

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 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: 72, 73, or 74; (ii) a VH complementarity determining region two (CDR2) having the amino acid sequence shown in SEQ ID NO: 75 or 76; and (iii) a VH complementarity determining region three (CDR3) having the amino acid sequence shown in SEQ ID NO: 77; and

(b) the VL region comprises (i) a VL complementarity determining region one (CDR1) having the amino acid sequence shown in SEQ ID NO: 162; (ii) a VL complementarity determining region two (CDR2) having the amino acid sequence shown in SEQ ID NO: 163; and (iii) a VL complementarity

determining region three (CDR3) having the amino acid sequence shown in SEQ ID NO: 164.

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 having the sequence shown in SEQ ID NO: 14; optionally comprising one or several conservative amino acid substitutions in residues that are not within a CDR; and

a light chain variable (VL) region having the sequence shown in SEQ ID NO: 13; optionally comprising one or several conservative amino acid substitutions in residues that are not within a CDR.

3. The FLT3 specific CAR of any one of claims 1 to 2, 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.

4. The FLT3 specific CAR of any one of claims 1 to 3, 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 α -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.

5. The FLT3 specific CAR of claim 1, wherein the FLT3 specific CAR comprises the amino acid sequence shown in SEQ ID NO: 234.

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

7. A polynucleotide comprising a nucleic acid sequence encoding the FLT3 specific CAR of any one of claims 1 to 6.

8. An expression vector comprising the polynucleotide of claim 7.

9. An engineered immune cell expressing a FLT3 specific CAR of any one of claims 1 to 6.

10. The engineered immune cell of claim 9, 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).

11. The engineered immune cell of any one of claims 9 to 10, 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.

12. The engineered immune cell of any one of claims 9 to 11, for use as a medicament.

13. The engineered immune cell for use according to claim 12, 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.

14. The engineered immune cell of any one of claims 9 to 11, or for use according to any one of claims 12 to 13, 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.

15. A population of cells comprising engineered immune cells of any one of claims 9 to 14, 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%.

16. 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 6; 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.

17. A pharmaceutical composition, comprising the engineered immune cells of any one of claims 9 to 11.

18. A combination therapy comprising the engineered immune cell for use according to claim 13 in conjunction with 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.

19. 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 4, 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).

20. A monoclonal antibody for use in a method of treatment of a subject, wherein the subject has already been pre-administered with engineered immune cells expressing a FLT3 specific chimeric antigen receptor (CAR) of claim 4, and wherein the monoclonal antibody, being specific for the epitope, is to be administered to the subject, the method being 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.

21. The method of claim 19, or the monoclonal antibody for use according to claim 20, 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.

22. The monoclonal antibody for use according to claim 20, 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.