

CHIMERIC ANTIGEN RECEPTORS TARGETING FLT3**CROSS REFERENCE TO RELATED APPLICATIONS**

[0001] The present application claims the benefit of priority to U.S. Provisional Application No. 62/514,634, filed on June 2, 2017, the contents of which are hereby incorporated by reference in their entirety.

[0002] The contents of U.S. Provisional Application Nos. 62/514,574 filed on June 2, 2017, and 62/660,908 filed on April 20, 2018, and a PCT Application entitled “ANTIBODIES SPECIFIC FOR FLT3 AND THEIR USES” filed on May 31, 2018, all of which disclose FLT3 specific antibodies, are hereby incorporated by reference in their entirety.

REFERENCE TO SEQUENCE LISTING

[0003] This application is being filed electronically via EFS-Web and includes an electronically submitted sequence listing in .txt format. The .txt file contains a sequence listing entitled ALGN_010_01WO_SeqList_ST25.txt” created on May 24, 2018, and having a size of 238 KB. The sequence listing contained in this .txt file is part of the specification and is incorporated herein by reference in its entirety.

FIELD

[0004] Provided herein are Fms-like tyrosine kinase 3 (FLT3)-specific antibodies, chimeric antigen receptors (CARs) that specifically bind to FLT3 (FLT3 CARs), polynucleotides encoding FLT3 CARs, and engineered immune cells comprising the FLT3-specific CARs (e.g. FLT3-specific CAR-T cells). The invention further relates to methods for engineering immune cells to express FLT3 specific CARs and methods of using the FLT3-specific antibodies and FLT3-specific CAR-T cells for treating conditions associated with FLT3 (e.g., malignant cells expressing FLT3).

BACKGROUND

[0005] Adoptive transfer of immune cells genetically modified to recognize malignancy-associated antigens is showing promise as a new approach to treating cancer (see, e.g., Brenner et al., Current Opinion in Immunology, 22(2): 251-257 (2010); Rosenberg et al., Nature Reviews

Cancer, 8(4): 299-308 (2008)). T cells can be genetically modified to express chimeric antigen receptors (CARs), fusion proteins comprised of an antigen recognition moiety and T cell activation domains (see, e.g., Eshhar et al., Proc. Natl. Acad. Sci. USA, 90(2): 720-724 (1993), and Sadelain et al., Curr. Opin. Immunol, 21(2): 215-223 (2009)).

[0006] FLT3 is an Acute Myeloid Leukemia (AML) target antigen, is over-expressed on AML patient blasts when compared to healthy cells, and is expressed on the majority of patient cells (Carow et al, Feb 2, 1996 Blood: 87(3); Birg et al. Nov 1992 Blood: 80(10)). FLT3 is a frequently mutated gene in AML patients, and mutations are associated with poor prognosis (Abu-Duhier et al. Br J Haematol. 2000 Oct;111(1):190-5, Yamamoto et al. April 15, 2001; Blood: 97 (8)). Small-molecule FLT3 inhibitors have shown activity in clinical trials; however, the responses are usually transient due to the acquisition of resistance. Additionally kinase inhibitors treat only a percentage of patients expressing the mutated form of FLT3, highlighting the urgent need for improved therapies.

[0007] Accordingly, there is a need for alternative treatments for cancer and in particular malignancies involving aberrant expression of FLT3. Provided herein are methods and compositions addressing this need.

SUMMARY

[0008] Provided herein are antibodies or antigen binding fragments thereof that specifically bind to Fms-like tyrosine kinase 3 (FLT3), chimeric antigen receptors (CARs) that specifically bind to FLT3, and engineered immune cells expressing such CARs (e.g. FLT3-specific CAR-T cells). Also provided are making such antibodies, CARs and immune cells. Further provided are using such antibodies, CARs and engineered immune cells, for example for the treatment of a condition associated with malignant cells expressing FLT3 (e.g., cancer). In one aspect, provided herein is an isolated antibody which specifically binds to FLT3, wherein the antibody comprises a heavy chain variable (VH) region having any one of the partial VH sequences listed in Table 2 and/or a light chain variable (VL) region having any one of the partial VL sequences listed in Table 2.

[0009] In another aspect, provided herein is an isolated antibody which specifically binds to FLT3, wherein the antibody comprises (a) a heavy chain variable (VH) region comprising complementarity determining region one (CDR1), CDR2, and CDR3 sequences listed in Table 3

and/or (b) a light chain variable (VL) region comprising CDR1, CDR2, and CDR3 sequences listed in Table 3.

[0010] Also provided herein are Chimeric antigen receptors (CARs) that bind to FLT3. It is demonstrated that the expression of FLT3 specific CARs in T cells is effective to activate the T cells upon contact with FLT3. The FLT3 specific CARs provided herein bind human FLT3 and exhibit cytotoxic activity upon contact with FLT3-expressing cells.

[0011] Accordingly, in another aspect, provided herein is a 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) binding to the extracellular domain of FLT3. In some embodiments, provided herein are FLT3 CARs comprising an extracellular ligand-binding domain, a first transmembrane domain, and an intracellular signaling domain, wherein the extracellular domain comprises a scFv binding to particular domains of the FLT3 target, e.g. binding to SEQ ID NO: 199 (Domain 1), SEQ ID NO: 200 (Domain 2), SEQ ID NO: 201 (Domain 3), SEQ ID NO: 254 (Domain 2-3), SEQ ID NO: 202 (Domain 4) or SEQ ID NO: 203 (Domain 5), SEQ ID NO: 254 (Domain 2-3) or SEQ ID NO: 202 (Domain 4).

[0012] In some embodiments, the FLT3 specific CARs comprise an extracellular domain comprising a scFv, wherein the scFv binds to Domain 4 of the FLT3 target (SEQ ID NO: 202).

[0013] In some embodiments, the FLT3 specific CARs comprise an extracellular domain comprising a scFv, wherein the scFv binds to Domain 2 of the FLT3 target (SEQ ID NO: 200).

[0014] The extracellular domain of FLT3 specific CARs comprises a scFv, wherein the scFv comprises a heavy chain variable (VH) region and a light chain variable (VL) region, the VH and VL regions each comprising three complementarity determining regions (CDRs) specific for FLT3.

[0015] In some embodiments, 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 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.

[0016] In some embodiments, provided are FLT3 specific CARs comprising a scFv, wherein the scFv comprises a 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.

[0017] In some embodiments, provided are FLT3 specific CARs comprising a scFv, wherein the scFv comprises a 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.

[0018] In some embodiments, provided are FLT3 specific CARs comprising a scFv comprising a VH region and a VL region, wherein the VH region has 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; and the VL region has the sequence shown in SEQ ID NOs: 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.

[0019] In some embodiments, the VH region comprises 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, or a variant thereof with one or several conservative amino acid substitutions in residues that are not within a CDR and/or the VL region comprises the amino acid 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, or a

variant thereof with one or several amino acid substitutions in amino acids that are not within a CDR.

[0020] In some embodiments, 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 a light chain variable region (VL) comprising the following CDRs: 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.

[0021] In some embodiments, 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.

[0022] In some embodiments, 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 a light chain variable region (VL) comprising the following CDRs: 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.

[0023] In some embodiments, 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.

[0024] In some embodiments, 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 a light chain variable region (VL) comprising the following CDRs: 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.

[0025] In some embodiments, 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.

[0026] In some embodiments, 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 a light chain variable region (VL) comprising the following CDRs: 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.

[0027] In some embodiments, 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.

[0028] In some embodiments, each CDR is defined in accordance with the Kabat definition, the Chothia definition, the combination of the Kabat definition and the Chothia definition, the AbM definition, or the contact definition of CDR.

[0029] In some embodiments, the intracellular signaling domain comprises a CD3 ζ signalling domain. In some embodiments, the intracellular signaling domain comprises a 4-1BB domain. In some embodiments, the FLT3 specific CAR comprises a second intracellular signaling domain. In some embodiments, the second intracellular signaling domain comprises a 4-1BB domain.

[0030] In some embodiments, the FLT3 specific CARs disclosed herein may comprise a stalk domain between the extracellular ligand-binding domain and the first transmembrane domain. In some embodiments, the stalk domain is selected from the group consisting of: a CD8 α hinge, an IgG1 hinge, and an Fc γ RIII α hinge. In some embodiments, the stalk domain is a human CD8 α hinge, a human IgG1 hinge, or a human Fc γ RIII α hinge.

[0031] In some embodiments, the FLT3 specific CARs disclosed herein may comprise one or more epitopes specific for one or more monoclonal antibodies. In some embodiments, the epitope specific for a monoclonal antibody is a CD20 epitope. In some embodiments, the CD20 epitope comprises the amino acid sequence shown in SEQ ID NO: 229 or SEQ ID NO: 230.

[0032] In some embodiments, the FLT3 specific CAR comprises the amino acid sequence shown in SEQ ID NO: 235, 236, 237 or 242. In some embodiments, the FLT3 specific CAR comprises the amino acid sequence shown in SEQ ID NO: 235. In some embodiments, the FLT3 specific CAR comprises the amino acid sequence shown in SEQ ID NO: 236.

[0033] In some embodiments, the first transmembrane domain comprises a CD8 α chain transmembrane domain.

[0034] In some embodiments, the FLT3 specific CAR can comprise another extracellular ligand-binding domain which is not specific for FLT3.

[0035] In some embodiments, the extracellular ligand-binding domain(s), the first transmembrane domain, and intracellular signaling domain(s) are on a single polypeptide.

[0036] In some embodiments, the CAR can comprise 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. In an exemplary embodiment, 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.

[0037] In some embodiments, the CAR can comprise 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.

[0038] In another aspect, provided herein is an isolated polynucleotide comprising a nucleic acid sequence encoding the FLT3 specific CAR described herein.

[0039] In another aspect, provided herein is an expression vector comprising the polynucleotide encoding the FLT3 specific CAR described herein.

[0040] In another aspect, provided herein is an engineered immune cell expressing at its cell surface membrane a FLT3 specific CAR described herein. In some embodiments, the engineered immune cell can comprise another CAR which is not specific for FLT3. In some embodiments, the engineered immune cell can comprise a polynucleotide encoding a suicide polypeptide. In some embodiments, the suicide polypeptide is RQR8.

[0041] In some embodiments, the engineered immune cell is derived from an inflammatory T-lymphocyte, a cytotoxic T-lymphocyte, a regulatory T-lymphocyte, or a helper T-lymphocyte.

[0042] In some embodiments, the engineered immune cell can comprise a disruption 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 such as for example programmed death-1 (PD-1).

[0043] In some embodiments, the engineered immune cell is obtained from a healthy donor. In some embodiments, the engineered immune cell is obtained from an individual afflicted with a disease or disorder.

[0044] In another aspect, provided herein is an engineered immune cell expressing at its cell surface membrane a FLT3 specific CAR as described herein for use as a medicament. In another aspect, provided herein is a FLT3 specific antibody for use as a medicament. In some embodiments, the medicament comprising the FLT3 specific CAR expressing immune cells or FLT3 specific antibodies is for use in treatment of a FLT3 associated disease or disorder. In one embodiment, the FLT3 associated disease or disorder is a cancer of hematopoietic origin, such as a lymphoma or leukemia. In some embodiments, the cancer is 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, Burkitt's lymphoma, marginal zone lymphoma, mantle cell lymphoma, large cell lymphoma, precursor B-lymphoblastic lymphoma, myeloid leukemia, Waldenstrom's macroglobulinemia, diffuse large B cell lymphoma, follicular lymphoma, marginal zone lymphoma, mucosa-associated lymphatic tissue lymphoma, small cell lymphocytic lymphoma, mantle cell 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, intravascular large B-cell lymphoma, 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, e.g. ALL or AML.

[0045] In one aspect, provided herein is a population of cells comprising engineered immune cells expressing FLT specific CARs described herein, wherein (ii) said population of cells comprises a percentage of stem cell memory and central memory cells greater than 20%, 30% or

40%, and/or (iii) said population of cells achieves a percentage lysis of FLT3 expressing cells greater than 10%, 20%, 30% or 40%,.

[0046] In another aspect, provided herein is a method of engineering an immune cell expressing any one of the FLT3 specific CARs described herein, the method comprising: providing an immune cell; and introducing into the cell at least one polynucleotide encoding said FLT3 specific CAR; whereby said immune cell expresses said FLT3 specific CAR.

[0047] In some embodiments, the method comprises providing an immune cell; introducing into the cell at least one polynucleotide encoding said FLT3 specific CAR; and introducing at least one polynucleotide encoding a CAR which is not specific for FLT3.

[0048] In another aspect, provided herein is a method of treating a subject suffering from a FLT3 associated disease or disorder, the method comprising: providing an immune cell expressing at the surface a FLT3 specific CAR as described herein; and administering said immune cells to said subject. The invention also provides methods of treating subjects suffering from a FLT3 associated disease or disorder, the method comprising providing FLT3 specific antibodies described herein and administering said antibodies to said subject.

[0049] In some embodiments, provided herein is a pharmaceutical composition comprising an engineered immune cell expressing FLT3 specific CARs as described herein. In other embodiments, the invention provides pharmaceutical compositions comprising any of the FLT3 specific antibodies described herein.

[0050] In another aspect, provided herein is a method of treating a condition associated with malignant cells expressing FLT3 in a subject comprising administering to a subject in need thereof an effective amount of the pharmaceutical composition comprising an engineered immune cell as described herein or FLT3 specific antibodies as described herein. In some embodiments, the condition is a cancer. In some embodiments, the cancer is a cancer of hematopoietic origin, such as a lymphoma or leukemia. In some embodiments the cancer is 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, Burkitt's lymphoma, marginal zone lymphoma, mantle cell lymphoma, large cell lymphoma, precursor B-lymphoblastic lymphoma, myeloid leukemia,

Waldenstrom's macroglobulinemia, diffuse large B cell lymphoma, follicular lymphoma, marginal zone lymphoma, mucosa-associated lymphatic tissue lymphoma, small cell lymphocytic lymphoma, mantle cell 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, intravascular large B-cell lymphoma, 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, e.g. ALL or AML.

[0051] In another aspect, provided herein is a method of inhibiting tumor growth or progression in a subject who has malignant cells expressing FLT3, comprising administering to the subject in need thereof an effective amount of the pharmaceutical composition comprising an engineered immune cell as described herein or a FLT3 specific antibody as described herein to the subject.

[0052] In another aspect, provided herein is a method of inhibiting metastasis of malignant cells expressing FLT3 in a subject, comprising administering to the subject in need thereof an effective amount of the pharmaceutical composition comprising an engineered immune cell as described herein or a FLT3 specific antibody as described herein to the subject.

[0053] In another aspect, provided herein is a method of inducing tumor regression in a subject who has malignant cells expressing FLT3, comprising administering to the subject in need thereof an effective amount of the pharmaceutical composition comprising an engineered immune cell as described herein or a FLT3 specific antibody as described herein to the subject.

[0054] In some embodiments, any of the above methods further comprises administering one or more additional therapies, such as for example, a monoclonal antibody and/or a chemotherapeutic. In some embodiments, the monoclonal antibody can be, for example, an antibody that binds to a checkpoint inhibitor such as, for example, an anti-PD-1 antibody or an

anti-PD-L1 antibody. In some embodiments, any of the above methods further comprises administering a Receptor Tyrosine Kinase inhibitor, an mTOR inhibitor, an epigenetic modulator, a proteasome inhibitor, an immunomodulatory agent such as lenalidomide, a Hedgehog inhibitor or an Isocitrate Dehydrogenase (IDH) inhibitors, to the subject.

BRIEF DESCRIPTION OF THE DRAWINGS

[0055] FIG. 1 shows representative FACS plots demonstrating efficient transduction of activated T cells with the P3A1 CAR construct relative to a non-transduced control, and the transduction efficiencies for all constructs as percentage of CAR-expressing T cells and mean fluorescence intensities (MFI).

[0056] FIG. 2 shows the expression of activation markers CD25 and 4-1BB in T cells transduced with the various CAR constructs assessed by flow cytometry.

[0057] FIG. 3 shows the phenotype of CAR-T cells at the end of the culture as determined by flow cytometry analysis of the expression of the CD62L and/or CD45RO markers.

[0058] FIG. 4 shows the overall experimental design of the long-term killing assay and the response of the different CAR-T cells to repeated exposure to target cells measured as fold-expansion and cytotoxic activity (percentage of lysis) on day 6.

[0059] FIG. 5 shows the antitumor activity of CAR-T cells expressing the P3A1 or P3E10 construct at two doses using an orthotopic mouse model of human AML. Bioluminescent signals quantified as total flux (photon/sec) are shown at different time points.

[0060] FIG. 6 shows the sensitivity of T cells expressing at cell surface FLT3 specific CAR comprising CD20 epitopes to complement-dependent-cytotoxicity (CDC) induced by the anti-CD20 antibody rituximab.

[0061] FIG. 7 shows the recovery of endogenous bone marrow progenitor cells in mice treated with anti-mouse FLT3 CAR T cells expressing CD20 epitopes and subsequently administered with rituximab.

DETAILED DESCRIPTION

[0062] In one aspect, the invention disclosed herein provides chimeric antigen receptors (CARs) and immune cells comprising CARs (e.g. CAR-T cells) that specifically bind to FLT3 (e.g., human FLT3). The invention also provides polynucleotides encoding these CARs, compositions comprising immune cells expressing these CARs, and methods of making and

using these CARs and CAR expressing immune cells. The invention also provides methods for treating a condition associated with FLT3 mediated pathologies in a subject, such as cancer mediated by malignant cells expressing FLT3, using the FLT3 specific CARs and immune cells expressing these CARs as described herein.

[0063] In one aspect, the invention disclosed herein provides antibodies that specifically bind to FLT3 (e.g., human FLT3). The invention also provides compositions comprising these antibodies and methods of using these antibodies. For example, provided herein are methods for treating a condition associated with FLT3-mediated pathologies in a subject, such as cancer, using these antibodies.

General Techniques

[0064] The practice of the invention will employ, unless otherwise indicated, conventional techniques of molecular biology (including recombinant techniques), microbiology, cell biology, biochemistry and immunology, which are within the skill of the art. Such techniques are explained fully in the literature, such as, Molecular Cloning: A Laboratory Manual, second edition (Sambrook et al., 1989) Cold Spring Harbor Press; Oligonucleotide Synthesis (M.J. Gait, ed., 1984); Methods in Molecular Biology, Humana Press; Cell Biology: A Laboratory Notebook (J.E. Cellis, ed., 1998) Academic Press; Animal Cell Culture (R.I. Freshney, ed., 1987); Introduction to Cell and Tissue Culture (J.P. Mather and P.E. Roberts, 1998) Plenum Press; Cell and Tissue Culture: Laboratory Procedures (A. Doyle, J.B. Griffiths, and D.G. Newell, eds., 1993-1998) J. Wiley and Sons; Methods in Enzymology (Academic Press, Inc.); Handbook of Experimental Immunology (D.M. Weir and C.C. Blackwell, eds.); Gene Transfer Vectors for Mammalian Cells (J.M. Miller and M.P. Calos, eds., 1987); Current Protocols in Molecular Biology (F.M. Ausubel et al., eds., 1987); PCR: The Polymerase Chain Reaction, (Mullis et al., eds., 1994); Current Protocols in Immunology (J.E. Coligan et al., eds., 1991); Short Protocols in Molecular Biology (Wiley and Sons, 1999); Immunobiology (C.A. Janeway and P. Travers, 1997); Antibodies (P. Finch, 1997); Antibodies: a practical approach (D. Catty., ed., IRL Press, 1988-1989); Monoclonal antibodies: a practical approach (P. Shepherd and C. Dean, eds., Oxford University Press, 2000); Using antibodies: a laboratory manual (E. Harlow and D. Lane (Cold Spring Harbor Laboratory Press, 1999); The Antibodies (M. Zanetti and J.D. Capra, eds., Harwood Academic Publishers, 1995).

Definitions

[0065] The term “extracellular ligand-binding domain” as used herein refers to a polypeptide that is capable of binding a ligand or capable of interacting with a cell surface molecule. For example, the extracellular ligand-binding domain may be chosen to recognize a ligand that acts as a cell surface marker on target cells associated with a particular disease state.

[0066] The term “stalk domain” or “hinge domain” are used interchangeably herein to refer to any polypeptide that functions to link the transmembrane domain to the extracellular ligand-binding domain. In particular, stalk domains are used to provide more flexibility and accessibility for the extracellular ligand-binding domain.

[0067] The term “intracellular signaling domain” refers to the portion of a protein which transduces the effector signal function signal and directs the cell to perform a specialized function.

[0068] A “co-stimulatory molecule” as used herein refers to the cognate binding partner on immune cells, e.g. T cells, that specifically binds with a co-stimulatory ligand, thereby mediating a co-stimulatory response by the cell, such as, but not limited to proliferation. Co-stimulatory molecules include, but are not limited to an MHC class I molecule, BTLA and Toll ligand receptor. Examples of costimulatory molecules include CD27, CD28, CD8, 4-1BB (CD137), OX40, CD30, CD40, PD-1, ICOS, lymphocyte function-associated antigen-1 (LFA-1), CD2, CD7, LIGHT, NKG2C, B7-H3 and a ligand that specifically binds with CD83 and the like.

[0069] A “co-stimulatory ligand” refers to a molecule on an antigen presenting cell that specifically binds a cognate co-stimulatory signal molecule on a T cell, thereby providing a signal which, in addition to the primary signal provided by, for instance, binding of a TCR/CD3 complex with an MHC molecule loaded with peptide, mediates a T cell response, including, but not limited to, proliferation activation, differentiation and the like. A co-stimulatory ligand can include but is not limited to CD7, B7-1 (CD80), B7-2 (CD86), PD-L1, PD-L2, 4-1BBL, OX40L, inducible costimulatory ligand (ICOS-L), intercellular adhesion molecule (ICAM, CD30L, CD40, CD70, CD83, HLA-G, MICA, M1CB, HVEM, lymphotoxin β receptor, 3/TR6, ILT3, ILT4, an agonist or antibody that binds Toll ligand receptor and a ligand that specifically binds with B7-H3. A co-stimulatory ligand also encompasses, inter alia, an antibody that specifically binds with a co-stimulatory molecule present on a T cell, such as but not limited to, CD27, CD28, 4-1BB, OX40, CD30, CD40, PD-1, ICOS, lymphocyte function-associated antigen-1 (LFA-1), CD2, CD7, LTGHT, NKG2C, B7-H3, a ligand that specifically binds with CD83.

[0070] An “antibody” is an immunoglobulin molecule capable of specific binding to a target, such as a carbohydrate, polynucleotide, lipid, polypeptide, etc., through at least one antigen recognition site, located in the variable region of the immunoglobulin molecule. As used herein, the term encompasses not only intact polyclonal or monoclonal antibodies, but also fragments thereof (such as Fab, Fab', F(ab')₂, Fv), single chain (scFv) and domain antibodies (including, for example, shark and camelid antibodies), and fusion proteins comprising an antibody, and any other modified configuration of the immunoglobulin molecule that comprises an antigen recognition site. An antibody includes an antibody of any class, such as IgG, IgA, IgE, IgD, or IgM (or sub-class thereof), and the antibody need not be of any particular class. Depending on the antibody amino acid sequence of the constant region of its heavy chains, immunoglobulins can be assigned to different classes. There are five major classes of immunoglobulins: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into subclasses (isotypes), e.g., IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2. The heavy-chain constant regions that correspond to the different classes of immunoglobulins are called alpha, delta, epsilon, gamma, and mu, respectively. The subunit structures and three-dimensional configurations of different classes of immunoglobulins are well known.

[0071] The term “antigen binding fragment” or “antigen binding portion” of an antibody, as used herein, refers to one or more fragments of an intact antibody that retain the ability to specifically bind to a given antigen (e.g., FLT3). Antigen binding functions of an antibody can be performed by fragments of an intact antibody. Examples of binding fragments encompassed within the term “antigen binding fragment” of an antibody include Fab; Fab'; F(ab')₂; an Fd fragment consisting of the VH and CH1 domains; an Fv fragment consisting of the VL and VH domains of a single arm of an antibody; a single domain antibody (dAb) fragment (Ward et al., Nature 341:544-546, 1989), and an isolated complementarity determining region (CDR).

[0072] An antibody, an antigen binding fragment, an antibody conjugate, or a polypeptide that “specifically binds” to a target (e.g., FLT3 protein) is a term well understood in the art, and methods to determine such specific binding are also well known in the art. A molecule is said to exhibit “specific binding” if it reacts or associates more frequently, more rapidly, with greater duration and/or with greater affinity with a particular cell or substance than it does with alternative cells or substances. An antibody “specifically binds” to a target if it binds with greater affinity, avidity, more readily, and/or with greater duration than it binds to other substances. For example, an antibody that specifically binds to a FLT3 epitope is an antibody

that binds this epitope with greater affinity, avidity, more readily, and/or with greater duration than it binds to other FLT3 epitopes or non-FLT3 epitopes. It is also understood that by reading this definition, for example, an antibody (or moiety or epitope) that specifically binds to a first target may or may not specifically bind to a second target. As such, “specific binding” does not necessarily require (although it can include) exclusive binding. Generally, but not necessarily, reference to binding means specific binding.

[0073] A “variable region” of an antibody refers to the variable region of the antibody light chain or the variable region of the antibody heavy chain, either alone or in combination. As known in the art, the variable regions of the heavy and light chain each consist of four framework regions (FR) connected by three complementarity determining regions (CDRs) also known as hypervariable regions. The CDRs in each chain are held together in close proximity by the FRs and, with the CDRs from the other chain, contribute to the formation of the antigen binding site of antibodies. There are at least two techniques for determining CDRs: (1) an approach based on cross-species sequence variability (i.e., Kabat et al. Sequences of Proteins of Immunological Interest, (5th ed., 1991, National Institutes of Health, Bethesda MD)); and (2) an approach based on crystallographic studies of antigen-antibody complexes (Al-lazikani et al., 1997, J. Molec. Biol. 273:927-948). As used herein, a CDR may refer to CDRs defined by either approach or by a combination of both approaches.

[0074] A “CDR” of a variable domain are amino acid residues within the variable region that are identified in accordance with the definitions of the Kabat, Chothia, the accumulation of both Kabat and Chothia, AbM, contact, and/or conformational definitions or any method of CDR determination well known in the art. Antibody CDRs may be identified as the hypervariable regions originally defined by Kabat et al. See, e.g., Kabat et al., 1992, Sequences of Proteins of Immunological Interest, 5th ed., Public Health Service, NIH, Washington D.C. The positions of the CDRs may also be identified as the structural loop structures originally described by Chothia and others. See, e.g., Chothia et al., Nature 342:877-883, 1989. Other approaches to CDR identification include the “AbM definition,” which is a compromise between Kabat and Chothia and is derived using Oxford Molecular’s AbM antibody modeling software (now Accelrys®), or the “contact definition” of CDRs based on observed antigen contacts, set forth in MacCallum et al., J. Mol. Biol., 262:732-745, 1996. In another approach, referred to herein as the “conformational definition” of CDRs, the positions of the CDRs may be identified as the residues that make enthalpic contributions to antigen binding. See, e.g., Makabe et al., Journal

of Biological Chemistry, 283:1156-1166, 2008. Still other CDR boundary definitions may not strictly follow one of the above approaches, but will nonetheless overlap with at least a portion of the Kabat CDRs, although they may be shortened or lengthened in light of prediction or experimental findings that particular residues or groups of residues or even entire CDRs do not significantly impact antigen binding. As used herein, a CDR may refer to CDRs defined by any approach known in the art, including combinations of approaches. The methods used herein may utilize CDRs defined according to any of these approaches. For any given embodiment containing more than one CDR, the CDRs may be defined in accordance with any of Kabat, Chothia, extended, AbM, contact, and/or conformational definitions.

[0075] As used herein, "monoclonal antibody" refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical except for possible naturally-occurring mutations that may be present in minor amounts. Monoclonal antibodies are highly specific, being directed against a single antigenic site. Furthermore, in contrast to polyclonal antibody preparations, which typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody is directed against a single determinant on the antigen. The modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the invention may be made by the hybridoma method first described by Kohler and Milstein, *Nature* 256:495, 1975, or may be made by recombinant DNA methods such as described in U.S. Pat. No. 4,816,567. The monoclonal antibodies may also be isolated from phage libraries generated using the techniques described in McCafferty et al., *Nature* 348:552-554, 1990, for example.

[0076] As used herein, "humanized" antibody refers to forms of non-human (e.g. murine) antibodies that are chimeric immunoglobulins, immunoglobulin chains, or fragments thereof (such as Fv, Fab, Fab', F(ab')₂ or other antigen binding subsequences of antibodies) that contain minimal sequence derived from non-human immunoglobulin. In one aspect, humanized antibodies are human immunoglobulins (recipient antibody) in which residues from a complementarity determining region (CDR) of the recipient are replaced by residues from a CDR of a non-human species (donor antibody) such as mouse, rat, or rabbit having the desired specificity, affinity, and capacity. In some instances, Fv framework region (FR) residues of the

human immunoglobulin are replaced by corresponding non-human residues. Furthermore, the humanized antibody may comprise residues that are found neither in the recipient antibody nor in the imported CDR or framework sequences, but are included to further refine and optimize antibody performance. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin and all or substantially all of the FR regions are those of a human immunoglobulin consensus sequence. The humanized antibody optimally also will comprise at least a portion of an immunoglobulin constant region or domain (Fc), typically that of a human immunoglobulin. Preferred are antibodies having Fc regions modified as described in WO 99/58572. Other forms of humanized antibodies have one or more CDRs (CDR L1, CDR L2, CDR L3, CDR H1, CDR H2, or CDR H3) which are altered with respect to the original antibody, which are also termed one or more CDRs “derived from” one or more CDRs from the original antibody.

[0077] As used herein, “human antibody” means an antibody having an amino acid sequence corresponding to that of an antibody produced by a human and/or which has been made using any of the techniques for making human antibodies known to those skilled in the art or disclosed herein. This definition of a human antibody includes antibodies comprising at least one human heavy chain polypeptide or at least one human light chain polypeptide. One such example is an antibody comprising murine light chain and human heavy chain polypeptides. Human antibodies can be produced using various techniques known in the art. In one embodiment, the human antibody is selected from a phage library, where that phage library expresses human antibodies (Vaughan et al., *Nature Biotechnology*, 14:309-314, 1996; Sheets et al., *Proc. Natl. Acad. Sci. (USA)* 95:6157-6162, 1998; Hoogenboom and Winter, *J. Mol. Biol.*, 227:381, 1991; Marks et al., *J. Mol. Biol.*, 222:581, 1991). Human antibodies can also be made by immunization of animals into which human immunoglobulin loci have been transgenically introduced in place of the endogenous loci, e.g., mice in which the endogenous immunoglobulin genes have been partially or completely inactivated. This approach is described in U.S. Pat. Nos. 5,545,807; 5,545,806; 5,569,825; 5,625,126; 5,633,425; and 5,661,016. Alternatively, the human antibody may be prepared by immortalizing human B lymphocytes that produce an antibody directed against a target antigen (such B lymphocytes may be recovered from an individual or from single cell cloning of the cDNA, or may have been immunized in vitro). See,

e.g., Cole et al. *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, p. 77, 1985; Boerner et al., *J. Immunol.*, 147 (1):86-95, 1991; and U.S. Pat. No. 5,750,373.

[0078] The term “chimeric antibody” is intended to refer to antibodies in which the variable region sequences are derived from one species and the constant region sequences are derived from another species, such as an antibody in which the variable region sequences are derived from a mouse antibody and the constant region sequences are derived from a human antibody.

[0079] A “monovalent antibody” comprises one antigen binding site per molecule (e.g., IgG or Fab). In some instances, a monovalent antibody can have more than one antigen binding sites, but the binding sites are from different antigens.

[0080] A “bivalent antibody” comprises two antigen binding sites per molecule (e.g., IgG). In some instances, the two binding sites have the same antigen specificities. However, bivalent antibodies may be bispecific.

[0081] Antibodies of the invention can be produced using techniques well known in the art, e.g., recombinant technologies, phage display technologies, synthetic technologies or combinations of such technologies or other technologies readily known in the art (see, for example, Jayasena, S.D., *Clin. Chem.*, 45: 1628-50, 1999 and Fellouse, F.A., et al, *J. Mol. Biol.*, 373(4):924-40, 2007).

[0082] As known in the art, “polynucleotide,” or “nucleic acid,” as used interchangeably herein, refer to chains of nucleotides of any length, and include DNA and RNA. The nucleotides can be deoxyribonucleotides, ribonucleotides, modified nucleotides or bases, and/or their analogs, or any substrate that can be incorporated into a chain by DNA or RNA polymerase. A polynucleotide may comprise modified nucleotides, such as methylated nucleotides and their analogs. If present, modification to the nucleotide structure may be imparted before or after assembly of the chain. The sequence of nucleotides may be interrupted by non-nucleotide components. A polynucleotide may be further modified after polymerization, such as by conjugation with a labeling component. Other types of modifications include, for example, “caps”, substitution of one or more of the naturally occurring nucleotides with an analog, internucleotide modifications such as, for example, those with uncharged linkages (e.g., methyl phosphonates, phosphotriesters, phosphoamidates, carbamates, etc.) and with charged linkages (e.g., phosphorothioates, phosphorodithioates, etc.), those containing pendant moieties, such as, for example, proteins (e.g., nucleases, toxins, antibodies, signal peptides, poly-L-lysine, etc.), those with intercalators (e.g., acridine, psoralen, etc.), those containing chelators (e.g., metals,

radioactive metals, boron, oxidative metals, etc.), those containing alkylators, those with modified linkages (e.g., alpha anomeric nucleic acids, etc.), as well as unmodified forms of the polynucleotide(s). Further, any of the hydroxyl groups ordinarily present in the sugars may be replaced, for example, by phosphonate groups, phosphate groups, protected by standard protecting groups, or activated to prepare additional linkages to additional nucleotides, or may be conjugated to solid supports. The 5' and 3' terminal OH can be phosphorylated or substituted with amines or organic capping group moieties of from 1 to 20 carbon atoms. Other hydroxyls may also be derivatized to standard protecting groups. Polynucleotides can also contain analogous forms of ribose or deoxyribose sugars that are generally known in the art, including, for example, 2'-O-methyl-, 2'-O-allyl, 2'-fluoro- or 2'-azido-ribose, carbocyclic sugar analogs, alpha- or beta-anomeric sugars, epimeric sugars such as arabinose, xyloses or lyxoses, pyranose sugars, furanose sugars, sedoheptuloses, acyclic analogs and abasic nucleoside analogs such as methyl riboside. One or more phosphodiester linkages may be replaced by alternative linking groups. These alternative linking groups include, but are not limited to, embodiments wherein phosphate is replaced by P(O)S("thioate"), P(S)S("dithioate"), (O)NR₂("amidate"), P(O)R, P(O)OR', CO or CH₂("formacetal"), in which each R or R' is independently H or substituted or unsubstituted alkyl (1-20 C) optionally containing an ether (-O-) linkage, aryl, alkenyl, cycloalkyl, cycloalkenyl or araldyl. Not all linkages in a polynucleotide need be identical. The preceding description applies to all polynucleotides referred to herein, including RNA and DNA.

[0083] As known in the art, a "constant region" of an antibody refers to the constant region of the antibody light chain or the constant region of the antibody heavy chain, either alone or in combination.

[0084] As used herein, "substantially pure" refers to material which is at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 97%, at least 98%, or even at least 99% pure (i.e., free from contaminants).

[0085] A "host cell" includes an individual cell or cell culture that can be or has been a recipient for vector(s) for incorporation of polynucleotide inserts. Host cells include progeny of a single host cell, and the progeny may not necessarily be completely identical (in morphology or in genomic DNA complement) to the original parent cell due to natural, accidental, or deliberate mutation. A host cell includes cells transfected in vivo with a polynucleotide(s) of this invention.

[0086] As known in the art, the term “Fc region” is used to define a C-terminal region of an immunoglobulin heavy chain. The “Fc region” may be a native sequence Fc region or a variant Fc region. Although the boundaries of the Fc region of an immunoglobulin heavy chain might vary, the human IgG heavy chain Fc region is usually defined to stretch from an amino acid residue at position Cys226, or from Pro230, to the carboxyl-terminus thereof. The numbering of the residues in the Fc region is that of the EU index as in Kabat. Kabat et al., *Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md., 1991. The Fc region of an immunoglobulin generally comprises two constant regions, CH2 and CH3.

[0087] As used in the art, “Fc receptor” and “FcR” describe a receptor that binds to the Fc region of an antibody. The preferred FcR is a native sequence human FcR. Moreover, a preferred FcR is one which binds an IgG antibody (a gamma receptor) and includes receptors of the FcγRI, FcγRII, and FcγRIII subclasses, including allelic variants and alternatively spliced forms of these receptors. FcγRII receptors include FcγRIIA (an “activating receptor”) and FcγRIIB (an “inhibiting receptor”), which have similar amino acid sequences that differ primarily in the cytoplasmic domains thereof. FcRs are reviewed in Ravetch and Kinet, *Ann. Rev. Immunol.*, 9:457-92, 1991; Capel et al., *Immunomethods*, 4:25-34, 1994; and de Haas et al., *J. Lab. Clin. Med.*, 126:330-41, 1995. “FcR” also includes the neonatal receptor, FcRn, which is responsible for the transfer of maternal IgGs to the fetus (Guyer et al., *J. Immunol.*, 117:587, 1976; and Kim et al., *J. Immunol.*, 24:249, 1994).

[0088] The term “compete”, as used herein with regard to an antibody, means that a first antibody, or an antigen binding fragment (or portion) thereof, binds to an epitope in a manner sufficiently similar to the binding of a second antibody, or an antigen binding portion thereof, such that the result of binding of the first antibody with its cognate epitope is detectably decreased in the presence of the second antibody compared to the binding of the first antibody in the absence of the second antibody. The alternative, where the binding of the second antibody to its epitope is also detectably decreased in the presence of the first antibody, can, but need not be the case. That is, a first antibody can inhibit the binding of a second antibody to its epitope without that second antibody inhibiting the binding of the first antibody to its respective epitope. However, where each antibody detectably inhibits the binding of the other antibody with its cognate epitope or ligand, whether to the same, greater, or lesser extent, the antibodies are said to “cross-compete” with each other for binding of their respective epitope(s). Both

competing and cross-competing antibodies are encompassed by the invention. Regardless of the mechanism by which such competition or cross-competition occurs (e.g., steric hindrance, conformational change, or binding to a common epitope, or portion thereof), the skilled artisan would appreciate, based upon the teachings provided herein, that such competing and/or cross-competing antibodies are encompassed and can be useful for the methods disclosed herein.

[0089] As used herein “autologous” means that cells, a cell line, or population of cells used for treating patients are originating from said patient.

[0090] As used herein “allogeneic” means that cells or population of cells used for treating patients are not originating from said patient but from a donor.

[0091] As used herein, “treatment” is an approach for obtaining beneficial or desired clinical results. For purposes of this invention, beneficial or desired clinical results include, but are not limited to, one or more of the following: reducing the proliferation of (or destroying) neoplastic or cancerous cells, inhibiting metastasis of neoplastic cells, shrinking or decreasing the size of FLT3 expressing tumor, remission of a FLT3 associated disease (e.g., cancer), decreasing symptoms resulting from a FLT3 associated disease (e.g., cancer), increasing the quality of life of those suffering from a FLT3 associated disease (e.g., cancer), decreasing the dose of other medications required to treat a FLT3 associated disease (e.g., cancer), delaying the progression of a FLT3 associated disease (e.g., cancer), curing a FLT3 associated disease (e.g., cancer), and/or prolong survival of patients having a FLT3 associated disease (e.g., cancer).

[0092] “Ameliorating” means a lessening or improvement of one or more symptoms as compared to not administering a FLT3 specific CAR or a FLT3 specific CAR-T-cell.

“Ameliorating” also includes shortening or reduction in duration of a symptom.

[0093] As used herein, an “effective dosage” or “effective amount” of drug, compound, or pharmaceutical composition is an amount sufficient to effect any one or more beneficial or desired results. For prophylactic use, beneficial or desired results include eliminating or reducing the risk, lessening the severity, or delaying the onset of the disease, including biochemical, histological and/or behavioral symptoms of the disease, its complications and intermediate pathological phenotypes presenting during development of the disease. For therapeutic use, beneficial or desired results include clinical results such as reducing incidence or amelioration of one or more symptoms of various FLT3 associated diseases or conditions (such as for example multiple myeloma), decreasing the dose of other medications required to treat the disease, enhancing the effect of another medication, and/or delaying the progression of

the FLT3 associated disease of patients. An effective dosage can be administered in one or more administrations. For purposes of this invention, an effective dosage of drug, compound, or pharmaceutical composition is an amount sufficient to accomplish prophylactic or therapeutic treatment either directly or indirectly. As is understood in the clinical context, an effective dosage of a drug, compound, or pharmaceutical composition may or may not be achieved in conjunction with another drug, compound, or pharmaceutical composition. Thus, an “effective dosage” may be considered in the context of administering one or more therapeutic agents, and a single agent may be considered to be given in an effective amount if, in conjunction with one or more other agents, a desirable result may be or is achieved.

[0094] An “individual”, “patient” or a “subject” are used interchangeably herein and is a mammal. Mammals include, but are not limited to, humans, monkeys, pigs, other farm animals, sport animals, pets, primates, horses, dogs, cats, rodents including mice, rats, guinea pigs, etc. An subject is a mammal and are used interchangeably herein. In some embodiments, the subject is a human. In some embodiments, the subject is a non-human primate. In some embodiments, the subject is a human or a monkey, e.g. a cynomolgus monkey.

[0095] As used herein, “vector” means a construct, which is capable of delivering, and, in some embodiments, expressing, one or more gene(s) or sequence(s) of interest in a host cell. Examples of vectors include, but are not limited to, viral vectors, naked DNA or RNA expression vectors, plasmid, cosmid or phage vectors, DNA or RNA expression vectors associated with cationic condensing agents, DNA or RNA expression vectors encapsulated in liposomes, and certain eukaryotic cells, such as producer cells.

[0096] As used herein, “expression control sequence” means a nucleic acid sequence that directs transcription of a nucleic acid. An expression control sequence can be a promoter, such as a constitutive or an inducible promoter, or an enhancer. The expression control sequence is operably linked to the nucleic acid sequence to be transcribed.

[0097] As used herein, “pharmaceutically acceptable carrier” or “pharmaceutical acceptable excipient” includes any material which, when combined with an active ingredient, allows the ingredient to retain biological activity and is non-reactive with the subject’s immune system. Examples include, but are not limited to, any of the standard pharmaceutical carriers such as a phosphate buffered saline solution, water, emulsions such as oil/water emulsion, and various types of wetting agents. Exemplary diluents for aerosol or parenteral administration include phosphate buffered saline (PBS) or normal (0.9%) saline. Compositions comprising such

carriers are formulated by well known conventional methods (see, for example, Remington's Pharmaceutical Sciences, 18th edition, A. Gennaro, ed., Mack Publishing Co., Easton, PA, 1990; and Remington, The Science and Practice of Pharmacy 21st Ed. Mack Publishing, 2005).

[0098] The term " k_{on} ", as used herein, refers to the rate constant for association of an antibody or scFv of a CAR to an antigen.

[0099] The term " k_{off} ", as used herein, refers to the rate constant for dissociation of an antibody or scFv of a CAR from the antibody/antigen complex.

[00100] The term " K_D ", as used herein, refers to the equilibrium dissociation constant of an antibody-antigen interaction or an scFv-antigen interaction.

[00101] Reference to "about" a value or parameter herein includes (and describes) embodiments that are directed to that value or parameter per se. For example, description referring to "about X" includes description of "X." Numeric ranges are inclusive of the numbers defining the range.

[00102] It is understood that wherever embodiments are described herein with the language "comprising," otherwise analogous embodiments described in terms of "consisting of" and/or "consisting essentially of" are also provided.

[00103] Where aspects or embodiments of the invention are described in terms of a Markush group or other grouping of alternatives, the invention encompasses not only the entire group listed as a whole, but each member of the group individually and all possible subgroups of the main group, but also the main group absent one or more of the group members. The invention also envisages the explicit exclusion of one or more of any of the group members in the claimed invention.

[00104] Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. In case of conflict, the present specification, including definitions, will control. Throughout this specification and claims, the word "comprise," or variations such as "comprises" or "comprising" will be understood to imply the inclusion of a stated integer or group of integers but not the exclusion of any other integer or group of integers. Unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular.

[00105] Exemplary methods and materials are described herein, although methods and materials similar or equivalent to those described herein can also be used in the practice or

testing of the invention. The materials, methods, and examples are illustrative only and not intended to be limiting.

FLT3 Antibodies

[00106] The present disclosure provides antibodies that bind to FLT3 [e.g., human FLT3 (e.g., accession number: NP_004110 or SEQ ID NO: 198)] and characterized by any one or more of the following characteristics: (a) treat, prevent, ameliorate one or more symptoms of a condition associated with malignant cells expressing FLT3 in a subject (e.g., cancer such as AML); (b) inhibit tumor growth or progression in a subject (who has a malignant tumor expressing FLT3); (c) inhibit metastasis of cancer (malignant) cells expressing FLT3 in a subject (who has one or more malignant cells expressing FLT3); (d) induce regression (e.g., long-term regression) of a tumor expressing FLT3; (e) exert cytotoxic activity in malignant cells expressing FLT3; (f) block FLT3 interaction with other yet to be identified factors; and/or (g) induce bystander effect that kill or inhibit growth of non-FLT3 expressing malignant cells in the vicinity.

[00107] In one aspect, provided is an isolated antibody which specifically binds to FLT3, wherein the antibody comprises (a) a heavy chain variable (VH) region comprising (i) a VH complementarity determining region one (CDR1) comprising the sequence shown in 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, 246, or 247; (ii) a VH CDR2 comprising the 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, 248, 249, 251, 252, 253, or 255; and (iii) a VH CDR3 comprising the sequence shown in SEQ ID NO: 42, 48, 53, 59, 65, 71, 77, 83, 89, 95, 101, 107, 113, 119, 125, 131, 137, 143, 245, 250, or 254; and/or a light chain variable (VL) region comprising (i) a VL CDR1 comprising the sequence shown in SEQ ID NO: 144, 147, 150, 153, 156, 159, 162, 165, 168, 171, 174, 177, 180, 183, 186, 189, 192, 195, 257, 261, 263, 265, 268, 270, 273, or 275; (ii) a VL CDR2 comprising the sequence shown in SEQ ID NO: 145, 148, 151, 154, 157, 160, 163, 166, 169, 172, 175, 178, 181, 184, 187, 190, 193, 196, 259, 266, or 271; and (iii) a VL CDR3 comprising the sequence shown in SEQ ID NO: 146, 149, 152, 155, 158, 161, 164, 167, 170, 173, 176, 179, 182, 185, 188, 191, 194, 197, 256, 258, 260, 262, 264, 267, 269, 272, or 274.

[00108] In another aspect, provided is an isolated antibody which specifically binds to FLT3, wherein the antibody comprises: a VH region comprising 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; and/or a VL region comprising 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.

[0100] In some embodiments, provided is an isolated antibody which specifically binds to FLT3, wherein the antibody comprises: a VH region comprising the sequence shown in SEQ ID NO: 275, 289, or 291; and/or a VL region comprising the sequence shown in SEQ ID NO: 274, 288, or 290.

[0101] In some embodiments, provided is an antibody having any one of partial light chain sequence as listed in Table 2 and/or any one of partial heavy chain sequence as listed in Table 2.

[0102] In some embodiments, the antibodies described herein comprise a constant region. In some embodiments, the antibodies described herein are of the human IgG1, IgG2 or IgG2Aa, IgG3, or IgG4 subclass. In some embodiments, the antibodies described herein comprise a glycosylated constant region. In some embodiments, the antibodies described herein comprise a constant region having decreased binding affinity to one or more human Fc gamma receptor(s).

[0103] The antibodies provided herein can encompass monoclonal antibodies, polyclonal antibodies, antibody fragments (e.g., Fab, Fab', F(ab')₂, Fv, Fc, etc.), chimeric antibodies, single chain (ScFv), and/or humanized antibodies. The antibodies may be murine, rat, human, or any other origin (including chimeric or humanized antibodies).

FLT3 specific CARs and Methods of Making Thereof

[0104] Provided herein are CARs that bind to FLT3 (e.g., human FLT3 (e.g., SEQ ID NO: 198) or accession number: NP_004110). FLT3 specific CARs provided herein include single chain CARs and multichain CARs. The CARs have the ability to redirect T cell specificity and reactivity toward FLT3 in a non-MHC-restricted manner, exploiting the antigen-binding properties of monoclonal antibodies. The non-MHC-restricted antigen recognition gives T cells expressing CARs the ability to recognize an antigen independent of antigen processing, thus bypassing a major mechanism of tumor escape.

[0105] In some embodiments, CARs provided herein comprise an extracellular ligand-binding domain (e.g., a single chain variable fragment (scFv)), a transmembrane domain, and an intracellular signaling domain. In some embodiments, the extracellular ligand-binding domain, transmembrane domain, and intracellular signaling domain are in one polypeptide, i.e., in a single chain. Multichain CARs and polypeptides are also provided herein. In some

embodiments, the multichain CARs comprise: a first polypeptide comprising a transmembrane domain and at least one extracellular ligand-binding domain, and a second polypeptide comprising a transmembrane domain and at least one intracellular signaling domain, wherein the polypeptides assemble together to form a multichain CAR.

[0106] In some embodiments, a FLT3 specific multichain CAR is based on the high affinity receptor for IgE (FcεRI). The FcεRI expressed on mast cells and basophiles triggers allergic reactions. FcεRI is a tetrameric complex composed of a single α subunit, a single β subunit, and two disulfide-linked γ subunits. The α subunit contains the IgE-binding domain. The β and γ subunits contain ITAMs that mediate signal transduction. In some embodiments, the extracellular domain of the FcRα chain is deleted and replaced by a FLT3 specific extracellular ligand-binding domain. In some embodiments, the multichain FLT3 specific CAR comprises an scFv that binds specifically to FLT3, the CD8α hinge, and the ITAM of the FcRβ chain. In some embodiments, the CAR may or may not comprise the FcRγ chain.

[0107] In some embodiments, the extracellular ligand-binding domain comprises an scFv comprising the light chain variable (VL) region and the heavy chain variable (VH) region of a target antigen specific monoclonal antibody joined by a flexible linker. Single chain variable region fragments are made by linking light and/or heavy chain variable regions by using a short linking peptide (Bird et al., Science 242:423-426, 1988). An example of a linking peptide is the GS linker having the amino acid sequence (GGGGS)₃ (SEQ ID NO: 232), which bridges approximately 3.5 nm between the carboxy terminus of one variable region and the amino terminus of the other variable region. Linkers of other sequences have been designed and used (Bird et al., 1988, supra). In general, linkers can be short, flexible polypeptides, e.g. comprised of about 20 or fewer amino acid residues. Linkers can in turn be modified for additional functions, such as attachment of drugs or attachment to solid supports. The single chain variants can be produced either recombinantly or synthetically. For synthetic production of scFv, an automated synthesizer can be used. For recombinant production of scFv, a suitable plasmid containing polynucleotide that encodes the scFv can be introduced into a suitable host cell, either eukaryotic, such as yeast, plant, insect or mammalian cells, or prokaryotic, such as E. coli. Polynucleotides encoding the scFv of interest can be made by routine manipulations such as ligation of polynucleotides. The resultant scFv can be isolated using standard protein purification techniques known in the art.

[0108] The FLT3 extracellular domain is composed of 5 immunoglobulin domains, with Domain 5 being closest in proximity to the cell membrane, and domain 1 being furthest out (in linear fashion; Verstraete, July 7, 2011; Blood: 118 (1)). The sequences of the five FLT3 extracellular domains are listed in Table 1 below.

Table 1

FLT3 extracellular domain No.	Sequence
1	NQDLPIKCVLINHKNNSSVGKSSSYPMVSESPEDLGALRPQSSGTVY EAAAVEVDVSASITLQVLVDAPGNISCLWVFKHSSLNCQPHFDLQNRGV VSMVILKMTETQA GEYLLFIQSEATNYTILFTVSIR (SEQ ID NO: 199)
2	NTLLYLRRPYFRKMENQDALVCISESVPEPIVEWVLCDSQGESCKEESPA VVKKEEKVLHELFGTDIRCCARNELGRECTRL (SEQ ID NO: 200)
3	FTIDLNQTPQTTLPLQLFLKVGELWIRCKAVHVNHGFGTLWELENKALEE GNYFEMSTYSTNRTMIRILFAFVSSVARNDTGYTCCSSSKHPSQSALVTIV E (SEQ ID NO: 201)
4	KGFINATNSSDYEDIDQYEEFCFSVRFKAYPQIRCTWTFSRKSPCEQKGL DNGYSISKFCNHHKHQPGYIFHAENDDAQFTKMFTLN (SEQ ID NO: 202)
5	IRRKPVLAELASASQASCFSDGYPLPSWTWKKCSDKSPNCTEEITEGVWN RKANRKVFGQWVSSSTLNMSEAIKGFVKCCAYNSLGTSCETILLNSPGP FPFIQDN (SEQ ID NO: 203)

[0109] Domain 2-3 comprises the sequence of Domain 2 and Domain 3 (SEQ ID NO: 254).

[0110] In some embodiments, the extracellular ligand-binding domain of FLT3 specific CARs described herein binds to Domain 1, 2, 3, 4 or 5 of the FLT3 extracellular domain.

[0111] In some embodiments, the extracellular ligand-binding domain of FLT3 specific CARs described herein binds to Domain 1 of the FLT3 extracellular domain.

[0112] In some embodiments, the extracellular ligand-binding domain of FLT3 specific CARs described herein binds to Domain 2 of the FLT3 extracellular domain.

[0113] In some embodiments, the extracellular ligand-binding domain of FLT3 specific CARs described herein binds to Domain 3 of the FLT3 extracellular domain.

[0114] In some embodiments, the extracellular ligand-binding domain of FLT3 specific CARs described herein binds to Domain 4 of the FLT3 extracellular domain.

[0115] In some embodiments, the extracellular ligand-binding domain of FLT3 specific CARs described herein binds to Domain 5 of the FLT3 extracellular domain.

[0116] In some embodiments, the extracellular ligand-binding domain of FLT3 specific CARs described herein binds to Domain 2-3 of the FLT3 extracellular domain.

[0117] In some embodiments, the extracellular ligand-binding domain of FLT3 specific CARs described herein binds to Domain 2-3 or 4 of the FLT3 extracellular domain.

[0118] In another aspect, provided is a CAR, which specifically binds to FLT3, wherein the CAR comprises an extracellular ligand-binding domain comprising: a 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; and/or a 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. In some embodiments, the VH and VL are linked together by a flexible linker. In some embodiments, a flexible linker comprises the amino acid sequence shown in SEQ ID NO: 232.

[0119] In another aspect, provided is a 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 Fv fragment (scFv) comprising a heavy chain variable (VH) region and a light chain variable (VL) region, wherein 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.

[0120] In another aspect, provided is a 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 Fv fragment (scFv) comprising a heavy chain variable (VH) region and a light chain variable (VL) region, wherein 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.

In another aspect, provided is a 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 Fv 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 (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.

[0121] In some embodiments, a CAR of the invention comprises an extracellular ligand-binding domain having any one of partial light chain sequence as listed in Table 2 and/or any one of partial heavy chain sequence as listed in Table 2.

[0122] In Table 2, the underlined sequences are CDR sequences according to Kabat and in bold according to Chothia, except for the heavy chain CDR2 sequences of P4F6, P4C7, P3A1, P5A3, P9B5, P9F1, P1B4, P1B11, P7H3, P3E10, P1A5, P5F7, P4H11, P15F7, P12B6, P8B6, P14G2, and P7F9, in which the Chothia CDR sequence is underlined and the Kabat CDR sequence is in bold.

Table 2

mAb	Light Chain	Heavy Chain
P4F6	EIVLTQSPGTLSSLSPGERATLSC <u>RASH</u> <u>SVSSYL</u> AWYQQKPGQAPRLLIY <u>GAS</u> <u>SRAT</u> GIPDRFSGSGSGTDFTLTISRLEP EDFAVYYC <u>QOYGSPPRT</u> FGQGTKVE IK (SEQ ID NO: 1)	QVQLVQSGAEVKKPGSSVKVSCKAS <u>GGTFGSYGIS</u> WVRQAPGQGLEWMG <u>GIPIFGT</u> VTYAQKFQGRVTITADES TRTAYMELSSLRSED TAVYYC <u>ARDS</u> <u>WSGATGASDT</u> WGQGTLLTVSS (SEQ ID NO: 2)
P4C7	EIVLTQSPGTLSSLSPGERATLSC <u>RASQ</u> <u>YVSASLL</u> AWYQQKPGQAPRLLIY <u>GA</u> <u>STRAT</u> GIPDRFSGSGSGTDFTLTISRLEP EPEDFAVYYC <u>QOYARSST</u> FGQGTKV EIK (SEQ ID NO: 3)	QVQLVQSGAEVKKPGSSVKVSCKAS <u>GGTFSSYTIS</u> WVRQAPGQGLEWMG <u>GIIPAFGI</u> ANYAQKFQGRVTITADK STSTAYMELSSLRSED TAVYYC <u>AKG</u> <u>GSYSLDYFDI</u> WGQGTLLTVSS (SEQ ID NO: 4)
P3A1	DIQMTQSPSSLSASVGDRVTITC <u>RAS</u> <u>QSISSYL</u> NWYQQKPGKAPKLLIY <u>AAS</u> <u>SLOS</u> GVPSRFSGSGSGTDFTLTISLQ PEDFATYYC <u>QOSYSTPLT</u> FGQGTKV	QVQLVQSGAEVKKPGSSVKVSCKAS <u>GGTFSSYDIS</u> WVRQAPGQGLEWMG <u>GIIPVSGR</u> ANYAQKFQGRVTITTDK STSTAYMELSSLRSED TAVYYC <u>CARY</u>

mAb	Light Chain	Heavy Chain
	EIK (SEQ ID NO: 5)	<u>RPTYWPLDY</u> WGQGLTVTVSS (SEQ ID NO: 6)
P5A3	QSALTQPASVSGSPGQSITISCT <u>TGTSS</u> <u>DVGGYNYV</u> SWYQQHPGKAPKLMY <u>EVSKRPS</u> GVPDRFSGSGKSGNTASLTV SGLQAEDEADYYC <u>SSYAGSNTVV</u> FG GGTKLTVL (SEQ ID NO: 7)	QVQLVQSGAEVKKPGSSVKVSCKAS <u>GGTFSSYY</u> IGWVRQAPGQGLEWMG <u>GIIPWFGT</u> TANYAQKFQGRVTITAD KSTNTAYMELSSLRSEDNAVYYCAA <u>DHHDSPSGYTSGGFDY</u> WGQGLTVTVSS (SEQ ID NO: 8)
P9B5	QSVLTQPPSASGTPGQRTISCS <u>SGSSS</u> <u>NIGSNYVY</u> WYQQLPGTAPKLLIY <u>RN</u> <u>NQRPS</u> GVPDRFSGSGKSGTSASLAISGL RSEDEADYYCA <u>AAWDDSLSGV</u> FGG GTKLTVL (SEQ ID NO: 9)	EVQLLESGGGLVQPGGSLRLSCAAS <u>GFIFASYAM</u> SWVRQAPGKGLEWVS <u>EISSSGGS</u> TTYADSVKGRFTISRDN KNTLYLQMNSLRAEDNAVYYCARD <u>RVMAGLGYPFDY</u> WGQGLTVTVSS (SEQ ID NO: 10)
P9F1	QSVLTQPPSASGTPGQRTISCS <u>SGSGS</u> <u>NIGSNYVY</u> WYQQLPGTAPKLLIY <u>RN</u> <u>NQRPS</u> GVPDRFSGSGKSGTSASLAISGL RSEDEADYYCA <u>AAWDGSLSRP</u> VFGTG TKLTVL (SEQ ID NO: 11)	EVQLLESGGGLVQPGGSLRLSCAAS <u>GFIFSSFAM</u> SWVRQAPGKGLEWVS <u>DISGSGASTY</u> YADSVKGRFTISRDN KNTLYLQMNSLRAEDNAVYYCASA <u>SGGSGSYWPYMDP</u> WGQGLTVTVSS (SEQ ID NO: 12)
P1B4	EIVLTQSPGTLSPGERATLSC <u>RASQ</u> <u>SVPNEQLA</u> WYQQKPGQAPRLLIY <u>DA</u> <u>SSRAT</u> GIPDRFSGSGSGTDFTLTISRLE PEDFAVYYC <u>QQYGSPLT</u> FGQGTVK EIK (SEQ ID NO: 13)	QVQLVQSGAEVKKPGSSVKVSCKAS <u>GGVFSRYAL</u> SWVRQAPGQGLEWM <u>GGIIPMLGF</u> FANYAQKFQGRVTITAD ESTSTAYMELSSLRSEDNAVYYCATL <u>DFGALDY</u> WGQGLTVTVSS (SEQ ID NO: 14)
P1B11	EIVLTQSPGTLSPGERATLSC <u>RASQ</u> <u>SVSSSELA</u> WYQQKPGQAPRLLIY <u>DAS</u> <u>SRAT</u> GIPDRFSGSGSGTDFTLTISRLEP EDFAVYYC <u>QQYDSSPLT</u> FGQGTVKE IK (SEQ ID NO: 15)	QVQLVQSGAEVKKPGSSVKVSCKAS <u>GGTFRSFDIS</u> WVRQAPGQGLEWMG <u>RIIPILGY</u> ANYAQKFQGRVTITADES TSTAYMELSSLRSEDNAVYYCAS <u>DL</u> <u>GAPWAGYPFDP</u> WGQGLTVTVSS

mAb	Light Chain	Heavy Chain
		(SEQ ID NO: 16)
P7H3	QSVLTQPPSVSVAPGKTARITC <u>GGNN</u> <u>IGSKSVHWY</u> QQKPGQAPVLVIY <u>YDS</u> <u>DRPS</u> GIPERFSGSNSGNTATLTISRVE AGDEADYYC <u>QVWDSSTAWV</u> FGGGT KLTVL (SEQ ID NO: 17)	EVQLLESGGGLVQPGGSLRLSCAAS <u>GFTFSSYAMHWVR</u> QAPGKGLEWVS <u>AI</u> <u>SGSGGSTYYADSVKGR</u> FTISRDN KNTLYLQMNSLRAEDTAVYYC <u>ARG</u> <u>TRWWWGDAFDH</u> WGQGTTLTVSS (SEQ ID NO: 18)
P3E10	EIVLTQSPGTLSPGERATLSC <u>RASQ</u> <u>SVPSSQLAWY</u> QQKPGQAPRLLIY <u>DA</u> <u>SSRAT</u> GIPDRFSGSGSGTDFTLTISRLE PEDFAVYYC <u>QQYGSSPLT</u> FGQGTKV EIK (SEQ ID NO: 19)	QVQLVQSGAEVKKPGSSVKVSCKAS <u>GGTFSSYAIQWVR</u> QAPGQGLEWMG <u>GI</u> <u>VGSWGLANYAQKFQGR</u> VTITTD KSTSTAYMELSSLRSEDTAVYYCAT <u>SAFGELAS</u> WGQGTTLTVSS (SEQ ID NO: 20)
PIA5	EIVLTQSPGTLSPGERATLSC <u>RASQ</u> <u>AVDSSDLAWY</u> QQKPGQAPRLLIY <u>DA</u> <u>YTRPS</u> GIPDRFSGSGSGTDFTLTISRLE PEDFAVYYC <u>QQYGSSPLT</u> FGGGTKL EIK (SEQ ID NO: 21)	QVQLVQSGAEVKKPGSSVKVSCKAS <u>GGVFSRYALSWVR</u> QAPGQGLEWM <u>GGIIPMLGFANYAQKFQGR</u> VTITAD ESTSTAYMELSSLRSEDTAVYYCAT <u>LDFGALDY</u> WGQGTTLTVSS (SEQ ID NO: 22)
P5F7	EIVLTQSPATLSLSPGERATLSC <u>RASQ</u> <u>SVSSNLAWY</u> QQKPGQAPRLLIY <u>DTF</u> <u>TRAT</u> GIPARFSGSGSGTDFTLTISRLE PEDFAVYYC <u>QQYGSSPPT</u> FGQGTRL EIK (SEQ ID NO: 23)	EVQLLESGGGLVQPGGSLRLSCAAS <u>GFTFSSYAMNWVR</u> QAPGKGLEWVS <u>SISGGGRSTYYADSVKGR</u> FTISRDN KNTLYLQMNSLRAEDTAVYYC <u>ARD</u> <u>LSPSDVGWGYGFDI</u> WGQGTTLTVS S (SEQ ID NO: 24)
P4H11	EIVLTQSPGTLSPGERATLSC <u>RASQ</u> <u>SVSNTYLAWY</u> QQKPGQAPRLLIY <u>DT</u> <u>SSRAT</u> GIPDRFSGSGSGTDFTLTISRLE PEDFAVYYC <u>QQYGSSLT</u> FGQGTKVE IK (SEQ ID NO: 25)	EVQLLESGGGLVQPGGSLRLSCAAS <u>GFTFSSYAMNWVR</u> QAPGKGLEWVS <u>SISGGGRSTYYADSVKGR</u> FTISRDN KNTLYLQMNSLRAEDTAVYYC <u>ARD</u> <u>LSPSDVGWGYGFDI</u> WGQGTTLTVS S (SEQ ID NO: 26)

mAb	Light Chain	Heavy Chain
P15F7	DIQMTQSPSSLSASVGDRVITITCRAS <u>OSISTYLN</u> WYQQKPGKAPKLLIYAAS <u>NLQSG</u> VPSRFSGSGSGTDFTLTISLQ PEDFATYYC <u>QOSYSIPLT</u> FGQGTKVE IK (SEQ ID NO: 27)	EVQLLES GGGLVQPGGSLRLSCAAS <u>GFTFN</u> NYAMNWVRQAPGKGLEWV <u>SVISGSGG</u> TTYADSVKGRFTISR NSKNTLYLQMNSLRAEDTAVYYCA <u>SGIWDLRY</u> WGQGTLLTVSS (SEQ ID NO: 28)
P12B6	EIVLTQSPGTLSPGERATLSCRASQ <u>IVSSSYLA</u> WYQQKPGQAPRLLIYGAS <u>SRAS</u> GIPDRFSGSGSGTDFTLTISRLEP EDFAVYYC <u>QOYGGSPYT</u> FGQGTKV EIK (SEQ ID NO: 29)	QVQLVQSGAEVKKPGSSVKVSCKAS <u>GGTFMSY</u> AISWVRQAPQGGLWWM <u>GGIPIFGI</u> ANYAQKFQGRVTITADK STSTAYMELSSLRSEDVAVYYCARE <u>TLIYPIPFEL</u> WGQGTLLTVSS (SEQ ID NO: 30)
P8B6	EIVLTQSPGTLSPGERATLSCRASQ <u>SVSHSYLA</u> WYQQKPGQAPRLLIYGA <u>SFRAA</u> GIPDRFSGSGSGTDFTLTISR EPEDFAVYYC <u>QOYGS</u> DPYTFGQG VEIK (SEQ ID NO: 31)	QVQLVQSGAEVKKPGSSVKVSCKAS <u>GGTFSSY</u> AVSWVRQAPQGGLWWM <u>GGIPIFGI</u> ANYAQKFQGRVTITADT STSTAYMELSSLRSEDVAVYYCAIEG <u>IGGDLRYDGYDA</u> WGQGTLLTVSS (SEQ ID NO: 32)
P14G2	DIQMTQSPSSLSASVGDRVITITCRAS <u>QSISSYLN</u> WYQQKPGKAPKLLIYDAS <u>DLOR</u> GVPSRFSGSGSGTDFTLTISLQ PEDFATYYC <u>QOSYNTPWT</u> FGQGTKV EIK (SEQ ID NO: 33)	EVQLLES GGGLVQPGGSLRLSCAAS <u>GFTFS</u> NYVMNWVRQAPGKGLEWV <u>SAISGSGA</u> TTYADSVKGRFTISR NSKNTLYLQMNSLRAEDTAVYYCV <u>SGLWAGGI</u> WGQGTLLTVSS (SEQ ID NO: 34)
P7F9	NFMLTQPHSVSESPGKTVTISCTRSSG <u>SIASNYVQ</u> WYQQKPGQAPVLVVD <u>DSDRPS</u> GIPERFSGSNSGNTATLTISR VEAGDEADYYC <u>QVWDSSSDHWV</u> FG GGTKLTVL (SEQ ID NO: 35)	EVQLLES GGGLVQPGGSLRLSCAAS <u>GFTFS</u> SYAMSWVRQAPGKGLEWVS <u>AIGGSGGS</u> TYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAMYYCAR <u>DYYAFSDPAYGGMDV</u> WGQGTLLT VSS (SEQ ID NO: 36)

mAb	Light Chain	Heavy Chain
P08B0 6EE	EIVLTQSPGTLSSLSPGERATLSC <u>RASQ</u> <u>SVSHSYLA</u> WYQQKPGQAPRLLIY <u>GA</u> <u>SFRAA</u> GIPDRFSGSGSGTDFTLTISRLE EPEDFAVYYC <u>QOYGSEPYT</u> FGQGTK VEIK (SEQ ID NO: 264)	QVQLVQSGAEVKKPGSSVKVSCKAS <u>GGTFSSYAVS</u> WVRQAPGQGLEWM <u>GGIPIFGI</u> ANYAQKFQGRVTITADTS TSTAYMELSSLRSEDTAVYYCA <u>IEGI</u> <u>GGDLRYEGYDA</u> WGQGTLLTVSS (SEQ ID NO: 265)
P04A0 4	EIVLTQSPGTLSSLSPGERATLSC <u>RASQ</u> <u>SVTSSQLA</u> WYQQKPGQAPRLLIY <u>DA</u> <u>SSRAT</u> GIPDRFSGSGSGTDFTLTISRLE PEDFAVYYC <u>QOYGSSLLIT</u> FGQGTK VEIK (SEQ ID NO: 266)	QVQLVQSGAEVKKPGSSVKVSCKAS <u>GGTFSSYYIT</u> WVRQAPGQGLEWMG <u>RIMPAFGW</u> TNYAQKFQGRVTITTDK STSTAYMELSSLRSEDTAVYYCAS <u>D</u> <u>EFGAFDV</u> WGQGTLLTVSS (SEQ ID NO: 267)
P01A0 5	EIVLTQSPGTLSSLSPGERATLSC <u>RASQ</u> <u>AVDSSDLA</u> WYQHKPGQAPRLLIY <u>DA</u> <u>YTRPS</u> GIPDRFSGSGSGTDFTLTISRLE PEDFAVYYC <u>QOYGSSPLT</u> FGGGTKL EIK (SEQ ID NO: 268)	QVQLVQSGAEVKKPGSSVKVSCKAS <u>GGVFSRYALS</u> WVRQAPGQGLEWM <u>GGIIPMLGF</u> ANYAQKFQGRVTITAD ESTSTAYMELSSLRSEDTAVYYCAT <u>LDFGALDY</u> WGQGTLLTVSS (SEQ ID NO: 269)
P08B0 3	DIVMTQSPGTLSSLSPGERATLSC <u>RAS</u> <u>QSVSSNLA</u> WYQQKPGQAPRLLIY <u>DA</u> <u>YTRAT</u> GIPARFSGSGSGTDFTLTISRLE EPEDFAVYYC <u>QOYGSPYT</u> FGQGTKV EIK (SEQ ID NO: 270)	QVQLVQSGAEVKKPGSSVKVSCKAS <u>GGTFSSYDIS</u> WVRQAPGQGLEWMG <u>RIIPSEGA</u> ANYAQKFQGRVTITADKS TSTAYMELSSLRSEDTAVYYCAT <u>DD</u> <u>GEGWTPPFGY</u> WGQGTLLTVSS (SEQ ID NO: 271)
P5F7	DIVMTQSPATLSSLSPGERATLSC <u>RAS</u> <u>QSVSSNLA</u> WYQQKPGQAPRLLIY <u>DT</u> <u>FTRAT</u> GIPARFSGSGSGTDFTLTISRLE EPEDFAVYYC <u>QOYGSSPPT</u> FGQGTR LEIK (SEQ ID NO: 272)	EVQLLESGGGLVQPGGSLRLSCAAS <u>GFTFSSYAMN</u> WVRQAPGKGLEWVS <u>SISGGGRSTYY</u> ADSVKGRFTISRDN KNTLYLQMNSLRAEDTAVYYCARD <u>LSPSDVGWGYGFDI</u> WGQGTLLTVS S (SEQ ID NO: 273)

mAb	Light Chain	Heavy Chain
P5F7g	EIVLTQSPATLSLSPGERATLSC <u>RASQ</u> <u>SVSSNLA</u> WYQQKPGQAPRLLIY <u>DTE</u> <u>TRAT</u> GIPARFSGSGSGTDFTLTISSELP EDFAVYYC <u>QOYGSSPPT</u> FGQGTRLEI K (SEQ ID NO: 274)	EVQLLESGGGLVQPGGSLRLSCAAS <u>GFTFSSY</u> AMNWVRQAPGKGLEWVS <u>SISGGGRSTYY</u> ADSVKGRFTISRDN KNTLYLQMNSLRAEDTAVYYC <u>ARD</u> <u>LSPSDVGWGYGFDI</u> WGQGTLLTVS S (SEQ ID NO: 275)
P10A0 2g	EIVLTQSPATLSLSPGERATLSC <u>RASQ</u> <u>DVSDLLA</u> WYQQKPGQAPRLLIY <u>DAY</u> <u>TRAT</u> GIPARFSGSGSGTDFTLTISSELP EDFAVYYC <u>QOYASSPIT</u> FGQGTRLEI K (SEQ ID NO: 276)	EVQLLESGGGLVQPGGSLRLSCAAS <u>GFTFSSY</u> AMNWVRQAPGKGLEWVS <u>SISGGGRSTYY</u> ADSVKGRFTISRDN KNTLYLQMNSLRAEDTAVYYC <u>ARD</u> <u>LSPSDVGWGYGFDI</u> WGQGTLLTVS S (SEQ ID NO: 277)
P10A0 4g	EIVLTQSPATLSLSPGERATLSC <u>RASQ</u> <u>KVSDLLA</u> WYQQKPGQAPRLLIY <u>DAY</u> <u>TRAT</u> GIPARFSGSGSGTDFTLTISSELP EDFAVYYC <u>QOYTGSPIT</u> FGQGTRLEI K (SEQ ID NO: 278)	EVQLLESGGGLVQPGGSLRLSCAAS <u>GFTFSSY</u> AMNWVRQAPGKGLEWVS <u>SISGGGRSTYY</u> ADSVKGRFTISRDN KNTLYLQMNSLRAEDTAVYYC <u>ARD</u> <u>LSPSDVGWGYGFDI</u> WGQGTLLTVS S (SEQ ID NO: 279)
P10A0 5g	EIVLTQSPATLSLSPGERATLSC <u>RASL</u> <u>SVSDLLA</u> WYQQKPGQAPRLLIY <u>DAY</u> <u>SRAT</u> GIPARFSGSGSGTDFTLTISSELP EDFAVYYC <u>QOYSSNPIT</u> FGQGTRLEI K (SEQ ID NO: 280)	EVQLLESGGGLVQPGGSLRLSCAAS <u>GFTFSSY</u> AMNWVRQAPGKGLEWVS <u>SISGGGRSTYY</u> ADSVKGRFTISRDN KNTLYLQMNSLRAEDTAVYYC <u>ARD</u> <u>LSPSDVGWGYGFDI</u> WGQGTLLTVS S (SEQ ID NO: 281)
P10A0 7g	EIVLTQSPATLSLSPGERATLSC <u>RASG</u> <u>SVSDLLA</u> WYQQKPGQAPRLLIY <u>DAY</u> <u>SRAT</u> GIPARFSGSGSGTDFTLTISSELP	EVQLLESGGGLVQPGGSLRLSCAAS <u>GFTFSSY</u> AMNWVRQAPGKGLEWVS <u>SISGGGRSTYY</u> ADSVKGRFTISRDN

mAb	Light Chain	Heavy Chain
	EDFAVYYC <u>QQYASYPIT</u> FGQGTRLEI K (SEQ ID NO: 282)	KNTLYLQMNSLRAEDTAVYYCARD <u>LSPSDVGWGYGFDI</u> WGQGTLVTVS S (SEQ ID NO: 283)
P10B0 3g	EIVLTQSPATLSLSPGERATLSC <u>RASQ</u> <u>SVSDLLA</u> WYQQKPGQAPRLLIY <u>DAF</u> <u>SRAT</u> GIPARFSGSGSGTDFTLTISSELP EDFAVYYC <u>QQYGTTPIT</u> FGQGTRLEI K (SEQ ID NO: 284)	EVQLLESGGGLVQPGGSLRLSCAAS <u>GFTFSSYAMN</u> WVRQAPGKGLEWVS <u>SISGGGRSTYYADSVKGR</u> FTISRDN KNTLYLQMNSLRAEDTAVYYCARD <u>LSPSDVGWGYGFDI</u> WGQGTLVTVS S (SEQ ID NO: 285)
P10B0 6g	EIVLTQSPATLSLSPGERATLSC <u>RASE</u> <u>SVSDLLA</u> WYQQKPGQAPRLLIY <u>DAY</u> <u>SRAT</u> GIPARFSGSGSGTDFTLTISSELP EDFAVYYC <u>QQYSASPIT</u> FGQGTRLEI K (SEQ ID NO: 286)	EVQLLESGGGLVQPGGSLRLSCAAS <u>GFTFSSYAMN</u> WVRQAPGKGLEWVS <u>SISGGGRSTYYADSVKGR</u> FTISRDN KNTLYLQMNSLRAEDTAVYYCARD <u>LSPSDVGWGYGFDI</u> WGQGTLVTVS S (SEQ ID NO: 287)
P5F7g 2	EIVLTQSPATLSLSPGERATLSC <u>RASQ</u> <u>SVSSNLA</u> WYQQKPGQAPRLLIY <u>DTF</u> <u>TRAT</u> GIPARFSGSGSGTDFTLTISSELP EDFAVYYC <u>QQYGSSPPT</u> FGQGTRLEI K (SEQ ID NO: 288)	EVQLLESGGGLVQPGGSLRLSCAAS <u>GFTFSSYAMN</u> WVRQAPGKGLEWVS <u>AISGGGRSTYYADSVKGR</u> FTISRDN KNTLYLQMNSLRAEDTAVYYCARD <u>LSPSDVGWGYGFDI</u> WGQGTLVTVS S (SEQ ID NO: 289)
P5F7g 3	EIVLTQSPATLSLSPGERATLSC <u>RASQ</u> <u>SVSSLLA</u> WYQQKPGQAPRLLIY <u>DAY</u> <u>TRAT</u> GIPARFSGSGSGTDFTLTISSELP EDFAVYYC <u>QQYTGSPIT</u> FGQGTRLEI K (SEQ ID NO: 290)	EVQLLESGGGLVQPGGSLRLSCAAS <u>GFTFSSYAMN</u> WVRQAPGKGLEWVS <u>AISGGGRSTYYADSVKGR</u> FTISRDN KNTLYLQMNSLRAEDTAVYYCARD <u>LSPSDVGWGYGFDI</u> WGQGTLVTVS S (SEQ ID NO: 291)

mAb	Light Chain	Heavy Chain
P5F7g 4	EIVLTQSPATLSLSPGERATLSC <u>RASQ</u> <u>SVSSLLAWYQQKPGQAPRLLIYDAY</u> <u>TRAT</u> GIPARFSGSGSGTDFTLTSSLEP EDFAVYYC <u>QOYTGSPIT</u> FGQGTRLEI K (SEQ ID NO: 292)	EVQLLESGGGLVQPGGSLRLSCAAS <u>GFTFSSYAMS</u> WVRQAPGKGLEWVS <u>AISGGGRSTYYADSVKGRFTISRDN</u> KNTLYLQMNSLRAEDTAVYYCARD <u>LSPSDVGWGYGFDI</u> WGQGTLLTVS S (SEQ ID NO: 293)

[0123] Also provided herein are CDR portions of antigen binding domains of antibodies to FLT3 or CDR portions of extracellular ligand-binding domains of CARs to FLT3 (including Chothia, Kabat CDRs, and CDR contact regions). Determination of CDR regions is well within the skill of the art. It is understood that in some embodiments, CDRs can be a combination of the Kabat and Chothia CDR (also termed “combined CRs” or “extended CDRs”). In some embodiments, the CDRs are the Kabat CDRs. In other embodiments, the CDRs are the Chothia CDRs. In other words, in embodiments with more than one CDR, the CDRs may be any of Kabat, Chothia, combination CDRs, or combinations thereof. Table 3 provides examples of CDR sequences provided herein.

Table 3

Heavy Chain			
mAb	CDRH1	CDRH2	CDRH3
P4F6	SYGIS (SEQ ID NO: 37) (Kabat); GGTFGSY (SEQ ID NO: 38) (Chothia); GGTFGSYGIS (SEQ ID NO: 39) (Extended)	GIPIFGTVTYAQK FQG (SEQ ID NO: 40) (Kabat); IPIFGT (SEQ ID NO: 41) (Chothia)	DSWSGATGASD T (SEQ ID NO: 42)
P4C7	SYTIS (SEQ ID NO: 43) (Kabat); GGTFSSY (SEQ ID NO: 44) (Chothia) GGTFSSYTIS (Extended)	GIIPAFGIANYAQK FQG (SEQ ID NO: 46) (Kabat); IPAFGI (SEQ ID NO: 47) (Chothia)	GGSYSLDYFDI (SEQ ID NO: 48)

	(SEQ ID NO: 45)		
P3A1	SYDIS (SEQ ID NO: 49) (Kabat); GGTFSSY (SEQ ID NO: 44) (Chothia); GGTFSSYDIS (SEQ ID NO: 50) (Extended)	GIIPVSGRANYAQ KFQG (SEQ ID NO: 51) (Kabat); IPVSGR (SEQ ID NO: 52) (Chothia)	VRPTYWPLDY (SEQ ID NO: 53)
P5A3	YYYIG (SEQ ID NO: 54) (Kabat); GGTFSSY (SEQ ID NO: 55) (Chothia); GGTFSSYYIG (SEQ ID NO: 56) (Extended)	GIIPWFGTANYAQ KFQG (SEQ ID NO: 57) (Kabat); IPWFGT (SEQ ID NO: 58) (Chothia)	DHHDSPSGYTSG GFDV (SEQ ID NO: 59)
P9B5	SYAMS (SEQ ID NO: 60) (Kabat); GFIFASY (SEQ ID NO: 61) (Chothia); GFIFASYAMS (SEQ ID NO: 62) (Extended)	EISSSGGSTTYADS VKG (SEQ ID NO: 63) (Kabat); SSSGGS (SEQ ID NO: 64) (Chothia)	DRVMAGLGYDP FDY (SEQ ID NO: 65)
P9F1	SFAMS (SEQ ID NO: 66) (Kabat); GFIFSSF (SEQ ID NO: 67) (Chothia); GFIFSSFAMS (SEQ ID NO: 68) (Extended)	DISGSGASTYYAD SVKG (SEQ ID NO: 69) (Kabat); SGSGAS (SEQ ID NO: 70) (Chothia)	ASGGSGSYWPY MDP (SEQ ID NO: 71)
P1B4	RYALS (SEQ ID NO: 72) (Kabat); GGVFSRY (SEQ ID NO: 73) (Chothia); GGVFSRYALS (SEQ ID NO: 74) (Extended)	GIIPMLGFANYAQ KFQG (SEQ ID NO: 75) (Kabat); IPMLGF (SEQ ID NO: 76) (Chothia)	LDFGALDY (SEQ ID NO: 77)
P1B11	SFDIS (SEQ ID NO: 78)	RIIPILGYANYAQK	DLGAPWAGYPF

	(Kabat); GGTFRSF (SEQ ID NO: 79) (Chothia); GGTFRSFDIS (SEQ ID NO: 80) (Extended)	FQG (SEQ ID NO: 81) (Kabat); IPILGY (SEQ ID NO: 82) (Chothia)	DP (SEQ ID NO: 83)
P7H3	SYAMH (SEQ ID NO: 84) (Kabat); GFTFSSY (SEQ ID NO: 85) (Chothia); GFTFSSYAMH (SEQ ID NO: 86) (Extended)	AISGSGGSTYYAD SVKG (SEQ ID NO: 87) (Kabat); SGSGGS (SEQ ID NO: 88) (Chothia)	GTRWWWGDAF DH (SEQ ID NO: 89)
P3E10	SYAIQ (SEQ ID NO: 90) (Kabat); GGTFSSY (SEQ ID NO: 91) (Chothia); GGTFSSYAIQ (SEQ ID NO: 92) (Extended)	GIVGSWGLANYA QKFQG (SEQ ID NO: 93) (Kabat); VGSWGL (SEQ ID NO: 94) (Chothia)	SAFGELAS (SEQ ID NO: 95)
P1A5	RYALS (SEQ ID NO: 96) (Kabat); GGVFSRY (SEQ ID NO: 97) (Chothia); GGVFSRYALS (SEQ ID NO: 98) (Extended)	GIIPMLGFANYAQ KFQG (SEQ ID NO: 99) (Kabat); IPMLGF (SEQ ID NO: 100) (Chothia)	LDFGALDY (SEQ ID NO: 101)
P5F7	SYAMN (SEQ ID NO: 102) (Kabat); GFTFSSY (SEQ ID NO: 103) (Chothia); GFTFSSYAMN (SEQ ID NO: 104) (Extended)	SISGGGRSTYYAD SVKG (SEQ ID NO: 105) (Kabat); SGGGRS (SEQ ID NO: 106) (Chothia)	DLSPSDVGWGY GFDI (SEQ ID NO: 107)
P4H11	SYAMN (SEQ ID NO: 108) (Kabat); GFTFSSY (SEQ ID NO: 111) (Kabat);	SISGGGRSTYYAD SVKG (SEQ ID NO: 111) (Kabat);	DLSPSDVGWGY GFDI (SEQ ID NO: 113)

	109) (Chothia); GFTFSSYAMN (SEQ ID NO: 110) (Extended)	SGGGRS (SEQ ID NO: 112) (Chothia)	
P15F7	NYAMN (SEQ ID NO: 114) (Kabat); GFTFNNY (SEQ ID NO: 115) (Chothia); GFTFNNYAMN (SEQ ID NO: 116) (Extended)	VISGSGGTTYAD SVKG (SEQ ID NO: 117) (Kabat); SGSGGT (SEQ ID NO: 118) (Chothia)	GIWDLRY (SEQ ID NO: 119)
P12B6	SYAIS (SEQ ID NO: 120) (Kabat); GGTFMSY (SEQ ID NO: 121) (Chothia); GGTFMSY AIS (SEQ ID NO: 122) (Extended)	GIPIFGIANYAQKF QG (SEQ ID NO: 123) (Kabat); IPIFGI (SEQ ID NO: 124) (Chothia)	ETLIYPIPFEL (SEQ ID NO: 125)
P8B6	SYAVS (SEQ ID NO: 126) (Kabat); GGTFSSY (SEQ ID NO: 127) (Chothia); GGTFSSYAVS (SEQ ID NO: 128) (Extended)	GIPIFGIANYAQKF QG (SEQ ID NO: 129) (Kabat); IPIFGI (SEQ ID NO: 130) (Chothia)	EGIGGDLRYDGY DA (SEQ ID NO: 131)
P14G2	NYVMN (SEQ ID NO: 132) (Kabat); GFTFSNY (SEQ ID NO: 133) (Chothia); GFTFSNYVMN (SEQ ID NO: 134) (Extended)	AISGSGATTYYAD SVKG (SEQ ID NO: 135) (Kabat); SGSGAT (SEQ ID NO: 136) (Chothia)	GLWAGGI (SEQ ID NO: 137)
P7F9	SYAMS (SEQ ID NO: 138) (Kabat); GFTFSSY (SEQ ID NO: 139) (Chothia); GFTFSSYAMS (SEQ ID NO: 140) (Extended)	AIGSGGSTYYAD SVKG (SEQ ID NO: 141) (Kabat); GGSGGS (SEQ ID NO: 142) (Chothia)	DYYAFSDPAYG GMDV (SEQ ID NO: 143)

	NO: 140) (Extended)		
P08B06EE	SYAVS (SEQ ID NO: 294) (Kabat); GGTFSSY (SEQ ID NO: 295) (Chothia); GGTFSSYAVS (SEQ ID NO: 296) (Extended)	GIPIFGIANYAQKF QG (SEQ ID NO: 297) (Kabat); IPIFGI (SEQ ID NO: 298) (Chothia)	EGIGGDLRYEGY DA (SEQ ID NO: 299)
P04A04	SYIIT (SEQ ID NO: 300) GGTFSSY (SEQ ID NO: 295) (Chothia) GGTFSSYIIT (SEQ ID NO: 301) (Extended)	RIMPAFGWTNYA QKFQG (SEQ ID NO: 302) (Kabat) MPAFGW (SEQ ID NO: 303) (Chothia)	DEFGAFDV (SEQ ID NO: 304)
P01A05	RYALS (SEQ ID NO: 305) (Kabat); GGVFSRY (SEQ ID NO: 306) (Chothia); GGVFSRYALS (SEQ ID NO: 307) (Extended)	IPMLGF (SEQ ID NO: 308) (Chothia) GGIIPMLGFANYA QKFQG (SEQ ID NO: 309) (Kabat)	LDFGALDY (SEQ ID NO: 3110)
P08B03	SYDIS (SEQ ID NO: 311) (Kabat); GGTFSSY (SEQ ID NO: 295) (Chothia); GGTFSSYDIS (SEQ ID NO: 312) (Extended)	RIIPSFGAANYAQK FQG (SEQ ID NO: 313) (Kabat) IPSFGA (SEQ ID NO: 314) (Chothia)	DDGEGWTPPFG Y (SEQ ID NO: 315)
P5F7g; P10A02g; P10A04g; P10A05g; P10A07g; P10B03g; P10B06g	SYAMN (SEQ ID NO: 316) (Kabat); GFTFSSY (SEQ ID NO: 317) (Chothia); GFTFSSYAMN (SEQ ID NO: 318) (Extended)	SISGGGRSTYYAD SVKG (SEQ ID NO: 319) (Kabat); SGGGRS (SEQ ID NO: 320) (Chothia)	DLSPSDVGWGY GFDI (SEQ ID NO: 321)
P5F7g2; P5F7g3	SYAMN (SEQ ID NO: 316) (Kabat);	AISGGGRSTYYAD SVKG (SEQ ID NO: 319) (Kabat);	DLSPSDVGWGY GFDI (SEQ ID NO: 321)

	GFTFSSY (SEQ ID NO: 317) (Chothia); GFTFSSYAMN (SEQ ID NO: 318) (Extended)	322) (Kabat); SGGGRS (SEQ ID NO: 320) (Chothia)	321)
P5F7g4	SYAMS (SEQ ID NO: 138) (Kabat); GFTFSSY (SEQ ID NO: 317) (Chothia); GFTFSSYAMS (SEQ ID NO: 140) (Extended)	AISGGGRSTYYAD SVKG (SEQ ID NO: 322) (Kabat); SGGGRS (SEQ ID NO: 320) (Chothia)	DLSPSDVGWGY GFDI (SEQ ID NO: 321)
Light Chain			
mAb	CDRL1	CDRL2	CDRL3
P4F6	RASHSVSSSYLA (SEQ ID NO: 144)	GASSRAT (SEQ ID NO: 145)	QQYGSPPT (SEQ ID NO: 146)
P4C7	RASQYVSASLLA (SEQ ID NO: 147)	GASTRAT (SEQ ID NO: 148)	QQYARSST (SEQ ID NO: 149)
P3A1	RASQSISSYLN (SEQ ID NO: 150)	AASSLQS (SEQ ID NO: 151)	QQSYSTPLT (SEQ ID NO: 152)
P5A3	TGTSSDVGGYNYVS (SEQ ID NO: 153)	EVSKRPS (SEQ ID NO: 154)	SSYAGSNTVV (SEQ ID NO: 155)
P9B5	SGSSNIGSNYVY (SEQ ID NO: 156)	RNNQRPS (SEQ ID NO: 157)	AAWDDSLSGVV (SEQ ID NO: 158)
P9F1	SGSGSNIGSNYVY (SEQ ID NO: 159)	RNNQRPS (SEQ ID NO: 160)	AAWDGSLSRPV (SEQ ID NO: 161)
P1B4	RASQSVNEQLA (SEQ ID NO: 162)	DASSRAT (SEQ ID NO: 163)	QQYGSPPLT (SEQ ID NO: 164)
P1B11	RASQSVSSSELA (SEQ ID NO: 165)	DASSRAT (SEQ ID NO: 166)	QQYDSSPLT (SEQ ID NO: 167)
P7H3	GGNNIGSKSVH (SEQ ID NO: 168)	YDSRPS (SEQ ID NO: 169)	QVWDSSTAWV (SEQ ID NO: 170)
P3E10	RASQSVSSQLA (SEQ ID NO: 171)	DASSRAT (SEQ ID NO: 172)	QQYGSSPLT

	NO: 171)	NO: 172)	(SEQ ID NO: 173)
P1A5	RASQAVDSSDLA (SEQ ID NO: 174)	DAYTRPS (SEQ ID NO: 175)	QQYGSSPLT (SEQ ID NO: 176)
P5F7	RASQSVSSNLA (SEQ ID NO: 177)	DTFTRAT (SEQ ID NO: 178)	QQYGSSPPT (SEQ ID NO: 179)
P4H11	RASQSVSNTYLA (SEQ ID NO: 180)	DTSSRAT (SEQ ID NO: 181)	QQYGSSLT (SEQ ID NO: 182)
P15F7	RASQSISTYLN (SEQ ID NO: 183)	AASNLQS (SEQ ID NO: 184)	QQSYSIPLT (SEQ ID NO: 185)
P12B6	RASQIVSSSYLA (SEQ ID NO: 186)	GASSRAS (SEQ ID NO: 187)	QQYGGSPYT (SEQ ID NO: 188)
P8B6	RASQSVSHSYLA (SEQ ID NO: 189)	GASFRAA (SEQ ID NO: 190)	QQYGSDPYT (SEQ ID NO: 191)
P14G2	RASQSISSYLN (SEQ ID NO: 192)	DASDLQR (SEQ ID NO: 193)	QQSYNTPWT (SEQ ID NO: 194)
P7F9	TRSSGSIASNYVQ (SEQ ID NO: 195)	DDSDRPS (SEQ ID NO: 196)	QVWDSSSDHWV (SEQ ID NO: 197)
P08B06EE	RASQSVSHSYLA (SEQ ID NO: 323)	GASFRAA (SEQ ID NO: 324)	QQYGSEPYT (SEQ ID NO: 325)
P04A04	RASQSVTSSQLA (SEQ ID NO: 326)	GASFRAA (SEQ ID NO: 324)	QQYGSSLLIT (SEQ ID NO: 327)
P01A05	RASQAVDSSDLA (SEQ ID NO: 328)	DAYTRPS (SEQ ID NO: 329)	QQYGSSPLT (SEQ ID NO: 330)
P08B03	RASQSVSSNLA (SEQ ID NO: 331)	DAYTRAT (SEQ ID NO: 332)	QQYGSPYT (SEQ ID NO: 333)
P5F7g	RASQSVSSNLA (SEQ ID NO: 331)	DTFTRAT (SEQ ID NO: 334)	QQYGSSPPT (SEQ ID NO: 335)
P10A02g	RASQDVSDLLA (SEQ ID NO: 336)	DAYTRAT (SEQ ID NO: 332)	QQYASSPIT (SEQ ID NO: 337)
P10A04g	RASQKVSDLLA (SEQ ID NO: 338)	DAYTRAT (SEQ ID NO: 332)	QQYTGSPIT (SEQ ID NO: 339)

P10A05g	RASLSVSDLLA (SEQ ID NO: 340)	DAYS RAT (SEQ ID NO: 341)	QQYSSNPIT (SEQ ID NO: 342)
P10A07g	RASGSVSDLLA (SEQ ID NO: 343)	DAYS RAT (SEQ ID NO: 341)	QQYASYPIT (SEQ ID NO: 344)
P10B03g	RASQSVSDLLA (SEQ ID NO: 345)	DAFS RAT (SEQ ID NO: 346)	QQYGTPPIT (SEQ ID NO: 347)
P10B06g	RASESVSDLLA (SEQ ID NO: 348)	DAYS RAT (SEQ ID NO: 341)	QQYSASPIT (SEQ ID NO: 349)
P5F7g2	RASQSVSSNLA (SEQ ID NO: 331)	DTFTRAT (SEQ ID NO: 334)	QQYGSSPPT (SEQ ID NO: 335)
P5F7g3; P5F7g4	RASQSVSSLLA (SEQ ID NO: 350)	DAYTRAT (SEQ ID NO: 332)	QQYTGSPIT (SEQ ID NO: 339)

[0124] The invention encompasses modifications to the CARs and polypeptides of the invention shown in Table 2, including functionally equivalent CARs having modifications which do not significantly affect their properties and variants which have enhanced or decreased activity and/or affinity. For example, the amino acid sequence may be mutated to obtain an antibody with the desired binding affinity to FLT3. Modification of polypeptides is routine practice in the art and need not be described in detail herein. Examples of modified polypeptides include polypeptides with conservative substitutions of amino acid residues, one or more deletions or additions of amino acids which do not significantly deleteriously change the functional activity, or which mature (enhance) the affinity of the polypeptide for its ligand, or use of chemical analogs.

[0125] Amino acid sequence insertions include amino- and/or carboxyl-terminal fusions ranging in length from one residue to polypeptides containing a hundred or more residues, as well as intrasequence insertions of single or multiple amino acid residues. Examples of terminal insertions include an antibody with an N-terminal methionyl residue or the antibody fused to an epitope tag. Other insertional variants of the antibody molecule include the fusion to the N- or C-terminus of the antibody of an enzyme or a polypeptide which increases the half-life of the antibody in the blood circulation.

[0126] Substitution variants have at least one amino acid residue in the antibody molecule removed and a different residue inserted in its place. The sites of greatest interest for

substitutional mutagenesis include the hypervariable regions, but FR alterations are also contemplated. Conservative substitutions are shown in Table 4 under the heading of “conservative substitutions.” If such substitutions result in a change in biological activity, then more substantial changes, denominated “exemplary substitutions” in Table 4, or as further described below in reference to amino acid classes, may be introduced and the products screened.

Table 4: Amino Acid Substitutions

Original Residue (naturally occurring amino acid)	Conservative Substitutions	Exemplary Substitutions
Ala (A)	Val	Val; Leu; Ile
Arg (R)	Lys	Lys; Gln; Asn
Asn (N)	Gln	Gln; His; Asp, Lys; Arg
Asp (D)	Glu	Glu; Asn
Cys (C)	Ser	Ser; Ala
Gln (Q)	Asn	Asn; Glu
Glu (E)	Asp	Asp; Gln
Gly (G)	Ala	Ala
His (H)	Arg	Asn; Gln; Lys; Arg
Ile (I)	Leu	Leu; Val; Met; Ala; Phe; Norleucine
Leu (L)	Ile	Norleucine; Ile; Val; Met; Ala; Phe
Lys (K)	Arg	Arg; Gln; Asn
Met (M)	Leu	Leu; Phe; Ile
Phe (F)	Tyr	Leu; Val; Ile; Ala; Tyr
Pro (P)	Ala	Ala
Ser (S)	Thr	Thr
Thr (T)	Ser	Ser
Trp (W)	Tyr	Tyr; Phe
Tyr (Y)	Phe	Trp; Phe; Thr; Ser
Val (V)	Leu	Ile; Leu; Met; Phe; Ala; Norleucine

[0127] In some embodiments, provided herein is a CAR comprising an extracellular ligand-binding domain that binds to FLT3 and competes for binding to FLT3 with a CAR described herein, including CAR comprising an extracellular domain comprising P4F6, P4C7, P3A1, P5A3, P9B5, P9F1, P1B4, P1B11, P7H3, P3E10, P1A5, P5F7, P4H11, P15F7, P12B6, P8B6, P14G2, P7F9, P08B06EE, P04A04, P01A05, P08B03, P5F7g, P10A02g, P10A04g, P10A05g, P10A07g, P10B03g, P10B06g, P5F7g2, P5F7g3, and P5F7g4.

[0128] In some embodiments, provided herein is a CAR, which specifically binds to FLT3, wherein the CAR comprises a VH region comprising a sequence shown in SEQ ID NO: 4; and/or a VL region comprising a sequence shown in SEQ ID NO: 3. In some embodiments, provided herein is a CAR, which specifically binds to FLT3, wherein the CAR comprises a VH region comprising a sequence shown in SEQ ID NO: 6; and/or a VL region comprising a sequence shown in SEQ ID NO: 5. In some embodiments, the invention provides a CAR, which specifically binds to FLT3, wherein the CAR comprises a VH region comprising a sequence shown in SEQ ID NO: 10; and/or a VL region comprising a sequence shown in SEQ ID NO: 9. In some embodiments, provided herein is a CAR, which specifically binds to FLT3, wherein the CAR comprises a VH region comprising a sequence shown in SEQ ID NO: 20; and/or a VL region comprising a sequence shown in SEQ ID NO: 19.

[0129] In some embodiments, the invention also provides CARs comprising CDR portions of antibodies to FLT3 antibodies based on CDR contact regions. CDR contact regions are regions of an antibody that imbue specificity to the antibody for an antigen. In general, CDR contact regions include the residue positions in the CDRs and Vernier zones which are constrained in order to maintain proper loop structure for the antibody to bind a specific antigen. See, e.g., Makabe et al., J. Biol. Chem., 283:1156-1166, 2007. Determination of CDR contact regions is well within the skill of the art.

[0130] The binding affinity (K_D) of the ligand binding domain of the FLT3 specific CAR as described herein to FLT3 (such as human FLT3 (e.g., (SEQ ID NO: 198) can be for example about 0.1 to about 1000 nM, for example between about 0.5nM to about 500nM, or for example between about 1nM to about 250nM. In some embodiments, the binding affinity is about any of 1000 nM, 750 nM, 500 nM, 400 nM, 300 nM, 250 nM, 200 nM, 100 nM, 90 nM, 80 nM, 70 nM, 60 nM, 50 nM, 45 nM, 40 nM, 35 nM, 30 nM, 25 nM, 20 nM, 19 nM, 18 nM, 17 nM, 16 nM, 15 nM, 10 nM, 8 nM, 7.5 nM, 7 nM, 6.5 nM, 6 nM, 5.5 nM, 5 nM, 4 nM, 3 nM, 2 nM, 1 nM, 0.5 nM, 0.3 nM or 0.1 nM.

[0131] In some embodiments, the binding affinity (K_D) of the scFv of the ligand binding domain of the FLT3 specific CAR as described herein to FLT3 is about 10nM to about 100 nM, about 10nM to about 90nM, about 10nM to about 80nM, about 20nM to about 70nM, about 25nM to about 75nM, or about 40nM to about 110nM. In one embodiment, the binding affinities of the scFv described in this paragraph are for human FLT3.

[0132] In some embodiments, the binding affinity (K_D) of the scFv of the ligand binding domain of the FLT3 specific CAR as described herein to domain 4 of the extracellular domain of human FLT3 is about 1nM to about 100nM, about 10nM to about 100nM, about 20nM to about 100nM, about 25nM to about 105nM, about 40nM to about 80nM, about 40nM to about 100nM, or about 50nM to about 100nM.

[0133] In some embodiments, the binding affinity (K_D) of the scFv of the ligand binding domain of the FLT3 specific CAR as described herein to domain 2-3 of the extracellular domain of human FLT3 is about 5nM to 30nM, about 5nM to about 25nM, about 10nM to about 30nM, about 10nM to about 40nM, or about 10nM to about 25nM.

[0134] In an exemplary embodiment, the K_D of the ScFv is measured as disclosed in Example 1.

[0135] In some embodiments, the binding affinity is less than about any of 1000 nM, 900 nM, 800 nM, 250 nM, 200 nM, 100 nM, 50 nM, 30 nM, 20 nM, 10 nM, 7.5 nM, 7 nM, 6.5 nM, 6 nM, 5 nM.

Monoclonal antibody-specific epitopes

[0136] In some embodiments, the extracellular domain of any one of the FLT3 specific CARs disclosed herein may comprise one or more epitopes specific for (i.e., specifically recognized by) a monoclonal antibody. These epitopes are also referred to herein as mAb-specific epitopes. In these embodiments, the extracellular domain comprises the VH and VL polypeptides that specifically bind to FLT3 and one or more epitopes that bind to one or more monoclonal antibodies (mAbs). CARs comprising the mAb-specific epitopes can be single-chain or multi-chain.

[0137] The inclusion of eptiopes specific for monoclonal antibodies in the extracellular domain of the CARs described herein allows sorting and depletion of engineered immune cells expressing the CARs. In some embodiments, this feature also promotes recovery of endogenous

FLT3 expressing cells that were depleted by administration of engineered immune cells expressing the CARs.

[0138] Accordingly, in some embodiments, the present invention relates to a method for sorting and/or depleting the engineered immune cells endowed with the CARs comprising mAb-specific epitopes and a method for promoting recovery of endogenous FLT3 expressing cells, such as bone marrow progenitor cells.

[0139] Several epitope-monoclonal antibody couples can be used to generate CARs comprising monoclonal antibody specific epitopes; in particular, those already approved for medical use, such as CD20 epitope/rituximab as a non-limiting example.

[0140] In some embodiments, the monoclonal antibody specific for the epitope may be conjugated with a cytotoxic drug. It is also possible to promote CDC cytotoxicity by using engineered antibodies on which are grafted component(s) of the complement system. In some embodiments, activation of the CAR-T cells can be modulated by depleting the cells using an antibody which recognizes the epitope.

[0141] The invention also encompasses methods for sorting the engineered immune cells endowed with the FLT3 specific CARs expressing the mAb-specific epitope(s) and therapeutic methods where the activation of the engineered immune cells endowed with these CARs is modulated by depleting the cells using an antibody that targets the external ligand binding domain of said CARs.

[0142] CARs comprising one or more epitopes specifically recognized by a monoclonal antibody are disclosed in WO2016/120216, which is hereby incorporated by reference in its entirety. The epitope can be selected from any number of epitopes known in the art. In some embodiments, the epitope can be a target of a monoclonal antibody approved for medical use, such as, for example without limitation, the CD20 epitope recognized by rituximab. In some embodiments, the epitope comprises the amino acid sequence shown in SEQ ID NO: 229. CPYSNPSLC (SEQ ID NO: 229)

[0143] In some embodiments, the epitope can be located between the scFv and the hinge of a CAR. In some embodiments, two instances of the same epitope, separated by linkers, may be used in the CAR. For example, a polypeptide comprising 2 copies of the epitope shown in SEQ ID NO: 229, separated by linkers, as shown in GSGGGGSCPYSNPSLCGSGGGGSCPYSNPSLCGSGGGGS (SEQ ID NO: 230), can be used within a CAR, located between the light chain variable region and the hinge.

[0144] In some embodiments, the extracellular binding domain of the CAR comprising the V_H and V_L polypeptides and the mAb-specific epitope(s) may have different structures depending on the position of insertion of the epitope and the use of linkers. For example, the extracellular binding domain of the FLT3 specific CAR comprising mAb-specific epitopes may have one of the following structures:

V₁-L₁-V₂-(L)_x-Epitope1-(L)_x;

V₁-L₁-V₂-(L)_x-Epitope1-(L)_x-Epitope2-(L)_x;

V₁-L₁-V₂-(L)_x-Epitope1-(L)_x-Epitope2-(L)_x-Epitope3-(L)_x;

(L)_x-Epitope1-(L)_x-V₁-L₁-V₂;

(L)_x-Epitope1-(L)_x-Epitope2-(L)_x-V₁-L₁-V₂;

Epitope1-(L)_x-Epitope2-(L)_x-Epitope3-(L)_x-V₁-L₁-V₂;

(L)_x-Epitope1-(L)_x-V₁-L₁-V₂-(L)_x-Epitope2-(L)_x;

(L)_x-Epitope1-(L)_x-V₁-L₁-V₂-(L)_x-Epitope2-(L)_x-Epitope3-(L)_x;

(L)_x-Epitope1-(L)_x-V₁-L₁-V₂-(L)_x-Epitope2-(L)_x-Epitope3-(L)_x-Epitope4-(L)_x;

(L)_x-Epitope1-(L)_x-Epitope2-(L)_x-V₁-L₁-V₂-(L)_x-Epitope3-(L)_x;

(L)_x-Epitope1-(L)_x-Epitope2-(L)_x-V₁-L₁-V₂-(L)_x-Epitope3-(L)_x-Epitope4-(L)_x;

V₁-(L)_x-Epitope1-(L)_x-V₂;

V₁-(L)_x-Epitope1-(L)_x-V₂-(L)_x-Epitope2-(L)_x;

V₁-(L)_x-Epitope1-(L)_x-V₂-(L)_x-Epitope2-(L)_x-Epitope3-(L)_x;

V₁-(L)_x-Epitope1-(L)_x-V₂-(L)_x-Epitope2-(L)_x-Epitope3-(L)_x-Epitope4-(L)_x;

(L)_x-Epitope1-(L)_x-V₁-(L)_x-Epitope2-(L)_x-V₂; or,

(L)_x-Epitope1-(L)_x-V₁-(L)_x-Epitope2-(L)_x-V₂-(L)_x-Epitope3-(L)_x;

wherein,

V₁ is V_L and V₂ is V_H or V₁ is V_H and V₂ is V_L;

L₁ is a linker suitable to link the V_H chain to the V_L chain;

L is a linker comprising glycine and serine residues, and each occurrence of L in the extracellular binding domain can be identical or different to other occurrence of L in the same extracellular binding domain, for example SGGGG, GGGGS or SGGGGS, and,

x is 0 or 1 and each occurrence of x is selected independently from the others; and,

Epitope 1, Epitope 2, Epitope 3 and Epitope 4 are mAb-specific epitopes and can be identical or different. In some embodiments, Epitope 1, Epitope 2 and Epitope 4 are a mAb-specific epitope

having an amino acid sequence of SEQ ID NO: 229 and Epitope 3 is a mAb-specific epitope having an amino acid sequence of SEQ ID NO: 231.

[0145] In some embodiments, the extracellular binding domain of the CAR comprises the following sequence

V₁-L₁-V₂-(L)_x-Epitope1-(L)_x-Epitope2-(L)_x; or,
(L)_x-Epitope1-(L)_x-V₁-L₁-V₂-(L)_x-Epitope2-(L)_x-Epitope3-(L)_x-Epitope4-(L)_x. wherein V₁, V₂, L₁, L, x and Epitope 1, Epitope 2, Epitope 3 and Epitope 4 are as defined above.

[0146] In some embodiments, any one of the FLT3 specific CARs disclosed herein may comprise one or more mAb-specific epitopes selected from 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 $\alpha\beta$ 7 epitope, a BAFF (B-cell activatin factor) epitope, an IL-1 β epitope, a RANKL epitope, a CTLA4 epitope, a CD34 epitope, an IL-12 epitope, and/or an IL-23 epitope.

[0147] In some embodiments, the FLT3 specific CARs disclosed herein may comprise one or more mAb-specific epitopes selected from epitopes 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 and/or ustekinumab.

[0148] In some embodiments, the FLT3 specific CARs comprise one or more mAb-specific epitopes selected from the epitopes disclosed in Table 5:

Table 5: Examples of mAb-specific epitopes that can be used in the extracellular binding domain of the FLT3 specific CARs of the invention such as for example mimotopes and epitope with their corresponding mAb

Rituximab		
Mimotope	SEQ ID NO: 229	CPYSNPSLC
Palivizumab		
Epitope	SEQ ID NO: 255	NSELLSLINDMPITNDQKKLMSNN
Cetuximab		

Mimotope 1	SEQ ID NO: 256	CQFDLSTRRLKC
Mimotope 2	SEQ ID NO: 257	CQYNLSSRALKC
Mimotope 3	SEQ ID NO: 258	CVWQRWQKSYVC
Mimotope 4	SEQ ID NO: 259	CMWDRFSRWYKC
Nivolumab		
Epitope 1	SEQ ID NO: 260	SFVLNWYRMSPSNQTDKLAAPEDR
Epitope 2	SEQ ID NO: 261	SGTYLCGAISLAPKAQIKE
QBEND-10		
Epitope	SEQ ID NO: 262	ELPTQGTFNSNVSTNVSPAKPTTTA
Alemtuzumab		
Epitope	SEQ ID NO: 263	GQNDTSQTSSPS

[0149] The intracellular signaling domain of a CAR according to the invention is responsible for intracellular signaling following the binding of extracellular ligand-binding domain to the target resulting in the activation of the immune cell and immune response. The intracellular signaling domain has the ability to activate of at least one of the normal effector functions of the immune cell in which the CAR is expressed. For example, the effector function of a T cell can be a cytolytic activity or helper activity including the secretion of cytokines.

[0150] In some embodiments, an intracellular signaling domain for use in a CAR can be the cytoplasmic sequences of, for example without limitation, the T cell receptor and co-receptors that act in concert to initiate signal transduction following antigen receptor engagement, as well as any derivative or variant of these sequences and any synthetic sequence that has the same functional capability. Intracellular signaling domains comprise two distinct classes of cytoplasmic signaling sequences: those that initiate antigen-dependent primary activation, and those that act in an antigen-independent manner to provide a secondary or co-stimulatory signal. Primary cytoplasmic signaling sequences can comprise signaling motifs which are known as immunoreceptor tyrosine-based activation motifs of ITAMs. ITAMs are well defined signaling motifs found in the intracytoplasmic tail of a variety of receptors that serve as binding sites for syk/zap70 class tyrosine kinases. Examples of ITAM used in the invention can include as non limiting examples those derived from TCR ζ , FcR γ , FcR β , FcR ϵ , CD3 γ , CD3 δ , CD3 ϵ , CD5, CD22, CD79a, CD79b and CD66d. In some embodiments, the intracellular signaling domain of the CAR can comprise the CD3 ζ signaling domain which has amino acid sequence with at least about 70%, for example at least 80%, or at least 90%, 95%, 97%, or 99% sequence identity with

an amino acid sequence shown in SEQ. ID NO: 210. In some embodiments the intracellular signaling domain of the CAR of the invention comprises a domain of a co-stimulatory molecule.

[0151] In some embodiments, the intracellular signaling domain of a CAR of the invention comprises a part of co-stimulatory molecule selected from the group consisting of fragment of 41BB (GenBank: AAA53133.) and CD28 (NP_006130.1). In some embodiments, the intracellular signaling domain of the CAR of the invention comprises amino acid sequence which comprises at least 70%, for example at least 80%, or at least 90%, 95%, 97%, or 99% sequence identity with an amino acid sequence shown in SEQ. ID NO: 209 and SEQ. ID NO: 213. In some embodiments, the intracellular signaling domain of the CAR of the invention comprises amino acid sequence which comprises at least 70%, for example at least 80%, or at least 90%, 95%, 97%, or 99% sequence identity with an amino acid sequence shown in SEQ. ID NO: 209 and SEQ. ID NO: 214.

[0152] CARs are expressed on the surface membrane of the cell. Thus, the CAR can comprise a transmembrane domain. Suitable transmembrane domains for a CAR disclosed herein have the ability to (a) be expressed at the surface of a cell, e.g. an immune cell such as, for example without limitation, lymphocyte cells or Natural killer (NK) cells, and (b) interact with the ligand-binding domain and intracellular signaling domain for directing cellular response of immune cell against a predefined target cell. The transmembrane domain can be derived either from a natural or from a synthetic source. The transmembrane domain can be derived from any membrane-bound or transmembrane protein. As non-limiting examples, the transmembrane polypeptide can be a subunit of the T cell receptor such as α , β , γ or δ , polypeptide constituting CD3 complex, IL-2 receptor p55 (α chain), p75 (β chain) or γ chain, subunit chain of Fc receptors, in particular Fc γ receptor III, or CD proteins. Alternatively, the transmembrane domain can be synthetic and can comprise predominantly hydrophobic residues such as leucine and valine. In some embodiments said transmembrane domain is derived from the human CD8 α chain (e.g., NP_001139345.1).

[0153] The transmembrane domain is linked to the extracellular ligand-binding domain by a stalk domain (also called hinge domain). A stalk domain may comprise up to 300 amino acids, e.g. 10 to 100 amino acids or 25 to 50 amino acids. Stalk region may be derived from all or part of naturally occurring molecules, such as from all or part of the extracellular region of CD8, CD4, or CD28, or from all or part of an antibody constant region. Alternatively the stalk domain may be a synthetic sequence that corresponds to a naturally occurring stalk sequence, or may be

an entirely synthetic stalk sequence. In some embodiments said stalk domain is a part of human CD8 α chain (e.g., NP_001139345.1). In another particular embodiment, said transmembrane and hinge domains comprise a part of human CD8 α chain, e.g. which comprises at least 70%, or at least 80%, or at least 90%, 95% 97%, or 99% sequence identity with amino acid sequence selected from the group consisting of SEQ ID NO: 206 and 208. In some embodiments, the stalk domain of FLT3 specific CARs described herein comprises a CD8 α hinge, an IgG1 hinge, or an Fc γ RIII α hinge. In some embodiments, the stalk domain comprises a human CD8 α hinge, a human IgG1 hinge, or a human Fc γ RIII α hinge. In some embodiments, CARs disclosed herein can comprise an extracellular ligand-binding domain that specifically binds FLT3, CD8 α human hinge and transmembrane domains, the CD3 ζ signaling domain, and 4-1BB signaling domain.

[0154] Table 6 provides exemplary sequences of domains which can be used in the CARs disclosed herein.

Table 6: Exemplary sequences of CAR Components

Domain	Amino Acid Sequence	SEQ ID NO:
CD8 α signal peptide	MALPVTALLPLALLHAARP	204
Fc γ RIII α hinge	GLAVSTISSFFPPGYQ	205
CD8 α hinge	TTTPAPRPPTAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACD	206
IgG1 hinge	EPKSPDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMIARTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK	207
CD8 α transmembrane (TM) domain	IYIWAPLAGTCGVLLLSLVITLYC	208

Domain	Amino Acid Sequence	SEQ ID NO:
41BB intracellular signaling domain (ISD)	KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEEGG CEL	209
CD3 ζ intracellular signaling domain (ISD)	RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDK RRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIG MKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR	210
Fc ϵ RI α -TM-IC (Fc ϵ RI α chain transmembrane and intracellular domain)	FFIPLLVVILFAVDTGLFISTQQQVTFLLKIKRTRKGFRL NPHPKPNPKNN	211
Fc ϵ RI β - Δ ITAM (Fc ϵ RI β chain without ITAM)	MDTESNRRANLALPQEPSSVPAFEVLEISPQEVSSGRLK SASSPPLHTWLTVLKKEQEFLGVTQILTAMICLCFGTVV CSVLDISHIEGDIFSSFKAGYPFWGAIFFSISGMLSISERR NATYLVRGSLGANTASSIAGGTGITILIINLKKSLEYIH SCQKFFETKCFMASFSTEIVVMMLFLTILGLGSAVSLTIC GAGEELKGKNKVP	212
41BB-IC (41BB co-stimulatory domain)	KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEEGG CEL	213
CD28-IC (CD28 co-stimulatory domain)	RSKRSRGGHSDYMNMTPRRPGPTRKHYQPYAPPRDFAA YRS	214
Fc ϵ RI γ -SP (signal peptide)	MIPAVVLLLLLLVEQAAA	215
Fc ϵ RI γ - Δ ITAM (Fc ϵ RI γ chain without ITAM)	LGEPQLCYILDAILFLYGIVLTLLYCRLKIQRKAAITSYE KS	216

Domain	Amino Acid Sequence	SEQ ID NO:
GSG-P2A (GSG-P2A ribosomal skip polypeptide)	GSGATNFSLLKQAGDVEENPGP	217
GSG-T2A (GSG-T2A ribosomal skip polypeptide)	GSGEGRGSLLTCGDVEENPGP	218

[0155] Downregulation or mutation of target antigens is commonly observed in cancer cells, creating antigen-loss escape variants. Thus, to offset tumor escape and render immune cell more specific to target, the FLT3 specific CAR can comprise one or more additional extracellular ligand-binding domains, to simultaneously bind different elements in target thereby augmenting immune cell activation and function. In some embodiments, the extracellular ligand-binding domains can be placed in tandem on the same transmembrane polypeptide, and optionally can be separated by a linker. In some embodiments, said different extracellular ligand-binding domains can be placed on different transmembrane polypeptides composing the CAR. In some embodiments, the invention relates to a population of CARs, each CAR comprising a different extracellular ligand-binding domain. In a particular, the invention relates to a method of engineering immune cells comprising providing an immune cell and expressing at the surface of the cell a population of CARs, each CAR comprising different extracellular ligand-binding domains. In another particular embodiment, the invention relates to a method of engineering an immune cell comprising providing an immune cell and introducing into the cell polynucleotides encoding polypeptides composing a population of CAR each one comprising different extracellular ligand-binding domains. By population of CARs, it is meant at least two, three, four, five, six or more CARs each one comprising different extracellular ligand-binding domains. The different extracellular ligand-binding domains according to the invention can for example simultaneously bind different elements in target thereby augmenting immune cell activation and function. The invention also relates to an isolated immune cell which comprises a population of CARs each one comprising different extracellular ligand-binding domains.

[0156] In another aspect, provided herein are polynucleotides encoding any of the CARs and polypeptides described herein. Polynucleotides can be made and expressed by procedures known in the art.

[0157] In another aspect, provided herein are compositions (such as a pharmaceutical compositions) comprising any of the cells of the invention. In some embodiments, the composition comprises a cell comprising a polynucleotide encoding any of the CARs described herein. In still other embodiments, the composition comprises either or both of the polynucleotides shown in SEQ ID NO: 245 and SEQ ID NO:246 below:

P3E10 heavy chain variable region

CAGGTGCAGCTGGTGCAGAGCGGAGCAGAGGTGAAGAAGCCAGGCAGCTCCGTGA
AGGTGTCCTGCAAGGCCTCTGGCGGCACATTCTCTAGCTACGCCATCCAGTGGGTGC
GGCAGGCACCAGGACAGGGCCTGGAGTGGATGGGAGGAATCGTGGGAAGCTGGGG
CCTGGCAAACCTACGCCCAGAAGTTTCAGGGCAGAGTGACCATCACACCGATAAGT
CTACAAGCACCGCCTATATGGAGCTGTCCTCTCTGAGGTCCGAGGACACAGCCGTGT
ACTATTGCGCCACCTCCGCCTTCGGCGAGCTGGCATCTTGGGGACAGGGCACACTG
GTGACCGTGAGCTCC (SEQ ID NO: 245)

P3E10 light chain variable region

GAGATCGTGCTGACACAGAGCCCAGGCACCCTGTCCCTGTCTCCAGGAGAGAGGGC
CACTGTCTGTAGGGCCAGCCAGTCCGTGCCTTCTAGCCAGCTGGCCTGGTACCA
GCAGAAGCCAGGCCAGGCCCCCAGACTGCTGATCTATGACGCCTCCTCTAGAGCCA
CAGGCATCCCAGATAGGTTCTCTGGCAGCGGCTCCGGCACCGACTTTACACTGACCA
TCTCCAGGCTGGAGCCCCGAGGATTTCCCGTGTACTATTGCCAGCAGTACGGCAGCT
CCCCTCTGACATTTGGCCAGGGCACCAAGGTGGAGATCAAGG (SEQ ID NO: 246)

[0158] In still other embodiments, the composition comprises either or both of the polynucleotides shown in SEQ ID NO: 247 and SEQ ID NO:248 below:

P3A1 heavy chain variable region

CAGGTGCAGCTGGTGCAGAGCGGAGCAGAGGTGAAGAAGCCCGGCAGCTCCGTGA
AGGTGTCCTGCAAGGCCAGCGGCGGCACATTCTCTAGCTACGATATCTCTTGGGTGA

GGCAGGCCCCAGGCCAGGGACTGGAGTGGATGGGAGGAATCATCCCCGTGAGCGG
AAGGGCAAACACTACGCACAGAAGTTTCAGGGCCGGGTGACCATCACCACAGACAAGT
CCACATCTACCGCCTATATGGAGCTGTCCTCTCTGAGAAGCGAGGATACAGCCGTGT
ACTATTGCGCCAGAGTGAGGCCTACCTACTGGCCACTGGACTATTGGGGCCAGGGC
AACTGGTGACCGTGAGCTCC (SEQ ID NO: 247)

P3A1 light chain variable region

GACATCCAGATGACCCAGTCCCCATCTAGCCTGAGCGCCTCCGTGGGCGATAGAGT
GACAATCACCTGTAGGGCCTCTCAGAGCATCTCCTCTTACCTGAATTGGTATCAGCA
GAAGCCCGGCAAGGCCCCCTAAGCTGCTGATCTACGCAGCAAGCTCCCTGCAGTCCG
GAGTGCCATCTCGGTTCTCCGGCTCTGGCAGCGGCACAGACTTTACACTGACCATCT
CTAGCCTGCAGCCTGAGGATTTGCGCACCTACTATTGCCAGCAGTCCTATTCTACAC
CACTGACCTTTGGCCAGGGCACAAAGGTGGAGATCAAG (SEQ ID NO: 248).

[0159] In still other embodiments, the composition comprises either or both of the polynucleotides shown in SEQ ID NO: 249 and SEQ ID NO:250 below:

P9B5 heavy chain variable region

GAGGTGCAGCTGCTGGAGAGCGGCGGCCTGGTGCAGCCTGGCGGCTCTCTGAG
ACTGAGCTGCGCAGCATCCGGCTTCATCTTTGCCTCTTACGCAATGAGCTGGGTGAG
GCAGGGCCCCTGGCAAGGGACTGGAGTGGGTGAGCGAGATCAGTCCTCTGGCGGCA
GCACCACATATGCCGACTCCGTGAAGGGCCGCTTACAATCAGCCGGGACAACCTCT
AAGAATACCCTGTACCTGCAGATGAACTCCCTGAGAGCCGAGGACACAGCCGTGTA
CTATTGCGCCAGAGATAGAGTGATGGCCGGCCTGGGCTATGACCCATTTGATTACTG
GGGCCAGGGCACACTGGTGACCGTGAGCTCC (SEQ ID NO: 249).

P9B5 light chain variable region

CAGAGCGTGCTGACCCAGCCACCTTCCGCCTCTGGAACACCCGGCCAGAGGGTGAC
CATCAGCTGTTCCGGCTCTAGCTCCAACATCGGCTCCAATTACGTGTATTGGTACCA
GCAGCTGCCCCGGCACAGCCCCTAAGCTGCTGATCTACAGAAACAATCAGAGGCCAT
CTGGCGTGCCCGACCGCTTCTCTGGCAGCAAGTCCGGCACCTCTGCCAGCCTGGCAA
TCAGCGGACTGCGGTCCGAGGACGAGGCCGATTACTATTGCGCAGCATGGGACGAT

TCCCTGTCTGGAGTGGTGTGTTGGCGGCGGCACAAAGCTGACCGTGCTG (SEQ ID NO: 250).

[0160] In still other embodiments, the composition comprises either or both of the polynucleotides shown in SEQ ID NO: 251 and SEQ ID NO:252 below:

P4C7 heavy chain variable region

CAGGTGCAGCTGGTGCAGAGCGGAGCAGAGGTGAAGAAGCCTGGCAGCTCCGTGA
AGGTGTCTTGCAAGGCCAGCGGCGGCACATTCTCTAGCTACACCATCTCCTGGGTGC
GGCAGGCCCCAGGCCAGGGACTGGAGTGGATGGGAGGAATCATCCCAGCCTTCGGC
ATCGCCAACACTACGCCCAGAAGTTTCAGGGCCGCGTGACAATCACCGCCGACAAGTC
CACATCTACCGCCTATATGGAGCTGTCTCTCTGCGGAGCGAGGATACCGCCGTGTA
CTATTGCGCCAAGGGCGGCAGCTACTCCCTGGACTATTTTGATATCTGGGGCCAGGG
CACACTGGTGACCGTGAGCTCC (SEQ ID NO: 251)

P4C7 light chain variable region

GAGATCGTGCTGACACAGTCCCCTGGCACCCCTGTCTCTGAGCCCAGGCGAGAGGGC
CACACTGTCCTGTAGGGCATCTCAGTACGTGTCCGCCTCTCTGCTGGCCTGGTATCA
GCAGAAGCCTGGCCAGGCCCCAAGACTGCTGATCTACGGAGCATCCACAAGAGCCA
CCGGCATCCCCGACAGGTTACGCGGCTCCGGCTCTGGAACCGACTTCACCTGACCA
TCTCTAGACTGGAGCCTGAGGACTTCGCCGTGTACTATTGCCAGCAGTATGCCAGGT
CTAGCACATTTGGCCAGGGCACCAAGGTGGAGATCAAG (SEQ ID NO: 252)

[0161] Expression vectors, and administration of polynucleotide compositions are further described herein.

[0162] In another aspect, provided herein is a method of making any of the polynucleotides described herein.

[0163] Polynucleotides complementary to any such sequences are also encompassed by the invention. Polynucleotides may be single-stranded (coding or antisense) or double-stranded, and may be DNA (genomic, cDNA or synthetic) or RNA molecules. RNA molecules include HnRNA molecules, which contain introns and correspond to a DNA molecule in a one-to-one manner, and mRNA molecules, which do not contain introns. Additional coding or non-coding

sequences may, but need not, be present within a polynucleotide of the invention, and a polynucleotide may, but need not, be linked to other molecules and/or support materials.

[0164] Polynucleotides may comprise a native sequence (i.e., an endogenous sequence that encodes an antibody or a portion thereof) or may comprise a variant of such a sequence. Polynucleotide variants contain one or more substitutions, additions, deletions and/or insertions such that the immunoreactivity of the encoded polypeptide is not diminished, relative to a native immunoreactive molecule. The effect on the immunoreactivity of the encoded polypeptide may generally be assessed as described herein. Variants generally exhibit at least about 70% identity, or at least about 80% identity, or even at least about 90% or 95% identity to a polynucleotide sequence that encodes a native antibody or a portion thereof.

[0165] Two polynucleotide or polypeptide sequences are said to be “identical” if the sequence of nucleotides or amino acids in the two sequences is the same when aligned for maximum correspondence as described below. Comparisons between two sequences are typically performed by comparing the sequences over a comparison window to identify and compare local regions of sequence similarity. A “comparison window” as used herein, refers to a segment of at least about 20 contiguous positions, usually 30 to about 75, or 40 to about 50, in which a sequence may be compared to a reference sequence of the same number of contiguous positions after the two sequences are optimally aligned.

[0166] Optimal alignment of sequences for comparison may be conducted using the Megalign program in the Lasergene suite of bioinformatics software (DNASTAR, Inc., Madison, WI), using default parameters. This program embodies several alignment schemes described in the following references: Dayhoff, M.O., 1978, A model of evolutionary change in proteins - Matrices for detecting distant relationships. In Dayhoff, M.O. (ed.) Atlas of Protein Sequence and Structure, National Biomedical Research Foundation, Washington DC Vol. 5, Suppl. 3, pp. 345-358; Hein J., 1990, Unified Approach to Alignment and Phylogenies pp. 626-645 Methods in Enzymology vol. 183, Academic Press, Inc., San Diego, CA; Higgins, D.G. and Sharp, P.M., 1989, CABIOS 5:151-153; Myers, E.W. and Muller W., 1988, CABIOS 4:11-17; Robinson, E.D., 1971, Comb. Theor. 11:105; Santou, N., Nes, M., 1987, Mol. Biol. Evol. 4:406-425; Sneath, P.H.A. and Sokal, R.R., 1973, Numerical Taxonomy the Principles and Practice of Numerical Taxonomy, Freeman Press, San Francisco, CA; Wilbur, W.J. and Lipman, D.J., 1983, Proc. Natl. Acad. Sci. USA 80:726-730.

[0167] Generally, the “percentage of sequence identity” is determined by comparing two optimally aligned sequences over a window of comparison of at least 20 positions, wherein the portion of the polynucleotide or polypeptide sequence in the comparison window may comprise additions or deletions (i.e., gaps) of 20 percent or less, usually 5 to 15 percent, or 10 to 12 percent, as compared to the reference sequences (which does not comprise additions or deletions) for optimal alignment of the two sequences. The percentage is calculated by determining the number of positions at which the identical nucleic acid bases or amino acid residue occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the reference sequence (i.e. the window size) and multiplying the results by 100 to yield the percentage of sequence identity.

[0168] Variants may also, or alternatively, be substantially homologous to a native gene, or a portion or complement thereof. Such polynucleotide variants are capable of hybridizing under moderately stringent conditions to a naturally occurring DNA sequence encoding a native antibody (or a complementary sequence).

[0169] Suitable “moderately stringent conditions” include prewashing in a solution of 5 X SSC, 0.5% SDS, 1.0 mM EDTA (pH 8.0); hybridizing at 50°C-65°C, 5 X SSC, overnight; followed by washing twice at 65°C for 20 minutes with each of 2X, 0.5X and 0.2X SSC containing 0.1 % SDS.

[0170] As used herein, “highly stringent conditions” or “high stringency conditions” are those that: (1) employ low ionic strength and high temperature for washing, for example 0.015 M sodium chloride/0.0015 M sodium citrate/0.1% sodium dodecyl sulfate at 50°C; (2) employ during hybridization a denaturing agent, such as formamide, for example, 50% (v/v) formamide with 0.1% bovine serum albumin/0.1% Ficoll/0.1% polyvinylpyrrolidone/50 mM sodium phosphate buffer at pH 6.5 with 750 mM sodium chloride, 75 mM sodium citrate at 42°C; or (3) employ 50% formamide, 5 x SSC (0.75 M NaCl, 0.075 M sodium citrate), 50 mM sodium phosphate (pH 6.8), 0.1% sodium pyrophosphate, 5 x Denhardt’s solution, sonicated salmon sperm DNA (50 µg/ml), 0.1% SDS, and 10% dextran sulfate at 42°C, with washes at 42°C in 0.2 x SSC (sodium chloride/sodium citrate) and 50% formamide at 55°C, followed by a high-stringency wash consisting of 0.1 x SSC containing EDTA at 55°C. The skilled artisan will recognize how to adjust the temperature, ionic strength, etc. as necessary to accommodate factors such as probe length and the like.

[0171] It will be appreciated by those of ordinary skill in the art that, as a result of the degeneracy of the genetic code, there are many nucleotide sequences that encode a polypeptide as described herein. Some of these polynucleotides bear minimal homology to the nucleotide sequence of any native gene. Nonetheless, polynucleotides that vary due to differences in codon usage are specifically contemplated by the invention. Further, alleles of the genes comprising the polynucleotide sequences provided herein are within the scope of the invention. Alleles are endogenous genes that are altered as a result of one or more mutations, such as deletions, additions and/or substitutions of nucleotides. The resulting mRNA and protein may, but need not, have an altered structure or function. Alleles may be identified using standard techniques (such as hybridization, amplification and/or database sequence comparison).

[0172] The polynucleotides of this invention can be obtained using chemical synthesis, recombinant methods, or PCR. Methods of chemical polynucleotide synthesis are well known in the art and need not be described in detail herein. One of skill in the art can use the sequences provided herein and a commercial DNA synthesizer to produce a desired DNA sequence.

[0173] For preparing polynucleotides using recombinant methods, a polynucleotide comprising a desired sequence can be inserted into a suitable vector, and the vector in turn can be introduced into a suitable host cell for replication and amplification, as further discussed herein. Polynucleotides may be inserted into host cells by any means known in the art. Cells are transformed by introducing an exogenous polynucleotide by direct uptake, endocytosis, transfection, F-mating or electroporation. Once introduced, the exogenous polynucleotide can be maintained within the cell as a non-integrated vector (such as a plasmid) or integrated into the host cell genome. The polynucleotide so amplified can be isolated from the host cell by methods well known within the art. See, e.g., Sambrook et al., 1989.

[0174] Alternatively, PCR allows reproduction of DNA sequences. PCR technology is well known in the art and is described in U.S. Patent Nos. 4,683,195, 4,800,159, 4,754,065 and 4,683,202, as well as PCR: The Polymerase Chain Reaction, Mullis et al. eds., Birkhäuser Press, Boston, 1994.

[0175] RNA can be obtained by using the isolated DNA in an appropriate vector and inserting it into a suitable host cell. When the cell replicates and the DNA is transcribed into RNA, the RNA can then be isolated using methods well known to those of skill in the art, as set forth in Sambrook et al., 1989, *supra*, for example.

[0176] Suitable cloning vectors may be constructed according to standard techniques, or may be selected from a large number of cloning vectors available in the art. While the cloning vector selected may vary according to the host cell intended to be used, useful cloning vectors will generally have the ability to self-replicate, may possess a single target for a particular restriction endonuclease, and/or may carry genes for a marker that can be used in selecting clones containing the vector. Suitable examples include plasmids and bacterial viruses, e.g., pUC18, pUC19, Bluescript (e.g., pBS SK+) and its derivatives, mp18, mp19, pBR322, pMB9, ColE1, pCR1, RP4, phage DNAs, and shuttle vectors such as pSA3 and pAT28. These and many other cloning vectors are available from commercial vendors such as BioRad, Strategene, and Invitrogen.

[0177] Expression vectors generally are replicable polynucleotide constructs that contain a polynucleotide according to the invention. It is implied that an expression vector must be replicable in the host cells either as episomes or as an integral part of the chromosomal DNA. Suitable expression vectors include but are not limited to plasmids, viral vectors, including adenoviruses, adeno-associated viruses, retroviruses, cosmids, and expression vector(s) disclosed in PCT Publication No. WO 87/04462. Vector components may generally include, but are not limited to, one or more of the following: a signal sequence; an origin of replication; one or more marker genes; suitable transcriptional controlling elements (such as promoters, enhancers and terminator). For expression (i.e., translation), one or more translational controlling elements are also usually required, such as ribosome binding sites, translation initiation sites, and stop codons.

[0178] The vectors containing the polynucleotides of interest can be introduced into the host cell by any of a number of appropriate means, including electroporation, transfection employing calcium chloride, rubidium chloride, calcium phosphate, DEAE-dextran, or other substances; microprojectile bombardment; lipofection; and infection (e.g., where the vector is an infectious agent such as vaccinia virus). The choice of introducing vectors or polynucleotides will often depend on features of the host cell.

[0179] A polynucleotide encoding a FLT3 specific CAR disclosed herein may exist in an expression cassette or expression vector (e.g., a plasmid for introduction into a bacterial host cell, or a viral vector such as a baculovirus vector for transfection of an insect host cell, or a plasmid or viral vector such as a lentivirus for transfection of a mammalian host cell). In some embodiments, a polynucleotide or vector can include a nucleic acid sequence encoding

ribosomal skip sequences such as, for example without limitation, a sequence encoding a 2A peptide. 2A peptides, which were identified in the Aphthovirus subgroup of picornaviruses, causes a ribosomal “skip” from one codon to the next without the formation of a peptide bond between the two amino acids encoded by the codons (see (Donnelly and Elliott 2001; Atkins, Wills et al. 2007; Doronina, Wu et al. 2008)). By “codon” is meant three nucleotides on an mRNA (or on the sense strand of a DNA molecule) that are translated by a ribosome into one amino acid residue. Thus, two polypeptides can be synthesized from a single, contiguous open reading frame within an mRNA when the polypeptides are separated by a 2A oligopeptide sequence that is in frame. Such ribosomal skip mechanisms are well known in the art and are known to be used by several vectors for the expression of several proteins encoded by a single messenger RNA.

[0180] To direct transmembrane polypeptides into the secretory pathway of a host cell, in some embodiments, a secretory signal sequence (also known as a leader sequence, prepro sequence or pre sequence) is provided in a polynucleotide sequence or vector sequence. The secretory signal sequence is operably linked to the transmembrane nucleic acid sequence, i.e., the two sequences are joined in the correct reading frame and positioned to direct the newly synthesized polypeptide into the secretory pathway of the host cell. Secretory signal sequences are commonly positioned 5' to the nucleic acid sequence encoding the polypeptide of interest, although certain secretory signal sequences may be positioned elsewhere in the nucleic acid sequence of interest (see, e.g., Welch et al., U.S. Patent No. 5,037,743; Holland et al., U.S. Patent No. 5,143,830). In some embodiments the signal peptide comprises the amino acid sequence shown in SEQ ID NO: 204 or 215. Those skilled in the art will recognize that, in view of the degeneracy of the genetic code, considerable sequence variation is possible among these polynucleotide molecules. In some embodiments, nucleic acid sequences of the invention are codon-optimized for expression in mammalian cells, e.g. for expression in primate (e.g. human or monkey) cells. Codon-optimization refers to the exchange in a sequence of interest of codons that are generally rare in highly expressed genes of a given species by codons that are generally frequent in highly expressed genes of such species, such codons encoding the amino acids as the codons that are being exchanged.

Methods of engineering an immune cell

[0181] Methods of preparing immune cells for use in immunotherapy are provided herein. In some embodiments, the methods comprise obtaining immune cells, introducing a CAR according to the invention into immune cells, and expanding the cells. In some embodiments, the invention relates to a method of engineering an immune cell comprising: providing a cell and expressing at the surface of the cell at least one CAR as described above. Methods for engineering immune cells are described in, for example, PCT Patent Application Publication Nos. WO/2014/039523, WO/2014/184741, WO/2014/191128, WO/2014/184744, and WO/2014/184143, each of which is incorporated herein by reference in its entirety. In some embodiments, the method comprises: transfecting the cell with at least one polynucleotide encoding CAR as described above, and expressing the polynucleotides in the cell.

[0182] Prior to engineering of cells, a source of cells can be obtained from a subject through a variety of non-limiting methods. Cells can be obtained from a number of non-limiting sources, including peripheral blood mononuclear cells, bone marrow, lymph node tissue, cord blood, thymus tissue, tissue from a site of infection, ascites, pleural effusion, spleen tissue, and tumors. In some embodiments, any number of T cell lines available and known to those skilled in the art, may be used. In some embodiments, cells can be derived from a healthy donor or from a donor suffering from a disease or disorder, for example, an individual diagnosed with cancer or from an individual diagnosed with an infection. In some embodiments, cells can be part of a mixed population of cells which present different phenotypic characteristics.

[0183] In some embodiments, the polynucleotides are present in lentiviral vectors for stable expression in the cells.

[0184] In some embodiments, the method can further comprise a step of genetically modifying a cell by disrupting or inactivating at least one gene expressing, for example without limitation, a component of the TCR, a target for an immunosuppressive agent, an HLA gene, and/or an immune checkpoint protein such as, for example, PDCD1 or CTLA-4. By disruption or inactivating a gene it is intended that the gene of interest is not expressed in a functional protein form. In some embodiments, the gene to be disrupted or inactivated is selected from the group consisting of, for example without limitation, TCR α , TCR β , CD52, glucocorticoid receptor (GR), deoxycytidine kinase (DCK), PD-1, and CTLA-4. In some embodiments, the method comprises inactivating one or more genes by introducing into the cells a rare-cutting endonuclease able to selectively inactivate a gene by selective DNA cleavage. In some

embodiments the rare-cutting endonuclease can be, for example, a transcription activator-like effector nuclease (TALE-nuclease) or Cas9 endonuclease.

[0185] In some embodiments, an additional catalytic domain is used with a rare-cutting endonuclease to enhance its capacity to inactivate targeted genes. For example, an additional catalytic domain can be a DNA end-processing enzyme. Non-limiting examples of DNA end-processing enzymes include 5-3' exonucleases, 3-5' exonucleases, 5-3' alkaline exonucleases, 5' flap endonucleases, helicases, phosphatase, hydrolases and template-independent DNA polymerases. Non-limiting examples of such catalytic domain comprise of a protein domain or catalytically active derivative of the protein domain selected from the group consisting of hExoI (EXO1_HUMAN), Yeast ExoI (EXO1_YEAST), *E. coli* ExoI, Human TREX2, Mouse TREX1, Human TREX1, Bovine TREX1, Rat TREX1, TdT (terminal deoxynucleotidyl transferase) Human DNA2, Yeast DNA2 (DNA2_YEAST). In some embodiments, an additional catalytic domain can have a 3'-5'-exonuclease activity, and In some embodiments, said additional catalytic domain is TREX, e.g. a TREX2 catalytic domain (WO2012/058458). In some embodiments, said catalytic domain is encoded by a single chain TREX polypeptide. The additional catalytic domain may be fused to a nuclease fusion protein or chimeric protein. In some embodiments, the additional catalytic domain is fused using, for example, a peptide linker.

[0186] In some embodiments, the method further comprises a step of introducing into cells an exogenous nucleic acid comprising at least a sequence homologous to a portion of the target nucleic acid sequence, such that homologous recombination occurs between the target nucleic acid sequence and the exogenous nucleic acid. In some embodiments, said exogenous nucleic acid comprises first and second portions which are homologous to region 5' and 3' of the target nucleic acid sequence, respectively. The exogenous nucleic acid may also comprise a third portion positioned between the first and the second portion which comprises no homology with the regions 5' and 3' of the target nucleic acid sequence. Following cleavage of the target nucleic acid sequence, a homologous recombination event is stimulated between the target nucleic acid sequence and the exogenous nucleic acid. In some embodiments, homologous sequences of at least about 50 bp, greater than about 100 bp, or greater than about 200 bp can be used within the donor matrix. The exogenous nucleic acid can be, for example without limitation, from about 200 bp to about 6000 bp, e.g. from about 1000 bp to about 2000 bp. Shared nucleic acid homologies are located in regions flanking upstream and downstream the site of the break, and the nucleic acid sequence to be introduced is located between the two arms.

[0187] In some embodiments, a nucleic acid successively comprises a first region of homology to sequences upstream of said cleavage; a sequence to inactivate a targeted gene selected from the group consisting of TCR α , TCR β , CD52, glucocorticoid receptor (GR), deoxycytidine kinase (DCK), and an immune checkpoint protein such as for example programmed death-1 (PD-1); and a second region of homology to sequences downstream of the cleavage. The polynucleotide introduction step can be simultaneous, before or after the introduction or expression of the rare-cutting endonuclease. Depending on the location of the target nucleic acid sequence wherein break event has occurred, such exogenous nucleic acid can be used to knock-out a gene, e.g. when exogenous nucleic acid is located within the open reading frame of the gene, or to introduce new sequences or genes of interest. Sequence insertions by using such exogenous nucleic acid can be used to modify a targeted existing gene, by correction or replacement of the gene (allele swap as a non-limiting example), or to up- or down-regulate the expression of the targeted gene (promoter swap as non-limiting example), the targeted gene correction or replacement. In some embodiments, inactivation of a gene selected from the group consisting of TCR α , TCR β , CD52, GR, DCK, and immune checkpoint proteins, can be done at a precise genomic location targeted by a specific TALE-nuclease, wherein said specific TALE-nuclease catalyzes a cleavage and wherein the exogenous nucleic acid successively comprising at least a region of homology and a sequence to inactivate one targeted gene selected from the group consisting of TCR α , TCR β , CD52, GR, DCK, immune checkpoint proteins which is integrated by homologous recombination. In some embodiments, several genes can be, successively or at the same time, inactivated by using several TALE-nucleases respectively and specifically targeting one defined gene and several specific polynucleotides for specific gene inactivation.

[0188] In some embodiments, the method comprises inactivation of one or more additional genes selected from the group consisting of TCR α , TCR β , CD52, GR, DCK, and immune checkpoint proteins. In some embodiments, inactivation of a gene can be accomplished by introducing into the cells at least one rare-cutting endonuclease such that the rare-cutting endonuclease specifically catalyzes cleavage in a targeted sequence of the cell genome; and optionally, introducing into the cells an exogenous nucleic acid successively comprising a first region of homology to sequences upstream of the cleavage, a sequence to be inserted in the genome of the cell, and a second region of homology to sequences downstream of the cleavage; wherein the introduced exogenous nucleic acid inactivates a gene and integrates at least one

exogenous polynucleotide sequence encoding at least one recombinant protein of interest. In some embodiments, the exogenous polynucleotide sequence is integrated within a gene encoding a protein selected from the group consisting of TCR α , TCR β , CD52, GR, DCK, and immune checkpoint protein.

[0189] In another aspect, a step of genetically modifying cells can comprise: modifying T cells by inactivating at least one gene expressing a target for an immunosuppressive agent, and; expanding the cells, optionally in presence of the immunosuppressive agent. An immunosuppressive agent is an agent that suppresses immune function by one of several mechanisms of action. An immunosuppressive agent can diminish the extent and/or voracity of an immune response. Non-limiting examples of immunosuppressive agents include calcineurin inhibitors, targets of rapamycin, interleukin-2 α -chain blockers, inhibitors of inosine monophosphate dehydrogenase, inhibitors of dihydrofolic acid reductase, corticosteroids, and immunosuppressive antimetabolites. Some cytotoxic immunosuppressants act by inhibiting DNA synthesis. Others may act through activation of T cells or by inhibiting the activation of helper cells. The methods according to the invention allow conferring immunosuppressive resistance to T cells for immunotherapy by inactivating the target of the immunosuppressive agent in T cells. As non-limiting examples, targets for immunosuppressive agent can be a receptor for an immunosuppressive agent such as for example without limitation CD52, glucocorticoid receptor (GR), FKBP family gene members, and cyclophilin family gene members.

[0190] In some embodiments, the genetic modification of the method involves expression, in provided cells to engineer, of one rare-cutting endonuclease such that the rare-cutting endonuclease specifically catalyzes cleavage in one targeted gene, thereby inactivating the targeted gene. In some embodiments, a method of engineering cells comprises at least one of the following steps: providing a T cell, such as from a cell culture or from a blood sample; selecting a gene in the T cell expressing a target for an immunosuppressive agent; introducing into the T cell a rare-cutting endonuclease able to selectively inactivate by DNA cleavage, e.g. by double-strand break the gene encoding a target for the immunosuppressive agent, and expanding the cells, optionally in presence of the immunosuppressive agent.

[0191] In some embodiments, the method comprises: providing a T cell, such as from a cell culture or from a blood sample; selecting a gene in the T cell wherein the gene expresses a target for an immunosuppressive agent; transfecting the T cell with nucleic acid encoding a rare-

cutting endonuclease able to selectively inactivate by DNA cleavage, for example by double-strand break the gene encoding a target for the immunosuppressive agent, and expressing the rare-cutting endonucleases into the T cells; and expanding the cells, optionally in presence of the immunosuppressive agent.

[0192] In some embodiments, the rare-cutting endonuclease specifically targets CD52 or GR. In some embodiments, the gene selected for inactivation encodes CD52, and the immunosuppressive treatment comprises a humanized antibody targeting CD52 antigen. In some embodiments, the gene selected for inactivation encodes GR, and the immunosuppressive treatment comprises a corticosteroid such as dexamethasone. In some embodiments, the gene selected for inactivation is a FKBP family gene member or a variant thereof and the immunosuppressive treatment comprises FK506, also known as Tacrolimus or fujimycin. In some embodiments, the FKBP family gene member is FKBP12 or a variant thereof. In some embodiments, gene selected for inactivation is a cyclophilin family gene member or a variant thereof and the immunosuppressive treatment comprises cyclosporine.

[0193] In some embodiments, the rare-cutting endonuclease can be, for example, a meganuclease, a zinc finger nuclease, or a TALE-nuclease (TALEN). In some embodiments, the rare-cutting endonuclease is a TALE-nuclease.

[0194] Also provided herein are methods of engineering T cells, suitable for immunotherapy, wherein the methods comprise: genetically modifying T cells by inactivating at least immune checkpoint protein. In some embodiments the immune checkpoint protein is, for example, PD-1 and/or CTLA-4. In some embodiments, methods of genetically modifying a cell comprises: modifying T cells by inactivating at least one immune checkpoint protein; and expanding the cells. Immune checkpoint proteins include, but are not limited to Programmed Death 1 (PD-1, also known as PDCD1 or CD279, accession number: NM_005018), Cytotoxic T-Lymphocyte Antigen 4 (CTLA-4, also known as CD152, GenBank accession number AF414120.1), LAG3 (also known as CD223, accession number: NM_002286.5), Tim3 (also known as HAVCR2, GenBank accession number: JX049979.1), BTLA (also known as CD272, accession number: NM_181780.3), BY55 (also known as CD160, GenBank accession number: CR541888.1), TIGIT (also known as VSTM3, accession number: NM_173799), B7H5 (also known as C10orf54, homolog of mouse vista gene, accession number: NM_022153.1), LAIR1 (also known as CD305, GenBank accession number: CR542051.1), SIGLEC10 (GeneBank accession number: AY358337.1), 2B4 (also known as CD244, accession number: NM_001166664.1),

which directly inhibit immune cells. For example, CTLA-4 is a cell-surface protein expressed on certain CD4 and CD8 T cells; when engaged by its ligands (B7-1 and B7-2) on antigen presenting cells, T cell activation and effector function are inhibited.

[0195] In some embodiments, said method to engineer cells comprises at least one of the following steps: providing a T cell, such as from a cell culture or from a blood sample; introducing into the T cell a rare-cutting endonuclease able to selectively inactivate by DNA cleavage, for example by double-strand break one gene encoding a immune checkpoint protein; and expanding the cells. In some embodiments, the method comprises: providing a T cell, such as from a cell culture or from a blood sample; transfecting said T cell with nucleic acid encoding a rare-cutting endonuclease able to selectively inactivate by DNA cleavage, for example by double-strand break a gene encoding a immune checkpoint protein; expressing the rare-cutting endonucleases into the T cells; expanding the cells. In some embodiments, the rare-cutting endonuclease specifically targets a gene selected from the group consisting of: PD-1, CTLA-4, LAG3, Tim3, BTLA, BY55, TIGIT, B7H5, LAIR1, SIGLEC10, 2B4, TCR α , and TCR β . In some embodiments, the rare-cutting endonuclease can be a meganuclease, a zinc finger nuclease or a TALE-nuclease. In some embodiments, the rare-cutting endonuclease is a TALE-nuclease.

[0196] In some embodiments, the present invention can be particularly suitable for allogeneic immunotherapy. In such embodiments, cells may be modified by a method comprising: inactivating at least one gene encoding a component of the T cell receptor (TCR) in T cells; and expanding the T cells. In some embodiments, the genetic modification of the method relies on the expression, in provided cells to engineer, of one rare-cutting endonuclease such that the rare-cutting endonuclease specifically catalyzes cleavage in one targeted gene thereby inactivating the targeted gene. In some embodiments, said method to engineer cells comprises at least one of the following steps: providing a T cell, such as from a cell culture or from a blood sample; introducing into the T cell a rare-cutting endonuclease able to selectively inactivate by DNA cleavage, for example by double-strand break at least one gene encoding a component of the T cell receptor (TCR), and expanding the cells.

[0197] In some embodiments, the method comprises: providing a T cell, such as from a cell culture or from a blood sample; transfecting said T cell with nucleic acid encoding a rare-cutting endonuclease able to selectively inactivate by DNA cleavage, for example by double-strand break at least one gene encoding a component of the T cell receptor (TCR); expressing the rare-

LLPVLCQAHGLTPQQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNGG
GRPAAE (SEQ ID NO: 220).

Repeat TRBC T01-L

LTPQQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNGGGKQALETVQR
LLPVLCQAHGLTPQQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNGG
GKQALETVQRLLPVLCQAHGLTPQQVVAIASNNGGGKQALETVQRLLPVLCQAHGLTPQ
QVVAIASNNGGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNNGGKQALETVQRLLPV
LCQAHGLTPEQVVAIASNIGGKQALETVQALLPVLCQAHGLTPQQVVAIASNNGGKQA
LETVQRLLPVLCQAHGLTPEQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPEQVVAI
ASHDGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNIGGKQALETVQALLPVLCQAH
GLTPQQVVAIASNNGGGKQALETVQRLLPVLCQAHGLTPEQVVAIASHDGGKQALETVQ
RLLPVLCQAHGLTPEQVVAIASNIGGKQALETVQALLPVLCQAHGLTPQQVVAIASNGG
GRPAAE (SEQ ID NO: 221).

Repeat TRBC T01-R

NPQRSTVWYLTTPQQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNGGG
KQALETVQRLLPVLCQAHGLTPQQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPQQ
VVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASHDGGKQALETVQRLLPVLC
QAHGLTPEQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNNGGGKQAL
ETVQRLLPVLCQAHGLTPQQVVAIASNNGGGKQALETVQRLLPVLCQAHGLTPQQVVAI
ASNNGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNNGGGKQALETVQRLLPVLCQA
HGLTPQQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNNGGKQALETV
QRLLPVLCQAHGLTPQQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPQQVVAIASN
GGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNNGGKQALETVQRLLPVLCQAHGLT
PQQVVAIASNNGGGRPAAE (SEQ ID NO: 222).

Repeat TRBC T02-L

LTPEQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPEQVVAIASHDGGKQALETVQR
LLPVLCQAHGLTPEQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNIGG
KQALETVQALLPVLCQAHGLTPEQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPEQ
VVAIASHDGGKQALETVQRLLPVLCQAHGLTPEQVVAIASHDGGKQALETVQRLLPVLC

CQAHGLTPQQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNIGGKQAL
ETVQALLPVLCQAHGLTPQQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPQQVVAI
ASNNGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNGGGKQALETVQRLLPVLCQA
HGLTPEQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNNGGKQALETV
QRLLPVLCQAHGLTPEQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPQQVVAIASN
GGGRPALE (SEQ ID NO: 223).

Repeat TRBC_T02-R

LTPQQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNNGGKQALETVQR
LLPVLCQAHGLTPQQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNIGG
KQALETVQALLPVLCQAHGLTPQQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQ
VVAIASNIGGKQALETVQALLPVLCQAHGLTPQQVVAIASNGGGKQALETVQRLLPVLC
QAHGLTPEQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNGGGKQAL
ETVQRLLPVLCQAHGLTPEQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPQQVVAI
ASNNGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNNGGKQALETVQRLLPVLCQA
HGLTPEQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNGGGKQALETV
QRLLPVLCQAHGLTPQQVVAIASNGGGKQALETVQRLLPVLCQAHGLTPQQVVAIASN
GGGRPALE (SEQ ID NO: 224).

Repeat CD52_T02-L

LTPQQVVAIASNGGGKQALETVQRLLPVLCQAHGLTPEQVVAIASHDGGKQALETVQR
LLPVLCQAHGLTPEQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNGG
GKQALETVQRLLPVLCQAHGLTPEQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPE
QVVAIASHDGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNGGGKQALETVQRLLPV
LCQAHGLTPEQVVAIASNIGGKQALETVQALLPVLCQAHGLTPEQVVAIASHDGGKQA
LETVQRLLPVLCQAHGLTPQQVVAIASNGGGKQALETVQRLLPVLCQAHGLTPEQVVA
IASHDGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNIGGKQALETVQALLPVLCQA
HGLTPEQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPEQVVAIASHDGGKQALETVQ
RLLPVLCQAHGLTPEQVVAIASNIGGKQALETVQALLPVLCQAHGLTPQQVVAIASNGG
GRPALE (SEQ ID NO: 225).

Repeat CD52_T02-R

LTPQQVVAIASNGGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNNGGKQALETVQR
 LLPVLCQAHGLTPEQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNGG
 GKQALETVQRLLPVLCQAHGLTPEQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPQ
 QVVAIASNGGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNGGGKQALETVQRLLPV
 LCQAHGLTPEQVVAIASNIGGKQALETVQALLPVLCQAHGLTPEQVVAIASHDGGKQA
 LETVQRLLPVLCQAHGLTPEQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPQQVVA
 IASNGGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNNGGKQALETVQRLLPVLCQA
 HGLTPQQVVAIASNGGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNIGGKQALETV
 QALLPVLCQAHGLTPEQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPQQVVAIASN
 GGGRPALE (SEQ ID NO: 226).

[0199] In another aspect, one another step of genetically modifying cell can be a method of expanding TCR α deficient T cells comprising introducing into the T cell pT α (also known as preTCR α) or a functional variant thereof and expanding the cells, optionally through stimulation of the CD3 complex. In some embodiments, the method comprises: a) transfecting the cells with nucleic acid encoding at least a fragment of pT α to support CD3 surface expression; b) expressing said pT α into the cells; and c) expanding the cells, optionally through stimulation of the CD3 complex.

[0200] Also provided are methods of preparing T cells for immunotherapy comprising steps of the method for expansion for T cell. In some embodiments, the pTα polynucleotide sequence can be introduced randomly or by homologous recombination. In some embodiments, the insertion can be associated with the inactivation of the TCRα gene.

[0201] Different functional variants of pTα can be used. A “functional variant” of the peptide refers to a molecule substantially similar to either the entire peptide or a fragment thereof. A “fragment” of the pTα or functional variant thereof refers to any subset of the molecule, that is, a shorter peptide than the full-length pTα. In some embodiments, pTα or functional variants can be, for example, full-length pTα or a C-terminal truncated pTα version. C-terminal truncated pTα lacks in C-terminal end one or more residues. As non limiting examples, C-terminal truncated pTα version lacks 18, 48, 62, 78, 92, 110 or 114 residues from the C-terminus of the protein. Amino acid sequence variants of the peptide can be prepared by mutations in the DNA which encodes the peptide. Such functional variants include, for example, deletions from, or insertions or substitutions of, residues within the amino acid sequence. Any combination of

deletion, insertion, and substitution may also be made to arrive at the final construct, provided that the final construct possesses the desired activity, in particular the restoration of a functional CD3 complex. In an exemplary embodiment, at least one mutation is introduced in the different pT α versions as described above to affect dimerization. As non limiting example, mutated residue can be at least W46R, D22A, K24A, R102A or R117A of the human pT α protein or aligned positions using CLUSTALW method on pT α family or homologue member. For example pT α or variant thereof as described above comprise the mutated residue W46R or the mutated residues D22A, K24A, R102A and R117A. In some embodiments, said pT α or variants are also fused to a signal-transducing domain such as CD28, OX40, ICOS, CD27, CD137 (4-1BB) and CD8 as non limiting examples. The extracellular domain of pT α or variants as described above can be fused to a fragment of the TCR α protein, particularly the transmembrane and intracellular domain of TCR α . pT α variants can also be fused to the intracellular domain of TCR α .

[0202] In some embodiments, pT α versions can be fused to an extracellular ligand-binding domain. In some embodiments, pT α or functional variant thereof is fused to a single chain antibody fragment (scFv) comprising the light and the heavy variable fragment of a target antigen specific monoclonal antibody joined by a flexible linker.

[0203] The term “TCR α deficient T cell” refers to an isolated T cell that lacks expression of a functional TCR α chain. This may be accomplished by different means, as non limiting examples, by engineering a T cell such that it does not express any functional TCR α on its cell surface or by engineering a T cell such that it produces very little functional TCR α chain on its surface or by engineering a T cell to express mutated or truncated form of TCR α chain. TCR α deficient cells can no longer be expanded through CD3 complex. Thus, to overcome this problem and to allow proliferation of TCR α deficient cells, pT α or functional variant thereof is introduced into the cells, thus restoring a functional CD3 complex. In some embodiments, the method further comprises introducing into said T cells rare-cutting endonucleases able to selectively inactivate by DNA cleavage one gene encoding one component of the T cell receptor (TCR). In some embodiments, the rare-cutting endonuclease is a TALE-nuclease.

[0204] In some embodiments, polynucleotides encoding polypeptides according to the present invention can be mRNA which is introduced directly into the cells, for example by electroporation. In some embodiments, cytoPulse technology can be used to transiently

permeabilize living cells for delivery of material into the cells. Parameters can be modified in order to determine conditions for high transfection efficiency with minimal mortality.

[0205] Also provided herein are methods of transfecting T cell. In some embodiments, the method comprises: contacting a T cell with RNA and applying to T cell an agile pulse sequence consisting of: (a) an electrical pulse with a voltage range from about 2250 to 3000 V per centimeter; (b) a pulse width of 0.1 ms; (c) a pulse interval of about 0.2 to 10 ms between the electrical pulses of step (a) and (b); (d) an electrical pulse with a voltage range from about 2250 to 3000 V with a pulse width of about 100 ms and a pulse interval of about 100 ms between the electrical pulse of step (b) and the first electrical pulse of step (c); and (e) four electrical pulses with a voltage of about 325 V with a pulse width of about 0.2 ms and a pulse interval of 2 ms between each of 4 electrical pulses. In some embodiments, a method of transfecting T cell comprising contacting said T cell with RNA and applying to T cell an agile pulse sequence comprising: (a) an electrical pulse with a voltage of about 2250, 2300, 2350, 2400, 2450, 2500, 2550, 2600, 2650, 2700, 2750, 2800, 2850, 2900 or 3000V per centimeter; (b) a pulse width of 0.1 ms; (c) and a pulse interval of about 0.2, 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 ms between the electrical pulses of step (a) and (b); (d) one electrical pulse with a voltage range from about 2250, of 2250, 2300, 2350, 2400, 2450, 2500, 2550, 2600, 2650, 2700, 2750, 2800, 2850, 2900 or 3000V with a pulse width of 100 ms and a pulse interval of 100 ms between the electrical pulse of step (b) and the first electrical pulse of step (c); and (e) 4 electrical pulses with a voltage of about 325 V with a pulse width of about 0.2 ms and a pulse interval of about 2 ms between each of 4 electrical pulses. Any values included in the value range described above are disclosed in the present application. Electroporation medium can be any suitable medium known in the art. In some embodiments, the electroporation medium has conductivity in a range spanning about 0.01 to about 1.0 milliSiemens.

[0206] In some embodiments, as non limiting examples, an RNA encodes a rare-cutting endonuclease, one monomer of the rare-cutting endonuclease such as half-TALE-nuclease, a CAR, at least one component of the multi-chain chimeric antigen receptor, a pTα or functional variant thereof, an exogenous nucleic acid, and/or one additional catalytic domain.

Engineered immune cells

[0207] The invention also provides engineered immune cells comprising any of the CAR polynucleotides described herein. In some embodiments, a CAR can be introduced into an

immune cell as a transgene via a plasmid vector. In some embodiments, the plasmid vector can also contain, for example, a selection marker which provides for identification and/or selection of cells which received the vector.

[0208] CAR polypeptides may be synthesized in situ in the cell after introduction of polynucleotides encoding the CAR polypeptides into the cell. Alternatively, CAR polypeptides may be produced outside of cells, and then introduced into cells. Methods for introducing a polynucleotide construct into cells are known in the art. In some embodiments, stable transformation methods can be used to integrate the polynucleotide construct into the genome of the cell. In other embodiments, transient transformation methods can be used to transiently express the polynucleotide construct, and the polynucleotide construct not integrated into the genome of the cell. In other embodiments, virus-mediated methods can be used. The polynucleotides may be introduced into a cell by any suitable means such as for example, recombinant viral vectors (e.g. retroviruses, adenoviruses), liposomes, and the like. Transient transformation methods include, for example without limitation, microinjection, electroporation or particle bombardment. Polynucleotides may be included in vectors, such as for example plasmid vectors or viral vectors.

[0209] Also provided herein are isolated cells and cell lines obtained by the above-described methods of engineering cells provided herein. In some embodiments, an isolated cell comprises at least one CAR as described above. In some embodiments, an isolated cell comprises a population of CARs, each CAR comprising different extracellular ligand-binding domains.

[0210] Also provided herein are isolated immune cells obtained according to any one of the methods described above. Any immune cell capable of expressing heterologous DNAs can be used for the purpose of expressing the CAR of interest. In some embodiments, the immune cell used for expressing any one of the CARs described herein is a T cell. In some embodiments, an immune cell used for expressing CARs can be derived from, for example without limitation, a stem cell. The stem cells can be adult stem cells, non-human embryonic stem cells, more particularly non-human stem cells, cord blood stem cells, progenitor cells, bone marrow stem cells, induced pluripotent stem cells, totipotent stem cells or hematopoietic stem cells. Representative human stem cells are CD34⁺ cells.

[0211] In some embodiments, the engineered immune cells expressing at their cell surface membrane a FLT3 specific CAR of the invention comprise a percentage of stem cell memory and central memory cells greater than 10%, 20%, 30%, 40%, 50%, or 60%. In some

embodiments, the engineered immune cells expressing at their cell surface membrane a FLT3 specific CAR of the invention comprise a percentage of stem cell memory and central memory cells of about 10% to about 60%, about 10% to about 50%, about 10% to about 40%, about 15% to about 50%, about 15% to about 40%, about 20% to about 60%, or about 20% to about 70%.

[0212] In some embodiments, the percentage of stem cell memory and central memory cells is measured as disclosed in Example 2d.

[0213] The immune cell used for expressing any one of the CARs described herein can also be a dendritic cell, killer dendritic cell, a mast cell, a NK-cell, a B-cell or a T cell selected from the group consisting of inflammatory T-lymphocytes, cytotoxic T-lymphocytes, regulatory T-lymphocytes or helper T-lymphocytes. In some embodiments, the cell can be derived from the group consisting of CD4⁺ T-lymphocytes and CD8⁺ T-lymphocytes.

[0214] In one embodiment, the immune cell is an inflammatory T-lymphocyte that expresses any one of the CARs described herein. In one embodiment, the immune cell is a cytotoxic T-lymphocyte that expresses any one of the CARs described herein. In one embodiment, the immune cell is a regulatory T-lymphocyte that expresses any one of the CARs described herein. In one embodiment, the immune cell is a helper T-lymphocyte that expresses any one of the CARs described herein.

[0215] Also provided herein are cell lines obtained from a transformed T cell according to any of the above-described methods. Also provided herein are modified cells resistant to an immunosuppressive treatment. In some embodiments, an isolated cell according to the invention comprises a polynucleotide encoding a CAR.

[0216] The immune cells of the invention can be activated and expanded, either prior to or after genetic modification of the T cells, using methods as generally described, for example without limitation, in U.S. Patents 6,352,694; 6,534,055; 6,905,680; 6,692,964; 5,858,358; 6,887,466; 6,905,681; 7,144,575; 7,067,318; 7,172,869; 7,232,566; 7,175,843; 5,883,223; 6,905,874; 6,797,514; 6,867,041; and U.S. Patent Application Publication No. 20060121005. T cells can be expanded in vitro or in vivo. Generally, the T cells of the invention can be expanded, for example, by contact with an agent that stimulates a CD3 TCR complex and a co-stimulatory molecule on the surface of the T cells to create an activation signal for the T cell. For example, chemicals such as calcium ionophore A23187, phorbol 12-myristate 13-acetate (PMA), or mitogenic lectins like phytohemagglutinin (PHA) can be used to create an activation signal for the T cell.

[0217] In some embodiments, T cell populations may be stimulated in vitro by contact with, for example, an anti-CD3 antibody, or antigen-binding fragment thereof, or an anti-CD2 antibody immobilized on a surface, or by contact with a protein kinase C activator (e.g., bryostatin) in conjunction with a calcium ionophore. For co-stimulation of an accessory molecule on the surface of the T cells, a ligand that binds the accessory molecule is used. For example, a population of T cells can be contacted with an anti-CD3 antibody and an anti-CD28 antibody, under conditions appropriate for stimulating proliferation of the T cells. Conditions appropriate for T cell culture include an appropriate media (e.g., Minimal Essential Media or RPMI Media 1640 or, X-vivo 5, (Lonza)) that may contain factors necessary for proliferation and viability, including serum (e.g., fetal bovine or human serum), interleukin-2 (IL-2), insulin, IFN- γ , IL-4, IL-7, GM-CSF, IL-10, IL-2, IL-15, TGF β , and TNF, or any other additives for the growth of cells known to the skilled artisan. Other additives for the growth of cells include, but are not limited to, surfactant, plasmanate, and reducing agents such as N-acetyl- cysteine and 2-mercaptoethanol. Media can include RPMI 1640, AIM-V, DMEM, MEM, α - MEM, F-12, X-Vivo 1, and X-Vivo 20, Optimizer, with added amino acids, sodium pyruvate, and vitamins, either serum-free or supplemented with an appropriate amount of serum (or plasma) or a defined set of hormones, and/or an amount of cytokine(s) sufficient for the growth and expansion of T cells. Antibiotics, e.g., penicillin and streptomycin, are included only in experimental cultures, not in cultures of cells that are to be infused into a subject. The target cells are maintained under conditions necessary to support growth, for example, an appropriate temperature (e.g., 37° C) and atmosphere (e.g., air plus 5% CO₂). T cells that have been exposed to varied stimulation times may exhibit different characteristics

[0218] In some embodiments, the cells of the invention can be expanded by co-culturing with tissue or cells. The cells can also be expanded in vivo, for example in the subject's blood after administering the cell into the subject.

[0219] In some embodiments, an isolated cell according to the present invention comprises one inactivated gene selected from the group consisting of CD52, GR, PD-1, CTLA-4, LAG3, Tim3, BTLA, BY55, TIGIT, B7H5, LAIR1, SIGLEC10, 2B4, HLA, TCR α and TCR β and/or expresses a CAR, a multi-chain CAR and/or a pT α transgene. In some embodiments, an isolated cell comprises polynucleotides encoding polypeptides comprising a multi-chain CAR. In some embodiments, the isolated cell according to the present invention comprises two inactivated genes selected from the group consisting of: CD52 and GR, CD52 and TCR α , CDR52 and

TCR β , GR and TCR α , GR and TCR β , TCR α and TCR β , PD-1 and TCR α , PD-1 and TCR β , CTLA-4 and TCR α , CTLA-4 and TCR β , LAG3 and TCR α , LAG3 and TCR β , Tim3 and TCR α , Tim3 and TCR β , BTLA and TCR α , BTLA and TCR β , BY55 and TCR α , BY55 and TCR β , TIGIT and TCR α , TIGIT and TCR β , B7H5 and TCR α , B7H5 and TCR β , LAIR1 and TCR α , LAIR1 and TCR β , SIGLEC10 and TCR α , SIGLEC10 and TCR β , 2B4 and TCR α , 2B4 and TCR β and/or expresses a CAR, a multi-chain CAR and a pT α transgene.

[0220] In some embodiments, TCR is rendered not functional in the cells according to the invention by inactivating TCR α gene and/or TCR β gene(s). In some embodiments, a method to obtain modified cells derived from an individual is provided, wherein the cells can proliferate independently of the major histocompatibility complex (MHC) signaling pathway. Modified cells, which can proliferate independently of the MHC signaling pathway, susceptible to be obtained by this method are encompassed in the scope of the present invention. Modified cells disclosed herein can be used in for treating individuals in need thereof against Host versus Graft (HvG) rejection and Graft versus Host Disease (GvHD); therefore in the scope of the present invention is a method of treating individuals in need thereof against Host versus Graft (HvG) rejection and Graft versus Host Disease (GvHD) comprising treating said individual by administering to said individual an effective amount of modified cells comprising inactivated TCR α and/or TCR β genes.

[0221] In some embodiments, the immune cells are engineered to be resistant to one or more chemotherapy drugs. The chemotherapy drug can be, for example, a purine nucleotide analogue (PNA), thus making the immune cell suitable for cancer treatment combining adoptive immunotherapy and chemotherapy. Exemplary PNAs include, for example, clofarabine, fludarabine, and cytarabine, alone or in combination. PNAs are metabolized by deoxycytidine kinase (dCK) into mono-, di-, and tri-phosphate PNA. Their tri-phosphate forms compete with ATP for DNA synthesis, act as pro-apoptotic agents, and are potent inhibitors of ribonucleotide reductase (RNR), which is involved in trinucleotide production. Provided herein are FLT3 specific CAR-T cells comprising an inactivated dCK gene. In some embodiments, the dCK knockout cells are made by transfection of T cells using polynucleotides encoding specific TAL-nuclease directed against dCK genes by, for example, electroporation of mRNA. The dCK knockout FLT3 specific CAR-T cells are resistant to PNAs, including for example clofarabine and/or fludarabine, and maintain T cell cytotoxic activity toward FLT3-expressing cells.

[0222] In some embodiments, isolated cells or cell lines of the invention can comprise a pTα or a functional variant thereof. In some embodiments, an isolated cell or cell line can be further genetically modified by inactivating the TCRα gene.

[0223] In some embodiments, the CAR-T cell comprises a polynucleotide encoding a suicide polypeptide, such as for example RQR8. See, e.g., WO2013153391A, which is hereby incorporated by reference in its entirety. In CAR-T cells comprising the polynucleotide, the suicide polypeptide is expressed at the surface of a CAR-T cell. In some embodiments, the suicide polypeptide comprises the amino acid sequence shown in SEQ ID NO: 227.

CPYSNPSLCSGGGGSELPTQGTFSNVSTNVSPAKPTTTACPYSNPSLCSGGGGSP
APRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLS
LVITLYCNHRNRRRVCKCPRPVV (SEQ ID NO: 227).

[0224] The suicide polypeptide may also comprise a signal peptide at the amino terminus. In some embodiments, the suicide polypeptide comprises the amino acid sequence shown in SEQ ID NO: 228.

MGTSLLCWMALCLLGADHADACPYSNPSLCSGGGGSELPTQGTFSNVSTNVSPAKPTT
TACPYSNPSLCSGGGGSPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIY
IWAPLAGTCGVLLLSLVITLYCNHRNRRRVCKCPRPVV (SEQ ID NO: 228).

[0225] When the suicide polypeptide is expressed at the surface of a CAR-T cell, binding of rituximab to the rituximab epitopes of the polypeptide causes lysis of the cell. More than one molecule of rituximab may bind per polypeptide expressed at the cell surface. Each rituximab epitope of the polypeptide may bind a separate molecule of rituximab. Deletion of FLT3 specific CAR-T cells may occur in vivo, for example by administering rituximab to a subject. The decision to delete the transferred cells may arise from undesirable effects being detected in the subject which are attributable to the transferred cells, such as for example, when unacceptable levels of toxicity are detected.

[0226] In some embodiments, upon administration to a patient, engineered immune cells expressing at their cell surface any one of the FLT3 specific CARs described herein may reduce, kill or lyse endogenous FLT3 expressing cells of the patient. In one embodiment, a percentage reduction or lysis of FLT3 expressing endogenous cells or cells of a cell line expressing FLT3 by engineered immune cells expressing any one of the FLT3 specific CARs described herein is at least about or greater than 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, or 95%. In one embodiment, a percentage reduction or lysis

of FLT3 expressing endogenous cells or cells of a cell line expressing FLT3 by engineered immune cells expressing any one of the FLT3 specific CARs described herein is about 5% to about 95%, about 10% to about 95%, about 10% to about 90%, about 10% to about 80%, about 10% to about 70%, about 10% to about 60%, about 10% to about 50%, about 10% to about 40%, about 20% to about 90%, about 20% to about 80%, about 20% to about 70%, about 20% to about 60%, about 20% to about 50%, about 25% to about 75%, or about 25% to about 60%. In one embodiment, the endogenous FLT3 expressing cells are endogenous FLT3 expressing bone marrow cells.

[0227] In one embodiment, the percent reduction or lysis of target cells, e.g., a cell line expressing FLT3, by engineered immune cells expressing at their cell surface membrane a FLT3 specific CAR of the invention can be measured using the assay disclosed in Example 2e.

Method for sorting CAR-positive immune cells

[0228] In one aspect, provided are methods for *in vitro* sorting of a population of immune cells, wherein a subset of the population of immune cells comprises engineered immune cells expressing any one of the FLT3 specific CARs comprising epitopes specific for monoclonal antibodies described herein. The method comprises contacting the population of immune cells with a monoclonal antibody specific for the epitopes and selecting the immune cells that bind to the monoclonal antibody to obtain a population of cells enriched in engineered immune cells expressing FLT3 specific CAR.

[0229] In some embodiments, said monoclonal antibody specific for said epitope is optionally conjugated to a fluorophore. In this embodiment, the step of selecting the cells that bind to the monoclonal antibody can be done by Fluorescence Activated Cell Sorting (FACS). In some embodiments, said monoclonal antibody specific for said epitope is optionally conjugated to a magnetic particle. In this embodiment, the step of selecting the cells that bind to the monoclonal antibody can be done by Magnetic Activated Cell Sorting (MACS).

[0230] In some embodiments, the extracellular binding domain of the CAR comprises a mAb-specific epitope of SEQ ID NO: 262. In some embodiments, the extracellular binding domain of the CAR comprises a mAb-specific epitope of SEQ ID NO: 262 and the antibody used to contact the population of immune cells is QBEND-10.

[0231] In some embodiments, the extracellular binding domain of the CAR comprises a mAb-specific epitope of SEQ ID NO: 229. In some embodiments, the extracellular binding domain of

the CAR comprises a mAb-specific epitope of SEQ ID NO: 229 and the antibody used to contact the population of immune cells is Rituximab.

[0232] In some embodiments, the population of FLT3 CAR-expressing immune cells obtained when using the method for *in vitro* sorting of immune cells described above, comprises at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, or 95% of FLT3 CAR-expressing immune cells. In some embodiments, the population of FLT3 CAR-expressing immune cells obtained when using the method for *in vitro* sorting of CAR-expressing immune cells described above, comprises at least 85% of FLT3 CAR-expressing immune cells.

[0233] In some embodiments, the mAbs used in the *in vitro* sorting method are previously bound onto a support such as a column or on beads such as routinely realized by the skilled in the art. In some embodiments, immune cells expressing CARs are T-cells.

[0234] According to the invention, cells to be administered to the recipient may be enriched *in vitro* from the source population. Methods of expanding source populations are well known in the art, and may include selecting cells that express an antigen such as CD34 antigen, using combinations of density centrifugation, immuno-magnetic bead purification, affinity chromatography, and fluorescent activated cell sorting, known to those skilled in the art.

[0235] Flow cytometry is widely used in the art and is a method well known to one of ordinary skill to sort and quantify specific cell types within a population of cells. In general, flow cytometry is a method for quantitating components or structural features of cells primarily by optical means. Since different cell types can be distinguished by quantitating structural features, flow cytometry and cell sorting can be used to count and sort cells of different phenotypes in a mixture.

[0236] A flow cytometric analysis involves two basic steps: 1) labeling selected cell types with one or more labeled markers, and 2) determining the number of labeled cells relative to the total number of cells in the population.

[0237] The primary method of labeling cell types is by binding labeled antibodies to markers expressed by the specific cell type. The antibodies are either directly labeled with a fluorescent compound or indirectly labeled using, for example, a fluorescent- labeled second antibody which recognizes the first antibody.

[0238] In some embodiments, the method used for sorting immune cells expressing a CAR is the Magnetic- Activated Cell Sorting (MACS).

[0239] Magnetic-activated cell sorting (MACS) is a method for separation of various cell populations depending on their surface antigens (CD molecules) by using superparamagnetic nanoparticles and columns. It takes a few simple steps to get pure cell populations. Cells in a single-cell suspension are magnetically labeled with microbeads. The sample is applied to a column composed of ferromagnetic spheres, which are covered with a cell-friendly coating allowing fast and gentle separation of cells. The unlabeled cells pass through while the magnetically labeled cells are retained within the column. The flow-through can be collected as the unlabeled cell fraction. After a short washing step, the column is removed from the separator, and the magnetically labeled cells are eluted from the column.

[0240] In some embodiments, the mAb used in the method for sorting immune cells expressing the CAR is chosen from 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 and/or ustekinumab. In some embodiments, said mAb is rituximab. In another embodiment, said mAb is QBEND-10.

Therapeutic applications

[0241] Isolated cells obtained by the methods described above, or cell lines derived from such isolated cells, expressing FLT3 specific CARs can be used as a medicament. Similarly, FLT 3 specific antibodies disclosed herein can be used as a medicament. In some embodiments, such a medicament can be used for treating a FLT3-associated disease or a condition. In some embodiments, the FLT3-associated disease or a condition comprises malignant cells expressing FLT3, for example, cancer. In some embodiments, the cancer is a cancer of hematopoietic origin, such as a lymphoma or leukemia. In some embodiments, the cancer is 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, Burkitt's lymphoma, marginal zone lymphoma, mantle cell lymphoma, large cell lymphoma, precursor B-

lymphoblastic lymphoma, myeloid leukemia, Waldenstrom's macroglobulinemia, diffuse large B cell lymphoma, follicular lymphoma, marginal zone lymphoma, mucosa-associated lymphatic tissue lymphoma, small cell lymphocytic lymphoma, mantle cell 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, intravascular large B-cell lymphoma, 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, or other hematopoietic cells related cancers. In an exemplary embodiment the cancer is AML. In an exemplary embodiment the cancer is ALL.

[0242] In some embodiments, an isolated cell according to the invention, or cell line derived from the isolated cells, or an antibody can be used in the manufacture of a medicament for treatment of a cancer in a subject in need thereof.

[0243] Also provided herein are methods for treating subjects. In some embodiments, the method comprises providing an immune cell of the invention to a subject in need thereof. In some embodiments, the method comprises a step of administering transformed immune cells of the invention to a subject in need thereof. In some embodiments, the method comprises administering an antibody of the invention to a subject in need thereof.

[0244] In some embodiments, T cells of the invention can undergo robust in vivo T cell expansion and can persist for an extended amount of time.

[0245] Methods of treatment of the invention can be ameliorating, curative or prophylactic. The method of the invention may be either part of an autologous immunotherapy or part of an allogeneic immunotherapy treatment. The invention is particularly suitable for allogeneic immunotherapy. T cells from donors can be transformed into non-alloreactive cells using standard protocols and reproduced as needed, thereby producing CAR-T cells which may be

administered to one or several subjects. Such CAR-T cell therapy can be made available as an “off the shelf” therapeutic product.

[0246] Cells that can be used with the disclosed methods are described in the previous section. Treatment can be used to treat subjects diagnosed with, for example, cancer. Cancers that may be treated include, for example without limitation, cancers that involve B lymphocytes, including any of the above-listed cancers. Types of cancers to be treated with the CARs and CAR-T cells of the invention include, but are not limited to certain leukemia or lymphoid malignancies. Adult tumors/cancers and pediatric tumors/cancers are also included. In some embodiments, the treatment can be in combination with one or more therapies against cancer selected from the group of antibodies therapy, chemotherapy, cytokines therapy, dendritic cell therapy, gene therapy, hormone therapy, laser light therapy and radiation therapy.

[0247] In some embodiments, treatment can be administered into subjects undergoing an immunosuppressive treatment. Indeed, the invention generally relies on cells or a population of cells, which have been made resistant to at least one immunosuppressive agent due to the inactivation of a gene encoding a receptor for such immunosuppressive agent. In this aspect, the immunosuppressive treatment should help the selection and expansion of the T cells according to the invention within the subject. The administration of the cells or population of cells according to the invention may be carried out in any convenient manner, including by aerosol inhalation, injection, ingestion, transfusion, implantation or transplantation. The compositions described herein may be administered to a subject subcutaneously, intradermally, intratumorally, intranodally, intramedullary, intramuscularly, by intravenous or intralymphatic injection, or intraperitoneally. In some embodiments, the cell compositions of the invention are administered by intravenous injection.

[0248] In some embodiments, the administration of the cells or population of cells can comprise administration of, for example, about 10^4 to about 10^9 cells per kg body weight including all integer values of cell numbers within those ranges. In some embodiments the administration of the cells or population of cells can comprise administration of about 10^5 to 10^6 cells per kg body weight including all integer values of cell numbers within those ranges. The cells or population of cells can be administered in one or more doses. In some embodiments, said effective amount of cells can be administered as a single dose. In some embodiments, said effective amount of cells can be administered as more than one dose over a period time. Timing of administration is within the judgment of managing physician and depends on the clinical

condition of the subject. The cells or population of cells may be obtained from any source, such as a blood bank or a donor. While individual needs vary, determination of optimal ranges of effective amounts of a given cell type for a particular disease or conditions within the skill of the art. An effective amount means an amount which provides a therapeutic or prophylactic benefit. The dosage administered will be dependent upon the age, health and weight of the recipient, kind of concurrent treatment, if any, frequency of treatment and the nature of the effect desired. In some embodiments, an effective amount of cells or composition comprising those cells are administered parenterally. In some embodiments, administration can be an intravenous administration. In some embodiments, administration can be directly done by injection within a tumor.

[0249] In some embodiments of the invention, cells are administered to a subject in conjunction with (e.g., before, simultaneously or following) any number of relevant treatment modalities, including but not limited to treatment with agents such as monoclonal antibody therapy, CCR2 antagonist (e.g., INC-8761), antiviral therapy, cidofovir and interleukin-2, Cytarabine (also known as ARA-C) or natalizumab treatment for MS patients or efalizumab treatment for psoriasis patients or other treatments for PML patients. In some embodiments, FLT3 specific CAR-T cells are administered to a subject in conjunction with one or more of the following: an anti-PD-1 antibody (e.g., nivolumab, pembrolizumab, or PF-06801591), an anti-PD-L1 antibody (e.g., avelumab, atezolizumab, or durvalumab), an anti-OX40 antibody (e.g., PF-04518600), an anti-4-1BB antibody (e.g., PF-05082566), an anti-MCSF antibody (e.g., PD-0360324), an anti-GITR antibody, and/or an anti-TIGIT antibody. In some embodiments, a FLT3 specific CAR comprising the amino acid sequence shown in SEQ ID NO: 236 is administered to a subject in conjunction with anti-PD-L1 antibody avelumab. In further embodiments, the T cells of the invention may be used in combination with chemotherapy, radiation, immunosuppressive agents, such as cyclosporin, azathioprine, methotrexate, mycophenolate, and FK506, antibodies, or other immunoablative agents such as CAMPATH, anti-CD3 antibodies or other antibody therapies, cytoxin, fludarabine, cyclosporin, FK506, rapamycin, mycophenolic acid, steroids, FR901228, cytokines, and/or irradiation. These drugs inhibit either the calcium dependent phosphatase calcineurin (cyclosporine and FK506) or inhibit the p70S6 kinase that is important for growth factor induced signaling (rapamycin) (Henderson, Naya et al. 1991; Liu, Albers et al. 1992; Bierer, Hollander et al. 1993). In further embodiments, the T cells of the invention may be used in combination with Receptor Tyrosine

Kinase inhibitors such as Midostaurin and Sunitinib, mTOR inhibitors such as Rapamycin and Everolimus, epigenetic modulators such as Vorinostat, proteasome inhibitors such as Bortezomib, immunomodulatory agents such as lenalidomide, Hedgehog inhibitors such as Erismodegib and PF-04449913 or Isocitrate Dehydrogenase (IDH) inhibitors such as AG-120 and AG-221. In a further embodiment, the cell compositions of the invention are administered to a subject in conjunction with (e.g., before, simultaneously or following) bone marrow transplantation, T cell ablative therapy using either chemotherapy agents such as, fludarabine, external-beam radiation therapy (XRT), cyclophosphamide, or antibodies such as OKT3 or CAMPATH. In some embodiments, the cell compositions of the invention are administered following B-cell ablative therapy such as agents that react with CD20, e.g., Rituxan. For example, In some embodiments, subjects may undergo standard treatment with high dose chemotherapy followed by peripheral blood stem cell transplantation. In certain embodiments, following the transplant, subjects receive an infusion of the expanded immune cells of the invention. In some embodiments, expanded cells are administered before or following surgery.

[0250] In some embodiments, provided are methods for depleting FLT3 specific CAR-expressing engineered immune cells from a subject administered with said cells. Depletion can be by inhibition or elimination.

[0251] In one aspect, a method for depleting engineered immune cells expressing a FLT3 specific CAR comprising an epitope specific for a monoclonal antibody comprises contacting said engineered immune cell with a monoclonal antibody specific for the epitope.

[0252] In some embodiments, a method for depleting from a subject administered with engineered immune cells expressing a FLT3 specific CAR comprising an epitope specific for a monoclonal antibody comprises administering to the subject a monoclonal antibody specific for the epitope. In these embodiments, administration of the monoclonal antibody specific for the epitope present in the extracellular domain of the CAR to the subject eliminates or inhibits the activity of engineered CAR-expressing immune cells from the subject. In one aspect, depletion of engineered CAR expressing immune cells allows for recovery of an endogenous population of FLT3 expressing cells.

[0253] In one aspect, the invention relates to a method for promoting recovery of endogenous FLT3-expressing cells in a subject administered with engineered immune cells expressing at cell surface a FLT3 specific CAR comprising an epitope specific for a monoclonal antibody, the method comprising administering a monoclonal antibody specific for the epitope to the subject.

In some embodiments, endogenous FLT3 expressing cells are endogenous FLT3 expressing bone marrow cells. In one aspect, the term “recovery” refers to increasing the number of endogenous FLT3 expressing cells. The number of endogenous FLT3 expressing cells may increase due to increase in proliferation of endogenous FLT3 expressing cells and/or due to reduction in elimination of endogenous FLT3 expressing cells by FLT3 CAR expressing engineered immune cells. In some embodiments, administration of the monoclonal antibody to the subject depletes the FLT3 CAR expressing engineered immune cells and increases the number of endogenous FLT3-expressing cells, e.g., endogenous FLT3-expressing bone marrow progenitor cells, in the subject. In one embodiment, administration of the monoclonal antibody to the subject increases the number of endogenous FLT3 expressing cells by at least about 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, or 95%, compared to the number of endogenous FLT3 expressing cells prior to administration of the monoclonal antibody.

[0254] In one aspect, provided is a method for treating a FLT3-mediated condition in a subject, the method comprising: (a) administering to the subject engineered immune cells expressing at cell surface FLT3 specific CARs comprising one or more epitopes specific for one or more monoclonal antibodies; and (b) subsequently depleting the engineered immune cells from the subject by administering one or more monoclonal antibodies specific for the epitope to the subject.

[0255] In some embodiments, the mAbs used in the method for depleting CAR-expressing engineered immune cells are selected from 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, ustekinumab, and combinations thereof.

[0256] In some embodiments, said epitope specific for a monoclonal antibody (mAb-specific epitope) is a CD20 epitope or mimotope, e.g. SEQ ID NO. 229 and the mAb specific for the epitope is rituximab.

[0257] In some embodiments, the step of administering a monoclonal antibody to the subject comprises infusing the subject with the monoclonal antibody. In some embodiments, the amount

of epitope-specific mAb administered to the subject is sufficient to eliminate at least 20%, 30%, 40%, 50%, 60%, 70%, 80% or 90% of the CAR-expressing immune cell in the subject.

[0258] In some embodiments, the step of administering a monoclonal antibody to the subject comprises infusing the subject with 375mg/m² of rituximab, once or several times weekly.

[0259] In some embodiments, when immune cells expressing a CAR comprising an mAb-specific epitope (CAR-expressing immune cells) are depleted in a CDC assay using epitope-specific mAb, the amount of viable CAR-expressing immune cells decreases, e.g. by at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80% or 90%.

[0260] In some embodiments, a cytotoxic drug is coupled to the epitope-specific mAbs which are used to deplete CAR-expressing immune cells. By combining targeting capabilities of monoclonal antibodies with the cell-killing ability of cytotoxic drugs, antibody-drug conjugate (ADC) allows a sensitive discrimination between healthy and diseased tissue when compared to the use of the drug alone. Market approvals were received for several ADCs; the technology for making them -particularly on linkers- is abundantly presented in the following prior art (Payne, G. (2003) *Cancer Cell* 3:207-212; Trail et al (2003) *Cancer Immunol. Immunother.* 52:328-337; Syrigos and Epenetos (1999) *Anticancer Research* 19:605-614; Niculescu-Duvaz and Springer (1997) *Adv. Drug Del. Rev.* 26:151-172; U.S. Pat. No. 4,975,278).

[0261] In some embodiments, the epitope-specific mAb to be infused is conjugated beforehand with a molecule able to promote complement dependent cytotoxicity (CDC). Therefore, the complement system helps or complements the ability of antibodies to clear pathogens from the organism. When stimulated by one of several, is triggered an activation cascade as a massive amplification of the response and activation of the cell-killing membrane attack complex. Different molecule may be used to conjugate the mAb, such as glycans [Courtois, A, Gac-Breton, S., Berthou, C, Guezennec, J., Bordron, A. and Boisset, C. (2012), Complement dependent cytotoxicity activity of therapeutic antibody fragments is acquired by immunogenic glycan coupling, *Electronic Journal of Biotechnology* ISSN: 0717-3458; <http://www.ejbiotechnology.info> DOI: 10.2225/voll5-issue5).

Kits

[0262] The invention also provides kits for use in the instant methods. Kits of the invention include one or more containers comprising a polynucleotide encoding a FLT3 specific CAR, an engineered immune cell comprising a polynucleotide encoding a FLT3 specific CAR, or a FLT

specific antibody, as described herein, and instructions for use in accordance with any of the methods of the invention described herein. Generally, these instructions comprise a description of administration of the engineered immune cell for the above described therapeutic treatments.

[0263] The instructions relating to the use of the engineered immune cells or antibodies as described herein generally include information as to dosage, dosing schedule, and route of administration for the intended treatment. The containers may be unit doses, bulk packages (e.g., multi-dose packages) or sub-unit doses. Instructions supplied in the kits of the invention are typically written instructions on a label or package insert (e.g., a paper sheet included in the kit), but machine-readable instructions (e.g., instructions carried on a magnetic or optical storage disk) are also acceptable.

[0264] The kits of this invention are in suitable packaging. Suitable packaging includes, but is not limited to, vials, bottles, jars, flexible packaging (e.g., sealed Mylar or plastic bags), and the like. Also contemplated are packages for use in combination with a specific device, such as an inhaler, nasal administration device (e.g., an atomizer) or an infusion device such as a minipump. A kit may have a sterile access port (for example the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). The container may also have a sterile access port (for example the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). At least one active agent in the composition is a FLT3 specific CAR or a FLT3 specific antibody. The container may further comprise a second pharmaceutically active agent.

[0265] Kits may optionally provide additional components such as buffers and interpretive information. Normally, the kit comprises a container and a label or package insert(s) on or associated with the container.

[0266] The following examples are offered for illustrative purposes only, and are not intended to limit the scope of the invention in any way. Indeed, various modifications of the invention in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description and fall within the scope of the appended claims.

[0267] Representative materials of the present invention were deposited in the American Type Culture Collection, 10801 University Boulevard, Manassas, Va. 20110-2209, USA, on June 1st, 2017. Vector P3E10-VL having ATCC Accession No. PTA-124228 is a polynucleotide encoding the P3E10 light chain variable region, and vector P3E10-VH having ATCC Accession No. PTA-124227 is a polynucleotide encoding the P3E10 heavy chain variable region. The

deposit was made under the provisions of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purpose of Patent Procedure and Regulations thereunder (Budapest Treaty). This assures maintenance of a viable culture of the deposit for 30 years from the date of deposit. The deposit will be made available by ATCC under the terms of the Budapest Treaty, and subject to an agreement between Pfizer, Inc. and ATCC, which assures permanent and unrestricted availability of the progeny of the culture of the deposit to the public upon issuance of the pertinent U.S. patent or upon laying open to the public of any U.S. or foreign patent application, whichever comes first, and assures availability of the progeny to one determined by the U.S. Commissioner of Patents and Trademarks to be entitled thereto according to 35 U.S.C. Section 122 and the Commissioner's rules pursuant thereto (including 37 C.F.R. Section 1.14 with particular reference to 886 OG 638).

[0268] The assignee of the present application has agreed that if a culture of the materials on deposit should die or be lost or destroyed when cultivated under suitable conditions, the materials will be promptly replaced on notification with another of the same. Availability of the deposited material is not to be construed as a license to practice the invention in contravention of the rights granted under the authority of any government in accordance with its patent laws.

EXAMPLES

Example 1: Determination of kinetics and affinity of human FLT3/ FLT3 antibodies interactions at 37°C

[0269] This example determines the kinetics and affinity of various anti-FLT3 antibodies at 37°C. All experiments were performed on a Biacore T200 surface Plasmon resonance biosensor (GE Lifesciences, Piscataway NJ).

[0270] The sensor chip preparation was performed at 25°C with a running buffer of 10 mM HEPES, 150 mM NaCl, 0.05% (v/v) Tween-20, pH 7.4. An anti-human Fc sensor chip was made by activating all flow cells of a Biacore CM4 sensor chip with a 1:1 (v/v) mixture of 400 mM EDC and 100 mM NHS for 7 minutes, at a flow rate of 10 µL/min. An anti-human Fc reagent (Goat Anti-Human IgG Fc, SouthernBiotech Catalog #2081-01) was diluted to 30 µg/mL in 10 mM Sodium Acetate pH 4.5 and injected on all flow cells for 7 minutes at 20 µL/min. All flow cells were blocked with 100 mM ethylenediamine in 150 mM Borate buffer pH 8.5 for 7 minutes at 10 µL/min.

[0271] The experiments were performed at 37°C using a running buffer of 10 mM HEPES, 150 mM NaCl, 0.05% (v/v) Tween-20, pH 7.4, 1 mg/mL BSA. FLT3 antibodies were captured from undiluted supernatants onto downstream flow cells (flow cells 2, 3 and 4) at a flow rate of 10 μ L/min for 1 minute. Different antibodies were captured on each flow cell. Flow cell 1 was used as a reference surface. Following capture of FLT3 antibodies, analyte (buffer, hFLT3) was injected at 30 μ L/min on all flow cells for two minutes. After the analyte injection, dissociation was monitored for 10 minutes followed by regeneration of all flow cells with three 1-minute injections of 75 mM Phosphoric Acid. For each captured FLT3 antibody, the following analyte injections were performed: buffer, 11 nM hFLT3, 33 nM hFLT3, 100 nM hFLT3 and 300 nM hFLT3. Buffer cycles were collected for each captured FLT3 antibody for double-referencing purposes (double-referencing as described in Myszk, D.G. Improving biosensor analysis. *J. Mol. Recognit.* **12**, 279-284 (1999)). The double-referenced sensorgrams were fit globally to a simple 1:1 Langmuir with mass transport binding model.

[0272] The kinetics and affinity parameters for tested anti-FLT3 antibodies are shown in Table 7. The antibodies shown in Table 7 share the same VH and VL regions as the CARs shown in Table 8 having the same name.

Table 7

		IgG format					scFv format			
					huFlt3	mFlt3				
	domain	k _a (1/Ms)	k _d (1/s)	t _{1/2} (min)	K _D (nM)	K _d (nM)	k _a (1/Ms)	k _d (1/s)	t _{1/2} (min)	K _D (nM)
P4F6	1	1,40E+05	3,50E-03	3,3	25	36,1	1,32E+05	1,99E-03	5,8	15,1
P4C7	1	1,60E+05	1,20E-03	9,3	7,7	402,4	1,21E+05	1,64E-03	7	13,6
P3A1	2-3	9,50E+04	6,00E-03	1,9	64	-	1,07E+05	1,67E-03	6,9	15,67
P5A3	2-3	9,80E+04	1,00E-02	1,2	102	19,3	8,10E+04	1,75E-02	0,7	216
P9B5	2-3	4,20E+04	4,70E-04	24,4	11	1,5	3,80E+04	6,87E-04	16,8	18,1
P9F1	2-3	1,80E+05	2,30E-02	0,5	127	-	2,26E+05	2,86E-02	0,4	126,5
P1B4	4	1,80E+05	5,80E-03	2	32	-	1,20E+05	3,27E-03	3,5	27,3
P1B11	4	1,20E+05	5,50E-03	2,1	45	-	8,47E+04	2,57E-03	4,5	30,3
P7H3	4	9,90E+05	1,80E-03	6,6	2	0,9	2,05E+05	1,87E-03	6,2	9,1
P3E10	4	1,80E+05	1,90E-02	0,6	106	-	1,72E+05	1,12E-02	1	65,1
P1A5	4	1,78E+06	3,47E-04	33	0,19	-	2,59E+05	2,92E-04	40	1,1

P4A4	4	1,16E+06	4,69E-04	25	0,4	-				
P1G12	4	6,17E+05	2,92E-04	40	0,47	6,5				
P4E5	4	1,20E+06	1,51E-04	77	0,13	18,9				
P5A4	4	5,53E+05	1,01E-04	114	0,18	-				
P5F7	4	6,36E+05	1,52E-04	76	0,24	-	1,89E+05	1,97E-04	59	1
P4H11	4	6,18E+04	1,44E-02	1	233	-	1,60E+05	9,33E-03	1	58,3
P15F7	5	1,40E+05	5,50E-03	2,1	38	-				
P12B6	5	1,10E+05	9,00E-03	1,3	84	-				
P7D3	5	7,10E+04	5,20E-03	2,2	72	-				
P7A6	5	3,40E+04	3,70E-04	31	11					
P8B6	5	9,30E+04	2,30E-04	51	2,5	-				
P14G2	5	1,40E+05	1,10E-03	10,7	8	-	9,35E+04	3,05E-04	37,9	3,3
P7F9	4	1,10E+05	1,30E-03	8,9	12	13,8	8,70E+04	2,41E-03	4,8	27,7

Example 2: Generation of FLT3 specific CAR-T cells

a) Plasmids

[0273] The following codon-optimized FLT3 CAR sequences listed in Table 8 below were, synthesized and subcloned into a lentiviral vector such as pLVX-EF1a-IRES-Puro (Clontech) using the EcoRI (5') and MluI (3') restriction sites (thus removing the IRES-Puro cassette).

Table 8: Exemplary FLT3 specific CARs

CAR	CAR Amino Acid Sequence	Components
P1A5	MALPVTALLLPLALLLHAARPQVQLVQSGAEVKKP GSSVKVSCASGGVFSRYALSWVRQAPGQGLEWM GGIIPMLGFANYAQKFQGRVTITADESTSTAYMELS SLRSEDTAVYYCATLDFGALDYWGQGLTVTVSSG GGGSGGGGSGGGGSEIVLTQSPGTLSPGERATLS CRASQAVDSSDLAWYQQKPGQAPRLLIYDAYTRPS GIPDRFSGSGSGTDFLTISRLEPEDFAVYYCQQYGS SPLTFGGGTKLEIKTTTPAPRPPTPAPTIASQPLSLRP EACRPAAGGAVHTRGLDFACDIYWAPLAGTCGVL LLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEED	CD8 α signal peptide; P1A5_VHVL VH; GS linker; P1A5_VHVL VL; CD8 α hinge; CD8 α TM domain; 41BB ISD;

CAR	CAR Amino Acid Sequence	Components
	GCSCRFPEEEEEGGCELRVKFSRSADAPAYQQGQNQ LYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKN PQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHD GLYQGLSTATKDTYDALHMQALPPR (SEQ ID NO: 233)	CD3 ζ ISD
P1B4	MALPVTALLLPLALLLHAARPQVQLVQSGAEVKKP GSSVKVSKASGGVFSRYALSWVRQAPGQGLEWM GGIIPMLGFANYAQKFQGRVTITADESTSTAYMELS SLRSEDTAVYYCATLDFGALDYWGQGLTVTVSSG GGGSGGGSGGGGSEIVLTQSPGTLSPGERATLS CRASQSVNEQLAWYQQKPGQAPRLLIYDASSRAT GIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYGS PPLTFGQGTKVEIKTTTPAPRPPTAPTIASQPLSLRP EACRPAAGGAVHTRGLDFACDIYWAPLAGTCGVL LLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEED GCSCRFPEEEEEGGCELRVKFSRSADAPAYQQGQNQ LYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKN PQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHD GLYQGLSTATKDTYDALHMQALPPR (SEQ ID NO: 234)	CD8 α signal peptide; P1B4_VHVL VH; GS linker; P1B4_VHVL VL; CD8 α hinge; CD8 α TM domain; 41BB ISD; CD3 ζ ISD
P3A1	MALPVTALLLPLALLLHAARPQVQLVQSGAEVKKP GSSVKVSKASGGTFSSYDISWVRQAPGQGLEWM GGIIPVSGRANYAQKFQGRVTITTDKSTSTAYMELS SLRSEDTAVYYCARVRPTYWPLDYWGQGLTVTVS SGGGSGGGSGGGGSDIQMTQSPSSLSASVGDRV TITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQ SGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSY STPLTFGQGTKVEIKTTTPAPRPPTAPTIASQPLSLR PEACRPAAGGAVHTRGLDFACDIYWAPLAGTCGV LLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEE	CD8 α signal peptide; P3A1_VHVL VH; GS linker; P3A1_VHVL VL; CD8 α hinge; CD8 α TM domain; 41BB ISD; CD3 ζ ISD

CAR	CAR Amino Acid Sequence	Components
	DGCSCRFPEEEEEGGCELRVKFSRSADAPAYQQGQN QLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRK NPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGH DGLYQGLSTATKDTYDALHMQALPPR (SEQ ID NO: 235)	
P3E10	MALPVTALLLPLALLLHAARPQVQLVQSGAEVKKP GSSVKVSKASGGTFSSYAIQWVRQAPGQGLEWM GGIVGSWGLANYAQKFQGRVTITTDKSTSTAYMEL SSLRSEDТАVYYCATSAFGELASWGQGLTVTVSSG GGGSGGGGSGGGGSEIVLTQSPGTLSPGERATLS CRASQSVPSQLAWYQQKPGQAPRLLIYDASSRAT GIPDRFSGSGSGTDFLTISRLEPEDFAVYYCQQYGS SPLTFGQGTKVEIKTTTPAPRPPTPAPTIASQPLSLRP EACRPAAGGAVHTRGLDFACDIYWAPLAGTCGVL LLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEED GCSCRFPEEEEEGGCELRVKFSRSADAPAYQQGQNQ LYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKN PQEGLYNELQKDKMAEAYSEIGMKGERRRGKGH GLYQGLSTATKDTYDALHMQALPPR (SEQ ID NO: 236)	CD8 α signal peptide; P3E10_VHVL VH; GS linker; P3E10_VHVL VL; CD8 α hinge; CD8 α TM domain; 41BB ISD; CD3 ζ ISD
P4C7	MALPVTALLLPLALLLHAARPQVQLVQSGAEVKKP GSSVKVSKASGGTFSSYTISWVRQAPGQGLEWMG GIIPAFGIANYAQKFQGRVTITADKSTSTAYMELSSL RSEDТАVYYCAKGGSYSLDYFDIWGQGLTVTVSSG GGGSGGGGSGGGGSEIVLTQSPGTLSPGERATLS CRASQYVSASLLAWYQQKPGQAPRLLIYGASTRAT GIPDRFSGSGSGTDFLTISRLEPEDFAVYYCQQYAR SSTFGQGTKVEIKTTTPAPRPPTPAPTIASQPLSLRPE ACRPAAGGAVHTRGLDFACDIYWAPLAGTCGVLL LSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDG	CD8 α signal peptide; P4C7_VHVL VH; GS linker; P4C7_VHVL VL; CD8 α hinge; CD8 α TM domain; 41BB ISD; CD3 ζ ISD

CAR	CAR Amino Acid Sequence	Components
	CSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQL YNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNP QEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDG LYQGLSTATKDTYDALHMQALPPR (SEQ ID NO: 237)	
P4H11	MALPVTALLLPLALLLHAARPEVQLLES GGGLVQP GGSLRLSCAASGFTFSSYAMNWVRQAPGKGLEWV SSISGGGRSTYYADSVKGRFTISRDN SKNTLYLQMN SLRAEDTAVYYCARDLSPSDVGWGYGFDIWGQGT LVTVSSGGGSGGGGSGGGGSEIVLTQSPGTL SLSP GERATLSCRASQSVSN TYLAWYQQKPGQAPRLLIY DTSSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVY YCQQYGSSLTFGQGTKVEIKTTTPAPRPPTPAPTIA QPLSLRPEACRPAAGGAVHTRGLDFACDIYWAPL AGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPV QTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAY QQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGG KPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERR RGKGHDGLYQGLSTATKDTYDALHMQALPPR (SEQ ID NO: 238)	CD8 α signal peptide; P4H11_VHVL VH; GS linker; P4H11_VHVL VL; CD8 α hinge; CD8 α TM domain; 41BB ISD; CD3 ζ ISD
P5F7	MALPVTALLLPLALLLHAARPEVQLLES GGGLVQP GGSLRLSCAASGFTFSSYAMNWVRQAPGKGLEWV SSISGGGRSTYYADSVKGRFTISRDN SKNTLYLQMN SLRAEDTAVYYCARDLSPSDVGWGYGFDIWGQGT LVTVSSGGGSGGGGSGGGGSEIVLTQSPATL SLSP GERATLSCRASQSVSSNLAWYQQKPGQAPRLLIYD TFTRATGIPARFSGSGSGTDFTLTISRLEPEDFAVY CQQYGSSPPTFGQGTRLEIKTTTPAPRPPTPAPTIA QPLSLRPEACRPAAGGAVHTRGLDFACDIYWAPL AGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPV	CD8 α signal peptide; P5F7_VHVL VH; GS linker; P5F7_VHVL VL; CD8 α hinge; CD8 α TM domain; 41BB ISD; CD3 ζ ISD

CAR	CAR Amino Acid Sequence	Components
	QTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAY QQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGG KPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERR RGKGHDGLYQGLSTATKDTYDALHMQALPPR (SEQ ID NO: 239)	
P7H3	MALPVTALLLPLALLLHAARPEVQLLES GGGLVQP GGSLRLSCAASGFTFSSYAMHWVRQAPGKGLEWV SAISGSGGSTYYADSVKGRFTISRDN SKNTLYLQMN SLRAEDTAVYYCARGTRWWWGDAFDHWGQGTL VTVSSGGGGSGGGSGGGGSQSVLTQPPSVSVAPG KTARITCGGNNIGSKSVHWYQQKPGQAPVLVIYYD SDRPSGIPERFSGSNSGNTATLTISRVEAGDEADYYC QVWDSSTAWVFGGGTKLTVLTTTPAPRPPTPAPTIA SQPLSLRPEACRPAAGGAVHTRGLDFACDIYWAPL AGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPV QTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAY QQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGG KPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERR RGKGHDGLYQGLSTATKDTYDALHMQALPPR (SEQ ID NO: 240)	CD8 α signal peptide; P7H3_VHVL VH; GS linker; P7H3_VHVL VL; CD8 α hinge; CD8 α TM domain; 41BB ISD; CD3 ζ ISD
P8B6	MALPVTALLLPLALLLHAARPQVQLVQSGAEVKKP GSSVKVSKASGFTFSSYAVSWVRQAPGQGLEWM GGIIPIFGIANYAQKFQGRVTITADTSTSTAYMELSS LRSEDTAVYYCAIEGIGGDLRYDGYDAWGQGTLV TVSSGGGGSGGGSGGGGSEIVLTQSPGTLSPGE RATLSCRASQSVSHSYLAWYQQKPGQAPRLLIYGA SFRAAGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYC QQYGSDPYTFGQGTKVEIKTTTPAPRPPTPAPTIA SQ PLSLRPEACRPAAGGAVHTRGLDFACDIYWAPLA GTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQ	CD8 α signal peptide; P8B6_VHVL VH; GS linker; P8B6_VHVL VL; CD8 α hinge; CD8 α TM domain; 41BB ISD; CD3 ζ ISD

CAR	CAR Amino Acid Sequence	Components
	TTQEEDGCSCRFPEEEEGGCEL RVKFSRSADAPAYQ QQQNQLYNELNLGRREEYDVLDKRRGRDPEMGGK PRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRR GKGHDGLYQGLSTATKDTYDALHMQALPPR (SEQ ID NO: 241)	
P9B5	MALPVTALLLPLALLLHAARPEVQLLESGGGLVQP GGSLRLSCAASGFIFASYAMSWVRQAPGKGLEWVS EISSSGGSTTYADSVKGRFTISRDN SKNTLYLQMNS LRAEDTAVYYCARDRV MAGLGYDPFDYWGQGTL VTVSSGGGGSGGGSGGGGSQSVLTQPPSASGTPG QRTVISCSSSNIGSNYVYWYQQLPGTAPKLLIYR NNQRPSGVPDRFSGSKSGTSASLAISGLRSEDEADY YCAAWDDSLSGVVFGGGTKLTVLTTTPAPRPPTPA PTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYI WAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPF MRPVQTTQEEDGCSCRFPEEEEGGCEL RVKFSRSA DAPAYQQQNQLYNELNLGRREEYDVLDKRRGRD PEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGM KGERRRGKGHDGLYQGLSTATKDTYDALHMQALP PR (SEQ ID NO: 242)	CD8 α signal peptide; P9B5_VHVL VH; GS linker; P9B5_VHVL VL; CD8 α hinge; CD8 α TM domain; 41BB ISD; CD3 ζ ISD
P12B6	MALPVTALLLPLALLLHAARPQVQLVQSGAEVKKP GSSVKVSCKASGGTFMSY AISWVRQAPGQGLEWM GGIPIFGIANYAQKFQGRVTITADKSTSTAYMELSS LRSEDTAVYYCARETLIYPIPFELWGQGTLVTVSSG GGSGGGGGSGGGGSEIVLTQSPGTLSPGERATLS CRASQIVSSSYLAWYQQKPGQAPRLLIYGASSRASG IPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQYGGG PYTFGQGKVEIKTTTPAPRPPTPA PTIASQPLSLRPE ACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLL LSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDG	CD8 α signal peptide; P12B6_VHVL VH; GS linker; P12B6_VHVL VL; CD8 α hinge; CD8 α TM domain; 41BB ISD; CD3 ζ ISD

CAR	CAR Amino Acid Sequence	Components
	CSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQL YNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNP QEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDG LYQGLSTATKDTYDALHMQALPPR (SEQ ID NO: 243)	
P14G2	MALPVTALLLPLALLLHAARPEVQLLESGGGLVQP GGSLRLSCAASGFTFSNYVMNWVRQAPGKGLEWV SAISGSGATTYYADSVKGRFTISRDN SKNTLYLQMN SLRAEDTAVYYCVSGLWAGGIWGQGLTVTVSSGG GGSGGGSGGGGSDIQMTQSPSSLSASVGDRVTTTC RASQSISSYLNWYQQKPGKAPKLLIYDASDLQRGV PSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYNTP WTFGQGTKVEIKTTTPAPRPPTPAPTIASQPLSLRPE ACRPAAGGAVHTRGLDFACDIYWAPLAGTCGVLL LSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDG CSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQL YNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNP QEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDG LYQGLSTATKDTYDALHMQALPPR (SEQ ID NO: 244)	CD8 α signal peptide; P14G2_VHVL VH; GS linker; P14G2_VHVL VL; CD8 α hinge; CD8 α TM domain; 41BB ISD; CD3 ζ ISD

[0274] In some experiments, the sequence encoding the CAR is preceded by a sequence encoding a fluorescent protein such as BFP (Blue Fluorescent Protein) and separated from such sequence by a sequence encoding a self-cleaving 2A peptide. Lentiviruses were produced using psPAX2, an HIV-1 gag-pol packaging plasmid, and pMD2.G, a VSV-G expression plasmid.

b) T cell activation and lentiviral transduction

[0275] Untouched T cells were isolated from human peripheral blood mononuclear cells (PBMCs) using the Pan T Cell isolation kit (Miltenyi Biotec) and activated for three days with antibodies against human CD2, CD3, and CD28 (T Cell activation/expansion kit - Miltenyi

Biotech). Lentiviral vectors (LV) were produced by transient transfection of sub-confluent HEK-293T/17 (American Type Culture Collection (ATCC)) cells in 6-well plates. Briefly, pLVX, psPAX2, and pMD2.G plasmids were transfected at a 4:3:1 ratio, respectively, using Lipofectamine 2000 (Invitrogen) following the manufacturer's instructions. The following day, the media was replaced with T cell culture medium (5% human AB serum in X-vivo-15 medium (Lonza)), and 48 h after transfection the LV supernatant was harvested and filtered through a 0.45 µm syringe filter (Millipore). Activated T cells were seeded at 0.25×10^6 cells/mL in T cell culture medium containing 40 ng/ml IL-2 and transduced by adding an equal volume of fresh LV supernatant. LVs comprising the following CAR constructs were used in these experiments: P1A5, P1B4, P3A1, P3E10, P4C7, P4H11, P7H3, P9B5, P12B6, and P14G2. Cells were cultured at 37°C and 5% CO₂ for three days and used for flow cytometry analysis or expanded in fresh T cell medium containing 20 ng/ml IL-2.

c) Measurement of the transduction efficiency by flow cytometry

[0276] To determine the transduction efficiency of the different FLT3 CAR lentiviral constructs, flow cytometry analysis was performed on T cells 72 h after transduction. Briefly, single cells were gated using forward scatter and side scatter parameters and the transduction efficiency calculated as the percentage of BFP positive cells. In other instances, CAR-expressing cells were detected by staining with anti-CD20 antibody rituximab, which binds the CD20 epitopes in the CAR molecule, followed by FITC-conjugated anti-human IgG. FIG. 1 shows that activated T cells were effectively transduced with the different FLT3 CAR constructs at varying efficiencies (30% to 70%).

d) FLT3FLT3 CAR-T cells differentiate during culture but maintain a high percentage of memory T cell subsets

[0277] Expression of the activation markers CD25 and 4-1BB (CD137) on CAR-T cells during culture has been associated with T cell exhaustion and increased differentiation towards effector memory phenotype (Long et al., 2015). In this experiment, T cells transduced with the P1A5, P1B4, P3A1, P3E10, P4C7, P4H11, P7H3, P9B5, P12B6, and P14G2 CARs were stained with CD25 and CD137 antibodies ten days post-stimulation and analyzed by flow cytometry. The degree of T cell activation was determined by the percentage of CAR-T cells positive for CD25 and 4-1BB. A non-transduced (NT) T cell population was included for comparison (FIG.

2). Expression of some FLT3FLT3 CARs (P1A5, P12B6 and P14G2) was associated with an “activated” state (FIG. 2) whereas expression of other FLT3FLT3 CARs including P9B5, P3E10, P4C7, and P3A1 resulted in levels of activation markers that were comparable with non-transduced cells (FIG. 2). Higher levels of activation marker expression translate into increased differentiation of CAR-T cells, which can be assessed by day 15 post-stimulation by measuring the expression of CD62L and CD45RO on their cell surface. In this experiment, CAR-T cells were stained with such markers using specific antibodies and classified into four different categories or subsets with increasing degree of differentiation: 1) CD62L^{HIGH} CD45RO^{LOW} stem cell memory (Tscm) cells; 2) CD62L^{HIGH} CD45RO^{HIGH} central memory (Tcm) cells; CD62L^{LOW} CD45RO^{HIGH} effector memory (Tem) cells; and CD62L^{LOW} CD45RO^{LOW} effector (Teff) cells. FIG. 3 shows representative FACS plots for P9B5 CAR-T cells (low differentiation) and P14G2 CAR-T cells (high differentiation) and the T cell subset composition of all the different FLT3FLT3 CAR-T cells on day 15 post-stimulation. CAR-T cells exhibiting an “activated” phenotype also showed a more differentiated phenotype (i.e. higher percentage of Tem cells and lower percentage of Tscm cells) by the end of the culture (FIG. 3). In particular, expression of P9B5, P3A1, P7H3, and P3E10 FLT3 CARs was associated with a high percentage of stem cell memory and central memory cells, which have been correlated with increased persistence and antitumor efficacy in ALL and CLL subjects (*Xu et al., Blood. 2014;123(24):3750-3759*).

e) In vitro proliferation and long-term killing activity assay

[0278] The capacity of CAR-T cells to proliferate and lyse target cells expressing the cognate antigen is an effective way to assess their activity in vitro. To monitor target cell viability by luminescence, FLT3-expressing Molm13 cells were stably transduced to express the luciferase gene. These Luc⁺ target cells were then incubated with FLT3 CAR-T cells and the killing activity as well as the proliferation of CAR-T cells was measured by luminescence and viable cell counting, respectively. For a more stringent assay designed to stress the effector cells (CAR-T cells), new target cells were added periodically to the initial culture and the long-term killing activity of CAR-T cells determined by luminescence at the end of the culture (FIG. 4).

Consistent with the higher proliferative capacity of stem cell memory and central memory T cells, P9B5, P3A1, P7H3, and P3E10 CAR-T cell populations showed higher target-dependent expansion and long-term cytotoxic activity in vitro whereas CAR-T cells with a higher degree of

differentiation such as P12B6 and P14G2 expanded poorly in the presence of target cells and had reduced killing activity.

f) Activity of FLT3 specific CAR-T cells in an orthotopic mouse assay

[0279] This example illustrates treatment of AML with FLT3 CAR-T cells using the Eol1 orthotopic model. *In vivo* efficacy study of FLT3 CAR-T cells was performed with Eol1 cells, expressing luciferase and GFP, in an orthotopic model. Three hundred thousand Eol1 Luc2AGFP cells were injected intravenously through the tail vein into 6-8 weeks old female CB17/SCID animals. Intraperitoneal injection of D-luciferin (Regis Technologies, Morton Grove, IL) (200uL per animal at 15mg/mL), followed by anesthesia with isoflurane and subsequent whole body bioluminescence imaging (BLI) enable monitoring of tumor burden. Bioluminescent signals emitted by the interaction between luciferase expressed by the tumor cells and luciferin were captured by imaging using an IVIS Spectrum CT (Perkin Elmer, MA) and quantified as total flux (photons/sec) using Living Image 4.4 (Caliper Life Sciences, Alameda, CA).

[0280] When the total flux reached an average of 30E6 for all animals (day 10 post tumor implant), the animals were randomized into three groups. Each group was administered one of the following cells: 1) non-transduced T cells used as a control, 2) FLT3 CAR-T cells expressing P3E10 ("P3E10 V1"), or 3) FLT3 CAR-T cells expressing P3A1 ("P3A1 v1") All of cells 1-3 possess TCR α . The FLT3 CAR-T cells were prepared as described in example above. FLT3 CAR constructs P3E10 and P3A1 are shown in Table 8 above. A single dose of 2.5 or 5 million control (NT) or FLT3 CAR-T (P3E10 or P3A1) cells were administered through bolus tail vein injection. Animals were terminated when they lose more than 15% of total body weight, an endpoint for Eol1 orthotopic models.

[0281] Results from the study are summarized in FIG. 5. A single dose of 5 million P3E10 or P3A1 FLT3 CAR-T cells or 2.5 million P3E10 FLT3 CAR-T cells resulted in lower total flux from days 13-43 post tumor implant as compared to the negative control. Thus, treatment with FLT3 CAR-T cells inhibited tumor progression as compared to the negative control.

[0282] These results demonstrate FLT3 CAR-T cells are effective to inhibit tumor progression.

Example 3: Depletion of FLT3 specific CAR T cells expressing CD20 epitopes

a) FLT3 specific CAR T cells expressing CD20 epitopes are sensitive to complement-dependent cytotoxicity (CDC) in the presence of rituximab

[0283] The sensitivity of FLT3 specific CAR T cells to complement in the presence of an anti-CD20 antibody was evaluated *in vitro* using a CDC assay. For this experiment, T cells transduced with a lentiviral construct for the expression of a FLT3 CAR containing CD20 epitopes in the extracellular domain were mixed with 25% of complement (AbD serotec) in the presence or absence of rituximab (100 µg/mL) and incubated at 37°C and 5% CO₂ for 4 hours. Depletion of FLT3 CAR-T cells was determined by flow cytometry analysis using biotinylated FLT3 protein. FIG. 6 shows that the cells expressing the FLT3 CAR and the CD20 epitopes were efficiently depleted in the presence of the antibody and complement, while control FLT3 specific CAR T cells that do not express the CD20 epitopes were minimally affected.

[0284] These results demonstrate that FLT3 specific CAR-T cells expressing CD20 epitopes can be depleted *in vitro* in the presence of rituximab and complement.

b) Depletion of FLT3 CAR T cells following rituximab administration allows recovery of FLT3-expressing bone marrow progenitors in NSG mice

[0285] The ability of the anti-CD20 antibody rituximab to mediate depletion of FLT3 specific CAR T cells expressing CD20 epitopes and facilitate bone marrow recovery was tested in mice. In this experiment, mice were treated with T cells expressing either a FLT3 CAR that can bind to mouse FLT3 protein on the surface of bone marrow progenitors (α -mouse FLT3 CAR Ts) and comprising a CD20 epitope recognized by rituximab or a control CAR, with negligible binding to mouse FLT3 protein. Flow cytometry analysis of bone marrow cells was used to demonstrate CAR T cell cytotoxic activity against FLT3-expressing hematopoietic progenitors, seen as a reduction of progenitors compared to mice that received control CAR T cells or to untreated mice (FIG. 7, Panel B). After confirmation of CAR T cell killing activity, mice were given rituximab for four consecutive days and circulating residual CAR T cells were enumerated by flow cytometry at day 13. FIG. 7, Panel C shows that the FLT3 CAR T cells in the blood of these mice were depleted compared to the control group, which did not receive rituximab. Finally, flow cytometry analysis of bone marrow cells demonstrated that only the mice that received rituximab (day 20) showed bone marrow recovery (FIG. 7, Panel D).

[0286] This experiment demonstrates that rituximab-dependent depletion of FLT3 CAR T cells that express CD20 epitopes mitigates damage to FLT3-expressing tissues such as bone marrow, allowing for rapid bone marrow recovery from residual progenitor cells.

[0287] Although the disclosed teachings have been described with reference to various applications, methods, kits, and compositions, it will be appreciated that various changes and modifications can be made without departing from the teachings herein and the claimed invention below. The foregoing examples are provided to better illustrate the disclosed teachings and are not intended to limit the scope of the teachings presented herein. While the present teachings have been described in terms of these exemplary embodiments, the skilled artisan will readily understand that numerous variations and modifications of these exemplary embodiments are possible without undue experimentation. All such variations and modifications are within the scope of the current teachings.

[0288] All references cited herein, including patents, patent applications, papers, text books, and the like, and the references cited therein, to the extent that they are not already, are hereby incorporated by reference in their entirety. In the event that one or more of the incorporated literature and similar materials differs from or contradicts this application, including but not limited to defined terms, term usage, described techniques, or the like, application controls.

[0289] The foregoing description and Examples detail certain specific embodiments of the invention and describes the best mode contemplated by the inventors. It will be appreciated, however, that no matter how detailed the foregoing may appear in text, the invention may be practiced in many ways and the invention should be construed in accordance with the appended claims and any equivalents thereof.

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) comprising 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.

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 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.

5. 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 the VH region comprises 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; and the VL region comprises 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.

6. 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, 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.

7. The FLT3 specific CAR of any one of claims 2-5, wherein the VH region comprises 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, or a variant thereof with one or several conservative amino acid substitutions in residues that are not within a CDR and/or the VL region comprises the amino acid 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, or a variant thereof with one or several amino acid substitutions in amino acids that are not within a CDR.

8. The FLT3 specific CAR of any one of claims 1-7, wherein 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.

9. The FLT3 specific CAR of any one of claims 1-7, wherein 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.

10. The FLT3 specific CAR of claim 8, 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.

11. The FLT3 specific CAR of claim 9, 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.

12. The FLT3 specific CAR of any one of claims 1-7, wherein 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.

13. The FLT3 specific CAR of claim 12, 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.

14. The FLT3 specific CAR of any one of claims 1-7, wherein 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.

15. The FLT3 specific CAR of claim 14, 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.
16. The FLT3 specific CAR of any one of claims 1 to 15, wherein the intracellular signaling domain comprises a CD3 ζ signalling domain.
17. The FLT3 specific CAR of any one of claims 1 to 15, wherein the intracellular signaling domain comprises a 4-1BB domain.
18. The FLT3 specific CAR of any one of claims 1 to 17, comprising a second intracellular signaling domain.
19. The FLT3 specific CAR of claim 18, wherein the second intracellular signaling domain comprises a 4-1BB domain.
20. The FLT3 specific CAR of any one of claims 1 to 19, comprising a stalk domain between the extracellular ligand-binding domain and the first transmembrane domain.
21. The FLT3 specific CAR of claim 20, 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.
22. The FLT3 specific CAR of any one of claims 1 to 21, comprising one or more epitopes specific for a monoclonal antibody.
23. The FLT3 specific CAR of claim 22, wherein the epitope is 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 activatin 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.
24. The FLT3 specific CAR of claim 22 or 23, wherein the epitope is 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.

25. The FLT3 specific CAR of any one of claims 22-24, wherein the epitope is a CD20 epitope.

26. The FLT3 specific CAR of claim 25, wherein the CD20 epitope comprises the amino acid sequence shown in SEQ ID NO: 229.

27. 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.

28. The FLT3 specific CAR of claim 1, wherein the FLT3 specific CAR comprises the amino acid sequence shown in SEQ ID NO: 235.

29. The FLT3 specific CAR of claim 1, wherein the FLT3 specific CAR comprises the amino acid sequence shown in SEQ ID NO: 236.

30. The FLT3 specific CAR of any one of claims 1 to 29, wherein the first transmembrane domain comprises a CD8 α chain transmembrane domain.

31. The FLT3 specific CAR of any one of claims 1 to 30, comprising a second extracellular ligand-binding domain that is not specific for FLT3.

32. The FLT3 specific CAR of any one of claims 1 to 31, wherein the extracellular ligand-binding domain(s), the first transmembrane domain, and intracellular signaling domain(s) are on a single polypeptide.

33. The FLT3 specific CAR of any one of claims 1 to 32, comprising 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.

34. The FLT3 specific CAR of claim 33, 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.
35. A polynucleotide comprising a nucleic acid sequence encoding the FLT3 specific CAR of any one of claims 1 to 34.
36. The polynucleotide of claim 35, wherein the polynucleotide comprises the nucleic acid sequence of the vector having ATCC Accession No. PTA-124227.
37. The polynucleotide of claim 35, wherein the polynucleotide comprises the nucleic acid sequence of the vector having ATCC Accession No. PTA-124228.
38. The polynucleotide of claim 35, wherein the polynucleotide comprises the nucleic acid sequence shown in SEQ ID NO: 253.
39. The polynucleotide of claim 35, wherein the polynucleotide comprises the nucleic acid sequence shown in SEQ ID NO: 248.
40. The polynucleotide of claim 35, wherein the polynucleotide comprises the nucleic acid sequence shown in SEQ ID NO: 247.
41. The polynucleotide of claim 35, wherein the polynucleotide comprises the nucleic acid sequence shown in SEQ ID NO: 246.
42. The polynucleotide of claim 35, wherein the polynucleotide comprises the nucleic acid sequence shown in SEQ ID NO: 245.
43. An expression vector comprising the polynucleotide of any one of claims 35 to 42.
44. An engineered immune cell expressing a FLT3 specific CAR of any one of claims 1 to 34.
45. The engineered immune cell of claim 44, comprising another CAR which is not specific for FLT3.

46. The engineered immune cell of claim 44 or 45, comprising a polynucleotide encoding a suicide polypeptide.
47. The engineered immune cell of claim 46, wherein the suicide polypeptide is RQR8.
48. The engineered immune cell of any one of claims 44 to 47, wherein the immune cell is derived from an inflammatory T-lymphocyte, a cytotoxic T-lymphocyte, a regulatory T-lymphocyte, or a helper T-lymphocyte.
49. The engineered immune cell of any one of claims 44 to 48, comprising 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.
50. The engineered immune cell of claim 49, wherein the immune checkpoint protein is programmed death-1 (PD-1).
51. The engineered immune cell of any one of claims 44 to 50, wherein the immune cell is obtained from a healthy donor.
52. The engineered immune cell of any one of claims 44 to 50, wherein the immune cell is obtained from a donor suffering from a disease or disorder.
53. The engineered immune cell of any one of claims 44 to 52 for use as a medicament.
54. The engineered immune cell of claim 53, wherein the medicament is for use in 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, Burkitt's lymphoma, marginal zone lymphoma, mantle cell lymphoma, large cell lymphoma, precursor B-lymphoblastic lymphoma, myeloid leukemia, Waldenstrom's macroglobulinemia, diffuse large B cell lymphoma, follicular lymphoma, marginal zone lymphoma, mucosa-associated lymphatic tissue lymphoma, small cell lymphocytic lymphoma, mantle cell 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, intravascular large B-cell lymphoma, 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.

55. The engineered immune cell of any one of claims 44 to 54, 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.

56. A population of cells comprising engineered immune cells of any one of claims 44 to 55, wherein

(ii) said population of cells comprises a percentage of stem cell memory and central memory cells greater than 20%, 30% or 40%, and/or

(iii) said population of cells achieves a percentage of lysis of FLT3 expressing cells greater than 10%, 20%, 30% or 40%.

57. 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 34;

whereby the immune cell expresses the FLT3 specific CAR.

58. The method of engineering an immune cell, comprising:

- a. providing an immune cell;
- b. introducing into the cell at least one polynucleotide encoding FLT3 specific CAR of any one of claims 1 to 34; and
- c. introducing into the cell at least one polynucleotide encoding for a CAR which is not specific for FLT3.

59. A method of treating a subject in need thereof, comprising:

- a. providing a population of immune cells expressing a FLT3 specific CAR of any one of claims 1 to 34; and
- b. administering said population of immune cells to said subject.

60. A pharmaceutical composition, comprising the engineered immune cells of any one of claims 44 to 52.

61. A method of treating a disease or disorder associated with FLT3 in a subject in need thereof, comprising administering to the subject an effective amount of the pharmaceutical composition of claim 60 or the engineered immune cells of any one of claims 44 to 52.

62. The method of claim 61, wherein the disease or disorder associated with FLT3 is a cancer.

63. The method of claim 62, wherein the cancer is selected from the group consisting of is a 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, Burkitt's lymphoma, marginal zone lymphoma, mantle cell lymphoma, large cell lymphoma, precursor B-lymphoblastic lymphoma, myeloid leukemia, Waldenstrom's macroglobulinemia, diffuse large B cell lymphoma, follicular lymphoma, marginal zone lymphoma, mucosa-associated lymphatic tissue lymphoma, small cell lymphocytic lymphoma, mantle cell 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, intravascular large B-cell lymphoma, 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.

64. A method of inhibiting tumor growth or progression in a subject who has malignant cells expressing FLT3, comprising administering to the subject an effective amount of the pharmaceutical composition of claim 60 or the engineered immune cells of any one of claims 44 to 52 to the subject.

65. A method of inhibiting metastasis of malignant cells expressing FLT3 in a subject in need thereof, comprising administering to the subject an effective amount of the pharmaceutical composition of claim 60 or the engineered immune cells of any one of claims 44 to 52 to the subject.

66. A method of inducing tumor regression in a subject who has malignant cells expressing FLT3, comprising administering to the subject an effective amount of the pharmaceutical composition of claim 60 or the engineered immune cells of any one of claims 44 to 52 to the subject.

67. The method of any one of claims 61 to 66, wherein the method comprises administering a nucleoside analog therapy to the subject.

68. The method of claim 67, wherein the nucleoside analog therapy is fludarabine or clofarabine.

69. The method of any one of claims 61 to 66, wherein the method comprises administering 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.

70. The method of any one of claims 61 to 66, wherein the method comprises administering a proteasome inhibitor, an immunomodulatory agent or a Hedgehog inhibitor or combination thereof to the subject.

71. The method of any one of claims 61 to 66, wherein the method comprises administering lenalidomide to the subject.

72. 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 any one of claims 22 to 26, 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.

73. The method of claim 72, wherein the monoclonal antibody specific for the epitope is conjugated to a fluorophore and optionally the step of selecting the cells that bind to the monoclonal antibody is done by Fluorescence Activated Cell Sorting (FACS).

74. The method of claim 72, wherein the monoclonal antibody specific for the epitope is conjugated to 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).

75. The method of any one of claims 72 to 74, wherein the epitope is a CD 20 epitope.

76. The method of claim 75, wherein the CD20 epitope has an amino acid sequence shown in SEQ ID NO: 229 and optionally, the monoclonal antibody is rituximab.

77. The method of any one of claims 72 to 76, wherein the population of cells enriched in engineered immune cell comprises at least 70% of FLT3 CAR-expressing immune cells.

78. A method for depleting from a subject engineered immune cells, wherein the subject has been administered engineered immune cells expressing a FLT3 specific chimeric antigen receptor (CAR) of any one of claims 22 to 26, the method comprising administering to the subject a monoclonal antibody specific for the epitope.

79. The method of claim 76, wherein the epitope is a CD20 epitope.
80. The method of claim 79, wherein the CD20 epitope has an amino acid sequence shown in SEQ ID NO: 229 and optionally, the monoclonal antibody is rituximab.
81. The method of any one of claims 78 to 80, wherein the monoclonal antibody is conjugated to a molecule that can activate the complement system.
82. The method of any one of claims 78 to 80, wherein a cytotoxic drug is coupled to the monoclonal antibody.
83. A method for promoting recovery of endogenous FLT3-expressing bone marrow progenitor cells in a subject administered with engineered immune cells expressing a FLT3 specific chimeric antigen receptor (CAR) of any one of claims 22 to 26, the method comprising administering a monoclonal antibody specific for the epitope to the patient.
84. The method of claim 83, wherein the epitope is a CD20 epitope.
85. The method of claim 84, wherein the CD20 epitope has an amino acid sequence shown in SEQ ID NO: 229 and optionally, the monoclonal antibody is rituximab.
86. The method of any one of claims 83 to 85, wherein the monoclonal antibody is conjugated to a molecule that can activate the complement system.
87. The method of any one of claims 83 to 85, wherein a cytotoxic drug is coupled to the monoclonal antibody.