

CLAIMS

1. A Fms-like tyrosine kinase 3 (FLT3) specific chimeric antigen receptor (CAR) comprising an extracellular ligand-binding domain, a first transmembrane domain, and an intracellular signaling domain, wherein the extracellular ligand-binding domain comprises a single chain variable fragment (scFv) comprising a heavy chain variable (VH) region and a light chain variable (VL) region, wherein the scFv binds to SEQ ID NO: 202 (Domain 4 of FLT3) or SEQ ID NO: 200 (Domain 2 of FLT3).

2. A Fms-like tyrosine kinase 3 (FLT3) specific chimeric antigen receptor (CAR) comprising an extracellular ligand-binding domain, a first transmembrane domain, and an intracellular signaling domain, wherein the extracellular domain comprises a single chain variable fragment (scFv) comprising a heavy chain variable (VH) region and a light chain variable (VL) region, wherein

(a) the VH region comprises (i) a VH complementarity determining region one (CDR1) having the amino acid sequence shown in SEQ ID NO: 37, 38, 39, 43, 44, 45, 49, 50, 54, 55, 56, 60, 61, 62, 66, 67, 68, 72, 73, 74, 78, 79, 80, 84, 85, 86, 90, 91, 92, 96, 97, 98, 102, 103, 104, 108, 109, 110, 114, 115, 116, 120, 121, 122, 126, 127, 128, 132, 133, 134, 138, 139, 140, 294, 295, 296, 300, 301, 305, 306, 307, 311, 312, 316, 317, or 318; (ii) a VH complementarity determining region two (CDR2) having the amino acid sequence shown in SEQ ID NO: 40, 41, 46, 47, 51, 52, 57, 58, 63, 64, 69, 70, 75, 76, 81, 82, 87, 88, 93, 94, 99, 100, 105, 106, 111, 112, 117, 118, 123, 124, 129, 130, 135, 136, 141, 142, 297, 298, 302, 303, 308, 309, 313, 314, 319, 320, or 322; and (iii) a VH complementarity determining region three (CDR3) having the amino acid sequence shown in SEQ ID NO: 42, 48, 53, 59, 65, 71, 77, 83, 89, 95, 101, 107, 113, 119, 125, 131, 137, 143, 299, 304, 310, 315, or 321; and/or

(b) the VL region comprises (i) a VL complementarity determining region one (CDR1) having the amino acid sequence shown in SEQ ID NO: 144, 147, 150, 153, 156, 159, 162, 165, 168, 171, 174, 177, 180, 183, 186, 189, 192, 195, 323, 326, 328, 331, 336, 338, 340, 343, 345, 348, or 350; (ii) a VL complementarity determining region two (CDR2) having the amino acid sequence shown in SEQ ID NO: 145, 148, 151, 154, 157, 160, 163, 166, 169, 172, 175, 178, 181, 184, 187, 190, 193, 196, 324, 329, 332, 334, 341, or 346; and (iii) a VL complementarity

determining region three (CDR3) having the amino acid sequence shown in SEQ ID NO: 146, 149, 152, 155, 158, 161, 164, 167, 170, 173, 176, 179, 182, 185, 188, 191, 194, 197, 325, 327, 330, 333, 335, 337, 339, 342, 344, 347, or 349.

3. A Fms-like tyrosine kinase 3 (FLT3) specific chimeric antigen receptor (CAR) comprising an extracellular ligand-binding domain, a first transmembrane domain, and an intracellular signaling domain, wherein the extracellular domain comprises a single chain variable fragment (scFv) comprises:

a heavy chain variable (VH) region having the sequence shown in SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 265, 267, 269, 271, 273, 275, 277, 279, 281, 283, 285, 287, 289, 291, or 293; optionally comprising one or several conservative amino acid substitutions in residues that are not within a CDR; and/or

a light chain variable (VL) region having the sequence shown in SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 264, 266, 268, 270, 272, 274, 276, 278, 280, 282, 284, 286, 288, 290, or 292; optionally comprising one or several conservative amino acid substitutions in residues that are not within a CDR.

4. A Fms-like tyrosine kinase 3 (FLT3) specific chimeric antigen receptor (CAR) comprising an extracellular ligand-binding domain, a first transmembrane domain, and an intracellular signaling domain, wherein the extracellular domain comprises a single chain variable fragment (scFv) comprising a heavy chain variable (VH) region and a light chain variable (VL) region, wherein

(a) the VH region comprises a VH CDR1 comprising the amino acid sequence shown in SEQ ID NO: 49, 44, or 50; a VH CDR2 comprising the amino acid sequence shown in SEQ ID NO: 51 or 52; and a VH CDR3 comprising the amino acid sequence shown in SEQ ID NO: 53; and the VL region comprises a VL CDR1 comprising the amino acid sequence shown in SEQ ID NO: 150; a VL CDR2 comprising the amino acid sequence shown in SEQ ID NO: 151; and a VL CDR3 comprising the amino acid sequence shown in SEQ ID NO: 152; optionally wherein the VH region comprises the amino acid sequence shown in SEQ ID NO: 6 and the VL region comprises the amino acid sequence shown in SEQ ID NO: 5; or

(b) the VH region comprises a VH CDR1 comprising the amino acid sequence shown in SEQ ID NO: 90, 91, or 92; a VH CDR2 comprising the amino acid sequence shown in SEQ ID NO: 93 or 94; and a VH CDR3 comprising the amino acid sequence shown in SEQ ID NO: 95; and the VL region comprises a VL CDR1 comprising the amino acid sequence shown in SEQ ID NO: 171; a VL CDR2 comprising the amino acid sequence shown in SEQ ID NO: 172; and a VL CDR3 comprising the amino acid sequence shown in SEQ ID NO: 173; optionally wherein the VH region comprises the amino acid sequence shown in SEQ ID NO: 20 and the VL region comprises the amino acid sequence shown in SEQ ID NO: 19.

5. The FLT3 specific CAR of any one of claims 1-3, wherein:

(a) the VH region comprises a VH CDR1 comprising the amino acid sequence shown in SEQ ID NO: 43, 44, or 45; a VH CDR2 comprising the amino acid sequence shown in SEQ ID NO: 46 or 47; and a VH CDR3 comprising the amino acid sequence shown in SEQ ID NO: 48; and the VL region comprises a VL CDR1 comprising the amino acid sequence shown in SEQ ID NO: 147; a VL CDR2 comprising the amino acid sequence shown in SEQ ID NO: 148; and a VL CDR3 comprising the amino acid sequence shown in SEQ ID NO: 149; optionally wherein the VH region comprises the amino acid sequence shown in SEQ ID NO: 4 and the VL region comprises the amino acid sequence shown in SEQ ID NO: 3; or

(b) the VH region comprises a VH CDR1 comprising the amino acid sequence shown in SEQ ID NO: 60, 61, or 62; a VH CDR2 comprising the amino acid sequence shown in SEQ ID NO: 63 or 64; and a VH CDR3 comprising the amino acid sequence shown in SEQ ID NO: 65; and the VL region comprises a VL CDR1 comprising the amino acid sequence shown in SEQ ID NO: 156; a VL CDR2 comprising the amino acid sequence shown in SEQ ID NO: 157; and a VL CDR3 comprising the amino acid sequence shown in SEQ ID NO: 158; optionally

wherein the VH region comprises the amino acid sequence shown in SEQ ID NO: 10 and the VL region comprises the amino acid sequence shown in SEQ ID NO: 9.

6. The FLT3 specific CAR of any one of claims 1 to 5, wherein:

(i) the intracellular signaling domain comprises a CD3 ζ signalling domain or a 4-1BB domain; and/or

(ii) the first transmembrane domain comprises a CD8 α chain transmembrane domain; and/or

(iii) the FLT3 specific CAR comprises a second intracellular signaling domain; optionally wherein the second intracellular signaling domain comprises a 4-1BB domain; and/or

(iv) the FLT3 specific CAR comprises a stalk domain between the extracellular ligand-binding domain and the first transmembrane domain; optionally wherein the stalk domain is selected from the group consisting of: a CD8 α hinge, an IgG1 hinge, and an Fc γ RIII α hinge; and/or

(v) the FLT3 specific CAR comprises a second extracellular ligand-binding domain that is not specific for FLT3.

7. The FLT3 specific CAR of any one of claims 1 to 6, comprising one or more epitopes specific for a monoclonal antibody; and optionally wherein the epitope is:

(i) selected from the group consisting of: a CD52 epitope, a CD20 epitope, a CD3 epitope, a CD41 epitope, a CD25 epitope, a CD30 epitope, an EGFR epitope, a TNF α epitope, a VEGF epitope, a complement protein C5 epitope, a CD11a epitope, a CD33 epitope, an alpha-4 integrin epitope, an IgE Fc region epitope, an RSV protein F epitope, an IL-6 receptor epitope, a HER2 receptor epitope, an integrin $\alpha_4\beta_7$ epitope, a BAFF (B-cell activating factor) epitope, an IL-1 β epitope, a RANKL epitope, a CTLA4 epitope, a CD34 epitope, an IL-12 epitope, an IL-23 epitope, and combinations thereof; and/or

(ii) an epitope specifically recognized by alemtuzumab, ibritumomab tiuxetan, muromonab-CD3, tositumomab, abciximab, basiliximab, brentuximab vedotin, cetuximab, infliximab, rituximab, bevacizumab, certolizumab pegol, daclizumab, eculizumab, efalizumab, gemtuzumab, natalizumab, omalizumab, palivizumab, ranibizumab, tocilizumab, trastuzumab, vedolizumab, adalimumab, belimumab, canakinumab, denosumab, golimumab, ipilimumab, ofatumumab, panitumumab, QBEND-10, or ustekinumab; and/or

(iii) a CD20 epitope; optionally wherein the CD20 epitope comprises the amino acid sequence shown in SEQ ID NO: 229.

8. The FLT3 specific CAR of claim 1, wherein the FLT3 specific CAR comprises the amino acid sequence shown in SEQ ID NO: 235, 236, 237 or 242.

9. The FLT3 specific CAR of any one of claims 1 to 8, wherein:

(i) the extracellular ligand-binding domain(s), the first transmembrane domain, and intracellular signaling domain(s) are on a single polypeptide; and/or

(ii) the FLT3 specific CAR comprises a second transmembrane domain, wherein the first transmembrane domain and the extracellular ligand-binding domain(s) are on a first polypeptide, and wherein the second transmembrane domain and the intracellular signaling domain(s) are on a second polypeptide, wherein the first transmembrane domain comprises a transmembrane domain from the α chain of the high-affinity IgE receptor (Fc ϵ RI) and the second transmembrane domain comprises a transmembrane domain from the γ or β chain of Fc ϵ RI; optionally comprising a third polypeptide comprising a third transmembrane domain fused to an intracellular signaling domain from a co-stimulatory molecule, wherein the third transmembrane domain comprises a transmembrane domain from the γ or β chain of Fc ϵ RI.

10. A polynucleotide comprising a nucleic acid sequence encoding the FLT3 specific CAR of any one of claims 1 to 9.

11. The polynucleotide of claim 10, wherein the polynucleotide comprises the nucleic acid sequence:

(i) of the vector having ATCC Accession No. PTA-124227 or of the vector having ATCC Accession No. PTA-124228; or

(ii) shown in SEQ ID NO: 253, 248, 247, 246, or 245.

12. An expression vector comprising the polynucleotide of any one of claims 10 to 11.

13. An engineered immune cell expressing a FLT3 specific CAR of any one of claims 1 to 9.

14. The engineered immune cell of claim 13, comprising

(i) another CAR which is not specific for FLT3; and/or

(ii) a polynucleotide encoding a suicide polypeptide; optionally wherein the suicide polypeptide is RQR8; and/or

(iii) a disruption of one or more endogenous genes, wherein the endogenous gene encodes TCR α , TCR β , CD52, glucocorticoid receptor (GR), deoxycytidine kinase (dCK), or an immune checkpoint protein; optionally wherein the immune checkpoint protein is programmed death-1 (PD-1).

15. The engineered immune cell of any one of claims 13 to 14, wherein the immune cell is

(i) derived from an inflammatory T-lymphocyte, a cytotoxic T-lymphocyte, a regulatory T-lymphocyte, or a helper T-lymphocyte; and/or

(ii) is obtained from a healthy donor or from a donor suffering from a disease or disorder.

16. The engineered immune cell of any one of claims 13 to 15, for use as a medicament.

17. The engineered immune cell for use according to claim 16, wherein the medicament is for use in:

(i) treatment of a disease or disorder associated with FLT3; optionally wherein the disease or disorder is a cancer; or

(ii) the inhibition of tumor growth or progression, or the induction of tumor regression, in a subject who has malignant cells expressing FLT3; or

(iii) the inhibition of metastasis of malignant cells expressing FLT3; or

(iv) treatment of a cancer selected from the group consisting of multiple myeloma, malignant plasma cell neoplasm, Hodgkin's lymphoma, nodular lymphocyte predominant Hodgkin's lymphoma, Kahler's disease and Myelomatosis, plasma cell leukemia, plasmacytoma, B-cell prolymphocytic leukemia, hairy cell leukemia, B-cell non-Hodgkin's lymphoma (NHL), acute myeloid leukemia (AML), chronic lymphocytic leukemia (CLL), acute lymphocytic leukemia (ALL), chronic myeloid leukemia (CML), follicular lymphoma, marginal zone lymphoma, mantle cell lymphoma, large cell lymphoma, precursor B-lymphoblastic lymphoma, myeloid leukemia, diffuse large B cell lymphoma, mucosa-associated lymphatic

tissue lymphoma, small cell lymphocytic lymphoma, Burkitt lymphoma, primary mediastinal (thymic) large B-cell lymphoma, lymphoplasmacytic lymphoma, waldenström macroglobulinemia, nodal marginal zone B cell lymphoma, splenic marginal zone lymphoma, intravascular large B-cell lymphoma, primary effusion lymphoma, lymphomatoid granulomatosis, T cell/histiocyte-rich large B-cell lymphoma, primary central nervous system lymphoma, primary cutaneous diffuse large B-cell lymphoma (leg type), EBV positive diffuse large B-cell lymphoma of the elderly, diffuse large B-cell lymphoma associated with inflammation, ALK-positive large B-cell lymphoma, plasmablastic lymphoma, large B-cell lymphoma arising in HHV8-associated multicentric Castleman disease, B-cell lymphoma unclassified with features intermediate between diffuse large B-cell lymphoma and Burkitt lymphoma, B-cell lymphoma unclassified with features intermediate between diffuse large B-cell lymphoma and classical Hodgkin lymphoma, and other hematopoietic cells related cancer.

18. The engineered immune cell of any one of claims 13 to 15, or for use according to any one of claims 16 to 17, wherein the FLT3 specific CAR comprises an extracellular domain comprising an ScFv,

- wherein the scFv binds to the extracellular domain of human FLT3 with a K_D comprised between 10nM and 80nM, or
- wherein the scFv binds to domain 4 of the extracellular domain of human FLT3 with a K_D comprised between 1nM and 100nM, or
- wherein the scFv binds to domain 2-3 of the extracellular domain of human FLT3 with a K_D comprised between 5nM and 30nM.

19. A population of cells comprising engineered immune cells of any one of claims 13 to 18, wherein

- (i) said population of cells comprises a percentage of stem cell memory and central memory cells greater than 20%, 30% or 40%, and/or
- (ii) said population of cells achieves a percentage of lysis of FLT3 expressing cells greater than 10%, 20%, 30% or 40%.

20. A method of engineering an immune cell expressing a FLT3 specific CAR, comprising:

- a. providing an immune cell; and
- b. introducing into the cell at least one polynucleotide encoding FLT3 specific CAR of any one of claims 1 to 9; and

c. optionally introducing into the cell at least one polynucleotide encoding for a CAR which is not specific for FLT3;
whereby the immune cell expresses the FLT3 specific CAR.

21. A pharmaceutical composition, comprising the engineered immune cells of any one of claims 13 to 15.

22. The engineered immune cell for use according to claim 17, wherein the use comprises administering:

(i) a nucleoside analog therapy to the subject; optionally wherein the nucleoside analog therapy is fludarabine or clofarabine; or

(ii) a Receptor Tyrosine Kinase inhibitor, an mTOR inhibitors, an epigenetic modulator, a proteasome inhibitor, an immunomodulatory agent, a Hedgehog inhibitor or an Isocitrate Dehydrogenase (IDH) inhibitors or combination thereof to the subject; or

(iii) a proteasome inhibitor, an immunomodulatory agent or a Hedgehog inhibitor or combination thereof to the subject; or

(iv) lenalidomide to the subject.

23. A method for *in vitro* sorting of a population of immune cells, wherein a subset of the population of immune cells comprises engineered immune cells expressing a FLT3 specific chimeric antigen receptor (CAR) of claim 7, the method comprising:

a. contacting the population of immune cells with a monoclonal antibody specific for the epitope; and
b. selecting the immune cells that bind to the monoclonal antibody to obtain a population of cells enriched in engineered immune cells; optionally wherein the population of cells enriched in engineered immune cell comprises at least 70% of FLT3 CAR-expressing immune cells; and optionally wherein

the monoclonal antibody specific for the epitope is conjugated to:

(i) a fluorophore and optionally the step of selecting the cells that bind to the monoclonal antibody is done by Fluorescence Activated Cell Sorting (FACS); or

(ii) a magnetic particle and optionally the step of selecting the cells that bind to the monoclonal antibody is done by Magnetic Activated Cell Sorting (MACS).

24. A monoclonal antibody for use in a method of treatment, wherein the subject has been administered engineered immune cells expressing a FLT3 specific chimeric antigen receptor (CAR) of claim 7, the method comprising administering to the subject the monoclonal antibody, wherein the monoclonal antibody is specific for the epitope; and wherein the method is for:

(i) depleting said engineered immune cells from the subject; or

(ii) for promoting recovery of endogenous FLT3-expressing bone marrow progenitor cells in the subject.

25. The method of claim 23, or the monoclonal antibody for use according to claim 24, wherein the epitope is a CD20 epitope; optionally wherein the CD20 epitope has an amino acid sequence shown in SEQ ID NO: 229; and optionally the monoclonal antibody is rituximab.

26. The monoclonal antibody for use according to claim 24, wherein the monoclonal antibody is:

(i) conjugated to a molecule that can activate the complement system; or

(ii) coupled to a cytotoxic drug is coupled to the monoclonal antibody.