

Consistent with these functions, the TAMs, especially macrophages with the M2 phenotype, is closely associated with worse clinical prognosis in many kinds of malignant tumors. Infiltrating TAMs themselves or polarization pathway of TAMs are considered as new therapeutic targets for the therapy of malignant tumors.

5

SUMMARY

The present disclosure relates, at least in part, to antibodies and fragments thereof that binds to VSIG4 (V-Set And Immunoglobulin Domain Containing 4; also referred to as CRIG or Z39Ig),
10 and methods of using such antibodies and antigen-binding fragments for treating cancer, inducing cytokine and/or chemokine secretion in macrophages, and conversion of M2 macrophages to M1 macrophages.

In one example, the present disclosure relates to an isolated humanized antibody or antigen-binding fragment includes: (a) a heavy chain CDR1 comprising the amino acid sequence of SEQ
15 ID NO: 17, a heavy chain CDR2 comprising the amino acid sequence of SEQ ID NO: 18, a heavy chain CDR3 sequence comprising the amino acid sequence of SEQ ID NO: 19; and (b) a light chain CDR1 comprising the amino acid sequence of SEQ ID NO: 20, a light chain CDR2 comprising the amino acid sequence of SEQ ID NO: 21, and a light chain CDR3 comprising the
20 amino acid sequence of SEQ ID NO: 22 or SEQ ID NO: 23.

In one aspect, the present disclosure refers to an isolated humanized antibody or antigen-binding fragment thereof, comprising:

- a. a heavy chain CDR1 comprising the amino acid sequence of SEQ ID NO: 17, a heavy chain CDR2 comprising the amino acid sequence of SEQ ID NO: 18, a heavy chain CDR3
25 sequence comprising the amino acid sequence of SEQ ID NO: 19; and
- b. a light chain CDR1 comprising the amino acid sequence of SEQ ID NO: 20, a light chain CDR2 comprising the amino acid sequence of SEQ ID NO: 21, and a light chain CDR3 comprising the amino acid sequence of SEQ ID NO: 22 or SEQ ID NO: 23.

In some examples, the antibody or antigen-binding fragment described herein may include any
30 one of: (a) a heavy chain variable domain comprising an amino acid sequence at least 80%, 90%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence of SEQ ID NO: 2, SEQ ID NO: 6, SEQ ID NO: 14, or SEQ ID NO: 16; (b) a light chain variable domain comprising an amino acid sequence at least 80%, 90%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence of SEQ ID NO: 4, SEQ ID NO: 8, SEQ ID NO: 10, or SEQ ID NO: 12; or (c) a
35 heavy chain variable domain comprising an amino acid sequence at least 80%, 90%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence of SEQ ID NO: 2, SEQ ID NO: 6, SEQ ID NO: 14, or SEQ ID NO: 16, and a light chain variable domain comprising an amino acid sequence at least 80%, 90%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence of SEQ ID NO: 4, SEQ ID NO: 8, SEQ ID NO: 10, or SEQ ID NO: 12.

In some examples, the antibody or antigen-binding fragment described herein may include any one of, a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO: 2, SEQ ID NO: 6, SEQ ID NO: 14, or SEQ ID NO: 16, a light chain variable domain comprising the amino acid sequence of SEQ ID NO: 4, SEQ ID NO: 8, SEQ ID NO: 10, or SEQ ID NO: 12, or a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO: 2, SEQ ID NO: 6, SEQ ID NO: 14, or SEQ ID NO: 16, and a light chain variable domain comprising the amino acid sequence of SEQ ID NO: 4, SEQ ID NO: 8, SEQ ID NO: 10, or SEQ ID NO: 12. In some examples, the antibody or antigen-binding fragment described herein may include a light chain CDR3 comprising the amino acid sequence of SEQ ID NO: 23.

In some examples, the antibody or antigen-binding fragment described herein may include a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO: 2 and a light chain variable domain comprising the amino acid sequence of SEQ ID NO: 4. In some examples, the antibody or antigen-binding fragment described herein may include a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO: 6 and a light chain variable domain comprising the amino acid sequence of SEQ ID NO: 8. In some examples, the antibody or antigen-binding fragment may include a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO: 6 and a light chain variable domain comprising the amino acid sequence of SEQ ID NO: 10. In some examples, the antibody or antigen-binding fragment may include a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO: 6 and a light chain variable domain comprising the amino acid sequence of SEQ ID NO: 12. In some examples, the antibody or antigen-binding fragment described herein may include a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO: 14 and a light chain variable domain comprising the amino acid sequence of SEQ ID NO: 10. In some examples, the antibody or antigen-binding fragment described herein may include a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO: 16 and a light chain variable domain comprising the amino acid sequence of SEQ ID NO: 12.

In some examples, the antibody or antigen-binding fragment described herein has a binding affinity (K_D) for a human V-Set And Immunoglobulin Domain Containing 4 (VSIG4) molecule of 1×10^{-7} to 1×10^{-9} . In some examples, the antibody or antigen-binding fragment described herein has a binding affinity (K_D) for a VSIG4 molecule of about 7.156×10^{-8} to about 7.636×10^{-9} . In some examples, the antibody or antigen-binding fragment described herein has a

binding affinity (K_D) for a VSIG4 molecule of about 7.156×10^{-8} , about 7.636×10^{-9} , about 7.952×10^{-9} , about 8.226×10^{-9} , or about 8.688×10^{-9} .

In another aspect, the present disclosure refers to a nucleic acid molecule encoding any one of the antibody or antigen-binding fragments described herein.

5 In another example, the present disclosure relates to a recombinant vector including any one of the nucleic acid molecules described herein. In another aspect, the present disclosure refers to a recombinant vector comprising the nucleic acid molecule as disclosed herein. In some examples, the recombinant vector described herein contains the nucleic acid molecule described herein that is operatively linked to a promoter. In some examples, the recombinant vector includes two
10 separate vectors, each comprising the nucleic acid sequence corresponding to the heavy chain and the light chain of the antibody or antigen-binding fragment provided herein.

In another example, the present disclosure provides a host cell including the nucleic acid molecule or the recombinant vector described herein. In another aspect, the present disclosure refers to a host cell comprising the nucleic acid molecule as disclosed herein or the recombinant
15 vector as disclosed herein. In some examples, the host cell is a mammalian cell, a yeast cell, or a bacterial cell. In some examples, the host cell is a cell selected from the group consisting of *E.coli*, *P.pastoris*, Sf9, COS, HEK293, Expi293, CHO-S, CHO-DG44, CHO-K1, and a mammalian lymphocyte. In some examples, the host cell is the Expi293 cell.

In another example, the present disclosure relates to a pharmaceutical composition including:
20 any one of the antibody or antigen-binding fragments described herein, any one of the nucleic acid molecules described herein, any one of the recombinant vectors described herein, or any one of the host cells described herein; and a pharmaceutically acceptable carrier.

In another aspect, the present disclosure refers to a pharmaceutical composition comprising:
25 the antibody or antigen-binding fragment as disclosed herein, the nucleic acid molecule as disclosed herein, the recombinant vector as disclosed herein, or the host cell as disclosed herein; and a pharmaceutically acceptable carrier.

By way of further example, in another example, the present disclosure relates to a method of treating a subject in need thereof, the method comprising the steps of: (a) administering to the
30 subject a composition that comprises or delivers any one of the antibody or antigen-binding fragments described herein, any one of the nucleic acid molecules described herein, any one of the recombinant vectors described herein, or any one of the host cells described herein, thereby treating a disease or a condition. In another aspect, the present disclosure refers to use of the antibody or antigen-binding fragment as disclosed herein, the nucleic acid molecule as disclosed
35 herein, the recombinant vector as disclosed herein, or the host cell as disclosed herein, in the manufacture of a medicament for treating a disease or a condition in a subject in need thereof. In some examples, the subject has, or is at risk for developing, cancer. In some examples, the cancer is selected from a bladder cancer, breast cancer, cervical cancer, colon cancer, endometrial cancer, esophageal cancer, fallopian tube cancer, gall bladder cancer, gastrointestinal cancer, head and neck cancer, hematological cancer, laryngeal cancer, liver
40 cancer, lung cancer, lymphoma, melanoma, mesothelioma, ovarian cancer, primary peritoneal cancer, salivary gland cancer, sarcoma, stomach cancer, thyroid cancer, pancreatic cancer, renal cell carcinoma, glioblastoma, and prostate cancer.

In some examples, the subject has been administered or will be administered one or more additional anticancer therapies selected from ionizing radiation, a chemotherapeutic agent, an antibody agent, and a cell-based therapy, such that the subject receives treatment with both. For example, in some examples, the one or more additional anticancer therapies can include an immune checkpoint inhibitor, IL-12, GM-CSF, an anti-CD4 agent, cisplatin, fluorouracil, doxorubicin, irinotecan, paclitaxel, indoleamine 2,3 -di oxygenase- 1 (IDO1) inhibitor, or cyclophosphamide.

In yet another example, the present disclosure also relates to a method of increasing secretion of cytokines or chemokines in M2 macrophages, the method including contacting the M2 macrophages with any one of the antibody or antigen-binding fragments described herein.

In another aspect, the present disclosure refers a method of increasing secretion of cytokines or chemokines in M2 macrophages comprising: a. contacting the M2 macrophages with the antibody or antigen-binding fragment as described herein.

In yet another example, the present disclosure relates to a method of inducing CD8⁺ T cell proliferation, the method including: (a) contacting an M2 macrophage with any one of the antibody or antigen-binding fragments described herein; and (b) co-incubating the M2 macrophage with CD8⁺ T cells.

In another aspect, the present disclosure refers to a method of inducing CD8⁺ T cell proliferation, the method comprising: a. contacting an M2 macrophage with the antibody or antigen-binding fragment as described herein; and b. co-incubating the M2 macrophage with a CD8⁺ T cells.

In yet another example, the present disclosure also relates to a method of converting an M2 macrophage into an M1 macrophage, the method including contacting the M2 macrophage with any one of the antibody or antigen-binding fragments described herein.

In another aspect, the present disclosure refers to a method of converting an M2 macrophage into an M1 macrophage, the method comprising contacting the M2 macrophage with the antibody or antigen-binding fragment as described herein.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure belongs.

Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present disclosure, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

Other features and advantages of the disclosure will be apparent from the following detailed description, and from the claims.

CLAIMS

What is claimed is:

1. An isolated humanized antibody or antigen-binding fragment thereof, comprising:
 - a. a heavy chain CDR1 comprising the amino acid sequence of SEQ ID NO: 17, a heavy chain CDR2 comprising the amino acid sequence of SEQ ID NO: 18, a heavy chain CDR3 sequence comprising the amino acid sequence of SEQ ID NO: 19; and
 - b. a light chain CDR1 comprising the amino acid sequence of SEQ ID NO: 20, a light chain CDR2 comprising the amino acid sequence of SEQ ID NO: 21, and a light chain CDR3 comprising the amino acid sequence of SEQ ID NO: 22 or SEQ ID NO: 23.
2. The antibody or antigen-binding fragment of claim 1, wherein the antibody or antigen-binding fragment comprises any one of the following:
 - a. a heavy chain variable domain comprising an amino acid sequence at least 80%, 90%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence of SEQ ID NO: 2, SEQ ID NO: 6, SEQ ID NO: 14, or SEQ ID NO: 16;
 - b. a light chain variable domain comprising an amino acid sequence at least 80%, 90%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence of SEQ ID NO: 4, SEQ ID NO: 8, SEQ ID NO: 10, or SEQ ID NO: 12; or
 - c. a heavy chain variable domain comprising an amino acid sequence at least 80%, 90%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence of SEQ ID NO: 2, SEQ ID NO: 6, SEQ ID NO: 14, or SEQ ID NO: 16, and a light chain variable domain comprising an amino acid sequence at least 80%, 90%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence of SEQ ID NO: 4, SEQ ID NO: 8, SEQ ID NO: 10, or SEQ ID NO: 12.
3. The antibody or antigen-binding fragment of any one of claims 1-2, wherein the antibody or antigen-binding fragment comprises any one of the following:

- 5
- a. a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO: 2, SEQ ID NO: 6, SEQ ID NO: 14, or SEQ ID NO: 16;
 - b. a light chain variable domain comprising the amino acid sequence of SEQ ID NO: 4, SEQ ID NO: 8, SEQ ID NO: 10, or SEQ ID NO: 12; or
 - c. a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO: 2, SEQ ID NO: 6, SEQ ID NO: 14, or SEQ ID NO: 16, and a light chain variable domain comprising the amino acid sequence of SEQ ID NO: 4, SEQ ID NO: 8, SEQ ID NO: 10, or SEQ ID NO: 12.
- 10
4. The antibody or antigen-binding fragment of claim 1, wherein the antibody wherein light chain CDR3 comprising the amino acid sequence of SEQ ID NO: 23.
- 15
5. The antibody or antigen-binding fragment of claim 2, wherein the antibody or antigen-binding fragment comprises a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO: 2 and a light chain variable domain comprising the amino acid sequence of SEQ ID NO: 4.
- 20
6. The antibody or antigen-binding fragment of claim 2, wherein the antibody or antigen-binding fragment comprises a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO: 6 and a light chain variable domain comprising the amino acid sequence of SEQ ID NO: 8.
- 25
7. The antibody or antigen-binding fragment of claim 2, wherein the antibody or antigen-binding fragment comprises a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO: 6 and a light chain variable domain comprising the amino acid sequence of SEQ ID NO: 10.
- 30
8. The antibody or antigen-binding fragment of claim 2, wherein the antibody or antigen-binding fragment comprises a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO: 6 and a light chain variable domain comprising the amino acid sequence of SEQ ID NO: 12.

- 5 9. The antibody or antigen-binding fragment of claim 2, wherein the antibody or antigen-binding fragment comprises a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO: 14 and a light chain variable domain comprising the amino acid sequence of SEQ ID NO: 10.
- 10 10. The antibody or antigen-binding fragment of claim 2, wherein the antibody or antigen-binding fragment comprises a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO: 16 and a light chain variable domain comprising the amino acid sequence of SEQ ID NO: 12.
- 15 11. The antibody or antigen-binding fragment of any one of claims 1-10, wherein the antibody or antigen-binding fragment has a binding affinity (K_D) for a human V-Set And Immunoglobulin Domain Containing 4 (VSIG4) molecule of 1×10^{-7} to 1×10^{-9} .
- 20 12. The antibody or antigen-binding fragment of any one of claims 1-11, wherein the antibody or antigen-binding fragment has a binding affinity (K_D) for a VSIG4 molecule of about 7.156×10^{-8} to about 7.636×10^{-9} .
- 25 13. The antibody or antigen-binding fragment of any one of claims 1-11, wherein the antibody or antigen-binding fragment has a binding affinity (K_D) for a VSIG4 molecule of about 7.156×10^{-8} , about 7.636×10^{-9} , about 7.952×10^{-9} , about 8.226×10^{-9} , or about 8.688×10^{-9} .
- 30 14. A nucleic acid molecule encoding the antibody or antigen-binding fragment of any one of claims 1-13.
15. A recombinant vector comprising the nucleic acid molecule of claim 14.
16. The recombinant vector of claim 15, wherein the nucleic acid molecule of claim 14 is operatively linked to a promoter.

17. The recombinant vector of claim 15 or 16, wherein the vector comprises two separate vectors, each comprising the nucleic acid sequence corresponding to the heavy chain and the light chain of the antibody or antigen-binding fragment.

5

18. A host cell comprising the nucleic acid molecule of claim 14 or the recombinant vector of any one of claims 15-17.

19. The host cell of claim 18, wherein the host cell is a mammalian cell, a yeast cell, or a bacterial cell.

10

20. The host cell of claim 19, wherein the host cell is a cell selected from the group consisting of *E.coli*, *P.pastoris*, Sf9, COS, HEK293, Expi293, CHO-S, CHO-DG44, CHO-K1, and a mammalian lymphocyte.

15

21. The host cell of claim 20, wherein the host cell is the Expi293 cell.

22. A pharmaceutical composition comprising:

20

the antibody or antigen-binding fragment of any one of claims 1-13, the nucleic acid molecule of claim 14, the recombinant vector of any one of claims 15-17, or the host cell of any one of claims 18-21; and
a pharmaceutically acceptable carrier.

23. Use of the antibody or antigen-binding fragment of any one of claims 1-13, the nucleic acid molecule of claim 14, the recombinant vector of any one of claims 15-17, or the host cell of any one of claims 18-21, in the manufacture of a medicament for treating a disease or a condition in a subject in need thereof.

25

24. The use of claim 23, wherein the subject has, or is at risk for developing, cancer.

30

25. The use of claim 24, where in the cancer is selected from a bladder cancer, breast cancer, cervical cancer, colon cancer, endometrial cancer, esophageal cancer, fallopian tube

cancer, gall bladder cancer, gastrointestinal cancer, head and neck cancer, hematological cancer, laryngeal cancer, liver cancer, lung cancer, lymphoma, melanoma, mesothelioma, ovarian cancer, primary peritoneal cancer, salivary gland cancer, sarcoma, stomach cancer, thyroid cancer, pancreatic cancer, renal cell carcinoma, glioblastoma, and prostate cancer.

26. The use of any one of claims 23-25, wherein the subject has been administered or will be administered one or more additional anticancer therapies selected from ionizing radiation, a chemotherapeutic agent, an antibody agent, and a cell-based therapy, such that the subject receives treatment with both.

27. The use of claim 26, wherein the one or more additional anticancer therapies comprise an immune checkpoint inhibitor, IL-12, GM-CSF, an anti-CD4 agent, cisplatin, fluorouracil, doxorubicin, irinotecan, paclitaxel, indoleamine 2,3 -di oxygenase- 1 (IDO1) inhibitor, or cyclophosphamide.

28. A method of increasing secretion of cytokines or chemokines in M2 macrophages comprising:

- a. Contacting the M2 macrophages with the antibody or antigen-binding fragment of any one of claims 1-13.

29. A method of inducing CD8⁺ T cell proliferation, the method comprising:

- a. contacting an M2 macrophage with the antibody or antigen-binding fragment of any one of claims 1-13; and
- b. co-incubating the M2 macrophage with a CD8⁺ T cells.

30. A method of converting an M2 macrophage into an M1 macrophage, the method comprising contacting the M2 macrophage with the antibody or antigen-binding fragment of any one of claims 1-13.