

with CD33 and CD123 CAR T cells suggest challenges both in terms of safety and efficacy, which are due to abundant expression of CD33 and CD123 in normal hematopoiesis and phenotypic heterogeneity in AML tumor cells. Accordingly, there are needs for a novel combinatorial CAR format for R/R AML that has the potential to provide improved safety and efficacy relative to alternative CAR therapies currently under clinical investigation.

3. SUMMARY OF THE INVENTION

The presently disclosed subject matter provides chimeric receptors that target ADGRE2, and chimeric receptors that target CLEC12A, cells comprising such chimeric receptors, and methods of using such cells for treatments, e.g., for treating acute myeloid leukemia (AML).

In one aspect, the presently disclosed subject matter provides a chimeric receptor, comprising an extracellular antigen-binding domain that binds to ADGRE2, a transmembrane domain, and an intracellular 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: 33 or a conservative modification thereof, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO: 34 or a conservative modification thereof, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 35 or a conservative modification thereof; and/or b) a light chain variable region comprising a CDR1 comprising the amino acid sequence set forth in SEQ ID NO: 36 or a conservative modification thereof, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO: 37 or a conservative modification thereof, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 38 or a conservative modification thereof.

-In one aspect, the presently disclosed subject matter provides a chimeric receptor, comprising an extracellular antigen-binding domain that binds to CLEC12A, a transmembrane domain, and an intracellular domain, wherein the extracellular antigen-binding domain comprises: a) a heavy chain variable region comprising: i) a CDR1 comprising the amino acid sequence set forth in SEQ ID NO: 69 or a conservative modification thereof, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO: 70 or a conservative modification thereof, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 71 or a conservative modification thereof; ii) a CDR1 comprising the amino acid sequence set forth in SEQ ID NO: 81 or a conservative modification thereof, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO: 82 or a conservative modification thereof, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 83 or a conservative modification thereof; iii) a CDR1 comprising the amino acid sequence set forth in SEQ ID NO: 89 or a conservative modification thereof, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO: 90 or a conservative modification thereof, and a CDR3 comprising the amino acid

sequence set forth in SEQ ID NO: 91 or a conservative modification thereof; iv) a CDR1 comprising the amino acid sequence set forth in SEQ ID NO: 89 or a conservative modification thereof, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO: 90 or a conservative modification thereof, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 98 or a conservative modification thereof; v) a CDR1 comprising the amino acid sequence set forth in SEQ ID NO: 81 or a conservative modification thereof, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO: 103 or a conservative modification thereof, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 83 or a conservative modification thereof; vi) a CDR1 comprising the amino acid sequence set forth in SEQ ID NO: 109 or a conservative modification thereof, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO: 103 or a conservative modification thereof, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 83 or a conservative modification thereof; vii) a CDR1 comprising the amino acid sequence set forth in SEQ ID NO: 89 or a conservative modification thereof, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO: 90 or a conservative modification thereof, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 114 or a conservative modification thereof; viii) a CDR1 comprising the amino acid sequence set forth in SEQ ID NO: 120 or a conservative modification thereof, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO: 121 or a conservative modification thereof, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 122 or a conservative modification thereof; or ix) a CDR1 comprising the amino acid sequence set forth in SEQ ID NO: 129 or a conservative modification thereof, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO: 130 or a conservative modification thereof, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 131 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: 72 or a conservative modification thereof, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO: 73 or a conservative modification thereof, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 74 or a conservative modification thereof; ii) a CDR1 comprising the amino acid sequence set forth in SEQ ID NO: 84 or a conservative modification thereof, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO: 73 or a conservative modification thereof, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 85 or a conservative modification thereof; iii) a CDR1 comprising the amino acid sequence set forth in SEQ ID NO: 92 or a conservative modification thereof, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO: 93 or a conservative modification thereof, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 94 or a conservative modification

thereof; iv)) a CDR1 comprising the amino acid sequence set forth in SEQ ID NO: 99 or a conservative modification thereof, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO: 93 or a conservative modification thereof, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 151 or a conservative modification thereof; v) a CDR1 comprising the amino acid sequence set forth in SEQ ID NO: 104 or a conservative modification thereof, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO: 73 or a conservative modification thereof, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 105 or a conservative modification thereof; vi) a CDR1 comprising the amino acid sequence set forth in SEQ ID NO: 110 or a conservative modification thereof, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO: 73 or a conservative modification thereof, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 85 or a conservative modification thereof; vii) a CDR1 comprising the amino acid sequence set forth in SEQ ID NO: 115 or a conservative modification thereof, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO: 93 or a conservative modification thereof, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 116 or a conservative modification thereof; viii) a CDR1 comprising the amino acid sequence set forth in SEQ ID NO: 123 or a conservative modification thereof, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO: 124 or a conservative modification thereof, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 125 or a conservative modification thereof; or ix) a CDR1 comprising the amino acid sequence set forth in SEQ ID NO: 132 or a conservative modification thereof, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO: 133 or a conservative modification thereof, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 134 or a conservative modification thereof.

In one aspect, the presently disclosed subject matter provides a cell comprising the chimeric receptor as described herein.

In one aspect, the presently disclosed subject matter provides a cell comprising a) a first chimeric receptor that is the chimeric receptor that is described herein, and b) a second chimeric receptor that is the chimeric receptor as described herein.

In one aspect, the presently disclosed subject matter provides a nucleic acid molecule encoding the chimeric receptor as described herein.

In one aspect, the presently disclosed subject matter provides a nucleic acid composition comprising a first nucleic acid molecule and a second nucleic acid molecule as described herein.

In one aspect, the presently disclosed subject matter provides a vector comprising the nucleic acid molecule as described herein.

In one aspect, the presently disclosed subject matter provides a vector comprising the nucleic acid composition as described herein.

In one aspect, the presently disclosed subject matter provides a cell comprising the nucleic acid molecule as described herein.

In one aspect, the presently disclosed subject matter provides a cell comprising the nucleic acid composition as described herein.

In one aspect, the presently disclosed subject matter provides a cell comprising the vector as described herein.

In one aspect, the presently disclosed subject matter provides a composition comprising the cell as described herein.

In one aspect, the presently disclosed subject matter provides a use of the cell or the composition as described herein in the manufacture of a medicament for reducing tumor burden in a subject.

In one aspect, the presently disclosed subject matter provides a use of the cell or the composition as described herein in the manufacture of a medicament for increasing or lengthening survival of a subject having a tumor.

In one aspect, the presently disclosed subject matter provides a use of the cell or the composition as described herein in the manufacture of a medicament for treating and/or preventing a tumor in a subject.

In one aspect, the presently disclosed subject matter provides a method for producing a cell comprising the chimeric receptor as described herein, comprising introducing into the cell a nucleic acid molecule that encodes the chimeric receptor as described herein.

In one aspect, the presently disclosed subject matter provides a method for producing a cell comprising the chimeric receptor as described herein and the chimeric receptor as described herein, comprising introducing into the cell a nucleic acid molecule that encodes the chimeric receptor as described herein and the chimeric receptor as described herein.

In one aspect, the presently disclosed subject matter provides a cell or the composition as described herein for use in therapy.

5 In one aspect, the presently disclosed subject matter provides chimeric receptors that target ADGRE2. In certain embodiments, the ADGRE2-targeted chimeric receptor comprises an extracellular antigen-binding domain that binds to ADGRE2, a transmembrane domain, and an intracellular domain. In certain 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: 33 or a conservative modification thereof, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO: 34 or a conservative modification

thereof, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 35 or a conservative modification thereof; and/or b) a light chain variable region comprising a CDR1 comprising the amino acid sequence set forth in SEQ ID NO: 36 or a conservative modification thereof, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO: 37 or a conservative modification thereof, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 38 or a conservative modification thereof.

In certain embodiments, the extracellular antigen-binding domain comprises a single-chain variable fragment (scFv), a Fab, or a F(ab)₂. In certain embodiments, the extracellular antigen-binding domain comprises an scFv. In certain embodiments, the scFv is a humanized scFv.

In certain embodiments, the heavy chain variable region comprises a CDR1 comprising the amino acid sequence set forth in SEQ ID NO: 33, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO: 34, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 35. In certain embodiments, the light chain variable region comprises a CDR1 comprising the amino acid sequence set forth in SEQ ID NO: 36, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO: 37, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 38. In certain embodiments, the heavy chain variable region comprises a CDR1 comprising the amino acid sequence set forth in SEQ ID NO: 33, a CDR2 comprising the