

CLAIMS

What is claimed is:

1. A vector comprising a backbone and a nucleic acid sequence encoding:
 - (1) a truncated epidermal growth factor receptor comprising at least one of HER1t, HER1t-1 or a functional variant thereof; 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 or CD28, or both;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 truncated epidermal growth factor receptor comprises a polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% 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 at least one of:
 - (a) a polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO:8 (hM195scFv);
 - (b) a polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NOs:9 and 10 (M2H12);
 - (c) a polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NOs:11 and 12 (DRB2); and
 - (d) a polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NOs:14 and 15 (My9-6).

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 a vector comprising a backbone and a nucleic acid sequence encoding
 - (1) a cell tag; and
 - (2) 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 or CD28, or both; and
 - (e) a CD3 zeta signaling domain.
9. The immune effector cell of claim 8, wherein the cell tag comprises HER1t, HER1t-1, CD20t-1 or CD20.
10. The immune effector cell of claim 9, wherein the cell tag comprises HER1t, and said HER1t comprises the polypeptide sequence of SEQ ID NO: 32.
11. The immune effector cell of claim 9, wherein the cell tag comprises HER1t-1, and said HER1t-1 comprises the polypeptide sequence of SEQ ID NO: 54
12. An immune effector cell comprising the vector of claim 1.
13. The immune effector cell of claim 12, wherein the immune effector cell is a T cell, or a Natural Killer (NK) cell.
14. Use of a cell genetically modified to express a CAR in the manufacture of a medicament for the stimulation of a T cell-mediated immune response to a target cell population or tissue in a human subject in need thereof, wherein the CAR comprises a
 - (a) a CD33 antigen binding domain;
 - (b) a stalk domain;
 - (c) a transmembrane domain;
 - (d) a costimulatory signaling domain comprising 4-1BB or CD28, or both;

- (e) a CD3 zeta signaling domain; and
 - (f) a truncated epidermal growth factor receptor (HER1t).
15. The use according to claim 14, wherein the human has been diagnosed with acute myeloid leukemia (AML).
16. The use according to claim 15, wherein the acute myeloid leukemia is relapsed or refractory AML.
17. The vector of claim 1, wherein the CAR comprises:
- (a) the CD33 antigen binding domain with the amino acid sequence of SEQ ID NO:8;
 - (b) the stalk domain with the amino acid sequence of SEQ ID NO:22;
 - (c) the costimulatory signaling domain comprising CD28 with the amino acid sequence of SEQ ID NO:28;
 - (d) a HER1 tag which comprises at least one of HER1t with the amino acid sequence of SEQ ID NO:32 and HER1t-1 with an amino acid sequence of SEQ ID NO: 54; and
 - (e) the CD3 zeta signaling domain with the amino acid sequence of SEQ ID NO: 26.
18. The vector of claim 1, wherein the CAR comprises:
- (a) the CD33 antigen binding domain with the amino acid sequence of SEQ ID NO:8;
 - (b) the stalk domain with the amino acid sequence of SEQ ID NO:22;
 - (c) the costimulatory signaling domain comprising 4-1BB with an amino acid sequence of SEQ ID NO:24;
 - (d) a HER1 tag which comprises at least one of HER1t with an amino acid sequence of SEQ ID NO:32 and HER1t-1 with an amino acid sequence of SEQ ID NO: 54; and
 - (e) the CD3 zeta signaling domain with an amino acid sequence of SEQ ID NO: 26.
19. A system for expressing a CAR in an immune effector cell, said system comprising one or more vectors according to claim 1, wherein the CAR comprises:
- (a) the CD33 antigen binding domain with an amino acid sequence of SEQ ID NO:8;
 - (b) the stalk domain with an amino acid sequence of SEQ ID NO:22;

- (c) the costimulatory signaling domain comprising CD28 with an amino acid sequence of SEQ ID NO:28, or the costimulatory signaling domain comprising 4-1BB with an amino acid sequence of SEQ ID NO:24;
 - (d) a HER1 tag which comprises the at least one of HER1t with an amino acid sequence of SEQ ID NO:32 and HER1t-1 with an amino acid sequence of SEQ ID NO: 54;
and
 - (e) the CD3 zeta signaling domain with an amino acid sequence of SEQ ID NO: 26.
20. The system of claim 19, wherein said immune effector cell is a T cell or NK cell.
21. The system of any one of claim 19, further comprising a nucleic acid encoding at least one additional gene.
22. The system of claim 21, wherein said additional gene comprises a cytokine.
23. The system of claim 22, wherein said cytokine comprises at least one of IL-2, IL-15, IL-12, IL-21, and a fusion of IL-15 and IL-15R□ □
24. A method of stimulating the proliferation and/or survival of engineered T-cells comprising:
- (a) obtaining a sample of cells from a subject, the sample comprising T-cells or T-cell progenitors;
 - (b) transfecting the cells with one or more vectors encoding an isolated nucleic acid as provided in claim 1 and a vector encoding a transposase , to provide a population of engineered CD33 CAR-expressing T-cells;
 - (c) and optionally, culturing the population of CD33CAR 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.