

hypervariable or complementarity determining regions (CDRs). When the TCR engages with antigenic peptide and MHC (peptide/MHC), the T cell becomes activated, enabling it to attack and destroy the target cell.

[0017] However, current therapies have shown varying levels of effectiveness with undesired side effects. Therefore, a need exists to identify novel and improved therapies for treating FLT3 related diseases and disorders.

SUMMARY OF THE INVENTION

[0018] The invention relates to engineered immune cells (such as CARs or TCRs), antigen binding molecules (including but not limited to, antibodies, scFvs, heavy and/or light chains, and CDRs of these antigen binding molecules) with specificity to FLT3.

[0019] The invention further relates to a novel CD28 sequence useful as costimulatory domains in these cells.

[0020] Chimeric antigen receptors of the invention typically comprise: (i) a FLT3 specific antigen binding molecule, (ii) one or more costimulatory domain, and (iii) one or more activating domain. It will be appreciated that each domain may be heterogeneous, thus comprised of sequences derived from different protein chains.

[0020a] In one aspect, the present inventions relates to a chimeric antigen receptor comprising an antigen binding molecule that specifically binds to FLT3, a costimulatory domain, and an activating domain that is a signaling domain of CD3 zeta, wherein the antigen binding molecule comprises:

- a) a variable heavy chain region comprising complementary determining regions (“CDRs”) 1, 2, and 3 with amino acid sequences SEQ ID NO:17, SEQ ID NO:18, and SEQ ID NO:19, respectively, and a variable light chain region comprising CDRs 1, 2, and 3 with amino acid sequences SEQ ID NO:22, SEQ ID NO:23, and SEQ ID NO:24, respectively; or
- b) a variable heavy chain region comprising complementary determining regions (“CDRs”) 1, 2, and 3 with amino acid sequences SEQ ID NO:97, SEQ ID NO:35, and SEQ ID NO:36, respectively, and a variable light chain region comprising CDRs 1, 2, and 3 with amino acid sequences SEQ ID NO:38, SEQ ID NO:39, and SEQ ID NO:40, respectively;

and the costimulatory domain is a CD28 costimulatory domain comprising a sequence that differs at no more than 10, 9, 8, 7, 6, 5, 4, 3, 2, 1, or 0 amino acid residues from the sequence of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, or SEQ ID NO: 8.

[0020b] In yet another aspect, the present invention relates to an isolated polypeptide comprising the amino acid sequence of SEQ ID NO: 64 or SEQ ID NO: 70.

[0021] In some examples, the disclosure relates to a chimeric antigen receptor comprising an antigen binding molecule that specifically binds to FLT3, wherein the antigen binding molecule comprises at least one of: (a) a variable heavy chain CDR1 comprising an amino acid sequence differing from that of SEQ ID NO: 17 by not more than 3, 2, 1, or 0 amino acid residues; (b) a variable heavy chain CDR2 comprising an amino acid sequence differing from that of SEQ ID NO:18 or SEQ ID NO:26 by not more than 3, 2, 1, or 0 amino acid residues; (c) a variable heavy chain CDR3 comprising an amino acid sequence differing from that of SEQ ID NOs SEQ ID NO: 19 or SEQ ID NO:27 by not more than 3, 2, 1, or 0 amino acid residues; (d) a variable light chain CDR1 comprising an amino acid sequence differing from that of SEQ ID NO:22 or SEQ ID NO:30 by not more than 3, 2, 1, or 0 amino acid residues; (e) a variable light chain CDR2 comprising an amino acid sequence differing from that of SEQ ID NO:23 or 31 by not more than 3, 2, 1, or 0 amino acid residues; (f) a variable light chain CDR3 comprising an amino acid sequence differing from that of SEQ ID:24 or SEQ ID NO:32 by not more than 3, 2, 1, or 0 amino acid residues.

[0026] In other examples, the disclosure relates to a chimeric antigen receptor wherein the costimulatory domain comprises SEQ ID NO: 2 and the activating domain comprises SEQ ID NO: 10.

[0027] In another aspect, the invention further relates to polynucleotides encoding the chimeric antigen receptors, and vectors comprising the polynucleotides. The vector can be, for example, a retroviral vector, a DNA vector, a plasmid, a RNA vector, an adenoviral vector, an adenovirus associated vector, a lentiviral vector, or any combination thereof. In another example, the disclosure further relates to immune cells comprising the vectors. In some examples, the lentiviral vector is a pGAR vector.

[0028] Exemplary immune cells include, but are not limited to T cells, tumor infiltrating lymphocytes (TILs), NK cells, TCR-expressing cells, dendritic cells, or NK-T cells. The T cells can be autologous, allogeneic, or heterologous. In yet another aspect, the invention relates to pharmaceutical compositions comprising the immune cells of described herein.

[0029] In certain examples, the disclosure relates to antigen binding molecules (and chimeric antigen receptors comprising these molecules) comprising at least one of:

- (a) a VH region differing from the amino acid sequence of the VH region of 10E3 by no more than 10, 9, 8, 7, 6, 5, 4, 3, 2, 1, or 0 amino acid residues and a VL region differing from the amino acid sequence of the VL region of 10E3 by no more than 10, 9, 8, 7, 6, 5, 4, 3, 2, 1, or 0 amino acid residues;
- (b) a VH region differing from the amino acid sequence of the VH region of 2E7 by no more than 10, 9, 8, 7, 6, 5, 4, 3, 2, 1, or 0 amino acid residues and a VL region differing from the amino acid sequence of the VL region of 2E7 by no more than 10, 9, 8, 7, 6, 5, 4, 3, 2, 1, or 0 amino acid residues;
- (c) a VH region differing from the amino acid sequence of the VH region of 8B5 by no more than 10, 9, 8, 7, 6, 5, 4, 3, 2, 1, or 0 amino acid residues and a VL region differing from the amino acid sequence of the VL region of 8B5 by no more than 10, 9, 8, 7, 6, 5, 4, 3, 2, 1, or 0 amino acid residues;
- (d) a VH region differing from the amino acid sequence of the VH region of 4E9 by no more than 10, 9, 8, 7, 6, 5, 4, 3, 2, 1, or 0 amino acid residues and a VL region differing

from the amino acid sequence of the VL region of 4E9 by no more than 10, 9, 8, 7, 6, 5, 4, 3, 2, 1, or 0 amino acid residues; and

(e) a VH region differing from the amino acid sequence of the VH region of 11F11 by no more than 10, 9, 8, 7, 6, 5, 4, 3, 2, 1, or 0 amino acid residues and a VL region differing from the amino acid sequence of the VL region of 10E3 by no more than 10, 9, 8, 7, 6, 5, 4, 3, 2, 1, or 0 amino acid residues;

and wherein the VH and VL region or regions are linked by at least one linker.

[0030] In other examples, the disclosure relates to antigen binding molecules (and chimeric antigen receptors comprising these molecules) wherein the linker comprises at least one of the scFv G4S linker and the scFv Whitlow linker.

[0031] In another aspect, the present invention relates to vectors encoding the polypeptides as disclosed herein and to immune cells comprising these polypeptides. Preferred immune cells include T cells, tumor infiltrating lymphocytes (TILs), NK cells, TCR-expressing cells, dendritic cells, or NK-T cells. The T cells may be autologous, allogeneic, or heterologous.

[0031a] In yet another aspect, the present invention relates to an isolated polynucleotide encoding a chimeric antigen receptor (CAR), said CAR comprising an antigen binding molecule that specifically binds to FLT3, wherein the antigen binding molecule comprises the polynucleotide sequence SEQ ID NO:63 or SEQ ID NO:69.

[0032] In other examples, the disclosure relates to isolated polynucleotides encoding a chimeric antigen receptor (CAR) or T cell receptor (TCR) comprising an antigen binding molecule that specifically binds to FLT3, wherein the antigen binding molecule comprises a variable heavy (V_H) chain CDR3 comprising an amino acid sequence of SEQ ID NO: 19 or SEQ ID NO:27. The polynucleotides may further comprise an activating domain. In preferred examples, the activating domain is CD3, more preferably CD3 zeta, more preferably the amino acid sequence set forth in SEQ ID NO: 9.

[0033] In other examples, the disclosure includes a costimulatory domain, such as CD28, CD28T, OX40, 4-1BB/CD137, CD2, CD3 (alpha, beta, delta, epsilon, gamma, zeta), CD4, CD5, CD7, CD9, CD16, CD22, CD27, CD30, CD 33, CD37, CD40, CD 45, CD64, CD80, CD86, CD134, CD137, CD154, PD-1, ICOS, lymphocyte function-associated antigen-1 (LFA-1 (CD11a/CD18), CD247, CD276 (B7-H3), LIGHT (tumor necrosis factor superfamily member 14; TNFSF14), NKG2C, Ig alpha (CD79a), DAP-10, Fc gamma receptor, MHC class I molecule, TNF, TNFr, integrin, signaling lymphocytic activation molecule, BTLA, Toll ligand receptor, ICAM-1, B7-H3, CDS, ICAM-1, GITR, BAFFR, LIGHT, HVEM (LIGHTR), KIRDS2, SLAMF7, NKp80 (KLRF1), NKp44, NKp30, NKp46, CD19, CD4, CD8alpha, CD8beta, IL-2R beta, IL-2R gamma, IL-7R alpha, ITGA4, VLA1, CD49a, ITGA4, IA4, CD49D, ITGA6, VLA-6, CD49f, ITGAD, CD11d, ITGAE, CD103, ITGAL, CD11a, LFA-1, ITGAM, CD11b, ITGAX, CD11c, ITGB1, CD29, ITGB2, CD18, LFA-1, ITGB7, NKG2D, TNFR2, TRANCE/RANKL, DNAM1 (CD226), SLAMF4 (CD244, 2B4),

CDR1, CDR2, CDR3, and variable light chain CDR1, CDR2, and CDR3 sequences as described herein.

[0038] Additional heavy and light chain variable domains and CDR polynucleotide and amino acid sequences suitable for use in FLT3-binding molecules according to the present disclosure are found in U.S. Provisional Application Number 62/199,944, filed on July 31, 2015.

[0038a] In yet another aspect, the present invention relates to the polynucleotide, polypeptide, chimeric antigen receptor, cell or pharmaceutical composition as disclosed herein for use in treating a disease or a disorder.

[0038b] In yet another aspect, the present invention relates to the use of the polynucleotide, polypeptide, chimeric antigen receptor, cell or pharmaceutical composition as disclosed herein in the manufacture of a medicament for treating cancer wherein the cancer is selected from the group consisting of acute myeloid leukemia (AML), chronic myelogenous leukemia (CML), chronic myelomonocytic leukemia (CMML), juvenile myelomonocytic leukemia, atypical chronic myeloid leukemia, acute promyelocytic leukemia (APL), acute monoblastic leukemia, acute erythroid leukemia, acute megakaryoblastic leukemia, myelodysplastic syndrome (MDS), myeloproliferative disorder, myeloid neoplasm, myeloid sarcoma), and inflammatory/autoimmune disease; wherein the inflammatory/autoimmune disease is selected from the group consisting of rheumatoid arthritis, psoriasis, allergies, asthma, Crohn's disease, IBD, IBS, fibromyalgia, mastocytosis, and Celiac disease.

ACAGAAACCAGGGAAAGCCCCTCAGCGCCTGCTCTATGCTGCATCCACTTTGCAA
AGTGGGGTCCCATCAAGGTTTCAGCGGCAGTGGATCTGGGACAGAATTCCTCTC
ACAATCAGCAGCCTGCAGCCTGAAGATTTTGCAACTTATTACTGTCTACAGTATA
ATACTTACCCGTGGACGTTTCGGTCAGGGAACGAAGGTGGAAATCAAACGA (SEQ
ID NO: 28)

[0237] Clone 2E7 LC AA (CDRs underlined)

DIQMTQSPSSLSASVGDRVTITCRASQDIRNDFGWYQQKPGKAPQRLLYAASTLQSG
VPSRFGSGSGTEFTLTISLQPEDFATYYCLQYNTYPWTFGQGTKVEIKR (SEQ ID
NO: 29)

[0238] Clone 2E7 LC AA CDR1: RASQDIRNDFG (SEQ ID NO: 30)

[0239] Clone 2E7 LC AA CDR2: AASTLQS (SEQ ID NO: 31)

[0240] Clone 2E7 LHC AA CDR3: LQYNTYPWT (SEQ ID NO: 32)

[0241] Clone 8B5 HC DNA

CAGATACAACTGGTGGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTCCCTG
AGACTCTCCTGTGTAGCGTCTGGATTCACCTTCAAGAACTATGGCATGCACTGGG
TCCGCCAGGCTCCAGGCAAGGGGCTGGAGTGGGTGGCAGTTATTTGGTATGATG
GAAGTAATGAATACTATGGAGACCCCGTGAAGGGCCGATTCACCATCTCCAGAG
ACAACTCCAAGAACATGTTGTATCTGCAAATGAACAGCCTGAGAGCCGATGACA
CGGCTGTGTATTACTGTGCGAGGTCGGGAATAGCAGTGGCTGGGGCCTTTGACTA
CTGGGGCCAGGGAACCCTGGTCACCGTCTCCTCA (SEQ ID NO: 33)

[0242] Clone 8B5 HC AA (CDRs underlined)

QIQLVESGGGVVQPGRSLRLSCVASGFTFKNYGMHWVRQAPGKGLEWVAVVIWYDG
SNEYYGDPVKGRFTISRDN SKNMLYLQMNSLRADDTAVYYCARSGIAVAGAFDYW
GQGT LVT VSS (SEQ ID NO: 34)

[0243] Clone 8B5 HC AA CDR1: NYGMH (SEQ ID NO: 97)

[0244] Clone 8B5 HC AA CDR2: VIWYDGSNEYYGDPVKG (SEQ ID NO: 35)

[0245] Clone 8B5 HC AA CDR3: SGIAVAGAFDY (SEQ ID NO: 36)

What is claimed:

1. A chimeric antigen receptor comprising an antigen binding molecule that specifically binds to FLT3, a costimulatory domain, and an activating domain that is a signaling domain of CD3 zeta, wherein the antigen binding molecule comprises:
 - a) a variable heavy chain region comprising complementary determining regions (“CDRs”) 1, 2, and 3 with amino acid sequences SEQ ID NO:17, SEQ ID NO:18, and SEQ ID NO:19, respectively, and a variable light chain region comprising CDRs 1, 2, and 3 with amino acid sequences SEQ ID NO:22, SEQ ID NO:23, and SEQ ID NO:24, respectively; or
 - b) a variable heavy chain region comprising complementary determining regions (“CDRs”) 1, 2, and 3 with amino acid sequences SEQ ID NO:97, SEQ ID NO:35, and SEQ ID NO:36, respectively, and a variable light chain region comprising CDRs 1, 2, and 3 with amino acid sequences SEQ ID NO:38, SEQ ID NO:39, and SEQ ID NO:40, respectively;and the costimulatory domain is a CD28 costimulatory domain comprising a sequence that differs at no more than 10, 9, 8, 7, 6, 5, 4, 3, 2, 1, or 0 amino acid residues from the sequence of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, or SEQ ID NO: 8.
2. The chimeric antigen receptor according to claim 1 wherein the CD3 zeta comprises a sequence that differs at no more than 10, 9, 8, 7, 6, 5, 4, 3, 2, 1, or 0 amino acid residues from the sequence of SEQ ID NO: 10.
3. The chimeric antigen receptor according to claim 1 wherein the costimulatory domain comprises a sequence that differs at no more than 10, 9, 8, 7, 6, 5, 4, 3, 2, 1, or 0 amino acid residues from the sequence of SEQ ID NO: 2 and the activating domain comprises a sequence that differs at no more than 10, 9, 8, 7, 6, 5, 4, 3, 2, 1, or 0 amino acid residues from the sequence of SEQ ID NO: 10.
4. A polynucleotide encoding the chimeric antigen receptor of claim 1.
5. A vector comprising the polynucleotide of claim 4.
6. The vector according to claim 5 which is a retroviral vector, a DNA vector, a plasmid, a RNA vector, an adenoviral vector, an adenovirus associated vector, a lentiviral vector, or any combination thereof.

7. An immune cell comprising the vector of claim 5.
8. The immune cell according to claim 7, wherein the immune cell is a T cell, tumor infiltrating lymphocyte (TIL), NK cell, TCR-expressing cell, dendritic cell, or NK-T cell.
9. The immune cell according to claim 8, wherein the cell is an autologous T cell.
10. The immune cell according to claim 8, wherein the cell is an allogeneic T cell.
11. The immune cell of claim 7, wherein the vector is introduced into a cell that is isolated from a patient's body or that is grown from a sample taken from a patient's body.
12. The immune cell of claim 7, wherein the vector is introduced into a cell that is isolated from a donor's body or that is grown from a sample taken from a patient's body.
13. A pharmaceutical composition comprising an immune cell of claim 7.
14. An isolated polypeptide comprising the amino acid sequence of SEQ ID NO:64 or SEQ ID NO:70.
15. A vector encoding the polypeptide of claim 14.
16. An immune cell comprising the polypeptide of claim 14.
17. The immune cell according to claim 16, wherein the immune cell is a T cell, tumor infiltrating lymphocyte (TIL), NK cell, TCR-expressing cell, dendritic cell, or NK-T cell.
18. The T cell of claim 17 that is an autologous T cell.
19. The T cell of claim 17 that is an allogeneic T cell.
20. An isolated polynucleotide encoding a chimeric antigen receptor (CAR), said CAR comprising an antigen binding molecule that specifically binds to FLT3, wherein the antigen binding molecule comprises the polynucleotide sequence SEQ ID NO:63 or SEQ ID NO:69.
21. The polynucleotide according to claim 4 or 20, the polypeptide according to claim 14, the chimeric antigen receptor according to claim 1, the cell according to claim 7 or 16, or the pharmaceutical composition according to claim 13 for use in treating a disease or a disorder.
22. Use of the polynucleotide according to claim 4 or 20, in the manufacture of a medicament for treating cancer wherein the cancer is selected from the group consisting of acute myeloid leukemia (AML), chronic myelogenous leukemia (CML), chronic myelomonocytic leukemia (CMML), juvenile myelomonocytic leukemia, atypical chronic myeloid leukemia, acute

promyelocytic leukemia (APL), acute monoblastic leukemia, acute erythroid leukemia, acute megakaryoblastic leukemia, myelodysplastic syndrome (MDS), myeloproliferative disorder, myeloid neoplasm, myeloid sarcoma), and inflammatory/autoimmune disease; wherein the inflammatory/autoimmune disease is selected from the group consisting of rheumatoid arthritis, psoriasis, allergies, asthma, Crohn's disease, IBD, IBS, fibromyalgia, mastocytosis, and Celiac disease.

23. Use of the polypeptide according to claim 14 in the manufacture of a medicament for treating cancer wherein the cancer is selected from the group consisting of acute myeloid leukemia (AML), chronic myelogenous leukemia (CML), chronic myelomonocytic leukemia (CMML), juvenile myelomonocytic leukemia, atypical chronic myeloid leukemia, acute promyelocytic leukemia (APL), acute monoblastic leukemia, acute erythroid leukemia, acute megakaryoblastic leukemia, myelodysplastic syndrome (MDS), myeloproliferative disorder, myeloid neoplasm, myeloid sarcoma), and inflammatory/autoimmune disease; wherein the inflammatory/autoimmune disease is selected from the group consisting of rheumatoid arthritis, psoriasis, allergies, asthma, Crohn's disease, IBD, IBS, fibromyalgia, mastocytosis, and Celiac disease.

24. Use of the chimeric antigen receptor according to claim 1 in the manufacture of a medicament for treating cancer wherein the cancer is selected from the group consisting of acute myeloid leukemia (AML), chronic myelogenous leukemia (CML), chronic myelomonocytic leukemia (CMML), juvenile myelomonocytic leukemia, atypical chronic myeloid leukemia, acute promyelocytic leukemia (APL), acute monoblastic leukemia, acute erythroid leukemia, acute megakaryoblastic leukemia, myelodysplastic syndrome (MDS), myeloproliferative disorder, myeloid neoplasm, myeloid sarcoma), and inflammatory/autoimmune disease; wherein the inflammatory/autoimmune disease is selected from the group consisting of rheumatoid arthritis, psoriasis, allergies, asthma, Crohn's disease, IBD, IBS, fibromyalgia, mastocytosis, and Celiac disease.

25. Use of the cell according to claim 7 or 16, in the manufacture of a medicament for treating cancer wherein the cancer is selected from the group consisting of acute myeloid leukemia (AML), chronic myelogenous leukemia (CML), chronic myelomonocytic leukemia (CMML), juvenile myelomonocytic leukemia, atypical chronic myeloid leukemia, acute promyelocytic leukemia (APL), acute monoblastic leukemia, acute erythroid leukemia, acute megakaryoblastic leukemia, myelodysplastic syndrome (MDS), myeloproliferative disorder,

myeloid neoplasm, myeloid sarcoma), and inflammatory/autoimmune disease; wherein the inflammatory/autoimmune disease is selected from the group consisting of rheumatoid arthritis, psoriasis, allergies, asthma, Crohn's disease, IBD, IBS, fibromyalgia, mastocytosis, and Celiac disease.

26. Use of the pharmaceutical composition according to claim 13 in the manufacture of a medicament for treating cancer wherein the cancer is selected from the group consisting of acute myeloid leukemia (AML), chronic myelogenous leukemia (CML), chronic myelomonocytic leukemia (CMML), juvenile myelomonocytic leukemia, atypical chronic myeloid leukemia, acute promyelocytic leukemia (APL), acute monoblastic leukemia, acute erythroid leukemia, acute megakaryoblastic leukemia, myelodysplastic syndrome (MDS), myeloproliferative disorder, myeloid neoplasm, myeloid sarcoma), and inflammatory/autoimmune disease; wherein the inflammatory/autoimmune disease is selected from the group consisting of rheumatoid arthritis, psoriasis, allergies, asthma, Crohn's disease, IBD, IBS, fibromyalgia, mastocytosis, and Celiac disease.

27. The use according to any of claims 21, 22, 23, 24 or 25 wherein the cancer is leukemia, lymphoma, or myeloma.

28. The use according to any of claims 21, 22, 23, 24 or 25, wherein the cancer is AML.

29. The lentiviral vector according to claim 6, wherein the lentiviral vector is a pGAR vector.