

CLAIMS

What is claimed is:

1. A vector comprising a backbone and a nucleic acid sequence encoding:
 - (1) a cell tag comprising an amino acid sequence of SEQ ID NO: 32 or 54 or a functional variant thereof that differs therefrom by conservative amino acid substitutions; 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 an amino acid sequence of SEQ ID NO: 32 or 54 or a functional variant thereof that differs therefrom by up to 20 conservative amino acid substitutions.
4. The vector of claim 1, wherein the cell tag comprises an amino acid sequence of SEQ ID NO: 32 or 54 or a functional variant thereof that differs therefrom by up to 10 conservative amino acid substitutions.
5. The vector of claim 1, wherein the cell tag comprises an amino acid sequence of SEQ ID NO: 32 or 54 or a functional variant thereof that differs therefrom by up to 5 conservative amino acid substitutions.
6. The vector of claim 1, wherein the cell tag comprises the amino acid sequence of SEQ ID NO: 32 or a functional variant thereof that differs therefrom by conservative amino acid substitutions.
7. The vector of claim 1, wherein the cell tag comprises the amino acid sequence of SEQ ID NO: 32 or a functional variant thereof that differs therefrom by up to 20 conservative amino acid substitutions.
8. The vector of claim 1, wherein the cell tag comprises the amino acid sequence of SEQ ID NO: 32.
9. The vector of claim 1, wherein the cell tag comprises the amino acid sequence of SEQ ID NO: 54 or a functional variant thereof that differs therefrom by conservative amino acid substitutions.
10. The vector of claim 1, wherein the cell tag comprises the amino acid sequence of SEQ ID NO: 54 or a functional variant thereof that differs therefrom by up to 20 conservative amino acid substitutions.

11. The vector of claim 1, wherein the cell tag comprises the amino acid sequence of SEQ ID NO: 54 or a functional variant thereof that differs therefrom by up to 10 conservative amino acid substitutions.
12. The vector of claim 1, wherein the cell tag comprises the amino acid sequence of SEQ ID NO: 54 or a functional variant thereof that differs therefrom by up to 5 conservative amino acid substitutions.
13. 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.
14. The vector of claim 1, wherein the costimulatory signaling domain comprises 4-1BB.
15. The vector of claim 1, wherein the costimulatory signaling domain comprises CD28.
16. The vector of claim 2, wherein the non-viral vector is a *Sleeping Beauty* transposon.
17. An immune effector cell comprising the vector of any one of claims 1 – 16, 24, and 26 - 29.
18. The immune effector cell of claim 17, further expressing a cytokine.
19. The immune effector cell of claim 18, 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.

20. The immune effector cell of claim 17, 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.
21. The immune effector cell of claim 20, wherein the fusion protein has at least 95% identity with the amino acid sequence of SEQ ID NO: 37.
22. The immune effector cell of claim 17, wherein the immune effector cell is a T cell or a Natural Killer (NK) cell.
23. Use of the cell of claim 22 in the manufacture of a medicament for the treatment of acute myeloid leukemia in a human subject in need thereof .
24. The vector of claim 1, wherein the cell tag comprises the amino acid sequence of SEQ ID NO: 54.
25. The use of claim 23, wherein the acute myeloid leukemia is relapsed or refractory AML.
26. 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 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.
27. 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; and
 - (e) the CD3 zeta signaling domain comprises the amino acid sequence of SEQ ID NO: 26.
28. 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: 54; and
 - (e) the CD3 zeta signaling domain comprises the amino acid sequence of SEQ ID NO: 26.
29. The vector of claim 28, wherein the costimulatory signaling domain comprises the amino acid sequence of SEQ ID NO: 28;
30. A system for expressing a CAR in an immune effector cell, the system comprising the vector of any one of claims 1–16, 24, and 26–29.
31. The system of claim 30, wherein the immune effector cell is a T cell or NK cell.
32. The system of claim 30, further comprising a nucleic acid encoding at least one additional gene.
33. The system of claim 32, wherein the additional gene comprises a cytokine.
34. The system of claim 32, wherein the additional gene encodes a fusion protein having at least 90% sequence identity with SEQ ID NO: 37.
35. 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 any one of claims 1–16, 24, and 26–29 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.

36. The immune effector cell of claim 22, wherein the T cell is a cytotoxic T lymphocyte (CTL), or a regulatory T cell.