SUMMARY

[0005] In one aspect, the present invention refers to a recombinant polypeptide comprising a sequence at least 90% identical to SEQ ID NO: 1, wherein the polypeptide comprises (i) an Arg at the position corresponding to position 14; (ii) an Ala at the position corresponding to position 33; (iii) a His at the position corresponding to position 115; (iv) an Arg at the position corresponding to position 136; (v) an Asp at the position corresponding to position 214; (vi) an Ala at the position corresponding to position 215; (vii) a Val at the position corresponding to position 216; (viii) a Gln at the position corresponding to position 217; (ix) a His at the position corresponding to position 243; (x) a His at the position corresponding to position 253; and (xi) an Arg at the position corresponding to position 255, said polypeptide having transposase activity. In a first example there is disclosed a recombinant polypeptide comprising or consisting of enhanced transposase activity in mammalian cells. In some examples, a polypeptide comprises or consists of a sequence at least 90% identical to SEQ ID NO: 1 (hSB110) or SEQ ID NO: 3 (hSB81). In certain examples, a polypeptide comprises or consists of a sequence of SEQ ID NO: 1 or SEQ ID NO: 3 or a sequence at least 90% identical to the full length of SEQ ID NO: 1 or SEQ ID NO:3 and exhibits transposase activity in mammalian cells. In a further examples, the polypeptide comprises or consists of a sequence at least 90% identical to SEQ ID NO: 1 or SEQ ID NO:3, exhibits transposase activity in mammalian cells, and comprises one or more of the following features: an Arg at the position corresponding to position 136 (in hSB110), a His at the position corresponding to position 253 (in hSB110), an Arg at the position corresponding to position 255 (in hSB110) and/or a Thr at the position corresponding to position 314 (in hSB110). In further examples, the preceding at least 90% identical polypeptide does not comprise the sequence of a naturally occurring transposase enzyme or does not comprise the sequence of SEQ ID NO: 5 (SB11), SEQ ID NO: 6 (SB10) or SEQ ID NO: 7 (SB100x). In some examples, a polypeptide comprises or consists of a sequence at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to SEQ ID NO: 1 (hSB110) or SEQ ID NO: 3 (hSB81). In some examples, a polypeptide comprises or consists of a sequence at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to SEQ ID NO: 1 (hSB110) or SEQ ID NO: 3 (hSB81) and exhibits transposase activity in mammalian cells. In some examples, a polypeptide comprises or consists of a sequence at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to SEQ ID NO: 1 (hSB110) or SEQ ID NO: 3 (hSB81), exhibits transposase activity in mammalian cells, and comprises or consists of one or more of the following features: an Arg at the position corresponding to position 136 (in hSB110), a His at the position corresponding to position 253 (in hSB110), an Arg at the position corresponding to

position 255 (in hSB110) and/or a Thr at the position corresponding to position 314 (in hSB110). In some examples, the preceding at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical polypeptide does not comprise the sequence of a naturally occurring transposase enzyme or does not comprise the sequence of SEQ ID NO: 5 (SB11), SEQ ID NO: 6 (SB10) or SEQ ID NO: 7 (SB100x). In yet further examples, a transposase polypeptide further comprises or consists of a heterologous polypeptide sequence fused to the N- or C-terminus of the transposase sequence. For example, the heterologous polypeptide sequence may comprise or consist of a reporter, a purification tag or a cell penetrating polypeptide (CPP). In further examples, the mammalian cell in which polypeptides disclosed herein exhibit transposase activity are human cells. In further examples, the human cells are immune cells. In further examples the human immune cells are T cells. In further examples, the T cells are T helper cells (T_H cells), cytotoxic T cells (T_c cells or CTLs), memory T cells (T_{CM} cells), effector T cells (T_{EM} cells), regulatory T cells (Treg cells; also known as suppressor T cells), natural killer T cells (NKT cells), mucosal associated invariant T cells, alpha-beta T cells (Tαβ cells), and/or gamma-delta T cells (Tγδ cells).

[0006] In yet a further examples, a polypeptide comprises or consists of a sequence at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to SEQ ID NO: 1 (hSB110) or SEQ ID NO: 3 (hSB81), comprises one or more of the following features: an Arg at the position corresponding to position 136 (in hSB110), a His at the position corresponding to position 253 (in hSB110), an Arg at the position corresponding to position 255 (in hSB110) and/or a Thr at the position corresponding to position 314 (in hSB110) and further comprises one or more of the following additional features: an Arg at the position corresponding to position 14 (in hSB110), an Ala at the position corresponding to position 33 (in hSB110), a His at the position corresponding to position 115 (in hSB110), an Asp at the position corresponding to position 214 (in hSB110), an Ala at the position corresponding to position 215 (in hSB110), a Val at the position corresponding to position 216 (in hSB110), a Gln at the position corresponding to position 217 (in hSB110), and/or a His at the position corresponding to position 243 (in hSB110). In some examples, the preceding at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical polypeptide does not comprise the sequence of a naturally occurring transposase enzyme or does not comprise the sequence of SEQ ID NO: 5 (SB11), SEQ ID NO: 6 (SB10) or SEQ ID NO: 7 (SB100x). In some examples, a polypeptide comprises 2, 3, 4, 5, 6, 7 or 8 of the sequence features selected from the group consisting of: an Arg at the position corresponding to position 14 (in hSB110), an Ala at the position corresponding to position 33 (in hSB110), a His at the position corresponding to position 115 (in hSB110), an Asp at the position corresponding to

position 214 (in hSB110), an Ala at the position corresponding to position 215 (in hSB110), a Val at the position corresponding to position 216 (in hSB110), a Gln at the position corresponding to position 217 (in hSB110), and a His at the position corresponding to position 243 (in hSB110). In further examples, the preceding at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical polypeptide comprises the sequence DAVQ at the positions corresponding to positions 214-217(in hSB110).

[0007] In some examples, the polypeptide comprises or consists of a sequence at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to SEQ ID NO: 1 (hSB110), comprises a Asn at the position corresponding to position 314 (in hSB110) and comprises one or more of the following features: an Arg at the position corresponding to position 136 (in hSB110), a His at the position corresponding to position 253 (in hSB110), and/or an Arg at the position corresponding to position 255 (in hSB110). In some examples, the preceding at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical polypeptide does not comprise the sequence of a naturally occurring transposase enzyme or does not comprise the sequence of SEQ ID NO: 5 (SB11), SEQ ID NO: 6 (SB10) or SEQ ID NO: 7 (SB100x). In specific examples, the polypeptide comprises or consists of the sequence of SEQ ID NO: 1.

[0008] In other examples, the polypeptide comprises or consists of a sequence at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to SEQ ID NO: 3 (hSB81), comprises a Thr at the position corresponding to position 314 (in hSB110) and comprises one or more of the following features: an Arg at the position corresponding to position 136 (in hSB110), a His at the position corresponding to position 253 (in hSB110), and/or an Arg at the position corresponding to position 255 (in hSB110). In some examples, the preceding at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical polypeptide does not comprise the sequence of a naturally occurring transposase enzyme or does not comprise the sequence of SEQ ID NO: 5 (SB11), SEQ ID NO: 6 (SB10) or SEQ ID NO: 7 (SB100x). In specific examples, the polypeptide comprises or consists of the sequence of SEQ ID NO: 3.

[0009] In another aspect, the present invention refers to a polynucleotide molecule comprising a sequence encoding the polypeptide as described herein. In a further example there is disclosed a polynucleotide molecule comprising or consisting of a sequence encoding a polypeptide according to the disclosure. The molecule may be a DNA expression vector in some examples. For example, the DNA expression vector may comprise a transposase coding sequence operably linked to a promoter for *in vitro* expression of the polypeptide (*e.g.,* a T7 or SP6 promoter) or a promoter for expression of the polypeptide in mammalian cells. In some

examples, the polynucleotide molecule may be a RNA or mRNA. In further examples, the RNA may comprise a 5'-cap, an IRES motif, a (heterologous) 5' UTR, a (heterologous) 3' UTR and/or a poly(A) sequence. The RNA may additionally comprise a poly(A) sequence of 20 to 300 nucleotides in some examples.

[0010] In one aspect, the present invention refers to a host cell comprising a polypeptide encoding the polypeptide as disclosed herein. In yet a further example there is disclosed a method of making a transposase polypeptide as described above, comprising transfecting a cell with a polynucleotide encoding a transposase polypeptide and expressing the polypeptide from the polynucleotide. In still a further example, there is disclosed a host cell comprising a polypeptide or a polynucleotide molecule of the disclosure. In some cases, the cell is a mammalian cell, such as a human cell. In certain examples, the cell is a stem cell or an induced pluripotent stem (iPS) cell. In further examples, the cell is a natural killer (NK) cell, a precursor of a NK cell, a T-cell, a precursor of a T-cell, or an immune cell. In some cases, the cell may comprise a RNA encoding a transposase polypeptide of the disclosure. In yet further examplesa population of cells, said cells comprising polypeptide or a polynucleotide molecule of the disclosure, is disclosed.

[0011] In another aspect, the present invention refers to an *in vitro* method of genetically engineering a cell comprising: (a) transfecting the cell with a nucleic acid encoding the polypeptide of claim 1; and a DNA encoding a selected genetic element flanked by transposon repeats; and (b) incubating the cell under conditions appropriate for transient or stable transposase activity, thereby integrating the selected genetic element in the genome of the cell and producing an engineered cell. In another example, a method is disclosed for genetically engineering a cell comprising: transfecting the cell with a transposase polypeptide as described above or a nucleic acid encoding the transposase polypeptide and a DNA vector comprising a sequence encoding a selected genetic element flanked by transposon repeats, then incubating the cell under conditions appropriate for (transient or stable) transposase activity, thereby integrating the selected genetic element in the genome of the cell and producing an engineered cell. In certain examples, a DNA vector encoding a selected genetic element flanked by transposon repeats further comprises a sequence encoding a transposase polypeptide of the disclosure. Thus, in certain examples, a method of the disclosure comprises transfecting a cell with a DNA vector comprising a sequence encoding a selected genetic element flanked by transposon repeats and a sequence encoding a transposase of the disclosure, which is under the control of a promoter sequence, then incubating the cell under conditions appropriate for transposase expression and activity, thereby integrating the selected genetic element in the genome of the cell and producing an engineered cell. In some examples, the method comprises the third step of isolating or culturing the engineered cell. In

some examples, the selected genetic element is a screenable or selectable marker. In further examples, the selected genetic element may encode an antibody, a inhibitory nucleic acid (*e.g.,* a small interfering RNA (siRNA)), a therapeutic polypeptide, a T-cell receptor (TCR), a chimeric antigen receptor (CAR), or an enhancer of immune cell function. In specific examples, the selected genetic element encodes a CAR or TCR. In still further examples, the selected genetic element may be a gene or a portion thereof that is used to replace or modify the corresponding gene from a cell (*e.g.,* to alter the sequence or expression of the gene or to "knock-out" gene expression in the cell). In some examples, the transfected cell is a mammalian cell such as human cell. In some cases, the cell may be a stem cell or an iPS cell. In certain examples, the cell may be an immune system cell or a precursor thereof, such as a NK cell, a T-cell, a precursor of a NK cell, or a precursor of a T-cell.

[0012] In some examples, transfecting cells may comprise use of a chemical-based transfection reagent, electroporation of the cells or other technologies providing delivery of nucleic acid and/or protein to the cytoplasm and nucleus of cells. For example, cells can be transfected using salt precipitates (*e.g.,* $CaPO_4$ precipitates), lipids (*e.g.,* charged or non-polar lipids), cationic polymers, PEG-complexes and/or protein complexes (*e.g.,* cationic polypeptides). In some examples, transfections may involve the use of liposomes, such as phospholipid liposomes (*e.g.,* liposomes that incorporate a glycerophospholipid or sphingolipids). In still further examples, cells may be transduced with a viral vector (*e.g.,* adenoviral, adeno-associated viral, retroviral (*e.g*., lentiviral) or vaccinia virus vector). In certain examples, a viral vector for use according to the disclosure is a non-integrating viral vector. A skilled artisan will recognize that, in certain examples, a transposase as disclosed herein may be delivered to cells together or separately from a nucleic acid encoding transposon repeats. For example, in certain examples, a transposase may be delivered to cells as a recombinant polypeptide using a protein transfection reagent and the nucleic acid molecule comprising the transposon repeats can be delivered using a nucleic acid transfection system or a viral vector. In further examples, an RNA encoding a transposase is co-transfected with a DNA comprising transposon repeats and a selected genetic element.

[0013] In further examples, the method additionally comprises transfecting a population of cells with a transposase polypeptide of the disclosure or a nucleic acid encoding the transposase polypeptide and a DNA vector comprising a sequence encoding a selected genetic element flanked by transposon repeats and then incubating the population under conditions appropriate for transposase activity, thereby integrating the selected genetic element in the genome of the cells and producing a population of engineered cells. In specific examples, the

method comprises transfecting a population of T-cells, or T-cell precursors, with a transposase polypeptide or a nucleic acid encoding the transposase polypeptide and a DNA vector comprising a sequence encoding a CAR flanked by transposon repeats and incubating the population under conditions appropriate for transposase activity, thereby integrating the CAR in the genome of the cells and producing a population of engineered T-cells, or T-cell precursors. In further examples, the method comprises transfecting a population of T-cells, or T-cell precursors, with a DNA vector comprising a sequence encoding a CAR flanked by transposon repeats and a sequence encoding a transposase of the disclosure(operably linked to a promoter) and incubating the population under conditions appropriate for transposase activity, thereby integrating the CAR in the genome of the cells and producing a population of engineered T-cells, or T-cell precursors. Culturing the engineered cells in a medium that selectively enhances proliferation of CAR-expressing T-cells may be additionally performed in some examples.

[0014] In yet a further example there is disclosed a method of providing a T-cell response in a human subject having a disease comprising first obtaining a population of engineered T-cells, or T-cell precursors, in accordance with the disclosure, optionally culturing the cells in a medium that selectively enhances proliferation of CAR-expressing T-cells, and then administering an effective amount of the CAR-expressing T-cells to the subject to provide a T-cell response.

[0015] Thus, in some examples, a method as disclosed herein comprises: (a) obtaining a sample of cells from the subject, the sample comprising T-cells or T-cell progenitors; (b) transfecting the cells with a DNA encoding a transposon-flanked chimeric antigen receptor (CAR) and a transposase of the disclosureeffective to integrate the DNA encoding the CAR into the genome of the cells, to provide a population of transgenic CAR-expressing cells; (c) optionally, culturing the population of transgenic CAR cells *ex vivo* in a medium that selectively enhances proliferation of CAR-expressing T-cells; and (d) administering an effective amount of the transgenic CAR cells to the subject to provide a T-cell response. Thus, in some examples, the transgenic CAR cells are cultured *ex vivo* for less than 21 days, such as for less than 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2 days or less. In certain examples, the CAR cells are cultured *ex vivo* no more that 3 to 5 days. In still further examples, steps (a)-(d) of the instant method (*i.e.,* obtaining cell samples to administering CAR T cells) are completed in no more than 21, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, or 5 days. In further examples, the sample of cells from the subject may be a sample of less than about 200 mls of a peripheral blood or umbilical cord blood. In some examples, the sample may be collected by apheresis. In certain examples, the sample is collected by a method that does not involved apheresis (*e.g.,* by

venipuncture). In still further examples, the sample of cells has an initial volume of less than 175 mls, less than about 175 mls, less than 150 mls, less than about 150 mls, less than 125 mls, less than about 125 mls, less than 100 mls, less than about 100 mls, less than 75 mls, less than about 75 mls, less than 50 mls, less than about 50 mls, less than 25 mls, or less than about 25 mls (*e.g.,* the sample of cells has an initial volume of between about 50 and about 200 mls, between about 50 and about 100 mls, or between about 100 and about 200 mls when obtained from the subject).

[0016] In some examples, methods disclosed herein concern transfecting the cells with a DNA encoding a chimeric antigen receptor (CAR) and a transposase. Methods of transfecting of cells are well known in the art, but in certain examples, highly efficient transfections methods such as electroporation are employed. For example, nucleic acids may be introduced into cells using a nucleofection apparatus. In certain examples, the transfection step does not involve infecting or transducing the cells with virus, which can cause genotoxicity and/or lead to an immune response against cells containing viral sequences in a treated subject.

[0017] Further examples concern transfecting cells with an expression vector encoding a CAR. A wide range of CAR constructs and expression vectors for the same are known in the art. For example, in some examples, the CAR expression vector is a DNA expression vector such as a plasmid, linear expression vector or an episome. In some examples, the vector comprises additional sequences, such as sequence that facilitate expression of the CAR, such a promoter, enhancer, poly-A signal, and/or one or more introns. In certain examples, the CAR coding sequence is flanked by transposon sequences, such that the presence of a transposase allows the coding sequence to integrate into the genome of the transfected cell.

[0018] As detailed *supra*, in certain examples, cells are further transfected with a transposase of the disclosurethat facilitates integration of a CAR coding sequence into the genome of the transfected cells. In some examples, the transposase is disclosed as DNA expression vector. In certain examples, the transposase is disclosed as an expressible RNA or a protein such that long-term expression of the transposase does not occur in the transgenic cells. For example, in some examples, the transposase is disclosed as encoded by an mRNA (*e.g.*, an mRNA comprising a cap and poly-A tail).

[0019] In still further examples, a transgenic CAR cell disclosed herein further comprises an expression vector for expression of a membrane-bound cytokine that stimulates proliferation and/or survival of T-cells. In particular, CAR cells comprising such cytokines can proliferate and/or persist with little or no *ex vivo* culture with activating and propagating cells (AaPCs) or artificial antigen presenting cells (aAPCs) due to the simulation provided by the

cytokine expression. Likewise, such CAR cells can proliferate *in vivo* even when large amounts of antigen recognized by the CAR is not present (*e.g.*, as in the case of a cancer patient in remission or a patient with minimal residual disease). In some examples, the CAR cells comprise a DNA or RNA expression vector for expression of a Cγ cytokine and elements (*e.g.,* a transmembrane domain) to provide surface expression of the cytokine. For example, the CAR cells can comprise membrane-bound versions of IL-7, IL-15 or IL-21. In some examples, the cytokine is tethered to the membrane by fusion of the cytokine coding sequence with the receptor for the cytokine. For example, a cell can comprise a vector for expression of a IL-15-IL-15Rα fusion protein. In still further examples, a vector encoding a membrane-bound Cγ cytokine is a DNA expression vector, such as vector integrated into the genome of the CAR cells or an extra-chromosomal vector (*e.g.*, and episomal vector). In still further examples, expression of the membrane-bound Cγ cytokine is under the control of an inducible promoter (*e.g.*, a drug inducible promoter) such that the expression of the cytokine in the CAR cells (and thereby the proliferation of the CAR cells) can be controlled by inducing or suppressing promoter activity.

[0020] Also disclosed herein are examples concerning obtaining a sample from a patient comprising NK cells, NKT cells, T-cells or T-cell progenitor cells. For example, in some cases, the sample is an umbilical cord blood sample, a peripheral blood sample (*e.g.*, a mononuclear cell fraction) or a sample from the subject comprising pluripotent stem cells. In some examples, a sample from the subject can be cultured to generate induced pluripotent stem (iPS) cells and these cells used to produce NK cells, NKT cells or T-cells. Cell samples may be cultured directly from the subject or may be cryopreserved prior to use. In some examples, obtaining a cell sample comprises collecting a cell sample. In other examples, the sample is obtained by a third party. In still further examples, a sample from a subject can be treated to purify or enrich the T-cells or T-cell progenitors in the sample. For example, the sample can be subjected to gradient purification, cell culture selection and/or cell sorting (*e.g., via* fluorescence-activated cell sorting (FACS)).

[0021] In some examples, a method disclosed herein further comprises using antigen presenting cells (*e.g.,* for expansion of engineered cells). For example, the antigen presenting cells can be dendritic cells, activating and propagating cells (AaPCs), or inactivated (*e.g.,* irradiated) artificial antigen presenting cells (aAPCs). Methods for producing such antigen presenting cells are known in the art and further detailed herein. Thus, in some examples, transgenic CAR cells are co-cultured with antigen presenting cells (*e.g.,* inactivated aAPCs) *ex vivo* for a limited period of time in order to expand the CAR cell population. The step of co-culturing CAR cells can be done in a medium that comprises, for example, interleukin-21 (IL-21) and/or interleukin-2 (IL-2). In some examples, the co-culturing is performed at a ratio of CAR

cells to antigen presenting cells of about 10:1 to about 1:10; about 3:1 to about 1:5; or about 1:1 to about 1:3. For example, the co-culture of CAR cells and antigen presenting cells can be at a ratio of about 1:1, about 1:2 or about 1:3.

[0022] In some examples, cells for culture of CAR cells such as AaPCs or aAPCs are engineered to express specific polypeptide to enhance growth of the CAR cells. For example, the cells can comprise (i) an antigen targeted by the CAR (*i.e.,* that is expressed on the transgenic CAR cells); (ii) CD64; (ii) CD86; (iii) CD137L; and/or (v) membrane-bound IL-15, expressed on the surface of the aAPCs. In some examples, the AaPCs or aAPCS comprise a CAR-binding antibody or fragment thereof expressed on the surface of the AaPCs or aAPCs. Preferably, AaPCs or aAPCs for use in the instant methods are tested for, and confirmed to be free of, infectious material and/or are tested and confirmed to be inactivated and non-proliferating.

[0023] While expansion on AaPCs or aAPCs can increase the number or concentration of CAR cells in a culture, this procedure is labor intensive and expensive. Moreover, in some examples, a subject in need of therapy should be re-infused with transgenic CAR cells in as short a time as possible. Thus, in some examples, *ex vivo* culturing the transgenic CAR cells is for no more than 14 days, no more than 7 days, or no more than 3 days. For example, the *ex vivo* culture (*e.g.*, culture in the presence of AaPCs or aAPCs) can be performed for less than one population doubling of the transgenic CAR cells. In still further examples, the transgenic cells are not cultured *ex vivo* in the presence of AaPCs or aAPCs.

[0024] In still further examples, a method disclosed herein comprises a step for enriching the cell population for CAR-expressing T-cells after transfection of the cells or after *ex vivo* expansion of the cells. For example, the enrichment step can comprise sorting of the cells (*e.g.*, via FACS), for example, by using an antigen bound by the CAR or a CAR-binding antibody. In still further examples, the enrichment step comprises depletion of the non-T-cells or depletion of cells that lack CAR expression. For example, $CD56^+$ cells can be depleted from a culture population. In yet further examples, a sample of CAR cells is preserved (or maintained in culture) when the cells are administered to the subject. For example, a sample may be cryopreserved for later expansion or analysis.

[0025] In certain examples, transgenic CAR cells are inactivated for expression of an endogenous T-cell receptor and/or endogenous HLA. For example, T cells can be engineered to eliminate expression of endogenous alpha/beta T-cell receptor (TCR). In specific examples, CAR^+ T cells are genetically modified to eliminate expression of TCR. In some examples, there is a disruption of the T-cell receptor α/β in CAR-expressing T cells using zinc finger nucleases

(ZFNs). In certain examples, the T-cell receptor αβ-chain in CAR-expressing T cells is knocked-out, for example, by using zinc finger nucleases.

[0026] As further detailed herein, CAR cells as disclosed herein can be used to treat a wide range of diseases and conditions. Essentially any disease that involves the specific or enhanced expression of a particular antigen can be treated by targeting CAR cells to the antigen. For example, autoimmune diseases, infections, and cancers can be treated with methods and/or compositions of the invention. These include cancers, such as primary, metastatic, recurrent, sensitive-to-therapy, refractory-to-therapy cancers (*e.g.*, chemo-refractory cancer). The cancer may be of the blood, lung, brain, colon, prostate, breast, liver, kidney, stomach, cervix, ovary, testes, pituitary gland, esophagus, spleen, skin, bone, and so forth (*e.g.*, B-cell lymphomas or a melanomas). In the case of cancer treatment CAR cells typically target a cancer cell antigen (also known as a tumor-associated antigen (TAA)).

[0027] In still further examples, transgenic CAR cells disclosed herein may be used to treat subject having minimal residual disease (*e.g.*, a subject having very low amounts of CAR-targeted antigen present), such as cancer patients that are in apparent remission. Using new highly sensitive diagnostic techniques, cancer-associated antigens (or cancer cells) can be detected in patients that do not exhibit overt cancer symptoms. Such patients may be treated by the instant methods to eliminate residual disease by use of antigen-targeted CAR cells. In certain examples, transgenic CAR cells for targeting of residual disease further comprise expression of a membrane-bound proliferative cytokine, as these cells will retain the ability to expand *in vivo* despite the low amount of target antigen.

[0028] The processes disclosed herein can be utilized to manufacture (*e.g.*, for clinical trials) CAR^+ T cells with binding specificity for various tumor antigens (*e.g.*, CD19, ROR1, CD56, EGFR, CD33, CD123, c-met, GD2). CAR^+ T cells generated using this technology can be used to treat patients with leukemias (*e.g.*, AML, ALL, CML), infections and/or solid tumors. For example, methods of the examplescan be used to treat cell proliferative diseases, fungal, viral, bacterial or parasitic infections. Pathogens that may be targeted include, without limitation, *Plasmodium*, trypanosome, *Aspergillus*, *Candida*, HSV, RSV, EBV, CMV, JC virus, BK virus, or Ebola pathogens. Further examples of antigens that can be targeted by CAR cells of the embodiments include, without limitation, CD19, CD20, carcinoembryonic antigen, alphafetoprotein, CA-125, 5T4, MUC-1, epithelial tumor antigen, melanoma-associated antigen, mutated p53, mutated ras, HER2/Neu, ERBB2, folate binding protein, HIV-1 envelope glycoprotein gp120, HIV-1 envelope glycoprotein gp41, GD2, CD123, CD23,CD30 , CD56, c-

Met, meothelin, GD3, HERV-K, IL-11Ralpha, kappa chain, lambda chain, CSPG4, ERBB2, EGFRvIII, or VEGFR2. In certain examples, methods of the disclosureconcern targeting of CD19 or HERV-K-expressing cells. For example, a HERV-K targeted CAR cell can comprise a CAR including the scFv sequence of monoclonal antibody 6H5. In still further examples, a CAR of the disclosure can be conjugated or fused with a cytokine, such as IL-2, IL-7, IL-15, IL-21 or a combination thereof.

[0029] In some examples, methods are described for treating an individual with a medical condition comprising the step of providing an effective amount of cells from the population of cells described herein, including more than once in some examples, such as at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or more days apart. In specific examples, the cancer is cancer of the bladder, blood, bone, bone marrow, brain, breast, colon, esophagus, gastrointestinal, gum, head, kidney, liver, lung, nasopharynx, neck, ovary, prostate, skin, stomach, testis, tongue, or uterus. In certain examples, the cancer is a lymphoma, leukemia, non-Hodgkin's lymphoma, acute lymphoblastic leukemia, chronic lymphoblastic leukemia, chronic lymphocytic leukemia, or B cell-associated autoimmune diseases.

[0030] As used herein in the specification and claims, "a" or "an" may mean one or more. As used herein in the specification and claims, when used in conjunction with the word "comprising", the words "a" or "an" may mean one or more than one. As used herein, in the specification and claim, "another" or "a further" may mean at least a second or more.

[0031] As used herein in the specification and claims, the term "about" is used to indicate that a value includes the inherent variation of error for the device, the method being employed to determine the value, or the variation that exists among the study subjects.

[0032] Other objects, features and advantages of the present invention will become apparent from the following detailed description. It should be understood, however, that the detailed description and the specific examples, while indicating certain embodiments of the invention, are given by way of illustration only, since various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from this detailed description.

BRIEF DESCRIPTION OF THE DRAWINGS

[0033] The following drawings form part of the present specification and are included to further demonstrate certain aspects of the present invention. The invention may be better understood by reference to one or more of these drawings in combination with the detailed description of specific embodiments presented herein.

[0034] FIG. 1: An exemplary strategy for transposase-based engineering of cells. In this example, engineered T-cells are produced, which express a CAR. T cells are co-transfected with a transposon DNA construct encoding a CAR flanked by transposon repeats along with a mRNA encoding the transposase. In this case, a 4D-NUCLEOFECTOR™ electroporation system (Lonza Group Ltd., Switzerland) is used for the transfection. Once introduced into the cells, the transposase is transiently expressed and mediates integration of the CAR construct into genomic DNA. The mRNA encoding the transposase is degraded within the cells, ensuring no log-term expression of transposase in the cells.

[0035] FIG. 2: Histograms show flow cytometry data of human T-cells transfected with CAR as outlined in FIG. 1. Results are compared between two recombinant transposases of the embodiments, hSB110 and hSB81, versus SB100x. Upper panels show the number of cells positive for CAR (y-axis) versus cells that have been rendered non-viable, as assessed by 7AAD (7-Aminoactinomycin D) staining (x-axis), 8-days post transfection. Lower panels show the number of cells positive for CAR expression (y-axis)

WHAT IS CLAIMED IS:

1. A recombinant polypeptide comprising a sequence at least 90% identical to SEQ ID NO: 1, wherein the polypeptide comprises

(i) an Arg at the position corresponding to position 14;
(ii) an Ala at the position corresponding to position 33;
(iii) a His at the position corresponding to position 115;
(iv) an Arg at the position corresponding to position 136;
(v) an Asp at the position corresponding to position 214;
(vi) an Ala at the position corresponding to position 215;
(vii) a Val at the position corresponding to position 216;
(viii) a Gln at the position corresponding to position 217;
(ix) a His at the position corresponding to position 243;
(x) a His at the position corresponding to position 253; and
(xi) an Arg at the position corresponding to position 255, said polypeptide having transposase activity.

2. The polypeptide of claim 1, comprising an Asn at the position corresponding to position 314.

3. The polypeptide of claim 2, wherein the polypeptide is at least 95% identical to the sequence of SEQ ID NO: 1.

4. The polypeptide of claim 2, comprising the sequence of SEQ ID NO: 1.

5. The polypeptide of claim 1, comprising a Thr at the position corresponding to position 314.

6. The polypeptide of claim 5, wherein the polypeptide is at least 90% identical to the sequence of SEQ ID NO: 3.

7. The polypeptide of claim 5, comprising the sequence of SEQ ID NO: 3.

8. A polynucleotide molecule comprising a sequence encoding the polypeptide of claim 1.

9. The polynucleotide molecule of claim 8, wherein the molecule is a mRNA.

10. The polynucleotide molecule of claim 8, wherein the molecule comprises a sequence at least 90% identical to SEQ ID NO: 2.

11. The polynucleotide molecule of claim 8, wherein the molecule comprises the sequence of SEQ ID NO: 2.

12. A host cell comprising a polypeptide encoding the polypeptide of claim 1.

13. The cell of claim 12, wherein the cell is a natural killer (NK) cell, a T-cell or a precursor of a NK cell or a precursor of a T-cell.

14. An *in vitro* method of genetically engineering a cell comprising:

(a) transfecting the cell with a nucleic acid encoding the polypeptide of claim 1; and a DNA encoding a selected genetic element flanked by transposon repeats; and

(b) incubating the cell under conditions appropriate for transient or stable transposase activity, thereby integrating the selected genetic element in the genome of the cell and producing an engineered cell.

15. The method of claim 14, wherein the selected genetic element encodes an antibody, a T-cell receptor (TCR), a chimeric antigen receptor (CAR).

16. The method of claim 15, wherein the selected genetic element encodes a CAR.

17. The method of claim 14, wherein transfecting the cell comprises electroporating the cell.

18. The polypeptide of claim 5, wherein the polypeptide is at least 95% identical to the sequence of SEQ ID NO: 3.

19. The polynucleotide molecule of claim 8, wherein the molecule comprises a sequence at least 90% identical to SEQ ID NO: 4.

20. The polynucleotide molecule of claim 8, wherein the molecule comprises the sequence of SEQ ID NO: 4