

We claim:

1. A CTLA4 binder comprising an immunoglobulin single variable domain (ISVD) that binds to human CTLA4 at one or more of the following residues:

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- 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.

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2. 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 GGTFSFYGMG (SEQ ID NO: 2);

15 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.

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3. 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 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.

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4. The CTLA4 binder of any one of claims 1-3 wherein the ISVD comprises the amino acid sequence:

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XVQLVESGGGVVQPGGSLRLSCAASGGTFSFYGMG

WFRQAPGKEREFVADIRTSAGRITYYADSVKGRFTISRDN SKNTVYLQMNSLRP EDTALYYCAAEP SGISGWDYWGQGT
LVTVSS, 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 AAADYKDH DGDYKDH D IDYKDDDDKGA AHHHHHH (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 any one of claims 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
 - 20 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;
 - 25 • 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
 - 30 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:

DVQLVESGGGVVQPGGSLRLSCAASGGTFSFYGMG

WFRQAPGKEREFVADIRTSAGRTYYADSVKGRFTISRDN SKNTVYLQMNSLRPEDTALYY

CAAEP SGISGWDYWGQGT LVTVSSGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSE

10 VQLVESGGGVVQPGGSLRLSCAASGGTFSFYGMGWFRQAPGKEREFVADIRTSAGRTYYA

DSVKGRFTISRDN SKNTVYLQMNSLRPEDTALYYCAAEP SGISGWDYWGQGT LVTVSSGG

GGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGVVQPGNSLRLSCAASGF

TFSSFGMSWVRQAPGKLEWVSSISGSGSDTLYADSVKGRFTISRDN AKTTLYLQMNSLR

PEDTALYYCTIGGSLSRSSQGT LVTVSSA (SEQ ID NO: 62).

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

DVQLVESGGGVVQPGGSLRLSCAASGGTFSFYGMG

WFRQAPGKEREFVADIRTSAGRTYYADSVKGRFTISRDN SKNTVYLQMNSLRPEDTALYY

CAAEP SGISGWDYWGQGT LVTVSSGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSE

20 VQLVESGGGVVQPGNSLRLSCAASGFTFSSFGMSWVRQAPGKLEWVSSISGSGSDTLYA

DSVKGRFTISRDN AKTTLYLQMNSLRPEDTALYYCTIGGSLSRSSQGT LVTVSSA (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 or 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.
- 5 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
10 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.
22. A CTLA4 binder produced by the method of claim 21.
- 15 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.
- 20 24. A method for enhancing an immune response in the body of a subject comprising administering an effective amount of the CTLA4 binder of any one of claims 1-15 to the subject optionally in association with a further therapeutic agent.
- 25 25. A method for treating or preventing cancer or an infectious disease in the body of a human subject comprising administering an effective amount of CTLA4 binder of any one of claims 1-15 optionally in association with a further therapeutic agent to the subject.
- 30 26. The method 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

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