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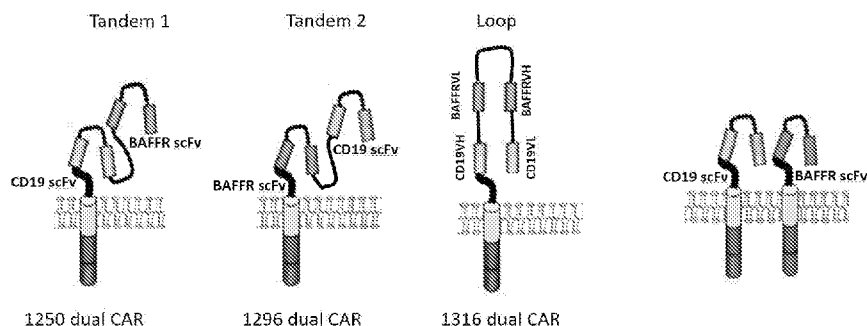
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(54) Title: BAFF-R/CD19 TARGETED CHIMERIC ANTIGEN RECEPTOR-MODIFIED T CELLS AND USE THEREOF

FIGURE 1



(57) Abstract: Chimeric antigen receptors targeting both BAFF-R and CD19 are described as are methods for their use.

BAFF-R/CD19 TARGETED CHIMERIC ANTIGEN RECEPTOR-MODIFIED T CELLS AND USE THEREOF

BACKGROUND

[001] Tumor-specific T cell based immunotherapies, including therapies employing engineered T cells, have been investigated for anti-tumor treatment.

[002] CD19 CAR-T cell therapy is effective in many patients with B-ALL and lymphoma. However, relapse due to antigen loss variants escaping CD19-directed CAR-T therapy can occur in up to 20-30% of patient. CD22 CAR-T cell therapy has been proposed as one alternative strategy to overcome relapse from CD19 antigen loss, as demonstrated by achievement of clinical responses in patients with CD19-negative B-ALL. However, CD22 expression density can vary particularly in mixed lineage leukemia (MLL) and its expression was reported to diminish after CD22-targeted therapy.

SUMMARY

[003] Described herein are methods for treating B cell malignancies, including B-ALL and lymphoma, using T cells expressing a chimeric antigen receptor (CAR) targeted to B cell activating factor receptor (BAFF-R) and CD19. In some cases, a single CAR targets both BAFF-R and CD19. In the case of a single CAR targeting both BAFF-R and CD19 the extracellular targeting portion can have a tandem format in which an scFv to one of the targets precedes the other scFv or a loop format in which an scFv to one of the two targets is disposed between the VL and VH domains of a scFv to the other target. In any of these formats, the CAR includes: a transmembrane domain (e.g., a CD8 transmembrane domain), a co-stimulatory domain (e.g., a 4-1BB co-stimulatory domain) and a CD3 zeta signaling domain. The CAR can also include a spacer sequence between, for example, the scFv domain(s) and the transmembrane domain, between the

transmembrane domain and the co-stimulatory domain, and/or between the co-stimulatory domain and the CD3 zeta signaling domain.

[004] Described herein is a nucleic acid molecule comprising a nucleotide sequence encoding a chimeric antigen receptor targeted to both BAFF-R and CD19, wherein the chimeric antigen receptor comprises, from amino to carboxy terminus: a targeting domain for targeting BAFF-R and CD19; a spacer domain; a transmembrane domain; a costimulatory domain; and a CD3 ζ signaling domain.

[005] In various embodiments: (1) the targeting domain comprises, from amino to carboxy terminus: a) a scFv targeted to BAFF-R and a scFv targeted to CD19; or b) a scFv targeted to CD19 and a scFv targeted to BAFF-R; (2) the targeting domain comprises, from amino to carboxy terminus: a) a VL domain of a CD19 scFv; a scFv targeted to BAFF-R; and a VH of the CD19 scFv; or b) a VH domain of a CD19 scFv; a scFv targeted to BAFF-R; and a VL of the CD19 scFv; and (3) the targeting domain comprises, from amino to carboxy terminus: a) a VL domain of a BAFF-R scFv; a scFv targeted to CD19; and a VH of the BAFF-R scFv; or b) a VH domain of a BAFF-R scFv; a scFv targeted to CD19; and a VL of the BAFF-R scFv.

[006] Also described herein is a nucleic acid molecule comprising a nucleotide sequence encoding a chimeric antigen receptor targeted to both BAFF-R and a chimeric antigen receptor targeted to CD19, wherein the chimeric antigen receptor targeted to BAFF-R comprises, from amino to carboxy terminus: a BAFF-R scFv; a spacer domain; a transmembrane domain; a costimulatory domain; and a CD3 ζ signaling domain; and the chimeric antigen receptor targeted to CD19 comprises, from amino to carboxy terminus: a CD19 scFv; a spacer domain; a transmembrane domain; a costimulatory domain; and a CD3 ζ signaling domain.

[007] In various embodiments: the costimulatory domain is selected from the group consisting of: a CD28 costimulatory domain or a variant thereof having 1-5 amino acid modifications, a 4-1BB costimulatory domain or a variant thereof having 1-5 amino acid modifications and an OX40 costimulatory domain or a variant thereof having 1-5 amino acid modifications; the transmembrane domain is selected from: a CD4 transmembrane

domain or variant thereof having 1-5 amino acid modifications, a CD8 transmembrane domain or variant thereof having 1-5 amino acid modifications, a CD28 transmembrane domain or a variant thereof having 1-5 amino acid modifications, and a CD3 ζ transmembrane domain or a variant thereof having 1-5 amino acid modifications; a costimulatory domain; and CD3 ζ signaling domain of a variant thereof having 1-5 amino acid modifications; and the spacer domain is selected from the group consisting of: IgG4 hinge (S $\rightarrow$ P), IgG4 hinge, IgG4 hinge (S228P)+linker, CD28 hinge, CD8 hinge-48aa, CD8 hinge-45aa, IgG4(HL-CH3), IgG4(L235E,N297Q), IgG4(S228P, L235E,N297Q), and IgG4(CH3), and a variant of each of IgG4 hinge (S $\rightarrow$ P), IgG4 hinge, IgG4 hinge (S228P)+linker, CD28 hinge, CD8 hinge-48aa, CD8 hinge-45aa, IgG4(HL-CH3), IgG4(L235E,N297Q), IgG4(S228P, L235E,N297Q), and IgG4(CH3) thereof having 1-5 amino acid modifications.

[008] In various embodiments: (1) the BAFF-R scFv includes a light chain variable region and a heavy chain variable region, wherein the light chain variable region includes: CDR L1 (SEQ ID NO:1), CDR L2 (SEQ ID NO:2) and CDR L3 (SEQ ID NO:3); and the heavy chain variable region includes: CDR H1 (SEQ ID NO:4), CDR H2 (SEQ ID NO:5), and CDR H3 (SEQ ID NO:6); (2) the BAFF-R scFv includes a light chain variable region and a heavy chain variable region, wherein the light chain variable region includes: CDR L1 (SEQ ID NO:7), CDR L2 (SEQ ID NO:8) and CDR L3 (SEQ ID NO:9); and the heavy chain variable region includes: CDR H1 (SEQ ID NO:10), CDR H2 (SEQ ID NO:11), and CDR H3 (SEQ ID NO:12); (3) the BAFF-R scFv includes: a heavy chain variable domain selected from: Chi90 HC, Hu90 HC-1, Hu90 HC-2, Hu90 HC-3, Chi55 HC, Hu55 HC-1, Hu55 HC-2, and Hu55 HC-3 and a light chain variable domain selected from: Chi90 LC, Hu90 LC-1, Hu90 LC-2, Hu90 LC-3, Chi55 LC, Hu55 LC-1, Hu55 LC-2, and Hu55 LC-3; (4) the BAFF-R scFv includes a light chain variable domain having an amino acid sequence selected from SEQ ID NOs: 17-20 and 25-28; (5) the BAFF-R scFv includes a heavy chain variable domain having an amino acid sequence selected from SEQ ID NOs: 13-16 and 21-24; (6) the CD19 scFv comprises a VL comprising the amino acid sequence
DIQMTQTSSLSASLGDRVTISCRASQDISKYLNWYQQKPDGTVKLLIYHTSRLH

SGVPSRFSGSGSGTDYSLTISNLEQEDIATYFC QQGNTLPYTFGGGKLEIT (SEQ ID NO: 30) and a VH comprising the amino acid sequence EVK LQESGPGLVAPSQSLSVTCTVSGVSLPDYG VSWIRQPPRKGLEWLGVWGSSETTYYN SALKSRLTIKD NSKSQVFLKMNSLQTD DTAIYYCAKHYYYGG SYAMDYWGQGTSVTVSS (SEQ ID NO: 31).

[009] Also described herein is a vector comprising one or more of the nucleic acid molecules described above. In various embodiments the vector is a lentiviral vector. Also described is a population of human T cells transduced by a vector described herein.

[0010] Also described is a method of treating cancer in a subject in need thereof comprising administering to a subject a therapeutically effective amount of a composition comprising a population of human T cells as described herein. In various embodiments of the method: the cancer is lymphoma, leukemia or myeloma; the lymphoma is mantle cell lymphoma, follicular lymphoma, diffuse large B-cell lymphoma, marginal zone lymphoma or Burkitt's lymphoma; the leukemia is acute lymphoblastic leukemia, chronic lymphocytic leukemia or hairy cell leukemia; the myeloma is multiple myeloma; the population of T cells are autologous or allogeneic to the patient; and the population of human T cells comprise cells comprise CD4⁺ cell and CD8⁺ cells.

[0011] In some embodiments, the BAFF-R scFv includes a light chain variable region and a heavy chain variable region, wherein the light chain variable region includes CDR L1 as set forth in SEQ ID NO:7, a CDR L2 as set forth in SEQ ID NO:8 and a CDR L3 as set forth in SEQ ID NO:9; and the heavy chain variable region includes a CDR H1 as set forth in SEQ ID NO:10, a CDR H2 as set forth in SEQ ID NO:11, and a CDR H3 as set forth in SEQ ID NO:12. In embodiments, the antibody is a humanized antibody.

[0012] In some embodiments, the BAFF-R scFv includes: CDR L1 of SEQ ID NO:1 or 7, CDR L2 of SEQ ID NO:2 or 8, CDR L3 of SEQ ID NO:3 or 9, CDR H1 of SEQ ID NO:4 or 10, CDR H2 of SEQ ID NO:5 or 11, and CDR H3 of SEQ ID NO:7 or 13.

[0013] In some embodiments, the portion of the BAFF-R scFv includes: the light chain variable domain of monoclonal antibody H90 and the heavy chain variable domain of

monoclonal antibody H90 or the light chain variable domain of monoclonal antibody H55 and the heavy chain variable domain of monoclonal antibody H55. The heavy and light chain variable domains can be joined by a linker of, 5-100, 10-50 or 10-20 amino acids (e.g., GGGGSGGGGSGGGGS).

[0014] In some embodiments, the BAFF-R scFv includes: a) a humanized variant of the light chain variable domain of monoclonal antibody H90 and a humanized variant of the heavy chain variable domain of monoclonal antibody H90; or b) a humanized variant of the light chain variable domain of monoclonal antibody H55 and a humanized variant of the heavy chain variable domain of monoclonal antibody H55. The heavy and light chain variable domains can be joined by a linker of 10-20 amino acids (e.g., GGGGSGGGGSGGGGS). In some cases, the humanized variant of the H90 light chain variable domain is selected from: Hu90 LC-1, Hu90 LC-2 and Hu90 LC-3 and the humanized variant of the H90 heavy chain variable domain is selected from: Hu90 HC-1, Hu90 HC-2 and Hu90 HC-3. In some cases, the humanized variant of the H55 light chain variable domain is selected from: Hu55 LC-1, Hu55 LC-2 and Hu55 LC-3 and the humanized variant of the H55 heavy chain variable domain is selected from: Hu55 HC-1, Hu55 HC-2 and Hu90 HC-3.

[0015] In embodiments, the BAFF-R scFv light chain variable region includes a serine at a position corresponding to Kabat position 7. In embodiments, the light chain variable region includes a proline at a position corresponding to Kabat position 8. In embodiments, the light chain variable region includes a valine at a position corresponding to Kabat position 15. In embodiments, the light chain variable region includes a threonine at a position corresponding to Kabat position 22. In embodiments, the light chain variable region includes a glutamine at a position corresponding to Kabat position 24. In embodiments, the light chain variable region includes a glycine at a position corresponding to Kabat position 41. In embodiments, the light chain variable region includes a lysine at a position corresponding to Kabat position 42. In embodiments, the light chain variable region includes an alanine at a position corresponding to Kabat position 43. In embodiments, the light chain variable region includes a proline at a position corresponding to Kabat position 44. In embodiments, the light chain variable

region includes a threonine at a position corresponding to Kabat position 56. In embodiments, the light chain variable region includes a threonine at a position corresponding to Kabat position 72. In embodiments, the light chain variable region includes a phenylalanine at a position corresponding to Kabat position 73. In embodiments, the light chain variable region includes a glutamine at a position corresponding to Kabat position 79. In embodiments, the light chain variable region includes a valine at a position corresponding to Kabat position 104.

[0016] In embodiments, the BAFF-R scFv the light chain variable region includes a serine at a position corresponding to Kabat position 7, a proline at a position corresponding to Kabat position 8, a valine at a position corresponding to Kabat position 15, a threonine at a position corresponding to Kabat position 22, a glutamine or a serine at a position corresponding to Kabat position 24, a glycine at a position corresponding to Kabat position 41, a lysine at a position corresponding to Kabat position 42, an alanine or a threonine at a position corresponding to Kabat position 43, a proline at a position corresponding to Kabat position 44, a threonine at a position corresponding to Kabat position 56, a threonine at a position corresponding to Kabat position 72, a phenylalanine or a lysine at a position corresponding to Kabat position 73, a glutamine at a position corresponding to Kabat position 79 or a valine at a position corresponding to Kabat position 104.

[0017] In embodiments, the BAFF-R scFv the light chain variable region includes a binding framework region residue that is a serine at a position corresponding to Kabat position 7, a proline at a position corresponding to Kabat position 8, a valine at a position corresponding to Kabat position 15, a threonine at a position corresponding to Kabat position 22, a glutamine or a serine at a position corresponding to Kabat position 24, a glycine at a position corresponding to Kabat position 41, a lysine at a position corresponding to Kabat position 42, an alanine or a threonine at a position corresponding to Kabat position 43, a proline at a position corresponding to Kabat position 44, a threonine at a position corresponding to Kabat position 56, a threonine at a position corresponding to Kabat position 72, a phenylalanine or a lysine at a position

corresponding to Kabat position 73, a glutamine at a position corresponding to Kabat position 79 or a valine at a position corresponding to Kabat position 104.

[0018] In embodiments, the BAFF-R scFv the heavy chain variable region includes a threonine or an alanine at a position corresponding to Kabat position 10. In embodiments, the heavy chain variable region includes a lysine at a position corresponding to Kabat position 11. In embodiments, the heavy chain variable region includes a valine at a position corresponding to Kabat position 12. In embodiments, the heavy chain variable region includes a threonine at a position corresponding to Kabat position 15. In embodiments, the heavy chain variable region includes a threonine at a position corresponding to Kabat position 19. In embodiments, the heavy chain variable region includes a threonine at a position corresponding to Kabat position 23. In embodiments, the heavy chain variable region includes a proline at a position corresponding to Kabat position 41. In embodiments, the heavy chain variable region includes an alanine at a position corresponding to Kabat position 44. In embodiments, the heavy chain variable region includes a proline or a threonine at a position corresponding to Kabat position 61. In embodiments, the heavy chain variable region includes an arginine at a position corresponding to Kabat position 66. In embodiments, the heavy chain variable region includes a threonine at a position corresponding to Kabat position 70. In embodiments, the heavy chain variable region includes a lysine at a position corresponding to Kabat position 75. In embodiments, the heavy chain variable region includes a valine at a position corresponding to Kabat position 79. In embodiments, the heavy chain variable region includes a threonine at a position corresponding to Kabat position 81. In embodiments, the heavy chain variable region includes a methionine at a position corresponding to Kabat position 82. In embodiments, the heavy chain variable region includes an asparagine at a position corresponding to Kabat position 82B. In embodiments, the heavy chain variable region includes a methionine at a position corresponding to Kabat position 82C. In embodiments, the heavy chain variable region includes a proline at a position corresponding to Kabat position 84. In embodiments, the heavy chain variable region includes a valine at a position corresponding to Kabat position 85. In embodiments, the heavy chain variable

region includes a lysine at a position corresponding to Kabat position 108. In embodiments, the heavy chain variable region includes a valine at a position corresponding to Kabat position 109.

[0019] In embodiments, the BAFF-R scFv heavy chain variable region includes a threonine or an alanine at a position corresponding to Kabat position 10, a lysine at a position corresponding to Kabat position 11, a valine at a position corresponding to Kabat position 12, a threonine at a position corresponding to Kabat position 15, a threonine at a position corresponding to Kabat position 19, a threonine at a position corresponding to Kabat position 23, a proline at a position corresponding to Kabat position 41, an alanine at a position corresponding to Kabat position 44, a proline, a serine or a threonine at a position corresponding to Kabat position 61, an arginine at a position corresponding to Kabat position 66, a threonine at a position corresponding to Kabat position 70, a lysine at a position corresponding to Kabat position 75, a valine at a position corresponding to Kabat position 79, a threonine or a lysine at a position corresponding to Kabat position 81, a methionine at a position corresponding to Kabat position 82, an asparagine at a position corresponding to Kabat position 82B, a methionine at a position corresponding to Kabat position 82C, a proline at a position corresponding to Kabat position 84, a valine at a position corresponding to Kabat position 85, a lysine at a position corresponding to Kabat position 108 or a valine at a position corresponding to Kabat position 109.

[0020] In embodiments, the BAFF-R scFv heavy chain variable region includes a binding framework region residue that is a threonine or an alanine at a position corresponding to Kabat position 10, a lysine at a position corresponding to Kabat position 11, a valine at a position corresponding to Kabat position 12, a threonine at a position corresponding to Kabat position 15, a threonine at a position corresponding to Kabat position 19, a threonine at a position corresponding to Kabat position 23, a proline at a position corresponding to Kabat position 41, an alanine at a position corresponding to Kabat position 44, a proline, a serine or a threonine at a position corresponding to Kabat position 61, an arginine at a position corresponding to Kabat position 66, a threonine at a position corresponding to Kabat position 70, a lysine at a position corresponding to Kabat position 75, a valine at a position corresponding to Kabat position 79, a threonine or a

lysine at a position corresponding to Kabat position 81, a methionine at a position corresponding to Kabat position 82, an asparagine at a position corresponding to Kabat position 82B, a methionine at a position corresponding to Kabat position 82C, a proline at a position corresponding to Kabat position 84, a valine at a position corresponding to Kabat position 85, a lysine at a position corresponding to Kabat position 108 or a valine at a position corresponding to Kabat position 109.

DESCRIPTION OF DRAWINGS

[0021] **FIGURE 1** depicts schematic drawings of two example of tandem constructs and one example of a loop construct for BAFF-R/CD19 dual CARs. Also shown is an example of bicistronic construct in which a single cell expresses two CARs, one targeted to BAFF-R and one targeted to CD19.

[0022] **FIGURE 2** depicts the amino acid sequence of an immature 1250 dual CAR, including signal sequence (SEQ ID NO:58). The mature CAR (SEQ ID NO:59) includes, from amino to carboxy terminus: a BAFF-R scFv, a linker, a CD19 scFv (derived from FMC63), an IgG4 (SmP/L235E,N297Q) spacer domain, a CD4 transmembrane domain, a 4-1BB cytoplasmic domain, a GGG linker, and a CD3 signaling domain.

[0023] **FIGURE 3** depicts the amino acid sequence of an immature 1296 dual CAR, including signal sequence (SEQ ID NO:60). The mature CAR (SEQ ID NO:61) includes, from amino to carboxy terminus: a CD19 scFv (derived from FMC63), a linker, a BAFF-R scFv, an IgG4 (SmP/L235E,N297Q) spacer domain, a CD4 transmembrane domain, a 4-1BB cytoplasmic domain, a GGG linker, and a CD3 signaling domain.

[0024] **FIGURE 4** depicts the amino acid sequence of an immature 1316 dual CAR, including signal sequence (SEQ ID NO:62). The mature CAR (SEQ ID NO:63) includes, from amino to carboxy terminus: a CD19 VL (derived from FMC63), a GGGS linker, a BAFF-R scFv, a CD19 VH (derived from FMC63), an IgG4 (SmP/L235E,N297Q) spacer domain, a CD4 transmembrane domain, a 4-1BB cytoplasmic domain, a GGG linker, and a CD3 signaling domain.

[0025] **FIGURE 5** depicts the amino acid sequence of: (A) an immature CD19 CAR, including signal sequence (SEQ ID NO:64) and B an immature BAFF-R CAR including a signal sequence (SEQ ID NO:66). The mature CD19 CAR (SEQ ID NO:65) includes, from amino to carboxy terminus: a CD19 scFv (derived from FMC63), an IgG4 (SmP/L235E,N297Q) spacer domain, a CD28 transmembrane domain, a CD28(GG) cytoplasmic domain, a GGG linker, and a CD3 signaling domain. The mature BAFF-R CAR (SEQ ID NO:67) includes, from amino to carboxy terminus: a BAFF-R scFv, an IgG4 (SmP/L235E,N297Q) spacer domain, a CD4 transmembrane domain, a 4-1BB cytoplasmic domain, a GGG linker, and a CD3 signaling domain.

[0026] **FIGURE 6** is a schematic drawing of an example of a vector for expressing a BAFF-R/CD19 dual CAR. BAFF-R and CD19 scFv elements can be varied to express the different arrangement of dual CARs. Truncated EGFR (EGFRt) is expressed as a selection marker.

[0027] **FIGURE 7** depicts the results of a study of dual CAR expression. T cells (Jurkat) were transduced with each CAR construct or an empty vector (mock). Cells were stained with Protein L- or EGFR-APC conjugated antibodies. Protein L targets the variable light chain of the scFv, and truncated EGFR is co-expressed by the CAR vector. Constructs that were able to properly express dual CARs were further examined. Dual CAR 1296 and dual CAR 1316 expressed intact CAR and EGFRt selection marker, whereas dual CAR 1250 failed to express intact CAR.

[0028] **FIGURE 8** depicts the result of a FACS assay assessing 1250 dual CAR T-cell degranulation following incubation with target cells. Targets cells are either BAFF-R single positive, CD19 single positive, or BAFF-R and CD19 double negative. Control BAFF-R single CAR and non-transduced T cells (non-CAR) were used as controls. The 1250 dual CAR failed to elicit response against BAFF-R-positive L cells suggesting BAFF-R-targeting scFv is not properly expressed.

[0029] **FIGURE 9** depicts the results of analysis related to development of cells lines used to develop a model for studying BAFF-R/CD19 dual CAR. FACS histograms of Nalm-6 knockout lines used for dual CAR model development. Nalm-6 B-ALL tumor

line was gene edited with CRISPR to knockout BAFF-R (left) or CD19 (right). Surface protein expression was confirmed by FACS staining with commercial BAFF-R and CD19 antibodies. Nalm-6 wildtype (WT) was used as controls.

[0030] FIGURE 10A-10B depicts the results of an analysis of the CTL function of BAFF-R/CD19 dual CAR. Graphs show calculated specific lysis are plotted from a cytotoxic T lymphocyte assay against Nalm-6 ALL tumor lines. Target cell line Nalm-6 (WT, CD19 knockout, or BAFF-R knockout variants) were labeled with chromium-51 and incubated with effector CAR T cells. CARs included BAFF-R/CD19 dual-targeting CARs: **A).** 1296 and 1250 or **B).** 1296 and 1316; controls in both panels include single-targeting CARs: BAFF-R CAR and CD19 CAR and non-transduced T cells (non-CAR, allogeneic control). All T cells were derived from a single healthy donor in each panel. Chromium released by target cells due to effector T cell function was measured by a gamma counter and calculated as a percentage of maximum possible release. Experiment was conducted in triplicate and analyzed by a Student's t-test; **A).** ** $P < 0.001$ compared between dual CARs; and **B).** ** $P < 0.001$ c/w non-CAR control. 1250 dual CAR CTL data suggests potential BAFF-R targeting deficiency.

[0031] FIGURE 11A-11B depicts the activity of BAFF-R/CD19 Dual CAR in BAFF-R-plus CD19-deficient mixed B-ALL tumor. **A)** Bioluminescence images of NSG mice following IV tumor challenge on day 0 with a mixture of 1×10^5 RFP-negative, luciferase-expressing Nalm-6-CD19KO plus 2.5×10^5 RFP-positive, luciferase-expressing Nalm-6-BAFF-RKO tumor cells. Groups of 5 tumor-bearing mice each were then randomly assigned to treatment with either 2.5×10^6 CD4 T_N CAR-T + 10^6 CD8 T_N 1296 or 1316 dual CAR T cells/mouse IV on day 10, as a single infusion. Non-transduced CD4/CD8 T cells from the same donor were used as allogeneic controls (non-CAR). **B)** Kaplan-Meier plots of overall survival are shown. Log-rank test compare experimental groups as shown. 1316 treatment conferred significant prolonged survival compared to 1296 treatment.

[0032] FIGURE 12 depicts the activity of 1316 BAFF-R/CD19 Dual CAR against knock-out tumors. FACS plots of BAFF-R CAR T cell functional potency as measured

by a CD107a degranulation assay. CD4 or CD8 BAFF-R CAR T cells were coincubated with either CD19⁺BAFF-R⁺ Nalm-6 or CD19⁺BAFF-R⁻ Nalm-6 lines. Single targeting CD19 or BAFF-R CAR T cells were used as controls.

[0033] FIGURE 13A-13B depicts the activity of T_{N/MEM} 1316 BAFF-R/CD19 Dual CAR in mixed B-ALL tumor. **A)** Bioluminescence images of NSG mice following IV tumor challenge on day 0 with a mixture of 1×10^5 RFP-negative, luciferase-expressing Nalm-6-CD19KO plus 1×10^5 RFP-positive, luciferase-expressing Nalm-6-BAFF-RKO tumor cells. Groups of 5 tumor-bearing mice each were then randomly assigned to treatment with 1316 dual CAR T cells/mouse IV on day 9, as a single infusion of either low dose (2.8×10^6 T_{N/MEM}), high dose (5.6×10^6 T_{N/MEM}), which yielded 1×10^6 and 2×10^6 BAFF-R CAR T cells, respectively. 2.5 or 5×10^6 non-transduced T_{N/MEM} cells from the same donor were used as allogeneic controls (non-CAR). **B)** Kaplan-Meier plots of overall survival are shown. Log-rank test compare experimental groups as shown. No significant difference in survival between the two dosing were observed.

DETAILED DESCRIPTION

[0034] The BAFF-R/CD19 dual CAR and BAFF-R/CD19 bicistronic CAR can employ any of a variety of BAFF-R scFv and CD19 scFv and, in the case of BAFF-R/CD19 dual CAR in which a first scFv is located between the variable domains of a second scFv, any of a variety of VL and VH can be used.

[0035] FIGURE 1 depicts schematic drawings of two example of tandem construct, one in which the BAFF-R scFv is amino terminal to the CD19 scFv and one in which the CD19 scFv is amino terminal to the BAFF-R scFv. Also depicted is one example of a loop construct for BAFF-R/CD29 dual CARs. In this example a BAFF-R is located between the CD19 VL (amino terminal to the scFv) and the CD19 VH domain (carboxy terminal to the scFv). Also shown is an example of bicistronic construct in which a single cell expresses two CARs, one targeted to BAFF-R and one targeted to CD19.

BAFF-R scFv Sequences

[0036] The BAFF-R scFv sequences used in BAFF-R/CD19 dual CAR can be derived from two monoclonal antibodies, Clone 90 and Clone 55 described in greater detail in US PCT/US2017/036181. For example, the VL can include the C90 CDR sequences or the C55 CDR sequences described below.

[0037] C90 CDR L1: ESVDNYGISF (SEQ ID NO:1)

[0038] C90 CDR L2: AAS (SEQ ID NO:2)

[0039] C90 CDR L3: QQSKEVPWT (SEQ ID NO:3)

[0040] C90 CDR H1: GDSITSGY (SEQ ID NO:4)

[0041] C90 CDR H2: ISYSGST (SEQ ID NO:5)

[0042] C90 CDR H3: ASPNYPFYAMDY (SEQ ID NO:6)

[0043] C55 CDR L1: QDISNY (SEQ ID NO:7)

[0044] C55 CDR L2: YTS (SEQ ID NO:8)

[0045] C55 CDR L3: FSELPWT (SEQ ID NO:9)

[0046] C55 CDR H1: GFSLSTSGMG (SEQ ID NO:10)

[0047] C55 CDR H2: IWWDDDK (SEQ ID NO:11)

[0048] C55 CDR H3: ARSFGYGLDY (SEQ ID NO:12)

[0049] Among the suitable heavy chain variable domains (VH) for use in the BAFF-R scFv of a dual CAR are the following heavy chain variable domains derived from monoclonal antibody Clone 90 (Described in greater detail in PCT/US2017/036181. Of these, Hu90 HC-1, HC-2 and HC-3 are humanized.

[0050] Chi90 HC:

MYRMQLLSIALSLALVTNSEVQLQESGPSLVKPSQTLTCSVTGDSITSGYWN

WIRKFPGNKLEYMGYISYSGSTYYNPSLKSRIITRDTSKNQYYLQLNSVTPEDTA
TYYCASPNYPFYAMDYWGQGTSTVTVSSDI (SEQ ID NO:13)

[0051] Hu90 HC-1:

MDPKGSLSWRILLFLSLAFELSYGQVQLQESGPGLVKPSQTLSTCTVSGDSITSG
YWNWIRQHPGKGLEIYIGYISYSGSTYYNPSLKSRTISRDTSKNQFSLKLSSVTA
ADTAVYYCASPNYPFYAMDYWGQGTLVTVSS (SEQ ID NO:14)

[0052] Hu90 HC-2:

MDPKGSLSWRILLFLSLAFELSYGEVQLQESGPGLVKPSQTLSTCTVSGDSITSG
YWNWIRQHPGKGLEIYIGYISYSGSTYYNPSLKSRTISRDTSKNQYSLKLSSVTA
ADTAVYYCASPNYPFYAMDYWGQGTLVTVSS (SEQ ID NO:15)

[0053] Hu90 HC-3:

MDPKGSLSWRILLFLSLAFELSYGEVQLQESGPGLVKPSETLSLTCSVSGDSITSG
YWNWIRQPPGKGLEIYIGYISYSGSTYYNPSLKSRTISRDTSKNQYSLRLSSVTAA
DTALYYCASPNYPFYAMDYWGQGTRVTVSS (SEQ ID NO:16)

[0054] Among the suitable light chain variable domains (VL) for use in the BAFF-R scFv of a dual CAR are the following heavy chain variable domains derived from monoclonal antibody Clone 90 (Described in greater detail in PCT/US2017/036181. Of these, Hu90 LC-1, LC-2 and LC-3 are humanized.

[0055] Chi90 LC:

MYRMQLLSIALSLALVTNSDIVLTQSPASLAVSLGQRATISCRASESVDNYGISF
MNWFQQKPGQPPKLLIYAASNQGSGVPARFSGSGSGTDFSLNIHPMEEDDTAMY
FCQQSKEVPWTFGGGTKLEIKTMEIKR (SEQ ID NO:17)

[0056] HuC90 LC-1:

METDTLLLWVLLLWVPGSTGEIVLTQSPATLSLSPGERATLSCRASESVDNYGISF
LNWFQQKPGQAPRLLIYAASNRATGIPARFSGSGSGTDFLTLSLEPEDFAVYYC
QQSKEVPWTFGGGTKVEIKRTV (SEQ ID NO:18)

[0057] Hu90 LC-2:

METDTLLLWVLLLWVPGSTGDIVLTQSPATLSLSPGERATLSCRASESDNYGIS
FMNWFQQKPGQAPRLLIYAASNRATGIPARFSGSGSGTDFTLTISSELPEDFAVYY
CQQSKEVPWTFGGGTKVEIKRTV (SEQ ID NO:19)

[0058] HuC90 LC-3:

METDTLLLWVLLLWVPGSTGDIVMTQSPSSLSASVGDRVITTCRASESDNYGIS
FMNWFQQKPGKAPKLLIYAASNLGSGVPSRFSGSGSGTDFTLTISLQPEDFATY
YCQQSKEVPWTFGQGTKVEIKRTV (SEQ ID NO:20)

[0059] Also among the suitable heavy chain variable domains (VH) for use in the BAFF-R scFv of a dual CAR are the following heavy chain variable domains derived from monoclonal antibody Clone 55 (described in greater detail in PCT/US2017/036181). Of these, Hu55 HC-1, HC-2 and HC-3 are humanized.

[0060] Chi55 HC:

MYRMQLLSIALSLALVTNSQVTLKESGPGILKPSQTLSTCSFSGFSLSTSGMGV
GWIRQPSGKGLEWLAHIWWDDDKYYNSSLKSHLTISKDTSRNQVFLKITSVDTA
DTATYYCARSFGYGLDYWGQGTLTVSSAS (SEQ ID NO:21)

[0061] Hu55 HC-1:

MDPKGSLSWRILLFLSLAFELSYGQVTLKESGPTLVKPTQTLTLTCTFSGFSLSTS
GMGVGWIRQPPGKALEWLAHIWWDDDKYYNPSLKSRLTITKDTSKNQVVLMT
NMDPVDATYYCARSFGYGLDYWGQGTLVTVSS (SEQ ID NO:22)

[0062] Hu55 HC-2:

MDPKGSLSWRILLFLSLAFELSYGQVTLKESGPTLVKPTQTLTLTCTFSGFSLSTS
GMGVGWIRQPPGKALEWLAHIWWDDDKYYNSSLKSRLTITKDTSKNQVVLMT
NMDPVDATYYCARSFGYGLDYWGQGTLVTVSS (SEQ ID NO:23)

[0063] Hu55 HC-3:

MDPKGSLSWRILLFLSLAFELSYGQVTLKESGPALVKPTQTLTLTCTFSGFSLSTS
GMGVGWIRQPPGKALEWLAHIWWDDDKYYNTSLKSRLTITKDTSKNQVVLKM
TNMDPVDATYYCARSFGYGLDYWGQGTLVTVSS (SEQ ID NO:24)

[0064] Also among the suitable light chain variable domains (VL) for use in the BAFF-R scFv of a dual CAR are the following heavy chain variable domains derived from monoclonal antibody Clone 90 (described in greater detail in PCT/US2017/036181). Of these, Hu55 LC-1, LC-2 and HC-3 are humanized.

[0065] Chi55 LC:

MYRMQLLSICIALSLALVTNSDIQMTQTTSSLSASLGDRVITSCSASQDISNYLNW
YQQKPDGTVKLLIYYTSSLHSGVPSRFSGSGSGTDYSLTISSLEPEDIATYYCHQFS
ELPWTFGGGKLEIKRT (SEQ ID NO:25)

[0066] Hu55 LC-1:

METDTLLLWVLLLWVPGSTGDIQMTQSPSSLSASVGDRVITCQASQDISNYLN
WYQQKPGKAPKLLIYYTSSLHTGVPSRFSGSGSGTDYFTISSLQPEDATYYCHQ
FSELPWTFGGGKVEIKRTV (SEQ ID NO:26)

[0067] Hu55 LC-2:

METDTLLLWVLLLWVPGSTGDIQMTQSPSSLSASVGDRVITCQASQDISNYLNW
YQQKPGKAPKLLIYYTSSLHTGVPSRFSGSGSGTDYTLTISSLQPEDATYYCHQF
SELPWTFGGGKVEIKRTV (SEQ ID NO:27)

[0068] Hu55 LC-3:

METDTLLLWVLLLWVPGSTGDIQMTQSPSSLSASVGDRVITCQASQDISNYLN
WYQQKPGKTPKLLIYYTSSLHTGVPSRFSGSGSGTDYTLTISSLQPEDATYYCHQ
FSELPWTFGGGKVEIKRTV (SEQ ID NO:28)

[0069] The VH and VL domains of the BAFF-R scFv can be modified. Thus, each of Hu90 LC-1, Hu90 LC-2, Hu90 LC-3, Hu90 HC-1, Hu90 HC-2 and Hu90 HC-3, Hu55 LC-1, Hu55 LC-2, Hu55 LC-3, Hu55 HC-1, Hu55 HC-2 and Hu90 HC-3 in a scFv can include 1, 2, 3, 4 or 5 single amino acid substitutions. In some cases, the substitutions are confined to the framework regions (FRs) rather than the CDRs. In some cases, the substitutions are conservative substitutions.

[0070] The position of CDRs and FRs may be defined by the Kabat numbering system (Kabat et al., Sequences of Proteins of Immunological Interest, Fifth Edition, U.S.

Department of Health and Human Services, U.S. Government Printing Office (1991)). Likewise, the positions occupied by individual residues within the light or the heavy chain of an antibody may be defined by the Kabat numbering system. Therefore, the location of residues required for binding within a humanized light chain and a humanized heavy chain of a humanized antibody may be defined by the position of the residue according to the Kabat numbering system as is well known in the art. As described above, a humanized antibody may be an antibody having CDRs from a donor antibody (e.g. mouse) and variable region framework (FR) from a human antibody. The framework regions (FRs) are said to hold the CDRs in place in a humanized antibody. Proceeding from the amino-terminus, these regions are designated FR L1, FR L2, FR L3, and FR L4 for the light chain and FR H1, FR H2, FR H3, and FR H4, for the heavy chain, respectively.

CD19 scFv Sequences

[0071] A variety of scFv targeting CD19 can be used in BAFF-R/CD19 dual CAR.

[0072] FMC63 scFv: IPDIQMTQTTSSLSASLGDRVTISCRASQDISKYLNWYQQ
KPDGTVKLLI YHTSRLHSGV PSRFSGSGSGTDYSLTISNLEQEDIATYFC
QQGNTLPYTFGGGTKLEITGSTSGSGKPGSGEGSTKGEVKLQESGPGLVA
PSQSLSVTCT VSGVSLPDYG VSWIRQPPRKGLEWLGVIWGSETTYYNAL
KSRLTIKDN SKSQVFLKMN SLQTDDTAIYYCAKHYYYGGSYAMDYWGQG
TSVTVSS (SEQ ID NO: 29).

[0073] FMC63 VL: DIQMTQTTSSLSASLGDRVTISCRASQDISKYLNWYQQ
KPDGTVKLLIYHTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIATYFC
QQGNTLPYTFGGGTKLEIT (SEQ ID NO: 30).

[0074] FMC63 VH: EVK LQESGPGLVAPSQSLSVTCTVSGVSLPDYG
VSWIRQPPRKGLEWLGVIWGSETTYYNALKSRLTIKDN SKSQVFLKMN
SLQTDDTAIYYCAKHYYYGGSYAMDYWGQGTSVTVSS (SEQ ID NO: 31).

[0075] Additional scFv that bind CD19 are described in US 2016/0152723 and in WO 2016/033570

Spacer Region

[0076] The dual CAR and bicistronic CAR described herein can include a spacer located between the targeting domain (e.g., the scFv) and the transmembrane domain. A variety of different spacers can be used. Some of them include at least portion of a human Fc region, for example a hinge portion of a human Fc region or a CH3 domain or variants thereof. Table 1 below provides various spacers that can be used in the CARs described herein.

Table 1: Examples of Spacers

Name	Length	Sequence
a3	3 aa	AAA
linker	10 aa	GGGSSGGGSG (SEQ ID NO:32)
IgG4 hinge (S→P) (S228P)	12 aa	ESKYGPPCPPCP (SEQ ID NO:33)
IgG4 hinge	12 aa	ESKYGPPCPSCP (SEQ ID NO:34)
IgG4 hinge (S228P)+ linker	22 aa	ESKYGPPCPPCPGGGSSGGGSG (SEQ ID NO:35)
CD28 hinge	39 aa	IEVMYPPPYLDNEKSNGTIIHVKGKHLCPSPFLPGPSKP (SEQ ID NO:36)
CD8 hinge-48aa	48 aa	AKPTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACD (SEQ ID NO:37)
CD8 hinge-45aa	45aa	TTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACD (SEQ ID NO:38)
IgG4(HL-CH3) (includes S228P in hinge)	129 aa	ESKYGPPCPPCPGGGSSGGGSGGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSV MHEALHNHYTQKSLSLSLGK (SEQ ID NO:39)
IgG4(L235E,N297Q)	229 aa	ESKYGPPCPSCPAPEFEGGPSVFLFPPKPKDTLMISRTPEVTCVWVDVSQEDPEVQFNWYVDGVEVHQAKTKPREEQFQSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSV MHEALHNHYTQKSLSLSLGK (SEQ ID NO:40)
IgG4(S228P, L235E,N297Q)	229 aa	ESKYGPPCPPCPAPEFEGGPSVFLFPPKPKDTLMISRTPEVTCVWVDVSQEDPEVQFNWYVDGVEVHQAKTKPREEQFQSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSV MHEALHNHYTQKSLSLSLGK (SEQ ID NO:41)
IgG4(CH3)	107 aa	GQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSV MHEALHNHYTQKSLSLSLGK (SEQ ID NO:42)

[0077] Some spacer regions include all or part of an immunoglobulin (e.g., IgG1, IgG2, IgG3, IgG4) hinge region, i.e., the sequence that falls between the CH1 and CH2 domains of an immunoglobulin, e.g., an IgG4 Fc hinge or a CD8 hinge. Some spacer regions include an immunoglobulin CH3 domain or both a CH3 domain and a CH2 domain. The immunoglobulin derived sequences can include one or more amino acid modifications, for example, 1, 2, 3, 4 or 5 substitutions, e.g., substitutions that reduce off-target binding.

[0078] The hinge/linker region can also comprise a IgG4 hinge region having the sequence ESKYGPPCPSCP (SEQ ID NO:34) or ESKYGPPCPPCP (SEQ ID NO:33).

[0079] The hinge/linker region can also comprise the sequence ESKYGPPCPPCP (SEQ ID NO:33) followed by the linker sequence GGGSSGGSG (SEQ ID NO:32) followed by IgG4 CH3 sequence

GQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTT
PPVLDSGDSFFLYSRLTVDKSRWQEGNVFSCSV MHEALHNHYTQKSLSLGLGK
(SEQ ID NO:41). Thus, the entire linker/spacer region can comprise the sequence:
ESKYGPPCPPCPGGGSSGGSGGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFY
PSDIAVEWESNGQPENNYKTTTPVLDSGDSFFLYSRLTVDKSRWQEGNVFSCSV
MHEALHNHYTQKSLSLGLGK (SEQ ID NO:__). In some cases, the spacer has 1,2,3,4,
or 5 single amino acid changes (e.g., conservative changes) compared to SEQ ID NO:__.
In some cases, the IgG4 Fc hinge/linker region that is mutated at two positions (L235E;
N297Q) in a manner that reduces binding by Fc receptors (FcRs).

Transmembrane Domain

[0080] A variety of transmembrane domains can be used in the dual CAR and biscistonic CAR described herein. Table 2 includes examples of suitable transmembrane domains. Where a spacer region is present, the transmembrane domain is located carboxy terminal to the spacer region.

Table 2: Examples of Transmembrane Domains

Name	Accession	Length	Sequence
CD3z	J04132.1	21 aa	LCYLLDGILFIYGVILTALFL (SEQ ID NO:43)
CD28	NM_006139	27aa	FWVLVVVGGVLACYSLLVTVAFIIFWV (SEQ ID NO:44)
CD28(M)	NM_006139	28aa	MFWVLVVVGGVLACYSLLVTVAFIIFWV (SEQ ID NO:45)
CD4	M35160	22aa	MALIVLGGVAGLLFIGLGIFF (SEQ ID NO:46)
CD8tm	NM_001768	21aa	IYIWAPLAGTCGVLLSLVIT (SEQ ID NO:47)
CD8tm2	NM_001768	23aa	IYIWAPLAGTCGVLLSLVITLY (SEQ ID NO:48)
CD8tm3	NM_001768	24aa	IYIWAPLAGTCGVLLSLVITLYC (SEQ ID NO:49)
41BB	NM_001561	27aa	IISFFLALTSTALLFLLFF LTLRFSVV (SEQ ID NO:50)

Costimulatory and CD3zeta Domain

[0081] The costimulatory domain can be any domain that is suitable for use with a CD3 ζ signaling domain. In some cases, the costimulatory domain is a CD28 costimulatory domain that includes a sequence that is at least 90%, at least 95%, at least 98% identical to or identical to: RSKRSRGGHSDYMNMTPRRPGPTRKHYPYAPPRDFAAYRS (SEQ ID NO:52; LL to GG amino acid change double underlined). In some cases, the CD28 co-signaling domain has 1, 2, 3, 4 of 5 amino acid changes (preferably conservative and preferably not in the underlined GG sequence) compared to SEQ ID NO:23. In some cases the co-signaling domain is a 4-1BB co-signaling domain that includes a sequence that is at least 90%, at least 95%, at least 98% identical to or identical to: KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCEL (SEQ ID NO:54). In some cases, the 4-1BB co-signaling domain has 1, 2, 3, 4 or 5 amino acid changes (preferably conservative) compared to SEQ ID NO:24.

[0082] The costimulatory domain(s) are located between the transmembrane domain and the CD3 ζ signaling domain. Table 3 includes examples of suitable costimulatory domains together with the sequence of the CD3 ζ signaling domain.

Table 3: CD3 ζ Domain and Examples of Costimulatory Domains

Name	Accession	Length	Sequence
CD3 ζ	J04132.1	113 aa	RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDRGRDPPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGGHDGLYQGLSTATKDTYDALHMQALP PR (SEQ ID NO:51)
CD28	NM_006139	42aa	RSKRSRLHSDYMNMTPRRPGPTRKHYPYAPPRDFAAYRS (SEQ ID NO:52)
CD28gg*	NM_006139	42aa	RSKRSRGHSDYMNMTPRRPGPTRKHYPYAPPRDFAAYRS (SEQ ID NO:53)
4-1BB	NM_001561	42 aa	KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCEL (SEQ ID NO:54)
OX40		42 aa	ALYLLRRDQRLPPDAHKKPPGGGSFRTPIQEEQADAHSTLAKI (SEQ ID NO:55)

[0083] In various embodiments: the costimulatory domain is selected from the group consisting of: a costimulatory domain depicted in Table 3 or a variant thereof having 1-5 (e.g., 1 or 2) amino acid modifications, a CD28 costimulatory domain or a variant thereof having 1-5 (e.g., 1 or 2) amino acid modifications, a 4-1BB costimulatory domain or a variant thereof having 1-5 (e.g., 1 or 2) amino acid modifications and an OX40 costimulatory domain or a variant thereof having 1-5 (e.g., 1 or 2) amino acid modifications. In certain embodiments, a 4-1BB costimulatory domain or a variant thereof having 1-5 (e.g., 1 or 2) amino acid modifications is present. In some embodiments there are two costimulatory domains, for example a CD28 co-stimulatory domain or a variant thereof having 1-5 (e.g., 1 or 2) amino acid modifications (e.g., substitutions) and a 4-1BB co-stimulatory domain or a variant thereof having 1-5 (e.g., 1 or 2) amino acid modifications (e.g., substitutions). In various embodiments the 1-5 (e.g., 1 or 2) amino acid modification are substitutions. The costimulatory domain is amino terminal to the CD3 ζ signaling domain and in some cases a short linker consisting of 2 –

10, e.g., 3 amino acids (e.g., GGG) is positioned between the costimulatory domain and the CD3 ζ signaling domain.

CD3 ζ Signaling Domain

[0084] The CD3 ζ Signaling domain can be any domain that is suitable for use with a CD3 ζ signaling domain. In some cases, the CD3 ζ signaling domain includes a sequence that is at least 90%, at least 95%, at least 98% identical to or identical to:

RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPGEMGGKPRRK
NPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDAL
HMQUALPPR (SEQ ID NO:51). In some cases, the CD3 ζ signaling has 1, 2, 3, 4 of 5 amino acid changes (preferably conservative) compared to SEQ ID NO:51.

Truncated EGFR

[0085] The CD3 ζ signaling domain can be followed by a ribosomal skip sequence (e.g., LEGGGEGRGSLTTCGDVEENPGPR; SEQ ID NO:56) and a truncated EGFR having a sequence that is at least 90%, at least 95%, at least 98% identical to or identical to:

LVTSLLLCELPHPAFLIPRKVCNGIGIGEFKDSLSINATNIKHFKNCTSSISGDLHIL
PVAFRGDSFTHTPPLDPQELDILKTVKEITGFLLIQAWPENRTDLHAFENLEIIRGR
TKQHGQFSLAVVSLNITSLGLRSLKEISDGDVVISGNKNLCYANTINWKKLFGTSG
QKTKIISNRGENSCKATGQVCHALCSPEGCWGPEPRDCVSCRNVSRGRECVDKC
NLLEGEPREFVENSECIQCHPECLPQAMNITCTGRGPDNCIQCAHYIDGPHCVKT
CPAGVMGENNTLVWKYADAGHVCHLCHPNCTYGCTGPGLEGCPNGPKIPSIA
TGMVGALLLLLVVALGIGLFM (SEQ ID NO:57). In some cases, the truncated EGFR has 1, 2, 3, 4 of 5 amino acid changes (preferably conservative) compared to SEQ ID NO:57.

[0086] An amino acid modification refers to an amino acid substitution, insertion, and/or deletion in a protein or peptide sequence. An "amino acid substitution" or "substitution" refers to replacement of an amino acid at a particular position in a parent peptide or protein sequence with another amino acid. A substitution can be made to change an amino acid in the resulting protein in a non-conservative manner (i.e., by changing the

codon from an amino acid belonging to a grouping of amino acids having a particular size or characteristic to an amino acid belonging to another grouping) or in a conservative manner (i.e., by changing the codon from an amino acid belonging to a grouping of amino acids having a particular size or characteristic to an amino acid belonging to the same grouping). Such a conservative change generally leads to less change in the structure and function of the resulting protein. The following are examples of various groupings of amino acids: 1) Amino acids with nonpolar R groups: Alanine, Valine, Leucine, Isoleucine, Proline, Phenylalanine, Tryptophan, Methionine; 2) Amino acids with uncharged polar R groups: Glycine, Serine, Threonine, Cysteine, Tyrosine, Asparagine, Glutamine; 3) Amino acids with charged polar R groups (negatively charged at pH 6.0): Aspartic acid, Glutamic acid; 4) Basic amino acids (positively charged at pH 6.0): Lysine, Arginine, Histidine (at pH 6.0). Another grouping may be those amino acids with phenyl groups: Phenylalanine, Tryptophan, and Tyrosine.

[0087] The CAR can include a sequence that is at least 90%, at least 95%, at least 98% identical to or identical to the mature amino acid sequence depicted in FIG 9 (SEQ ID Nos: 29-40), either including or excluding the GMCSFRa signal sequence and either including or excluding the T2A ribosomal skip sequence and the truncated EGFRt).

[0088] In some cases, the CAR can be produced using a vector in which the CAR open reading frame is followed by a T2A ribosome skip sequence and a truncated EGFR (EGFRt), which lacks the cytoplasmic signaling tail. In this arrangement, co-expression of EGFRt provides an inert, non-immunogenic surface marker that allows for accurate measurement of gene modified cells, and enables positive selection of gene-modified cells, as well as efficient cell tracking of the therapeutic T cells in vivo following adoptive transfer. Efficiently controlling proliferation to avoid cytokine storm and off-target toxicity is an important hurdle for the success of T cell immunotherapy. The EGFRt incorporated in the CAR lentiviral vector can act as suicide gene to ablate the CAR+ T cells in cases of treatment-related toxicity.

[0089] The CAR described herein can be produced by any means known in the art, though preferably it is produced using recombinant DNA techniques. Nucleic acids

encoding the several regions of the chimeric receptor can be prepared and assembled into a complete coding sequence by standard techniques of molecular cloning known in the art (genomic library screening, overlapping PCR, primer-assisted ligation, site-directed mutagenesis, etc.) as is convenient. The resulting coding region is preferably inserted into an expression vector and used to transform a suitable expression host cell line, preferably a T lymphocyte cell line, and most preferably an autologous T lymphocyte cell line.

[0090] Various T cell subsets isolated from the patient can be transduced with a vector for CAR expression. Central memory T cells are one useful T cell subset. Central memory T cell can be isolated from peripheral blood mononuclear cells (PBMC) by selecting for CD45RO⁺/CD62L⁺ cells, using, for example, the CliniMACS® device to immunomagnetically select cells expressing the desired receptors. The cells enriched for central memory T cells can be activated with anti-CD3/CD28, transduced with, for example, a lentiviral vector that directs the expression of the CAR as well as a non-immunogenic surface marker for in vivo detection, ablation, and potential ex vivo selection. The activated/genetically modified central memory T cells can be expanded in vitro with IL-2/IL-15 and then cryopreserved.

Example 1: Preparation of T Cell Populations for Expression of Dual CAR

[0091] The following T cell populations can be prepared for expression of BAFF-CD19 dual CAR: CD4⁺ naïve T cells (CD4⁺ T_N), CD8⁺ naïve T cells (CD8⁺ T_N), CD8⁺ central memory T cells (CD8⁺ T_{CM}), CD8⁺ memory stem cells (CD8⁺ MSC) and Pan T cells (Pan T). Briefly, 5 mL of a blood sample are added to 5 mL histopaque-1077 (Sigma Aldrich). The mixture is centrifuged for 20 min at 2500 RPM (room temperature (RT), no brake). The middle peripheral blood mononuclear cell (PBMC) layer is collected, washed with 50 mL PBS (Corning), centrifuged for 5 min at 1500 RPM (RT). The collected cells are combined with 10 mL RBC lysis buffer (Qiagen) and incubated for 7 min. The cells are then washed with PBS and centrifuged for 5 min (1500 RPM, RT).

[0092] Various T cell populations can be prepared using the following kits available from StemCell Technologies, Inc. using the manufacturer's instructions: EasySep™ Human

Naïve CD4⁺ T Cell Enrichment Kit (CD4⁺ T_N), EasySep™ Human Naïve CD8⁺ T Cell Enrichment Kit (CD8⁺ T_N), and EasySep™ Human T Cell Enrichment Kit (Pan T). CD8⁺ T_{CM} can be prepared by isolating CD8⁺ T cells using the EasySep™ Human CD8⁺ T Cell Enrichment Kit from StemCell Technologies, Inc. using the manufacturer's instructions and then stained with: CD8-PerCP-Cy5.5, CD45 RO-APC, and CD62L-PE. The stained cells are then sorted to isolate CD8⁺/CD45⁺/CD62L⁺ triple positive cells. CD8⁺ memory stem cells (CD8⁺ MSC) can be generated from CD8⁺ T_N using the culture conditions shown in Table 4. The other T cell populations can be cultured as indicated in Table 4.

TABLE 4				
Population	Media	Serum	Cytokines	Additional Supplement
CD4 ⁺ T _N , CD8 ⁺ T _N , CD8 ⁺ T _{CM} , Pan T	X-VIVO 15 (Lonza)	10% human Ab serum (Valley Biomedical)	100 U/mL hIL-2	100 U/mL penicillin 100 µg/mL streptomycin
CD8 ⁺ MSC	AIM-V (Thermo Fisher)	5% human Ab serum (Valley Biomedical)	5 ng/mL IL-7 30 ng/mL IL-21 (Cellgenix)	2 mM glutamax (Thermo Fisher Scientific) 5 mM TWS119 (Cayman Chemical)

Example 2: Sequence of BAFF-R/CD19 Dual CAR

[0093] A variety of BAFF-R/CD19 dual CAR were prepared. The 1250 dual CAR (SEQ ID NO:59) includes, from amino to carboxy terminus: a BAFF-R scFv, a linker, a CD19 scFv (derived from FMC63), an IgG4 (SmP/L235E,N297Q) spacer domain, a CD4 transmembrane domain, a 41BB cytoplasmic domain, a GGG linker, and a CD3 zeta signaling domain. (FIGURE 2). The 1296 dual CAR (SEQ ID NO:61) includes, from amino to carboxy terminus: a CD19 scFv (derived from FMC63), a linker, a BAFF-R scFv, an IgG4 (SmP/L235E,N297Q) spacer domain, a CD4 transmembrane domain, a 41BB cytoplasmic domain, a GGG linker, and a CD3 zeta signaling domain (FIGURE 3). The 1316 dual CAR (SEQ ID NO:63) includes, from amino to carboxy terminus: a CD19 VL (derived from FMC63), a GGG linker, a BAFF-R scFv, a CD19 VH (derived from FMC63), an IgG4 (SmP/L235E,N297Q) spacer domain, a CD4 transmembrane domain, a 41BB cytoplasmic domain, a GGG linker, and a CD3 zeta signaling domain.

[0094] A bisectronic CAR expresses a BAFF-R CAR and a CD19 CAR using the lentiviral vector. The two CAR are expressed in the same T cell. The mature CD19 CAR (SEQ ID NO:65) can include, from amino to carboxy terminus: a CD19 scFv (derived from FMC63), an IgG4 (SmP/L235E,N297Q) spacer domain, a CD28 transmembrane domain, a CD28(GG) cytoplasmic domain, a GGG linker, and a CD3 signaling domain. The mature BAFF-R CAR (SEQ ID NO:67) can include, from amino to carboxy terminus: a BAFF-R scFv, an IgG4 (SmP/L235E,N297Q) spacer domain, a CD4 transmembrane domain, a 4-1BB cytoplasmic domain, a GGG linker, and a CD3 signaling domain.

Example 2: Preparation of Lentiviral Vectors Expressing BAFF-R/CD19 Dual CAR

[0095] FIGURE 5 is a schematic diagram of the lentiviral vector used to express 1250 dual CAR. The 1296 dual CAR, and the 1316 dual CAR were produced using similar lentiviral vectors (with replacement of the svFv portion).

Example 3: Preparation of CAR Expressing Cells

[0096] Cells were activated in preparation for transduction with lentiviral vectors expressing a dual CAR by combining the cells with CD3/CD28 human T-cell activation beads (Thermo Fisher) at a 1:1 bead to cell ratio and incubating overnight (humidified, 5% CO₂, 37 °C). After incubation, the cells were counted and distributed 1×10^6 cell/well in a 48-well plate. Cells were infected at an MOI of 1. In each case the total culture media was supplemented to 250 μ L, centrifuged for 30 min (800 g, RT). Cells are incubated overnight (humidified, 5% CO₂, 37 °C) and then cultured for 10 days in the media indicated in Table 1. Cultures of CD4⁺ T_N, CD8⁺ T_N, CD8⁺ T_{CM}, and Pan T cells included CD3/CD28 beads at a 1:1 cell to bead ratio. The culture of CD8⁺ T MSC did not include CD3/CD28 beads. Expression of the CAR was assessed by the percentage of GFP positive cells using flow cytometry.

[0097] Example 4: Expression of CAR

[0098] T cells (Jurkat) were transduced with each CAR construct or an empty vector. To assess expression of the CAR, cells were stained with Protein L- or EGFR-APC

conjugated antibodies. Protein L targets the variable light chain of the scFv, and truncated EGFR is co-expressed by the CAR vector. Constructs that were able to properly express dual CARs were further examined. As can be seen in **FIGURE 7**, Dual CAR 1296 and dual CAR 1316 expressed intact CAR and EGFRt selection marker, whereas dual CAR 1250 failed to express intact CAR.

[0099] Example 5: In Vitro Cell Killing by BAFF-R Targeted CAR T Cells

[00100] An *in vitro* assay using various T cell populations expressing 1296 Dual CAR, 1316 Dual CAR, CD19 CAR or BAFF-R CAR cells. Target cell line Nalm-6 (WT, CD19 knockout, or BAFF-R knockout variants) were labeled with chromium-31 and incubated with effector CAR T cells. CARs included BAFF-R/CD19 dual-targeting CARs: 1296 and 1316; single-targeting CARs: BAFF-R CAR and CD19 CAR. Non-transduced T cells (non-CAR) were used as an allogeneic control. All T cells were derived from a single healthy donor. Chromium released by target cells due to effector T cell function was measured by a gamma counter and calculated as a percentage of maximum possible release. As can be seen in **FIGURE 8**, 1250 dual CAR failed to elicit response against BAFF-R-positive L cells suggesting BAFF-R-targeting scFv is not properly expressed.

[00101] Example 6: BAFF-R- and CD19-deficient Murine Model

[00102] Nalm-6 B-ALL tumor line was gene edited with CRISPR to knockout BAFF-R or CD19. Surface protein expression was confirmed by FACS staining with commercial BAFF-R and CD19 antibodies. Nalm-6 wild-type (WT) was used as controls. As shown in **FIGURE 9**, FACS analysis confirms the knockout.

[00103] Example 7: CTL Function of Dual CAR T Cells

[00104] In this study, the results of which are shown in **FIGURE 10**, target cell lines Nalm-6, WT, CD19 knockout, or BAFF-R knockout variants, were labeled with chromium-51 and incubated with effector CAR T cells. CARs included BAFF-R/CD19 dual-targeting CARs. The 1250 dual CAR CTL data suggests potential BAFF-R targeting deficiency.

[00105] Example 8: Activity of BAFF-R/CD19 Dual CAR in BAFF-R-positive CD19-negative mixed B-ALL tumor

[00106] **FIGURE 11A-11B** shows the results of a study examining the impact of 1316 Dual CAR and 1296NSG mice following IV tumor challenge on day 0 with a mixture of 1×10^5 RFP-negative, luciferase-expressing Nalm-6-CD19KO plus 2.5×10^5 RFP-positive, luciferase-expressing Nalm-6-BAFF-RKO tumor cells. Groups of 5 tumor-bearing mice each were then randomly assigned to treatment with either 2.5×10^6 CD4 T_N CAR-T + 10^6 CD8 T_N 1296 or 1316 dual CAR T cells/mouse IV on day 10, as a single infusion. Non-transduced CD4/CD8 T cells from the same donor were used as allogeneic controls (non-CAR). As can be seen, 1316 treatment conferred significant prolonged survival compared to 1296 treatment.

[00107] Example 9: Degranulation of 1316 BAFF-R/CD19 Dual CAR against knock-out tumors

[00108] A CD107a degranulation was used to assess Dual CAR 1316 potency against knockout tumors. CD4 or CD8 BAFF-R CAR T cells were incubated with either CD19-BAFF-R⁺ Nalm-6 or CD19⁺BAFF-R⁻ Nalm-6 lines. Single targeting CD19 or BAFF-R CAR T cells were used as controls.

[00109] Example 10: Activity of TN/MEM 1316 BAFF-R/CD19 Dual CAR in mixed B-ALL tumor

[00110] NSG mice were challenged on day 0 with a mixture of 1×10^5 RFP-negative, luciferase-expressing Nalm-6-CD19KO plus 1×10^5 RFP-positive, luciferase-expressing Nalm-6-BAFF-RKO tumor cells. Groups of 5 tumor-bearing mice each were then randomly assigned to treatment with 1316 dual CAR T cells/mouse IV on day 9, as a single infusion of either low dose (2.8×10^6 TN/MEM), high dose (5.6×10^6 TN/MEM), which yielded 1×10^6 and 2×10^6 BAFF-R CAR T cells, respectively. 2.5 or 5×10^6 non-transduced TN/MEM cells from the same donor were used as allogeneic controls (non-CAR). 1316 Dual CAR conferred significant prolonged survival. No significant difference in survival between the two dosing were observed.

WHAT IS CLAIMED IS:

1. A nucleic acid molecule comprising a nucleotide sequence encoding a chimeric antigen receptor targeted to both BAFF-R and CD19, wherein the chimeric antigen receptor comprises, a targeting domain comprising, in any order, a VL domain of a CD19-binding scFv, a VH domain of a CD19-binding scFv, a VL domain of a BAFF-R-binding scFv, the VH domain of a BAFF-R-binding scFv; followed by: a spacer domain, a transmembrane domain, a costimulatory domain and a CD3 ζ signaling domain.

2. A nucleic acid molecule comprising a nucleotide sequence encoding a chimeric antigen receptor targeted to both BAFF-R and CD19, wherein the targeting domain comprises, a targeting domain comprising, from amino to carboxy terminus: a) a scFv targeted to BAFF-R and a scFv targeted to CD19; or b) a scFv targeted to CD19 and a scFv targeted to BAFF-R; a spacer domain; a transmembrane domain; a costimulatory domain; and a CD3 ζ signaling domain.

3. The nucleic acid molecule of claim 1, wherein the targeting domain comprises, from amino terminus to carboxy terminus: a VL domain of a CD19-binding scFv, a VH domain of a BAFF-R-binding scFv, a VL domain of a BAFF-R-binding scFv, and a VH domain of a CD19-binding scFv.

4. The nucleic acid molecule of claim 1, wherein the targeting domain comprises, from amino terminus to carboxy terminus: a VH domain of a CD19-binding scFv, a VH domain of a BAFF-R-binding scFv, a VL domain of a BAFF-R-binding scFv, and a VL domain of a CD19-binding scFv.

5. The nucleic acid molecule of claim 1, wherein the targeting domain comprises, from amino terminus to carboxy terminus: a VL domain of a CD19-binding scFv, a VL domain of a BAFF-R-binding scFv, a VH domain of a BAFF-R-binding scFv, and a VH domain of a CD19-binding scFv.

6. The nucleic acid molecule of claim 1, wherein the targeting domain comprises, from amino terminus to carboxy terminus: a VH domain of a CD19-binding scFv, a VL

domain of a BAFF-R-binding scFv, a VH domain of a BAFF-R-binding scFv, and a VL domain of a CD19-binding scFv.

7. The nucleic acid molecule of any of the forgoing claims wherein the BAFF-R VH comprises an amino acid sequence selected from the group consisting of: SEQ ID NOs: 13-16 and the BAFF-R VL comprises an amino acid sequence selected from the group consisting of: SEQ ID NOs: 17-20.

8. The nucleic acid molecule of any of the forgoing claims wherein the BAFF-R VH comprises an amino acid sequence selected from the group consisting of: SEQ ID NOs: 21-24 and the BAFF-R VL comprises an amino acid sequence selected from the group consisting of: SEQ ID NOs: 25-28.

9. The nucleic acid molecule of claim 1, wherein the chimeric antigen receptor comprises an amino acid sequence selected from SEQ ID NOS: 58-63.

10. The nucleic acid molecule of any of the forgoing claims wherein a linker comprising 4-15 amino acids between each of the VL and VH domains.

11. The nucleic acid molecule of claim 9, wherein the linkers include only G and S.

12. The nucleic acid molecule of claim 1, wherein the targeting domain comprises, from amino to carboxy terminus: a) a VL domain of a CD19 scFv; a scFv targeted to BAFF-R; and a VH of the CD19 scFv; or b) a VH domain of a CD19 scFv; a scFv targeted to BAFF-R; and a VL of the CD19 scFv.

13. The nucleic acid molecule of claim 1, wherein the targeting domain comprises, from amino to carboxy terminus: a) a VL domain of a BAFF-R scFv; a scFv targeted to CD19; and a VH of the BAFF-R scFv; or b) a VH domain of a BAFF-R scFv; a scFv targeted to CD19; and a VL of the BAFF-R scFv.

14. A nucleic acid molecule comprising a nucleotide sequence encoding a chimeric antigen receptor targeted to both BAFF-R and a chimeric antigen receptor targeted to CD19, wherein the chimeric antigen receptor targeted to BAFF-R comprises, from amino

to carboxy terminus: a BAFF-R scFv; a spacer domain; a transmembrane domain; a costimulatory domain; and a CD3 ζ signaling domain; and the chimeric antigen receptor targeted to CD19 comprises, from amino to carboxy terminus: a CD19 scFv; a spacer domain; a transmembrane domain; a costimulatory domain; and a CD3 ζ signaling domain

15. The nucleic acid molecule of any of claims the forgoing claims, wherein the costimulatory domain is selected from the group consisting of: a CD28 costimulatory domain or a variant thereof having 1-5 amino acid modifications, a 4-1BB costimulatory domain or a variant thereof having 1-5 amino acid modifications and an OX40 costimulatory domain or a variant thereof having 1-5 amino acid modifications.

16. The nucleic acid molecule of any of any of claims the forgoing claims, wherein the transmembrane domain is selected from: a CD4 transmembrane domain or variant thereof having 1-5 amino acid modifications, a CD8 transmembrane domain or variant thereof having 1-5 amino acid modifications, a CD28 transmembrane domain or a variant thereof having 1-5 amino acid modifications, and a CD3 ζ transmembrane domain or a variant thereof having 1-5 amino acid modifications; a costimulatory domain; and CD3 ζ signaling domain of a variant thereof having 1-5 amino acid modifications.

17. The nucleic acid molecule any of claims the forgoing claims, wherein the spacer domain is selected from the group consisting of: IgG4 hinge (S $\rightarrow$ P), IgG4 hinge, IgG4 hinge (S228P)+linker, CD28 hinge, CD8 hinge-48aa, CD8 hinge-45aa, IgG4(HL-CH3), IgG4(L235E,N297Q), IgG4(S228P, L235E,N297Q), and IgG4(CH3), and a variant of each of IgG4 hinge (S $\rightarrow$ P), IgG4 hinge, IgG4 hinge (S228P)+linker, CD28 hinge, CD8 hinge-48aa, CD8 hinge-45aa, IgG4(HL-CH3), IgG4(L235E,N297Q), IgG4(S228P, L235E,N297Q), and IgG4(CH3) thereof having 1-5 amino acid modifications.

18. The nucleic acid molecule any of claims the forgoing claims, wherein the BAFF-R scFv includes a light chain variable region and a heavy chain variable region, wherein the light chain variable region includes: CDR L1 (SEQ ID NO:1), CDR L2 (SEQ ID NO:2) and CDR L3 (SEQ ID NO:3); and the heavy chain variable region includes: CDR H1 (SEQ ID NO:4), CDR H2 (SEQ ID NO:5), and CDR H3 (SEQ ID NO:6).

19. The nucleic acid molecule of any of claims the forgoing claims, wherein the BAFF-R scFv includes a light chain variable region and a heavy chain variable region, wherein the light chain variable region includes: CDR L1 (SEQ ID NO:7), CDR L2 (SEQ ID NO:8) and CDR L3 (SEQ ID NO:9); and the heavy chain variable region includes: CDR H1 (SEQ ID NO:10), CDR H2 (SEQ ID NO:11), and CDR H3 (SEQ ID NO:12).

20. The nucleic acid molecule any of claims the forgoing claims, wherein the BAFF-R scFv includes: a heavy chain variable domain selected from: Chi90 HC, Hu90 HC-1, Hu90 HC-2, Hu90 HC-3, Chi55 HC, Hu55 HC-1, Hu55 HC-2, and Hu55 HC-3 and a light chain variable domain selected from: Chi90 LC, Hu90 LC-1, Hu90 LC-2, Hu90 LC-3, Chi55 LC, Hu55 LC-1, Hu55 LC-2, and Hu55 LC-3.

21. The nucleic acid molecule of any of claims the forgoing claims, wherein the BAFF-R scFv includes a light chain variable domain having an amino acid sequence selected from SEQ ID NOs: 17-20 and 25-28.

22. The nucleic acid molecule any of claims the forgoing claims, wherein the BAFF-R scFv includes a heavy chain variable domain having an amino acid sequence selected from SEQ ID NOs: 13-16 and 21-24.

23. The nucleic acid molecule of any of claims the forgoing claims, wherein the CD19 scFv comprises a VL comprising the amino acid sequence
 DIQMTQTSSLSASLGDRVTISCRASQDISKYLNWYQQ
 KPDGTVKLLIYHTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIATYFC
 QQGNTLPYTFGGGTKLEIT (SEQ ID NO: 30) and a VH comprising the amino acid sequence
 EVK LQESGPGLVAPSQSLSVTCTVSGVSLPDYG
 VSWIRQPPRKGLEWLGVIWGSETTYNSALKSRLLTIKDN SKSQVFLKMN
 SLQTDDTAIYYCAKHYYYGG SYAMDYWGQGTSVTVSS (SEQ ID NO: 31).

24. A vector comprising the nucleic acid molecule of any of the forgoing claim.

25. The vector of claim 24, wherein the vector is a lentiviral vector.

26. A population of human T cells or NK cells transduced by the vector of claim 25.

27. A method of treating cancer in a subject in need thereof comprising administering to a subject a therapeutically effective amount of a composition comprising the population of human T cells claim 17, thereby treating cancer in the subject.

28. The method of claim 27, wherein the cancer is lymphoma, leukemia or myeloma.

29. The method of claim 28, wherein the lymphoma is mantle cell lymphoma, follicular lymphoma, diffuse large B-cell lymphoma, marginal zone lymphoma or Burkitt's lymphoma.

30. The method of claim 28, wherein the leukemia is acute lymphoblastic leukemia, chronic lymphocytic leukemia or hairy cell leukemia.

31. The method of claim 19, wherein the myeloma is multiple myeloma.

32. The method of any of claims 27-31, wherein the population of T cells are autologous or allogeneic to the patient.

33. The method of any of claims 27-32, wherein the population of human T cells comprise cells comprise CD4⁺ cell and CD8⁺ cells.

FIGURE 1

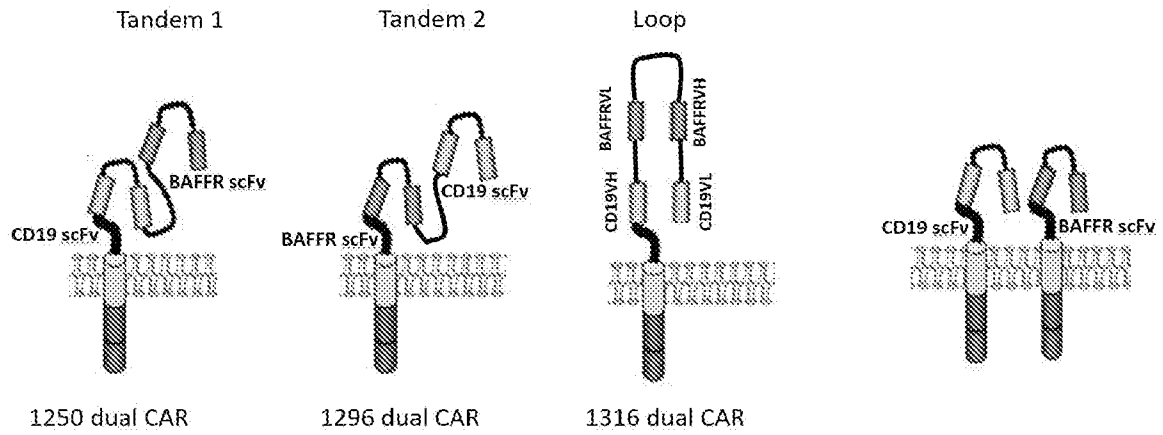


FIGURE 2

BAFFscFv-(g4s)4-CD19R-IgG4(L235E,N297Q)-41BB-Zeta

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1   MLLLVTSLLL CELPHPAFLI IPEVQLQESG PGLVKPSQTL SLTCTVSGDS
    GMCSFRa signal peptide   BAFFscFv

51  ITSGYWNWIR QHPGKGGLEYI GYISYSGSTY YNPSLKSRVT ISRDTSKNQY
101 SLKLSSVTAA DTAVYYCASP NYPFYAMDYW GQGTLLTVSS GGGGSGGGGS
151 GGGGSDIVLT QSPATLSLSP GERATLSCRA SESVDNYGIS FMNWFQQKPG
201 QAPRLLIYAA SNRATGIPAR FSGSGSGTDF TLTISSELEPE DFAVYYCQQS
251 KEVPWTFGGG TKVEIKRGGG GSGGGGSGGG GSGGGGSDIQ MTQTTSSLSA
    (g4s) 4                      CD19RscFv

301 SLGDRVITIS RASQDISKYL NWWQQKPDGT VKLLIYHTSR LHSGVPSRFS
351 GSGSGTDYSL TISNLEQEDI ATYFCQQGNT LPYTFGGGTK LEITGSTSGS
401 GKPGSGEGST KGEVKLQESG PGLVAPSQSL SVTCTVSGVS LPDYGVSWIR
451 QPPRKGLEWL GVIWGSETTY YNSALKSRLT IIKDNSKSQV FLKMNSLQTD
501 DTAIYYCAKH YYYGGSYAMD YWQOGTSVTV SSESKEYGPC PPCPAPEFEG
    IgG4SmP/L235E,N297Q

551 GPSVFLFPPK PKDTLMISRT PEVTCVVVDV SQEDPEVQFN WYVDGVEVHN
601 AKTKPREEQF QSTYRVSVSL TVLHQDWLNG KEYKCKVSNK GLPSSIEKTI
651 SKAKGQPREP QVYTLPPSQE EMTKNQVSLT CLVKGFYPSD IAVEWESNGQ
701 PENNYKTTPP VLDSGGSFFL YSRLTVDKSR WQEGNVFSCS VMHEALHNHY
751 TQKSLSLSLG KMALIVLGGV AGLLLFIGLG IFFKRGRKKL LYIFKQPFMR
    CD4 transmembrane          41BB cyto

801 PVQTTQEEDG CSCRFPEEEE GGCELGGGRV KFSRSADAPA YQQGQNQLYN
    CD3 Zeta

851 ELNLGRREEY DVLDKRRGRD PEMGGKPRRK NPQEGLYNEL QKDKMAEAYS
901 EIGMKGERRR GKGHDGLYQG LSTATKDTYD ALHMQALPPR

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FIGURE 3

CD19R-(g4s)4-BAFFscFv-IgG4(L235E,N297Q)-41BB-Zeta

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1      MLLLVTSLLL CELPHPAFLI IPDIQMTQTT SLSASLGDR VTISCRASQD
      GMCFSRa signal peptide  CD19RscFv
51     ISKYLNWYQQ KPDGTVKLLI YHTSRLHSGV PSRFSGSGSG TDYSLTISNL
101    EQEDIATYFC QQGNTLPYTF GGGTKLEITG STSGSGKPGS GEGSTKGEVK
151    LQESGPGLVA PSQSLSVTCT VSGVSLPDYG VSWIRQPPRK GLEWLGVWIG
201    SETTYYNALS KSRLTIKDN SKSQVFLKMN SLQTDDTAIY YCAKHYYYGG
251    SYAMDYWGQG TSVTVSSGGG GSGGGGSGGG GSGGGGSEVQ LQESGPGLVK
      (g4s)4                      BAFFscFv
301    PSQTLSTCT VSGDSITSGY WNWIRQHSGK GLEYIGYISY SGSTYYNPSL
351    KSRVTISRDT SKNQYSLKLS SVTAADTAVY YCASPNYPFY AMDYWGQGTI
401    VTVSSGGGGS GGGGSGGGGS DIVLTQSPAT LSLSPGERAT LSCRASESVD
451    NYGISFMNWF QQKPGQAPRL LIYAASNRAI GIPARFSGSG SGTDFTLTIS
501    SLEPEDFAVY YCQQSKEVPW TFGGGTKVEI KRESKYGPPC PPCPAPEFEG

      IgG4 (SmP) (L235E,N297Q)
551    GPSVFLFPPK PKDTLMISRT PEVTCVVVDV SQEDPEVQFN WYVDGVEVHN
601    AKTKPREEQF QSTYRVVSVL TVLHQDWLNG KEYKCKVSNK GLPSSIEKTI
651    SKAKGQPREP QVYTLPPSQE EMTKNQVSLT CLVKGFYPSD IAVEWESNGQ
701    PENNYKTPPP VLDSGDSFLL YSRLTVDKSR WQEGNVFSCS VMHEALHNHY
751    TQKSLSLSLG KMALIVLGGV AGLLLFIGLG IFFKRGRKKL LYIFKQPFMR
      CD4 transmembrane          41BB cyto
801    PVQTTQEEDG CSCRFPETEE GGCELGGGRV KFSRSADAPA YQQGQNQLYN
      CD3 Zeta
851    ELNLGRREEY DVLDKRRGRD PEMGGKPRRK NPQEGLYNEL QKDKMAEAYS
901    EIGMKGERRR GKGDHGLYQG LSTATKDTYD ALHMQALPPR

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FIGURE 4

CD19VL-BAFFVH-linker-BAFFVL-CD19RVH-IgG4(L235E,N297Q)-CD4tm-41BB-Zeta

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1  MLLLVTSLLL CELPHPAFLL IPDIQMTQTT SSLSASLGDR VTISCRASQD
   GMCSFRa signal peptide CD19R VL
51  ISKYLNWYQQ KPDGTVKLLI YHTSRLHSGV PSRFSGSGSG TDYSLTISNL
101 EQEDIATYFC QQGNTLPYTF GGGTKLEITG GGGSEVQLQE SGPGLVKPSQ
      (g4s) BAFFscFv VH
151 TLSLTCTVSG DSITSGYWNW IRQHPGKGLE YIGYISYSGS TYYNPSLKSR
201 VTISRDTSKN QYSLKLSSVT AADTAVYYCA SPNYPFYAMD YWGQGLVTV
251 SSGSTSGSGK PGSGEGSTKG DIVLTQSPAT LSLSPGERAT LSCRASESVD
   Linker BAFFscFv VL
301 NYGISFMNWF QQKPGQAPRL LIYAASNRAT GIPARFSGSG SGTDFTLTIS
351 SLEPEDFAVY YCQQSKEVPW TFGGGTKVEI KRGGGGSEVK LQESGPGLVA
      (g4s)CD19RscFv VH
401 PSQSLSVTCT VSGVSLPDYG VSWIRQPPRK GLEWLGVIWG SETTYYNSAL
451 KSRLTIKDN SKSQVFLKMN SLQTDDTAIY YCAKHYYYGG SYAMDYWGQG
501 TSVTVSSESK YGPPCPPCPA PEFEGGPSVF LFPPKPKDTL MISRTPEVTC
      IgG4 (SmP) (L235E,N297Q)
551 VVVDVSQEDP EVQFNWYVDG VEVHNAKTKP REEQFQSTYR VVSVLTVLHQ
601 DWLNGKEYKC KVSNGKLPSS IEKTISKAKG QPREPQVYTL PPSQEEMTKN
651 QVSLTCLVKG FYPSDIAVEW ESNGQPENNY KTPPVLDSD GSFFLYSRLT
701 VDKSRWQEGN VFSCSVMHEA LHNHYTQKSL SLSLGKMALI VLGGVAGLLL
      CD4 transmembrane
751 FIGLGIFFKR GRKKLLYIFK QPFMRPVQTT QEEDGCSCRF PEEEEGGCEL
      41BB cyto
801 GGGRVKFSRS ADAPAYQQGQ NQLYNELNLG RREEYDVLDK RRGRDPEMGG
      CD3zeta
851 KPRRKNPQEG LYNELQKDKM AEAYSEIGMK GERRRGKGHD GLYQGLSTAT
901 KDTYDALHMQ ALPPR

```

FIGURE 5

CD19R-IgG4(L235E,N297Q)-CD28tm-CD28gg-Zeta_BAFFscFv-IgG4(L235E,N297Q)-41BB-Zeta
bicistronic

CD19RscFv-IgG4(L235E, N297Q)-CD28tm-CD28gg-Zeta

```

1  MLLLVTSLLL CELPHPAFLI IPDIQMTQTT SLSASLGDR VTISCRASQD
   GMCFSRa signal peptide CD19RscFv
51  ISKYLNWYQQ KPDGTVKLLI YHTSRLHSGV PSRFSGSGSG TDYSLTISNL
101 EQEDIATYFC QQGNTLPYTF GGGTKLEITG STSGSGKPGS GEGSTKGEVK
151 LQESGPGGLVA PSQSLSVTCT VSGVSLPDYG VSWIRQPPRK GLEWLGVWIG
201 SETTYYNALS KSRLTIKDN SKSQVFLKMN SLQTDITAIY YCAKHYYYGG
251 SYAMDYWGQG TSVTVSSESK YGPPCPPCPA PEFEGGPSVF LFPPKPKDTL
   IgG4 (SmP) (L235E,N297Q)
301 MISRTPEVTC VVDVVSQEDP EVQFNWYVDG VEVHNAKTKP REEQFQSTYR
351 VVSVLTVLHQ DWLNGKEYKC KVSNGKLPSS IEKTISKAKG QPREPQVYTL
401 PPSQEEMTKN QVSLTCLVKG FYPSDIAVEW ESNQOPENNY KTTTPVLDSG
451 GSFFLYSRLT VDKSRWQEGN VFSCSVMEHA LHNHYTQKSL SLSLGKMFVV
   CD28
501 LVVVGVLVAC YSLLVTVAFI IFWVRSKRSR GGHSYMNMT PRRPGPTRKH
   Transmembrane CD28gg
551 YQPYAPPRDF AAYRSGGGRV KFSRSADAPA YQQGQNQLYN ELNLGRREEY
   CD3 Zeta
601 DVLDKRRGRD PEMGGKPRRK NPQEGLYNEL QKDKMAEAYS EIGMKGERRR
651 GKGDGLYQG LSTATKDTYD ALHMQALPPR

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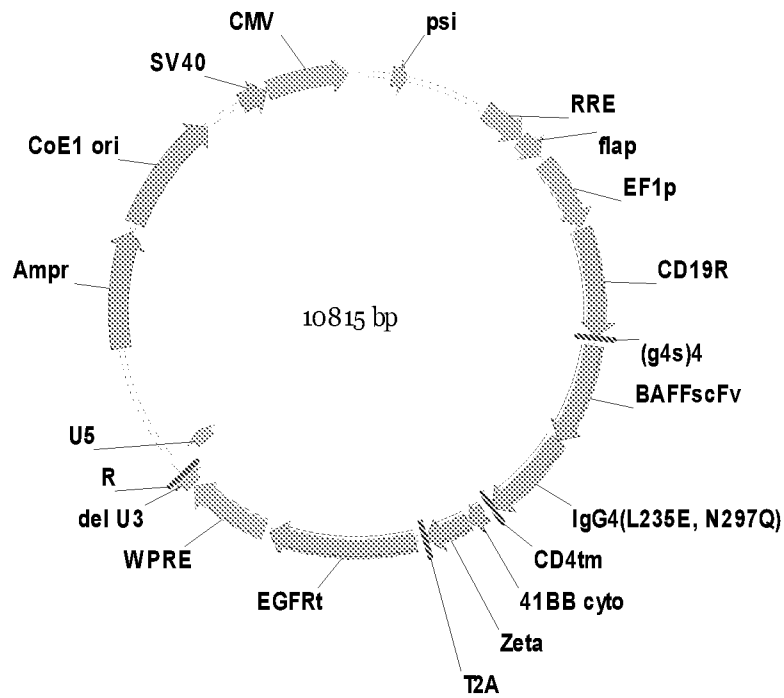
BAFFscFv-IgG4(L235E, N297Q)-CD4tm-41BB-Zeta

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1  MLLLVTSLLL CELPHPAFLI IPEVQLQESG PGLVKPSQTL SLTCTVSGDS
   GMCFSRa signal peptide BAFFscFv
51  ITSGYWNWIR QHPGKGLIYI GYISYSGSTY YNPSLKSRVT ISRDTSKNQY
101 SLKLSSVTAA DTAVYYCASP NYPFYAMDYW GQGTLVTVSS GGGSGGGGS
151 GGGGSDIVLT QSPATLSLSP GERATLSCRA SESVDNYGIS FMNWFQKPG
201 QAPRLLIYAA SNRATGIPAR FSGSGSGTDF TLTSSLEPE DFAVYYCQQS
251 KEVPWTFGGG TKVEIKRESK YGPPCPPCPA PEFEGGPSVF LFPPKPKDTL
   IgG4 (SmP) (L235E,N297Q)
301 MISRTPEVTC VVDVVSQEDP EVQFNWYVDG VEVHNAKTKP REEQFQSTYR
351 VVSVLTVLHQ DWLNGKEYKC KVSNGKLPSS IEKTISKAKG QPREPQVYTL
401 PPSQEEMTKN QVSLTCLVKG FYPSDIAVEW ESNQOPENNY KTTTPVLDSG
451 GSFFLYSRLT VDKSRWQEGN VFSCSVMEHA LHNHYTQKSL SLSLGKMALI
   CD4
501 VLGGVAGLLL FIGLGIFFKR GRKKLLYIFK QPFMRPVQTT QEEDGCSCRF
   Transmembrane 41BB cyto
551 PEEEEGGCEL GGRVKFSRS ADAPAYQQGQ NQLYNELNLG RREEYDVLDK
   CD3 Zeta
601 RRGRDPPEMGG KPRRKNPQEG LYNELQKDKM AEAYSEIGMK GERRRGKGD
651 GLYQGLSTAT KDTYDALHMQ ALPPR

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FIGURE 6



hiCAR expression in transduced Jurkat cells

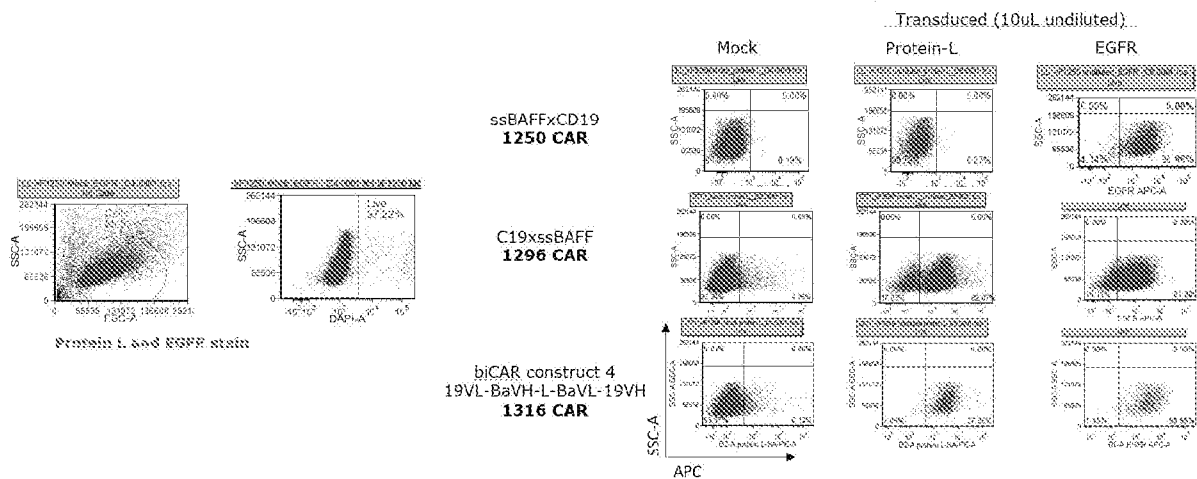


FIGURE 8

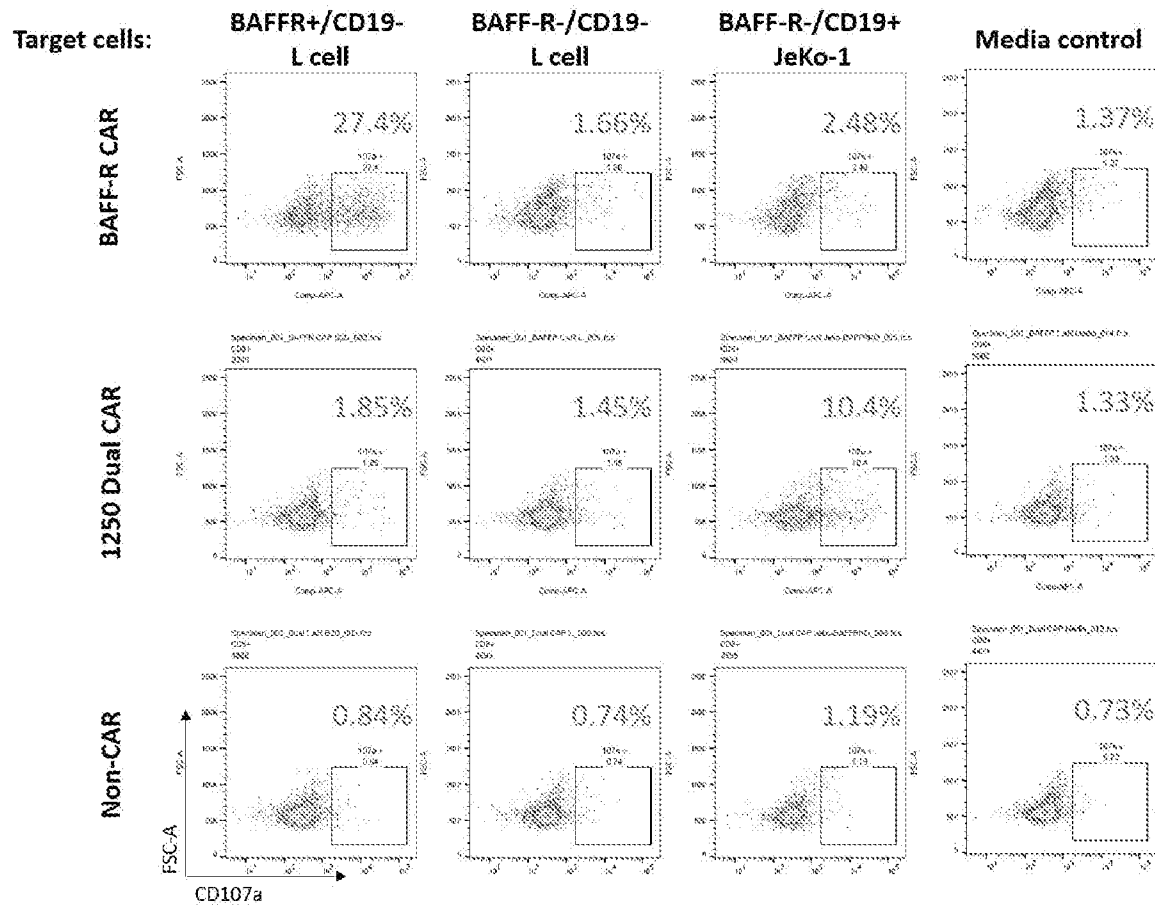


FIGURE 9

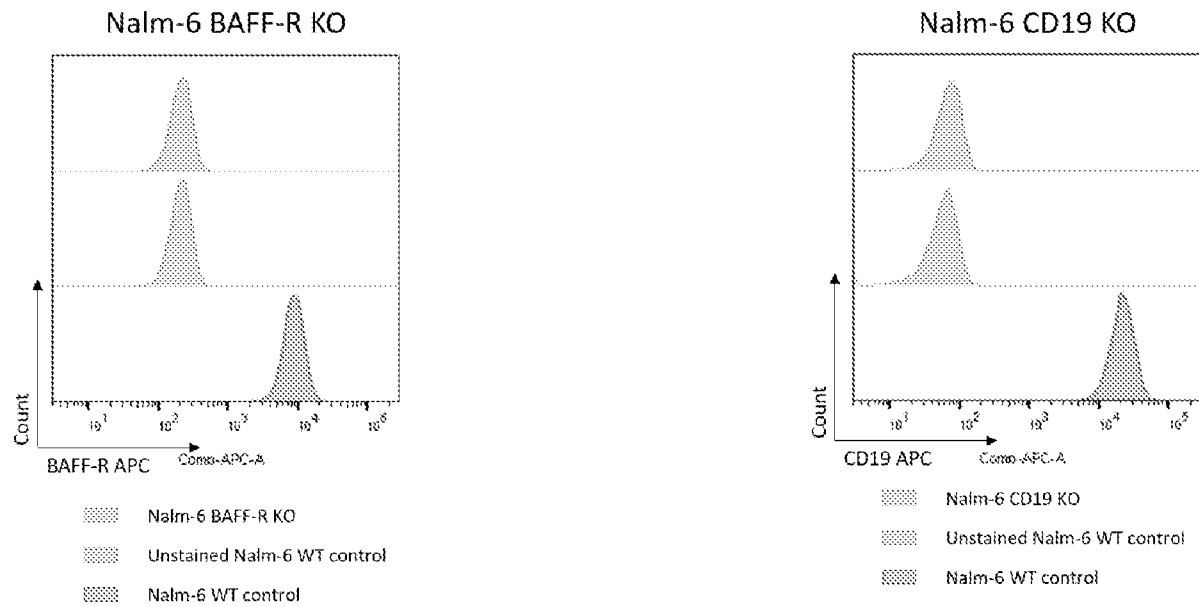


FIGURE 10

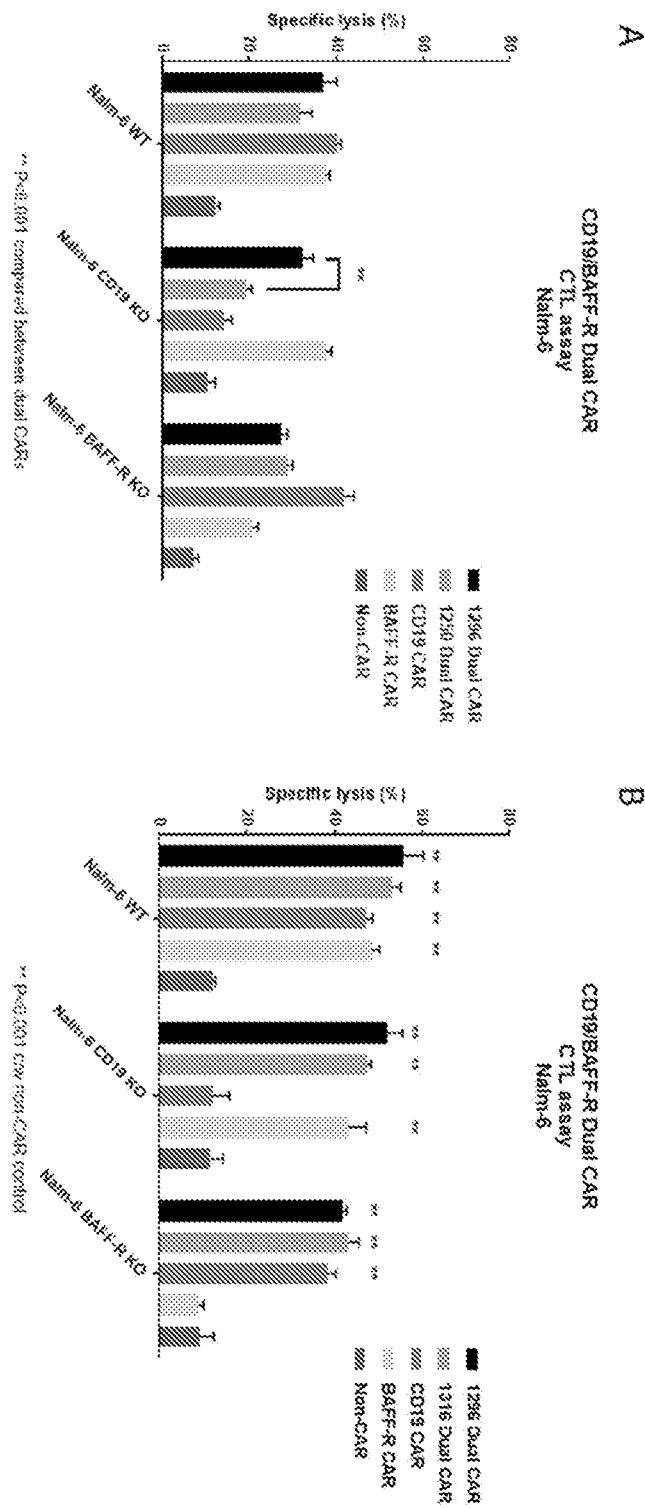
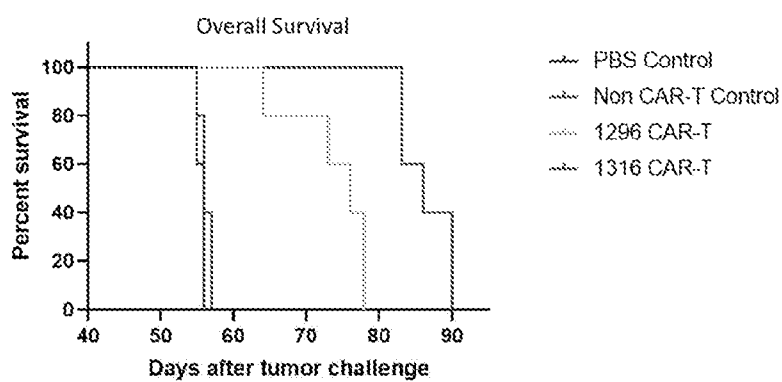
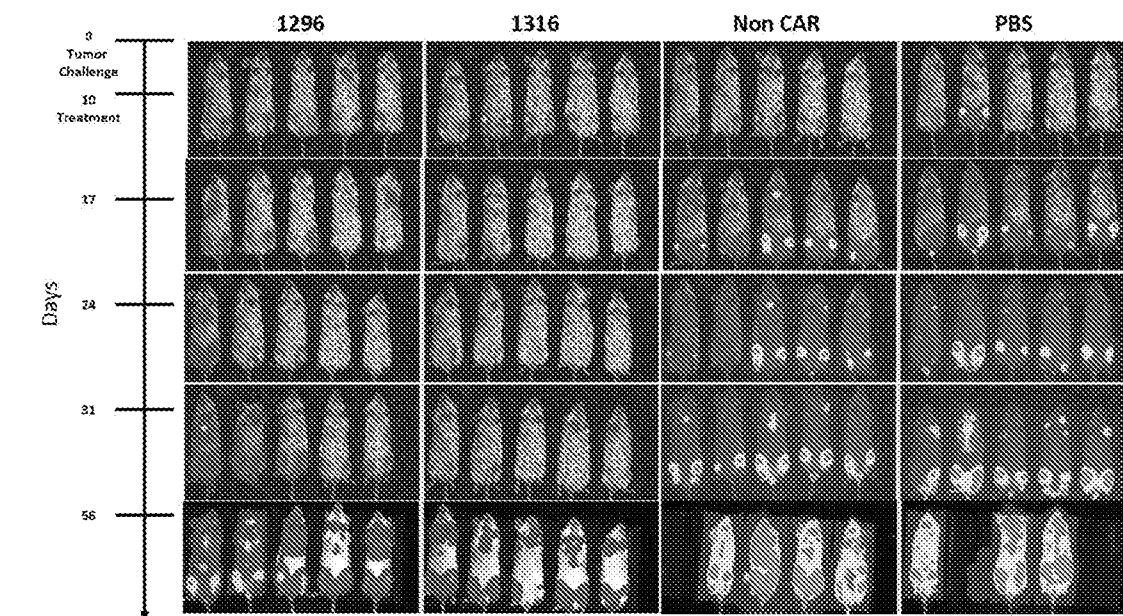


FIGURE 11



Compared groups		P value
Non CAR	1296	<0.01
Non CAR	1316	<0.01
1296	1316	<0.01

FIGURE 12

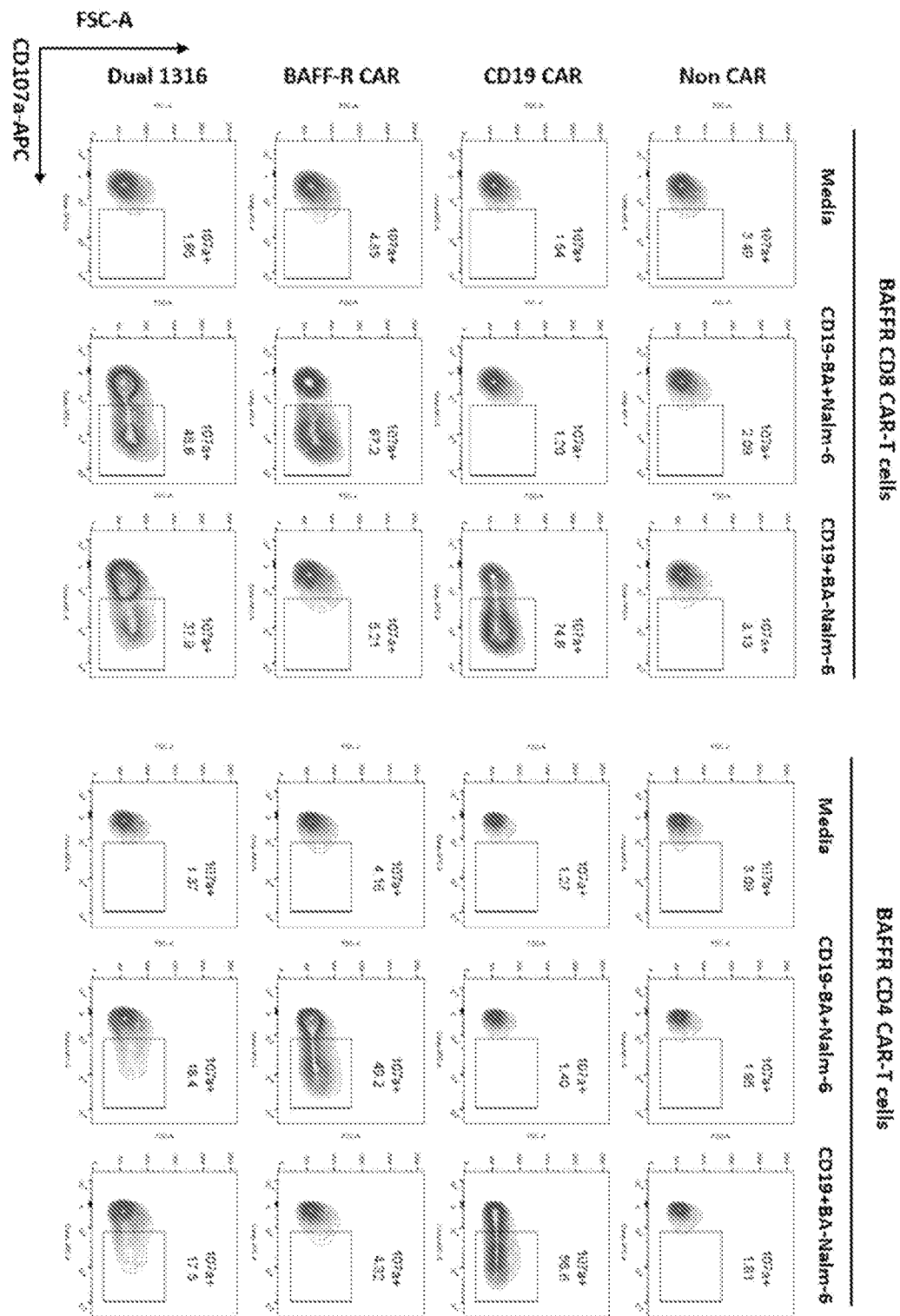
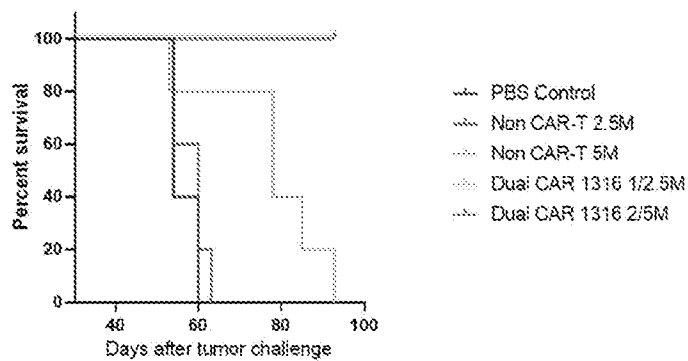
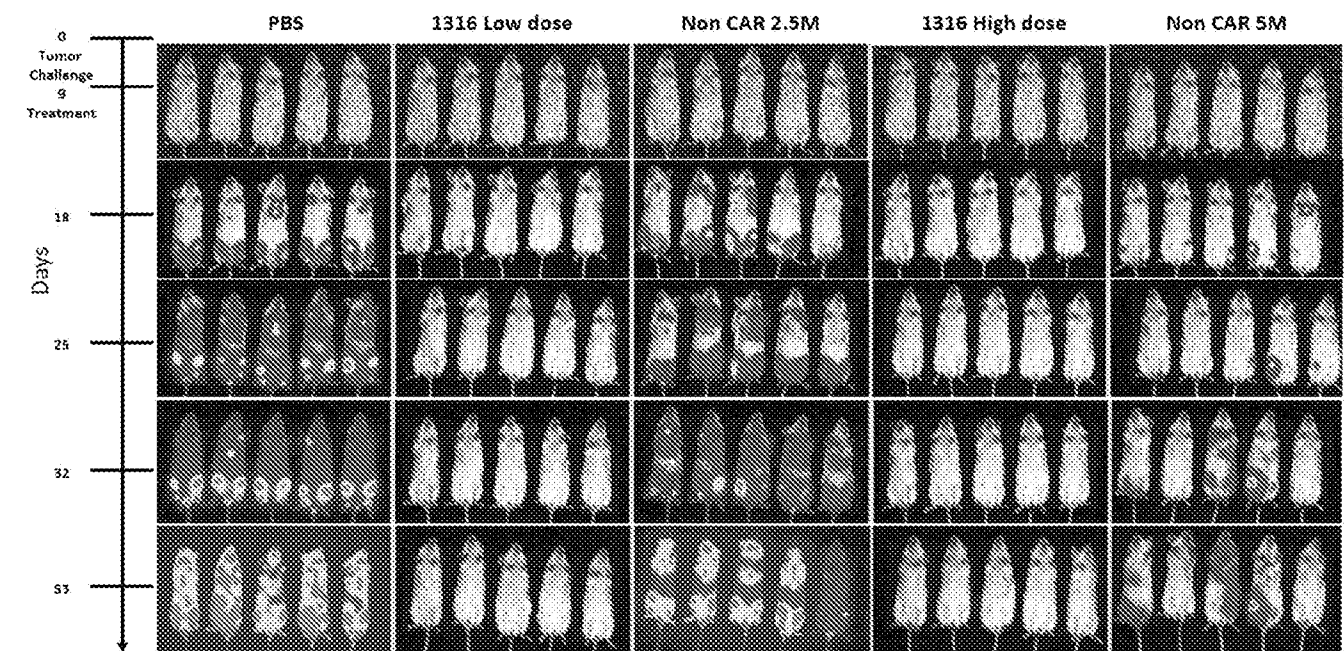


FIGURE 13



Compared groups		P value
Non CAR 2.5M	1316, 1/2.5M	<0.05
Non CAR 5	1316, 2/5M	<0.05
1316, 1/2.5M	1316, 2/5M	n.s.

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2020/019082

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K14/725 C07K16/28
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, WPI Data, BIOSIS, Sequence Search, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 2018/213337 A1 (US HEALTH [US]) 22 November 2018 (2018-11-22) abstract paragraph [0021] paragraph [0036] paragraph [0097] examples 4, 7 figure 24 sequences 15, 16	1-33
Y	WO 2017/214167 A1 (HOPE CITY [US]; UNIV TEXAS [US]) 14 December 2017 (2017-12-14) abstract example 10 sequences 20, 16, 30, 32, 24, 26, 28, 14, 18, 22, 33-37 ----- -/--	1-33



Further documents are listed in the continuation of Box C.



See patent family annex.

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Date of the actual completion of the international search

24 June 2020

Date of mailing of the international search report

01/07/2020

Name and mailing address of the ISA/

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Authorized officer

Mundel, Christophe

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2020/019082

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:
- a. ☐ forming part of the international application as filed:
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- ☐ on paper or in the form of an image file.
- b. ☐ furnished together with the international application under PCT Rule 13~~ter~~.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.
- c. ☒ furnished subsequent to the international filing date for the purposes of international search only:
- ☒ in the form of an Annex C/ST.25 text file (Rule 13~~ter~~.1(a)).
- ☐ on paper or in the form of an image file (Rule 13~~ter~~.1(b) and Administrative Instructions, Section 713).
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3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2020/019082

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 2016/149578 A1 (US HEALTH [US]) 22 September 2016 (2016-09-22) abstract paragraph [0018] figure 5 -----	1-33
Y	NICE TURAZZI ET AL: "Engineered T cells towards TNFRSF13C (BAFFR): a novel strategy to efficiently target B-cell acute lymphoblastic leukaemia", BRITISH JOURNAL OF HAEMATOLOGY, vol. 182, no. 6, 1 September 2018 (2018-09-01), pages 939-943, XP055706525, GB ISSN: 0007-1048, DOI: 10.1111/bjh.14899 abstract page 942, right-hand column, line 27 - line 38 -----	1-33

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US2020/019082

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