

CLAIMS

What is claimed is:

1. A vector comprising a backbone and a nucleic acid sequence encoding:
 - (1) a cell tag comprising: (a) truncated epidermal growth factor receptor or (b) a full-length or truncated CD20; and
 - (2) a chimeric antigen receptor (CAR), wherein the CAR comprises:
 - (a) a CD33 antigen binding domain;
 - (b) a stalk domain;
 - (c) a transmembrane domain;
 - (d) a costimulatory signaling domain comprising 4-1BB and/or CD28; and
 - (e) a CD3 zeta signaling domain.
2. The vector of claim 1, wherein the vector is a lentivirus vector, a retroviral vector, or a non-viral vector.
3. The vector of claim 1, wherein the cell tag comprises a polypeptide having at least 90% identity with the amino acid sequence of SEQ ID NO: 32 or SEQ ID NO: 54.
4. The vector of claim 1, wherein the CD33 antigen binding domain comprises the amino acid sequence of at least one of SEQ ID NOs: 1, 3, 8–14, or a functional variant or fragment thereof that differs therefrom by conservative amino acid substitutions.
5. The vector of claim 1, wherein the costimulatory signaling domain comprises 4-1BB.
6. The vector of claim 1, wherein the costimulatory signaling domain comprises CD28.
7. The vector of claim 2, wherein the non-viral vector is a *Sleeping Beauty* transposon.
8. An immune effector cell comprising the vector of any one of claims 1–7, 15, 17, and 18.
9. The immune effector cell of claim 8, further expressing a cytokine.
10. The immune effector cell of claim 9, wherein the cytokine comprises IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-14, IL-15, IL-16, IL-17, IL-18, IL-19, IL-20, IL-21, IL-22,

IL-23, IL-24, IL-25, IL-26, IL-27, IL-28, IL-29, IL-30, IL-31, IL-32, or IL-33, or a functional variant or fragment thereof.

11. The immune effector cell of claim 8, further expressing a fusion protein comprising IL-15, or a functional variant or fragment thereof, and IL-15R α , or a functional variant or fragment thereof.

12. The immune effector cell of claim 11, wherein the fusion protein has at least 95% identity with the amino acid sequence of SEQ ID NO: 37.

13. The immune effector cell of claim 8, wherein the immune effector cell is a T cell or a Natural Killer (NK) cell.

14. Use of the cell of claim 13 in the manufacture of a medicament for the treatment of acute myeloid leukemia in a human subject in need thereof.

15. The vector of claim 1, wherein the cell tag comprises a polypeptide having at least 90% identity with the amino acid sequence of SEQ ID NO: 54.

16. The use of claim 14, wherein the acute myeloid leukemia is relapsed or refractory AML.

17. The vector of claim 1, wherein:

(a) the CD33 antigen binding domain comprises the amino acid sequence of SEQ ID NO: 8, or a functional variant or fragment thereof that differs therefrom by conservative amino acid substitutions;

(b) the stalk domain comprises a polypeptide having at least 90% identity with the amino acid sequence of SEQ ID NO: 22;

(c) the costimulatory signaling domain comprises a polypeptide having at least 90% identity with the amino acid sequence of SEQ ID NO: 24 or 28;

(d) the cell tag comprises a polypeptide having at least 90% identity with the amino acid sequence of SEQ ID NO: 32 or 54; and

(e) the CD3 zeta signaling domain comprises a polypeptide having at least 90% identity with the amino acid sequence of SEQ ID NO: 26.

18. The vector of claim 1, wherein:

(a) the CD33 antigen binding domain comprises the amino acid sequence of SEQ ID NO: 8;

- (b) the stalk domain comprises the amino acid sequence of SEQ ID NO: 22;
 - (c) the costimulatory signaling domain comprises the amino acid sequence of SEQ ID NO: 24 or 28;
 - (d) the cell tag comprises the amino acid sequence of SEQ ID NO: 32 or 54; and
 - (e) the CD3 zeta signaling domain comprises the amino acid sequence of SEQ ID NO: 26.
19. A system for expressing a CAR in an immune effector cell, the system comprising the vector of claim 17 or 18.
20. The system of claim 19, wherein the immune effector cell is a T cell or NK cell.
21. The system of claim 19, further comprising a nucleic acid encoding at least one additional gene.
22. The system of claim 21, wherein the additional gene comprises a cytokine.
23. The system of claim 21, wherein the additional gene encodes a fusion protein having at least 90% sequence identity with SEQ ID NO: 37.
24. A method of stimulating the proliferation and/or survival of engineered T-cells, the method comprising:
- (a) transfecting T-cells or T-cell progenitors obtained from a subject with the vector of claim 1 and a vector encoding a transposase, to provide a population of engineered CD33 CAR-expressing T-cells; and
 - (b) optionally, culturing the population of CD33 CAR-expressing T-cells *ex vivo* for 2 days or less.
25. The immune effector cell of claim 13, wherein the T cell is a cytotoxic T lymphocyte (CTL), or a regulatory T cell.