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(54) Title: COMPOSITIONS AND METHODS FOR TREATING CANCER WITH ANTI-CD123 IMMUNOTHERAPY


FIGURE 1A

(57) Abstract: Chimeric antigen receptors containing CD123 antigen binding domains are disclosed. Nucleic acids, recombinant expression vectors, host cells, antigen binding fragments, and pharmaceutical compositions, relating to the chimeric antigen receptors are also disclosed. Methods of treating or preventing cancer in a subject, and methods of making chimeric antigen receptor T cells are also disclosed.

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# COMPOSITIONS AND METHODS FOR TREATING CANCER WITH ANTI-CD123 IMMUNOTHERAPY

## CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of U.S. Utility Application No. 17/685,132, filed on March 2, 2022 and U.S. Utility Application No. 18/154,209, filed on January 13, 2023. The entire contents of each of which are incorporated herein by reference.

## SEQUENCE LISTING

This application contains a Sequence Listing that has been submitted electronically as an XML file named Sequence_Listing. The XML file, created on February 28, 2023, is 114,458 bytes in size. The material in the XML file is hereby incorporated by reference in its entirety.

## FIELD OF THE DISCLOSURE

This application relates to the field of cancer, particularly to CD123 antigen binding domains and chimeric antigen receptors (CARs) containing such CD123 antigen binding domains and methods of use thereof.

## BACKGROUND

Cancer is one of the most deadly threats to human health. In the U.S. alone, cancer affects nearly 1.3 million new patients each year, and is the second leading cause of death after cardiovascular disease, accounting for approximately 1 in 4 deaths. Solid tumors are responsible for most of those deaths. Although there have been significant advances in the medical treatment of certain cancers, the overall 5-year survival rate for all cancers has improved only by about 10% in the past 20 years. Cancers, or malignant tumors, metastasize and grow rapidly in an uncontrolled manner, making treatment extremely difficult.

AML is a devastating disease with overall survival rate of only 26%. While young patients tend to have a better prognosis for treatment in AML, the five year survival in older patients may be as low as only 5%. First line treatment of AML involves multiple rounds of chemotherapy, (*i.e.* induction, consolidation) which bear high risk of toxicity. If hematopoietic stem cell transplant is

performed after the 1st remission, the 5 year disease-free survival rate is only 30-50% (http://www.cancer.ca/en/cancer-information/cancer-type/leukemia-acute-myelogenous-aml/prognosis-and-survival/survival-statistics/?region=on). In addition, AML patients with high disease burden may not be candidates for bone marrow transplant, and minimal residual disease pre-transplant correlates with AML relapse. The present 1st line induction/consolidation therapy often fails to achieve MDR-negative remission to sufficiently reduce tumor burden, thus the risk of AML relapse after 1st line therapy with or without BMT remains high (1) Biol Blood Marrow Transplant. 2006 Jun;12(6):691-2., Leukemia burden and outcome of allogeneic transplant in acute myelogenous leukemia., Kamble RT, Hjortsvang E, Selby GB; (2) Leuk Lymphoma. 2015 May;56(5):1353-61. Impact of pre-transplant disease burden on the outcome of allogeneic hematopoietic stem cell transplant in refractory and relapsed acute myeloid leukemia: a single-center study. Tian H *et al.*). PBDCN is a rare myeloid neoplasm that is classified as a subtype of AML and is sometimes treated as AML with induction and consolidation chemotherapy, and sometimes as ALL. BMT is often administered at 1st remission. However, there are currently no ongoing clinical trials for PBDCN, and no approved 1st line treatment. (Leukemia Lymphoma Society, https://www.lls.org/leukemia/blastic-plasmacytoid-dendritic-cell-neoplasm). Therefore, better therapeutic modalities are urgently needed for CD123+ malignancies.

CAR approaches targeting CD123 are superior to chemotherapy because they may achieve better efficacy in eliminating CD123+ tumor cells and tumor stem cells, and because they avoid the toxicities associated with chemotherapy. Importantly, CAR T cells are expected to be more efficient than chemotherapy in eliminating minimal residual disease, resulting in better long-term treatment prognosis. Furthermore, CAR123 may be used for tumor debulking as a bridge to transplant, as may help patient with high tumor burden become eligible for BMT.

CAR123 represents an improvement over prior art because unique human ScFv (hereinafter "hScFv") sequences are used in the CAR design, as opposed to murine-derived ScFvs employed in CAR designs elsewhere. Mouse-derived sequences carry the risk of immunogenicity, and may induce allergic or anaphylactic responses in patients, leading to CAR T elimination, or life-threatening anaphylaxis.

Chimeric Antigen Receptors (CARs) are hybrid molecules comprising three essential units: (1) an extracellular antigen-binding motif, (2) linking/transmembrane motifs, and (3) intracellular T-cell signaling motifs (Long AH, Haso WM, Orentas RJ. Lessons learned from a highly-active CD22-specific chimeric antigen receptor. Oncoimmunology. 2013; 2 (4):e23621). The antigen-binding motif of a CAR is commonly fashioned after a single chain Fragment variable (ScFv), the minimal binding domain of an immunoglobulin (Ig) molecule. Alternate antigen-binding motifs,

such as receptor ligands (*i.e.*, IL-13 has been engineered to bind tumor expressed IL-13 receptor), intact immune receptors, library-derived peptides, and innate immune system effector molecules (such as NKG2D) also have been engineered. Alternate cell targets for CAR expression (such as NK or gamma-delta T cells) are also under development (Brown CE *et al.* Clin Cancer Res. 2012;18(8):2199–209; Lehner M *et al.* PLoS One. 2012; 7 (2):e31210). There remains significant work with regard to defining the most active T-cell population to transduce with CAR vectors, determining the optimal culture and expansion techniques, and defining the molecular details of the CAR protein structure itself.

The linking motifs of a CAR can be a relatively stable structural domain, such as the constant domain of IgG, or designed to be an extended flexible linker. Structural motifs, such as those derived from IgG constant domains, can be used to extend the ScFv binding domain away from the T-cell plasma membrane surface. This may be important for some tumor targets where the binding domain is particularly close to the tumor cell surface membrane (such as for the disialoganglioside GD2; Orentas *et al.*, unpublished observations). To date, the signaling motifs used in CARs always include the CD3-ζ chain because this core motif is the key signal for T cell activation. The first reported second-generation CARs featured CD28 signaling domains and the CD28 transmembrane sequence. This motif was used in third-generation CARs containing CD137 (4-1BB) signaling motifs as well (Zhao Y *et al.* J Immunol. 2009; 183 (9): 5563–74). With the advent of new technology, the activation of T cells with beads linked to anti-CD3 and anti-CD28 antibody, and the presence of the canonical "signal 2" from CD28 was no longer required to be encoded by the CAR itself. Using bead activation, third-generation vectors were found to be not superior to second-generation vectors in *in vitro* assays, and they provided no clear benefit over second-generation vectors in mouse models of leukemia (Haso W, Lee DW, Shah NN, Stetler-Stevenson M, Yuan CM, Pastan IH, Dimitrov DS, Morgan RA, FitzGerald DJ, Barrett DM, Wayne AS, Mackall CL, Orentas RJ. Anti-CD22-chimeric antigen receptors targeting B cell precursor acute lymphoblastic leukemia, Blood. 2013; 121 (7):1165–74; Kochenderfer JN *et al.* Blood. 2012; 119 (12):2709–20). This is borne out by the clinical success of CD19-specific CARs that are in a second generation CD28/CD3-ζ (Lee DW *et al.* American Society of Hematology Annual Meeting. New Orleans, LA; December 7-10, 2013) and a CD137/CD3-ζ signaling format (Porter DL *et al.* N Engl J Med. 2011; 365 (8): 725–33). In addition to CD137, other tumor necrosis factor receptor superfamily members such as OX40 also are able to provide important persistence signals in CAR-transduced T cells (Yvon E *et al.* Clin Cancer Res. 2009;15(18):5852–60). Equally important are the culture conditions under which the CAR T-cell populations were cultured.

T-cell-based immunotherapy has become a new frontier in synthetic biology; multiple promoters and gene products are envisioned to steer these highly potent cells to the tumor microenvironment, where T cells can both evade negative regulatory signals and mediate effective tumor killing. The elimination of unwanted T cells through the drug-induced dimerization of inducible caspase 9 constructs with AP1903 demonstrates one way in which a powerful switch that can control T-cell populations can be initiated pharmacologically (Di Stasi A *et al.* N Engl J Med. 2011;365(18):1673–83). The creation of effector T-cell populations that are immune to the negative regulatory effects of transforming growth factor-β by the expression of a decoy receptor further demonstrates that degree to which effector T cells can be engineered for optimal antitumor activity (Foster AE *et al.* J Immunother. 2008;31(5):500–5). Thus, while it appears that CARs can trigger T-cell activation in a manner similar to an endogenous T-cell receptor, a major impediment to the clinical application of this technology to date has been limited *in vivo* expansion of CAR+ T cells, rapid disappearance of the cells after infusion, and disappointing clinical activity. Accordingly, there is an urgent and long felt need in the art for discovering novel compositions and methods for treatment of AML using an approach that can exhibit specific and efficacious anti-tumor effect without the aforementioned shortcomings (i.e. high toxicity, insufficient efficacy).

In addition, natural killer (NK) cell-based cancer immunotherapy has been gaining momentum in the past years (Shimasaki, N., Jain, A. & Campana, D. NK cells for cancer immunotherapy. Nat Rev Drug Discov 19, 200–218 (2020)). Human haploidentical NK cells were shown to be amenable to adoptive transfer and expansion in pediatric and adult cancer patients. (Miller, J. S. et al. Blood 105, 3051–3057 (2005); Rubnitz, J. E. et al. J. Clin. Oncol. 28, 955–959 (2010)). Moreover, second-generation CD19-CAR NK cells generated ex-vivo were effective in killing B Cell ALL (Imai, C., Iwamoto, S. & Campana, D. Blood 106, 376–383 (2005). NK cells activity in vivo may be further enhanced by expression of IL-15, IL-12, IL-18, or other cytokine variants stimulating autonomous growth, cytotoxicity, and prolonged effector function (Imamura, M. et al. *Blood* **124**, 1081–1088 (2014)); Ni, J., Miller, M., Stojanovic, A., Garbi, N. & Cerwenka, A. J. Exp. Med. 209, 2351–2365 (2012). By secreting chemoattractants such as CCL5, XCL1 and FLT3L, NK cells are capable of attracting dendritic cells to tumor sites, and thus promote tumor microenvironment favorable for tumor control by the immune system (Bottcher, J. P. et al. Cell 172, 1022–1037 (2018); Barry, K. C. et al. A natural killer-dendritic cell axis defines checkpoint therapy-responsive tumor microenvironments. Nat. Med. 24, 1178–1191 (2018)). In addition, NK cells may be rendered more effective against tumors by manipulation of culture conditions. For example, NK cell activation with cytokines IL-12, IL-15 and IL-18 enhanced NK cell anti-AML responses (Romee, R. et al. Sci. Transl. Med. 8, 357ra123 (2016)).

Clinical generation of NK cells from cord blood hematopoietic progenitors is feasible (Spanholtz, J. et al. PLOS ONE 6, e20740 (2011); Knorr, D. A. et al. Stem Cell Transl. Med. 2, 274–283 (2013)) Similarly, CAR NK cells may be derived by differentiation form iPSCs expressing CAR (Li, Y., Hermanson, D. L., Moriarity, B. S. & Kaufman, D. S. Cell Stem Cell 23, 181–192 (2018). While methods for generating highly effective NK cells and CAR NK cells for cancer therapy are continuing to evolve, significant strides have been made in this field in the past years (Granzin, M. et al.. Oncoimmunology 5, e1219007 (2016)). Taken together, these findings demonstrate the emerging potential of CAR NK cells in cancer immunotherapy.

The present invention addresses these needs by providing CAR compositions and therapeutic methods that can be used to treat cancers and other diseases and/or conditions. In particular, the present invention, as disclosed and described herein, provides CARs that may be used in the treatment of diseases, disorders or conditions associated with dysregulated expression of CD123 and which CARs contain CD123 antigen binding domains that exhibit a high surface expression on transduced T cells and NK cells, exhibit a high degree of cytolysis, and transduced T cell *in vivo* expansion and persistence.

## SUMMARY

Novel anti-CD123 antibodies or antigen binding domains thereof and chimeric antigen receptors (CARs) that contain such CD123 antigen binding domains are provided herein, as well as host cells (*e.g.*, T cells) expressing the receptors, and nucleic acid molecules encoding the receptors. CAR may consist either of a single molecule expressed on the effector cell surface, or a CAR comprised of an effector cell-expressed signaling module and a soluble targeting module, such as when the soluble targeting module binds to the cell-expressed signaling module, a complete functional CAR is formed. The CARs exhibit a high surface expression on transduced T cells, with a high degree of cytolysis and transduced T cell expansion and persistence *in vivo*. Methods of using the disclosed CARs, host cells, and nucleic acid molecules are also provided, for example, to treat a cancer in a subject.

Thus, in one aspect, an isolated polynucleotide encoding a human anti-CD123 antibody or a fragment thereof is provided comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 1, 3, 5, 7, 9, 11, 15, 17, 19, 21, 23, 25, 69, 71, 77, and 87.

In one embodiment, an isolated polynucleotide encoding a fully human anti-CD123 antibody or a fragment thereof is provided, wherein the antibody or a fragment thereof comprises a

fragment selected from the group consisting of an Fab fragment, an $F(ab')_2$ fragment, an Fv fragment, and a single chain Fv (ScFv).

In one embodiment, an isolated polynucleotide encoding a fully human anti-CD123 antibody or a fragment thereof is provided, wherein the antibody or a fragment thereof comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 4, 6, 8, 10, 12, 16, 18, 20, 22, 24, 26, 70, 72, 78, and 88.

In one aspect, an isolated nucleic acid molecule encoding a chimeric antigen receptor (CAR) is provided comprising, from N-terminus to C-terminus, at least one CD123 antigen binding domain encoded by a nucleotide sequence comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 1, 3, 5, 7, 9, 11, 15, 17, 19, 21, 23, 25, 69, 71, 77, and 87, at least one transmembrane domain, and at least one intracellular signaling domain.

In one embodiment, an isolated nucleic acid molecule encoding the CAR is provided wherein the encoded extracellular CD123 antigen binding domain comprises at least one single chain variable fragment of an antibody that binds to CD123.

In another embodiment, an isolated nucleic acid molecule encoding the CAR is provided wherein the encoded extracellular CD123 antigen binding domain comprises at least one heavy chain variable region of an antibody that binds to CD123.

In one embodiment, the targeting domain of the CAR is expressed separately in the form of monoclonal antibody, ScFv Fab, Fab'2 and is containing an antigen-targeting domain comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 1, 3, 5, 7, 9, 11, 15, 17, 19, 21, 23, 25, 69, 71, 77, and 87, coupled to an additional binding tag or epitope, whereas the effector-cell expressed component of the CAR contains a binding domain specifically directed to bind the tag or epitope expressed on the soluble CAR module, such as specific binding on the soluble component of the CAR to the cell bound component of the CAR forms the full functional CAR structure.

In another embodiment, the targeting domain of the CAR is expressed separately in the form of a monoclonal antibody, ScFv Fab, Fab'2 and contains an antigen-targeting domain comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 1, 3, 5, 7, 9, 11, 15, 17, 19, 21, 23, 25, 69, 71, 77, and 87, and an additional ScFv, whereas the effector-cell expressed component of the CAR contains a tag or epitope specifically reactive with the additional ScFv expressed on the soluble CAR module, such as specific binding on the soluble component of the CAR to the cell bound component of the CAR forms the full functional CAR structure.

In yet another embodiment, an isolated nucleic acid molecule encoding the CAR is provided wherein the encoded CAR extracellular CD123 antigen binding domain further comprises at least one lipocalin-based antigen binding antigen (anticalins) that binds to CD123.

In one embodiment, an isolated nucleic acid molecule is provided wherein the encoded extracellular CD123 antigen binding domain is connected to the transmembrane domain by a linker domain.

In another embodiment, an isolated nucleic acid molecule encoding the CAR is provided wherein the encoded CD123 extracellular antigen binding domain is preceded by a sequence encoding a leader or signal peptide.

In yet another embodiment, an isolated nucleic acid molecule encoding the CAR is provided comprising at least one CD123 antigen binding domain encoded by a nucleotide sequence comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 1, 3, 5, 7, 9, 11, 15, 17, 19, 21, 23, 25, 69, 71, 77, and 87, and wherein the CAR additionally encodes an extracellular antigen binding domain targets an antigen that includes, but is not limited to, CD19, CD20, CD22, ROR1, mesothelin, CD33, CD38, CD138, BCMA (CD269), GPC2, GPC3, FGFR4, c-Met, PSMA, Glycolipid F77, EGFRvIII, GD-2, NY-ESO-1 TCR, MAGE A3 TCR, or any combination thereof.

In certain embodiments, an isolated nucleic acid molecule encoding the CAR is provided wherein the additionally encoded extracellular antigen binding domain comprises an anti-CD19 ScFv antigen binding domain, an anti-CD20 ScFv antigen binding domain, an anti-CD22 ScFv antigen binding domain, an anti-ROR1 ScFv antigen binding domain, an anti-mesothelin ScFv antigen binding domain, an anti-CD33 ScFv antigen binding domain, an anti-CD38 ScFv antigen binding domain, an anti-CD123 (IL3RA) ScFv antigen binding domain, an anti-CD138 ScFv antigen binding domain, an anti-BCMA (CD269) ScFv antigen binding domain, an anti-GPC2 ScFv antigen binding domain, an anti-GPC3 ScFv antigen binding domain, an anti-FGFR4 ScFv antigen binding domain, an anti-c-Met ScFv antigen binding domain, an anti-PMSA ScFv antigen binding domain, an anti-glycolipid F77 ScFv antigen binding domain, an anti-EGFRvIII ScFv antigen binding domain, an anti-GD-2 ScFv antigen binding domain, an anti-NY-ESo-1 TCR ScFv antigen binding domain, an anti-MAGE A3 TCR ScFv antigen binding domain, or an amino acid sequence with 85%, 90%, 95%, 96%, 97%, 98% or 99% identity thereof, or any combination thereof.

In one aspect, the CARs provided herein further comprise a linker or spacer domain.

In one embodiment, an isolated nucleic acid molecule encoding the CAR is provided wherein the extracellular CD123 antigen binding domain, the intracellular signaling domain, or both are connected to the transmembrane domain by a linker or spacer domain.

In one embodiment, an isolated nucleic acid molecule encoding the CAR is provided wherein the encoded linker domain is derived from the extracellular domain of CD8 or CD28, and is linked to a transmembrane domain.

In another embodiment, an isolated nucleic acid molecule encoding the CAR is provided wherein the encoded CAR further comprises a transmembrane domain that comprises a transmembrane domain of a protein selected from the group consisting of the alpha, beta or zeta chain of the T-cell receptor, CD28, CD3 epsilon, CD45, CD4, CD5, CD8, CD9, CD16, CD22, CD33, CD37, CD64, CD80, CD86, CD134, CD137 and CD154, or a combination thereof.

In yet another embodiment, an isolated nucleic acid molecule encoding the CAR is provided wherein the encoded intracellular signaling domain further comprises a CD3 zeta intracellular domain.

In one embodiment, an isolated nucleic acid molecule encoding the CAR is provided wherein the encoded intracellular signaling domain is arranged on a C-terminal side relative to the CD3 zeta intracellular domain.

In another embodiment, an isolated nucleic acid molecule encoding the CAR is provided wherein the encoded at least one intracellular signaling domain comprises a costimulatory domain, a primary signaling domain, or a combination thereof.

In further embodiments, an isolated nucleic acid molecule encoding the CAR is provided wherein the encoded at least one costimulatory domain comprises a functional signaling domain of OX40, CD70, CD27, CD28, CD5, ICAM-1, LFA-1 (CD11a/CD18), ICOS (CD278), DAP10, DAP12, and 4-1BB (CD137), or a combination thereof.

In one embodiment, an isolated nucleic acid molecule encoding the CAR is provided that further contains a leader sequence or signal peptide wherein the leader or signal peptide nucleotide sequence comprises the nucleotide sequence of SEQ ID NO: 13, SEQ ID NO: 39, SEQ ID NO: 41, or SEQ ID NO: 43.

In yet another embodiment, an isolated nucleic acid molecule encoding the CAR is provided wherein the encoded leader sequence comprises the amino acid sequence of SEQ ID NO: 14 SEQ ID NO: 40, SEQ ID NO: 42, or SEQ ID NO: 44.

In one aspect, a chimeric antigen receptor (CAR) is provided herein comprising, from N-terminus to C-terminus, at least one CD123 antigen binding domain, at least one transmembrane domain, and at least one intracellular signaling domain.

In one embodiment, a CAR is provided wherein the extracellular CD123 antigen binding domain comprises at least one single chain variable fragment of an antibody that binds to the

antigen, or at least one heavy chain variable region of an antibody that binds to the antigen, or a combination thereof.

In another embodiment, a CAR is provided wherein the at least one transmembrane domain comprises a transmembrane domain of a protein selected from the group consisting of the alpha, beta or zeta chain of the T-cell receptor, CD28, CD3 epsilon, CD45, CD4, CD5, CD8, CD9, CD16, CD22, CD33, CD37, CD64, CD80, CD86, CD134, CD137 and CD154, or a combination thereof.

In some embodiments, the CAR is provided wherein CAR additionally encodes an extracellular antigen binding domain comprising CD19, CD20, CD22, ROR1, mesothelin, CD33, CD38, CD123 (IL3RA), CD138, BCMA (CD269), GPC2, GPC3, FGFR4, c-Met, PSMA, Glycolipid F77, EGFRvIII, GD-2, NY-ESO-1 TCR, MAGE A3 TCR, or an amino acid sequence with 85%, 90%, 95%, 96%, 97%, 98% or 99% identity thereof, or any combination thereof.

In one embodiment, the CAR is provided wherein the extracellular antigen binding domain comprises an anti-CD19 ScFv antigen binding domain, an anti-CD20 ScFv antigen binding domain, an anti-CD22 ScFv antigen binding domain, an anti-ROR1 ScFv antigen binding domain, an anti-mesothelin ScFv antigen binding domain, an anti-CD33 ScFv antigen binding domain, an anti-CD38 ScFv antigen binding domain, an anti-CD123 (IL3RA) ScFv antigen binding domain, an anti-CD138 ScFv antigen binding domain, an anti-BCMA (CD269) ScFv antigen binding domain, an anti-GPC2 ScFv antigen binding domain, an anti-GPC3 ScFv antigen binding domain, an anti-FGFR4 ScFv antigen binding domain, an anti-c-Met ScFv antigen binding domain, an anti-PMSA ScFv antigen binding domain, an anti-glycolipid F77 ScFv antigen binding domain, an anti-EGFRvIII ScFv antigen binding domain, an anti-GD-2 ScFv antigen binding domain, an anti-NY-ESo-1 TCR ScFv antigen binding domain, an anti-MAGE A3 TCR ScFv antigen binding domain, or an amino acid sequence with 85%, 90%, 95%, 96%, 97%, 98% or 99% identity thereof, or any combination thereof.

In another embodiment, the CAR is provided wherein the extracellular antigen binding domain comprises an immunoglobulin variable heavy chain only (VH) anti-CD19 antigen binding domain, an anti-CD20 VH antigen binding domain, an anti-CD22 VH antigen binding domain, an anti-ROR1 VH antigen binding domain, an anti-mesothelin VH antigen binding domain, an anti-CD33 VH antigen binding domain, an anti-CD38 VH antigen binding domain, an anti-CD123 (IL3RA) VH antigen binding domain, an anti-CD138 VH antigen binding domain, an anti-BCMA (CD269) VH antigen binding domain, an anti-GPC2 VH antigen binding domain, an anti-GPC3 VH antigen binding domain, an anti-FGFR4 VH antigen binding domain, an anti-c-Met VH antigen binding domain, an anti-PMSA VH antigen binding domain, an anti-glycolipid F77 VH antigen binding domain, an anti-EGFRvIII VH antigen binding domain, an anti-GD-2 VH antigen binding

domain, an anti-NY-ESO-1 TCR VH antigen binding domain, an anti-MAGE A3 TCR VH antigen binding domain, or an amino acid sequence with 85%, 90%, 95%, 96%, 97%, 98% or 99% identity thereof, or any combination thereof.

In another embodiment, the CAR is provided wherein the extracellular antigen binding domain comprises a protein or a peptide (P) sequence capable of specifically binding target antigen, which may be derived from a natural or a synthetic sequence comprising anti-CD19 P antigen binding domain, an anti-CD20 P antigen binding domain, an anti-CD22 P antigen binding domain, an anti-ROR1 P antigen binding domain, an anti-mesothelin P antigen binding domain, an anti-CD33 P antigen binding domain, an anti-CD38 P antigen binding domain, an anti-CD123 (IL3RA) P antigen binding domain, an anti-CD138 P antigen binding domain, an anti-BCMA (CD269) P antigen binding domain, an anti-GPC2 P antigen binding domain, an anti-GPC3 P antigen binding domain, an anti-FGFR4 P antigen binding domain, an anti-c-Met P antigen binding domain, an anti-PMSA P antigen binding domain, an anti-glycolipid F77 P antigen binding domain, an anti-EGFRvIII P antigen binding domain, an anti-GD-2 P antigen binding domain, an anti-NY-ESO-1 TCR P antigen binding domain, an anti-MAGE A3 TCR P antigen binding domain, or an amino acid sequence with 85%, 90%, 95%, 96%, 97%, 98% or 99% identity thereof, or any combination thereof. In another embodiment, a CAR is provided wherein the at least one intracellular signaling domain comprises a costimulatory domain and a primary signaling domain.

In yet another embodiment, a CAR is provided wherein the at least one intracellular signaling domain comprises a costimulatory domain comprising a functional signaling domain of a protein selected from the group consisting of OX40, CD70, CD27, CD28, CD5, ICAM-1, LFA-1 (CD11a/CD18), ICOS (CD278), DAP10, DAP12, and 4-1BB (CD137), or a combination thereof.

In one embodiment, the nucleic acid sequence encoding a CAR comprises the nucleic acid sequence of SEQ ID NO: 1. In one embodiment, the nucleic acid sequence encodes a CAR comprising the amino acid sequence of SEQ ID NO: 2.

In another embodiment, the nucleic acid sequence encoding a CAR comprises the nucleic acid sequence of SEQ ID NO: 3. In one embodiment, the nucleic acid sequence encodes a CAR comprising the amino acid sequence of SEQ ID NO: 4.

In another embodiment, the nucleic acid sequence encoding a CAR comprises the nucleic acid sequence of SEQ ID NO: 5. In one embodiment, the nucleic acid sequence encodes a CAR comprising the amino acid sequence of SEQ ID NO: 6.

In another embodiment, the nucleic acid sequence encoding a CAR comprises the nucleic acid sequence of SEQ ID NO: 7. In one embodiment, the nucleic acid sequence encodes a CAR comprising the amino acid sequence of SEQ ID NO: 8.

In another embodiment, the nucleic acid sequence encoding a CAR comprises the nucleic acid sequence of SEQ ID NO: 9. In one embodiment, the nucleic acid sequence encodes a CAR comprising the amino acid sequence of SEQ ID NO: 10.

In another embodiment, the nucleic acid sequence encoding a CAR comprises the nucleic acid sequence of SEQ ID NO: 11. In one embodiment, the nucleic acid sequence encodes a CAR comprising the amino acid sequence of SEQ ID NO: 12.

In another embodiment, the nucleic acid sequence encoding a CAR comprises the nucleic acid sequence of SEQ ID NO: 15. In one embodiment, the nucleic acid sequence encodes a CAR comprising the amino acid sequence of SEQ ID NO: 16.

In another embodiment, the nucleic acid sequence encoding a CAR comprises the nucleic acid sequence of SEQ ID NO: 17. In one embodiment, the nucleic acid sequence encodes a CAR comprising the amino acid sequence of SEQ ID NO: 18.

In another embodiment, the nucleic acid sequence encoding a CAR comprises the nucleic acid sequence of SEQ ID NO: 19. In one embodiment, the nucleic acid sequence encodes a CAR comprising the amino acid sequence of SEQ ID NO: 20.

In another embodiment, the nucleic acid sequence encoding a CAR comprises the nucleic acid sequence of SEQ ID NO: 21. In one embodiment, the nucleic acid sequence encodes a CAR comprising the amino acid sequence of SEQ ID NO: 22.

In another embodiment, the nucleic acid sequence encoding a CAR comprises the nucleic acid sequence of SEQ ID NO: 23. In one embodiment, the nucleic acid sequence encodes a CAR comprising the amino acid sequence of SEQ ID NO: 24.

In another embodiment, the nucleic acid sequence encoding a CAR comprises the nucleic acid sequence of SEQ ID NO: 25. In one embodiment, the nucleic acid sequence encodes a CAR comprising the amino acid sequence of SEQ ID NO: 26.

In another embodiment, the nucleic acid sequence encoding a CAR comprises the nucleic acid sequence of SEQ ID NO: 69. In one embodiment, the nucleic acid sequence encodes a CAR comprising the amino acid sequence of SEQ ID NO: 70.

In another embodiment, the nucleic acid sequence encoding a CAR comprises the nucleic acid sequence of SEQ ID NO: 71. In one embodiment, the nucleic acid sequence encodes a CAR comprising the amino acid sequence of SEQ ID NO: 72.

In another embodiment, the nucleic acid sequence encoding a CAR comprises the nucleic acid sequence of SEQ ID NO: 77. In one embodiment, the nucleic acid sequence encodes a CAR comprising the amino acid sequence of SEQ ID NO: 78.

In another embodiment, the nucleic acid sequence encoding a CAR comprises the nucleic acid sequence of SEQ ID NO: 87. In one embodiment, the nucleic acid sequence encodes a CAR comprising the amino acid sequence of SEQ ID NO: 88.

In one aspect, the CARs disclosed herein are modified to express or contain a detectable marker for use in diagnosis, monitoring, and/or predicting the treatment outcome such as progression free survival of cancer patients or for monitoring the progress of such treatment.

In one embodiment, the nucleic acid molecule encoding the disclosed CARs can be contained in a vector, such as a viral vector. The vector is a DNA vector, an RNA vector, a plasmid vector, a cosmid vector, a herpes virus vector, a measles virus vector, a lentivirus vector, adenoviral vector, adeno-associated viral vector, baculovirus vector, foamy virus vector, or a retrovirus vector, or a combination thereof.

In certain embodiments, the lentiviral vectors encoding one or more of the CARs disclosed herein may be used to produce the genomic material packaged into pseudotyped lentiviral particles. In one embodiment, the pseudotyped lentiviral particles comprise Vesicular Stomatitis Virus-Envelope Glycoprotein (VSV-G) pseudotyped lentiviral vector particles. In another embodiment, the pseudotyped lentiviral particles comprise Baboon Envelope Glycoprotein Pseudotyped Vector (BaEV-G) pseudotyped lentiviral vector particles. In yet another embodiment, the pseudotyped lentiviral particles comprise Feline Endogenous Retrovirus Envelop Glycoprotein RD114 (RD114-G).

In certain embodiments, the vector further comprises a promoter wherein the promoter is an inducible promoter, a tissue specific promoter, a constitutive promoter, a suicide promoter or any combination thereof.

In yet another embodiment, the vector expressing the CAR can be further modified to include one or more operative elements to control the expression of CAR T cells, or to eliminate CAR-T cells by virtue of a suicide switch. The suicide switch can include, for example, an apoptosis inducing signaling cascade or a drug that induces cell death. In a preferred embodiment, the vector expressing the CAR can be further modified to express an enzyme such as thymidine kinase (TK) or cytosine deaminase (CD).

In another aspect, host cells including the nucleic acid molecule encoding the CAR are also provided. In some embodiments, the host cell is a T cell, such as a primary T cell obtained from a subject. In one embodiment, the host cell is a $CD8^+$ T cell.

In yet another aspect, a pharmaceutical composition is provided comprising an anti-tumor effective amount of a population of human T cells, wherein the T cells comprise a nucleic acid sequence that encodes a chimeric antigen receptor (CAR), wherein the CAR comprises at least one

extracellular antigen binding domain comprising a CD123 antigen binding domain comprising the amino acid sequence selected from the group consisting of SEQ ID NOs: 70, 72, 78, and 88; at least one linker domain; at least one transmembrane domain; and at least one intracellular signaling domain, wherein the T cells are T cells of a human having a cancer. The cancer includes, *inter alia*, a hematological cancer such as leukemia (*e.g.*, chronic lymphocytic leukemia (CLL), acute lymphocytic leukemia (ALL), or chronic myelogenous leukemia (CML), lymphoma (*e.g.*, mantle cell lymphoma, non-Hodgkin's lymphoma or Hodgkin's lymphoma)) or multiple myeloma, or a combination thereof.

In one embodiment, a pharmaceutical composition is provided wherein the at least one transmembrane domain of the CAR contains a transmembrane domain of a protein selected from the group consisting of the alpha, beta or zeta chain of the T-cell receptor, CD28, CD3 epsilon, CD45, CD4, CD5, CD8, CD9, CD16, CD22, Mesothelin, CD33, CD37, CD64, CD80, CD86, CD134, CD137 and CD154, or a combination thereof.

In another embodiment, a pharmaceutical composition is provided wherein the human cancer includes an adult carcinoma comprising oral and pharynx cancer (tongue, mouth, pharynx, head and neck), digestive system cancers (esophagus, stomach, small intestine, colon, rectum, anus, liver, interhepatic bile duct, gallbladder, pancreas), respiratory system cancers (larynx, lung and bronchus), bones and joint cancers, soft tissue cancers, skin cancers (melanoma, basal and squamous cell carcinoma), pediatric tumors (neuroblastoma, rhabdomyosarcoma, osteosarcoma, Ewing's sarcoma), tumors of the central nervous system (brain, astrocytoma, glioblastoma, glioma), and cancers of the breast, the genital system (uterine cervix, uterine corpus, ovary, vulva, vagina, prostate, testis, penis, endometrium), the urinary system (urinary bladder, kidney and renal pelvis, ureter), the eye and orbit, the endocrine system (thyroid), and the brain and other nervous system, or any combination thereof.

In yet another embodiment, a pharmaceutical composition is provided comprising an anti-tumor effective amount of a population of human T cells of a human having a cancer wherein the cancer is a refractory cancer non-responsive to one or more chemotherapeutic agents. The cancer includes hematopoietic cancer, myelodysplastic syndrome pancreatic cancer, head and neck cancer, cutaneous tumors, minimal residual disease (MRD) in acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML), adult B cell malignancies including, CLL (Chronic lymphocytic leukemia), CML (chronic myelogenous leukemia), non-Hodgkin's lymphoma (NHL), pediatric B cell malignancies (including B lineage ALL (acute lymphocytic leukemia)), multiple myeloma lung cancer, breast cancer, ovarian cancer, prostate cancer, colon cancer, melanoma or other hematological cancer and solid tumors, or any combination thereof.

In another aspect, methods of making CAR-containing T cells (hereinafter "CAR-T cells") are provided. The methods include transducing a T cell with a vector or nucleic acid molecule encoding a disclosed CAR that specifically binds CD123, thereby making the CAR-T cell.

In yet another aspect, a method of generating a population of RNA-engineered cells is provided that comprises introducing an *in vitro* transcribed RNA or synthetic RNA of a nucleic acid molecule encoding a disclosed CAR into a cell of a subject, thereby generating a CAR cell.

In yet another aspect, a method for diagnosing a disease, disorder or condition associated with the expression of CD123 on a cell, is provided comprising a) contacting the cell with a human anti-CD123 antibody or fragment thereof, wherein the antibody or a fragment thereof comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 70, 72, 78, and 88; and b) detecting the presence of CD123 wherein the presence of CD123 diagnoses for the disease, disorder or condition associated with the expression of CD123.

In one embodiment, the disease, disorder or condition associated with the expression of CD123 is cancer including hematopoietic cancer, myelodysplastic syndrome pancreatic cancer, head and neck cancer, cutaneous tumors, minimal residual disease (MRD) in acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML), adult B cell malignancies including, CLL (chronic lymphocytic leukemia), CML (chronic myelogenous leukemia), non-Hodgkin's lymphoma (NHL), pediatric B cell malignancies (including B lineage ALL (acute lymphocytic leukemia)), multiple myeloma lung cancer, breast cancer, ovarian cancer, prostate cancer, colon cancer, melanoma or other hematological cancer and solid tumors, or any combination thereof.

In another embodiment, a method of diagnosing, prognosing, or determining risk of a CD123-related disease in a mammal, is provided comprising detecting the expression of CD123 in a sample derived from the mammal comprising: a) contacting the sample with a human anti-CD123 antibody or fragment thereof, wherein the antibody or a fragment thereof comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 70, 72, 78, and 88; and b) detecting the presence of CD123 wherein the presence of CD123 diagnoses for a CD123-related disease in the mammal.

In another embodiment, a method of inhibiting CD123-dependent T cell inhibition, is provided comprising contacting a cell with a human anti-CD123 antibody or fragment thereof, wherein the antibody or a fragment thereof comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 70, 72, 78, and 88. In one embodiment, the cell is selected from the group consisting of a CD123-expressing tumor cell, a tumor-associated macrophage, and any combination thereof.

In another embodiment, a method of blocking T-cell inhibition mediated by a CD123-expressing cell and altering the tumor microenvironment to inhibit tumor growth in a mammal, is provided comprising administering to the mammal an effective amount of a composition comprising an isolated anti-CD123 antibody or fragment thereof, wherein the antibody or a fragment thereof comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 70, 72, 78, and 88. In one embodiment, the cell is selected from the group consisting of a CD123-expressing tumor cell, a tumor-associated macrophage, and any combination thereof.

In another embodiment, a method of inhibiting, suppressing or preventing immunosuppression of an anti-tumor or anti-cancer immune response in a mammal, is provided comprising administering to the mammal an effective amount of a composition comprising an isolated anti-CD123 antibody or fragment thereof, wherein the antibody or a fragment thereof comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 70, 72, 78, and 88. In one embodiment, the antibody or fragment thereof inhibits the interaction between a first cell with a T cell, wherein the first cell is selected from the group consisting of a CD123-expressing tumor cell, a tumor-associated macrophage, and any combination thereof.

In another aspect, a method is provided for inducing an anti-tumor immunity in a mammal comprising administering to the mammal a therapeutically effective amount of a T cell transduced with vector or nucleic acid molecule encoding a disclosed CAR.

In another embodiment, a method of treating or preventing cancer in a mammal is provided comprising administering to the mammal one or more of the disclosed CARs, in an amount effective to treat or prevent cancer in the mammal. The method includes administering to the subject a therapeutically effective amount of host cells expressing a disclosed CAR that specifically binds CD123 and/or one or more of the aforementioned antigens, under conditions sufficient to form an immune complex of the antigen binding domain on the CAR and the extracellular domain of CD123 and/or one or more of the aforementioned antigens in the subject.

In yet another embodiment, a method is provided for treating a mammal having a disease, disorder or condition associated with an elevated expression of a tumor antigen, the method comprising administering to the subject a pharmaceutical composition comprising an anti-tumor effective amount of a population of T cells, wherein the T cells comprise a nucleic acid sequence that encodes a chimeric antigen receptor (CAR), wherein the CAR includes at least one extracellular CD123 antigen binding domain comprising the amino acid sequence of SEQ ID NOs: 70, 72, 78, or 88, or any combination thereof, at least one linker or spacer domain, at least one transmembrane domain, at least one intracellular signaling domain, and wherein the T cells are T cells of the subject having cancer.

In yet another embodiment, a method is provided for treating cancer in a subject in need thereof comprising administering to the subject a pharmaceutical composition comprising an anti-tumor effective amount of a population of T cells, wherein the T cells comprise a nucleic acid sequence that encodes a chimeric antigen receptor (CAR), wherein the CAR comprises at least one CD123 antigen binding domain comprising the amino acid sequence of SEQ ID NOs: 70, 72, 78, or 88, or any combination thereof, at least one linker or spacer domain, at least one transmembrane domain, at least one intracellular signaling domain, wherein the T cells are T cells of the subject having cancer. In some embodiments of the aforementioned methods, the at least one transmembrane domain comprises a transmembrane the alpha, beta or zeta chain of the T-cell receptor, CD28, CD3 epsilon, CD45, CD4, CD5, CD8, CD9, CD16, CD22, Mesothelin, CD33, CD37, CD64, CD80, CD86, CD134, CD137 and CD154, or a combination thereof.

In yet another embodiment, a method is provided for generating a persisting population of genetically engineered T cells in a human diagnosed with cancer. In one embodiment, the method comprises administering to a human a T cell genetically engineered to express a CAR wherein the CAR comprises at least one CD123 antigen binding domain comprising the amino acid sequence of SEQ ID NOs: 70, 72, 78, or 88, or any combination thereof; at least one transmembrane domain; and at least one intracellular signaling domain wherein the persisting population of genetically engineered T cells, or the population of progeny of the T cells, persists in the human for at least one month, two months, three months, four months, five months, six months, seven months, eight months, nine months, ten months, eleven months, twelve months, two years, or three years after administration.

In one embodiment, the progeny T cells in the human comprise a memory T cell. In another embodiment, the T cell is an autologous T cell.

In all of the aspects and embodiments of methods described herein, any of the aforementioned cancers, diseases, disorders or conditions associated with an elevated expression of a tumor antigen that may be treated or prevented or ameliorated using one or more of the CARs disclosed herein.

In yet another aspect, a kit is provided for making a chimeric antigen receptor T-cell as described *supra* or for preventing, treating, or ameliorating any of the cancers, diseases, disorders or conditions associated with an elevated expression of a tumor antigen in a subject as described *supra*, comprising a container comprising any one of the nucleic acid molecules, vectors, host cells, or compositions disclosed *supra* or any combination thereof, and instructions for using the kit.

It will be understood that the CARs, host cells, nucleic acids, and methods are useful beyond the specific aspects and embodiments that are described in detail herein. The foregoing features

and advantages of the disclosure will become more apparent from the following detailed description, which proceeds with reference to the accompanying figures.

## BRIEF DESCRIPTION OF THE FIGURES

**FIGURES 1A-1C** depict CD123 CAR structure, surface expression and cell viability in human primary T cells. (FIGURE 1A) Anti-CD123 CAR constructs were generated by linking the single chain fragment variable sequence (scFv) targeting CD123 in frame to CD8 hinge (H) and transmembrane domain (TM), the 4-1BB (CD137) co-stimulatory domain and the CD3 zeta activation domain. (FIGURE 1B) T cells were activated with TransAct CD3/CD28 reagent in the presence of IL-2, and transduced with lentiviral vectors encoding CAR123 constructs. On culture day 7, viable transduced T cells (7-AAD negative) were assayed for CAR surface expression using CD123 Fc followed by anti-Fc-AF647. Percentage of CAR T-positive populations in relation to non-transduced T cell control. (FIGURE 1C) CAR T cell viability was measured by trypan blue exclusion (Vi-Cell) at culture day 3 and day 7. The CD33 CAR construct (1906) was included as control.

**FIGURE 2** depicts CD123 surface expression level on MOLM14, KG-1a, RS4;11, 293T and A431tumor cell lines. Representative flow histograms are shown.

**FIGURES 3A-3C** depict the analysis of tumor cell lysis induced by CAR123 constructs in vitro. Luciferase-based cytotoxicity assays were performed using MOL14 (FIGURE 3A), KG1a (FIGURE 3B) and 293T (FIGURE 3C) target cell lines stably expression firefly luciferase. CAR T cells and tumor cells were co-incubated overnight at the indicated effector to target (E:T) ratios: 2.5:1, 5:1, or 10:1. Percentage specific target lysis was assessed by luminometry.

**FIGURES 4A-4C** depict CAR T cytokine release in response to leukemia cell lines. Cytokine production by CAR T cells, listed on the x-axis, upon overnight co-culture with the MOLM14 leukemia line at the E:T ratio of 10:1, was measured by ELISA. Bars represent

mean + SD of replicate samples. Data are representative of three independent experiments performed with CAR T cells from three separate donors.

**FIGURES 5A AND 5B** depict the CAR constructs tested in the two *in vivo* studies. (FIGURE 5A) CD123 CAR candidate D0126 and control CAR 33 LTG1906 were included in the first animal study, (FIGURE 5B) CD123 CAR candidate D0131 was added to D0126 and LTG1906 in the second animal study. Tumor alone (TA) and untransduced T cells (UTD) groups were included as controls in both studies.

**FIGURES 6A-6D** depict the *in vivo* activity of CAR T constructs in the first animal study. NSG mice were injected *i.v.* with MOLM14-luciferase cells on Day 0, and treated with $5x10^6$/mouse T cells or UTD on day seven. Six mice per CAR T treatment group and control group were studied. (FIGURE 6A) Representative time course bioluminescent images of tumor burden in mice. (FIGURE 6B) Time course of tumor growth based on mouse whole body bioluminescence (radiance) were quantified and individually plotted as shown, n= 6, (FIGURE 6C) Percentage change in body weight was recorded every other day, n= 6, mean ± SEM. (FIGURE 6D) Survival curves of mice following CAR T treatment and controls.

**FIGURES 7A-7C** depict human T cells detected in mouse blood during the first *in vivo* study. Total human T cells in mouse peripheral blood were measured at: day 14 (FIGURE 7A), day 22 (FIGURE 7B) and day 33 (FIGURE 7C) by volumetric flow cytometry, and normalized using CountBright beads. All surviving mice are depicted. Results are shown as scatter dot plots. Lines indicate group means.

**FIGURES 8A-8D** depict the *in vivo* activity of CAR T constructs in the second *in vivo* study. NSG mice were injected *i.v.* with MOLM14-luciferase cells on Day 0, and administrated with $5x10^6$/mouse T cells on day seven. FIGURE.8A shows representative time course bioluminescent images of the tumor burden in each group. (FIGURE 8B) Time course plot of tumor growth based on mouse whole body bioluminescence (radiance), n= 6, mean ± SEM. (FIGURE 8C) Percentage of body weight change was recorded every other day, n= 6, mean ± SEM. FIGURE 8D Survival curve of CAR T and control groups overtime is shown.

**FIGURES 9A-9E** depict human T cell detected in mouse peripheral blood throughout the second animal study. The total human T cells numbers were measured by flow cytometry at:

day 2 (FIGURE 9A), day 14 (FIGURE 9B), day 21 (FIGURE 9C), day 28 (FIGURE 9D) and day 42 (FIGURE 9E), and quantified with CountBright beads. Results are presented as scatter dot plots. Lines indicate group means.

**FIGURES 10A-10B** depict NK cell isolation and generation of target cells for CD123-CAR. (FIGURE 10A) Isolation and purity of NK cells. (FIGURE 10B) Target cells were generated by overexpressing CD123 on the RS4-11 cell line. CD123 transduced RS4-11 were sorted, and limited dilution was performed to generate homogenous CD123 expressing RS4-11 cells.

**FIGURE 11** depicts CD123-CAR binders expressed on transduced primary NK cells. Primary NK cells were isolated and cultured in a medium with IL-2, IL-15, and IL-1β for two days. On Day 3, activated NK cells were separately transduced with 13 different lentiviral vectors containing different CD123-CAR. CD123-CAR expressions on NK cells were detected on day 8 after transduction.

**FIGURES 12A-12B** depict the expression and cytotoxicity of CD123-CAR. (FIGURE 12A) NK cells were transduced with a lentiviral vector containing CD123-CAR constructs D0126 and Z32. CD123-CAR expressions were determined on Day 8 after transduction. (FIGURE 12B) Cytotoxicity of CD123-CAR-NK cells was determined using RS411-CD123 target cells. Results represented 3 independent experiments.

**FIGURES 13A-13B** depict the specific killing of CD123-CAR NK cells towards target cells expressing CD123. (FIGURE 13A) NK cells were transduced with different volumes of the lentiviral vectors containing CD123-CAR. CD123-CAR expressions were detected on Day 8 after transduction. (FIGURE 13B) Cytotoxicity of CD123-CAR-NK cells was determined using RS411-CD123 target cells. The effector and target ratio used for the cytotoxicity experiments was 1:1.

**FIGURES 14A-14B** depict impact of CD123-CAR on NK cell expansion and viability. (FIGURE 14A) NK cells were transduced with CD123-CAR constructs D0126 and Z32 and expanded parallel with untransduced NK cells. (FIGURE 14B) NK cells viability were determined at the different time point in culture after transduced with CD123-CAR. D1, D3, D5, D8, and D11 indicate one day, three days, five days, eight days and eleven days after transduction. UTD indicates untransduced NK cells; Z32 indicates CD123 binder Z32; D0126 indicates CD123 binder D0126.

# DETAILED DESCRIPTION

## Definitions

As used herein, the singular forms "a," "an," and "the," refer to both the singular as well as plural, unless the context clearly indicates otherwise. For example, the term "an antigen" includes single or plural antigens and can be considered equivalent to the phrase "at least one antigen." As used herein, the term "comprises" means "includes." Thus, "comprising an antigen" means "including an antigen" without excluding other elements. The phrase "and/or" means "and" or "or." It is further to be understood that any and all base sizes or amino acid sizes, and all molecular weight or molecular mass values, given for nucleic acids or polypeptides are approximate, and are provided for descriptive purposes, unless otherwise indicated. Although many methods and materials similar or equivalent to those described herein can be used, particular suitable methods and materials are described below. In case of conflict, the present specification, including explanations of terms, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting. To facilitate review of the various embodiments, the following explanations of terms are provided:

The term "about" when referring to a measurable value such as an amount, a temporal duration, and the like, is meant to encompass variations of .+-.20% or in some instances .+-.10%, or in some instances .+-.5%, or in some instances .+-.1%, or in some instances .+-.0.1% from the specified value, as such variations are appropriate to perform the disclosed methods.

Unless otherwise noted, the technical terms herein are used according to conventional usage. Definitions of common terms in molecular biology can be found in Benjamin Lewin, *Genes VII*, published by Oxford University Press, 1999; Kendrew *et al.* (eds.), *The Encyclopedia of Molecular Biology*, published by Blackwell Science Ltd., 1994; and Robert A. Meyers (ed.), *Molecular Biology and Biotechnology: a Comprehensive Desk Reference*, published by VCH Publishers, Inc., 1995; and other similar references.

The present disclosure provides for CD123 antibodies or fragments thereof as well as chimeric antigen receptors (CARs) having such CD123 antigen binding domains. The enhancement of the functional activity of the CAR directly relates to the enhancement of functional activity of the CAR-expressing T cell. As a result of one or more of these modifications, the CARs exhibit both a high degree of cytokine-induced cytolysis and cell surface expression on transduced T cells, along with an increased level of *in vivo* T cell expansion and persistence of the transduced CAR-expressing T cell.

The unique ability to combine functional moieties derived from different protein domains has been a key innovative feature of Chimeric Antigen Receptors (CARs). The choice of each of these protein domains is a key design feature, as is the way in which they are specifically combined. Each design domain is an essential component that can be used across different CAR platforms to engineer the function of lymphocytes. For example, the choice of the extracellular binding domain can make an otherwise ineffective CAR be effective.

The invariable framework components of the immunoglobulin-derived protein sequences used to create the extracellular antigen binding domain of a CAR can either be entirely neutral, or they can self-associate and drive the T cell to a state of metabolic exhaustion, thus making the therapeutic T cell expressing that CAR far less effective. This occurs independently of the antigen binding function of this CAR domain. Furthermore, the choice of the intracellular signaling domain(s) also can govern the activity and the durability of the therapeutic lymphocyte population used for immunotherapy. While the ability to bind target antigen and the ability to transmit an activation signal to the T cell through these extracellular and intracellular domains, respectively, are important CAR design aspects, what has also become apparent is that the choice of the source of the extracellular antigen binding fragments can have a significant effect on the efficacy of the CAR and thereby have a defining role for the function and clinical utility of the CAR.

Surprisingly and unexpectedly it has now been discovered that use of an entirely human antigen binding domain in a CAR, rather than using mouse-derived antigen binding fragments which are prone to induce anti-mouse immune response and CAR T elimination in a host (*c.f.*, the UPenn-sponsored clinical trial using mouse derived SS1 ScFv sequence, NCT02159716), may also determine the functional activity of a CAR-expressing T cell.

The CARs disclosed herein are expressed at a high level in a cell. A cell expressing the CAR has a high *in vivo* proliferation rate, produces large amounts of cytokines, and has a high cytotoxic activity against a cell having, on its surface, a CD123 antigen to which a CAR binds. The use of a human extracellular CD123 antigen binding domain results in generation of a CAR that functions better *in vivo*, while avoiding the induction of anti-CAR immunity in the host immune response and the killing of the CAR T cell population. The CARs expressing the entirely human extracellular CD123 ScFv antigen binding domain exhibit superior activities/properties including i) prevention of poor CAR T persistence and function as seen with mouse-derived binding sequences; ii) lack of regional (*i.e.* intrapleural) delivery of the CAR to be efficacious; and iii) ability to generate CAR T cell designs based both on binders with high and low affinity to CD123. This latter property allows investigators to better tune efficacy vs toxicity, and/or tissue specificity of the CAR T product, since lower-affinity binders may have higher specificity to tumors vs normal tissues due

to higher expression of CD123 on tumors than normal tissue, which may prevent on-target off tumor toxicity and bystander cell killing.

What follows is a detailed description of the inventive CARs including a description of their extracellular CD123 antigen binding domain, the transmembrane domain and the intracellular domain, along with additional description of the CARs, antibodies and antigen binding fragments thereof, conjugates, nucleotides, expression, vectors, and host cells, methods of treatment, compositions, and kits employing the disclosed CARs.

### A. Chimeric Antigen Receptors (CARs)

The CARs disclosed herein comprise at least one CD123 antigen binding domain capable of binding to CD123, at least one transmembrane domain, and at least one intracellular domain.

A chimeric antigen receptor (CAR) is an artificially constructed hybrid protein or polypeptide containing the antigen binding domains of an antibody (*e.g.*, single chain variable fragment (ScFv)) linked to T-cell signaling domains via the transmembrane domain. Characteristics of CARs include their ability to redirect T-cell specificity and reactivity toward a selected target in a non-MHC-restricted manner, and exploiting the antigen-binding properties of monoclonal antibodies. The non-MHC-restricted antigen recognition gives T cells expressing CARs the ability to recognize antigen independent of antigen processing, thus bypassing a major mechanism of tumor escape. Moreover, when expressed in T-cells, CARs advantageously do not dimerize with endogenous T cell receptor (TCR) alpha and beta chains.

As disclosed herein, the intracellular T cell signaling domains of the CARs can include, for example, a T cell receptor signaling domain, a T cell costimulatory signaling domain, or both. The T cell receptor signaling domain refers to a portion of the CAR comprising the intracellular domain of a T cell receptor, such as, for example, and not by way of limitation, the intracellular portion of the CD3 zeta protein. The costimulatory signaling domain refers to a portion of the CAR comprising the intracellular domain of a costimulatory molecule, which is a cell surface molecule other than an antigen receptor or their ligands that are required for an efficient response of lymphocytes to antigen.

#### 1. Extracellular Domain

In one embodiment, the CAR comprises a target-specific binding element otherwise referred to as an antigen binding domain or moiety. The choice of domain depends upon the type and

number of ligands that define the surface of a target cell. For example, the antigen binding domain may be chosen to recognize a ligand that acts as a cell surface marker on target cells associated with a particular disease state. Thus, examples of cell surface markers that may act as ligands for the antigen binding domain in the CAR include those associated with viral, bacterial and parasitic infections, autoimmune disease and cancer cells.

In one embodiment, the CAR can be engineered to target a tumor antigen of interest by way of engineering a desired antigen binding domain that specifically binds to an antigen on a tumor cell. Tumor antigens are proteins that are produced by tumor cells that elicit an immune response, particularly T-cell mediated immune responses. The selection of the antigen binding domain will depend on the particular type of cancer to be treated. Tumor antigens include, for example, a glioma-associated antigen, carcinoembryonic antigen (CEA), .beta.-human chorionic gonadotropin, alphafetoprotein (AFP), lectin-reactive AFP, thyroglobulin, RAGE-1, MN-CA IX, human telomerase reverse transcriptase, RU1, RU2 (AS), intestinal carboxyl esterase, mut hsp70-2, M-CSF, prostase, prostate-specific antigen (PSA), PAP, NY-ESO-1, LAGE-1a, p53, prostein, PSMA, Her2/neu, survivin and telomerase, prostate-carcinoma tumor antigen-1 (PCTA-1), MAGE, ELF2M, neutrophil elastase, ephrinB2, CD22, insulin growth factor (IGF)-I, IGF-II, IGF-I receptor and CD123. The tumor antigens disclosed herein are merely included by way of example. The list is not intended to be exclusive and further examples will be readily apparent to those of skill in the art.

In one embodiment, the tumor antigen comprises one or more antigenic cancer epitopes associated with a malignant tumor. Malignant tumors express a number of proteins that can serve as target antigens for an immune attack. These molecules include, but are not limited to, tissue-specific antigens such as MART-1, tyrosinase and GP 100 in melanoma and prostatic acid phosphatase (PAP) and prostate-specific antigen (PSA) in prostate cancer. Other target molecules belong to the group of transformation-related molecules such as the oncogene HER-2/Neu/ErbB-2. Yet another group of target antigens are onco-fetal antigens such as carcinoembryonic antigen (CEA). In B-cell lymphoma the tumor-specific idiotype immunoglobulin constitutes a truly tumor-specific immunoglobulin antigen that is unique to the individual tumor. B-cell differentiation antigens such as CD19, CD20 and CD37 are other candidates for target antigens in B-cell lymphoma. Some of these antigens (CEA, HER-2, CD19, CD20, idiotype) have been used as targets for passive immunotherapy with monoclonal antibodies with limited success.

In one preferred embodiment, the tumor antigen is CD123 and the tumors associated with expression of CD123 comprise lung mesothelioma, ovarian, and pancreatic cancers that express high levels of the extracellular protein CD123, or any combination thereof.

The type of tumor antigen may also be a tumor-specific antigen (TSA) or a tumor-associated antigen (TAA). A TSA is unique to tumor cells and does not occur on other cells in the body. A TAA is not unique to a tumor cell and instead is also expressed on a normal cell under conditions that fail to induce a state of immunologic tolerance to the antigen. The expression of the antigen on the tumor may occur under conditions that enable the immune system to respond to the antigen. TAAs may be antigens that are expressed on normal cells during fetal development when the immune system is immature and unable to respond or they may be antigens that are normally present at extremely low levels on normal cells but which are expressed at much higher levels on tumor cells.

Non-limiting examples of TSAs or TAAs include the following: Differentiation antigens such as MART-1/MelanA (MART-I), gp100 (Pmel 17), tyrosinase, TRP-1, TRP-2 and tumor-specific multi-lineage antigens such as MAGE-1, MAGE-3, BAGE, GAGE-1, GAGE-2, p15; overexpressed embryonic antigens such as CEA; overexpressed oncogenes and mutated tumor-suppressor genes such as p53, Ras, HER-2/neu; unique tumor antigens resulting from chromosomal translocations; such as BCR-ABL, E2A-PRL, H4-RET, IGH-IGK, MYL-RAR; and viral antigens, such as the Epstein Barr virus antigens EBVA and the human papillomavirus (HPV) antigens E6 and E7. Other large, protein-based antigens include TSP-180, MAGE-4, MAGE-5, MAGE-6, RAGE, NY-ESO, p185erbB2, p180erbB-3, c-met, nm-23H1, PSA, TAG-72, CA 19-9, CA 72-4, CAM 17.1, NuMa, K-ras, beta-Catenin, CDK4, Mum-1, p 15, p 16, 43-9F, 5T4, 791Tgp72, alpha-fetoprotein, beta-HCG, BCA225, BTAA, CA 125, CA 15-3\CA 27.29\BCAA, CA 195, CA 242, CA-50, CAM43, CD68\P1, CO-029, FGF-5, G250, Ga733\EpCAM, HTgp-175, M344, MA-50, MG7-Ag, MOV18, NB/70K, NY-CO-1, RCAS1, SDCCAG16, TA-90\Mac-2 binding protein\cyclophilin C-associated protein, TAAL6, TAG72, TLP, and TPS.

In one embodiment, the antigen binding domain portion of the CAR targets an antigen that includes but is not limited to CD19, CD20, CD22, ROR1, CD123, CD33, c-Met, PSMA, Glycolipid F77, EGFRvIII, GD-2, MY-ESO-1 TCR, MAGE A3 TCR, and the like.

In a preferred embodiment, the antigen binding domain portion of the CAR targets the extracellular CD123 antigen.

In one preferred embodiment, the isolated nucleic acid molecule encoding the extracellular CD123 MT-16 antigen binding domain comprises a nucleotide sequence of SEQ ID NO: 69, or a sequence with 85%, 90%, 95%, 96%, 97%, 98% or 99% identity thereof. In one embodiment, an isolated nucleic acid molecule is provided wherein the encoded extracellular CD123 MT-16 antigen binding domain comprises an amino acid sequence of SEQ ID NO: 70, or an amino acid sequence

with 85%, 90%, 95%, 96%, 97%, 98% or 99% identity to an amino acid sequence of SEQ ID NO: 70.

In one preferred embodiment, the isolated nucleic acid molecule encoding the extracellular CD123 MT-32 antigen binding domain comprises a nucleotide sequence of SEQ ID NO: 71, or a sequence with 85%, 90%, 95%, 96%, 97%, 98% or 99% identity thereof. In one embodiment, an isolated nucleic acid molecule is provided wherein the encoded extracellular CD123 MT-32 antigen binding domain comprises an amino acid sequence of SEQ ID NO: 72, or an amino acid sequence with 85%, 90%, 95%, 96%, 97%, 98% or 99% identity to an amino acid sequence of SEQ ID NO: 72.

In one preferred embodiment, the isolated nucleic acid molecule encoding the extracellular CD123 Z16 antigen binding domain comprises a nucleotide sequence of SEQ ID NO: 77, or a sequence with 85%, 90%, 95%, 96%, 97%, 98% or 99% identity thereof. In one embodiment, an isolated nucleic acid molecule is provided wherein the encoded extracellular CD123 Z16 antigen binding domain comprises an amino acid sequence of SEQ ID NO: 78, or an amino acid sequence with 85%, 90%, 95%, 96%, 97%, 98% or 99% identity to an amino acid sequence of SEQ ID NO: 78.

In one preferred embodiment, the isolated nucleic acid molecule encoding the extracellular CD123 Z32 antigen binding domain comprises a nucleotide sequence of SEQ ID NO: 87, or a sequence with 85%, 90%, 95%, 96%, 97%, 98% or 99% identity thereof. In one embodiment, an isolated nucleic acid molecule is provided wherein the encoded extracellular CD123 Z32 antigen binding domain comprises an amino acid sequence of SEQ ID NO: 88, or an amino acid sequence with 85%, 90%, 95%, 96%, 97%, 98% or 99% identity to an amino acid sequence of SEQ ID NO: 88.

The generation and binding characteristics of the specific CD123 variable heavy chain only and ScFv antigen binding fragments or antigen binders described herein is shown in Example 1.

In the various embodiments of the CD123-specific CARs disclosed herein, the general scheme is set forth in FIGS. 1A-1C and includes, from the N-terminus to the C-terminus, a signal or leader peptide, anti-CD123 ScFv, extracellular linker, CD8 transmembrane, 4-1BB, CD3 zeta, wherein the bolded text represents the cloning sites for linking domains.

In one embodiment, the nucleic acid sequence encoding a CAR comprises the nucleic acid sequence of SEQ ID NO: 1, and encodes the CAR comprising the amino acid sequence as set forth in SEQ ID NO: 2.

In one embodiment, the nucleic acid sequence encoding a CAR comprises the nucleic acid sequence of SEQ ID NO: 3, or a sequence with 85%, 90%, 95%, 96%, 97%, 98% or 99% identity

thereof, and encodes the CAR comprising the amino acid sequence as set forth in SEQ ID NO: 4 or a sequence with 85%, 90%, 95%, 96%, 97%, 98% or 99% identity thereof.

In another embodiment, the nucleic acid sequence encoding a CAR comprises the nucleic acid sequence of SEQ ID NO: 5, and encodes the CAR comprising the amino acid sequence as set forth in SEQ ID NO: 6.

In another embodiment, the nucleic acid sequence encoding a CAR comprises the nucleic acid sequence of SEQ ID NO: 7 or a sequence with 85%, 90%, 95%, 96%, 97%, 98% or 99% identity thereof, and encodes the CAR comprising the amino acid sequence as set forth in SEQ ID NO: 8 or a sequence with 85%, 90%, 95%, 96%, 97%, 98% or 99% identity thereof.

In another embodiment, the nucleic acid sequence encoding a CAR comprises the nucleic acid sequence of SEQ ID NO: 9, and encodes the CAR comprising the amino acid sequence as set forth in SEQ ID NO: 10.

In another embodiment, the nucleic acid sequence encoding a CAR comprises the nucleic acid sequence of SEQ ID NO: 11 or a sequence with 85%, 90%, 95%, 96%, 97%, 98% or 99% identity thereof, and encodes the CAR comprising the amino acid sequence as set forth in SEQ ID NO: 12 or a sequence with 85%, 90%, 95%, 96%, 97%, 98% or 99% identity thereof.

In yet another embodiment, the nucleic acid sequence encoding a CAR comprises the nucleic acid sequence of SEQ ID NO: 15, and encodes the CAR comprising the amino acid sequence as set forth in SEQ ID NO: 16.

In yet another embodiment, the nucleic acid sequence encoding a CAR comprises the nucleic acid sequence of SEQ ID NO: 17 or a sequence with 85%, 90%, 95%, 96%, 97%, 98% or 99% identity thereof, and encodes the CAR comprising the amino acid sequence as set forth in SEQ ID NO: 18 or a sequence with 85%, 90%, 95%, 96%, 97%, 98% or 99% identity thereof.

In yet another embodiment, the nucleic acid sequence encoding a CAR comprises the nucleic acid sequence of SEQ ID NO: 19, and encodes the CAR comprising the amino acid sequence as set forth in SEQ ID NO: 20.

In yet another embodiment, the nucleic acid sequence encoding a CAR comprises the nucleic acid sequence of SEQ ID NO: 21 or a sequence with 85%, 90%, 95%, 96%, 97%, 98% or 99% identity thereof, and encodes the CAR comprising the amino acid sequence as set forth in SEQ ID NO: 22 or a sequence with 85%, 90%, 95%, 96%, 97%, 98% or 99% identity thereof.

In yet another embodiment, the nucleic acid sequence encoding a CAR comprises the nucleic acid sequence of SEQ ID NO: 23, and encodes the CAR comprising the amino acid sequence as set forth in SEQ ID NO: 24.

In yet another embodiment, the nucleic acid sequence encoding a CAR comprises the nucleic acid sequence of SEQ ID NO: 25 or a sequence with 85%, 90%, 95%, 96%, 97%, 98% or 99% identity thereof, and encodes the CAR comprising the amino acid sequence as set forth in SEQ ID NO: 26 or a sequence with 85%, 90%, 95%, 96%, 97%, 98% or 99% identity thereof.

The development of anti-CD123 CAR T cells incorporating single chain fragment variable (ScFv) sequences reactive with CD123 antigen, is described in Examples 3 and 4 *infra*, and the generation of NK cells expressing CD123 CAR constructs is shown in Example 5 *infra*.

Example 3 describes the generation and *in vitro* evaluation of CAR T cells targeting the CD123 antigen for the treatment of AML.

Lentiviral vectors encoding the CD123 CAR constructs were used for CAR transduction into human primary T cells at multiplicity of infection (MOI) of 40. Different CD123 CAR construct exhibited different level of expression ranging from 0-80% (n=4 donors), FIGURE 1B. CAR D0126, D0127, D0131, D0132, D0133 and D0134 exhibited similar or higher surface expression than positive CAR 123 control LTG2078; while CAR D0130 had slightly lower surface expression, followed by D0129 and D0128, while D0125 had lowest expression in multiple donors. Cell viability was examined at day 3 and day 7 after T cell activation, as showed in FIGURE 1C. All the CD123 CAR T cells showed improved or equivalent viability compared with control CAR LTG2078.

Target-specific cytotoxicity of CD123 CARs *in vitro*, was evaluated against CD123-positive leukemic lines (MOLM14, KG1a, RS4;11) and CD123-negative non-leukemic lines (293T and A431). CAR-T cells were co-incubated with MOLM14, KG-1a or 293T cell lines at effector to target ratios 2.5:1; 5:1 and 10:1. After overnight co-incubation, cultures were analyzed in luminescence based *in vitro* killing assays. Most CAR123 constructs-expressing primary T cell lines lysed MOL14-CD123+, while three CD123 CAR lines, D0125, D0128 and D0129, lacked target lytic capability (Figure 3A). Similarly, KG-1a-CD123+ target cells were killed by most CAR T constructs, except for D0125, D0128 and D0129 (Figure 3B). The control CD33 CAR LTG1906 exhibited high cytotoxicity toward MOLM14 ($CD33^{High}$) and low lytic potency towards KG1a ($CD33^{Low}$), in agreement with the CD123 expression levels. Furthermore, no killing above background of CD123 negative 293T cell line (Figure 3C) was observed, demonstrating the robust target-specific cytotoxic function of all CD123 CAR constructs, except for CAR D0125, D0128, and D0129.

Production of the T cell homeostatic and pro-inflammatory cytokines IL-2, IFNγ, and TNFα by the CD123 CARs, and control constructs CAR LTG2078 and CD33 CAR LTG1906,

was examined by ELISA in culture supernatants after overnight co-incubation of CAR T cells with MOLM14 target line at an E:T ratio of 10 (Figure 4A-4C). Specific target induced cytokine release was detected by comparison of each CAR T group incubated with target cells to the respective CAR T alone experimental group, and also comparing the target co-incubated CAR T groups to the previously characterized CAR123 control LTG2078. While CAR123 control LTG2078 and CAR33 control LTG1906 elaborated cytokines after co-incubation with MOLM14 target cells, most test CD123 CAR T constructs have not produced significant increases in IFNγ, TNFα, or IL-2 cytokines after overnight co-culture with MOLM14 cells. One exception was CAR123 D0127, which elaborated IFNγ, and TNFα levels even in the absence of target cells (T cells alone group), indicating tumor-independent cytokine response. This effect could not be anticipated form previous experiments, and it demonstrates the non-obviousness of the present invention. Excluding CAR123 D0127, cytokine response of the CD123 CAR constructs evaluated herein was comparable to the non-transduced T cells (UTD) control, suggesting low risk of inducing cytokine – mediated adverse effects, such as cytokine release syndrome (CRS) and immune effector cell-associated neurotoxicity syndrome (ICANS).

NSG (NOD.Cg-Prkdc$^{scid}$ Il2rg*tm1Wjl*/SzJ) mouse MOLM14 xenograft AML model was used to further explore the *in vivo* tumor rejection functionality of the two top CAR123 candidates D0126 and D0131. Two animal studies using CAR T cells derived from separate healthy donors were performed, one focusing on CAR D0126 (Figure 5A) and the other comparing between CAR123 constructs D0126 and D0131 (Figure 5B). The previously characterized CAR LTG1906, targeting the CD33 antigen on MOLM14 tumor cells, was included as a comparative control.

In the first *in vivo* study, CD123 CAR D0126 was compared with the previously characterized CD33 CAR-T construct LTG1906, and control experimental groups tumor alone (TA) and untransduced T cells (UTD) were also included. Tumor growth kinetics was monitored by *in vivo* imaging system (IVIS) overtime (Figure 6A and 6B). As MOLM14 tumors express both CD123 and CD33 antigens, treatment groups dosed with CAR D0126, targeting the CD123 antigen, as well as the comparator group dosed with the CAR LTG1906, targeting the CD33 antigen, showed robust tumor rejection compared to tumor alone (TA) and UTD control groups. Five of six mice in each group demonstrated complete tumor rejection, and only one mouse per group had residual tumor cells at study conclusion (Figure 6B). Notably, both CAR D0126 and CAR LTG1906 - treated groups showed no body weight loss (Figure 6C), thus no CAR-related toxicity was detected in this model. CARs D0126 and LTG1906 both mediated complete survival to study termination at day 36 (6 out of 6 mice survived), while the tumor alone (TA), and UTD

control groups succumbed to high-burden disseminated disease by day 15 (Figure 6D). Mouse peripheral blood was sampled at days 14, 22 and 33. Human T cells were detected in all groups (Figure 7A, 7B, 7C). Moreover, CAR D0126 and LTG1906 T cells were detected in the peripheral blood of mice at the end of the study, demonstrating high persistence of the CD123 CAR candidate D0126, and the comparative control CAR33 LTG1906 T cells.

In the second animal study, CD123 CAR D0131 was included in addition to CAR D0126. Tumor progression is shown in Figure 8A. Similarly to the first animal study, CAR D0126 demonstrated strong anti-tumor potency, and tumors were rejected in four out of six mice. CAR123 D0131 manifested weaker anti-tumor activity as compared with CAR123 D0126 (Figure 8A and 8B). The best survival effect was detected in the CAR D0126- treated group, with four of the six mice surviving to the extended study termination day, day 56, and remaining completely tumor-free (Figure 8D). The total T cells in the peripheral blood were monitored in this study. As expected, human T cells were detected in the mice' peripheral blood two days after CAR T cell or UTD administration in all groups except the TA negative control (Figure 9A). The T cell amounts increased in all CAR T groups overtime, suggesting T cell expansion (Figure 9B), and persistence throughout days 21, 28 and 42 (Figure 9C, 9D, 9E). On study day 42, the CAR123 D0126 group had the highest number of T cells (Figure 9E), indicating the greatest T cell expansion and persistence among CAR constructs tested in this experiment.

In summary, the CD123 CAR T cell candidate D0126 efficiently eliminated tumors in NSG mice engrafted with MOLM-14 cells in two *in vivo* studies utilizing T cells from different human donors, and demonstrated efficient tumor clearance, CAR T persistence and prolonged survival in the MOLM14 AML xenograft mouse model (Figure 9A).

CAR NK cells targeting CD123 (Example 5, *infra*) were generated by transfection of primary NK cells from healthy donors using lentiviral vectors pseudotyped with Baboon envelope protein (BaEV-LV). Primary NK cells were isolated from PBMCs by magnetic separation resulting in pure cell populations (Figure 10A). NK-resistant RS4-11 target cell line stably transduced with CD123 protein was used to test CD123-CAR functionality (Figure 10B).

NK cells were activated by cultivation in NK MACS medium containing IL-2/IL-15/IL-1β for two days, followed by transduction with BaEV pseudotyped lentiviral vectors (BaEV -LV), resulting in efficient transduction of primary NK cells. Transduction of NK cells with lentiviral vectors containing different CD123-CAR constructs resulted in differential expression of CD123-CAR at the surface of NK cells (Figure 11). Among the thirteen CD123-CARs, Z32 and D0126 CAR constructs were the best for transducing NK cells, and yielded transduction efficiency of

51.55% and 61.37%, respectively. Based on these expression results, we have selected CAR constructs Z32 and D0126 for further analysis.

Activated NK cells were transduced with BaEV pseudotyped lentiviral vector containing CD123-CAR Z32 (Z32-BaEV-LV) and D0126 (D0126-BaEV-LV). CD123-CAR expression for Z32 and D0126 was 70.5% and 64.19%, respectively (Figure 12A). In addition, the cytotoxicity of the CD123-CAR-expressing NK cells was tested against target cells RS4-11-CD123. RS4;11 cells expressing CD123 (Figure 10B) are insensitive to NK cell natural cytotoxicity. Consequently, non-transduced NK cells could not kill RS4;11-CD123 cells, whereas both CD123-CAR (Z32 and D0126) NK cells killed RS4;11-CD123 very efficiently, demonstrating the high functionality and specificity of the generated CD123-CAR NK cells (Figure 12B).

Next, the specificity of CD123-CAR toward CD123 antigen was confirmed by serial dilution. NK cells were transduced with different amounts of lentiviral vectors containing CD123-CAR. As expected, the higher quantity of CD123-CAR-LV showed higher expression of CD123-CAR (Figure 13A). Finally, the cytotoxicity of differentially expressing CD123-CAR NK cells was tested against RS4-11-CD123 cells at the same effector-target ratio (Figure 13B). The highest expressing CD123-CAR-NK cells showed the highest killing, and the lowest expressing CD123-CAR-NK cells showed the lowest killing confirmed the specificity of CD123-CAR toward CD123 antigen. Finally, expression of CD123-CAR has no adverse effect on NK expansion and viability. Primary NK cells were isolated, activated, and transduced with Z32 and D0126, followed by expansion for 13 days. Untransduced NK cells were used as control. The expansion of untransduced, Z32 transduced, and D0126 transduced NK cells was 61 fold, 49 fold, and 42 fold, respectively (Figure 14A). There were no significant differences in cell viability among untransduced, Z32- transduced, and D0126- transduced NK cells (Figure 14B), suggesting that the CD123-CARs have no adverse effect on NK cell viability.

Taken together, these results demonstrate successful generation of CAR-T and CAR-NK cells targeting the CD123 antigen for the treatment of cancer.

Without being intended to limit to any particular mechanism of action, it is believed that possible reasons for the enhanced therapeutic function associated with the exemplary CARs of the invention include, for example, and not by way of limitation, a) improved lateral movement within the plasma membrane allowing for more efficient signal transduction, b) superior location within plasma membrane microdomains, such as lipid rafts, and greater ability to interact with transmembrane signaling cascades associated with T cell activation, c) superior location within the plasma membrane by preferential movement away from dampening or down-modulatory interactions, such as less proximity to or interaction with phosphatases such as CD45, and d)

superior assembly into T cell receptor signaling complexes (*i.e.* the immune synapse), or any combination thereof.

While the disclosure has been illustrated with an exemplary extracellular CD123 variable heavy chain only and ScFv antigen binding domains, other nucleotide and/or amino acid variants within the CD123 variable heavy chain only and ScFv antigen binding domains may be used to derive the CD123 antigen binding domains for use in the CARs described herein.

Depending on the desired antigen to be targeted, the CAR can be additionally engineered to include the appropriate antigen binding domain that is specific to the desired antigen target. For example, if CD19 is the desired antigen that is to be targeted, an antibody for CD19 can be used as the antigen bind domain incorporation into the CAR.

In one exemplary embodiment, the antigen binding domain portion of the CAR additionally targets CD19. Preferably, the antigen binding domain in the CAR is anti-CD19 ScFv, wherein the nucleic acid sequence of the anti-CD19 ScFv comprises the sequence set forth in SEQ ID NO: 37. In one embodiment, the anti-CD19 ScFv comprises the nucleic acid sequence that encodes the amino acid sequence of SEQ ID NO: 37. In another embodiment, the anti-CD19 ScFv portion of the CAR comprises the amino acid sequence set forth in SEQ ID NO: 38.

In one aspect of the present invention, there is provided a CAR capable of binding to a non-TSA or non-TAA including, for example and not by way of limitation, an antigen derived from Retroviridae (*e.g.* human immunodeficiency viruses such as HIV-1 and HIV-LP), Picornaviridae (*e.g.* poliovirus, hepatitis A virus, enterovirus, human coxsackievirus, rhinovirus, and echovirus), rubella virus, coronavirus, vesicular stomatitis virus, rabies virus, ebola virus, parainfluenza virus, mumps virus, measles virus, respiratory syncytial virus, influenza virus, hepatitis B virus, parvovirus, Adenoviridae, Herpesviridae [*e.g.* type 1 and type 2 herpes simplex virus (HSV), varicella-zoster virus, cytomegalovirus (CMV), and herpes virus], Poxviridae (*e.g.* smallpox virus, vaccinia virus, and pox virus), or hepatitis C virus, or any combination thereof.

In another aspect of the present invention, there is provided a CAR capable of binding to an antigen derived from a bacterial strain of Staphylococci, Streptococcus, Escherichia coli, Pseudomonas, or Salmonella. Particularly, there is provided a CAR capable of binding to an antigen derived from an infectious bacterium, for example, Helicobacter pyloris, Legionella pneumophilia, a bacterial strain of Mycobacteria sps. (*e.g.* M. tuberculosis, M. avium, M. intracellulare, M. kansaii, or M. gordonea), Staphylococcus aureus, Neisseria gonorrhoeae, Neisseria meningitides, Listeria monocytogenes, Streptococcus pyogenes, Group A Streptococcus, Group B Streptococcus (Streptococcus agalactiae), Streptococcus pneumoniae, or Clostridium tetani, or a combination thereof.

## 2. Transmembrane Domain

With respect to the transmembrane domain, the CAR comprises one or more transmembrane domains fused to the extracellular CD33 antigen binding domain of the CAR.

The transmembrane domain may be derived either from a natural or from a synthetic source. Where the source is natural, the domain may be derived from any membrane-bound or transmembrane protein.

Transmembrane regions of particular use in the CARs described herein may be derived from (*i.e.* comprise at least the transmembrane region(s) of) the alpha, beta or zeta chain of the T-cell receptor, CD28, CD3 epsilon, CD45, CD4, CD5, CD8, CD9, CD16, CD22, mesothelin, CD33, CD37, CD64, CD80, CD86, CD134, CD137, CD154. Alternatively the transmembrane domain may be synthetic, in which case it will comprise predominantly hydrophobic residues such as leucine and valine. Preferably a triplet of phenylalanine, tryptophan and valine will be found at each end of a synthetic transmembrane domain. Optionally, a short oligo- or polypeptide linker, preferably between 2 and 10 amino acids in length may form the linkage between the transmembrane domain and the cytoplasmic signaling domain of the CAR. A glycine-serine doublet provides a particularly suitable linker.

In one embodiment, the transmembrane domain that naturally is associated with one of the domains in the CAR is used in addition to the transmembrane domains described *supra*.

In some instances, the transmembrane domain can be selected by amino acid substitution to avoid binding of such domains to the transmembrane domains of the same or different surface membrane proteins to minimize interactions with other members of the receptor complex.

In one embodiment, the transmembrane domain in the CAR of the invention is the CD8 transmembrane domain. In one embodiment, the CD8 transmembrane domain comprises the nucleic acid sequence of SEQ ID NO: 27. In one embodiment, the CD8 transmembrane domain comprises the nucleic acid sequence that encodes the amino acid sequence of SEQ ID NO: 28. In another embodiment, the CD8 transmembrane domain comprises the amino acid sequence of SEQ ID NO: 28.

In one embodiment, the encoded transmembrane domain comprises an amino acid sequence having at least one, two or three modifications (*e.g.*, substitutions) but not more than 20, 10 or 5

modifications (*e.g.*, substitutions) of an amino acid sequence of SEQ ID NO:28, or a sequence with 95-99% identity to an amino acid sequence of SEQ ID NO:28.

In some instances, the transmembrane domain of the CAR comprises the CD8.alpha.hinge domain. In one embodiment, the CD8 hinge domain comprises the nucleic acid sequence of SEQ ID NO: 29. In one embodiment, the CD8 hinge domain comprises the nucleic acid sequence that encodes the amino acid sequence of SEQ ID NO: 30. In another embodiment, the CD8 hinge domain comprises the amino acid sequence of SEQ ID NO: 30, or a sequence with 95-99% identify thereof.

In one embodiment, an isolated nucleic acid molecule is provided wherein the encoded linker domain is derived from the extracellular domain of CD8, and is linked to the transmembrane CD8 domain, the transmembrane CD28 domain, or a combination thereof.

In one embodiment, the transmembrane domain in the CAR of the invention is the TNFRSF19 transmembrane domain. In one embodiment, the TNFRSF19 transmembrane domain comprises the nucleic acid sequence of SEQ ID NO: 51. In one embodiment, the TNFRSF19 transmembrane domain comprises the nucleic acid sequence that encodes the amino acid sequence of SEQ ID NO: 52. In another embodiment, the TNFRSF19 transmembrane domain comprises the amino acid sequence of SEQ ID NO: 52.

In one embodiment, the encoded transmembrane domain comprises an amino acid sequence having at least one, two or three modifications (*e.g.*, substitutions) but not more than 20, 10 or 5 modifications (*e.g.*, substitutions) of an amino acid sequence of SEQ ID NO: 52, or a sequence with 95-99% identity to an amino acid sequence of SEQ ID NO: 52.

### 3. Spacer Domain

In the CAR, a spacer domain, also termed hinge domain, can be arranged between the extracellular domain and the transmembrane domain, or between the intracellular domain and the transmembrane domain. The spacer domain means any oligopeptide or polypeptide that serves to link the transmembrane domain with the extracellular domain and/or the transmembrane domain with the intracellular domain. The spacer domain comprises up to 300 amino acids, preferably 10 to 100 amino acids, and most preferably 25 to 50 amino acids.

In several embodiments, the linker can include a spacer element, which, when present, increases the size of the linker such that the distance between the effector molecule or the detectable marker and the antibody or antigen binding fragment is increased. Exemplary spacers are known to the person of ordinary skill, and include those listed in U.S. Pat. Nos. 7,964,5667, 7,498,298,

6,884,869, 6,323,315, 6,239,104, 6,034,065, 5,780,588, 5,665,860, 5,663,149, 5,635,483, 5,599,902, 5,554,725, 5,530,097, 5,521,284, 5,504,191, 5,410,024, 5,138,036, 5,076,973, 4,986,988, 4,978,744, 4,879,278, 4,816,444, and 4,486,414, as well as U.S. Pat. Pub. Nos. 20110212088 and 20110070248, each of which is incorporated by reference herein in its entirety.

The spacer domain preferably has a sequence that promotes binding of a CAR with an antigen and enhances signaling into a cell. Examples of an amino acid that is expected to promote the binding include cysteine, a charged amino acid, and serine and threonine in a potential glycosylation site, and these amino acids can be used as an amino acid constituting the spacer domain.

As the spacer domain, the entire or a part of amino acid numbers 118 to 178 (SEQ ID NO: 31) which is a hinge region of CD8.alpha. (NCBI RefSeq: NP.sub.--001759.3), amino acid numbers 135 to 195 of CD8.beta. (GenBank: AAA35664.1), amino acid numbers 315 to 396 of CD4 (NCBI RefSeq: NP.sub.--000607.1), or amino acid numbers 137 to 152 of CD28 (NCBI RefSeq: NP.sub.--006130.1) can be used. Also, as the spacer domain, a part of a constant region of an antibody H chain or L chain (CH1 region or CL region, for example, a peptide having an amino acid sequence shown in SEQ ID NO: 32) can be used. Further, the spacer domain may be an artificially synthesized sequence.

In addition, an entire or a part of amino acids comprising the constant region of a human IgG4 (UniProt ID: P01861), including CH1, (amino acid numbers 1-98), hinge, SEQ ID NO: 80, and the corresponding nucleotide SEQ ID NO:79, (amino acid numbers 99-110), CH2, amino acid SEQ ID NO: 82 and corresponding nucleotide SEQ ID NO: 81, (amino acid numbers 111-220) and CH3, SEQ ID NO:84 and corresponding nucleotide SEQ ID NO: 83, (amino acid numbers 221-327) or a combination thereof, such as IgG4 Hinge CH2 CH3 domain, SEQ ID NO: 86, and the corresponding nucleotide SEQ ID NO: 85, can be used.

In one embodiment, the spacer domain of the CAR comprises the TNFRSF19 hinge domain which comprises the nucleic acid sequence of SEQ ID NO: 53. In one embodiment, the TNFRSF19 hinge domain comprises the nucleic acid sequence that encodes the amino acid sequence of SEQ ID NO: 54. In another embodiment, the TNFRSF19 hinge domain comprises the amino acid sequence of SEQ ID NO: 54, or a sequence with 95-99% identify thereof.

In one embodiment, the spacer domain of the CAR comprises the TNFRSF19 truncated hinge domain comprises the nucleic acid sequence of SEQ ID NO: 55. In one embodiment, the TNFRSF19 truncated hinge domain comprises the nucleic acid sequence that encodes the amino acid sequence of SEQ ID NO: 56. In another embodiment, the TNFRSF19 truncated hinge domain comprises the amino acid sequence of SEQ ID NO: 56, or a sequence with 95-99% identify thereof.

In one embodiment, the TNFRSF19 hinge and transmembrane domains comprise the nucleic acid sequence of SEQ ID NO: 49. In one embodiment, the TNFRSF19 hinge and transmembrane domains comprise the nucleic acid sequence that encodes the amino acid sequence of SEQ ID NO: 50. In another embodiment, the TNFRSF19 hinge and transmembrane domains comprise the amino acid sequence of SEQ ID NO: 50, or a sequence with 95-99% identify thereof.

In one embodiment, a CD8a hinge domain is fused to a TNFRSF19 transmembrane domain comprising the nucleic acid sequence of SEQ ID NO: 57. In one embodiment, the CD8a hinge domain is fused to a TNFRSF19 transmembrane domain comprises the nucleic acid sequence that encodes the amino acid sequence of SEQ ID NO: 58. In another embodiment, the CD8a hinge domain is fused to a TNFRSF19 transmembrane domain comprises the amino acid sequence of SEQ ID NO: 58, or a sequence with 95-99% identify thereof.

Further, in the CAR, a signal peptide sequence, also termed leader peptide, can be linked to the N-terminus. The signal peptide sequence exists at the N-terminus of many secretory proteins and membrane proteins, and has a length of 15 to 30 amino acids. Since many of the protein molecules mentioned above as the intracellular domain have signal peptide sequences, the signal peptides can be used as a signal peptide for the CAR. In one embodiment, the signal peptide comprises the amino acid sequence shown in SEQ ID NO: 14).

In one embodiment, the CD8 alpha leader peptide, is comprising the nucleic acid sequence of SEQ ID NO: 43. In one embodiment, CD8 alpha leader peptide comprises the nucleic acid sequence that encodes the amino acid sequence of SEQ ID NO: 44. In another embodiment, the CD8a hinge domain is fused to a TNFRSF19 transmembrane domain comprises the amino acid sequence of SEQ ID NO: 44, or a sequence with 95-99% identify thereof.

In another embodiment, the GMCSF leader peptide, is comprising the nucleic acid sequence of SEQ ID NO: 39. In one embodiment, the GMCSF leader peptide, comprises the nucleic acid sequence that encodes the amino acid sequence of SEQ ID NO: 40. In another embodiment, the CD8a hinge domain is fused to a TNFRSF19 transmembrane domain comprises the amino acid sequence of SEQ ID NO: 40, or a sequence with 95-99% identify thereof.

In another embodiment, the TNFRSF19 leader peptide is comprising the nucleic acid sequence of SEQ ID NO: 41. In one embodiment, TNFRSF19 leader peptide, and CD8 alpha leader peptide comprises the nucleic acid sequence that encodes the amino acid sequence of SEQ ID NO: 42. In another embodiment, the CD8a hinge domain is fused to a TNFRSF19 transmembrane domain comprises the amino acid sequence of SEQ ID NO: 42, or a sequence with 95-99% identify thereof.

In one embodiment, a tag sequence encoding a truncated sequence of epidermal growth factor receptor (tEGFR) is comprising the nucleic acid sequence of SEQ ID NO: 67. In one embodiment, tEGFR comprises the nucleic acid sequence that encodes the amino acid sequence of SEQ ID NO: 68. In another embodiment, the tEGFR tag comprises the amino acid sequence of SEQ ID NO: 68, or a sequence with 95-99% identify thereof.

In one embodiment, a furin recognition site and downstream T2A self-cleaving peptide sequence, designed for simultaneous bicistronic expression of the tag sequence and the CAR sequence, is comprising the nucleic acid sequence of SEQ ID NO: 65. In one embodiment, furin and T2A sequence comprises the nucleic acid sequence that encodes the amino acid sequence of SEQ ID NO: 66. In another embodiment, the tEGFR tag comprises the amino acid sequence of SEQ ID NO: 66 or a sequence with 95-99% identify thereof.

In one embodiment, an upstream furin recognition site and T2A self-cleaving peptide sequence and a furin recognition downstream site, designed for simultaneous bicistronic expression of the tag sequence and the CAR sequence, is comprising the nucleic acid sequence of SEQ ID NO: 67. In one embodiment, furin and T2A sequence comprises the nucleic acid sequence that encodes the amino acid sequence of SEQ ID NO: 68. In another embodiment, the tEGFR tag comprises the amino acid sequence of SEQ ID NO: 68 or a sequence with 95-99% identify thereof.

In one embodiment, the targeting domain of the CAR is expressed separately in the form of monoclonal antibody, ScFv Fab, Fab'2 and is containing at binding tag or epitope, whereas the effector-cell expressed component of the CAR contains a binding domain specifically directed to bind the tag or epitope expressed on the soluble CAR module, such as specific binding on the soluble component of the CAR to the cell bound component forms the full functional CAR structure.

### 4. Intracellular Domain

The cytoplasmic domain or otherwise the intracellular signaling domain of the CAR is responsible for activation of at least one of the normal effector functions of the immune cell in which the CAR has been placed in. The term "effector function" refers to a specialized function of a cell. Effector function of a T cell, for example, may be cytolytic activity or helper activity including the secretion of cytokines. Thus the term "intracellular signaling domain" refers to the portion of a protein which transduces the effector function signal and directs the cell to perform a specialized function. While usually the entire intracellular signaling domain can be employed, in many cases it is not necessary to use the entire chain. To the extent that a truncated portion of the intracellular

signaling domain is used, such truncated portion may be used in place of the intact chain as long as it transduces the effector function signal. The term intracellular signaling domain is thus meant to include any truncated portion of the intracellular signaling domain sufficient to transduce the effector function signal.

Preferred examples of intracellular signaling domains for use in the CAR include the cytoplasmic sequences of the T cell receptor (TCR) and co-receptors that act in concert to initiate signal transduction following antigen receptor engagement, as well as any derivative or variant of these sequences and any synthetic sequence that has the same functional capability.

It is known that signals generated through the TCR alone are insufficient for full activation of the T cell and that a secondary or co-stimulatory signal is also required. Thus, T cell activation can be said to be mediated by two distinct classes of cytoplasmic signaling sequence: those that initiate antigen-dependent primary activation through the TCR (primary cytoplasmic signaling sequences) and those that act in an antigen-independent manner to provide a secondary or co-stimulatory signal (secondary cytoplasmic signaling sequences).

Primary cytoplasmic signaling sequences regulate primary activation of the TCR complex either in a stimulatory way, or in an inhibitory way. Primary cytoplasmic signaling sequences that act in a stimulatory manner may contain signaling motifs which are known as immunoreceptor tyrosine-based activation motifs or ITAMs.

Examples of ITAM containing primary cytoplasmic signaling sequences that are of particular use in the CARs disclosed herein include those derived from TCR zeta (CD3 Zeta), FcR gamma, FcR beta, CD3 gamma, CD3 delta, CD3 epsilon, CD5, CD22, CD79a, CD79b, and CD66d. Specific, non-limiting examples, of the ITAM include peptides having sequences of amino acid numbers 51 to 164 of CD3.zeta. (NCBI RefSeq: NP.sub.--932170.1), amino acid numbers 45 to 86 of Fc.epsilon.RI.gamma. (NCBI RefSeq: NP.sub.--004097.1), amino acid numbers 201 to 244 of Fc.epsilon.RI.beta. (NCBI RefSeq: NP.sub.--000130.1), amino acid numbers 139 to 182 of CD3.gamma. (NCBI RefSeq: NP.sub.--000064.1), amino acid numbers 128 to 171 of CD3 .delta. (NCBI RefSeq: NP.sub.--000723.1), amino acid numbers 153 to 207 of CD3.epsilon. (NCBI RefSeq: NP.sub.--000724.1), amino acid numbers 402 to 495 of CD5 (NCBI RefSeq: NP.sub.--055022.2), amino acid numbers 707 to 847 of 0022 (NCBI RefSeq: NP.sub.--001762.2), amino acid numbers 166 to 226 of CD79a (NCBI RefSeq: NP.sub.--001774.1), amino acid numbers 182 to 229 of CD79b (NCBI RefSeq: NP.sub.--000617.1), and amino acid numbers 177 to 252 of CD66d (NCBI RefSeq: NP.sub.--001806.2), and their variants having the same function as these peptides have. The amino acid number based on amino acid sequence information of NCBI RefSeq ID or GenBank described herein is numbered based on the full length of the precursor (comprising a

signal peptide sequence etc.) of each protein. In one embodiment, the cytoplasmic signaling molecule in the CAR comprises a cytoplasmic signaling sequence derived from CD3 zeta.

In a preferred embodiment, the intracellular domain of the CAR can be designed to comprise the CD3-zeta signaling domain by itself or combined with any other desired cytoplasmic domain(s) useful in the context of the CAR. For example, the intracellular domain of the CAR can comprise a CD3 zeta chain portion and a costimulatory signaling region. The costimulatory signaling region refers to a portion of the CAR comprising the intracellular domain of a costimulatory molecule. A costimulatory molecule is a cell surface molecule other than an antigen receptor or their ligands that is required for an efficient response of lymphocytes to an antigen. Examples of such costimulatory molecules include CD27, CD28, 4-1BB (CD137), OX40, CD30, CD40, PD-1, ICOS, lymphocyte function-associated antigen-1 (LFA-1), CD2, CD7, LIGHT, NKG2C, B7-H3, and a ligand that specifically binds with CD83, and the like. Specific, non-limiting examples, of such costimulatory molecules include peptides having sequences of amino acid numbers 236 to 351 of CD2 (NCBI RefSeq: NP.sub.--001758.2), amino acid numbers 421 to 458 of CD4 (NCBI RefSeq: NP.sub.--000607.1), amino acid numbers 402 to 495 of CD5 (NCBI RefSeq: NP.sub.--055022.2), amino acid numbers 207 to 235 of CD8.alpha. (NCBI RefSeq: NP.sub.--001759.3), amino acid numbers 196 to 210 of CD83 (GenBank: AAA35664.1), amino acid numbers 181 to 220 of CD28 (NCBI RefSeq: NP.sub.--006130.1), amino acid numbers 214 to 255 of CD137 (4-1BB, NCBI RefSeq: NP.sub.--001552.2), amino acid numbers 241 to 277 of CD134 (OX40, NCBI RefSeq: NP.sub.--003318.1), and amino acid numbers 166 to 199 of ICOS (NCBI RefSeq: NP.sub.--036224.1), and their variants having the same function as these peptides have. Thus, while the disclosure herein is exemplified primarily with 4-1BB as the co-stimulatory signaling element, other costimulatory elements are within the scope of the disclosure.

The cytoplasmic signaling sequences within the cytoplasmic signaling portion of the CAR may be linked to each other in a random or specified order. Optionally, a short oligo- or polypeptide linker, preferably between 2 and 10 amino acids in length may form the linkage. A glycine-serine doublet provides a particularly suitable linker.

In one embodiment, the intracellular domain is designed to comprise the signaling domain of CD3-zeta and the signaling domain of CD28. In another embodiment, the intracellular domain is designed to comprise the signaling domain of CD3-zeta and the signaling domain of 4-1BB. In yet another embodiment, the intracellular domain is designed to comprise the signaling domain of CD3-zeta and the signaling domain of CD28 and 4-1BB.

In one embodiment, the intracellular domain in the CAR is designed to comprise the signaling domain of 4-1BB and the signaling domain of CD3-zeta, wherein the signaling domain of

4-1BB comprises the nucleic acid sequence set forth in SEQ ID NO: 33 or SEQ ID NO: 73 and the signaling domain of CD3-zeta comprises the nucleic acid sequence set forth in SEQ ID NO: 35, SEQ ID NO: 47, SEQ ID NO: 61, or SEQ ID NO: 75.

In one embodiment, the intracellular domain in the CAR is designed to comprise the signaling domain of 4-1BB and the signaling domain of CD3-zeta, wherein the signaling domain of 4-1BB comprises the nucleic acid sequence that encodes the amino acid sequence of SEQ ID NO: 34, or SEQ ID NO: 74, respectively and the signaling domain of CD3-zeta comprises the nucleic acid sequence that encodes the amino acid sequence of SEQ ID NO: 36, or SEQ ID NO: 48, SEQ ID NO: 62, or SEQ ID NO 76.

In one embodiment, the intracellular domain in the CAR is designed to comprise the signaling domain of 4-1BB and the signaling domain of CD3-zeta, wherein the signaling domain of 4-1BB comprises the amino acid sequence set forth in SEQ ID NO: 34, or SEQ ID NO: 74, respectively and the signaling domain of CD3-zeta comprises the amino acid sequence set forth in SEQ ID NO: 36, SEQ ID NO: 48, SEQ ID NO: 62, or SEQ ID NO: 76, respectively.

In one embodiment, the intracellular domain in the CAR is designed to comprise the signaling domain of CD28 and the signaling domain of CD3-zeta, wherein the signaling domain of CD28 comprises the nucleic acid sequence set forth in SEQ ID NO: 45, or SEQ ID NO: 59, respectively, and the signaling domain of CD3-zeta comprises the nucleic acid sequence set forth in SEQ ID NO: 35, SEQ ID NO: 47, or SEQ ID NO: 61, respectively.

In one embodiment, the intracellular domain in the CAR is designed to comprise the signaling domain of CD28 and the signaling domain of CD3-zeta, wherein the signaling domain of CD28 comprises the nucleic acid sequence that encodes the amino acid sequence of SEQ ID NO: 46, or SEQ ID NO: 60, respectively and the signaling domain of CD3-zeta comprises the nucleic acid sequence that encodes the amino acid sequence of SEQ ID NO: 36, or SEQ ID NO: 48, or SEQ ID NO: 62.

In one embodiment, the intracellular domain in the CAR is designed to comprise the signaling domain of CD28 and the signaling domain of CD3-zeta, wherein the signaling domain of CD28 comprises the amino acid sequence set forth in SEQ ID NO: 46, or SEQ ID NO: 60, respectively and the signaling domain of CD3-zeta comprises the amino acid sequence set forth in SEQ ID NO: 36, SEQ ID NO: 48, or SEQ ID NO: 62, respectively.

### 5. Additional Description of CARs

Also expressly included within the scope of the invention are functional portions of the CARs disclosed herein. The term "functional portion" when used in reference to a CAR refers to any part or fragment of one or more of the CARs disclosed herein, which part or fragment retains the biological activity of the CAR of which it is a part (the parent CAR). Functional portions encompass, for example, those parts of a CAR that retain the ability to recognize target cells, or detect, treat, or prevent a disease, to a similar extent, the same extent, or to a higher extent, as the parent CAR. In reference to the parent CAR, the functional portion can comprise, for instance, about 10%, 25%, 30%, 50%, 68%, 80%, 90%, 95%, or more, of the parent CAR.

The functional portion can comprise additional amino acids at the amino or carboxy terminus of the portion, or at both termini, which additional amino acids are not found in the amino acid sequence of the parent CAR. Desirably, the additional amino acids do not interfere with the biological function of the functional portion, *e.g.*, recognize target cells, detect cancer, treat or prevent cancer, etc. More desirably, the additional amino acids enhance the biological activity, as compared to the biological activity of the parent CAR.

Included in the scope of the disclosure are functional variants of the CARs disclosed herein. The term "functional variant" as used herein refers to a CAR, polypeptide, or protein having substantial or significant sequence identity or similarity to a parent CAR, which functional variant retains the biological activity of the CAR of which it is a variant. Functional variants encompass, for example, those variants of the CAR described herein (the parent CAR) that retain the ability to recognize target cells to a similar extent, the same extent, or to a higher extent, as the parent CAR. In reference to the parent CAR, the functional variant can, for instance, be at least about 30%, 50%, 75%, 80%, 90%, 98% or more identical in amino acid sequence to the parent CAR.

A functional variant can, for example, comprise the amino acid sequence of the parent CAR with at least one conservative amino acid substitution. Alternatively or additionally, the functional variants can comprise the amino acid sequence of the parent CAR with at least one non-conservative amino acid substitution. In this case, it is preferable for the non-conservative amino acid substitution to not interfere with or inhibit the biological activity of the functional variant. The non-conservative amino acid substitution may enhance the biological activity of the functional variant, such that the biological activity of the functional variant is increased as compared to the parent CAR.

Amino acid substitutions of the CARs are preferably conservative amino acid substitutions. Conservative amino acid substitutions are known in the art, and include amino acid substitutions in

which one amino acid having certain physical and/or chemical properties is exchanged for another amino acid that has the same or similar chemical or physical properties. For instance, the conservative amino acid substitution can be an acidic/negatively charged polar amino acid substituted for another acidic/negatively charged polar amino acid (*e.g.*, Asp or Glu), an amino acid with a nonpolar side chain substituted for another amino acid with a nonpolar side chain (*e.g.*, Ala, Gly, Val, He, Leu, Met, Phe, Pro, Trp, Cys, Val, etc.), a basic/positively charged polar amino acid substituted for another basic/positively charged polar amino acid (*e.g.* Lys, His, Arg, etc.), an uncharged amino acid with a polar side chain substituted for another uncharged amino acid with a polar side chain (*e.g.*, Asn, Gin, Ser, Thr, Tyr, etc.), an amino acid with a beta-branched side-chain substituted for another amino acid with a beta-branched side-chain (*e.g.*, He, Thr, and Val), an amino acid with an aromatic side-chain substituted for another amino acid with an aromatic side chain (*e.g.*, His, Phe, Trp, and Tyr), etc.

The CAR can consist essentially of the specified amino acid sequence or sequences described herein, such that other components, *e.g.*, other amino acids, do not materially change the biological activity of the functional variant.

The CARs (including functional portions and functional variants) can be of any length, *i.e.*, can comprise any number of amino acids, provided that the CARs (or functional portions or functional variants thereof) retain their biological activity, *e.g.*, the ability to specifically bind to antigen, detect diseased cells in a mammal, or treat or prevent disease in a mammal, etc. For example, the CAR can be about 50 to about 5000 amino acids long, such as 50, 70, 75, 100, 125, 150, 175, 200, 300, 400, 500, 600, 700, 800, 900, 1000 or more amino acids in length.

The CARs (including functional portions and functional variants of the invention) can comprise synthetic amino acids in place of one or more naturally-occurring amino acids. Such synthetic amino acids are known in the art, and include, for example, aminocyclohexane carboxylic acid, norleucine, -amino n-decanoic acid, homoserine, S-acetylaminomethyl-cysteine, trans-3- and trans-4-hydroxyproline, 4-aminophenylalanine, 4- nitrophenylalanine, 4-chlorophenylalanine, 4-carboxyphenylalanine, β-phenylserine β-hydroxyphenylalanine, phenylglycine, a-naphthylalanine, cyclohexylalanine, cyclohexylglycine, indoline-2-carboxylic acid, 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid, aminomalonic acid, aminomalonic acid monoamide, N'-benzyl-N'-methyl-lysine, N',N'-dibenzyl-lysine, 6-hydroxylysine, ornithine, -aminocyclopentane carboxylic acid, a-aminocyclohexane carboxylic acid, a-aminocycloheptane carboxylic acid, a-(2-amino-2-norbornane)-carboxylic acid, γ-diaminobutyric acid, β-diaminopropionic acid, homophenylalanine, and a-tert-butylglycine.

The CARs (including functional portions and functional variants) can be glycosylated, amidated, carboxylated, phosphorylated, esterified, N-acylated, cyclized via, *e.g.*, a disulfide bridge, or converted into an acid addition salt and/or optionally dimerized or polymerized, or conjugated.

The CARs (including functional portions and functional variants thereof) can be obtained by methods known in the art. The CARs may be made by any suitable method of making polypeptides or proteins. Suitable methods of *de novo* synthesizing polypeptides and proteins are described in references, such as Chan *et al.*, Fmoc Solid Phase Peptide Synthesis, Oxford University Press, Oxford, United Kingdom, 2000; Peptide and Protein Drug Analysis, ed. Reid, R., Marcel Dekker, Inc., 2000; Epitope Mapping, ed. Westwood *et al.*, Oxford University Press, Oxford, United Kingdom, 2001; and U.S. Patent 5,449,752. Also, polypeptides and proteins can be recombinantly produced using the nucleic acids described herein using standard recombinant methods. See, for instance, Sambrook *et al.*, Molecular Cloning: A Laboratory Manual, 3rd ed., Cold Spring Harbor Press, Cold Spring Harbor, NY 2001; and Ausubel *et al.*, Current Protocols in Molecular Biology, Greene Publishing Associates and John Wiley & Sons, NY, 1994. Further, some of the CARs (including functional portions and functional variants thereof) can be isolated and/or purified from a source, such as a plant, a bacterium, an insect, a mammal, *e.g.*, a rat, a human, etc. Methods of isolation and purification are well-known in the art. Alternatively, the CARs described herein (including functional portions and functional variants thereof) can be commercially synthesized by companies. In this respect, the CARs can be synthetic, recombinant, isolated, and/or purified.

### A. Antibodies and Antigen Binding Fragments

One embodiment further provides a CAR, a T cell expressing a CAR, an antibody, or antigen binding domain or portion thereof, which specifically binds to one or more of the antigens disclosed herein. As used herein, a "T cell expressing a CAR," or a "CAR T cell" means a T cell expressing a CAR, and has antigen specificity determined by, for example, the antibody-derived targeting domain of the CAR.

As used herein, and "antigen binding domain" can include an antibody and antigen binding fragments thereof. The term "antibody" is used herein in the broadest sense and encompasses various antibody structures, including but not limited to monoclonal antibodies, polyclonal antibodies, multi-specific antibodies (*e.g.*, bispecific antibodies), and antigen binding fragments thereof, so long as they exhibit the desired antigen-binding activity. Non-limiting examples of antibodies include, for example, intact immunoglobulins and variants and fragments thereof known in the art that retain binding affinity for the antigen.

A "monoclonal antibody" is an antibody obtained from a population of substantially homogeneous antibodies, *i.e.*, the individual antibodies comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts. Monoclonal antibodies are highly specific, being directed against a single antigenic epitope. The modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. In some examples, a monoclonal antibody is an antibody produced by a single clone of B lymphocytes or by a cell into which nucleic acid encoding the light and heavy variable regions of the antibody of a single antibody (or an antigen binding fragment thereof) have been transfected, or a progeny thereof. In some examples monoclonal antibodies are isolated from a subject. Monoclonal antibodies can have conservative amino acid substitutions which have substantially no effect on antigen binding or other immunoglobulin functions. Exemplary methods of production of monoclonal antibodies are known, for example, see Harlow & Lane, Antibodies, A Laboratory Manual, 2nd ed. Cold Spring Harbor Publications, New York (2013).

Typically, an immunoglobulin has heavy (H) chains and light (L) chains interconnected by disulfide bonds. Immunoglobulin genes include the kappa, lambda, alpha, gamma, delta, epsilon and mu constant region genes, as well as the myriad immunoglobulin variable domain genes. There are two types of light chain, lambda (λ) and kappa (κ). There are five main heavy chain classes (or isotypes) which determine the functional activity of an antibody molecule: IgM, IgD, IgG, IgA and IgE.

Each heavy and light chain contains a constant region (or constant domain) and a variable region (or variable domain; see, *e.g.*, Kindt *et al.* Kuby Immunology, 6$^{th}$ ed., W.H. Freeman and Co., page 91 (2007).) In several embodiments, the heavy and the light chain variable regions combine to specifically bind the antigen. In additional embodiments, only the heavy chain variable region is required. For example, naturally occurring camelid antibodies consisting of a heavy chain only are functional and stable in the absence of light chain (see, *e.g.*, Hamers-Casterman *et al.*, Nature, 363:446-448, 1993; Sheriff *et al.*, Nat. Struct. Biol., 3:733-736, 1996). References to "VH" or "VH" refer to the variable region of an antibody heavy chain, including that of an antigen binding fragment, such as Fv, ScFv, dsFv or Fab. References to "VL" or "VL" refer to the variable domain of an antibody light chain, including that of an Fv, ScFv, dsFv or Fab.

Light and heavy chain variable regions contain a "framework" region interrupted by three hypervariable regions, also called "complementarity-determining regions" or "CDRs" (see, *e.g.*, Kabat *et al.*, Sequences of Proteins of Immunological Interest, U.S. Department of Health and

Human Services, 1991). The sequences of the framework regions of different light or heavy chains are relatively conserved within a species. The framework region of an antibody, that is the combined framework regions of the constituent light and heavy chains, serves to position and align the CDRs in three-dimensional space.

The CDRs are primarily responsible for binding to an epitope of an antigen. The amino acid sequence boundaries of a given CDR can be readily determined using any of a number of well-known schemes, including those described by Kabat *et al.* ("Sequences of Proteins of Immunological Interest," 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD, 1991; "Kabat" numbering scheme), Al-Lazikani *et al.*, (JMB 273,927-948, 1997; "Chothia" numbering scheme), and Lefranc *et al.* ("IMGT unique numbering for immunoglobulin and T cell receptor variable domains and Ig superfamily V-like domains," Dev. Comp. Immunol., 27:55-77, 2003; "IMGT" numbering scheme). The CDRs of each chain are typically referred to as CDR1, CDR2, and CDR3 (from the N-terminus to C-terminus), and are also typically identified by the chain in which the particular CDR is located. Thus, a VH CDR3 is the CDR3 from the variable domain of the heavy chain of the antibody in which it is found, whereas a VL CDR1 is the CDR1 from the variable domain of the light chain of the antibody in which it is found. Light chain CDRs are sometimes referred to as LCDR1, LCDR2, and LCDR3. Heavy chain CDRs are sometimes referred to as HCDR1, HCDR2, and HCDR3.

An "antigen binding fragment" is a portion of a full length antibody that retains the ability to specifically recognize the cognate antigen, as well as various combinations of such portions. Non-limiting examples of antigen binding fragments include Fv, Fab, Fab', Fab'-SH, F(ab')2; diabodies; linear antibodies; single-chain antibody molecules (*e.g.* ScFv); and multi-specific antibodies formed from antibody fragments. Antibody fragments include antigen binding fragments either produced by the modification of whole antibodies or those synthesized de novo using recombinant DNA methodologies (see, *e.g.*, Kontermann and Dubel (Ed), Antibody Engineering, Vols. 1-2, 2nd Ed., Springer Press, 2010).

A single-chain antibody (ScFv) is a genetically engineered molecule containing the VH and VL domains of one or more antibody(ies) linked by a suitable polypeptide linker as a genetically fused single chain molecule (see, for example, Bird *et al.*, Science, 242:423 426, 1988; Huston *et al.*, Proc. Natl. Acad. Sci., 85:5879 5883, 1988; Ahmad *et al.*, Clin. Dev. Immunol., 2012, doi:10.1155/2012/980250; Marbry, IDrugs, 13:543-549, 2010). The intramolecular orientation of the VH-domain and the VL-domain in a ScFv, is typically not decisive for ScFvs. Thus, ScFvs with both possible arrangements (VH-domain-linker domain-VL-domain; VL-domain-linker domain-VH-domain) may be used.

In a dsFv, the heavy and light chain variable chains have been mutated to introduce a disulfide bond to stabilize the association of the chains. Diabodies also are included, which are bivalent, bispecific antibodies in which VH and VL domains are expressed on a single polypeptide chain, but using a linker that is too short to allow for pairing between the two domains on the same chain, thereby forcing the domains to pair with complementary domains of another chain and creating two antigen binding sites (see, for example, Holliger *et al.*, Proc. Natl. Acad. Sci., 90:6444 6448, 1993; Poljak *et al.*, Structure, 2:1121 1123, 1994).

Antibodies also include genetically engineered forms such as chimeric antibodies (such as humanized murine antibodies) and heteroconjugate antibodies (such as bispecific antibodies). See also, Pierce Catalog and Handbook, 1994-1995 (Pierce Chemical Co., Rockford, IL); Kuby, J., Immunology, 3rd Ed., W.H. Freeman & Co., New York, 1997.

Non-naturally occurring antibodies can be constructed using solid phase peptide synthesis, can be produced recombinantly, or can be obtained, for example, by screening combinatorial libraries consisting of variable heavy chains and variable light chains as described by Huse *et al.*, Science 246:1275-1281 (1989), which is incorporated herein by reference. These and other methods of making, for example, chimeric, humanized, CDR-grafted, single chain, and bifunctional antibodies, are well known to those skilled in the art (Winter and Harris, Immunol. Today 14:243-246 (1993); Ward *et al.*, Nature 341:544-546 (1989); Harlow and Lane, *supra*, 1988; Hilyard *et al.*, Protein Engineering: A practical approach (IRL Press 1992); Borrabeck, Antibody Engineering, 2d ed. (Oxford University Press 1995); each of which is incorporated herein by reference).

An "antibody that binds to the same epitope" as a reference antibody refers to an antibody that blocks binding of the reference antibody to its antigen in a competition assay by 50% or more, and conversely, the reference antibody blocks binding of the antibody to its antigen in a competition assay by 50% or more. Antibody competition assays are known, and an exemplary competition assay is provided herein.

A "humanized" antibody or antigen binding fragment includes a human framework region and one or more CDRs from a non-human (such as a mouse, rat, or synthetic) antibody or antigen binding fragment. The non-human antibody or antigen binding fragment providing the CDRs is termed a "donor," and the human antibody or antigen binding fragment providing the framework is termed an "acceptor." In one embodiment, all the CDRs are from the donor immunoglobulin in a humanized immunoglobulin. Constant regions need not be present, but if they are, they can be substantially identical to human immunoglobulin constant regions, such as at least about 85-90%, such as about 95% or more identical. Hence, all parts of a humanized antibody or antigen binding

fragment, except possibly the CDRs, are substantially identical to corresponding parts of natural human antibody sequences.

A "chimeric antibody" is an antibody which includes sequences derived from two different antibodies, which typically are of different species. In some examples, a chimeric antibody includes one or more CDRs and/or framework regions from one human antibody and CDRs and/or framework regions from another human antibody.

A "fully human antibody" or "human antibody" is an antibody which includes sequences from (or derived from) the human genome, and does not include sequence from another species. In some embodiments, a human antibody includes CDRs, framework regions, and (if present) an Fc region from (or derived from) the human genome. Human antibodies can be identified and isolated using technologies for creating antibodies based on sequences derived from the human genome, for example by phage display or using transgenic animals (see, *e.g.*, Barbas *et al.* Phage display: A Laboratory Manuel. 1st Ed. New York: Cold Spring Harbor Laboratory Press, 2004. Print.; Lonberg, Nat. Biotech., 23: 1117-1125, 2005; Lonenberg, Curr. Opin. Immunol., 20:450-459, 2008).

An antibody may have one or more binding sites. If there is more than one binding site, the binding sites may be identical to one another or may be different. For instance, a naturally-occurring immunoglobulin has two identical binding sites, a single-chain antibody or Fab fragment has one binding site, while a bispecific or bifunctional antibody has two different binding sites.

Methods of testing antibodies for the ability to bind to any functional portion of the CAR are known in the art and include any antibody-antigen binding assay, such as, for example, radioimmunoassay (RIA), ELISA, Western blot, immunoprecipitation, and competitive inhibition assays (see, *e.g.*, Janeway *et al.*, *infra*, U.S. Patent Application Publication No. 2002/0197266 Al, and U.S. Patent No. 7,338,929).

Also, a CAR, a T cell expressing a CAR, an antibody, or antigen binding portion thereof, can be modified to comprise a detectable label, such as, for instance, a radioisotope, a fluorophore (*e.g.*, fluorescein isothiocyanate (FITC), phycoerythrin (PE)), an enzyme (*e.g.*, alkaline phosphatase, horseradish peroxidase), and element particles (*e.g.*, gold particles).

### B. Conjugates

A CAR, a T cell expressing a CAR, or monoclonal antibodies, or antigen binding fragments thereof, specific for one or more of the antigens disclosed herein, can be conjugated to an agent, such as an effector molecule or detectable marker, using any number of means known to those of

skill in the art. Both covalent and noncovalent attachment means may be used. Conjugates include, but are not limited to, molecules in which there is a covalent linkage of an effector molecule or a detectable marker to an antibody or antigen binding fragment that specifically binds one or more of the antigens disclosed herein. One of skill in the art will appreciate that various effector molecules and detectable markers can be used, including (but not limited to) chemotherapeutic agents, anti-angiogenic agents, toxins, radioactive agents such as $^{125}I$, $^{32}P$, $^{14}C$, $^{3}H$ and $^{35}S$ and other labels, target moieties and ligands, etc.

The choice of a particular effector molecule or detectable marker depends on the particular target molecule or cell, and the desired biological effect. Thus, for example, the effector molecule can be a cytotoxin that is used to bring about the death of a particular target cell (such as a tumor cell).

The procedure for attaching an effector molecule or detectable marker to an antibody or antigen binding fragment varies according to the chemical structure of the effector. Polypeptides typically contain a variety of functional groups; such as carboxylic acid (COOH), free amine (-$NH_2$) or sulfhydryl (-SH) groups, which are available for reaction with a suitable functional group on an antibody to result in the binding of the effector molecule or detectable marker. Alternatively, the antibody or antigen binding fragment is derivatized to expose or attach additional reactive functional groups. The derivatization may involve attachment of any of a number of known linker molecules such as those available from Pierce Chemical Company, Rockford, IL. The linker can be any molecule used to join the antibody or antigen binding fragment to the effector molecule or detectable marker. The linker is capable of forming covalent bonds to both the antibody or antigen binding fragment and to the effector molecule or detectable marker. Suitable linkers are well known to those of skill in the art and include, but are not limited to, straight or branched-chain carbon linkers, heterocyclic carbon linkers, or peptide linkers. Where the antibody or antigen binding fragment and the effector molecule or detectable marker are polypeptides, the linkers may be joined to the constituent amino acids through their side groups (such as through a disulfide linkage to cysteine) or to the alpha carbon amino and carboxyl groups of the terminal amino acids.

In several embodiments, the linker can include a spacer element, which, when present, increases the size of the linker such that the distance between the effector molecule or the detectable marker and the antibody or antigen binding fragment is increased. Exemplary spacers are known to the person of ordinary skill, and include those listed in U.S. Pat. Nos. 7,964,5667, 7,498,298, 6,884,869, 6,323,315, 6,239,104, 6,034,065, 5,780,588, 5,665,860, 5,663,149, 5,635,483, 5,599,902, 5,554,725, 5,530,097, 5,521,284, 5,504,191, 5,410,024, 5,138,036, 5,076,973,

4,986,988, 4,978,744, 4,879,278, 4,816,444, and 4,486,414, as well as U.S. Pat. Pub. Nos. 20110212088 and 20110070248, each of which is incorporated by reference herein in its entirety.

In some embodiments, the linker is cleavable under intracellular conditions, such that cleavage of the linker releases the effector molecule or detectable marker from the antibody or antigen binding fragment in the intracellular environment. In yet other embodiments, the linker is not cleavable and the effector molecule or detectable marker is released, for example, by antibody degradation. In some embodiments, the linker is cleavable by a cleaving agent that is present in the intracellular environment (for example, within a lysosome or endosome or caveolea). The linker can be, for example, a peptide linker that is cleaved by an intracellular peptidase or protease enzyme, including, but not limited to, a lysosomal or endosomal protease. In some embodiments, the peptide linker is at least two amino acids long or at least three amino acids long. However, the linker can be 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or 15 amino acids long, such as 1-2, 1-3, 2-5, 3-10, 3-15, 1-5, 1-10, 1-15 amino acids long. Proteases can include cathepsins B and D and plasmin, all of which are known to hydrolyze dipeptide drug derivatives resulting in the release of active drug inside target cells (see, for example, Dubowchik and Walker, 1999, Pharm. Therapeutics 83:67-123). For example, a peptide linker that is cleavable by the thiol-dependent protease cathepsin-B, can be used (for example, a Phenylalanine -Leucine or a Glycine- Phenylalanine -Leucine-Glycine linker). Other examples of such linkers are described, for example, in U.S. Pat. No. 6,214,345, incorporated herein by reference. In a specific embodiment, the peptide linker cleavable by an intracellular protease is a Valine-Citruline linker or a Phenylalanine-Lysine linker (see, for example, U.S. Pat. No. 6,214,345, which describes the synthesis of doxorubicin with the Valine-Citruline linker).

In other embodiments, the cleavable linker is pH-sensitive, *i.e.*, sensitive to hydrolysis at certain pH values. Typically, the pH-sensitive linker is hydrolyzable under acidic conditions. For example, an acid-labile linker that is hydrolyzable in the lysosome (for example, a hydrazone, semicarbazone, thiosemicarbazone, cis-aconitic amide, orthoester, acetal, ketal, or the like) can be used. (See, for example, U.S. Pat. Nos. 5,122,368; 5,824,805; 5,622,929; Dubowchik and Walker, 1999, Pharm. Therapeutics 83:67-123; Neville *et al.*, 1989, Biol. Chem. 264:14653-14661.) Such linkers are relatively stable under neutral pH conditions, such as those in the blood, but are unstable at below pH 5.5 or 5.0, the approximate pH of the lysosome. In certain embodiments, the hydrolyzable linker is a thioether linker (such as, for example, a thioether attached to the therapeutic agent via an acylhydrazone bond (see, for example, U.S. Pat. No. 5,622,929).

In other embodiments, the linker is cleavable under reducing conditions (for example, a disulfide linker). A variety of disulfide linkers are known in the art, including, for example, those that can be formed using SATA (N-succinimidyl-S-acetylthioacetate), SPDP (N-succinimidyl-3-(2-

pyridyldithio)propionate), SPDB (N-succinimidyl-3-(2-pyridyldithio)butyrate) and SMPT (N-succinimidyl-oxycarbonyl-alpha-methyl-alpha-(2-pyridyl-dithio)toluene)-, SPDB and SMPT. (See, for example, Thorpe *et al.*, 1987, Cancer Res. 47:5924-5931; Wawrzynczak *et al.*, In Immunoconjugates: Antibody Conjugates in Radioimagery and Therapy of Cancer (C. W. Vogel ed., Oxford U. Press, 1987); Phillips *et al.*, Cancer Res. 68:92809290, 2008). See also U.S. Pat. No. 4,880,935.)

In yet other specific embodiments, the linker is a malonate linker (Johnson *et al.*, 1995, Anticancer Res. 15:1387-93), a maleimidobenzoyl linker (Lau *et al.*, 1995, Bioorg-Med-Chem. 3(10):1299-1304), or a 3'-N-amide analog (Lau *et al.*, 1995, Bioorg-Med-Chem. 3(10):1305-12).

In yet other embodiments, the linker is not cleavable and the effector molecule or detectable marker is released by antibody degradation. (See U.S. Publication No. 2005/0238649 incorporated by reference herein in its entirety).

In several embodiments, the linker is resistant to cleavage in an extracellular environment. For example, no more than about 20%, no more than about 15%, no more than about 10%, no more than about 5%, no more than about 3%, or no more than about 1% of the linkers, in a sample of conjugate, are cleaved when the conjugate is present in an extracellular environment (for example, in plasma). Whether or not a linker is resistant to cleavage in an extracellular environment can be determined, for example, by incubating the conjugate containing the linker of interest with plasma for a predetermined time period (for example, 2, 4, 8, 16, or 24 hours) and then quantitating the amount of free effector molecule or detectable marker present in the plasma. A variety of exemplary linkers that can be used in conjugates are described in WO 2004-010957, U.S. Publication No. 2006/0074008, U.S. Publication No. 20050238649, and U.S. Publication No. 2006/0024317, each of which is incorporated by reference herein in its entirety.

In several embodiments, conjugates of a CAR, a T cell expressing a CAR, an antibody, or antigen binding portion thereof, and one or more small molecule toxins, such as a calicheamicin, maytansinoids, dolastatins, auristatins, a trichothecene, and CC1065, and the derivatives of these toxins that have toxin activity, are provided.

Maytansine compounds suitable for use as maytansinoid toxin moieties are well known in the art, and can be isolated from natural sources according to known methods, produced using genetic engineering techniques (see Yu *et al.* (2002) PNAS 99:7968-7973), or maytansinol and maytansinol analogues prepared synthetically according to known methods. Maytansinoids are mitototic inhibitors which act by inhibiting tubulin polymerization. Maytansine was first isolated from the east African shrub Maytenus serrata (U.S. Pat. No. 3,896,111). Subsequently, it was discovered that certain microbes also produce maytansinoids, such as maytansinol and C-3

maytansinol esters (U.S. Pat. No. 4,151,042). Synthetic maytansinol and derivatives and analogues thereof are disclosed, for example, in U.S. Pat. Nos. 4,137,230; 4,248,870; 4,256,746; 4,260,608; 4,265,814; 4,294,757; 4,307,016; 4,308,268; 4,308,269; 4,309,428; 4,313,946; 4,315,929; 4,317,821; 4,322,348; 4,331,598; 4,361,650; 4,364,866; 4,424,219; 4,450,254; 4,362,663; and 4,371,533, each of which is incorporated herein by reference. Conjugates containing maytansinoids, methods of making same, and their therapeutic use are disclosed, for example, in U.S. Pat. Nos. 5,208,020; 5,416,064; 6,441,163 and European Patent EP 0 425 235 B1, the disclosures of which are hereby expressly incorporated by reference.

Additional toxins can be employed with a CAR, a T cell expressing a CAR, an antibody, or antigen binding portion thereof. Exemplary toxins include Pseudomonas exotoxin (PE), ricin, abrin, diphtheria toxin and subunits thereof, ribotoxin, ribonuclease, saporin, and calicheamicin, as well as botulinum toxins A through F. These toxins are well known in the art and many are readily available from commercial sources (for example, Sigma Chemical Company, St. Louis, MO). Contemplated toxins also include variants of the toxins (see, for example, see, U.S. Patent Nos. 5,079,163 and 4,689,401).

Saporin is a toxin derived from Saponaria officinalis that disrupts protein synthesis by inactivating the 60S portion of the ribosomal complex (Stirpe *et al.*, Bio/Technology, 10:405-412, 1992). However, the toxin has no mechanism for specific entry into cells, and therefore requires conjugation to an antibody or antigen binding fragment that recognizes a cell-surface protein that is internalized in order to be efficiently taken up by cells.

Diphtheria toxin is isolated from Corynebacterium diphtheriae. Typically, diphtheria toxin for use in immunotoxins is mutated to reduce or to eliminate non-specific toxicity. A mutant known as CRM107, which has full enzymatic activity but markedly reduced non-specific toxicity, has been known since the 1970's (Laird and Groman, J. Virol. 19:220, 1976), and has been used in human clinical trials. See, U.S. Patent No. 5,792,458 and U.S. Patent No. 5,208,021.

Ricin is the lectin RCA60 from Ricinus communis (Castor bean). For examples of ricin, see, U.S. Patent No. 5,079,163 and U.S. Patent No. 4,689,401. Ricinus communis agglutinin (RCA) occurs in two forms designated $RCA_{60}$ and $RCA_{120}$ according to their molecular weights of approximately 65 and 120 kD, respectively (Nicholson & Blaustein, J. Biochim. Biophys. Acta 266:543, 1972). The A chain is responsible for inactivating protein synthesis and killing cells. The B chain binds ricin to cell-surface galactose residues and facilitates transport of the A chain into the cytosol (Olsnes *et al.*, Nature 249:627-631, 1974 and U.S. Patent No. 3,060,165).

Ribonucleases have also been conjugated to targeting molecules for use as immunotoxins (see Suzuki *et al.*, Nat. Biotech. 17:265-70, 1999). Exemplary ribotoxins such as α-sarcin and

restrictocin are discussed in, for example Rathore *et al.*, Gene 190:31-5, 1997; and Goyal and Batra, Biochem. 345 Pt 2:247-54, 2000. Calicheamicins were first isolated from Micromonospora echinospora and are members of the enediyne antitumor antibiotic family that cause double strand breaks in DNA that lead to apoptosis (see, for example Lee *et al.*, J. Antibiot. 42:1070-87,1989). The drug is the toxic moiety of an immunotoxin in clinical trials (see, for example, Gillespie *et al.*, Ann. Oncol. 11:735-41, 2000).

Abrin includes toxic lectins from Abrus precatorius. The toxic principles, abrin a, b, c, and d, have a molecular weight of from about 63 and 67 kD and are composed of two disulfide-linked polypeptide chains A and B. The A chain inhibits protein synthesis; the B chain (abrin-b) binds to D-galactose residues (see, Funatsu *et al.*, Agr. Biol. Chem. 52:1095, 1988; and Olsnes, Methods Enzymol. 50:330-335, 1978).

A CAR, a T cell expressing a CAR, monoclonal antibodies, antigen binding fragments thereof, specific for one or more of the antigens disclosed herein, can also be conjugated with a detectable marker; for example, a detectable marker capable of detection by ELISA, spectrophotometry, flow cytometry, microscopy or diagnostic imaging techniques (such as computed tomography (CT), computed axial tomography (CAT) scans, magnetic resonance imaging (MRI), nuclear magnetic resonance imaging NMRI), magnetic resonance tomography (MTR), ultrasound, fiberoptic examination, and laparoscopic examination). Specific, non-limiting examples of detectable markers include fluorophores, chemiluminescent agents, enzymatic linkages, radioactive isotopes and heavy metals or compounds (for example super paramagnetic iron oxide nanocrystals for detection by MRI). For example, useful detectable markers include fluorescent compounds, including fluorescein, fluorescein isothiocyanate, rhodamine, 5-dimethylamine-1-napthalenesulfonyl chloride, phycoerythrin, lanthanide phosphors and the like. Bioluminescent markers are also of use, such as luciferase, Green fluorescent protein (GFP), Yellow fluorescent protein (YFP). A CAR, a T cell expressing a CAR, an antibody, or antigen binding portion thereof, can also be conjugated with enzymes that are useful for detection, such as horseradish peroxidase, β-galactosidase, luciferase, alkaline phosphatase, glucose oxidase and the like. When a CAR, a T cell expressing a CAR, an antibody, or antigen binding portion thereof, is conjugated with a detectable enzyme, it can be detected by adding additional reagents that the enzyme uses to produce a reaction product that can be discerned. For example, when the agent horseradish peroxidase is present the addition of hydrogen peroxide and diaminobenzidine leads to a colored reaction product, which is visually detectable. A CAR, a T cell expressing a CAR, an antibody, or antigen binding portion thereof, may also be conjugated with biotin, and detected

through indirect measurement of avidin or streptavidin binding. It should be noted that the avidin itself can be conjugated with an enzyme or a fluorescent label.

A CAR, a T cell expressing a CAR, an antibody, or antigen binding portion thereof, may be conjugated with a paramagnetic agent, such as gadolinium. Paramagnetic agents such as superparamagnetic iron oxide are also of use as labels. Antibodies can also be conjugated with lanthanides (such as europium and dysprosium), and manganese. An antibody or antigen binding fragment may also be labeled with a predetermined polypeptide epitopes recognized by a secondary reporter (such as leucine zipper pair sequences, binding sites for secondary antibodies, metal binding domains, epitope tags).

A CAR, a T cell expressing a CAR, an antibody, or antigen binding portion thereof, can also be conjugated with a radiolabeled amino acid. The radiolabel may be used for both diagnostic and therapeutic purposes. For instance, the radiolabel may be used to detect one or more of the antigens disclosed herein and antigen expressing cells by x-ray, emission spectra, or other diagnostic techniques. Further, the radiolabel may be used therapeutically as a toxin for treatment of tumors in a subject, for example for treatment of a neuroblastoma. Examples of labels for polypeptides include, but are not limited to, the following radioisotopes or radionucleotides: $^{3}H$, $^{14}C$, $^{15}N$, $^{35}S$, $^{90}Y$, $^{99}Tc$, $^{111}In$, $^{125}I$, $^{131}I$.

Means of detecting such detectable markers are well known to those of skill in the art. Thus, for example, radiolabels may be detected using photographic film or scintillation counters, fluorescent markers may be detected using a photodetector to detect emitted illumination. Enzymatic labels are typically detected by providing the enzyme with a substrate and detecting the reaction product produced by the action of the enzyme on the substrate, and colorimetric labels are detected by simply visualizing the colored label.

### C. Nucleotides, Expression, Vectors, and Host Cells

Further provided by an embodiment of the invention is a nucleic acid comprising a nucleotide sequence encoding any of the CARs, an antibody, or antigen binding portion thereof, described herein (including functional portions and functional variants thereof). The nucleic acids of the invention may comprise a nucleotide sequence encoding any of the leader sequences, antigen binding domains, transmembrane domains, and/or intracellular T cell signaling domains described herein.

In some embodiments, the nucleotide sequence may be codon-modified. Without being bound to a particular theory, it is believed that codon optimization of the nucleotide sequence

increases the translation efficiency of the mRNA transcripts. Codon optimization of the nucleotide sequence may involve substituting a native codon for another codon that encodes the same amino acid, but can be translated by tRNA that is more readily available within a cell, thus increasing translation efficiency. Optimization of the nucleotide sequence may also reduce secondary mRNA structures that would interfere with translation, thus increasing translation efficiency.

In an embodiment of the invention, the nucleic acid may comprise a codon-modified nucleotide sequence that encodes the antigen binding domain of the inventive CAR. In another embodiment of the invention, the nucleic acid may comprise a codon-modified nucleotide sequence that encodes any of the CARs described herein (including functional portions and functional variants thereof).

"Nucleic acid" as used herein includes "polynucleotide," "oligonucleotide," and "nucleic acid molecule," and generally means a polymer of DNA or RNA, which can be single-stranded or double-stranded, synthesized or obtained (*e.g.*, isolated and/or purified) from natural sources, which can contain natural, non-natural or altered nucleotides, and which can contain a natural, non-natural or altered internucleotide linkage, such as a phosphoroamidate linkage or a phosphorothioate linkage, instead of the phosphodiester found between the nucleotides of an unmodified oligonucleotide. In some embodiments, the nucleic acid does not comprise any insertions, deletions, inversions, and/or substitutions. However, it may be suitable in some instances, as discussed herein, for the nucleic acid to comprise one or more insertions, deletions, inversions, and/or substitutions.

A recombinant nucleic acid may be one that has a sequence that is not naturally occurring or has a sequence that is made by an artificial combination of two otherwise separated segments of sequence. This artificial combination is often accomplished by chemical synthesis or, more commonly, by the artificial manipulation of isolated segments of nucleic acids, *e.g.*, by genetic engineering techniques, such as those described in Sambrook *et al.*, *supra.* The nucleic acids can be constructed based on chemical synthesis and/or enzymatic ligation reactions using procedures known in the art. See, for example, Sambrook *et al.*, *supra*, and Ausubel *et al.*, *supra.* For example, a nucleic acid can be chemically synthesized using naturally occurring nucleotides or variously modified nucleotides designed to increase the biological stability of the molecules or to increase the physical stability of the duplex formed upon hybridization (*e.g.*, phosphorothioate derivatives and acridine substituted nucleotides). Examples of modified nucleotides that can be used to generate the nucleic acids include, but are not limited to, 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xanthine, 4-acetylcytosine, 5-(carboxyhydroxymethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1 -methylinosine, 2,2-

dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-substituted adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid methylester, 3- (3-amino-3-N-2-carboxypropyl) uracil, and 2,6-diaminopurine. Alternatively, one or more of the nucleic acids of the invention can be purchased from companies, such as Integrated DNA Technologies (Coralville, IA, USA).

The nucleic acid can comprise any isolated or purified nucleotide sequence which encodes any of the CARs or functional portions or functional variants thereof. Alternatively, the nucleotide sequence can comprise a nucleotide sequence which is degenerate to any of the sequences or a combination of degenerate sequences.

An embodiment also provides an isolated or purified nucleic acid comprising a nucleotide sequence which is complementary to the nucleotide sequence of any of the nucleic acids described herein or a nucleotide sequence which hybridizes under stringent conditions to the nucleotide sequence of any of the nucleic acids described herein.

The nucleotide sequence which hybridizes under stringent conditions may hybridize under high stringency conditions. By "high stringency conditions" is meant that the nucleotide sequence specifically hybridizes to a target sequence (the nucleotide sequence of any of the nucleic acids described herein) in an amount that is detectably stronger than non-specific hybridization. High stringency conditions include conditions which would distinguish a polynucleotide with an exact complementary sequence, or one containing only a few scattered mismatches from a random sequence that happened to have a few small regions (*e.g.*, 3-10 bases) that matched the nucleotide sequence. Such small regions of complementarity are more easily melted than a full-length complement of 14-17 or more bases, and high stringency hybridization makes them easily distinguishable. Relatively high stringency conditions would include, for example, low salt and/or high temperature conditions, such as provided by about 0.02-0.1 M NaCl or the equivalent, at temperatures of about 50-70 °C. Such high stringency conditions tolerate little, if any, mismatch between the nucleotide sequence and the template or target strand, and are particularly suitable for detecting expression of any of the inventive CARs. It is generally appreciated that conditions can be rendered more stringent by the addition of increasing amounts of formamide.

Also provided is a nucleic acid comprising a nucleotide sequence that is at least about 70% or more, *e.g.*, about 80%, about 90%, about 91 %, about 92%, about 93%, about 94%, about 95%,

about 96%, about 97%, about 98%, or about 99% identical to any of the nucleic acids described herein.

In an embodiment, the nucleic acids can be incorporated into a recombinant expression vector. In this regard, an embodiment provides recombinant expression vectors comprising any of the nucleic acids. For purposes herein, the term "recombinant expression vector" means a genetically-modified oligonucleotide or polynucleotide construct that permits the expression of an mRNA, protein, polypeptide, or peptide by a host cell, when the construct comprises a nucleotide sequence encoding the mRNA, protein, polypeptide, or peptide, and the vector is contacted with the cell under conditions sufficient to have the mRNA, protein, polypeptide, or peptide expressed within the cell. The vectors are not naturally-occurring as a whole.

However, parts of the vectors can be naturally-occurring. The recombinant expression vectors can comprise any type of nucleotides, including, but not limited to DNA and RNA, which can be single-stranded or double- stranded, synthesized or obtained in part from natural sources, and which can contain natural, non-natural or altered nucleotides. The recombinant expression vectors can comprise naturally-occurring or non-naturally-occurring internucleotide linkages, or both types of linkages. Preferably, the non-naturally occurring or altered nucleotides or internucleotide linkages do not hinder the transcription or replication of the vector.

In an embodiment, the recombinant expression vector can be any suitable recombinant expression vector, and can be used to transform or transfect any suitable host cell. Suitable vectors include those designed for propagation and expansion or for expression or both, such as plasmids and viruses. The vector can be selected from the group consisting of the pUC series (Fermentas Life Sciences, Glen Burnie, MD), the pBluescript series (Stratagene, LaJolla, CA), the pET series (Novagen, Madison, WI), the pGEX series (Pharmacia Biotech, Uppsala, Sweden), and the pEX series (Clontech, Palo Alto, CA).

Bacteriophage vectors, such as λΰTIO, λϋTI 1, λZapII (Stratagene), EMBL4, and λNMI 149, also can be used. Examples of plant expression vectors include pBIOl, pBI101.2, pBHOl .3, pBI121 and pBIN19 (Clontech). Examples of animal expression vectors include pEUK-Cl, pMAM, and pMAMneo (Clontech). The recombinant expression vector may be a viral vector, *e.g.*, a retroviral vector or a lentiviral vector. A lentiviral vector is a vector derived from at least a portion of a lentivirus genome, including especially a self-inactivating lentiviral vector as provided in Milone *et al.*, Mol. Ther. 17(8): 1453-1464 (2009). Other examples of lentivirus vectors that may be used in the clinic, include, for example, and not by way of limitation, the LENTIVECTOR.RTM. gene delivery technology from Oxford BioMedica plc, the

LENTIMAX.TM. vector system from Lentigen and the like. Nonclinical types of lentiviral vectors are also available and would be known to one skilled in the art.

A number of transfection techniques are generally known in the art (see, *e.g.*, Graham *et al.*, Virology, 52: 456-467 (1973); Sambrook *et al.*, *supra*; Davis *et al.*, Basic Methods in Molecular Biology, Elsevier (1986); and Chu *et al*, Gene, 13: 97 (1981).

Transfection methods include calcium phosphate co-precipitation (see, *e.g.*, Graham *et al.*, *supra*), direct micro injection into cultured cells (see, *e.g.*, Capecchi, Cell, 22: 479-488 (1980)), electroporation (see, *e.g.*, Shigekawa *et al.*, BioTechniques, 6: 742-751 (1988)), liposome mediated gene transfer (see, *e.g.*, Mannino *et al.*, BioTechniques, 6: 682-690 (1988)), lipid mediated transduction (see, *e.g.*, Feigner *et al.*, Proc. Natl. Acad. Sci. USA, 84: 7413-7417 (1987)), and nucleic acid delivery using high velocity microprojectiles (see, *e.g.*, Klein *et al*, Nature, 327: 70-73 (1987)).

In an embodiment, the recombinant expression vectors can be prepared using standard recombinant DNA techniques described in, for example, Sambrook *et al.*, *supra*, and Ausubel *et al.*, *supra*. Constructs of expression vectors, which are circular or linear, can be prepared to contain a replication system functional in a prokaryotic or eukaryotic host cell. Replication systems can be derived, *e.g.*, from ColEl, 2 μ plasmid, λ, SV40, bovine papilloma virus, and the like.

The recombinant expression vector may comprise regulatory sequences, such as transcription and translation initiation and termination codons, which are specific to the type of host cell (*e.g.*, bacterium, fungus, plant, or animal) into which the vector is to be introduced, as appropriate, and taking into consideration whether the vector is DNA- or RNA-based. The recombinant expression vector may comprise restriction sites to facilitate cloning.

The recombinant expression vector can include one or more marker genes, which allow for selection of transformed or transfected host cells. Marker genes include biocide resistance, *e.g.*, resistance to antibiotics, heavy metals, etc., complementation in an auxotrophic host to provide prototrophy, and the like. Suitable marker genes for the inventive expression vectors include, for instance, neomycin/G418 resistance genes, hygromycin resistance genes, histidinol resistance genes, tetracycline resistance genes, and ampicillin resistance genes.

The recombinant expression vector can comprise a native or nonnative promoter operably linked to the nucleotide sequence encoding the CAR (including functional portions and functional variants thereof), or to the nucleotide sequence which is complementary to or which hybridizes to the nucleotide sequence encoding the CAR. The selection of promoters, *e.g.*, strong, weak, inducible, tissue-specific and developmental-specific, is within the ordinary skill of the artisan. Similarly, the combining of a nucleotide sequence with a promoter is also within the skill of the

artisan. The promoter can be a non-viral promoter or a viral promoter, *e.g.*, a cytomegalovirus (CMV) promoter, an SV40 promoter, an RSV promoter, or a promoter found in the long-terminal repeat of the murine stem cell virus.

The recombinant expression vectors can be designed for either transient expression, for stable expression, or for both. Also, the recombinant expression vectors can be made for constitutive expression or for inducible expression.

Further, the recombinant expression vectors can be made to include a suicide gene. As used herein, the term "suicide gene" refers to a gene that causes the cell expressing the suicide gene to die. The suicide gene can be a gene that confers sensitivity to an agent, *e.g.*, a drug, upon the cell in which the gene is expressed, and causes the cell to die when the cell is contacted with or exposed to the agent. Suicide genes are known in the art (see, for example, Suicide Gene Therapy: Methods and Reviews, Springer, Caroline J. (Cancer Research UK Centre for Cancer Therapeutics at the Institute of Cancer Research, Sutton, Surrey, UK), Humana Press, 2004) and include, for example, the Herpes Simplex Virus (HSV) thymidine kinase (TK) gene, cytosine daminase, purine nucleoside phosphorylase, and nitroreductase.

An embodiment further provides a host cell comprising any of the recombinant expression vectors described herein. As used herein, the term "host cell" refers to any type of cell that can contain the inventive recombinant expression vector. The host cell can be a eukaryotic cell, *e.g.*, plant, animal, fungi, or algae, or can be a prokaryotic cell, *e.g.*, bacteria or protozoa. The host cell can be a cultured cell or a primary cell, *i.e.*, isolated directly from an organism, *e.g.*, a human. The host cell can be an adherent cell or a suspended cell, *i.e.*, a cell that grows in suspension. Suitable host cells are known in the art and include, for instance, DH5a E. coli cells, Chinese hamster ovarian cells, monkey VERO cells, COS cells, HEK293 cells, and the like. For purposes of amplifying or replicating the recombinant expression vector, the host cell may be a prokaryotic cell, *e.g.*, a DH5a cell. For purposes of producing a recombinant CAR, the host cell may be a mammalian cell. The host cell may be a human cell. While the host cell can be of any cell type, can originate from any type of tissue, and can be of any developmental stage, the host cell may be a peripheral blood lymphocyte (PBL) or a peripheral blood mononuclear cell (PBMC). The host cell may be a T cell.

For purposes herein, the T cell can be any T cell, such as a cultured T cell, *e.g.*, a primary T cell, or a T cell from a cultured T cell line, *e.g.*, Jurkat, SupTl, etc., or a T cell obtained from a mammal. If obtained from a mammal, the T cell can be obtained from numerous sources, including but not limited to blood, bone marrow, lymph node, the thymus, or other tissues or fluids. T cells can also be enriched for or purified. The T cell may be a human T cell. The T cell may be a T cell

isolated from a human. The T cell can be any type of T cell and can be of any developmental stage, including but not limited to, $CD4^+/CD8^+$ double positive T cells, $CD4^+$ helper T cells, *e.g.*, Th1 and Th2 cells, $CD8^+$ T cells (*e.g.*, cytotoxic T cells), tumor infiltrating cells, memory T cells, memory stem cells, *i.e.* Tscm, naive T cells, and the like. The T cell may be a $CD8^+$ T cell or a $CD4^+$ T cell.

In an embodiment, the CARs as described herein can be used in suitable non-T cells. Such cells are those with an immune-effector function, such as, for example, NK cells, and T-like cells generated from pluripotent stem cells.

Also provided by an embodiment is a population of cells comprising at least one host cell described herein. The population of cells can be a heterogeneous population comprising the host cell comprising any of the recombinant expression vectors described, in addition to at least one other cell, *e.g.*, a host cell (*e.g.*, a T cell), which does not comprise any of the recombinant expression vectors, or a cell other than a T cell, *e.g.*, a B cell, a macrophage, a neutrophil, an erythrocyte, a hepatocyte, an endothelial cell, an epithelial cell, a muscle cell, a brain cell, etc. Alternatively, the population of cells can be a substantially homogeneous population, in which the population comprises mainly host cells (*e.g.*, consisting essentially of) comprising the recombinant expression vector. The population also can be a clonal population of cells, in which all cells of the population are clones of a single host cell comprising a recombinant expression vector, such that all cells of the population comprise the recombinant expression vector. In one embodiment of the invention, the population of cells is a clonal population comprising host cells comprising a recombinant expression vector as described herein.

CARs (including functional portions and variants thereof), nucleic acids, recombinant expression vectors, host cells (including populations thereof), and antibodies (including antigen binding portions thereof), can be isolated and/or purified. For example, a purified (or isolated) host cell preparation is one in which the host cell is more pure than cells in their natural environment within the body. Such host cells may be produced, for example, by standard purification techniques. In some embodiments, a preparation of a host cell is purified such that the host cell represents at least about 50%, for example at least about 70%, of the total cell content of the preparation. For example, the purity can be at least about 50%, can be greater than about 60%, about 70% or about 80%, or can be about 100%.

### D. Methods of Treatment

It is contemplated that the CARs disclosed herein can be used in methods of treating or preventing a disease in a mammal. In this regard, an embodiment provides a method of treating or

preventing cancer in a mammal, comprising administering to the mammal the CARs, the nucleic acids, the recombinant expression vectors, the host cells, the population of cells, the antibodies and/or the antigen binding portions thereof, and/or the pharmaceutical compositions in an amount effective to treat or prevent cancer in the mammal.

An embodiment further comprises lymphodepleting the mammal prior to administering the CARs disclosed herein. Examples of lymphodepletion include, but may not be limited to, nonmyeloablative lymphodepleting chemotherapy, myeloablative lymphodepleting chemotherapy, total body irradiation, etc.

For purposes of the methods, wherein host cells or populations of cells are administered, the cells can be cells that are allogeneic or autologous to the mammal. Preferably, the cells are autologous to the mammal. As used herein, allogeneic means any material derived from a different animal of the same species as the individual to whom the material is introduced. Two or more individuals are said to be allogeneic to one another when the genes at one or more loci are not identical. In some aspects, allogeneic material from individuals of the same species may be sufficiently unlike genetically to interact antigenically. As used herein, "autologous" means any material derived from the same individual to whom it is later to be re-introduced into the individual.

The mammal referred to herein can be any mammal. As used herein, the term "mammal" refers to any mammal, including, but not limited to, mammals of the order Rodentia, such as mice and hamsters, and mammals of the order Logomorpha, such as rabbits. The mammals may be from the order Carnivora, including Felines (cats) and Canines (dogs). The mammals may be from the order Artiodactyla, including Bovines (cows) and Swines (pigs) or of the order Perssodactyla, including Equines (horses). The mammals may be of the order Primates, Ceboids, or Simoids (monkeys) or of the order Anthropoids (humans and apes). Preferably, the mammal is a human.

With respect to the methods, the cancer can be any cancer, including any of acute lymphocytic cancer, acute myeloid leukemia, alveolar rhabdomyosarcoma, bladder cancer (*e.g.*, bladder carcinoma), bone cancer, brain cancer (*e.g.*, meduloblastoma), breast cancer, cancer of the anus, anal canal, or anorectum, cancer of the eye, cancer of the intrahepatic bile duct, cancer of the joints, cancer of the neck, gallbladder, or pleura, cancer of the nose, nasal cavity, or middle ear, cancer of the oral cavity, cancer of the vulva, chronic lymphocytic leukemia, chronic myeloid cancer, colon cancer, esophageal cancer, cervical cancer, fibrosarcoma, gastrointestinal carcinoid tumor, head and neck cancer (*e.g.*, head and neck squamous cell carcinoma), Hodgkin lymphoma, hypopharynx cancer, kidney cancer, larynx cancer, leukemia, liquid tumors, liver cancer, lung cancer (*e.g.*, non-small cell lung carcinoma and lung adenocarcinoma), lymphoma, mesothelioma, mastocytoma, melanoma, multiple myeloma, nasopharynx cancer, non-Hodgkin lymphoma, B-

chronic lymphocytic leukemia, hairy cell leukemia, acute lymphocytic leukemia (ALL), and Burkitt's lymphoma, ovarian cancer, pancreatic cancer, peritoneum, omentum, and mesentery cancer, pharynx cancer, prostate cancer, rectal cancer, renal cancer, skin cancer, small intestine cancer, soft tissue cancer, solid tumors, synovial sarcoma, gastric cancer, testicular cancer, thyroid cancer, and ureter cancer.

The terms "treat," and "prevent" as well as words stemming therefrom, as used herein, do not necessarily imply 100% or complete treatment or prevention. Rather, there are varying degrees of treatment or prevention of which one of ordinary skill in the art recognizes as having a potential benefit or therapeutic effect. In this respect, the methods can provide any amount or any level of treatment or prevention of cancer in a mammal.

Furthermore, the treatment or prevention provided by the method can include treatment or prevention of one or more conditions or symptoms of the disease, *e.g.*, cancer, being treated or prevented. Also, for purposes herein, "prevention" can encompass delaying the onset of the disease, or a symptom or condition thereof.

Another embodiment provides a method of detecting the presence of cancer in a mammal, comprising: (a) contacting a sample comprising one or more cells from the mammal with the CARs, the nucleic acids, the recombinant expression vectors, the host cells, the population of cells, the antibodies, and/or the antigen binding portions thereof, or the pharmaceutical compositions, thereby forming a complex, (b) and detecting the complex, wherein detection of the complex is indicative of the presence of cancer in the mammal.

The sample may be obtained by any suitable method, *e.g.*, biopsy or necropsy. A biopsy is the removal of tissue and/or cells from an individual. Such removal may be to collect tissue and/or cells from the individual in order to perform experimentation on the removed tissue and/or cells. This experimentation may include experiments to determine if the individual has and/or is suffering from a certain condition or disease-state. The condition or disease may be, *e.g.*, cancer.

With respect to an embodiment of the method of detecting the presence of a proliferative disorder, *e.g.*, cancer, in a mammal, the sample comprising cells of the mammal can be a sample comprising whole cells, lysates thereof, or a fraction of the whole cell lysates, *e.g.*, a nuclear or cytoplasmic fraction, a whole protein fraction, or a nucleic acid fraction. If the sample comprises whole cells, the cells can be any cells of the mammal, *e.g.*, the cells of any organ or tissue, including blood cells or endothelial cells.

The contacting can take place *in vitro* or *in vivo* with respect to the mammal. Preferably, the contacting is *in vitro*.

Also, detection of the complex can occur through any number of ways known in the art. For instance, the CARs disclosed herein, polypeptides, proteins, nucleic acids, recombinant expression vectors, host cells, populations of cells, or antibodies, or antigen binding portions thereof, described herein, can be labeled with a detectable label such as, for instance, a radioisotope, a fluorophore (*e.g.*, fluorescein isothiocyanate (FITC), phycoerythrin (PE)), an enzyme (*e.g.*, alkaline phosphatase, horseradish peroxidase), and element particles (*e.g.*, gold particles) as disclosed *supra*.

Methods of testing a CAR for the ability to recognize target cells and for antigen specificity are known in the art. For instance, Clay *et al.*, J. Immunol, 163: 507-513 (1999), teaches methods of measuring the release of cytokines (*e.g.*, interferon-γ, granulocyte/monocyte colony stimulating factor (GM-CSF), tumor necrosis factor a (TNF-a) or interleukin 2 (IL-2)). In addition, CAR function can be evaluated by measurement of cellular cytotoxicity, as described in Zhao *et al*, J. Immunol , 174: 4415-4423 (2005).

Another embodiment provides for the use of the CARs, nucleic acids, recombinant expression vectors, host cells, populations of cells, antibodies, or antigen binding portions thereof, and/or pharmaceutical compositions of the invention, for the treatment or prevention of a proliferative disorder, *e.g.*, cancer, in a mammal. The cancer may be any of the cancers described herein.

Any method of administration can be used for the disclosed therapeutic agents, including local and systemic administration. For example topical, oral, intravascular such as intravenous, intramuscular, intraperitoneal, intranasal, intradermal, intrathecal and subcutaneous administration can be used. The particular mode of administration and the dosage regimen will be selected by the attending clinician, taking into account the particulars of the case (for example the subject, the disease, the disease state involved, and whether the treatment is prophylactic). In cases in which more than one agent or composition is being administered, one or more routes of administration may be used; for example, a chemotherapeutic agent may be administered orally and an antibody or antigen binding fragment or conjugate or composition may be administered intravenously. Methods of administration include injection for which the CAR, CAR T Cell, conjugates, antibodies, antigen binding fragments, or compositions are provided in a nontoxic pharmaceutically acceptable carrier such as water, saline, Ringer's solution, dextrose solution, 5% human serum albumin, fixed oils, ethyl oleate, or liposomes. In some embodiments, local administration of the disclosed compounds can be used, for instance by applying the antibody or antigen binding fragment to a region of tissue from which a tumor has been removed, or a region suspected of being prone to tumor development. In some embodiments, sustained intra-tumoral (or near-tumoral) release of the pharmaceutical preparation that includes a therapeutically effective amount of the

antibody or antigen binding fragment may be beneficial. In other examples, the conjugate is applied as an eye drop topically to the cornea, or intravitreally into the eye.

The disclosed therapeutic agents can be formulated in unit dosage form suitable for individual administration of precise dosages. In addition, the disclosed therapeutic agents may be administered in a single dose or in a multiple dose schedule. A multiple dose schedule is one in which a primary course of treatment may be with more than one separate dose, for instance 1-10 doses, followed by other doses given at subsequent time intervals as needed to maintain or reinforce the action of the compositions. Treatment can involve daily or multi-daily doses of compound(s) over a period of a few days to months, or even years. Thus, the dosage regime will also, at least in part, be determined based on the particular needs of the subject to be treated and will be dependent upon the judgment of the administering practitioner.

Typical dosages of the antibodies or conjugates can range from about 0.01 to about 30 mg/kg, such as from about 0.1 to about 10 mg/kg.

In particular examples, the subject is administered a therapeutic composition that includes one or more of the conjugates, antibodies, compositions, CARs, CAR T cells or additional agents, on a multiple daily dosing schedule, such as at least two consecutive days, 10 consecutive days, and so forth, for example for a period of weeks, months, or years. In one example, the subject is administered the conjugates, antibodies, compositions or additional agents for a period of at least 30 days, such as at least 2 months, at least 4 months, at least 6 months, at least 12 months, at least 24 months, or at least 36 months.

In some embodiments, the disclosed methods include providing surgery, radiation therapy, and/or chemotherapeutics to the subject in combination with a disclosed antibody, antigen binding fragment, conjugate, CAR or T cell expressing a CAR (for example, sequentially, substantially simultaneously, or simultaneously). Methods and therapeutic dosages of such agents and treatments are known to those skilled in the art, and can be determined by a skilled clinician. Preparation and dosing schedules for the additional agent may be used according to manufacturer's instructions or as determined empirically by the skilled practitioner. Preparation and dosing schedules for such chemotherapy are also described in Chemotherapy Service, (1992) Ed., M. C. Perry, Williams & Wilkins, Baltimore, MD.

In some embodiments, the combination therapy can include administration of a therapeutically effective amount of an additional cancer inhibitor to a subject. Non-limiting examples of additional therapeutic agents that can be used with the combination therapy include microtubule binding agents, DNA intercalators or cross-linkers, DNA synthesis inhibitors, DNA and RNA transcription inhibitors, antibodies, enzymes, enzyme inhibitors, gene regulators, and

angiogenesis inhibitors. These agents (which are administered at a therapeutically effective amount) and treatments can be used alone or in combination. For example, any suitable anti-cancer or anti-angiogenic agent can be administered in combination with the CARS, CAR- T cells, antibodies, antigen binding fragment, or conjugates disclosed herein. Methods and therapeutic dosages of such agents are known to those skilled in the art, and can be determined by a skilled clinician.

Additional chemotherapeutic agents include, but are not limited to alkylating agents, such as nitrogen mustards (for example, chlorambucil, chlormethine, cyclophosphamide, ifosfamide, and melphalan), nitrosoureas (for example, carmustine, fotemustine, lomustine, and streptozocin), platinum compounds (for example, carboplatin, cisplatin, oxaliplatin, and BBR3464), busulfan, dacarbazine, mechlorethamine, procarbazine, temozolomide, thiotepa, and uramustine; antimetabolites, such as folic acid (for example, methotrexate, pemetrexed, and raltitrexed), purine (for example, cladribine, clofarabine, fludarabine, mercaptopurine, and tioguanine), pyrimidine (for example, capecitabine), cytarabine, fluorouracil, and gemcitabine; plant alkaloids, such as podophyllum (for example, etoposide, and teniposide), taxane (for example, docetaxel and paclitaxel), vinca (for example, vinblastine, vincristine, vindesine, and vinorelbine); cytotoxic/antitumor antibiotics, such as anthracycline family members (for example, daunorubicin, doxorubicin, epirubicin, idarubicin, mitoxantrone, and valrubicin), bleomycin, rifampicin, hydroxyurea, and mitomycin; topoisomerase inhibitors, such as topotecan and irinotecan; monoclonal antibodies, such as alemtuzumab, bevacizumab, cetuximab, gemtuzumab, rituximab, panitumumab, pertuzumab, and trastuzumab; photosensitizers, such as aminolevulinic acid, methyl aminolevulinate, porfimer sodium, and verteporfin; and other agents , such as alitretinoin, altretamine, amsacrine, anagrelide, arsenic trioxide, asparaginase, axitinib, bexarotene, bevacizumab, bortezomib, celecoxib, denileukin diftitox, erlotinib, estramustine, gefitinib, hydroxycarbamide, imatinib, lapatinib, pazopanib, pentostatin, masoprocol, mitotane, pegaspargase, tamoxifen, sorafenib, sunitinib, vemurafinib, vandetanib, and tretinoin. Selection and therapeutic dosages of such agents are known to those skilled in the art, and can be determined by a skilled clinician.

The combination therapy may provide synergy and prove synergistic, that is, the effect achieved when the active ingredients used together is greater than the sum of the effects that results from using the compounds separately. A synergistic effect may be attained when the active ingredients are: (1) co-formulated and administered or delivered simultaneously in a combined, unit dosage formulation; (2) delivered by alternation or in parallel as separate formulations; or (3) by some other regimen. When delivered in alternation, a synergistic effect may be attained when the compounds are administered or delivered sequentially, for example by different injections in

separate syringes. In general, during alternation, an effective dosage of each active ingredient is administered sequentially, *i.e.* serially, whereas in combination therapy, effective dosages of two or more active ingredients are administered together.

In one embodiment, an effective amount of an antibody or antigen binding fragment that specifically binds to one or more of the antigens disclosed herein or a conjugate thereof is administered to a subject having a tumor following anti-cancer treatment. After a sufficient amount of time has elapsed to allow for the administered antibody or antigen binding fragment or conjugate to form an immune complex with the antigen expressed on the respective cancer cell, the immune complex is detected. The presence (or absence) of the immune complex indicates the effectiveness of the treatment. For example, an increase in the immune complex compared to a control taken prior to the treatment indicates that the treatment is not effective, whereas a decrease in the immune complex compared to a control taken prior to the treatment indicates that the treatment is effective.

### E. Biopharmaceutical Compositions

Biopharmaceutical or biologics compositions (hereinafter, "compositions") are provided herein for use in gene therapy, immunotherapy and/or cell therapy that include one or more of the disclosed CARs, or T cells expressing a CAR, antibodies, antigen binding fragments, conjugates, CARs, or T cells expressing a CAR that specifically bind to one or more antigens disclosed herein, in a carrier (such as a pharmaceutically acceptable carrier). The compositions can be prepared in unit dosage forms for administration to a subject. The amount and timing of administration are at the discretion of the treating clinician to achieve the desired outcome. The compositions can be formulated for systemic (such as intravenous) or local (such as intra-tumor) administration. In one example, a disclosed CARs, or T cells expressing a CAR, antibody, antigen binding fragment, conjugate, is formulated for parenteral administration, such as intravenous administration. Compositions including a CAR, or T cell expressing a CAR, a conjugate, antibody or antigen binding fragment as disclosed herein are of use, for example, for the treatment and detection of a tumor, for example, and not by way of limitation, a neuroblastoma. In some examples, the compositions are useful for the treatment or detection of a carcinoma. The compositions including a CAR, or T cell expressing a CAR, a conjugate, antibody or antigen binding fragment as disclosed herein are also of use, for example, for the detection of pathological angiogenesis.

The compositions for administration can include a solution of the CAR, or T cell expressing a CAR, conjugate, antibody or antigen binding fragment dissolved in a pharmaceutically acceptable carrier, such as an aqueous carrier. A variety of aqueous carriers can be used, for example, buffered

saline and the like. These solutions are sterile and generally free of undesirable matter. These compositions may be sterilized by conventional, well known sterilization techniques. The compositions may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions such as pH adjusting and buffering agents, toxicity adjusting agents, adjuvant agents, and the like, for example, sodium acetate, sodium chloride, potassium chloride, calcium chloride, sodium lactate and the like. The concentration of a CAR, or T cell expressing a CAR, antibody or antigen binding fragment or conjugate in these formulations can vary widely, and will be selected primarily based on fluid volumes, viscosities, body weight and the like in accordance with the particular mode of administration selected and the subject's needs. Actual methods of preparing such dosage forms for use in in gene therapy, immunotherapy and/or cell therapy are known, or will be apparent, to those skilled in the art.

A typical composition for intravenous administration includes about 0.01 to about 30 mg/kg of antibody or antigen binding fragment or conjugate per subject per day (or the corresponding dose of a CAR, or T cell expressing a CAR, conjugate including the antibody or antigen binding fragment). Actual methods for preparing administrable compositions will be known or apparent to those skilled in the art and are described in more detail in such publications as *Remington's Pharmaceutical Science, 19th ed.*, Mack Publishing Company, Easton, PA (1995).

A CAR, or T cell expressing a CAR, antibodies, antigen binding fragments, or conjugates may be provided in lyophilized form and rehydrated with sterile water before administration, although they are also provided in sterile solutions of known concentration. The CARs, or T cells expressing a CAR, antibody or antigen binding fragment or conjugate solution is then added to an infusion bag containing 0.9% sodium chloride, USP, and in some cases administered at a dosage of from 0.5 to 15 mg/kg of body weight. Considerable experience is available in the art in the administration of antibody or antigen binding fragment and conjugate drugs; for example, antibody drugs have been marketed in the U.S. since the approval of RITUXAN® in 1997. A CAR, or T cell expressing a CAR, antibodies, antigen binding fragments and conjugates thereof can be administered by slow infusion, rather than in an intravenous push or bolus. In one example, a higher loading dose is administered, with subsequent, maintenance doses being administered at a lower level. For example, an initial loading dose of 4 mg/kg antibody or antigen binding fragment (or the corresponding dose of a conjugate including the antibody or antigen binding fragment) may be infused over a period of some 90 minutes, followed by weekly maintenance doses for 4-8 weeks of 2 mg/kg infused over a 30 minute period if the previous dose was well tolerated.

Controlled release parenteral formulations can be made as implants, oily injections, or as particulate systems. For a broad overview of protein delivery systems see, Banga, A.J., *Therapeutic*

*Peptides and Proteins: Formulation, Processing, and Delivery Systems*, Technomic Publishing Company, Inc., Lancaster, PA, (1995). Particulate systems include microspheres, microparticles, microcapsules, nanocapsules, nanospheres, and nanoparticles. Microcapsules contain the therapeutic protein, such as a cytotoxin or a drug, as a central core. In microspheres, the therapeutic is dispersed throughout the particle. Particles, microspheres, and microcapsules smaller than about 1 μm are generally referred to as nanoparticles, nanospheres, and nanocapsules, respectively. Capillaries have a diameter of approximately 5 μm so that only nanoparticles are administered intravenously. Microparticles are typically around 100 μm in diameter and are administered subcutaneously or intramuscularly. See, for example, Kreuter, J., *Colloidal Drug Delivery Systems*, J. Kreuter, ed., Marcel Dekker, Inc., New York, NY, pp. 219-342 (1994); and Tice & Tabibi, *Treatise on Controlled Drug Delivery*, A. Kydonieus, ed., Marcel Dekker, Inc. New York, NY, pp. 315-339, (1992).

Polymers can be used for ion-controlled release of the CARs, or T cells expressing a CAR, antibody or antigen binding fragment or conjugate compositions disclosed herein. Various degradable and nondegradable polymeric matrices for use in controlled drug delivery are known in the art (Langer, *Accounts Chem. Res.* 26:537-542, 1993). For example, the block copolymer, polaxamer 407, exists as a viscous yet mobile liquid at low temperatures but forms a semisolid gel at body temperature. It has been shown to be an effective vehicle for formulation and sustained delivery of recombinant interleukin-2 and urease (Johnston *et al., Pharm. Res.* 9:425-434, 1992; and Pec *et al., J. Parent. Sci. Tech.* 44(2):58-65, 1990). Alternatively, hydroxyapatite has been used as a microcarrier for controlled release of proteins (Ijntema *et al.*, *Int. J. Pharm.*112:215-224, 1994). In yet another aspect, liposomes are used for controlled release as well as drug targeting of the lipid-capsulated drug (Betageri *et al., Liposome Drug Delivery Systems*, Technomic Publishing Co., Inc., Lancaster, PA (1993)). Numerous additional systems for controlled delivery of therapeutic proteins are known (see U.S. Patent No. 5,055,303; U.S. Patent No. 5,188,837; U.S. Patent No. 4,235,871; U.S. Patent No. 4,501,728; U.S. Patent No. 4,837,028; U.S. Patent No. 4,957,735; U.S. Patent No. 5,019,369; U.S. Patent No. 5,055,303; U.S. Patent No. 5,514,670; U.S. Patent No. 5,413,797; U.S. Patent No. 5,268,164; U.S. Patent No. 5,004,697; U.S. Patent No. 4,902,505; U.S. Patent No. 5,506,206; U.S. Patent No. 5,271,961; U.S. Patent No. 5,254,342 and U.S. Patent No. 5,534,496).

### F. Kits

In one aspect, kits employing the CARs disclosed herein are also provided. For example, kits for treating a tumor in a subject, or making a CAR T cell that expresses one or more of the CARs disclosed herein. The kits will typically include a disclosed antibody, antigen binding fragment, conjugate, nucleic acid molecule, CAR or T cell expressing a CAR as disclosed herein. More than one of the disclosed antibodies, antigen binding fragments, conjugates, nucleic acid molecules, CARs or T cells expressing a CAR can be included in the kit.

The kit can include a container and a label or package insert on or associated with the container. Suitable containers include, for example, bottles, vials, syringes, etc. The containers may be formed from a variety of materials such as glass or plastic. The container typically holds a composition including one or more of the disclosed antibodies, antigen binding fragments, conjugates, nucleic acid molecules, CARs or T cells expressing a CAR. In several embodiments the container may have a sterile access port (for example the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). A label or package insert indicates that the composition is used for treating the particular condition.

The label or package insert typically will further include instructions for use of a disclosed antibodies, antigen binding fragments, conjugates, nucleic acid molecules, CARs or T cells expressing a CAR, for example, in a method of treating or preventing a tumor or of making a CAR T cell. The package insert typically includes instructions customarily included in commercial packages of therapeutic products that contain information about the indications, usage, dosage, administration, contraindications and/or warnings concerning the use of such therapeutic products. The instructional materials may be written, in an electronic form (such as a computer diskette or compact disk) or may be visual (such as video files). The kits may also include additional components to facilitate the particular application for which the kit is designed. Thus, for example, the kit may additionally contain means of detecting a label (such as enzyme substrates for enzymatic labels, filter sets to detect fluorescent labels, appropriate secondary labels such as a secondary antibody, or the like). The kits may additionally include buffers and other reagents routinely used for the practice of a particular method. Such kits and appropriate contents are well known to those of skill in the art.

## EXAMPLES

This invention is further illustrated by the following examples, which are not to be construed in any way as imposing limitations upon the scope thereof. On the contrary, it is to be clearly understood that resort may be had to various other embodiments, modifications, and equivalents thereof which, after reading the description herein, may suggest themselves to those skilled in the art without departing from the spirit of the present invention and/or the scope of the appended claims.

## EXAMPLE 1

### Isolation of human CD123-Specific Antibodies from a Fully Human Yeast Display scFv Library

This example describes the derivation of fully human binding sequences targeting the CD123 antigen from a yeast display library.

### MATERIALS AND METHODS:

A large yeast display human naive single chain variable fragment (scFv) antibody library was used to isolate anti-human CD123 antibodies described herein. The library was constructed using a collection of human antibody gene repertoires from more than 60 individuals. Three rounds of magnetic-activated cell sorting (MACS) were performed to enrich human scFv binders to the recombinant human CD123-Fc. For the first round of yeast library panning, the yeast display scFv library ($5x10^{10}$ cells) was incubated with 5 μg/mL CD123-Fc in 15ml PBSA (consisting of 0.1% Bovine Serum Albumin (BSA) in Dulbecco's phosphate-buffered saline (PBS) buffer), at room temperature on a rotator for 1.5 hours. After two times washing with 25ml PBSA, the yeast library mix was incubated with 100 μL Protein G microbeads (Miltenyi Biotec) at room temperature on a rotator for 30 minutes. After one time washing, the library mix was resuspended in 50 ml of PBSA and loaded onto the MACS cell separation column (LS column). After three times washing with 10ml PBSA. The yeast displayed scFv binders to the column were then eluted two times with 2 ml PBSA. These eluted yeast cells were combined and then resuspended into 50ml SDCAA medium (20 g D-glucose, 6.7 g BD DifcoTM Yeast Nitrogen Base without Amino Acids, 5 g BactoTM Casamino Acids, 5.4 g Na2.HPO4, and 8.56 g $NaH_2PO_4.H_2O$ in 1 L water) and amplified with

shaking at 225 rpm at 30°C for 20 hours. The amplified pool was then induced in SGCAA medium (consisting of the same composition of SDCAA medium, but containing galactose instead of glucose), with shaking at 225 rpm at 30°C for another 16 hours and used for next round of panning. The same process was repeated two more times to enrich the CD123-Fc specific binders.

To further enrich the binders with higher affinity and better specificity, FACS based sorting was employed to isolate the strongest binders from the pool. The induced pool was incubated with 1μg/ml of CD123-Fc at room temperature for 1 hour and then stained with Anti-c-Myc-Alexa 488 and Goat anti-Hu-Fc PE conjugates, the top 1% of the pool with the highest PE versus FITC signal was gated and sorted. The sorted pool was amplified in SDCAA medium and yeast plasmid DNA was extracted and transformed into bacterial for single clone DNA sequencing. Two unique sequences were identified and designated as MT-16 and MT-32, cloned into CAR constructs for expression in CAR-T or CAR-NK format constructs for expression in CAR-T or CAR-NK format, for further function characterizations.

## EXAMPLE 2

### Isolation of human CD123-Specific Antibodies from a Fully Human Phage Display InfinityOne scFv Library

This example describes the derivation of fully human binding sequences targeting the CD123 antigen from a phage display library.

### MATERIALS AND METHODS:

### Production of Human Phage-Displayed ScFv CD123-Specific Antibodies

A naïve human scFv (recombinant single chain fragment variable of immunoglobulin) phage display library (approximate diversity, $7x10^{10}$ unique specificities), constructed from peripheral blood B cells of 121 healthy donors (F. Tomszak, unpublished data) was used for selection of scFvs specific for recombinant human CD123. Amplified libraries of $10^{12}$ phage-displayed ScFv were incubated with 1 μg of coated CD123 in 100μl volume in one well of a 96-well plate for 2 h at room temperature during the first, second and third rounds of biopanning, respectively. After each round of incubation, the wells were washed 10 times for the first round, 20 times for the second round and 30 times for the third round with phosphate-buffered saline containing 0.05% Tween 20 (PBST) to remove nonspecifically bound phage. Antigen binding phage were eluted with 100 μl 10μg/ml

Trypsin diluted in PBS and mixed with TG1 competent cells for 1 hour at 37°C, and the phage was amplified from the infected cells and used in the next round of biopanning. After the third round of biopanning, 376 clones were randomly picked from the infected TG1 cells and each inoculated into 150 µl 2YT medium containing 100 µg/ml ampicillin and 200 mM glucose in 96-well plates by using the automated colony picking system (Molecular Devices, QPix 460) and were incubated at 37°C overnight in a shaker at 300 rpm. Next day 10 µl of the bacterial cultures were used to inoculate 150 µl 2YT medium containing 100 µg/ml ampicillin and 50 µM isopropyl-β-d-thiogalactopyranoside in 96-well plates and the plates were further incubated at 30°C overnight in a shaker at 300 rpm. The scFv supernatants were mixed with 2% BSA in PBST containing 1:2500 diluted horseradish peroxidase-conjugated recombinant monoclonal mouse anti-c-myc antibody at a1:1 volume ratio and used for enzyme-linked immunosorbent assay (ELISA) to identify clones of phage displaying scFvs with high CD123 binding affinity. The supernatants were incubated for 1 h at room temperature with recombinant human CD123 coated at 30 ng per well in 384-well plates and washed three times with PBST, (after overnight incubation at 4°C it was blocked with 2% BSA in PBS containing 0.05% Tween 20 and washed three times with PBS containing 0.05% Tween 20.) After incubation the 3,3,′5,5′-tetramethylbenzidine (TMB) substrate was added, and solution absorbance at 450 nm (A450) measured. Clones that bound to CD123 with a signal to noise ratio of >5 were selected for further characterization.

**Flow Cytometry-based binding assay.**

Identified binders from ELISA were tested on CD123-positive the cell line MOLM-13. The CD123-negative cell line Jeko-1 CD20KO eGFP served as negative control. For positive control staining REAL270 (alpha CD123) and REAL116 (alpha CD123) antibodies were used, respectively. Soluble scFvs were expressed as described above. The bacterial pellets were disrupted by incubation with 0.3 mL TE buffer (10 mM Tris-HCl containing 1 mM EDTA, pH 8.0 at 37°C)/well at 37°C, 250 rpm for 18 hours. Cultures were centrifuged at 4000xg, RT for 20 minutes and the supernatant was transferred to a fresh microtiter plate.

For cell staining cell number and viability were determined at MACSQuantX. A cell suspension containing needed cell number were centrifuged at 300xg, 4°C for 10 minutes and supernatant was discarded. Cells are resuspended by addition of PEB buffer (1xPBS + 2mM EDTA, 0,5% BSA pH 7.4 at RT) to a concentration of 1E+06 cells/mL. One hundred thousand cells per cell line were added to each well of 96-well V-bottom plate and the plate were centrifuged at 1300xg, 4°C for 2 minutes. The supernatant was discarded, cells were resuspended in 100 µL of supernatant from periplasmatic preparation and cells were incubated for 10 minutes on ice. Cells were washed

by addition of 100 µL PEB followed by centrifugation (1300 x g, 4°C for 2 minutes), twice. Fifty microliter of secondary (Anti-His-APC conjugated antibody) or REAL antibody (diluted 1:50 in PEB buffer) per well were added and the plate is incubated for 10 minutes in the dark at 4°C. Cells were washed by addition of 100 µL PEB followed by centrifugation (1300xg, 4°C for 2 minutes), twice. Propidium iodide was diluted 1:100 in Fixing solution (1xPBS + 2mM EDTA + 1% PFA + 0.3% MeOH + 3% NaAzide) and washed cells were resuspended in 50 µL of the mixture. Signals are measured at MACSQuantX. Signals were analyzed using FlowLogic software. Statistical analysis was performed with VORTEX software.

**RESULTS:**

Based upon the results of the ELISA binding assay, ten unique scFv clones specific for recombinant human CD123 and MOLM13 cells were identified (Table 1). The corresponding scFv sequences were incorporated as binder domains into CAR constructs for further analysis in CAR-T and CAR-NK format.

Table 1.

| | ScFv designation |
|---|---|
| 1 | MB31-A01 |
| 2 | MB31-C01 |
| 3 | MB35-E02 |
| 4 | MB36-A05 |
| 5 | MB40-F08 |
| 6 | MB40-H08 |
| 7 | MB42-D03 |
| 8 | MB42-E02 |
| 9 | MB42-E12 |
| 10 | MB44-H01 |

## EXAMPLE 3

### Development of CD123-targeting CAR T cell constructs

Few treatment options exist for AML, and treatment-associated toxicities and post-treatment disease relapse are common. Moreover, immunotherapies employing non-human sequences, such as mouse-derived antibodies, may result in therapy rejection or adverse reactions in patients. In order to develop a new CAR T treatment for AML, fifteen CD123-targeting CAR T constructs incorporating fully human ScFv targeting domains were designed and evaluated for anti-tumor activity.

### MATERIALS AND METHODS:

**Cell lines used to demonstrate CAR activity**

Acute myeloid leukemia cell line MOLM-14 was purchased from the German Collection of Microorganisms and Cell Lines (DSMZ, Braunschweig Germany). Other cell lines, myelogenous leukemia line KG-1a, acute lymphocytic leukemia line RS4;11, epidermoid carcinoma line A431 and 293T cell line were purchased from American Tissue Culture Collection (ATCC, Manassas, VA). The MOLM14 cell line was cultured in RPMI-1640 Medium (ATCC) supplemented with 20% heat-inactivated fetal bovine serum (FBS). The KG-1a line was cultured in IMDM Medium supplemented with 20% FBS. The A431 line was cultured in DMEM Medium (ATCC) supplemented with 10% heat inactivated FBS. The 293T cells were cultured in Dynamis™ medium (Thermo Fisher Scientific, Grand Island, NY) with 4mM L-Glutamine (Lonza, Morristown, NJ). Each cell line was prepared as a single-cell clone of luciferase-expressing cell line by stably transducing wild-type tumor lines with lentiviral vector encoding firefly luciferase (Lentigen Technology, Inc., Gaithersburg, MD).

**Generation of CAR constructs and Lentiviral Vector production**

The human anti-CD123 chimeric antigen receptor (CAR) constructs were generated from various single chain variable fragment (ScFv) sequences targeting the extracellular domain of human CD123/IL-3 receptor α. Each scFv sequence was linked in frame to CD8 hinge, 4-1BB costimulatory domain, and CD3-ζ activating domain sequences. The comparator CD33-targeting CAR sequence was generated in a similar manner, except that a heavy chain only variable domain (VH_4) was used as a targeting domain instead of an scFv. The VH_4 sequence was linked in

frame to CD8 hinge, 4-1BB costimulatory domain, and CD3-ζ activating domain sequences (Schneider, Dina et al. "A Unique Human Immunoglobulin Heavy Chain Variable Domain-Only CD33 CAR for the Treatment of Acute Myeloid Leukemia." Frontiers in Oncology vol. 8 539. 22 Nov. 2018, doi:10.3389/fonc.2018.00539). Leader peptide derived from the human GMCSFR1 was included in all CAR constructs to facilitate trafficking to T cell membrane. CAR sequences were cloned into a Lentiviral Vector (LV) expression cassette under the control of the human EF-1α promoter (Lentigen Technology Inc., Gaithersburg, MD). Lentiviral particles were generated by transient transfection of HEK 293T cells, pelleted by centrifugation and stored at -80°C until transduction.

**Primary T cell preparation and transduction**

Healthy donor primary T cells were isolated either from leukapheresis collections (AllCells, Alameda, CA) or from processed buffy coats (Oklahoma Blood Institute, Tulsa, OK), obtained with donors' written consent. The CD4-positive and CD8-positive human T cells were purified via positive selection using a 1:1 mixture of CD4 and CD8 Microbeads (Miltenyi Biotec, Bergisch Gladbach, Germany) according to manufacturer's protocol. Purified T cells were cultured in serum free TexMACS medium supplemented with either 30 IU/ml IL-2 at a density of 1x $10^6$ cells/ml, and activated with CD3/CD28 MACS® GMP T Cell TransAct reagent (Miltenyi Biotec). Further, activated T cells were transduced on day 1 with lentiviral vector particles encoding CAR constructs. On day 3, and every 2-3 days thereafter, cultures were supplemented with fresh TexMACS medium containing 30 IU/ml IL-2, until harvest on day 8-10. Where noted, TexMACS medium supplemented with 970 IU/ml IL-7 and 90 IU/ml IL-15 was used.

**CD123 surface expression on tumor cell lines**

CD123 surface expression was determine in an array of tumor lines by flow cytometry using anti-CD123 antibody clone AC145 (Miltenyi Biotec, Bergisch Gladbach, Germany), and negative gating was based on the cognate isotype control. The CD123 surface expression density on target cell lines was evaluated by QuantiBRITE Phycoerythrin (PE) beads (BD Biosciences, San Jose, CA) based on the antibodies bound per cell (ABC) method as per manufacturer's protocol. Briefly, beads conjugated to the PE fluorophore at four different densities served to generate a standard curve, and tumor cells stained with anti CD123 antibodies conjugated to PE were acquired under identical settings. The ABC value was extrapolated for each tumor cell line based on the standard curve.

**Flow cytometric analysis of CAR surface expression**

Half million CAR T cells were washed two times in cold AutoMACS buffer supplemented with 0.5% bovine serum albumin (Miltenyi Biotec, Bergisch Gladbach, Germany) and stained with 2.5 ug/ml CD123-Fc peptide (Novoprotein, Summit, NJ), followed by anti Fc-AF647 conjugate (Jackson ImmunoResearch, West Grove, PA). The 7-Aminoactinomycin D staining (7-AAD, BD Biosciences, San Jose, CA) was added to exclude dead cells. Non-transduced cells (UTD) were used as a negative control. Cells were washed twice with AutoMACS buffer supplemented with 0.5% bovine serum albumin, resuspended in 200 ul staining buffer and acquired by flow cytometry. Flow cytometric analysis was performed on a MACSQuant® 10 Analyzer (Miltenyi Biotec), and data plots were generated using FlowJo software (Ashland, OR).

**CAR T cell cytotoxicity and cytokine assay**

To assess CAR T cell mediated cytotoxicity, $5x10^3$ tumor target cells stably transduced with firefly luciferase were combined with CAR T cells at the indicated effector to target ratios and incubated overnight at 37°C with 5% $CO_2$. SteadyGlo reagent (Promega, Madison WI) was added to each well and the resulting luminescence quantified as counts per second (sample CPS). Target only wells (max CPS) and target only wells plus 1% Tween-20 (min CPS) were used to determine assay range. Percent specific lysis was calculated as: (1-(sample CPS-min CPS)/(max CPS-min CPS)). For cytokine release analysis, $5\times10^4$ effectors and $5\times10^3$ targets were co-cultured overnight, and supernatants from co-cultures were removed and analyzed by ELISA (eBioscience, San Diego, CA) for IFNγ, TNFα and IL-2 concentration. Three technical replicates were performed for each condition, and each experiment was repeated using CAR T cells generated from at least three healthy donors.

**Results**

Example 3 data describes the generation and *in vitro* evaluation of CAR T cells targeting the CD123 antigen for the treatment of AML.

Schematic representations of the tandem CAR constructs targeting the CD123 antigen are shown in FIGURE 1A. CAR 123 is comprised of a fully human binder (InfinityOne), linked in frame to CD8 hinge and transmembrane domain, 4-1BB co-stimulatory domain and CD3ζ activation domain. Ten scFv sequences were selected for evaluation in the CAR format based on flow cytometric binding analysis of the cognate soluble binders to target lines with and without CD123 expression. CAR variants D0125-D0134 were constructed (TABLE 2). CAR sequences

were further incorporated into a third-generation lentiviral vectors and transduced into human primary T cells at saturation, to generate the CD123 CAR T cells under the control of the mammalian EF-1α promoter. Previously evaluated CAR control constructs, targeting CD123 (LTG2078) and CD33 (LTG1906) were also included (TABLE 3). Un-transduced T cells derived from same donor as the CAR -expressing cells (UTD) were used as a negative control.

**Table 2. CD123 CAR constructs**

| Construct Number | ScFv | Construct designation |
|---|---|---|
| D0125 | CD123 (MB31-A01) | EF-1a-CD123 MB31-A01CD8 BBz |
| D0126 | CD123 (MB31-C01) | EF-1a-CD123 MB31-C01CD8 BBz |
| D0127 | CD123 (MB35-E02) | EF-1a-CD123 MB35-E02 CD8 BBz |
| D0128 | CD123 (MB36-A05) | EF-1a-CD123 MB36-A05 CD8 BBz |
| D0129 | CD123 (MB40-F08) | EF-1a-CD123 MB40-F08 CD8 BBz |
| D0130 | CD123 (MB40-H08) | EF-1a-CD123 MB40-H08 CD8 BBz |
| D0131 | CD123 (MB42-D03) | EF-1a-CD123 MB42-D03 CD8 BBz |
| D0132 | CD123 (MB42-E02) | EF-1a-CD123 MB42-E02 CD8 BBz |
| D0133 | CD123 (MB42-E12) | EF-1a-CD123 MB42-E12 CD8 BBz |
| D0134 | CD123 (MB44-H01) | EF-1a-CD123 MB44 -H01CD8 BBz |

**Table 3. Single-targeting CAR controls**

| Construct Number | scFv | Construct designation |
|---|---|---|
| LTG2078 | M12306 | EF-1α CD123 CD8 BBz |
| LTG1906 | CD33_4 | EF-1α CD33 CD8 BBz |

Lentiviral vectors encoding the CD123 CAR constructs were used for CAR transduction into human primary T cells at multiplicity of infection (MOI) of 40. CAR surface expression of transduced T cells by flow cytometry using recombinant IL3R-alpha Fc-tagged, followed by staining with anti-Fc Alexa Flour 647. Different CD123 CAR construct exhibited different level of expression ranging from 0-80% (n=4 donors), FIGURE 1B. CAR D0126, D0127, D0131, D0132, D0133 and D0134 exhibited similar or higher surface expression than positive CAR 123 control LTG2078; while CAR D0130 had slightly lower surface expression, followed by D0129 and D0128, while D0125 had lowest expression in multiple donors. Cell viability was examined at day 3 and day 7 after T cell activation, as showed in FIGURE 1C. All the CD123 CAR T cells showed improved or equivalent viability compared with control CAR LTG2078.

To evaluate the target specific cytotoxicity of CD123 CARs *in vitro*, leukemic lines (MOLM14, KG1a, RS4;11) and non-leukemic lines (293T and A431) were evaluated for surface

CD123 expression by flow cytometry with CD123 specific antibodies. As shown in Figure 2, 99% MOLM14, and 66% KG-1a human AML tumor cell lines express CD123, whereas the human B-ALL line RS4;11 has only limited CD123 expression. By contrast, 293T and A431 have no CD123 expression. Therefore, lines MOLM14 and KG-1a were selected as target cell lines, and 293T as negative control cell line for CAR T cells functional evaluation.

Human primary T cells were transduced with lentiviral vectors encoding CAR constructs and expanded in culture to day 8. CAR-T cells were co-incubated with MOLM14, KG-1a or 293T cell lines at effector to target ratios 2.5:1; 5:1 and 10:1. After overnight co-incubation, cultures were analyzed in a luminescence based *in vitro* killing assays. Most CAR123 constructs-expressing primary T cell lines lysed MOL14-CD123+ with varied potency, while three CD123 CAR lines, D0125, D0128 and D0129, lacked target lytic capability (Figure 3A). Similarly, KG-1a-CD123+ target cells were killed by most CAR T constructs, except for D0125, D0128 and D0129 (Figure 3B). The CD33 CAR LTG1906 exhibited high cytotoxicity toward MOLM14 ($CD33^{High}$) and low lytic potency towards KG1a ($CD33^{Low}$), in agreement with the CD123 expression levels. Furthermore, no killing above background of CD123 negative 293T cell line (Figure 3C) was observed, demonstrating the robust target-specific cytotoxic function of all CD123 CAR constructs, except for CAR D0125, D0128, and D0129.

Production of the T cell homeostatic and pro-inflammatory cytokines IL-2, IFNγ, and TNFα by the CD123 CARs, and control constructs CAR LTG2078 and CD33 CAR LTG1906, was examined by ELISA in culture supernatants after overnight co-incubation of CAR T cells with MOLM14 target line at an E:T ratio of 10 (Figure 4A-4C). Specific target induced cytokine release was detected by comparison of each CAR T group incubated with target cells to the respective CAR T alone experimental group, and also comparing the target co-incubated CAR T groups to the previously characterized CAR123 control LTG2078. While CAR123 control LTG2078 and CAR33 control LTG1906 elaborated cytokines after co-incubation with MOLM14 target cells, most test CD123 CAR T constructs have not produced significant increases in IFNγ, TNFα, or IL-2 cytokines after overnight co-culture with MOLM14 cells. One exception was CAR123 D0127, which elaborated IFNγ, and TNFα levels even in the absence of target cells (T cells alone group), indicating tumor-independent cytokine response. This effect could not be anticipated form previous experiments, and it demonstrates the non-obviousness of the present invention. Excluding CAR123 D0127, cytokine response of the CD123 CAR constructs evaluated herein was comparable to the non-transduced T cells (UTD) control, suggesting low risk of

inducing cytokine – mediated adverse effects, such as cytokine release syndrome (CRS) and immune effector cell-associated neurotoxicity syndrome (ICANS).

Among all CAR123 constructs, D0126 showed the highest transduction efficiency and viability as well as best cytotoxic function against CD123+ tumor cells among this set of CAR constructs. Another CAR123 construct, D0131, also demonstrated high CAR transduction efficiency and viability, but moderate target cell killing activity *in vitro*. Therefore, CAR123 constructs D0126 and D0131 were selected for further evaluation *in vivo*.

## EXAMPLE 4

### Evaluation of the anti-tumor function of CD123-taregting CAR T cells in a mouse MOLM14 xenograft model

This example describes the evaluation of the CD123 – targeting CAR T cells incorporating scFv sequences derived from the InfinityOne library *in vivo*.

### MATERIALS AND METHODS:

#### Cell Lines

Acute myeloid leukemia cell line MOLM-14 was purchased from the German Collection of Microorganisms and Cell Lines (DSMZ, Braunschweig Germany). The MOLM14 cell line was stably transduced with firefly luciferase gene and cultured in RPMI-1640 Medium (ATCC) supplemented with 20% heat-inactivated fetal bovine serum (FBS).

#### In vivo analysis of CAR T function

Animal experiments were performed in compliance with the applicable laws, regulations and guidelines of the National Institutes of Health (NIH) and with the approval of MI Bioresearch (Ann Arbor, MI) Animal Care and Use Committee. In this study, the function of CD123-targeting CAR T cells was assessed in NSG (NOD.Cg-Prkdc$^{cid}$Il2rg$^{tm1Wjl}$/SzJ) mice *in vivo*. Six to eight week old female NSG mice, 6 per group, were injected with 1.0 x $10^6$ MOLM-14 CD123$^+$ AML cells into the tail vein on day 0. Tumor burden was determined by IVIS bioluminescent imaging on day 4, and mice were then randomized to groups with equal mean tumor burden, and 5.0 × $10^6$ CAR T$^+$ cells/mouse (normalized for transduction efficiency) were administered on study day 5. Tumor

regression was determined by bioluminescent imaging on days 14, 21, 28, 35, 42, 49 using a Xenogen IVIS-200 instrument (Perkin Elmer, Shelton, Connecticut). Images were analyzed using Living Image, version 4.1, software (Perkin Elmer) and the bioluminescent signal flux for each mouse was expressed as average radiance (photons per second per $cm^2$ per steradian). Survival was recorded and analyzed at the end of the study. To determine the presence of CAR T and tumor cells, peripheral blood was collected from all animals on study day 14, 21, 28 and 42. The absolute numbers of blood CAR T cell and MOLM-14 tumor cells were determined by flow cytometry.

**Flow cytometric analysis of CAR T and tumor cells in mouse blood**

Seventy microliters of mouse blood was collected on study day 14, 21, 28 and 42, and analyzed for CAR T and MOLM-14 tumor cell number by flow cytometry. Red Blood Cells were then lysed with Red Blood Cell Lysis Solution (BD BioScience, San Jose, CA) as per manufacturer's instructions, the remaining lymphocytes were stained with anti-human CD45, anti-human CD3 (Miltenyi Biotec), anti-human CD8 (Miltenyi Biotec), anti-human CD123 (Miltenyi Biotec), and 7-AAD (BD Biosciences, San Jose, CA) and then analyzed by flow cytometry. Dead cells were excluded from analysis by 7-AAD staining. To obtain direct counts of human T cell and MOLM-14 in blood, the MACSQuant 10 volumetric function was utilized, and CountBright Absolute Counting Beads (ThermoFischer Scientific, Waltham, MA) were used to account for sample loss during processing, as per manufacturer's protocol.

**RESULTS:**

NSG MOLM14 xenograft AML model was used to further explore the *in vivo* tumor rejection functionality of the two top CAR123 candidates D0126 and D0131. Two animal studies using CAR T cells derived from separate healthy donors were performed, one focusing on CAR D0126 (Figure 5A) and the other comparing between CAR123 constructs D0126 and D0131 (Figure 5B). The previously characterized CAR LTG1906, targeting the CD33 antigen on MOLM14 tumor cells, was included as a comparative control.

In the first *in vivo* study, CD123 CAR D0126 was compared with the previously characterized CD33 CAR-T construct LTG1906, and control experimental groups tumor alone (TA) and untransduced T cells (UTD) were also included. CAR-T cells were generated by transduction with lentiviral vectors encoding CAR D0126 and CAR LTG1906 and subsequent culture expansion in TexMACS medium supplemented with 30 IU/ml IL2. MOLM14-Luc cells were used as target line. MOLM14-Luc cells, $1 \times 10^6$, were injected intravenously (i.v.) into each NSG mouse. Tumor growth was evaluated by IVIS imaging on day 6, and then mice were

randomized into experimental groups. On day seven, $5\times 10^6$ human CAR+ T cells or UTD cells per mouse were administrated by tail vein injection. Tumor growth kinetics was monitored by in vivo imaging system (IVIS) overtime (Figure 6A and 6B). As MOLM14 tumors express both CD123 and CD33 antigens, treatment groups dosed with CAR D0126, targeting the CD123 antigen, as well as the comparator group dosed with the CAR LTG1906, targeting the CD33 antigen, showed robust tumor rejection compared to tumor alone (TA) and UTD control groups. Five of six mice in each group demonstrated complete tumor rejection, and only one mouse per group had residual tumor cells at study conclusion (Figure 6B). Notably, both CAR D0126 and CAR LTG1906 - treated groups showed no body weight loss (Figure 6C), thus no CAR-related toxicity was detected in this model. CARs D0126 and LTG1906 both mediated complete survival to study termination at day 36 (6 out of 6 mice survived), while the tumor alone (TA), and UTD control groups succumbed to high-burden disseminated disease by day 15 (Figure 6D). Mouse peripheral blood was sampled at days 14, 22 and 33. Human T cells were detected in all groups (Figure 7A, 7B, 7C). Moreover, CAR D0126 and LTG1906 T cells were detected in the peripheral blood of mice at the end of the study, demonstrating high persistence of the CD123 CAR candidate D0126, and the comparative control CAR33 LTG1906 T cells.

In the second animal study, CD123 CAR D0131 was included in addition to CAR D0126. CAR T cells in this study were generated from peripheral blood T cells of a different donor from the one used in the first in vivo study. T cells were transduced and expanded with TexMACS medium supplemented with 970 IU/ml IL-7 and 90 IU/ml IL-15. Tumor progression in each group is shown in Figure 8A. Similarly to the first animal study, CAR D0126 demonstrated strong anti-tumor potency, and tumors were rejected in four out of six mice. CAR123 D0131 manifested weaker anti-tumor activity as compared with CAR123 D0126 (Figure 8A and 8B). Although no significant body weight loss was observed in the CAR T treated groups (Figure 8C), mice death was observed in all groups. The best survival effect was detected in the CAR D0126- treated group, with four of the six mice surviving to the extended study termination day, day 56, and remaining completely tumor-free (Figure 8D). The total T cells in the peripheral blood were monitored in this study. As expected, human T cells were detected in the mice' peripheral blood two days after CAR T cell or UTD administration in all groups except the TA negative control (Figure 9A). The T cell amounts increased in all CAR T groups overtime, suggesting T cell expansion (Figure 9B), and persistence throughout days 21, 28 and 42 (Figure 9C, 9D, 9E). On study day 42, the CAR123 D0126 group had the highest number of T cells (Figure 9E), indicating the greatest T cell expansion and persistence among CAR constructs tested in this experiment.

In summary, the CD123 CAR candidate D0126 efficiently eliminated tumors in NSG mice engrafted with MOLM-14 cells in two *in vivo* studies utilizing T cells from different human donors, and demonstrated efficient tumor clearance, CAR T persistence and prolonged survival in the MOLM14 AML xenograft mouse model (Figure 9A). Therefore, CAR123 D0126 was identified as lead candidate for the development of CD123-targeting CAR T therapy for the treatment of CD123-positive malignancies.

## EXAMPLE 5

### Development of CD123-taregting CAR NK cells

This example describes the generation of CAR NK cells by lentiviral transduction.

### MATERIALS AND METHODS:

#### Lentiviral vector constructs and production

Each CD123-CAR was comprised of CD123 scFv binder, CD8 hinge and transmembrane domains, a 4-1BB transactivation domain and a CD3 zeta signaling domain. Constructs were cloned into a third-generation lentiviral plasmid backbone (Lentigen) under the control of a human EF-1α promoter. Lentiviral vector (LV) containing supernatants were generated by transient transfection of HEK 293T cells, as previously described (Kuroda Het al., J Virol Methods. (2009) 157:113–21). For pseudotyping the lentiviral vectors, a modified BaEV envelope glycoprotein was used as described previously(Girard-Gagnepain A et al., Blood. (2014) 124:1221–31). LV containing supernatants were stored at −80°C and titers were determined on NK-92 cells.

#### Primary NK Cell Separation

For isolation of NK cells from buffy coats, peripheral blood mononuclear cell (PBMC) preparation was performed by standard density-gradient centrifugation using Ficoll-Paque PLUS (GE Healthcare). Resting NK cells were enriched from PBMCs by depleting the non-NK cell population using the NK cell isolation kit for human cells (Miltenyi Biotec).

#### Cell Culture and Transduction

NK cells were cultured at $10^6$ cells/mL in NK MACS medium with 5% human AB serum, 500 U/mL IL-2 (Miltenyi Biotec), 10 ng/mL IL-15 (Miltenyi Biotec), and 10 ng/mL IL-1β (Miltenyi

Biotec) . After 2 days of culture, NK cells were transduced as previously described (Bari R, Granzin M, et al., Front Immunol. (2019) 10:2001).

Briefly, NK cells were suspended at $5 \times 10^5$ cells/mL in 200 µL serum-free culture medium containing 10 µg/mL Vectofusin-1 and up to 50 µL LV supernatant for transduction. After spinoculation at 400g for 2 h, the cells were cultured with the LV for 24 h in cell culture incubator. The cell culture medium was then exchanged with fresh complete cell culture medium containing 5% human AB serum, 500 U/mL IL-2, and 10 ng/mL IL-15. Transduction efficiency was determined by flow cytometry from day 3 post-transduction onwards. The transduced NK cells were spun down every 3 days, counted, and the cell number adjusted to 0.5 million cells/ml in fresh complete NK cell culture medium (5% human AB serum, 500 U/mL IL-2, and 10 ng/mL IL-15) for long-term culture.

**RESULTS:**

**Primary NK cells were efficiently transduced with CD123-CAR using baboon envelope glycoprotein-pseudotyped lentiviral vector.**

We have generated thirteen CD123-CAR constructs containing CD123 binders, CD8 hinge and transmembrane domains, a 4-1BB transactivation domain, and a CD3 zeta signaling domain (TABLE 4). These CD123-CAR constructs were cloned into a third-generation lentiviral plasmid backbone (Lentigen) under the control of a human EF-1α promoter. We have shown in our earlier publication that a modified baboon envelope glycoprotein-pseudotyped lentiviral vector (BaEV) can efficiently transduce NK cells (Bari R et al., Front Immunol. (2019) 10:2001). All of the listed 13 lentiviral vectors (LV) containing CD123-CAR were pseudotyped with BaEV, and viral vectors were generated by transient transfection of HEK 293T cells.

**Table 4. List of CD123 CARs and binders**

| Construct Number | ScFv | Construct designation | SsFv source library |
|---|---|---|---|
| Z16 | CD123 (Z16) | EF-1a-CD123 Z16-CD8 BBz | yeast |
| LTG2078, control | CD123 (Z23) | EF-1a-CD123 Z23-CD8 BBz | yeast |
| Z32 | CD123 (Z32) | EF-1a-CD123 Z32-CD8 BBz | yeast |
| D0125 | CD123 (MB31-A01) | EF-1a-CD123 MB31-A01CD8 BBz | phage |
| D0126 | CD123 (MB31-C01) | EF-1a-CD123 MB31-C01CD8 BBz | phage |
| D0127 | CD123 (MB35-E02) | EF-1a-CD123 MB35-E02 CD8 BBz | phage |
| D0128 | CD123 (MB36-A05) | EF-1a-CD123 MB36-A05 CD8 BBz | phage |
| D0129 | CD123 (MB40-F08) | EF-1a-CD123 MB40-F08 CD8 BBz | phage |
| D0130 | CD123 (MB40-H08) | EF-1a-CD123 MB40-H08 CD8 BBz | phage |
| D0131 | CD123 (MB42-D03) | EF-1a-CD123 MB42-D03 CD8 BBz | phage |
| D0132 | CD123 (MB42-E02) | EF-1a-CD123 MB42-E02 CD8 BBz | phage |
| D0133 | CD123 (MB42-E12) | EF-1a-CD123 MB42-E12 CD8 BBz | phage |
| D0134 | CD123 (MB44-H01) | EF-1a-CD123 MB44 -H01CD8 BBz | phage |

Primary NK cells were isolated from PBMCs by magnetic separation resulting in pure cell populations (Figure 10A). Most of the cell lines, specifically acute myeloid leukemia (AML) cells, are sensitive to the natural cytotoxicity of NK cells, thus not suitable for testing the cytotoxicity of CAR-NK cells. However, RS4-11 cell lines are known to insensitive to NK cell natural cytotoxicity. Therefore, many NK cell research laboratories, including ours, routinely use RS4-11 as target cells to test CAR-NK cell functionality. To use the RS4-11 as a target cells to test CD123-CAR functionality, a daughter RS4-11 cell line stably expressing CD123 was generated (Figure 10B).

NK cells were activated by cultivation in NK MACS medium containing IL-2/IL-15/IL-1β for two days, followed by transduction with BaEV pseudotyped lentiviral vectors (BaEV -LV), resulting in efficient efficiency transduction of primary NK cells. Transduction of NK cells with lentiviral vectors containing different CD123-CAR constructs resulted in differential expression of CD123-CAR at the surface of NK cells (Figure 11). Among the thirteen CD123-CARs, Z32 and D0126 binders were the best for transducing NK cells, and yielded transduction efficiency of 51.55% and 61.37%, respectively. Based on these expression results, we have selected CAR constructs Z32 and D0126 for further analysis.

**CD123-CAR NK cells efficiently and specifically kill target cells expressing CD123.**

Activated NK cells were transduced with BaEV pseudotyped lentiviral vector containing CD123-CAR Z32 (Z32-BaEV-LV) and D0126 (D0126-BaEV-LV). CD123-CAR expression for Z32 and D0126 was 70.5% and 64.19%, respectively (Figure 12A). In addition, the

cytotoxicity of the CD123-CAR-expressing NK cells was tested against target cells RS4-11-CD123. RS4;11 cells expressing CD123 (Figure 10B) are insensitive to NK cell natural cytotoxicity. Consequently, non-transduced NK cells could not kill RS4;11-CD123 cells, whereas both CD123-CAR (Z32 and D0126) NK cells killed RS4;11-CD123 very efficiently, demonstrating the high functionality and specificity of the generated CD123-CAR NK cells (Figure 12B).

Next, the specificity of CD123-CAR toward CD123 antigen was confirmed by serial dilution. NK cells were transduced with different amounts of lentiviral vectors containing CD123-CAR. As expected, the higher quantity of CD123-CAR-LV showed higher expression of CD123-CAR (Figure 13A). Finally, the cytotoxicity of differentially expressing CD123-CAR NK cells was tested against RS4-11-CD123 cells at the same effector-target ratio (Figure 13B). The highest expressing CD123-CAR-NK cells showed the highest killing, and the lowest expressing CD123-CAR-NK cells showed the lowest killing confirmed the specificity of CD123-CAR toward CD123 antigen.

**Expression of CD123-CAR has no adverse effect on NK expansion and viability.**

Primary NK cells were isolated, activated, and transduced with Z32 and D0126, followed by expansion for 13 days. Untransduced NK cells were used as control. The expansion of untransduced, Z32 transduced, and D0126 transduced NK cells was 61 fold, 49 fold, and 42 fold, respectively (Figure 14A). The experiment was started with equal NK cell number for each condition. Some of the NK cells lost during the transduction process may explain the differences in cell expansion between untransduced and transduced cells. However, the expansion difference between NK cells transduced with lentiviral vectors encoding the Z32 and D0126-CARs was negligible. The viability of NK cells on day 3, day 5, day 8, and Day 11 (Figure 5B) was checked as well. There were no significant differences in cell viability among untransduced, Z32-transduced, and D0126- transduced NK cells (Figure 14B), suggesting that the CD123-CARs have no adverse effect on NK cell viability.

## REFERENCE TO THE SEQUENCE LISTING

This application contains a Sequence Listing electronically submitted to the United States Patent and Trademark Office via a PDF file entitled “Sequence Listing". The Sequence Listing is incorporated by reference.

## SEQUENCES OF THE DISCLOSURE

The nucleic and amino acid sequences listed below are shown using standard letter abbreviations for nucleotide bases, and either single-letter or three-letter code for amino acids, as defined in 37 C.F.R. 1.822. Only one strand of each nucleic acid sequence is shown, but the complementary strand is understood as included by any reference to the displayed strand. In the accompanying sequence listing:

**SEQ ID NO: 1** nucleotide sequence of CAR D0125 CD123 MB31-A01 CD8 BBz

ATGCTCTTGCTCGTGACTTCTTTGCTTTTGTGCGAACTTCCGCACCCAGCCTTCCTT
TTGATACCTCAGATACAATTGGTACAGTCTGGAGCCGAGGTTAAGAAGCCGGGAT
CTTCCGTCAAAGTGTCCTGTAAAGCCTCTGGGGGCACCTTCTCTTCCTACGCAATT
AGTTGGGTGAGACAAGCTCCAGGTCAGGGTTTGGAGTGGATGGGAGGGATAATC
CCGATATTCGGGACAGCAAACTACGCCCAGAAATTTCAAGGGCGCGTAACGATA
ACAGCTGACGAGTCCACATCTACGGCATACATGGAGTTGAGTTCTCTGAGGAGTG
AGGACACAGCTGTATATTACTGCGCGCGGGGAAGCGGAGAACTTCTCTACGCAA
GTTATTATTATTACTACATGGATGTCTGGGGTAAGGGCACTACCGTAACAGTTTC
AAGTGGAGGTGGTGGTTCTGGTGGGGGAGGTAGCGGCGGCGGGGGTTCCCAATC
CGCACTCACGCAGCCTGCCTCTGTTTCAGGATCACCGGGACAGTCTATAACAATC
AGTTGTACTGGCACCAGTTCAGATGTCGGGGGGTATAACTACGTTTCATGGTACC
AACAACACCCAGGAAAGGCACCAGAACTCATGATATATGACGTGTCAAACCGAC
CGTCTGGCGTATCTAACCGATTTAGTGGCTCCAAGTCTGGTAATACCGCGTCACT
GACAATCAGCGGGTTGCAGGCTGAGGATGAAGCTGACTACTATTGTAGTTCCTAC
ACCAGCTCTAGTACTCCTGTTGTCTTCGGCGGGGGCACTAAGCTCACAGTATTGG
CGGCCGCAACGACCACTCCTGCACCCCGCCCTCCGACTCCGGCCCCAACCATTGC
CAGCCAGCCCCTGTCCCTGCGGCCGGAAGCCTGCAGACCGGCTGCCGGCGGAGC
CGTCCATACCCGGGGACTGGATTTCGCCTGCGATATCTATATCTGGGCACCACTC

GCCGGAACCTGTGGAGTGCTGCTGCTGTCCCTTGTGATCACCCTGTACTGCAAGC
GCGGACGGAAGAAACTCTTGTACATCTTCAAGCAGCCGTTCATGCGCCCTGTGCA
AACCACCCAAGAAGAGGACGGGTGCTCCTGCCGGTTCCCGGAAGAGGAAGAGGG
CGGCTGCGAACTGCGCGTGAAGTTTTCCCGGTCCGCCGACGCTCCGGCGTACCAG
CAGGGGCAAAACCAGCTGTACAACGAACTTAACCTCGGTCGCCGGGAAGAATAT
GACGTGCTGGACAAGCGGCGGGGAAGAGATCCCGAGATGGGTGGAAAGCCGCG
GCGGAAGAACCCTCAGGAGGGCTTGTACAACGAGCTGCAAAAGGACAAAATGGC
CGAAGCCTACTCCGAGATTGGCATGAAGGGAGAGCGCAGACGCGGGAAGGGAC
ACGATGGACTGTACCAGGGACTGTCAACCGCGACTAAGGACACTTACGACGCCC
TGCACATGCAGGCCCTGCCCCCGCGCTAA

**SEQ ID NO: 2** amino acid sequence of CAR D0125 CD123 MB31-A01 CD8 BBz

MLLLVTSLLLCELPHPAFLLIPQIQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWV
RQAPGQGLEWMGGIIPIFGTANYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVY
YCARGSGELLYASYYYYYMDVWGKGTTVTVSSGGGGSGGGGSGGGGSQSALTQPA
SVSGSPGQSITISCTGTSSDVGGYNYVSWYQQHPGKAPELMIYDVSNRPSGVSNRFSG
SKSGNTASLTISGLQAEDEADYYCSSYTSSSTPVVFGGGTKLTVLAAATTTPAPRPPTP
APTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYC
KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQ
GQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEA
YSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

**SEQ ID NO: 3** nucleotide sequence of CAR D0126 CD123 MB31-C01 CD8 BBz

ATGCTCTTGCTCGTGACTTCTTTGCTTTTGTGCGAACTTCCGCACCCAGCCTTCCTT
TTGATACCTGAAGTACAGCTCCTCGAATCTGGCGGTGGTCTCGTTAAGCCTGGTGG
GTCCCTTAGACTCTCTTGTGCAGCGAGCGGTTTCACCTTCAGCAACGCTTGGATGA
GTTGGGTCCGCCAGGCGCCTGGAAAGGGCCTCGAATGGGTTGGTCGGATAAAAAG
CAAGACGGATGGAGGGACCACAGATTACGCGGCGCCGGTGAAAGGTCGGTTCACA
ATTTCAAGGGATGACTCAAAAAATACTTTGTATCTGCAAATGAATTCCCTCAAGAC
GGAAGATACTGCAGTCTATTATTGCACAACCGGTTTGCTCTGGTTTGGCACTCGCA
ATTATTACTATGGCATGGATGTATGGGGCCAAGGAACGACCGTCACTGTTTCAAGT
GGAGGTGGCGGGAGCGGAGGAGGGGGCTCCGGAGGTGGCGGTTCTCAATCAGCA

CTTACTCAGCCAGCTTCAGTCAGTGGTTCCCCGGGCCAATCCATCACCATTTCATG
CACCGGCACATCAAGTGATGTTGGTGGCTACAATTACGTGAGTTGGTATCAGCAAC
ATCCAGGAAAGGCTCCTAAGCTTGTAATTTATGATGTATCCAATCGGCCTTCTGGG
CTTAGCAATCGCTTTTCCGGATCTAAATCAGGCAATACTGCGTCCCTTACCATAAG
CGGGCTTCAAGCCGAAGATGAAGCAGATTACTATTGTAACTCCTACGCTGGGAGC
GGTTCATGGGTATTTGGAGGCGGTACGAAGTTGACTGTCTTGGCGGCCGCAACGA
CCACTCCTGCACCCCGCCCTCCGACTCCGGCCCCAACCATTGCCAGCCAGCCCCTG
TCCCTGCGGCCGGAAGCCTGCAGACCGGCTGCCGGCGGAGCCGTCCATACCCGGG
GACTGGATTTCGCCTGCGATATCTATATCTGGGCACCACTCGCCGGAACCTGTGGA
GTGCTGCTGCTGTCCCTTGTGATCACCCTGTACTGCAAGCGCGGACGGAAGAAACT
CTTGTACATCTTCAAGCAGCCGTTCATGCGCCCTGTGCAAACCACCCAAGAAGAGG
ACGGGTGCTCCTGCCGGTTCCCGGAAGAGGAAGAGGGCGGCTGCGAACTGCGCGT
GAAGTTTTCCCGGTCCGCCGACGCTCCGGCGTACCAGCAGGGGCAAAACCAGCTG
TACAACGAACTTAACCTCGGTCGCCGGGAAGAATATGACGTGCTGGACAAGCGGC
GGGGAAGAGATCCCGAGATGGGTGGAAAGCCGCGGCGGAAGAACCCTCAGGAGG
GCTTGTACAACGAGCTGCAAAAGGACAAAATGGCCGAAGCCTACTCCGAGATTGG
CATGAAGGGAGAGCGCAGACGCGGGAAGGGACACGATGGACTGTACCAGGGACT
GTCAACCGCGACTAAGGACACTTACGACGCCCTGCACATGCAGGCCCTGCCCCCG
CGCTAA

**SEQ ID NO: 4** amino acid sequence of CAR D0126 CD123 MB31-C01 CD8 BBz

MLLLVTSLLLCELPHPAFLLIPEVQLLESGGGLVKPGGSLRLSCAASGFTFSNAWMSW
VRQAPGKGLEWVGRIKSKTDGGTTDYAAPVKGRFTISRDDSKNTLYLQMNSLKTED
TAVYYCTTGLLWFGTRNYYYGMDVWGQGTTVTVSSGGGGSGGGGSGGGGSQSAL
TQPASVSGSPGQSITISCTGTSSDVGGYNYVSWYQQHPGKAPKLVIYDVSNRPSGLSN
RFSGSKSGNTASLTISGLQAEDEADYYCNSYAGSGSWVFGGGTKLTVLAAATTTPAP
RPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVI
TLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPA
YQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDK
MAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

**SEQ ID NO: 5** nucleotide sequence of CAR D0127 CD123 MB35-E02 CD8 BBz

ATGCTCTTGCTCGTGACTTCTTTGCTTTTGTGCGAACTTCCGCACCCAGCCTT
CCTTTTGATACCTCAAGTTCAGCTGGTCCAGAGCGGCGCCGAGGTAAAAAA
GCCAGGCTCTTCTGTAAAGGTGTCCTGTAAGGCCAGTGGAGGCACTTTTTC
CTCCTACGCAATCTCATGGGTCCGACAAGCACCTGGTCAAGGACTGGAATG
GATGGGCGGTATCATCCCGATCTTTGGTACTGCTAACTATGCGCAGAAGTT
CCAGGGTAGGGTGACCATAACCGCAGATGAGAGTACATCCACTGCCTATAT
GGAGCTCAGTAGCCTGAGGTCTGAGGATACTGCCGTTTACTATTGTGCACG
CCACGGCGGGATGGCAACAATGCTCCCTTACGGAGCATTTGACATCTGGGG
TCAAGGTACAATGGTAACTGTATCATCTGGCGGTGGCGGTAGTGGTGGGGG
AGGCAGCGGAGGTGGGGGCAGTGATATACGACTGACGCAATCTCCCTCTA
GCCTGAGTGCCAGTGTCGGAGATCGGGTCACAATCACATGCCGGGCTAGTC
AGGGTATCAGTAGCTATCTTAATTGGTACCAACAAAAACCAGGAAAAGCA
CCGAAACTGCTCATTTATGCAGCTTCTCGGTTGCAATCTGGAGTCCCAAGCC
GGTTTAGTGGAAGTGGCAGTGGGACGGACTTTACCTTGACTATATCCTCAT
TGCAACCTGAGGATTTCGCTACTTATTACTGCCAACAATCTTACTCCACGAG
TCTTACGTTCGGTGGGGGCACGAAAGTGGAGATCAAAGCGGCCGCAACGA
CCACTCCTGCACCCCGCCCTCCGACTCCGGCCCCAACCATTGCCAGCCAGC
CCCTGTCCCTGCGGCCGGAAGCCTGCAGACCGGCTGCCGGCGGAGCCGTCC
ATACCCGGGGACTGGATTTCGCCTGCGATATCTATATCTGGGCACCACTCG
CCGGAACCTGTGGAGTGCTGCTGCTGTCCCTTGTGATCACCCTGTACTGCAA
GCGCGGACGGAAGAAACTCTTGTACATCTTCAAGCAGCCGTTCATGCGCCC
TGTGCAAACCACCCAAGAAGAGGACGGGTGCTCCTGCCGGTTCCCGGAAG
AGGAAGAGGGCGGCTGCGAACTGCGCGTGAAGTTTTCCCGGTCCGCCGAC
GCTCCGGCGTACCAGCAGGGGCAAAACCAGCTGTACAACGAACTTAACCTC
GGTCGCCGGGAAGAATATGACGTGCTGGACAAGCGGCGGGGAAGAGATCC
CGAGATGGGTGGAAAGCCGCGGCGGAAGAACCCTCAGGAGGGCTTGTACA
ACGAGCTGCAAAAGGACAAAATGGCCGAAGCCTACTCCGAGATTGGCATG
AAGGGAGAGCGCAGACGCGGGAAGGGACACGATGGACTGTACCAGGGACT
GTCAACCGCGACTAAGGACACTTACGACGCCCTGCACATGCAGGCCCTGCC
CCCGCGCTAA

**SEQ ID NO: 6** amino acid sequence of CAR D0127 CD123 MB35-E02 CD8 BBz

MLLLVTSLLLCELPHPAFLLIPQVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISW
VRQAPGQGLEWMGGIIPIFGTANYAQKFQGRVTITADESTSTAYMELSSLRSEDTAV
YYCARHGGMATMLPYGAFDIWGQGTMVTVSSGGGGSGGGGSGGGGSDIRLTQSPSS
LSASVGDRVTITCRASQGISSYLNWYQQKPGKAPKLLIYAASRLQSGVPSRFSGSGSG
TDFTLTISSLQPEDFATYYCQQSYSTSLTFGGGTKVEIKAAATTTPAPRPPTPAPTIASQ
PLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKL
LYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYN
ELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMK
GERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

**SEQ ID NO: 7** nucleotide sequence of CAR D0128 CD123 MB36-A05 CD8 BBz

ATGCTCTTGCTCGTGACTTCTTTGCTTTTGTGCGAACTTCCGCACCCAGCCTTCCTT
TTGATACCTCAAGTCCAGCTCGTTCAGAGTGGTGCAGAGGTGAAGAAGCCCGGCT
CATCTGTGAAAGTGTCATGCAAAGCAAGCGGCGGGACCTTCAGCAGTTACGCGAT
CTCCTGGGTACGACAAGCCCCCGGCCAGGGCCTGGAATGGATGGGAGGGATCATT
CCGATTTTCGGTACAGCAAACTATGCACAAAAATTTCAGGGGAGAGTTACGATAA
CTGCAGACAAGAGCACTTCAACGGCATACATGGAGCTTTCATCATTGCGCTCCGAG
GACACGGCTGTTTACTACTGCGCTCGAGGGGGACGGAACTCTTACTATTATTACTA
CATGGACGTGTGGGGCAAAGGGACAACGGTGACGGTAAGTAGTGGGGGAGGCGG
AAGCGGTGGTGGGGGAAGTGGAGGCGGTGGGTCACAGTCAGCCCTCACACAACCG
GCCTCTGTCTCAGGGAGTCCAGGACAGAGTATTACTATAAGCTGCACTGGGACATC
CTCAGACGTCGGCGGTTATAATTATGTTTCCTGGTACCAACAACATCCCGGGAAGG
CTCCCAAGCTGATGATATACGAAGTGAGTAATCGACCCTCTGGCGTGAGCAATCG
ATTCTCTGGGAGTAAGAGTGGCAACACTGCGAGTCTTACGATTTCTGGCCTGCAGG
CGGAAGACGAAGCCGATTATTACTGTAGCAGCTACACTTCAAGCTCCCCTGTTGTT
TTCGGTGGCGGCACTAAACTTACCGTGCTTGCGGCCGCAACGACCACTCCTGCACC
CCGCCCTCCGACTCCGGCCCCAACCATTGCCAGCCAGCCCCTGTCCCTGCGGCCGG
AAGCCTGCAGACCGGCTGCCGGCGGAGCCGTCCATACCCGGGGACTGGATTTCGC
CTGCGATATCTATATCTGGGCACCACTCGCCGGAACCTGTGGAGTGCTGCTGCTGT
CCCTTGTGATCACCCTGTACTGCAAGCGCGGACGGAAGAAACTCTTGTACATCTTC
AAGCAGCCGTTCATGCGCCCTGTGCAAACCACCCAAGAAGAGGACGGGTGCTCCT

GCCGGTTCCCGGAAGAGGAAGAGGGCGGCTGCGAACTGCGCGTGAAGTTTTCCCG
GTCCGCCGACGCTCCGGCGTACCAGCAGGGGCAAAACCAGCTGTACAACGAACTT
AACCTCGGTCGCCGGGAAGAATATGACGTGCTGGACAAGCGGCGGGGAAGAGAT
CCCGAGATGGGTGGAAAGCCGCGGCGGAAGAACCCTCAGGAGGGCTTGTACAAC
GAGCTGCAAAAGGACAAAATGGCCGAAGCCTACTCCGAGATTGGCATGAAGGGA
GAGCGCAGACGCGGGAAGGGACACGATGGACTGTACCAGGGACTGTCAACCGCG
ACTAAGGACACTTACGACGCCCTGCACATGCAGGCCCTGCCCCCGCGCTAA

**SEQ ID NO: 8** amino acid sequence of CAR D0128 CD123 MB36-A05 CD8 BBz

MLLLVTSLLLCELPHPAFLLIPQVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISW
VRQAPGQGLEWMGGIIPIFGTANYAQKFQGRVTITADKSTSTAYMELSSLRSEDTAV
YYCARGGRNSYYYYYMDVWGKGTTVTVSSGGGGSGGGGSGGGGSQSALTQPASVS
GSPGQSITISCTGTSSDVGGYNYVSWYQQHPGKAPKLMIYEVSNRPSGVSNRFSGSKS
GNTASLTISGLQAEDEADYYCSSYTSSSPVVFGGGTKLTVLAAATTTPAPRPPTPAPTI
ASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGR
KKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQ
LYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEI
GMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

**SEQ ID NO: 9** nucleotide sequence of CAR D0129 CD123 MB40-F08 CD8 BBz

ATGCTCTTGCTCGTGACTTCTTTGCTTTTGTGCGAACTTCCGCACCCAGCCTTCCTT
TTGATACCTCAGGTTCAGCTCGTTCAAAGCGGAGCTGAAGTTAAAAAACCTGGGTC
TTCTGTCAAGGTAAGTTGCAAAGCATCCGGAGGCACGTTTTCTTCCTATGCAATAA
GTTGGGTCCGGCAAGCACCTGGTCAGGGATTGGAATGGATGGGTGGTATTATACC
AATATTCGGAACGGCGAACTACGCACAGAAGTTTCAAGGCAGGGTAACTATTACC
GCGGACGAGTCTACCTCAACAGCGTATATGGAACTGAGCAGTCTCAGATCAGAAG
ATACCGCAGTTTATTACTGCGCTCGGGGGTCTGGAGAGCTTCTCTATGCATCCTAC
TACTACTATTATATGGACGTATGGGGCAAGGGTACCACCGTTACCGTGTCTTCTGG
AGGTGGCGGATCTGGAGGTGGAGGATCCGGTGGGGGAGGCAGCCAATCTGCACTG
ACTCAACCCGCGTCCGTGAGCGGATCCCCTGGGCAATCAATAACAATCTCTTGCAC
GGGGACCTCATCTGATGTTGGTGGATATAATTACGTCAGCTGGTACCAACAACACC
CCGGTAAGGCTCCGAAGCTGATGATTTACGAAGTGAGTAATCGCCCAAGTGGTGT

AAGCAACAGATTCTCAGGCTCAAAGAGTGGGAACACTGCGTCCCTGACTATCTCA
GGCCTCCAGGCTGAGGACGAAGCAGATTATTACTGTTCTTCATACACCAGTAGTAG
TCCTTTGGTCTTCGGCACCGGCACCAAGGTAACTGTACTGGCGGCCGCAACGACCA
CTCCTGCACCCCGCCCTCCGACTCCGGCCCCAACCATTGCCAGCCAGCCCCTGTCC
CTGCGGCCGGAAGCCTGCAGACCGGCTGCCGGCGGAGCCGTCCATACCCGGGGAC
TGGATTTCGCCTGCGATATCTATATCTGGGCACCACTCGCCGGAACCTGTGGAGTG
CTGCTGCTGTCCCTTGTGATCACCCTGTACTGCAAGCGCGGACGGAAGAAACTCTT
GTACATCTTCAAGCAGCCGTTCATGCGCCCTGTGCAAACCACCCAAGAAGAGGAC
GGGTGCTCCTGCCGGTTCCCGGAAGAGGAAGAGGGCGGCTGCGAACTGCGCGTGA
AGTTTTCCCGGTCCGCCGACGCTCCGGCGTACCAGCAGGGGCAAAACCAGCTGTA
CAACGAACTTAACCTCGGTCGCCGGGAAGAATATGACGTGCTGGACAAGCGGCGG
GGAAGAGATCCCGAGATGGGTGGAAAGCCGCGGCGGAAGAACCCTCAGGAGGGC
TTGTACAACGAGCTGCAAAAGGACAAAATGGCCGAAGCCTACTCCGAGATTGGCA
TGAAGGGAGAGCGCAGACGCGGGAAGGGACACGATGGACTGTACCAGGGACTGT
CAACCGCGACTAAGGACACTTACGACGCCCTGCACATGCAGGCCCTGCCCCCGCG
CTAA

**SEQ ID NO: 10** amino acid sequence of CAR D0129 CD123 MB40-F08 CD8 BBz

MLLLVTSLLLCELPHPAFLLIPQVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISW
VRQAPGQGLEWMGGIIPIFGTANYAQKFQGRVTITADESTSTAYMELSSLRSEDTAV
YYCARGSGELLYASYYYYYMDVWGKGTTVTVSSGGGGSGGGGSGGGGSQSALTQP
ASVSGSPGQSITISCTGTSSDVGGYNYVSWYQQHPGKAPKLMIYEVSNRPSGVSNRFS
GSKSGNTASLTISGLQAEDEADYYCSSYTSSSPLVFGTGTKVTVLAAATTTPAPRPPTP
APTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYC
KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQ
GQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEA
YSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

**SEQ ID NO: 11** nucleotide sequence of CAR D0130 CD123 MB40-H08 CD8 BBz

ATGCTCTTGCTCGTGACTTCTTTGCTTTTGTGCGAACTTCCGCACCCAGCCTT
CCTTTTGATACCTCAGGTTCAGCTGGTACAGTCCGGCGCAGAGGTTAAAAA
GCCAGGAAGCTCCGTGAAGGTTTCATGCAAGGCATCCGGTGGTACATTCTC

ATCATATGCGATCAGTTGGGTCCGGCAGGCTCCCGGCCAGGGATTGGAGTG
GATGGGAGGGATAATCCCCATATTTGGCACAGCAAATTACGCTCAAAAATT
TCAAGGTAGAGTAACGATAACTGCGGATGAATCTACTAGCACGGCGTATAT
GGAACTGAGTAGTCTCCGGAGCGAGGATACAGCGGTTTACTACTGCGCTAG
GAATGAATGGTACTCCTATTATTACTACTACATGGGTGTGTGGGGTAAAGG
AACTACTGTTACGGTGTCCAGTGGAGGAGGAGGTAGCGGAGGTGGAGGAT
CAGGCGGTGGGGGCTCCCAAAGTGCGCTTACACAACCTGCAAGCGTATCAG
GTTCCCCAGGGCAATCAATTACAATAAGCTGCACGGGTACCTCCAGTGATG
TCGGAGGTTACAACTACGTGTCATGGTACCAGCAACATCCAGGCAAGGCAC
CAAAACTTATGATCTACGAAGTCAGCAACAGACCCAGCGGTGTAAGCAAT
AGGTTTAGCGGATCTAAGTCCGGTAATACTGCTTCTCTGACAATCTCAGGA
CTCCAAGCCGAGGACGAAGCTGATTACTACTGCTCATCATACACCAGTAGC
TCTACACTGGTGGTGTTCGGAGGGGGAACGAAGCTTACCGTACTGGCGGCC
GCAACGACCACTCCTGCACCCCGCCCTCCGACTCCGGCCCCAACCATTGCC
AGCCAGCCCCTGTCCCTGCGGCCGGAAGCCTGCAGACCGGCTGCCGGCGGA
GCCGTCCATACCCGGGGACTGGATTTCGCCTGCGATATCTATATCTGGGCA
CCACTCGCCGGAACCTGTGGAGTGCTGCTGCTGTCCCTTGTGATCACCCTGT
ACTGCAAGCGCGGACGGAAGAAACTCTTGTACATCTTCAAGCAGCCGTTCA
TGCGCCCTGTGCAAACCACCCAAGAAGAGGACGGGTGCTCCTGCCGGTTCC
CGGAAGAGGAAGAGGGCGGCTGCGAACTGCGCGTGAAGTTTTCCCGGTCC
GCCGACGCTCCGGCGTACCAGCAGGGGCAAAACCAGCTGTACAACGAACT
TAACCTCGGTCGCCGGGAAGAATATGACGTGCTGGACAAGCGGCGGGGAA
GAGATCCCGAGATGGGTGGAAAGCCGCGGCGGAAGAACCCTCAGGAGGGC
TTGTACAACGAGCTGCAAAAGGACAAAATGGCCGAAGCCTACTCCGAGAT
TGGCATGAAGGGAGAGCGCAGACGCGGGAAGGGACACGATGGACTGTACC
AGGGACTGTCAACCGCGACTAAGGACACTTACGACGCCCTGCACATGCAG
GCCCTGCCCCCGCGCTAA

**SEQ ID NO: 12** amino acid sequence of CAR D0130 CDAR123 MB40-H08 CD8 BBz

MLLLVTSLLLCELPHPAFLLIPQVQLVQSGAEVKKPGSSVKVSCKASGGTFSSY
AISWVRQAPGQGLEWMGGIIPIFGTANYAQKFQGRVTITADESTSTAYMELSSL
RSEDTAVYYCARNEWYSYYYYYMGVWGKGTTVTVSSGGGGSGGGGSGGGG
SQSALTQPASVSGSPGQSITISCTGTSSDVGGYNYVSWYQQHPGKAPKLMIYEV

SNRPSGVSNRFSGSKSGNTASLTISGLQAEDEADYYCSSYTSSSTLVVFGGGTK
LTVLAAATTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYI
WAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFP
EEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDP
EMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLS
TATKDTYDALHMQALPPR

**SEQ ID NO: 13** nucleotide sequence of leader/signal peptide sequence

atgctgctgctggtgaccagcctgctgctgtgcgaactgccgcatccggcgtttctgctgattccg

**SEQ ID NO: 14** amino acid sequence of leader/signal peptide sequence

MLLLVTSLLLCELPHPAFLLIP

**SEQ ID NO: 15** nucleotide sequence of CAR D0131 CD123 MB42-D03 CD8 BBz

ATGCTCTTGCTCGTGACTTCTTTGCTTTTGTGCGAACTTCCGCACCCAGCCTT
CCTTTTGATACCTCAAGTTCAACTTGTACAATCCGGAGCAGAAGTAAAAAA
ACCCGGGGCCAGCGTAAAAGTTTCCTGTAAAGCTAGCGGCTACACATTCAC
TAGCTACGGCATCTCCTGGGTACGCCAAGCGCCAGGACAAGGCCTCGAATG
GATGGGATGGATTAGCGCTTACAACGGTAATACCAATTATGCACAAAAGCT
GCAAGGACGGGTTACGATGACAACAGACACGAGCACGAGTACGGCCTATA
TGGAGCTGAGAAGTCTTCGAAGTGATGACACTGCAGTATATTACTGTGCCC
GCGGAGCGTACTACGATTTTTGGAGCAGTTACAGCTGGTTTGATCCCTGGG
GGCAGGGGACCCTGGTTACTGTTAGCTCAGGTGGGGGGGGCTCAGGAGGT
GGAGGAAGCGGGGGTGGAGGATCTAGTTATGTTCTTACCCAGCCGCCTTCT
GTCAGTGTGGCCCCTGGTAAGACAGCCAGGATAACCTGTGGTGGGAATTCA
ATTGGCAGCAAATCAGTACAGTGGTACCAACAAAAACCCGGACAAGCCCC
CGTTTTGGTCATATATGATGATAGCGATAGGCCTTCTGGAATCCCGGAGAG
GTTTTCAGGATCAAATAGCGGGAACACCGCCACATTGACCATAAGTCGAGT
CGAGGCGGGCGACGAAGCTGACTATTATTGTCAAGTGTGGGATAGCTCTAG
TGATGTGGTATTCGGTGGGGGGACCAAATTGACAGTCTTGGCGGCCGCAAC
GACCACTCCTGCACCCCGCCCTCCGACTCCGGCCCCAACCATTGCCAGCCA

GCCCCTGTCCCTGCGGCCGGAAGCCTGCAGACCGGCTGCCGGCGGAGCCGT
CCATACCCGGGGACTGGATTTCGCCTGCGATATCTATATCTGGGCACCACT
CGCCGGAACCTGTGGAGTGCTGCTGCTGTCCCTTGTGATCACCCTGTACTGC
AAGCGCGGACGGAAGAAACTCTTGTACATCTTCAAGCAGCCGTTCATGCGC
CCTGTGCAAACCACCCAAGAAGAGGACGGGTGCTCCTGCCGGTTCCCGGAA
GAGGAAGAGGGCGGCTGCGAACTGCGCGTGAAGTTTTCCCGGTCCGCCGA
CGCTCCGGCGTACCAGCAGGGGCAAAACCAGCTGTACAACGAACTTAACCT
CGGTCGCCGGGAAGAATATGACGTGCTGGACAAGCGGCGGGGAAGAGATC
CCGAGATGGGTGGAAAGCCGCGGCGGAAGAACCCTCAGGAGGGCTTGTAC
AACGAGCTGCAAAAGGACAAAATGGCCGAAGCCTACTCCGAGATTGGCAT
GAAGGGAGAGCGCAGACGCGGGAAGGGACACGATGGACTGTACCAGGGA
CTGTCAACCGCGACTAAGGACACTTACGACGCCCTGCACATGCAGGCCCTG
CCCCCGCGCTAA

**SEQ ID NO: 16** amino acid sequence of CAR D0131 CD123 MB42-D03 CD8 BBz

MLLLVTSLLLCELPHPAFLLIPQVQLVQSGAEVKKPGASVKVSCKASGYTFTSY
GISWVRQAPGQGLEWMGWISAYNGNTNYAQKLQGRVTMTTDTSTSTAYMEL
RSLRSDDTAVYYCARGAYYDFWSSYSWFDPWGQGTLVTVSSGGGGSGGGGS
GGGGSSYVLTQPPSVSVAPGKTARITCGGNSIGSKSVQWYQQKPGQAPVLVIY
DDSDRPSGIPERFSGSNSGNTATLTISRVEAGDEADYYCQVWDSSSDVVFGGG
TKLTVLAAATTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDI
YIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCR
FPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGR
DPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQG
LSTATKDTYDALHMQALPPR

**SEQ ID NO: 17** nucleotide sequence of CAR D0132 CD123 MB42-E02 CD8 BBz

ATGCTCTTGCTCGTGACTTCTTTGCTTTTGTGCGAACTTCCGCACCCAGCCTT
CCTTTTGATACCTCAGGTACAACTTGTCCAATCCGGTGCCGAAGTCAAGAA
ACCTGGAGCATCCGTAAAGGTCAGCTGCAAAGCCAGCGGGTATACCTTCAC
GAGTTATGGAATCTCTTGGGTCAGACAAGCGCCAGGCCAAGGGCTGGAAT
GGATGGGATGGATAAGCGCATACAATGGCAACACAAATTATGCTCAGAAA

CTGCAAGGTCGCGTTACCATGACCACCGACACATCAACGTCCACCGCCTAT
ATGGAGCTTAGAAGCTTGCGAAGTGACGACACAGCCGTGTATTATTGCGCT
CGGGGTGCTTATTATGACTTCTGGTCTGGTTACTCTTGGTTTGATCCTTGGG
GTCAAGGCACGCTTGTGACGGTATCCTCAGGAGGCGGCGGAAGTGGAGGG
GGTGGATCAGGTGGTGGTGGAAGCCAATCAGTACTCACTCAGCCACCAAGT
GTATCAGTGGCTCCAGGTCAGACCGCGCGGATACCGTGTGGAGGAAACAA
CATCGGGTCAAAGGGCGTACATTGGTACCAGCAGAAGTCTGGACAAGCTCC
CGTTATGGTGGTGTACGATGACTCAGACAGGCCATCCGGCATCCCTGAGCG
GTTCAGCGGTTCTAATTCAGGAAATACAGCAACATTGACCATCAGCAGGGT
CGAAGCCGGTGACGAGGCGGACTATTATTGTCAGGTCTGGGATTCAAGCGG
CGACCTTGTTTTGTTTGGGGGTGGAACTAAACTGACCGTACTGGCGGCCGC
AACGACCACTCCTGCACCCCGCCCTCCGACTCCGGCCCCAACCATTGCCAG
CCAGCCCCTGTCCCTGCGGCCGGAAGCCTGCAGACCGGCTGCCGGCGGAGC
CGTCCATACCCGGGGACTGGATTTCGCCTGCGATATCTATATCTGGGCACC
ACTCGCCGGAACCTGTGGAGTGCTGCTGCTGTCCCTTGTGATCACCCTGTAC
TGCAAGCGCGGACGGAAGAAACTCTTGTACATCTTCAAGCAGCCGTTCATG
CGCCCTGTGCAAACCACCCAAGAAGAGGACGGGTGCTCCTGCCGGTTCCCG
GAAGAGGAAGAGGGCGGCTGCGAACTGCGCGTGAAGTTTTCCCGGTCCGC
CGACGCTCCGGCGTACCAGCAGGGGCAAAACCAGCTGTACAACGAACTTA
ACCTCGGTCGCCGGGAAGAATATGACGTGCTGGACAAGCGGCGGGGAAGA
GATCCCGAGATGGGTGGAAAGCCGCGGCGGAAGAACCCTCAGGAGGGCTT
GTACAACGAGCTGCAAAAGGACAAAATGGCCGAAGCCTACTCCGAGATTG
GCATGAAGGGAGAGCGCAGACGCGGGAAGGGACACGATGGACTGTACCAG
GGACTGTCAACCGCGACTAAGGACACTTACGACGCCCTGCACATGCAGGCC
CTGCCCCCGCGCTAA

**SEQ ID NO: 18** amino acid sequence of CAR D0132 CD123 MB42-E02 CD8 BBz

MLLLVTSLLLCELPHPAFLLIPQVQLVQSGAEVKKPGASVKVSCKASGYTFTSY
GISWVRQAPGQGLEWMGWISAYNGNTNYAQKLQGRVTMTTDTSTSTAYMEL
RSLRSDDTAVYYCARGAYYDFWSGYSWFDPWGQGTLVTVSSGGGGSGGGGS
GGGGSQSVLTQPPSVSVAPGQTARIPCGGNNIGSKGVHWYQQKSGQAPVMVV
YDDSDRPSGIPERFSGSNSGNTATLTISRVEAGDEADYYCQVWDSSGDLVLFG
GGTKLTVLAAATTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFA

CDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGC
SCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKR
RGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGL
YQGLSTATKDTYDALHMQALPPR

**SEQ ID NO: 19** nucleotide sequence of CAR D0133 CD123 MB42-E12 CD8 BBz

ATGCTCTTGCTCGTGACTTCTTTGCTTTTGTGCGAACTTCCGCACCCAGCCTT
CCTTTTGATACCTCAGGTGCAACTGGTTCAATCTGGCGCCGAAGTAAAAAA
ACCGGGCGCCAGCGTTAAAGTATCCTGTAAAGCGAGCGGCTACACATTTAC
CAGCTATGGCATCTCATGGGTGAGACAAGCGCCCGGCCAAGGACTGGAAT
GGATGGGGTGGATCAGCGCCTACAATGGGAACACTAACTACGCACAGAAG
CTGCAAGGCCGGGTTACCATGACGACCGATACGAGTACCTCAACAGCGTAC
ATGGAACTTCGAAGTCTGCGCAGTGACGACACCGCAGTATACTACTGCGCC
CGAGGAGCGTACTACGACTTCTGGTCCAGCTACTCTTGGTTTGACCCGTGG
GGCCAAGGAACACTCGTAACAGTATCCAGTGGAGGAGGCGGGTCAGGTGG
CGGTGGTTCAGGCGGTGGCGGGTCATCTTATGTTCTCACTCAGCCCCCATCC
GTGTCCGTAGCGCCAGGGAAAACAGCCCGGATTACGTGCGGGGGAAATAA
TATAGGCAGCAAGAGCGTTCATTGGTATCAACAAAAGCCAGGGCAGGCAC
CGGTCTTGGTGGTCTACGACGACAGTGATCGGCCCTCAGGAATTCCTGAAA
GATTCTCAGGGTCAAATTCTGGCAACACGGCGACGCTTACAATAAGCAGGG
TCGAGGCAGGAGACGAAGCCGATTATTACTGCCAGGTATGGGATTCCTCTT
CTGACCATGTGGTGTTTGGCGGTGGCACAAAGCTCACGGTCTTGGCGGCCG
CAACGACCACTCCTGCACCCCGCCCTCCGACTCCGGCCCCAACCATTGCCA
GCCAGCCCCTGTCCCTGCGGCCGGAAGCCTGCAGACCGGCTGCCGGCGGAG
CCGTCCATACCCGGGGACTGGATTTCGCCTGCGATATCTATATCTGGGCAC
CACTCGCCGGAACCTGTGGAGTGCTGCTGCTGTCCCTTGTGATCACCCTGTA
CTGCAAGCGCGGACGGAAGAAACTCTTGTACATCTTCAAGCAGCCGTTCAT
GCGCCCTGTGCAAACCACCCAAGAAGAGGACGGGTGCTCCTGCCGGTTCCC
GGAAGAGGAAGAGGGCGGCTGCGAACTGCGCGTGAAGTTTTCCCGGTCCG
CCGACGCTCCGGCGTACCAGCAGGGGCAAAACCAGCTGTACAACGAACTT
AACCTCGGTCGCCGGGAAGAATATGACGTGCTGGACAAGCGGCGGGGAAG
AGATCCCGAGATGGGTGGAAAGCCGCGGCGGAAGAACCCTCAGGAGGGCT
TGTACAACGAGCTGCAAAAGGACAAAATGGCCGAAGCCTACTCCGAGATT

GGCATGAAGGGAGAGCGCAGACGCGGGAAGGGACACGATGGACTGTACCA
GGGACTGTCAACCGCGACTAAGGACACTTACGACGCCCTGCACATGCAGGC
CCTGCCCCCGCGCTAA

**SEQ ID NO: 20** amino acid sequence of CAR D0133 CD123 MB42-E12 CD8 BBz

MLLLVTSLLLCELPHPAFLLIPQVQLVQSGAEVKKPGASVKVSCKASGYTFTSY
GISWVRQAPGQGLEWMGWISAYNGNTNYAQKLQGRVTMTTDTSTSTAYMEL
RSLRSDDTAVYYCARGAYYDFWSSYSWFDPWGQGTLVTVSSGGGGSGGGGS
GGGGSSYVLTQPPSVSVAPGKTARITCGGNNIGSKSVHWYQQKPGQAPVLVV
YDDSDRPSGIPERFSGSNSGNTATLTISRVEAGDEADYYCQVWDSSSDHVVFG
GGTKLTVLAAATTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFA
CDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGC
SCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKR
RGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGL
YQGLSTATKDTYDALHMQALPPR

**SEQ ID NO: 21** nucleotide sequence of CAR D0134 CD123 MB44-H01 CD8 BBz

ATGCTCTTGCTCGTGACTTCTTTGCTTTTGTGCGAACTTCCGCACCCAGCCTT
CCTTTTGATACCTCAGGTTCAACTCGTTCAAAGCGGGGCTGAAGTTAAAAA
GCCGGGGTCTAGCGTTAAGGTTTCCTGTAAAGCGTCTGGAGGAACTTTTTC
CTCCTACGCCATTAGCTGGGTACGACAAGCTCCAGGACAGGGTCTCGAGTG
GATGGGTGGGATAATTCCGATCTTTGGAACTGCGAATTACGCCCAGCGATT
CCAAGGCCGAGTTACGATTACTGCTGACGAGAGTACGTCTACCGCATACAT
GGAATTGAGTTCTCTTCGGTCAGAAGATACAGCGGTATACTACTGCGCTAG
GGGCCTCGGCACTAGTTACTACTATTACTATATGGATGTATGGGGCAAGGG
CACAACTGTGACTGTTTCTAGCGGTGGCGGCGGGTCCGGTGGTGGTGGAAG
CGGTGGCGGAGGGTCACAGTCAGTACTCACTCAGCCACCGAGTGCGTCTGG
CTCACCAGGACAATCTGTAACCATTAGTTGCACAGGCACTAGCTCTGATGT
TGGGGGCTACAATTATGTCTCCTGGTACCAACAACACCCCGGAAAAGCGCC
GAAGCTGATGATCTACGAGGTGAGTAATAGACCTAGTGGTGTTAGTAACAG
GTTCTCAGGCTCTAAGTCCGGTAACACCGCGTCTCTCACTATATCTGGCCTT
CAAGCTGAGGACGAGGCAGACTATTATTGCAGCTCATACACCTCAAGCAGT

ACCCCCGTTGTGTTTGGTGGCGGTACCAAATTGACTGTGCTGGCGGCCGCA
ACGACCACTCCTGCACCCCGCCCTCCGACTCCGGCCCCAACCATTGCCAGC
CAGCCCCTGTCCCTGCGGCCGGAAGCCTGCAGACCGGCTGCCGGCGGAGCC
GTCCATACCCGGGGACTGGATTTCGCCTGCGATATCTATATCTGGGCACCA
CTCGCCGGAACCTGTGGAGTGCTGCTGCTGTCCCTTGTGATCACCCTGTACT
GCAAGCGCGGACGGAAGAAACTCTTGTACATCTTCAAGCAGCCGTTCATGC
GCCCTGTGCAAACCACCCAAGAAGAGGACGGGTGCTCCTGCCGGTTCCCGG
AAGAGGAAGAGGGCGGCTGCGAACTGCGCGTGAAGTTTTCCCGGTCCGCC
GACGCTCCGGCGTACCAGCAGGGGCAAAACCAGCTGTACAACGAACTTAA
CCTCGGTCGCCGGGAAGAATATGACGTGCTGGACAAGCGGCGGGGAAGAG
ATCCCGAGATGGGTGGAAAGCCGCGGCGGAAGAACCCTCAGGAGGGCTTG
TACAACGAGCTGCAAAAGGACAAAATGGCCGAAGCCTACTCCGAGATTGG
CATGAAGGGAGAGCGCAGACGCGGGAAGGGACACGATGGACTGTACCAGG
GACTGTCAACCGCGACTAAGGACACTTACGACGCCCTGCACATGCAGGCCC
TGCCCCCGCGCTAA

**SEQ ID NO: 22** amino acid sequence of CAR D0134 CD123 MB44-H01 CD8 BBz

MLLLVTSLLLCELPHPAFLLIPQVQLVQSGAEVKKPGSSVKVSCKASGGTFSSY
AISWVRQAPGQGLEWMGGIIPIFGTANYAQRFQGRVTITADESTSTAYMELSSL
RSEDTAVYYCARGLGTSYYYYYMDVWGKGTTVTVSSGGGGSGGGGSGGGGS
QSVLTQPPSASGSPGQSVTISCTGTSSDVGGYNYVSWYQQHPGKAPKLMIYEV
SNRPSGVSNRFSGSKSGNTASLTISGLQAEDEADYYCSSYTSSSTPVVFGGGTKL
TVLAAATTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIW
APLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPE
EEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPE
MGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLST
ATKDTYDALHMQALPPR

**SEQ ID NO: 23** nucleotide sequence of CAR LTG2078 CD123 M12306 CD8 BBz

ATGCTGCTGCTGGTGACCAGCCTGCTGCTGTGCGAACTGCCGCATCCGGCG
TTTCTGCTGATTCCGGAAGTCCAATTGGTGCAGAGCGGATCCGAACTTAAG
AAACCTGGCGCGAGCGTGAAAGTGTCCTGCAAGGCCTCCGGAGGGACTTTC

TCGTCGTACGCCATTAGCTGGGTCCGCCAAGCTCCTGGCCAAGGCCTGGAG
TGGATGGGCGGGATTATCCCCATCTTCGGGACTGCGAACTACGCCCAGAAG
TTTCAGGGCCGGGTCACTATCACCGCCGACGAATCAACCTCGACCGCCTAC
ATGGAACTGTCCTCGCTTCGGTCCGAGGATACTGCCGTGTACTATTGTGCCT
CAACGGCCAGACGCGGATGGGACACCGCTGGTCCGCTCGATTACTGGGGCC
AGGGAACCCTCGTGACCGTCAGCTCCGGAGGAGGAGGCTCCGGTGGTGGA
GGATCCGGGGGTGGTGGATCCGACATCCAAATGACCCAGTCCCCCTCGTCC
CTGAGCGCCTCTGTGGGCGACAGAGTGACAATTGCATGCAGGGCCTCACAG
ACTATCTCCCGCTACCTGAACTGGTACCAGCAGAAGCCAGGAAAGGCCCCT
AAGCTGCTCATCTACGCTGCGTCCTCGCTCCAATCCGGGGTGTCCTCACGGT
TTTCCGGATCGGGTTCCGGCACCGAGTTCACCCTGACCATCAGCAGCCTGC
AGCCCGAGGACTTCGCAACCTACTTCTGCCAGCAAACCTACTCCCCGCCGA
TTACGTTCGGACAGGGGACTCGGCTGGAAATCAAGGCGGCCGCAACTACC
ACCCCTGCCCCTCGGCCGCCGACTCCGGCCCCAACCATCGCAAGCCAACCC
CTCTCCTTGCGCCCCGAAGCTTGCCGCCCGGCCGCGGGTGGAGCCGTGCAT
ACCCGGGGGCTGGACTTTGCCTGCGATATCTACATTTGGGCCCCGCTGGCC
GGCACTTGCGGCGTGCTCCTGCTGTCGCTGGTCATCACCCTTTACTGCAAGA
GGGGCCGGAAGAAGCTGCTTTACATCTTCAAGCAGCCGTTCATGCGGCCCG
TGCAGACGACTCAGGAAGAGGACGGATGCTCGTGCAGATTCCCTGAGGAG
GAAGAGGGGGGATGCGAACTGCGCGTCAAGTTCTCACGGTCCGCCGACGC
CCCCGCATATCAACAGGGCCAGAATCAGCTCTACAACGAGCTGAACCTGGG
AAGGAGAGAGGAGTACGACGTGCTGGACAAGCGACGCGGACGCGACCCGG
AGATGGGGGGGAAACCACGGCGGAAAAACCCTCAGGAAGGACTGTACAAC
GAACTCCAGAAAGACAAGATGGCGGAAGCCTACTCAGAAATCGGGATGAA
GGGAGAGCGGAGGAGGGGAAAGGGTCACGACGGGCTGTACCAGGGACTG
AGCACCGCCACTAAGGATACCTACGATGCCTTGCATATGCAAGCACTCCCA
CCCCGGTAG

**SEQ ID NO: 24** amino acid sequence of CAR LTG2078 CD123 M12306 CD8 BBz

MLLLVTSLLLCELPHPAFLLIPEVQLVQSGSELKKPGASVKVSCKASGGTFSSY
AISWVRQAPGQGLEWMGGIIPIFGTANYAQKFQGRVTITADESTSTAYMELSSL
RSEDTAVYYCASTARRGWDTAGPLDYWGQGTLVTVSSGGGGSGGGGSGGGG
SDIQMTQSPSSLSASVGDRVTIACRASQTISRYLNWYQQKPGKAPKLLIYAASS

LQSGVSSRFSGSGSGTEFTLTISSLQPEDFATYFCQQTYSPPITFGQGTRLEIKAA
ATTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAG
TCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGG
CELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKP
RRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDT
YDALHMQALPPR

**SEQ ID NO: 25** nucleotide sequence of CAR LTG1906 CD33_4 CD8 BBz

ATGCTGCTGCTGGTGACCAGCCTGCTGCTGTGCGAACTGCCGCATCCGGCG
TTTCTGCTGATTCCGGAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTA
CAGCCTGGAGGGTCCCTGAGACTCTCCTGTGCAGCCTCTGGATTCACCTTCA
GTAGCTATGGCATGAGCTGGGTCCGCCAGGCTCCAAGACAAGGGCTTGAGT
GGGTGGCCAACATAAAGCAAGATGGAAGTGAGAAATACTATGCGGACTCA
GTGAAGGGCCGATTCACCATCTCCAGAGACAATTCCAAGAACACGCTGTAT
CTGCAAATGAACAGCCTGAGAGCCGAGGACACAGCCACGTATTACTGTGC
GAAAGAAAATGTGGACTGGGGCCAGGGCACCCTGGTCACCGTCTCCTCAGC
GGCCGCAACTACCACCCCTGCCCCTCGGCCGCCGACTCCGGCCCCAACCAT
CGCAAGCCAACCCCTCTCCTTGCGCCCCGAAGCTTGCCGCCCGGCCGCGGG
TGGAGCCGTGCATACCCGGGGGCTGGACTTTGCCTGCGATATCTACATTTG
GGCCCCGCTGGCCGGCACTTGCGGCGTGCTCCTGCTGTCGCTGGTCATCAC
CCTTTACTGCAAGAGGGGCCGGAAGAAGCTGCTTTACATCTTCAAGCAGCC
GTTCATGCGGCCCGTGCAGACGACTCAGGAAGAGGACGGATGCTCGTGCA
GATTCCCTGAGGAGGAAGAGGGGGGATGCGAACTGCGCGTCAAGTTCTCA
CGGTCCGCCGACGCCCCCGCATATCAACAGGGCCAGAATCAGCTCTACAAC
GAGCTGAACCTGGGAAGGAGAGAGGAGTACGACGTGCTGGACAAGCGACG
CGGACGCGACCCGGAGATGGGGGGGAAACCACGGCGGAAAAACCCTCAGG
AAGGACTGTACAACGAACTCCAGAAAGACAAGATGGCGGAAGCCTACTCA
GAAATCGGGATGAAGGGAGAGCGGAGGAGGGGAAAGGGTCACGACGGGC
TGTACCAGGGACTGAGCACCGCCACTAAGGATACCTACGATGCCTTGCATA
TGCAAGCACTCCCACCCCGGTAG

**SEQ ID NO: 26** amino acid sequence of CAR LTG1906 CD33_4 CD8 BBz

MLLLVTSLLLCELPHPAFLLIPEVQLVESGGGLVQPGGSLRLSCAASGFTFSSYG
MSWVRQAPRQGLEWVANIKQDGSEKYYADSVKGRFTISRDNSKNTLYLQMNS
LRAEDTATYYCAKENVDWGQGTLVTVSSAAATTTPAPRPPTPAPTIASQPLSLR
PEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKL
LYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQN
QLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAE
AYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

**SEQ ID NO: 27** nucleotide sequence of DNA CD8 transmembrane domain

atctacatct gggcgccctt ggccgggact tgtggggtcc ttctcctgtc actggttatc acccttttact gc

**SEQ ID NO: 28** amino acid sequence of CD8 transmembrane domain

Ile Trp Ala Pro Leu Ala Gly Thr Cys Gly Val Leu Leu Leu Ser Leu
Val Ile Thr Leu Tyr Cys

**SEQ ID NO: 29** nucleotide sequence of DNA CD8 hinge domain

accacgacgc cagcgccgcg accaccaaca ccggcgccca ccatcgcgtc gcagcccctg
tccctgcgcc cagaggcgtg ccggccagcg gcgggggggcg cagtgcacac gagggggctg
gacttcgcct gtgat

**SEQ ID NO: 30** amino acid sequence of CD8 hinge domain

Thr Thr Thr Pro Ala Pro Arg Pro Pro Thr Pro Ala Pro Thr Ile Ala
Ser Gln Pro Leu Ser Leu Arg Pro Glu Ala Cys Arg Pro Ala Ala Gly
Gly Ala Val His Thr Arg Gly Leu Asp Phe Ala Cys Asp Ile Tyr

**SEQ ID NO: 31** amino acid sequence of amino acid numbers 118 to 178 hinge region of CD8.alpha. (NCBI RefSeq: NP.sub.--001759.3)

Lys Arg Gly Arg Lys Lys Leu Leu Tyr Ile Phe Lys Gln Pro Phe Met
Arg Pro Val Gln Thr Thr Gln Glu Glu Asp Gly Cys Ser Cys Arg Phe
Pro Glu Glu Glu Glu Gly Gly Cys Glu Leu

**SEQ ID NO: 32** amino acid sequence of Human IgG CL sequence

Gly Gln Pro Lys Ala Ala Pro Ser Val Thr Leu Phe Pro Pro Ser Ser
Glu Glu Leu Gln Ala Asn Lys Ala Thr Leu Val Cys Leu Ile Ser Asp
Phe Tyr Pro Gly Ala Val Thr Val Ala Trp Lys Ala Asp Ser Ser Pro
Val Lys Ala Gly Val Glu Thr Thr Thr Pro Ser Lys Gln Ser Asn Asn
Lys Tyr Ala Ala Ser Ser Tyr Leu Ser Leu Thr Pro Glu Gln Trp Lys
Ser His Arg Ser Tyr Ser Cys Gln Val Thr His Glu Gly Ser Thr Val
Glu Lys Thr Val Ala Pro Thr Glu Cys Ser

**SEQ ID NO: 33** nucleotide sequence of DNA signaling domain of 4-1BB

aaacggggca gaaagaaact cctgtatata ttcaaacaac catttatgag accagtacaa
actactcaag aggaagatgg ctgtagctgc cgatttccag aagaagaaga aggaggatgt
gaactg

**SEQ ID NO: 34** amino acid sequence of signaling domain of 4-1BB

Lys Arg Gly Arg Lys Lys Leu Leu Tyr Ile Phe Lys Gln Pro Phe Met
Arg Pro Val Gln Thr Thr Gln Glu Glu Asp Gly Cys Ser Cys Arg Phe
Pro Glu Glu Glu Glu Gly Gly Cys Glu Leu

**SEQ ID NO: 35** nucleotide sequence of DNA signaling domain of CD3-zeta

agagtgaagt tcagcaggag cgcagacgcc cccgcgtaca agcagggcca gaaccagctc
tataacgagc tcaatctagg acgaagagag gagtacgatg ttttggacaa gagacgtggc
cgggaccctg agatgggggg aaagccgaga aggaagaacc ctcaggaagg cctgtacaat
gaactgcaga aagataagat ggcggaggcc tacagtgaga ttgggatgaa aggcgagcgc
cggaggggca aggggcacga tggcctttac cagggtctca gtacagccac caaggacacc
tacgacgccc ttcacatgca ggccctgccc cctcgc

**SEQ ID NO: 36** amino acid sequence of CD3zeta

Arg Val Lys Phe Ser Arg Ser Ala Asp Ala Pro Ala Tyr Lys Gln Gly
Gln Asn Gln Leu Tyr Asn Glu Leu Asn Leu Gly Arg Arg Glu Glu Tyr
Asp Val Leu Asp Lys Arg Arg Gly Arg Asp Pro Glu Met Gly Gly Lys
Pro Arg Arg Lys Asn Pro Gln Glu Gly Leu Tyr Asn Glu Leu Gln Lys
Asp Lys Met Ala Glu Ala Tyr Ser Glu Ile Gly Met Lys Gly Glu Arg
Arg Arg Gly Lys Gly His Asp Gly Leu Tyr Gln Gly Leu Ser Thr Ala
Thr Lys Asp Thr Tyr Asp Ala Leu His Met Gln Ala Leu Pro Pro Arg

**SEQ ID NO: 37** nucleotide sequence of ScFv CD 19

gacatccaga tgacacagac tacatcctcc ctgtctgcct ctctgggaga cagagtcaccatcagttgca gggcaagtca ggacattagt aaatatttaa attggtatca gcagaaacca gatggaactg ttaaactcct gatctaccat acatcaagat tacactcagg agtcccatca aggttcagtg gcagtgggtc tggaacagat tattctctca ccattagcaa cctggagcaa gaagatattg ccacttactt ttgccaacag ggtaatacgc ttccgtacac gttcggaggg gggaccaagc tggagatcac aggtggcggt ggctcgggcg gtggtgggtc gggtggcggc ggatctgagg tgaaactgca ggagtcagga cctggcctgg tggcgccctc acagagcctg tccgtcacat gcactgtctc aggggtctca ttacccgact atggtgtaag ctggattcgc cagcctccac gaaagggtct ggagtggctg ggagtaatat ggggtagtga aaccacatac tataattcag ctctcaaatc cagactgacc atcatcaagg acaactccaa gagccaagtt ttcttaaaaa tgaacagtct gcaaactgat gacacagcca tttactactg tgccaaacat tattactacg gtggtagcta tgctatggac tactggggcc aaggaacctc agtcaccgtc tcctca

**SEQ ID NO: 38** amino acid sequence of ScFv CD 19

Asp Ile Gln Met Thr Gln Thr Thr Ser Ser Leu Ser Ala Ser Leu Gly Asp Arg Val Thr Ile Ser
Cys Arg Ala Ser Gln Asp Ile Ser Lys Tyr Leu Asn Trp Tyr Gln Gln Lys Pro Asp Gly Thr Val
Lys Leu Leu Ile Tyr His Thr Ser Arg Leu His Ser Gly Val Pro Ser Arg Phe Ser Gly Ser Gly
Ser Gly Thr Asp Tyr Ser Leu Thr Ile Ser Asn Leu Glu Gln Glu Asp Ile Ala Thr Tyr Phe Cys
Gln Gln Gly Asn Thr Leu Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Thr Gly Gly Gly
Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu Val Lys Leu Gln Glu Ser Gly Pro Gly
Leu Val Ala Pro Ser Gln Ser Leu Ser Val Thr Cys Thr Val Ser Gly Val Ser Leu Pro Asp Tyr
Gly Val Ser Trp Ile Arg Gln Pro Pro Arg Lys Gly Leu Glu Trp Leu Gly Val Ile Trp Gly Ser
Glu Thr Thr Tyr Tyr Asn Ser Ala Leu Lys Ser Arg Leu Thr Ile Ile Lys Asp Asn Ser Lys Ser

Gln Val Phe Leu Lys Met Asn Ser Leu Gln Thr Asp Asp Thr Ala Ile Tyr Tyr Cys Ala Lys His Tyr Tyr Tyr Gly Gly Ser Tyr Ala Met Asp Tyr Trp Gly Gln Gly Thr Ser Val Thr Val Ser Ser

**SEQ ID NO: 39** nucleotide sequence of GMCSF leader peptide

ATGCTGCTGCTGGTGACCAGCCTGCTGCTGTGCGAACTGCCGCATCCGGCG
TTTCTGCTGATTCCG

**SEQ ID NO: 40** amino acid sequence of GMCSF leader peptide

MLLLVTSLLLCELPHPAFLLIP

**SEQ ID NO: 41** nucleotide sequence of TNFRSF19 leader peptide

GGCTCTGAAAGTGCTGTTGGAACAAGAAAAGACCTTCTTCACCTTGCTCGT
GTTGCTGGGGTACCTGTCCTGCAAAGTCACCTGT

**SEQ ID NO: 42** amino acid sequence of TNFRSF19 leader peptide

MALKVLLEQEKTFFTLLVLLGYLSCKVTC

**SEQ ID NO: 43** nucleotide sequence of CD8 alpha leader peptide

atggcgctgccggtgaccgcgctgctgctgccgctggcgctgctgctgcatgcggcgcgc
ccg

**SEQ ID NO: 44** amino acid sequence of CD8 alpha leader peptide

MALPVTALLLPLALLLHAARP

**SEQ ID NO: 45** nucleotide sequence of CD28 co-stimulatory domain

CGGTCGAAGAGGTCCAGACTCTTGCACTCCGACTACATGAACATGACTCCT
AGAAGGCCCGGACCCACTAGAAAGCACTACCAGCCGTACGCCCCTCCTCGG
GATTTCGCCGCATACCGG TCC

**SEQ ID NO: 46** amino acid sequence of CD28 co-stimulatory domain

RSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRS

**SEQ ID NO: 47** nucleotide sequence of CD3 zeta activation domain

AGAGTGAAGTTCAGCCGCTCAGCCGATGCACCGGCCTACCAGCAGGGACA
GAACCAGCTCTACAACGAGCTCAACCTGGGTCGGCGGGAAGAATATGACGT
GCTGGACAAACGGCGCGGCAGAGATCCGGAGATGGGGGGAAAGCCGAGGA
GGAAGAACCCTCAAGAGGGCCTGTACAACGAACTGCAGAAGGACAAGATG
GCGGAAGCCTACTCCGAGATCGGCATGAAGGGAGAACGCCGGAGAGGGAA
GGGTCATGACGGACTGTACCAGGGCCTGTCAACTGCCACTAAGGACACTTA
CGATGCGCTCCATATGCAAGCTTTGCCCCCGCGG

**SEQ ID NO: 48** amino acid sequence of CD3 zeta activation domain

RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRR
KNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYD
ALHMQALPPR

**SEQ ID NO: 49** nucleotide sequence of TNFRSF19 hinge and transmembrane domain (transmembrane domain underlined)

GCGGCCGCGGTCGGATTCCAAGACATGGAATGCGTGCCCTGCGGCGACCCG
CCACCTCCTTACGAGCCGCACTGCGCATCGAAGGTCAACCTCGTGAAGATC
GCGAGCACCGCGTCCTCACCCCGGGATACTGCTCTGGCCGCCGTGATTTGTT
CCGCCTTGGCCACCGTGCTTCTGGCCCTGCTGATCCTCTGTGTGATC

**SEQ ID NO: 50** amino acid sequence of TNFRSF19 hinge and transmembrane domain (transmembrane domain underlined)

A A A V G F Q D M E C V P C G D P P P P Y E P H C A S K V N L V K I A S T A
S S P R D T A L A A V I C S A L A T V L L A L L I L C V I

**SEQ ID NO: 51** nucleotide sequence of TNFRSF19 transmembrane domain
GCCGCCGTGATTTGTTCCGCCTTGGCCACCGTGCTTCTGGCCCTGCTGATCC
TCTGTGTGATC

**SEQ ID NO: 52** amino acid sequence of TNFRSF19 transmembrane domain

A A V I C S A L A T V L L A L L I L C V I

**SEQ ID NO: 53** nucleotide sequence of TNFRSF19 hinge domain

GCGGCCGCGGTCGGATTCCAAGACATGGAATGCGTGCCCTGCGGCGACCCG
CCACCTCCTTACGAGCCGCACTGCGCATCGAAGGTCAACCTCGTGAAGATC
GCGAGCACCGCGTCCTCACCCCGGGATACTGCTCTG

**SEQ ID NO: 54** amino acid sequence of TNFRSF19 hinge domain

A A A V G F Q D M E C V P C G D P P P P Y E P H C A S K V N L V K I A S T A S
S P R D T A L

**SEQ ID NO: 55** nucleotide sequence of truncated TNFRSF19 hinge domain

TACGAGCCTCACTGCGCCAGCAAAGTCAACTTGGTGAAGATCGCGAGCACT
GCCTCGTCCCCTCGGGACACTGCTCTGGC

**SEQ ID NO: 56** amino acid sequence of truncated TNFRSF19 hinge domain

Y E P H C A S K V N L V K I A S T A S S P R D T A L

**SEQ ID NO: 57** nucleotide sequence of CD8a hinge domain fused to TNFRSF19 transmembrane domain(transmembrane sequence underlined)

GCGGCCGCGCCCGCCCCTCGGCCCCCGACTCCTGCCCCGACGATCGCTTCCC
AACCTCTCTCGCTGCGCCCGGAAGCATGCCGGCCCGCCGCCGGTGGCGCTG
TCCACACTCGCGGACTGGACTTTGATACCGCACTGGCGGCCGTGATCTGTA
GCGCCCTGGCCACCGTGCTGCTGGCGCTGCTCATCCTTTGCGTGATCTACTG
CAAGCGGCAGCCTAGG

**SEQ ID NO: 58** amino acid sequence of CD8a hinge domain fused to TNFRSF19 transmembrane domain (transmembrane sequence underlined)

A A A P A P R P P T P A P T I A S Q P L S L R P E A C R P A A G G A V H T R G
L D F D T A L A A V I C S A L A T V L L A L L I L C V I Y C K R Q P R

**SEQ ID NO: 59** nucleotide sequence of CD28 co-stimulatory domain

CGGTCGAAGAGGTCCAGACTCTTGCACTCCGACTACATGAACATGACTCCT
AGAAGGCCCGGACCCACTAGAAAGCACTACCAGCCGTACGCCCCTCCTCGG
GATTTCGCCGCATACCGGTCC

**SEQ ID NO: 60** amino acid sequence of CD28 co-stimulatory domain

RSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRS

**SEQ ID NO: 61** nucleotide sequence of CD3 zeta version 2

cgcgtgaaatttagccgcagcgcggatgcgccggcgtatcagcagggccagaaccagctg
tataacgaactgaacctgggccgccgcgaagaatatgatgtgctggataaacgccgcggc
cgcgatccggaaatgggcggcaaaccgcgccgcaaaaacccgcaggaaggcctgtataac
gaactgcagaaagataaaatggcggaagcgtatagcgaaattggcatgaaaggcgaacgc
cgccgcggcaaaggccatgatggcctgtatcagggcctgagcaccgcgaccaaagatacc
tatgatgcgctgcatatgcaggcgctgccgccgcgc

**SEQ ID NO: 62** amino acid sequence of CD3 zeta version 2

R V K F S R S A D A P A Y Q Q G Q N Q L Y N E L N L G R R E
E Y D V L D K R R G R D P E M G G K P R R K N P Q E G L Y N
E L Q K D K M A E A Y S E I G M K G E R R R G K G H D G L Y
Q G L S T A T K D T Y D A L H M Q A L P P R

**SEQ ID NO: 63** nucleotide sequence of Furin P2A Furin

CGCGCGAAACGCAGCGGCAGCGGCGCGACCAACTTTAGCCTGCTGAAACA
GGCGGGCGAT GTGGAAGAAAACCCGGGCCCGCGAGCAAAGAGG

**SEQ ID NO: 64** amino acid sequence of Furin P2A Furin (furin sequence underlined)

RAKRSGSGATNFSLLKQAGDVEENPGPRAKR

**SEQ ID NO: 65** nucleotide sequence of Furin T2A

AGAGCTAAACGCTCTGGGTCTGGTGAAGGACGAGGTAGCCTTCTTACGTGC
GGAGACGTGGAGGAAAACCCAGGACCC

**SEQ ID NO: 66** amino acid sequence of Furin T2A (furin sequence underlined)

RAKRSGSGEGRGSLLTCGDVEENPGP

**SEQ ID NO: 67** nucleotide sequence of truncated EGFR (tEGFR) tag

AGGAAGGTTTGCAATGGAATCGGTATAGGGGAGTTTAAGGATTCACTTAGC
ATAAACGCTACTAATATTAAACACTTCAAAAACTGTACGAGTATAAGTGGA
GATCTTCACATTTTGCCGGTTGCATTCCGAGGCGATTCATTCACCCACACGC
CACCGCTTGACCCACAAGAATTGGATATTCTTAAAACCGTTAAAGAAATAA
CGGGGTTTTTGCTCATTCAAGCGTGGCCAGAAAATCGCACTGACCTCCATG
CTTTCGAGAACCTGGAGATTATAAGAGGACGAACTAAGCAGCATGGTCAAT
TCTCCCTTGCTGTGGTCAGCCTGAACATCACCAGTCTTGGTTTGCGGTCCCT
CAAGGAAATTTCAGATGGAGATGTCATCATAAGCGGCAACAAGAATTTGTG
CTATGCAAATACCATAAACTGGAAAAAACTGTTTGGCACTTCCGGCCAGAA
AACCAAGATTATTTCAAATCGGGGTGAGAACAGCTGCAAAGCCACCGGCCA
GGTTTGTCATGCCTTGTGCTCTCCGGAAGGCTGTTGGGGGCCAGAACCCAG
GGACTGCGTCAGTTGCAGAAACGTCTCAAGAGGCCGCGAATGCGTTGACAA
GTGTAACCTCCTTGAGGGTGAGCCACGAGAGTTTGTTGAGAACAGCGAGTG
TATACAATGTCACCCTGAATGTTTGCCCCAGGCTATGAATATAACCTGCACA
GGCCGCGGGCCTGATAACTGCATCCAGTGTGCTCATTACATAGATGGACCT
CACTGTGTGAAAACCTGCCCGGCCGGAGTTATGGGAGAAAACAACACTCTG
GTGTGGAAATACGCTGATGCAGGCCACGTGTGCCACCTTTGTCACCCGAAT
TGTACATATGGGTGTACCGGTCCTGGACTTGAAGGTTGCCCTACCAATGGC
CCTAAAATACCCAGTATCGCAACTGGCATGGTAGGCGCTCTTCTCTTGCTCT
TGGTAGTTGCTCTCGGCATAGGTCTTTTTATG

**SEQ ID NO: 68** amino acid sequence of truncated EGFR (tEGFR) tag

RKVCNGIGIGEFKDSLSINATNIKHFKNCTSISGDLHILPVAFRGDSFTHTPPLDP
QELDILKTVKEITGFLLIQAWPENRTDLHAFENLEIIRGRTKQHGQFSLAVVSLN
ITSLGLRSLKEISDGDVIISGNKNLCYANTINWKKLFGTSGQKTKIISNRGENSCK
ATGQVCHALCSPEGCWGPEPRDCVSCRNVSRGRECVDKCNLLEGEPREFVENS
ECIQCHPECLPQAMNITCTGRGPDNCIQCAHYIDGPHCVKTCPAGVMGENNTL
VWKYADAGHVCHLCHPNCTYGCTGPGLEGCPTNGPKIPSIATGMVGALLLLL
VVALGIGLFM

**SEQ ID NO: 69** nucleotide sequence of CD123 binder MT-16

CAGGTCCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGGTCCTCGGTG
AAGGTCTCCTGCAAGGCTTCTGGAGGCACCTTCAGCAGCTATGCTATCAGCTGGG
TGCGACAGGCCCCTGGACAAGGGCTTGAGTGGATGGGAGGGATCATCCCTATCTT
TGGTACAGCAAACTACGCACAGAAGTTCCAGGGCAGAGTCACGATTACCGCGGA

CGAATCCACGAGCACAGCCTACACGGAGCTGAGCAGCCTGAGATCTGAGGACAC
GGCCGTGTATTACTGTGCGAGAGCCCGGTTGGGAGGAGCTTTTGATATCTGGGGC
CAAGGGACAATGGTCACCGTCTCTTCAGGAGGTGGCGGGTCTGGTGGAGGCGGT
AGCGGTGGTGGCGGATCCCAGTCTGTGCTGACGCAGCCGCCCTCAGTGTCTGCGG
CCCCAGGACAGAAGGTCACCATCTCCTGCTCTGGAGGCAGCTCCAACATTGGCAA
TCATTATGTGTCCTGGTATCAGCAGCTCCCAGGAGCAGCCCCCAAACTCCTCATTT
ATGACGATAATAAGCGACCCTCAGGGATTCCTGACCGATTCTCTGGCTCCAGGTC
TGGCACGTCAGCCACCCTGGGCATCACCGGACTCCAGAGTGGGGACGAGGCCGA
TTATTACTGCGGAGCATGGGATAGTAGTCTTGCTGCTCATGTCTTCGGAACTGGG
ACCAAGGTCACCGTCCTAGGT

**SEQ ID NO: 70** amino acid sequence of CD123 binder MT-16

QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGGIIP
IFGTANYAQKFQGRVTITADESTSTAYTELSSLRSEDTAVYYCARARLGGAFDI
WGQGTMVTVSSGGGGSGGGGSGGGGSQSVLTQPPSVSAAPGQKVTISCSGGSS
NIGNHYVSWYQQLPGAAPKLLIYDDNKRPSGIPDRFSGSRSGTSATLGITGLQS
GDEADYYCGAWDSSLAAHVFGTGTKVTVLG

**SEQ ID NO: 71** nucleotide sequence of CD123 binder MT-32

CAGGTACAGCTGCAGCAGTCAGGTCCAGGACTGGTGAAGCCCTCGCAGACC
CTCTCACTCACCTGTGCCATCTCCGGGGACAGTGTCTCTAGCAACAGTGCTG
CTTGGAACTGGATCAGGCAGTCCCCATCGAGAGGCCTTGAGTGGCTGGGAA
GGACATACTACAGGTCCAAGTGGTATAATGATTATGCAGTATCTGTGAAAA
GTCGAATAACCATCAACCCAGACACATCCAAGAACCAGTTCTCCCTGCAGC
TGAACTCTGTGACTCCCGAGGACATGGCTGTGTATTACTGTGCAAGAGGCG
TTGATAGTAGCTTTGACTACTGGGGCCAGGGAACCCTGGTCACCGTCTCCTC
AGGAGGTGGCGGGTCTGGTGGAGGCGGTAGCGGTGGTGGCGGATCCCAGT
CTGTCGTGACGCAGCCGCCCTCAGTGTCTGCGGCCCCAGGACAGAGCGTCA
CCATCTCCTGTTCTGGAAGCAGTTCCACCGTTGGCGATAATTATGTGTCCTG
GTACCAGCAACTCCCAGGAACAGCCCCCAAACTCCTCATTTTTGACGATTAT
AAACGACCCTCAGGGGTTCCTGACCGATTCTCTGGCTCCCAGTCTGGCACCT
CAGCCTCCCTGGTCATCACTGGTCTCCAGGCAGAAGATGAGGCTGATTATT
ACTGCCAGTCTTATGACAGCAGCCTGAGTGGTTATGTCTTCGGGCCTGGGA
CCAAGGTCACCGTCCTAGGT

**SEQ ID NO: 72** amino acid sequence of CD123 binder MT-32

QVQLQQSGPGLVKPSQTLSLTCAISGDSVSSNSAAWNWIRQSPSRGLEWLGRT
YYRSKWYNDYAVSVKSRITINPDTSKNQFSLQLNSVTPEDMAVYYCARGVDSS
FDYWGQGTLVTVSSGGGGSGGGGSGGGGSQSVVTQPPSVSAAPGQSVTISCSG
SSSTVGDNYVSWYQQLPGTAPKLLIFDDYKRPSGVPDRFSGSQSGTSASLVITG
LQAEDEADYYCQSYDSSLSGYVFGPGTKVTVLG

**SEQ ID NO: 73** nucleotide sequence of DNA signaling domain of 4-1BB

AAGCGCGGACGGAAGAAACTCTTGTACATCTTCAAGCAGCCGTTCATGCGC
CCTGTGCAAACCACCCAAGAAGAGGACGGGTGCTCCTGCCGGTTCCCGGAA
GAGGAAGAGGGCGGCTGCGAACTG

**SEQ ID NO: 74** amino acid sequence of DNA signaling domain of 4-1BB

KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCEL

**SEQ ID NO: 75** nucleotide sequence of DNA signaling domain of CD3z

CGCGTGAAGTTTTCCCGGTCCGCCGACGCTCCGGCGTACCAGCAGGGGCAA
AACCAGCTGTACAACGAACTTAACCTCGGTCGCCGGGAAGAATATGACGTG
CTGGACAAGCGGCGGGGAAGAGATCCCGAGATGGGTGGAAAGCCGCGGCG
GAAGAACCCTCAGGAGGGCTTGTACAACGAGCTGCAAAAGGACAAAATGG
CCGAAGCCTACTCCGAGATTGGCATGAAGGGAGAGCGCAGACGCGGGAAG
GGACACGATGGACTGTACCAGGGACTGTCAACCGCGACTAAGGACACTTAC
GACGCCCTGCACATGCAGGCCCTGCCCCCGCGC

**SEQ ID NO: 76** amino acid sequence of DNA signaling domain of CD3z

RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRR
KNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYD
ALHMQALPPR

**SEQ ID NO: 77** nucleotide sequence of CAR123 Z16

ATGCTCTTGCTCGTGACTTCTTTGCTTTTGTGCGAACTTCCGCACCCAGCCTT
CCTTTTGATACCTCAGGTCCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAA
GCCTGGGTCCTCGGTGAAGGTCTCCTGCAAGGCTTCTGGAGGCACCTTCAG
CAGCTATGCTATCAGCTGGGTGCGACAGGCCCCTGGACAAGGGCTTGAGTG
GATGGGAGGGATCATCCCTATCTTTGGTACAGCAAACTACGCACAGAAGTT
CCAGGGCAGAGTCACGATTACCGCGGACGAATCCACGAGCACAGCCTACA
CGGAGCTGAGCAGCCTGAGATCTGAGGACACGGCCGTGTATTACTGTGCGA
GAGCCCGGTTGGGAGGAGCTTTTGATATCTGGGGCCAAGGGACAATGGTCA
CCGTCTCTTCAGGAGGTGGCGGGTCTGGTGGAGGCGGTAGCGGTGGTGGCG
GATCCCAGTCTGTGCTGACGCAGCCGCCCTCAGTGTCTGCGGCCCCAGGAC
AGAAGGTCACCATCTCCTGCTCTGGAGGCAGCTCCAACATTGGCAATCATT
ATGTGTCCTGGTATCAGCAGCTCCCAGGAGCAGCCCCCAAACTCCTCATTTA

TGACGATAATAAGCGACCCTCAGGGATTCCTGACCGATTCTCTGGCTCCAG
GTCTGGCACGTCAGCCACCCTGGGCATCACCGGACTCCAGAGTGGGGACGA
GGCCGATTATTACTGCGGAGCATGGGATAGTAGTCTTGCTGCTCATGTCTTC
GGAACTGGGACCAAGGTCACCGTCCTgGGTGCGGCCGCAACGACCACTCCT
GCACCCCGCCCTCCGACTCCGGCCCCAACCATTGCCAGCCAGCCCCTGTCCC
TGCGGCCGGAAGCCTGCAGACCGGCTGCCGGCGGAGCCGTCCATACCCGGG
GACTGGATTTCGCCTGCGATATCTATATCTGGGCACCACTCGCCGGAACCTG
TGGAGTGCTGCTGCTGTCCCTTGTGATCACCCTGTACTGCAAGCGCGGACG
GAAGAAACTCTTGTACATCTTCAAGCAGCCGTTCATGCGCCCTGTGCAAAC
CACCCAAGAAGAGGACGGGTGCTCCTGCCGGTTCCCGGAAGAGGAAGAGG
GCGGCTGCGAACTGCGCGTGAAGTTTTCCCGGTCCGCCGACGCTCCGGCGT
ACCAGCAGGGGCAAAACCAGCTGTACAACGAACTTAACCTCGGTCGCCGG
GAAGAATATGACGTGCTGGACAAGCGGCGGGGAAGAGATCCCGAGATGGG
TGGAAAGCCGCGGCGGAAGAACCCTCAGGAGGGCTTGTACAACGAGCTGC
AAAAGGACAAAATGGCCGAAGCCTACTCCGAGATTGGCATGAAGGGAGAG
CGCAGACGCGGGAAGGGACACGATGGACTGTACCAGGGACTGTCAACCGC
GACTAAGGACACTTACGACGCCCTGCACATGCAGGCCCTGCCCCCGCGC

**SEQ ID NO: 78** amino acid sequence of CAR123 Z16

MLLLVTSLLLCELPHPAFLLIPQVQLVQSGAEVKKPGSSVKVSCKASGGTFSSY
AISWVRQAPGQGLEWMGGIIPIFGTANYAQKFQGRVTITADESTSTAYTELSSL
RSEDTAVYYCARARLGGAFDIWGQGTMVTVSSGGGGSGGGGSGGGGSQSVLT
QPPSVSAAPGQKVTISCSGGSSNIGNHYVSWYQQLPGAAPKLLIYDDNKRPSGI
PDRFSGSRSGTSATLGITGLQSGDEADYYCGAWDSSLAAHVFGTGTKVTVLGA
AATTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLA
GTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEG
GCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGG
KPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATK
DTYDALHMQALPPR

**SEQ ID NO: 79** nucleotide sequence of human IgG4 hinge

GAGAGCAAATACGGGCCGCCATGTCCCCCGTGTCCG

**SEQ ID NO: 80** amino acid sequence of human IgG4 hinge

ESKYGPPCPPCP

**SEQ ID NO: 81** nucleotide sequence of human IgG4 CH2 domain

GCACCACCAGTTGCTGGCCCTAGTGTCTTCTTGTTCCCTCCCAAGCCCAAAG
ACACCTTGATGATTTCCAGAACTCCTGAGGTTACCTGCGTTGTCGTAGATGT
TTCTCAGGAGGACCCAGAGGTCCAATTTAACTGGTACGTTGATGGGGTGGA
AGTTCACAATGCGAAGACAAAGCCGCGGGAAGAACAATTTCAGTCCACTTA
CCGGGTTGTCAGCGTTCTGACGGTATTGCATCAAGACTGGCTTAATGGAAA
GGAATATAAGTGTAAGGTGTCCAACAAAGGTTTGCCGAGCAGTATTGAGAA
GACCATATCAAAGGCGAAG

**SEQ ID NO: 82 amino acid** sequence of human IgG4 CH2 domain

APPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYV
DGVEVHNAKTKPREEQFQSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSS
IEKTISKA K

**SEQ ID NO: 83** nucleotide sequence of human IgG4 CH3 domain

GGGCAGCCGCGCGAGCCACAAGTTTACACTTTGCCGCCATCTCAAGAGGAA
ATGACTAAAAACCAGGTATCCTTGACATGCCTCGTAAAAGGATTTTATCCA
TCTGATATTGCTGTGGAATGGGAGTCTAACGGGCAGCCGGAAAATAATTAC
AAAACTACACCACCTGTGCTCGATTCAGATGGAAGTTTCTTCCTTTACAGTA
GACTTACGGTGGACAAATCTAGGTGGCAGGAAGGGAATGTGTTTAGTTGTA
GTGTAATGCACGAGGCACTTCATAACCACTATACACAGAAGTCACTGAGTT
TGAGTCTTGGCAAA

**SEQ ID NO: 84** amino acid sequence of human IgG4 CH3 domain

GQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT
TPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLG
K

**SEQ ID NO: 85** nucleotide sequence of human IgG4 hinge CH2 CH3 domain

GAGAGCAAATACGGGCCGCCATGTCCCCCGTGTCCGGCACCACCAGTTGCTGGCC
CTAGTGTCTTCTTGTTCCCTCCCAAGCCCAAAGACACCTTGATGATTTCCAGAACTC
CTGAGGTTACCTGCGTTGTCGTAGATGTTTCTCAGGAGGACCCAGAGGTCCAATTT
AACTGGTACGTTGATGGGGTGGAAGTTCACAATGCGAAGACAAAGCCGCGGGAAG
AACAATTTCAGTCCACTTACCGGGTTGTCAGCGTTCTGACGGTATTGCATCAAGAC
TGGCTTAATGGAAAGGAATATAAGTGTAAGGTGTCCAACAAAGGTTTGCCGAGCA
GTATTGAGAAGACCATATCAAAGGCGAAGGGGCAGCCGCGCGAGCCACAAGTTTA
CACTTTGCCGCCATCTCAAGAGGAAATGACTAAAAACCAGGTATCCTTGACATGCC
TCGTAAAAGGATTTTATCCATCTGATATTGCTGTGGAATGGGAGTCTAACGGGCAG
CCGGAAAATAATTACAAAACTACACCACCTGTGCTCGATTCAGATGGAAGTTTCTT
CCTTTACAGTAGACTTACGGTGGACAAATCTAGGTGGCAGGAAGGGAATGTGTTT
AGTTGTAGTGTAATGCACGAGGCACTTCATAACCACTATACACAGAAGTCACTGA
GTTTGAGTCTTGGCAAA

**SEQ ID NO: 86** amino acid sequence of human IgG4 hinge CH2 CH3 domain

ESKYGPPCPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWY
VDGVEVHNAKTKPREEQFQSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKT

ISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT
TPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK

**SEQ ID NO: 87** nucleotide sequence of CAR123 Z32

ATGCTCTTGCTCGTGACTTCTTTGCTTTTGTGCGAACTTCCGCACCCAGCCTTCCTT
TTGATACCTCAGGTACAGCTGCAGCAGTCAGGTCCAGGACTGGTGAAGCCCTCGC
AGACCCTCTCACTCACCTGTGCCATCTCCGGGGACAGTGTCTCTAGCAACAGTGCT
GCTTGGAACTGGATCAGGCAGTCCCCATCGAGAGGCCTTGAGTGGCTGGGAAGGA
CATACTACAGGTCCAAGTGGTATAATGATTATGCAGTATCTGTGAAAAGTCGAATA
ACCATCAACCCAGACACATCCAAGAACCAGTTCTCCCTGCAGCTGAACTCTGTGAC
TCCCGAGGACATGGCTGTGTATTACTGTGCAAGAGGCGTTGATAGTAGCTTTGACT
ACTGGGGCCAGGGAACCCTGGTCACCGTCTCCTCAGGAGGTGGCGGGTCTGGTGG
AGGCGGTAGCGGTGGTGGCGGATCCCAGTCTGTCGTGACGCAGCCGCCCTCAGTG
TCTGCGGCCCCAGGACAGAGCGTCACCATCTCCTGTTCTGGAAGCAGTTCCACCGT
TGGCGATAATTATGTGTCCTGGTACCAGCAACTCCCAGGAACAGCCCCCAAACTCC
TCATTTTTGACGATTATAAACGACCCTCAGGGGTTCCTGACCGATTCTCTGGCTCCC
AGTCTGGCACCTCAGCCTCCCTGGTCATCACTGGTCTCCAGGCAGAAGATGAGGCT
GATTATTACTGCCAGTCTTATGACAGCAGCCTGAGTGGTTATGTCTTCGGGCCTGG
GACCAAGGTCACCGTCCTGGGTGCGGCCGCAACGACCACTCCTGCACCCCGCCCTC
CGACTCCGGCCCCAACCATTGCCAGCCAGCCCCTGTCCCTGCGGCCGGAAGCCTGC
AGACCGGCTGCCGGCGGAGCCGTCCATACCCGGGGACTGGATTTCGCCTGCGATA
TCTATATCTGGGCACCACTCGCCGGAACCTGTGGAGTGCTGCTGCTGTCCCTTGTG
ATCACCCTGTACTGCAAGCGCGGACGGAAGAAACTCTTGTACATCTTCAAGCAGC
CGTTCATGCGCCCTGTGCAAACCACCCAAGAAGAGGACGGGTGCTCCTGCCGGTT
CCCGGAAGAGGAAGAGGGCGGCTGCGAACTGCGCGTGAAGTTTTCCCGGTCCGCC
GACGCTCCGGCGTACCAGCAGGGGCAAAACCAGCTGTACAACGAACTTAACCTCG
GTCGCCGGGAAGAATATGACGTGCTGGACAAGCGGCGGGGAAGAGATCCCGAGA
TGGGTGGAAAGCCGCGGCGGAAGAACCCTCAGGAGGGCTTGTACAACGAGCTGCA
AAAGGACAAAATGGCCGAAGCCTACTCCGAGATTGGCATGAAGGGAGAGCGCAG
ACGCGGGAAGGGACACGATGGACTGTACCAGGGACTGTCAACCGCGACTAAGGA
CACTTACGACGCCCTGCACATGCAGGCCCTGCCCCCGCGC

**SEQ ID NO: 88** amino acid sequence of sequence of CAR123 Z32

MLLLVTSLLLCELPHPAFLLIPQVQLQQSGPGLVKPSQTLSLTCAISGDSVSSNS
AAWNWIRQSPSRGLEWLGRTYYRSKWYNDYAVSVKSRITINPDTSKNQFSLQL
NSVTPEDMAVYYCARGVDSSFDYWGQGTLVTVSSGGGGSGGGGSGGGGSQS
VVTQPPSVSAAPGQSVTISCSGSSSTVGDNYVSWYQQLPGTAPKLLIFDDYKRP
SGVPDRFSGSQSGTSASLVITGLQAEDEADYYCQSYDSSLSGYVFGPGTKVTVL
GAAATTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAP
LAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEE
EGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMG
GKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTAT
KDTYDALHMQALPPR

WHAT IS CLAIMED IS:

1. An isolated nucleic acid molecule encoding a chimeric antigen receptor (CAR) comprising at least one extracellular antigen binding domain comprising a CD123 antigen binding domain encoded by a nucleotide sequence comprising SEQ ID NO: 69, 71, 77, or 87, at least one transmembrane domain, and at least one intracellular signaling domain.

2. The isolated nucleic acid molecule of claim 1, wherein the encoded at least one CD123 antigen binding domain comprises at least one single chain variable fragment of an antibody that binds to CD123.

3. The isolated nucleic acid molecule of claim 1, wherein the encoded at least one CD123 antigen binding domain comprises at least one heavy chain variable region of an antibody that binds to CD123.

4. The isolated nucleic acid molecule of claim 1, wherein the encoded at least one CD123 antigen binding domain, the at least one intracellular signaling domain, or both are connected to the transmembrane domain by a linker or spacer domain.

5. The isolated nucleic acid molecule of claim 4, wherein the encoded linker or spacer domain is derived from the extracellular domain of CD8, TNFRSF19, or CD28, and is linked to a transmembrane domain.

6. The isolated nucleic acid molecule of claim 1, wherein the encoded extracellular CD123 antigen binding domain is preceded by a leader nucleotide sequence encoding a leader peptide.

7. The isolated nucleic acid molecule of claim 6, wherein the leader nucleotide sequence comprises a nucleotide sequence comprising SEQ ID NO: 13 encoding the leader amino acid sequence of SEQ ID NO: 14, or SEQ ID NO: 39 encoding the leader amino acid sequence of SEQ ID NO: 40, or SEQ ID NO: 41 encoding the leader amino acid sequence of SEQ ID NO: 42, or SEQ ID NO: 43 encoding the leader amino acid sequence of SEQ ID NO: 44.

8. The isolated nucleic acid molecule of claim 1, wherein the transmembrane domain comprises a transmembrane domain of a protein comprising the alpha, beta or zeta chain of the T-cell receptor, CD8, CD28, CD3 epsilon, CD45, CD4, CD5, CD8, CD9, CD16, CD22, CD33, CD37, CD64, CD80, CD83, CD86, CD134, CD137, CD154, and TNFRSF19, or any combination thereof.

9. The isolated nucleic acid molecule of claim 1, wherein the nucleic acid sequence encoding the extracellular CD123 antigen binding domain comprises a nucleic sequence comprising SEQ ID NO: 69, 71, 77, or 87, or a sequence with 85%, 90%, 95%, 96%, 97%, 98% or 99% identity thereof.

10. The isolated nucleic acid molecule of claim 1, wherein the encoded at least one intracellular signaling domain further comprises a CD3 zeta intracellular domain.

11. The isolated nucleic acid molecule of claim 10, wherein the encoded at least one intracellular signaling domain is arranged on a C-terminal side relative to the CD3 zeta intracellular domain.

12. The isolated nucleic acid molecule of claim 1, wherein the encoded at least one intracellular signaling domain comprises a costimulatory domain, a primary signaling domain, or any combination thereof.

13. The isolated nucleic acid molecule of claim 12, wherein the encoded at least one costimulatory domain comprises a functional signaling domain of OX40, CD70, CD27, CD28, CD5, ICAM-1, LFA-1 (CD11a/CD18), ICOS (CD278), DAP10, DAP12, and 4-1BB (CD137), or any combination thereof.

14. A chimeric antigen receptor (CAR) encoded by the isolated nucleic acid molecule of claim 1.

15. The CAR of claim 14, comprising at least one extracellular antigen binding domain comprising a CD123 antigen binding domain comprising the amino acid sequence of

SEQ ID NO: 70, 72, 78, or 88, at least one transmembrane domain, and at least one intracellular signaling domain.

16. The CAR of claim 15, wherein the CD123 antigen binding domain comprises at least one single chain variable fragment of an antibody that binds to CD123.

17. The CAR of claim 15, wherein the CD123 antigen binding domain comprises at least one heavy chain variable region of an antibody that binds to CD123.

18. The CAR of claim 15, wherein the transmembrane domain comprises a transmembrane domain of a protein comprising the alpha, beta or zeta chain of the T-cell receptor, CD8, CD28, CD3 epsilon, CD45, CD4, CD5, CD8, CD9, CD16, CD22, CD33, CD37, CD64, CD80, CD86, CD134, CD137, CD154, and TNFRSF19 or any combination thereof.

19. The CAR of claim 18, wherein the CD8 transmembrane domain comprises the amino acid sequence of SEQ ID NO: 27, or an amino acid sequence with 85%, 90%, 95%, 96%, 97%, 98% or 99% identity to an amino acid sequence of SEQ ID NO: 28.

20. The CAR of claim 15, wherein the at least one extracellular antigen binding domain comprising a CD123 antigen binding domain comprising the amino acid sequence of SEQ ID NO: 70, 72, 78, or 88, and the at least one intracellular signaling domain, or both are connected to the transmembrane domain by a linker or spacer domain.

21. The CAR of claim 20, wherein the linker or spacer domain is derived from the extracellular domain of CD8, TNFRSF19, IgG4, or CD28, and is linked to a transmembrane domain.

22. The CAR of claim 17, wherein the at least one intracellular signaling domain comprises a costimulatory domain and a primary signaling domain.

23. The CAR of claim 22, wherein the at least one intracellular signaling domain comprises a costimulatory domain comprising a functional signaling domain of a protein selected from the group consisting of OX40, CD70, CD27, CD28, CD5, ICAM-1, LFA-1

(CD11a/CD18), ICOS (CD278), DAP10, DAP12, and 4-1BB (CD137), or a combination thereof.

24. A vector comprising a nucleic acid molecule of claim 1.

25. The vector of claim 24, wherein the vector is selected from the group consisting of a DNA vector, an RNA vector, a plasmid vector, a cosmid vector, a herpes virus vector, a measles virus vector, a lentivirus vector, adenoviral vector, or a retrovirus vector, or a combination thereof.

26. The vector of claim 24, further comprising a promoter.

27. The vector of claim 26, wherein the promoter is an inducible promoter, a constitutive promoter, a tissue specific promoter, a suicide promoter or any combination thereof.

28. A cell comprising the vector of claim 24.

29. The cell of claim 28, wherein the cell is a T cell.

30. The cell of claim 28, wherein the T cell is a $CD8^{+}$ T cell.

31. The cell of claim 28, wherein the cell is a human cell.

32. A method of making a cell comprising transducing a T cell with a vector of claim 24.

33. A method of generating a population of RNA-engineered cells comprising introducing an *in vitro* transcribed RNA or synthetic RNA into a cell, where the RNA comprises a nucleic acid molecule of claim 1.

34. A method of providing an anti-tumor immunity in a mammal comprising administering to the mammal an effective amount of a cell of claim 28.

35. A method of treating or preventing cancer in a mammal, comprising administering to the mammal the CAR of claim 15, in an amount effective to treat or prevent cancer in the mammal.

36. A pharmaceutical composition comprising an anti-tumor effective amount of a population of human T cells, wherein the T cells comprise a nucleic acid sequence that encodes a chimeric antigen receptor (CAR), wherein the CAR comprises at least one extracellular antigen binding domain comprising a CD123 antigen binding domain comprising the amino acid sequence of SEQ ID NO: 70, 72, 78, or 88, at least one linker domain, at least one transmembrane domain, at least one intracellular signaling domain, and wherein the T cells are T cells of a human having a cancer.

37. The pharmaceutical composition of claim 36, wherein the at least one transmembrane domain comprises a transmembrane domain of a protein comprising the alpha, beta or zeta chain of the T-cell receptor, CD8, CD28, CD3 epsilon, CD45, CD4, CD5, CD8, CD9, CD16, CD22, CD33, CD37, CD64, CD80, CD86, CD134, CD137 and CD154, or any combination thereof.

38. The pharmaceutical composition of claim 36, wherein the T cells are T cells of a human having a hematological cancer.

39. The pharmaceutical composition of claim 38, wherein the hematological cancer is leukemia or lymphoma.

40. The pharmaceutical composition of claim 39, wherein the leukemia is acute myeloid leukemia (AML), blastic plasmacytoid dendritic cell neoplasm (BPDCN), chronic myelogenous leukemia (CML), chronic lymphocytic leukemia (CLL), acute lymphoblastic T cell leukemia (T-ALL), or acute lymphoblastic B cell leukemia (B-ALL).

41. The pharmaceutical composition of claim 39, wherein the lymphoma is mantle cell lymphoma, non-Hodgkin's lymphoma or Hodgkin's lymphoma.

42. The pharmaceutical composition of claim 38, wherein the hematological cancer is multiple myeloma.

43. The pharmaceutical composition of claim 36, wherein the human cancer includes an adult carcinoma comprising oral and pharynx cancer (tongue, mouth, pharynx, head and neck), digestive system cancers (esophagus, stomach, small intestine, colon, rectum, anus, liver, interhepatic bile duct, gallbladder, pancreas), respiratory system cancers (larynx, lung and bronchus), bones and joint cancers, soft tissue cancers, skin cancers (melanoma, basal and squamous cell carcinoma), pediatric tumors (neuroblastoma, rhabdomyosarcoma, osteosarcoma, Ewing's sarcoma), tumors of the central nervous system (brain, astrocytoma, glioblastoma, glioma), and cancers of the breast, the genital system (uterine cervix, uterine corpus, ovary, vulva, vagina, prostate, testis, penis, endometrium), the urinary system (urinary bladder, kidney and renal pelvis, ureter), the eye and orbit, the endocrine system (thyroid), and the brain and other nervous system, or any combination thereof.

44. A method of treating a mammal having a disease, disorder or condition associated with an elevated expression of a tumor antigen, the method comprising administering to the subject a pharmaceutical composition comprising an anti-tumor effective amount of a population of T cells, wherein the T cells comprise a nucleic acid sequence that encodes a chimeric antigen receptor (CAR), wherein the CAR comprises at least one extracellular antigen binding domain comprising a CD123 antigen binding domain comprising the amino acid sequence of SEQ ID NO: 70, 72, 78, or 88, at least one linker or spacer domain, at least one transmembrane domain, at least one intracellular signaling domain, wherein the T cells are T cells of the subject having cancer.

45. A method of treating cancer in a subject in need thereof, the method comprising administering to the subject a pharmaceutical composition comprising an anti-tumor effective amount of a population of T cells, wherein the T cells comprise a nucleic acid sequence that encodes a chimeric antigen receptor (CAR), wherein the CAR comprises at least one extracellular antigen binding domain comprising a CD123 antigen binding domain comprising the amino acid sequence of SEQ ID NO: 70, 72, 78, or 88, at least one linker or spacer domain, at least one transmembrane domain, at least one

intracellular signaling domain, wherein the T cells are T cells of the subject having cancer.

46. The method of claim 44 or 45, wherein the at least one transmembrane domain comprises a transmembrane domain of a protein comprising the alpha, beta or zeta chain of the T-cell receptor, CD8, CD28, CD3 epsilon, CD45, CD4, CD5, CD8, CD9, CD16, CD22, CD33, CD37, CD64, CD80, CD86, CD134, CD137 and CD154, or any combination thereof.

47. A process for producing a chimeric antigen receptor-expressing cell, the process comprising introducing the isolated nucleic acid of claim 1 into a cell.

48. The process for producing a chimeric antigen receptor-expressing cell according to claim 47, wherein the cell is a T cell or a cell population containing a T cell.


FIGURE 1A

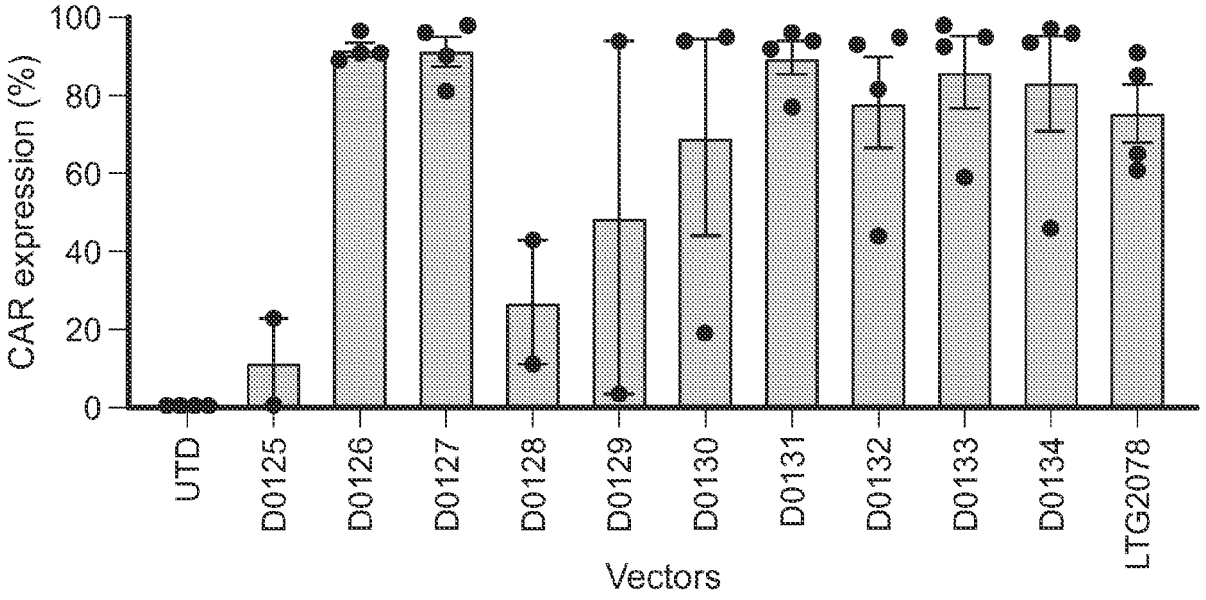


FIGURE 1B

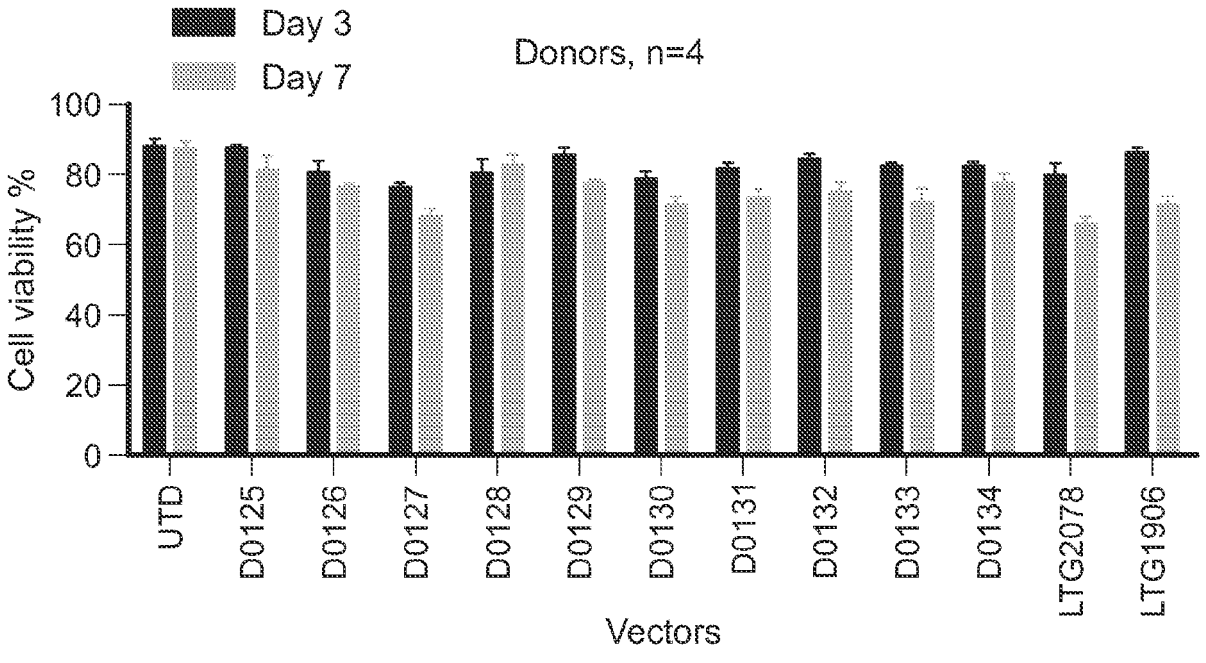


FIGURE 1C

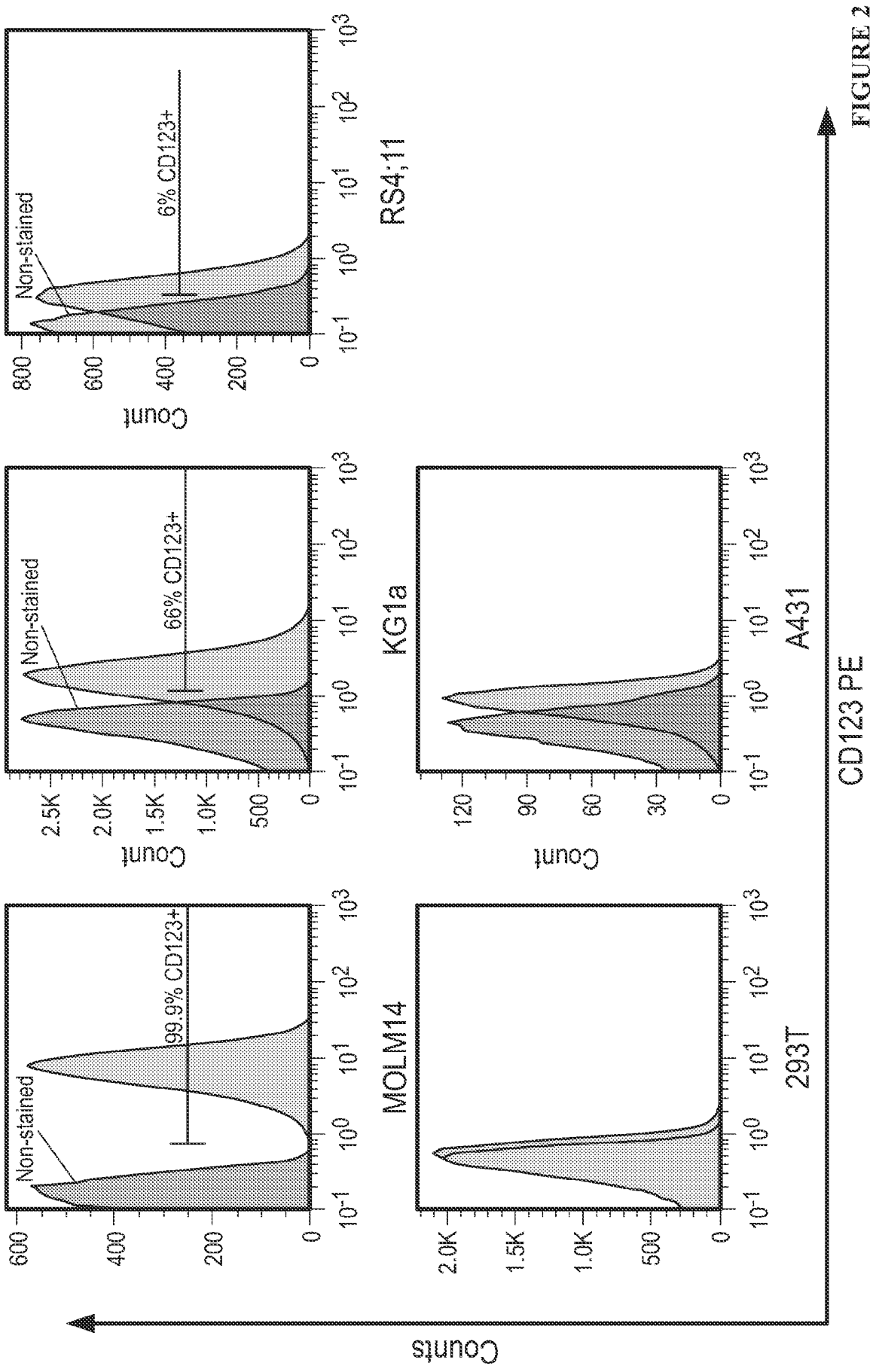
Counts
CD123 PE
293T
MOLM14
A431
KG1a
RS4;11
Count
Non-stained
99.9% CD123+
66% CD123+
6% CD123+

FIGURE 2

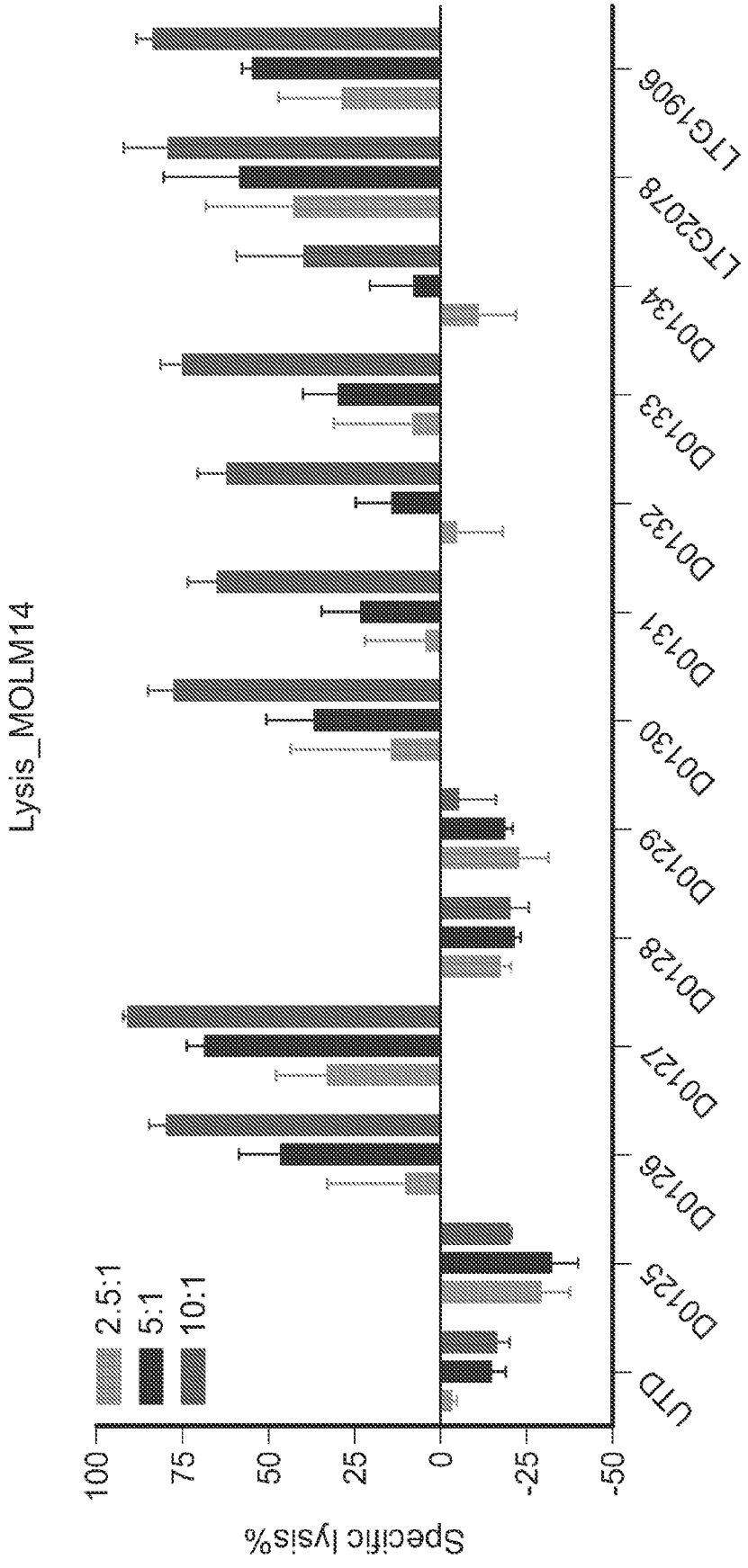
Lysis_MOLM14
Specific lysis%
100
75
50
25
0
-25
-50
2.5:1
5:1
10:1
UTD
D0125
D0126
D0127
D0128
D0129
D0130
D0131
D0132
D0133
D0134
LTG2078
LTG1906
Vectors

FIGURE 3A

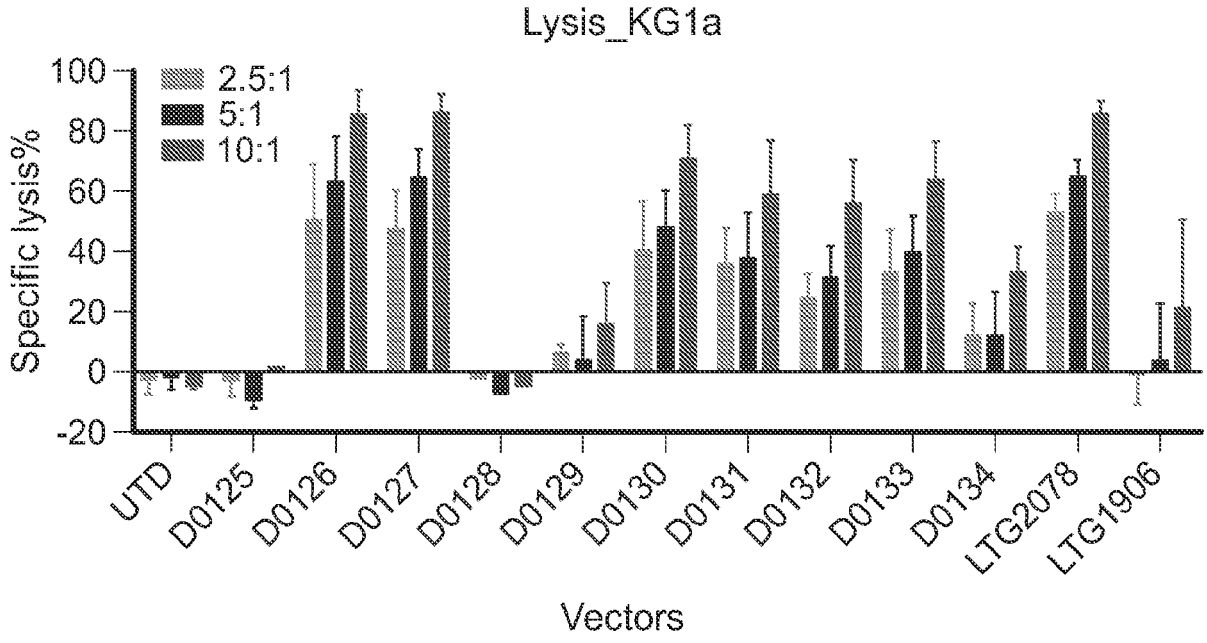


FIGURE 3B

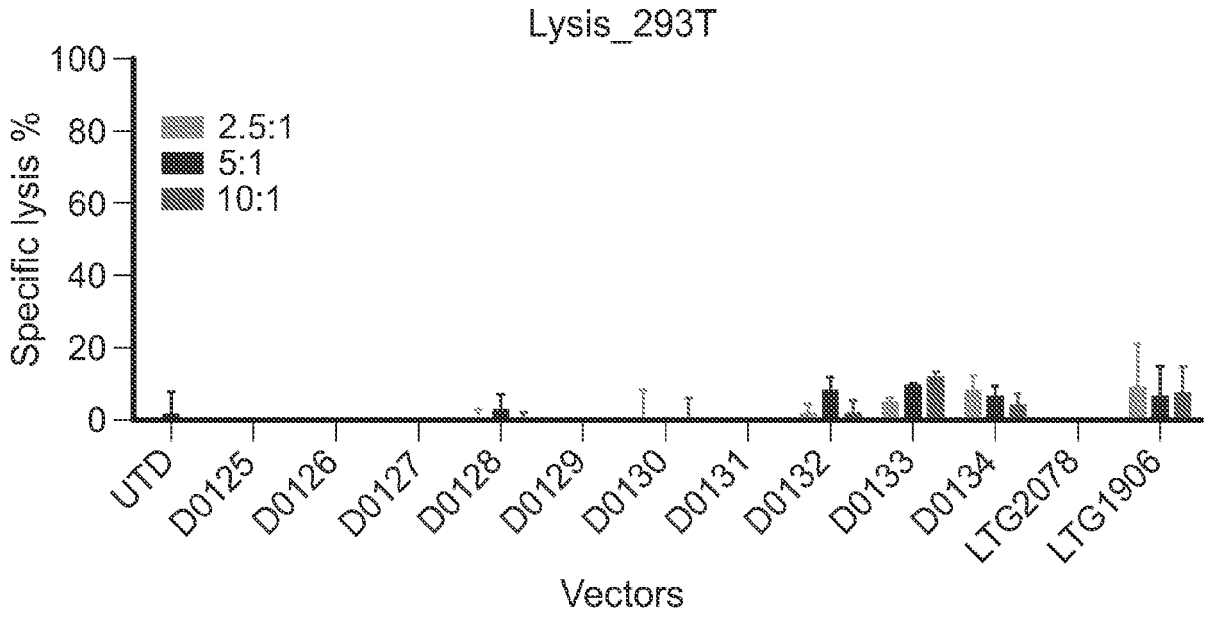


FIGURE 3C

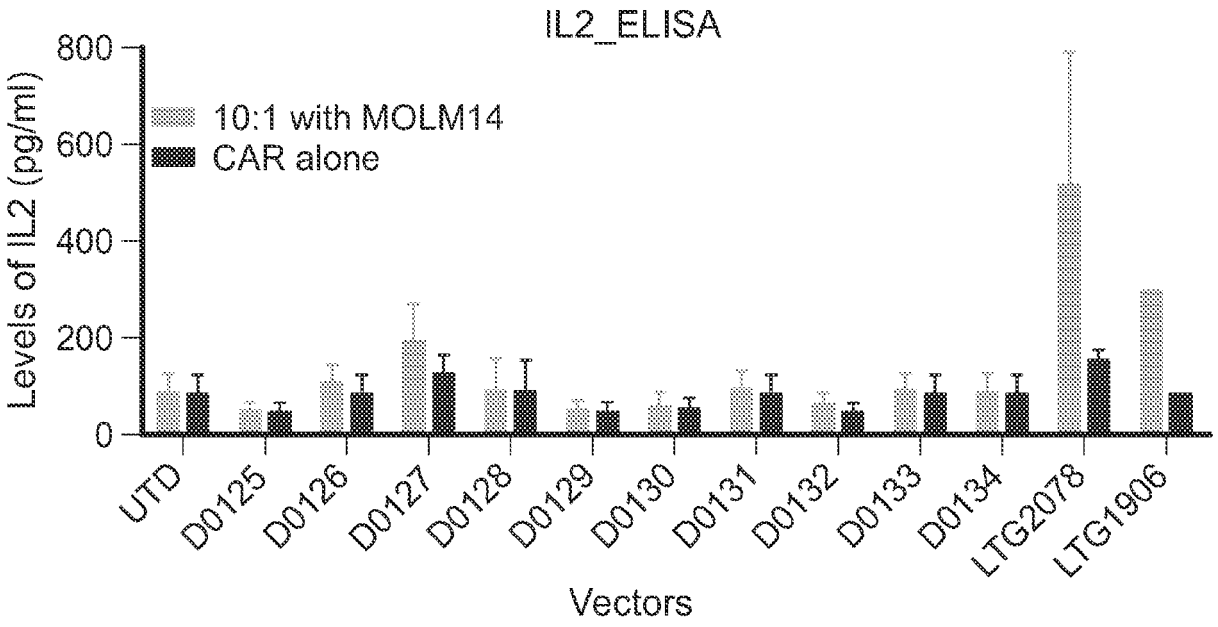


**FIGURE 4A**

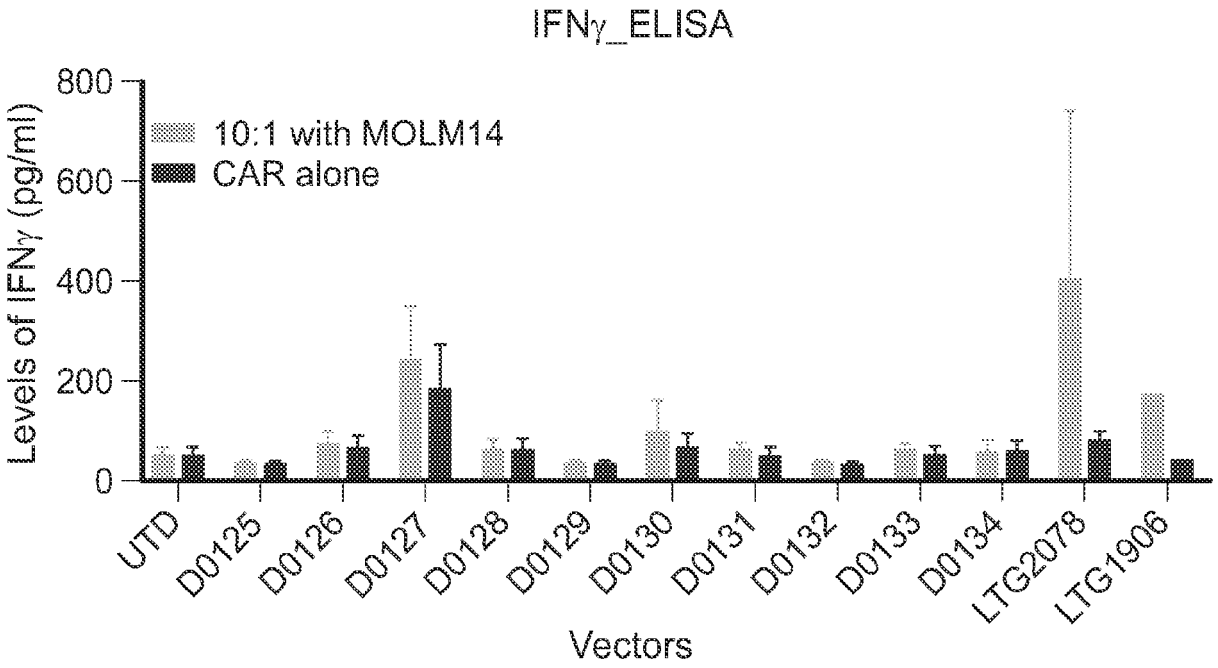


**FIGURE 4B**

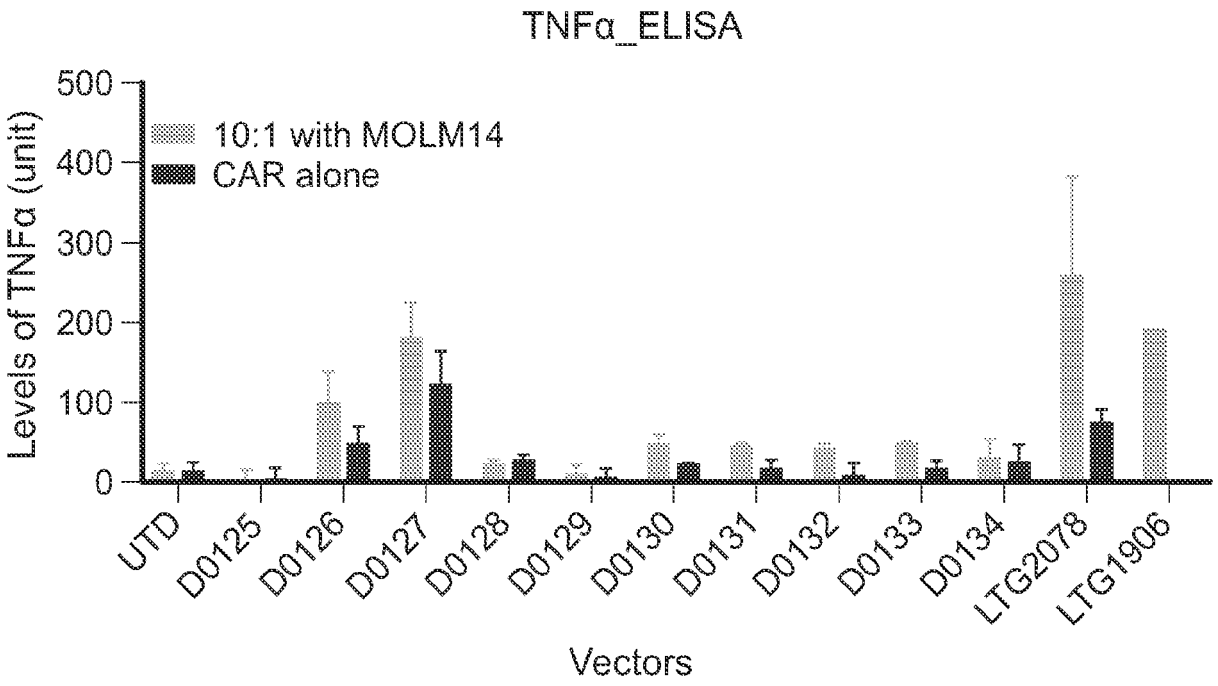


FIGURE 4C

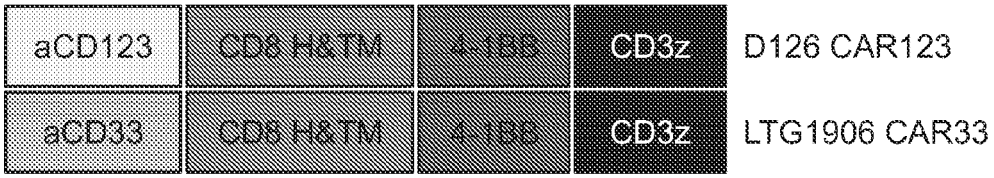


FIGURE 5A

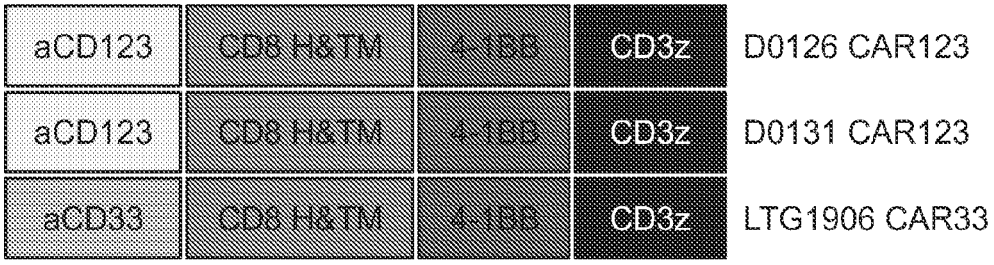


FIGURE 5B

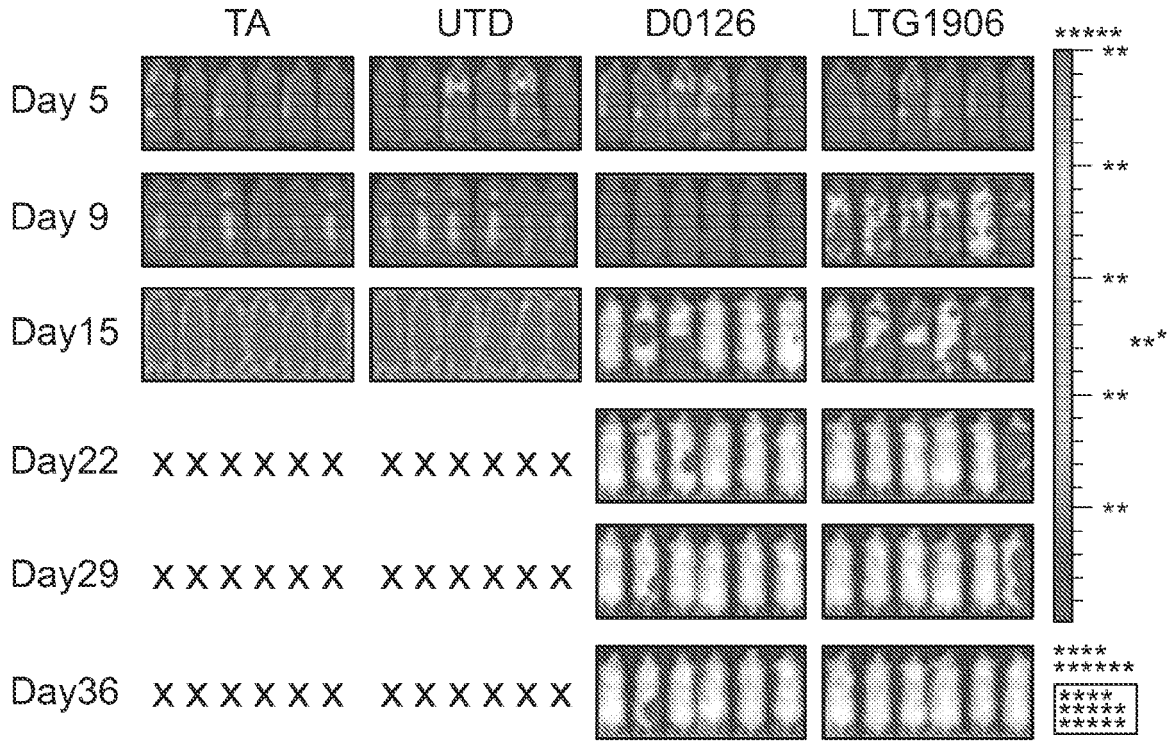


FIGURE 6A

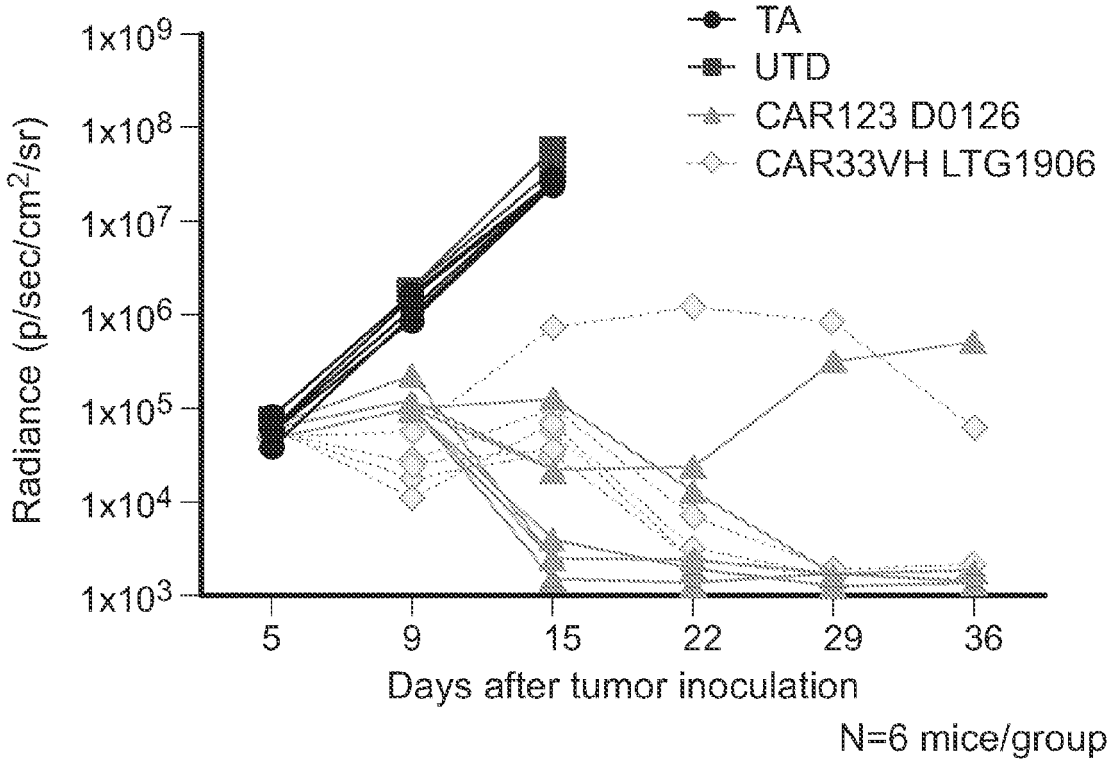


FIGURE 6B

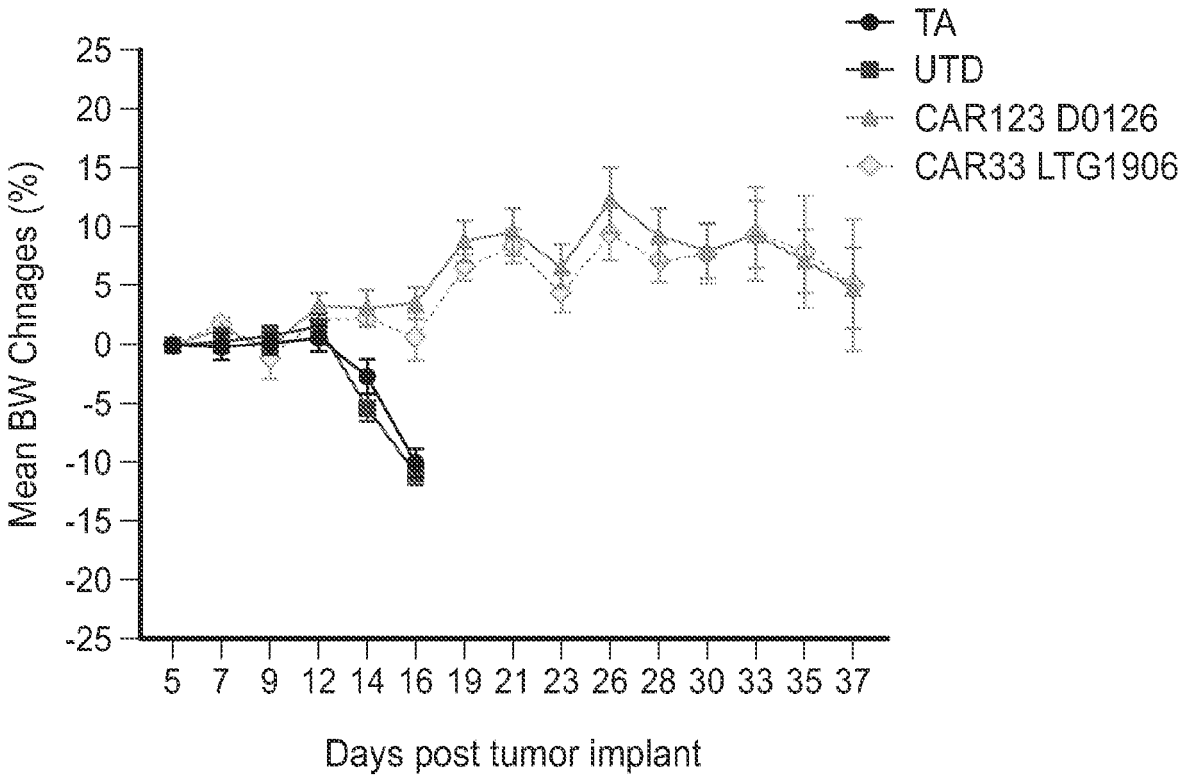


FIGURE 6C

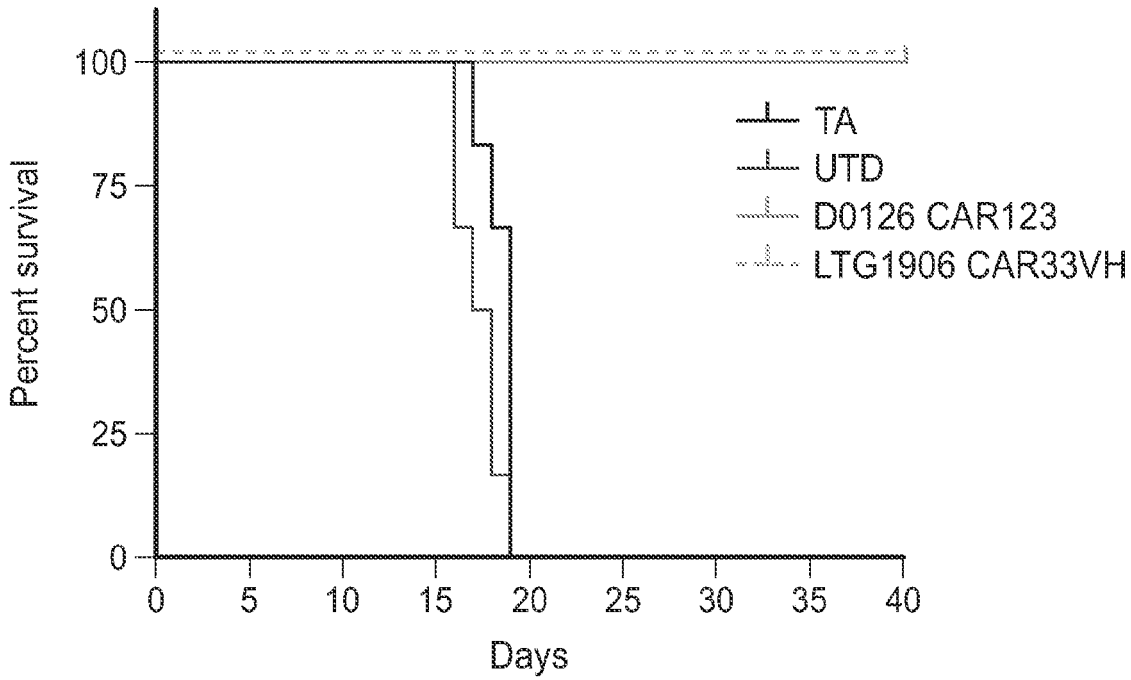


FIGURE 6D

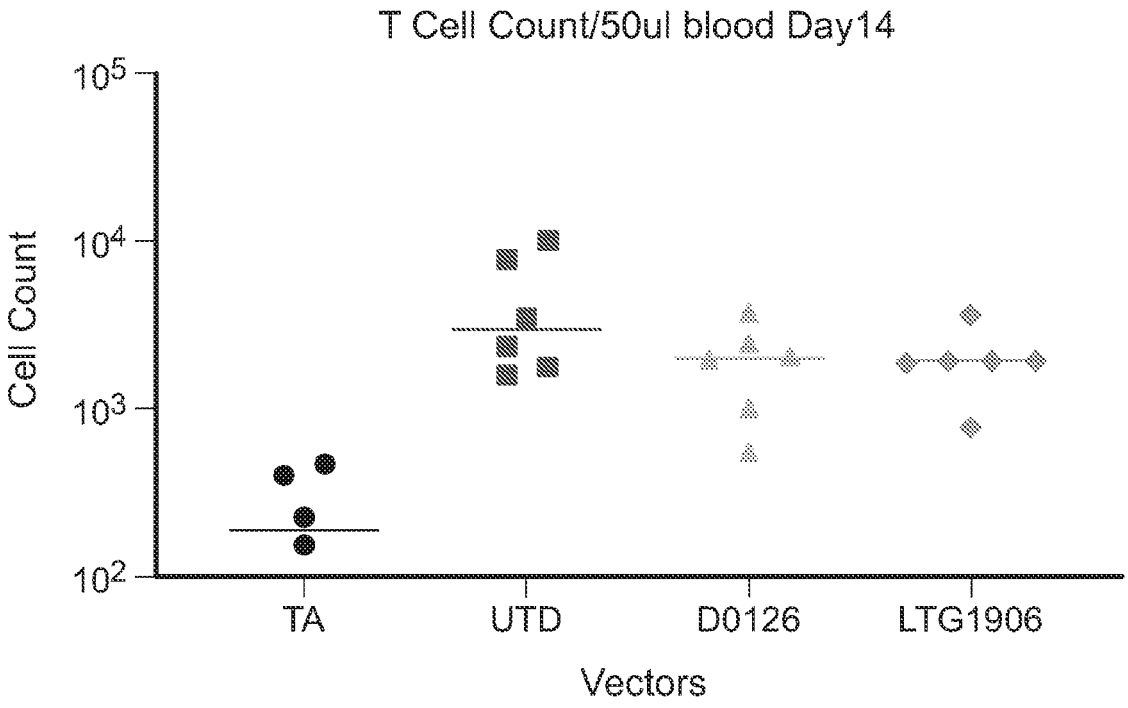


FIGURE 7A

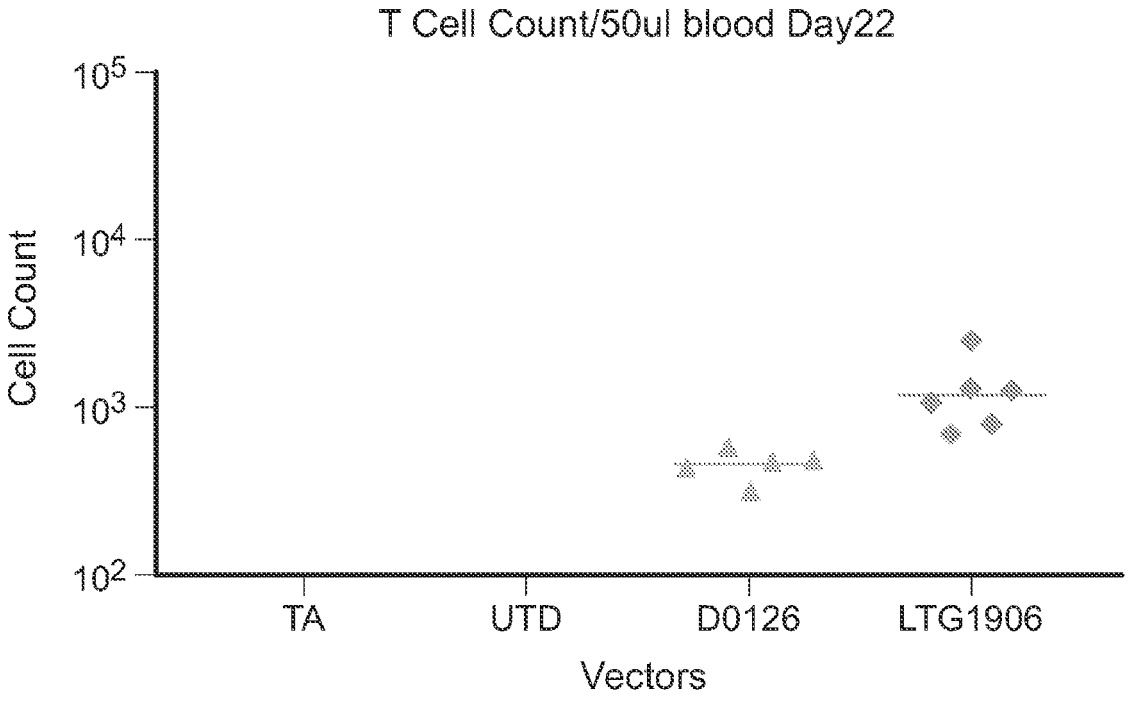


FIGURE 7B

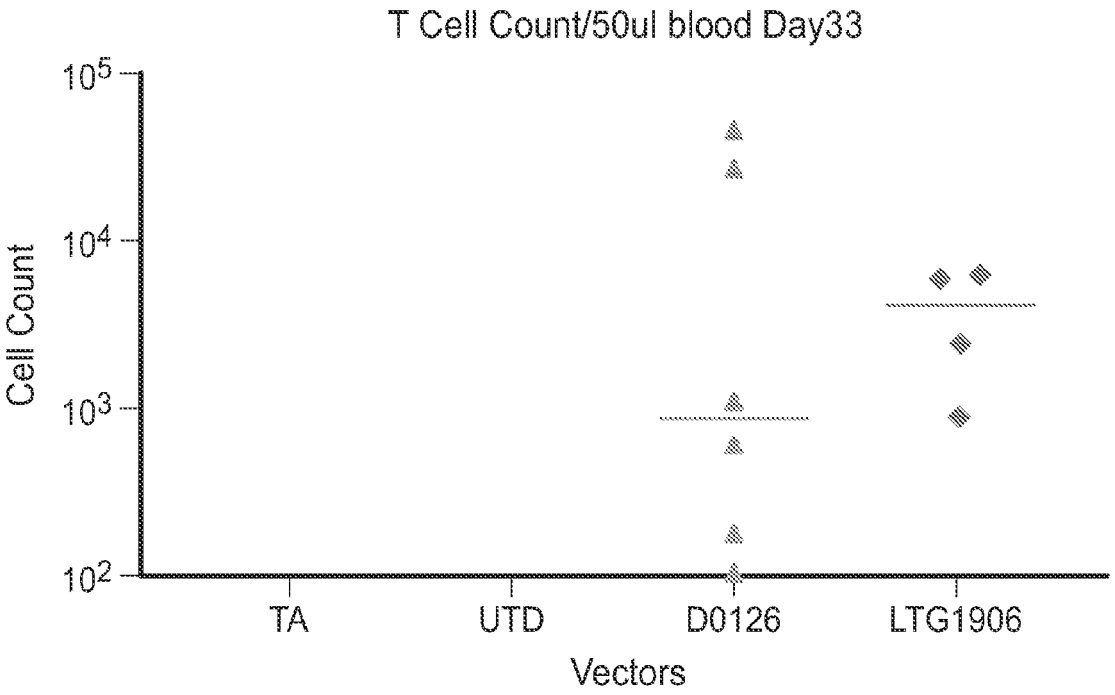


FIGURE 7C

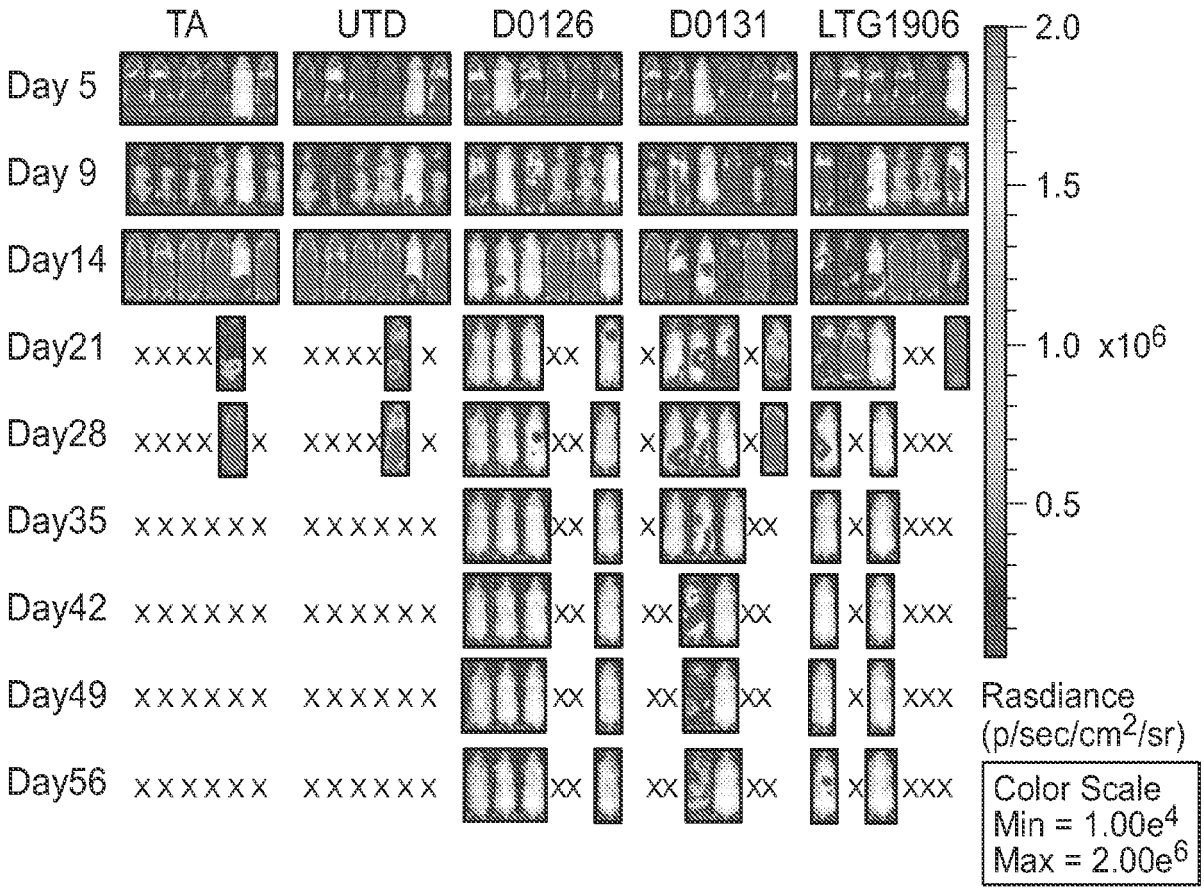


FIGURE 8A

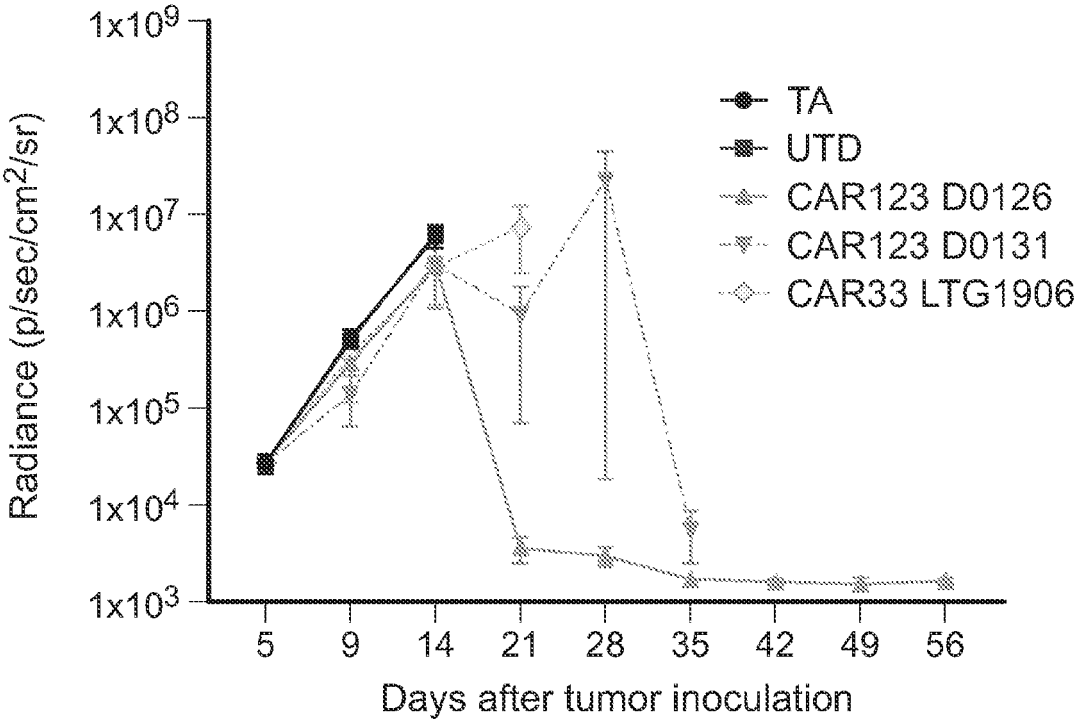


FIGURE 8B

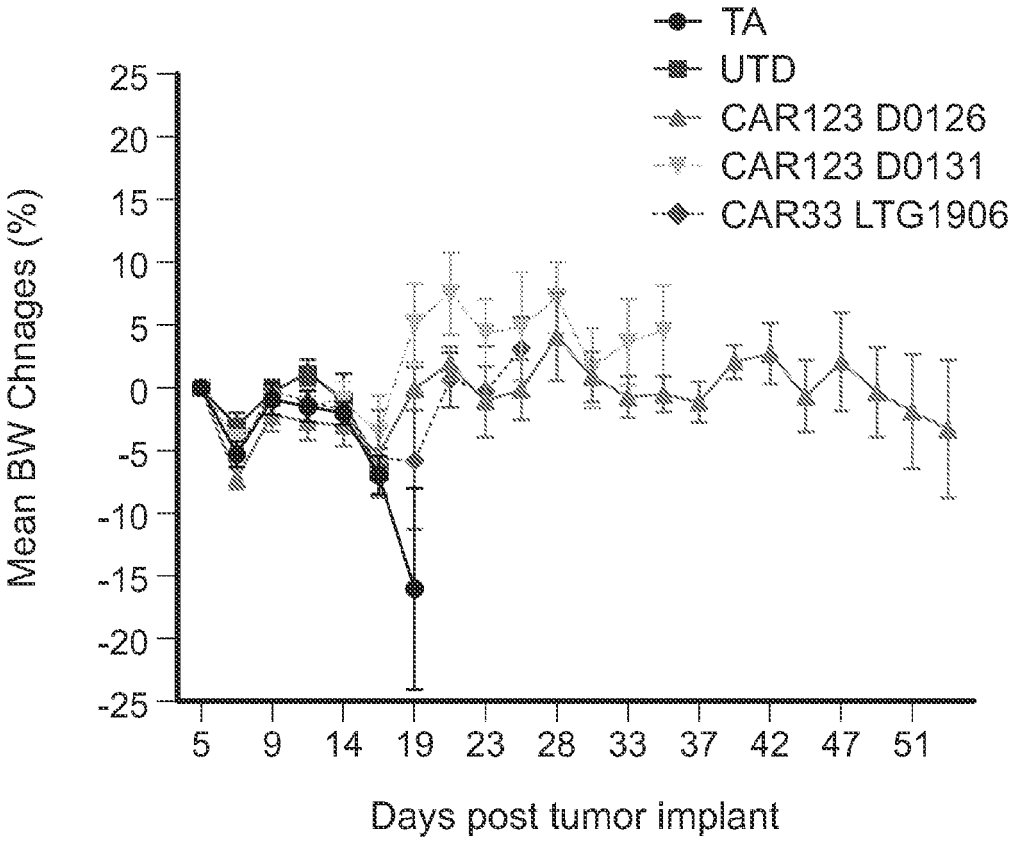


FIGURE 8C

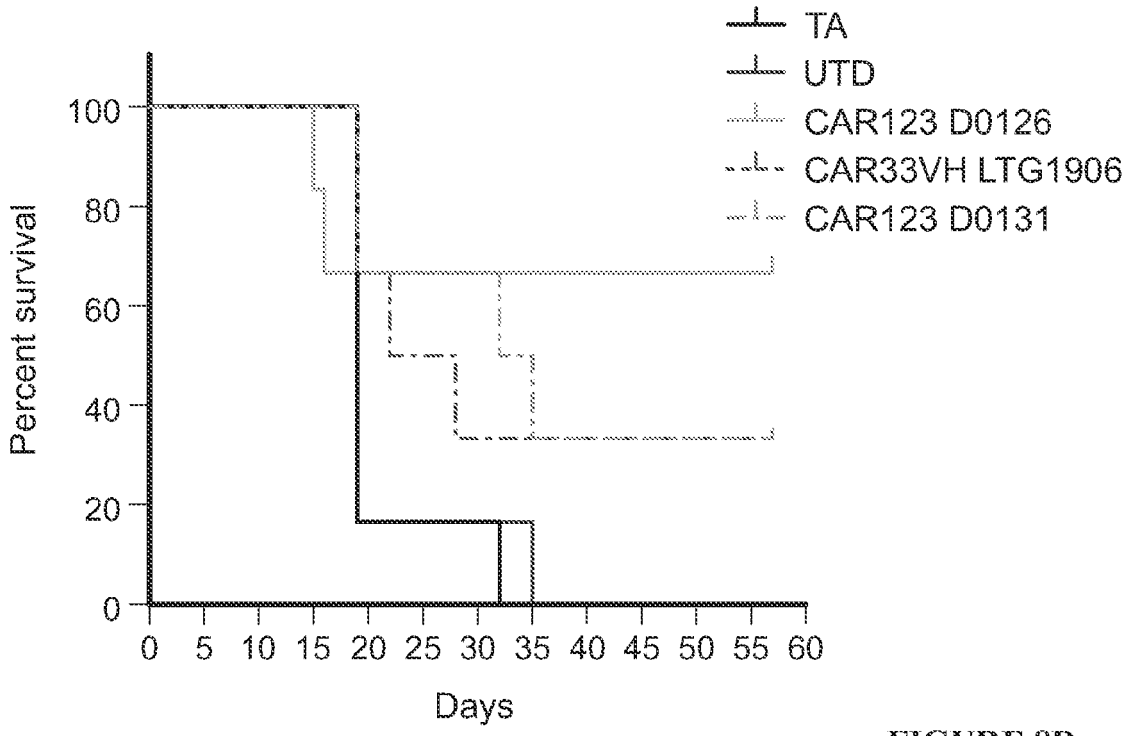


FIGURE 8D

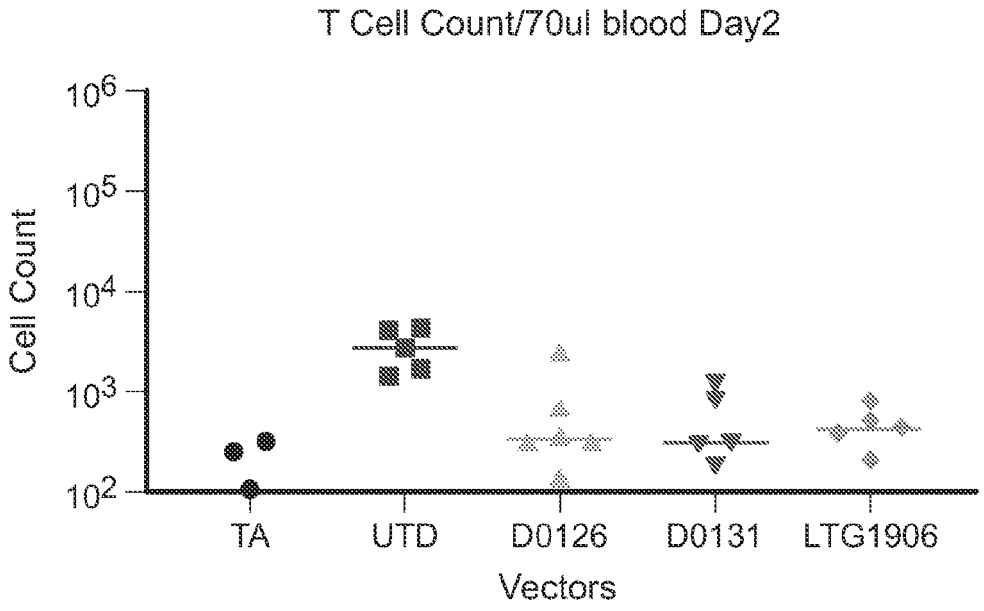


FIGURE 9A

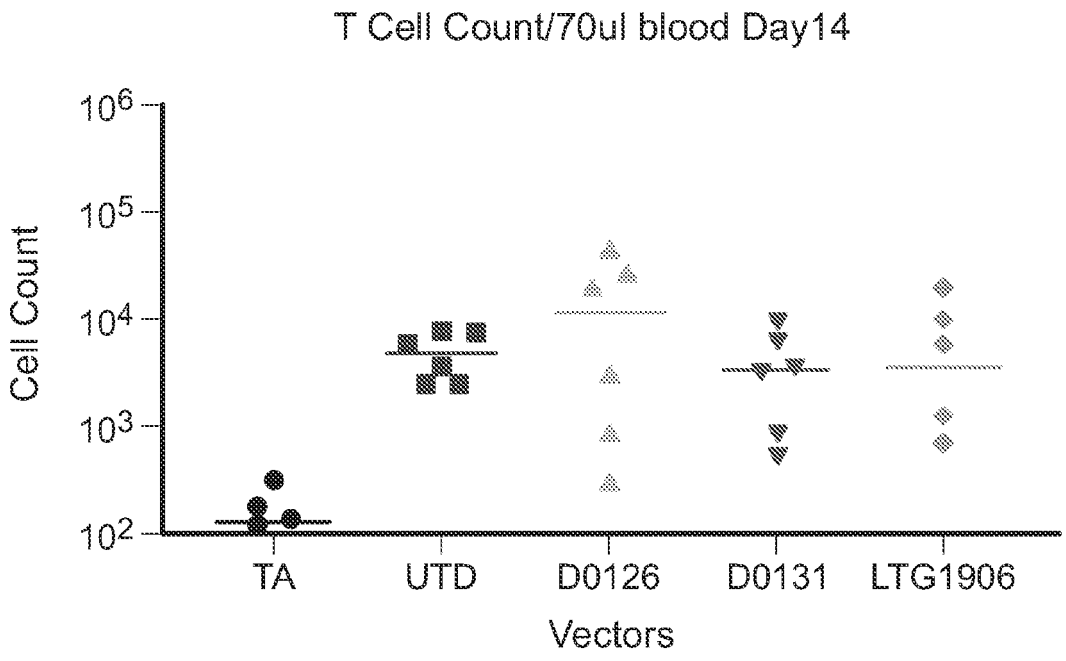


FIGURE 9B

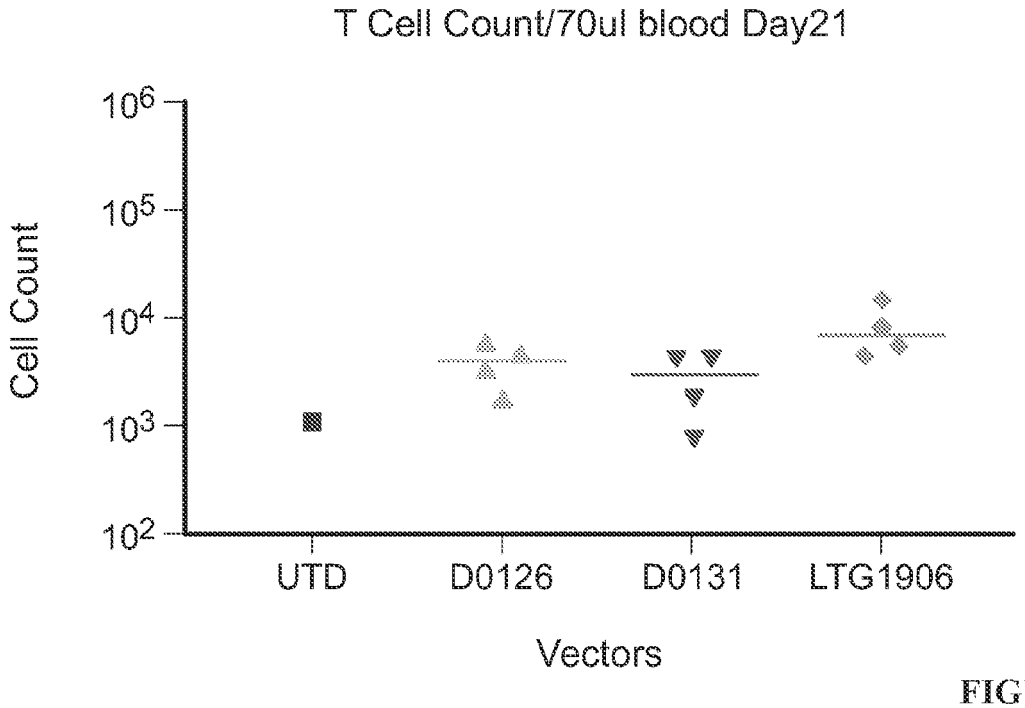


FIGURE 9C

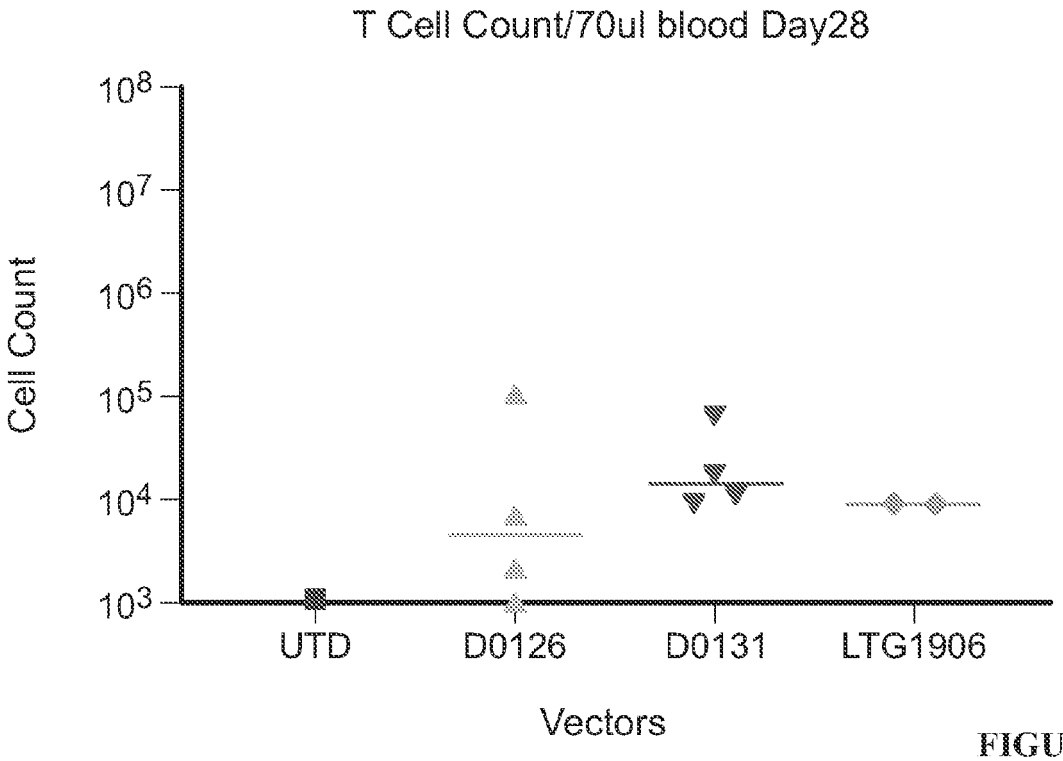


FIGURE 9D

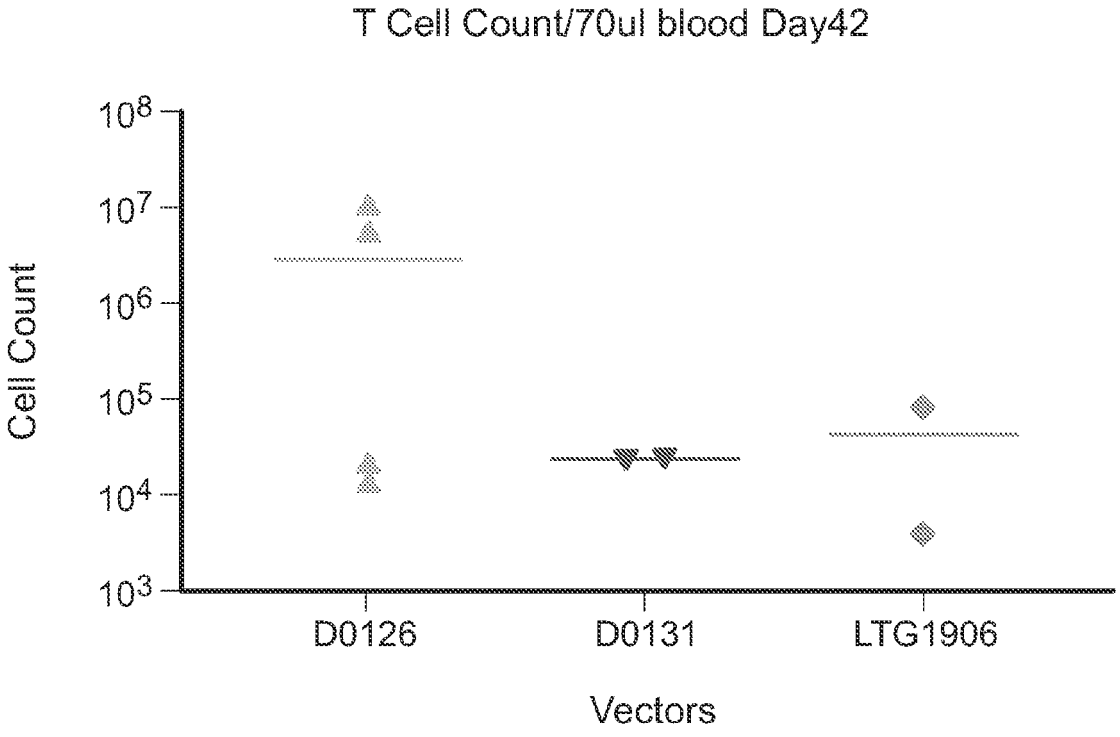


FIGURE 9E

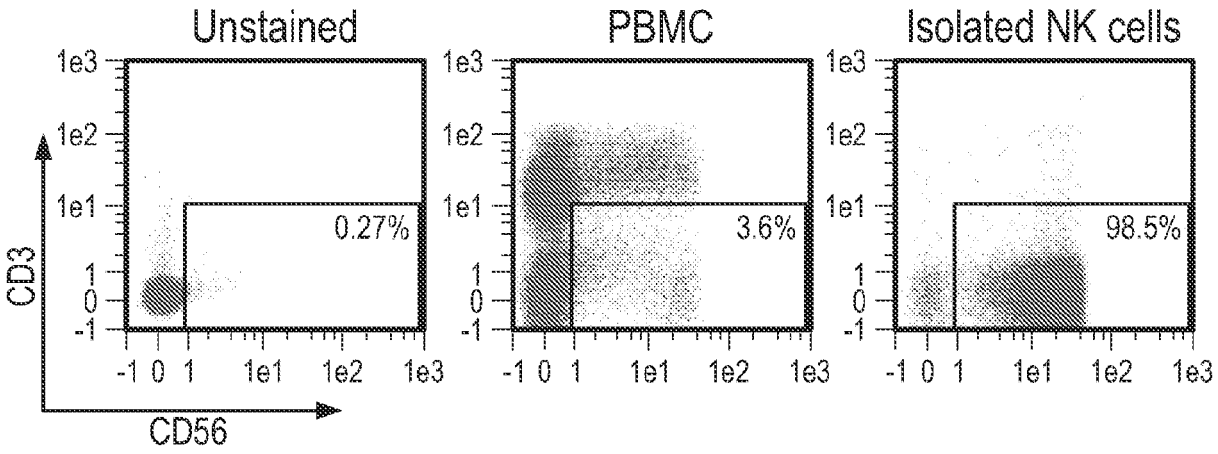


**FIGURE 10A**

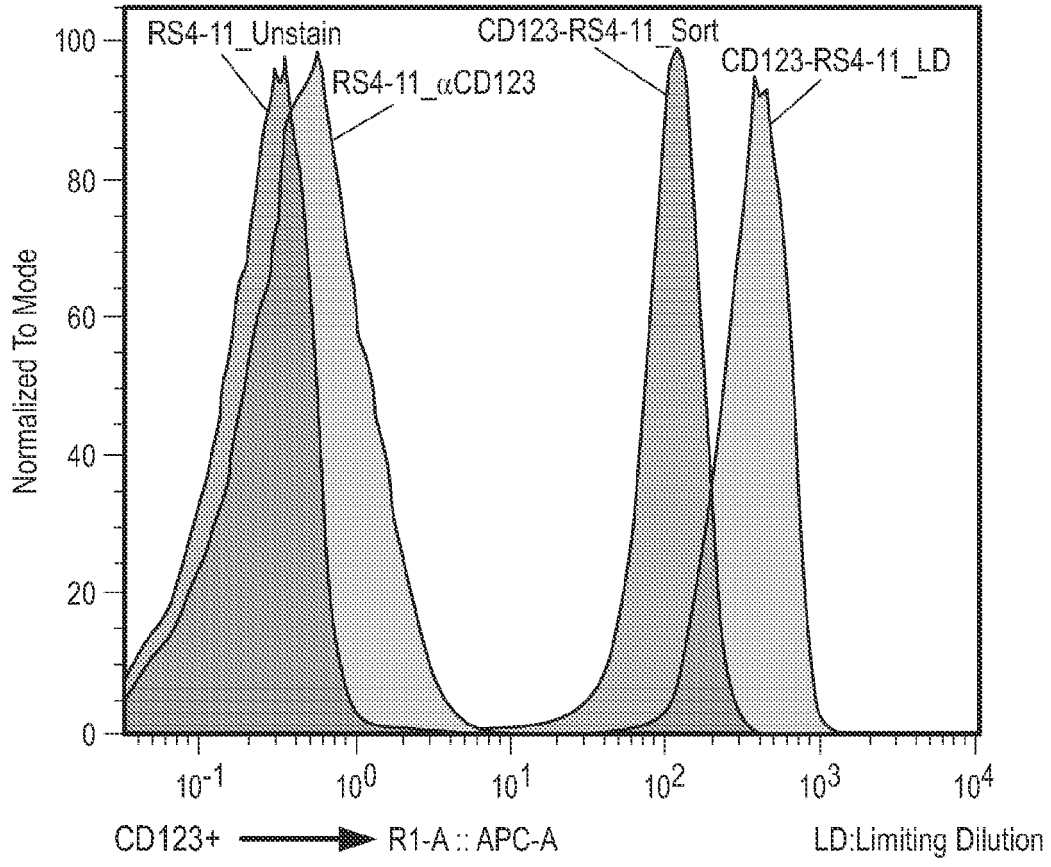


**FIGURE 10B**

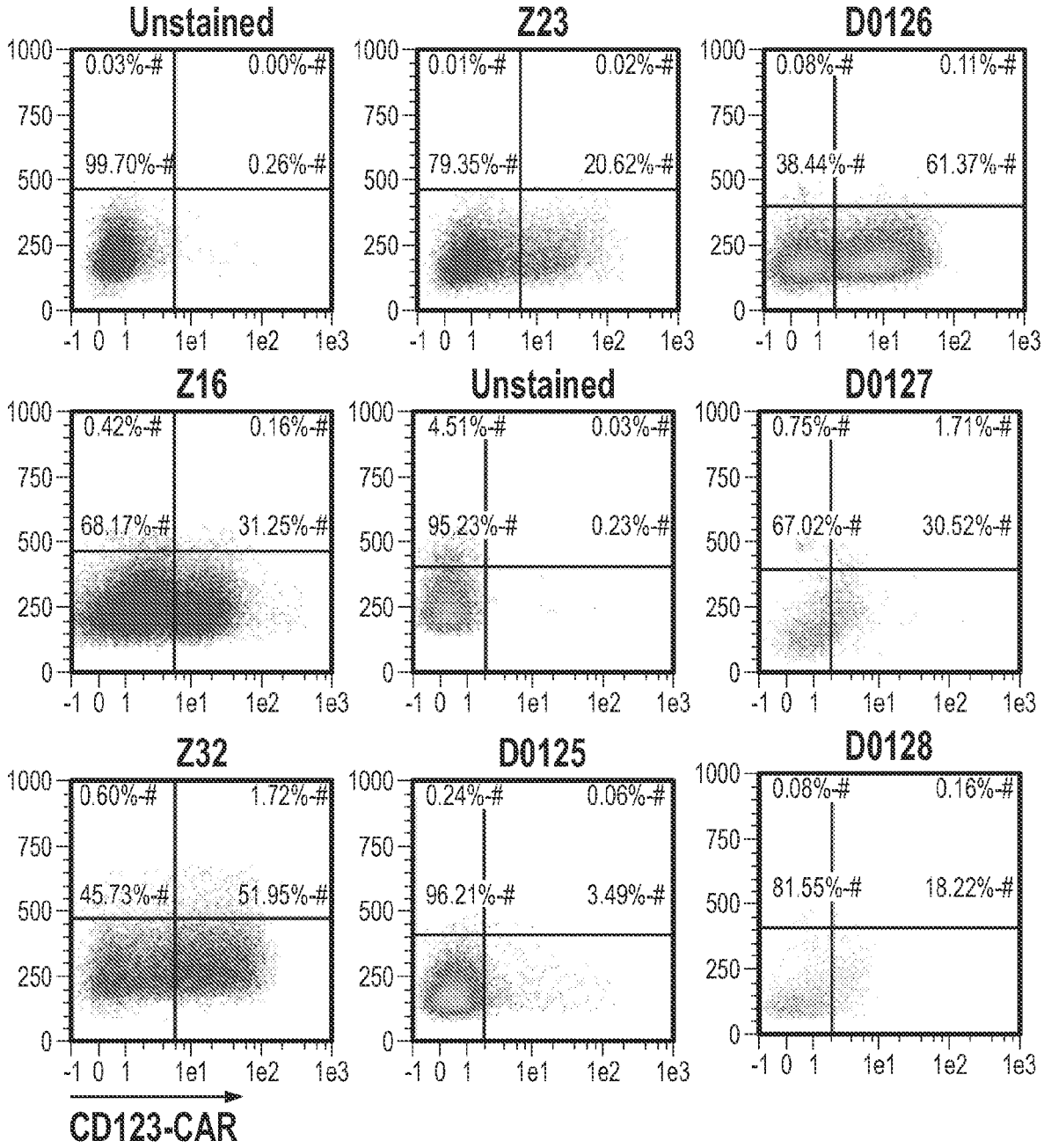
Unstained
0.03%-#
0.00%-#
99.70%-#
0.26%-#
Z23
0.01%-#
0.02%-#
79.35%-#
20.62%-#
D0126
0.08%-#
0.11%-#
38.44%-#
61.37%-#
Z16
0.42%-#
0.16%-#
68.17%-#
31.25%-#
Unstained
4.51%-#
0.03%-#
95.23%-#
0.23%-#
D0127
0.75%-#
1.71%-#
67.02%-#
30.52%-#
Z32
0.60%-#
1.72%-#
45.73%-#
51.95%-#
D0125
0.24%-#
0.06%-#
96.21%-#
3.49%-#
D0128
0.08%-#
0.16%-#
81.55%-#
18.22%-#
1000
750
500
250
0
-1 0 1 1e1 1e2 1e3
CD123-CAR

FIGURE 11

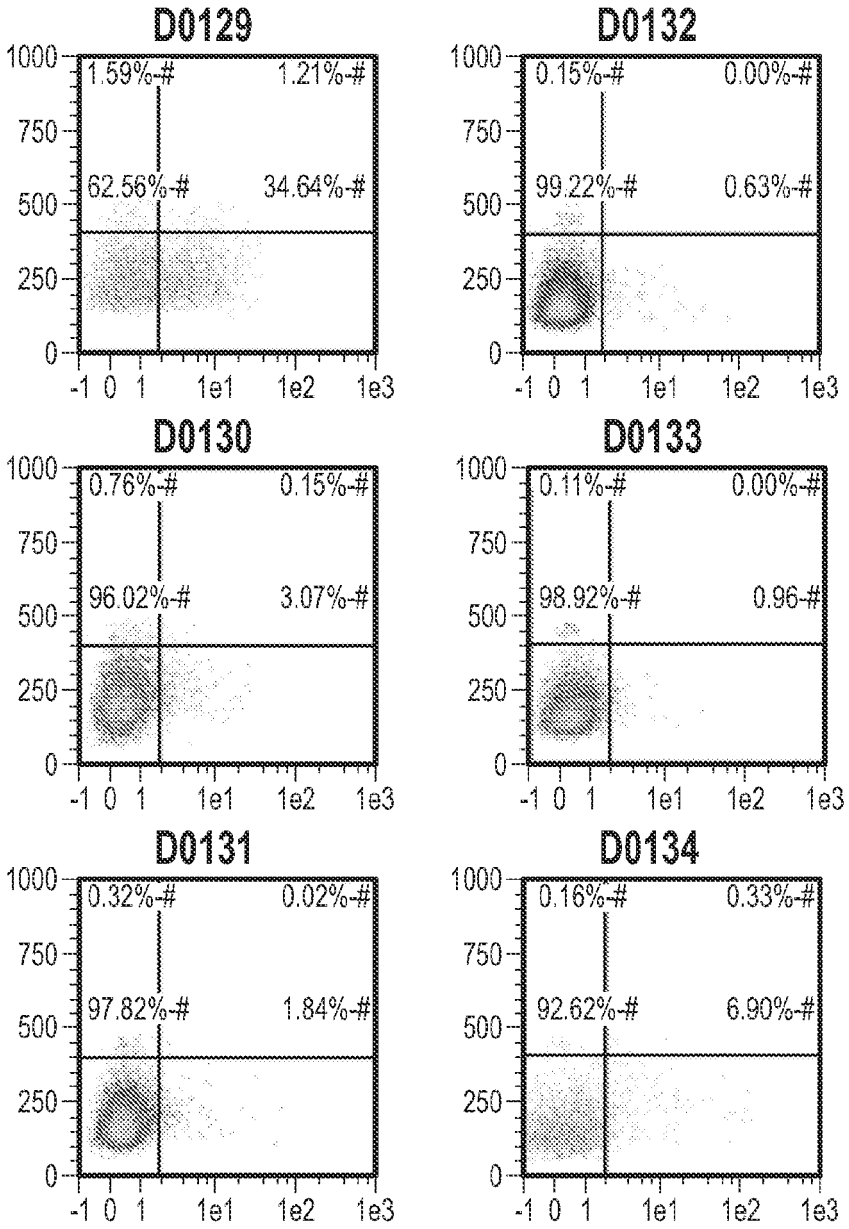


FIGURE 11 (Cont.)

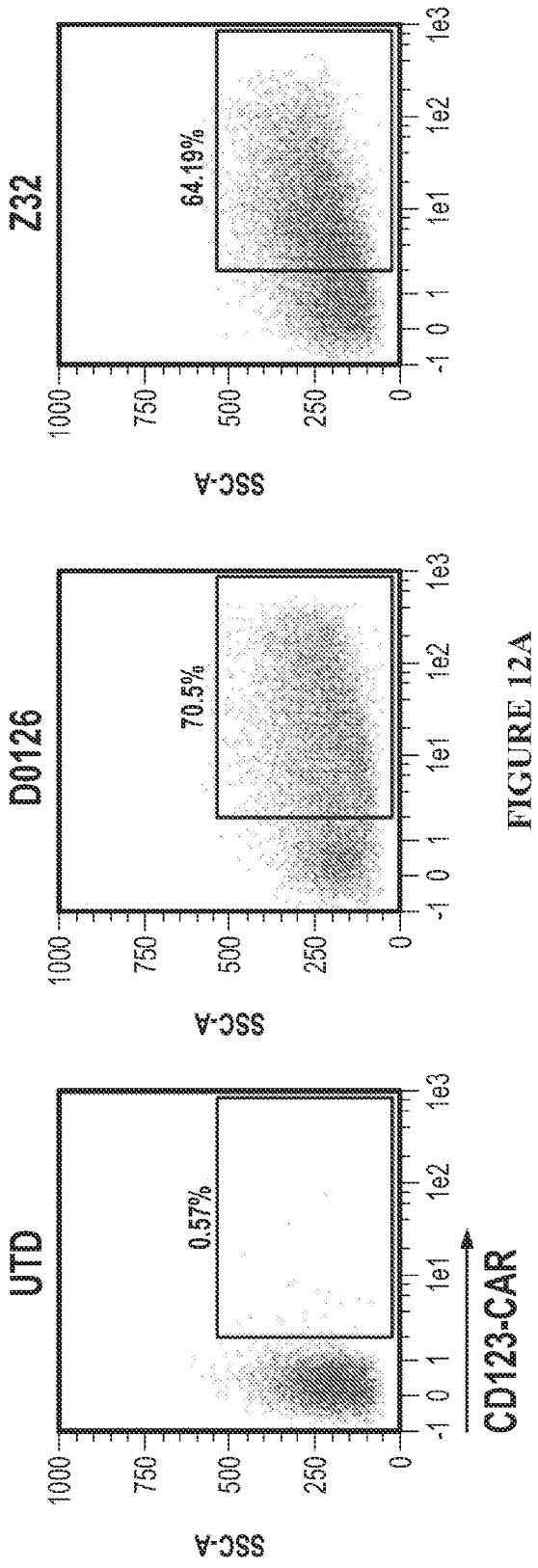
UTD
0.57%
Z32
64.19%
D0126
70.5%
SSC-A
1000
750
500
250
0
-1 0 1 1e1 1e2 1e3
CD123-CAR

FIGURE 12A

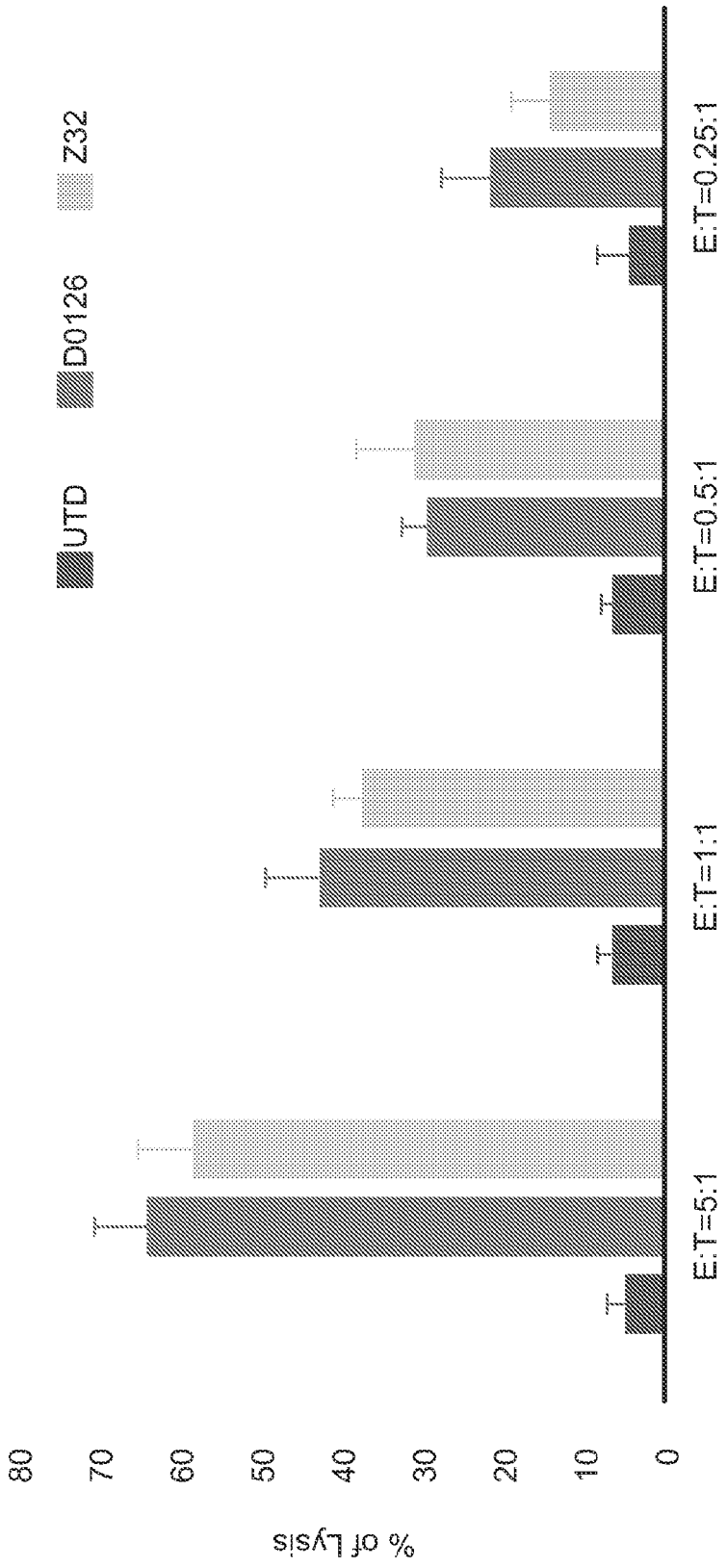
UTD
D0126
Z32
% of Lysis
0
10
20
30
40
50
60
70
80
E:T=5:1
E:T=1:1
E:T=0.5:1
E:T=0.25:1

FIGURE 12B

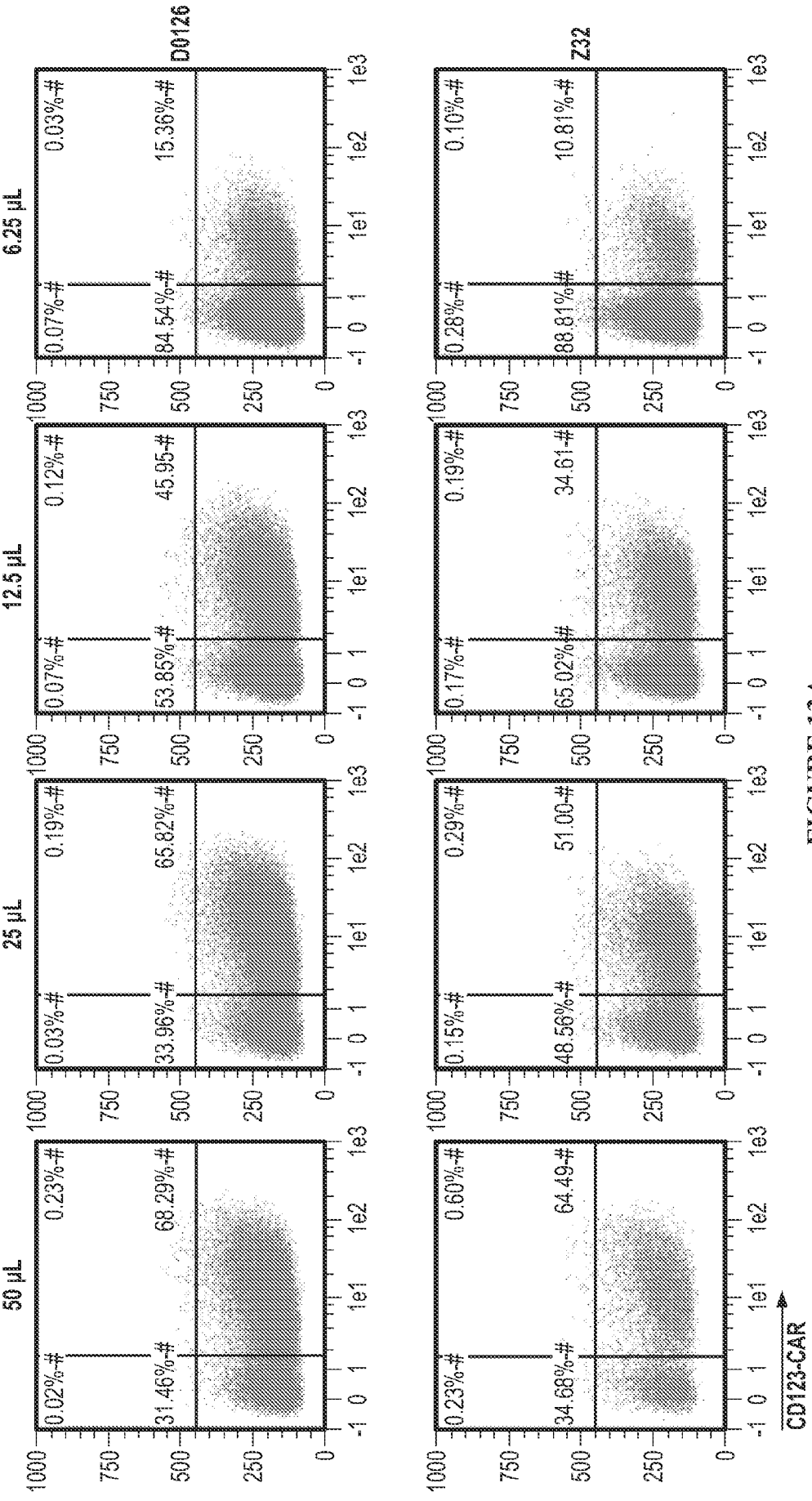
D0126
Z32
6.25 µL
12.5 µL
25 µL
50 µL
0.07%-#
84.54%-#
0.03%-#
15.36%-#
0.12%-#
53.85%-#
45.95-#
0.07%-#
0.19%-#
33.96%-#
65.82%-#
0.03%-#
0.23%-#
31.46%-#
68.29%-#
0.02%-#
0.28%-#
88.81%#
0.10%-#
10.81%-#
0.19%-#
65.02%#
34.61-#
0.17%-#
0.29%-#
48.56%-#
51.00-#
0.15%-#
0.60%-#
34.68%-#
64.49-#
0.23%-#
1000
750
500
250
0
-1 0 1 1e1 1e2 1e3
CD123-CAR

FIGURE 13A

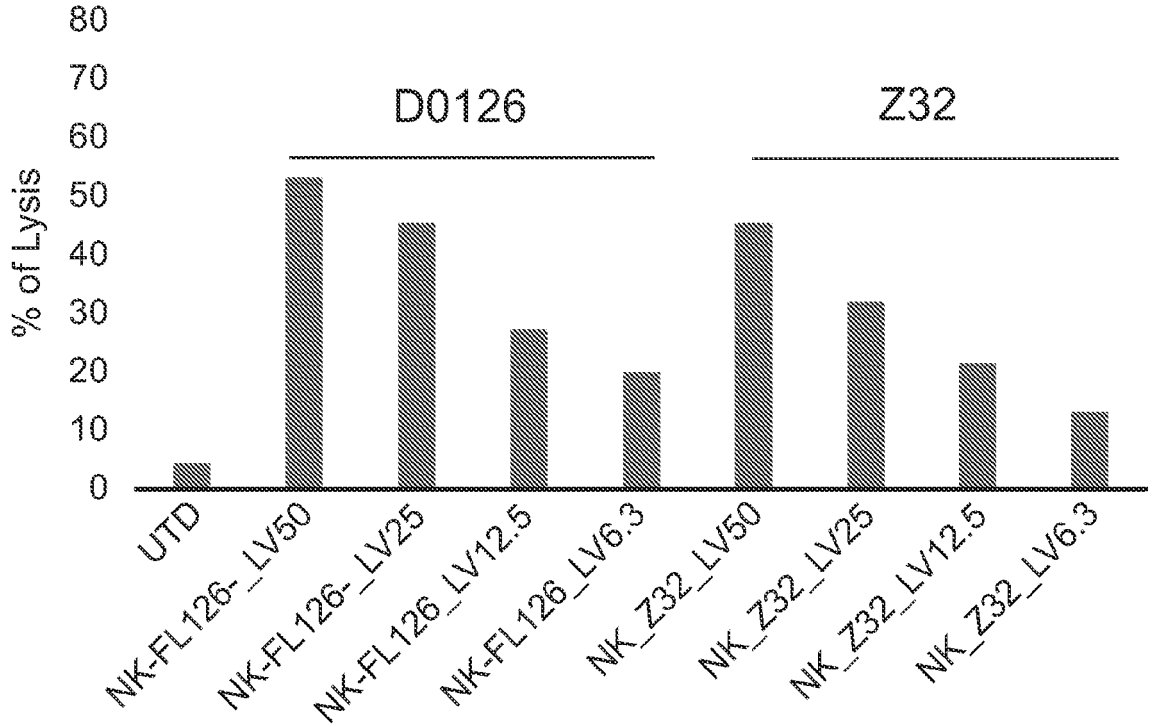


**FIGURE 13B**

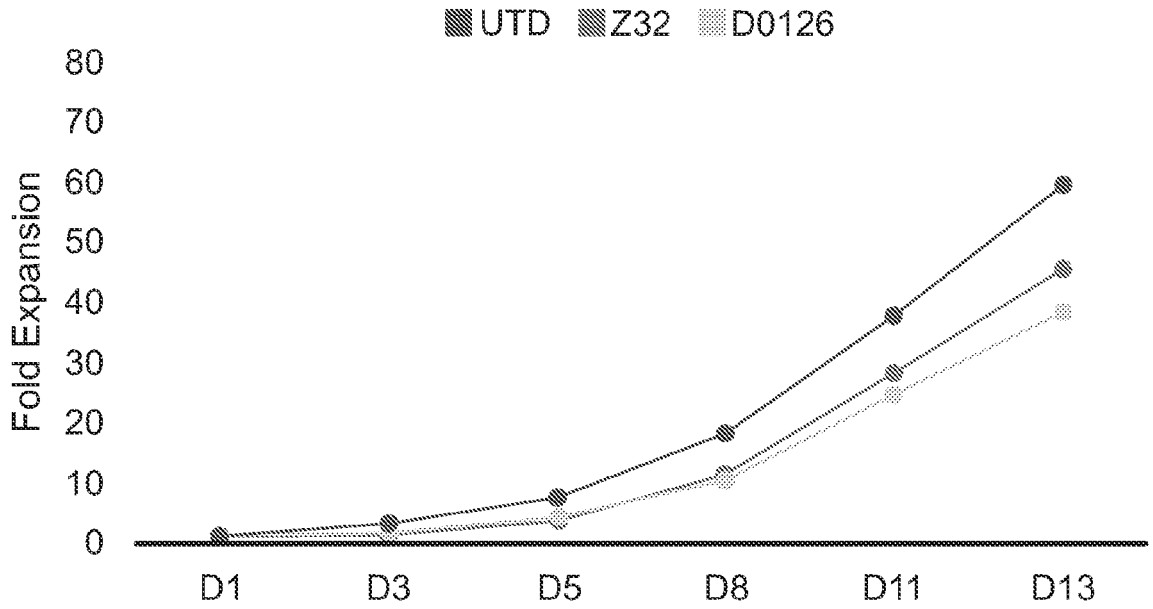


**FIGURE 14A**

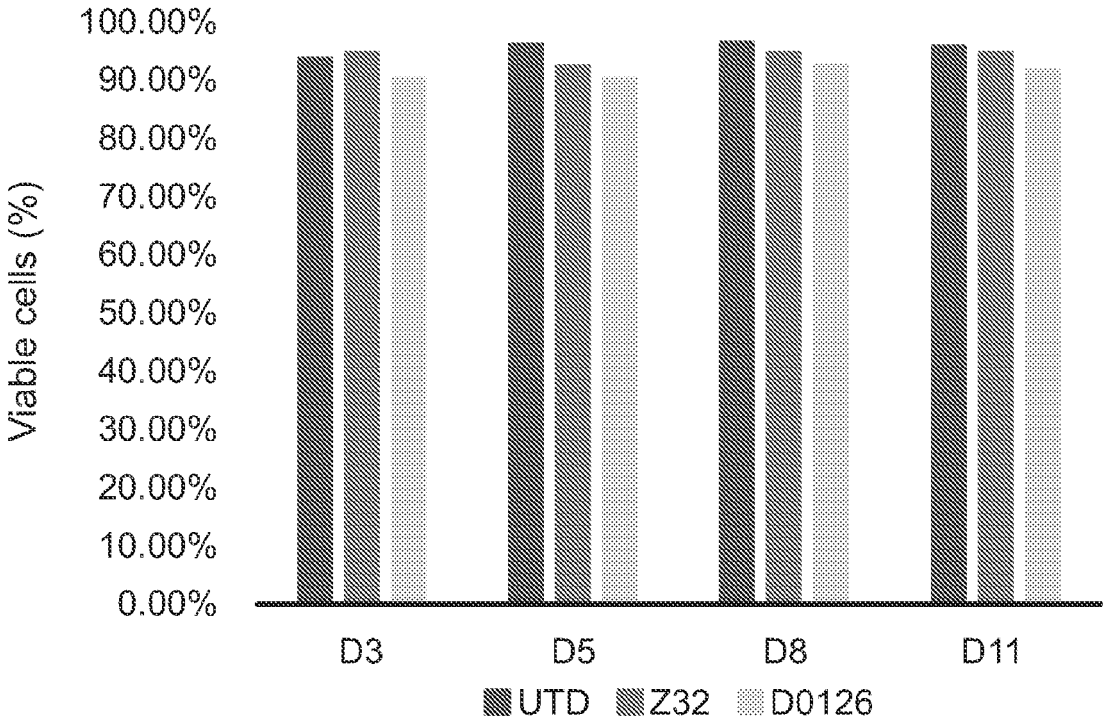
100.00%
90.00%
80.00%
70.00%
60.00%
50.00%
40.00%
30.00%
20.00%
10.00%
0.00%
Viable cells (%)
D3
D5
D8
D11
UTD
Z32
D0126

**FIGURE 14B**

# Sequence Listing

| | | |
|---|---|---|
| **1** | **Sequence Listing Information** | |
| 1-1 | File Name | 42449-0078WO1_SL_ST26.xml |
| 1-2 | DTD Version | V1_3 |
| 1-3 | Software Name | WIPO Sequence |
| 1-4 | Software Version | 2.2.0 |
| 1-5 | Production Date | 2023-02-28 |
| 1-6 | Original free text language code | en |
| 1-7 | Non English free text language code | |
| **2** | **General Information** | |
| 2-1 | Current application: IP Office | WO |
| 2-2 | Current application: Application number | |
| 2-3 | Current application: Filing date | |
| 2-4 | Current application: Applicant file reference | 42449-0078WO1 |
| 2-5 | Earliest priority application: IP Office | US |
| 2-6 | Earliest priority application: Application number | 17/685,132 |
| 2-7 | Earliest priority application: Filing date | 2022-03-02 |
| 2-8en | Applicant name | Lentigen Technology, Inc. |
| 2-8 | Applicant name: Name Latin | |
| 2-9en | Inventor name | |
| 2-9 | Inventor name: Name Latin | |
| 2-10en | Invention title | Compositions and Methods for Treating Cancer with Anti-CD123 Immunotherapy |
| 2-11 | Sequence Total Quantity | 88 |

| | | |
|---|---|---|
| **3-1** | **Sequences** | |
| 3-1-1 | Sequence Number [ID] | 1 |
| 3-1-2 | Molecule Type | DNA |
| 3-1-3 | Length | 1503 |
| 3-1-4 | Features Location/ | **source 1..1503** |
| | Qualifiers | mol_type=other DNA |
| | | organism=synthetic construct |
| | NonEnglishQualifier Value | |
| 3-1-5 | Residues | atgctcttgc tcgtgacttc tttgcttttg tgcgaacttc cgcacccagc cttccttttg 60<br>atacctcaga tacaattggt acagtctgga gccgaggtta agaagccggg atcttccgtc 120<br>aaagtgtcct gtaaagcctc tgggggcacc ttctcttcct acgcaattag ttgggtgaga 180<br>caagctccag gtcagggttt ggagtggatg ggagggataa tcccgatatt cgggacagca 240<br>aactacgccc agaaatttca agggcgcgta acgataacag ctgacgagtc cacatctacg 300<br>gcatacatgg agttgagttc tctgaggagt gaggacacag ctgtatatta ctgcgcgcgg 360<br>ggaagcggag aacttctcta cgcaagttat tattattact acatggatgt ctggggtaag 420<br>ggcactaccg taacagtttc aagtggaggt ggtggttctg gtgggggagg tagcggcggc 480<br>gggggttccc aatccgcact cacgcagcct gcctctgttt caggatcacc gggacagtct 540<br>ataacaatca gttgtactgg caccagttca gatgtcgggg ggtataacta cgtttcatgg 600<br>taccaacaac acccaggaaa ggcaccagaa ctcatgatat atgacgtgtc aaaccgaccg 660<br>tctggcgtat ctaaccgatt tagtggctcc aagtctggta ataccgcgtc actgacaatc 720<br>agcgggttgc aggctgagga tgaagctgac tactattgta gttcctacac cagctctagt 780<br>actcctgttg tcttcggcgg gggcactaag ctcacagtat tggcggccgc aacgaccact 840<br>cctgcacccc gccctccgac tccggcccca accattgcca gccagcccct gtccctgcgg 900<br>ccggaagcct gcagaccggc tgccggcgga gccgtccata cccggggact ggatttcgcc 960<br>tgcgatatct atatctgggc accactcgcc ggaacctgtg gagtgctgct gctgtccctt 1020<br>gtgatcaccc tgtactgcaa gcgcggacgg aagaaactct tgtacatctt caagcagccg 1080<br>ttcatgcgcc ctgtgcaaac cacccaagaa gaggacgggt gctcctgccg gttcccggaa 1140<br>gaggaagagg gcggctgcga actgcgcgtg aagttttccc ggtccgccga cgctccggcg 1200<br>taccagcagg ggcaaaacca gctgtacaac gaacttaacc tcggtcgccg ggaagaatat 1260<br>gacgtgctgg acaagcggcg gggaagagat cccgagatgg gtggaaagcc gcggcggaag 1320<br>aaccctcagg agggcttgta caacgagctg caaaaggaca aaatggccga agcctactcc 1380<br>gagattggca tgaagggaga gcgcagacgc gggaagggac acgatggact gtaccaggga 1440<br>ctgtcaaccg cgactaagga cacttacgac gccctgcaca tgcaggccct gcccccgcgc 1500<br>taa 1503 |
| **3-2** | **Sequences** | |
| 3-2-1 | Sequence Number [ID] | 2 |
| 3-2-2 | Molecule Type | AA |
| 3-2-3 | Length | 500 |
| 3-2-4 | Features Location/ | **source 1..500** |
| | Qualifiers | mol_type=protein |
| | | organism=synthetic construct |
| | NonEnglishQualifier Value | |
| 3-2-5 | Residues | MLLLVTSLLL CELPHPAFLL IPQIQLVQSG AEVKKPGSSV KVSCKASGGT FSSYAISWVR 60<br>QAPGQGLEWM GGIIPIFGTA NYAQKFQGRV TITADESTST AYMELSSLRS EDTAVYYCAR 120<br>GSGELLYASY YYYYMDVWGK GTTVTVSSGG GGSGGGGSGG GGSQSALTQP ASVSGSPGQS 180<br>ITISCTGTSS DVGGYNYVSW YQQHPGKAPE LMIYDVSNRP SGVSNRFSGS KSGNTASLTI 240<br>SGLQAEDEAD YYCSSYTSSS TPVVFGGGTK LTVLAAATTT PAPRPPTPAP TIASQPLSLR 300<br>PEACRPAAGG AVHTRGLDFA CDIYIWAPLA GTCGVLLLSL VITLYCKRGR KKLLYIFKQP 360<br>FMRPVQTTQE EDGCSCRFPE EEEGGCELRV KFSRSADAPA YQQGQNQLYN ELNLGRREEY 420<br>DVLDKRRGRD PEMGGKPRRK NPQEGLYNEL QKDKMAEAYS EIGMKGERRR GKGHDGLYQG 480<br>LSTATKDTYD ALHMQALPPR 500 |
| **3-3** | **Sequences** | |
| 3-3-1 | Sequence Number [ID] | 3 |
| 3-3-2 | Molecule Type | DNA |
| 3-3-3 | Length | 1503 |
| 3-3-4 | Features Location/ | **source 1..1503** |
| | Qualifiers | mol_type=other DNA |
| | | organism=synthetic construct |
| | NonEnglishQualifier Value | |
| 3-3-5 | Residues | atgctcttgc tcgtgacttc tttgcttttg tgcgaacttc cgcacccagc cttccttttg 60<br>atacctgaag tacagctcct cgaatctggc ggtggtctcg ttaagcctgg tgggtccctt 120<br>agactctctt gtgcagcgag cggtttcacc ttcagcaacg cttggatgag ttgggtccgc 180<br>caggcgcctg gaaagggcct cgaatgggtt ggtcggataa aaagcaagac ggatggaggg 240<br>accacagatt acgcggcgcc ggtgaaaggt cggttcacaa tttcaaggga tgactcaaaa 300<br>aatactttgt atctgcaaat gaattccctc aagacggaag atactgcagt ctattattgc 360 |

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|---|---|---|
| | | acaaccggtt tgctctggtt tggcactcgc aattattact atggcatgga tgtatggggc 420<br>caaggaacga ccgtcactgt ttcaagtgga ggtggcggga gcggaggagg gggctccgga 480<br>ggtggcggtt ctcaatcagc acttactcag ccagcttcag tcagtggttc cccgggccaa 540<br>tccatcacca tttcatgcac cggcacatca agtgatgttg gtggctacaa ttacgtgagt 600<br>tggtatcagc aacatccagg aaaggctcct aagcttgtaa tttatgatgt atccaatcgg 660<br>ccttctgggc ttagcaatcg cttttccgga tctaaatcag gcaatactgc gtcccttacc 720<br>ataagcgggc ttcaagccga agatgaagca gattactatt gtaactccta cgctgggagc 780<br>ggttcatggg tatttggagg cggtacgaag ttgactgtct tggcggccgc aacgaccact 840<br>cctgcacccc gccctccgac tccggcccca accattgcca gccagcccct gtccctgcgg 900<br>ccggaagcct gcagaccggc tgccggcgga gccgtccata cccggggact ggatttcgcc 960<br>tgcgatatct atatctgggc accactcgcc ggaacctgtg gagtgctgct gctgtccctt 1020<br>gtgatcaccc tgtactgcaa gcgcggacgg aagaaactct tgtacatctt caagcagccg 1080<br>ttcatgcgcc ctgtgcaaac cacccaagaa gaggacgggt gctcctgccg gttcccggaa 1140<br>gaggaagagg gcggctgcga actgcgcgtg aagttttccc ggtccgccga cgctccggcg 1200<br>taccagcagg ggcaaaacca gctgtacaac gaacttaacc tcggtcgccg ggaagaatat 1260<br>gacgtgctgg acaagcggcg gggaagagat cccgagatgg gtggaaagcc gcggcggaag 1320<br>aaccctcagg agggcttgta caacgagctg caaaaggaca aaatggccga agcctactcc 1380<br>gagattggca tgaagggaga gcgcagacgc gggaagggac acgatggact gtaccaggga 1440<br>ctgtcaaccg cgactaagga cacttacgac gccctgcaca tgcaggccct gcccccgcgc 1500<br>taa 1503 |
| **3-4** | **Sequences** | |
| 3-4-1 | Sequence Number [ID] | 4 |
| 3-4-2 | Molecule Type | AA |
| 3-4-3 | Length | 500 |
| 3-4-4 | Features Location/ | **source 1..500** |
| | Qualifiers | mol_type=protein |
| | | organism=synthetic construct |
| | NonEnglishQualifier Value | |
| 3-4-5 | Residues | MLLLVTSLLL CELPHPAFLL IPEVQLLESG GGLVKPGGSL RLSCAASGFT FSNAWMSWVR 60<br>QAPGKGLEWV GRIKSKTDGG TTDYAAPVKG RFTISRDDSK NTLYLQMNSL KTEDTAVYYC 120<br>TTGLLWFGTR NYYYGMDVWG QGTTVTVSSG GGGSGGGGSG GGGSQSALTQ PASVSGSPGQ 180<br>SITISCTGTS SDVGGYNYVS WYQQHPGKAP KLVIYDVSNR PSGLSNRFSG SKSGNTASLT 240<br>ISGLQAEDEA DYYCNSYAGS GSWVFGGGTK LTVLAAATTT PAPRPPTPAP TIASQPLSLR 300<br>PEACRPAAGG AVHTRGLDFA CDIYIWAPLA GTCGVLLLSL VITLYCKRGR KKLLYIFKQP 360<br>FMRPVQTTQE EDGCSCRFPE EEEGGCELRV KFSRSADAPA YQQGQNQLYN ELNLGRREEY 420<br>DVLDKRRGRD PEMGGKPRRK NPQEGLYNEL QKDKMAEAYS EIGMKGERRR GKGHDGLYQG 480<br>LSTATKDTYD ALHMQALPPR 500 |
| **3-5** | **Sequences** | |
| 3-5-1 | Sequence Number [ID] | 5 |
| 3-5-2 | Molecule Type | DNA |
| 3-5-3 | Length | 1485 |
| 3-5-4 | Features Location/ | **source 1..1485** |
| | Qualifiers | mol_type=other DNA |
| | | organism=synthetic construct |
| | NonEnglishQualifier Value | |
| 3-5-5 | Residues | atgctcttgc tcgtgacttc tttgcttttg tgcgaacttc cgcacccagc cttccttttg 60<br>atacctcaag ttcagctggt ccagagcggc gccgaggtaa aaaagccagg ctcttctgta 120<br>aaggtgtcct gtaaggccag tggaggcact ttttcctcct acgcaatctc atgggtccga 180<br>caagcacctg gtcaaggact ggaatggatg ggcggtatca tcccgatctt tggtactgct 240<br>aactatgcgc agaagttcca gggtagggtg accataaccg cagatgagag tacatccact 300<br>gcctatatgg agctcagtag cctgaggtct gaggatactg ccgtttacta ttgtgcacgc 360<br>cacggcggga tggcaacaat gctcccttac ggagcatttg acatctgggg tcaaggtaca 420<br>atggtaactg tatcatctgg cggtggcggt agtggtgggg gaggcagcgg aggtgggggc 480<br>agtgatatac gactgacgca atctccctct agcctgagtg ccagtgtcgg agatcgggtc 540<br>acaatcacat gccgggctag tcagggtatc agtagctatc ttaattggta ccaacaaaaa 600<br>ccaggaaaag caccgaaact gctcatttat gcagcttctc ggttgcaatc tggagtccca 660<br>agccggttta gtggaagtgg cagtgggacg gactttacct tgactatatc ctcattgcaa 720<br>cctgaggatt tcgctactta ttactgccaa caatcttact ccacgagtct tacgttcggt 780<br>gggggcacga aagtggagat caaagcggcc gcaacgacca ctcctgcacc ccgccctccg 840<br>actccggccc caaccattgc cagccagccc ctgtccctgc ggccggaagc ctgcagaccg 900<br>gctgccggcg gagccgtcca tacccgggga ctggatttcg cctgcgatat ctatatctgg 960<br>gcaccactcg ccggaacctg tggagtgctg ctgctgtccc ttgtgatcac cctgtactgc 1020<br>aagcgcggac ggaagaaact cttgtacatc ttcaagcagc cgttcatgcg ccctgtgcaa 1080<br>accacccaag aagaggacgg gtgctcctgc cggttcccgg aagaggaaga gggcggctgc 1140<br>gaactgcgcg tgaagttttc ccggtccgcc gacgctccgg cgtaccagca ggggcaaaac 1200 |

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|---|---|---|
| | | cagctgtaca acgaacttaa cctcggtcgc cgggaagaat atgacgtgct ggacaagcgg 1260<br>cggggaagag atcccgagat gggtggaaag ccgcggcgga agaaccctca ggagggcttg 1320<br>tacaacgagc tgcaaaagga caaaatggcc gaagcctact ccgagattgg catgaaggga 1380<br>gagcgcagac gcgggaaggg acacgatgga ctgtaccagg gactgtcaac cgcgactaag 1440<br>gacacttacg acgccctgca catgcaggcc ctgcccccgc gctaa 1485 |
| **3-6** | **Sequences** | |
| 3-6-1 | Sequence Number [ID] | 6 |
| 3-6-2 | Molecule Type | AA |
| 3-6-3 | Length | 494 |
| 3-6-4 | Features Location/ | **source 1..494** |
| | Qualifiers | mol_type=protein |
| | | organism=synthetic construct |
| | NonEnglishQualifier Value | |
| 3-6-5 | Residues | MLLLVTSLLL CELPHPAFLL IPQVQLVQSG AEVKKPGSSV KVSCKASGGT FSSYAISWVR 60<br>QAPGQGLEWM GGIIPIFGTA NYAQKFQGRV TITADESTST AYMELSSLRS EDTAVYYCAR 120<br>HGGMATMLPY GAFDIWGQGT MVTVSSGGGG SGGGGSGGGG SDIRLTQSPS SLSASVGDRV 180<br>TITCRASQGI SSYLNWYQQK PGKAPKLLIY AASRLQSGVP SRFSGSGSGT DFTLTISSLQ 240<br>PEDFATYYCQ QSYSTSLTFG GGTKVEIKAA ATTTPAPRPP TPAPTIASQP LSLRPEACRP 300<br>AAGGAVHTRG LDFACDIYIW APLAGTCGVL LLSLVITLYC KRGRKKLLYI FKQPFMRPVQ 360<br>TTQEEDGCSC RFPEEEEGGC ELRVKFSRSA DAPAYQQGQN QLYNELNLGR REEYDVLDKR 420<br>RGRDPEMGGK PRRKNPQEGL YNELQKDKMA EAYSEIGMKG ERRRGKGHDG LYQGLSTATK 480<br>DTYDALHMQA LPPR 494 |
| **3-7** | **Sequences** | |
| 3-7-1 | Sequence Number [ID] | 7 |
| 3-7-2 | Molecule Type | DNA |
| 3-7-3 | Length | 1488 |
| 3-7-4 | Features Location/ | **source 1..1488** |
| | Qualifiers | mol_type=other DNA |
| | | organism=synthetic construct |
| | NonEnglishQualifier Value | |
| 3-7-5 | Residues | atgctcttgc tcgtgacttc tttgcttttg tgcgaacttc cgcacccagc cttccttttg 60<br>atacctcaag tccagctcgt tcagagtggt gcagaggtga agaagcccgg ctcatctgtg 120<br>aaagtgtcat gcaaagcaag cggcgggacc ttcagcagtt acgcgatctc ctgggtacga 180<br>caagcccccg gccagggcct ggaatggatg ggagggatca ttccgatttt cggtacagca 240<br>aactatgcac aaaaatttca ggggagagtt acgataactg cagacaagag cacttcaacg 300<br>gcatacatgg agctttcatc attgcgctcc gaggacacgg ctgtttacta ctgcgctcga 360<br>gggggacgga actcttacta ttattactac atggacgtgt ggggcaaagg gacaacggtg 420<br>acggtaagta gtgggggagg cggaagcggt ggtgggggaa gtggaggcgg tgggtcacag 480<br>tcagccctca cacaaccggc ctctgtctca gggagtccag gacagagtat tactataagc 540<br>tgcactggga catcctcaga cgtcggcggt tataattatg tttcctggta ccaacaacat 600<br>cccgggaagg ctcccaagct gatgatatac gaagtgagta atcgaccctc tggcgtgagc 660<br>aatcgattct ctgggagtaa gagtggcaac actgcgagtc ttacgatttc tggcctgcag 720<br>gcggaagacg aagccgatta ttactgtagc agctacactt caagctcccc tgttgttttc 780<br>ggtggcggca ctaaacttac cgtgcttgcg gccgcaacga ccactcctgc accccgccct 840<br>ccgactccgg ccccaaccat tgccagccag cccctgtccc tgcggccgga agcctgcaga 900<br>ccggctgccg gcggagccgt ccatacccgg ggactggatt tcgcctgcga tatctatatc 960<br>tgggcaccac tcgccggaac ctgtggagtg ctgctgctgt cccttgtgat caccctgtac 1020<br>tgcaagcgcg gacggaagaa actcttgtac atcttcaagc agccgttcat gcgccctgtg 1080<br>caaaccaccc aagaagagga cgggtgctcc tgccggttcc cggaagagga agagggcggc 1140<br>tgcgaactgc gcgtgaagtt ttcccggtcc gccgacgctc cggcgtacca gcaggggcaa 1200<br>aaccagctgt acaacgaact taacctcggt cgccgggaag aatatgacgt gctggacaag 1260<br>cggcggggaa gagatcccga gatgggtgga aagccgcggc ggaagaaccc tcaggagggc 1320<br>ttgtacaacg agctgcaaaa ggacaaaatg gccgaagcct actccgagat tggcatgaag 1380<br>ggagagcgca gacgcgggaa gggacacgat ggactgtacc agggactgtc aaccgcgact 1440<br>aaggacactt acgacgccct gcacatgcag gccctgcccc cgcgctaa 1488 |
| **3-8** | **Sequences** | |
| 3-8-1 | Sequence Number [ID] | 8 |
| 3-8-2 | Molecule Type | AA |
| 3-8-3 | Length | 495 |
| 3-8-4 | Features Location/ | **source 1..495** |
| | Qualifiers | mol_type=protein |
| | | organism=synthetic construct |
| | NonEnglishQualifier Value | |
| 3-8-5 | Residues | MLLLVTSLLL CELPHPAFLL IPQVQLVQSG AEVKKPGSSV KVSCKASGGT FSSYAISWVR 60<br>QAPGQGLEWM GGIIPIFGTA NYAQKFQGRV TITADKSTST AYMELSSLRS EDTAVYYCAR 120 |

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| | | GGRNSYYYYY MDVWGKGTTV TVSSGGGGSG GGGSGGGGSQ SALTQPASVS GSPGQSITIS 180<br>CTGTSSDVGG YNYVSWYQQH PGKAPKLMIY EVSNRPSGVS NRFSGSKSGN TASLTISGLQ 240<br>AEDEADYYCS SYTSSSPVVF GGGTKLTVLA AATTTPAPRP PTPAPTIASQ PLSLRPEACR 300<br>PAAGGAVHTR GLDFACDIYI WAPLAGTCGV LLLSLVITLY CKRGRKKLLY IFKQPFMRPV 360<br>QTTQEEDGCS CRFPEEEEGG CELRVKFSRS ADAPAYQQGQ NQLYNELNLG RREEYDVLDK 420<br>RRGRDPEMGG KPRRKNPQEG LYNELQKDKM AEAYSEIGMK GERRRGKGHD GLYQGLSTAT 480<br>KDTYDALHMQ ALPPR 495 |
| **3-9** | **Sequences** | |
| 3-9-1 | Sequence Number [ID] | 9 |
| 3-9-2 | Molecule Type | DNA |
| 3-9-3 | Length | 1500 |
| 3-9-4 | Features Location/ | **source 1..1500** |
| | Qualifiers | mol_type=other DNA |
| | | organism=synthetic construct |
| | NonEnglishQualifier Value | |
| 3-9-5 | Residues | atgctcttgc tcgtgacttc tttgcttttg tgcgaacttc cgcacccagc cttccttttg 60<br>atacctcagg ttcagctcgt tcaaagcgga gctgaagtta aaaaacctgg gtcttctgtc 120<br>aaggtaagtt gcaaagcatc cggaggcacg ttttcttcct atgcaataag ttgggtccgg 180<br>caagcacctg gtcagggatt ggaatggatg ggtggtatta taccaatatt cggaacggcg 240<br>aactacgcac agaagtttca aggcagggta actattaccg cggacgagtc tacctcaaca 300<br>gcgtatatgg aactgagcag tctcagatca gaagataccg cagtttatta ctgcgctcgg 360<br>gggtctggag agcttctcta tgcatcctac tactactatt atatggacgt atggggcaag 420<br>ggtaccaccg ttaccgtgtc ttctggaggt ggcggatctg gaggtggagg atccggtggg 480<br>ggaggcagcc aatctgcact gactcaaccc gcgtccgtga gcggatcccc tgggcaatca 540<br>ataacaatct cttgcacggg gacctcatct gatgttggtg gatataatta cgtcagctgg 600<br>taccaacaac accccggtaa ggctccgaag ctgatgattt acgaagtgag taatcgccca 660<br>agtggtgtaa gcaacagatt ctcaggctca aagagtggga acactgcgtc cctgactatc 720<br>tcaggcctcc aggctgagga cgaagcagat tattactgtt cttcatacac cagtagtagt 780<br>cctttggtct tcggcaccgg caccaaggta actgtactgg cggccgcaac gaccactcct 840<br>gcaccccgcc ctccgactcc ggccccaacc attgccagcc agcccctgtc cctgcggccg 900<br>gaagcctgca gaccggctgc cggcggagcc gtccataccc ggggactgga tttcgcctgc 960<br>gatatctata tctgggcacc actcgccgga acctgtggag tgctgctgct gtcccttgtg 1020<br>atcaccctgt actgcaagcg cggacggaag aaactcttgt acatcttcaa gcagccgttc 1080<br>atgcgccctg tgcaaaccac ccaagaagag gacgggtgct cctgccggtt cccggaagag 1140<br>gaagagggcg gctgcgaact gcgcgtgaag ttttcccggt ccgccgacgc tccggcgtac 1200<br>cagcaggggc aaaaccagct gtacaacgaa cttaacctcg gtcgccggga agaatatgac 1260<br>gtgctggaca agcggcgggg aagagatccc gagatgggtg gaaagccgcg gcggaagaac 1320<br>cctcaggagg gcttgtacaa cgagctgcaa aaggacaaaa tggccgaagc ctactccgag 1380<br>attggcatga agggagagcg cagacgcggg aagggacacg atggactgta ccagggactg 1440<br>tcaaccgcga ctaaggacac ttacgacgcc ctgcacatgc aggccctgcc cccgcgctaa 1500 |
| **3-10** | **Sequences** | |
| 3-10-1 | Sequence Number [ID] | 10 |
| 3-10-2 | Molecule Type | AA |
| 3-10-3 | Length | 499 |
| 3-10-4 | Features Location/ | **source 1..499** |
| | Qualifiers | mol_type=protein |
| | | organism=synthetic construct |
| | NonEnglishQualifier Value | |
| 3-10-5 | Residues | MLLLVTSLLL CELPHPAFLL IPQVQLVQSG AEVKKPGSSV KVSCKASGGT FSSYAISWVR 60<br>QAPGQGLEWM GGIIPIFGTA NYAQKFQGRV TITADESTST AYMELSSLRS EDTAVYYCAR 120<br>GSGELLYASY YYYYMDVWGK GTTVTVSSGG GGSGGGGSGG GGSQSALTQP ASVSGSPGQS 180<br>ITISCTGTSS DVGGYNYVSW YQQHPGKAPK LMIYEVSNRP SGVSNRFSGS KSGNTASLTI 240<br>SGLQAEDEAD YYCSSYTSSS PLVFGTGTKV TVLAAATTTP APRPPTPAPT IASQPLSLRP 300<br>EACRPAAGGA VHTRGLDFAC DIYIWAPLAG TCGVLLLSLV ITLYCKRGRK KLLYIFKQPF 360<br>MRPVQTTQEE DGCSCRFPEE EEGGCELRVK FSRSADAPAY QQGQNQLYNE LNLGRREEYD 420<br>VLDKRRGRDP EMGGKPRRKN PQEGLYNELQ KDKMAEAYSE IGMKGERRRG KGHDGLYQGL 480<br>STATKDTYDA LHMQALPPR 499 |
| **3-11** | **Sequences** | |
| 3-11-1 | Sequence Number [ID] | 11 |
| 3-11-2 | Molecule Type | DNA |
| 3-11-3 | Length | 1491 |
| 3-11-4 | Features Location/ | **source 1..1491** |
| | Qualifiers | mol_type=other DNA |
| | | organism=synthetic construct |
| | NonEnglishQualifier Value | |

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| 3-11-5 | Residues | atgctcttgc tcgtgacttc tttgcttttg tgcgaacttc cgcacccagc cttccttttg 60<br>atacctcagg ttcagctggt acagtccggc gcagaggtta aaaagccagg aagctccgtg 120<br>aaggtttcat gcaaggcatc cggtggtaca ttctcatcat atgcgatcag ttgggtccgg 180<br>caggctcccg gccagggatt ggagtggatg ggagggataa tccccatatt tggcacagca 240<br>aattacgctc aaaaatttca aggtagagta acgataactg cggatgaatc tactagcacg 300<br>gcgtatatgg aactgagtag tctccggagc gaggatacag cggtttacta ctgcgctagg 360<br>aatgaatggt actcctatta ttactactac atgggtgtgt ggggtaaagg aactactgtt 420<br>acggtgtcca gtggaggagg aggtagcgga ggtggaggat caggcggtgg gggctcccaa 480<br>agtgcgctta cacaacctgc aagcgtatca ggttccccag ggcaatcaat tacaataagc 540<br>tgcacgggta cctccagtga tgtcggaggt tacaactacg tgtcatggta ccagcaacat 600<br>ccaggcaagg caccaaaact tatgatctac gaagtcagca acagacccag cggtgtaagc 660<br>aataggttta gcggatctaa gtccggtaat actgcttctc tgacaatctc aggactccaa 720<br>gccgaggacg aagctgatta ctactgctca tcatacacca gtagctctac actggtggtg 780<br>ttcggagggg gaacgaagct taccgtactg gcggccgcaa cgaccactcc tgcaccccgc 840<br>cctccgactc cggccccaac cattgccagc cagcccctgt ccctgcggcc ggaagcctgc 900<br>agaccggctg ccggcggagc cgtccatacc cggggactgg atttcgcctg cgatatctat 960<br>atctgggcac cactcgccgg aacctgtgga gtgctgctgc tgtcccttgt gatcaccctg 1020<br>tactgcaagc gcggacggaa gaaactcttg tacatcttca agcagccgtt catgcgccct 1080<br>gtgcaaacca cccaagaaga ggacgggtgc tcctgccggt tcccggaaga ggaagagggc 1140<br>ggctgcgaac tgcgcgtgaa gttttcccgg tccgccgacg ctccggcgta ccagcagggg 1200<br>caaaaccagc tgtacaacga acttaacctc ggtcgccggg aagaatatga cgtgctggac 1260<br>aagcggcggg gaagagatcc cgagatgggt ggaaagccgc ggcggaagaa ccctcaggag 1320<br>ggcttgtaca acgagctgca aaaggacaaa atggccgaag cctactccga gattggcatg 1380<br>aagggagagc gcagacgcgg gaagggacac gatggactgt accagggact gtcaaccgcg 1440<br>actaaggaca cttacgacgc cctgcacatg caggccctgc ccccgcgcta a 1491 |
| **3-12** | **Sequences** | |
| 3-12-1 | Sequence Number [ID] | 12 |
| 3-12-2 | Molecule Type | AA |
| 3-12-3 | Length | 496 |
| 3-12-4 | Features Location/ Qualifiers<br><br><br>NonEnglishQualifier Value | **source 1..496**<br>mol_type=protein<br>organism=synthetic construct |
| 3-12-5 | Residues | MLLLVTSLLL CELPHPAFLL IPQVQLVQSG AEVKKPGSSV KVSCKASGGT FSSYAISWVR 60<br>QAPGQGLEWM GGIIPIFGTA NYAQKFQGRV TITADESTST AYMELSSLRS EDTAVYYCAR 120<br>NEWYSYYYYY MGVWGKGTTV TVSSGGGGSG GGGSGGGGSQ SALTQPASVS GSPGQSITIS 180<br>CTGTSSDVGG YNYVSWYQQH PGKAPKLMIY EVSNRPSGVS NRFSGSKSGN TASLTISGLQ 240<br>AEDEADYYCS SYTSSSTLVV FGGGTKLTVL AAATTTPAPR PPTPAPTIAS QPLSLRPEAC 300<br>RPAAGGAVHT RGLDFACDIY IWAPLAGTCG VLLLSLVITL YCKRGRKKLL YIFKQPFMRP 360<br>VQTTQEEDGC SCRFPEEEEG GCELRVKFSR SADAPAYQQG QNQLYNELNL GRREEYDVLD 420<br>KRRGRDPEMG GKPRRKNPQE GLYNELQKDK MAEAYSEIGM KGERRRGKGH DGLYQGLSTA 480<br>TKDTYDALHM QALPPR 496 |
| **3-13** | **Sequences** | |
| 3-13-1 | Sequence Number [ID] | 13 |
| 3-13-2 | Molecule Type | DNA |
| 3-13-3 | Length | 66 |
| 3-13-4 | Features Location/ Qualifiers<br><br><br>NonEnglishQualifier Value | **source 1..66**<br>mol_type=other DNA<br>organism=synthetic construct |
| 3-13-5 | Residues | atgctgctgc tggtgaccag cctgctgctg tgcgaactgc cgcatccggc gtttctgctg 60<br>attccg 66 |
| **3-14** | **Sequences** | |
| 3-14-1 | Sequence Number [ID] | 14 |
| 3-14-2 | Molecule Type | AA |
| 3-14-3 | Length | 22 |
| 3-14-4 | Features Location/ Qualifiers<br><br><br>NonEnglishQualifier Value | **source 1..22**<br>mol_type=protein<br>organism=synthetic construct |
| 3-14-5 | Residues | MLLLVTSLLL CELPHPAFLL IP 22 |
| **3-15** | **Sequences** | |
| 3-15-1 | Sequence Number [ID] | 15 |
| 3-15-2 | Molecule Type | DNA |
| 3-15-3 | Length | 1485 |

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| 3-15-4 | Features Location/ | **source 1..1485** |
| | Qualifiers | mol_type=other DNA |
| | | organism=synthetic construct |
| | NonEnglishQualifier Value | |
| 3-15-5 | Residues | atgctcttgc tcgtgacttc tttgcttttg tgcgaacttc cgcacccagc cttccttttg 60<br>atacctcaag ttcaacttgt acaatccgga gcagaagtaa aaaaacccgg ggccagcgta 120<br>aaagtttcct gtaaagctag cggctacaca ttcactagct acggcatctc ctgggtacgc 180<br>caagcgccag gacaaggcct cgaatggatg ggatggatta gcgcttacaa cggtaatacc 240<br>aattatgcac aaaagctgca aggacgggtt acgatgacaa cagacacgag cacgagtacg 300<br>gcctatatgg agctgagaag tcttcgaagt gatgacactg cagtatatta ctgtgcccgc 360<br>ggagcgtact acgatttttg gagcagttac agctggtttg atccctgggg gcaggggacc 420<br>ctggttactg ttagctcagg tgggggggc tcaggaggtg gaggaagcgg gggtggagga 480<br>tctagttatg ttcttaccca gccgccttct gtcagtgtgg cccctggtaa gacagccagg 540<br>ataacctgtg gtgggaattc aattggcagc aaatcagtac agtggtacca acaaaaaccc 600<br>ggacaagccc ccgttttggt catatatgat gatagcgata ggccttctgg aatcccggag 660<br>aggttttcag gatcaaatag cgggaacacc gccacattga ccataagtcg agtcgaggcg 720<br>ggcgacgaag ctgactatta ttgtcaagtg tgggatagct ctagtgatgt ggtattcggt 780<br>gggggacca aattgacagt cttggcggcc gcaacgacca ctcctgcacc ccgccctccg 840<br>actccggccc caaccattgc cagccagccc ctgtccctgc ggccggaagc ctgcagaccg 900<br>gctgccggcg gagccgtcca tacccgggga ctggatttcg cctgcgatat ctatatctgg 960<br>gcaccactcg ccggaacctg tggagtgctg ctgctgtccc ttgtgatcac cctgtactgc 1020<br>aagcgcggac ggaagaaact cttgtacatc ttcaagcagc cgttcatgcg ccctgtgcaa 1080<br>accacccaag aagaggacgg gtgctcctgc cggttcccgg aagaggaaga gggcggctgc 1140<br>gaactgcgcg tgaagttttc ccggtccgcc gacgctccgg cgtaccagca ggggcaaaac 1200<br>cagctgtaca acgaacttaa cctcggtcgc cgggaagaat atgacgtgct ggacaagcgg 1260<br>cggggaagag atcccgagat gggtggaaag ccgcggcgga agaaccctca ggagggcttg 1320<br>tacaacgagc tgcaaaagga caaaatggcc gaagcctact ccgagattgg catgaaggga 1380<br>gagcgcagac gcgggaaggg acacgatgga ctgtaccagg gactgtcaac cgcgactaag 1440<br>gacacttacg acgccctgca catgcaggcc ctgcccccgc gctaa 1485 |
| **3-16** | **Sequences** | |
| 3-16-1 | Sequence Number [ID] | 16 |
| 3-16-2 | Molecule Type | AA |
| 3-16-3 | Length | 494 |
| 3-16-4 | Features Location/ | **source 1..494** |
| | Qualifiers | mol_type=protein |
| | | organism=synthetic construct |
| | NonEnglishQualifier Value | |
| 3-16-5 | Residues | MLLLVTSLLL CELPHPAFLL IPQVQLVQSG AEVKKPGASV KVSCKASGYT FTSYGISWVR 60<br>QAPGQGLEWM GWISAYNGNT NYAQKLQGRV TMTTDTSTST AYMELRSLRS DDTAVYYCAR 120<br>GAYYDFWSSY SWFDPWGQGT LVTVSSGGGG SGGGGSGGGG SSYVLTQPPS VSVAPGKTAR 180<br>ITCGGNSIGS KSVQWYQQKP GQAPVLVIYD DSDRPSGIPE RFSGSNSGNT ATLTISRVEA 240<br>GDEADYYCQV WDSSSDVVFG GGTKLTVLAA ATTTPAPRPP TPAPTIASQP LSLRPEACRP 300<br>AAGGAVHTRG LDFACDIYIW APLAGTCGVL LLSLVITLYC KRGRKKLLYI FKQPFMRPVQ 360<br>TTQEEDGCSC RFPEEEEGGC ELRVKFSRSA DAPAYQQGQN QLYNELNLGR REEYDVLDKR 420<br>RGRDPEMGGK PRRKNPQEGL YNELQKDKMA EAYSEIGMKG ERRRGKGHDG LYQGLSTATK 480<br>DTYDALHMQA LPPR 494 |
| **3-17** | **Sequences** | |
| 3-17-1 | Sequence Number [ID] | 17 |
| 3-17-2 | Molecule Type | DNA |
| 3-17-3 | Length | 1488 |
| 3-17-4 | Features Location/ | **source 1..1488** |
| | Qualifiers | mol_type=other DNA |
| | | organism=synthetic construct |
| | NonEnglishQualifier Value | |
| 3-17-5 | Residues | atgctcttgc tcgtgacttc tttgcttttg tgcgaacttc cgcacccagc cttccttttg 60<br>atacctcagg tacaacttgt ccaatccggt gccgaagtca agaaacctgg agcatccgta 120<br>aaggtcagct gcaaagccag cgggtatacc ttcacgagtt atggaatctc ttgggtcaga 180<br>caagcgccag gccaagggct ggaatggatg ggatggataa gcgcatacaa tggcaacaca 240<br>aattatgctc agaaactgca aggtcgcgtt accatgacca ccgacacatc aacgtccacc 300<br>gcctatatgg agcttagaag cttgcgaagt gacgacacag ccgtgtatta ttgcgctcgg 360<br>ggtgcttatt atgacttctg gtctggttac tcttggtttg atccttgggg tcaaggcacg 420<br>cttgtgacgg tatcctcagg aggcggcgga agtggagggg gtggatcagg tggtggtgga 480<br>agccaatcag tactcactca gccaccaagt gtatcagtgg ctccaggtca gaccgcgcgg 540<br>ataccgtgtg gaggaaacaa catcgggtca aagggcgtac attggtacca gcagaagtct 600<br>ggacaagctc ccgttatggt ggtgtacgat gactcagaca ggccatccgg catccctgag 660 |

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|---|---|---|
| | | cggttcagcg gttctaattc aggaaataca gcaacattga ccatcagcag ggtcgaagcc 720<br>ggtgacgagg cggactatta ttgtcaggtc tgggattcaa gcggcgacct tgttttgttt 780<br>gggggtggaa ctaaactgac cgtactggcg gccgcaacga ccactcctgc accccgccct 840<br>ccgactccgg ccccaaccat tgccagccag cccctgtccc tgcggccgga agcctgcaga 900<br>ccggctgccg gcggagccgt ccatacccgg ggactggatt tcgcctgcga tatctatatc 960<br>tgggcaccac tcgccggaac ctgtggagtg ctgctgctgt cccttgtgat caccctgtac 1020<br>tgcaagcgcg gacggaagaa actcttgtac atcttcaagc agccgttcat gcgccctgtg 1080<br>caaaccaccc aagaagagga cgggtgctcc tgccggttcc cggaagagga agagggcggc 1140<br>tgcgaactgc gcgtgaagtt ttcccggtcc gccgacgctc cggcgtacca gcaggggcaa 1200<br>aaccagctgt acaacgaact taacctcggt cgccgggaag aatatgacgt gctggacaag 1260<br>cggcggggaa gagatcccga gatgggtgga aagccgcggc ggaagaaccc tcaggagggc 1320<br>ttgtacaacg agctgcaaaa ggacaaaatg gccgaagcct actccgagat tggcatgaag 1380<br>ggagagcgca gacgcgggaa gggacacgat ggactgtacc agggactgtc aaccgcgact 1440<br>aaggacactt acgacgccct gcacatgcag gccctgcccc cgcgctaa 1488 |
| **3-18** | **Sequences** | |
| 3-18-1 | Sequence Number [ID] | 18 |
| 3-18-2 | Molecule Type | AA |
| 3-18-3 | Length | 495 |
| 3-18-4 | Features Location/ Qualifiers<br><br>NonEnglishQualifier Value | **source 1..495**<br>mol_type=protein<br>organism=synthetic construct |
| 3-18-5 | Residues | MLLLVTSLLL CELPHPAFLL IPQVQLVQSG AEVKKPGASV KVSCKASGYT FTSYGISWVR 60<br>QAPGQGLEWM GWISAYNGNT NYAQKLQGRV TMTTDTSTST AYMELRSLRS DDTAVYYCAR 120<br>GAYYDFWSGY SWFDPWGQGT LVTVSSGGGG SGGGGSGGGG SQSVLTQPPS VSVAPGQTAR 180<br>IPCGGNNIGS KGVHWYQQKS GQAPVMVVYD DSDRPSGIPE RFSGSNSGNT ATLTISRVEA 240<br>GDEADYYCQV WDSSGDLVLF GGGTKLTVLA AATTTPAPRP PTPAPTIASQ PLSLRPEACR 300<br>PAAGGAVHTR GLDFACDIYI WAPLAGTCGV LLLSLVITLY CKRGRKKLLY IFKQPFMRPV 360<br>QTTQEEDGCS CRFPEEEEGG CELRVKFSRS ADAPAYQQGQ NQLYNELNLG RREEYDVLDK 420<br>RRGRDPEMGG KPRRKNPQEG LYNELQKDKM AEAYSEIGMK GERRRGKGHD GLYQGLSTAT 480<br>KDTYDALHMQ ALPPR 495 |
| **3-19** | **Sequences** | |
| 3-19-1 | Sequence Number [ID] | 19 |
| 3-19-2 | Molecule Type | DNA |
| 3-19-3 | Length | 1488 |
| 3-19-4 | Features Location/ Qualifiers<br><br>NonEnglishQualifier Value | **source 1..1488**<br>mol_type=other DNA<br>organism=synthetic construct |
| 3-19-5 | Residues | atgctcttgc tcgtgacttc tttgcttttg tgcgaacttc cgcacccagc cttccttttg 60<br>atacctcagg tgcaactggt tcaatctggc gccgaagtaa aaaaaccggg cgccagcgtt 120<br>aaagtatcct gtaaagcgag cggctacaca tttaccagct atggcatctc atgggtgaga 180<br>caagcgcccg gccaaggact ggaatggatg gggtggatca gcgcctacaa tgggaacact 240<br>aactacgcac agaagctgca aggccgggtt accatgacga ccgatacgag tacctcaaca 300<br>gcgtacatgg aacttcgaag tctgcgcagt gacgacaccg cagtatacta ctgcgcccga 360<br>ggagcgtact acgacttctg gtccagctac tcttggtttg acccgtgggg ccaaggaaca 420<br>ctcgtaacag tatccagtgg aggaggcggg tcaggtggcg gtggttcagg cggtggcggg 480<br>tcatcttatg ttctcactca gccccccatcc gtgtccgtag cgccagggaa aacagcccgg 540<br>attacgtgcg ggggaaataa tataggcagc aagagcgttc attggtatca acaaaagcca 600<br>gggcaggcac cggtcttggt ggtctacgac gacagtgatc ggccctcagg aattcctgaa 660<br>agattctcag ggtcaaattc tggcaacacg gcgacgctta caataagcag ggtcgaggca 720<br>ggagacgaag ccgattatta ctgccaggta tgggattcct cttctgacca tgtggtgttt 780<br>ggcggtggca caaagctcac ggtcttggcg gccgcaacga ccactcctgc accccgccct 840<br>ccgactccgg ccccaaccat tgccagccag cccctgtccc tgcggccgga agcctgcaga 900<br>ccggctgccg gcggagccgt ccatacccgg ggactggatt tcgcctgcga tatctatatc 960<br>tgggcaccac tcgccggaac ctgtggagtg ctgctgctgt cccttgtgat caccctgtac 1020<br>tgcaagcgcg gacggaagaa actcttgtac atcttcaagc agccgttcat gcgccctgtg 1080<br>caaaccaccc aagaagagga cgggtgctcc tgccggttcc cggaagagga agagggcggc 1140<br>tgcgaactgc gcgtgaagtt ttcccggtcc gccgacgctc cggcgtacca gcaggggcaa 1200<br>aaccagctgt acaacgaact taacctcggt cgccgggaag aatatgacgt gctggacaag 1260<br>cggcggggaa gagatcccga gatgggtgga aagccgcggc ggaagaaccc tcaggagggc 1320<br>ttgtacaacg agctgcaaaa ggacaaaatg gccgaagcct actccgagat tggcatgaag 1380<br>ggagagcgca gacgcgggaa gggacacgat ggactgtacc agggactgtc aaccgcgact 1440<br>aaggacactt acgacgccct gcacatgcag gccctgcccc cgcgctaa 1488 |
| **3-20** | **Sequences** | |

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|---|---|---|
| 3-20-1 | Sequence Number [ID] | 20 |
| 3-20-2 | Molecule Type | AA |
| 3-20-3 | Length | 495 |
| 3-20-4 | Features Location/ | **source 1..495** |
| | Qualifiers | mol_type=protein |
| | | organism=synthetic construct |
| | NonEnglishQualifier Value | |
| 3-20-5 | Residues | MLLLVTSLLL CELPHPAFLL IPQVQLVQSG AEVKKPGASV KVSCKASGYT FTSYGISWVR 60<br>QAPGQGLEWM GWISAYNGNT NYAQKLQGRV TMTTDTSTST AYMELRSLRS DDTAVYYCAR 120<br>GAYYDFWSSY SWFDPWGQGT LVTVSSGGGG SGGGGSGGGG SSYVLTQPPS VSVAPGKTAR 180<br>ITCGGNNIGS KSVHWYQQKP GQAPVLVVYD DSDRPSGIPE RFSGSNSGNT ATLTISRVEA 240<br>GDEADYYCQV WDSSSDHVVF GGGTKLTVLA AATTTPAPRP PTPAPTIASQ PLSLRPEACR 300<br>PAAGGAVHTR GLDFACDIYI WAPLAGTCGV LLLSLVITLY CKRGRKKLLY IFKQPFMRPV 360<br>QTTQEEDGCS CRFPEEEEGG CELRVKFSRS ADAPAYQQGQ NQLYNELNLG RREEYDVLDK 420<br>RRGRDPEMGG KPRRKNPQEG LYNELQKDKM AEAYSEIGMK GERRRGKGHD GLYQGLSTAT 480<br>KDTYDALHMQ ALPPR 495 |
| **3-21** | **Sequences** | |
| 3-21-1 | Sequence Number [ID] | 21 |
| 3-21-2 | Molecule Type | DNA |
| 3-21-3 | Length | 1491 |
| 3-21-4 | Features Location/ | **source 1..1491** |
| | Qualifiers | mol_type=other DNA |
| | | organism=synthetic construct |
| | NonEnglishQualifier Value | |
| 3-21-5 | Residues | atgctcttgc tcgtgacttc tttgcttttg tgcgaacttc cgcacccagc cttccttttg 60<br>atacctcagg ttcaactcgt tcaaagcggg gctgaagtta aaaagccggg gtctagcgtt 120<br>aaggtttcct gtaaagcgtc tggaggaact ttttcctcct acgccattag ctgggtacga 180<br>caagctccag gacagggtct cgagtggatg ggtgggataa ttccgatctt tggaactgcg 240<br>aattacgccc agcgattcca aggccgagtt acgattactg ctgacgagag tacgtctacc 300<br>gcatacatgg aattgagttc tcttcggtca gaagatacag cggtatacta ctgcgctagg 360<br>ggcctcggca ctagttacta ctattactat atggatgtat ggggcaaggg cacaactgtg 420<br>actgtttcta gcggtggcgg cgggtccggt ggtggtggaa gcggtggcgg agggtcacag 480<br>tcagtactca ctcagccacc gagtgcgtct ggctcaccag gacaatctgt aaccattagt 540<br>tgcacaggca ctagctctga tgttgggggc tacaattatg tctcctggta ccaacaacac 600<br>cccggaaaag cgccgaagct gatgatctac gaggtgagta atagacctag tggtgttagt 660<br>aacaggttct caggctctaa gtccggtaac accgcgtctc tcactatatc tggccttcaa 720<br>gctgaggacg aggcagacta ttattgcagc tcatacacct caagcagtac ccccgttgtg 780<br>tttggtggcg gtaccaaatt gactgtgctg gcggccgcaa cgaccactcc tgcaccccgc 840<br>cctccgactc cggccccaac cattgccagc cagcccctgt ccctgcggcc ggaagcctgc 900<br>agaccggctg ccggcggagc cgtccatacc cggggactgg atttcgcctg cgatatctat 960<br>atctgggcac cactcgccgg aacctgtgga gtgctgctgc tgtcccttgt gatcaccctg 1020<br>tactgcaagc gcggacggaa gaaactcttg tacatcttca agcagccgtt catgcgccct 1080<br>gtgcaaacca cccaagaaga ggacgggtgc tcctgccggt tcccggaaga ggaagagggc 1140<br>ggctgcgaac tgcgcgtgaa gttttcccgg tccgccgacg ctccggcgta ccagcagggg 1200<br>caaaaccagc tgtacaacga acttaacctc ggtcgccggg aagaatatga cgtgctggac 1260<br>aagcggcggg gaagagatcc cgagatgggt ggaaagccgc ggcggaagaa ccctcaggag 1320<br>ggcttgtaca acgagctgca aaaggacaaa atggccgaag cctactccga gattggcatg 1380<br>aagggagagc gcagacgcgg gaagggacac gatggactgt accagggact gtcaaccgcg 1440<br>actaaggaca cttacgacgc cctgcacatg caggccctgc ccccgcgcta a 1491 |
| **3-22** | **Sequences** | |
| 3-22-1 | Sequence Number [ID] | 22 |
| 3-22-2 | Molecule Type | AA |
| 3-22-3 | Length | 496 |
| 3-22-4 | Features Location/ | **source 1..496** |
| | Qualifiers | mol_type=protein |
| | | organism=synthetic construct |
| | NonEnglishQualifier Value | |
| 3-22-5 | Residues | MLLLVTSLLL CELPHPAFLL IPQVQLVQSG AEVKKPGSSV KVSCKASGGT FSSYAISWVR 60<br>QAPGQGLEWM GGIIPIFGTA NYAQRFQGRV TITADESTST AYMELSSLRS EDTAVYYCAR 120<br>GLGTSYYYYY MDVWGKGTTV TVSSGGGGSG GGGSGGGGSQ SVLTQPPSAS GSPGQSVTIS 180<br>CTGTSSDVGG YNYVSWYQQH PGKAPKLMIY EVSNRPSGVS NRFSGSKSGN TASLTISGLQ 240<br>AEDEADYYCS SYTSSSTPVV FGGGTKLTVL AAATTTPAPR PPTPAPTIAS QPLSLRPEAC 300<br>RPAAGGAVHT RGLDFACDIY IWAPLAGTCG VLLLSLVITL YCKRGRKKLL YIFKQPFMRP 360<br>VQTTQEEDGC SCRFPEEEEG GCELRVKFSR SADAPAYQQG QNQLYNELNL GRREEYDVLD 420 |

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|---|---|---|
| | | KRRGRDPEMG GKPRRKNPQE GLYNELQKDK MAEAYSEIGM KGERRRGKGH DGLYQGLSTA 480<br>TKDTYDALHM QALPPR 496 |
| **3-23** | **Sequences** | |
| 3-23-1 | Sequence Number [ID] | 23 |
| 3-23-2 | Molecule Type | DNA |
| 3-23-3 | Length | 1482 |
| 3-23-4 | Features Location/ | **source 1..1482** |
| | Qualifiers | mol_type=other DNA |
| | | organism=synthetic construct |
| | NonEnglishQualifier Value | |
| 3-23-5 | Residues | atgctgctgc tggtgaccag cctgctgctg tgcgaactgc cgcatccggc gtttctgctg 60<br>attccggaag tccaattggt gcagagcgga tccgaactta agaaacctgg cgcgagcgtg 120<br>aaagtgtcct gcaaggcctc cggagggact ttctcgtcgt acgccattag ctgggtccgc 180<br>caagctcctg gccaaggcct ggagtggatg ggcgggatta tccccatctt cgggactgcg 240<br>aactacgccc agaagtttca gggccgggtc actatcaccg ccgacgaatc aacctcgacc 300<br>gcctacatgg aactgtcctc gcttcggtcc gaggatactg ccgtgtacta ttgtgcctca 360<br>acggccagac gcggatggga caccgctggt ccgctcgatt actggggcca gggaaccctc 420<br>gtgaccgtca gctccggagg aggaggctcc ggtggtggag gatccggggg tggtggatcc 480<br>gacatccaaa tgacccagtc cccctcgtcc ctgagcgcct ctgtgggcga cagagtgaca 540<br>attgcatgca gggcctcaca gactatctcc cgctacctga actggtacca gcagaagcca 600<br>ggaaaggccc ctaagctgct catctacgct gcgtcctcgc tccaatccgg ggtgtcctca 660<br>cggttttccg gatcgggttc cggcaccgag ttcaccctga ccatcagcag cctgcagccc 720<br>gaggacttcg caacctactt ctgccagcaa acctactccc cgccgattac gttcggacag 780<br>gggactcggc tggaaatcaa ggcggccgca actaccaccc ctgcccctcg gccgccgact 840<br>ccggccccaa ccatcgcaag ccaacccctc tccttgcgcc ccgaagcttg ccgcccggcc 900<br>gcgggtggag ccgtgcatac ccgggggctg gactttgcct gcgatatcta catttgggcc 960<br>ccgctggccg gcacttgcgg cgtgctcctg ctgtcgctgg tcatcaccct ttactgcaag 1020<br>aggggccgga agaagctgct ttacatcttc aagcagccgt tcatgcggcc cgtgcagacg 1080<br>actcaggaag aggacggatg ctcgtgcaga ttccctgagg aggaagaggg gggatgcgaa 1140<br>ctgcgcgtca agttctcacg gtccgccgac gcccccgcat atcaacaggg ccagaatcag 1200<br>ctctacaacg agctgaacct gggaaggaga gaggagtacg acgtgctgga caagcgacgc 1260<br>ggacgcgacc cggagatggg ggggaaacca cggcggaaaa accctcagga aggactgtac 1320<br>aacgaactcc agaaagacaa gatggcggaa gcctactcag aaatcgggat gaagggagag 1380<br>cggaggaggg gaaagggtca cgacgggctg taccagggac tgagcaccgc cactaaggat 1440<br>acctacgatg ccttgcatat gcaagcactc ccaccccggt ag 1482 |
| **3-24** | **Sequences** | |
| 3-24-1 | Sequence Number [ID] | 24 |
| 3-24-2 | Molecule Type | AA |
| 3-24-3 | Length | 493 |
| 3-24-4 | Features Location/ | **source 1..493** |
| | Qualifiers | mol_type=protein |
| | | organism=synthetic construct |
| | NonEnglishQualifier Value | |
| 3-24-5 | Residues | MLLLVTSLLL CELPHPAFLL IPEVQLVQSG SELKKPGASV KVSCKASGGT FSSYAISWVR 60<br>QAPGQGLEWM GGIIPIFGTA NYAQKFQGRV TITADESTST AYMELSSLRS EDTAVYYCAS 120<br>TARRGWDTAG PLDYWGQGTL VTVSSGGGGS GGGGSGGGGS DIQMTQSPSS LSASVGDRVT 180<br>IACRASQTIS RYLNWYQQKP GKAPKLLIYA ASSLQSGVSS RFSGSGSGTE FTLTISSLQP 240<br>EDFATYFCQQ TYSPPITFGQ GTRLEIKAAA TTTPAPRPPT PAPTIASQPL SLRPEACRPA 300<br>AGGAVHTRGL DFACDIYIWA PLAGTCGVLL LSLVITLYCK RGRKKLLYIF KQPFMRPVQT 360<br>TQEEDGCSCR FPEEEEGGCE LRVKFSRSAD APAYQQGQNQ LYNELNLGRR EEYDVLDKRR 420<br>GRDPEMGGKP RRKNPQEGLY NELQKDKMAE AYSEIGMKGE RRRGKGHDGL YQGLSTATKD 480<br>TYDALHMQAL PPR 493 |
| **3-25** | **Sequences** | |
| 3-25-1 | Sequence Number [ID] | 25 |
| 3-25-2 | Molecule Type | DNA |
| 3-25-3 | Length | 1086 |
| 3-25-4 | Features Location/ | **source 1..1086** |
| | Qualifiers | mol_type=other DNA |
| | | organism=synthetic construct |
| | NonEnglishQualifier Value | |
| 3-25-5 | Residues | atgctgctgc tggtgaccag cctgctgctg tgcgaactgc cgcatccggc gtttctgctg 60<br>attccggagg tgcagctggt ggagtctggg ggaggcttgg tacagcctgg agggtccctg 120<br>agactctcct gtgcagcctc tggattcacc ttcagtagct atggcatgag ctgggtccgc 180<br>caggctccaa gacaagggct tgagtgggtg gccaacataa agcaagatgg aagtgagaaa 240<br>tactatgcgg actcagtgaa gggccgattc accatctcca gagacaattc caagaacacg 300 |

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|---|---|---|
| | | ctgtatctgc aaatgaacag cctgagagcc gaggacacag ccacgtatta ctgtgcgaaa 360<br>gaaaatgtgg actggggcca gggcaccctg gtcaccgtct cctcagcggc cgcaactacc 420<br>acccctgccc ctcggccgcc gactccggcc ccaaccatcg caagccaacc cctctccttg 480<br>cgccccgaag cttgccgccc ggccgcgggt ggagccgtgc atacccgggg gctggacttt 540<br>gcctgcgata tctacatttg ggccccgctg gccggcactt gcggcgtgct cctgctgtcg 600<br>ctggtcatca ccctttactg caagaggggc cggaagaagc tgctttacat cttcaagcag 660<br>ccgttcatgc ggcccgtgca gacgactcag gaagaggacg gatgctcgtg cagattccct 720<br>gaggaggaag agggggatg cgaactgcgc gtcaagttct cacggtccgc cgacgccccc 780<br>gcatatcaac agggccagaa tcagctctac aacgagctga acctgggaag gagagaggag 840<br>tacgacgtgc tggacaagcg acgcggacgc gacccggaga tgggggggaa accacggcgg 900<br>aaaaaccctc aggaaggact gtacaacgaa ctccagaaag acaagatggc ggaagcctac 960<br>tcagaaatcg ggatgaaggg agagcggagg aggggaaagg gtcacgacgg gctgtaccag 1020<br>ggactgagca ccgccactaa ggatacctac gatgccttgc atatgcaagc actcccaccc 1080<br>cggtag 1086 |
| **3-26** | **Sequences** | |
| 3-26-1 | Sequence Number [ID] | 26 |
| 3-26-2 | Molecule Type | AA |
| 3-26-3 | Length | 361 |
| 3-26-4 | Features Location/ | **source 1..361** |
| | Qualifiers | mol_type=protein |
| | | organism=synthetic construct |
| | NonEnglishQualifier Value | |
| 3-26-5 | Residues | MLLLVTSLLL CELPHPAFLL IPEVQLVESG GGLVQPGGSL RLSCAASGFT FSSYGMSWVR 60<br>QAPRQGLEWV ANIKQDGSEK YYADSVKGRF TISRDNSKNT LYLQMNSLRA EDTATYYCAK 120<br>ENVDWGQGTL VTVSSAAATT TPAPRPPTPA PTIASQPLSL RPEACRPAAG GAVHTRGLDF 180<br>ACDIYIWAPL AGTCGVLLLS LVITLYCKRG RKKLLYIFKQ PFMRPVQTTQ EEDGCSCRFP 240<br>EEEEGGCELR VKFSRSADAP AYQQGQNQLY NELNLGRREE YDVLDKRRGR DPEMGGKPRR 300<br>KNPQEGLYNE LQKDKMAEAY SEIGMKGERR RGKGHDGLYQ GLSTATKDTY DALHMQALPP 360<br>R 361 |
| **3-27** | **Sequences** | |
| 3-27-1 | Sequence Number [ID] | 27 |
| 3-27-2 | Molecule Type | DNA |
| 3-27-3 | Length | 72 |
| 3-27-4 | Features Location/ | **source 1..72** |
| | Qualifiers | mol_type=other DNA |
| | | organism=synthetic construct |
| | NonEnglishQualifier Value | |
| 3-27-5 | Residues | atctacatct gggcgccctt ggccgggact tgtggggtcc ttctcctgtc actggttatc 60<br>acccttact gc 72 |
| **3-28** | **Sequences** | |
| 3-28-1 | Sequence Number [ID] | 28 |
| 3-28-2 | Molecule Type | AA |
| 3-28-3 | Length | 22 |
| 3-28-4 | Features Location/ | **source 1..22** |
| | Qualifiers | mol_type=protein |
| | | organism=synthetic construct |
| | NonEnglishQualifier Value | |
| 3-28-5 | Residues | IWAPLAGTCG VLLLSLVITL YC 22 |
| **3-29** | **Sequences** | |
| 3-29-1 | Sequence Number [ID] | 29 |
| 3-29-2 | Molecule Type | DNA |
| 3-29-3 | Length | 135 |
| 3-29-4 | Features Location/ | **source 1..135** |
| | Qualifiers | mol_type=other DNA |
| | | organism=synthetic construct |
| | NonEnglishQualifier Value | |
| 3-29-5 | Residues | accacgacgc cagcgccgcg accaccaaca ccggcgccca ccatcgcgtc gcagcccctg 60<br>tccctgcgcc cagaggcgtg ccggccagcg gcgggggcgc cagtgcacac gagggggctg 120<br>gacttcgcct gtgat 135 |
| **3-30** | **Sequences** | |
| 3-30-1 | Sequence Number [ID] | 30 |
| 3-30-2 | Molecule Type | AA |
| 3-30-3 | Length | 47 |
| 3-30-4 | Features Location/ | **source 1..47** |
| | Qualifiers | mol_type=protein |

| | | |
|---|---|---|
| | | organism=synthetic construct |
| | NonEnglishQualifier Value | |
| 3-30-5 | Residues | TTTPAPRPPT PAPTIASQPL SLRPEACRPA AGGAVHTRGL DFACDIY 47 |
| **3-31** | **Sequences** | |
| 3-31-1 | Sequence Number [ID] | 31 |
| 3-31-2 | Molecule Type | AA |
| 3-31-3 | Length | 42 |
| 3-31-4 | Features Location/ | **source 1..42** |
| | Qualifiers | mol_type=protein |
| | | organism=Homo sapiens |
| | NonEnglishQualifier Value | |
| 3-31-5 | Residues | KRGRKKLLYI FKQPFMRPVQ TTQEEDGCSC RFPEEEEGGC EL 42 |
| **3-32** | **Sequences** | |
| 3-32-1 | Sequence Number [ID] | 32 |
| 3-32-2 | Molecule Type | AA |
| 3-32-3 | Length | 106 |
| 3-32-4 | Features Location/ | **source 1..106** |
| | Qualifiers | mol_type=protein |
| | | organism=Homo sapiens |
| | NonEnglishQualifier Value | |
| 3-32-5 | Residues | GQPKAAPSVT LFPPSSEELQ ANKATLVCLI SDFYPGAVTV AWKADSSPVK AGVETTTPSK 60<br>QSNNKYAASS YLSLTPEQWK SHRSYSCQVT HEGSTVEKTV APTECS 106 |
| **3-33** | **Sequences** | |
| 3-33-1 | Sequence Number [ID] | 33 |
| 3-33-2 | Molecule Type | DNA |
| 3-33-3 | Length | 126 |
| 3-33-4 | Features Location/ | **source 1..126** |
| | Qualifiers | mol_type=other DNA |
| | | organism=synthetic construct |
| | NonEnglishQualifier Value | |
| 3-33-5 | Residues | aaacggggca gaaagaaact cctgtatata ttcaaacaac catttatgag accagtacaa 60<br>actactcaag aggaagatgg ctgtagctgc cgatttccag aagaagaaga aggaggatgt 120<br>gaactg 126 |
| **3-34** | **Sequences** | |
| 3-34-1 | Sequence Number [ID] | 34 |
| 3-34-2 | Molecule Type | AA |
| 3-34-3 | Length | 42 |
| 3-34-4 | Features Location/ | **source 1..42** |
| | Qualifiers | mol_type=protein |
| | | organism=synthetic construct |
| | NonEnglishQualifier Value | |
| 3-34-5 | Residues | KRGRKKLLYI FKQPFMRPVQ TTQEEDGCSC RFPEEEEGGC EL 42 |
| **3-35** | **Sequences** | |
| 3-35-1 | Sequence Number [ID] | 35 |
| 3-35-2 | Molecule Type | DNA |
| 3-35-3 | Length | 336 |
| 3-35-4 | Features Location/ | **source 1..336** |
| | Qualifiers | mol_type=other DNA |
| | | organism=synthetic construct |
| | NonEnglishQualifier Value | |
| 3-35-5 | Residues | agagtgaagt tcagcaggag cgcagacgcc cccgcgtaca agcagggcca gaaccagctc 60<br>tataacgagc tcaatctagg acgaagagag gagtacgatg ttttggacaa gagacgtggc 120<br>cgggaccctg agatgggggg aaagccgaga aggaagaacc ctcaggaagg cctgtacaat 180<br>gaactgcaga aagataagat ggcggaggcc tacagtgaga ttgggatgaa aggcgagcgc 240<br>cggaggggca aggggcacga tggcctttac cagggtctca gtacagccac caaggacacc 300<br>tacgacgccc ttcacatgca ggccctgccc cctcgc 336 |
| **3-36** | **Sequences** | |
| 3-36-1 | Sequence Number [ID] | 36 |
| 3-36-2 | Molecule Type | AA |
| 3-36-3 | Length | 112 |
| 3-36-4 | Features Location/ | **source 1..112** |
| | Qualifiers | mol_type=protein |
| | | organism=synthetic construct |
| | NonEnglishQualifier Value | |

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|---|---|---|
| 3-36-5 | Residues | RVKFSRSADA PAYKQGQNQL YNELNLGRRE EYDVLDKRRG RDPEMGGKPR RKNPQEGLYN 60<br>ELQKDKMAEA YSEIGMKGER RRGKGHDGLY QGLSTATKDT YDALHMQALP PR 112 |
| **3-37** | **Sequences** | |
| 3-37-1 | Sequence Number [ID] | 37 |
| 3-37-2 | Molecule Type | DNA |
| 3-37-3 | Length | 726 |
| 3-37-4 | Features Location/ | **source 1..726** |
| | Qualifiers | mol_type=other DNA |
| | | organism=synthetic construct |
| | NonEnglishQualifier Value | |
| 3-37-5 | Residues | gacatccaga tgacacagac tacatcctcc ctgtctgcct ctctgggaga cagagtcacc 60<br>atcagttgca gggcaagtca ggacattagt aaatatttaa attggtatca gcagaaacca 120<br>gatggaactg ttaaactcct gatctaccat acatcaagat tacactcagg agtcccatca 180<br>aggttcagtg gcagtgggtc tggaacagat tattctctca ccattagcaa cctggagcaa 240<br>gaagatattg ccacttactt ttgccaacag ggtaatacgc ttccgtacac gttcggaggg 300<br>gggaccaagc tggagatcac aggtggcggt ggctcgggcg gtggtgggtc gggtggcggc 360<br>ggatctgagg tgaaactgca ggagtcagga cctggcctgg tggcgccctc acagagcctg 420<br>tccgtcacat gcactgtctc aggggtctca ttacccgact atggtgtaag ctggattcgc 480<br>cagcctccac gaaagggtct ggagtggctg ggagtaatat ggggtagtga aaccacatac 540<br>tataattcag ctctcaaatc cagactgacc atcatcaagg acaactccaa gagccaagtt 600<br>ttcttaaaaa tgaacagtct gcaaactgat gacacagcca tttactactg tgccaaacat 660<br>tattactacg gtggtagcta tgctatggac tactggggcc aaggaacctc agtcaccgtc 720<br>tcctca 726 |
| **3-38** | **Sequences** | |
| 3-38-1 | Sequence Number [ID] | 38 |
| 3-38-2 | Molecule Type | AA |
| 3-38-3 | Length | 242 |
| 3-38-4 | Features Location/ | **source 1..242** |
| | Qualifiers | mol_type=protein |
| | | organism=synthetic construct |
| | NonEnglishQualifier Value | |
| 3-38-5 | Residues | DIQMTQTTSS LSASLGDRVT ISCRASQDIS KYLNWYQQKP DGTVKLLIYH TSRLHSGVPS 60<br>RFSGSGSGTD YSLTISNLEQ EDIATYFCQQ GNTLPYTFGG GTKLEITGGG GSGGGGSGGG 120<br>GSEVKLQESG PGLVAPSQSL SVTCTVSGVS LPDYGVSWIR QPPRKGLEWL GVIWGSETTY 180<br>YNSALKSRLT IIKDNSKSQV FLKMNSLQTD DTAIYYCAKH YYYGGSYAMD YWGQGTSVTV 240<br>SS 242 |
| **3-39** | **Sequences** | |
| 3-39-1 | Sequence Number [ID] | 39 |
| 3-39-2 | Molecule Type | DNA |
| 3-39-3 | Length | 66 |
| 3-39-4 | Features Location/ | **source 1..66** |
| | Qualifiers | mol_type=other DNA |
| | | organism=synthetic construct |
| | NonEnglishQualifier Value | |
| 3-39-5 | Residues | atgctgctgc tggtgaccag cctgctgctg tgcgaactgc cgcatccggc gtttctgctg 60<br>attccg 66 |
| **3-40** | **Sequences** | |
| 3-40-1 | Sequence Number [ID] | 40 |
| 3-40-2 | Molecule Type | AA |
| 3-40-3 | Length | 22 |
| 3-40-4 | Features Location/ | **source 1..22** |
| | Qualifiers | mol_type=protein |
| | | organism=synthetic construct |
| | NonEnglishQualifier Value | |
| 3-40-5 | Residues | MLLLVTSLLL CELPHPAFLL IP 22 |
| **3-41** | **Sequences** | |
| 3-41-1 | Sequence Number [ID] | 41 |
| 3-41-2 | Molecule Type | DNA |
| 3-41-3 | Length | 85 |
| 3-41-4 | Features Location/ | **source 1..85** |
| | Qualifiers | mol_type=other DNA |
| | | organism=synthetic construct |
| | NonEnglishQualifier Value | |
| 3-41-5 | Residues | ggctctgaaa gtgctgttgg aacaagaaaa gaccttcttc accttgctcg tgttgctggg 60<br>gtacctgtcc tgcaaagtca cctgt 85 |

| | | | |
|---|---|---|---|
| **3-42** | **Sequences** | | |
| 3-42-1 | Sequence Number [ID] | 42 | |
| 3-42-2 | Molecule Type | AA | |
| 3-42-3 | Length | 29 | |
| 3-42-4 | Features Location/ | **source 1..29** | |
| | Qualifiers | mol_type=protein | |
| | | organism=synthetic construct | |
| | NonEnglishQualifier Value | | |
| 3-42-5 | Residues | MALKVLLEQE KTFFTLLVLL GYLSCKVTC | 29 |
| **3-43** | **Sequences** | | |
| 3-43-1 | Sequence Number [ID] | 43 | |
| 3-43-2 | Molecule Type | DNA | |
| 3-43-3 | Length | 63 | |
| 3-43-4 | Features Location/ | **source 1..63** | |
| | Qualifiers | mol_type=other DNA | |
| | | organism=synthetic construct | |
| | NonEnglishQualifier Value | | |
| 3-43-5 | Residues | atggcgctgc cggtgaccgc gctgctgctg ccgctggcgc tgctgctgca tgcggcgcgc | 60 |
| | | ccg | 63 |
| **3-44** | **Sequences** | | |
| 3-44-1 | Sequence Number [ID] | 44 | |
| 3-44-2 | Molecule Type | AA | |
| 3-44-3 | Length | 21 | |
| 3-44-4 | Features Location/ | **source 1..21** | |
| | Qualifiers | mol_type=protein | |
| | | organism=synthetic construct | |
| | NonEnglishQualifier Value | | |
| 3-44-5 | Residues | MALPVTALLL PLALLLHAAR P | 21 |
| **3-45** | **Sequences** | | |
| 3-45-1 | Sequence Number [ID] | 45 | |
| 3-45-2 | Molecule Type | DNA | |
| 3-45-3 | Length | 123 | |
| 3-45-4 | Features Location/ | **source 1..123** | |
| | Qualifiers | mol_type=other DNA | |
| | | organism=synthetic construct | |
| | NonEnglishQualifier Value | | |
| 3-45-5 | Residues | cggtcgaaga ggtccagact cttgcactcc gactacatga acatgactcc tagaaggccc | 60 |
| | | ggacccacta gaaagcacta ccagccgtac gcccctcctc gggatttcgc cgcataccgg | 120 |
| | | tcc | 123 |
| **3-46** | **Sequences** | | |
| 3-46-1 | Sequence Number [ID] | 46 | |
| 3-46-2 | Molecule Type | AA | |
| 3-46-3 | Length | 41 | |
| 3-46-4 | Features Location/ | **source 1..41** | |
| | Qualifiers | mol_type=protein | |
| | | organism=synthetic construct | |
| | NonEnglishQualifier Value | | |
| 3-46-5 | Residues | RSKRSRLLHS DYMNMTPRRP GPTRKHYQPY APPRDFAAYR S | 41 |
| **3-47** | **Sequences** | | |
| 3-47-1 | Sequence Number [ID] | 47 | |
| 3-47-2 | Molecule Type | DNA | |
| 3-47-3 | Length | 336 | |
| 3-47-4 | Features Location/ | **source 1..336** | |
| | Qualifiers | mol_type=other DNA | |
| | | organism=synthetic construct | |
| | NonEnglishQualifier Value | | |
| 3-47-5 | Residues | agagtgaagt tcagccgctc agccgatgca ccggcctacc agcagggaca gaaccagctc | 60 |
| | | tacaacgagc tcaacctggg tcggcgggaa gaatatgacg tgctggacaa acggcgcggc | 120 |
| | | agagatccgg agatgggggg aaagccgagg aggaagaacc ctcaagaggg cctgtacaac | 180 |
| | | gaactgcaga aggacaagat ggcggaagcc tactccgaga tcggcatgaa gggagaacgc | 240 |
| | | cggagaggga agggtcatga cggactgtac cagggcctgt caactgccac taaggacact | 300 |
| | | tacgatgcgc tccatatgca agctttgccc ccgcgg | 336 |
| **3-48** | **Sequences** | | |
| 3-48-1 | Sequence Number [ID] | 48 | |
| 3-48-2 | Molecule Type | AA | |

| | | |
|---|---|---|
| 3-48-3 | Length | 112 |
| 3-48-4 | Features Location/ | **source 1..112** |
| | Qualifiers | mol_type=protein |
| | | organism=synthetic construct |
| | NonEnglishQualifier Value | |
| 3-48-5 | Residues | RVKFSRSADA PAYQQGQNQL YNELNLGRRE EYDVLDKRRG RDPEMGGKPR RKNPQEGLYN 60<br>ELQKDKMAEA YSEIGMKGER RRGKGHDGLY QGLSTATKDT YDALHMQALP PR 112 |
| **3-49** | **Sequences** | |
| 3-49-1 | Sequence Number [ID] | 49 |
| 3-49-2 | Molecule Type | DNA |
| 3-49-3 | Length | 201 |
| 3-49-4 | Features Location/ | **source 1..201** |
| | Qualifiers | mol_type=other DNA |
| | | organism=synthetic construct |
| | NonEnglishQualifier Value | |
| 3-49-5 | Residues | gcggccgcgg tcggattcca agacatggaa tgcgtgccct gcggcgaccc gccacctcct 60<br>tacgagccgc actgcgcatc gaaggtcaac ctcgtgaaga tcgcgagcac cgcgtcctca 120<br>ccccgggata ctgctctggc cgccgtgatt tgttccgcct tggccaccgt gcttctggcc 180<br>ctgctgatcc tctgtgtgat c 201 |
| **3-50** | **Sequences** | |
| 3-50-1 | Sequence Number [ID] | 50 |
| 3-50-2 | Molecule Type | AA |
| 3-50-3 | Length | 67 |
| 3-50-4 | Features Location/ | **source 1..67** |
| | Qualifiers | mol_type=protein |
| | | organism=synthetic construct |
| | NonEnglishQualifier Value | |
| 3-50-5 | Residues | AAAVGFQDME CVPCGDPPPP YEPHCASKVN LVKIASTASS PRDTALAAVI CSALATVLLA 60<br>LLILCVI 67 |
| **3-51** | **Sequences** | |
| 3-51-1 | Sequence Number [ID] | 51 |
| 3-51-2 | Molecule Type | DNA |
| 3-51-3 | Length | 63 |
| 3-51-4 | Features Location/ | **source 1..63** |
| | Qualifiers | mol_type=other DNA |
| | | organism=synthetic construct |
| | NonEnglishQualifier Value | |
| 3-51-5 | Residues | gccgccgtga tttgttccgc cttggccacc gtgcttctgg ccctgctgat cctctgtgtg 60<br>atc 63 |
| **3-52** | **Sequences** | |
| 3-52-1 | Sequence Number [ID] | 52 |
| 3-52-2 | Molecule Type | AA |
| 3-52-3 | Length | 21 |
| 3-52-4 | Features Location/ | **source 1..21** |
| | Qualifiers | mol_type=protein |
| | | organism=synthetic construct |
| | NonEnglishQualifier Value | |
| 3-52-5 | Residues | AAVICSALAT VLLALLILCV I 21 |
| **3-53** | **Sequences** | |
| 3-53-1 | Sequence Number [ID] | 53 |
| 3-53-2 | Molecule Type | DNA |
| 3-53-3 | Length | 138 |
| 3-53-4 | Features Location/ | **source 1..138** |
| | Qualifiers | mol_type=other DNA |
| | | organism=synthetic construct |
| | NonEnglishQualifier Value | |
| 3-53-5 | Residues | gcggccgcgg tcggattcca agacatggaa tgcgtgccct gcggcgaccc gccacctcct 60<br>tacgagccgc actgcgcatc gaaggtcaac ctcgtgaaga tcgcgagcac cgcgtcctca 120<br>ccccgggata ctgctctg 138 |
| **3-54** | **Sequences** | |
| 3-54-1 | Sequence Number [ID] | 54 |
| 3-54-2 | Molecule Type | AA |
| 3-54-3 | Length | 46 |
| 3-54-4 | Features Location/ | **source 1..46** |
| | Qualifiers | mol_type=protein |

| | | |
|---|---|---|
| | | organism=synthetic construct |
| | NonEnglishQualifier Value | |
| 3-54-5 | Residues | AAAVGFQDME CVPCGDPPPP YEPHCASKVN LVKIASTASS PRDTAL 46 |
| **3-55** | **Sequences** | |
| 3-55-1 | Sequence Number [ID] | 55 |
| 3-55-2 | Molecule Type | DNA |
| 3-55-3 | Length | 80 |
| 3-55-4 | Features Location/ | **source 1..80** |
| | Qualifiers | mol_type=other DNA |
| | | organism=synthetic construct |
| | NonEnglishQualifier Value | |
| 3-55-5 | Residues | tacgagcctc actgcgccag caaagtcaac ttggtgaaga tcgcgagcac tgcctcgtcc 60 |
| | | cctcgggaca ctgctctggc 80 |
| **3-56** | **Sequences** | |
| 3-56-1 | Sequence Number [ID] | 56 |
| 3-56-2 | Molecule Type | AA |
| 3-56-3 | Length | 26 |
| 3-56-4 | Features Location/ | **source 1..26** |
| | Qualifiers | mol_type=protein |
| | | organism=synthetic construct |
| | NonEnglishQualifier Value | |
| 3-56-5 | Residues | YEPHCASKVN LVKIASTASS PRDTAL 26 |
| **3-57** | **Sequences** | |
| 3-57-1 | Sequence Number [ID] | 57 |
| 3-57-2 | Molecule Type | DNA |
| 3-57-3 | Length | 222 |
| 3-57-4 | Features Location/ | **source 1..222** |
| | Qualifiers | mol_type=other DNA |
| | | organism=synthetic construct |
| | NonEnglishQualifier Value | |
| 3-57-5 | Residues | gcggccgcgc ccgcccctcg gccccgact cctgccccga cgatcgcttc ccaacctctc 60 |
| | | tcgctgcgcc cggaagcatg ccggcccgcc gccggtggcg ctgtccacac tcgcggactg 120 |
| | | gactttgata ccgcactggc ggccgtgatc tgtagcgccc tggccaccgt gctgctggcg 180 |
| | | ctgctcatcc tttgcgtgat ctactgcaag cggcagccta gg 222 |
| **3-58** | **Sequences** | |
| 3-58-1 | Sequence Number [ID] | 58 |
| 3-58-2 | Molecule Type | AA |
| 3-58-3 | Length | 74 |
| 3-58-4 | Features Location/ | **source 1..74** |
| | Qualifiers | mol_type=protein |
| | | organism=synthetic construct |
| | NonEnglishQualifier Value | |
| 3-58-5 | Residues | AAAPAPRPPT PAPTIASQPL SLRPEACRPA AGGAVHTRGL DFDTALAAVI CSALATVLLA 60 |
| | | LLILCVIYCK RQPR 74 |
| **3-59** | **Sequences** | |
| 3-59-1 | Sequence Number [ID] | 59 |
| 3-59-2 | Molecule Type | DNA |
| 3-59-3 | Length | 123 |
| 3-59-4 | Features Location/ | **source 1..123** |
| | Qualifiers | mol_type=other DNA |
| | | organism=synthetic construct |
| | NonEnglishQualifier Value | |
| 3-59-5 | Residues | cggtcgaaga ggtccagact cttgcactcc gactacatga acatgactcc tagaaggccc 60 |
| | | ggacccacta gaaagcacta ccagccgtac gcccctcctc gggatttcgc cgcataccgg 120 |
| | | tcc 123 |
| **3-60** | **Sequences** | |
| 3-60-1 | Sequence Number [ID] | 60 |
| 3-60-2 | Molecule Type | AA |
| 3-60-3 | Length | 41 |
| 3-60-4 | Features Location/ | **source 1..41** |
| | Qualifiers | mol_type=protein |
| | | organism=synthetic construct |
| | NonEnglishQualifier Value | |
| 3-60-5 | Residues | RSKRSRLLHS DYMNMTPRRP GPTRKHYQPY APPRDFAAYR S 41 |
| **3-61** | **Sequences** | |

| | | |
|---|---|---|
| 3-61-1 | Sequence Number [ID] | 61 |
| 3-61-2 | Molecule Type | DNA |
| 3-61-3 | Length | 336 |
| 3-61-4 | Features Location/ | **source 1..336** |
| | Qualifiers | mol_type=other DNA |
| | | organism=synthetic construct |
| | NonEnglishQualifier Value | |
| 3-61-5 | Residues | cgcgtgaaat ttagccgcag cgcggatgcg ccggcgtatc agcagggcca gaaccagctg 60<br>tataacgaac tgaacctggg ccgccgcgaa gaatatgatg tgctggataa acgccgcggc 120<br>cgcgatccgg aaatgggcgg caaaccgcgc cgcaaaaacc cgcaggaagg cctgtataac 180<br>gaactgcaga aagataaaat ggcggaagcg tatagcgaaa ttggcatgaa aggcgaacgc 240<br>cgccgcggca aaggccatga tggcctgtat cagggcctga gcaccgcgac caaagatacc 300<br>tatgatgcgc tgcatatgca ggcgctgccg ccgcgc 336 |
| **3-62** | **Sequences** | |
| 3-62-1 | Sequence Number [ID] | 62 |
| 3-62-2 | Molecule Type | AA |
| 3-62-3 | Length | 112 |
| 3-62-4 | Features Location/ | **source 1..112** |
| | Qualifiers | mol_type=protein |
| | | organism=synthetic construct |
| | NonEnglishQualifier Value | |
| 3-62-5 | Residues | RVKFSRSADA PAYQQGQNQL YNELNLGRRE EYDVLDKRRG RDPEMGGKPR RKNPQEGLYN 60<br>ELQKDKMAEA YSEIGMKGER RRGKGHDGLY QGLSTATKDT YDALHMQALP PR 112 |
| **3-63** | **Sequences** | |
| 3-63-1 | Sequence Number [ID] | 63 |
| 3-63-2 | Molecule Type | DNA |
| 3-63-3 | Length | 93 |
| 3-63-4 | Features Location/ | **source 1..93** |
| | Qualifiers | mol_type=other DNA |
| | | organism=synthetic construct |
| | NonEnglishQualifier Value | |
| 3-63-5 | Residues | cgcgcgaaac gcagcggcag cggcgcgacc aactttagcc tgctgaaaca ggcgggcgat 60<br>gtggaagaaa acccgggccc gcgagcaaag agg 93 |
| **3-64** | **Sequences** | |
| 3-64-1 | Sequence Number [ID] | 64 |
| 3-64-2 | Molecule Type | AA |
| 3-64-3 | Length | 31 |
| 3-64-4 | Features Location/ | **source 1..31** |
| | Qualifiers | mol_type=protein |
| | | organism=synthetic construct |
| | NonEnglishQualifier Value | |
| 3-64-5 | Residues | RAKRSGSGAT NFSLLKQAGD VEENPGPRAK R 31 |
| **3-65** | **Sequences** | |
| 3-65-1 | Sequence Number [ID] | 65 |
| 3-65-2 | Molecule Type | DNA |
| 3-65-3 | Length | 78 |
| 3-65-4 | Features Location/ | **source 1..78** |
| | Qualifiers | mol_type=other DNA |
| | | organism=synthetic construct |
| | NonEnglishQualifier Value | |
| 3-65-5 | Residues | agagctaaac gctctgggtc tggtgaagga cgaggtagcc ttcttacgtg cggagacgtg 60<br>gaggaaaacc caggaccc 78 |
| **3-66** | **Sequences** | |
| 3-66-1 | Sequence Number [ID] | 66 |
| 3-66-2 | Molecule Type | AA |
| 3-66-3 | Length | 26 |
| 3-66-4 | Features Location/ | **source 1..26** |
| | Qualifiers | mol_type=protein |
| | | organism=synthetic construct |
| | NonEnglishQualifier Value | |
| 3-66-5 | Residues | RAKRSGSGEG RGSLLTCGDV EENPGP 26 |
| **3-67** | **Sequences** | |
| 3-67-1 | Sequence Number [ID] | 67 |
| 3-67-2 | Molecule Type | DNA |
| 3-67-3 | Length | 1005 |

| | | |
|---|---|---|
| 3-67-4 | Features Location/ | **source 1..1005** |
| | Qualifiers | mol_type=other DNA |
| | | organism=synthetic construct |
| | NonEnglishQualifier Value | |
| 3-67-5 | Residues | aggaaggttt gcaatggaat cggtataggg gagtttaagg attcacttag cataaacgct 60<br>actaatatta aacacttcaa aaactgtacg agtataagtg gagatcttca cattttgccg 120<br>gttgcattcc gaggcgattc attcacccac acgccaccgc ttgacccaca agaattggat 180<br>attcttaaaa ccgttaaaga aataacgggg tttttgctca ttcaagcgtg gccagaaaat 240<br>cgcactgacc tccatgcttt cgagaacctg gagattataa gaggacgaac taagcagcat 300<br>ggtcaattct cccttgctgt ggtcagcctg aacatcacca gtcttggttt gcggtccctc 360<br>aaggaaattt cagatggaga tgtcatcata agcggcaaca agaatttgtg ctatgcaaat 420<br>accataaact ggaaaaaact gtttggcact tccggccaga aaaccaagat tatttcaaat 480<br>cggggtgaga acagctgcaa agccaccggc caggtttgtc atgccttgtg ctctccggaa 540<br>ggctgttggg ggccagaacc cagggactgc gtcagttgca gaaacgtctc aagaggccgc 600<br>gaatgcgttg acaagtgtaa cctccttgag ggtgagccac gagagtttgt tgagaacagc 660<br>gagtgtatac aatgtcaccc tgaatgtttg ccccaggcta tgaatataac ctgcacaggc 720<br>cgcgggcctg ataactgcat ccagtgtgct cattacatag atggacctca ctgtgtgaaa 780<br>acctgcccgg ccggagttat gggagaaaac aacactctgg tgtggaaata cgctgatgca 840<br>ggccacgtgt gccacctttg tcacccgaat tgtacatatg ggtgtaccgg tcctggactt 900<br>gaaggttgcc ctaccaatgg ccctaaaata cccagtatcg caactggcat ggtaggcgct 960<br>cttctcttgc tcttggtagt tgctctcggc ataggtcttt ttatg 1005 |
| **3-68** | **Sequences** | |
| 3-68-1 | Sequence Number [ID] | 68 |
| 3-68-2 | Molecule Type | AA |
| 3-68-3 | Length | 335 |
| 3-68-4 | Features Location/ | **source 1..335** |
| | Qualifiers | mol_type=protein |
| | | organism=synthetic construct |
| | NonEnglishQualifier Value | |
| 3-68-5 | Residues | RKVCNGIGIG EFKDSLSINA TNIKHFKNCT SISGDLHILP VAFRGDSFTH TPPLDPQELD 60<br>ILKTVKEITG FLLIQAWPEN RTDLHAFENL EIIRGRTKQH GQFSLAVVSL NITSLGLRSL 120<br>KEISDGDVII SGNKNLCYAN TINWKKLFGT SGQKTKIISN RGENSCKATG QVCHALCSPE 180<br>GCWGPEPRDC VSCRNVSRGR ECVDKCNLLE GEPREFVENS ECIQCHPECL PQAMNITCTG 240<br>RGPDNCIQCA HYIDGPHCVK TCPAGVMGEN NTLVWKYADA GHVCHLCHPN CTYGCTGPGL 300<br>EGCPTNGPKI PSIATGMVGA LLLLLVVALG IGLFM 335 |
| **3-69** | **Sequences** | |
| 3-69-1 | Sequence Number [ID] | 69 |
| 3-69-2 | Molecule Type | DNA |
| 3-69-3 | Length | 732 |
| 3-69-4 | Features Location/ | **source 1..732** |
| | Qualifiers | mol_type=other DNA |
| | | organism=synthetic construct |
| | NonEnglishQualifier Value | |
| 3-69-5 | Residues | caggtccagc tggtgcagtc tggggctgag gtgaagaagc ctgggtcctc ggtgaaggtc 60<br>tcctgcaagg cttctggagg caccttcagc agctatgcta tcagctgggt gcgacaggcc 120<br>cctggacaag ggcttgagtg gatgggaggg atcatcccta tctttggtac agcaaactac 180<br>gcacagaagt tccagggcag agtcacgatt accgcggacg aatccacgag cacagcctac 240<br>acggagctga gcagcctgag atctgaggac acggccgtgt attactgtgc gagagcccgg 300<br>ttgggaggag cttttgatat ctggggccaa gggacaatgg tcaccgtctc ttcaggaggt 360<br>ggcgggtctg gtggaggcgg tagcggtggt ggcggatccc agtctgtgct gacgcagccg 420<br>ccctcagtgt ctgcggcccc aggacagaag gtcaccatct cctgctctgg aggcagctcc 480<br>aacattggca atcattatgt gtcctggtat cagcagctcc caggagcagc ccccaaactc 540<br>ctcatttatg acgataataa gcgaccctca gggattcctg accgattctc tggctccagg 600<br>tctggcacgt cagccaccct gggcatcacc ggactccaga gtggggacga ggccgattat 660<br>tactgcggag catgggatag tagtcttgct gctcatgtct tcggaactgg gaccaaggtc 720<br>accgtcctag gt 732 |
| **3-70** | **Sequences** | |
| 3-70-1 | Sequence Number [ID] | 70 |
| 3-70-2 | Molecule Type | AA |
| 3-70-3 | Length | 244 |
| 3-70-4 | Features Location/ | **source 1..244** |
| | Qualifiers | mol_type=protein |
| | | organism=synthetic construct |
| | NonEnglishQualifier Value | |

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|---|---|---|
| 3-70-5 | Residues | QVQLVQSGAE VKKPGSSVKV SCKASGGTFS SYAISWVRQA PGQGLEWMGG IIPIFGTANY 60<br>AQKFQGRVTI TADESTSTAY TELSSLRSED TAVYYCARAR LGGAFDIWGQ GTMVTVSSGG 120<br>GGSGGGGSGG GGSQSVLTQP PSVSAAPGQK VTISCSGGSS NIGNHYVSWY QQLPGAAPKL 180<br>LIYDDNKRPS GIPDRFSGSR SGTSATLGIT GLQSGDEADY YCGAWDSSLA AHVFGTGTKV 240<br>TVLG 244 |
| **3-71** | **Sequences** | |
| 3-71-1 | Sequence Number [ID] | 71 |
| 3-71-2 | Molecule Type | DNA |
| 3-71-3 | Length | 738 |
| 3-71-4 | Features Location/ | **source 1..738** |
| | Qualifiers | mol_type=other DNA |
| | | organism=synthetic construct |
| | NonEnglishQualifier Value | |
| 3-71-5 | Residues | caggtacagc tgcagcagtc aggtccagga ctggtgaagc cctcgcagac cctctcactc 60<br>acctgtgcca tctccgggga cagtgtctct agcaacagtg ctgcttggaa ctggatcagg 120<br>cagtccccat cgagaggcct tgagtggctg ggaaggacat actacaggtc caagtggtat 180<br>aatgattatg cagtatctgt gaaaagtcga ataaccatca acccagacac atccaagaac 240<br>cagttctccc tgcagctgaa ctctgtgact cccgaggaca tggctgtgta ttactgtgca 300<br>agaggcgttg atagtagctt tgactactgg ggccagggaa ccctggtcac cgtctcctca 360<br>ggaggtggcg ggtctggtgg aggcggtagc ggtggtggcg gatcccagtc tgtcgtgacg 420<br>cagccgccct cagtgtctgc ggccccagga cagagcgtca ccatctcctg ttctggaagc 480<br>agttccaccg ttggcgataa ttatgtgtcc tggtaccagc aactcccagg aacagccccc 540<br>aaactcctca tttttgacga ttataaacga ccctcagggg ttcctgaccg attctctggc 600<br>tcccagtctg gcacctcagc ctccctggtc atcactggtc tccaggcaga agatgaggct 660<br>gattattact gccagtctta tgacagcagc ctgagtggtt atgtcttcgg gcctgggacc 720<br>aaggtcaccg tcctaggt 738 |
| **3-72** | **Sequences** | |
| 3-72-1 | Sequence Number [ID] | 72 |
| 3-72-2 | Molecule Type | AA |
| 3-72-3 | Length | 246 |
| 3-72-4 | Features Location/ | **source 1..246** |
| | Qualifiers | mol_type=protein |
| | | organism=synthetic construct |
| | NonEnglishQualifier Value | |
| 3-72-5 | Residues | QVQLQQSGPG LVKPSQTLSL TCAISGDSVS SNSAAWNWIR QSPSRGLEWL GRTYYRSKWY 60<br>NDYAVSVKSR ITINPDTSKN QFSLQLNSVT PEDMAVYYCA RGVDSSFDYW GQGTLVTVSS 120<br>GGGGSGGGGS GGGGSQSVVT QPPSVSAAPG QSVTISCSGS SSTVGDNYVS WYQQLPGTAP 180<br>KLLIFDDYKR PSGVPDRFSG SQSGTSASLV ITGLQAEDEA DYYCQSYDSS LSGYVFGPGT 240<br>KVTVLG 246 |
| **3-73** | **Sequences** | |
| 3-73-1 | Sequence Number [ID] | 73 |
| 3-73-2 | Molecule Type | DNA |
| 3-73-3 | Length | 126 |
| 3-73-4 | Features Location/ | **source 1..126** |
| | Qualifiers | mol_type=other DNA |
| | | organism=synthetic construct |
| | NonEnglishQualifier Value | |
| 3-73-5 | Residues | aagcgcggac ggaagaaact cttgtacatc ttcaagcagc cgttcatgcg ccctgtgcaa 60<br>accacccaag aagaggacgg gtgctcctgc cggttcccgg aagaggaaga gggcggctgc 120<br>gaactg 126 |
| **3-74** | **Sequences** | |
| 3-74-1 | Sequence Number [ID] | 74 |
| 3-74-2 | Molecule Type | AA |
| 3-74-3 | Length | 42 |
| 3-74-4 | Features Location/ | **source 1..42** |
| | Qualifiers | mol_type=protein |
| | | organism=synthetic construct |
| | NonEnglishQualifier Value | |
| 3-74-5 | Residues | KRGRKKLLYI FKQPFMRPVQ TTQEEDGCSC RFPEEEEGGC EL 42 |
| **3-75** | **Sequences** | |
| 3-75-1 | Sequence Number [ID] | 75 |
| 3-75-2 | Molecule Type | DNA |
| 3-75-3 | Length | 336 |
| 3-75-4 | Features Location/ | **source 1..336** |
| | Qualifiers | mol_type=other DNA |

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|---|---|---|
| | | organism=synthetic construct |
| | NonEnglishQualifier Value | |
| 3-75-5 | Residues | cgcgtgaagt tttcccggtc cgccgacgct ccggcgtacc agcaggggca aaaccagctg 60<br>tacaacgaac ttaacctcgg tcgccgggaa gaatatgacg tgctggacaa gcggcgggga 120<br>agagatcccg agatgggtgg aaagccgcgg cggaagaacc ctcaggaggg cttgtacaac 180<br>gagctgcaaa aggacaaaat ggccgaagcc tactccgaga ttggcatgaa gggagagcgc 240<br>agacgcggga agggacacga tggactgtac cagggactgt caaccgcgac taaggacact 300<br>tacgacgccc tgcacatgca ggccctgccc ccgcgc 336 |
| **3-76** | **Sequences** | |
| 3-76-1 | Sequence Number [ID] | 76 |
| 3-76-2 | Molecule Type | AA |
| 3-76-3 | Length | 112 |
| 3-76-4 | Features Location/ | **source 1..112** |
| | Qualifiers | mol_type=protein |
| | | organism=synthetic construct |
| | NonEnglishQualifier Value | |
| 3-76-5 | Residues | RVKFSRSADA PAYQQGQNQL YNELNLGRRE EYDVLDKRRG RDPEMGGKPR RKNPQEGLYN 60<br>ELQKDKMAEA YSEIGMKGER RRGKGHDGLY QGLSTATKDT YDALHMQALP PR 112 |
| **3-77** | **Sequences** | |
| 3-77-1 | Sequence Number [ID] | 77 |
| 3-77-2 | Molecule Type | DNA |
| 3-77-3 | Length | 1476 |
| 3-77-4 | Features Location/ | **source 1..1476** |
| | Qualifiers | mol_type=other DNA |
| | | organism=synthetic construct |
| | NonEnglishQualifier Value | |
| 3-77-5 | Residues | atgctcttgc tcgtgacttc tttgcttttg tgcgaacttc cgcacccagc cttccttttg 60<br>atacctcagg tccagctggt gcagtctggg gctgaggtga agaagcctgg gtcctcggtg 120<br>aaggtctcct gcaaggcttc tggaggcacc ttcagcagct atgctatcag ctgggtgcga 180<br>caggcccctg gacaagggct tgagtggatg ggagggatca tccctatctt tggtacagca 240<br>aactacgcac agaagttcca gggcagagtc acgattaccg cggacgaatc cacgagcaca 300<br>gcctacacgg agctgagcag cctgagatct gaggacacgg ccgtgtatta ctgtgcgaga 360<br>gcccggttgg gaggagcttt tgatatctgg ggccaaggga caatggtcac cgtctcttca 420<br>ggaggtggcg ggtctggtgg aggcggtagc ggtggtggcg gatcccagtc tgtgctgacg 480<br>cagccgccct cagtgtctgc ggccccagga cagaaggtca ccatctcctg ctctggaggc 540<br>agctccaaca ttggcaatca ttatgtgtcc tggtatcagc agctcccagg agcagccccc 600<br>aaactcctca tttatgacga taataagcga ccctcaggga ttcctgaccg attctctggc 660<br>tccaggtctg gcacgtcagc caccctgggc atcaccggac tccagagtgg ggacgaggcc 720<br>gattattact gcggagcatg ggatagtagt cttgctgctc atgtcttcgg aactgggacc 780<br>aaggtcaccg tcctgggtgc ggccgcaacg accactcctg caccccgccc tccgactccg 840<br>gccccaacca ttgccagcca gcccctgtcc ctgcggccgg aagcctgcag accggctgcc 900<br>ggcggagccg tccatacccg gggactggat ttcgcctgcg atatctatat ctgggcacca 960<br>ctcgccggaa cctgtggagt gctgctgctg tcccttgtga tcaccctgta ctgcaagcgc 1020<br>ggacggaaga aactcttgta catcttcaag cagccgttca tgcgccctgt gcaaaccacc 1080<br>caagaagagg acgggtgctc ctgccggttc ccggaagagg aagagggcgg ctgcgaactg 1140<br>cgcgtgaagt tttcccggtc cgccgacgct ccggcgtacc agcaggggca aaaccagctg 1200<br>tacaacgaac ttaacctcgg tcgccgggaa gaatatgacg tgctggacaa gcggcgggga 1260<br>agagatcccg agatgggtgg aaagccgcgg cggaagaacc ctcaggaggg cttgtacaac 1320<br>gagctgcaaa aggacaaaat ggccgaagcc tactccgaga ttggcatgaa gggagagcgc 1380<br>agacgcggga agggacacga tggactgtac cagggactgt caaccgcgac taaggacact 1440<br>tacgacgccc tgcacatgca ggccctgccc ccgcgc 1476 |
| **3-78** | **Sequences** | |
| 3-78-1 | Sequence Number [ID] | 78 |
| 3-78-2 | Molecule Type | AA |
| 3-78-3 | Length | 492 |
| 3-78-4 | Features Location/ | **source 1..492** |
| | Qualifiers | mol_type=protein |
| | | organism=synthetic construct |
| | NonEnglishQualifier Value | |
| 3-78-5 | Residues | MLLLVTSLLL CELPHPAFLL IPQVQLVQSG AEVKKPGSSV KVSCKASGGT FSSYAISWVR 60<br>QAPGQGLEWM GGIIPIFGTA NYAQKFQGRV TITADESTST AYTELSSLRS EDTAVYYCAR 120<br>ARLGGAFDIW GQGTMVTVSS GGGGSGGGGS GGGGSQSVLT QPPSVSAAPG QKVTISCSGG 180<br>SSNIGNHYVS WYQQLPGAAP KLLIYDDNKR PSGIPDRFSG SRSGTSATLG ITGLQSGDEA 240<br>DYYCGAWDSS LAAHVFGTGT KVTVLGAAAT TTPAPRPPTP APTIASQPLS LRPEACRPAA 300<br>GGAVHTRGLD FACDIYIWAP LAGTCGVLLL SLVITLYCKR GRKKLLYIFK QPFMRPVQTT 360 |

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|---|---|---|
| | | QEEDGCSCRF PEEEEGGCEL RVKFSRSADA PAYQQGQNQL YNELNLGRRE EYDVLDKRRG 420<br>RDPEMGGKPR RKNPQEGLYN ELQKDKMAEA YSEIGMKGER RRGKGHDGLY QGLSTATKDT 480<br>YDALHMQALP PR 492 |
| **3-79** | **Sequences** | |
| 3-79-1 | Sequence Number [ID] | 79 |
| 3-79-2 | Molecule Type | DNA |
| 3-79-3 | Length | 36 |
| 3-79-4 | Features Location/ | **source 1..36** |
| | Qualifiers | mol_type=genomic DNA |
| | | organism=Homo sapiens |
| | NonEnglishQualifier Value | |
| 3-79-5 | Residues | gagagcaaat acgggccgcc atgtcccccg tgtccg 36 |
| **3-80** | **Sequences** | |
| 3-80-1 | Sequence Number [ID] | 80 |
| 3-80-2 | Molecule Type | AA |
| 3-80-3 | Length | 12 |
| 3-80-4 | Features Location/ | **source 1..12** |
| | Qualifiers | mol_type=protein |
| | | organism=Homo sapiens |
| | NonEnglishQualifier Value | |
| 3-80-5 | Residues | ESKYGPPCPP CP 12 |
| **3-81** | **Sequences** | |
| 3-81-1 | Sequence Number [ID] | 81 |
| 3-81-2 | Molecule Type | DNA |
| 3-81-3 | Length | 327 |
| 3-81-4 | Features Location/ | **source 1..327** |
| | Qualifiers | mol_type=genomic DNA |
| | | organism=Homo sapiens |
| | NonEnglishQualifier Value | |
| 3-81-5 | Residues | gcaccaccag ttgctggccc tagtgtcttc ttgttccctc ccaagcccaa agacaccttg 60<br>atgatttcca gaactcctga ggttacctgc gttgtcgtag atgtttctca ggaggaccca 120<br>gaggtccaat ttaactggta cgttgatggg gtggaagttc acaatgcgaa gacaaagccg 180<br>cgggaagaac aatttcagtc cacttaccgg gttgtcagcg ttctgacggt attgcatcaa 240<br>gactggctta atggaaagga atataagtgt aaggtgtcca acaaaggttt gccgagcagt 300<br>attgagaaga ccatatcaaa ggcgaag 327 |
| **3-82** | **Sequences** | |
| 3-82-1 | Sequence Number [ID] | 82 |
| 3-82-2 | Molecule Type | AA |
| 3-82-3 | Length | 109 |
| 3-82-4 | Features Location/ | **source 1..109** |
| | Qualifiers | mol_type=protein |
| | | organism=Homo sapiens |
| | NonEnglishQualifier Value | |
| 3-82-5 | Residues | APPVAGPSVF LFPPKPKDTL MISRTPEVTC VVVDVSQEDP EVQFNWYVDG VEVHNAKTKP 60<br>REEQFQSTYR VVSVLTVLHQ DWLNGKEYKC KVSNKGLPSS IEKTISKAK 109 |
| **3-83** | **Sequences** | |
| 3-83-1 | Sequence Number [ID] | 83 |
| 3-83-2 | Molecule Type | DNA |
| 3-83-3 | Length | 321 |
| 3-83-4 | Features Location/ | **source 1..321** |
| | Qualifiers | mol_type=genomic DNA |
| | | organism=Homo sapiens |
| | NonEnglishQualifier Value | |
| 3-83-5 | Residues | gggcagccgc gcgagccaca agtttacact ttgccgccat ctcaagagga aatgactaaa 60<br>aaccaggtat ccttgacatg cctcgtaaaa ggattttatc catctgatat tgctgtggaa 120<br>tgggagtcta acgggcagcc ggaaaataat tacaaaacta caccacctgt gctcgattca 180<br>gatggaagtt tcttccttta cagtagactt acggtggaca aatctaggtg gcaggaaggg 240<br>aatgtgttta gttgtagtgt aatgcacgag gcacttcata accactatac acagaagtca 300<br>ctgagtttga gtcttggcaa a 321 |
| **3-84** | **Sequences** | |
| 3-84-1 | Sequence Number [ID] | 84 |
| 3-84-2 | Molecule Type | AA |
| 3-84-3 | Length | 107 |
| 3-84-4 | Features Location/ | **source 1..107** |
| | Qualifiers | mol_type=protein |

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|---|---|---|
| | | organism=Homo sapiens |
| | NonEnglishQualifier Value | |
| 3-84-5 | Residues | GQPREPQVYT LPPSQEEMTK NQVSLTCLVK GFYPSDIAVE WESNGQPENN YKTTPPVLDS 60<br>DGSFFLYSRL TVDKSRWQEG NVFSCSVMHE ALHNHYTQKS LSLSLGK 107 |
| **3-85** | **Sequences** | |
| 3-85-1 | Sequence Number [ID] | 85 |
| 3-85-2 | Molecule Type | DNA |
| 3-85-3 | Length | 684 |
| 3-85-4 | Features Location/ | **source 1..684** |
| | Qualifiers | mol_type=other DNA |
| | | organism=synthetic construct |
| | NonEnglishQualifier Value | |
| 3-85-5 | Residues | gagagcaaat acgggccgcc atgtcccccg tgtccggcac caccagttgc tggccctagt 60<br>gtcttcttgt tccctcccaa gcccaaagac accttgatga tttccagaac tcctgaggtt 120<br>acctgcgttg tcgtagatgt ttctcaggag gacccagagg tccaatttaa ctggtacgtt 180<br>gatggggtgg aagttcacaa tgcgaagaca aagccgcggg aagaacaatt tcagtccact 240<br>taccgggttg tcagcgttct gacggtattg catcaagact ggcttaatgg aaaggaatat 300<br>aagtgtaagg tgtccaacaa aggtttgccg agcagtattg agaagaccat atcaaaggcg 360<br>aaggggcagc cgcgcgagcc acaagtttac actttgccgc catctcaaga ggaaatgact 420<br>aaaaaccagg tatccttgac atgcctcgta aaaggatttt atccatctga tattgctgtg 480<br>gaatgggagt ctaacgggca gccggaaaat aattacaaaa ctacaccacc tgtgctcgat 540<br>tcagatggaa gtttcttcct ttacagtaga cttacggtgg acaaatctag gtggcaggaa 600<br>gggaatgtgt ttagttgtag tgtaatgcac gaggcacttc ataaccacta tacacagaag 660<br>tcactgagtt tgagtcttgg caaa 684 |
| **3-86** | **Sequences** | |
| 3-86-1 | Sequence Number [ID] | 86 |
| 3-86-2 | Molecule Type | AA |
| 3-86-3 | Length | 228 |
| 3-86-4 | Features Location/ | **source 1..228** |
| | Qualifiers | mol_type=protein |
| | | organism=synthetic construct |
| | NonEnglishQualifier Value | |
| 3-86-5 | Residues | ESKYGPPCPP CPAPPVAGPS VFLFPPKPKD TLMISRTPEV TCVVVDVSQE DPEVQFNWYV 60<br>DGVEVHNAKT KPREEQFQST YRVVSVLTVL HQDWLNGKEY KCKVSNKGLP SSIEKTISKA 120<br>KGQPREPQVY TLPPSQEEMT KNQVSLTCLV KGFYPSDIAV EWESNGQPEN NYKTTPPVLD 180<br>SDGSFFLYSR LTVDKSRWQE GNVFSCSVMH EALHNHYTQK SLSLSLGK 228 |
| **3-87** | **Sequences** | |
| 3-87-1 | Sequence Number [ID] | 87 |
| 3-87-2 | Molecule Type | DNA |
| 3-87-3 | Length | 1482 |
| 3-87-4 | Features Location/ | **source 1..1482** |
| | Qualifiers | mol_type=other DNA |
| | | organism=synthetic construct |
| | NonEnglishQualifier Value | |
| 3-87-5 | Residues | atgctcttgc tcgtgacttc tttgcttttg tgcgaacttc cgcacccagc cttccttttg 60<br>atacctcagg tacagctgca gcagtcaggt ccaggactgg tgaagccctc gcagaccctc 120<br>tcactcacct gtgccatctc cggggacagt gtctctagca acagtgctgc ttggaactgg 180<br>atcaggcagt ccccatcgag aggccttgag tggctgggaa ggacatacta caggtccaag 240<br>tggtataatg attatgcagt atctgtgaaa agtcgaataa ccatcaaccc agacacatcc 300<br>aagaaccagt tctccctgca gctgaactct gtgactcccg aggacatggc tgtgtattac 360<br>tgtgcaagag gcgttgatag tagctttgac tactggggcc agggaaccct ggtcaccgtc 420<br>tcctcaggag gtggcgggtc tggtggaggc ggtagcggtg gtggcggatc ccagtctgtc 480<br>gtgacgcagc cgccctcagt gtctgcggcc ccaggacaga gcgtcaccat ctcctgttct 540<br>ggaagcagtt ccaccgttgg cgataattat gtgtcctggt accagcaact cccaggaaca 600<br>gcccccaaac tcctcatttt tgacgattat aaacgaccct caggggttcc tgaccgattc 660<br>tctggctccc agtctggcac ctcagcctcc ctggtcatca ctggtctcca ggcagaagat 720<br>gaggctgatt attactgcca gtcttatgac agcagcctga gtggttatgt cttcgggcct 780<br>gggaccaagg tcaccgtcct gggtgcggcc gcaacgacca ctcctgcacc ccgccctccg 840<br>actccggccc caaccattgc cagccagccc ctgtccctgc ggccggaagc ctgcagaccg 900<br>gctgccggcg gagccgtcca tacccgggga ctggatttcg cctgcgatat ctatatctgg 960<br>gcaccactcg ccggaacctg tggagtgctg ctgctgtccc ttgtgatcac cctgtactgc 1020<br>aagcgcggac ggaagaaact cttgtacatc ttcaagcagc cgttcatgcg ccctgtgcaa 1080<br>accacccaag aagaggacgg gtgctcctgc cggttcccgg aagaggaaga gggcggctgc 1140<br>gaactgcgcg tgaagttttc ccggtccgcc gacgctccgg cgtaccagca ggggcaaaac 1200<br>cagctgtaca acgaacttaa cctcggtcgc cgggaagaat atgacgtgct ggacaagcgg 1260 |

| | | |
|---|---|---|
| | | cggggaagag atcccgagat gggtggaaag ccgcggcgga agaaccctca ggagggcttg 1320<br>tacaacgagc tgcaaaagga caaaatggcc gaagcctact ccgagattgg catgaaggga 1380<br>gagcgcagac gcgggaaggg acacgatgga ctgtaccagg gactgtcaac cgcgactaag 1440<br>gacacttacg acgccctgca catgcaggcc ctgcccccgc gc 1482 |
| **3-88** | **Sequences** | |
| 3-88-1 | Sequence Number [ID] | 88 |
| 3-88-2 | Molecule Type | AA |
| 3-88-3 | Length | 494 |
| 3-88-4 | Features Location/ | **source 1..494** |
| | Qualifiers | mol_type=protein |
| | | organism=synthetic construct |
| | NonEnglishQualifier Value | |
| 3-88-5 | Residues | MLLLVTSLLL CELPHPAFLL IPQVQLQQSG PGLVKPSQTL SLTCAISGDS VSSNSAAWNW 60<br>IRQSPSRGLE WLGRTYYRSK WYNDYAVSVK SRITINPDTS KNQFSLQLNS VTPEDMAVYY 120<br>CARGVDSSFD YWGQGTLVTV SSGGGGSGGG GSGGGGSQSV VTQPPSVSAA PGQSVTISCS 180<br>GSSSTVGDNY VSWYQQLPGT APKLLIFDDY KRPSGVPDRF SGSQSGTSAS LVITGLQAED 240<br>EADYYCQSYD SSLSGYVFGP GTKVTVLGAA ATTTPAPRPP TPAPTIASQP LSLRPEACRP 300<br>AAGGAVHTRG LDFACDIYIW APLAGTCGVL LLSLVITLYC KRGRKKLLYI FKQPFMRPVQ 360<br>TTQEEDGCSC RFPEEEEGGC ELRVKFSRSA DAPAYQQGQN QLYNELNLGR REEYDVLDKR 420<br>RGRDPEMGGK PRRKNPQEGL YNELQKDKMA EAYSEIGMKG ERRRGKGHDG LYQGLSTATK 480<br>DTYDALHMQA LPPR 494 |