

CLAIMS

What is claimed is:

1. A chimeric antigen receptor (CAR), comprising an extracellular antigen-binding domain, a transmembrane domain and an intracellular signaling domain, wherein the extracellular antigen-binding domain comprises:

a heavy ~~light~~ chain complementarity determining ~~variable~~ region 1 (CDR1) having the ~~comprising an~~ amino acid sequence of SEQ ID NO: ~~66,73 and~~ a heavy chain CDR2 having the ~~variable region comprising an~~ amino acid sequence of SEQ ID NO: ~~67, and a heavy chain~~ CDR3 having the amino acid sequence of SEQ ID NO: ~~68;72,~~

a heavy chain complementarity determining region 1 (CDR1) having the amino acid sequence of SEQ ID NO: 58, a heavy chain CDR2 having the amino acid sequence of SEQ ID NO: 59, and a heavy chain CDR3 having the amino acid sequence of SEQ ID NO: 60;

a heavy chain complementarity determining region 1 (CDR1) having the amino acid sequence of SEQ ID NO: 39, a heavy chain CDR2 having the amino acid sequence of SEQ ID NO: 40, and a heavy chain CDR3 having the amino acid sequence of SEQ ID NO: 41;

a heavy chain CDR1 having the amino acid sequence of SEQ ID NO: 42, a heavy chain CDR2 having the amino acid sequence of SEQ ID NO: 43, and a heavy chain CDR3 having the amino acid sequence of SEQ ID NO: 44; or

a heavy chain CDR1 having the amino acid sequence of SEQ ID NO: 45, a heavy chain CDR2 having the amino acid sequence of SEQ ID NO: 46, and a heavy chain CDR3 having the amino acid sequence of SEQ ID NO: 47;

wherein the extracellular antigen-binding domain binds the ~~anti~~-G protein receptor family C group 5 member D (GPC5D) antigen.

2. The CAR of claim 1, wherein

the extracellular antigen-binding domain comprising the heavy chain CDR1 having the amino acid sequence of SEQ ID NO: 66, the heavy chain CDR2 having the

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amino acid sequence of SEQ ID NO: 67, and the heavy chain CDR3 having the amino acid sequence of SEQ ID NO: 68, and further comprises a light chain CDR1 having the amino acid sequence of SEQ ID NO: 69, a light chain CDR2 having the amino acid sequence of SEQ ID NO: 70, and a light chain CDR3 having the amino acid sequence of SEQ ID NO: 71;

the extracellular antigen binding domain comprising the heavy chain CDR1 having the amino acid sequence of SEQ ID NO: 58, the heavy chain CDR2 having the amino acid sequence of SEQ ID NO: 59, and the heavy chain CDR3 having the amino acid sequence of SEQ ID NO: 60, and further comprises a light chain CDR1 having the amino acid sequence of SEQ ID NO: 61, a light chain CDR2 having the amino acid sequence of SEQ ID NO: 62, and a light chain CDR3 having the amino acid sequence of SEQ ID NO: 63;

the extracellular antigen binding domain comprising the heavy chain CDR1 having the amino acid sequence of SEQ ID NO: 39, the heavy chain CDR2 having the amino acid sequence of SEQ ID NO: 40, and the heavy chain CDR3 having the amino acid sequence of SEQ ID NO: 41, and further comprises a light chain CDR1 having the amino acid sequence of SEQ ID NO: 48, a light chain CDR2 having the amino acid sequence of SEQ ID NO: 49, and a light chain CDR3 having the amino acid sequence of SEQ ID NO: 50;

the extracellular antigen binding domain comprising the heavy chain CDR1 having the amino acid sequence of SEQ ID NO: 42, the heavy chain CDR2 having the amino acid sequence of SEQ ID NO: 43, and the heavy chain CDR3 having the amino acid sequence of SEQ ID NO: 44, and further comprises a light chain CDR1 having the amino acid sequence of SEQ ID NO: 51, a light chain CDR2 having the amino acid sequence of SEQ ID NO: 52, and a light chain CDR3 having the amino acid sequence of SEQ ID NO: 53; or

the extracellular antigen binding domain comprising the heavy chain CDR1 having the amino acid sequence of SEQ ID NO: 45, the heavy chain CDR2 having the amino acid sequence of SEQ ID NO: 46, and the heavy chain CDR3 having the amino acid sequence of SEQ ID NO: 47, and further comprises a light chain CDR1 having the amino acid sequence of SEQ ID NO: 54, a light chain CDR2 having the amino acid

sequence of SEQ ID NO: 55, and a light chain CDR3 having the amino acid sequence of SEQ ID NO: 56.

6. The CAR of claim 1 or 2, wherein the extracellular antigen-binding domain comprises a light chain variable region (LCVR) comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 73, 65, 1, 3 and 5, or a heavy chain variable region (HCVR) comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 72, 64, 2, 4, and 6, or a combination of a LCVR comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 73, 65, 1, 3 and 5, and a HCVR comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 72, 64, 2, 4, and 6.

7. The CAR of claims 1-3, wherein the extracellular antigen-binding domain comprises:
a light chain variable region comprising an amino acid sequence of SEQ ID NO: 73 and a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 72;
a light chain variable region comprising an amino acid sequence of SEQ ID NO: 65 and a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 64;
a light chain variable region comprising an amino acid sequence of SEQ ID NO: 1 and a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 2;
a light chain variable region comprising an amino acid sequence of SEQ ID NO: 3 and a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 4;
or
a light chain variable region comprising an amino acid sequence of SEQ ID NO: 5 and a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 6.

The CAR of any of claims 1-4, wherein the extracellular antigen-binding domain comprises a single-chain variable fragment (scFv).

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9. ~~3.~~ The CAR of claim ~~52~~, wherein the scFv comprises a linker polypeptide between the light chain variable region and the heavy chain variable region.

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4. The CAR of claim ~~63~~, wherein the linker polypeptide comprises an amino acid sequence of SEQ ID NO: 7.

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5. The CAR of any of claims ~~5-72-4~~, wherein the scFv comprises an amino acid sequence selected from the group consisting of SEQ ID NOS: 78, ~~77, 75, 76, 8, 9, 10, 24, 25,~~ and ~~2677.~~

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6. The CAR of any of claims 1-~~85~~, wherein the extracellular antigen-binding domain comprises a signal polypeptide.

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7. The CAR of claims ~~96~~, wherein the signal polypeptide comprises an amino acid sequence of SEQ ID NO: 11.

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8. The CAR of any of claims 1-~~107~~, wherein the intracellular signaling domain comprises a polypeptide component selected from the group consisting of a TNF receptor superfamily member 9 (CD137) component, a T-cell surface glycoprotein CD3 zeta chain (CD3z) component, a cluster of differentiation (CD27) component, a cluster of differentiation superfamily member component, and a combination thereof.

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9. The CAR of claim ~~148~~, wherein the CD137 component comprises an amino acid sequence of SEQ ID NO: 12.

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10. The CAR of claim ~~148~~, wherein the CD3z component comprises an amino acid sequence of SEQ ID NO: 13.

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11. The CAR of claim ~~148~~, wherein the intracellular signaling domain comprises an amino acid sequence of SEQ ID NO: 14.

~~19.~~ 12. The CAR of any of claims 1-~~14~~11, wherein the transmembrane domain comprises a CD8a transmembrane region (CD8a-TM) polypeptide.

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13. The CAR of claim ~~15~~12, wherein the CD8a-TM polypeptide comprises an amino acid sequence of SEQ ID NO: 15.

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14. The CAR of any of claims 1-~~16~~13, further comprising a hinge region linking the transmembrane domain to the extracellular antigen-binding domain.

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15. The CAR of claim ~~17~~14, wherein the hinge region is a CD8a-hinge region.

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16. The CAR of claim ~~18~~15, wherein the CD8a-hinge region comprises an amino acid sequence of SEQ ID NO: 16.

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17. The CAR of any of claims 1-~~19~~16, wherein the extracellular antigen-binding domain comprises an amino acid sequence selected from the group consisting of SEQ ID NOS: 82, 81, 80, 79, ~~17, 18, 19, 20, 21, and 22~~ and 81.

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18. The CAR of any of claims 1-~~20~~17, wherein the CAR comprises an amino acid sequence selected from the group consisting of SEQ ID NOS: 86, 85, ~~83, 84, 27, 28, 29, 30, 31 and 32~~, and 85.

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19. An isolated lymphocyte expressing the CAR of any of claims 1-~~21~~18.

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20. The isolated lymphocyte of claim ~~22~~19, wherein the lymphocyte is a T lymphocyte.

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21. The isolated lymphocyte of claim ~~23~~20, wherein the T lymphocyte is a naïve T cell.

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22. The isolated lymphocyte of claim ~~23~~20, wherein the T lymphocyte is a memory stem T cell.

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23. The isolated lymphocyte of claim ~~23~~20, wherein the T lymphocyte is a central memory T cell.

24. The isolated lymphocyte of any of claims ~~23~~20 to ~~26~~23, wherein the T lymphocyte is CD4+.

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25. The isolated lymphocyte of any of claims ~~23~~20 to ~~26~~23, wherein the T lymphocyte is CD8+.

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26. The isolated lymphocyte of any of claims ~~23~~20 to ~~26~~23, wherein the T lymphocyte is CD4+ and CD8+.

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27. An isolated nucleic acid molecule encoding the CAR of any of claims 1-~~29~~18.

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28. The isolated nucleic acid molecule of claim ~~30~~27, wherein the nucleic acid molecule comprises a nucleic acid sequence selected from the group consisting of SEQ ID NOS: 90, ~~89, 87, 88, 33, 34, 35, 36, 37, and 38~~ and 89.

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29. The isolated nucleic acid molecule of claim ~~30~~27, wherein the nucleic acid molecule comprises a nucleotide sequence that is at least about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to a nucleotide sequence of SEQ ID NOS: 90, ~~89, 87, 88, 33, 34, 35, 36, 37, and 38~~ and 89.

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30. A vector comprising the nucleic acid molecule of any of claims ~~30-32~~27-29.

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31. A cell expressing the nucleic acid molecule of any of claims ~~30-32~~27-29.

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32. A pharmaceutical composition, comprising
an effective amount of the lymphocyte of any of claims ~~22-29~~19-26.

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33. A pharmaceutical composition, comprising
an effective amount of the lymphocyte of any of claims ~~22-29~~19-26 and a
pharmaceutically acceptable excipient.

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~~42.~~ 34. The CAR of any of claims 1-~~24~~18, the lymphocyte of any of claims 19-26, or the
pharmaceutical composition of claims ~~35~~32 or ~~36~~33 for
use in therapy.

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~~Use of the~~ 35. The CAR of any of claims 1-~~24~~18, the lymphocyte of any of claims 19-26, or the
pharmaceutical composition of claims ~~35 or 36 in the manufacture of a medicament~~32 or
33 ~~for the treatment of~~ use in a method of treating a subject having cancer.

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~~Use of~~ 36. The lymphocyte of any of claims 19-26, or the pharmaceutical composition of
claims 32 or 33 for use in a method of treating a subject having cancer, wherein the method
~~comprising~~ comprises:

45. ~~administering~~ a therapeutically effective amount of the lymphocyte of any of claims ~~22-29~~
~~in the manufacture of a medicament for the treatment of a subject having cancer,~~
to be administered to a subject in need thereof, whereby the lymphocyte induces
killing of cancer cells in the subject.

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~~The use of claim 38 or 39~~ 37. The CAR, the lymphocyte, or the pharmaceutical composition for
use in a method of claim 35 or the lymphocyte or the pharmaceutical composition for use
in a method of claim 36, wherein the cancer is selected from the group consisting of a lung
cancer, a gastric cancer, a colon cancer, a hepatocellular carcinoma, a renal cell carcinoma,
a bladder urothelial carcinoma, a metastatic melanoma, a breast cancer, an ovarian cancer,
a cervical cancer, a head and neck cancer, a pancreatic cancer, a glioma, a glioblastoma,
and a non-Hodgkin's lymphoma (NHL), an acute lymphocytic leukemia (ALL), a chronic
lymphocytic leukemia (CLL), a chronic myelogenous leukemia (CML), a smoldering

multiple myeloma (SMM), a multiple myeloma (MM), an acute myeloid leukemia (AML), and combinations thereof.

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38. The CAR, the lymphocyte, or the pharmaceutical composition for use in a method of claim 38, 39 or 40 35 or 37 or the lymphocyte or the pharmaceutical composition for use in a method of claim 36, wherein the cancer is multiple myeloma.

42. Use of the lymphocyte of any of claims 22-29 in the manufacture of a medicament for the 39.
An in vitro method of targeted killing of a cancer cell, the method comprising:

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43. contacting the cancer cell with the lymphocyte of any of claims 19-26,
whereby the lymphocyte induces killing of the cancer cell.

40. The use method of claim 42 39, wherein the cancer cell is selected from the group consisting of

a lung cancer, a gastric cancer, a colon cancer, a hepatocellular carcinoma, a renal cell carcinoma, a bladder urothelial carcinoma, a metastatic melanoma, a breast cancer, an ovarian cancer, a cervical cancer, a head and neck cancer, a pancreatic cancer, a glioma, a glioblastoma, and a non-Hodgkin's lymphoma (NHL), an acute lymphocytic leukemia (ALL), a chronic lymphocytic leukemia (CLL), a chronic myelogenous leukemia (CML), a smoldering multiple myeloma (SMM), a multiple myeloma (MM), an acute myeloid leukemia (AML), and combinations thereof

44. 41. The use method of claim 42 40, wherein the cancer cell is a multiple myeloma cell.

45. A 42. The CAR of any of claims 1-18 for use in a method of detecting the presence of cancer in a subject in vitro, comprising:

- (a) contacting a cell sample obtained from the subject with the CAR of claim any of claims 1-18, thereby forming a CAR-cell complex, and
- (b) detecting the complex, wherein detection of the complex is indicative of the presence of cancer in the subject.

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46. A therapeutically effective amount of the lymphocyte of any of claims 22-29 for use in therapy, whereby the lymphocyte induces killing of cancer cells in the subject.

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