

CHIMERIC ANTIGEN RECEPTORS TARGETING CD19 AND USE THEREOF

1. CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims priority to U.S. Provisional Patent Application
5 Serial No. 63/015,362, filed April 24, 2020, and U.S. Provisional Patent Application
Serial No. 63/073,133, filed September 1, 2020, the contents of each of which are
incorporated by reference in their entirety, and to each of which priority is claimed.

2. TECHNICAL FIELD

The presently disclosed subject matter provides methods for treating
10 neoplasm (e.g., cancer) using cells comprising a chimeric antigen receptor (CAR) that
specifically targets CD19.

3. BACKGROUND

Cell-based immunotherapy is a therapy with curative potential for the
treatment of cancer. T-cells and other immune cells may be modified to target tumor
15 antigens through the introduction of genetic material coding for artificial or synthetic
receptors for antigen, termed Chimeric Antigen Receptors (CARs), specific to selected
antigens. Targeted T-cell therapy using CARs has shown recent clinical success in
treating hematologic malignancies.

T-cells expressing CD19-specific CARs with binding domains from
20 murine monoclonal antibodies have been shown to treat B-cell malignancies. T-cell
mediated immune responses specific for murine scFv antigen-binding domain of the
CAR can develop in human patients and result in premature elimination of CAR T-cells
increasing the risk of tumor relapse. Thus, there are needs for improved CD19-targeted
CARs with reduced toxicity and immunogenicity and/or improved safety and efficacy.

4. SUMMARY-OF-THE-INVENTION

The presently disclosed subject matter provides CD19-targeted chimeric
antigen receptors (CARs), cells comprising the CD19-targeted CARs, and uses of the
cells for treatments, e.g., for treating a neoplasm.

In one aspect, the present disclosure refers to a chimeric antigen receptor (CAR),
30 comprising an extracellular antigen-binding domain that specifically binds to CD19, a
transmembrane domain, and an intracellular signaling domain, wherein the extracellular
antigen-binding domain comprises:

a) a heavy chain variable region comprising a CDR1 comprising the amino acid

sequence set forth in SEQ ID NO: 7 or a conservative modification thereof, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO: 8 or a conservative modification thereof, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 9 or a conservative modification thereof; and

5 b) a light chain variable region comprising:

i) a CDR1 comprising the amino acid sequence set forth in SEQ ID NO: 11 or a conservative modification thereof, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO: 12 or a conservative modification thereof, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 13 or a conservative
10 modification thereof;

ii) a CDR1 comprising the amino acid sequence set forth in SEQ ID NO: 16 or a conservative modification thereof, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO: 12 or a conservative modification thereof, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 17 or a conservative
15 modification thereof;

iii) a CDR1 comprising the amino acid sequence set forth in SEQ ID NO: 11 or a conservative modification thereof, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO: 20 or a conservative modification thereof, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 21 or a conservative
20 modification thereof;

iv) a CDR1 comprising the amino acid sequence set forth in SEQ ID NO: 11 or a conservative modification thereof, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO: 12 or a conservative modification thereof, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 57 or a conservative
25 modification thereof;

v) a CDR1 comprising the amino acid sequence set forth in SEQ ID NO: 11 or a conservative modification thereof, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO: 12 or a conservative modification thereof, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 59 or a conservative
30 modification thereof;

vi) a CDR1 comprising the amino acid sequence set forth in SEQ ID NO: 16 or a conservative modification thereof, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO: 12 or a conservative modification thereof, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 61 or a conservative

modification thereof;

vii) a CDR1 comprising the amino acid sequence set forth in SEQ ID NO: 11 or a conservative modification thereof, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO: 12 or a conservative modification thereof, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 63 or a conservative modification thereof; or

viii) a CDR1 comprising the amino acid sequence set forth in SEQ ID NO: 11 or a conservative modification thereof, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO: 65 or a conservative modification thereof, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 66 or a conservative modification thereof.

In another aspect, the present disclosure refers to a cell comprising the CAR as described herein.

In another aspect, the present disclosure refers to a nucleic acid molecule encoding the CAR as described herein.

In another aspect, the present disclosure refers to a vector comprising the nucleic acid molecule as described herein.

In yet another aspect, the present disclosure refers to a cell expressing the nucleic acid molecule as described herein.

In yet another aspect, the present disclosure refers to a composition comprising the cell as described herein.

In yet another aspect, the present disclosure refers to a use 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, treating and/or preventing a neoplasm in a subject, and/or increasing or lengthening survival of a subject having a neoplasm.

In yet another aspect, the present disclosure refers to a method for producing a cell comprising the CAR as described herein, comprising introducing into the cell a nucleic acid molecule that encodes the CAR as described herein.

In yet another aspect, the present disclosure refers to a kit for reducing tumor burden in a subject, treating and/or preventing a neoplasm in a subject, and/or increasing or lengthening survival of a subject having a neoplasm, comprising the cell as described herein, optionally wherein the kit further comprises written instructions for using the cell for reducing tumor burden in a subject, treating and/or preventing a

neoplasm in a subject, and/or increasing or lengthening survival of a subject having a neoplasm.

In yet another aspect, the present disclosure refers to the cell as described herein or the composition as described herein for use in therapy.

5 In certain embodimentsexamples, the CAR comprises an extracellular antigen-binding domain that specifically binds to CD19, a transmembrane domain, and an intracellular signaling domain. In certain embodimentsexamples, the extracellular antigen-binding domain comprises a single-chain variable fragment (scFv), a Fab, or a F(ab)₂. In certain embodimentsexamples, the extracellular antigen-binding domain
10 comprises a scFv. In certain

~~embodiments~~examples, the scFv is a human scFv. In certain examples~~embodiments~~, the extracellular antigen-binding domain comprises (a) a heavy chain variable region comprising a CDR1 comprising the amino acid sequence set forth in SEQ ID NO: 7 or a conservative modification thereof, a CDR2 comprising the amino acid sequence set forth
5 in SEQ ID NO: 8 or a conservative modification thereof, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 9 or a conservative modification thereof; and/or (b) a light chain variable region comprising:

i) a CDR1 comprising the amino acid sequence set forth in SEQ ID NO: 11 or a conservative modification thereof, a CDR2 comprising the amino acid sequence
10 set forth in SEQ ID NO: 12 or a conservative modification thereof, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 13 or a conservative modification thereof;

ii) a CDR1 comprising the amino acid sequence set forth in SEQ ID NO: 16 or a conservative modification thereof, a CDR2 comprising the amino acid sequence
15 set forth in SEQ ID NO: 12 or a conservative modification thereof, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 17 or a conservative modification thereof;

iii) a CDR1 comprising the amino acid sequence set forth in SEQ ID NO: 11 or a conservative modification thereof, a CDR2 comprising the amino acid sequence
20 set forth in SEQ ID NO: 20 or a conservative modification thereof, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 21 or a conservative modification thereof;

iv) a CDR1 comprising the amino acid sequence set forth in SEQ ID NO: 11 or a conservative modification thereof, a CDR2 comprising the amino acid sequence
25 set forth in SEQ ID NO: 12 or a conservative modification thereof, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 57 or a conservative modification thereof;

v) a CDR1 comprising the amino acid sequence set forth in SEQ ID NO: 11 or a conservative modification thereof, a CDR2 comprising the amino acid sequence
30 set forth in SEQ ID NO: 12 or a conservative modification thereof, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 59 or a conservative modification thereof;

vi) a CDR1 comprising the amino acid sequence set forth in SEQ ID NO: 16 or a conservative modification thereof, a CDR2 comprising the amino acid sequence

set forth in SEQ ID NO: 12 or a conservative modification thereof, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 61 or a conservative modification thereof;

vii) a CDR1 comprising the amino acid sequence set forth in SEQ ID NO: 11 or a conservative modification thereof, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO: 12 or a conservative modification thereof, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 63 or a conservative modification thereof; or

viii) a CDR1 comprising the amino acid sequence set forth in SEQ ID NO: 11 or a conservative modification thereof, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO: 65 or a conservative modification thereof, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 66 or a conservative modification thereof.

In certain example embodiments, the heavy chain variable region comprises a CDR1 comprising the amino acid sequence set forth in SEQ ID NO: 7, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO: 8, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 9.

In certain embodiment examples, the light chain variable region comprises a CDR1 comprising the amino acid sequence set forth in SEQ ID NO: 11, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO: 12, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 13.

In certain embodiment examples, the light chain variable region comprises a CDR1 comprising the amino acid sequence set forth in SEQ ID NO: 16, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO: 12, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 17.

In certain embodiment examples, the light chain variable region comprises a CDR1 comprising the amino acid sequence set forth in SEQ ID NO: 11, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO: 20, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 21.

In certain embodiment examples, the light chain variable region comprises a CDR1 comprising the amino acid sequence set forth in SEQ ID NO: 11, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO: 12, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 57.

In certain embodimentsexamples, the light chain variable region comprises a CDR1 comprising the amino acid sequence set forth in SEQ ID NO: 11, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO: 12, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 59.

5 In certain embodimentsexamples, the light chain variable region comprises a CDR1 comprising the amino acid sequence set forth in SEQ ID NO: 16, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO: 12, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 61.

10 In certain embodimentsexamples, the light chain variable region comprises a CDR1 comprising the amino acid sequence set forth in SEQ ID NO: 11, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO: 12, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 63.

15 In certain embodimentsexamples, the light chain variable region comprises a CDR1 comprising the amino acid sequence set forth in SEQ ID NO: 11, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO: 65, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 66.

20 In certain embodimentsexamples, the heavy chain variable region comprises a CDR1 comprising the amino acid sequence set forth in SEQ ID NO: 7, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO: 8, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 9; and the light chain variable region comprises a CDR1 comprising the amino acid sequence set forth in SEQ ID NO: 11, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO: 12, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 13.

25 In certain embodimentsexamples, the heavy chain variable region comprises a CDR1 comprising the amino acid sequence set forth in SEQ ID NO: 7, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO: 8, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 9; and the light chain variable region comprises a CDR1 comprising the amino acid sequence set forth in SEQ ID NO: 16, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO: 12, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 17.

30 In certain embodimentsexamples, the heavy chain variable region comprises a CDR1 comprising the amino acid sequence set forth in SEQ ID NO: 7, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO: 8, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 9; and the light chain variable region

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comprises a CDR1 comprising the amino acid sequence set forth in SEQ ID NO: 11, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO: 20, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 21.

5 In certain embodimentsexamples, the heavy chain variable region comprises a CDR1 comprising the amino acid sequence set forth in SEQ ID NO: 7, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO: 8, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 9; and the light chain variable region comprises a CDR1 comprising the amino acid sequence set forth in SEQ ID NO: 11, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO: 12, 10 and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 57.

15 In certain embodimentsexamples, the heavy chain variable region comprises a CDR1 comprising the amino acid sequence set forth in SEQ ID NO: 7, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO: 8, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 9; and the light chain variable region comprises a CDR1 comprising the amino acid sequence set forth in SEQ ID NO: 11, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO: 12, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 59.

20 In certain embodimentsexamples, the heavy chain variable region comprises a CDR1 comprising the amino acid sequence set forth in SEQ ID NO: 7, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO: 8, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 9; and the light chain variable region comprises a CDR1 comprising the amino acid sequence set forth in SEQ ID NO: 16, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO: 12, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 61.

25 In certain embodimentsexamples, the heavy chain variable region comprises a CDR1 comprising the amino acid sequence set forth in SEQ ID NO: 7, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO: 8, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 9; and the light chain variable region comprises a CDR1 comprising the amino acid sequence set forth in SEQ ID NO: 11, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO: 12, 30 and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 63.

35 In certain embodimentsexamples, the heavy chain variable region comprises a CDR1 comprising the amino acid sequence set forth in SEQ ID NO: 7, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO: 8, and a CDR3 comprising

the amino acid sequence set forth in SEQ ID NO: 9; and the light chain variable region comprises a CDR1 comprising the amino acid sequence set forth in SEQ ID NO: 11, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO: 65, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 66.

5 In certain embodimentsexamples, the heavy chain variable region comprises an amino acid sequence that is at least about 80%, about 81%, about 82%, about 83%, about 84%, about 85%, about 86%, about 87%, about 88%, about 89%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, or about 99% identical or homologous to the amino acid
10 sequence set forth in SEQ ID NO: 10. In certain embodimentsexamples, the heavy chain variable region comprises the amino acid sequence set forth in SEQ ID NO: 10.

In certain embodimentsexamples, the light chain variable region comprises an amino acid sequence that is at least about 80%, about 81%, about 82%, about 83%, about 84%, about 85%, about 86%, about 87%, about 88%, about 89%,
15 about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98% or about 99% identical or homologous to the amino acid sequence set forth in SEQ ID NO: 14, SEQ ID NO: 18, SEQ ID NO: 22, SEQ ID NO: 58, SEQ ID NO: 60, SEQ ID NO: 62, SEQ ID NO: 64, or SEQ ID NO: 67. In certain
20 embodimentsexamples, the light chain variable region comprises the amino acid sequence set forth in SEQ ID NO: 14, SEQ ID NO: 18, SEQ ID NO: 22 SEQ ID NO: 58, SEQ ID NO: 60, SEQ ID NO: 62, SEQ ID NO: 64, or SEQ ID NO: 67. In certain
embodimentsexamples, the light chain variable region comprises the amino acid sequence set forth in SEQ ID NO: 14, SEQ ID NO: 18, or SEQ ID NO: 22.

In certain embodimentsexamples, the heavy chain variable region
25 comprising an amino acid sequence that is at least about 80%, about 81%, about 82%, about 83%, about 84%, about 85%, about 86%, about 87%, about 88%, about 89%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98% or about 99% identical or homologous to the amino acid sequence set forth in SEQ ID NO: 10; and the light chain variable region comprises an amino acid
30 sequence that is at least about 80%, about 81%, about 82%, about 83%, about 84%, about 85%, about 86%, about 87%, about 88%, about 89%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98% or about 99% identical or homologous to the amino acid sequence set forth in SEQ ID NO: 14, SEQ ID NO: 18, SEQ ID NO: 22, SEQ ID NO: 58, SEQ ID NO: 60, SEQ ID NO:
35 62, SEQ ID NO: 64, or SEQ ID NO: 67. In certain embodimentsexamples, the heavy chain variable region comprises the

amino acid sequence set forth in SEQ ID NO: 10; and the light chain variable region comprises the amino acid sequence set forth in SEQ ID NO: 14, SEQ ID NO: 18, SEQ ID NO: 22, SEQ ID NO: 58, SEQ ID NO: 60, SEQ ID NO: 62, SEQ ID NO: 64, or SEQ ID NO: 67. In certain embodimentsexamples, the heavy chain variable region comprises the amino acid sequence set forth in SEQ ID NO: 10; and the light chain variable region comprises the amino acid sequence set forth in SEQ ID NO: 14, SEQ ID NO: 18, or SEQ ID NO: 22.

In certain embodimentsexamples, the heavy chain variable region comprises the amino acid sequence set forth in SEQ ID NO: 10, and the light chain variable region comprises the amino acid sequence set forth in SEQ ID NO: 14.

In certain embodimentsexamples, the heavy chain variable region comprises the amino acid sequence set forth in SEQ ID NO: 10, and the light chain variable region comprises the amino acid sequence set forth in SEQ ID NO: 18.

In certain embodimentsexamples, the heavy chain variable region comprises the amino acid sequence set forth in SEQ ID NO: 10, and the light chain variable region comprises the amino acid sequence set forth in SEQ ID NO: 22.

In certain embodimentsexamples, the heavy chain variable region comprises the amino acid sequence set forth in SEQ ID NO: 10, and the light chain variable region comprises the amino acid sequence set forth in SEQ ID NO: 58.

In certain embodimentsexamples, the heavy chain variable region comprises the amino acid sequence set forth in SEQ ID NO: 10, and the light chain variable region comprises the amino acid sequence set forth in SEQ ID NO: 60.

In certain embodimentsexamples, the heavy chain variable region comprises the amino acid sequence set forth in SEQ ID NO: 10, and the light chain variable region comprises the amino acid sequence set forth in SEQ ID NO: 62.

In certain embodimentsexamples, the heavy chain variable region comprises the amino acid sequence set forth in SEQ ID NO: 10, and the light chain variable region comprises the amino acid sequence set forth in SEQ ID NO: 64.

In certain embodimentsexamples, the heavy chain variable region comprises the amino acid sequence set forth in SEQ ID NO: 10, and the light chain variable region comprises the amino acid sequence set forth in SEQ ID NO: 67.

In certain embodimentsexamples, the extracellular antigen-binding domain comprises a linker between the heavy chain variable region and the light chain variable region. In certain embodimentsexamples, the linker consists of the amino acid sequence set forth

in SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, or SEQ ID NO: 4. In certain embodimentsexamples, the heavy chain variable region and the light chain variable region are positioned from the N- to the C-terminus: V_L-V_H.

5 In certain embodimentsexamples, the extracellular antigen-binding domain comprises or is a scFv, which comprises or consists of the amino acid sequence set forth in SEQ ID NO: 15, SEQ ID NO: 19, or SEQ ID NO: 23.

10 In certain embodimentsexamples, 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, or a BTLA polypeptide. In certain embodimentsexamples, the transmembrane domain comprises a CD28 polypeptide.

15 In certain embodimentsexamples, the intracellular signaling domain comprises a CD3ζ polypeptide. In certain embodimentsexamples, the CD3ζ polypeptide is a modified CD3ζ polypeptide. In certain embodimentsexamples, the modified CD3ζ polypeptide comprises a native ITAM1, an ITAM2 variant consisting of two loss-of-function mutations, and an ITAM3 consisting of two loss-of-function mutations. In certain embodimentsexamples, the native ITAM1 consists of the amino acid sequence set forth in SEQ ID NO: 31. In certain embodimentsexamples, the ITAM2 variant consists of the amino acid sequence set forth in SEQ ID NO: 37. In certain embodimentsexamples, the ITAM3 variant consists of the amino acid sequence set forth in SEQ ID NO: 41. In certain embodimentsexamples, the modified CD3ζ polypeptide comprising or consists of the amino acid sequence set forth in SEQ ID NO: 43.

25 In certain embodimentsexamples, the intracellular signaling domain further comprises at least one co-stimulatory signaling region. In certain embodimentsexamples, 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. In certain embodimentsexamples, the at least one co-stimulatory signaling region comprises an intracellular domain of CD28 or a portion thereof, an intracellular domain of 4-1BB or a portion thereof, an intracellular domain of OX40 or a portion thereof, an intracellular domain of ICOS or a portion thereof, or an intracellular domain of DAP-10 or a portion thereof. In certain embodimentsexamples, the at least one co-stimulatory signaling region comprises a CD28 polypeptide.

35 In certain embodimentsexamples, the CAR is expressed from a vector. In certain embodimentsexamples, the vector is a retroviral vector.

The presently disclosed subject matter further provides cells comprising the CARs disclosed herein. In certain embodimentsexamples, the cell is transduced with the CAR. In certain embodimentsexamples, the CAR is constitutively expressed on the surface of the cell. In certain embodimentsexamples, the cell is an immunoresponsive cell. In certain embodimentsexamples, the cell is a cell of the lymphoid lineage or a cell of the myeloid lineage. In certain embodimentsexamples, the cell is selected from the group consisting of a T cell, a Natural Killer (NK) cell, a stem cell from which lymphoid cells may be differentiated, and a stem cell from which myeloid cells may be differentiated. In certain embodimentsexamples, the cell is a T cell. In certain embodimentsexamples, the T cell is selected from the group consisting of helper T cells, cytotoxic T cells, memory T cells, regulatory T cells, tumor-infiltrating lymphocyte (TIL), Natural Killer T cells, mucosal associated invariant T cells, and $\gamma\delta$ T cells. In certain embodimentsexamples, the cell is a NK cell. In certain embodimentsexamples, the NK cell is derived from a stem cell. In certain embodimentsexamples, the stem cell is a pluripotent stem cell. In certain embodimentsexamples, the pluripotent stem cell is an embryoid stem cell or an induced pluripotent stem cell.

Furthermore, the presently disclosed subject matter provides nucleic acid molecules encoding the CAR disclosed herein. In certain embodimentsexamples, the nucleic acid molecule further comprises a promoter that is operably linked to the CAR. The promoter can be endogenous or exogenous. In certain embodimentsexamples, the promoter is an exogenous promoter. In certain embodimentsexamples, the exogenous promoter is selected from the group consisting of an elongation factor (EF)-1 promoter, a cytomegalovirus immediate-early promoter (CMV) promoter, a simian virus 40 early promoter (SV40) promoter, a phosphoglycerate kinase (PGK) promoter, a metallothionein promoter, and Ubiquitin C promoter. In certain embodimentsexamples, the promoter is an endogenous promoter. In certain embodimentsexamples, the endogenous promoter is selected from a TCR alpha promoter, a TCR beta promoter, and a beta 2-microglobulin promoter. In certain embodimentsexamples, the promoter is an inducible promoter. In certain embodimentsexamples, the inducible promoter is selected from the group consisting of a NFAT transcriptional response element (TRE) promoter, a CD69 promoter, a CD25 promoter, an IL-2 promoter, a 4-1BB promoter, a PD1 promoter, and a LAG3 promoter.

The presently disclosed subject matter also provides vectors comprising the nucleic acid molecule disclosed herein. In certain embodimentsexamples, the vector is a retroviral vector.

The presently disclosed subject matter further provides cells expressing the nucleic acid molecule disclosed herein. In certain embodimentsexamples, the cell is a T cell or Natural Killer (NK) cell.

5 The presently disclosed subject matter provides compositions comprising the cell disclosed herein. In certain embodimentsexamples, the composition is a pharmaceutical composition further comprising a pharmaceutically acceptable carrier. In certain embodimentsexamples, the composition comprises between about 1×10^6 and about 5×10^8 cells. In certain embodimentsexamples, the composition comprises between about 1×10^6 and about 1×10^8 cells. In certain embodimentsexamples, the
10 composition comprises between about 1×10^6 and about 5×10^7 cells. In certain embodimentsexamples, the composition comprises about 2.5×10^7 cells.

The presently disclosed subject matter further provides various methods of using the presently disclosed cells. The presently disclosed subject matter provides methods of reducing tumor burden in a subject. In certain embodimentsexamples, the
15 method comprises administering to the subject the cell or the composition disclosed herein. In certain embodimentsexamples, the method reduces the number of tumor cells, reduces tumor size, and/or eradicates the tumor in the subject.

The presently disclosed subject matter provides methods of increasing or lengthening survival of a subject having a neoplasm. In certain embodimentsexamples,
20 the method comprises administering to the subject the cell or the composition disclosed herein.

The presently disclosed subject matter provides methods of treating and/or preventing a neoplasm in a subject. In certain embodimentsexamples, the method comprises administering to the subject the cell or the composition disclosed herein.

25 The presently disclosed cells and compositions can be used in a therapy. In certain embodimentsexamples, the presently disclosed cells and compositions are used in treating and/or preventing a neoplasm in a subject. In certain embodimentsexamples, the presently disclosed cells and compositions are used in increasing or lengthening survival of a subject having a neoplasm. In certain embodimentsexamples, the presently
30 disclosed cells and compositions are used in treating and/or preventing a neoplasm in a subject.

In certain embodimentsexamples, the tumor and/or neoplasm is associated with CD19. In certain embodimentsexamples, the tumor and/or neoplasm is a blood cancer. In certain embodimentsexamples, the blood cancer is selected from the group
35 consisting of multiple myeloma, leukemia, and lymphomas. In certain embodimentsexamples, the leukemia is selected

from the group consisting of include acute myeloid leukemia (AML), chronic myeloid leukemia (CML), acute lymphocytic leukemia (ALL), chronic lymphocytic leukemia (CLL), acute promyelocytic leukemia (APL), mixed-phenotype acute leukemia (MLL), hairy cell leukemia, and B cell prolymphocytic leukemia. In certain

5 ~~embodiments~~examples, the lymphoma is Hodgkin's lymphoma or non-Hodgkin's lymphoma. In certain ~~embodiments~~examples, the tumor and/or neoplasm is a B cell malignancy. In certain ~~embodiments~~examples, the B cell malignancy is selected from the group consisting of B cell non-Hodgkin lymphomas (NHL), B cell Hodgkin's lymphomas, B cell acute lymphocytic leukemia (ALL), B cell chronic lymphocytic leukemia (CLL),
10 multiple myeloma (MM), CLL with Richter's transformation, and CNS lymphoma. In certain ~~embodiments~~examples, the tumor and/or neoplasm is B cell lymphoma. In certain ~~embodiments~~examples, the B cell lymphoma is relapsed or refractory (R/R) B cell lymphoma. In certain ~~embodiments~~examples, the subject is a human subject.

Furthermore, the presently disclosed subject matter provides methods for
15 producing a cell comprising the CAR disclosed herein. In certain ~~embodiments~~examples, the method comprises introducing into the cell a nucleic acid molecule that encodes the CAR disclosed herein.

Furthermore, the presently disclosed subject matter provides kits for reducing tumor burden in a subject, treating and/or preventing a neoplasm in a subject,
20 and/or increasing or lengthening survival of a subject having a neoplasm. In certain ~~embodiments~~examples, the kit comprises the cell disclosed herein. In certain ~~embodiments~~examples, the kit further comprises written instructions for using the cell for reducing tumor burden in a subject, treating and/or preventing a neoplasm in a subject, and/or increasing or lengthening survival of a subject having a neoplasm.

25 **5. BRIEF DESCRIPTION OF THE DRAWINGS**

Figures 1A and 1B depict cytotoxicity of T cells comprising CD19-targeted CARs against CD19-expressing cells. Figure 1A shows the killing against CD19-expressing Raji cancer cell lines. Figure 1B shows the killing against CD19 knock-out Raji cells. Positive control is 1928z-1XX CAR.

30 Figures 2A and 2B depict cytotoxicity of T cells comprising CD19-targeted CARs against CD19-expressing cells measured by flow based killing assessment. Figure 2A shows the killing against CD19-expressing Raji cancer cell lines. Figure 2B shows the killing against CD19 knock-out Raji cells. Positive control is 1928z-1XX CAR.