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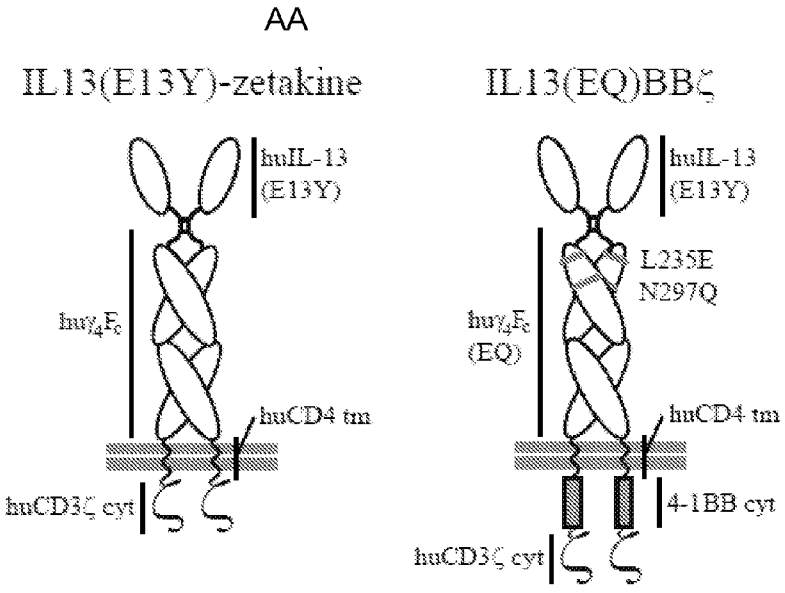
[Continued on next page]

(54) Title: COSTIMULATORY CHIMERIC ANTIGEN RECEPTOR T CELLS TARGETING IL13Rα2

FIGURE 1



(57) Abstract: Chimeric transmembrane immunoreceptors (CAR) which include an extracellular domain that includes IL-13 or a variant thereof that binds interleukin-13Rα2 (IL13Rα2), a transmembrane region, a costimulatory domain and an intracellular signaling domain are described.

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Costimulatory Chimeric Antigen Receptor T Cells Targeting IL13Rα2

BACKGROUND

[001] Tumor-specific T cell based immunotherapies, including therapies employing engineered T cells, have been investigated for anti-tumor treatment. In some cases the T cells used in such therapies do not remain active *in vivo* for a long enough period. In some cases, the tumor-specificity of the T cells is relatively low. Therefore, there is a need in the art for tumor-specific cancer therapies with longer term anti-tumor functioning.

[002] Malignant gliomas (MG), which include anaplastic astrocytoma (AA-grade III) and glioblastoma (GBM-grade IV), have an incidence rate of approximately 20,000 new cases diagnosed annually in the United States. According to the American Brain Tumor Association total prevalence of individuals living with a malignant brain tumor, based on United States 2010 census data, is roughly 140,000 persons. Although MG is a rare disease, it is highly aggressive and heterogeneous with respect to its malignant behavior and nearly uniformly lethal. Current standard-of-care therapies for high-grade MG yield only short term benefits, and these brain tumors are virtually incurable. Indeed, even with modern surgical and radiotherapeutic techniques, which often exacerbate the already severe morbidities imposed by location in the central nervous system (CNS), the 5-year survival rates are quite low. Furthermore, for the majority of patients who relapse with disease, there are few therapeutic options. Thus, there is a significant need for more effective therapies, particularly for those patients that have recurred/progressed following frontline therapies, and participation of this patient population in clinical trials is warranted.

[003] Adoptive T cell therapy (ACT) utilizing chimeric antigen receptor (CAR) engineered T cells may provide a safe and effective way to reduce recurrence rates of MG, since CAR T cells can be engineered to specifically recognize antigenically-distinct tumor populations (Cartellieri et al. 2010 *J Biomed Biotechnol* 2010:956304; Ahmed et

al. 2010 *Clin Cancer Res* 16:474; Sampson et al. 2014 *Clin Cancer Res* 20:972; Brown et al. 2013 Clin Cancer Res 2012 18:2199; Chow et al. 2013 *Mol Ther* 21:629), and T cells can migrate through the brain parenchyma to target and kill infiltrative malignant cells (Hong et al. 2010 *Clin Cancer Res* 16:4892; Brown et al. 2007 *J Immunol* 179:3332; Hong et al. 2010 *Clin Cancer Res* 16:4892; Yaghoubi 2009 Nat Clin PRact Oncol 6:53). Preclinical studies have demonstrated that IL13Rα2-targeting CAR+ T cells exhibit potent major histocompatibility complex (MHC)-independent, IL13Rα2-specific cytolytic activity against both stem-like and differentiated glioma cells, and induce regression of established glioma xenografts *in vivo* (Kahlon et al. 2004 *Cancer Res* 64:9160; Brown et al. 2012 *Clin Cancer Res* 18:2199).

SUMMARY

[004] Described herein are chimeric transmembrane immunoreceptors (chimeric antigen receptors or "CARs") which comprise an extracellular domain, a transmembrane region and an intracellular signaling domain. The extracellular domain is made up of an IL-13 ligand that binds interleukin-13Rα2 (IL13Rα2) and, optionally, a spacer, comprising, for example a portion human Fc domain. The transmembrane portion includes a CD4 transmembrane domain, a CD8 transmembrane domain, a CD28 transmembrane domain, a CD3 transmembrane domain or a 4IBB transmembrane domain. The intracellular signaling domain includes the signaling domain from the zeta chain of the human CD3 complex (CD3ζ) and one or more costimulatory domains, e.g., a 4-1BB costimulatory domain. The extracellular domain enables the CAR, when expressed on the surface of a T cell, to direct T cell activity to those cells expressing IL13Rα2, a receptor expressed on the surface of tumor cells, including glioma. Importantly, the IL13Rα2 binding portion of the CAR includes an amino acid modification, such as an E13Y mutation, that increases binding specificity. The inclusion of a costimulatory domain, such as the 4-1BB (CD137) costimulatory domain in series with CD3ζ in the intracellular region enables the T cell to receive co-stimulatory signals. T cells, for example, patient-specific, autologous T cells can be engineered to express the CARs described herein and the engineered cells can be expanded and used in ACT. Various T cell subsets can be used. In addition, the CAR can be expressed in other immune cells such as NK cells. Where a patient is treated with an

immune cell expressing a CAR described herein the cell can be an autologous or allogenic T cell. In some cases the cells used are CD4+ and CD8+ central memory T cells (T_{CM}), which are CD45RO+CD62L+, and the use of such cells can improve long-term persistence of the cells after adoptive transfer compared to the use of other types of patient-specific T cells.

[005] Described herein is a nucleic acid molecule encoding a chimeric antigen receptor (CAR)r, wherein the chimeric antigen receptor comprises: human IL-13 or a variant thereof having 1-10 (e.g., 1 or 2) amino acid modifications; a transmembrane domain selected from: a CD4 transmembrane domain or variant thereof having 1-10 (e.g., 1 or 2) amino acid modifications, a CD8 transmembrane domain or variant thereof having 1-10 (e.g., 1 or 2) amino acid modifications, a CD28 transmembrane domain or a variant thereof having 1-10 (e.g., 1 or 2) amino acid modifications, and a CD3ζ transmembrane domain or a variant thereof having 1-10 (e.g., 1 or 2) amino acid modifications; a costimulatory domain; and CD3 ζ signaling domain of a variant thereof having 1-10 (e.g., 1 or 2) amino acid modifications.

[006] In various embodiments the costimulatory domain is selected from the group consisting of: a CD28 costimulatory domain or a variant thereof having 1-10 (e.g., 1 or 2) amino acid modifications, a 4-IBB costimulatory domain or a variant thereof having 1-10 (e.g., 1 or 2) amino acid modifications and an OX40 costimulatory domain or a variant thereof having 1-10 (e.g., 1 or 2) amino acid modifications. In certain embodiments, a 4IBB costimulatory domain or a variant thereof having 1-10 (e.g., 1 or 2) amino acid modifications in present.

[007] Additional embodiment the CAR comprises: a variant of a human IL13 having 1-10 amino acid modification that increase binding specificity for IL13Rα2 versus IL13Rα1; the human IL-13 or variant thereof is an IL-13 variant comprising the amino acid sequence of SEQ ID NO:3 with 1 to 5 amino acid modifications, provided that the amino acid at position 11 of SEQ ID NO:3 other than E; two different costimulatory domains selected from the group consisting of: a CD28 costimulatory domain or a variant thereof having 1-10 (e.g., 1 or 2) amino acid modifications, a 4IBB costimulatory domain

or a variant thereof having 1-10 (e.g., 1 or 2) amino acid modifications and an OX40 costimulatory domain or a variant thereof having 1-10 (e.g., 1 or 2) amino acid modifications; two different costimulatory domains selected from the group consisting of: a CD28 costimulatory domain or a variant thereof having 1-2 amino acid modifications, a 4IBB costimulatory domain or a variant thereof having 1-2 amino acid modifications and an OX40 costimulatory domain or a variant thereof having 1-2 amino acid modifications; human IL-13 or a variant thereof having 1-2 amino acid modifications; a transmembrane domain selected from: a CD4 transmembrane domain or variant thereof having 1-2 amino acid modifications, a CD8 transmembrane domain or variant thereof having 1-2 amino acid modifications, a CD28 transmembrane domain or a variant thereof having 1-2 amino acid modifications, and a CD3ζ transmembrane domain or a variant thereof having 1-2 amino acid modifications; a costimulatory domain; and CD3ζ signaling domain of a variant thereof having 1-2 amino acid modifications; a spacer region located between the IL-13 or variant thereof and the transmembrane domain (e.g., the spacer region comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 4, 14-20, 50 and 52); the spacer comprises an IgG hinge region; the spacer region comprises 10-150 amino acids; the 4-1BB signaling domain comprises the amino acid sequence of SEQ ID NO:6; the CD3ζ signaling domain comprises the amino acid sequence of SEQ ID NO:7; and a linker of 3 to 15 amino acids that is located between the costimulatory domain and the CD3 ζ signaling domain or variant thereof. In certain embodiments where there are two costimulatory domains, one is an 4-IBB costimulatory domain and the other a costimulatory domain selected from: CD28 and CD28gg

[008] In some embodiments: nucleic acid molecule expresses a polypeptide comprising an amino acid sequence selected from SEQ ID NOs: 10, 31-48 and 52; the chimeric antigen receptor comprises a IL-13/IgG4/CD4t/41-BB region comprising the amino acid of SEQ ID NO:11 and a CD3 ζ signaling domain comprising the amino acid sequence of SEQ ID NO:7; and the chimeric antigen receptor comprises the amino acid sequence of SEQ ID NOs: 10, 31-48 and 52.

[009] Also disclosed is a population of human T cells transduced by a vector comprising an expression cassette encoding a chimeric antigen receptor, wherein

chimeric antigen receptor comprises: human IL-13 or a variant thereof having 1-10 amino acid modifications; a transmembrane domain selected from: a CD4 transmembrane domain or variant thereof having 1-10 amino acid modifications, a CD8 transmembrane domain or variant thereof having 1-10 amino acid modifications, a CD28 transmembrane domain or a variant thereof having 1-10 amino acid modifications, and a CD3ζ transmembrane domain or a variant thereof having 1-10 amino acid modifications; a costimulatory domain; and CD3 ζ signaling domain of a variant thereof having 1-10 amino acid modifications. In various embodiments: the population of human T cells comrpise a vector expressing a chimeric antigen receptor comprising an amino acid sequence selected from SEQ ID NOs: 10, 31-48 and 52; the population of human T cells are comprises of central memory T cells (Tcm cells) (e.g., at least 20%, 30%, 40%, 50% 60%, 70%, 80% of the cells are Tcm cells; at least 15%, 20%, 25%, 30%, 35% of the Tcm cells are CD4+ and at least 15%, 20%, 25%, 30%, 35% of the Tcm cells are CD8+ cells).

[0010] Also described is a method of treating cancer in a patient comprising administering a population of autologous or allogeneic human T cells (e.g., autologous or allogenic T cells comprising Tcm cells, e.g., at least 20%, 30%, 40%, 50% 60%, 70%, 80% of the cells are Tcm cells; at least 15%, 20%, 25%, 30%, 35% of the Tcm cells are CD4+ and at least 15%, 20%, 25%, 30%, 35% of the Tcm cells are CD8+ cells) transduced by a vector comprising an expression cassette encoding a chimeric antigen receptor, wherein chimeric antigen receptor comprises an amino acid sequence selected from SEQ ID NOs: 10, 31-48 and 52. In various embodiments: the population of human T cells comprise central memory T cells; the cancer is glioblastoma; and the transduced human T cells where prepared by a method comprising obtaining T cells from the patient, treating the T cells to isolate central memory T cells, and transducing at least a portion of the central memory cells to with a viral vector comprising an expression cassette encoding a chimeric antigen receptor, wherein chimeric antigen receptor comprises an amino acid sequence selected from SEQ ID NOs: 10, 31-48 and 52.

[0011] Also described is: a nucleic acid molecule encoding an polypeptide comprising an amino acid sequence that is at least 95% identical to an amino acid sequence selected

from SEQ ID NO:10 and SEQ ID NOs: 10, 31-48 and 52; a nucleic acid molecule encoding an polypeptide comprising an amino acid sequence that is identical to an amino acid sequence selected from SEQ ID NO: 10, 31-48 and 52 except for the presence of no more than 5 amino acid substitutions, deletions or insertions; a nucleic acid molecule encoding an polypeptide comprising an amino acid sequence that is identical to an amino acid sequence selected from SEQ ID NO:10 and SEQ ID NOs: 10, 31-48 and 52 except for the presence of no more than 5 amino acid substitutions; and a nucleic acid molecule encoding an polypeptide comprising an amino acid sequence that is identical to an amino acid sequence selected from SEQ ID NO:10 and SEQ ID NOs: 10, 31-48 and 52 except for the presence of no more than 2 amino acid substitutions.

[0012] Certain CAR described herein, for example, the IL13(EQ)BBζ CAR and the IL13(EQ)CD28- BBζ CAR, have certain beneficial characteristics compared to certain other IL13-targeted CAR. For example, they have improved selectivity for IL13Rα, elicit lower Th2 cytokine production, particularly lower IL13 production.

[0013] T cells expressing a CAR targeting IL13Rα2 can be useful in treatment of cancers such as glioblastoma, as well as other cancer that expresses IL13Rα2 which include but are not limited to medulloblastoma, breast cancer, head and neck cancer, kidney cancer, ovarian cancer and Kaposi's sarcoma. Thus, this disclosure includes methods for treating cancer using T cells expressing a CAR described herein.

[0014] This disclosure also nucleic acid molecules that encode any of the CARs described herein (e.g., vectors that include a nucleic acid sequence encoding one of the CARs) and isolated T lymphocytes that express any of the CARs described herein.

[0015] The CAR described herein can include a spacer region located between the IL13 domain 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:14)
IgG4 hinge (S→P) (S228P)	12 aa	ESKYGPPCPPCP (SEQ ID NO:15)
IgG4 hinge	12 aa	ESKYGPPCPSCP (SEQ ID NO:52)
IgG4 hinge + linker	22 aa	ESKYGPPCPPCPGGGSSGGGSG (SEQ ID NO:16)
CD28 hinge	39 aa	IEVMYPPPYLDNEKSNGTIIHVKGKHL CPSPLFPGPSKP (SEQ ID NO:17)
CD8 hinge-48aa	48 aa	AKPTTTPAPRPPTPAPTIASQPLSLRPE ACRPAAGGAVHTRGLDFACD (SEQ ID NO:18)
CD8 hinge-45aa	45aa	TTTPAPRPPTPAPTIASQPLSLRPEACR PAAGGAVHTRGLDFACD (SEQ ID NO:19)
IgG4(HL-CH3)	129 aa	ESKYGPPCPPCPGGGSSGGGSGGQPR EPQVYTLPPSQEEMTKNQVSLTCLVK GFYPSDIAVEWESNGQPENNYKTTPP VLDSDGSFFLYSRLTVDKSRWQEGNV FSCSVMHEALHNHYTQKSLSLSLGK (SEQ ID NO:20)
IgG4(L235E,N297Q)	229 aa	ESKYGPPCPSCPAPEFEGGPSVFLFPPK PKDTLMISRTPEVTCVVVDVSQEDPE VQFNWYVDGVEVHQAKTKPREEQFN STYRVVSVLTVLHQDWLNGKEYKCK VSNKGLPSSIEKTISKAKGQPREPQVY TLPPSQEEMTKNQVSLTCLVKGFYPS DIAVEWESNGQPENNYKTTPPVLDSD GSFFLYSRLTVDKSRWQEGNVFSCSV MHEALHNHYTQKSLSLSLGK (SEQ ID NO:4)
IgG4(S228P, L235E,N297Q)	229 aa	ESKYGPPCPPCPAPEFEGGPSVFLFPPK PKDTLMISRTPEVTCVVVDVSQEDPE VQFNWYVDGVEVHQAKTKPREEQFN STYRVVSVLTVLHQDWLNGKEYKCK

		VSNKGLPSSIEKTISKAKGQPREPQVY TLPPSQEEMTKNQVSLTCLVKGFYPS DIAVEWESNGQPENNYKTTPPVLDSD GSFFLYSRLTVDKSRWQEGNVFSCSV MHEALHNHYTQKSLSLSLGK (SEQ ID NO:51)
IgG4(CH3)	107 aa	GQPREPQVYTLPPSQEEMTKNQVSLT CLVKGFYPSDIAVEWESNGQPENNYK TTPPVLDSDGSFFLYSRLTVDKSRWQ EGNVFSCSVMHEALHNHYTQKSLSLS LGK (SEQ ID NO:50)

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 ore more amino acid modifications, for example, 1, 2, 3, 4 or 5 substitutions, e.g., substitutions that reduce off-target binding.

[0016] 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.

[0017] In certain embodiments, the spacer is derived from an IgG1, IgG2, IgG3, or IgG4 that includes one or more amino acid residues substituted with an amino acid residue different from that present in an unmodified spacer. The one or more substituted amino acid residues are selected from, but not limited to one or more amino acid residues at positions 220, 226, 228, 229, 230, 233, 234, 235, 234, 237, 238, 239, 243, 247, 267, 268, 280, 290, 292, 297, 298, 299, 300, 305, 309, 218, 326, 330, 331, 332, 333, 334, 336, 339, or a combination thereof. In this numbering scheme, described in greater detail below, the first amino acid in the IgG4(L235E,N297Q) spacer in Table 1 is 219 and the first amino acid in the IgG4(HL-CH3) spacer in Table 1 is 219 as is the first amino acid in the IgG hinge sequence and the IgG4 hinge linker (HL) sequence in Table 1

[0018] In some embodiments, the modified spacer is derived from an IgG1, IgG2, IgG3, or IgG4 that includes, but is not limited to, one or more of the following amino acid residue substitutions: C220S, C226S, S228P, C229S, P230S, E233P, V234A, L234V, L234F, L234A, L235A, L235E, G236A, G237A, P238S, S239D, F243L, P247I, S267E, H268Q, S280H, K290S, K290E, K290N, R292P, N297A, N297Q, S298A, S298G, S298D, S298V, T299A, Y300L, V305I, V309L, E318A, K326A, K326W, K326E, L328F, A330L, A330S, A331S, P331S, I332E, E333A, E333S, E333S, K334A, A339D, A339Q, P396L, or a combination thereof.

[0019] In certain embodiments, the modified spacer is derived from IgG4 region that includes one or more amino acid residues substituted with an amino acid residue different from that present in an unmodified region. The one or more substituted amino acid residues are selected from, but not limited to, one or more amino acid residues at positions 220, 226, 228, 229, 230, 233, 234, 235, 234, 237, 238, 239, 243, 247, 267, 268, 280, 290, 292, 297, 298, 299, 300, 305, 309, 218, 326, 330, 331, 332, 333, 334, 336, 339, or a combination thereof.

[0020] In some embodiments, the modified spacer is derived from an IgG4 region that includes, but is not limited to, one or more of the following amino acid residue

substitutions: 220S, 226S, 228P, 229S, 230S, 233P, 234A, 234V, 234F, 234A, 235A, 235E, 236A, 237A, 238S, 239D, 243L, 247I, 267E, 268Q, 280H, 290S, 290E, 290N, 292P, 297A, 297Q, 298A, 298G, 298D, 298V, 299A, 300L, 305I, 309L, 318A, 326A, 326W, 326E, 328F, 330L, 330S, 331S, 331S, 332E, 333A, 333S, 333S, 334A, 339D, 339Q, 396L, or a combination thereof, wherein the amino acid in the unmodified spacer is substituted with the above identified amino acids at the indicated position.

[0021] For amino acid positions in immunoglobulin discussed herein, numbering is according to the EU index or EU numbering scheme (Kabat et al. 1991 Sequences of Proteins of Immunological Interest, 5th Ed., United States Public Health Service, National Institutes of Health, Bethesda, hereby entirely incorporated by reference). The EU index or EU index as in Kabat or EU numbering scheme refers to the numbering of the EU antibody (Edelman et al. 1969 *Proc Natl Acad Sci USA* 63:78-85).

[0022] A variety of transmembrane domains can be used in CAR directed against IL13Ra2. Table 2 includes examples of suitable transmembrane domains. Where a spacer domain is present, the transmembrane domain is located carboxy terminal to the spacer domain.

Table 2: Examples of Transmembrane Domains

Name	Accession	Length	Sequence
CD3z	J04132.1	21 aa	LCYLLDGILFIYGVILTALFL (SEQ ID NO:21)
CD28	NM_006139	27aa	FWVLVVVGGVLACYSLLVTVAFIIFWV (SEQ ID NO:22)
CD28(M)	NM_006139	28aa	MFWVLVVVGGVLACYSLLVTVAFIIFWV (SEQ ID NO:22)
CD4	M35160	22aa	MALIVLGGVAGLLLFIGLGIFF (SEQ ID NO:5)
CD8tm	NM_001768	21aa	IYIWAPLAGTCGVLLLSLVIT (SEQ ID NO:23)

CD8tm2	NM_001768	23aa	IYIWAPLAGTCGVLLLSLVITLY (SEQ ID NO:24)
CD8tm3	NM_001768	24aa	IYIWAPLAGTCGVLLLSLVITLYC (SEQ ID NO:25)
41BB	NM_001561	27aa	IISFFLALTSTALLFLLFF LTLRFSVV (SEQ ID NO:26)

Many of the CAR described herein include one or more (e.g., two) costimulatory domains. The costimulatory domain(s) are located between the transmembrane domain and the CD3ζ signaling domain. Table 3 includes examples of suitable costimulatory domains together with the sequence of the CD3ζ signaling domain.

Table 3: Examples of Costimulatory Domains

Name	Accession	Length	Sequence
CD3ζ	J04132.1	113 aa	RVKFSRSADAPAYQQGQNQLYNELNLGR REEYDVLDKRRGRDPEMGGKPRRKNPQ EGLYNELQKDKMAEAYSEIGMKGERRR GKGHDGLYQGLSTATKDTYDALHMQAL PPR
CD28	NM_006139	42aa	RSKRSRLLHSDYMNMTPRRPGPTRKHYQ PYAPPRDFAAYRS (SEQ ID NO: 27)
CD28gg*	NM_006139	42aa	RSKRSRGGHSDYMNMTPRRPGPTRKHY QPYAPPRDFAAYRS (SEQ ID NO:28)
41BB	NM_001561	42 aa	KRGRKKLLYIFKQPFMRPVQTTQEEDGC SCRFPEEEEGGCEL (SEQ ID NO:29)
OX40		42 aa	ALYLLRRDQRLPPDAHKPPGGGSFRTPIQ EEQADAHSTLAKI (SEQ ID NO:30)

DESCRIPTION OF DRAWINGS

[0023] Figure 1 is a schematic depiction of IL13(E13Y)-zetakine CAR (Left) composed of the IL13Rα2-specific human IL-13 variant (huIL-13(E13Y)), human IgG4 Fc spacer (huγ4Fc), human CD4 transmembrane (huCD4 tm), and human CD3ζ chain cytoplasmic

(huCD3ζ cyt) portions as indicated. Also depicted is a IL13(EQ)BBζ CAR which is the same as the IL13(E13Y)-zetakine with the exception of the two point mutations, L235E and N297Q indicated in red, that are located in the CH2 domain of the IgG4 spacer, and the addition of a costimulatory 4-1BB cytoplasmic domain (4-1BB cyt).

[0024] Figures 2A-C depict certain vectors an open reading frames. **A** is a diagram of the cDNA open reading frame of the 2670 nucleotide IL13(EQ)BBZ-T2ACD19t construct, where the IL13Rα2-specific ligand IL13(E13Y), IgG4(EQ) Fc hinge, CD4 transmembrane, 4-1BB cytoplasmic signaling, three-glycine linker, and CD3ζ cytoplasmic signaling domains of the IL13(EQ)BBZ CAR, as well as the T2A ribosome skip and truncated CD19 sequences are indicated. The human GM-CSF receptor alpha and CD19 signal sequences that drive surface expression of the IL13(EQ)BBζ CAR and CD19t are also indicated. **B** is a diagram of the sequences flanked by long terminal repeats (indicated by 'R') that will integrate into the host genome. **C** is a map of the IL13(EQ)BBZ-T2A-CD19t_epHIV7 plasmid.

[0025] Figure 3 depicts the construction of pHIV7.

[0026] Figure 4 depicts the elements of pHIV7.

[0027] Figure 5 depicts a production scheme for IL13(EQ)BBζ/CD19t+ T_{CM}.

[0028] Figures 6A-C depicts the results of flow cytometric analysis of surface transgene and T cell marker expression. IL13(EQ)BBζ/CD19t+ T_{CM} HD006.5 and HD187.1 were co-stained with anti-IL13-PE and anti-CD8-FITC to detect CD8+ CAR+ and CD4+ (i.e., CD8 negative) CAR+ cells (**A**), or anti-CD19-PE and anti-CD4-FITC to detect CD4+ CD19t+ and CD8+ (i.e., CD4 negative) CAR+ cells (**B**). IL13(EQ)BBζ/CD19t+ T_{CM} HD006.5 and HD187.1 stained with fluorochromeconjugatedanti-CD3, TCR, CD4, CD8, CD62L and CD28 (grey histograms) or isotype controls (black histograms) (**C**). In all cases the percentages based on viable lymphocytes (DAPI negative) stained above isotype.

[0029] Figures 7A-B depict the *in vitro* functional characterization of IL13Rα2-specific effector function of IL13(EQ)BBZ+ T_{CM}. IL13(EQ)BBZ/CD19t+ T_{CM} HD006.5 and

HD187.1 were used as effectors in a 6-hour ^{51}Cr release assay using a 10:1 E:T ratio based on CD19t expression. The IL13Rα2-positive tumor targets were K562 engineered to express IL13Rα2 (K562-IL13Rα2) and primary glioma line PBT030-2, and the IL13Rα2-negative tumor target control was K562 parental line (**A**). IL13(EQ)BBZ/CD19t+ T_{CM} HD006.5 and HD187.1 were evaluated for antigen-dependent cytokine production following overnight co-culture at a 10:1 E:T ratio with IL13Rα2-positive and negative targets. Cytokine levels were measured using the Bio-Plex Pro Human Cytokine TH1/TH2 Assay kit and INF-γ are reported (**B**).

[0030] Figures 8A-C depict the result of studies demonstrating the regression of established glioma tumor xenografts after adoptive transfer of IL13(EQ)BBζ/CD19t+ T_{CM}. EGFP-ffLuc+ PBT030-2 tumor cells (1×10^5) were stereotactically implanted into the right forebrain of NSG mice. On day 5, mice received either $2x10^6$ IL13(EQ)BBζ/CD19t+ T_{CM} ($1.1x10^6$ CAR+; n=6), $2x10^6$ mock TCM (no CAR; n=6) or PBS (n=6). Representative mice from each group showing relative tumor burden using Xenogen Living Image (**A**). Quantification of ffLuc flux (photons/sec) shows that IL13(EQ)BBζ/CD19t+ T_{CM} induce tumor regression as compared to mock-transduced T_{CM} and PBS (#p<0.02, *p<0.001, repeated measures ANOVA) (**B**). Kaplan Meier survival curve (n=6 per group) demonstrating significantly improved survival (p=0.0008; log-rank test) for mice treated with IL13(EQ)BBζ/CD19t+ T_{CM} (**C**)

[0031] Figures 9A-C depict the results of studies comparing ant-tumor efficacy of IL13(EQ)BBZ T_{CM} and IL13-zetakine CTL clones. EGFP-ffLuc+ PBT030-2 TSs (1×10^5) were stereotactically implanted into the right forebrain of NSG mice. On day 8, mice received either $1.6x10^6$ mock T_{CM} (no CAR), 1.0×10^6 CAR+ IL13(EQ)BBζ T_{CM} (1.6×10^6 total T cells; 63% CAR), $1.0x10^6$ IL13-zetakine CD8+ CTL cl. 2D7 (clonal CAR+), or no treatment (n=6 per group). Representative mice from each group showing relative tumor burden using Xenogen Living Image (**A**). Linear regression lines of natural log of ffLuc flux (photons/sec) over time, P-values are for group by time interaction comparisons (**B**). Kaplan Meier survival analysis (n= 6 per group) demonstrate significantly improved survival (p=0.02; log-rank test) for mice treated with IL13(EQ)BBζ T_{CM} as compared to IL13-zetakine CD8+ CTL cl. 2D7 (**C**).

[0032] Figures 10A-C depict the results of studies comparing ant-tumor efficacy of IL13(EQ)BBζ T_{CM} and IL13-zetakine CTL clones. EGFP-ffLuc+ PBT030-2 TSs (1×10^5) were stereotactically implanted into the right forebrain of NSG mice. On day 8, mice received either $1.3x10^6$ mock T_{CM} (no CAR; n=6), 1.0, 0.3 or $0.1x10^6$ CAR+ IL13(EQ)BBζ T_{CM} (78% CAR+; n=6-7), 1.0, 0.3 or $0.1x10^6$ IL13-zetakine CD8+ CTL cl. 2D7 (clonal CAR+; n=6-7), or no treatment (n=5). Xenogen imaging of representative mice from each group showing relative tumor burden (**A**). Linear regression lines of natural log of ffLuc flux (photons/sec) shows that IL13(EQ)BBζ T_{CM} achieve superior tumor regression as compared to first-generation IL13-zetakine CTL cl. 2D7, mock T_{CM} and tumor only (**B**). Average flux per group at day 27 post tumor injection demonstrating that the $0.1x10^6$ IL13(EQ)BBζ T_{CM} dose outperforms the ten-fold higher $1.0x10^6$ dose of IL13-zetakine CD8+ CTL cl. 2D7 ($p = 0.043$; Welch two sample t- test) (**C**).

[0033] Figure 11 depicts the results of studies demonstrating IL13(EQ)BBζ Tcm display improved persistence compared IL13-zetakine CTL clones. CD3 immunohistochemistry evaluating T cell persistence at the tumor site 7-days post T cell infusion. Significant numbers of T cells are detected for IL13(EQ)BBζ Tcm (top panel). By contrast, very few viable CD3+ IL13-zetakine T cells are detected (bottom panel).

[0034] Figures 12A-D depict the results of experiments comparing route of CAR+ T cell delivery (i.c. versus i.v.) for large established tumors. EGFP-ffLuc+ PBT030-2 TSs (1×10^5) were implanted into the right forebrain of NSG mice. On days 19 and 26, mice were injected i.v. through the tail vein with either $5x10^6$ CAR+ IL13(EQ)BBζ+ Tcm ($11.8x10^6$ total cells; n=4), or mock Tcm ($11.8x10^6$ cells; n=4). Alternatively, on days 19, 22, 26 and 29 mice were injected i.c. with either $1x10^6$ CAR+ IL13(EQ)BBζ+ Tcm ($2.4x10^6$ total cells; n=4), or mock Tcm ($2.4x10^6$ cells; n=5). Average ffLuc flux (photons/sec) over time shows that i.c. delivered IL13(EQ)BBζ Tcm mediates tumor regression of day 19 tumors. By comparison, i.v. delivered T cells do not shown reduction in tumor burden as compared to untreated or mock Tcm controls (**A**). Kaplan Meier survival curve demonstrates improved survival for mice treated i.c. IL13(EQ)BBZ Tcm as compared to mice treated with i.v. administered CAR+ Tcm ($p = 0.0003$ log rank test) (**B**). Representative H&E and CD3 IHC of mice treated i.v. (**C**) versus i.c. (**D**) with

IL13(EQ)BBZ+ Tcm. CD3+ T cells were only detected in the i.c. treated group, with no CD3+ cells detected in the tumor or surrounding brain parenchyma for i.v. treated mice.

[0035] Figures 13A-B depict the results of studies showing that CAR+ T cell injected intracranially, either intratumoral (i.c.t.) or intraventricular (i.c.v.), can traffic to tumors on the opposite hemisphere. EGFP-ffLuc+ PBT030-2 TSs (1×105) were stereotactically implanted into the right and left forebrains of NSG mice. On day 6, mice were injected i.c. at the right tumor site with 1.0x106 IL13(EQ)BBζ+ Tcm (1.6x106 total cells; 63% CAR; n=4). Schematic of multifocal glioma experimental model (**A**). CD3 IHC showing T cells infiltrating both the right and left tumor sites (**B**).

[0036] Figures 14A-C depict the results of a series of studies evaluating costimulatory domains of IL13Rα2-specific CAR. Schematic of IL13Ra2-specific CAR constructs comparing various intracellular endo/signaling domains, including the first generation CD3z CAR lacking costimulation, versus second generation CARs incorporating either 4-1BB or CD28, versus a third generation CAR containing both CD28 and 41BB. All CAR cassettes also contain the T2A ribosomal skip and truncated CD19 (CD19t) sequences as a marker for transduced cells (**A**). CD4 and CD8 TCM were lentivirally transduced and CAR-expressing T cells were immunomagnetically enriched via anti-CD19. CD19 and IL13 (i.e., CAR) expression levels as measured by flow cytometry (**B**). Stability of each CAR construct was determined by dividing the CAR (IL13) mean flourescence intenstity (MFI) by that of the transduction marker (CD19t) (**C**). The 4-1BB containing CARs demonstrated the lowest expression levels as compared to the CD19t transduction marker.

[0037] Figures 15A-B depict the results of studies demonstrating that IL13Rα2-specific CAR containing the 4-1BB costimulatory domain produce less Th1 and Th2 cytokines. The ability of the indicated mock-transduced or CAR-expressing T cells to kill IL13Rα2-expressing PBT030-2 tumor cell targets was determined in a 4-hour 51Cr-release assay at the indicated effector:target ratios. Mean % chromium release + S.D. of triplicate wells are depicted (**A**). As expected, mock-transduced T cells did not efficiently lyse the targets. In contrast, all CAR-expressing T cells lysed the tumor cells in a similar manner.

The indicated mock-transduced or CAR-expressing T cells were co-cultured overnight with IL13Rα2-expressing PBT030-2 tumor cells at a 10:1 ratio and supernatants were analyzed for IL-13 and IFN-γ levels by cytometric bead array (**B**). Means + S.D. of triplicate wells are depicted. Interestingly, T cells expressing the zeta, 41BB-zeta or CD28-41BB-zeta CARs exhibited lower antigen-stimulated cytokine production than T cells expressing the CD28-zeta CAR.

[0038] Figures 16A-C depict the results of a series of studies of the in vivo efficacy of IL13Rα2-specific CARs. NSG mice received an intracranial injection of ffLuc+ PBT030-2 tumor cells on day 0, and were randomized into 6 groups (n = 9-10 mice per group) for i.c. treatment with either PBS (Tumor Only), mock-transduced T cells or T cells expressing the indicated IL13Rα2-specific CAR on day 8. Quantitative bioluminescence imaging was then carried out to monitor tumor growth over time. Bioluminescence images for representative mice in each group (**A**). Mean + S.E. of total flux levels of luciferase activity over time in each group (**B**). Flux levels for each mouse at Day 27. All groups treated with IL13Rα2-specific CAR T cells, except those treated with T cells expressing the CD28-CAR, show statistically-significant reduction in tumor volume compared to mice treated with mock-transduced T cells (**C**)

[0039] Figure 17 depicts the amino acid sequence of IL13(EQ)BBζ/CD19t+ (SEQ ID NO:10).

[0040] Figure 18 depicts a sequence comparison of IL13(EQ)41BBζ[IL13{EQ}41BBζ T2A-CD19t_epHIV7; pF02630] (SEQ ID NO:12) and CD19Rop_epHIV7 (pJ01683) (SEQ ID NO:13).

[0041] Figure 19 depicts the amino acid sequence of IL13(EmY)-CD8h3-CD8tm2-41BB Zeta (SEQ ID NO:31 with GMSCFRa signal peptide; SEQ ID NO:39 without GMSCFRa signal peptide).

[0042] Figure 20 depicts the amino acid sequence of IL13(EmY)-CD8h3-CD28tm-CD28gg-41BB-Zeta (SEQ ID NO:32 with GMSCFRa signal peptide; SEQ ID NO:40 without GMSCFRa signal peptide).

[0043] Figure 21 depicts the amino acid sequence of IL13(EmY)-IgG4(HL-CH3)-CD4tm-41BB-Zeta (SEQ ID NO:33 with GMSCFRa signal peptide; SEQ ID NO:41 without GMSCFRa signal peptide).

[0044] Figure 22 depicts the amino acid sequence of IL13(EmY)-IgG4(L235E,N297Q)-CD8tm-41BB-Zeta (SEQ ID NO:34 with GMSCFRa signal peptide; SEQ ID NO:42 without GMSCFRa signal peptide).

[0045] Figure 23 depicts the amino acid sequence of IL13(EmY)-Linker-CD28tm-CD28gg-41BB-Zeta (SEQ ID NO:35 with GMSCFRa signal peptide; SEQ ID NO:43without GMSCFRa signal peptide).

[0046] Figure 24 depicts the amino acid sequence of IL13(EmY)-HL-CD28m-CD28gg-41BB-Zeta (SEQ ID NO:36 with GMSCFRa signal peptide; SEQ ID NO:44 without GMSCFRa signal peptide).

[0047] Figure 25 depicts the amino acid sequence of IL13(EmY)-IgG4(HL-CH3)-CD28tm-CD28gg-41BB-Zeta (SEQ ID NO:37 with GMSCFRa signal peptide; SEQ ID NO:45 without GMSCFRa signal peptide).

[0048] Figure 26 depicts the amino acid sequence of IL13(EmY) IgG4(L235E,N297Q)-CD28tm-CD28gg-41BB-Zeta (SEQ ID NO:38 with GMSCFRa signal peptide; SEQ ID NO:46 without GMSCFRa signal peptide).

[0049] Figure 27 depicts the amino acid sequence of IL13(EmY)-CD8h3-CD8tm-41BB Zeta (SEQ ID NO:47 with GMSCFRa signal peptide; SEQ ID NO:48 without GMSCFRa signal peptide).

[0050]

DETAILED DESCRIPTION

[0051] Described below is the structure, construction and characterization of various IL13Rα2-specific chimeric antigen receptors. A chimeric antigen (CAR) is a recombinant

biomolecule that contains, at a minimum, an extracellular recognition domain, a transmembrane region, and an intracellular signaling domain. The term "antigen," therefore, is not limited to molecules that bind antibodies, but to any molecule that can bind specifically to a target. For example, a CAR can include a ligand that specifically binds a cell surface receptor. The extracellular recognition domain (also referred to as the extracellular domain or simply by the recognition element which it contains) comprises a recognition element that specifically binds to a molecule present on the cell surface of a target cell. The transmembrane region anchors the CAR in the membrane. The intracellular signaling domain comprises the signaling domain from the zeta chain of the human CD3 complex and optionally comprises one or more costimulatory signaling domains. CARs can both to bind antigen and transduce T cell activation, independent of MHC restriction. Thus, CARs are “universal” immunoreceptors which can treat a population of patients with antigen-positive tumors irrespective of their HLA genotype. Adoptive immunotherapy using T lymphocytes that express a tumor-specific CAR can be a powerful therapeutic strategy for the treatment of cancer.

[0052] One IL13Rα2-specific CAR described herein is referred to as IL13(EQ)BBζ. This CAR includes a variety of important features including: a IL13α2 ligand having an amino acid change that improves specificity of biding to IL13α2; the domain of CD137 (4-1BB) in series with CD3ζ to provide beneficial costimulation; and an IgG4 Fc region that is mutated at two sites within the CH2 region (L235E; N297Q) in a manner that reduces binding by Fc receptors (FcRs). Other CAR described herein contain a second costimulatory domain.

[0053] In some cases the CAR described herein, including the IL13(EQ)BBζ 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 CD19 (CD19t), which lacks the cytoplasmic signaling tail (truncated at amino acid 323). In this arrangement, co-expression of CD19t 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 and/or imaging of the therapeutic T cells in vivo following adoptive transfer. Co-expression of CD19t provides a marker for immunological targeting of the

transduced cells in vivo using clinically available antibodies and/or immunotoxin reagents to selectively delete the therapeutic cells, and thereby functioning as a suicide switch.

[0054] Gliomas, express IL13 receptors, and in particular, high-affinity IL13 receptors. However, unlike the IL13 receptor, glioma cells overexpress a unique IL13Rα2 chain capable of binding IL13 independently of the requirement for IL4Rβ or γc44. Like its homolog IL4, IL13 has pleotropic immunoregulatory activity outside the CNS. Both IL13 and IL4 stimulate IgE production by B lymphocytes and suppress pro-inflammatory cytokine production by macrophages.

[0055] Detailed studies using autoradiography with radiolabeled IL13 have demonstrated abundant IL13 binding on nearly all malignant glioma tissues studied. This binding is highly homogeneous within tumor sections and in single cell analysis. However, molecular probe analysis specific for IL13Rα2 mRNA did not detect expression of the glioma-specific receptor by normal brain elements and autoradiography with radiolabeled IL13 also could not detect specific IL13 binding in the normal CNS. These studies suggest that the shared IL13Rα1/IL4β/γc receptor is not expressed detectably in the normal CNS. Therefore, IL13Rα2 is a very specific cell-surface target for glioma and is a suitable target for a CAR designed for treatment of a glioma.

[0056] Binding of IL13-based therapeutic molecules to the broadly expressed IL13Rα1/IL4β/γc receptor complex, however, has the potential of mediating undesired toxicities to normal tissues outside the CNS, and thus limits the systemic administration of these agents. An amino acid substitution in the IL13 alpha helix A at amino acid 13 of tyrosine for the native glutamic acid selectively reduces the affinity of IL13 to the IL13Rα1/IL4β/γc receptor. Binding of this mutant (termed IL13(E13Y)) to IL13Rα2, however, was increased relative to wild-type IL13. Thus, this minimally altered IL13 analog simultaneously increases IL13's specificity and affinity for glioma cells. Therefore, CAR described herein include an IL13 containing a mutation (E to Y or E to some other amino acid such as K or R or L or V) at amino acid 13 (according to the numbering of Debinski et al. 1999 *Clin Cancer Res* 5:3143s). IL13 having the natural

sequence also may be used, however, and can be useful, particularly in situations where the modified T cells are to be locally administered, such as by injection directly into a tumor mass.

[0057] 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, 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.

[0058] Various T cell subsets isolated from the patient, including unselected PBMC or enriched CD3 T cells or enriched CD3 or memory T cell subsets, 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 SIN lentiviral vector that directs the expression of an IL13Rα2-specific CAR (e.g., IL13(EQ)BBζ) as well as a truncated human CD19 (CD19t), a non-immunogenic surface marker for both in vivo detection 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: Construction and Structure of an IL13Rα2-specific CAR

[0059] The structure of a useful IL13Rα2-specific CAR is described below. The codon optimized CAR sequence contains a membrane-tethered IL-13 ligand mutated at a single site (E13Y) to reduce potential binding to IL13Rα1, an IgG4 Fc spacer containing two mutations (L235E; N297Q) that greatly reduce Fc receptor-mediated recognition models,

a CD4 transmembrane domain, a costimulatory 4-1BB cytoplasmic signaling domain, and a CD3ζ cytoplasmic signaling domain. A T2A ribosome skip sequence separates this IL13(EQ)BBζ CAR sequence from CD19t, an inert, non-immunogenic cell surface detection/selection marker. This T2A linkage results in the coordinate expression of both IL13(EQ)BBζ and CD19t from a single transcript. **Figure 1A** is a schematic drawing of the 2670 nucleotide open reading frame encoding the IL13(EQ)BBZ-T2ACD19t construct. In this drawing, the IL13Rα2-specific ligand IL13(E13Y), IgG4(EQ) Fc, CD4 transmembrane, 4-1BB cytoplasmic signaling, three-glycine linker, and CD3ζ cytoplasmic signaling domains of the IL13(EQ)BBZ CAR, as well as the T2A ribosome skip and truncated CD19 sequences are all indicated. The human GM-CSF receptor alpha and CD19 signal sequences that drive surface expression of the IL13(EQ)BBZ CAR and CD19t are also indicated. Thus, the IL13(EQ)BBZ-T2ACD19t construct includes a IL13Rα2-specific, hinge-optimized, costimulatory chimeric immunoreceptor sequence (designated IL13(EQ)BBZ), a ribosome-skip T2A sequence, and a CD19t sequence.

[0060] The IL13(EQ)BBZ sequence was generated by fusion of the human GM-CSF receptor alpha leader peptide with IL13(E13Y) ligand 5 L235E/N297Q-modified IgG4 Fc hinge (where the double mutation interferes with FcR recognition), CD4 transmembrane, 4-1BB cytoplasmic signaling domain, and CD3ζ cytoplasmic signaling domain sequences. This sequence was synthesized de novo after codon optimization. The T2A sequence was obtained from digestion of a T2A-containing plasmid. The CD19t sequence was obtained from that spanning the leader peptide sequence to the transmembrane components (i.e., basepairs 1-972) of a CD19-containing plasmid. All three fragments, 1) IL13(EQ)BBZ, 2) T2A, and 3) CD19t, were cloned into the multiple cloning site of the epHIV7 lentiviral vector. When transfected into appropriate cells, the vector integrates the sequence depicted schematically in **Figure 1B** into the host cells genome. **Figure 1C** provides a schematic drawing of the 9515 basepair IL13(EQ)BBZ-T2A-CD19t _epHIV7 plasmid itself.

[0061] As shown schematically in **Figure 2**, IL13(EQ)BBZ CAR differs in several important respects from a previously described IL13Rα2-specific CAR referred to as IL13(E13Y)-zetakine (Brown et al. 2012 *Clinical Cancer Research* 18:2199). The

IL13(E13Y)-zetakine is composed of the IL13Rα2-specific human IL-13 mutein (huIL-13(E13Y)), human IgG4 Fc spacer (huγ4Fc), human CD4 transmembrane (huCD4 tm), and human CD3ζ chain cytoplasmic (huCD3ζ cyt) portions as indicated. In contrast, the IL13(EQ)BBζ) has two point mutations, L235E and N297Q that are located in the CH2 domain of the IgG4 spacer, and a costimulatory 4-1BB cytoplasmic domain (4-1BB cyt).

Example 2: Construction and Structure of epHIV7 used for Expression of an IL13Rα2-specific CAR

[0062] The pHIV7 plasmid is the parent plasmid from which the clinical vector IL13(EQ)BBZ-T2A-CD19t_epHIV7 was derived in the T cell Therapeutics Research Laboratory (TCTRL) at City of Hope (COH). The epHIV7 vector used for expression of the CAR was produced from pHIV7 vector. Importantly, this vector uses the human EF1 promoter to drive expression of the CAR. Both the 5' and 3' sequences of the vector were derived from pv653RSN as previously derived from the HXBc2 provirus. The polypurine tract DNA flap sequences (cPPT) were derived from HIV-1 strain pNL4-3 from the NIH AIDS Reagent Repository. The woodchuck post-transcriptional regulatory element (WPRE) sequence was previously described.

[0063] Construction of pHIV7 is schematically depicted in Figure 3. Briefly, pv653RSN, containing 653 bp from gag-pol plus 5' and 3' long-terminal repeats (LTRs) with an intervening SL3-neomycin phosphotransferase gene (Neo), was subcloned into pBluescript, as follows: In Step 1, the sequences from 5' LTR to rev-responsive element (RRE) made p5'HIV-1 51, and then the 5' LTR was modified by removing sequences upstream of the TATA box, and ligated first to a CMV enhancer and then to the SV40 origin of replication (p5'HIV-2). In Step 2, after cloning the 3' LTR into pBluescript to make p3'HIV-1, a 400-bp deletion in the 3' LTR enhancer/promoter was made to remove cis-regulatory elements in HIV U3 and form p3'HIV-2. In Step 3, fragments isolated from the p5'HIV-3 and p3'HIV-2 were ligated to make pHIV-3. In Step 4, the p3'HIV-2 was further modified by removing extra upstream HIV sequences to generate p3'HIV-3 and a 600-bp BamHI-SalI fragment containing WPRE was added to p3'HIV-3 to make the p3'HIV-4. In Step 5, the pHIV-3 RRE was reduced in size by PCR and ligated to a 5'

fragment from pHIV-3 (not shown) and to the p3'HIV-4, to make pHIV-6. In Step 6, a 190-bp BglII-BamHI fragment containing the cPPT DNA flap sequence from HIV-1 pNL4-3 (55) was amplified from pNL4-3 and placed between the RRE and the WPRE sequences in pHIV6 to make pHIV-7. This parent plasmid pHIV7-GFP (GFP, green fluorescent protein) was used to package the parent vector using a four-plasmid system.

[0064] A packaging signal, psi ψ, is required for efficient packaging of viral genome into the vector. The RRE and WPRE enhance the RNA transcript transport and expression of the transgene. The flap sequence, in combination with WPRE, has been demonstrated to enhance the transduction efficiency of lentiviral vector in mammalian cells.

[0065] The helper functions, required for production of the viral vector), are divided into three separate plasmids to reduce the probability of generation of replication competent lentivirus via recombination: 1) pCgp encodes the gag/pol protein required for viral vector assembly; 2) pCMV-Rev2 encodes the Rev protein, which acts on the RRE sequence to assist in the transportation of the viral genome for efficient packaging; and 3) pCMV-G encodes the glycoprotein of the vesiculo-stomatitis virus (VSV), which is required for infectivity of the viral vector.

[0066] There is minimal DNA sequence homology between the pHIV7 encoded vector genome and the helper plasmids. The regions of homology include a packaging signal region of approximately 600 nucleotides, located in the gag/pol sequence of the pCgp helper plasmid; a CMV promoter sequence in all three helper plasmids; and a RRE sequence in the helper plasmid pCgp. It is highly improbable that replication competent recombinant virus could be generated due to the homology in these regions, as it would require multiple recombination events. Additionally, any resulting recombinants would be missing the functional LTR and tat sequences required for lentiviral replication.

[0067] The CMV promoter was replaced by the EF1α-HTLV promoter (EF1p), and the new plasmid was named epHIV7 (**Figure 4**). The EF1p has 563 bp and was introduced into epHIV7 using NruI and NheI, after the CMV promoter was excised.

[0068] The lentiviral genome, excluding gag/pol and rev that are necessary for the pathogenicity of the wild-type virus and are required for productive infection of target cells, has been removed from this system. In addition, the IL13(EQ)BBZ-T2ACD19t_epHIV7 vector construct does not contain an intact 3'LTR promoter, so the resulting expressed and reverse transcribed DNA proviral genome in targeted cells will have inactive LTRs. As a result of this design, no HIV-I derived sequences will be transcribed from the provirus and only the therapeutic sequences will be expressed from their respective promoters. The removal of the LTR promoter activity in the SIN vector is expected to significantly reduce the possibility of unintentional activation of host genes (56). Table 4 summarizes the various regulator elements present in IL13(EQ)BBZ-T2ACD19t_epHIV7.

Table 4 Functional elements of IL13(EQ)41BBZ-T2A-CD19t_epHIV7

Regulatory Elements and Genes	Location (Nucleotide Numbers)	Comments
U5	87-171	5' Unique sequence
psi	233-345	Packaging signal
RRE	957-1289	Rev-responsive element
flap	1290-1466	Contains polypurine track sequence and central termination sequence to facilitate nuclear import of pre-integration complex
EF1p Promoter	1524-2067	EF1-alpha Eukaryotic Promoter sequence driving expression of CD19Rop
IL13-IgG4 (EQ)-41BB-Zeta-T2A-CD19t	2084-4753	Therapeutic insert
WPRE	4790-5390	Woodchuck hepatitis virus derived regulatory element to enhance viral RNA transportation
delU3	5405-5509	3' U3 with deletion to generate SIN vector
R	5510-5590	Repeat sequence within LTR
U5	5591-5704	3' U5 sequence in LTR
Amp^R	6540-7398	Ampicillin-resistance gene
CoE1 ori	7461-8342	Replication origin of plasmid
SV40 ori	8639-8838	Replication origin of SV40
CMV promoter	8852-9451	CMV promoter to generate viral

Table 4 Functional elements of IL13(EQ)41BBZ-T2A-CD19t_epHIV7		
Regulatory Elements and Genes	**Location (Nucleotide Numbers)**	**Comments**
		genome RNA
R	9507-86	Repeat sequence within LTR

Example 3: Production of Vectors for Transduction of Patient T Cells

[0069] For each plasmid (IL13(EQ)BBZ-T2A-CD19t_epHIV7; pCgp; pCMV-G; and pCMV-Rev2), a seed bank is generated, which is used to inoculate the fermenter to produce sufficient quantities of plasmid DNA. The plasmid DNA is tested for identity, sterility and endotoxin prior to its use in producing lentiviral vector.

[0070] Briefly, cells were expanded from the 293T working cell (WCB), which has been tested to confirm sterility and the absence of viral contamination. A vial of 293T cells from the 293T WCB was thawed. Cells were grown and expanded until sufficient numbers of cells existed to plate an appropriate number of 10 layer cell factories (CFs) for vector production and cell train maintenance. A single train of cells can be used for production.

[0071] The lentiviral vector was produced in sub-batches of up to 10 CFs. Two sub-batches can be produced in the same week leading to the production of approximately 20 L of lentiviral supernatant/week. The material produced from all sub-batches were pooled during the downstream processing phase, in order to produce one lot of product. 293T cells were plated in CFs in 293T medium (DMEM with 10% FBS). Factories were placed in a 37°C incubator and horizontally leveled in order to get an even distribution of the cells on all the layers of the CF. Two days later, cells were transfected with the four lentiviral plasmids described above using the CaPO4 method, which involves a mixture of Tris:EDTA, 2M CaCl2, 2X HBS, and the four DNA plasmids. Day 3 after transfection, the supernatant containing secreted lentiviral vectors was collected, purified and concentrated. After the supernatant was removed from the CFs, End-of-Production Cells were collected from each CF. Cells were trypsinized from each factory and collected by centrifugation. Cells were resuspended in freezing medium and

cryopreserved. These cells were later used for replication-competent lentivirus (RCL) testing.

[0072] To purify and formulate vectors crude supernatant was clarified by membrane filtration to remove the cell debris. The host cell DNA and residual plasmid DNA were degraded by endonuclease digestion (Benzonase®). The viral supernatant was clarified of cellular debris using a 0.45 μm filter. The clarified supernatant was collected into a pre-weighed container into which the Benzonase® is added (final concentration 50 U/mL). The endonuclease digestion for residual plasmid DNA and host genomic DNA as performed at 37°C for 6 h. The initial tangential flow ultrafiltration (TFF) concentration of the endonuclease-treated supernatant was used to remove residual low molecular weight components from the crude supernatant, while concentrating the virus ~20 fold. The clarified endonuclease-treated viral supernatant was circulated through a hollow fiber cartridge with a NMWCO of 500 kD at a flow rate designed to maintain the shear rate at ~4,000 sec-1 or less, while maximizing the flux rate. Diafiltration of the nuclease-treated supernatant was initiated during the concentration process to sustain the cartridge performance. An 80% permeate replacement rate was established, using 4% lactose in PBS as the diafiltration buffer. The viral supernatant was brought to the target volume, representing a 20-fold concentration of the crude supernatant, and the diafiltration was continued for 4 additional exchange volumes, with the permeate replacement rate at 100%.

[0073] Further concentration of the viral product was accomplished by using a high speed centrifugation technique. Each sub-batch of the lentivirus was pelleted using a Sorvall RC-26 plus centrifuge at 6000 RPM (6,088 RCF) at 6oC for 16-20 h. The viral pellet from each sub-batch was then reconstituted in a 50 mL volume with 4% lactose in PBS. The reconstituted pellet in this buffer represents the final formulation for the virus preparation. The entire vector concentration process resulted in a 200-fold volume reduction, approximately. Following the completion of all of the sub-batches, the material was then placed at -80oC, while samples from each sub-batch were tested for sterility. Following confirmation of sample sterility, the sub-batches were rapidly thawed at 37oC with frequent agitation. The material was then pooled and manually aliquoted in the Class

II Type A/B3 biosafety cabinet in the viral vector suite. A fill configuration of 1 mL of the concentrated lentivirus in sterile USP class 6, externally threaded O-ring cryovials was used. Center for Applied Technology Development (CATD)'s Quality Systems (QS) at COH released all materials according to the Policies and Standard Operating Procedures for the CBG and in compliance with current Good Manufacturing Practices (cGMPs).

[0074] To ensure the purity of the lentiviral vector preparation, it was tested for residual host DNA contaminants, and the transfer of residual host and plasmid DNA. Among other tests, vector identity was evaluated by RT-PCR to ensure that the correct vector is present. All release criteria were met for the vector intended for use in this study.

Example 4: Preparation of T cells Suitable for Use in ACT

[0075] T lymphocytes are obtained from a patient by leukopheresis, and the appropriate allogenic or autologous T cell subset, for example, Central Memory T cells (T_{CM}), are genetically altered to express the CAR, then administered back to the patient by any clinically acceptable means, to achieve anti-cancer therapy.

[0076] An outline of the manufacturing strategy for T_{CM} is depicted in **Figure 8** (Manufacturing schema for IL13(EQ)BBζ/CD19t+ T_{CM}). Specifically, apheresis products obtained from consented research participants are ficolled, washed and incubated overnight. Cells are then depleted of monocyte, regulatory T cell and naïve T cell populations using GMP grade anti-CD14, anti-CD25 and anti-CD45RA reagents (Miltenyi Biotec) and the CliniMACS^{TM} separation device. Following depletion, negative fraction cells are enriched for CD62L+ T_{CM} cells using DREG56-biotin (COH clinical grade) and anti-biotin microbeads (Miltenyi Biotec) on the CliniMACSTM separation device.

[0077] Following enrichment, T_{CM} cells are formulated in complete X-Vivo15 plus 50 IU/mL IL-2 and 0.5 ng/mL IL-15 and transferred to a Teflon cell culture bag, where they are stimulated with Dynal ClinEx™ Vivo CD3/CD28 beads. Up to five days after stimulation, cells are transduced with IL13(EQ)BBZ-T2A-CD19t_epHIV7 lentiviral

vector at a multiplicity of infection (MOI) of 1.0 to 0.3. Cultures are maintained for up to 42 days with addition of complete X-Vivo15 and IL-2 and IL-15 cytokine as required for cell expansion (keeping cell density between $3x10^5$ and $2x10^6$ viable cells/mL, and cytokine supplementation every Monday, Wednesday and Friday of culture). Cells typically expand to approximately 10^9 cells under these conditions within 21 days. At the end of the culture period cells are harvested, washed twice and formulated in clinical grade cryopreservation medium (Cryostore CS5, BioLife Solutions).

[0078] On the day(s) of T cell infusion, the cryopreserved and released product is thawed, washed and formulated for re-infusion. The cryopreserved vials containing the released cell product are removed from liquid nitrogen storage, thawed, cooled and washed with a PBS/2% human serum albumin (HSA) Wash Buffer. After centrifugation, the supernatant is removed and the cells resuspended in a Preservative-Free Normal Saline (PFNS)/ 2% HSA infusion diluent. Samples are removed for quality control testing.

[0079] Two qualification runs on cells procured from healthy donors were performed using the manufacturing platform described above. Each preclinical qualification run product was assigned a human donor (HD) number – HD006.5 and HD187.1. Importantly, as shown in Table 5, these qualification runs expanded >80 fold within 28 days and the expanded cells expressed the IL13(EQ)BBγ/CD19t transgenes.

Table 5: Summary of Expression Data from Pre-clinical Qualification Run Product

Cell Product	CAR	CD19	CD4+	CD8+	Fold Expansion
HD006.5	20%	22%	24%	76%	84-fold (28 days)
Hd187.1	18%	25%	37%	63%	259-fold (28 days)

Example 5: Flow cytometric analysis of surface transgene and T cell marker expression in IL13(EQ)BBγ/CD19t+T_{CM}

[0080] The two preclinical qualification run products described in Example 4 were used in pre-clinical studies to as described below. **Figures 6A-C** depict the results of flow cytometric analysis of surface transgene and T cell marker expression. IL13(EQ)BBγ/CD19t+ T_{CM} HD006.5 and HD187.1 were co-stained with anti-IL13-PE and anti-CD8-FITC to detect CD8+ CAR+ and CD4+ (i.e., CD8 negative) CAR+ cells (**Figure 6A**), or anti-CD19-PE and anti-CD4-FITC to detect CD4+ CD19t+ and CD8+ (i.e., CD4 negative) CAR+ cells (**Figure 6B**). IL13(EQ)BBγ/CD19t+ T_{CM} HD006.5 and HD187.1 were stained with fluorochrome-conjugated anti-CD3, TCR, CD4, CD8, CD62L and CD28 (grey histograms) or isotype controls (black histograms). (**Figure 6C**). In each of **Figures 6A-C**, the percentages indicated are based on viable lymphocytes (DAPI negative) stained above isotype.

Example 6: Effector Activity of IL13(EQ)BBγ/CD19t+ T_{CM}

[0081] The effector activity of IL13(EQ)BBζ/CD19t+ T_{CM} was assessed and the results of this analysis are depicted in **Figures 7A-B**. Briefly, IL13(EQ)BBγ/CD19t+ T_{CM} HD006.5 and HD187.1 were used as effectors in a 6-hour 51Cr-release assay using a 10E:1T ratio based on CD19t expression. The IL13Rα2-positive tumor targets were K562 engineered to express IL13Rα2 (K562-IL13Rα2) and primary glioma line PBT030-2, and the IL13Rα2-negative tumor target control was the K562 parental line (**Figure 7A**). IL13(EQ)BBγ/CD19t+ HD006.5 and HD187.1 were evaluated for antigen-dependent cytokine production following overnight co-culture at a 10E:1T ratio with the same IL13Rα2-positive and negative targets as described in above. Cytokine levels were measured using the Bio-Plex Pro Human Cytokine TH1/TH2 Assay kit and INF-γ levels are depicted (**Figure 7B**).

Example 7: *In vivo* Anti-tumor Activity of IL13(EQ)BBγ/CD19t+ T_{CM}

[0082] The studies described below demonstrate that IL13(EQ)BBγ/CD19t+ T_{CM} exhibit anti-tumor efficacy in *in vivo* mouse models. Specifically, we have evaluated the anti-tumor potency of IL13(EQ)BBγ/CD19t+ T_{CM} against the IL13Rα2+ primary low-passage glioblastoma tumor sphere line PBT030-2, which has been engineered to express both EGFP and firefly luciferase (ffLuc) reporter genes (PBT030-2 EGFP:ffLuc) (**6**). A panel

of primary lines (PBT) from patient glioblastoma specimens grown as tumor spheres (TSs) in serum-free media. These expanded TS lines exhibit stem cell-like characteristics, including expression of stem cell markers, multilineage differentiation and capacity to initiate orthotopic tumors in immunocompromised mice (NSG) at low cell numbers. The PBT030-2 EGFP:ffLuc TS-initiated xenograft model ($0.1x10^6$ cells; 5 day engraftment) has been previously used to evaluate in vivo anti-tumor activity in NSG mice of IL13Rα2-specific CAR expressing T cells, whereby three injections of $2x10^6$ cytolytic T lymphocytes (CTLs) over a course of 2 weeks were shown to reduce tumor growth. However, in those experiments the majority of the PBT030-2 tumors eventually recurred. By comparison, a single injection of IL13(EQ)BBγ/CD19t+ T_{CM} ($1.1x10^6$ CAR+ T_{CM}; $2x10^6$ total TCM) exhibited robust anti-tumor activity against PBT030-2 EGFP:ffLuc TS-initiated tumors ($0.1x10^6$ cells; 5 day engraftment) as shown in **Figures 8A-C**. As compared to NSG mice treated with either PBS or mock transduced T_{CM} (no CAR), IL13(EQ)BBγ/CD19t+ T_{CM} significantly reduce ffLuc flux ($p < 0.001$ at >18-days) and significantly improve survival ($p = 0.0008$).

[0083] Briefly, EGFP-ffLuc+ PBT030-2 tumor cells (1×10^5) were stereotactically implanted into the right forebrain of NSG mice. On day 5, mice received either $2x10^6$ IL13(EQ)BBγ/CD19t+ T_{CM} (1.1x106 CAR+; n=6), $2x10^6$ mock T_{CM} (no CAR; n=6) or PBS (n=6). **Figure 8A** depicts representative mice from each group showing relative tumor burden using Xenogen Living Image. Quantification of ffLuc flux (photons/sec) shows that IL13(EQ)BBζ/CD19t+ T_{CM} induce tumor regression as compared to mock-transduced T_{CM} and PBS (#$p<0.02$, *$p<0.001$, repeated measures ANOVA) (**Figure 8B**). As shown in **Figure 8C**, a Kaplan Meier survival curve (n=6 per group) demonstrates significantly improved survival ($p=0.0008$; log-rank test) for mice treated with IL13(EQ)BBγ/CD19t+ T_{CM}.

Example 8: Comparison of IL13(EQ)BBζ+ Tcm and Non-Tcm IL13-zetakine CD8+ CTL Clones in Antitumor Efficacy and T cell Persistence

[0084] The studies described below compare IL13(EQ)BBζ+ Tcm and a previously created IL13Rα2-specific human CD8+ CTLs (IL13-zetakine CD8+ CTL (described in

Brown et al. 2012 *Clin Cancer Res* 18:2199 and Kahlon et al. 2004 *Cancer Res* 64:9160). The IL13-zetakine uses a CD3ζ stimulatory domain, lacks a co-stimulatory domain and uses the same IL13 variant as IL13(EQ)BBζ+.

[0085] A panel of primary lines (PBT) from patient glioblastoma specimens grown as tumor spheres (TSs) in serum-free media was generated (Brown et al. 2012 *Clin Cancer Res* 18:2199; Brown et al. 2009 *Cancer Res* 69:8886). These expanded TS lines exhibit stem cell-like characteristics, including expression of stem cell markers, multi-lineage differentiation and capacity to initiate orthotopic tumors in immunocompromised mice (NSG) at low cell numbers. The IL13Rα2+ primary low-passage glioblastoma TS line PBT030-2, which has been engineered to express both EGFP and firefly luciferase (ffLuc) reporter genes (PBT030-2 EGFP:ffLuc) (Brown et al. 2012 *Clin Cancer Res* 18:2199) was used for the experiments outlined below.

[0086] First, a single dose ($1x10^6$ CAR T cells) of IL13(EQ)BBζ+ Tcm product was compared to IL13-zetakine CD8+ CTL clones evaluated against day 8 PBT030-2 EGFP:ffuc TS-initiated xenografts ($0.1x10^6$ cells). While both IL13Rα2-specific CAR T cells (IL13-zetakine CTL and IL13(EQ)BBζ Tcm) demonstrated antitumor activity against established PBT030-2 tumors as compared to untreated and mock Tcm (CAR-negative) controls (**Figures 9A** and **9B**), IL13(EQ)BBZ+ Tcm mediated significantly improved survival and durable tumor remission with mice living >150 days as compared to our first-generation IL13-zetakine CD8+ CTL clones (**Figure 9C**).

[0087] To further compare the therapeutic effectiveness of these two IL13Rα2-CAR T cell products, a dose titration of 1.0, 0.3 and $0.1x10^6$ CAR T cells against day 8 PBT030-2 EGFP:ffuc TS-initiated tumors was performed (**Figures 10A-C**). The highest dose ($1x10^6$) of IL13-zetakine CD8+ CTL cl. 2D7 mediated antitumor responses as measured by Xenogen flux in 3 of 6 animals (**Figure 10C**), but no significant antitumor responses were observed at lower CAR T cell doses. By comparison, injection of IL13(EQ)BBζ+ Tcm product mediated complete tumor regression in the majority of mice at all dose levels, including treatment with as few as $0.1x10^6$ CAR T cells. These data demonstrate that IL13(EQ)BBζ+ Tcm is at least 10-fold more potent than IL13-zetakine CD8+ CTL

clones in antitumor efficacy. The improved anti-tumor efficacy of is due to improved T cell persistence in the tumor microenvironment. Evaluation of CD3+ T cells 7-days post i.c. injection revealed significant numbers of IL13(EQ)BBζ+ Tcm in the tumor microenvironment, whereas very few first-generation IL13-zeta CTLs were present (**Figure 11**).

Example 9: Comparison of CAR T cell delivery route for treatment of large TS-initiated PBT tumors

[0088] Described below are studies that compare the route of delivery, intraveneous (i.v.) or intracranial (i.c.), on antitumor activity against invasive primary PBT lines. In pilot studies (data not shown), it was unexpectedly observed that i.v. administered IL13(EQ)BBζ+ Tcm provided no therapeutic benefit as compared to PBS for the treatment of small (day 5) PBT030-2 EGFP:ffLuc tumors. This is in contrast to the robust therapeutic efficacy observed with i.c. administered CAR+ T cells. Reasoning that day 5 PBT030-2 tumors may have been too small to recruit therapeutic T cells from the periphery, a comparison was made of i.v. versus i.c. delivery against larger day 19 PBT030-2 EGFP:ffLuc tumors. For these studies, PBT030-2 engrafted mice were treated with either two i.v. infusions (5×10^6 CAR+ Tcm; days 19 and 26) or four i.c. infusions (1×10^6 CAR+ Tcm; days 19, 22, 26 and 29) of IL13(EQ)BBZ+ Tcm, or mock Tcm (no CAR). Here too no therapeutic benefit as monitored by Xenogen imaging or Kaplan-Meier survival analysis for i.v. administered CAR+ T cells (**Figures 12A** and **12B**). In contrast, potent antitumor activity was observed for i.c. administered IL13(EQ)BBζ+ Tcm (**Figures 12A-B**). Next, brains from a cohort of mice 7 days post T cell injection were harvested and evaluated for CD3+ human T cells by IHC. Surprisingly, for mice treated i.v. with either mock Tcm or IL13(EQ)BBζ Tcm there were no detectable CD3+ human T cells in the tumor or in others mouse brain regions where human T cells typically reside (i.e. the leptomeninges) (**Figure 12C**), suggesting a deficit in tumor tropism. This is in contrast to the significant number of T cells detected in the i.c. treated mice (**Figure 12D**).

[0089] Tumor derived cytokines, particularly MCP-1/CCL2, are important in recruiting T cells to the tumor. Thus, PBT030-2 tumor cells were evaluated and it was found that this line produces high levels of MCP-1/CCL2 comparable to U251T cells (data not shown), a glioma line previously shown to attract i.v. administered effector CD8+ T cells to i.c. engrafted tumors. Malignant gliomas are highly invasive tumors and are often multi-focal in presentation. The studies described above establish that IL13BBZ T_{CM} can eliminate infiltrated tumors such as PBT030-2, and mediate long-term durable antitumor activity. The capacity of intracranially delivered CAR T cells to traffic to multifocal disease was also examined. For this study PBT030-2 EGFP:ffLuc TSs were implanted in both the left and right hemispheres (**Figure 13A**) and CAR+ T cells were injected only at the right tumor site. Encouragingly, for all mice evaluated (n=3) we detected T cells by CD3 IHC 7-days post T cell infusion both at the site of injection (i.e. right tumor), as well within the tumor on the left hemisphere (**Figure 13B**). These findings provide evidence that CAR+ T cells are able to traffic to and infiltrate tumor foci at distant sites. Similar findings were also observed in a second tumor model using the U251T glioma cell line (data not shown).

Example 10: Comparison of Costimulatory Domains

[0090] A series of studies were conducted to evaluate various costimulatory domains. The various CAR evaluated are depicted schematically in **Figure 14A** and included a first generation CD3ζ CAR lacking a costimulatory domain, two second generation CARs incorporating either a 4-1BB costimulatory domain or a CD28 costimulatory domain, and a third generation CAR containing both a CD28 costimulatory domain and 41BB costimulatory domain. All CAR constructs also contain the T2A ribosomal skip sequence and a truncated CD19 (CD19t) sequence as a marker for transduced cells.

[0091] CD4 and CD8 T_{CM} were lentivirally transduced and CAR-expressing T cells were immunomagnetically enriched via anti-CD19. CD19 and IL13 (i.e., CAR) expression levels as measured by flow cytometry. The results are shown in **Figure 14B**. Stability of each CAR construct was determined by dividing the CAR (IL13) mean flourescence intenstity (MFI) by that of the transduction marker (CD19t) (**Figure 14C**).

The two CAR including a 4-1BB costimulatory domain exhibited the lowest expression levels as compared to the CD19t transduction marker.

[0092] The ability of the indicated mock-transduced or CAR-expressing T cells to kill IL13Rα2-expressing PBT030-2 tumor cell targets was determined in a 4-hour ^{51}Cr-release assay at the indicated effector:target ratios. The results of this study are in **Figure 15A** (mean % chromium release ± S.D. of triplicate wells are depicted). As expected, mock-transduced T cells did not efficiently lyse the targets. In contrast, all CAR-expressing T cells lysed the tumor cells in a similar manner. **Figure 15B** depicts the results of a study in which the indicated mock-transduced or CAR-expressing T cells were co-cultured overnight with IL13Rα2-expressing PBT030-2 tumor cells at a 10:1 ratio and supernatants were analyzed for IL-13 and IFN-γ levels by cytometric bead array. Interestingly, T cells expressing the zeta, 41BB-zeta or CD28-41BB-zeta CARs exhibited lower antigen-stimulated cytokine production than T cells expressing the CD28-zeta CAR.

[0093] The in vivo efficacy of the various CAR was examined as follows. Briefly, NSG mice received an intracranial injection of ffLuc+ PBT030-2 tumor cells on day 0, and were randomized into 6 groups (n = 9-10 mice per group) for i.c. treatment with either PBS (Tumor Only), mock-transduced T cells or T cells expressing the indicated IL13Rα2-specific CAR on day 8. Quantitative bioluminescence imaging was then carried out to monitor tumor growth over time. Bioluminescence images for representative mice in each group (**Figure 16A**). Flux levels for each mouse at Day 27 (**Figure 16B**). All groups treated with IL13Rα2-specific CAR T cells, except those treated with T cells expressing the CD28-CAR, show statistically-significant reduction in tumor volume compared to mice treated with mock-transduced T cells (**Figure 16C**).

Example 11: Amino acid Sequence of IL13(EQ)BBζ/CD19t

[0094] The complete amino acid sequence of IL13(EQ)BBζ/CD19t is depicted in **Figure 17**. The entire sequence (SEQ ID NO:1) includes: a 22 amino acid GMCSF signal peptide (SEQ ID NO:2), a 112 amino acid IL-13 sequence (SEQ ID NO:3; amino acid substitution E13Y shown in bold); a 229 amino acid IgG4 sequence (SEQ ID NO:4; with

amino acid substitutions L235E and N297Q shown in bold); a 22 amino acid CD4 transmembrane sequence (SEQ ID NO:5); a 42 amino acid 4-1BB sequence (SEQ ID NO:6); a 3 amino acid Gly linker; a 112 amino acid CD3ζ sequence (SEQ ID NO:7); a 24 amino acid T2A sequence (SEQ ID NO:8); and a 323 amino acid CD19t sequence (SEQ ID NO:9).

[0095] The mature chimeric antigen receptor sequence (SEQ ID NO:10) includes: a 112 amino acid IL-13 sequence (SEQ ID NO:3; amino acid substitution E13Y shown in bold); a 229 amino acid IgG4 sequence (SEQ ID NO:4; with amino acid substitutions L235E and N297Q shown in bold); at 22 amino acid CD4 sequence (SEQ ID NO:5); a 42 amino acid 4-1BB sequence (SEQ ID NO:6); a 3 amino acid Gly linker; and a 112 amino acid CD3ζ sequence (SEQ ID NO:7). Within this CAR sequence (SEQ ID NO:10) is the IL-13/IgG4/CD4t/41-BB sequence (SEQ ID NO:11), which includes: a 112 amino acid IL-13 sequence (SEQ ID NO:3; amino acid substitution E13Y shown in bold); a 229 amino acid IgG4 sequence (SEQ ID NO:4; with amino acid substitutions L235E and N297Q shown in bold); at 22 amino acid CD4 sequence (SEQ ID NO:5); and a 42 amino acid 4-1BB sequence (SEQ ID NO:6). The IL13/IgG4/CD4t/4-1BB sequence (SEQ ID NO:11) can be joined to the 112 amino acid CD3ζ sequence (SEQ ID NO:7) by a linker such as a Gly Gly Gly linker. The CAR sequence (SEQ ID NO:10) can be preceded by a 22 amino acid GMCSF signal peptide (SEQ ID NO:2).

[0096] **Figure 18** depicts a comparison of the sequences of IL13(EQ)41BBζ[IL13{EQ}41BBζ T2A-CD19t_epHIV7; pF02630] (SEQ ID NO:12) and CD19Rop_epHIV7 (pJ01683) (SEQ ID NO:13).

Example 12: Amino acid Sequence of IL13(EQ)BBζ/CD19t

[0097] **Figures 19-26** depict the amino acid sequences of additional CAR directed against IL13Rα2 in each case the various domains are labelled except for the GlyGlyGly spacer located between certain intracellular domains. Each includes human IL13 with and Glu to Tyr (SEQ ID NO:3; amino acid substitution E13Y shown in highlighted). In the expression vector used to express these CAR, the amino acid sequence expressed can include a 24 amino acid T2A sequence (SEQ ID NO:8); and a 323 amino acid CD19t

sequence (SEQ ID NO:9) to permit coordinated expression of a truncated CD19 sequence on the surface of CAR-expressing cells.

[0098] A panel of CAR comprising human IL13(E13Y) domain, a CD28 tm domain, a CD28gg costimulatory domain, a 4-1BB costimulatory domain, and a CD3ζ domain CAR backbone and including either a HL (22 amino acids) spacer, a CD8 hinge (48 amino acids) spacer, IgG4-HL-CH3 (129 amino acids) spacer or a IgG4(EQ) (229 amino acids) spacer were tested for their ability to mediate IL13Ra2-specific killing as evaluated in a 72-hour co-culture assay. With the exception of HL (22 amino acids) which appeared to have poor CAR expression in this system, all were active.

WHAT IS CLAIMED IS:

1. A nucleic acid molecule encoding a chimeric antigen receptor, wherein the chimeric antigen receptor comprises: human IL-13 or a variant thereof having 1-10 amino acid modifications; a transmembrane domain selected from: a CD4 transmembrane domain or variant thereof having 1-10 amino acid modifications, a CD8 transmembrane domain or variant thereof having 1-10 amino acid modifications, a CD28 transmembrane domain or a variant thereof having 1-10 amino acid modifications, and a CD3ζ transmembrane domain or a variant thereof having 1-10 amino acid modifications; a costimulatory domain; and CD3 ζ signaling domain of a variant thereof having 1-10 amino acid modifications.

2. The nucleic acid molecule of claim 1 wherein the costimulatory domain is selected from the group consisting of: a CD28 costimulatory domain or a variant thereof having 1-10 amino acid modifications, a 4IBB costimulatory domain or a variant thereof having 1-10 amino acid modifications and an OX40 costimulatory domain or a variant thereof having 1-10 amino acid modifications.

3. The nucleic acid molecule of claim 1 comprising a variant of a human IL13 having 1-10 amino acid modification that increase binding specificity for IL13Rα2 versus IL13Rα1.

4. The nucleic acid molecule of claim 1 wherein the human IL-13 or variant thereof is an IL-13 variant comprising the amino acid sequence of SEQ ID NO:3 with 1 to 5 amino acid modifications, provided that the amino acid at position 11 of SEQ ID NO:3 other than E.

5. The nucleic acid molecule of claim 2 wherein the chimeric antigen receptor comprises two different costimulatory domains selected from the group consisting of: a CD28 costimulatory domain or a variant thereof having 1-10 amino acid modifications, a 4IBB costimulatory domain or a variant thereof having 1-10 amino acid modifications and an OX40 costimulatory domain or a variant thereof having 1-10 amino acid modifications.

6. The nucleic acid molecule of claim 5 wherein the chimeric antigen receptor comprises two different costimulatory domains selected from the group consisting of: a CD28 costimulatory domain or a variant thereof having 1-2 amino acid modifications, a 4IBB costimulatory domain or a variant thereof having 1-2 amino acid modifications and an OX40 costimulatory domain or a variant thereof having 1-2 amino acid modifications.

7. The nucleic acid molecule of claim 1 wherein the chimeric antigen receptor comprises: human IL-13 or a variant thereof having 1-2 amino acid modifications; a transmembrane domain selected from: a CD4 transmembrane domain or variant thereof having 1-2 amino acid modifications, a CD8 transmembrane domain or variant thereof having 1-2 amino acid modifications, a CD28 transmembrane domain or a variant thereof having 1-2 amino acid modifications, and a CD3ζ transmembrane domain or a variant thereof having 1-2 amino acid modifications; a costimulatory domain; and CD3 ζ signaling domain of a variant thereof having 1-2 amino acid modifications.

8. The nucleic acid molecule of claim 1 comprising a spacer region located between the IL-13 or variant thereof and the transmembrane domain.

9. The nucleic acid molecule of claim 6 wherein the spacer region comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 4, 14-20, 50 and 521.

10. The nucleic acid molecule of claim 6 wherein the spacer comprises an IgG hinge region.

11. The nucleic acid molecule of claim 6 wherein the spacer comprises 10-150 amino acids.

12. The nucleic acid molecule of claim 2 wherein the 4-1BB signaling domain comprises the amino acid sequence of SEQ ID NO:6.

13. The nucleic acid molecule of claim 1 wherein the CD3ζ signaling domain comprises the amino acid sequence of SEQ ID NO:7.

14. The nucleic acid molecule of claim 1 wherein a linker of 3 to 15 amino acids is located between the costimulatory domain and the CD3 ζ signaling domain or variant thereof.

15. The nucleic acid molecule of claim 1 wherein the nucleic acid molecule expresses a polypeptide comprising an amino acid sequence selected from SEQ ID NOs: 10, 31-48 and 52.

16. The nucleic acid molecule of claim 1 wherein the chimeric antigen receptor comprises a IL-13/IgG4/CD4t/41-BB region comprising the amino acid of SEQ ID NO:11 and a CD3 ζ signaling domain comprising the amino acid sequence of SEQ ID NO:7.

17. The nucleic acid molecule of claim 14 wherein the chimeric antigen receptor comprises the amino acid sequence of SEQ ID NOs: 10, 31-48 and 52.

18. A population of human T cells transduced by a vector comprising an expression cassette encoding a chimeric antigen receptor, wherein chimeric antigen receptor comprises: human IL-13 or a variant thereof having 1-10 amino acid modifications; a transmembrane domain selected from: a CD4 transmembrane domain or variant thereof having 1-10 amino acid modifications, a CD8 transmembrane domain or variant thereof having 1-10 amino acid modifications, a CD28 transmembrane domain or a variant thereof having 1-10 amino acid modifications, and a CD3ζ transmembrane domain or a variant thereof having 1-10 amino acid modifications; a costimulatory domain; and CD3 ζ signaling domain of a variant thereof having 1-10 amino acid modifications.

19. A population of human T cells comprising a vector expressing a chimeric antigen receptor comprising an amino acid sequence selected from SEQ ID NOs: 10, 31-48 and 52.

20. The population of human T cells of claim 16 wherein the T cells are comprised of a population of central memory T cells.

21. A method of treating cancer in a patient comprising administering a population of autologous or allogeneic human T cells transduced by a vector comprising an expression cassette encoding a chimeric antigen receptor, wherein chimeric antigen receptor comprises an amino acid sequence selected from SEQ ID NOs: 10, 31-48 and 52.

22. The method of claim 19 wherein the population of human T cells comprise central memory T cells.

23. The method claim 19 wherein the cancer is glioblastoma.

24. The method of claim 20 wherein the transduced human T cells where prepared by a method comprising obtaining T cells from the patient, treating the T cells to isolate central memory T cells, and transducing at least a portion of the central memory cells to with a viral vector comprising an expression cassette encoding a chimeric antigen receptor, wherein chimeric antigen receptor comprises an amino acid sequence selected from SEQ ID NOs: 10, 31-48 and 52.

25. A nucleic acid molecule encoding an polypeptide comprising an amino acid sequence that is at least 95% identical to an amino acid sequence selected from SEQ ID NO:10 and SEQ ID NOs: 10, 31-48 and 52.

26. A nucleic acid molecule encoding an polypeptide comprising an amino acid sequence that is identical to an amino acid sequence selected from SEQ ID NO: 10, 31-48 and 52 except for the presence of no more than 5 amino acid substitutions, deletions or insertions.

27. A nucleic acid molecule encoding an polypeptide comprising an amino acid sequence that is identical to an amino acid sequence selected from SEQ ID NO:10 and SEQ ID NOs: 10, 31-48 and 52 except for the presence of no more than 5 amino acid substitutions.

28. A nucleic acid molecule encoding an polypeptide comprising an amino acid sequence that is identical to an amino acid sequence selected from SEQ ID NO:10 and

SEQ ID NOs: 10, 31-48 and 52 except for the presence of no more than 2 amino acid substitutions.

FIGURE 1

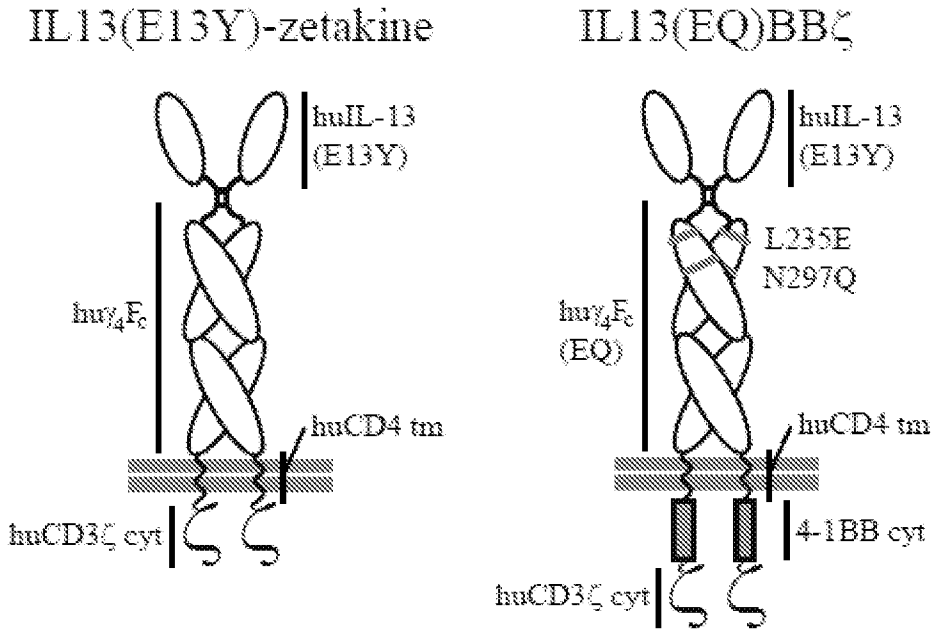

IL13(E13Y)-zetakine
huIL-13
(E13Y)
huγ4Fc
huCD4 tm
huCD3ζ cyt
IL13(EQ)BBζ
huIL-13
(E13Y)
L235E
N297Q
huγ4Fc
(EQ)
huCD4 tm
4-1BB cyt
huCD3ζ cyt

FIGURE 2

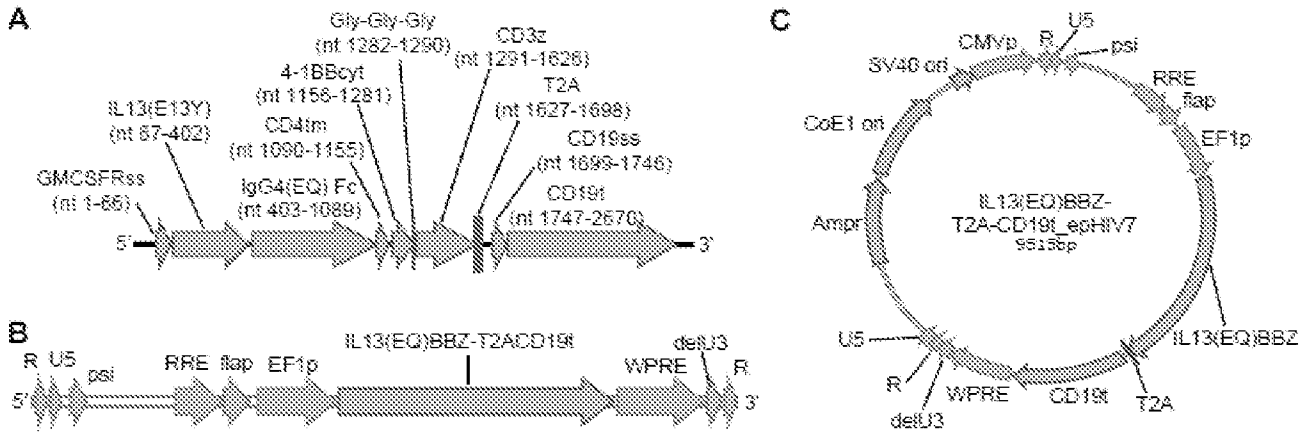

A
Gly-Gly-Gly
(nt 1282-1290)
CD3z
(nt 1291-1626)
4-1BBcyt
(nt 1156-1281)
T2A
(nt 1627-1698)
IL13(E13Y)
(nt 67-402)
CD4tm
(nt 1090-1155)
CD19ss
(nt 1699-1746)
GMCSFRss
(nt 1-66)
IgG4(EQ) Fc
(nt 403-1089)
CD19t
(nt 1747-2670)
5'
3'
B
R U5
psi
RRE
flap
EF1p
IL13(EQ)BBZ-T2ACD19t
WPRE
delU3
R
5'
3'
C
CMVp
R
U5
psi
SV40 ori
RRE
flap
CoE1 ori
EF1p
Ampr
IL13(EQ)BBZ-
T2A-CD19t_epHIV7
9513bp
U5
IL13(EQ)BBZ
R
WPRE
CD19t
T2A
delU3

FIGURE 3

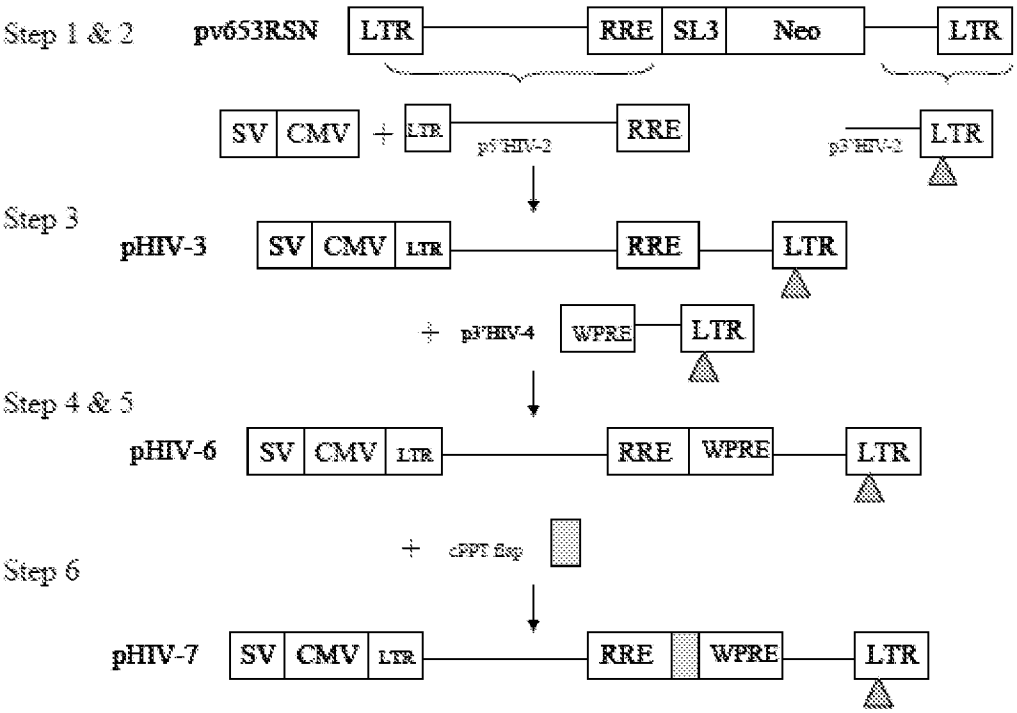

Step 1 & 2
pv653RSN
LTR
RRE
SL3
Neo
LTR
SV
CMV
LTR
RRE
LTR
Step 3
pHIV-3
SV
CMV
LTR
RRE
LTR
WPRE
LTR
Step 4 & 5
pHIV-6
SV
CMV
LTR
RRE
WPRE
LTR
Step 6
pHIV-7
SV
CMV
LTR
RRE
WPRE
LTR

FIGURE 4

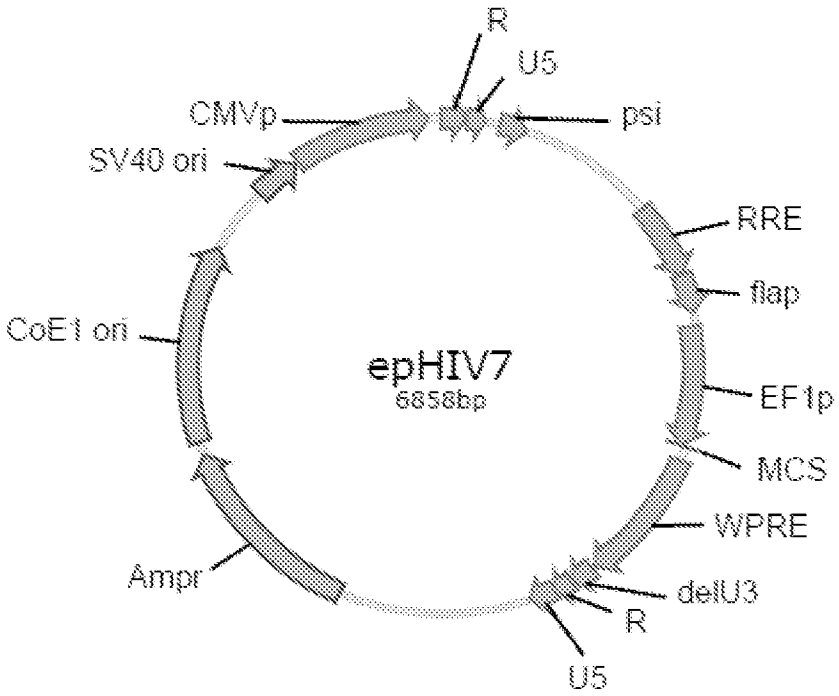

R
U5
CMVp
psi
SV40 ori
RRE
flap
CoE1 ori
epHIV7
6858bp
EF1p
MCS
WPRE
Ampr
delU3
R
U5

FIGURE 5

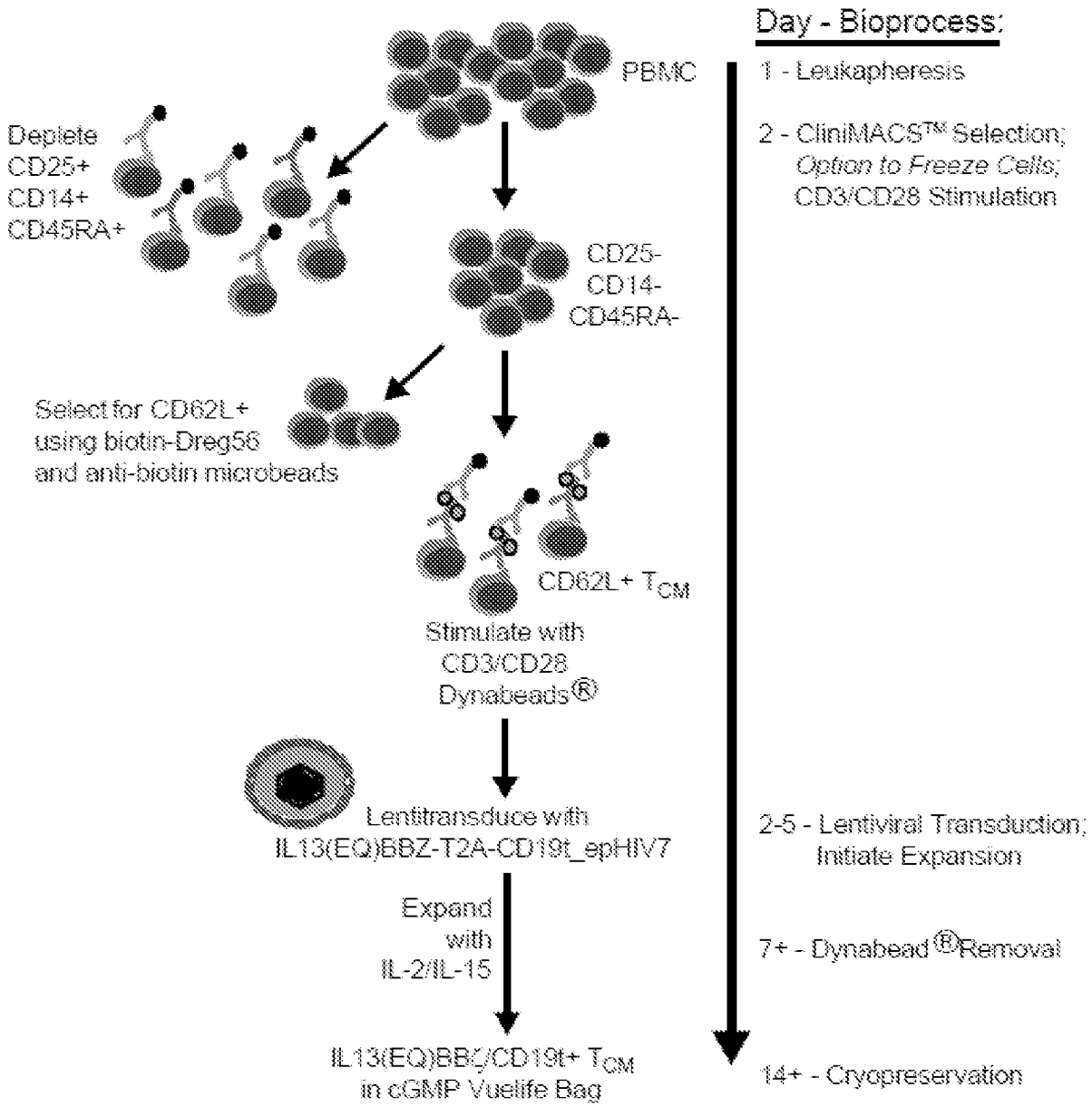

Day - Bioprocess:
PBMC
1 - Leukapheresis
Deplete
CD25+
CD14+
CD45RA+
2 - CliniMACS™ Selection;
Option to Freeze Cells;
CD3/CD28 Stimulation
CD25-
CD14-
CD45RA-
Select for CD62L+
using biotin-Dreg56
and anti-biotin microbeads
CD62L+ TCM
Stimulate with
CD3/CD28
Dynabeads®
Lentitransduce with
IL13(EQ)BBZ-T2A-CD19t_epHIV7
2-5 - Lentiviral Transduction;
Initiate Expansion
Expand
with
IL-2/IL-15
7+ - Dynabead® Removal
IL13(EQ)BBζ/CD19t+ TCM
in cGMP Vuelife Bag
14+ - Cryopreservation

FIGURE 6

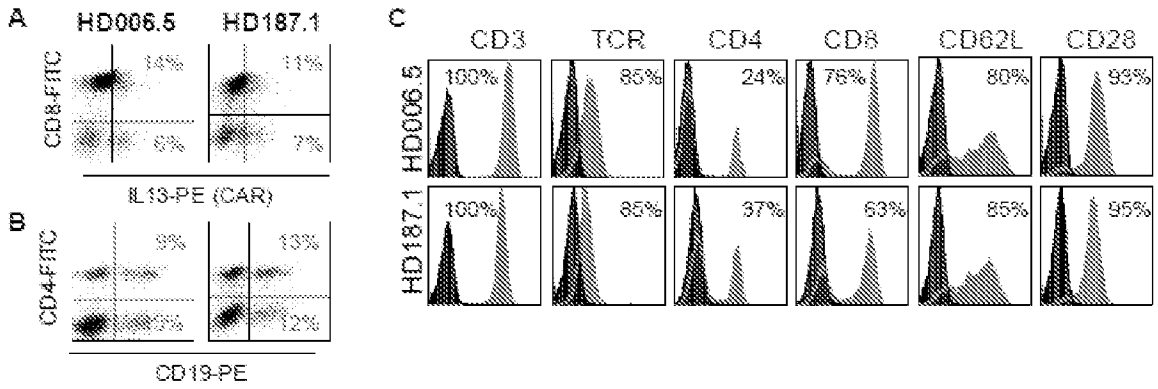
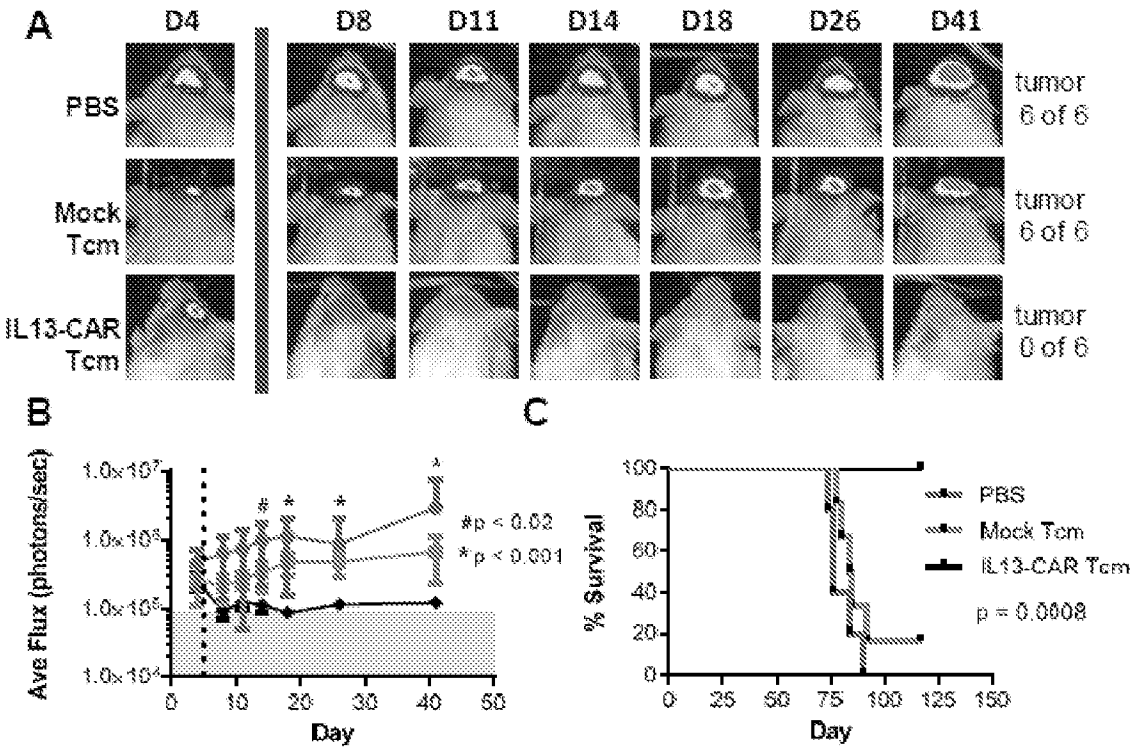

A
HD006.5
HD187.1
CD8-FITC
14%
6%
11%
7%
IL13-PE (CAR)
B
CD4-FITC
9%
13%
12%
CD19-PE
C
CD3
TCR
CD4
CD8
CD62L
CD28
HD006.5
100%
85%
24%
76%
80%
93%
HD187.1
100%
85%
37%
63%
85%
95%

FIGURE 7


A
> 9x
> 3x
% Specific Lysis
40
30
20
10
0
HD006.5
HD187.1
K562
K562-IL13Rα2
PBT030-2
B
4000
3000
2000
1000
0
INF-γ (pg/ml)
HD006.5
HD187.1

FIGURE 8


A
D4
D8
D11
D14
D18
D26
D41
PBS
Mock Tcm
IL13-CAR Tcm
tumor 6 of 6
tumor 6 of 6
tumor 0 of 6
B
Ave Flux (photons/sec)
1.0x10^7
1.0x10^6
1.0x10^5
1.0x10^4
0
10
20
30
40
50
Day
#p < 0.02
*p < 0.001
C
% Survival
100
80
60
40
20
0
0
25
50
75
100
125
150
Day
PBS
Mock Tcm
IL13-CAR Tcm
p = 0.0008

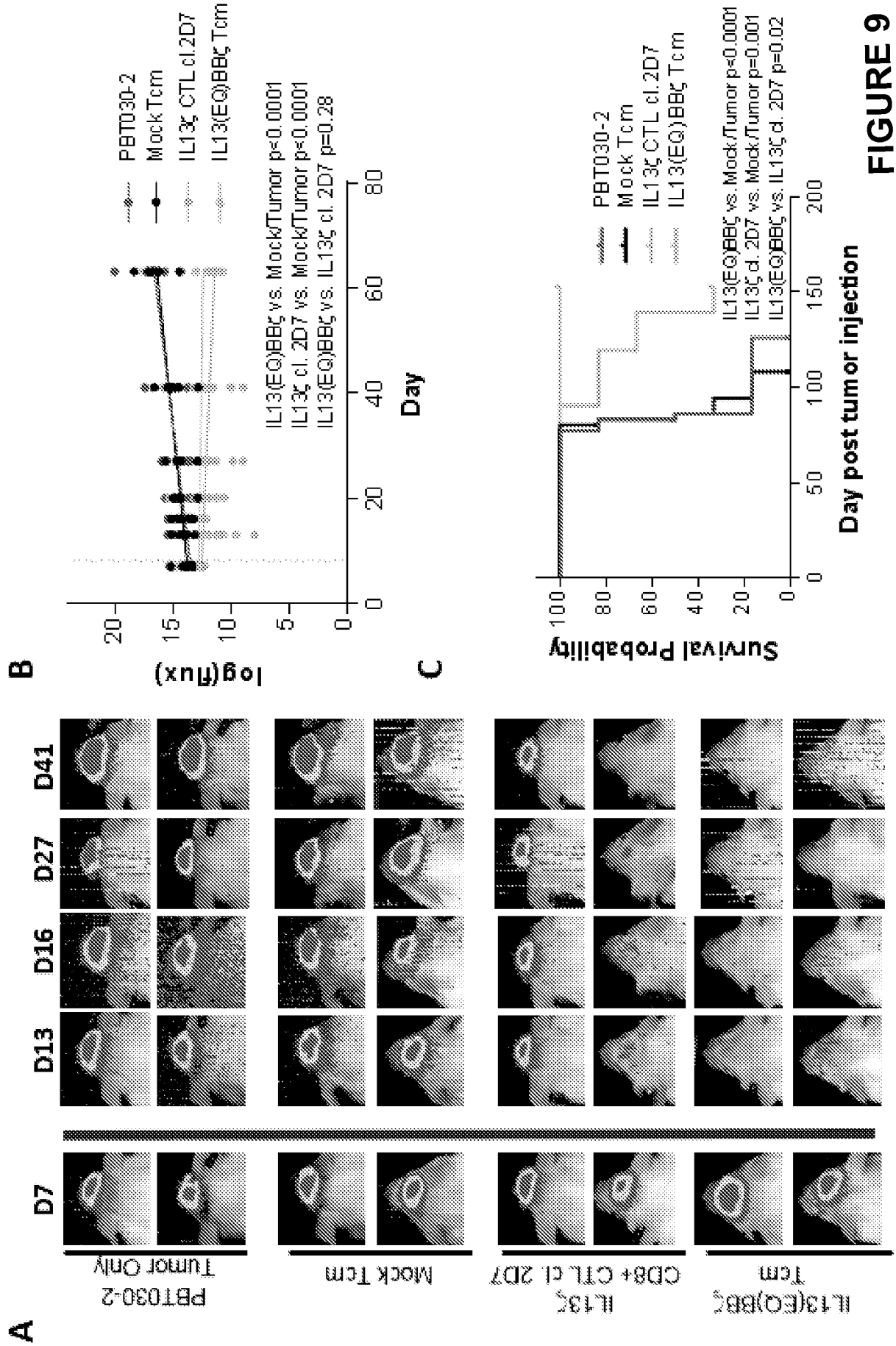

A
D7
D13
D16
D27
D41
PBT030-2 Tumor Only
Mock Tcm
CD8+ CTL cl 2D7
IL13ζ
IL13(EQ)BBζ Tcm
B
log(Flux)
PBT030-2
Mock Tcm
IL13ζ CTL cl.2D7
IL13(EQ)BBζ Tcm
IL13(EQ)BBζ vs. Mock/Tumor p<0.0001
IL13ζ cl. 2D7 vs. Mock/Tumor p<0.0001
IL13(EQ)BBζ vs. IL13ζ cl. 2D7 p=0.28
Day
C
Survival Probability
Day post tumor injection
IL13(EQ)BBζ vs. Mock/Tumor p<0.0001
IL13ζ cl. 2D7 vs. Mock/Tumor p=0.001
IL13(EQ)BBζ vs. IL13ζ cl. 2D7 p=0.02

FIGURE 9

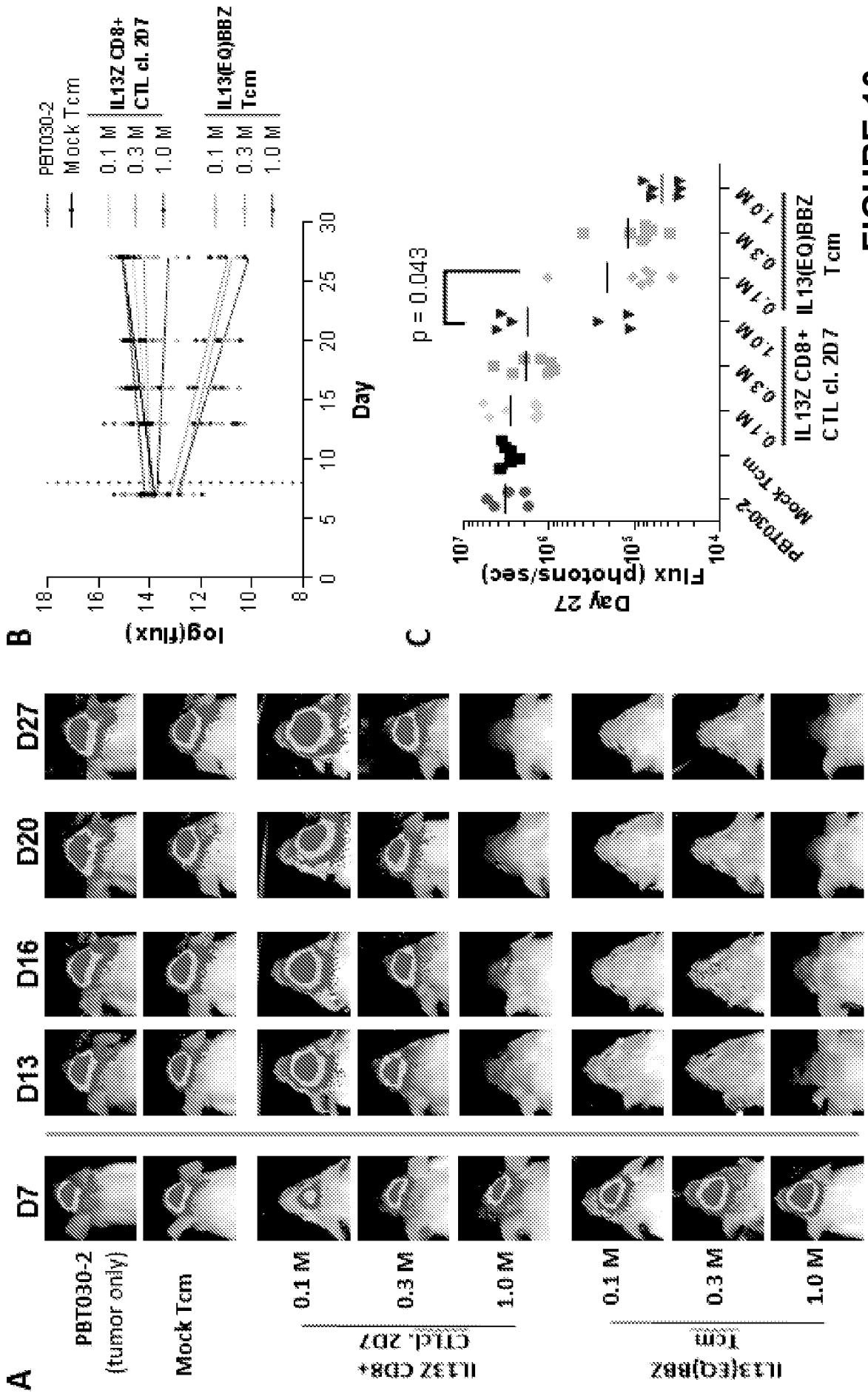

A
D7
D13
D16
D20
D27
PBT030-2 (tumor only)
Mock Tcm
0.1 M
0.3 M
1.0 M
IL13Z CD8+ CTLd. 2D7
IL13(EQ)BBZ Tcm
B
PBT030-2
Mock Tcm
0.1 M
0.3 M
1.0 M
IL13Z CD8+ CTL cl. 2D7
IL13(EQ)BBZ Tcm
log(flux)
Day
C
p = 0.043
Day 27
Flux (photons/sec)
PBT030-2
Mock Tcm
0.1M
0.3M
1.0M
IL13Z CD8+ CTL cl. 2D7
IL13(EQ)BBZ Tcm

FIGURE 10

FIGURE 11

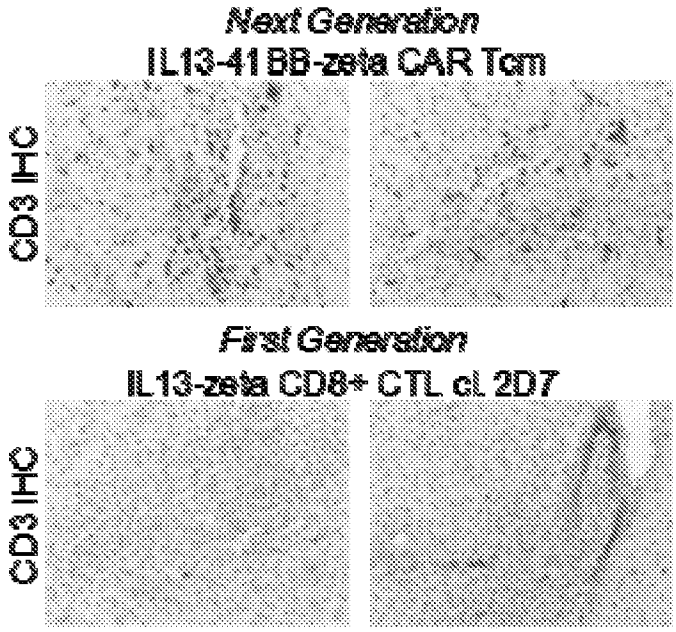
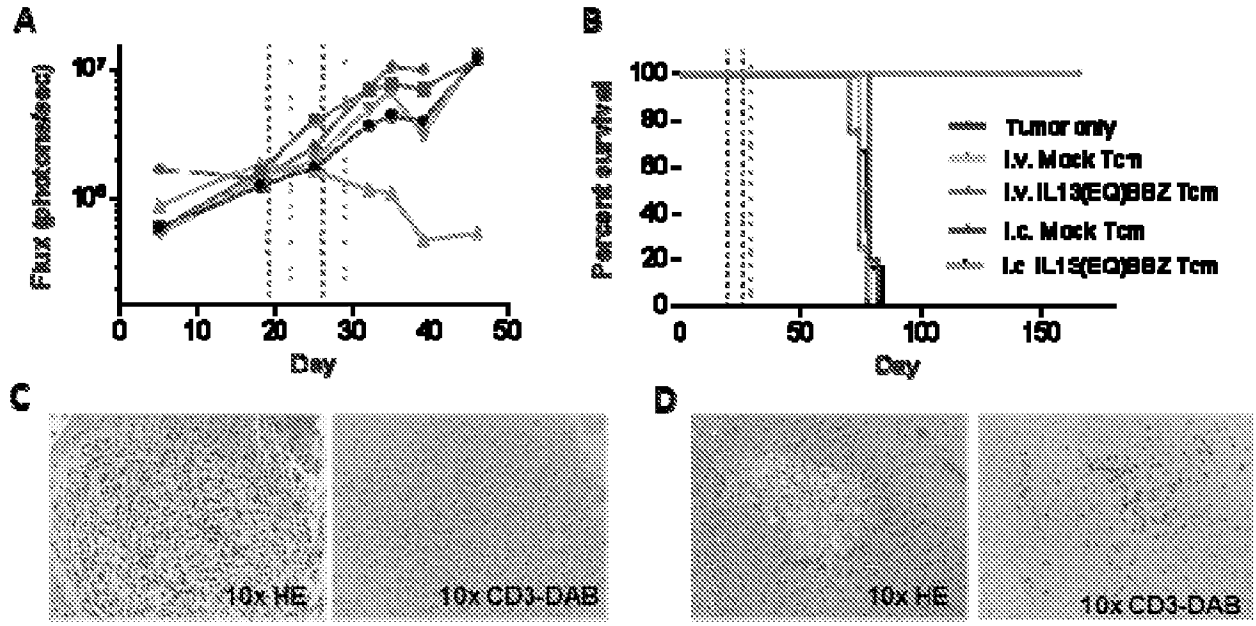

Next Generation
IL13-41BB-zeta CAR Tcm
CD3 IHC
First Generation
IL13-zeta CD8+ CTL cl 2D7
CD3 IHC

FIGURE 12


A
Flux (photons/sec)
10^7
10^6
0
10
20
30
40
50
Day
B
Percent survival
100
80
60
40
20
0
0
50
100
150
Day
Tumor only
I.v. Mock Tcm
I.v. IL13(EQ)BBZ Tcm
I.c. Mock Tcm
I.c IL13(EQ)BBZ Tcm
C
10x HE
10x CD3-DAB
D
10x HE
10x CD3-DAB

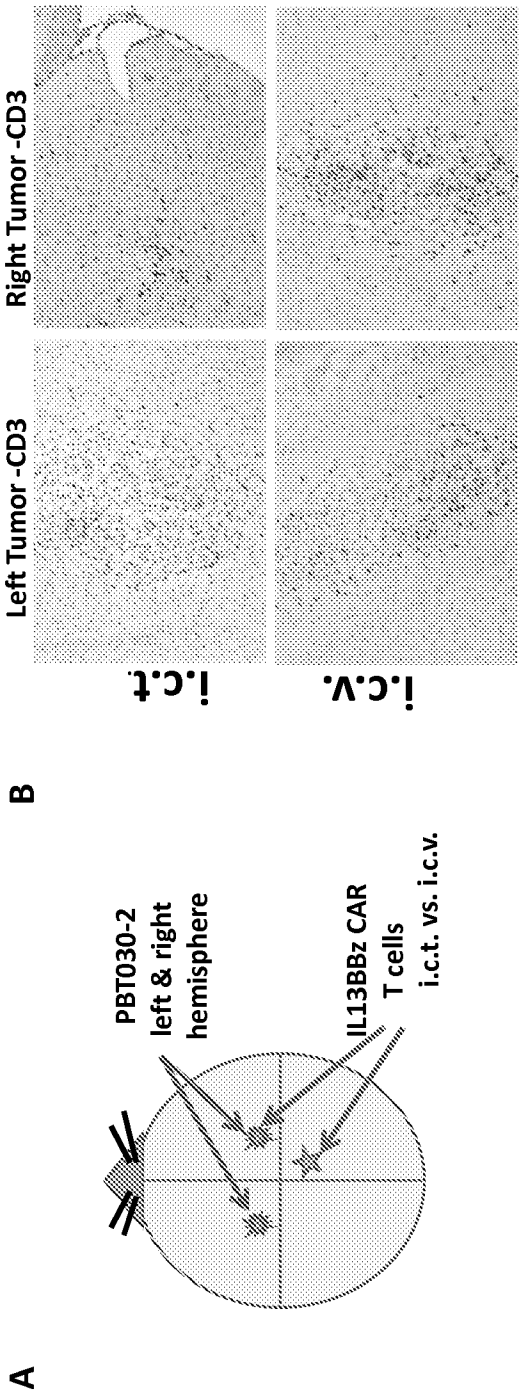

A
PBT030-2
left & right
hemisphere
IL13BBz CAR
T cells
i.c.t. vs. i.c.v.
B
Left Tumor -CD3
Right Tumor -CD3
i.c.t.
i.c.v.

FIGURE 13

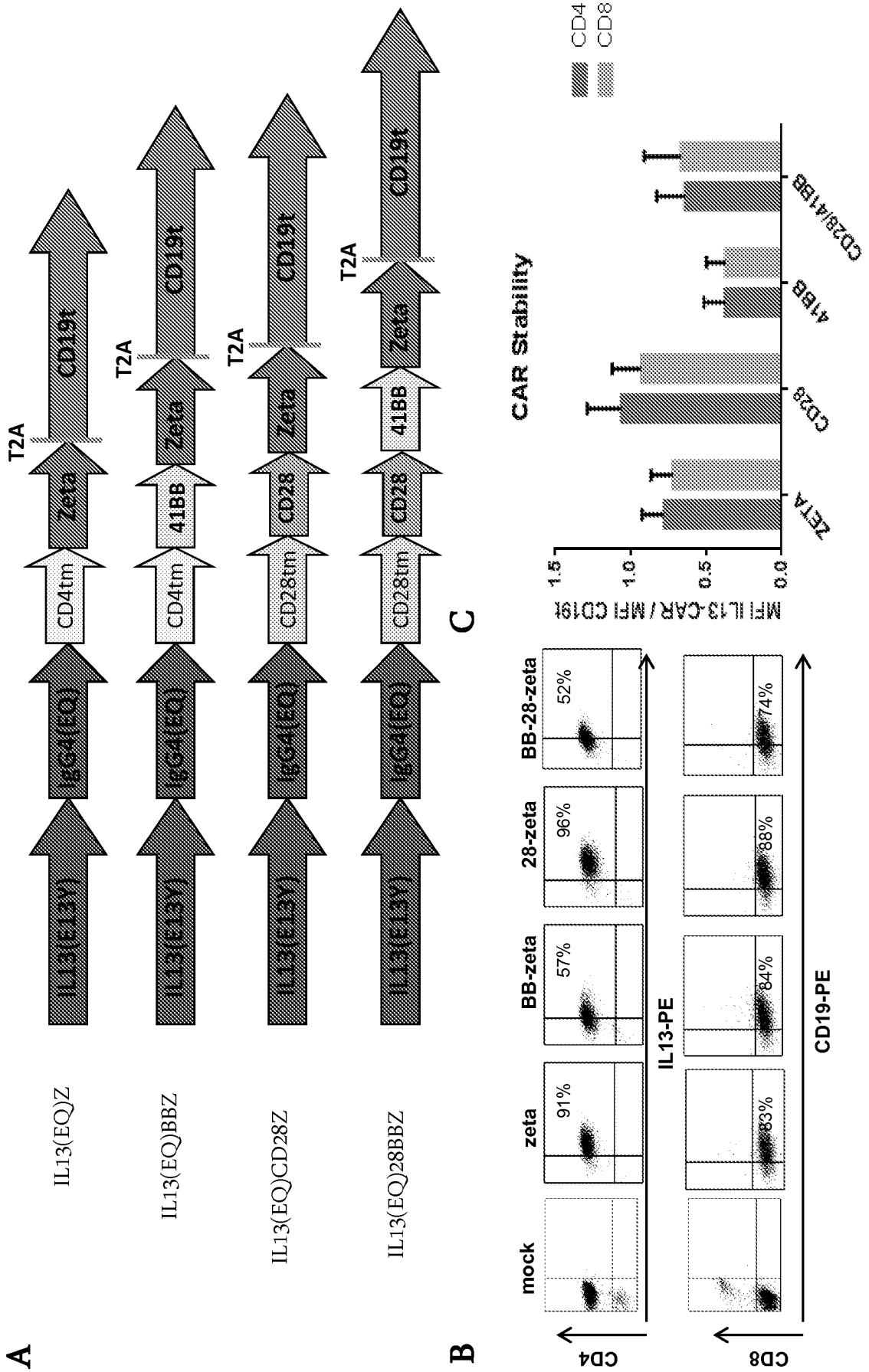

A
IL13(EQ)Z
IL13(EQ)BBZ
IL13(EQ)CD28Z
IL13(EQ)28BBZ
IL13(E13Y)
IgG4(EQ)
CD4tm
CD28tm
41BB
CD28
Zeta
T2A
CD19t
B
mock
zeta
BB-zeta
28-zeta
BB-28-zeta
91%
57%
96%
52%
83%
84%
88%
74%
IL13-PE
CD19-PE
CD4
CD8
C
CAR Stability
CD4
CD8
MFI IL13-CAR / MFI CD19t
1.5
1.0
0.5
0.0
ZETA
CD28
4-1BB
CD28/4-1BB

FIGURE 14

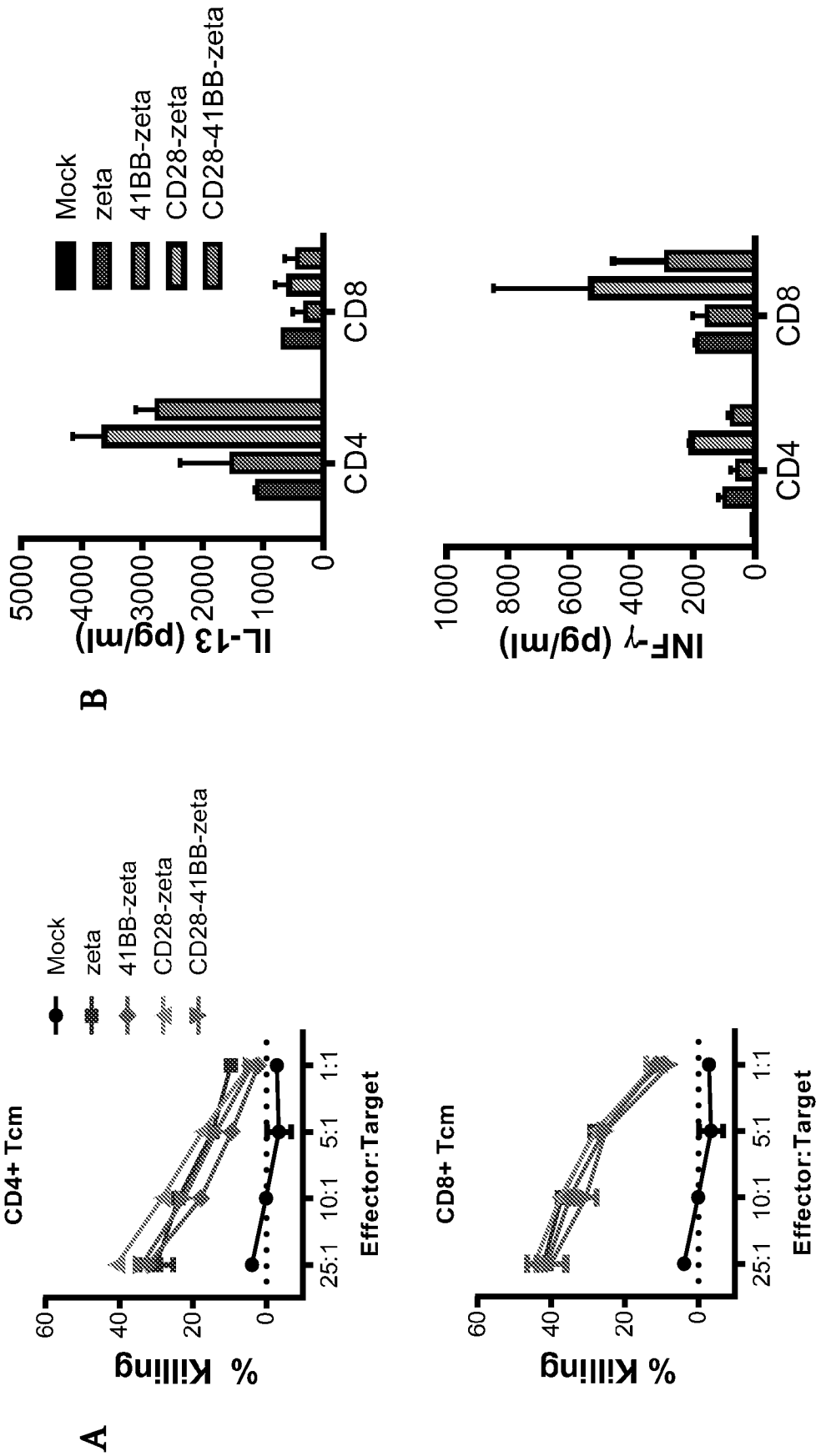

A
CD4+ Tcm
% Killing
Effector:Target
25:1
10:1
5:1
1:1
Mock
zeta
41BB-zeta
CD28-zeta
CD28-41BB-zeta
CD8+ Tcm
B
IL-13 (pg/ml)
INF-γ (pg/ml)
CD4
CD8

FIGURE 15

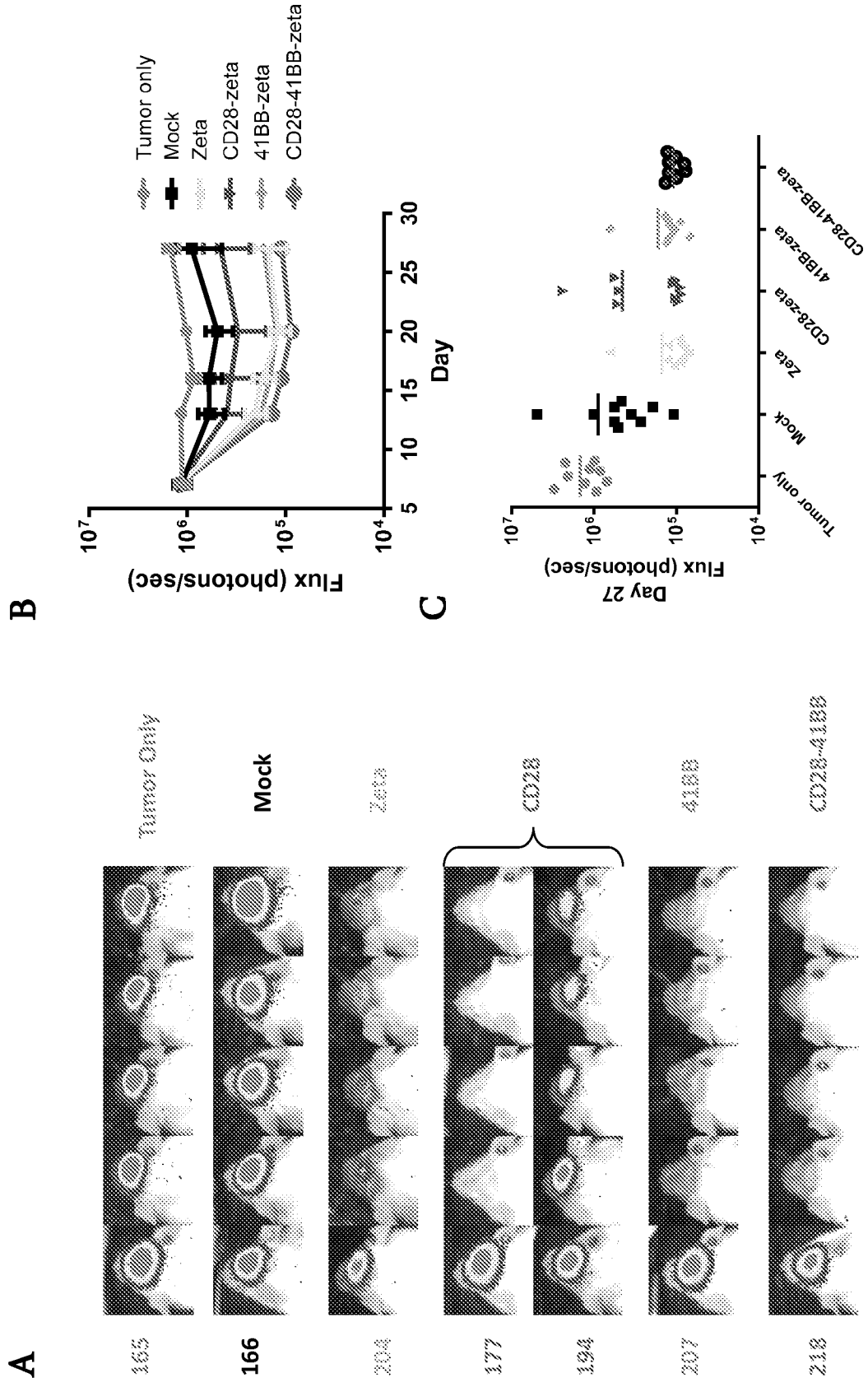

A
Tumor Only
Mock
Zeta
CD28
41BB
CD28-41BB
165
166
204
177
194
207
218
B
Tumor only
Mock
Zeta
CD28-zeta
41BB-zeta
CD28-41BB-zeta
Flux (photons/sec)
Day
C
Day 27
Flux (photons/sec)
Tumor only
Mock
Zeta
CD28-zeta
41BB-zeta
CD28-41BB-zeta

FIGURE 16

FIGURE 17

MLLLVTSLLLCELPHPAFLLIPGPVPPSTALRYLIEELVNITQNQKAPLCNGSMVWSINLTAGM

GMCSFRa signal peptide (22 aa) IL13 (112 aa)

YCAALESLINVSGCSAIEKTQRMLSGFCPHKVSAGQFSSLHVRDTKIEVAQFVKDLLLHLKKLF

REGRFNESKYGPPCPPCPAPEF**E**GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQF

IgG4(L235E, N297Q in bold) (229 aa)

NWYVDGVEVHNAKTKPREEQF**Q**STYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTIS

KAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVL

DSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGKMALIVLGGVAGLL

CD4tm (22 aa)

LFIGLGIFFKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELGGGRVKFSRSADA

41BB (42 aa) Gly3 Zeta (112 aa)

PAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAE

AYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPRLEGGGEGRGSLLTCGDV

T2A (24 aa)

EENPGPRMPPPRLLFFLLFLTPMEVRPEEPLVVKVEEGDNAVLQCLKGTSDGPTQQLTWSRE

CD19t (323 aa)

SPLKPFLKLSLGLPGLGIHMRPLAIWLFIFNVSQQMGGFYLCQPGPPSEKAWQPGWTVNVE

GSGELFRWNVSDLGGLGCGLKNRSSEGPSSPSGKLMSPKLYVWAKDRPEIWEGEPPCVPPR

DSLNQSLSQDLTMAPGSTLWLSCGVPPDSVSRGPLSWTHVHPKGPKSLLSLELKDDRPARD

MWVMETGLLLPRATAQDAGKYYCHRGNLTMSFHLEITARPVLWHWLLRTGGWKVSAVTL

AYLIFCLCSLVGILHLQRALVLRRKR

FIGURE 18

Yellow highlighting indicates the IL-13 optimized codon region including the GMCSF signal sequence (IL13op).
highlighting indicates the IgG4 optimized codon region (IgG4op[L235E, N297Q]).
highlighting indicates the two anticipated amino acid changes within the IgG4 hinge region(L235E and N297Q).
highlighting indicates the CD4 transmembrane optimized codon region.
highlighting indicates the 41BB cytoplasmic signaling region (41BB cyto).
highlighting indicates the 3 glycine linkers (g3).
Gray Highlighting indicates the CD3 zeta optimized codon region (zeta op).
highlighting indicates the T2A sequence (T2A).
highlighting Indicates the truncated CD19 sequence (CD19t).

```
                          1                                                  50
IL13(EQ)41BBZeta      (1) GTTAGACCAGATCTGAGCCTGGGAGCTCTCTGGCTAACTAGGGAACCCAC
 CD19Rop_epHIV7       (1) GTTAGACCAGATCTGAGCCTGGGAGCTCTCTGGCTAACTAGGGAACCCAC
       Consensus      (1) GTTAGACCAGATCTGAGCCTGGGAGCTCTCTGGCTAACTAGGGAACCCAC
                          51                                                 100
IL13(EQ)41BBZeta     (51) TGCTTAAGCCTCAATAAAGCTTGCCTTGAGTGCTTCAAGTAGTGTGTGCC
 CD19Rop_epHIV7      (51) TGCTTAAGCCTCAATAAAGCTTGCCTTGAGTGCTTCAAGTAGTGTGTGCC
       Consensus     (51) TGCTTAAGCCTCAATAAAGCTTGCCTTGAGTGCTTCAAGTAGTGTGTGCC
                         101                                                 150
IL13(EQ)41BBZeta    (101) CGTCTGTTGTGTGACTCTGGTAACTAGAGATCCCTCAGACCCTTTTAGTC
 CD19Rop_epHIV7     (101) CGTCTGTTGTGTGACTCTGGTAACTAGAGATCCCTCAGACCCTTTTAGTC
       Consensus    (101) CGTCTGTTGTGTGACTCTGGTAACTAGAGATCCCTCAGACCCTTTTAGTC
                         151                                                 200
IL13(EQ)41BBZeta    (151) AGTGTGGAAAATCTCTAGCAGTGGCGCCCGAACAGGGACTTGAAAGCGAA
 CD19Rop_epHIV7     (151) AGTGTGGAAAATCTCTAGCAGTGGCGCCCGAACAGGGACTTGAAAGCGAA
       Consensus    (151) AGTGTGGAAAATCTCTAGCAGTGGCGCCCGAACAGGGACTTGAAAGCGAA
                         201                                                 250
IL13(EQ)41BBZeta    (201) AGGGAAACCAGAGGAGCTCTCTCGACGCAGGACTCGGCTTGCTGAAGCGC
 CD19Rop_epHIV7     (201) AGGGAAACCAGAGGAGCTCTCTCGACGCAGGACTCGGCTTGCTGAAGCGC
       Consensus    (201) AGGGAAACCAGAGGAGCTCTCTCGACGCAGGACTCGGCTTGCTGAAGCGC
                         251                                                 300
IL13(EQ)41BBZeta    (251) GCACGGCAAGAGGCGAGGGGCGGCGACTGGTGAGTACGCCAAAAATTTTG
 CD19Rop_epHIV7     (251) GCACGGCAAGAGGCGAGGGGCGGCGACTGGTGAGTACGCCAAAAATTTTG
       Consensus    (251) GCACGGCAAGAGGCGAGGGGCGGCGACTGGTGAGTACGCCAAAAATTTTG
                         301                                                 350
IL13(EQ)41BBZeta    (301) ACTAGCGGAGGCTAGAAGGAGAGAGATGGGTGCGAGAGCGTCAGTATTAA
 CD19Rop_epHIV7     (301) ACTAGCGGAGGCTAGAAGGAGAGAGATGGGTGCGAGAGCGTCAGTATTAA
       Consensus    (301) ACTAGCGGAGGCTAGAAGGAGAGAGATGGGTGCGAGAGCGTCAGTATTAA
                         351                                                 400
IL13(EQ)41BBZeta    (351) GCGGGGGAGAATTAGATCGATGGGAAAAAATTCGGTTAAGGCCAGGGGGA
 CD19Rop_epHIV7     (351) GCGGGGGAGAATTAGATCGATGGGAAAAAATTCGGTTAAGGCCAGGGGGA
       Consensus    (351) GCGGGGGAGAATTAGATCGATGGGAAAAAATTCGGTTAAGGCCAGGGGGA
                         401                                                 450
IL13(EQ)41BBZeta    (401) AAGAAAAAATATAAATTAAAACATATAGTATGGGCAAGCAGGGAGCTAGA
 CD19Rop_epHIV7     (401) AAGAAAAAATATAAATTAAAACATATAGTATGGGCAAGCAGGGAGCTAGA
       Consensus    (401) AAGAAAAAATATAAATTAAAACATATAGTATGGGCAAGCAGGGAGCTAGA
                         451                                                 500
```

```
IL13(EQ)41BBZeta   (451) ACGATTCGCAGTTAATCCTGGCCTGTTAGAAACATCAGAAGGCTGTAGAC
 CD19Rop_epHIV7    (451) ACGATTCGCAGTTAATCCTGGCCTGTTAGAAACATCAGAAGGCTGTAGAC
       Consensus   (451) ACGATTCGCAGTTAATCCTGGCCTGTTAGAAACATCAGAAGGCTGTAGAC
                         501                                             550
IL13(EQ)41BBZeta   (501) AAATACTGGGACAGCTACAACCATCCCTTCAGACAGGATCAGAAGAACTT
 CD19Rop_epHIV7    (501) AAATACTGGGACAGCTACAACCATCCCTTCAGACAGGATCAGAAGAACTT
       Consensus   (501) AAATACTGGGACAGCTACAACCATCCCTTCAGACAGGATCAGAAGAACTT
                         551                                             600
IL13(EQ)41BBZeta   (551) AGATCATTATATAATACAGTAGCAACCCTCTATTGTGTGCATCAAAGGAT
 CD19Rop_epHIV7    (551) AGATCATTATATAATACAGTAGCAACCCTCTATTGTGTGCATCAAAGGAT
       Consensus   (551) AGATCATTATATAATACAGTAGCAACCCTCTATTGTGTGCATCAAAGGAT
                         601                                             650
IL13(EQ)41BBZeta   (601) AGAGATAAAAGACACCAAGGAAGCTTTAGACAAGATAGAGGAAGAGCAAA
 CD19Rop_epHIV7    (601) AGAGATAAAAGACACCAAGGAAGCTTTAGACAAGATAGAGGAAGAGCAAA
       Consensus   (601) AGAGATAAAAGACACCAAGGAAGCTTTAGACAAGATAGAGGAAGAGCAAA
                         651                                             700
IL13(EQ)41BBZeta   (651) ACAAAAGTAAGAAAAAAGCACAGCAAGCAGCAGCTGACACAGGACACAGC
 CD19Rop_epHIV7    (651) ACAAAAGTAAGAAAAAAGCACAGCAAGCAGCAGCTGACACAGGACACAGC
       Consensus   (651) ACAAAAGTAAGAAAAAAGCACAGCAAGCAGCAGCTGACACAGGACACAGC
                         701                                             750
IL13(EQ)41BBZeta   (701) AATCAGGTCAGCCAAAATTACCCTATAGTGCAGAACATCCAGGGGCAAAT
 CD19Rop_epHIV7    (701) AATCAGGTCAGCCAAAATTACCCTATAGTGCAGAACATCCAGGGGCAAAT
       Consensus   (701) AATCAGGTCAGCCAAAATTACCCTATAGTGCAGAACATCCAGGGGCAAAT
                         751                                             800
IL13(EQ)41BBZeta   (751) GGTACATCAGGCCATATCACCTAGAACTTTAAATGCATGGGTAAAAGTAG
 CD19Rop_epHIV7    (751) GGTACATCAGGCCATATCACCTAGAACTTTAAATGCATGGGTAAAAGTAG
       Consensus   (751) GGTACATCAGGCCATATCACCTAGAACTTTAAATGCATGGGTAAAAGTAG
                         801                                             850
IL13(EQ)41BBZeta   (801) TAGAAGAGAAGGCTTTCAGCCCAGAAGTGATACCCATGTTTTCAGCATTA
 CD19Rop_epHIV7    (801) TAGAAGAGAAGGCTTTCAGCCCAGAAGTGATACCCATGTTTTCAGCATTA
       Consensus   (801) TAGAAGAGAAGGCTTTCAGCCCAGAAGTGATACCCATGTTTTCAGCATTA
                         851                                             900
IL13(EQ)41BBZeta   (851) TCAGAAGGAGCCACCCCACAAGATTTAAACACCATGCTAAACACAGTGGG
 CD19Rop_epHIV7    (851) TCAGAAGGAGCCACCCCACAAGATTTAAACACCATGCTAAACACAGTGGG
       Consensus   (851) TCAGAAGGAGCCACCCCACAAGATTTAAACACCATGCTAAACACAGTGGG
                         901                                             950
IL13(EQ)41BBZeta   (901) GGGACATCAAGCAGCCATGCAAATGTTAAAAGAGACCATCAATGAGGAAG
 CD19Rop_epHIV7    (901) GGGACATCAAGCAGCCATGCAAATGTTAAAAGAGACCATCAATGAGGAAG
       Consensus   (901) GGGACATCAAGCAGCCATGCAAATGTTAAAAGAGACCATCAATGAGGAAG
                         951                                            1000
IL13(EQ)41BBZeta   (951) CTGCAGGCAAAGAGAAGAGTGGTGCAGAGAGAAAAAAGAGCAGTGGGAAT
 CD19Rop_epHIV7    (951) CTGCAGGCAAAGAGAAGAGTGGTGCAGAGAGAAAAAAGAGCAGTGGGAAT
       Consensus   (951) CTGCAGGCAAAGAGAAGAGTGGTGCAGAGAGAAAAAAGAGCAGTGGGAAT
                        1001                                            1050
IL13(EQ)41BBZeta  (1001) AGGAGCTTTGTTCCTTGGGTTCTTGGGAGCAGCAGGAAGCACTATGGGCG
 CD19Rop_epHIV7   (1001) AGGAGCTTTGTTCCTTGGGTTCTTGGGAGCAGCAGGAAGCACTATGGGCG
       Consensus  (1001) AGGAGCTTTGTTCCTTGGGTTCTTGGGAGCAGCAGGAAGCACTATGGGCG
                        1051                                            1100
IL13(EQ)41BBZeta  (1051) CAGCGTCAATGACGCTGACGGTACAGGCCAGACAATTATTGTCTGGTATA
 CD19Rop_epHIV7   (1051) CAGCGTCAATGACGCTGACGGTACAGGCCAGACAATTATTGTCTGGTATA
       Consensus  (1051) CAGCGTCAATGACGCTGACGGTACAGGCCAGACAATTATTGTCTGGTATA
                        1101                                            1150
IL13(EQ)41BBZeta  (1101) GTGCAGCAGCAGAACAATTTGCTGAGGGCTATTGAGGCGCAACAGCATCT
 CD19Rop_epHIV7   (1101) GTGCAGCAGCAGAACAATTTGCTGAGGGCTATTGAGGCGCAACAGCATCT
       Consensus  (1101) GTGCAGCAGCAGAACAATTTGCTGAGGGCTATTGAGGCGCAACAGCATCT
```

```
                          1151                                          1200
IL13(EQ)41BBZeta   (1151) GTTGCAACTCACAGTCTGGGGCATCAAGCAGCTCCAGGCAAGAATCCTGG
 CD19Rop_epHIV7    (1151) GTTGCAACTCACAGTCTGGGGCATCAAGCAGCTCCAGGCAAGAATCCTGG
       Consensus   (1151) GTTGCAACTCACAGTCTGGGGCATCAAGCAGCTCCAGGCAAGAATCCTGG
                          1201                                          1250
IL13(EQ)41BBZeta   (1201) CTGTGGAAAGATACCTAAAGGATCAACAGCTCCTGGGGATTTGGGGTTGC
 CD19Rop_epHIV7    (1201) CTGTGGAAAGATACCTAAAGGATCAACAGCTCCTGGGGATTTGGGGTTGC
       Consensus   (1201) CTGTGGAAAGATACCTAAAGGATCAACAGCTCCTGGGGATTTGGGGTTGC
                          1251                                          1300
IL13(EQ)41BBZeta   (1251) TCTGGAAAACTCATTTGCACCACTGCTGTGCCTTGGATCTACAAATGGCA
 CD19Rop_epHIV7    (1251) TCTGGAAAACTCATTTGCACCACTGCTGTGCCTTGGATCTACAAATGGCA
       Consensus   (1251) TCTGGAAAACTCATTTGCACCACTGCTGTGCCTTGGATCTACAAATGGCA
                          1301                                          1350
IL13(EQ)41BBZeta   (1301) GTATTCATCCACAATTTTAAAAGAAAAGGGGGGATTGGGGGGTACAGTGC
 CD19Rop_epHIV7    (1301) GTATTCATCCACAATTTTAAAAGAAAAGGGGGGATTGGGGGGTACAGTGC
       Consensus   (1301) GTATTCATCCACAATTTTAAAAGAAAAGGGGGGATTGGGGGGTACAGTGC
                          1351                                          1400
IL13(EQ)41BBZeta   (1351) AGGGGAAAGAATAGTAGACATAATAGCAACAGACATACAAACTAAAGAAT
 CD19Rop_epHIV7    (1351) AGGGGAAAGAATAGTAGACATAATAGCAACAGACATACAAACTAAAGAAT
       Consensus   (1351) AGGGGAAAGAATAGTAGACATAATAGCAACAGACATACAAACTAAAGAAT
                          1401                                          1450
IL13(EQ)41BBZeta   (1401) TACAAAAACAAATTACAAAAATTCAAAATTTTCGGGTTTATTACAGGGAC
 CD19Rop_epHIV7    (1401) TACAAAAACAAATTACAAAAATTCAAAATTTTCGGGTTTATTACAGGGAC
       Consensus   (1401) TACAAAAACAAATTACAAAAATTCAAAATTTTCGGGTTTATTACAGGGAC
                          1451                                          1500
IL13(EQ)41BBZeta   (1451) AGCAGAGATCCAGTTTGGGGATCAATTGCATGAAGAATCTGCTTAGGGTT
 CD19Rop_epHIV7    (1451) AGCAGAGATCCAGTTTGGGGATCAATTGCATGAAGAATCTGCTTAGGGTT
       Consensus   (1451) AGCAGAGATCCAGTTTGGGGATCAATTGCATGAAGAATCTGCTTAGGGTT
                          1501                                          1550
IL13(EQ)41BBZeta   (1501) AGGCGTTTTGCGCTGCTTCGCGAGGATCTGCGATCGCTCCGGTGCCCGTC
 CD19Rop_epHIV7    (1501) AGGCGTTTTGCGCTGCTTCGCGAGGATCTGCGATCGCTCCGGTGCCCGTC
       Consensus   (1501) AGGCGTTTTGCGCTGCTTCGCGAGGATCTGCGATCGCTCCGGTGCCCGTC
                          1551                                          1600
IL13(EQ)41BBZeta   (1551) AGTGGGCAGAGCGCACATCGCCCACAGTCCCCGAGAAGTTGGGGGGAGGG
 CD19Rop_epHIV7    (1551) AGTGGGCAGAGCGCACATCGCCCACAGTCCCCGAGAAGTTGGGGGGAGGG
       Consensus   (1551) AGTGGGCAGAGCGCACATCGCCCACAGTCCCCGAGAAGTTGGGGGGAGGG
                          1601                                          1650
IL13(EQ)41BBZeta   (1601) GTCGGCAATTGAACCGGTGCCTAGAGAAGGTGGCGCGGGGTAAACTGGGA
 CD19Rop_epHIV7    (1601) GTCGGCAATTGAACCGGTGCCTAGAGAAGGTGGCGCGGGGTAAACTGGGA
       Consensus   (1601) GTCGGCAATTGAACCGGTGCCTAGAGAAGGTGGCGCGGGGTAAACTGGGA
                          1651                                          1700
IL13(EQ)41BBZeta   (1651) AAGTGATGTCGTGTACTGGCTCCGCCTTTTTCCCGAGGGTGGGGGAGAAC
 CD19Rop_epHIV7    (1651) AAGTGATGTCGTGTACTGGCTCCGCCTTTTTCCCGAGGGTGGGGGAGAAC
       Consensus   (1651) AAGTGATGTCGTGTACTGGCTCCGCCTTTTTCCCGAGGGTGGGGGAGAAC
                          1701                                          1750
IL13(EQ)41BBZeta   (1701) CGTATATAAGTGCAGTAGTCGCCGTGAACGTTCTTTTTCGCAACGGGTTT
 CD19Rop_epHIV7    (1701) CGTATATAAGTGCAGTAGTCGCCGTGAACGTTCTTTTTCGCAACGGGTTT
       Consensus   (1701) CGTATATAAGTGCAGTAGTCGCCGTGAACGTTCTTTTTCGCAACGGGTTT
                          1751                                          1800
IL13(EQ)41BBZeta   (1751) GCCGCCAGAACACAGCTGAAGCTTCGAGGGGCTCGCATCTCTCCTTCACG
 CD19Rop_epHIV7    (1751) GCCGCCAGAACACAGCTGAAGCTTCGAGGGGCTCGCATCTCTCCTTCACG
       Consensus   (1751) GCCGCCAGAACACAGCTGAAGCTTCGAGGGGCTCGCATCTCTCCTTCACG
                          1801                                          1850
IL13(EQ)41BBZeta   (1801) CGCCCGCCGCCCTACCTGAGGCCGCCATCCACGCCGGTTGAGTCGCGTTC
 CD19Rop_epHIV7    (1801) CGCCCGCCGCCCTACCTGAGGCCGCCATCCACGCCGGTTGAGTCGCGTTC
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      Consensus  (1801) CGCCCGCCGCCCTACCTGAGGCCGCCATCCACGCCGGTTGAGTCGCGTTC
                        1851                                          1900
IL13(EQ)41BBZeta (1851) TGCCGCCTCCCGCCTGTGGTGCCTCCTGAACTGCGTCCGCCGTCTAGGTA
 CD19Rop_epHIV7  (1851) TGCCGCCTCCCGCCTGTGGTGCCTCCTGAACTGCGTCCGCCGTCTAGGTA
      Consensus  (1851) TGCCGCCTCCCGCCTGTGGTGCCTCCTGAACTGCGTCCGCCGTCTAGGTA
                        1901                                          1950
IL13(EQ)41BBZeta (1901) AGTTTAAAGCTCAGGTCGAGACCGGGCCTTTGTCCGGCGCTCCCTTGGAG
 CD19Rop_epHIV7  (1901) AGTTTAAAGCTCAGGTCGAGACCGGGCCTTTGTCCGGCGCTCCCTTGGAG
      Consensus  (1901) AGTTTAAAGCTCAGGTCGAGACCGGGCCTTTGTCCGGCGCTCCCTTGGAG
                        1951                                          2000
IL13(EQ)41BBZeta (1951) CCTACCTAGACTCAGCCGGCTCTCCACGCTTTGCCTGACCCTGCTTGCTC
 CD19Rop_epHIV7  (1951) CCTACCTAGACTCAGCCGGCTCTCCACGCTTTGCCTGACCCTGCTTGCTC
      Consensus  (1951) CCTACCTAGACTCAGCCGGCTCTCCACGCTTTGCCTGACCCTGCTTGCTC
                        2001                                          2050
IL13(EQ)41BBZeta (2001) AACTCTACGTCTTTGTTTCGTTTTCTGTTCTGCGCCGTTACAGATCCAAG
 CD19Rop_epHIV7  (2001) AACTCTACGTCTTTGTTTCGTTTTCTGTTCTGCGCCGTTACAGATCCAAG
      Consensus  (2001) AACTCTACGTCTTTGTTTCGTTTTCTGTTCTGCGCCGTTACAGATCCAAG
                        2051                                          2100
IL13(EQ)41BBZeta (2051) CTGTGACCGGCGCCTACGGCTAGCGCCGCCACCATGCTGCTGCTGGTGAC
 CD19Rop_epHIV7  (2051) CTGTGACCGGCGCCTACGGCTAGCGCCGCCACCATGCTGCTGCTGGTGAC
      Consensus  (2051) CTGTGACCGGCGCCTACGGCTAGCGCCGCCACCATGCTGCTGCTGGTGAC
                        2101                                          2150
IL13(EQ)41BBZeta (2101) CAGCCTGCTGCTGTGCGAGCTGCCCCACCCCGCCTTTCTGCTGATCCCTG
 CD19Rop_epHIV7  (2101) CAGCCTGCTGCTGTGCGAGCTGCCCCACCCCGCCTTTCTGCTGATCCCCG
      Consensus  (2101) CAGCCTGCTGCTGTGCGAGCTGCCCCACCCCGCCTTTCTGCTGATCCC G
                        2151                                          2200
IL13(EQ)41BBZeta (2151) GC--CCCG-TGCCCCCTAGCACCGCC---CTGCGCTACCTGATCGAGGAA
 CD19Rop_epHIV7  (2151) ACATCCAGATGACCCAGACCACCTCCAGCCTGAGCGCCAGCCTGGGCGAC
      Consensus  (2151)  C  CC G TG CCC  A CACC CC   CTG GC  C    T G  GA
                        2201                                          2250
IL13(EQ)41BBZeta (2195) CTGGTGA------------------------ACATCACCCAGAACCAGAA
 CD19Rop_epHIV7  (2201) CGGGTGACCATCAGCTGCCGGGCCAGCCAGGACATCAGCAAGTACCTGAA
      Consensus  (2201) C GGTGA                        ACATCA C AG ACC GAA
                        2251                                          2300
IL13(EQ)41BBZeta (2221) --------------AGCCC-------CC---------CTGTGCAAC----
 CD19Rop_epHIV7  (2251) CTGGTATCAGCAGAAGCCCGACGGCACCGTCAAGCTGCTGATCTACCACA
      Consensus  (2251)               AGCCC       CC         CTG  C AC
                        2301                                          2350
IL13(EQ)41BBZeta (2237) ------GGCAGCAT---GGTGTG---------------------------
 CD19Rop_epHIV7  (2301) CCAGCCGGCTGCACAGCGGCGTGCCCAGCCGGTTTAGCGGCAGCGGCTCC
      Consensus  (2301)       GGC GCA    GG GTG
                        2351                                          2400
IL13(EQ)41BBZeta (2251) -----------------GAGCATC---AACCTG-----------------
 CD19Rop_epHIV7  (2351) GGCACCGACTACAGCCTGACCATCTCCAACCTGGAACAGGAAGATATCGC
      Consensus  (2351)                   GA CATC   AACCTG
                        2401                                          2450
IL13(EQ)41BBZeta (2264) -ACC-------GCCGGCATGT------ACTG-----------TGCCGCC-
 CD19Rop_epHIV7  (2401) CACCTACTTTTGCCAGCAGGGCAACACACTGCCCTACACCTTTGGCGGCG
      Consensus  (2401)  ACC       GCC GCA G       ACTG           TG CG C
                        2451                                          2500
IL13(EQ)41BBZeta (2288) --------CTGGAAA------GCCTGATCAACGTGAGCGGCT--------
 CD19Rop_epHIV7  (2451) GAACAAAGCTGGAAATCACCGGCAGCACCTCCGGCAGCGGCAAGCCTGGC
      Consensus  (2451)         CTGGAAA      GC   A C  CG  AGCGGC
                        2501                                          2550
IL13(EQ)41BBZeta (2316) ----------GCAGCGCCATCG---------------AGAAAA-------
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 CD19Rop_epHIV7   (2501) AGCGGCGAGGGCAGCACCAAGGGCGAGGTGAAGCTGCAGGAAAGCGGCCC
       Consensus  (2501)           GCAGC CCA  G               AG AAA
                         2551                                          2600
IL13(EQ)41BBZeta  (2334) -------------CCCAGCG------------------------------
 CD19Rop_epHIV7   (2551) TGGCCTGGTGGCCCCCAGCCAGAGCCTGAGCGTGACCTGCACCGTGAGCG
       Consensus  (2551)              CCCAGC
                         2601                                          2650
IL13(EQ)41BBZeta  (2341) ----GATGCTGTCCGGCTTCTGC------------------CCCCACAAG
 CD19Rop_epHIV7   (2601) GCGTGAGCCTGCCCGACTACGGCGTGAGCTGGATCCGGCAGCCCCCCAGG
       Consensus  (2601)     GA  CTG CCG CT C GC                  CCCC CA G
                         2651                                          2700
IL13(EQ)41BBZeta  (2369) ----------------------------GTGTCCGCCGGAC-----AGTT
 CD19Rop_epHIV7   (2651) AAGGGCCTGGAATGGCTGGGCGTGATCTGGGGCAGCGAGACCACCTACTA
       Consensus  (2651)                               G G C GC  GAC     A T
                         2701                                          2750
IL13(EQ)41BBZeta  (2386) CAGCAGCCTGC--ACGTGCGGG-------------------ACACCAAGA
 CD19Rop_epHIV7   (2701) CAACAGCGCCCTGAAGAGCCGGCTGACCATCATCAAGGACAACAGCAAGA
       Consensus  (2701) CA CAGC   C  A G GC GG                    ACA CAAGA
                         2751                                          2800
IL13(EQ)41BBZeta  (2415) TCGAGGTGGCCCAGTTCGTGAAGGACCTGCTG-----------------C
 CD19Rop_epHIV7   (2751) GCCAGGTGTTCCTGAAGATGAACAGCCTGCAGACCGACGACACCGCCATC
       Consensus  (2751)  C AGGTG  CC G    TGAA   CCTGC G                 C
                         2801                                          2850
IL13(EQ)41BBZeta  (2448) TGCACCTG----AAGAA--------------GCTGTTCCG----GGA---
 CD19Rop_epHIV7   (2801) TACTACTGCGCCAAGCACTACTACTACGGCGGCAGCTACGCCATGGACTA
       Consensus  (2801) T C  CTG    AAG A              GC G T CG    GGA
                         2851                                          2900
IL13(EQ)41BBZeta  (2473) ---GGGCCGGTTCAAC------------------
 CD19Rop_epHIV7   (2851) CTGGGGCCAGGGCACCAGCGTGACCGTGAGCAGCGAGAGCAAGTACGGCC
       Consensus  (2851)    GGGCC G  CA C                  GAGAGCAAGTACGGCC
                         2901                                          2950
IL13(EQ)41BBZeta  (2502)
 CD19Rop_epHIV7   (2901) CTCCCTGCCCCCCTTGCCCTGCCCCCGAGTTCCTGGGCGGACCCAGCGTG
       Consensus  (2901) CTCCCTGCCCCCCTTGCCCTGCCCC GAGTTC  GGGCGGACCCAGCGTG
                         2951                                          3000
IL13(EQ)41BBZeta  (2552)
 CD19Rop_epHIV7   (2951) TTCCTGTTCCCCCCCAAGCCCAAGGACACCCTGATGATCAGCCGGACCCC
       Consensus  (2951) TTCCTGTTCCCCCCCAAGCCCAAGGACACCCTGATGATCAGCCGGACCCC
                         3001                                          3050
IL13(EQ)41BBZeta  (2602)
 CD19Rop_epHIV7   (3001) CGAGGTGACCTGCGTGGTGGTGGACGTGAGCCAGGAAGATCCCGAGGTCC
       Consensus  (3001)  GAGGTGACCTGCGTGGTGGTGGACGTGAGCCAGGAAGATCC GAGGTCC
                         3051                                          3100
IL13(EQ)41BBZeta  (2652)
 CD19Rop_epHIV7   (3051) AGTTCAATTGGTACGTGGACGGCGTGGAGGTGCACAACGCCAAGACCAAG
       Consensus  (3051) AGTTCAATTGGTACGTGGACGGCGTGGAGGTGCACAACGCCAAGACCAAG
                         3101                                          3150
IL13(EQ)41BBZeta  (2702)
 CD19Rop_epHIV7   (3101) CCCAGGGAAGAGCAGTTCAACAGCACCTACCGGGTGGTGTCCGTGCTGAC
       Consensus  (3101) CCCAGGGAAGAGCAGTTC A AGCACCTACCGGGTGGTGTCCGTGCTGAC
                         3151                                          3200
IL13(EQ)41BBZeta  (2752)
 CD19Rop_epHIV7   (3151) CGTGCTGCACCAGGACTGGCTGAACGGCAAAGAATACAAGTGCAAGGTGT
       Consensus  (3151) CGTGCTGCACCAGGACTGGCTGAACGGCAAAGAATACAAGTGCAAGGTGT
                         3201                                          3250
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IL13(EQ)41BBZeta  (2802)
 CD19Rop_epHIV7   (3201) CCAACAAGGGCCTGCCCAGCAGCATCGAGAAAACCATCAGCAAGGCCAAG
       Consensus  (3201) CCAACAAGGGCCTGCCCAGCAGCATCGAGAAAACCATCAGCAAGGCCAAG
                         3251                                            3300
IL13(EQ)41BBZeta  (2852)
 CD19Rop_epHIV7   (3251) GGCCAGCCTCGGGAGCCCCAGGTGTACACCCTGCCCCCTTCCCAGGAAGA
       Consensus  (3251) GGCCAGCCTCGGGAGCCCCAGGTGTACACCCTGCCCCCTTCCCAGGAAGA
                         3301                                            3350
IL13(EQ)41BBZeta  (2902)
 CD19Rop_epHIV7   (3301) GATGACCAAGAATCAGGTGTCCCTGACCTGCCTGGTGAAGGGCTTCTACC
       Consensus  (3301) GATGACCAAGAATCAGGTGTCCCTGACCTGCCTGGTGAAGGGCTTCTACC
                         3351                                            3400
IL13(EQ)41BBZeta  (2952)
 CD19Rop_epHIV7   (3351) CCAGCGACATCGCCGTGGAGTGGGAGAGCAACGGCCAGCCCGAGAACAAC
       Consensus  (3351) CCAGCGACATCGCCGTGGAGTGGGAGAGCAACGGCCAGCCCGAGAACAAC
                         3401                                            3450
IL13(EQ)41BBZeta  (3002) TACAAGACCACCCCCCCTGTGCTGGACAGCGACGGCAGCTTCTTCCTGTA
 CD19Rop_epHIV7   (3401) TACAAGACCACCCCCCCTGTGCTGGACAGCGACGGCAGCTTCTTCCTGTA
       Consensus  (3401) TACAAGACCACCCCCCCTGTGCTGGACAGCGACGGCAGCTTCTTCCTGTA
                         3451                                            3500
IL13(EQ)41BBZeta  (3052) CAGCAGGCTGACCGTGGACAAGAGCCGGTGGCAGGAAGGCAACGTCTTTA
 CD19Rop_epHIV7   (3451) CAGCAGGCTGACCGTGGACAAGAGCCGGTGGCAGGAAGGCAACGTCTTTA
       Consensus  (3451) CAGCAGGCTGACCGTGGACAAGAGCCGGTGGCAGGAAGGCAACGTCTTTA
                         3501                                            3550
IL13(EQ)41BBZeta  (3102) GCTGCAGCGTGATGCACGAGGCCCTGCACAACCACTACACCCAGAAGAGC
 CD19Rop_epHIV7   (3501) GCTGCAGCGTGATGCACGAGGCCCTGCACAACCACTACACCCAGAAGAGC
       Consensus  (3501) GCTGCAGCGTGATGCACGAGGCCCTGCACAACCACTACACCCAGAAGAGC
                         3551                                            3600
IL13(EQ)41BBZeta  (3152) CTGTCCCTGAGCCTGGGCAAG
 CD19Rop_epHIV7   (3551) CTGTCCCTGAGCCTGGGCAAGATGGCCCTGATCGTGCTGGGCGGCGTGGC
       Consensus  (3551) CTGTCCCTGAGCCTGGGCAAGATGGCCCTGATCGTGCTGGGCGGCGTGGC
                         3601                                            3650
IL13(EQ)41BBZeta  (3202)
 CD19Rop_epHIV7   (3601) CGGGCTGCTGCTGTTCATCGGCCTGGGCATCTTTTTC-------------
       Consensus  (3601) CGGGCTGCTGCTGTTCATCGGCCTGGGCATCTTTTTC
                         3651                                            3700
IL13(EQ)41BBZeta  (3252)
 CD19Rop_epHIV7   (3638) ----------------------------C----------------------
       Consensus  (3651)                             C
                         3701                                            3750
IL13(EQ)41BBZeta  (3302)
 CD19Rop_epHIV7   (3639) ------------------------------------------------
       Consensus  (3701)
                         3751                                            3800
IL13(EQ)41BBZeta  (3352)                       CGGGTGAAGTTCAGCCGGTCCGCCGACG
 CD19Rop_epHIV7   (3639) -----------------------GGGTGAAGTTCAGCCGGTCCGCCGACG
       Consensus  (3751)                        GGGTGAAGTTCAGCCGGTCCGCCGACG
                         3801                                            3850
IL13(EQ)41BBZeta  (3402) CCCCTGCCTACCAGCAGGGCCAGAACCAGCTGTACAACGAGCTGAACCTG
 CD19Rop_epHIV7   (3666) CCCCTGCCTACCAGCAGGGCCAGAACCAGCTGTACAACGAGCTGAACCTG
       Consensus  (3801) CCCCTGCCTACCAGCAGGGCCAGAACCAGCTGTACAACGAGCTGAACCTG
                         3851                                            3900
IL13(EQ)41BBZeta  (3452) GGCAGGCGGGAGGAATACGACGTGCTGGACAAGCGGAGAGGCCGGGACCC
 CD19Rop_epHIV7   (3716) GGCAGGCGGGAGGAATACGACGTGCTGGACAAGCGGAGAGGCCGGGACCC
       Consensus  (3851) GGCAGGCGGGAGGAATACGACGTGCTGGACAAGCGGAGAGGCCGGGACCC
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                          3901                                                3950
IL13(EQ)41BBZeta  (3502) TGAGATGGGCGGCAAGCCTCGGCGGAAGAACCCCCAGGAAGGCCTGTATA
 CD19Rop_epHIV7   (3766) TGAGATGGGCGGCAAGCCCAGGCGGAAGAACCCTCAGGAAGGCCTGTATA
       Consensus  (3901) TGAGATGGGCGGCAAGCC  GGCGGAAGAACCC CAGGAAGGCCTGTATA
                          3951                                                4000
IL13(EQ)41BBZeta  (3552) ACGAACTGCAGAAAGACAAGATGGCCGAGGCCTACAGCGAGATCGGCATG
 CD19Rop_epHIV7   (3816) ACGAACTGCAGAAAGACAAGATGGCCGAGGCCTACAGCGAGATCGGCATG
       Consensus  (3951) ACGAACTGCAGAAAGACAAGATGGCCGAGGCCTACAGCGAGATCGGCATG
                          4001                                                4050
IL13(EQ)41BBZeta  (3602) AAGGGCGAGCGGAGGCGGGGCAAGGGCCACGACGGCCTGTATCAGGGCCT
 CD19Rop_epHIV7   (3866) AAGGGCGAGCGGCGGAGGGGCAAGGGCCACGACGGCCTGTACCAGGGCCT
       Consensus  (4001) AAGGGCGAGCGG GG GGGGCAAGGGCCACGACGGCCTGTA CAGGGCCT
                          4051                                                4100
IL13(EQ)41BBZeta  (3652) GTCCACCGCCACCAAGGATACCTACGACGCCCTGCACATGCAGGCCCTGC
 CD19Rop_epHIV7   (3916) GAGCACCGCCACCAAGGATACCTACGACGCCCTGCACATGCAGGCCCTGC
       Consensus  (4051) G  CACCGCCACCAAGGATACCTACGACGCCCTGCACATGCAGGCCCTGC
                          4101                                                4150
IL13(EQ)41BBZeta  (3702) CCCCAAGG
 CD19Rop_epHIV7   (3966) CCCC----------------------------------------------
       Consensus  (4101) CCCC
                          4151                                                4200
IL13(EQ)41BBZeta  (3752)
 CD19Rop_epHIV7   (3970) ----------------------------------------------------
       Consensus  (4151)
                          4201                                                4250
IL13(EQ)41BBZeta  (3802)
 CD19Rop_epHIV7   (3970) ----------------------------------------------------
       Consensus  (4201)
                          4251                                                4300
IL13(EQ)41BBZeta  (3852)
 CD19Rop_epHIV7   (3970) ----------------------------------------------------
       Consensus  (4251)
                          4301                                                4350
IL13(EQ)41BBZeta  (3902)
 CD19Rop_epHIV7   (3970) ----------------------------------------------------
       Consensus  (4301)
                          4351                                                4400
IL13(EQ)41BBZeta  (3952)
 CD19Rop_epHIV7   (3970) ----------------------------------------------------
       Consensus  (4351)
                          4401                                                4450
IL13(EQ)41BBZeta  (4002)
 CD19Rop_epHIV7   (3970) ----------------------------------------------------
       Consensus  (4401)
                          4451                                                4500
IL13(EQ)41BBZeta  (4052)
 CD19Rop_epHIV7   (3970) ----------------------------------------------------
       Consensus  (4451)
                          4501                                                4550
IL13(EQ)41BBZeta  (4102)
 CD19Rop_epHIV7   (3970) ----------------------------------------------------
       Consensus  (4501)
                          4551                                                4600
IL13(EQ)41BBZeta  (4152)
 CD19Rop_epHIV7   (3970) ----------------------------------------------------
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       Consensus  (4551)
                          4601                                                4650
IL13(EQ)41BBZeta  (4202)
 CD19Rop_epHIV7   (3970) --------------------------------------------------
       Consensus  (4601)
                          4651                                                4700
IL13(EQ)41BBZeta  (4252)
 CD19Rop_epHIV7   (3970) --------------------------------------------------
       Consensus  (4651)
                          4701                                                4750
IL13(EQ)41BBZeta  (4302)
 CD19Rop_epHIV7   (3970) ----------------------------C------AGG-----------
       Consensus  (4701)                             C      AGG
                          4751                                                4800
IL13(EQ)41BBZeta  (4352)
 CD19Rop_epHIV7   (3974) --------------------------------T-----------------
       Consensus  (4751)
                          4801                                                4850
IL13(EQ)41BBZeta  (4402)
 CD19Rop_epHIV7   (3975) --------------------------------------------------
       Consensus  (4801)
                          4851                                                4900
IL13(EQ)41BBZeta  (4452)
 CD19Rop_epHIV7   (3975) --------------------------------------------------
       Consensus  (4851)
                          4901                                                4950
IL13(EQ)41BBZeta  (4502)
 CD19Rop_epHIV7   (3975) --------------------------------------------------
       Consensus  (4901)
                          4951                                                5000
IL13(EQ)41BBZeta  (4552)
 CD19Rop_epHIV7   (3975) --------------------------------------------------
       Consensus  (4951)
                          5001                                                5050
IL13(EQ)41BBZeta  (4602)
 CD19Rop_epHIV7   (3975) --------------------------------------------------
       Consensus  (5001)
                          5051                                                5100
IL13(EQ)41BBZeta  (4652)
 CD19Rop_epHIV7   (3975) --------------------------------------------------
       Consensus  (5051)
                          5101                                                5150
IL13(EQ)41BBZeta  (4702)
 CD19Rop_epHIV7   (3975) --------------------------------------------------
       Consensus  (5101)
                          5151                                                5200
IL13(EQ)41BBZeta  (4752)   TCTAGACCCGGGCTGCAGGAATTCGATATCAAGCTTATCGATAATCAA
 CD19Rop_epHIV7   (3975) ------GACCCGGGCTGCAGGAATTCGATATCAAGCTTATCGATAATCAA
       Consensus  (5151)       GACCCGGGCTGCAGGAATTCGATATCAAGCTTATCGATAATCAA
                          5201                                                5250
IL13(EQ)41BBZeta  (4802) CCTCTGGATTACAAAATTTGTGAAAGATTGACTGGTATTCTTAACTATGT
 CD19Rop_epHIV7   (4019) CCTCTGGATTACAAAATTTGTGAAAGATTGACTGGTATTCTTAACTATGT
       Consensus  (5201) CCTCTGGATTACAAAATTTGTGAAAGATTGACTGGTATTCTTAACTATGT
                          5251                                                5300
IL13(EQ)41BBZeta  (4852) TGCTCCTTTTACGCTATGTGGATACGCTGCTTTAATGCCTTTGTATCATG
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 CD19Rop_epHIV7   (4069) TGCTCCTTTTACGCTATGTGGATACGCTGCTTTAATGCCTTTGTATCATG
       Consensus  (5251) TGCTCCTTTTACGCTATGTGGATACGCTGCTTTAATGCCTTTGTATCATG
                         5301                                          5350
IL13(EQ)41BBZeta  (4902) CTATTGCTTCCCGTATGGCTTTCATTTTCTCCTCCTTGTATAAATCCTGG
 CD19Rop_epHIV7   (4119) CTATTGCTTCCCGTATGGCTTTCATTTTCTCCTCCTTGTATAAATCCTGG
       Consensus  (5301) CTATTGCTTCCCGTATGGCTTTCATTTTCTCCTCCTTGTATAAATCCTGG
                         5351                                          5400
IL13(EQ)41BBZeta  (4952) TTGCTGTCTCTTTATGAGGAGTTGTGGCCCGTTGTCAGGCAACGTGGCGT
 CD19Rop_epHIV7   (4169) TTGCTGTCTCTTTATGAGGAGTTGTGGCCCGTTGTCAGGCAACGTGGCGT
       Consensus  (5351) TTGCTGTCTCTTTATGAGGAGTTGTGGCCCGTTGTCAGGCAACGTGGCGT
                         5401                                          5450
IL13(EQ)41BBZeta  (5002) GGTGTGCACTGTGTTTGCTGACGCAACCCCCACTGGTTGGGGCATTGCCA
 CD19Rop_epHIV7   (4219) GGTGTGCACTGTGTTTGCTGACGCAACCCCCACTGGTTGGGGCATTGCCA
       Consensus  (5401) GGTGTGCACTGTGTTTGCTGACGCAACCCCCACTGGTTGGGGCATTGCCA
                         5451                                          5500
IL13(EQ)41BBZeta  (5052) CCACCTGTCAGCTCCTTTCCGGGACTTTCGCTTTCCCCCTCCCTATTGCC
 CD19Rop_epHIV7   (4269) CCACCTGTCAGCTCCTTTCCGGGACTTTCGCTTTCCCCCTCCCTATTGCC
       Consensus  (5451) CCACCTGTCAGCTCCTTTCCGGGACTTTCGCTTTCCCCCTCCCTATTGCC
                         5501                                          5550
IL13(EQ)41BBZeta  (5102) ACGGCGGAACTCATCGCCGCCTGCCTTGCCCGCTGCTGGACAGGGGCTCG
 CD19Rop_epHIV7   (4319) ACGGCGGAACTCATCGCCGCCTGCCTTGCCCGCTGCTGGACAGGGGCTCG
       Consensus  (5501) ACGGCGGAACTCATCGCCGCCTGCCTTGCCCGCTGCTGGACAGGGGCTCG
                         5551                                          5600
IL13(EQ)41BBZeta  (5152) GCTGTTGGGCACTGACAATTCCGTGGTGTTGTCGGGGAAATCATCGTCCT
 CD19Rop_epHIV7   (4369) GCTGTTGGGCACTGACAATTCCGTGGTGTTGTCGGGGAAATCATCGTCCT
       Consensus  (5551) GCTGTTGGGCACTGACAATTCCGTGGTGTTGTCGGGGAAATCATCGTCCT
                         5601                                          5650
IL13(EQ)41BBZeta  (5202) TTCCTTGGCTGCTCGCCTGTGTTGCCACCTGGATTCTGCGCGGGACGTCC
 CD19Rop_epHIV7   (4419) TTCCTTGGCTGCTCGCCTGTGTTGCCACCTGGATTCTGCGCGGGACGTCC
       Consensus  (5601) TTCCTTGGCTGCTCGCCTGTGTTGCCACCTGGATTCTGCGCGGGACGTCC
                         5651                                          5700
IL13(EQ)41BBZeta  (5252) TTCTGCTACGTCCCTTCGGCCCTCAATCCAGCGGACCTTCCTTCCCGCGG
 CD19Rop_epHIV7   (4469) TTCTGCTACGTCCCTTCGGCCCTCAATCCAGCGGACCTTCCTTCCCGCGG
       Consensus  (5651) TTCTGCTACGTCCCTTCGGCCCTCAATCCAGCGGACCTTCCTTCCCGCGG
                         5701                                          5750
IL13(EQ)41BBZeta  (5302) CCTGCTGCCGGCTCTGCGGCCTCTTCCGCGTCTTCGCCTTCGCCCTCAGA
 CD19Rop_epHIV7   (4519) CCTGCTGCCGGCTCTGCGGCCTCTTCCGCGTCTTCGCCTTCGCCCTCAGA
       Consensus  (5701) CCTGCTGCCGGCTCTGCGGCCTCTTCCGCGTCTTCGCCTTCGCCCTCAGA
                         5751                                          5800
IL13(EQ)41BBZeta  (5352) CGAGTCGGATCTCCCTTTGGGCCGCCTCCCCGCATCGATACCGTCGACTA
 CD19Rop_epHIV7   (4569) CGAGTCGGATCTCCCTTTGGGCCGCCTCCCCGCATCGATACCGTCGACTA
       Consensus  (5751) CGAGTCGGATCTCCCTTTGGGCCGCCTCCCCGCATCGATACCGTCGACTA
                         5801                                          5850
IL13(EQ)41BBZeta  (5402) GCCGTACCTTTAAGACCAATGACTTACAAGGCAGCTGTAGATCTTAGCCA
 CD19Rop_epHIV7   (4619) GCCGTACCTTTAAGACCAATGACTTACAAGGCAGCTGTAGATCTTAGCCA
       Consensus  (5801) GCCGTACCTTTAAGACCAATGACTTACAAGGCAGCTGTAGATCTTAGCCA
                         5851                                          5900
IL13(EQ)41BBZeta  (5452) CTTTTTAAAAGAAAAGGGGGGACTGGAAGGGCTAATTCACTCCCAAAGAA
 CD19Rop_epHIV7   (4669) CTTTTTAAAAGAAAAGGGGGGACTGGAAGGGCTAATTCACTCCCAAAGAA
       Consensus  (5851) CTTTTTAAAAGAAAAGGGGGGACTGGAAGGGCTAATTCACTCCCAAAGAA
                         5901                                          5950
IL13(EQ)41BBZeta  (5502) GACAAGATCTGCTTTTTGCCTGTACTGGGTCTCTCTGGTTAGACCAGATC
 CD19Rop_epHIV7   (4719) GACAAGATCTGCTTTTTGCCTGTACTGGGTCTCTCTGGTTAGACCAGATC
       Consensus  (5901) GACAAGATCTGCTTTTTGCCTGTACTGGGTCTCTCTGGTTAGACCAGATC
                         5951                                          6000
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IL13(EQ)41BBZeta  (5552) TGAGCCTGGGAGCTCTCTGGCTAACTAGGGAACCCACTGCTTAAGCCTCA
 CD19Rop_epHIV7   (4769) TGAGCCTGGGAGCTCTCTGGCTAACTAGGGAACCCACTGCTTAAGCCTCA
       Consensus  (5951) TGAGCCTGGGAGCTCTCTGGCTAACTAGGGAACCCACTGCTTAAGCCTCA
                         6001                                              6050
IL13(EQ)41BBZeta  (5602) ATAAAGCTTGCCTTGAGTGCTTCAAGTAGTGTGTGCCCGTCTGTTGTGTG
 CD19Rop_epHIV7   (4819) ATAAAGCTTGCCTTGAGTGCTTCAAGTAGTGTGTGCCCGTCTGTTGTGTG
       Consensus  (6001) ATAAAGCTTGCCTTGAGTGCTTCAAGTAGTGTGTGCCCGTCTGTTGTGTG
                         6051                                              6100
IL13(EQ)41BBZeta  (5652) ACTCTGGTAACTAGAGATCCCTCAGACCCTTTTAGTCAGTGTGGAAAATC
 CD19Rop_epHIV7   (4869) ACTCTGGTAACTAGAGATCCCTCAGACCCTTTTAGTCAGTGTGGAAAATC
       Consensus  (6051) ACTCTGGTAACTAGAGATCCCTCAGACCCTTTTAGTCAGTGTGGAAAATC
                         6101                                              6150
IL13(EQ)41BBZeta  (5702) TCTAGCAGAATTCGATATCAAGCTTATCGATACCGTCGACCTCGAGGGGG
 CD19Rop_epHIV7   (4919) TCTAGCAGAATTCGATATCAAGCTTATCGATACCGTCGACCTCGAGGGGG
       Consensus  (6101) TCTAGCAGAATTCGATATCAAGCTTATCGATACCGTCGACCTCGAGGGGG
                         6151                                              6200
IL13(EQ)41BBZeta  (5752) GGCCCGGTACCCAATTCGCCCTATAGTGAGTCGTATTACAATTCACTGGC
 CD19Rop_epHIV7   (4969) GGCCCGGTACCCAATTCGCCCTATAGTGAGTCGTATTACAATTCACTGGC
       Consensus  (6151) GGCCCGGTACCCAATTCGCCCTATAGTGAGTCGTATTACAATTCACTGGC
                         6201                                              6250
IL13(EQ)41BBZeta  (5802) CGTCGTTTTACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAACTTA
 CD19Rop_epHIV7   (5019) CGTCGTTTTACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAACTTA
       Consensus  (6201) CGTCGTTTTACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAACTTA
                         6251                                              6300
IL13(EQ)41BBZeta  (5852) ATCGCCTTGCAGCACATCCCCCTTTCGCCAGCTGGCGTAATAGCGAAGAG
 CD19Rop_epHIV7   (5069) ATCGCCTTGCAGCACATCCCCCTTTCGCCAGCTGGCGTAATAGCGAAGAG
       Consensus  (6251) ATCGCCTTGCAGCACATCCCCCTTTCGCCAGCTGGCGTAATAGCGAAGAG
                         6301                                              6350
IL13(EQ)41BBZeta  (5902) GCCCGCACCGATCGCCCTTCCCAACAGTTGCGCAGCCTGAATGGCGAATG
 CD19Rop_epHIV7   (5119) GCCCGCACCGATCGCCCTTCCCAACAGTTGCGCAGCCTGAATGGCGAATG
       Consensus  (6301) GCCCGCACCGATCGCCCTTCCCAACAGTTGCGCAGCCTGAATGGCGAATG
                         6351                                              6400
IL13(EQ)41BBZeta  (5952) GAAATTGTAAGCGTTAATATTTTGTTAAAATTCGCGTTAAATTTTTGTTA
 CD19Rop_epHIV7   (5169) GAAATTGTAAGCGTTAATATTTTGTTAAAATTCGCGTTAAATTTTTGTTA
       Consensus  (6351) GAAATTGTAAGCGTTAATATTTTGTTAAAATTCGCGTTAAATTTTTGTTA
                         6401                                              6450
IL13(EQ)41BBZeta  (6002) AATCAGCTCATTTTTTAACCAATAGGCCGAAATCGGCAAAATCCCTTATA
 CD19Rop_epHIV7   (5219) AATCAGCTCATTTTTTAACCAATAGGCCGAAATCGGCAAAATCCCTTATA
       Consensus  (6401) AATCAGCTCATTTTTTAACCAATAGGCCGAAATCGGCAAAATCCCTTATA
                         6451                                              6500
IL13(EQ)41BBZeta  (6052) AATCAAAAGAATAGACCGAGATAGGGTTGAGTGTTGTTCCAGTTTGGAAC
 CD19Rop_epHIV7   (5269) AATCAAAAGAATAGACCGAGATAGGGTTGAGTGTTGTTCCAGTTTGGAAC
       Consensus  (6451) AATCAAAAGAATAGACCGAGATAGGGTTGAGTGTTGTTCCAGTTTGGAAC
                         6501                                              6550
IL13(EQ)41BBZeta  (6102) AAGAGTCCACTATTAAAGAACGTGGACTCCAACGTCAAAGGGCGAAAAAC
 CD19Rop_epHIV7   (5319) AAGAGTCCACTATTAAAGAACGTGGACTCCAACGTCAAAGGGCGAAAAAC
       Consensus  (6501) AAGAGTCCACTATTAAAGAACGTGGACTCCAACGTCAAAGGGCGAAAAAC
                         6551                                              6600
IL13(EQ)41BBZeta  (6152) CGTCTATCAGGGCGATGGCCCACTACGTGAACCATCACCCTAATCAAGTT
 CD19Rop_epHIV7   (5369) CGTCTATCAGGGCGATGGCCCACTACGTGAACCATCACCCTAATCAAGTT
       Consensus  (6551) CGTCTATCAGGGCGATGGCCCACTACGTGAACCATCACCCTAATCAAGTT
                         6601                                              6650
IL13(EQ)41BBZeta  (6202) TTTTGGGGTCGAGGTGCCGTAAAGCACTAAATCGGAACCCTAAAGGGAGC
 CD19Rop_epHIV7   (5419) TTTTGGGGTCGAGGTGCCGTAAAGCACTAAATCGGAACCCTAAAGGGAGC
       Consensus  (6601) TTTTGGGGTCGAGGTGCCGTAAAGCACTAAATCGGAACCCTAAAGGGAGC
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                          6651                                          6700
IL13(EQ)41BBZeta   (6252) CCCCGATTTAGAGCTTGACGGGGAAAGCCGGCGAACGTGGCGAGAAAGGA
 CD19Rop_epHIV7    (5469) CCCCGATTTAGAGCTTGACGGGGAAAGCCGGCGAACGTGGCGAGAAAGGA
       Consensus   (6651) CCCCGATTTAGAGCTTGACGGGGAAAGCCGGCGAACGTGGCGAGAAAGGA
                          6701                                          6750
IL13(EQ)41BBZeta   (6302) AGGGAAGAAAGCGAAAGGAGCGGGCGCTAGGGCGCTGGCAAGTGTAGCGG
 CD19Rop_epHIV7    (5519) AGGGAAGAAAGCGAAAGGAGCGGGCGCTAGGGCGCTGGCAAGTGTAGCGG
       Consensus   (6701) AGGGAAGAAAGCGAAAGGAGCGGGCGCTAGGGCGCTGGCAAGTGTAGCGG
                          6751                                          6800
IL13(EQ)41BBZeta   (6352) TCACGCTGCGCGTAACCACCACACCCGCCGCGCTTAATGCGCCGCTACAG
 CD19Rop_epHIV7    (5569) TCACGCTGCGCGTAACCACCACACCCGCCGCGCTTAATGCGCCGCTACAG
       Consensus   (6751) TCACGCTGCGCGTAACCACCACACCCGCCGCGCTTAATGCGCCGCTACAG
                          6801                                          6850
IL13(EQ)41BBZeta   (6402) GGCGCGTCAGGTGGCACTTTTCGGGGAAATGTGCGCGGAACCCCTATTTG
 CD19Rop_epHIV7    (5619) GGCGCGTCAGGTGGCACTTTTCGGGGAAATGTGCGCGGAACCCCTATTTG
       Consensus   (6801) GGCGCGTCAGGTGGCACTTTTCGGGGAAATGTGCGCGGAACCCCTATTTG
                          6851                                          6900
IL13(EQ)41BBZeta   (6452) TTTATTTTTCTAAATACATTCAAATATGTATCCGCTCATGAGACAATAAC
 CD19Rop_epHIV7    (5669) TTTATTTTTCTAAATACATTCAAATATGTATCCGCTCATGAGACAATAAC
       Consensus   (6851) TTTATTTTTCTAAATACATTCAAATATGTATCCGCTCATGAGACAATAAC
                          6901                                          6950
IL13(EQ)41BBZeta   (6502) CCTGATAAATGCTTCAATAATATTGAAAAAGGAAGAGTATGAGTATTCAA
 CD19Rop_epHIV7    (5719) CCTGATAAATGCTTCAATAATATTGAAAAAGGAAGAGTATGAGTATTCAA
       Consensus   (6901) CCTGATAAATGCTTCAATAATATTGAAAAAGGAAGAGTATGAGTATTCAA
                          6951                                          7000
IL13(EQ)41BBZeta   (6552) CATTTCCGTGTCGCCCTTATTCCCTTTTTTGCGGCATTTTGCCTTCCTGT
 CD19Rop_epHIV7    (5769) CATTTCCGTGTCGCCCTTATTCCCTTTTTTGCGGCATTTTGCCTTCCTGT
       Consensus   (6951) CATTTCCGTGTCGCCCTTATTCCCTTTTTTGCGGCATTTTGCCTTCCTGT
                          7001                                          7050
IL13(EQ)41BBZeta   (6602) TTTTGCTCACCCAGAAACGCTGGTGAAAGTAAAAGATGCTGAAGATCAGT
 CD19Rop_epHIV7    (5819) TTTTGCTCACCCAGAAACGCTGGTGAAAGTAAAAGATGCTGAAGATCAGT
       Consensus   (7001) TTTTGCTCACCCAGAAACGCTGGTGAAAGTAAAAGATGCTGAAGATCAGT
                          7051                                          7100
IL13(EQ)41BBZeta   (6652) TGGGTGCACGAGTGGGTTACATCGAACTGGATCTCAACAGCGGTAAGATC
 CD19Rop_epHIV7    (5869) TGGGTGCACGAGTGGGTTACATCGAACTGGATCTCAACAGCGGTAAGATC
       Consensus   (7051) TGGGTGCACGAGTGGGTTACATCGAACTGGATCTCAACAGCGGTAAGATC
                          7101                                          7150
IL13(EQ)41BBZeta   (6702) CTTGAGAGTTTTCGCCCCGAAGAACGTTTTCCAATGATGAGCACTTTTAA
 CD19Rop_epHIV7    (5919) CTTGAGAGTTTTCGCCCCGAAGAACGTTTTCCAATGATGAGCACTTTTAA
       Consensus   (7101) CTTGAGAGTTTTCGCCCCGAAGAACGTTTTCCAATGATGAGCACTTTTAA
                          7151                                          7200
IL13(EQ)41BBZeta   (6752) AGTTCTGCTATGTGGCGCGGTATTATCCCGTATTGACGCCGGGCAAGAGC
 CD19Rop_epHIV7    (5969) AGTTCTGCTATGTGGCGCGGTATTATCCCGTATTGACGCCGGGCAAGAGC
       Consensus   (7151) AGTTCTGCTATGTGGCGCGGTATTATCCCGTATTGACGCCGGGCAAGAGC
                          7201                                          7250
IL13(EQ)41BBZeta   (6802) AACTCGGTCGCCGCATACACTATTCTCAGAATGACTTGGTTGAGTACTCA
 CD19Rop_epHIV7    (6019) AACTCGGTCGCCGCATACACTATTCTCAGAATGACTTGGTTGAGTACTCA
       Consensus   (7201) AACTCGGTCGCCGCATACACTATTCTCAGAATGACTTGGTTGAGTACTCA
                          7251                                          7300
IL13(EQ)41BBZeta   (6852) CCAGTCACAGAAAAGCATCTTACGGATGGCATGACAGTAAGAGAATTATG
 CD19Rop_epHIV7    (6069) CCAGTCACAGAAAAGCATCTTACGGATGGCATGACAGTAAGAGAATTATG
       Consensus   (7251) CCAGTCACAGAAAAGCATCTTACGGATGGCATGACAGTAAGAGAATTATG
                          7301                                          7350
IL13(EQ)41BBZeta   (6902) CAGTGCTGCCATAACCATGAGTGATAACACTGCGGCCAACTTACTTCTGA
 CD19Rop_epHIV7    (6119) CAGTGCTGCCATAACCATGAGTGATAACACTGCGGCCAACTTACTTCTGA
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       Consensus  (7301) CAGTGCTGCCATAACCATGAGTGATAACACTGCGGCCAACTTACTTCTGA
                         7351                                          7400
IL13(EQ)41BBZeta  (6952) CAACGATCGGAGGACCGAAGGAGCTAACCGCTTTTTTGCACAACATGGGG
 CD19Rop_epHIV7   (6169) CAACGATCGGAGGACCGAAGGAGCTAACCGCTTTTTTGCACAACATGGGG
       Consensus  (7351) CAACGATCGGAGGACCGAAGGAGCTAACCGCTTTTTTGCACAACATGGGG
                         7401                                          7450
IL13(EQ)41BBZeta  (7002) GATCATGTAACTCGCCTTGATCGTTGGGAACCGGAGCTGAATGAAGCCAT
 CD19Rop_epHIV7   (6219) GATCATGTAACTCGCCTTGATCGTTGGGAACCGGAGCTGAATGAAGCCAT
       Consensus  (7401) GATCATGTAACTCGCCTTGATCGTTGGGAACCGGAGCTGAATGAAGCCAT
                         7451                                          7500
IL13(EQ)41BBZeta  (7052) ACCAAACGACGAGCGTGACACCACGATGCCTGTAGCAATGGCAACAACGT
 CD19Rop_epHIV7   (6269) ACCAAACGACGAGCGTGACACCACGATGCCTGTAGCAATGGCAACAACGT
       Consensus  (7451) ACCAAACGACGAGCGTGACACCACGATGCCTGTAGCAATGGCAACAACGT
                         7501                                          7550
IL13(EQ)41BBZeta  (7102) TGCGCAAACTATTAACTGGCGAACTACTTACTCTAGCTTCCCGGCAACAA
 CD19Rop_epHIV7   (6319) TGCGCAAACTATTAACTGGCGAACTACTTACTCTAGCTTCCCGGCAACAA
       Consensus  (7501) TGCGCAAACTATTAACTGGCGAACTACTTACTCTAGCTTCCCGGCAACAA
                         7551                                          7600
IL13(EQ)41BBZeta  (7152) TTAATAGACTGGATGGAGGCGGATAAAGTTGCAGGACCACTTCTGCGCTC
 CD19Rop_epHIV7   (6369) TTAATAGACTGGATGGAGGCGGATAAAGTTGCAGGACCACTTCTGCGCTC
       Consensus  (7551) TTAATAGACTGGATGGAGGCGGATAAAGTTGCAGGACCACTTCTGCGCTC
                         7601                                          7650
IL13(EQ)41BBZeta  (7202) GGCCCTTCCGGCTGGCTGGTTTATTGCTGATAAATCTGGAGCCGGTGAGC
 CD19Rop_epHIV7   (6419) GGCCCTTCCGGCTGGCTGGTTTATTGCTGATAAATCTGGAGCCGGTGAGC
       Consensus  (7601) GGCCCTTCCGGCTGGCTGGTTTATTGCTGATAAATCTGGAGCCGGTGAGC
                         7651                                          7700
IL13(EQ)41BBZeta  (7252) GTGGGTCTCGCGGTATCATTGCAGCACTGGGGCCAGATGGTAAGCCCTCC
 CD19Rop_epHIV7   (6469) GTGGGTCTCGCGGTATCATTGCAGCACTGGGGCCAGATGGTAAGCCCTCC
       Consensus  (7651) GTGGGTCTCGCGGTATCATTGCAGCACTGGGGCCAGATGGTAAGCCCTCC
                         7701                                          7750
IL13(EQ)41BBZeta  (7302) CGTATCGTAGTTATCTACACGACGGGGAGTCAGGCAACTATGGATGAACG
 CD19Rop_epHIV7   (6519) CGTATCGTAGTTATCTACACGACGGGGAGTCAGGCAACTATGGATGAACG
       Consensus  (7701) CGTATCGTAGTTATCTACACGACGGGGAGTCAGGCAACTATGGATGAACG
                         7751                                          7800
IL13(EQ)41BBZeta  (7352) AAATAGACAGATCGCTGAGATAGGTGCCTCACTGATTAAGCATTGGTAAC
 CD19Rop_epHIV7   (6569) AAATAGACAGATCGCTGAGATAGGTGCCTCACTGATTAAGCATTGGTAAC
       Consensus  (7751) AAATAGACAGATCGCTGAGATAGGTGCCTCACTGATTAAGCATTGGTAAC
                         7801                                          7850
IL13(EQ)41BBZeta  (7402) TGTCAGACCAAGTTTACTCATATATACTTTAGATTGATTTAAAACTTCAT
 CD19Rop_epHIV7   (6619) TGTCAGACCAAGTTTACTCATATATACTTTAGATTGATTTAAAACTTCAT
       Consensus  (7801) TGTCAGACCAAGTTTACTCATATATACTTTAGATTGATTTAAAACTTCAT
                         7851                                          7900
IL13(EQ)41BBZeta  (7452) TTTTAATTTAAAAGGATCTAGGTGAAGATCCTTTTTGATAATCTCATGAC
 CD19Rop_epHIV7   (6669) TTTTAATTTAAAAGGATCTAGGTGAAGATCCTTTTTGATAATCTCATGAC
       Consensus  (7851) TTTTAATTTAAAAGGATCTAGGTGAAGATCCTTTTTGATAATCTCATGAC
                         7901                                          7950
IL13(EQ)41BBZeta  (7502) CAAAATCCCTTAACGTGAGTTTTCGTTCCACTGAGCGTCAGACCCCGTAG
 CD19Rop_epHIV7   (6719) CAAAATCCCTTAACGTGAGTTTTCGTTCCACTGAGCGTCAGACCCCGTAG
       Consensus  (7901) CAAAATCCCTTAACGTGAGTTTTCGTTCCACTGAGCGTCAGACCCCGTAG
                         7951                                          8000
IL13(EQ)41BBZeta  (7552) AAAAGATCAAAGGATCTTCTTGAGATCCTTTTTTTCTGCGCGTAATCTGC
 CD19Rop_epHIV7   (6769) AAAAGATCAAAGGATCTTCTTGAGATCCTTTTTTTCTGCGCGTAATCTGC
       Consensus  (7951) AAAAGATCAAAGGATCTTCTTGAGATCCTTTTTTTCTGCGCGTAATCTGC
                         8001                                          8050
IL13(EQ)41BBZeta  (7602) TGCTTGCAAACAAAAAAACCACCGCTACCAGCGGTGGTTTGTTTGCCGGA
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 CD19Rop_epHIV7   (6819) TGCTTGCAAACAAAAAAACCACCGCTACCAGCGGTGGTTTGTTTGCCGGA
       Consensus  (8001) TGCTTGCAAACAAAAAAACCACCGCTACCAGCGGTGGTTTGTTTGCCGGA
                         8051                                          8100
IL13(EQ)41BBZeta  (7652) TCAAGAGCTACCAACTCTTTTTCCGAAGGTAACTGGCTTCAGCAGAGCGC
 CD19Rop_epHIV7   (6869) TCAAGAGCTACCAACTCTTTTTCCGAAGGTAACTGGCTTCAGCAGAGCGC
       Consensus  (8051) TCAAGAGCTACCAACTCTTTTTCCGAAGGTAACTGGCTTCAGCAGAGCGC
                         8101                                          8150
IL13(EQ)41BBZeta  (7702) AGATACCAAATACTGTTCTTCTAGTGTAGCCGTAGTTAGGCCACCACTTC
 CD19Rop_epHIV7   (6919) AGATACCAAATACTGTTCTTCTAGTGTAGCCGTAGTTAGGCCACCACTTC
       Consensus  (8101) AGATACCAAATACTGTTCTTCTAGTGTAGCCGTAGTTAGGCCACCACTTC
                         8151                                          8200
IL13(EQ)41BBZeta  (7752) AAGAACTCTGTAGCACCGCCTACATACCTCGCTCTGCTAATCCTGTTACC
 CD19Rop_epHIV7   (6969) AAGAACTCTGTAGCACCGCCTACATACCTCGCTCTGCTAATCCTGTTACC
       Consensus  (8151) AAGAACTCTGTAGCACCGCCTACATACCTCGCTCTGCTAATCCTGTTACC
                         8201                                          8250
IL13(EQ)41BBZeta  (7802) AGTGGCTGCTGCCAGTGGCGATAAGTCGTGTCTTACCGGGTTGGACTCAA
 CD19Rop_epHIV7   (7019) AGTGGCTGCTGCCAGTGGCGATAAGTCGTGTCTTACCGGGTTGGACTCAA
       Consensus  (8201) AGTGGCTGCTGCCAGTGGCGATAAGTCGTGTCTTACCGGGTTGGACTCAA
                         8251                                          8300
IL13(EQ)41BBZeta  (7852) GACGATAGTTACCGGATAAGGCGCAGCGGTCGGGCTGAACGGGGGGTTCG
 CD19Rop_epHIV7   (7069) GACGATAGTTACCGGATAAGGCGCAGCGGTCGGGCTGAACGGGGGGTTCG
       Consensus  (8251) GACGATAGTTACCGGATAAGGCGCAGCGGTCGGGCTGAACGGGGGGTTCG
                         8301                                          8350
IL13(EQ)41BBZeta  (7902) TGCACACAGCCCAGCTTGGAGCGAACGACCTACACCGAACTGAGATACCT
 CD19Rop_epHIV7   (7119) TGCACACAGCCCAGCTTGGAGCGAACGACCTACACCGAACTGAGATACCT
       Consensus  (8301) TGCACACAGCCCAGCTTGGAGCGAACGACCTACACCGAACTGAGATACCT
                         8351                                          8400
IL13(EQ)41BBZeta  (7952) ACAGCGTGAGCTATGAGAAAGCGCCACGCTTCCCGAAGGGAGAAAGGCGG
 CD19Rop_epHIV7   (7169) ACAGCGTGAGCTATGAGAAAGCGCCACGCTTCCCGAAGGGAGAAAGGCGG
       Consensus  (8351) ACAGCGTGAGCTATGAGAAAGCGCCACGCTTCCCGAAGGGAGAAAGGCGG
                         8401                                          8450
IL13(EQ)41BBZeta  (8002) ACAGGTATCCGGTAAGCGGCAGGGTCGGAACAGGAGAGCGCACGAGGGAG
 CD19Rop_epHIV7   (7219) ACAGGTATCCGGTAAGCGGCAGGGTCGGAACAGGAGAGCGCACGAGGGAG
       Consensus  (8401) ACAGGTATCCGGTAAGCGGCAGGGTCGGAACAGGAGAGCGCACGAGGGAG
                         8451                                          8500
IL13(EQ)41BBZeta  (8052) CTTCCAGGGGGAAACGCCTGGTATCTTTATAGTCCTGTCGGGTTTCGCCA
 CD19Rop_epHIV7   (7269) CTTCCAGGGGGAAACGCCTGGTATCTTTATAGTCCTGTCGGGTTTCGCCA
       Consensus  (8451) CTTCCAGGGGGAAACGCCTGGTATCTTTATAGTCCTGTCGGGTTTCGCCA
                         8501                                          8550
IL13(EQ)41BBZeta  (8102) CCTCTGACTTGAGCGTCGATTTTTGTGATGCTCGTCAGGGGGGCGGAGCC
 CD19Rop_epHIV7   (7319) CCTCTGACTTGAGCGTCGATTTTTGTGATGCTCGTCAGGGGGGCGGAGCC
       Consensus  (8501) CCTCTGACTTGAGCGTCGATTTTTGTGATGCTCGTCAGGGGGGCGGAGCC
                         8551                                          8600
IL13(EQ)41BBZeta  (8152) TATGGAAAAACGCCAGCAACGCGGCCTTTTTACGGTTCCTGGCCTTTTGC
 CD19Rop_epHIV7   (7369) TATGGAAAAACGCCAGCAACGCGGCCTTTTTACGGTTCCTGGCCTTTTGC
       Consensus  (8551) TATGGAAAAACGCCAGCAACGCGGCCTTTTTACGGTTCCTGGCCTTTTGC
                         8601                                          8650
IL13(EQ)41BBZeta  (8202) TGGCCTTTTGCTCACATGTTCTTTCCTGCGTTATCCCCTGATTCTGTGGA
 CD19Rop_epHIV7   (7419) TGGCCTTTTGCTCACATGTTCTTTCCTGCGTTATCCCCTGATTCTGTGGA
       Consensus  (8601) TGGCCTTTTGCTCACATGTTCTTTCCTGCGTTATCCCCTGATTCTGTGGA
                         8651                                          8700
IL13(EQ)41BBZeta  (8252) TAACCGTATTACCGCCTTTGAGTGAGCTGATACCGCTCGCCGCAGCCGAA
 CD19Rop_epHIV7   (7469) TAACCGTATTACCGCCTTTGAGTGAGCTGATACCGCTCGCCGCAGCCGAA
       Consensus  (8651) TAACCGTATTACCGCCTTTGAGTGAGCTGATACCGCTCGCCGCAGCCGAA
                         8701                                          8750
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IL13(EQ)41BBZeta  (8302) CGACCGAGCGCAGCGAGTCAGTGAGCGAGGAAGCGGAAGAGCGCCCAATA
 CD19Rop_epHIV7   (7519) CGACCGAGCGCAGCGAGTCAGTGAGCGAGGAAGCGGAAGAGCGCCCAATA
       Consensus  (8701) CGACCGAGCGCAGCGAGTCAGTGAGCGAGGAAGCGGAAGAGCGCCCAATA
                         8751                                              8800
IL13(EQ)41BBZeta  (8352) CGCAAACCGCCTCTCCCCGCGCGTTGGCCGATTCATTAATGCAGCTGGCA
 CD19Rop_epHIV7   (7569) CGCAAACCGCCTCTCCCCGCGCGTTGGCCGATTCATTAATGCAGCTGGCA
       Consensus  (8751) CGCAAACCGCCTCTCCCCGCGCGTTGGCCGATTCATTAATGCAGCTGGCA
                         8801                                              8850
IL13(EQ)41BBZeta  (8402) CGACAGGTTTCCCGACTGGAAAGCGGGCAGTGAGCGCAACGCAATTAATG
 CD19Rop_epHIV7   (7619) CGACAGGTTTCCCGACTGGAAAGCGGGCAGTGAGCGCAACGCAATTAATG
       Consensus  (8801) CGACAGGTTTCCCGACTGGAAAGCGGGCAGTGAGCGCAACGCAATTAATG
                         8851                                              8900
IL13(EQ)41BBZeta  (8452) TGAGTTAGCTCACTCATTAGGCACCCCAGGCTTTACACTTTATGCTTCCG
 CD19Rop_epHIV7   (7669) TGAGTTAGCTCACTCATTAGGCACCCCAGGCTTTACACTTTATGCTTCCG
       Consensus  (8851) TGAGTTAGCTCACTCATTAGGCACCCCAGGCTTTACACTTTATGCTTCCG
                         8901                                              8950
IL13(EQ)41BBZeta  (8502) GCTCGTATGTTGTGTGGAATTGTGAGCGGATAACAATTTCACACAGGAAA
 CD19Rop_epHIV7   (7719) GCTCGTATGTTGTGTGGAATTGTGAGCGGATAACAATTTCACACAGGAAA
       Consensus  (8901) GCTCGTATGTTGTGTGGAATTGTGAGCGGATAACAATTTCACACAGGAAA
                         8951                                              9000
IL13(EQ)41BBZeta  (8552) CAGCTATGACCATGATTACGCCAAGCTCGAAATTAACCCTCACTAAAGGG
 CD19Rop_epHIV7   (7769) CAGCTATGACCATGATTACGCCAAGCTCGAAATTAACCCTCACTAAAGGG
       Consensus  (8951) CAGCTATGACCATGATTACGCCAAGCTCGAAATTAACCCTCACTAAAGGG
                         9001                                              9050
IL13(EQ)41BBZeta  (8602) AACAAAAGCTGGAGCTCCACCGCGGTGGCGGCCTCGAGGTCGAGATCCGG
 CD19Rop_epHIV7   (7819) AACAAAAGCTGGAGCTCCACCGCGGTGGCGGCCTCGAGGTCGAGATCCGG
       Consensus  (9001) AACAAAAGCTGGAGCTCCACCGCGGTGGCGGCCTCGAGGTCGAGATCCGG
                         9051                                              9100
IL13(EQ)41BBZeta  (8652) TCGACCAGCAACCATAGTCCCGCCCCTAACTCCGCCCATCCCGCCCCTAA
 CD19Rop_epHIV7   (7869) TCGACCAGCAACCATAGTCCCGCCCCTAACTCCGCCCATCCCGCCCCTAA
       Consensus  (9051) TCGACCAGCAACCATAGTCCCGCCCCTAACTCCGCCCATCCCGCCCCTAA
                         9101                                              9150
IL13(EQ)41BBZeta  (8702) CTCCGCCCAGTTCCGCCCATTCTCCGCCCCATGGCTGACTAATTTTTTTT
 CD19Rop_epHIV7   (7919) CTCCGCCCAGTTCCGCCCATTCTCCGCCCCATGGCTGACTAATTTTTTTT
       Consensus  (9101) CTCCGCCCAGTTCCGCCCATTCTCCGCCCCATGGCTGACTAATTTTTTTT
                         9151                                              9200
IL13(EQ)41BBZeta  (8752) ATTTATGCAGAGGCCGAGGCCGCCTCGGCCTCTGAGCTATTCCAGAAGTA
 CD19Rop_epHIV7   (7969) ATTTATGCAGAGGCCGAGGCCGCCTCGGCCTCTGAGCTATTCCAGAAGTA
       Consensus  (9151) ATTTATGCAGAGGCCGAGGCCGCCTCGGCCTCTGAGCTATTCCAGAAGTA
                         9201                                              9250
IL13(EQ)41BBZeta  (8802) GTGAGGAGGCTTTTTTGGAGGCCTAGGCTTTTGCAAAAAGCTTCGACGGT
 CD19Rop_epHIV7   (8019) GTGAGGAGGCTTTTTTGGAGGCCTAGGCTTTTGCAAAAAGCTTCGACGGT
       Consensus  (9201) GTGAGGAGGCTTTTTTGGAGGCCTAGGCTTTTGCAAAAAGCTTCGACGGT
                         9251                                              9300
IL13(EQ)41BBZeta  (8852) ATCGATTGGCTCATGTCCAACATTACCGCCATGTTGACATTGATTATTGA
 CD19Rop_epHIV7   (8069) ATCGATTGGCTCATGTCCAACATTACCGCCATGTTGACATTGATTATTGA
       Consensus  (9251) ATCGATTGGCTCATGTCCAACATTACCGCCATGTTGACATTGATTATTGA
                         9301                                              9350
IL13(EQ)41BBZeta  (8902) CTAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCATAGCCCATAT
 CD19Rop_epHIV7   (8119) CTAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCATAGCCCATAT
       Consensus  (9301) CTAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCATAGCCCATAT
                         9351                                              9400
IL13(EQ)41BBZeta  (8952) ATGGAGTTCCGCGTTACATAACTTACGGTAAATGGCCCGCCTGGCTGACC
 CD19Rop_epHIV7   (8169) ATGGAGTTCCGCGTTACATAACTTACGGTAAATGGCCCGCCTGGCTGACC
       Consensus  (9351) ATGGAGTTCCGCGTTACATAACTTACGGTAAATGGCCCGCCTGGCTGACC
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                          9401                                                9450
IL13(EQ)41BBZeta   (9002) GCCCAACGACCCCCGCCCATTGACGTCAATAATGACGTATGTTCCCATAG
 CD19Rop_epHIV7    (8219) GCCCAACGACCCCCGCCCATTGACGTCAATAATGACGTATGTTCCCATAG
       Consensus   (9401) GCCCAACGACCCCCGCCCATTGACGTCAATAATGACGTATGTTCCCATAG
                          9451                                                9500
IL13(EQ)41BBZeta   (9052) TAACGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGAGTATTTACGG
 CD19Rop_epHIV7    (8269) TAACGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGAGTATTTACGG
       Consensus   (9451) TAACGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGAGTATTTACGG
                          9501                                                9550
IL13(EQ)41BBZeta   (9102) TAAACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCC
 CD19Rop_epHIV7    (8319) TAAACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCC
       Consensus   (9501) TAAACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCC
                          9551                                                9600
IL13(EQ)41BBZeta   (9152) CCCTATTGACGTCAATGACGGTAAATGGCCCGCCTGGCATTATGCCCAGT
 CD19Rop_epHIV7    (8369) CCCTATTGACGTCAATGACGGTAAATGGCCCGCCTGGCATTATGCCCAGT
       Consensus   (9551) CCCTATTGACGTCAATGACGGTAAATGGCCCGCCTGGCATTATGCCCAGT
                          9601                                                9650
IL13(EQ)41BBZeta   (9202) ACATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTC
 CD19Rop_epHIV7    (8419) ACATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTC
       Consensus   (9601) ACATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTC
                          9651                                                9700
IL13(EQ)41BBZeta   (9252) ATCGCTATTACCATGGTGATGCGGTTTTGGCAGTACATCAATGGGCGTGG
 CD19Rop_epHIV7    (8469) ATCGCTATTACCATGGTGATGCGGTTTTGGCAGTACATCAATGGGCGTGG
       Consensus   (9651) ATCGCTATTACCATGGTGATGCGGTTTTGGCAGTACATCAATGGGCGTGG
                          9701                                                9750
IL13(EQ)41BBZeta   (9302) ATAGCGGTTTGACTCACGGGGATTTCCAAGTCTCCACCCCATTGACGTCA
 CD19Rop_epHIV7    (8519) ATAGCGGTTTGACTCACGGGGATTTCCAAGTCTCCACCCCATTGACGTCA
       Consensus   (9701) ATAGCGGTTTGACTCACGGGGATTTCCAAGTCTCCACCCCATTGACGTCA
                          9751                                                9800
IL13(EQ)41BBZeta   (9352) ATGGGAGTTTGTTTTGGCACCAAAATCAACGGGACTTTCCAAAATGTCGT
 CD19Rop_epHIV7    (8569) ATGGGAGTTTGTTTTGGCACCAAAATCAACGGGACTTTCCAAAATGTCGT
       Consensus   (9751) ATGGGAGTTTGTTTTGGCACCAAAATCAACGGGACTTTCCAAAATGTCGT
                          9801                                                9850
IL13(EQ)41BBZeta   (9402) AACAACTCCGCCCCATTGACGCAAATGGGCGGTAGGCGTGTACGGAATTC
 CD19Rop_epHIV7    (8619) AACAACTCCGCCCCATTGACGCAAATGGGCGGTAGGCGTGTACGGAATTC
       Consensus   (9801) AACAACTCCGCCCCATTGACGCAAATGGGCGGTAGGCGTGTACGGAATTC
                          9851                                                9900
IL13(EQ)41BBZeta   (9452) GGAGTGGCGAGCCCTCAGATCCTGCATATAAGCAGCTGCTTTTTGCCTGT
 CD19Rop_epHIV7    (8669) GGAGTGGCGAGCCCTCAGATCCTGCATATAAGCAGCTGCTTTTTGCCTGT
       Consensus   (9851) GGAGTGGCGAGCCCTCAGATCCTGCATATAAGCAGCTGCTTTTTGCCTGT
                          9901      9914
IL13(EQ)41BBZeta   (9502) ACTGGGTCTCTCTG
 CD19Rop_epHIV7    (8719) ACTGGGTCTCTCTG
       Consensus   (9901) ACTGGGTCTCTCTG
```

FIGURE 19

IL13(EmY)-CD8h3-CD8tm2-41BB-Zeta

MLLLVTSLLLCELPHPAFLLIPGPVPPSTALRYLIEELVNITQNQKAPLCNGSMVWSINLTAGM

GMCSFRa signal peptide IL13(EmY)

YCAALESLINVSGCSAIEKTQRMLSGFCPHKVSAGQFSSLHVRDTKIEVAQFVKDLLLHLKKLF

REGRFNAKPTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAG

CD8hinge (48 aa) CD8tm(2)

TCGVLLLSLVITLYKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELGGGRVKFS

4-1BB cyto CD3ζ

RSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQK

DKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

GMCSFRa signal peptide
IL13(EmY)
CD8hinge
CD8 transmembrane (2)
4-1BB cyto
(Gly)3
Zeta

FIGURE 20

IL13(EmY)-CD8h3-CD28tm-CD28gg-41BB-Zeta

MLLLVTSLLLCELPHPAFLLIPGPVPPSTALRYLIEELVNITQNQKAPLCNGSMVWSINLTAGM

GMCSFRa signal peptide IL13(EmY)

YCAALESLINVSGCSAIEKTQRMLSGFCPHKVSAGQFSSLHVRDTKIEVAQFVKDLLLHLKKLF

REGRFNAKPTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDFWVLVVVG

CD8 hinge (48 aa) CD28tm

GVLACYSLLVTVAFIIFWVRSKRSRGGHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSG

CD28gg

GGKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELGGGRVKFSRSADAPAYQ

4-1BB cyto CD3ζ

QGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEI

GMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

GMCSFRa signal peptide
IL13(EmY)
CD8hinge
CD28 transmembrane
CD28gg
4-1BB cyto
(Gly)3
Zeta

FIGURE 21

IL13(EmY)-IgG4(HL-CH3)-CD4tm-41BB-Zeta

MLLLVTSLLLCELPHPAFLLIPGPVPPSTALRYLIEELVNITQNQKAPLCNGSMVWSINLTAGM
GMCSFRa signal peptide IL13(EmY)

YCAALESLINVSGCSAIEKTQRMLSGFCPHKVSAGQFSSLHVRDTKIEVAQFVKDLLLHLKKLF

REGRFNESKYGPPCPPCPGGGSSGGGSGGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFY
IgG4Hinge Linker IgG4-CH3

PSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHN

HYTQKSLSLSLGKMALIVLGGVAGLLLFIGLGIFFKRGRKKLLYIFKQPFMRPVQTTQEEDGCS
CD4 tm 4-1BB cyto

CRFPEEEEGGCELGGGRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPE
CD3ζ

MGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDA

LHMQALPPR

GMCSFRa signal peptide
IL13(EmY)
IgG4Hinge
Linker
IgG4-Fc-CH3
CD4 transmembrane
4-1BB cyto
(Gly)3
Zeta

FIGURE 22

IL13(EmY)-IgG4(L235E,N297Q)-CD8tm-41BB-Zeta

MLLLVTSLLLCELPHPAFLLIPGPVPPSTALRYLIEELVNITQNQKAPLCNGSMVWSINLTAGM
GMCSFRa signal peptide IL13(EmY)

YCAALESLINVSGCSAIEKTQRMLSGFCPHKVSAGQFSSLHVRDTKIEVAQFVKDLLLHLKKLF

REGRFNESKYGPPCPPCPAPEFEGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQF
IGgG4-Fc(SmP)

NWYVDGVEVHNAKTKPREEQFQSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTIS

KAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVL

DSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGKIYIWAPLAGTCGV
CD8 tm

LLLSLVITKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELGGGRVKFSRSADAP
4-1BB cyto CD3ζ

AYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEA

YSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

GMCSFRa signal peptide
IL13(EmY)
IgG4-Fc(SmP)
CD8 transmembrane
4-1BB cyto
(Gly)3
Zeta

FIGURE 23

IL13(EmY)-Linker-CD28tm-CD28gg-41BB-Zeta

MLLLVTSLLLCELPHPAFLLIPGPVPPSTALRYLIEELVNITQNQKAPLCNGSMVWSINLTAGM
GMCSFRa signal peptide IL13(EmY)

YCAALESLINVSGCSAIEKTQRMLSGFCPHKVSAGQFSSLHVRDTKIEVAQFVKDLLLHLKKLF

REGRFNGGGSSGGGSGMFWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRGGHSDYMNM
Linker CD28(M) tm CD28gg

TPRRPGPTRKHYQPYAPPRDFAAYRSGGGKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFP
4-1BB cyto

EEEEGGCELGGGRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGK
CD3ζ

PRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQ

ALPPR

GMCSFRa signal peptide
IL13(EmY)
Linker
CD28(M) transmembrane
CD28gg
4-1BB cyto
(Gly)3
Zeta

FIGURE 24

IL13(EmY)-HL-CD28m-CD28gg-41BB-Zeta

MLLLVTSLLLCELPHPAFLLIPGPVPPSTALRYLIEELVNITQNQKAPLCNGSMVWSINLTAGM
GMCSFRa signal peptide IL13(EmY)

YCAALESLINVSGCSAIEKTQRMLSGFCPHKVSAGQFSSLHVRDTKIEVAQFVKDLLLHLKKLF

REGRFNESKYGPPCPPCPGGGSSGGGSGMFWVLVVVGGVLACYSLLVTVAFIIFWVRSKRS
IgG4Hinge Linker CD28(M) tm
CD28gg

RGGHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSGGGKRGRKKLLYIFKQPFMRPVQT
4-1BB cyto

TQEEDGCSCRFPEEEEGGCELGGGRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDK
CD3ζ

RRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTA

TKDTYDALHMQALPPR

GMCSFRa signal peptide
IL13(EmY)
IgG4Hinge
Linker
CD28(M) transmembrane
CD28gg
4-1BB cyto
(Gly)3
Zeta

Figure 25

IL13(EmY)-IgG4(HL-CH3)-CD28tm-CD28gg-41BB-Zeta

MLLLVTSLLLCELPHPAFLLIPGPVPPSTALRYLIEELVNITQNQKAPLCNGSMVWSINLTAGM
GMCSFRa signal peptide IL13(EmY)

YCAALESLINVSGCSAIEKTQRMLSGFCPHKVSAGQFSSLHVRDTKIEVAQFVKDLLLHLKKLF

REGRFNESKYGPPCPRCPGGGSSGGGSGGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFY
IgG4Hinge Linker IgG4 CH3

PSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHN

HYTQKSLSLSLGKMFWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRGGHSDYMNMTPRRP
CD28(M) tm CD28gg

GPTRKHYQPYAPPRDFAAYRSGGGKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEG
4-1BB cyto

GCELGGGRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRK
CD3ζ

NPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPP

R

GMCSFRa signal peptide
IL13(EmY)
IgG4Hinge
Linker
IgG4 CH3
CD28 transmembrane
CD28gg
4-1BB cyto
(Gly)3
Zeta

FIGURE 26

IL13(EmY)-IgG4(L235E,N297Q)-CD28tm-CD28gg-41BB-Zeta

MLLLVTSLLLCELPHPAFLLIPGPVPPSTALRYLIEELVNITQNQKAPLCNGSMVWSINLTAGM
GMCSFRa signal peptide IL13(EmY)

YCAALESLINVSGCSAIEKTQRMLSGFCPHKVSAGQFSSLHVRDTKIEVAQFVKDLLLHLKKLF

REGRFNESKYGPPCPPCPAPEFEGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQF
IgG4-Fc(L235E,N297Q)

NWYVDGVEVHNAKTKPREEQFQSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTIS

KAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVL

DSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGKMFWVLVVVGGV
CD28(M) tm

LACYSLLVTVAFIIFWVRSKRSRGGHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSGGG
CD28gg

KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELGGGRVKFSRSADAPAYQQG
4-1BB cyto CD3ζ

QNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIG

MKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

GMCSFRa signal peptide
IL13(EmY)
IgG4-Fc(L235E,N297Q)
CD28 (M) transmembrane
CD28gg
(Gly)3
4-1BB cyto
(Gly)3
Zeta

FIGURE 27

IL13(EmY)-CD8h3-CD8tm-41BB-Zeta

MLLLVTSLLLCELPHPAFLLIPGPVPPSTALRYLIEELVNITQNQKAPLCNGSMVWSINLTAGM

GMCSFRa signal peptide IL13(EmY)

YCAALESLINVSGCSAIEKTQRMLSGFCPHKVSAGQFSSLHVRDTKIEVAQFVKDLLLHLKKLF

REGRFNAKPTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAG

CD8hinge (48 aa) CD8tm

TCGVLLLSLVITGGGKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELGGGRVK

4-1BB cyto CD3ζ

FSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQ

KDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

GMCSFRa signal peptide
IL13(EmY)
CD8hinge
CD8 transmembrane
(Gly)3
4-1BB cyto
(Gly)3
Zeta

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2015/051089

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K14/715 C07K14/54 C07K19/00 C12N15/867
ADD. C12N5/0783

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 C12N

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, EMBL, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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X	S. KONG ET AL: "Suppression of Human Glioma Xenografts with Second-Generation IL13R-Specific Chimeric Antigen Receptor-Modified T Cells", CLINICAL CANCER RESEARCH, vol. 18, no. 21, 1 November 2012 (2012-11-01), pages 5949-5960, XP055242650, US ISSN: 1078-0432, DOI: 10.1158/1078-0432.CCR-12-0319 whole document, especially page 5950 right column paragraph 3. ----- -/--	1-4,7,8,10,11

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
20 January 2016	29/01/2016

Name and mailing address of the ISA/	Authorized officer
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1

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2015/051089

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