

WFRQAPGKEREVADIRTSAGRTYYADSVKGRFTISRDNKNTVYLQMNSLRPEDTALYY
CAAEPGSGISGWDYWGQGLTVTVSSGGGGSGGGSGGGSGGGSGGGSGGGSGGGSGGGGSE
VQLVESGGGVVQPGGSLRLSCAASGGTFSFYGMGWFRQAPGKEREVADIRTSAGRTYYA
DSVKGRFTISRDNKNTVYLQMNSLRPEDTALYYCAAEPGSGISGWDYWGQGLTVTVSSGG
5 GGGSGGGSGGGSGGGSGGGSGGGSGGGSGGGSGGGSEVQLVESGGGVVQPGNSLRLSCAASGF
TFSSFGMSWVRQAPGKLEWVSSISGSGSDTLYADSVKGRFTISRDNKNTTLYLQMNSLR
PEDTALYYCTIGGSLRSSQGLTVTVSSA (SEQ ID NO: 62); or

DVQLVESGGGVVQPGGSLRLSCAASGGTFSFYGMG

WFRQAPGKEREVADIRTSAGRTYYADSVKGRFTISRDNKNTVYLQMNSLRPEDTALYY
10 CAAEPGSGISGWDYWGQGLTVTVSSGGGGSGGGSGGGSGGGSGGGSGGGSGGGSGGGGSE
VQLVESGGGVVQPGNSLRLSCAASGFTFSFGMSWVRQAPGKLEWVSSISGSGSDTLYA
DSVKGRFTISRDNKNTTLYLQMNSLRPEDTALYYCTIGGSLRSSQGLTVTVSSA (SEQ ID NO: 64).

In an aspect, there is provided a CTLA4 binder comprising one or more
immunoglobulin single variable domains (ISVDs) that bind to human CTLA4, comprising the
15 amino acid sequence:

DVQLVESGGGVVQPGGSLRLSCAASGGTFSFYGMGWFRQAPGKEREVADIRTSAGRTYYADSVKGRFTISRDN
KNTVYLQMNSLRPEDTALYYCAAEPGSGISGWDYWGQGLTVTVSSGGGGSGGGSGGGSGGGSGGGSGGGSGGGSG
GGGSEVQLVESGGGVVQPGGSLRLSCAASGGTFSFYGMGWFRQAPGKEREVADIRTSAGRTYYADSVKGRFTIS
RDNSKNTVYLQMNSLRPEDTALYYCAAEPGSGISGWDYWGQGLTVTVSSGGGGSGGGSGGGSGGGSGGGSGGGSGG
20 GGGSGGGSEVQLVESGGGVVQPGNSLRLSCAASGFTFSFGMSWVRQAPGKLEWVSSISGSGSDTLYADSVKGR
FTISRDNKNTTLYLQMNSLRPEDTALYYCTIGGSLRSSQGLTVTVSSA (SEQ ID NO: 62).

In an aspect, there is provided a CTLA4 binder comprising one or more
immunoglobulin single variable domains (ISVDs) that bind to human CTLA4, comprising the
amino acid sequence:

DVQLVESGGGVVQPGGSLRLSCAASGGTFSFYGMGWFRQAPGKEREVADIRTSAGRTYYADSVKGRFTISRDN
KNTVYLQMNSLRPEDTALYYCAAEPGSGISGWDYWGQGLTVTVSSGGGGSGGGSGGGSGGGSGGGSGGGSGGGSG
GGGSEVQLVESGGGVVQPGNSLRLSCAASGFTFSFGMSWVRQAPGKLEWVSSISGSGSDTLYA
25 DSVKGRFTISRDNKNTTLYLQMNSLRPEDTALYYCTIGGSLRSSQGLTVTVSSA (SEQ ID NO: 64).

The present disclosure also provides a binder (*e.g.*, an antibody) that cross-blocks a
30 CTLA4 binder set forth herein from binding to 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 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 an *in vitro* method for preventing CTLA4 from binding to CD80 or CD86 comprising contacting CTLA4 with the CTLA4 binder as defined herein optionally in association with a further therapeutic agent. 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 of cancer or an infectious disease, wherein optionally the

We claim:

1. A CTLA4 binder comprising one or more immunoglobulin single variable domains (ISVDs) that bind to human CTLA4, wherein the ISVD comprises the amino acid sequence:

5 XVQLVESGGGVVQPGGSLRLSCAASGGTFSFYGMG
WFRQAPGKEREFVADIRTSAGRTYYADSVKGRFTISRDN SKNTVY LQMNSLRPEDTALYYCAAEP SGISGWDYWG
QGTLVTVSS, wherein X is D or E (SEQ ID NO: 60); optionally comprising a half-life extender
and/or a C-terminal extender.

10 2. A CTLA4 binder comprising one or more immunoglobulin single variable domains
(ISVDs) that bind to human CTLA4, wherein the ISVD comprises the amino acid sequence
selected from SEQ ID NOs: 93-109; optionally lacking the signal sequence (amino acids
AAADYKDHDGDYKDHDIDYKDDDDKGA AHHHHHH (amino acids 120-153 of SEQ ID NO: 93))
thereof.

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3. The CTLA4 binder of claim 1 or 2 fused to a half-life extender.

4. The CTLA4 binder of claim 2, wherein the half-life extender is an ISVD that binds to
human serum albumin.

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5. The CTLA4 binder of claim 4 wherein the ISVD that binds to human serum albumin
comprises the amino acid sequence SEQ ID NO: 66.

6. A CTLA4 binder of claim 1 or 2 comprising:

- 25 • 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;
- 30 • a peptide linker;
- a half-life extender; and, optionally,
- a C-terminal extension alanine.

7. A CTLA4 binder of claim 1 or 2 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.

8. The CTLA4 binder of claim 6 or 7 wherein the peptide linker comprises the amino acid sequence GGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGG (SEQ ID NO: 65).

9. A CTLA4 binder comprising one or more immunoglobulin single variable domains (ISVDs) that bind to human CTLA4, comprising the amino acid sequence:

DVQLVESGGGVVQPGGSLRLSCAASGGTFSFYGMG
WFRQAPGKEREFVADIRTSAGRTYYADSVKGRFTISRDN SKNTVYLQMNSLRPEDTALYY
CAAEP SGISGWDYWGQGLTVTVSSGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSE
VQLVESGGGVVQPGGSLRLSCAASGGTFSFYGMGWFRQAPGKEREFVADIRTSAGRTYYA
DSVKGRFTISRDN SKNTVYLQMNSLRPEDTALYYCAAEP SGISGWDYWGQGLTVTVSSGG
GGSGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGVVQPGNSLRLSCAASGF
TFSSFGMSWVRQAPGKLEWVSSISGSGSDTLYADSVKGRFTISRDN AKTTLYLQMNSLR
PEDTALYYCTIGGSLSRSSQGTLTVTVSSA (SEQ ID NO: 62).

10. A CTLA4 binder comprising one or more immunoglobulin single variable domains (ISVDs) that bind to human CTLA4, comprising the amino acid sequence:

DVQLVESGGGVVQPGGSLRLSCAASGGTFSFYGMG
WFRQAPGKEREFVADIRTSAGRTYYADSVKGRFTISRDN SKNTVYLQMNSLRPEDTALYY
CAAEP SGISGWDYWGQGLTVTVSSGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSE
VQLVESGGGVVQPGNSLRLSCAASGFTFSSFGMSWVRQAPGKLEWVSSISGSGSDTLYA
DSVKGRFTISRDN AKTTLYLQMNSLRPEDTALYYCTIGGSLSRSSQGTLTVTVSSA (SEQ ID NO: 64).

11. The CTLA4 binder of claims 1 or 2 which is multivalent.

12. An injection device or vessel that comprises the CTLA4 binder of any one of claims 1-11 optionally in association with a further therapeutic agent.

13. A polynucleotide encoding the CTLA4 binder of any one of claims 1-11.

14. The polynucleotide of claim 13 which comprises the nucleotide sequence of SEQ ID NO: 61 or 63.
- 5 15. A vector comprising the polynucleotide of claim 13 or 14.
16. A host cell comprising the polynucleotide or vector of any one of claims 13-15.
17. A method for making the CTLA4 binder of any one of claims 1-11 comprising
10 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.
- 15 18. A CTLA4 binder produced by the method of claim 17.
19. An *in vitro* method for preventing CTLA4 from binding to CD80 or CD86 comprising contacting CTLA4 with the CTLA4 binder of any one of claims 1-11 optionally in association with a further therapeutic agent.
- 20 20. A CTLA4 binder of any one of claims 1-11 for use in enhancing an immune response in the body of a subject optionally in association with a further therapeutic agent.
21. Use of a CTLA4 binder of any one of claims 1-11 in the manufacture of a medicament for
25 the treatment of cancer or an infectious disease, wherein optionally the medicament is to be administered with a further therapeutic agent.
22. The use of claim 21 wherein the cancer is metastatic cancer, a solid tumor, a hematologic cancer, leukemia, lymphoma, osteosarcoma, rhabdomyosarcoma, neuroblastoma, kidney
30 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
5 cancer, endometrial cancer, carcinoid cancer or liver cancer, breast cancer or gastric cancer.

23. The use of claim 21 wherein the infectious disease is a bacterial infection, a viral infection or a fungal infection.

10 24. The use of any one of claims 21-23 wherein the medicament is to be administered with a further therapeutic agent or a therapeutic procedure.