid sequence selected from the group consisting of SEQ ID NOs: 9-40. The present disclosure also provides a multispecific binder comprising a CTLA4 binding moiety (*e.g.*, an ISVD such as a Nanobody) that binds to

CTLA4 which is linked to one or more molecules that bind to an epitope that is not the epitope to which the CTLA4 binding moiety binds (*e.g.*, PD1, CTLA4, LAG3, BTLA and/or CD27).

5 The present disclosure also provides any such CTLA4 binder, polypeptide, immunoglobulin single variable domain (ISVD) or multispecific binder which is in association with a further therapeutic agent.

10 The present disclosure also provides an injection device (*e.g.*, hypodermic needle and syringe) or vessel that comprises the CTLA4 binder, immunoglobulin single variable domain (ISVD), Nanobody, polypeptide or multispecific binder optionally in association with a further therapeutic agent.

The present disclosure also provides a polynucleotide encoding the CTLA4 binder, immunoglobulin single variable domain (ISVD), Nanobody, polypeptide or multispecific binder, *e.g.*, which is in a vector. The present disclosure also provides a host cell (*e.g.*, a CHO cell or *Pichia* cell) comprising the polynucleotide or vector.

15 The present disclosure also provides a method for making an CTLA4 binder, immunoglobulin single variable domain (ISVD), Nanobody, polypeptide or multispecific binder comprising introducing a polynucleotide encoding the CTLA4 binder, immunoglobulin single variable domain (ISVD), Nanobody, polypeptide or multispecific binder into a host cell (*e.g.*, a CHO cell or *Pichia* cell) and culturing the host cell in a medium under conditions
20 favorable to expression of said immunoglobulin from said polynucleotide and, optionally, purifying the immunoglobulin from said host cell and/or said medium. Any immunoglobulin single variable domain (ISVD), Nanobody, polypeptide, multispecific binder or CTLA4 binder produced by such a method.

25 The present disclosure also provides a method for preventing CTLA4 on a T-cell from binding to CD80 and/or CD86 on an antigen-presenting cell comprising contacting said CTLA4 with an immunoglobulin single variable domain (ISVD), Nanobody, polypeptide, multispecific binder or CTLA4 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 comprising administering an effective amount of an immunoglobulin
30 single variable domain (ISVD), Nanobody, polypeptide, multispecific binder or CTLA4 binder to the subject (*e.g.*, mammal such as a human) optionally in association with a further therapeutic agent. The present disclosure also provides a method for treating or preventing cancer or an infectious disease in the

body of a subject comprising administering an effective amount of an immunoglobulin single variable domain (ISVD), Nanobody, polypeptide, multispecific binder or CTLA4 binder optionally in association with a further therapeutic agent to the subject. In an embodiment, 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 myelofibrosis, soft tissue sarcoma, thyroid cancer, endometrial cancer, carcinoid cancer or liver cancer, breast cancer or gastric cancer. In an embodiment, the infectious disease is a bacterial infection, a viral infection or a fungal infection. For example, in an embodiment, the subject is administered a further therapeutic agent or a therapeutic procedure in association with the immunoglobulin single variable domain (ISVD), Nanobody, polypeptide, multispecific binder or CTLA4 binder.

The present disclosure provides a CTLA4 binder (*e.g.*, a multivalent binder) comprising an immunoglobulin single variable domain (ISVD) that binds to human CTLA4 by contacting human CTLA4 at one or more of the following residues: VRVTVL (amino acids 33 – 38 of SEQ ID NO: 110), ADSQVTEVC (amino acids 41-49 of SEQ ID NO: 110) and CKVELMYPPPYLG (amino acids 93-106 of SEQ ID NO: 110); wherein the ISVD comprises a mutation at residues 11 (*e.g.*, L11V) and 89 (*e.g.*, V89L) wherein said residue numbers are Kabat residue numbers.

The present disclosure also provides a CTLA4 binder (*e.g.*, a multivalent binder) comprising an immunoglobulin single variable domain (ISVD) that binds to CTLA4 comprising the amino acid sequence set forth in SEQ ID NO: 1 but comprising one or more mutations at a position selected from the group consisting of E1, L11, A14, Q45, A74, N73, K83, V89, M96 or Q108L (*e.g.*, E1D, L11V, A14P, Q45R, A74S, N73X (wherein X is S, V, G, R, Q, M, H, T, D, E, W, F, K, A, Y or P), K83R, V89L, M96P, Q108L); wherein said residue numbers are Kabat residue numbers;

and, optionally, a half-life extender (*e.g.*, ALB11002) and/or a C-terminal extender (*e.g.*, an Alanine). In an aspect, there is provided a CTLA4 binder comprising an immunoglobulin single variable domain (ISVD) that binds to CTLA4 comprising the amino acid sequence set forth in SEQ ID NO: 1 but comprising one or more mutations selected from the group

5 consisting of E1D, L11V, A14P, Q45R, A74S, N73X (wherein X is S, V, G, R, Q, M, H, T, D, E, W, F, K, A, Y or P), K83R, V89L, M96P, Q108L; wherein said residue numbers are Kabat residue numbers; and, optionally, a half-life extender and/or a C-terminal extender. For example, in an embodiment, the the ISVD comprises the amino acid

sequence: X V Q L V E S G G G V V Q P G G S L R L S C A A S G G T F S F Y G M G

10 W F R Q A P G K E R E F V A D I R T S A G R T Y Y A D S V K G R F T I S R D N S K N T V Y L Q M N S L R P E D T A L Y Y C A A E P S G I S G W D Y W G Q G T L V T V S S, wherein X is D or E (SEQ ID NO: 60); optionally comprising a half-life extender (*e.g.*, ALB11002) and/or a C-terminal extender (*e.g.*, an Alanine). In an embodiment, the CTLA4 binder comprises an ISVD that includes the amino acid sequence selected from SEQ ID NOs: 93-109; optionally lacking amino acids A A A D Y K D H D G D Y K D H D I D Y K D D D D K G A A H H H H H H thereof. In an embodiment, the

CTLA4 binder comprises an ISVD that binds to CTLA4 comprising the amino acid sequence set forth in SEQ ID NO: 60 wherein X is D or E; a peptide linker (*e.g.*, G G G G S G G G S G G G S G G G S G G G S G G G S G G G S (SEQ ID NO: 65)); an ISVD that binds to CTLA4 comprising the amino acid sequence set forth in SEQ ID NO: 60 wherein X is D or E;

20 a peptide linker; a half-life extender; and, optionally, a C-terminal extension alanine; or wherein the CTLA4 binder comprises an ISVD that binds to CTLA4 comprising the amino acid sequence set forth in SEQ ID NO: 60 wherein X is D or E; a peptide linker; a half-life extender; and, optionally, a C-terminal extension alanine. In an embodiment, the CTLA4 binder comprises the amino acid sequence:

25 D V Q L V E S G G G V V Q P G G S L R L S C A A S G G T F S F Y G M G

W F R Q A P G K E R E F V A D I R T S A G R T Y Y A D S V K G R F T I S R D N S K N T V Y L Q M N S L R P E D T A L Y Y C A A E P S G I S G W D Y W G Q G T L V T V S S G G G S G G G S G G G S G G G S G G G S G G G S G G G S E V Q L V E S G G G V V Q P G N S L R L S C A A S G F T F S S F G M S W V R Q A P G K G L E W V S S I S G S G S D T L Y A D S V K G R F T I S R D N A K T T L Y L Q M N S L R P E D T A L Y Y C T I G G S L S R S S Q G T L V T V S S A (SEQ ID NO: 62); or

D V Q L V E S G G G V V Q P G G S L R L S C A A S G G T F S F Y G M G

35 W F R Q A P G K E R E F V A D I R T S A G R T Y Y A D S V K G R F T I S R D N S K N T V Y L Q M N S L R P E D T A L Y Y C A A E P S G I S G W D Y W G Q G T L V T V S S G G G S G G G S G G G S G G G S G G G S G G G S G G G S E V Q L V E S G G G V V Q P G N S L R L S C A A S G F T F S S F G M S W V R Q A P G K G L E W V S S I S G S G S D T L Y A

DSVKGRFTISRDNAKTTLYLQMNSLRPEDTALYYCTIGGSLSRSSQGLVTVSSA (SEQ ID NO: 64).

The present disclosure also provides a binder (*e.g.*, an antibody) that cross-blocks a CTLA4 binder set forth herein from binding to CTLA4. In another aspect, there is provided a binder that cross-blocks a CTLA4 binder as disclosed herein from binding to human CTLA4.

The present disclosure, in another aspect, provides an injection device or vessel that comprises any CTLA4 binder set forth herein (*e.g.*, comprising the amino acid sequence set forth in SEQ ID NO: 62 or 64) optionally in association with a further therapeutic agent.

The present disclosure, in another aspect, provides a polynucleotide (*e.g.*, DNA) encoding any CTLA4 binder set forth herein (*e.g.*, comprising the amino acid sequence set forth in SEQ ID NO: 62 or 64); *e.g.*, comprising the nucleotide sequence of SEQ ID NO: 61 or 63; or, in another aspect, a vector comprising such a polynucleotide; or, in another aspect, a host cell comprising such a polynucleotide or vector.

The present disclosure, in another aspect, provides a method for making the CTLA4 binder set forth herein (*e.g.*, comprising the amino acid sequence set forth in SEQ ID NO: 62 or 64) comprising introducing a polynucleotide encoding the CTLA4 binder into a host cell (*e.g.*, a CHO cell or *Pichia* cell) and culturing the host cell in a medium under conditions favorable to expression of said CTLA4 binder from said polynucleotide and, optionally, purifying the CTLA4 binder from said host cell and/or said medium. The present disclosure, in another aspect, also provides for a CTLA4 binder produced by such a method.

The present disclosure, in another aspect, also provides a method for preventing CTLA4 from binding to CD80 or CD86 (*e.g.*, in the body of a subject) comprising contacting said CTLA4 with the CTLA4 binder (*e.g.*, comprising the amino acid sequence set forth in SEQ ID NO: 62 or 64) optionally in association with a further therapeutic agent; as well as a method for enhancing an immune response in the body of a subject (*e.g.*, a human) comprising administering an effective amount of the CTLA4 binder (*e.g.*, comprising the amino acid sequence set forth in SEQ ID NO: 62 or 64) to the subject optionally in association with a further therapeutic agent (*e.g.*, pembrolizumab). In another aspect, there is provided a CTLA4 binder as disclosed herein for use in enhancing an immune response in the body of a subject optionally in association with a further therapeutic agent. In addition, the present disclosure provides a method for treating or preventing cancer or an infectious disease in the body of a subject comprising administering an effective amount of CTLA4 binder (*e.g.*, comprising the amino acid sequence set forth in SEQ ID NO: 62 or 64) optionally in association with a further therapeutic agent (*e.g.*, pembrolizumab) to the subject. In another aspect, there is provided use of a CTLA4 binder as disclosed herein in the manufacture of a medicament for the treatment or prevention of cancer or an infectious disease, optionally in association with a further therapeutic agent. In an embodiment

, 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 myelofibrosis, soft tissue sarcoma, thyroid cancer, endometrial cancer, carcinoid cancer or liver cancer, breast cancer or gastric cancer; or when the infectious disease is a bacterial infection, a viral infection or a fungal infection. In an embodiment, the subject is administered a further therapeutic agent (*e.g.*, pembrolizumab) or a therapeutic procedure in association with the CTLA4 binder .

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. CTLA4 binder sequences.

Figure 3 (A-B). Alignment of 11F1 sequence with that of the SEQ ID NOs: 8-43.

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 (A-B). bFACS analysis of Nanobody F023700912, F023700925 or control nanobody (IRR00051; anti-HER2/ERBB2 (bivalent anti-HER2 with 35GS connected to albumin binder)) binding to (A) Jurkat and (B) CHO-K1 cells expressing hCTLA4.

Figure 6 (A-B). Competition between Nanobody F023700912 or ipilimumab and (A) CD80 or (B) CD86 for binding to human CTLA4 expressed on CHO-K1 cells.

Figure 7 (A-H). Specificity assessment of Nanobody F023700912, F023700925 or control nanobody (IRR00051) for binding to BTLA, CD8, PD1, CTLA4, LAG3, CD28 or control cells. Binding of the Nanobodies was determined to (A) negative control L cells, (B) negative control CHO-K1 cells, (C) huCD28+ L cells, (D) huCD8alpha+ L cells, (E) huLag-3+ CHO-K1 cells, (F) huBTLA+ CHO-K1 cells, (G) huCTLA-4+ CHO-K cells, and (H) huPD-1+ CHO-K1 cells.

WFRQAPGKEREFVADIRTSAGRTYYADSVKGRFTISRDN SKNTVYLQMNSLRPEDTALYYCAAEP SGISGW DYWG QGTLVTVSS, wherein X is D or E (SEQ ID NO: 60); optionally comprising a half-life extender and/or a C-terminal extender.

- 5 5. The CTLA4 binder of any one of claims 1-4 wherein the ISVD comprises the amino acid sequence selected from SEQ ID NOs: 93-109; optionally lacking the signal sequence (amino acids AAADYKDHDGDYKDHDIDYKDDDDKGAHHHHHH (amino acids 120-153 of SEQ ID NO: 93)) thereof.
- 10 6. The CTLA4 binder of any one of claims 1-5 fused to a half-life extender.
7. The CTLA4 binder of claim 5, wherein the half-life extender is an ISVD that binds to human serum albumin.
- 15 8. The CTLA4 binder of claim 7 wherein the ISVD that binds to human serum albumin is ALB11002.
9. A CTLA4 binder of any one of claims 1-5 comprising:
- an ISVD that binds to CTLA4 comprising the amino acid sequence set forth in SEQ ID NO: 60 wherein X is D or E;
 - a peptide linker;
 - an ISVD that binds to CTLA4 comprising the amino acid sequence set forth in SEQ ID NO: 60 wherein X is D or E;
 - a peptide linker;
 - a half-life extender; and, optionally,
 - a C-terminal extension alanine.
10. A CTLA4 binder of any one of claims 1-5 comprising:
- an ISVD that binds to CTLA4 comprising the amino acid sequence set forth in SEQ ID NO: 60 wherein X is D or E;
 - a peptide linker;

- a half-life extender; and, optionally,
- a C-terminal extension alanine.

11. The CTLA4 binder of any one of claims 9-10 wherein the peptide linker comprises the amino acid sequence GGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGG (SEQ ID NO: 65).

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12. The CTLA4 binder of any one of claims 1-11 comprising the amino acid sequence:

DVQLVESGGGVVQPGGSLRLSCAASGDTFSFYGMG

WFRQAPGKEREVADIRTSAGRTYYADSVKGRFTISRDNKNTVYLQMNSLRPEDTALYY

CAAEPGSGISGWDYWGQGLTVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSE

10 VQLVESGGGVVQPGGSLRLSCAASGDTFSFYGMGWFRQAPGKEREVADIRTSAGRTYYA

DSVKGRFTISRDNKNTVYLQMNSLRPEDTALYYCAAEPGSGISGWDYWGQGLTVTVSSGG

GGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGVVQPGNSLRLSCAASGF

TFSSFGMSWVRQAPGKGLEWVSSISGSGSDTLYADSVKGRFTISRDNKNTLYLQMNSLR

PEDTALYYCTIGGSLRSSQGTLTVTVSSA (SEQ ID NO: 62).

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13. The CTLA4 binder of any one of claims 1-11 comprising the amino acid sequence:

DVQLVESGGGVVQPGGSLRLSCAASGDTFSFYGMG

WFRQAPGKEREVADIRTSAGRTYYADSVKGRFTISRDNKNTVYLQMNSLRPEDTALYY

CAAEPGSGISGWDYWGQGLTVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSE

20 VQLVESGGGVVQPGNSLRLSCAASGDTFSFGMSWVRQAPGKGLEWVSSISGSGSDTLYA

DSVKGRFTISRDNKNTLYLQMNSLRPEDTALYYCTIGGSLRSSQGTLTVTVSSA (SEQ ID NO: 64).

14. The CTLA4 binder of any one of claims 1-5 which is multivalent.

25 15. A binder that cross-blocks a CTLA4 binder of any one of claims 1-14 from binding to human CTLA4.

16. An injection device or vessel that comprises the CTLA4 binder of any one of claims 1-15 optionally in association with a further therapeutic agent.

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17. A polynucleotide encoding the CTLA4 binder of any one of claims 1-15.

18. The polynucleotide of claim 17 which comprises the nucleotide sequence of SEQ ID NO: 61 or 63.

19. A vector comprising the polynucleotide of any one of claims 17-18.

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20. A host cell comprising the polynucleotide or vector of any one of claims 17-19.

21. A method for making the CTLA4 binder of any one of claims 1-15 comprising introducing a polynucleotide encoding the CTLA4 binder into a host cell and culturing the host cell in a medium under conditions favorable to expression of said CTLA4 binder from said polynucleotide and, optionally, purifying the CTLA4 binder from said host cell and/or said medium.

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22. A CTLA4 binder produced by the method of claim 21.

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23. A method for preventing CTLA4 from binding to CD80 or CD86 comprising contacting CTLA4 with the CTLA4 binder of any one of claims 1-15 optionally in association with a further therapeutic agent.

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24. A CTLA4 binder of any one of claims 1-15 for use in enhancing an immune response in the body of a subject optionally in association with a further therapeutic agent.

25. Use of a CTLA4 binder of any one of claims 1-15 in the manufacture of a medicament for the treatment or prevention of cancer or an infectious disease, optionally in association with a further therapeutic agent.

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26. The use of claim 25 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

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- 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,
- 5 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.
- 10 27. The use of claim 25 wherein the infectious disease is a bacterial infection, a viral infection or a fungal infection.
28. The use of any one of claims 25-27 further comprising a therapeutic agent or a therapeutic procedure in association with the CTLA4 binder.