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(54) Title: CS1 TARGETED CHIMERIC ANTIGEN RECEPTOR-MODIFIED T CELLS

(57) Abstract: Chimeric antigen receptors for use in treating malignant melanoma and other cancers expressing CS1 are described.

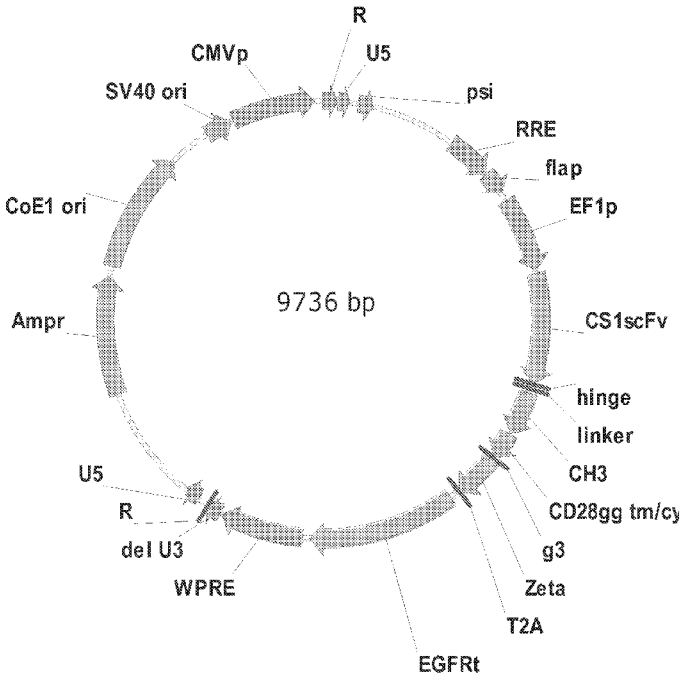

FIGURE 1

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CS1 TARGETED CHIMERIC ANTIGEN RECEPTOR-MODIFIED T CELLS

CROSS-REFERENCE TO RELATED APPLICATIONS

[001] This application claims the benefit of U.S. Non-Provisional Application No. 62/088,423, filed December 5, 2014, entitled "USE OF CENTRAL MEMORY DERIVED-CS1 CHIMERIC ANTIGEN RECEPTORMODIFIED T CELLS TO TREAT MULTIPLE MYELOMA", the contents of which are incorporated herein in its entirety.

BACKGROUND

[002] 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 antitumor activity 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.

[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 various cancers, since CAR T cells can be engineered to specifically recognize antigenically-distinct tumor populations in an MHC-independent manner.

[004] Multiple myeloma (MM) is a B cell malignancy characterized by clonal expansion of plasma cells. MM accounts for approximately 1 percent of all cancers and slightly more than 10 percent of hematologic malignancies in the United States. In the United States alone, approximately 20,000 new cases will be diagnosed this year and over 11,000 people will die from this disease. Current therapies for MM often induce remission, but nearly all patients eventually relapse and die.

[005] CS1 is a cell surface glycoprotein of the signaling lymphocyte activation molecule (SLAM) receptor family that is highly and selectively expressed on normal plasma cells and MM cells, with lower expression on NK cells and little or no expression on normal tissues. Elotuzumabc (HuLuc63), a humanized CS1 monoclonal antibody given together with bortezomib in patients with relapsed MM produces ≥ PR in 48% of patients. The high expression of CS1 on MM cells, coupled with its restriction to plasma cells in normal tissue, makes CS1 a reasonable target for CAR T cell therapy (Hsi et al. 2008 *Clin Cancer Res* 14:2775).

SUMMARY

[006] Described herein are CARs which comprise an extracellular domain, a transmembrane domain and an intracellular signaling domain. The extracellular domain includes a CS1-specific scFv region or a variant thereof and, optionally, a spacer, comprising, for example, a portion of human Fc domain. The extracellular domain enables the CAR, when expressed on the surface of a T cell, to direct T cell activity to cells expressing CS1, a receptor expressed on the surface of MM. The transmembrane domain includes, for example, a CD4 transmembrane domain, a CD8 transmembrane domain, a CD28 transmembrane domain, or a CD3 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, for example, a 4-1BB costimulatory domain. 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, including both alpha beta T cells and gamma delta T cells, 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 T cell or an allogenic T cell. In some cases the cells used are a cell population that includes both CD4+ and CD8+ central memory T cells (T_{CM}), which are CD62L+, CCR7+, CD45RO+, and CD45RA-. The cell population can include other types of T cells as well.

[007] The CS1 CAR described herein has certain beneficial characteristics, e.g., persistence and enhanced antitumor activity following adoptive transfer.

[008] T cells expressing a CAR targeting CS1 can be useful in treatment of cancers such as MM, as well as other cancers that express CS1. Thus, this disclosure includes methods for treating CS1 expressing cancer using T cells expressing a CAR described herein.

[009] Described herein is a nucleic acid molecule encoding a CAR comprising: a CS1 scFv (e.g., EVQLVESGGGLVQPGGSLRLSCAASGFDFSRYWMSWVRQAPGKGLEWIGEINP DSSTINYAPSLKDKFIISRDNAKNSLYLQMNSLRAEDTAVYYCARPDGNYWYFD VWGQGTLVTVSSGSTSGGGSGGGSGGGGSSDIQMTQSPSSLSASVGDRVTITCK ASQDVGIAVAWYQQKPGKVPKLLIYWASTRHTGVPDRFSGSGSGTDFTLTISSLQ PEDVATYYCQQYSSYPYTFGQGTKVEIK; SEQ ID NO:1) or a variant thereof having 1-5 (e.g., 1 or 2) amino acid modifications (e.g., substitutions); a transmembrane domain selected from: a CD4 transmembrane domain or variant thereof having 1-5 (e.g., 1 or 2) amino acid modifications (e.g., substitutions), a CD8 transmembrane domain or variant thereof having 1-5 (e.g., 1 or 2) amino acid modifications (e.g., substitutions), a CD28 transmembrane domain or a variant thereof having 1-5 (e.g., 1 or 2) amino acid modifications (e.g., substitutions), and a CD3ζ transmembrane domain or a variant thereof having 1-5 (e.g., 1 or 2) amino acid modifications (e.g., substitutions); a costimulatory domain (e.g., a CD28 co-stimulatory domain or a variant thereof having 1-5 (e.g., 1 or 2) amino acid modifications (e.g., substitutions); or a 4-1BB co-stimulatory domain or a variant thereof having 1-5 (e.g., 1 or 2) amino acid modifications (e.g., substitutions); or both a CD28 co-stimulatory domain or a variant thereof having 1-5 (e.g., 1 or 2) amino acid modifications (e.g., substitutions) and a 4-1BB co-stimulatory domain or a variant thereof having 1-5 (e.g., 1 or 2) amino acid modifications (e.g., substitutions); and a CD3ζ signaling domain or a variant thereof having 1-5 (e.g., 1 or 2) amino acid modifications.

[0010] 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 cells that express any of the CARs described herein.

[0011] Described herein is a nucleic acid molecule encoding a chimeric antigen receptor, wherein chimeric antigen receptor comprises: a CS1 scFv; a spacer region; a CD28 or CD4 transmembrane domain, a CD28 costimulatory domain or a 4-IBB costimulatory domain, an optional GlyGlyGly linker, and a CD3 ζ signaling domain.

[0012] In one embodiment, the CS1 CAR consists of or comprises the amino acid sequence of any of SEQ ID NOs:31, 34, 37, 40, 43, and 46 (mature CAR lacking a signal sequence) or the CS1 CAR consists of or comprises the amino acid sequence of any of SEQ ID NOs:30, 33, 36, 39, 42, and 45 (immature CAR having a GMCSFRa signal sequence). The CAR and can be expressed in a form that includes a signal sequence, e.g., a human GM-CSF receptor alpha signal sequence (MLLLVTSLLLCELPHPAFLLIP; SEQ ID NO:26). The CAR can be expressed with additional sequences that are useful for monitoring expression, for example a T2A skip sequence and a truncated EGFRt. Thus, the CAR can comprise or consist of the amino acid sequence of any of SEQ ID Nos: 29-46 or can comprise or consist of an amino acid sequence that is at least 95%, 96%, 97%, 98% or 99% identical to any of SEQ ID Nos: 29-46. The CAR can comprise or consist of the amino acid sequence of any of SEQ ID Nos: 29-46 with up to 1, 2, 3, 4 or 5 amino acid changes (preferably conservative amino acid changes).

[0013] Also disclosed is a population of human T cells transduced by a vector comprising an expression cassette encoding a CS1 chimeric antigen receptor described herein (e.g., a CAR that comprises or consists of the amino acid sequence of any of SEQ ID Nos: 29-46 or an amino acid sequence that is at least 95%, 96%, 97%, 98% or 99% identical to any of SEQ ID Nos: 29-46 or the amino acid sequence of any of SEQ ID Nos: 29-46 with up to 1, 2, 3, 4 or 5 amino acid changes (preferably conservative amino acid changes).

[0014] In various embodiments: the population of human T cells are central memory T cells (Tcm), e.g., CD8+/CD4+ Tcm.

[0015] 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.

CS1 ScFv Domain

[0016] The CS1 ScFv domain can be any ScFv that binds CS1. In some cases the CS1 ScFv domain includes a sequence that is at least 90%, at least 95%, at least 98% identical to or identical to SEQ ID NO:1. In some cases the CS1 scFv has 1, 2, 3, 4 of 5 amino acid changes (preferably conservative) compared to SEQ ID NO:1. The ScFv can be a humanized ScFv.

Spacer Region

[0017] The CAR described herein can include a spacer region located between the CS1targeting domain (i.e., a CS1 ScFv or variant thereof) 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:2)
IgG4 hinge (S→P) (S228P)	12 aa	ESKYGPPCPPCP (SEQ ID NO:3)
IgG4 hinge	12 aa	ESKYGPPCPSCP (SEQ ID NO:4)
IgG4 hinge (S228P)+ linker	22 aa	ESKYGPPCPPCPGGGSSGGGSG (SEQ ID NO:5)
CD28 hinge	39 aa	IEVMYPPPYLDNEKSNGTIIHVKGKHL CPSPLFPGPSKP (SEQ ID NO:6)
CD8 hinge-48aa	48 aa	AKPTTTPAPRPPTPAPTIASQPLSLRPE ACRPAAGGAVHTRGLDFACD (SEQ ID NO:7)
CD8 hinge-45aa	45aa	TTTPAPRPPTPAPTIASQPLSLRPEACR PAAGGAVHTRGLDFACD (SEQ ID NO:8)
IgG4(HL-CH3) (includes S228P in hinge)	129 aa	ESKYGPPCPPCPGGGSSGGGSGGQPR EPQVYTLPPSQEEMTKNQVSLTCLVK GFYPSDIAVEWESNGQPENNYKTTPP VLDSDGSFFLYSRLTVDKSRWQEGNV FSCSVMHEALHNHYTQKSLSLSLGK (SEQ ID NO:9)
IgG4(L235E,N297Q)	229 aa	ESKYGPPCPSCPAPEFEGGPSVFLFPPK PKDTLMISRTPEVTCVVVDVSQEDPE VQFNWYVDGVEVHQAKTKPREEQFQ STYRVVSVLTVLHQDWLNGKEYKCK VSNKGLPSSIEKTISKAKGQPREPQVY TLPPSQEEMTKNQVSLTCLVKGFYPS DIAVEWESNGQPENNYKTTPPVLDSD GSFFLYSRLTVDKSRWQEGNVFSCSV

		MHEALHNHYTQKSLSLSLGK (SEQ ID NO:10)
IgG4(S228P, L235E,N297Q)	229 aa	ESKYGPPCPPCPAPEFEGGPSVFLFPPK PKDTLMISRTPEVTCVVVDVSQEDPE VQFNWYVDGVEVHQAKTKPREEQFQ STYRVVSVLTVLHQDWLNGKEYKCK VSNKGLPSSIEKTISKAKGQPREPQVY TLPPSQEEMTKNQVSLTCLVKGFYPS DIAVEWESNGQPENNYKTTPPVLDSD GSFFLYSRLTVDKSRWQEGNVFSCSV MHEALHNHYTQKSLSLSLGK (SEQ ID NO:11)
IgG4(CH3)	107 aa	GQPREPQVYTLPPSQEEMTKNQVSLT CLVKGFYPSDIAVEWESNGQPENNYK TTPPVLDSDGSFFLYSRLTVDKSRWQ EGNVFSCSVMHEALHNHYTQKSLSLS LGK (SEQ ID NO:12)

[0018] 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.

[0019] In certain embodiments the spacer is a hinge/linger 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 hinge. 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 hinge of the hinge/liker 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.

[0021] In some embodiments, the modified hinge is derived from a human IgG4 hinge/CH2/CH3 region having the amino acid sequence of SEQ ID NO: 10 or 11 or an amino acid sequence that is at least 90%, at least 95%, at least 98% identical to SEQ ID NO:10 or 11.

[0022] In certain embodiments, the modified hinge is derived from IgG4 that includes one or more amino acid residues substituted with an amino acid residue different from that present in an unmodified hinge. 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.

[0023] In some embodiments, the modified hinge is derived from an IgG4 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 hinge is substituted with the above identified amino acids at the indicated position. In one instance the sequence includes the following amino acid changes S228P, L235E and N297Q.

[0024] 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).

[0025] The hinge/linker region can also comprise a IgG4 hinge region having the sequence ESKYGPPCPSCP (SEQ ID NO:4) or ESKYGPPCPPCP (SEQ ID NO:3).

[0026] The hinge/linger region can also comprise the sequence ESKYGPPCPPCP (SEQ ID NO:3) followed by the linker sequence GGGSSGGGSG (SEQ ID NO:2) followed by IgG4 CH3 sequence GQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTT PPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK (SEQ ID NO:12). Thus, the entire linker/spacer region can comprise the sequence: ESKYGPPCPPCPGGGSSGGGSGGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFY PSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSV MHEALHNHYTQKSLSLSLGK (SEQ ID NO:11). In some cases the spacer has 1,2,3,4, or 5 single amino acid changes (e.g., conservative changes) compared to SEQ ID NO:11. In some cases, the IgG4 Fc hinge/linker region that is mutated at two positions (L235E; N297Q) in a manner that reduces binding by Fc receptors (FcRs).

Transmembrane Region

[0027] A variety of transmembrane domains can be used in the. **Table 2** includes examples of suitable transmembrane domains. Where a spacer region is present, the transmembrane domain is located carboxy terminal to the spacer region.

Table 2: Examples of Transmembrane Domains

Name	Accession	Length	Sequence
CD3z	J04132.1	21 aa	LCYLLDGILFIYGVILTALFL (SEQ ID NO:13)
CD28	NM_006139	27aa	FWVLVVVGGVLACYSLLVTVAFIIFWV (SEQ ID NO:14)

CD28(M)	NM_006139	28aa	MFWVLVVVGGVLACYSLLVTVAFIIFWV (SEQ ID NO:15)
CD4	M35160	22aa	MALIVLGGVAGLLLFIGLGIFF (SEQ ID NO:16)
CD8tm	NM_001768	21aa	IYIWAPLAGTCGVLLLSLVIT (SEQ ID NO:17)
CD8tm2	NM_001768	23aa	IYIWAPLAGTCGVLLLSLVITLY (SEQ ID NO:18)
CD8tm3	NM_001768	24aa	IYIWAPLAGTCGVLLLSLVITLYC (SEQ ID NO:19)
41BB	NM_001561	27aa	IISFFLALTSTALLFLLFF LTLRFSVV (SEQ ID NO:20)

Costimulatory Domain

[0028] The costimulatory domain can be any domain that is suitable for use with a CD3ζ signaling domain. In some cases the costimulatory domain is a CD28 costimulatory domain that includes a sequence that is at least 90%, at least 95%, at least 98% identical to or identical to: RSKRSRGGHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRS (SEQ ID NO:23; LL to GG amino acid change double underlined). In some cases the CD28 co-signaling domain has 1, 2, 3, 4 of 5 amino acid changes (preferably conservative and preferably not in the underlined GG sequence) compared to SEQ ID NO:23. In some cases the co-signaling domain is a 4-1BB co-signaling domain that includes a sequence that is at least 90%, at least 95%, at least 98% identical to or identical to: KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCEL (SEQ ID NO:24). In some cases the 4-1BB co-signaling domain has 1, 2, 3, 4 of 5 amino acid changes (preferably conservative) compared to SEQ ID NO:24.

[0029] 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: CD3ζ Domain and Examples of Costimulatory Domains

Name	Accession	Length	Sequence
CD3ζ	J04132.1	113 aa	RVKFSRSADAPAYQQGQNQLYNELNLGR REEYDVLDKRRGRDPEMGGKPRRKNPQ EGLYNELQKDKMAEAYSEIGMKGERRR GKGHDGLYQGLSTATKDTYDALHMQAL PPR (SEQ ID NO:21)
CD28	NM_006139	42aa	RSKRSRLLHSDYMNMTPRRPGPTRKHYQ PYAPPRDFAAYRS (SEQ ID NO: 22)
CD28gg*	NM_006139	42aa	RSKRSRGGHSDYMNMTPRRPGPTRKHY QPYAPPRDFAAYRS (SEQ ID NO:23)
41BB	NM_001561	42 aa	KRGRKKLLYIFKQPFMRPVQTTQEEDGC SCRFPEEEEGGCEL (SEQ ID NO:24)
OX40		42 aa	ALYLLRRDQRLPPDAHKPPGGGSFRTPIQ EEQADAHSTLAKI (SEQ ID NO:25)

[0030] In various embodiments: the costimulatory domain is selected from the group consisting of: a costimulatory domain depicted in **Table 3** or a variant thereof having 1-5 (e.g., 1 or 2) amino acid modifications, a CD28 costimulatory domain or a variant thereof having 1-5 (e.g., 1 or 2) amino acid modifications, a 4-1BB costimulatory domain or a variant thereof having 1-5 (e.g., 1 or 2) amino acid modifications and an OX40 costimulatory domain or a variant thereof having 1-5 (e.g., 1 or 2) amino acid modifications. In certain embodiments, a 4-1BB costimulatory domain or a variant thereof having 1-5 (e.g., 1 or 2) amino acid modifications in present. In some embodiments there are two costimulatory domains, for example a CD28 co-stimulatory domain or a variant thereof having 1-5 (e.g., 1 or 2) amino acid modifications (e.g., substitutions) and a 4-1BB co-stimulatory domain or a variant thereof having 1-5 (e.g., 1 or 2) amino acid modifications (e.g., substitutions). In various embodiments the 1-5 (e.g., 1 or 2) amino acid modification are substitutions. The costimulatory domain is amino terminal to the CD3ζ signaling domain and in some cases a short linker consisting of 2 – 10, e.g., 3 amino acids (e.g., GGG) is positioned between the costimulatory domain and the CD3ζ signaling domain.

CD3ζ Signaling Domain

[0031] The CD3ζ Signaling domain can be any domain that is suitable for use with a CD3ζ signaling domain. In some cases the CD3ζ signaling domain includes a sequence that is at least 90%, at least 95%, at least 98% identical to or identical to: RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRK NPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDAL HMQALPPR (SEQ ID NO:21). In some cases the CD3ζ signaling has 1, 2, 3, 4 of 5 amino acid changes (preferably conservative) compared to SEQ ID NO:21.

Truncated EGFR

[0032] The CD3ζ signaling domain can be followed by a ribosomal skip sequence (e.g., LEGGGEGRGSLLTCGDVEENPGPR; SEQ ID NO:27) and a truncated EGFR having a sequence that is at least 90%, at least 95%, at least 98% identical to or identical to: LVTSLLLCELPHPAFLLIPRKVCNGIGIGEFKDSLSINATNIKHFKNCTSISGDLHIL PVAFRGDSFTHTPPLDPQELDILKTVKEITGFLLIQAWPENRTDLHAFENLEIIRGR TKQHGQFSLAVVSLNITSLGLRSLKEISDGDVIISGNKNLCYANTINWKKLFGTSG QKTKIISNRGENSCKATGQVCHALCSPEGCWGPEPRDCVSCRNVSRGRECVDKC NLLEGEPREFVENSECIQCHPECLPQAMNITCTGRGPDNCIQCAHYIDGPHCVKT CPAGVMGENNTLVWKYADAGHVCHLCHPNCTYGCTGPGLEGCPTNGPKIPSIA TGMVGALLLLLVVALGIGLFM (SEQ ID NO:28). In some cases the truncated EGFR has 1, 2, 3, 4 of 5 amino acid changes (preferably conservative) compared to SEQ ID NO:28.

CS1 CAR

[0033] The CS1 CAR can include a sequence that is at least 90%, at least 95%, at least 98% identical to or identical to the amino acid sequence depicted in Figure 2, Figure 6, Figure 7, Figure 8. Figure 9 or Figure 10 (SEQ ID Nos: 29-46; either including or excluding the GMCSFRa signal sequence and either including or excluding the T2A ribosomal skip sequence and the truncated EGFRt).

[0034] Among the CAR targeting CS1 described herein are those summarized in **Table 4** in which the spacer region, transmembrane domain and costimulatory domain(s) for each CAR are indicated.

Table 4: Examples of CAR Targeting CS1

Name	SEQ ID NO*	FIG	Spacer	TM	Costimulatory Domain(s)
CS1scFv-IgG4(HL-CH3)-CD28tm-CD28gg-Zeta-T2A-EGFRt.	29//30//31	**2**	IgG4(HL-CH3)	CD28	CD28GG
CS1scFv-IgG4(HL-CH3)-CD4tm-41BB-Zeta-T2A-EGFRt.	32//33//34	**6**	IgG4(HL-CH3)	CD4	4-IBB
CS1scFv-IgG4(L235E,N297Q)-CD4tm-41BB-Zeta-T2A-EGFRt.	35//36//37	**7**	IgG4(L235E,N297Q)	CD4	4-IBB
CS1scFv-IgG4(L235E, N297Q)-CD28tm-CD28gg-Zeta-T2A-EGFRt	38//39//40	**8**	IgG4(L235E,N297Q)	CD28	CD28GG
CS1scFv-Linker-CD4tm-41BB-Zeta-T2A-EGFRt.	41//42//43	**9**	L	CD4	4-IBB
CS1scFv-Linker-CD28tm-CD28gg-Zeta-T2A-EGFRt	44//45//46	**10**	L	CD28	CD28GG

*SEQ ID NOs for: entire sequence depicted including GMCSFRa signal sequence, T2A and EGFRt //sequence including GMCSFRa signal sequence but excluding T2A and EGFRt // sequence for sequence excluding GMCSFRa signal sequence, T2A and EGFRt.

DESCRIPTION OF DRAWINGS

[0035] Figure 1 is a schematic depiction of a CS1 CAR expressing lentiviral vector (CS1scFv-IgG4(HL-CH3)-CD28gg-Zeta(CO)-T2A-EGFRt_epHIV7). The CS1 CAR construct includes: a GMCSF signal sequence, CS1 scFv, IgG4 hinge region, linker, CH3 domain, a CD28 co-stimulatory domain and CD3ζ Signaling domain. The CAR construct

is followed by a T2A ribosomal skip sequence, and then suicide gene EGFRt coding sequence. The CAR and EGFRt molecules are expressed from a single transcript.

[0036] Figure 2 depicts the amino acid sequence of a CS1 CAR that includes signal peptide, a ribosomal skip sequence and an EGFRt (SEQ ID NO:29).

[0037] Figure 3 is a pair of graphs depicting the results of studies showing that CS1 CAR re-directed Tcm exhibited cytotoxicity against MM cells. Cytotoxicity of the propagated CS1 CAR T cells was evaluated using 4-hour 51Cr release assays after co-culture with 51Cr-labeled target cells. OKT3 expressing LCLs were used as positive controls since they engage all TCRs, and CS1-negative AML cells (KG1a) were used as negative controls. CS1 CAR, but not un-engineered mock T cells showed specific cytotoxicity against MM cells.

[0038] Figure 4 depicts the results of studies showing that CS1 CAR re-directed Tcm cells exhibited effector function in response to stimulation of MM cells. CS1 CAR T cells (10^5) were co-cultured 6 hours in 96-well tissue culture plates with 10^5 of MM.1S cells as stimulators. 107a degranulation and intracellular IFNgamma production were analyzed with flow cytrometry. The majority of the CAR T cells identified by Erbitux were induced to degranulate after engagement with MM cells and IFNgamma positive cells were detected in respond to antigen stimulation.

[0039] Figure 5 depicts the results of studies showing that CS1 CAR re-directed Tcm cells eradicate multiple myeloma *in vivo*. Approximately $2x10^6$ Firefly luciferase expressing MM.1S cells were inoculated into NSG mice via Intra-tibial injection. 7 days after tumor inoculation, $1x10^6$ CS-1 CAR T cells were infused into the tumor bearing mice by intravenous injection. Tumor burdens were monitored with Xenogen® imaging once a week. Mice that received un-engineered cells were used as control. CS1 CAR T cells completely eradicated MM tumor 14 days post T cell infusion, while un-engineered T cells have no effects on tumor inhibition.

[0040] Figure 6 depicts the amino acid sequence of CS1scFv-IgG4(HL-CH3)-CD4tm-41BB-Zeta-T2A-EGFRt (SEQ ID NO:32).

[0041] Figure 7 depicts the amino acid sequence of CS1scFv-IgG4(L235E,N297Q)-CD4tm-41BB-Zeta-T2A-EGFRt (SEQ ID NO:35).

[0042] Figure 8 depicts the amino acid sequence of CS1scFv-IgG4(L235E, N297Q)-CD28tm-CD28gg-Zeta-T2A-EGFRt (SEQ ID NO:38).

[0043] Figure 9 depicts the amino acid sequence of CS1scFv-Linker-CD4tm-41BB-Zeta-T2A-EGFRt (SEQ ID NO:41).

[0044] Figure 10 depicts the amino acid sequence of CS1scFv-Linker-CD28tm-CD28gg-Zeta-T2A-EGFRt (SEQ ID NO:44).

[0045] Figure 11 is the complete nucleotide sequence of CS1scFv-IgG4(HL-CH3)-CD28gg-Zeta-T2A-EGFRt_epHIV7 (SEQ ID NO: 47).

[0046] Figure 12 depicts the results of studies showing that CS1 CAR re-directed Tcm cells eradicate multiple myeloma in vivo. 2x106 GFPffluc+ MM.1S cells were inoculated via Intra-tibial injection into NSG mice on day -7. 1x106 central memory T cell (Tcm) derived CS1 CAR+ T cells were intravenously infused into the tumor bearing mice on day 0. Mice received no T cells or un-transduced Tcm from the same donor were used as negative controls. Tumor signals were monitored by biophotonic imaging. Means±SEM of phonton/sec from multiple mice are depicted. The CAR were those of Figure 2 (CH2 CD28); Figure 6 (CH2 4IBB); Figure 8 (EQ CD28); Figure 7 (EQ 4IBB); Figure 10 (L CD28) and Figure 9 (L CD4 IBB).

DETAILED DESCRIPTION

[0047] Described below is the structure, construction and characterization of several CS1-specific chimeric antigen receptors ("CAR"). A CAR is a recombinant biomolecule that contains 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 any receptor. "Antigen" thus refers to the recognition domain of the CAR. 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 co-stimulatory 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.

[0048] In some cases, the CS1 CAR can be produced using a vector in which the CAR open reading frame is followed by a T2A ribosome skip sequence and a truncated EGFR (EGFRt), which lacks the cytoplasmic signaling tail. In this arrangement, co-expression of EGFRt provides an inert, non-immunogenic surface marker that allows for accurate measurement of gene modified cells, and enables positive selection of gene-modified cells, as well as efficient cell tracking of the therapeutic T cells in vivo following adoptive transfer. Efficiently controlling proliferation to avoid cytokine storm and off-target toxicity is an important hurdle for the success of T cell immunotherapy. The EGFRt incorporated in the CS1CAR lentiviral vector can act as suicide gene to ablate the CAR+ T cells in cases of treatment-related toxicity.

[0049] The CAR described herein can be produced by any means known in the art, though preferably it is produced using recombinant DNA techniques. Nucleic acids encoding the several regions of the chimeric receptor can be prepared and assembled into a complete coding sequence by standard techniques of molecular cloning known in the art (genomic library screening, overlapping PCR, primer-assisted ligation, site-directed mutagenesis, etc.) as is convenient. The resulting coding region is preferably inserted into an expression vector and used to transform a suitable expression host cell line, preferably a T lymphocyte cell line, and most preferably an autologous T lymphocyte cell line.

[0050] Various T cell subsets isolated from the patient can be transduced with a vector for CAR expression. Central memory T cells are one useful T cell subset. Central memory T cell can be isolated from peripheral blood mononuclear cells (PBMC) by selecting for CD45RO+/CD62L+ cells, using, for example, the CliniMACS® device to immunomagnetically select cells expressing the desired receptors. The cells enriched for central memory T cells can be activated with anti-CD3/CD28, transduced with, for example, a lentiviral vector that directs the expression of an CS1 CAR as well as a non-immunogenic surface marker for in vivo detection, ablation, and potential ex vivo selection. The activated/genetically modified CS1 central memory T cells can be expanded *in vitro* with IL-2/IL-15 and then cryopreserved.

Example 1: Construction and Structure of epHIV7 used for Expression of CS1-specific CAR

[0051] The pHIV7 plasmid is a parent plasmid from which the clinical vectors expressing a CS1 CAR can be derived. The epHIV7 vector used for expression of the CAR was produced from pHIV7 vector (Wang et al. 2011 *Blood* 118:1255). 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.

[0052] Construction of pHIV7 was carried out as follows. 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.

[0053] 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.

[0054] 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.

[0055] 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.

[0056] The CMV promoter was replaced by the EF1α-HTLV promoter (EF1p), and the new plasmid was named epHIV7. The EF1p has 563 bp and was introduced into epHIV7 using NruI and NheI, after the CMV promoter was excised.

[0057] 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, 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. **Table 5** summarizes the various regulator elements present in epHIV7.

[0058] **Figure 1** is a schematic depiction of CS1 CAR (CS1scFv-IgG4(HL-CH3)-CD28gg-Zeta(CO)-T2A-EGFRt_epHIV7), a lentiviral vector containing the CAR construct composed of CS1 scFv, IgG4 hinge region, linker , a CD28 costimulatory domain and CD3ζ Signaling domain. The CAR construct is followed by a T2A ribosomal skip sequence, and then suicide gene EGFRt coding sequence. The CAR and EGFRt molecules are expressed from a single transcript. The entire nucleotide sequence of the vector is presented in **Figure 11** and **Table 5** presents position of various elements of the vector.

Table 5: Functional elements of CS1 CAR_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

Table 5: Functional elements of CS1 CAR_epHIV7		
Regulatory Elements and Genes	**Location (Nucleotide Numbers)**	**Comments**
EF1p Promoter	1524-2067	EF1-alpha Eukaryotic Promoter sequence driving expression of CD19Rop
	2084-4963	Therapeutic insert
WPRE	5011-5611	Woodchuck hepatitis virus derived regulatory element to enhance viral RNA transportation
delU3	5626-5730	3' U3 with deletion to generate SIN vector
R	5731-5811	Repeat sequence within LTR
U5	5812-5925	3' U5 sequence in LTR
Amp^R	6761-7619	Ampicillin-resistance gene
CoE1 ori	7682-8563	Replication origin of plasmid
SV40 ori	8860-=9059	Replication origin of SV40
CMV promoter	9073-9672	CMV promoter to generate viral genome RNA
R	9728-86	Repeat sequence within LTR

Example 2: Production of Vectors for Transduction of Patient T Cells

[0059] For each plasmid (CS1 CAR_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.

[0060] Briefly, cells are 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 is thawed. Cells are 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.

[0061] The lentiviral vector was produced in sub-batches of up to 10 CFs. Two subbatches 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.

[0062] 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%.

[0063] 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).

[0064] To ensure the purity of the lentiviral vector preparation, it is tested for residual host DNA contaminants, and the transfer of residual host and plasmid DNA. Among other tests, vector identity is evaluated by RT-PCR to ensure that the correct vector is present. All release criteria are met for the vector intended for use in this study.

Example 3: Preparation of Tcm cells Suitable for Use in ACT

[0065] T lymphocytes are obtained from a patient by leukopheresis, and the appropriate allogenic or autologous T cell subset, for example, Central Memory T cells (Tcm), are genetically altered to express the CAR, then administered back to the patient by any clinically acceptable means, to achieve anti-cancer therapy.

[0066] Tcm that are CD8+ are isolated essentially as described in Wang et al. (*J Immunology* 35:689, 2012). Briefly, on the day of leukapheresis, PBMC were isolated by density gradient centrifugation over Ficoll-Paque followed by two washes in PBS/EDTA. PBMC were then washed once in PBS, resuspended in X Vivo15 media containing 10%

fetal calf serum (FCS), transferred to a 300 cc transfer bag, and stored on a 3-D rotator overnight at room temperature (RT). The following day, up to $5x10^9$ PBMC were incubated in a 300 cc transfer bag with clinical grade anti-CD4 (2.5 mL), anti-CD14 (1.25 mL), and anti-CD45RA (2.5 mL) microbeads (Miltenyi Biotec) for 30 minutes at RT in X Vivo15 containing 10% FCS. CD4+, CD14+ and CD45RA+ cells were then immediately depleted using the CliniMACS™ depletion mode according to the manufacturer's instructions (Miltenyi Biotec). After centrifugation, the unlabeled negative fraction of cells was resuspended in CliniMACS™ PBS/EDTA buffer (Miltenyi Biotec) containing 0.5% human serum albumin (HSA) and then labeled with clinical grade biotinylated-DREG56 mAb (COHNMC CBG) at 0.1mg/106 cells for 30 minutes at RT. The cells were then washed and resuspended in a final volume of 100 mL CliniMACS™ PBS/EDTA containing 0.5% HSA and transferred into a new 300 cc transfer bag. After 30 minutes incubation with 1.25 mL anti-biotin microbeads (Miltenyi Biotec), the CD62L+ fraction of PBMC (CD8+ TCM) was purified with positive selection on CliniMACS™ according to the manufacturer's instructions, and resuspended in X Vivo15 containing 10% FCS.

[0067] Tcm that are CD8+/CD4+ are prepared using a modification of the forgoing process by modifying the CD4+, CD14+ and CD45RA+ selection to a CD14+ and CD45RA+ selection. The method uses a two-step process on the CliniMACS™ device to first deplete CD14+ and CD45RA+ cells, then to positively select CD62L+ cells. This modified platform generates $50x10^6$ bulk Tcm from a single leukapheresis.

[0068] Following enrichment, Tcm 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 lentiviral vector encoding CS1 CAR at a multiplicity of infection (MOI) of about 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.

[0069] On the day(s) of T cell infusion, the cryopreserved and released product will be thawed, washed and formulated for re-infusion. The cryopreserved vials containing the released cell product will be removed from liquid nitrogen storage, thawed, cooled and washed with a PBS/2% human serum albumin (HSA) Wash Buffer. After centrifugation, the supernatant will be removed and the cells resuspended in a Preservative-Free Normal Saline (PFNS)/ 2% HSA infusion diluent. Samples will be removed for quality control testing.

Example 4: Amino acid Sequence of CS1 CAR (CS1scFv-IgG4(HL-CH3)-CD28tm-CD28gg-Zeta-T2A-EGFRt)

[0070] The complete amino acid sequence of CS1scFv-IgG4(HL-CH3)-CD28tm-CD28gg-Zeta-T2A-EGFRt is depicted in **Figure 2**. The entire sequence (SEQ ID NO:29) includes: a 22 amino acid GMCSF signal peptide (SEQ ID NO:26), a CS1 scFv sequence (SEQ ID NO:1); a IgG4 hinge sequence (SEQ ID NO:3; with amino acid substitutions S to P shaded); a 10 amino acid linker (SEQ ID NO:2); IgG4 CH3 sequence (SEQ ID NO:12); a 28 amino acid CD28 transmembrane domain sequence (SEQ ID NO:14); a CD28gg co-stimulatory domain sequence (SEQ ID NO:23; LL to GG amino acid changes highlighted); a 3 amino acid Gly linker; a 112 amino acid CD3ζ sequence (SEQ ID NO:21); a 24 amino acid T2A skip sequence (SEQ ID NO:27); and EGFRt sequence (SEQ ID NO:28).

Example 5: Activity of CS1 CAR

[0071] Cytotoxicity of the propagated CS1 CAR T cells expressing the CAR shown in **Figure 2** was evaluated using 4-hour 51Cr release assays after co-culture with 51Cr-labeled MM cells (MM.1S). As shown in **Figure 3**, the engineered CS1 CAR T cells exhibit specific and efficient killing of MM cells, while un-transduced mock T cells has no cytocoxicity to MM cells. When co-cultured with MM cells, the engineered CS1 CAR Tcm-mediated strong effector function as indicated by 107a degranulation and

IFNgamma as shown in **Figure 4**. Upon adoptively transferred into MM tumor bearing NSG mice, the CS1 specific T cells exhibited efficient antitumor activity as shown in **Figure 5**.

[0072] In another study with additional CS1 CAR (**Figure 2** and **Figures 6-10**) $2x10^6$ GFPffluc+ MM.1S cells were inoculated via Intra-tibial injection into NSG mice on day -7. $1x10^6$ central memory T cell (Tcm) derived CS1 CAR+ T cells were intravenously infused into the tumor bearing mice on day 0. Mice received no T cells or un-transduced Tcm from the same donor were used as negative controls. Tumor signals were monitored by biophotonic imaging. Means±SEM of phonton/sec from multiple mice are depicted. The results of this analysis are shown in **Figure 12**.

WHAT IS CLAIMED IS:

1. A nucleic acid molecule encoding a chimeric antigen receptor, wherein the chimeric antigen receptor comprises: a CS1 scFv; an optional spacer region; a transmembrane domain; a co-signaling domain; and CD3 ζ signaling domain.

2. The nucleic acid molecule of claim 1 wherein the chimeric antigen receptor further comprises a linker sequence of 1 to 15 amino acids located between the co-signaling domain and the CD3 ζ signaling domain.

3. The nucleic acid molecule of claim 1 wherein the co-signaling domain is selected from a CD28 co-signaling domain and a 4-IBB co-signaling domain.

4. The nucleic acid molecule of claim 1 wherein the transmembrane domain is selected from a CD28 transmembrane domain and a CD4 transmembrane domain.

5. The nucleic acid molecule of claim 1 wherein the CS1 scFv comprises the amino acid sequence of SEQ ID NO:1.

6. The nucleic acid molecule of claim 2 wherein the linker sequence comprises or consists of 3-10 consecutive Gly.

7. The nucleic acid molecule of claim 1 wherein the spacer region comprises at least 10 contiguous amino acids of an IgG constant region or hinge region.

8. The nucleic acid molecule of claim 7 wherein the IgG is IgG4.

9. The nucleic acid of claim 1 wherein the spacer region comprises an IgG4 CD3 domain.

10. The nucleic acid molecule of claim 1 wherein the spacer region comprises an IgG4 Fc domain or a variant thereof.

11. The nucleic acid molecule of claim 1 wherein the spacer region comprises or consists of 4-12 amino acids.

12. The nucleic acid molecule of claim 1 wherein the spacer region is selected from the group consisting of: the sequence ESKYGPPCPPCPGGGSSGGGSG and the sequence GGGSSGGGSG.

13. The nucleic acid molecule of claim 1 wherein the spacer region is selected from the group consisting of: a) an IgG4 Fc domain; b) an IgG4 hinge region, a linker sequence, and an IgG4 CH3 domain; and c) a linker sequence.

14. The nucleic acid molecule of claim 1 wherein spacer region is selected from a spacer region comprising the amino acid sequence of any of SEQ ID Nos: 2-12.

15. The nucleic acid molecule of claim 1 wherein the IgG4 Fc hinge region comprises the amino acid sequence of SEQ ID NO:3 or 4.

16. The nucleic acid molecule of claim 3 wherein the CD28 co-signaling domain comprises the amino acid sequence of SEQ ID NO:22 or 23

17. The nucleic acid molecule of claim 3 wherein the 4-1BB co-signaling domain comprises the amino acid sequence of SEQ ID NO:24.

18. The nucleic acid molecule of claim 1 wherein the CD3 ζ signaling domain comprises the amino acid sequence of SEQ ID NO:21.

19. The nucleic acid molecule of claim 1 wherein the CD28 transmembrane domain comprises SEQ ID NO:14 or 15.

20. The nucleic acid molecule of claim 1 wherein the CD4 transmembrane domain comprises SEQ ID NO:16.

21. The nucleic acid molecule of claim 1 wherein the CD3ζ signaling domain comprising the amino acid sequence of SEQ ID NO:21.

22. The nucleic acid molecule of claim 1 wherein the chimeric antigen receptor comprises: a CS1 scFv; a spacer; CD28 transmembrane domain; a CD28 co-signaling domain; and CD3 ζ signaling domain.

23. The nucleic acid molecule of claim 22 wherein the chimeric antigen receptor comprises a linker amino acid sequence located between the CD28 co-signaling domain and the CD3 ζ signaling domain.

24. The nucleic acid molecule of claim 23 wherein the linker amino acid sequence is GlyGlyGly.

25. The nucleic acid molecule of claim 1 wherein the spacer comprises an IgG4 hinge region, a linker sequence and an IgG4 CH3 domain.

26. The nucleic acid molecule of claim 22 wherein the IgG4 hinge region comprises SEQ ID NO:6

27. The nucleic acid molecule of claim 22 wherein the linker sequence comprises SEQ ID NO:7.

28. The nucleic acid molecule of claim 22 wherein the IgG4 CH3 domain comprises SEQ ID NO:8.

29. The nucleic acid molecule of claim 22 wherein the linker region comprises SEQ ID NO:25.

30. The nucleic acid molecule of claim 22 wherein the chimeric antigen receptor comprises the amino acid sequence of SEQ ID NO:1.

31. The nucleic acid molecule of claim 1 wherein the chimeric antigen receptor comprises an amino acid sequence 90% or 95% identical to SEQ ID NO:1.

32. The nucleic acid molecule of claim 1 wherein the sequence encoding the chimeric antigen receptor is immediately preceded by a nucleic acid sequence encoding a signal sequence.

33. The nucleic acid molecule of claim 32 wherein the signal sequence comprises SEQ ID NO:4.

34. The nucleic acid molecule of claim 1 wherein the chimeric antigen receptor comprises: a CS1 scFv; a hinge/linker region; CD4 transmembrane domain; a 4-1BB co-signaling domain; and CD3 ζ signaling domain.

35. The nucleic acid molecule of claim 34 wherein the chimeric antigen receptor comprises a linker amino acid sequence located between the 4-1BB co-signaling domain and the CD3 ζ signaling domain.

36. The nucleic acid molecule of claim 35 wherein the linker amino acid sequence is GlyGlyGly.

37. The nucleic acid molecule of claim 34 wherein the hinge/linker region comprises an IgG4 hinge region, a linker sequence and an IgG4 CH3 domain.

38. The nucleic acid molecule of claim 34 wherein the IgG4 hinge region comprises SEQ ID NO: 3 or 4.

39. The nucleic acid molecule of claim 34 wherein the spacer sequence comprises SEQ ID NO: 2.

40. The nucleic acid molecule of claim 1 wherein the spacer comprises an amino acid sequence selected from SEQ ID NOs: 9-12.

41. The nucleic acid molecule of claim 1 wherein the spacer region comprises an amino acid sequence selected from SEQ ID NOs: 2 -12.

42. The nucleic acid molecule of claim 34 wherein the chimeric antigen receptor comprises the amino acid sequence of SEQ ID NO:18.

43. The nucleic acid molecule of claim 1 wherein the chimeric antigen receptor comprises the amino acid sequence 90% or 95% identical to any of SEQ ID NO:29-46.

44. The nucleic acid molecule of claim 1 wherein the sequence encoding the chimeric antigen receptor is immediately preceded by a nucleic acid sequence encoding a signal sequence.

45. The nucleic acid molecule of claim 44 wherein the signal sequence comprises SEQ ID NO:26.

46. The nucleic acid molecule of claim 1 wherein the chimeric antigen receptor comprises: a CS1 scFv; a spacer; CD4 transmembrane domain; a 4-1BB co-signaling domain; and CD3 ζ signaling domain.

47. The nucleic acid molecule of claim 46 wherein the chimeric antigen receptor comprises a linker amino acid sequence located between the 4-1BB co-signaling domain and the CD3 ζ signaling domain.

48. The nucleic acid molecule of claim 47 wherein the linker amino acid sequence is GlyGlyGly.

49. The nucleic acid molecule of claim 46 wherein the hinge/linker region comprises an IgG4 hinge-CH2-CH3 region.

50. The nucleic acid molecule of claim 46 wherein the IgG4 hinge-CH2-CH3 region comprises SEQ ID NO:__.

51. The nucleic acid molecule of claim 46 wherein the chimeric antigen receptor comprises the amino acid sequence of SEQ ID NO:__.

52. The nucleic acid molecule of claim 46 wherein the chimeric antigen receptor comprises the amino acid sequence 90% or 95% identical to SEQ ID NO:__.

53. The nucleic acid molecule of claim 46 wherein the sequence encoding the chimeric antigen receptor is immediately preceded by a nucleic acid sequence encoding a signal sequence.

54. The nucleic acid molecule of claim 53 wherein the signal sequence comprises SEQ ID NO:26.

55. A population of human T cells transduced by a vector comprising an expression cassette encoding a chimeric antigen receptor, wherein chimeric antigen receptor

comprises: a CS1 scFv; an optional spacer region; a transmembrane domain; a co-signaling domain; and CD3 ζ signaling domain.

56. The population of human T cells of claim 55 wherein the chimeric antigen receptor comprises: a CS1 scFv; a spacer; CD28 transmembrane domain; a CD28 co-signaling domain; and CD3 ζ signaling domain.

57. The population of human T cells of claim 55 wherein the chimeric antigen receptor comprises: a CS1 scFv; a spacer; CD4 transmembrane domain; a 4-1BB co-signaling domain; and CD3 ζ signaling domain.

58. The population of human T cells of claim 55 wherein the chimeric antigen receptor comprises: a CS1 scFv; a spacer a spacer comprising an amino acid sequence selected from SEQ ID Nos:2-5 and 9-12; CD4 transmembrane domain; a 4-1BB co-signaling domain; and CD3 ζ signaling domain.

59. The population of human T cells of claim 55 wherein the chimeric antigen receptor comprises: a CS1 scFv; a spacer a spacer comprising an amino acid sequence selected from SEQ ID Nos:2-5 and 9-12; CD28 transmembrane domain; a CD28 co-signaling domain; and CD3 ζ signaling domain.

60. The population of human T cells of claim 55 wherein the chimeric antigen receptor comprises: a CS1 scFv; a spacer a spacer comprising an amino acid sequence selected from SEQ ID Nos:2-5 and 9-12; CD4 transmembrane domain; a 4-1BB co-signaling domain; and CD3 ζ signaling domain.

61. The population of human T cells of claim 55 wherein the chimeric antigen receptor comprises: a spacer a spacer comprising an amino acid sequence selected from SEQ ID Nos:2-5 and 9-12; CD28 transmembrane domain; a CD28 co-signaling domain; and CD3 ζ signaling domain.

62. The population of human T cells of claim 55 wherein the chimeric antigen receptor comprises an amino acid sequence at least 90% or 95% identical to an amino

acid sequence selected from: SEQ ID NOs: 30, 31, 33, 34, 36, 37, 39, 40, 42, 43, 44 and 45.

63. The population of human T cells of claim 55 wherein the chimeric antigen receptor comprises an amino acid sequence identical to an amino acid sequence selected from: SEQ ID NOs: 30, 31, 33, 34, 36, 37, 39, 40, 42, 43, 44 and 45.

64. The population of human T cells of claim 55 wherein the chimeric antigen receptor comprises an amino acid sequence identical to an amino acid sequence selected from: SEQ ID NOs: 30, 31, 33, 34, 36, 37, 39, 40, 42, 43, 44 and 45, each with more that 5 amino acid substitutions.

65. The population of human T cells of claim 55 wherein at least 20%, 30%, 40%, 50%, 60%, 70% or 80% of the transduced human T cells are central memory T cells.

66. The population of human T cells of claim 55 wherein at least 10% or 20% of the transduced central memory T cells are CD4+.

67. The population of human T cells of claim 55 wherein at least 10% or 20% of the transduced central memory T cells are CD8+.

68. The population of human T cells of claim 55 wherein at least 10% of the central memory T cells are CD4+ and at least 10% are CD8+.

69. The population of human T cells of claim 55 wherein at least 80% of the transduced human T cells are CD4+ or CD8+ and CD62L+.

70. A method of treating cancer comprising administering to a patient in need thereof a pharmaceutical composition comprising the human T cells of any of claims 55-69.

71. The method of claim 70 wherein the population of human T cells are autologous to the patient.

72. The method of claim 70 wherein the population of human T cells are allogenic to the patient.

73. The method of claim 70 wherein the transduced human T cells are prepared by a method comprising obtaining T cells from the patient or obtaining T cells allogenic to the patient, treating the obtained T cells to isolate a population of cells enriched for CD4+/CD8+ central memory T cells, and transducing at least a portion of the isolated population of cells to with a viral vector comprising an expression cassette encoding a chimeric antigen receptor, wherein chimeric antigen receptor comprises: a CS1 scFv; a spacer; a transmembrane domain; a co-signaling domain; and CD3 ζ signaling domain.

74. The method of claim 73 wherein the chimeric antigen receptor comprises: a CS1 scFv; a spacer; CD28 transmembrane domain; a CD28 co-signaling domain; and CD3 ζ signaling domain.

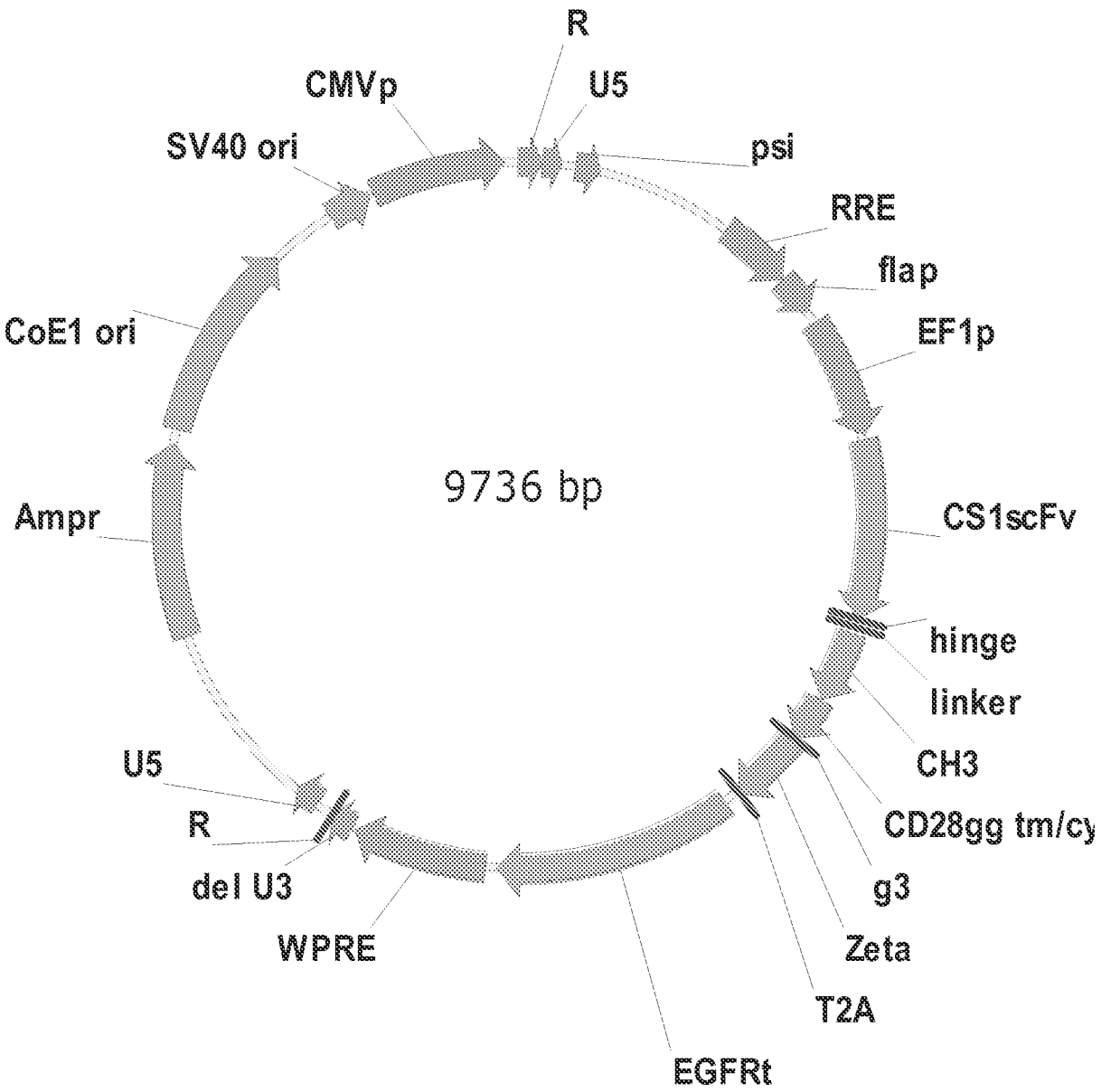
75. The method of claim 73 wherein the chimeric antigen receptor comprises: a CS1 scFv; a spacer; CD4 transmembrane domain; a 4-1BB co-signaling domain; and CD3 ζ signaling domain.

76. The method of claim 73 wherein the chimeric antigen receptor comprises: a CS1 scFv; a spacer; CD 28 domain; a 4-1BB co-signaling domain; and CD3 ζ signaling domain.

77. The method of claim 73 wherein the chimeric antigen receptor comprises: a CS1 scFv; a spacer comprising an amino acid sequence selected from SEQ ID Nos:2-5 and 9-12; CD28 transmembrane domain; a CD28 co-signaling domain; and CD3 ζ signaling domain.

78. The method of claim 73 wherein the chimeric antigen receptor comprises: a CS1 scFv; a a spacer comprising an amino acid sequence selected from SEQ ID Nos:2-5 and 9-12; CD4 transmembrane domain; a 4-1BB co-signaling domain; and CD3 ζ signaling domain.

79. The method of claim 73 wherein the chimeric antigen receptor comprises: a spacer comprising an amino acid sequence selected from SEQ ID Nos:2-5 and 9-12; CD28 transmembrane domain; a CD28 co-signaling domain; and CD3 ζ signaling domain.


R
U5
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RRE
flap
EF1p
CS1scFv
hinge
linker
CH3
CD28gg tm/cy
g3
Zeta
T2A
EGFRt
WPRE
del U3
R
U5
Ampr
CoE1 ori
SV40 ori
CMVp
9736 bp

FIGURE 1

MLLLVTSLLLCELPHPAFLLIPEVQLVESGGGLVQPGGSLRLSCAASGFDFSRYWMSWVRQA

GMCSFRa signal peptide (22 aa) CS1scFv (aa)

PGKGLEWIGEINPDSSTINYAPSLKDKFIISRDNAKNSLYLQMNSLRAEDTAVYYCARPDGNY

WYFDVWGQGTLVTVSSGSTSGGGSGGGSGGGGSSDIQMTQSPSSLSASVGDRVTITCKAS

QDVGIAVAWYQQKPGKVPKLLIYWASTRHTGVPDRFSGSGSGTDFTLTISSLQPEDVATYYC

QQYSSYPYTFGQGTKVEIKESKYGPPCPPCPGGGSSGGGSGGQPREPQVYTLPPSQEEMTK

IgG4-Hinge (12 aa) Linker (10 aa) IgG4-CH3

NQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGN

VFSCSVMHEALHNHYTQKSLSLSLGKMFWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRGG

CD28 transmembrane (28 aa) CD28 (41 aa)

HSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSGGGRVKFSRSADAPAYQQGQNQLYNE

Gly3 CD3 Zeta (112 aa)

LNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRG

KGHDGLYQGLSTATKDTYDALHMQALPPRLEGGGEGRGSLLTCGDVEENPGPRMLLLVTSL

T2A (24 aa) EGFRt

LLCELPHPAFLLIPRKVCNGIGIGEFKDSLSINATNIKHFKNCTSISGDLHILPVAFRGDSFTHTP

PLDPQELDILKTVKEITGFLLIQAWPENRTDLHAFENLEIIRGRTKQHGQFSLAVVSLNITSLGL

RSLKEISDGDVIISGNKNLCYANTINWKKLFGTSGQKTKIISNRGENSCKATGQVCHALCSPEG

CWGPEPRDCVSCRNVSRGRECVDKCNLLEGEPREFVENSECIQCHPECLPQAMNITCTGRG

PDNCIQCAHYIDGPHCVKTCPAGVMGENNTLVWKYADAGHVCHLCHPNCTYGCTGPGLE

GCPTNGPKIPSIATGMVGALLLLLVVALGIGLFM

FIGURE 2

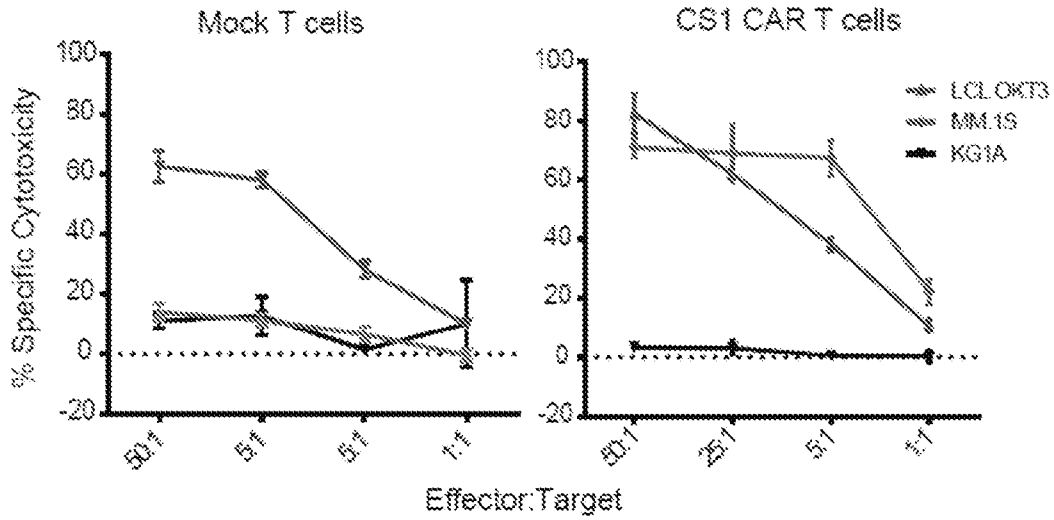
Mock T cells
CS1 CAR T cells
% Specific Cytotoxicity
100
80
60
40
20
0
-20
50:1
25:1
5:1
1:1
Effector:Target
LCL OKT3
MM.1S
KG1A

FIGURE 3

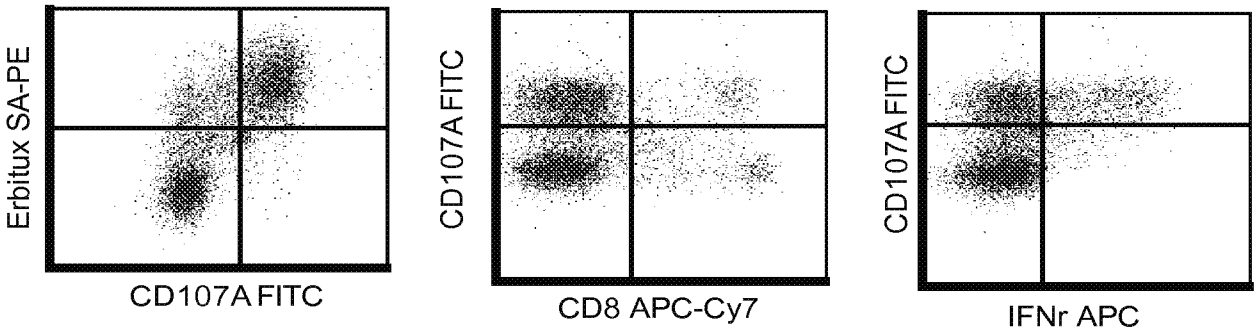
Erbitux SA-PE
CD107A FITC
CD107A FITC
CD8 APC-Cy7
CD107A FITC
IFNr APC

FIGURE 4

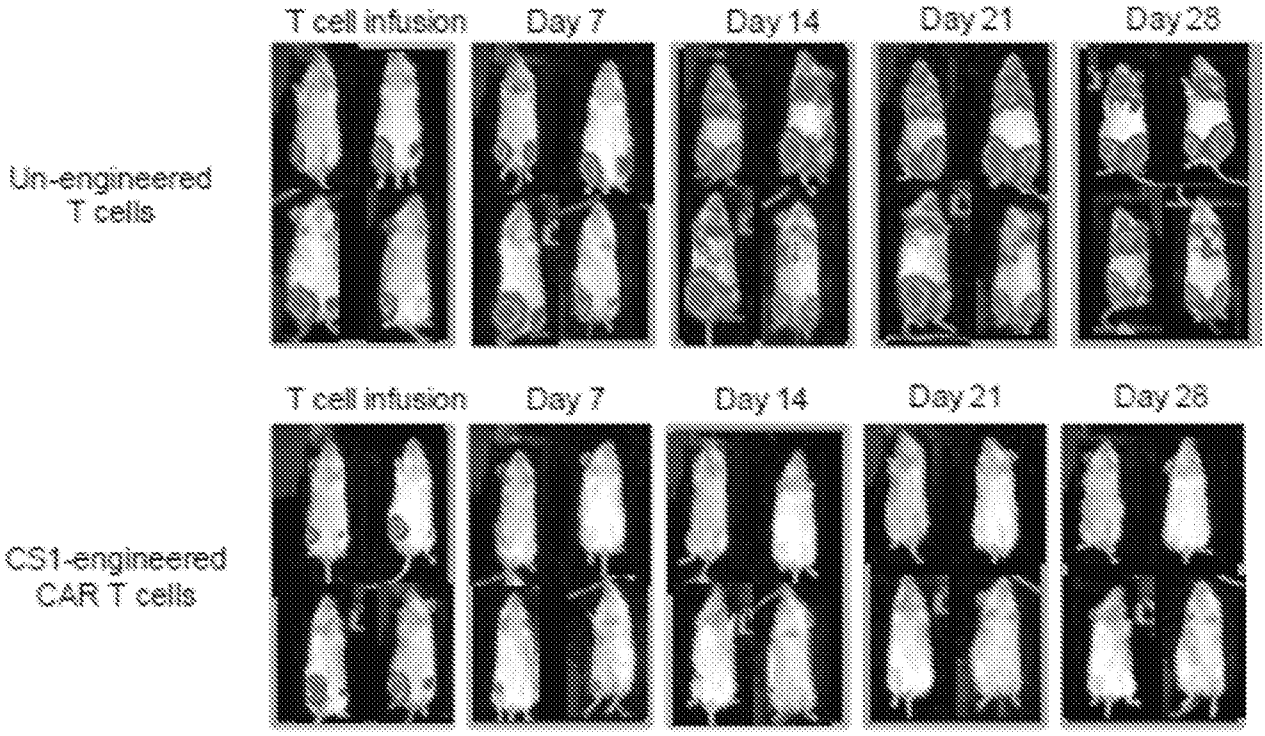
T cell infusion
Day 7
Day 14
Day 21
Day 28
Un-engineered T cells
T cell infusion
Day 7
Day 14
Day 21
Day 28
CS1-engineered CAR T cells

FIGURE 5

MLLLVTSLLLCELPHPAFLLIPEVQLVESGGGLVQPGGSLRLSCAASGFDFSRYWMSWVRQA

GMCSFRa signal CS1scFv

PGKGLEWIGEINPDSSTINYAPSLKDKFIISRDNAKNSLYLQMNSLRAEDTAVYYCARPDGNY
WYFDVWGQGTLVTVSSGSTSGGGSGGGSGGGGSSDIQMTQSPSSLSASVGDRVTITCKAS
QDVGIAVAWYQQKPGKVPKLLIYWASTRHTGVPDRFSGSGSGTDFTLTISSLQPEDVATYYC
QQYSSYPYTFGQGTKVEIKESKYGPPCPPCPGGGSSGGGSGGQPREPQVYTLPPSQEEMTK

IgG4 hinge linker IgG4

NQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGN
VFSCSVMHEALHNHYTQKSLSLSLGKMALIVLGGVAGLLLFIGLGIFFKRGRKKLLYIFKQPFM

CD4tm 4-1BB

RPVQTTQEEDGCSCRFPEEEEGGCELGGGRVKFSRSADAPAYQQGQNQLYNELNLGRREEY

Gly3 Zeta

DVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQ
GLSTATKDTYDALHMQALPPRLEGGGEGRGSLLTCGDVEENPGPRMLLLVTSLLLCELPHPA

T2A EGFRt

FLLIPRKVCNGIGIGEFKDSLSINATNIKHFKNCTSISGDLHILPVAFRGDSFTHTPPLDPQELDIL
KTVKEITGFLLIQAWPENRTDLHAFENLEIIRGRTKQHGQFSLAVVSLNITSLGLRSLKEISDGD
VIISGNKNLCYANTINWKKLFGTSGQKTKIISNRGENSCKATGQVCHALCSPEGCWGPEPRD
CVSCRNVSRGRECVDKCNLLEGEPREFVENSECIQCHPECLPQAMNITCTGRGPDNCIQCAH
YIDGPHCVKTCPAGVMGENNTLVWKYADAGHVCHLCHPNCTYGCTGPGLEGCPTNGPKIP
SIATGMVGALLLLLVVALGIGLFM

Figure 6

MLLLVTSLLLCELPHPAFLLIPEVQLVESGGGLVQPGGSLRLSCAASGFDFSRYWMSWVRQA

GMSCFRa CS1scFV

PGKGLEWIGEINPDSSTINYAPSLKDKFIISRDNAKNSLYLQMNSLRAEDTAVYYCARPDGNY
WYFDVWGQGTLVTVSSGSTSGGGSGGGSGGGGSSDIQMTQSPSSLSASVGDRVTITCKAS
QDVGIAVAWYQQKPGKVPKLLIYWASTRHTGVPDRFSGSGSGTDFTLTISSLQPEDVATYYC
QQYSSYPYTFGQGTKVEIKESKYGPPCPPCPAPEFEGGPSVFLFPPKPKDTLMISRTPEVTCVV

IgG4

VDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFQSTYRVVSVLTVLHQDWLNGKEYKCK
VSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNG
QPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK

MALIVLGGVAGLLLFIGLGIFFKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCEL

CD4tm 4-1BB

GGGRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQE

Gly3 Zeta

GLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPRLEGG

T2A

GEGRGSLLTCGDVEENPGPRMLLLVTSLLLCELPHPAFLLIPRKVCNGIGIGEFKDSLSINATNI

EGFt

KHFKNCTSISGDLHILPVAFRGDSFTHTPPLDPQELDILKTVKEITGFLLIQAWPENRTDLHAFE
NLEIIRGRTKQHGQFSLAVVSLNITSLGLRSLKEISDGDVIISGNKNLCYANTINWKKLFGTSGQ
KTKIISNRGENSCKATGQVCHALCSPEGCWGPEPRDCVSCRNVSRGRECVDKCNLLEGEPRE
FVENSECIQCHPECLPQAMNITCTGRGPDNCIQCAHYIDGPHCVKTCPAGVMGENNTLVW
KYADAGHVCHLCHPNCTYGCTGPGLEGCPTNGPKIPSIATGMVGALLLLLVVALGIGLFM

Figure 7

MLLLVTSLLLCELPHPAFLLIPEVQLVESGGGLVQPGGSLRLSCAASGFDFSRYWMSWVRQA

GMCSFRa CS1scFv

PGKGLEWIGEINPDSSTINYAPSLKDKFIISRDNAKNSLYLQMNSLRAEDTAVYYCARPDGNY
WYFDVWGQGTLVTVSSGSTSGGGSGGGSGGGGSSDIQMTQSPSSLSASVGDRVTITCKAS
QDVGIAVAWYQQKPGKVPKLLIYWASTRHTGVPDRFSGSGSGTDFTLTISSLQPEDVATYYC
QQYSSYPYTFGQGTKVEIKESKYGPPCPPCPAPEFEGGPSVFLFPPKPKDTLMISRTPEVTCVV

IgG4

VDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFQSTYRVVSVLTVLHQDWLNGKEYKCK
VSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNG
QPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK
MFWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRGGHSDYMNMTPRRPGPTRKHYQPYAP

CD28tm CD28cyto

PRDFAAYRSGGGRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGG

Gly3 Zeta

KPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHM
QALPPRLEGGGEGRGSLLTCGDVEENPGPRMLLLVTSLLLCELPHPAFLLIPRKVCNGIGIGEF

T2A EGFRt

KDSLSINATNIKHFKNCTSISGDLHILPVAFRGDSFTHTPPLDPQELDILKTVKEITGFLLIQAWP
ENRTDLHAFENLEIIRGRTKQHGQFSLAVVSLNITSLGLRSLKEISDGDVIISGNKNLCYANTIN
WKKLFGTSGQKTKIISNRGENSCKATGQVCHALCSPEGCWGPEPRDCVSCRNVSRGRECVD
KCNLLEGEPREFVENSECIQCHPECLPQAMNITCTGRGPDNCIQCAHYIDGPHCVKTCPAGV
MGENNTLVWKYADAGHVCHLCHPNCTYGCTGPGLEGCPTNGPKIPSIATGMVGALLLLLV
VALGIGLFM

Figure 8

MLLLVTSLLLCELPHPAFLLIPEVQLVESGGGLVQPGGSLRLSCAASGFDFSRYWMSWVRQA

GMCSFa CS1scFv

PGKGLEWIGEINPDSSTINYAPSLKDKFIISRDNAKNSLYLQMNSLRAEDTAVYYCARPDGNY
WYFDVWGQGTLVTVSSGSTSGGGSGGGSGGGGSSDIQMTQSPSSLSASVGDRVTITCKAS
QDVGIAVAWYQQKPGKVPKLLIYWASTRHTGVPDRFSGSGSGTDFTLTISSLQPEDVATYYC
QQYSSYPYTFGQGTKVEIKGGGSSGGGSGMALIVLGGVAGLLLFIGLGIFFKRGRKKLLYIFKQ

linker CD4tm 4-1BB

PFMRPVQTTQEEDGCSCRFPEEEEGGCELGGGRVKFSRSADAPAYQQGQNQLYNELNLGR

Glv3 Zeta

REEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHD
GLYQGLSTATKDTYDALHMQALPPRLEGGGEGRGSLLTCGDVEENPGPRMLLLVTSLLLCEL

T2A EGFRt

PHPAFLLIPRKVCNGIGIGEFKDSLSINATNIKHFKNCTSISGDLHILPVAFRGDSFTHTPPLDPQ
ELDILKTVKEITGFLLIQAWPENRTDLHAFENLEIIRGRTKQHGQFSLAVVSLNITSLGLRSLKEI
SDGDVIISGNKNLCYANTINWKKLFGTSGQKTKIISNRGENSCKATGQVCHALCSPEGCWGP
EPRDCVSCRNVSRGRECVDKCNLLEGEPREFVENSECIQCHPECLPQAMNITCTGRGPDNCI
QCAHYIDGPHCVKTCPAGVMGENNTLVWKYADAGHVCHLCHPNCTYGCTGPGLEGCPTN
GPKIPSIATGMVGALLLLLVVALGIGLFM

Figure 9

MLLLVTSLLLCELPHPAFLLIPEVQLVESGGGLVQPGGSLRLSCAASGFDFSRYWMSWVRQA

GMCSFRa CS1scFv

PGKGLEWIGEINPDSSTINYAPSLKDKFIISRDNAKNSLYLQMNSLRAEDTAVYYCARPDGNY
WYFDVWGQGTLVTVSSGSTSGGGSGGGSGGGGSSDIQMTQSPSSLSASVGDRVTITCKAS
QDVGIAVAWYQQKPGKVPKLLIYWASTRHTGVPDRFSGSGSGTDFTLTISSLQPEDVATYYC
QQYSSYPYTFGQGTKVEIKGGGSSGGGSGMFWVLVVVGGVLACYSLLVTVAFIIFWVRSKRS

Linker CD28tm CD28cyto

RGGHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSGGGRVKFSRSADAPAYQQGQNQ

Gly3 Zeta

LYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGE
RRRGKGHDGLYQGLSTATKDTYDALHMQALPPRLEGGGEGRGSLLTCGDVEENPGPRMLL

T2A EGFRt

LVTSLLLCELPHPAFLLIPRKVCNGIGIGEFKDSLSINATNIKHFKNCTSISGDLHILPVAFRGDSF
THTPPLDPQELDILKTVKEITGFLLIQAWPENRTDLHAFENLEIIRGRTKQHGQFSLAVVSLNIT
SLGLRSLKEISDGDVIISGNKNLCYANTINWKKLFGTSGQKTKIISNRGENSCKATGQVCHALC
SPEGCWGPEPRDCVSCRNVSRGRECVDKCNLLEGEPREFVENSECIQCHPECLPQAMNITC
TGRGPDNCIQCAHYIDGPHCVKTCPAGVMGENNTLVWKYADAGHVCHLCHPNCTYGCTG
PGLEGCPTNGPKIPSIATGMVGALLLLLVVALGIGLFM

Figure 10

```
   1 GTTAGACCAG ATCTGAGCCT GGGAGCTCTC TGGCTAACTA GGGAACCCAC TGCTTAAGCC
     CAATCTGGTC TAGACTCGGA CCCTCGAGAG ACCGATTGAT CCCTTGGGTG ACGAATTCGG
  61 TCAATAAAGC TTGCCTTGAG TGCTTCAAGT AGTGTGTGCC CGTCTGTTGT GTGACTCTGG
     AGTTATTTCG AACGGAACTC ACGAAGTTCA TCACACACGG GCAGACAACA CACTGAGACC
 121 TAACTAGAGA TCCCTCAGAC CCTTTTAGTC AGTGTGGAAA ATCTCTAGCA GTGGCGCCCG
     ATTGATCTCT AGGGAGTCTG GGAAAATCAG TCACACCTTT TAGAGATCGT CACCGCGGGC
 181 AACAGGGACT TGAAAGCGAA AGGGAAACCA GAGGAGCTCT CTCGACGCAG GACTCGGCTT
     TTGTCCCTGA ACTTTCGCTT TCCCTTTGGT CTCCTCGAGA GAGCTGCGTC CTGAGCCGAA
 241 GCTGAAGCGC GCACGGCAAG AGGCGAGGGG CGGCGACTGG TGAGTACGCC AAAAATTTTG
     CGACTTCGCG CGTGCCGTTC TCCGCTCCCC GCCGCTGACC ACTCATGCGG TTTTTAAAAC
 301 ACTAGCGGAG GCTAGAAGGA GAGAGATGGG TGCGAGAGCG TCAGTATTAA GCGGGGGAGA
     TGATCGCCTC CGATCTTCCT CTCTCTACCC ACGCTCTCGC AGTCATAATT CGCCCCCTCT
 361 ATTAGATCGA TGGGAAAAAA TTCGGTTAAG GCCAGGGGGA AAGAAAAAAT ATAAATTAAA
     TAATCTAGCT ACCCTTTTTT AAGCCAATTC CGGTCCCCCT TTCTTTTTTA TATTTAATTT
 421 ACATATAGTA TGGGCAAGCA GGGAGCTAGA ACGATTCGCA GTTAATCCTG GCCTGTTAGA
     TGTATATCAT ACCCGTTCGT CCCTCGATCT TGCTAAGCGT CAATTAGGAC CGGACAATCT
 481 AACATCAGAA GGCTGTAGAC AAATACTGGG ACAGCTACAA CCATCCCTTC AGACAGGATC
     TTGTAGTCTT CCGACATCTG TTTATGACCC TGTCGATGTT GGTAGGGAAG TCTGTCCTAG
 541 AGAAGAACTT AGATCATTAT ATAATACAGT AGCAACCCTC TATTGTGTGC ATCAAAGGAT
     TCTTCTTGAA TCTAGTAATA TATTATGTCA TCGTTGGGAG ATAACACACG TAGTTTCCTA
 601 AGAGATAAAA GACACCAAGG AAGCTTTAGA CAAGATAGAG GAAGAGCAAA ACAAAAGTAA
     TCTCTATTTT CTGTGGTTCC TTCGAAATCT GTTCTATCTC CTTCTCGTTT TGTTTTCATT
 661 GAAAAAAGCA CAGCAAGCAG CAGCTGACAC AGGACACAGC AATCAGGTCA GCCAAAATTA
     CTTTTTTCGT GTCGTTCGTC GTCGACTGTG TCCTGTGTCG TTAGTCCAGT CGGTTTTAAT
 721 CCCTATAGTG CAGAACATCC AGGGGCAAAT GGTACATCAG GCCATATCAC CTAGAACTTT
     GGGATATCAC GTCTTGTAGG TCCCCGTTTA CCATGTAGTC CGGTATAGTG GATCTTGAAA
 781 AAATGCATGG GTAAAAGTAG TAGAAGAGAA GGCTTTCAGC CCAGAAGTGA TACCCATGTT
     TTTACGTACC CATTTTCATC ATCTTCTCTT CCGAAAGTCG GGTCTTCACT ATGGGTACAA
 841 TTCAGCATTA TCAGAAGGAG CCACCCCACA AGATTTAAAC ACCATGCTAA ACACAGTGGG
     AAGTCGTAAT AGTCTTCCTC GGTGGGGGTG TCTAAATTTG TGGTACGATT TGTGTCACCC
 901 GGGACATCAA GCAGCCATGC AAATGTTAAA AGAGACCATC AATGAGGAAG CTGCAGGCAA
     CCCTGTAGTT CGTCGGTACG TTTACAATTT TCTCTGGTAG TTACTCCTTC GACGTCCGTT
 961 AGAGAAGAGT GGTGCAGAGA GAAAAAAGAG CAGTGGGAAT AGGAGCTTTG TTCCTTGGGT
     TCTCTTCTCA CCACGTCTCT CTTTTTTCTC GTCACCCTTA TCCTCGAAAC AAGGAACCCA
1021 TCTTGGGAGC AGCAGGAAGC ACTATGGGCG CAGCGTCAAT GACGCTGACG GTACAGGCCA
     AGAACCCTCG TCGTCCTTCG TGATACCCGC GTCGCAGTTA CTGCGACTGC CATGTCCGGT
1081 GACAATTATT GTCTGGTATA GTGCAGCAGC AGAACAATTT GCTGAGGGCT ATTGAGGCGC
     CTGTTAATAA CAGACCATAT CACGTCGTCG TCTTGTTAAA CGACTCCCGA TAACTCCGCG
1141 AACAGCATCT GTTGCAACTC ACAGTCTGGG GCATCAAGCA GCTCCAGGCA AGAATCCTGG
     TTGTCGTAGA CAACGTTGAG TGTCAGACCC CGTAGTTCGT CGAGGTCCGT TCTTAGGACC
1201 CTGTGGAAAG ATACCTAAAG GATCAACAGC TCCTGGGGAT TTGGGGTTGC TCTGGAAAAC
     GACACCTTTC TATGGATTTC CTAGTTGTCG AGGACCCCTA AACCCCAACG AGACCTTTTG
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     AGTAAACGTG GTGACGACAC GGAACCTAGA TGTTTACCGT CATAAGTAGG TGTTAAAATT
1321 AAGAAAAGGG GGGATTGGGG GGTACAGTGC AGGGGAAAGA ATAGTAGACA TAATAGCAAC
     TTCTTTTCCC CCCTAACCCC CCATGTCACG TCCCCTTTCT TATCATCTGT ATTATCGTTG
1381 AGACATACAA ACTAAAGAAT TACAAAAACA AATTACAAAA ATTCAAAATT TTCGGGTTTA
     TCTGTATGTT TGATTTCTTA ATGTTTTTGT TTAATGTTTT TAAGTTTTAA AAGCCCAAAT
1441 TTACAGGGAC AGCAGAGATC CAGTTTGGGG ATCAATTGCA TGAAGAATCT GCTTAGGGTT
     AATGTCCCTG TCGTCTCTAG GTCAAACCCC TAGTTAACGT ACTTCTTAGA CGAATCCCAA
1501 AGGCGTTTTG CGCTGCTTCG CGAGGATCTG CGATCGCTCC GGTGCCCGTC AGTGGGCAGA
     TCCGCAAAAC GCGACGAAGC GCTCCTAGAC GCTAGCGAGG CCACGGGCAG TCACCCGTCT
1561 GCGCACATCG CCCACAGTCC CCGAGAAGTT GGGGGGAGGG GTCGGCAATT GAACCGGTGC
     CGCGTGTAGC GGGTGTCAGG GGCTCTTCAA CCCCCCTCCC CAGCCGTTAA CTTGGCCACG
1621 CTAGAGAAGG TGGCGCGGGG TAAACTGGGA AAGTGATGTC GTGTACTGGC TCCGCCTTTT
     GATCTCTTCC ACCGCGCCCC ATTTGACCCT TTCACTACAG CACATGACCG AGGCGGAAAA
1681 TCCCGAGGGT GGGGGAGAAC CGTATATAAG TGCAGTAGTC GCCGTGAACG TTCTTTTTCG
     AGGGCTCCCA CCCCCTCTTG GCATATATTC ACGTCATCAG CGGCACTTGC AAGAAAAAGC
1741 CAACGGGTTT GCCGCCAGAA CACAGCTGAA GCTTCGAGGG GCTCGCATCT CTCCTTCACG
     GTTGCCCAAA CGGCGGTCTT GTGTCGACTT CGAAGCTCCC CGAGCGTAGA GAGGAAGTGC
1801 CGCCCGCCGC CCTACCTGAG GCCGCCATCC ACGCCGGTTG AGTCGCGTTC TGCCGCCTCC
     GCGGGCGGCG GGATGGACTC CGGCGGTAGG TGCGGCCAAC TCAGCGCAAG ACGGCGGAGG
1861 CGCCTGTGGT GCCTCCTGAA CTGCGTCCGC CGTCTAGGTA AGTTTAAAGC TCAGGTCGAG
     GCGGACACCA CGGAGGACTT GACGCAGGCG GCAGATCCAT TCAAATTTCG AGTCCAGCTC
1921 ACCGGGCCTT TGTCCGGCGC TCCCTTGGAG CCTACCTAGA CTCAGCCGGC TCTCCACGCT
     TGGCCCGGAA ACAGGCCGCG AGGGAACCTC GGATGGATCT GAGTCGGCCG AGAGGTGCGA
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1981 TTGCCTGACC CTGCTTGCTC AACTCTACGT CTTTGTTTCG TTTTCTGTTC TGCGCCGTTA
     AACGGACTGG GACGAACGAG TTGAGATGCA GAAACAAAGC AAAAGACAAG ACGCGGCAAT
2041 CAGATCCAAG CTGTGACCGG CGCCTACGGC TAGCGCCGCC ACCATGCTGC TGCTCGTGAC
     GTCTAGGTTC GACACTGGCC GCGGATGCCG ATCGCGGCGG TGGTACGACG ACGAGCACTG
2101 ATCTCTGCTG CTGTGCGAGC TGCCCCACCC CGCCTTTCTG CTGATTCCTG AGGTGCAGCT
     TAGAGACGAC GACACGCTCG ACGGGGTGGG GCGGAAAGAC GACTAAGGAC TCCACGTCGA
2161 GGTGGAAAGC GGCGGAGGAC TGGTGCAGCC TGGCGGATCT CTGAGACTGA GCTGTGCCGC
     CCACCTTTCG CCGCCTCCTG ACCACGTCGG ACCGCCTAGA GACTCTGACT CGACACGGCG
2221 CAGCGGCTTC GACTTCAGCC GGTACTGGAT GAGCTGGGTG CGCCAGGCCC CTGGCAAAGG
     GTCGCCGAAG CTGAAGTCGG CCATGACCTA CTCGACCCAC GCGGTCCGGG GACCGTTTCC
2281 CCTGGAATGG ATCGGCGAGA TCAACCCCGA CAGCAGCACC ATCAACTACG CCCCCAGCCT
     GGACCTTACC TAGCCGCTCT AGTTGGGGCT GTCGTCGTGG TAGTTGATGC GGGGGTCGGA
2341 GAAGGACAAG TTCATCATCA GCCGGGACAA CGCCAAGAAC AGCCTGTACC TGCAGATGAA
     CTTCCTGTTC AAGTAGTAGT CGGCCCTGTT GCGGTTCTTG TCGGACATGG ACGTCTACTT
2401 CTCCCTGCGG GCCGAGGACA CCGCCGTGTA CTATTGCGCC AGACCCGACG GCAACTACTG
     GAGGGACGCC CGGCTCCTGT GGCGGCACAT GATAACGCGG TCTGGGCTGC CGTTGATGAC
2461 GTACTTCGAC GTGTGGGGCC AGGGCACCCT CGTGACAGTG TCTAGCGGCA GCACAAGCGG
     CATGAAGCTG CACACCCCGG TCCCGTGGGA GCACTGTCAC AGATCGCCGT CGTGTTCGCC
2521 AGGCGGATCT GGCGGAGGAT CAGGCGGGGG AGGATCCAGC GATATCCAGA TGACCCAGAG
     TCCGCCTAGA CCGCCTCCTA GTCCGCCCCC TCCTAGGTCG CTATAGGTCT ACTGGGTCTC
2581 CCCCAGCAGC CTGTCTGCCA GCGTGGGCGA CAGAGTGACC ATCACATGCA AGGCCAGCCA
     GGGGTCGTCG GACAGACGGT CGCACCCGCT GTCTCACTGG TAGTGTACGT TCCGGTCGGT
2641 GGACGTGGGA ATCGCCGTGG CCTGGTATCA GCAGAAACCC GGCAAGGTGC CCAAGCTGCT
     CCTGCACCCT TAGCGGCACC GGACCATAGT CGTCTTTGGG CCGTTCCACG GGTTCGACGA
2701 GATCTACTGG GCCAGCACCA GACACACCGG CGTGCCCGAT AGATTTTCCG GCAGCGGCTC
     CTAGATGACC CGGTCGTGGT CTGTGTGGCC GCACGGGCTA TCTAAAAGGC CGTCGCCGAG
2761 CGGCACCGAC TTCACCCTGA CAATCAGCTC CCTGCAGCCT GAGGACGTGG CCACCTACTA
     GCCGTGGCTG AAGTGGGACT GTTAGTCGAG GGACGTCGGA CTCCTGCACC GGTGGATGAT
2821 CTGCCAGCAG TACAGCAGCT ACCCCTACAC CTTCGGACAG GGCACCAAGG TGGAAATCAA
     GACGGTCGTC ATGTCGTCGA TGGGGATGTG GAAGCCTGTC CCGTGGTTCC ACCTTTAGTT
2881 AGAGTCTAAG TACGGCCCTC CCTGCCCCCC TTGTCCAGGC GGCGGATCTT CCGGAGGAGG
     TCTCAGATTC ATGCCGGGAG GGACGGGGGG AACAGGTCCG CCGCCTAGAA GGCCTCCTCC
2941 AAGCGGAGGC CAGCCCAGAG AACCTCAGGT GTACACACTG CCCCCTAGCC AGGAAGAGAT
     TTCGCCTCCG GTCGGGTCTC TTGGAGTCCA CATGTGTGAC GGGGGATCGG TCCTTCTCTA
3001 GACCAAGAAT CAGGTGTCCC TGACATGCCT CGTGAAGGGC TTCTACCCCT CCGATATCGC
     CTGGTTCTTA GTCCACAGGG ACTGTACGGA GCACTTCCCG AAGATGGGGA GGCTATAGCG
3061 CGTGGAATGG GAGAGCAACG GCCAGCCTGA GAACAACTAC AAGACCACCC CCCCTGTGCT
     GCACCTTACC CTCTCGTTGC CGGTCGGACT CTTGTTGATG TTCTGGTGGG GGGGACACGA
3121 GGACAGCGAC GGCTCATTCT TCCTGTACAG CAGGCTGACC GTGGACAAGA GCCGGTGGCA
     CCTGTCGCTG CCGAGTAAGA AGGACATGTC GTCCGACTGG CACCTGTTCT CGGCCACCGT
3181 GGAAGGCAAC GTGTTCAGCT GCTCCGTGAT GCACGAGGCC CTGCACAACC ACTACACCCA
     CCTTCCGTTG CACAAGTCGA CGAGGCACTA CGTGCTCCGG GACGTGTTGG TGATGTGGGT
3241 GAAGTCCCTG AGCCTGTCCC TGGGCAAGAT GTTCTGGGTG CTGGTGGTCG TGGGCGGCGT
     CTTCAGGGAC TCGGACAGGG ACCCGTTCTA CAAGACCCAC GACCACCAGC ACCCGCCGCA
3301 GCTGGCCTGT TATAGCCTGC TCGTGACCGT GGCCTTCATC ATCTTTTGGG TGCGCAGCAA
     CGACCGGACA ATATCGGACG AGCACTGGCA CCGGAAGTAG TAGAAAACCC ACGCGTCGTT
3361 GCGGAGCAGA GGCGGCCACA GCGACTACAT GAACATGACC CCCAGACGGC CAGGCCCCAC
     CGCCTCGTCT CCGCCGGTGT CGCTGATGTA CTTGTACTGG GGGTCTGCCG GTCCGGGGTG
3421 CCGGAAACAC TATCAGCCTT ACGCCCCTCC CAGAGACTTC GCCGCTTATC GGTCCGGCGG
     GGCCTTTGTG ATAGTCGGAA TGCGGGGAGG GTCTCTGAAG CGGCGAATAG CCAGGCCGCC
3481 AGGGCGGGTG AAGTTCAGCA GAAGCGCCGA CGCCCCTGCC TACCAGCAGG GCCAGAATCA
     TCCCGCCCAC TTCAAGTCGT CTTCGCGGCT GCGGGGACGG ATGGTCGTCC CGGTCTTAGT
3541 GCTGTACAAC GAGCTGAACC TGGGCAGAAG GGAAGAGTAC GACGTCCTGG ATAAGCGGAG
     CGACATGTTG CTCGACTTGG ACCCGTCTTC CCTTCTCATG CTGCAGGACC TATTCGCCTC
3601 AGGCCGGGAC CCTGAGATGG GCGGCAAGCC TCGGCGGAAG AACCCCCAGG AAGGCCTGTA
     TCCGGCCCTG GGACTCTACC CGCCGTTCGG AGCCGCCTTC TTGGGGGTCC TTCCGGACAT
3661 TAACGAACTG CAGAAAGACA AGATGGCCGA GGCCTACAGC GAGATCGGCA TGAAGGGCGA
     ATTGCTTGAC GTCTTTCTGT TCTACCGGCT CCGGATGTCG CTCTAGCCGT ACTTCCCGCT
3721 GCGGAGGCGG GGCAAGGGCC ACGACGGCCT GTATCAGGGC CTGTCCACCG CCACCAAGGA
     CGCCTCCGCC CCGTTCCCGG TGCTGCCGGA CATAGTCCCG GACAGGTGGC GGTGGTTCCT
3781 TACCTACGAC GCCCTGCACA TGCAGGCCCT GCCCCCAAGG CTCGAGGGCG GCGGAGAGGG
     ATGGATGCTG CGGGACGTGT ACGTCCGGGA CGGGGGTTCC GAGCTCCCGC CGCCTCTCCC
3841 CAGAGGAAGT CTTCTAACAT GCGGTGACGT GGAGGAGAAT CCCGGCCCTA GGATGCTTCT
     GTCTCCTTCA GAAGATTGTA CGCCACTGCA CCTCCTCTTA GGGCCGGGAT CCTACGAAGA
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3901 CCTGGTGACA AGCCTTCTGC TCTGTGAGTT ACCACACCCA GCATTCCTCC TGATCCCACG
     GGACCACTGT TCGGAAGACG AGACACTCAA TGGTGTGGGT CGTAAGGAGG ACTAGGGTGC
3961 CAAAGTGTGT AACGGAATAG GTATTGGTGA ATTTAAAGAC TCACTCTCCA TAAATGCTAC
     GTTTCACACA TTGCCTTATC CATAACCACT TAAATTTCTG AGTGAGAGGT ATTTACGATG
4021 GAATATTAAA CACTTCAAAA ACTGCACCTC CATCAGTGGC GATCTCCACA TCCTGCCGGT
     CTTATAATTT GTGAAGTTTT TGACGTGGAG GTAGTCACCG CTAGAGGTGT AGGACGGCCA
4081 GGCATTTAGG GGTGACTCCT TCACACATAC TCCTCCTCTG GATCCACAGG AACTGGATAT
     CCGTAAATCC CCACTGAGGA AGTGTGTATG AGGAGGAGAC CTAGGTGTCC TTGACCTATA
4141 TCTGAAAACC GTAAAGGAAA TCACAGGGTT TTTGCTGATT CAGGCTTGGC CTGAAAACAG
     AGACTTTTGG CATTTCCTTT AGTGTCCCAA AAACGACTAA GTCCGAACCG GACTTTTGTC
4201 GACGGACCTC CATGCCTTTG AGAACCTAGA AATCATACGC GGCAGGACCA AGCAACATGG
     CTGCCTGGAG GTACGGAAAC TCTTGGATCT TTAGTATGCG CCGTCCTGGT TCGTTGTACC
4261 TCAGTTTTCT CTTGCAGTCG TCAGCCTGAA CATAACATCC TTGGGATTAC GCTCCCTCAA
     AGTCAAAAGA GAACGTCAGC AGTCGGACTT GTATTGTAGG AACCCTAATG CGAGGGAGTT
4321 GGAGATAAGT GATGGAGATG TGATAATTTC AGGAAACAAA AATTTGTGCT ATGCAAATAC
     CCTCTATTCA CTACCTCTAC ACTATTAAAG TCCTTTGTTT TTAAACACGA TACGTTTATG
4381 AATAAACTGG AAAAAACTGT TTGGGACCTC CGGTCAGAAA ACCAAAATTA TAAGCAACAG
     TTATTTGACC TTTTTTGACA AACCCTGGAG GCCAGTCTTT TGGTTTTAAT ATTCGTTGTC
4441 AGGTGAAAAC AGCTGCAAGG CCACAGGCCA GGTCTGCCAT GCCTTGTGCT CCCCCGAGGG
     TCCACTTTTG TCGACGTTCC GGTGTCCGGT CCAGACGGTA CGGAACACGA GGGGGCTCCC
4501 CTGCTGGGGC CCGGAGCCCA GGGACTGCGT CTCTTGCCGG AATGTCAGCC GAGGCAGGGA
     GACGACCCCG GGCCTCGGGT CCCTGACGCA GAGAACGGCC TTACAGTCGG CTCCGTCCCT
4561 ATGCGTGGAC AAGTGCAACC TTCTGGAGGG TGAGCCAAGG GAGTTTGTGG AGAACTCTGA
     TACGCACCTG TTCACGTTGG AAGACCTCCC ACTCGGTTCC CTCAAACACC TCTTGAGACT
4621 GTGCATACAG TGCCACCCAG AGTGCCTGCC TCAGGCCATG AACATCACCT GCACAGGACG
     CACGTATGTC ACGGTGGGTC TCACGGACGG AGTCCGGTAC TTGTAGTGGA CGTGTCCTGC
4681 GGGACCAGAC AACTGTATCC AGTGTGCCCA CTACATTGAC GGCCCCCACT GCGTCAAGAC
     CCCTGGTCTG TTGACATAGG TCACACGGGT GATGTAACTG CCGGGGGTGA CGCAGTTCTG
4741 CTGCCCGGCA GGAGTCATGG GAGAAAACAA CACCCTGGTC TGGAAGTACG CAGACGCCGG
     GACGGGCCGT CCTCAGTACC CTCTTTTGTT GTGGGACCAG ACCTTCATGC GTCTGCGGCC
4801 CCATGTGTGC CACCTGTGCC ATCCAAACTG CACCTACGGA TGCACTGGGC CAGGTCTTGA
     GGTACACACG GTGGACACGG TAGGTTTGAC GTGGATGCCT ACGTGACCCG GTCCAGAACT
4861 AGGCTGTCCA ACGAATGGGC CTAAGATCCC GTCCATCGCC ACTGGGATGG TGGGGGCCCT
     TCCGACAGGT TGCTTACCCG GATTCTAGGG CAGGTAGCGG TGACCCTACC ACCCCCGGGA
4921 CCTCTTGCTG CTGGTGGTGG CCCTGGGGAT CGGCCTCTTC ATGTGAGCGG CCGCTCTAGA
     GGAGAACGAC GACCACCACC GGGACCCCTA GCCGGAGAAG TACACTCGCC GGCGAGATCT
4981 CCCGGGCTGC AGGAATTCGA TATCAAGCTT ATCGATAATC AACCTCTGGA TTACAAAATT
     GGGCCCGACG TCCTTAAGCT ATAGTTCGAA TAGCTATTAG TTGGAGACCT AATGTTTTAA
5041 TGTGAAAGAT TGACTGGTAT TCTTAACTAT GTTGCTCCTT TTACGCTATG TGGATACGCT
     ACACTTTCTA ACTGACCATA AGAATTGATA CAACGAGGAA AATGCGATAC ACCTATGCGA
5101 GCTTTAATGC CTTTGTATCA TGCTATTGCT TCCCGTATGG CTTTCATTTT CTCCTCCTTG
     CGAAATTACG GAAACATAGT ACGATAACGA AGGGCATACC GAAAGTAAAA GAGGAGGAAC
5161 TATAAATCCT GGTTGCTGTC TCTTTATGAG GAGTTGTGGC CCGTTGTCAG GCAACGTGGC
     ATATTTAGGA CCAACGACAG AGAAATACTC CTCAACACCG GGCAACAGTC CGTTGCACCG
5221 GTGGTGTGCA CTGTGTTTGC TGACGCAACC CCCACTGGTT GGGGCATTGC CACCACCTGT
     CACCACACGT GACACAAACG ACTGCGTTGG GGGTGACCAA CCCCGTAACG GTGGTGGACA
5281 CAGCTCCTTT CCGGGACTTT CGCTTTCCCC CTCCCTATTG CCACGGCGGA ACTCATCGCC
     GTCGAGGAAA GGCCCTGAAA GCGAAAGGGG GAGGGATAAC GGTGCCGCCT TGAGTAGCGG
5341 GCCTGCCTTG CCCGCTGCTG GACAGGGGCT CGGCTGTTGG GCACTGACAA TTCCGTGGTG
     CGGACGGAAC GGGCGACGAC CTGTCCCCGA GCCGACAACC CGTGACTGTT AAGGCACCAC
5401 TTGTCGGGGA AATCATCGTC CTTTCCTTGG CTGCTCGCCT GTGTTGCCAC CTGGATTCTG
     AACAGCCCCT TTAGTAGCAG GAAAGGAACC GACGAGCGGA CACAACGGTG GACCTAAGAC
5461 CGCGGGACGT CCTTCTGCTA CGTCCCTTCG GCCCTCAATC CAGCGGACCT TCCTTCCCGC
     GCGCCCTGCA GGAAGACGAT GCAGGGAAGC CGGGAGTTAG GTCGCCTGGA AGGAAGGGCG
5521 GGCCTGCTGC CGGCTCTGCG GCCTCTTCCG CGTCTTCGCC TTCGCCCTCA GACGAGTCGG
     CCGGACGACG GCCGAGACGC CGGAGAAGGC GCAGAAGCGG AAGCGGGAGT CTGCTCAGCC
5581 ATCTCCCTTT GGGCCGCCTC CCCGCATCGA TACCGTCGAC TAGCCGTACC TTTAAGACCA
     TAGAGGGAAA CCCGGCGGAG GGGCGTAGCT ATGGCAGCTG ATCGGCATGG AAATTCTGGT
5641 ATGACTTACA AGGCAGCTGT AGATCTTAGC CACTTTTTAA AAGAAAAGGG GGGACTGGAA
     TACTGAATGT TCCGTCGACA TCTAGAATCG GTGAAAAATT TTCTTTTCCC CCCTGACCTT
5701 GGGCTAATTC ACTCCCAAAG AAGACAAGAT CTGCTTTTTG CCTGTACTGG GTCTCTCTGG
     CCCGATTAAG TGAGGGTTTC TTCTGTTCTA GACGAAAAAC GGACATGACC CAGAGAGACC
5761 TTAGACCAGA TCTGAGCCTG GGAGCTCTCT GGCTAACTAG GGAACCCACT GCTTAAGCCT
     AATCTGGTCT AGACTCGGAC CCTCGAGAGA CCGATTGATC CCTTGGGTGA CGAATTCGGA
5821 CAATAAAGCT TGCCTTGAGT GCTTCAAGTA GTGTGTGCCC GTCTGTTGTG TGACTCTGGT
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      GTTATTTCGA ACGGAACTCA CGAAGTTCAT CACACACGGG CAGACAACAC ACTGAGACCA
5881  AACTAGAGAT CCCTCAGACC CTTTTAGTCA GTGTGGAAAA TCTCTAGCAG AATTCGATAT
      TTGATCTCTA GGGAGTCTGG GAAAATCAGT CACACCTTTT AGAGATCGTC TTAAGCTATA
5941  CAAGCTTATC GATACCGTCG ACCTCGAGGG GGGGCCCGGT ACCCAATTCG CCCTATAGTG
      GTTCGAATAG CTATGGCAGC TGGAGCTCCC CCCCGGGCCA TGGGTTAAGC GGGATATCAC
6001  AGTCGTATTA CAATTCACTG GCCGTCGTTT TACAACGTCG TGACTGGGAA AACCCTGGCG
      TCAGCATAAT GTTAAGTGAC CGGCAGCAAA ATGTTGCAGC ACTGACCCTT TTGGGACCGC
6061  TTACCCAACT TAATCGCCTT GCAGCACATC CCCCTTTCGC CAGCTGGCGT AATAGCGAAG
      AATGGGTTGA ATTAGCGGAA CGTCGTGTAG GGGGAAAGCG GTCGACCGCA TTATCGCTTC
6121  AGGCCCGCAC CGATCGCCCT TCCCAACAGT TGCGCAGCCT GAATGGCGAA TGGAAATTGT
      TCCGGGCGTG GCTAGCGGGA AGGGTTGTCA ACGCGTCGGA CTTACCGCTT ACCTTTAACA
6181  AAGCGTTAAT ATTTTGTTAA AATTCGCGTT AAATTTTTGT TAAATCAGCT CATTTTTTAA
      TTCGCAATTA TAAAACAATT TTAAGCGCAA TTTAAAAACA ATTTAGTCGA GTAAAAAATT
6241  CCAATAGGCC GAAATCGGCA AAATCCCTTA TAAATCAAAA GAATAGACCG AGATAGGGTT
      GGTTATCCGG CTTTAGCCGT TTTAGGGAAT ATTTAGTTTT CTTATCTGGC TCTATCCCAA
6301  GAGTGTTGTT CCAGTTTGGA ACAAGAGTCC ACTATTAAAG AACGTGGACT CCAACGTCAA
      CTCACAACAA GGTCAAACCT TGTTCTCAGG TGATAATTTC TTGCACCTGA GGTTGCAGTT
6361  AGGGCGAAAA ACCGTCTATC AGGGCGATGG CCCACTACGT GAACCATCAC CCTAATCAAG
      TCCCGCTTTT TGGCAGATAG TCCCGCTACC GGGTGATGCA CTTGGTAGTG GGATTAGTTC
6421  TTTTTTGGGG TCGAGGTGCC GTAAAGCACT AAATCGGAAC CCTAAAGGGA GCCCCCGATT
      AAAAAACCCC AGCTCCACGG CATTTCGTGA TTTAGCCTTG GGATTTCCCT CGGGGGCTAA
6481  TAGAGCTTGA CGGGGAAAGC CGGCGAACGT GGCGAGAAAG GAAGGGAAGA AAGCGAAAGG
      ATCTCGAACT GCCCCTTTCG GCCGCTTGCA CCGCTCTTTC CTTCCCTTCT TTCGCTTTCC
6541  AGCGGGCGCT AGGGCGCTGG CAAGTGTAGC GGTCACGCTG CGCGTAACCA CCACACCCGC
      TCGCCCGCGA TCCCGCGACC GTTCACATCG CCAGTGCGAC GCGCATTGGT GGTGTGGGCG
6601  CGCGCTTAAT GCGCCGCTAC AGGGCGCGTC AGGTGGCACT TTTCGGGGAA ATGTGCGCGG
      GCGCGAATTA CGCGGCGATG TCCCGCGCAG TCCACCGTGA AAAGCCCCTT TACACGCGCC
6661  AACCCCTATT TGTTTATTTT TCTAAATACA TTCAAATATG TATCCGCTCA TGAGACAATA
      TTGGGGATAA ACAAATAAAA AGATTTATGT AAGTTTATAC ATAGGCGAGT ACTCTGTTAT
6721  ACCCTGATAA ATGCTTCAAT AATATTGAAA AAGGAAGAGT ATGAGTATTC AACATTTCCG
      TGGGACTATT TACGAAGTTA TTATAACTTT TTCCTTCTCA TACTCATAAG TTGTAAAGGC
6781  TGTCGCCCTT ATTCCCTTTT TTGCGGCATT TTGCCTTCCT GTTTTTGCTC ACCCAGAAAC
      ACAGCGGGAA TAAGGGAAAA AACGCCGTAA AACGGAAGGA CAAAAACGAG TGGGTCTTTG
6841  GCTGGTGAAA GTAAAAGATG CTGAAGATCA GTTGGGTGCA CGAGTGGGTT ACATCGAACT
      CGACCACTTT CATTTTCTAC GACTTCTAGT CAACCCACGT GCTCACCCAA TGTAGCTTGA
6901  GGATCTCAAC AGCGGTAAGA TCCTTGAGAG TTTTCGCCCC GAAGAACGTT TTCCAATGAT
      CCTAGAGTTG TCGCCATTCT AGGAACTCTC AAAAGCGGGG CTTCTTGCAA AAGGTTACTA
6961  GAGCACTTTT AAAGTTCTGC TATGTGGCGC GGTATTATCC CGTATTGACG CCGGGCAAGA
      CTCGTGAAAA TTTCAAGACG ATACACCGCG CCATAATAGG GCATAACTGC GGCCCGTTCT
7021  GCAACTCGGT CGCCGCATAC ACTATTCTCA GAATGACTTG GTTGAGTACT CACCAGTCAC
      CGTTGAGCCA GCGGCGTATG TGATAAGAGT CTTACTGAAC CAACTCATGA GTGGTCAGTG
7081  AGAAAAGCAT CTTACGGATG GCATGACAGT AAGAGAATTA TGCAGTGCTG CCATAACCAT
      TCTTTTCGTA GAATGCCTAC CGTACTGTCA TTCTCTTAAT ACGTCACGAC GGTATTGGTA
7141  GAGTGATAAC ACTGCGGCCA ACTTACTTCT GACAACGATC GGAGGACCGA AGGAGCTAAC
      CTCACTATTG TGACGCCGGT TGAATGAAGA CTGTTGCTAG CCTCCTGGCT TCCTCGATTG
7201  CGCTTTTTTG CACAACATGG GGGATCATGT AACTCGCCTT GATCGTTGGG AACCGGAGCT
      GCGAAAAAAC GTGTTGTACC CCCTAGTACA TTGAGCGGAA CTAGCAACCC TTGGCCTCGA
7261  GAATGAAGCC ATACCAAACG ACGAGCGTGA CACCACGATG CCTGTAGCAA TGGCAACAAC
      CTTACTTCGG TATGGTTTGC TGCTCGCACT GTGGTGCTAC GGACATCGTT ACCGTTGTTG
7321  GTTGCGCAAA CTATTAACTG GCGAACTACT TACTCTAGCT TCCCGGCAAC AATTAATAGA
      CAACGCGTTT GATAATTGAC CGCTTGATGA ATGAGATCGA AGGGCCGTTG TTAATTATCT
7381  CTGGATGGAG GCGGATAAAG TTGCAGGACC ACTTCTGCGC TCGGCCCTTC CGGCTGGCTG
      GACCTACCTC CGCCTATTTC AACGTCCTGG TGAAGACGCG AGCCGGGAAG GCCGACCGAC
7441  GTTTATTGCT GATAAATCTG GAGCCGGTGA GCGTGGGTCT CGCGGTATCA TTGCAGCACT
      CAAATAACGA CTATTTAGAC CTCGGCCACT CGCACCCAGA GCGCCATAGT AACGTCGTGA
7501  GGGGCCAGAT GGTAAGCCCT CCCGTATCGT AGTTATCTAC ACGACGGGGA GTCAGGCAAC
      CCCCGGTCTA CCATTCGGGA GGGCATAGCA TCAATAGATG TGCTGCCCCT CAGTCCGTTG
7561  TATGGATGAA CGAAATAGAC AGATCGCTGA GATAGGTGCC TCACTGATTA AGCATTGGTA
      ATACCTACTT GCTTTATCTG TCTAGCGACT CTATCCACGG AGTGACTAAT TCGTAACCAT
7621  ACTGTCAGAC CAAGTTTACT CATATATACT TTAGATTGAT TTAAAACTTC ATTTTTAATT
      TGACAGTCTG GTTCAAATGA GTATATATGA AATCTAACTA AATTTTGAAG TAAAAATTAA
7681  TAAAAGGATC TAGGTGAAGA TCCTTTTTGA TAATCTCATG ACCAAAATCC CTTAACGTGA
      ATTTTCCTAG ATCCACTTCT AGGAAAAACT ATTAGAGTAC TGGTTTTAGG GAATTGCACT
7741  GTTTTCGTTC CACTGAGCGT CAGACCCCGT AGAAAAGATC AAAGGATCTT CTTGAGATCC
      CAAAAGCAAG GTGACTCGCA GTCTGGGGCA TCTTTTCTAG TTTCCTAGAA GAACTCTAGG
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7801 TTTTTTTCTG CGCGTAATCT GCTGCTTGCA AACAAAAAAA CCACCGCTAC CAGCGGTGGT
     AAAAAAAGAC GCGCATTAGA CGACGAACGT TTGTTTTTTT GGTGGCGATG GTCGCCACCA
7861 TTGTTTGCCG GATCAAGAGC TACCAACTCT TTTTCCGAAG GTAACTGGCT TCAGCAGAGC
     AACAAACGGC CTAGTTCTCG ATGGTTGAGA AAAAGGCTTC CATTGACCGA AGTCGTCTCG
7921 GCAGATACCA AATACTGTTC TTCTAGTGTA GCCGTAGTTA GGCCACCACT TCAAGAACTC
     CGTCTATGGT TTATGACAAG AAGATCACAT CGGCATCAAT CCGGTGGTGA AGTTCTTGAG
7981 TGTAGCACCG CCTACATACC TCGCTCTGCT AATCCTGTTA CCAGTGGCTG CTGCCAGTGG
     ACATCGTGGC GGATGTATGG AGCGAGACGA TTAGGACAAT GGTCACCGAC GACGGTCACC
8041 CGATAAGTCG TGTCTTACCG GGTTGGACTC AAGACGATAG TTACCGGATA AGGCGCAGCG
     GCTATTCAGC ACAGAATGGC CCAACCTGAG TTCTGCTATC AATGGCCTAT TCCGCGTCGC
8101 GTCGGGCTGA ACGGGGGGTT CGTGCACACA GCCCAGCTTG GAGCGAACGA CCTACACCGA
     CAGCCCGACT TGCCCCCCAA GCACGTGTGT CGGGTCGAAC CTCGCTTGCT GGATGTGGCT
8161 ACTGAGATAC CTACAGCGTG AGCTATGAGA AAGCGCCACG CTTCCCGAAG GGAGAAAGGC
     TGACTCTATG GATGTCGCAC TCGATACTCT TTCGCGGTGC GAAGGGCTTC CCTCTTTCCG
8221 GGACAGGTAT CCGGTAAGCG GCAGGGTCGG AACAGGAGAG CGCACGAGGG AGCTTCCAGG
     CCTGTCCATA GGCCATTCGC CGTCCCAGCC TTGTCCTCTC GCGTGCTCCC TCGAAGGTCC
8281 GGGAAACGCC TGGTATCTTT ATAGTCCTGT CGGGTTTCGC CACCTCTGAC TTGAGCGTCG
     CCCTTTGCGG ACCATAGAAA TATCAGGACA GCCCAAAGCG GTGGAGACTG AACTCGCAGC
8341 ATTTTTGTGA TGCTCGTCAG GGGGGCGGAG CCTATGGAAA AACGCCAGCA ACGCGGCCTT
     TAAAAACACT ACGAGCAGTC CCCCCGCCTC GGATACCTTT TTGCGGTCGT TGCGCCGGAA
8401 TTTACGGTTC CTGGCCTTTT GCTGGCCTTT TGCTCACATG TTCTTTCCTG CGTTATCCCC
     AAATGCCAAG GACCGGAAAA CGACCGGAAA ACGAGTGTAC AAGAAAGGAC GCAATAGGGG
8461 TGATTCTGTG GATAACCGTA TTACCGCCTT TGAGTGAGCT GATACCGCTC GCCGCAGCCG
     ACTAAGACAC CTATTGGCAT AATGGCGGAA ACTCACTCGA CTATGGCGAG CGGCGTCGGC
8521 AACGACCGAG CGCAGCGAGT CAGTGAGCGA GGAAGCGGAA GAGCGCCCAA TACGCAAACC
     TTGCTGGCTC GCGTCGCTCA GTCACTCGCT CCTTCGCCTT CTCGCGGGTT ATGCGTTTGG
8581 GCCTCTCCCC GCGCGTTGGC CGATTCATTA ATGCAGCTGG CACGACAGGT TTCCCGACTG
     CGGAGAGGGG CGCGCAACCG GCTAAGTAAT TACGTCGACC GTGCTGTCCA AAGGGCTGAC
8641 GAAAGCGGGC AGTGAGCGCA ACGCAATTAA TGTGAGTTAG CTCACTCATT AGGCACCCCA
     CTTTCGCCCG TCACTCGCGT TGCGTTAATT ACACTCAATC GAGTGAGTAA TCCGTGGGGT
8701 GGCTTTACAC TTTATGCTTC CGGCTCGTAT GTTGTGTGGA ATTGTGAGCG GATAACAATT
     CCGAAATGTG AAATACGAAG GCCGAGCATA CAACACACCT TAACACTCGC CTATTGTTAA
8761 TCACACAGGA AACAGCTATG ACCATGATTA CGCCAAGCTC GAAATTAACC CTCACTAAAG
     AGTGTGTCCT TTGTCGATAC TGGTACTAAT GCGGTTCGAG CTTTAATTGG GAGTGATTTC
8821 GGAACAAAAG CTGGAGCTCC ACCGCGGTGG CGGCCTCGAG GTCGAGATCC GGTCGACCAG
     CCTTGTTTTC GACCTCGAGG TGGCGCCACC GCCGGAGCTC CAGCTCTAGG CCAGCTGGTC
8881 CAACCATAGT CCCGCCCCTA ACTCCGCCCA TCCCGCCCCT AACTCCGCCC AGTTCCGCCC
     GTTGGTATCA GGGCGGGGAT TGAGGCGGGT AGGGCGGGGA TTGAGGCGGG TCAAGGCGGG
8941 ATTCTCCGCC CCATGGCTGA CTAATTTTTT TTATTTATGC AGAGGCCGAG GCCGCCTCGG
     TAAGAGGCGG GGTACCGACT GATTAAAAAA AATAAATACG TCTCCGGCTC CGGCGGAGCC
9001 CCTCTGAGCT ATTCCAGAAG TAGTGAGGAG GCTTTTTTGG AGGCCTAGGC TTTTGCAAAA
     GGAGACTCGA TAAGGTCTTC ATCACTCCTC CGAAAAAACC TCCGGATCCG AAAACGTTTT
9061 AGCTTCGACG GTATCGATTG GCTCATGTCC AACATTACCG CCATGTTGAC ATTGATTATT
     TCGAAGCTGC CATAGCTAAC CGAGTACAGG TTGTAATGGC GGTACAACTG TAACTAATAA
9121 GACTAGTTAT TAATAGTAAT CAATTACGGG GTCATTAGTT CATAGCCCAT ATATGGAGTT
     CTGATCAATA ATTATCATTA GTTAATGCCC CAGTAATCAA GTATCGGGTA TATACCTCAA
9181 CCGCGTTACA TAACTTACGG TAAATGGCCC GCCTGGCTGA CCGCCCAACG ACCCCCGCCC
     GGCGCAATGT ATTGAATGCC ATTTACCGGG CGGACCGACT GGCGGGTTGC TGGGGGCGGG
9241 ATTGACGTCA ATAATGACGT ATGTTCCCAT AGTAACGCCA ATAGGGACTT TCCATTGACG
     TAACTGCAGT TATTACTGCA TACAAGGGTA TCATTGCGGT TATCCCTGAA AGGTAACTGC
9301 TCAATGGGTG GAGTATTTAC GGTAAACTGC CCACTTGGCA GTACATCAAG TGTATCATAT
     AGTTACCCAC CTCATAAATG CCATTTGACG GGTGAACCGT CATGTAGTTC ACATAGTATA
9361 GCCAAGTACG CCCCCTATTG ACGTCAATGA CGGTAAATGG CCCGCCTGGC ATTATGCCCA
     CGGTTCATGC GGGGGATAAC TGCAGTTACT GCCATTTACC GGGCGGACCG TAATACGGGT
9421 GTACATGACC TTATGGGACT TTCCTACTTG GCAGTACATC TACGTATTAG TCATCGCTAT
     CATGTACTGG AATACCCTGA AAGGATGAAC CGTCATGTAG ATGCATAATC AGTAGCGATA
9481 TACCATGGTG ATGCGGTTTT GGCAGTACAT CAATGGGCGT GGATAGCGGT TTGACTCACG
     ATGGTACCAC TACGCCAAAA CCGTCATGTA GTTACCCGCA CCTATCGCCA AACTGAGTGC
9541 GGGATTTCCA AGTCTCCACC CCATTGACGT CAATGGGAGT TTGTTTTGGC ACCAAAATCA
     CCCTAAAGGT TCAGAGGTGG GGTAACTGCA GTTACCCTCA AACAAAACCG TGGTTTTAGT
9601 ACGGGACTTT CCAAAATGTC GTAACAACTC CGCCCCATTG ACGCAAATGG GCGGTAGGCG
     TGCCCTGAAA GGTTTTACAG CATTGTTGAG GCGGGGTAAC TGCGTTTACC CGCCATCCGC
9661 TGTACGGAAT TCGGAGTGGC GAGCCCTCAG ATCCTGCATA TAAGCAGCTG CTTTTTGCCT
     ACATGCCTTA AGCCTCACCG CTCGGGAGTC TAGGACGTAT ATTCGTCGAC GAAAAACGGA
9721 GTACTGGGTC TCTCTG
     CATGACCCAG AGAGAC
```

Figure 11 (sheet 5 of 5)

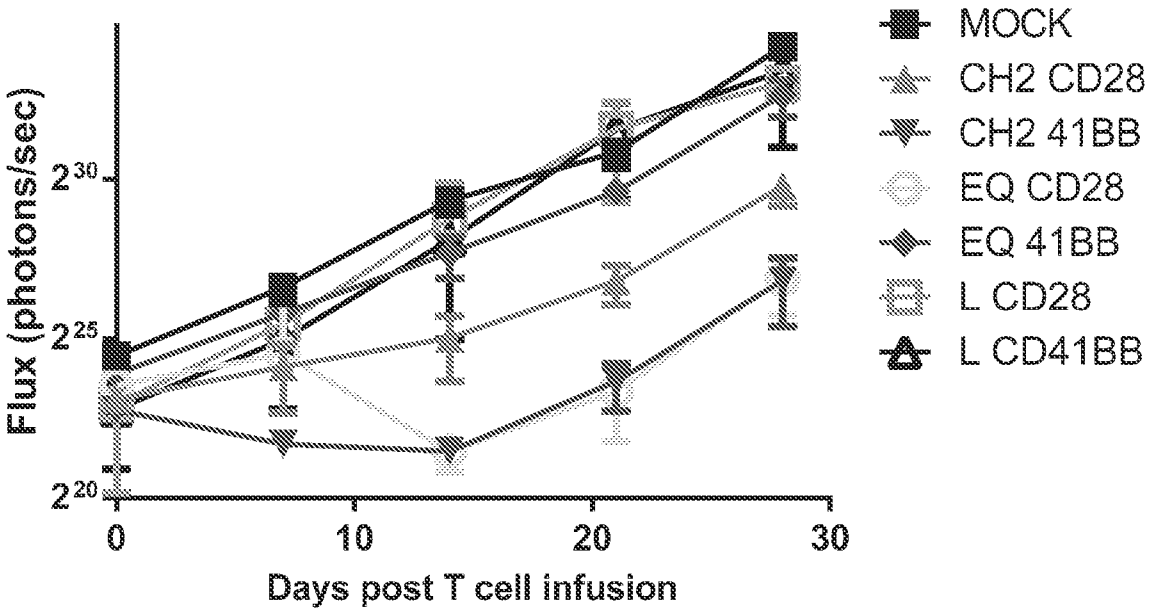
Flux (photons/sec)
2^{30}
2^{25}
2^{20}
0
10
20
30
Days post T cell infusion
MOCK
CH2 CD28
CH2 41BB
EQ CD28
EQ 41BB
L CD28
L CD41BB

Figure 12

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2015/064303

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K14/35 A61K35/14 C07K16/28 C07K16/46 C07K19/00
C12N5/0783
ADD.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07K C12P C12N A61K

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, BIOSIS, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2014/179759 A1 (OHIO STATE INNOVATION FOUNDATION [US]) 6 November 2014 (2014-11-06) claims 1-7, 13, 15-18, 20,; figures 1A, 13A; example 1; table 1 p. 1 li. 20-p. 2 li. 19, p. 10 li. 17-p. 11 li. 5, p. 11 li. 27-28, p. 2 li. 20-23, p. 23 li. 15-31, p. 17 li. 14-16, p. 9 li. 19-24, p. 10 li. 15-16, p. 20 li. 27-31, p. 22 li. 11-15, p. 13 li. 17-19, p. 12 li. 1-30, p. 12 li. 33-p. 13 li. 3, p. 13li. 7-9 -/--	1-4, 6-13, 22-25, 32, 34-37, 44, 46-49, 53, 55-57, 65-76

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

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
14 April 2016	25/04/2016

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Landré, Julien

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2015/064303

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	J CHU ET AL: "CS1-specific chimeric antigen receptor (CAR)-engineered natural killer cells enhance in vitro and in vivo antitumor activity against human multiple myeloma", LEUKEMIA, vol. 28, no. 4, 26 September 2013 (2013-09-26), pages 917-927, XP55133640, ISSN: 0887-6924, DOI: 10.1038/leu.2013.279 the whole document	8-10,13, 25,37,49
X,P	WO 2015/166056 A1 (CELLECTIS [FR]) 5 November 2015 (2015-11-05) the whole document	1-4, 6-13, 22-25, 32, 34-37, 44, 46-49, 53, 55-57, 65-76
X,P	WO 2015/121454 A1 (CELLECTIS [FR]) 20 August 2015 (2015-08-20) the whole document	1-4, 6-13, 22-25, 32, 34-37, 44, 46-49, 53, 55-57, 65-76
Y	WO 2013/123061 A1 (SEATTLE CHILDREN S HOSPITAL D B A SEATTLE CHILDREN S RES INST [US]) 22 August 2013 (2013-08-22) the whole document	8-10,13, 25,37,49

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2015/064303

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: 5, 14-21, 26-31, 33, 38-43, 45, 50-52, 54, 58-64, 77-79
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
see FURTHER INFORMATION sheet PCT/ISA/210

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2015/064303

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2014179759 A1	06-11-2014	AU 2014259675 A1	12-11-2015
		CA 2910666 A1	06-11-2014
		CN 105377897 A	02-03-2016
		EP 2992020 A1	09-03-2016
		KR 20160003071 A	08-01-2016
		US 2016075784 A1	17-03-2016
		WO 2014179759 A1	06-11-2014
WO 2015166056 A1	05-11-2015	NONE	
WO 2015121454 A1	20-08-2015	NONE	
WO 2013123061 A1	22-08-2013	AU 2013221672 A1	07-08-2014
		CA 2861491 A1	22-08-2013
		EP 2814846 A1	24-12-2014
		HK 1205144 A1	11-12-2015
		JP 2015513394 A	14-05-2015
		US 2015038684 A1	05-02-2015
		WO 2013123061 A1	22-08-2013

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box II.2

Claims Nos.: 5, 14-21, 26-31, 33, 38-43, 45, 50-52, 54, 58-64, 77-79

The specific sequences of claims 5, 14-21, 26-31, 33, 38-43, 45, 50-52, 54, 58-64, 77-79 have, according to PCT Rule13ter.1.d, not been searched since the Sequence Listing as present in the description does not comply with WIPO Standard ST 25 prescribed in the administrative instructions under Rule 5.2. The Sequence Listing has been furnished neither in paper form nor in machine readable form as provided for in the same instructions and the applicant has not remedied the disclosed deficiencies within the time limit fixed in the invitation pursuant to PCT Rule 13ter.1 .a.

The applicant's attention is drawn to the fact that claims relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure. If the application proceeds into the regional phase before the EPO, the applicant is reminded that a search may be carried out during examination before the EPO (see EPO Guidelines C-IV, 7.2), should the problems which led to the Article 17(2) declaration be overcome.