

for AML is hampered by the lack of suitable targets. Accordingly, there are needs for novel therapeutic strategies to design CARs targeting antigens that are highly expressed in AML cells and limited expression in normal tissues for treating AML, and for strategies capable of inducing potent cancer eradication with minimal toxicity and immunogenicity.

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3. SUMMARY

In one aspect, provided herein is an antigen-recognizing receptor, comprising an extracellular antigen-binding domain, a transmembrane domain, and an intracellular signaling domain, wherein the extracellular antigen-binding domain specifically binds to CD371 and comprises a heavy chain variable region comprising a CDR1 comprising the amino acid sequence set forth in SEQ ID NO: 28, CDR2 comprising the amino acid sequence set forth in SEQ ID NO: 29, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 30; and a light chain variable region comprising a CDR1 comprising the amino acid sequence set forth in SEQ ID NO: 31, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO: 32, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 33.

In another aspect, provided herein is a cell comprising the antigen-recognizing receptor as described herein.

In another aspect, provided herein is a nucleic acid molecule encoding the antigen-recognizing receptor as described herein.

20 In another aspect, provided herein is a vector comprising the nucleic acid molecule as described herein.

In another aspect, provided herein is a host cell expressing the nucleic acid molecule as described herein.

25 In another aspect, provided herein is a composition comprising the cell as described herein.

In another aspect, provided herein is use of an effective amount of the cell as described herein or the composition as described herein in the manufacture of a medicament for reducing tumor burden in a subject, wherein an effective amount of the cell as described herein or the composition as described herein is to be administered to the subject, wherein the cell is a T cell or an NK cell.

30 In another aspect, provided herein is use of an effective amount of the cell as described herein or the composition as described herein in the manufacture of a medicament for increasing or lengthening survival of a subject having a tumor or neoplasm, wherein an effective amount of the cell as described herein or the composition as described herein is to be administered to the subject, wherein the cell is a T cell or an NK cell.

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In another aspect, provided herein is use of an effective amount of the cell as described herein or the composition as described herein in the manufacture of a medicament for treating tumor or neoplasm in a subject, wherein an effective amount of the cell as described herein or the composition as described herein is to be administered to the subject,
5 wherein the cell is a T cell or an NK cell.

In yet another aspect, provided herein is a method for producing a cell comprising an antigen-recognizing receptor as described herein, comprising introducing into the cell a nucleic acid molecule that encodes the antigen-recognizing receptor.

In yet another aspect, provided herein is a kit for reducing tumor burden in a subject, treating a tumor or neoplasm in a subject, and/or increasing or lengthening survival of a
10 subject having a tumor or neoplasm, comprising the cell as described herein.

In yet another aspect, provided herein is the cell as described herein or the composition as described herein for use in therapy, wherein the cell is a T cell or an NK cell.

15 The presently disclosed subject matter provides antigen-recognizing receptors that specifically target CD371 and cells comprising such CD371-targeted antigen-recognizing receptors. The presently disclosed subject matter further provides uses of the CD371-targeted antigen-recognizing receptors for treatment.

The presently disclosed subject matter provides an antigen-recognizing receptor,
20 comprising an extracellular antigen-binding domain, a transmembrane domain, and an intracellular signaling domain, wherein the extracellular antigen-binding domain specifically binds to CD371. In certain examples, the extracellular antigen-binding domain is a single-chain variable fragment (scFv). In certain examples, the extracellular antigen-binding domain is a human scFv. In certain examples, the extracellular antigen-binding domain is a
25 Fab, which is optionally crosslinked. In certain examples, the extracellular antigen-binding domain is a F(ab)₂. In certain examples, one or more of the scFv, Fab and F(ab)₂ are comprised in a fusion protein with a heterologous sequence to form the extracellular antigen-binding domain.

In certain examples, the extracellular antigen-binding domain comprises:

30 (a) a heavy chain variable region CDR1 comprising the amino acid sequence set forth in SEQ ID NO: 28 or a conservative modification thereof; a heavy chain variable region CDR2 comprising the amino acid sequence set forth in SEQ ID NO: 29 or a conservative modification thereof; and a heavy chain variable region CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 30 or a conservative modification thereof;

(b) a heavy chain variable region CDR1 comprising the amino acid sequence set forth in SEQ ID NO: 34 or a conservative modification thereof; a heavy chain variable region CDR2 comprising the amino acid sequence set forth in SEQ ID NO: 35 or a conservative modification thereof; and a heavy chain variable region CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 36 or a conservative modification thereof;

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WHAT IS CLAIMED IS:

1. An antigen-recognizing receptor, comprising an extracellular antigen-binding domain, a transmembrane domain, and an intracellular signaling domain, wherein the extracellular antigen-binding domain specifically binds to CD371 and comprises a heavy
5 chain variable region comprising a CDR1 comprising the amino acid sequence set forth in SEQ ID NO: 28, CDR2 comprising the amino acid sequence set forth in SEQ ID NO: 29, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 30; and a light chain variable region comprising a CDR1 comprising the amino acid sequence set forth in SEQ ID NO: 31, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO: 32, and a
10 CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 33.
2. The antigen-recognizing receptor of claim 1, wherein the extracellular antigen-binding domain is a single-chain variable fragment (scFv).
3. The antigen-recognizing receptor of claim 2, wherein the extracellular antigen-binding domain is a human scFv.
- 15 4. The antigen-recognizing receptor of claim 1, wherein the extracellular antigen-binding domain is a Fab, which is optionally crosslinked.
5. The antigen-recognizing receptor of claim 1, wherein the extracellular antigen-binding domain is a F(ab)₂.
6. The antigen-recognizing receptor of any one of claims 2-5, wherein one or more of
20 the scFv, Fab and F(ab)₂ are comprised in a fusion protein with a heterologous sequence to form the extracellular antigen-binding domain.
7. The antigen-recognizing receptor of any one of claims 1-6, wherein the extracellular antigen-binding domain comprises a heavy chain variable region comprising an amino acid sequence that is at least 80% identical to the amino acid sequence set forth in SEQ ID NO: 1.
- 25 8. The antigen-recognizing receptor of any one of claims 1-6, wherein the extracellular antigen-binding domain comprises a heavy chain variable region comprising amino acids having a sequence set forth in SEQ ID NO: 1.

9. The antigen-recognizing receptor of any one of claims 1-8, wherein the extracellular antigen-binding domain comprises a light chain variable region comprising an amino acid sequence that is at least 80% identical to the amino acid sequence set forth in SEQ ID NO: 2.
10. The antigen-recognizing receptor of any one of claims 1-8, wherein the extracellular antigen-binding domain comprises a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 2.
11. The antigen-recognizing receptor of any one of claims 1-10, wherein the extracellular antigen-binding domain comprises: (a) a heavy chain variable region comprising an amino acid sequence that is at least 80% identical to the amino acid sequence selected set forth in SEQ ID NO: 1; and (b) a light chain variable region comprising an amino acid sequence that is at least 80% identical to the amino acid sequence set forth in SEQ ID NO: 2.
12. The antigen-recognizing receptor of any one of claims 1-10, wherein the extracellular antigen-binding domain comprises a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 1; and a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 2.
13. The antigen-recognizing receptor of any one of claims 1-12, wherein the extracellular antigen-binding domain comprises a linker between a heavy chain variable region and a light chain variable region of the extracellular antigen-binding domain.
14. The antigen-recognizing receptor of claim 13, wherein the linker has the amino acid sequence set forth in SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 91, SEQ ID NO: 92, SEQ ID NO: 93, or SEQ ID NO: 94.
15. The antigen-recognizing receptor of claim 13, wherein the linker has the amino acid sequence set forth in SEQ ID NO: 13 or SEQ ID NO: 14.
16. The antigen-recognizing receptor of any one of claims 1-15, wherein the extracellular antigen-binding domain comprises a signal peptide that is covalently joined to the 5' terminus of the extracellular antigen-binding domain.

17. The antigen-recognizing receptor of any one of claims 1-16, wherein the extracellular antigen-binding domain comprises a heavy chain variable region and a light chain variable region, which are positioned from the N- to the C-terminus: V_H-V_L.
18. The antigen-recognizing receptor of any one of claims 1-17, wherein the extracellular antigen-binding domain comprises an amino acid sequence that is at least 80% identical to the amino acid sequence set forth in SEQ ID NO: 16 or SEQ ID NO: 107.
19. The antigen-recognizing receptor of any one of claims 1-18, wherein the extracellular antigen-binding domain comprises the amino acid sequence set forth in SEQ ID NO: 16 or SEQ ID NO: 107.
20. The antigen-recognizing receptor of any one of claims 1-19, wherein the extracellular antigen-binding domain binds to CD371 with a dissociation constant (K_d) of 1×10^{-8} M or more.
21. The antigen-recognizing receptor of any one of claims 1-20, wherein the transmembrane domain comprises a CD8 polypeptide, a CD28 polypeptide, a CD3ζ polypeptide, a CD4 polypeptide, a 4-1BB polypeptide, an OX40 polypeptide, an ICOS polypeptide, a CTLA-4 polypeptide, a PD-1 polypeptide, a LAG-3 polypeptide, a 2B4 polypeptide, a BTLA polypeptide, or a combination thereof.
22. The antigen-recognizing receptor of claim 21, wherein the transmembrane domain comprises a CD28 polypeptide.
23. The antigen-recognizing receptor of any one of claims 1-22, wherein the intracellular signaling domain comprises a CD3ζ polypeptide.
24. The antigen-recognizing receptor of any one of claims 1-23, wherein the intracellular signaling domain further comprises at least one co-stimulatory signaling region.
25. The antigen-recognizing receptor of claim 24, wherein the at least one co-stimulatory signaling region comprises a CD28 polypeptide, a 4-1BB polypeptide, an OX40 polypeptide, an ICOS polypeptide, a DAP-10 polypeptide, or a combination thereof.

26. The antigen-recognizing receptor of claim 24 or 25, wherein the at least one co-stimulatory signaling region comprises a CD28 polypeptide or a 4-1BB polypeptide.
27. The antigen-recognizing receptor of any one of claims 1-26, wherein the antigen-recognizing receptor is a CAR.
- 5 28. The antigen-recognizing receptor of any one of claims 1-27, wherein the antigen-recognizing receptor is recombinantly expressed.
29. The antigen-recognizing receptor of any one of claims 1-28, wherein the antigen-recognizing receptor is expressed from a vector.
30. The antigen-recognizing receptor of claim 29, wherein the vector is a γ -retroviral
10 rector.
31. A cell comprising the antigen-recognizing receptor of any one of claims 1-30.
32. The cell of claim 31, wherein the cell is transduced with the antigen-recognizing receptor.
33. The cell of claim 31 or 32, wherein the antigen-recognizing receptor is constitutively
15 expressed on the surface of the cell.
34. The cell of any one of claims 31-33, wherein the cell is engineered to express a cytokine.
35. The cell of claim 34, wherein the cell comprises an exogenous polypeptide of the cytokine.
- 20 36. The cell of claim 34 or 35, wherein the cell comprises a nucleic acid molecule encoding the cytokine.
37. The cell of any one of claims 34-36, wherein the cytokine is selected from the group consisting of IL-18, IL-33, IL-36, and combinations thereof.
38. The cell of any one of claims 34-37, wherein the cytokine is IL-18.

39. The cell of any one of claims 31-38, wherein the cell is an immunoresponsive cell.
40. The cell of any one of claims 31-39, wherein the cell is a cell of the lymphoid lineage or a cell of the myeloid lineage.
41. The cell of any one of claims 31-40, wherein the cell is selected from the group
5 consisting of a T cell, a Natural Killer (NK) cell, and a stem cell from which lymphoid cells may be differentiated.
42. The cell of any one of claims 31-41, wherein the cell is a T cell.
43. The cell of claim 41 or 42, wherein the T cell is a cytotoxic T lymphocyte (CTL) or a regulatory T cell.
- 10 44. The cell of claim 43, wherein the stem cell is a pluripotent stem cell.
45. The cell of claim 44, wherein the pluripotent stem cell is an embryoid stem cell or an induced pluripotent stem cell.
46. A nucleic acid molecule encoding the antigen-recognizing receptor of any one of claims 1-31.
- 15 47. The nucleic acid molecule of claim 46, wherein the nucleic acid molecule comprises the nucleotide sequence set forth in SEQ ID NO: 22.
48. A vector comprising the nucleic acid molecule of claim 46 or 47.
49. The vector of claim 48, wherein the vector is a γ -retroviral vector.
50. A host cell expressing the nucleic acid molecule of claim 46 or 47.
- 20 51. The host cell of claim 50, wherein the host cell is a T cell.
52. A composition comprising the cell of any one of claims 31-45.
53. The composition of claim 53, which is a pharmaceutical composition further comprising a pharmaceutically acceptable carrier.

54. Use of an effective amount of the cell of any one of claims 31-45 or the composition of claim 52 or 53 in the manufacture of a medicament for reducing tumor burden in a subject, wherein an effective amount of the cell of any one of claims 31-45 or the composition of claim 52 or 53 is to be administered to the subject, wherein the cell is a T cell or an NK cell.
- 5 55. The use of claim 54, wherein the medicament reduces the number of tumor cells and/or reduces tumor size in the subject
56. Use of an effective amount of the cell of any one of claims 31-45 or the composition of claim 52 or 53 in the manufacture of a medicament for increasing or lengthening survival of a subject having a tumor or neoplasm, wherein an effective amount of the cell of any one
10 of claims 31-45 or the composition of claim 52 or 53 is to be administered to the subject, wherein the cell is a T cell or an NK cell.
57. Use of an effective amount of the cell of any one of claims 31-45 or the composition of claim 52 or 53 in the manufacture of a medicament for treating tumor or neoplasm in a subject, wherein an effective amount of the cell of any one of claims 31-45 or the
15 composition of claim 52 or 53 is to be administered to the subject, wherein the cell is a T cell or an NK cell.
58. The use of any one of claims 54-57, wherein the tumor or neoplasm is selected from the group consisting of acute myeloid leukemia (AML), multiple myeloma, Non-Hodgkin's Lymphoma, Hodgkin's Lymphoma, Chronic Lymphocytic Leukemia (CLL), glioblastoma,
20 myelodysplastic syndrome (MDS), and chronic myelogenous leukemia (CML).
59. The use of any one of claims 54-58, wherein the tumor or neoplasm is acute myeloid leukemia (AML).
60. A method for producing a cell comprising an antigen-recognizing receptor of any one of claims 1-30, comprising introducing into the cell a nucleic acid molecule that encodes the
25 antigen-recognizing receptor.

61. A kit for reducing tumor burden in a subject, treating a tumor or neoplasm in a subject, and/or increasing or lengthening survival of a subject having a tumor or neoplasm, comprising the cell of any one of claims 31-45.
62. The kit of claim 61, wherein the kit further comprises written instructions for using
5 the cell for reducing tumor burden in a subject, treating a tumor or neoplasm in a subject, and/or increasing or lengthening survival of a subject having a tumor or neoplasm.
63. The cell of any one of claims 31-45 or the composition of claim 52 or 53 for use in therapy, wherein the cell is a T cell or an NK cell.