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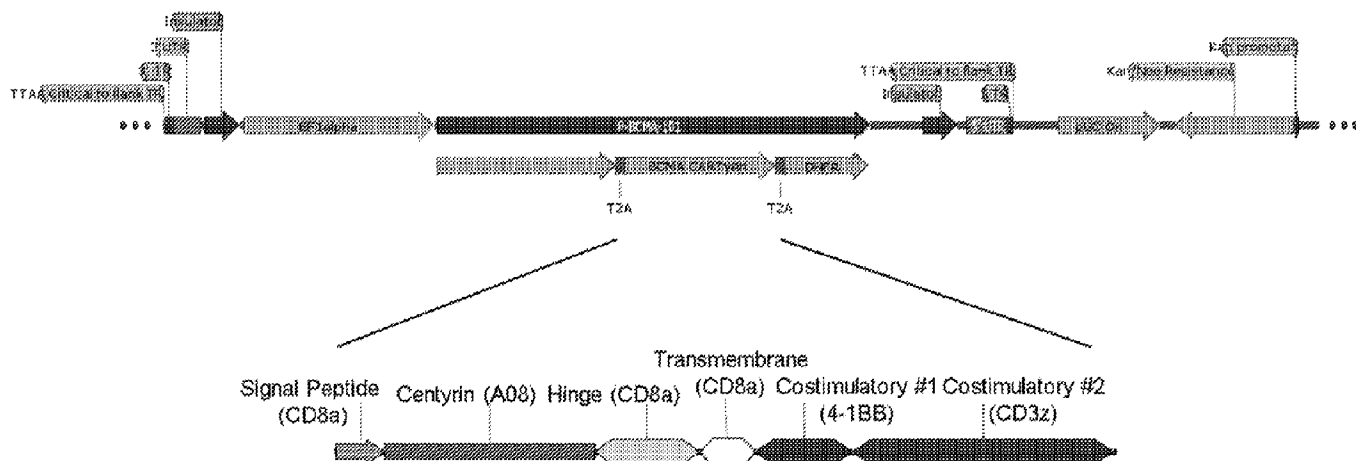
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(54) Titre : RECEPTEURS ANTIGENIQUES CHIMERIQUES ET LEURS PROCEDES D'UTILISATION
(54) Title: CHIMERIC ANTIGEN RECEPTORS AND METHODS FOR USE

FIGURE 1



(57) **Abrégé/Abstract:**

Disclosed are chimeric antigen receptors (CARs) comprising Centyrins (i.e. CARTyrins), transposons encoding CARs and CARTyrins of the disclosure, cells modified to express CARs and CARTyrins of the disclosure, as well as methods of making and methods of using same for adoptive cell therapy.

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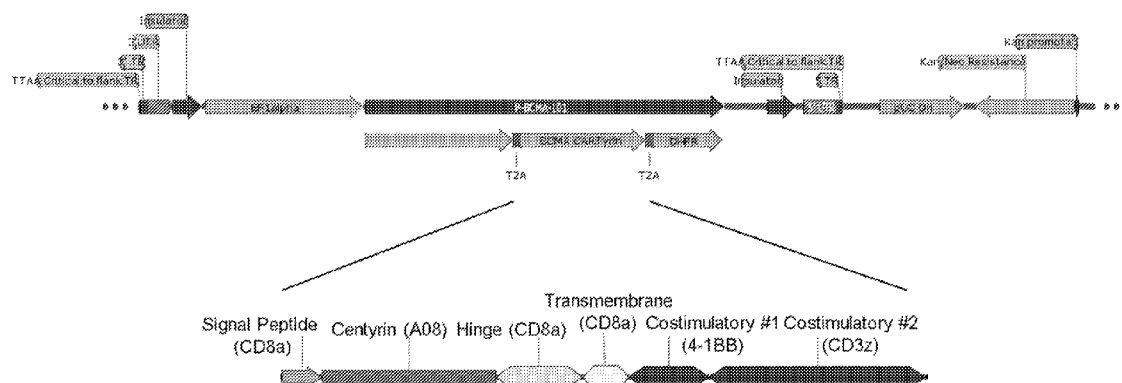


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(54) Title: CHIMERIC ANTIGEN RECEPTORS AND METHODS FOR USE

FIGURE 1



(57) Abstract: Disclosed are chimeric antigen receptors (CARs) comprising Centyrins (i.e. CARTyrins), transposons encoding CARs and CARTyrins of the disclosure, cells modified to express CARs and CARTyrins of the disclosure, as well as methods of making and methods of using same for adoptive cell therapy.

[Continued on next page]

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- *with sequence listing part of description (Rule 5.2(a))*

CHIMERIC ANTIGEN RECEPTORS AND METHODS FOR USE

RELATED APPLICATIONS

[01] This application claims the benefit of provisional applications USSN 62/362,746, filed July 15, 2016, USSN 62/405,180, filed October 6, 2016 and USSN 62/503,127, filed on May 8, 2017, the contents of each of which are herin incorporated by reference in their entirety.

INCORPORATION OF SEQUENCE LISTING

[02] The contents of the text file named “POTH-008_001WO_SeqList”, which was created on July 13, 2017 and is 55 KB in size, are hereby incorporated by reference in their entirety.

FIELD OF THE DISCLOSURE

[03] The disclosure is directed to molecular biology, and more, specifically, to chimeric antigen receptors, transposons containing one or more CARs, as well as methods of making and using the same.

BACKGROUND

[04] There has been a long-felt but unmet need in the art for a method of directing the specificity of an immune cell without using traditional antibody sequences or fragments thereof. The disclosure provides a superior chimeric antigen receptor.

SUMMARY

[05] The disclosure provides a chimeric antigen receptor (CAR) comprising: (a) an ectodomain comprising an antigen recognition region, wherein the antigen recognition region comprises at least one Centyrin; (b) a transmembrane domain, and (c) an endodomain comprising at least one costimulatory domain. As used throughout the disclosure, a CAR comprising a Centyrin is referred to as a CARTyrin. In certain embodiments, the antigen recognition region may comprise two Centyrins to produce a bi-specific or tandem CAR. In certain embodiments, the antigen recognition region may comprise three Centyrins to produce a tri-specific CAR. In certain embodiments, the ectodomain may further comprise a signal peptide. Alternatively, or in addition, in certain embodiments, the ectodomain may

further comprise a hinge between the antigen recognition region and the transmembrane domain.

[06] The disclosure provides a chimeric antigen receptor (CAR) comprising: (a) an ectodomain comprising an antigen recognition region, wherein the antigen recognition region comprises at least one protein scaffold or antibody mimetic; (b) a transmembrane domain, and (c) an endodomain comprising at least one costimulatory domain. In certain embodiments, the antigen recognition region may comprise two scaffold proteins or antibody mimetics to produce a bi-specific or tandem CAR. In certain embodiments, the antigen recognition region may comprise three protein scaffolds or antibody mimetics to produce a tri-specific CAR. In certain embodiments, the ectodomain may further comprise a signal peptide. Alternatively, or in addition, in certain embodiments, the ectodomain may further comprise a hinge between the antigen recognition region and the transmembrane domain.

[07] In certain embodiments of the CARs of the disclosure, the signal peptide may comprise a sequence encoding a human CD2, CD3 δ , CD3 ϵ , CD3 γ , CD3 ζ , CD4, CD8 α , CD19, CD28, 4-1BB or GM-CSFR signal peptide. In certain embodiments of the CARs of the disclosure, the signal peptide may comprise a sequence encoding a human CD8 α signal peptide. The human CD8 α signal peptide may comprise an amino acid sequence comprising MALPVTALLPLALLHAARP (SEQ ID NO: 3). The human CD8 α signal peptide may comprise an amino acid sequence comprising MALPVTALLPLALLHAARP (SEQ ID NO: 3) or a sequence having at least 70%, 80%, 90%, 95%, or 99% identity to the amino acid sequence comprising MALPVTALLPLALLHAARP (SEQ ID NO: 3). The human CD8 α signal peptide may be encoded by a nucleic acid sequence comprising atggcactgccagtcaccgccctgctgctgcctctggctgctgctgcacgcagctagacca (SEQ ID NO: 45).

[08] In certain embodiments of the CARs of the disclosure, the transmembrane domain may comprise a sequence encoding a human CD2, CD3 δ , CD3 ϵ , CD3 γ , CD3 ζ , CD4, CD8 α , CD19, CD28, 4-1BB or GM-CSFR transmembrane domain. In certain embodiments of the CARs of the disclosure, the transmembrane domain may comprise a sequence encoding a human CD8 α transmembrane domain. The CD8 α transmembrane domain may comprise an amino acid sequence comprising IYIWAPLAGTCGVLLSLVITLYC (SEQ ID NO: 4) or a sequence having at least 70%, 80%, 90%, 95%, or 99% identity to the amino acid sequence comprising IYIWAPLAGTCGVLLSLVITLYC (SEQ ID NO: 4). The CD8 α transmembrane domain may be encoded by the nucleic acid sequence comprising atctacatttgggcaccactggccgggacctgtggagtgtgctgctgagcctggatcacactgtactgc (SEQ ID NO: 5).

[009] In certain embodiments of the CARs of the disclosure, the endodomain may comprise a human CD3 ζ endodomain.

[010] In certain embodiments of the CARs of the disclosure, the at least one costimulatory domain may comprise a human 4-1BB, CD28, CD40, ICOS, MyD88, OX-40 intracellular segment, or any combination thereof. In certain embodiments of the CARs of the disclosure, the at least one costimulatory domain may comprise a CD28 and/or a 4-1BB costimulatory domain. The CD28 costimulatory domain may comprise an amino acid sequence comprising RVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQE GLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPP R (SEQ ID NO: 6) or a sequence having at least 70%, 80%, 90%, 95%, or 99% identity to the amino acid sequence comprising

RVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQE GLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPP R (SEQ ID NO: 6). The CD28 costimulatory domain may be encoded by the nucleic acid sequence comprising

cgcgtgaagtttagtcgatcagcagatgccccagcttacaacaggacagaccagctgtataacgagctgaatctgggccgccga gaggaatatgacgtgctggataagcggagaggacgcgacccccgaatgggaggcaagcccaggcgcaaaaacctcaggaagg cctgtataacgagctgcagaaggacaaaatggcagaagcctattctgagatcggcatgaagggggagcgacggagaggcaaagg gcacgatgggctgtaccagggaactgagcaccgccacaaaggacacctatgatgctctgcatatgcaggcactgcctccaagg

(SEQ ID NO: 7). The 4-1BB costimulatory domain may comprise an amino acid sequence comprising KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEEGGCEL (SEQ ID NO: 8) or a sequence having at least 70%, 80%, 90%, 95%, or 99% identity to the amino acid sequence comprising KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEEGGCEL (SEQ ID NO: 8). The 4-1BB costimulatory domain may be encoded by the nucleic acid sequence comprising

aagagaggcaggaagaaactgctgtatatTTTcaacagccctcatgcgccccgtgcagactaccaggaggaagacgggtgctcc tgcgattccctgaggaagaggaaggcggtgtgagctg (SEQ ID NO: 9). The 4-1BB costimulatory domain may be located between the transmembrane domain and the CD28 costimulatory domain.

[011] In certain embodiments of the CARs of the disclosure, the hinge may comprise a sequence derived from a human CD8 α , IgG4, and/or CD4 sequence. In certain embodiments of the CARs of the disclosure, the hinge may comprise a sequence derived from a human CD8 α sequence. The hinge may comprise a human CD8 α amino acid sequence comprising TTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACD (SEQ ID NO: 10) or a

sequence having at least 70%, 80%, 90%, 95%, or 99% identity to the amino acid sequence comprising TTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACD (SEQ ID NO: 10). The human CD8 α hinge amino acid sequence may be encoded by the nucleic acid sequence comprising

actaccacaccagcacctagaccaccaactccagctccaaccatcgcgagtcagcccctgagtctgagacctgaggcctgcaggccagctgcaggaggagctgtgcacaccaggggcctggacttcgcctgcgac (SEQ ID NO: 11).

[012] Centyrins of the disclosure may comprise a protein scaffold, wherein the scaffold is capable of specifically binding an antigen. Centyrins of the disclosure may comprise a protein scaffold comprising a consensus sequence of at least one fibronectin type III (FN3) domain, wherein the scaffold is capable of specifically binding an antigen. The at least one fibronectin type III (FN3) domain may be derived from a human protein. The human protein may be Tenascin-C. The consensus sequence may comprise

LPAPKNLVVSEVTEDSLRLSWTAPDAAFDSFLIQYQSEKVGGEAINLTVPGSERSYDL
TGLKPGTEYTVSIYGVKGGHRSNPLSAEFTT (SEQ ID NO: 1) or

MLPAPKNLVVSEVTEDSLRLSWTAPDAAFDSFLIQYQSEKVGGEAINLTVPGSERSYD
LTGLKPGTEYTVSIYGVKGGHRSNPLSAEFTT (SEQ ID NO: 13). The consensus sequence may be encoded by a nucleic acid sequence comprising

atgctgcctgcacaaagaacctgggtgtctcatgtgacagaggatagtgccagactgtcatggactgctcccgacgcagccttcg
atagttttatcatcgtgtaccgggagaacatcgaaaccggcgaggccattgtcctgacagtgcagggtccgaacgctcttatgacctg
acagatctgaagcccggaactgagtactatgtgcagatcgccggcgctcaaaggaggcaatatcagcttccctctgtccgcaatcttcac
caca (SEQ ID NO: 14). The consensus sequence may be modified at one or more positions

within (a) a A-B loop comprising or consisting of the amino acid residues TEDS (SEQ ID NO: 15) at positions 13-16 of the consensus sequence; (b) a B-C loop comprising or consisting of the amino acid residues TAPDAAF (SEQ ID NO: 16) at positions 22-28 of the consensus sequence; (c) a C-D loop comprising or consisting of the amino acid residues SEKVGE (SEQ ID NO: 17) at positions 38-43 of the consensus sequence; (d) a D-E loop comprising or consisting of the amino acid residues GSER (SEQ ID NO: 18) at positions 51-54 of the consensus sequence; (e) a E-F loop comprising or consisting of the amino acid residues GLKPG (SEQ ID NO: 19) at positions 60-64 of the consensus sequence; (f) a F-G loop comprising or consisting of the amino acid residues KGGHRSN (SEQ ID NO: 20) at positions 75-81 of the consensus sequence; or (g) any combination of (a)-(f). Centyrins of the disclosure may comprise a consensus sequence of at least 5 fibronectin type III (FN3) domains, at least 10 fibronectin type III (FN3) domains or at least 15 fibronectin type III (FN3) domains. The scaffold may bind an antigen with at least one affinity selected from a

K_D of less than or equal to $10^{-9}M$, less than or equal to $10^{-10}M$, less than or equal to $10^{-11}M$, less than or equal to $10^{-12}M$, less than or equal to $10^{-13}M$, less than or equal to $10^{-14}M$, and less than or equal to $10^{-15}M$. The K_D may be determined by surface plasmon resonance.

[013] The disclosure provides an anti-BCMA CARTyrin (referred to herein as A08) have an amino acid sequence comprising:

MGVQVETISPGDGRTPKRGQTCVVHYTGMLEDGKKVDSSRDRNKPFFKFMLGKQE
 VIRGWEEGVAQMSVGQRAKLISPDIAYGATGHPGIIPPHATLVFDVELLKLEGGGG
 SGFGDVGALESRLGNADLAYILSMEPCGHCLINNPNFCRESGLRTRTGSNIDCEKLR
 RRFSSLHFMVEVKGDLTAKKMVLALLELAQQDHGALDCCVVVILSHGCQASHLQFP
 GAVYGTGDCPVSVKIVNIFNGTSCPSLGGKPKLFFIQACGGEQKDHGFEVASTSPED
 ESPGSNPEPDATPFQEGLRTFDQLDAISSLPSPDIFVSYSTFPGFVSWRDPKSGSWYV
 ETLDDIFEQWAHSEDLQSLLLRVANAVSVKGIYKQMPGCFNLRKKLFFKTSGSSEG
 RGSLLTCGDVEENPGPMALPVTALLLPLALLLHAARPMLPAPKNLVVSRITEDSAR
LSWTAPDAAFDSFPIRYIETLIWGEAIWLDVPGSERSYDLTGLKPGTEYAVVITG
VKGGRFSSPLVASFTTTTTTPAPRPPTPAPTASQPLSLRPEACRPAAGGAVHTRGLDF
 ACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCR
 FPEEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDRRGRDP
 GGMKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDT
 YDALHMQALPPRGSGEGRGSLTTCGDVEENPGPMVGS LNCIVAVSQNMGIGKNGDF
 PWPPLRNESRYFQRM TTTSSVEGKQNLVIMGKKTWFSIPEKNRPLKGRINLVLSREL
 KEPPQGAHFLSRSLDDALKLTEQPELANKVDMVWIVGGSSVYKEAMNHPGHLKLFV
 TRIMQDFESDTFFPEIDLEKYKLLPEYPGVLSDVQEEKGIKYKFEVYEKND (SEQ ID

NO: 21, the bolded sequence comprising the amino acid sequence of the A08 Centyrin). In certain embodiments, the A08 CARTyrin of the disclosure may be encoded by a nucleic acid sequence comprising:

atgggggtccaggtcgagactattcaccaggggatggggaacatttcaaaaaggggccagactgcgtcgtgcattacaccggg
 atgctggaggacgggaagaaagtggacagctccagggatcgcaacaagccctcaagttcatgctggaaagcaggaagtatcc
 gaggatgggaggaaggcgtggcacagatgtcagtcggccagcgggccaactgaccattagccctgactacgcttatggagcaac
 agggcaccagggatcattccccctcatgccaccctggcttctgatgtggaactgctgaagctggagggaggaggaggatccggatt
 tggggacgtgggggcccctggagtctctgcgaggaaatgccgatctgcttacatcctgagcatggaacctgcccactgtctgatc
 attaacaatgtgaacttctgcagagaaagcggactgcgaacacggactggctccaatattgactgtgagaagctgcggagaaggtct
 ctagtctgcactttatgtgcaagtgaaggggatctgaccgccaagaaaatggtgctggccctgctggagctggctcagcaggacc
 atggagctcttgattgctgcgtggtcgtgatcctgtcccacgggtgccaggcttctcatctgcagttccccggagcaggttacggaaca
 gacggctgtcctgtcagcgtggagaagatcgtcaacatctcaacggcacttctgcccctagtctggggggaagccaaaactgttcttt

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cctgaaccagatgcaactccattccaggaggactgaggacctttgaccagctggatgctatctcaagcctgcccactcctagtacat
tttcgtgtcttacagtaccttcccaggctttgtctcatggcgcgcatcccaagtcaggagctggtacgtggagacactggacgacatctt
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cgtggaggaaaaccaggaccaatggcactgccagtcaccgacctgctgctgcctctggctctgctgctgcacgcagctagaccaat
gctgctgcaccaaagaacctggtggtgagccggtcacagaggactccgacagctgtcttgaccgcccctgacgccccttcg
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cgccccgtgcagactaccaggaggaagacgggtgctcctgtcgtattccctgaggaagagggaaggcggtgtgagctgcgctga
agtttagtcgatcagcagatgccccagcttacaacaggggacagaaccagctgtataacgagctgaatctgggcccggagaggaa
atgacgtgctggataagcggagaggacgcgaccccgaaatgggaggcaagcccaggcgcaaaaacctcaggaaggcctgtata
acgagctgcagaaggacaaaatggcagaagcctattctgagatcggcagtaagggggagcgacggagaggcaaggggcacgatg
ggctgtaccagggactgagcaccgcccacaaaggacacatgatgctctgcataatgcaggcactgcctccaaggggaagtggagaa
ggacgaggatcactgctgacatgcggcgacgtggaggaaaacctggcccaatggtcggtctctgaattgtatcgtcgcctgagt
cagaacatgggcattgggaagaatggcgatttccatggccacctctgcgcaacgagtcgagatacttccagcggtgacaactacct
cctctgtggaagggaaacagaatctggtcatcatgggaaagaaaacttgggtcagcattccagagaagaacggcccctgaaaggca
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ccggccatctgaaactgttcgtcacacggatcatgcaggacttgagagcgatactttcttctgaaattgacctggagaagtacaaact
gctgcccgaatatctggcgtgctgtccgatgtccaggaagaaaggcatcaatacaagttcgaggtctatgagaagaatgac
(SEQ ID NO: 22, this sequence is also referred to herein as the open reading frame (ORF) of
P-BMCA-101).

[014] The disclosure provides a composition comprising the CAR of the disclosure and at least one pharmaceutically acceptable carrier.

[015] The disclosure provides a transposon comprising the CAR of the disclosure.

[016] Transposons of the disclosure may comprise a selection gene for identification, enrichment and/or isolation of cells that express the transposon. Exemplary selection genes encode any gene product (e.g. transcript, protein, enzyme) essential for cell viability and survival. Exemplary selection genes encode any gene product (e.g. transcript, protein, enzyme) essential for conferring resistance to a drug challenge against which the cell is

sensitive (or which could be lethal to the cell) in the absence of the gene product encoded by the selection gene. Exemplary selection genes encode any gene product (e.g. transcript, protein, enzyme) essential for viability and/or survival in a cell media lacking one or more nutrients essential for cell viability and/or survival in the absence of the selection gene. Exemplary selection genes include, but are not limited to, *neo* (conferring resistance to neomycin), DHFR (encoding Dihydrofolate Reductase and conferring resistance to Methotrexate), TYMS (encoding Thymidylate Synthetase), MGMT (encoding O(6)-methylguanine-DNA methyltransferase), multidrug resistance gene (MDR1), ALDH1 (encoding Aldehyde dehydrogenase 1 family, member A1), FRNCF, RAD51C (encoding RAD51 Paralog C), GCS (encoding glucosylceramide synthase), and NKX2.2 (encoding NK2 Homeobox 2).

[017] Transposons of the disclosure may comprise an inducible proapoptotic polypeptide comprising (a) a ligand binding region, (b) a linker, and (c) a proapoptotic polypeptide, wherein the inducible proapoptotic polypeptide does not comprise a non-human sequence. In certain embodiments, the non-human sequence comprises a restriction site. In certain embodiments, the ligand binding region may be a multimeric ligand binding region. Inducible proapoptotic polypeptides of the disclosure may also be referred to as an “iC9 safety switch”. In certain embodiments, transposons of the disclosure may comprise an inducible caspase polypeptide comprising (a) a ligand binding region, (b) a linker, and (c) a caspase polypeptide, wherein the inducible proapoptotic polypeptide does not comprise a non-human sequence. In certain embodiments, transposons of the disclosure may comprise an inducible caspase polypeptide comprising (a) a ligand binding region, (b) a linker, and (c) a caspase polypeptide, wherein the inducible proapoptotic polypeptide does not comprise a non-human sequence. In certain embodiments, transposons of the disclosure may comprise an inducible caspase polypeptide comprising (a) a ligand binding region, (b) a linker, and (c) a truncated caspase 9 polypeptide, wherein the inducible proapoptotic polypeptide does not comprise a non-human sequence. In certain embodiments of the inducible proapoptotic polypeptides, inducible caspase polypeptides or truncated caspase 9 polypeptides of the disclosure, the ligand binding region may comprise a FK506 binding protein 12 (FKBP12) polypeptide. In certain embodiments, the amino acid sequence of the ligand binding region that comprise a FK506 binding protein 12 (FKBP12) polypeptide may comprise a modification at position 36 of the sequence. The modification may be a substitution of valine (V) for phenylalanine (F) at position 36 (F36V). In certain embodiments, the FKBP12 polypeptide is encoded by an amino acid sequence comprising

GVQVETISPGDGRTFPKRGQTCVVHYTGMLLEDGKKVDSSRDRNKPFFKMLGKQEVI
RGWEEGVAQMSVGQRAKLTISPDYAYGATGHPGIIPPHATLVFDVELLKLE (SEQ ID
NO: 23). In certain embodiments, the FKBP12 polypeptide is encoded by a nucleic acid
sequence comprising

GGGGTCCAGGTCGAGACTATTTACCAGGGGATGGGCGAACATTTCCAAAAAGG
GGCCAGACTTGCGTCGTGCATTACACCGGGATGCTGGAGGACGGGAAGAAAGTG
GACAGCTCCAGGGATCGCAACAAGCCCTTCAAGTTCATGCTGGGAAAGCAGGAA
GTGATCCGAGGATGGGAGGAAGGCGTGGCACAGATGTCAGTCGGCCAGCGGGCC
AAACTGACCATTAGCCCTGACTACGCTTATGGAGCAACAGGCCACCCAGGGATC
ATTCCCCCTCATGCCACCCTGGTCTTCGAT GTGGAAGCTGCTGAAGCTGGAG (SEQ
ID NO: 24). In certain embodiments, the induction agent specific for the ligand binding
region may comprise a FK506 binding protein 12 (FKBP12) polypeptide having a
substitution of valine (V) for phenylalanine (F) at position 36 (F36V) comprises AP20187
and/or AP1903, both synthetic drugs.

[018] In certain embodiments of the inducible proapoptotic polypeptides, inducible
caspase polypeptides or truncated caspase 9 polypeptides of the disclosure, the linker region
is encoded by an amino acid comprising GGGGS (SEQ ID NO: 25) or a nucleic acid
sequence comprising GGAGGAGGAGGATCC (SEQ ID NO: 26). In certain embodiments,
the nucleic acid sequence encoding the linker does not comprise a restriction site.

[019] In certain embodiments of the truncated caspase 9 polypeptides of the disclosure, the
truncated caspase 9 polypeptide is encoded by an amino acid sequence that does not comprise
an arginine (R) at position 87 of the sequence. Alternatively, or in addition, in certain
embodiments of the inducible proapoptotic polypeptides, inducible caspase polypeptides or
truncated caspase 9 polypeptides of the disclosure, the truncated caspase 9 polypeptide is
encoded by an amino acid sequence that does not comprise an alanine (A) at position 282 the
sequence. In certain embodiments of the inducible proapoptotic polypeptides, inducible
caspase polypeptides or truncated caspase 9 polypeptides of the disclosure, the truncated
caspase 9 polypeptide is encoded by an amino acid comprising

GFGDVGALESLRGNADLAYISLMEPCGHCLIINNPNFCRESGLRTRTGSNIDCEKLRR
RFSSLHFMVEVKGDLTAKKMVLALLELAQQDHGALDCCVVVILSHGCQASHLQFPG
AVYGTGDCPVSVEKIVNIFNGTSCPSLGGKPKLFFIQACGGEQKDHGFEVASTSPEDE
SPGSNPEPDATPFQEGLRTFDQLDAISSLPTPSDIFVSYSTFGFVSWRDPKSGSWYVE
TLDDIFEQWAHSEDLQSLLLRVANAVSVKGIYKQMPGCNFLRKKLFFKTS (SEQ ID
NO: 27) or a nucleic acid sequence comprising

TTTGGGGACGTGGGGGGCCCTGGAGTCTCTGCGAGGAAATGCCGATCTGGCTTACA
 TCCTGAGCATGGAACCCTGCGGCCACTGTCTGATCATTAAACAATGTGAACTTCTG
 CAGAGAAAGCGGACTGCGAACACGGACTGGCTCCAATATTGACTGTGAGAAGCT
 GCGGAGAAGGTTCTCTAGTCTGCACTTTATGGTTCGAAGTGAAAGGGGATCTGACC
 GCCAAGAAAATGGTGCTGGCCCTGCTGGAGCTGGCTCAGCAGGACCATGGAGCT
 CTGGATTGCTGCGTGCTGCTGATCCTGTCCCACGGGTGCCAGGCTTCTCATCTGC
 AGTTCCCCGGAGCAGTGTACGGAACAGACGGCTGTCTGTGAGCGTGGAGAAGA
 TCGTCAACATCTTCAACGGCACTTCTTGCCCTAGTCTGGGGGGAAAGCCAAAAC
 GTTCTTTATCCAGGCCTGTGGCGGGGAACAGAAAGATCACGGCTTCGAGGTGGC
 CAGCACCAGCCCTGAGGACGAATCACCAGGGAGCAACCCTGAACCAGATGCAAC
 TCCATTCCAGGAGGGACTGAGGACCTTTGACCAGCTGGATGCTATCTCAAGCCTG
 CCCACTCCTAGTGACATTTTCGTGTCTTACAGTACCTTCCCAGGCTTTGTCTCATG
 GCGCGATCCCAAGTCAGGGAGCTGGTACGTGGAGACACTGGACGACATCTTTGA
 ACAGTGGGCCCATTGAGAGGACCTGCAGAGCCTGCTGCTGCGAGTGGCAAACGC
 TGTCTCTGTGAAGGGCATCTACAAACAGATGCCCGGGTGCTTCAATTTTCTGAGA
 AAGAACTGTTCTTTAAGACTTCC (SEQ ID NO: 28).

[020] In certain embodiments of the inducible proapoptotic polypeptides, wherein the polypeptide comprises a truncated caspase 9 polypeptide, the inducible proapoptotic polypeptide is encoded by an amino acid sequence comprising

GVQVETISPGDGRTPKRGQTCVVHYTGMLLEDGKKVDSSRDRNKPFFKMLGKQEVI
 RGWEEGVAQMSVGQRAKLISPDIAYGATGHPGIIPPHATLVFDVELLKLEGGGGGS
 GFVDVGALESLRGNADLAYISLMEPCGHCLIINNPNFCRESGLRTRTGSNIDCEKLRR
 RFSSLHFMVEVKGDLTAKKMVLALLELAQQDHGALDCCVVVILSHGCQASHLQFPG
 AVYGTGDCPVSVKIVNIFNGTSCPSLGGKPKLFFIQACGGEQKDHGFEVASTSPEDE
 SPGSNPEPDATPFQEGLRTFDQLDAISSLPTPSDIFVSYSTFPGFVSWRDPKSGSWYVE
 TLDDIFEQWAHSEDLQSLLLRVANAVSVKGIYKQMPGCNFLRKKLFFKTS (SEQ ID
 NO: 29) or the nucleic acid sequence comprising

GGGGTCCAGGTCGAGACTATTTACCAGGGGATGGGCGAACATTTCCAAAAAGG
 GGCCAGACTTGCGTCGTGCATTACACCGGGATGCTGGAGGACGGGAAGAAAGTG
 GACAGCTCCAGGGATCGCAACAAGCCCTTCAAGTTCATGCTGGGAAAGCAGGAA
 GTGATCCGAGGATGGGAGGAAGGCGTGGCACAGATGTCAGTCGGCCAGCGGGCC
 AAAGTACCATTAGCCCTGACTACGCTTATGGAGCAACAGGCCACCCAGGGATC
 ATTCCCCCTCATGCCACCCTGGTCTTCGATGTGGAAGCTGCTGAAGCTGGAGGGAG
 GAGGAGGATCCGAATTTGGGGACGTGGGGGGCCCTGGAGTCTCTGCGAGGAAATG

CCGATCTGGCTTACATCCTGAGCATGGAACCCTGCGGCCACTGTCTGATCATTAA
 CAATGTGAACTTCTGCAGAGAAAGCGGACTGCGAACACGGACTGGCTCCAATAT
 TGA CTGTGAGAAGCTGCGGAGAAGGTTCTCTAGTCTGCACTTTATGGTCAAGTG
 AAAGGGGATCTGACCGCCAAGAAAATGGTGCTGGCCCTGCTGGAGCTGGCTCAG
 CAGGACCATGGAGCTCTGGATTGCTGCGTGGTCGTGATCCTGTCCCACGGGTGCC
 AGGCTTCTCATCTGCAGTTCCCCGGAGCAGTGTACGGAACAGACGGCTGTCTGT
 CAGCGTGGAGAAGATCGTCAACATCTTCAACGGCACTTCTTGCCCTAGTCTGGGG
 GGAAAGCCAAAAGTGTCTTTATCCAGGCCTGTGGCGGGGAACAGAAAGATCAC
 GGCTTCGAGGTGGCCAGCACCAGCCCTGAGGACGAATCACCAGGGAGCAACCCT
 GAACCAGATGCAACTCCATTCCAGGAGGGACTGAGGACCTTTGACCAGCTGGAT
 GCTATCTCAAGCCTGCCCACCTAGTGACATTTTCGTGTCTTACAGTACCTTCCC
 AGGCTTTGTCTCATGGCGCGATCCCAAGTCAGGGAGCTGGTACGTGGAGACACT
 GGACGACATCTTTGAACAGTGGGCCCATTGAGAGGACCTGCAGAGCCTGCTGCT
 GCGAGTGGCAAACGCTGTCTCTGTGAAGGGCATCTACAAACAGATGCCCGGGTG
 CTTCAATTTTCTGAGAAAGAAACTGTTCTTTAAGACTTCC (SEQ ID NO: 30).

[021] Transposons of the disclosure may comprise at least one self-cleaving peptide(s) located, for example, between one or more of a protein scaffold, Centyrin or CARTyrin of the disclosure and a selection gene of the disclosure. Transposons of the disclosure may comprise at least one self-cleaving peptide(s) located, for example, between one or more of a protein scaffold, Centyrin or CARTyrin of the disclosure and an inducible proapoptotic polypeptide of the disclosure. Transposons of the disclosure may comprise at least two self-cleaving peptide(s), a first self-cleaving peptide located, for example, upstream or immediately upstream of an inducible proapoptotic polypeptide of the disclosure and a second first self-cleaving peptide located, for example, downstream or immediately upstream of an inducible proapoptotic polypeptide of the disclosure.

[022] The at least one self-cleaving peptide may comprise, for example, a T2A peptide, GSG-T2A peptide, an E2A peptide, a GSG-E2A peptide, an F2A peptide, a GSG-F2A peptide, a P2A peptide, or a GSG-P2A peptide. A T2A peptide may comprise an amino acid sequence comprising EGRGSLLTCGDVEENPGP (SEQ ID NO: 31) or a sequence having at least 70%, 80%, 90%, 95%, or 99% identity to the amino acid sequence comprising EGRGSLLTCGDVEENPGP (SEQ ID NO: 31). A GSG-T2A peptide may comprise an amino acid sequence comprising GSGEGRGSLLTCGDVEENPGP (SEQ ID NO: 32) or a sequence having at least 70%, 80%, 90%, 95%, or 99% identity to the amino acid sequence comprising GSGEGRGSLLTCGDVEENPGP (SEQ ID NO: 32). A GSG-T2A peptide may comprise a

nucleic acid sequence comprising

ggatctggagagggaaggggaagcctgctgacctgtggagacgtggaggaaaaccaggacca (SEQ ID NO: 33). An

E2A peptide may comprise an amino acid sequence comprising

QCTNYALLKLAGDVESNPGP (SEQ ID NO: 34) or a sequence having at least 70%, 80%, 90%, 95%, or 99% identity to the amino acid sequence comprising

QCTNYALLKLAGDVESNPGP (SEQ ID NO: 34). A GSG-E2A peptide may comprise an amino acid sequence comprising GSGQCTNYALLKLAGDVESNPGP (SEQ ID NO: 35) or a sequence having at least 70%, 80%, 90%, 95%, or 99% identity to the amino acid sequence comprising GSGQCTNYALLKLAGDVESNPGP (SEQ ID NO: 35). An F2A peptide may

comprise an amino acid sequence comprising VKQTLNFDLLKLAGDVESNPGP (SEQ ID NO: 36) or a sequence having at least 70%, 80%, 90%, 95%, or 99% identity to the amino acid sequence comprising VKQTLNFDLLKLAGDVESNPGP (SEQ ID NO: 36). A GSG-

F2A peptide may comprise an amino acid sequence comprising

GSGVKQTLNFDLLKLAGDVESNPGP (SEQ ID NO: 37) or a sequence having at least 70%, 80%, 90%, 95%, or 99% identity to the amino acid sequence comprising

GSGVKQTLNFDLLKLAGDVESNPGP (SEQ ID NO: 37). A P2A peptide may comprise an amino acid sequence comprising ATNFSLLKQAGDVEENPGP (SEQ ID NO: 38) or a sequence having at least 70%, 80%, 90%, 95%, or 99% identity to the amino acid sequence comprising ATNFSLLKQAGDVEENPGP (SEQ ID NO: 38). A GSG-P2A peptide may comprise an amino acid sequence comprising GSGATNFSLLKQAGDVEENPGP (SEQ ID NO: 39) or a sequence having at least 70%, 80%, 90%, 95%, or 99% identity to the amino acid sequence comprising GSGATNFSLLKQAGDVEENPGP (SEQ ID NO: 39).

[023] Transposons of the disclosure may comprise a first and a second self-cleaving peptide, the first self-cleaving peptide located, for example, upstream of one or more of a protein scaffold, Centyrin or CARTyrin of the disclosure the second self-cleaving peptide located, for example, downstream of the one or more of a protein scaffold, Centyrin or CARTyrin of the disclosure. The first and/or the second self-cleaving peptide may comprise, for example, a T2A peptide, GSG-T2A peptide, an E2A peptide, a GSG-E2A peptide, an F2A peptide, a GSG-F2A peptide, a P2A peptide, or a GSG-P2A peptide. A T2A peptide may comprise an amino acid sequence comprising EGRGSLTCDGVEENPGP (SEQ ID NO: 31) or a sequence having at least 70%, 80%, 90%, 95%, or 99% identity to the amino acid sequence comprising EGRGSLTCDGVEENPGP (SEQ ID NO: 31). A GSG-T2A peptide may comprise an amino acid sequence comprising GSGEGRGSLTCDGVEENPGP (SEQ ID NO: 32) or a sequence having at least 70%, 80%, 90%, 95%, or 99% identity to the

amino acid sequence comprising GSGEGRGSLTCDVEENPGP (SEQ ID NO: 32). A GSG-T2A peptide may comprise a nucleic acid sequence comprising ggatctggagagggaaggggaagcctgctgacctgtggagacgtggagaaaaccaggacca (SEQ ID NO: 33). An E2A peptide may comprise an amino acid sequence comprising QCTNYALLKLAGDVESNPGP (SEQ ID NO: 34) or a sequence having at least 70%, 80%, 90%, 95%, or 99% identity to the amino acid sequence comprising QCTNYALLKLAGDVESNPGP (SEQ ID NO: 34). A GSG-E2A peptide may comprise an amino acid sequence comprising GSGQCTNYALLKLAGDVESNPGP (SEQ ID NO: 35) or a sequence having at least 70%, 80%, 90%, 95%, or 99% identity to the amino acid sequence comprising GSGQCTNYALLKLAGDVESNPGP (SEQ ID NO: 35). An F2A peptide may comprise an amino acid sequence comprising VKQTLNFDLLKLAGDVESNPGP (SEQ ID NO: 36) or a sequence having at least 70%, 80%, 90%, 95%, or 99% identity to the amino acid sequence comprising VKQTLNFDLLKLAGDVESNPGP (SEQ ID NO: 36). A GSG-F2A peptide may comprise an amino acid sequence comprising GSGVKQTLNFDLLKLAGDVESNPGP (SEQ ID NO: 37) or a sequence having at least 70%, 80%, 90%, 95%, or 99% identity to the amino acid sequence comprising GSGVKQTLNFDLLKLAGDVESNPGP (SEQ ID NO: 37). A P2A peptide may comprise an amino acid sequence comprising ATNFSLLKQAGDVEENPGP (SEQ ID NO: 38) or a sequence having at least 70%, 80%, 90%, 95%, or 99% identity to the amino acid sequence comprising ATNFSLLKQAGDVEENPGP (SEQ ID NO: 38). A GSG-P2A peptide may comprise an amino acid sequence comprising GSGATNFSLLKQAGDVEENPGP (SEQ ID NO: 39) or a sequence having at least 70%, 80%, 90%, 95%, or 99% identity to the amino acid sequence comprising GSGATNFSLLKQAGDVEENPGP (SEQ ID NO: 39).

[024] The disclosure provides a composition comprising the transposon the disclosure. In certain embodiments, the composition may further comprise a plasmid comprising a sequence encoding a transposase enzyme. The sequence encoding a transposase enzyme may be an mRNA sequence.

[025] Transposons of the disclosure may comprise piggyBac transposons. In certain embodiments of this method, the transposon is a plasmid DNA transposon with a sequence encoding the chimeric antigen receptor flanked by two cis-regulatory insulator elements. In certain embodiments, the transposon is a piggyBac transposon. Transposase enzymes of the disclosure may include piggyBac transposases or compatible enzymes. In certain embodiments, and, in particular, those embodiments wherein the transposon is a piggyBac transposon, the transposase is a piggyBacTM or a Super piggyBacTM (SPB) transposase. In

certain embodiments, and, in particular, those embodiments wherein the transposase is a Super piggyBac™ (SPB) transposase, the sequence encoding the transposase is an mRNA sequence.

[026] In certain embodiments of the methods of the disclosure, the transposase enzyme is a piggyBac™ (PB) transposase enzyme. The piggyBac (PB) transposase enzyme may comprise or consist of an amino acid sequence at least 75%, 80%, 85%, 90%, 95%, 99% or any percentage in between identical to:

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1  MGSSLDDEHI  LSALLQSDDE  LVGEDSDSEI  SDHVSDDVQ  SDTEEAFIDE  VHEVQPTSSG
61  SEILDEQNVI  EQPGSSLASN  RILTLPQRTI  RGKNKHCWST  SKSTRRSRVS  ALNIVRSQRG
121 PTRMCRNIYD  PLLCFKLFFT  DEIISEIVKW  TNAEISLKRR  ESMTGATFRD  TNEDEIYAFF
181 GILVMTAVRK  DNHMSTDDL  DRSLSMVYVS  VMSRDRFDFL  IRCLRMDDKS  IRPTLRENDV
241 FTPVRKIIDL  FIHQCIQNYT  PGAHLTIDEQ  LLGFRGRCPF  RMYIPNKPSK  YGIKILMMCD
301 SGYKYMINGM  PYLGRGTQTN  GVPLGEYYVK  ELSKPVHGSC  RNITCDNWFT  SIPLAKNLLQ
361 EPYKLTIVGT  VRSNKREIPE  VLKNSRSRPV  GTSMFCDGDP  LTLVSYKPKP  AKMVYLLSSC
421 DEDASINEST  GKPQMVMYYN  QTKGGVDTLD  QMCSVMTCSR  KTNRWPMALL  YGMINIACIN
481 SFIIYSHNVS  SKGEKVQSRK  KFMRNLYMSL  TSSFMRKRLE  APTLKRYLRD  NISNILPNEV
541 PGTSDDSTEE  PVMKKRTYCT  YCPSKIRKKA  NASCKKCKKV  ICREHNIDMC  QSCF (SEQ ID NO:
12) .

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[027] In certain embodiments of the methods of the disclosure, the transposase enzyme is a piggyBac™ (PB) transposase enzyme that comprises or consists of an amino acid sequence having an amino acid substitution at one or more of positions 30, 165, 282, or 538 of the sequence:

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1  MGSSLDDEHI  LSALLQSDDE  LVGEDSDSEI  SDHVSDDVQ  SDTEEAFIDE  VHEVQPTSSG
61  SEILDEQNVI  EQPGSSLASN  RILTLPQRTI  RGKNKHCWST  SKSTRRSRVS  ALNIVRSQRG
121 PTRMCRNIYD  PLLCFKLFFT  DEIISEIVKW  TNAEISLKRR  ESMTGATFRD  TNEDEIYAFF
181 GILVMTAVRK  DNHMSTDDL  DRSLSMVYVS  VMSRDRFDFL  IRCLRMDDKS  IRPTLRENDV
241 FTPVRKIIDL  FIHQCIQNYT  PGAHLTIDEQ  LLGFRGRCPF  RMYIPNKPSK  YGIKILMMCD
301 SGYKYMINGM  PYLGRGTQTN  GVPLGEYYVK  ELSKPVHGSC  RNITCDNWFT  SIPLAKNLLQ
361 EPYKLTIVGT  VRSNKREIPE  VLKNSRSRPV  GTSMFCDGDP  LTLVSYKPKP  AKMVYLLSSC
421 DEDASINEST  GKPQMVMYYN  QTKGGVDTLD  QMCSVMTCSR  KTNRWPMALL  YGMINIACIN
481 SFIIYSHNVS  SKGEKVQSRK  KFMRNLYMSL  TSSFMRKRLE  APTLKRYLRD  NISNILPNEV
541 PGTSDDSTEE  PVMKKRTYCT  YCPSKIRKKA  NASCKKCKKV  ICREHNIDMC  QSCF (SEQ ID NO:
12) .

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[028] In certain embodiments, the transposase enzyme is a piggyBac™ (PB) transposase enzyme that comprises or consists of an amino acid sequence having an amino acid substitution at two or more of positions 30, 165, 282, or 538 of the sequence of SEQ ID NO: 12. In certain embodiments, the transposase enzyme is a piggyBac™ (PB) transposase

enzyme that comprises or consists of an amino acid sequence having an amino acid substitution at three or more of positions 30, 165, 282, or 538 of the sequence of SEQ ID NO:

12. In certain embodiments, the transposase enzyme is a piggyBac™ (PB) transposase enzyme that comprises or consists of an amino acid sequence having an amino acid substitution at each of the following positions 30, 165, 282, and 538 of the sequence of SEQ ID NO: 12. In certain embodiments, the amino acid substitution at position 30 of the sequence of SEQ ID NO: 12 is a substitution of a valine (V) for an isoleucine (I). In certain embodiments, the amino acid substitution at position 165 of the sequence of SEQ ID NO: 12 is a substitution of a serine (S) for a glycine (G). In certain embodiments, the amino acid substitution at position 282 of the sequence of SEQ ID NO: 12 is a substitution of a valine (V) for a methionine (M). In certain embodiments, the amino acid substitution at position 538 of the sequence of SEQ ID NO: 12 is a substitution of a lysine (K) for an asparagine (N).

[029] In certain embodiments of the methods of the disclosure, the transposase enzyme is a Super piggyBac™ (sPBo) transposase enzyme. In certain embodiments, the Super piggyBac™ (sPBo) transposase enzymes of the disclosure may comprise or consist of the amino acid sequence of the sequence of SEQ ID NO: 12 wherein the amino acid substitution at position 30 is a substitution of a valine (V) for an isoleucine (I), the amino acid substitution at position 165 is a substitution of a serine (S) for a glycine (G), the amino acid substitution at position 282 is a substitution of a valine (V) for a methionine (M), and the amino acid substitution at position 538 is a substitution of a lysine (K) for an asparagine (N). In certain embodiments, the Super piggyBac™ (sPBo) transposase enzyme may comprise or consist of an amino acid sequence at least 75%, 80%, 85%, 90%, 95%, 99% or any percentage in between identical to:

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1  MGSSLDDEHI  LSALLQSDDE  LVGEDSDSEV  SDHVSEDDVQ  SDTEEFIDE  VHEVQPTSSG
61  SEILDEQNVI  EQPGSSLASN  RILTLPQRTI  RGKNKHCWST  SKSTRRSRVS  ALNIVRSQRG
121 PTRMCRNIYD  PLLCFKLFFT  DEIISEIVKW  TNAEISLKRR  ESMTSATFRD  TNEDEIYAFF
181 GILVMTAVRK  DNHMSTDDL  DRSLSMVYVS  VMSRDRFDL  IRCLRMDDKS  IRPTLRENDV
241 FTPVRKIWDL  FIHQCIQNYT  PGAHLTIDEQ  LLGFRGRCPF  RVYIPNKPSK  YGIKILMMCD
301 SGTKYMINGM  PYLGRGTQTN  GVPLGEYYVK  ELSKPVHGSC  RNITCDNWFT  SIPLAKNLLQ
361 EPYKLTIVGT  VRSNKREIPE  VLKNSRSRPV  GTSMFCFDGP  LTLVSYKPKP  AKMVYLLSSC
421 DEDASINEST  GKPQMVMYYN  QTKGGVDTLD  QMCVMTCSR  KTNRWPMALL  YGMINIACIN
481 SFIIYSHNVS  SKGEKVQSRK  KFMRNLYMSL  TSSFMRKRLE  APTLKRYLRD  NISNILPKEV
541 PGTSDDSTEE  PVMKKRTYCT  YCPSKIRRKA  NASCKKCKKV  ICREHNIDMC  QSCF (SEQ ID NO:
2) .

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[030] In certain embodiments of the methods of the disclosure, including those embodiments wherein the transposase comprises the above-described mutations at positions

30, 165, 282 and/or 538, the piggyBac™ or Super piggyBac™ transposase enzyme may further comprise an amino acid substitution at one or more of positions 3, 46, 82, 103, 119, 125, 177, 180, 185, 187, 200, 207, 209, 226, 235, 240, 241, 243, 258, 296, 298, 311, 315, 319, 327, 328, 340, 421, 436, 456, 470, 486, 503, 552, 570 and 591 of the sequence of SEQ ID NO: 12 or SEQ ID NO: 2. In certain embodiments, including those embodiments wherein the transposase comprises the above-described mutations at positions 30, 165, 282 and/or 538, the piggyBac™ or Super piggyBac™ transposase enzyme may further comprise an amino acid substitution at one or more of positions 46, 119, 125, 177, 180, 185, 187, 200, 207, 209, 226, 235, 240, 241, 243, 296, 298, 311, 315, 319, 327, 328, 340, 421, 436, 456, 470, 485, 503, 552 and 570. In certain embodiments, the amino acid substitution at position 3 of SEQ ID NO: 12 or SEQ ID NO: 2 is a substitution of an asparagine (N) for a serine (S). In certain embodiments, the amino acid substitution at position 46 of SEQ ID NO: 12 or SEQ ID NO: 2 is a substitution of a serine (S) for an alanine (A). In certain embodiments, the amino acid substitution at position 46 of SEQ ID NO: 12 or SEQ ID NO: 2 is a substitution of a threonine (T) for an alanine (A). In certain embodiments, the amino acid substitution at position 82 of SEQ ID NO: 12 or SEQ ID NO: 2 is a substitution of a tryptophan (W) for an isoleucine (I). In certain embodiments, the amino acid substitution at position 103 of SEQ ID NO: 12 or SEQ ID NO: 2 is a substitution of a proline (P) for a serine (S). In certain embodiments, the amino acid substitution at position 119 of SEQ ID NO: 12 or SEQ ID NO: 2 is a substitution of a proline (P) for an arginine (R). In certain embodiments, the amino acid substitution at position 125 of SEQ ID NO: 12 or SEQ ID NO: 2 is a substitution of an alanine (A) a cysteine (C). In certain embodiments, the amino acid substitution at position 125 of SEQ ID NO: 12 or SEQ ID NO: 2 is a substitution of a leucine (L) for a cysteine (C). In certain embodiments, the amino acid substitution at position 177 of SEQ ID NO: 12 or SEQ ID NO: 2 is a substitution of a lysine (K) for a tyrosine (Y). In certain embodiments, the amino acid substitution at position 177 of SEQ ID NO: 12 or SEQ ID NO: 2 is a substitution of a histidine (H) for a tyrosine (Y). In certain embodiments, the amino acid substitution at position 180 of SEQ ID NO: 12 or SEQ ID NO: 2 is a substitution of a leucine (L) for a phenylalanine (F). In certain embodiments, the amino acid substitution at position 180 of SEQ ID NO: 12 or SEQ ID NO: 2 is a substitution of an isoleucine (I) for a phenylalanine (F). In certain embodiments, the amino acid substitution at position 180 of SEQ ID NO: 12 or SEQ ID NO: 2 is a substitution of a valine (V) for a phenylalanine (F). In certain embodiments, the amino acid substitution at position 185 of SEQ ID NO: 12 or SEQ ID NO: 2 is a substitution of a leucine (L) for a methionine (M). In certain embodiments, the amino

acid substitution at position 187 of SEQ ID NO: 12 or SEQ ID NO: 2 is a substitution of a glycine (G) for an alanine (A). In certain embodiments, the amino acid substitution at position 200 of SEQ ID NO: 12 or SEQ ID NO: 2 is a substitution of a tryptophan (W) for a phenylalanine (F). In certain embodiments, the amino acid substitution at position 207 of SEQ ID NO: 12 or SEQ ID NO: 2 is a substitution of a proline (P) for a valine (V). In certain embodiments, the amino acid substitution at position 209 of SEQ ID NO: 12 or SEQ ID NO: 2 is a substitution of a phenylalanine (F) for a valine (V). In certain embodiments, the amino acid substitution at position 226 of SEQ ID NO: 12 or SEQ ID NO: 2 is a substitution of a phenylalanine (F) for a methionine (M). In certain embodiments, the amino acid substitution at position 235 of SEQ ID NO: 12 or SEQ ID NO: 2 is a substitution of an arginine (R) for a leucine (L). In certain embodiments, the amino acid substitution at position 240 of SEQ ID NO: 12 or SEQ ID NO: 12 is a substitution of a lysine (K) for a valine (V). In certain embodiments, the amino acid substitution at position 241 of SEQ ID NO: 12 or SEQ ID NO: 2 is a substitution of a leucine (L) for a phenylalanine (F). In certain embodiments, the amino acid substitution at position 243 of SEQ ID NO: 12 or SEQ ID NO: 2 is a substitution of a lysine (K) for a proline (P). In certain embodiments, the amino acid substitution at position 258 of SEQ ID NO: 12 or SEQ ID NO: 2 is a substitution of a serine (S) for an asparagine (N). In certain embodiments, the amino acid substitution at position 296 of SEQ ID NO: 12 or SEQ ID NO: 2 is a substitution of a tryptophan (W) for a leucine (L). In certain embodiments, the amino acid substitution at position 296 of SEQ ID NO: 12 or SEQ ID NO: 2 is a substitution of a tyrosine (Y) for a leucine (L). In certain embodiments, the amino acid substitution at position 296 of SEQ ID NO: 12 or SEQ ID NO: 2 is a substitution of a phenylalanine (F) for a leucine (L). In certain embodiments, the amino acid substitution at position 298 of SEQ ID NO: 12 or SEQ ID NO: 2 is a substitution of a leucine (L) for a methionine (M). In certain embodiments, the amino acid substitution at position 298 of SEQ ID NO: 12 or SEQ ID NO: 2 is a substitution of an alanine (A) for a methionine (M). In certain embodiments, the amino acid substitution at position 298 of SEQ ID NO: 12 or SEQ ID NO: 2 is a substitution of a valine (V) for a methionine (M). In certain embodiments, the amino acid substitution at position 311 of SEQ ID NO: 12 or SEQ ID NO: 2 is a substitution of an isoleucine (I) for a proline (P). In certain embodiments, the amino acid substitution at position 311 of SEQ ID NO: 12 or SEQ ID NO: 2 is a substitution of a valine for a proline (P). In certain embodiments, the amino acid substitution at position 315 of SEQ ID NO: 12 or SEQ ID NO: 2 is a substitution of a lysine (K) for an arginine (R). In certain embodiments, the amino acid substitution at position 319 of SEQ ID NO: 12 or SEQ ID NO: 2 is a

substitution of a glycine (G) for a threonine (T). In certain embodiments, the amino acid substitution at position 327 of SEQ ID NO: 12 or SEQ ID NO: 2 is a substitution of an arginine (R) for a tyrosine (Y). In certain embodiments, the amino acid substitution at position 328 of SEQ ID NO: 12 or SEQ ID NO: 2 is a substitution of a valine (V) for a tyrosine (Y). In certain embodiments, the amino acid substitution at position 340 of SEQ ID NO: 12 or SEQ ID NO: 2 is a substitution of a glycine (G) for a cysteine (C). In certain embodiments, the amino acid substitution at position 340 of SEQ ID NO: 12 or SEQ ID NO: 2 is a substitution of a leucine (L) for a cysteine (C). In certain embodiments, the amino acid substitution at position 421 of SEQ ID NO: 12 or SEQ ID NO: 2 is a substitution of a histidine (H) for the aspartic acid (D). In certain embodiments, the amino acid substitution at position 436 of SEQ ID NO: 12 or SEQ ID NO: 2 is a substitution of an isoleucine (I) for a valine (V). In certain embodiments, the amino acid substitution at position 456 of SEQ ID NO: 12 or SEQ ID NO: 2 is a substitution of a tyrosine (Y) for a methionine (M). In certain embodiments, the amino acid substitution at position 470 of SEQ ID NO: 12 or SEQ ID NO: 2 is a substitution of a phenylalanine (F) for a leucine (L). In certain embodiments, the amino acid substitution at position 485 of SEQ ID NO: 12 or SEQ ID NO: 2 is a substitution of a lysine (K) for a serine (S). In certain embodiments, the amino acid substitution at position 503 of SEQ ID NO: 12 or SEQ ID NO: 2 is a substitution of a leucine (L) for a methionine (M). In certain embodiments, the amino acid substitution at position 503 of SEQ ID NO: 12 or SEQ ID NO: 2 is a substitution of an isoleucine (I) for a methionine (M). In certain embodiments, the amino acid substitution at position 552 of SEQ ID NO: 12 or SEQ ID NO: 2 is a substitution of a lysine (K) for a valine (V). In certain embodiments, the amino acid substitution at position 570 of SEQ ID NO: 12 or SEQ ID NO: 2 is a substitution of a threonine (T) for an alanine (A). In certain embodiments, the amino acid substitution at position 591 of SEQ ID NO: 12 or SEQ ID NO: 2 is a substitution of a proline (P) for a glutamine (Q). In certain embodiments, the amino acid substitution at position 591 of SEQ ID NO: 12 or SEQ ID NO: 2 is a substitution of an arginine (R) for a glutamine (Q).

[031] In certain embodiments of the methods of the disclosure, including those embodiments wherein the transposase comprises the above-described mutations at positions 30, 165, 282 and/or 538, the piggyBac™ transposase enzyme may comprise or the Super piggyBac™ transposase enzyme may further comprise an amino acid substitution at one or more of positions 103, 194, 372, 375, 450, 509 and 570 of the sequence of SEQ ID NO: 12 or SEQ ID NO: 2. In certain embodiments of the methods of the disclosure, including those embodiments wherein the transposase comprises the above-described mutations at positions

30, 165, 282 and/or 538, the piggyBac™ transposase enzyme may comprise or the Super piggyBac™ transposase enzyme may further comprise an amino acid substitution at two, three, four, five, six or more of positions 103, 194, 372, 375, 450, 509 and 570 of the sequence of SEQ ID NO: 12 or SEQ ID NO: 2. In certain embodiments, including those embodiments wherein the transposase comprises the above-described mutations at positions 30, 165, 282 and/or 538, the piggyBac™ transposase enzyme may comprise or the Super piggyBac™ transposase enzyme may further comprise an amino acid substitution at positions 103, 194, 372, 375, 450, 509 and 570 of the sequence of SEQ ID NO: 12 or SEQ ID NO: 2. In certain embodiments, the amino acid substitution at position 103 of SEQ ID NO: 12 or SEQ ID NO: 2 is a substitution of a proline (P) for a serine (S). In certain embodiments, the amino acid substitution at position 194 of SEQ ID NO: 12 or SEQ ID NO: 2 is a substitution of a valine (V) for a methionine (M). In certain embodiments, the amino acid substitution at position 372 of SEQ ID NO: 12 or SEQ ID NO: 2 is a substitution of an alanine (A) for an arginine (R). In certain embodiments, the amino acid substitution at position 375 of SEQ ID NO: 12 or SEQ ID NO: 2 is a substitution of an alanine (A) for a lysine (K). In certain embodiments, the amino acid substitution at position 450 of SEQ ID NO: 12 or SEQ ID NO: 2 is a substitution of an asparagine (N) for an aspartic acid (D). In certain embodiments, the amino acid substitution at position 509 of SEQ ID NO: 12 or SEQ ID NO: 2 is a substitution of a glycine (G) for a serine (S). In certain embodiments, the amino acid substitution at position 570 of SEQ ID NO: 12 or SEQ ID NO: 2 is a substitution of a serine (S) for an asparagine (N). In certain embodiments, the piggyBac™ transposase enzyme may comprise a substitution of a valine (V) for a methionine (M) at position 194 of SEQ ID NO: 12. In certain embodiments, including those embodiments wherein the piggyBac™ transposase enzyme may comprise a substitution of a valine (V) for a methionine (M) at position 194 of SEQ ID NO: 12, the piggyBac™ transposase enzyme may further comprise an amino acid substitution at positions 372, 375 and 450 of the sequence of SEQ ID NO: 12 or SEQ ID NO: 2. In certain embodiments, the piggyBac™ transposase enzyme may comprise a substitution of a valine (V) for a methionine (M) at position 194 of SEQ ID NO: 12, a substitution of an alanine (A) for an arginine (R) at position 372 of SEQ ID NO: 12, and a substitution of an alanine (A) for a lysine (K) at position 375 of SEQ ID NO: 12. In certain embodiments, the piggyBac™ transposase enzyme may comprise a substitution of a valine (V) for a methionine (M) at position 194 of SEQ ID NO: 12, a substitution of an alanine (A) for an arginine (R) at position 372 of SEQ ID NO: 12, a substitution of an alanine (A) for a lysine (K) at position

375 of SEQ ID NO: 12 and a substitution of an asparagine (N) for an aspartic acid (D) at position 450 of SEQ ID NO: 12.

[032] The disclosure provides a vector comprising the CAR of the disclosure. In certain embodiments, the vector is a viral vector. The vector may be a recombinant vector.

[033] Viral vectors of the disclosure may comprise a sequence isolated or derived from a retrovirus, a lentivirus, an adenovirus, an adeno-associated virus or any combination thereof. The viral vector may comprise a sequence isolated or derived from an adeno-associated virus (AAV). The viral vector may comprise a recombinant AAV (rAAV). Exemplary adeno-associated viruses and recombinant adeno-associated viruses of the disclosure comprise two or more inverted terminal repeat (ITR) sequences located in cis next to a sequence encoding a protein scaffold, Centyrin or CARTyrin of the disclosure. Exemplary adeno-associated viruses and recombinant adeno-associated viruses of the disclosure include, but are not limited to all serotypes (e.g. AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, and AAV9). Exemplary adeno-associated viruses and recombinant adeno-associated viruses of the disclosure include, but are not limited to, self-complementary AAV (scAAV) and AAV hybrids containing the genome of one serotype and the capsid of another serotype (e.g. AAV2/5, AAV-DJ and AAV-DJ8). Exemplary adeno-associated viruses and recombinant adeno-associated viruses of the disclosure include, but are not limited to, rAAV-LK03.

[034] Viral vectors of the disclosure may comprise a selection gene. The selection gene may encode a gene product essential for cell viability and survival. The selection gene may encode a gene product essential for cell viability and survival when challenged by selective cell culture conditions. Selective cell culture conditions may comprise a compound harmful to cell viability or survival and wherein the gene product confers resistance to the compound. Exemplary selection genes of the disclosure may include, but are not limited to, *neo* (conferring resistance to neomycin), DHFR (encoding Dihydrofolate Reductase and conferring resistance to Methotrexate), TYMS (encoding Thymidylate Synthetase), MGMT (encoding O(6)-methylguanine-DNA methyltransferase), multidrug resistance gene (MDR1), ALDH1 (encoding Aldehyde dehydrogenase 1 family, member A1), FRNCF, RAD51C (encoding RAD51 Paralog C), GCS (encoding glucosylceramide synthase), NKX2.2 (encoding NK2 Homeobox 2) or any combination thereof.

[035] Viral vectors of the disclosure may comprise an inducible proapoptotic polypeptide comprising (a) a ligand binding region, (b) a linker, and (c) a proapoptotic polypeptide, wherein the inducible proapoptotic polypeptide does not comprise a non-human sequence. In certain embodiments, the non-human sequence comprises a restriction site. In certain

embodiments, the ligand binding region may be a multimeric ligand binding region. Inducible proapoptotic polypeptides of the disclosure may also be referred to as an “iC9 safety switch”. In certain embodiments, viral vectors of the disclosure may comprise an inducible caspase polypeptide comprising (a) a ligand binding region, (b) a linker, and (c) a caspase polypeptide, wherein the inducible proapoptotic polypeptide does not comprise a non-human sequence. In certain embodiments, viral vectors of the disclosure may comprise an inducible caspase polypeptide comprising (a) a ligand binding region, (b) a linker, and (c) a caspase polypeptide, wherein the inducible proapoptotic polypeptide does not comprise a non-human sequence. In certain embodiments, viral vectors of the disclosure may comprise an inducible caspase polypeptide comprising (a) a ligand binding region, (b) a linker, and (c) a truncated caspase 9 polypeptide, wherein the inducible proapoptotic polypeptide does not comprise a non-human sequence. In certain embodiments of the inducible proapoptotic polypeptides, inducible caspase polypeptides or truncated caspase 9 polypeptides of the disclosure, the ligand binding region may comprise a FK506 binding protein 12 (FKBP12) polypeptide. In certain embodiments, the amino acid sequence of the ligand binding region that comprise a FK506 binding protein 12 (FKBP12) polypeptide may comprise a modification at position 36 of the sequence. The modification may be a substitution of valine (V) for phenylalanine (F) at position 36 (F36V). In certain embodiments, the FKBP12 polypeptide is encoded by an amino acid sequence comprising

GVQVETISPGDGRTPKRGQTCVVHYTGMLLEDGKKVDSSDRNKPFFKMLGKQEVI
RGWEEGVAQMSVGQRAKLISPDIAYGATGHPGIIPPHATLVFDVELLKLE (SEQ ID
NO: 23). In certain embodiments, the FKBP12 polypeptide is encoded by a nucleic acid
sequence comprising

GGGGTCCAGGTCGAGACTATTTACACAGGGGATGGGCGAACATTTCCAAAAAGG
GGCCAGACTTGCGTCGTGCATTACACCGGGATGCTGGAGGACGGGAAGAAAGTG
GACAGCTCCAGGGATCGCAACAAGCCCTTCAAGTTCATGCTGGGAAAGCAGGAA
GTGATCCGAGGATGGGAGGAAGGCGTGGCACAGATGTCAGTCGGCCAGCGGGCC
AAACTGACCATTAGCCCTGACTACGCTTATGGAGCAACAGGCCACCCAGGGATC
ATTCCCCCTCATGCCACCCTGGTCTTCGAT GTGGAAGTCTGAAGCTGGAG (SEQ
ID NO: 24). In certain embodiments, the induction agent specific for the ligand binding

region may comprise a FK506 binding protein 12 (FKBP12) polypeptide having a substitution of valine (V) for phenylalanine (F) at position 36 (F36V) comprises AP20187 and/or AP1903, both synthetic drugs.

[036] In certain embodiments of the inducible proapoptotic polypeptides, inducible caspase polypeptides or truncated caspase 9 polypeptides of the disclosure, the linker region is encoded by an amino acid comprising GGGGS (SEQ ID NO: 25) or a nucleic acid sequence comprising GGAGGAGGAGGATCC (SEQ ID NO: 26). In certain embodiments, the nucleic acid sequence encoding the linker does not comprise a restriction site.

[037] In certain embodiments of the truncated caspase 9 polypeptides of the disclosure, the truncated caspase 9 polypeptide is encoded by an amino acid sequence that does not comprise an arginine (R) at position 87 of the sequence. Alternatively, or in addition, in certain embodiments of the inducible proapoptotic polypeptides, inducible caspase polypeptides or truncated caspase 9 polypeptides of the disclosure, the truncated caspase 9 polypeptide is encoded by an amino acid sequence that does not comprise an alanine (A) at position 282 the sequence. In certain embodiments of the inducible proapoptotic polypeptides, inducible caspase polypeptides or truncated caspase 9 polypeptides of the disclosure, the truncated caspase 9 polypeptide is encoded by an amino acid comprising

GFQDVGALESLRGNADLAYISLMEPCGHCLIINNPNFCRESGLRTRTGSNIDCEKLRR
RFSLLHFMVEVKGDLTAKKMLALLELAQQDHGALDCCVVVILSHGCQASHLQFPG
AVYGTGDCPVSVKIVNIFNGTSCPSLGGKPKLFFIQACGGEQKDHGFEVASTSPEDE
SPGSNPEPDATPFQEGLRTFDQLDAISSLPTPSDIFVSYSTFPGFVSWRDPKSGSWYVE
TLDDIFEQWAHSEDLQSLLLRVANAVSVKGIYKQMPGCNFLRKKLFFKTS (SEQ ID
NO: 27) or a nucleic acid sequence comprising

TTTGGGGACGTGGGGGCCCTGGAGTCTCTGCGAGGAAATGCCGATCTGGCTTACA
TCCTGAGCATGGAACCCTGCGGCCACTGTCTGATCATTACAATGTGAACCTCTG
CAGAGAAAGCGGACTGCGAACACGGACTGGCTCCAATATTGACTGTGAGAAGCT
GCGGAGAAGGTTCTCTAGTCTGCACTTTATGGTCGAAGTGAAAGGGGATCTGACC
GCCAAGAAAATGGTGCTGGCCCTGCTGGAGCTGGCTCAGCAGGACCATGGAGCT
CTGGATTGCTGCGTGGTCGTGATCCTGTCCACGGGTGCCAGGCTTCTCATCTGC
AGTTCCCCGGAGCAGTGTACGGAACAGACGGCTGTCTGTGACGTGGAGAAGA
TCGTCAACATCTTCAACGGCACTTCTTGCCCTAGTCTGGGGGGAAAGCCAAACT
GTTCTTTATCCAGGCCTGTGGCGGGGAACAGAAAGATCACGGCTTCGAGGTGGC
CAGCACCAGCCCTGAGGACGAATCACCAGGGAGCAACCCTGAACCAGATGCAAC
TCCATTCCAGGAGGGACTGAGGACCTTTGACCAGCTGGATGCTATCTCAAGCCTG
CCCACTCCTAGTGACATTTTCGTGTCTTACAGTACCTTCCCAGGCTTTGTCTCATG
GCGCGATCCCAAGTCAGGGAGCTGGTACGTGGAGACACTGGACGACATCTTTGA
ACAGTGGGCCCATTACAGAGGACCTGCAGAGCCTGCTGCTGCGAGTGGCAAACGC

TGTCTCTGTGAAGGGCATCTACAAACAGATGCCCCGGGTGCTTCAATTTTCTGAGA
AAGAAACTGTTCTTTAAGACTTCC (SEQ ID NO: 28).

[038] In certain embodiments of the inducible proapoptotic polypeptides, wherein the polypeptide comprises a truncated caspase 9 polypeptide, the inducible proapoptotic polypeptide is encoded by an amino acid sequence comprising

GVQVETISPGDGRTFPKRQTCVVHYTGMLLEDGKKVDSSRDRNKPFKFMLGKQEV
RGWEEGVAQMSVGQRAKLISPDIYAYGATGHPGIIPPHATLVFDVELLKLEGGGGGS
GFGDVGALSLRGNADLAYISLMEPCGHCLINNPNFCRESGLRTRTGSNIDCEKLRR
RFSSLHFMVEVKGDLTAKKMVLALLELAQQDHGALDCCVVVILSHGCQASHLQFPG
AVYGTGDCPVSVKIVNIFNGTSCPSLGGKPKLFFIQACGGEQKDHGFEVASTSPEDE
SPGSNPEPDATPFQEGLRTFDQLDAISSLPTSDIFVSYSTFPGFVSWRDPKSGSWYVE
TLDDIFEQWAHSEDLQSLLLRVANAVSVKGIYKQMPGCNFLRKKLFFKTS (SEQ ID

NO: 29) or the nucleic acid sequence comprising

GGGGTCCAGGTCGAGACTATTTACACAGGGGATGGGCGAACATTTCCAAAAAGG
GGCCAGACTTGCGTCGTGCATTACACCGGGATGCTGGAGGACGGGAAGAAAGTG
GACAGCTCCAGGGATCGCAACAAGCCCTTCAAGTTCATGCTGGGAAAGCAGGAA
GTGATCCGAGGATGGGAGGAAGGCGTGGCACAGATGTCAGTCGGCCAGCGGGCC
AAACTGACCATTAGCCCTGACTACGCTTATGGAGCAACAGGCCACCCAGGGATC
ATTCCCCCTCATGCCACCCTGGTCTTCGATGTGGAAGTCTGAAGCTGGAGGGAG
GAGGAGGATCCGAATTTGGGGACGTGGGGGCCCTGGAGTCTCTGCGAGGAAATG
CCGATCTGGCTTACATCCTGAGCATGGAACCCTGCGGCCACTGTCTGATCATTAA
CAATGTGAACTTCTGCAGAGAAAGCGGACTGCGAACACGGACTGGCTCCAATAT
TGACTGTGAGAAGCTGCGGAGAAGGTTCTCTAGTCTGCACTTTATGGTCGAAGTG
AAAGGGGATCTGACCGCCAAGAAAATGGTGCTGGCCCTGCTGGAGCTGGCTCAG
CAGGACCATGGAGCTCTGGATTGCTGCGTGGTCGTGATCCTGTCCCACGGGTGCC
AGGCTTCTCATCTGCAGTTCCCCGGAGCAGTGTACGGAACAGACGGCTGTCTGT
CAGCGTGGAGAAGATCGTCAACATCTTCAACGGCACTTCTTGCCCTAGTCTGGGG
GGAAAGCCAAAAGTGTCTTTATCCAGGCCTGTGGCGGGGAACAGAAAGATCAC
GGCTTCGAGGTGGCCAGCACCAGCCCTGAGGACGAATCACCAGGGAGCAACCCT
GAACCAGATGCAACTCCATTCCAGGAGGGACTGAGGACCTTTGACCAGCTGGAT
GCTATCTCAAGCCTGCCCACTCCTAGTGACATTTTCGTGTCTTACAGTACCTTCCC
AGGCTTTGTCTCATGGCGCGATCCCAAGTCAGGGAGCTGGTACGTGGAGACACT
GGACGACATCTTTGAACAGTGGGCCCATTGAGAGGACCTGCAGAGCCTGCTGCT

GCGAGTGGCAAACGCTGTCTCTGTGAAGGGCATCTACAAACAGATGCCCCGGGTG
CTTCAATTTTCTGAGAAAGAACTGTTCTTTAAGACTTCC (SEQ ID NO: 30).

[039] Viral vectors of the disclosure may comprise at least one self-cleaving peptide. In some embodiments, the vector may comprise at least one self-cleaving peptide and wherein a self-cleaving peptide is located between a CAR and a selection gene. In some embodiments, the vector may comprise at least one self-cleaving peptide and wherein a first self-cleaving peptide is located upstream of a CAR and a second self-cleaving peptide is located downstream of a CAR. Viral vectors of the disclosure may comprise at least one self-cleaving peptide(s) located, for example, between one or more of a protein scaffold, Centyrin or CARTyrin of the disclosure and an inducible proapoptotic polypeptide of the disclosure. Viral vectors of the disclosure may comprise at least two self-cleaving peptide(s), a first self-cleaving peptide located, for example, upstream or immediately upstream of an inducible proapoptotic polypeptide of the disclosure and a second first self-cleaving peptide located, for example, downstream or immediately upstream of an inducible proapoptotic polypeptide of the disclosure. The self-cleaving peptide may comprise, for example, a T2A peptide, GSG-T2A peptide, an E2A peptide, a GSG-E2A peptide, an F2A peptide, a GSG-F2A peptide, a P2A peptide, or a GSG-P2A peptide. A T2A peptide may comprise an amino acid sequence comprising EGRGSLTTCGDVEENPGP (SEQ ID NO: 31) or a sequence having at least 70%, 80%, 90%, 95%, or 99% identity to the amino acid sequence comprising EGRGSLTTCGDVEENPGP (SEQ ID NO: 31). A GSG-T2A peptide may comprise an amino acid sequence comprising GSGEGRGSLTTCGDVEENPGP (SEQ ID NO: 32) or a sequence having at least 70%, 80%, 90%, 95%, or 99% identity to the amino acid sequence comprising GSGEGRGSLTTCGDVEENPGP (SEQ ID NO: 32). A GSG-T2A peptide may comprise a nucleic acid sequence comprising ggatctggagaggggaaggggaagcctgctgacctgtggagacgtggaggaaaaccaggacca (SEQ ID NO: 33). An E2A peptide may comprise an amino acid sequence comprising QCTNYALLKLAGDVESNPGP (SEQ ID NO: 34) or a sequence having at least 70%, 80%, 90%, 95%, or 99% identity to the amino acid sequence comprising QCTNYALLKLAGDVESNPGP (SEQ ID NO: 34). A GSG-E2A peptide may comprise an amino acid sequence comprising GSGQCTNYALLKLAGDVESNPGP (SEQ ID NO: 35) or a sequence having at least 70%, 80%, 90%, 95%, or 99% identity to the amino acid sequence comprising GSGQCTNYALLKLAGDVESNPGP (SEQ ID NO: 35). An F2A peptide may comprise an amino acid sequence comprising VKQTLNFDLLKLAGDVESNPGP (SEQ ID NO: 36) or a sequence having at least 70%, 80%, 90%, 95%, or 99% identity to the amino

acid sequence comprising VKQTLNFDLLKLAGDVESNPGP (SEQ ID NO: 36). A GSG-F2A peptide may comprise an amino acid sequence comprising GSGVKQTLNFDLLKLAGDVESNPGP (SEQ ID NO: 37) or a sequence having at least 70%, 80%, 90%, 95%, or 99% identity to the amino acid sequence comprising GSGVKQTLNFDLLKLAGDVESNPGP (SEQ ID NO: 37). A P2A peptide may comprise an amino acid sequence comprising ATNFSLLKQAGDVEENPGP (SEQ ID NO: 38) or a sequence having at least 70%, 80%, 90%, 95%, or 99% identity to the amino acid sequence comprising ATNFSLLKQAGDVEENPGP (SEQ ID NO: 38). A GSG-P2A peptide may comprise an amino acid sequence comprising GSGATNFSLLKQAGDVEENPGP (SEQ ID NO: 39) or a sequence having at least 70%, 80%, 90%, 95%, or 99% identity to the amino acid sequence comprising GSGATNFSLLKQAGDVEENPGP (SEQ ID NO: 39).

[040] The disclosure provides a vector comprising the CAR of the disclosure. In certain embodiments, the vector is a nanoparticle. Exemplary nanoparticle vectors of the disclosure include, but are not limited to, nucleic acids (e.g. RNA, DNA, synthetic nucleotides, modified nucleotides or any combination thereof), amino acids (L-amino acids, D-amino acids, synthetic amino acids, modified amino acids, or any combination thereof), polymers (e.g. polymersomes), micelles, lipids (e.g. liposomes), organic molecules (e.g. carbon atoms, sheets, fibers, tubes), inorganic molecules (e.g. calcium phosphate or gold) or any combination thereof. A nanoparticle vector may be passively or actively transported across a cell membrane.

[041] Nanoparticle vectors of the disclosure may comprise a selection gene. The selection gene may encode a gene product essential for cell viability and survival. The selection gene may encode a gene product essential for cell viability and survival when challenged by selective cell culture conditions. Selective cell culture conditions may comprise a compound harmful to cell viability or survival and wherein the gene product confers resistance to the compound. Exemplary selection genes of the disclosure may include, but are not limited to, *neo* (conferring resistance to neomycin), DHFR (encoding Dihydrofolate Reductase and conferring resistance to Methotrexate), TYMS (encoding Thymidylate Synthetase), MGMT (encoding O(6)-methylguanine-DNA methyltransferase), multidrug resistance gene (MDR1), ALDH1 (encoding Aldehyde dehydrogenase 1 family, member A1), FRNCF, RAD51C (encoding RAD51 Paralog C), GCS (encoding glucosylceramide synthase), NKX2.2 (encoding NK2 Homeobox 2) or any combination thereof.

[042] Nanoparticle vectors of the disclosure may comprise an inducible proapoptotic polypeptide comprising (a) a ligand binding region, (b) a linker, and (c) a proapoptotic

polypeptide, wherein the inducible proapoptotic polypeptide does not comprise a non-human sequence. In certain embodiments, the non-human sequence comprises a restriction site. In certain embodiments, the ligand binding region may be a multimeric ligand binding region. Inducible proapoptotic polypeptides of the disclosure may also be referred to as an “iC9 safety switch”. In certain embodiments, nanoparticle vectors of the disclosure may comprise an inducible caspase polypeptide comprising (a) a ligand binding region, (b) a linker, and (c) a caspase polypeptide, wherein the inducible proapoptotic polypeptide does not comprise a non-human sequence. In certain embodiments, nanoparticle vectors of the disclosure may comprise an inducible caspase polypeptide comprising (a) a ligand binding region, (b) a linker, and (c) a caspase polypeptide, wherein the inducible proapoptotic polypeptide does not comprise a non-human sequence. In certain embodiments, nanoparticle vectors of the disclosure may comprise an inducible caspase polypeptide comprising (a) a ligand binding region, (b) a linker, and (c) a truncated caspase 9 polypeptide, wherein the inducible proapoptotic polypeptide does not comprise a non-human sequence. In certain embodiments of the inducible proapoptotic polypeptides, inducible caspase polypeptides or truncated caspase 9 polypeptides of the disclosure, the ligand binding region may comprise a FK506 binding protein 12 (FKBP12) polypeptide. In certain embodiments, the amino acid sequence of the ligand binding region that comprise a FK506 binding protein 12 (FKBP12) polypeptide may comprise a modification at position 36 of the sequence. The modification may be a substitution of valine (V) for phenylalanine (F) at position 36 (F36V). In certain embodiments, the FKBP12 polypeptide is encoded by an amino acid sequence comprising GVVQVETISPGDGRTPKRGQTCVVHYTGMLLEDGKKVDSSRDRNKPFKFMGLGKQEVI RGWEEGVAQMSVGQRAKLTISPDYAYGATGHPGIIPPHATLVFDVELLKLE (SEQ ID NO: 23). In certain embodiments, the FKBP12 polypeptide is encoded by a nucleic acid sequence comprising

GGGGTCCAGGTCGAGACTATTTACACAGGGGATGGGCGAACATTTCCAAAAAGG
GGCCAGACTTGCGTCGTGCATTACACCGGGATGCTGGAGGACGGGAAGAAAGTG
GACAGCTCCAGGGATCGCAACAAGCCCTTCAAGTTCATGCTGGGAAAGCAGGAA
GTGATCCGAGGATGGGAGGAAGGCGTGGCACAGATGTCAGTCGGCCAGCGGGCC
AAACTGACCATTAGCCCTGACTACGCTTATGGAGCAACAGGCCACCCAGGGATC
ATTCCCCCTCATGCCACCCTGGTCTTCGAT GTGGAAGTCTGAAGCTGGAG (SEQ
ID NO: 24). In certain embodiments, the induction agent specific for the ligand binding region may comprise a FK506 binding protein 12 (FKBP12) polypeptide having a

substitution of valine (V) for phenylalanine (F) at position 36 (F36V) comprises AP20187 and/or AP1903, both synthetic drugs.

[043] In certain embodiments of the inducible proapoptotic polypeptides, inducible caspase polypeptides or truncated caspase 9 polypeptides of the disclosure, the linker region is encoded by an amino acid comprising GGGGS (SEQ ID NO: 25) or a nucleic acid sequence comprising GGAGGAGGAGGATCC (SEQ ID NO: 26). In certain embodiments, the nucleic acid sequence encoding the linker does not comprise a restriction site.

[044] In certain embodiments of the truncated caspase 9 polypeptides of the disclosure, the truncated caspase 9 polypeptide is encoded by an amino acid sequence that does not comprise an arginine (R) at position 87 of the sequence. Alternatively, or in addition, in certain embodiments of the inducible proapoptotic polypeptides, inducible caspase polypeptides or truncated caspase 9 polypeptides of the disclosure, the truncated caspase 9 polypeptide is encoded by an amino acid sequence that does not comprise an alanine (A) at position 282 the sequence. In certain embodiments of the inducible proapoptotic polypeptides, inducible caspase polypeptides or truncated caspase 9 polypeptides of the disclosure, the truncated caspase 9 polypeptide is encoded by an amino acid comprising

GFGDVGALESLRGNADLAYISLMEPCGHCLIINNWNFCRESGLRTRTGSNIDCEKLRR
RFSSLHFMVEVKGDLTAKKMVLALLELAQQDHGALDCCVVVILSHGCQASHLQFPG
AVYGTGDCPVSVVEKIVNIFNGTSCPSLGGKPKLFFIQACGGEQKDHGFEVASTSPEDE
SPGSNPEPDATPFQEGLRTFDQLDAISSLPTPSDIFVSYSTFPGFVSWRDPKSGSWYVE
TLDDIFEQWAHSEDLQSLLLRVANAVSVKGIYKQMPGCNFLRKKLFFKTS (SEQ ID
NO: 27) or a nucleic acid sequence comprising

TTTGGGGACGTGGGGGCCCTGGAGTCTCTGCGAGGAAATGCCGATCTGGCTTACA
TCCTGAGCATGGAACCCTGCGGCCACTGTCTGATCATTAAACAATGTGAACCTCTG
CAGAGAAAGCGGACTGCGAACACGGACTGGCTCCAATATTGACTGTGAGAAGCT
GCGGAGAAGGTTCTCTAGTCTGCACTTTATGGTCGAAGTGAAAGGGGATCTGACC
GCCAAGAAAATGGTGCTGGCCCTGCTGGAGCTGGCTCAGCAGGACCATGGAGCT
CTGGATTGCTGCGTGGTCGTGATCCTGTCCACGGGTGCCAGGCTTCTCATCTGC
AGTTCCCCGGAGCAGTGTACGGAACAGACGGCTGTCCTGTCAGCGTGGAAGA
TCGTCAACATCTTCAACGGCACTTCTTGCCCTAGTCTGGGGGGAAAGCCAAA
GTTCTTTATCCAGGCCTGTGGCGGGGAACAGAAAGATCACGGCTTCGAGGTGGC
CAGCACCAGCCCTGAGGACGAATCACCAGGGAGCAACCCTGAACCAGATGCAAC
TCCATTCCAGGAGGGACTGAGGACCTTTGACCAGCTGGATGCTATCTCAAGCCTG
CCCACTCCTAGTGACATTTTCGTGTCTTACAGTACCTTCCCAGGCTTTGTCTCATG

GCGCGATCCCAAGTCAGGGAGCTGGTACGTGGAGACACTGGACGACATCTTTGA
ACAGTGGGCCCATTACAGAGGACCTGCAGAGCCTGCTGCTGCGAGTGGCAAACGC
TGTCTCTGTGAAGGGCATCTACAAACAGATGCCCCGGGTGCTTCAATTTTCTGAGA
AAGAAACTGTTCTTTAAGACTTCC (SEQ ID NO: 28).

[045] In certain embodiments of the inducible proapoptotic polypeptides, wherein the polypeptide comprises a truncated caspase 9 polypeptide, the inducible proapoptotic polypeptide is encoded by an amino acid sequence comprising

GVQVETISPGDGRTPKRGQTCVVHYTGMLLEDGKKVDSSRDRNKPFFKMLGKQEVI
RGWEEGVAQMSVGQRAKLTI SPDYAYGATGHPGIIPPHATLVFDVELLKLEGGGGGS
GFGDVGALES LRGNADLAYISLMEPCGHCLIINN VNFCRESGLRTRTGSNIDCEKLRR
RFSSLHFMVEVKGDLTAKKMVLALLELAQQDHGALDCCVVVILSHGCQASHLQFPG
AVYGTGDCPVSV EKIVNIFNGTSCPSLGGKPKLFFIQACGGEQKDHGFEVASTSPEDE
SPGSNPEPDATPFQEGLRTFDQLDAISSLPTPSDIFVSYSTFPGFVSWRDPKSGSWYVE
TLDDIFEQWAHSEDLQSLLLRVANAVSVKGIYKQMPGCNFLRKKLFFKTS (SEQ ID

NO: 29) or the nucleic acid sequence comprising

GGGGTCCAGGTCGAGACTATTTACACAGGGGATGGGCGAACATTTCCAAAAAGG
GGCCAGACTTGCGTCGTGCATTACACCGGGATGCTGGAGGACGGGAAGAAAGTG
GACAGCTCCAGGGATCGCAACAAGCCCTTCAAGTTCATGCTGGGAAAGCAGGAA
GTGATCCGAGGATGGGAGGAAGGCGTGGCACAGATGTCAGTCGGCCAGCGGGCC
AAACTGACCATTAGCCCTGACTACGCTTATGGAGCAACAGGCCACCCAGGGATC
ATTCCCCCTCATGCCACCCTGGTCTTCGATGTGGAAGCTGCTGAAGCTGGAGGGAG
GAGGAGGATCCGAATTTGGGGACGTGGGGGCCCTGGAGTCTCTGCGAGGAAATG
CCGATCTGGCTTACATCCTGAGCATGGAACCCTGCGGCCACTGTCTGATCATTAA
CAATGTGAACTTCTGCAGAGAAAGCGGACTGCGAACACGGACTGGCTCCAATAT
TGACTGTGAGAAGCTGCGGAGAAGGTTCTCTAGTCTGCACTTTATGGTCGAAGTG
AAAGGGGATCTGACCGCCAAGAAAATGGTGCTGGCCCTGCTGGAGCTGGCTCAG
CAGGACCATGGAGCTCTGGATTGCTGCGTGGTCGTGATCCTGTCCACGGGTGCC
AGGCTTCTCATCTGCAGTTCCCCGGAGCAGTGTACGGAACAGACGGCTGTCTGT
CAGCGTGGAGAAGATCGTCAACATCTTCAACGGCACTTCTTGCCCTAGTCTGGGG
GGAAAGCCAAAAGTGTCTTTATCCAGGCCTGTGGCGGGGAACAGAAAGATCAC
GGCTTCGAGGTGGCCAGCACCAGCCCTGAGGACGAATCACCAGGGAGCAACCCT
GAACCAGATGCAACTCCATTCCAGGAGGGACTGAGGACCTTTGACCAGCTGGAT
GCTATCTCAAGCCTGCCCACTCCTAGTGACATTTTCGTGTCTTACAGTACCTTCCC
AGGCTTTGTCTCATGGCGCGATCCCAAGTCAGGGAGCTGGTACGTGGAGACACT

GGACGACATCTTTGAACAGTGGGCCCATTGAGAGGACCTGCAGAGCCTGCTGCT
GCGAGTGGCAAACGCTGTCTCTGTGAAGGGCATCTACAAACAGATGCCCCGGGTG
CTTCAATTTTCTGAGAAAGAACTGTTCTTTAAGACTTCC (SEQ ID NO: 30).

[046] Nanoparticle vectors of the disclosure may comprise at least one self-cleaving peptide. In some embodiments, the nanoparticle vector may comprise at least one self-cleaving peptide and wherein a self-cleaving peptide is located between a CAR and the nanoparticle. In some embodiments, the nanoparticle vector may comprise at least one self-cleaving peptide and wherein a first self-cleaving peptide is located upstream of a CAR and a second self-cleaving peptide is located downstream of a CAR. In some embodiments, the nanoparticle vector may comprise at least one self-cleaving peptide and wherein a first self-cleaving peptide is located between a CAR and the nanoparticle and a second self-cleaving peptide is located downstream of the CAR. In some embodiments, the nanoparticle vector may comprise at least one self-cleaving peptide and wherein a first self-cleaving peptide is located between a CAR and the nanoparticle and a second self-cleaving peptide is located downstream of the CAR, for example, between the CAR and a selection gene. Nanoparticle vectors of the disclosure may comprise at least one self-cleaving peptide(s) located, for example, between one or more of a protein scaffold, Centyrin or CARTyrin of the disclosure and an inducible proapoptotic polypeptide of the disclosure. Nanoparticle vectors of the disclosure may comprise at least two self-cleaving peptide(s), a first self-cleaving peptide located, for example, upstream or immediately upstream of an inducible proapoptotic polypeptide of the disclosure and a second first self-cleaving peptide located, for example, downstream or immediately upstream of an inducible proapoptotic polypeptide of the disclosure. The self-cleaving peptide may comprise, for example, a T2A peptide, GSG-T2A peptide, an E2A peptide, a GSG-E2A peptide, an F2A peptide, a GSG-F2A peptide, a P2A peptide, or a GSG-P2A peptide. A T2A peptide may comprise an amino acid sequence comprising EGRGSLLTCGDVEENPGP (SEQ ID NO: 31) or a sequence having at least 70%, 80%, 90%, 95%, or 99% identity to the amino acid sequence comprising EGRGSLLTCGDVEENPGP (SEQ ID NO: 31). A GSG-T2A peptide may comprise an amino acid sequence comprising GSGEGRGSLLTCGDVEENPGP (SEQ ID NO: 32) or a sequence having at least 70%, 80%, 90%, 95%, or 99% identity to the amino acid sequence comprising GSGEGRGSLLTCGDVEENPGP (SEQ ID NO: 32). A GSG-T2A peptide may comprise a nucleic acid sequence comprising ggatctggagaggggaaggggaagcctgctgacgtgtggagacgtggaggaaaaccaggacca (SEQ ID NO: 33). An E2A peptide may comprise an amino acid sequence comprising

QCTNYALLKLAGDVESNPGP (SEQ ID NO: 34) or a sequence having at least 70%, 80%, 90%, 95%, or 99% identity to the amino acid sequence comprising

QCTNYALLKLAGDVESNPGP (SEQ ID NO: 34). A GSG-E2A peptide may comprise an amino acid sequence comprising GSGQCTNYALLKLAGDVESNPGP (SEQ ID NO: 35) or a sequence having at least 70%, 80%, 90%, 95%, or 99% identity to the amino acid sequence comprising GSGQCTNYALLKLAGDVESNPGP (SEQ ID NO: 35). An F2A peptide may comprise an amino acid sequence comprising VKQTLNFDLLKLAGDVESNPGP (SEQ ID NO: 36) or a sequence having at least 70%, 80%, 90%, 95%, or 99% identity to the amino acid sequence comprising VKQTLNFDLLKLAGDVESNPGP (SEQ ID NO: 36). A GSG-F2A peptide may comprise an amino acid sequence comprising

GSGVKQTLNFDLLKLAGDVESNPGP (SEQ ID NO: 37) or a sequence having at least 70%, 80%, 90%, 95%, or 99% identity to the amino acid sequence comprising

GSGVKQTLNFDLLKLAGDVESNPGP (SEQ ID NO: 37). A P2A peptide may comprise an amino acid sequence comprising ATNFSLLKQAGDVEENPGP (SEQ ID NO: 38) or a sequence having at least 70%, 80%, 90%, 95%, or 99% identity to the amino acid sequence comprising ATNFSLLKQAGDVEENPGP (SEQ ID NO: 38). A GSG-P2A peptide may comprise an amino acid sequence comprising GSGATNFSLLKQAGDVEENPGP (SEQ ID NO: 39) or a sequence having at least 70%, 80%, 90%, 95%, or 99% identity to the amino acid sequence comprising GSGATNFSLLKQAGDVEENPGP (SEQ ID NO: 39).

[047] The disclosure provides a composition comprising a vector of the disclosure.

[048] The disclosure provides a cell comprising a CAR of the disclosure. The disclosure provides a cell comprising a transposon of the disclosure. In certain embodiments, the cell comprising a CAR, a transposon, or a vector of the disclosure may express a CAR on the cell surface. The cell may be any type of cell. Preferably, the cell is an immune cell. The immune cell may be a T-cell, a Natural Killer (NK) cell, a Natural Killer (NK)-like cell (e.g. a Cytokine Induced Killer (CIK) cell), a hematopoietic progenitor cell, a peripheral blood (PB) derived T cell or an umbilical cord blood (UCB) derived T-cell. Preferably, the immune cell is a T-cell. The cell may be an artificial antigen presenting cell, which, optionally, may be used to stimulate and expand a modified immune cell or T cell of the disclosure. The cell may be a tumor cell, which, optionally, may be used as an artificial or modified antigen presenting cell.

[049] Modified cells of the disclosure that may be used for adoptive therapy may be autologous or allogeneic.

[050] The disclosure provides a method for expressing a chimeric antigen receptor (CAR) on the surface of a cell, comprising: (a) obtaining a cell population; (b) contacting the cell population to a composition comprising a CAR of the disclosure or a sequence encoding the CAR, under conditions sufficient to transfer the CAR across a cell membrane of at least one cell in the cell population, thereby generating a modified cell population; (c) culturing the modified cell population under conditions suitable for integration of the transposon; and (d) expanding and/or selecting at least one cell from the modified cell population that express the CAR on the cell surface.

[051] In certain embodiments of this method of expressing a CAR, the cell population may comprise leukocytes and/ or CD4⁺ and CD8⁺ leukocytes. The cell population may comprise CD4⁺ and CD8⁺ leukocytes in an optimized ratio. The optimized ratio of CD4⁺ to CD8⁺ leukocytes does not naturally occur *in vivo*. The cell population may comprise a tumor cell.

[052] In certain embodiments of this method of expressing a CAR, a transposon or vector comprises the CAR or the sequence encoding the CAR.

[053] In certain embodiments of this method of expressing a CAR, the conditions sufficient to transfer the sequence encoding the CAR across a cell membrane of at least one cell in the cell population comprise nucleofection.

[054] In certain embodiments of this method of expressing a CAR, wherein the conditions sufficient to transfer the sequence encoding the CAR across a cell membrane of at least one cell in the cell population comprise at least one of an application of one or more pulses of electricity at a specified voltage, a buffer, and one or more supplemental factor(s). In certain embodiments, the buffer may comprise PBS, HBSS, OptiMEM, BTXpress, Amaxa Nucleofector, Human T cell nucleofection buffer or any combination thereof. In certain embodiments, the one or more supplemental factor(s) may comprise (a) a recombinant human cytokine, a chemokine, an interleukin or any combination thereof; (b) a salt, a mineral, a metabolite or any combination thereof; (c) a cell medium; (d) an inhibitor of cellular DNA sensing, metabolism, differentiation, signal transduction, one or more apoptotic pathway(s) or combinations thereof; and (e) a reagent that modifies or stabilizes one or more nucleic acids. The recombinant human cytokine, the chemokine, the interleukin or any combination thereof may comprise IL2, IL7, IL12, IL15, IL21, IL1, IL3, IL4, IL5, IL6, IL8, CXCL8, IL9, IL10, IL11, IL13, IL14, IL16, IL17, IL18, IL19, IL20, IL22, IL23, IL25, IL26, IL27, IL28, IL29, IL30, IL31, IL32, IL33, IL35, IL36, GM-CSF, IFN-gamma, IL-1 alpha/IL-1F1, IL-1 beta/IL-1F2, IL-12 p70, IL-12/IL-35 p35, IL-13, IL-17/IL-17A, IL-17A/F Heterodimer, IL-17F, IL-18/IL-1F4, IL-23, IL-24, IL-32, IL-32 beta, IL-32 gamma, IL-33, LAP (TGF-beta 1),

Lymphotoxin-alpha/TNF-beta, TGF-beta, TNF-alpha, TRANCE/TNFSF11/RANK L or any combination thereof. The salt, the mineral, the metabolite or any combination thereof may comprise HEPES, Nicotinamide, Heparin, Sodium Pyruvate, L-Glutamine, MEM Non-Essential Amino Acid Solution, Ascorbic Acid, Nucleosides, FBS/FCS, Human serum, serum-substitute, anti-biotics, pH adjusters, Earle's Salts, 2-Mercaptoethanol, Human transferrin, Recombinant human insulin, Human serum albumin, Nucleofector PLUS Supplement, KCL, MgCl₂, Na₂HPO₄, NaH₂PO₄, Sodium lactobionate, Mannitol, Sodium succinate, Sodium Chloride, CNa, Glucose, Ca(NO₃)₂, Tris/HCl, K₂HPO₄, KH₂PO₄, Polyethylenimine, Poly-ethylene-glycol, Poloxamer 188, Poloxamer 181, Poloxamer 407, Poly-vinylpyrrolidone, Pop313, Crown-5, or any combination thereof. The cell medium may comprise PBS, HBSS, OptiMEM, DMEM, RPMI 1640, AIM-V, X-VIVO 15, CellGro DC Medium, CTS OpTimizer T Cell Expansion SFM, TexMACS Medium, PRIME-XV T Cell Expansion Medium, ImmunoCult-XF T Cell Expansion Medium or any combination thereof. The inhibitor of cellular DNA sensing, metabolism, differentiation, signal transduction, one or more apoptotic pathway(s) or combinations thereof comprise inhibitors of TLR9, MyD88, IRAK, TRAF6, TRAF3, IRF-7, NF-KB, Type 1 Interferons, pro-inflammatory cytokines, cGAS, STING, Sec5, TBK1, IRF-3, RNA pol III, RIG-1, IPS-1, FADD, RIP1, TRAF3, AIM2, ASC, Caspase1, Pro-IL1B, PI3K, Akt, Wnt3A, inhibitors of glycogen synthase kinase-3 β (GSK-3 β) (e.g. TWS119), Bafilomycin, Chloroquine, Quinacrine, AC-YVAD-CMK, Z-VAD-FMK, Z-IETD-FMK or any combination thereof. The reagent that modifies or stabilizes one or more nucleic acids comprises a pH modifier, a DNA-binding protein, a lipid, a phospholipid, CaPO₄, a net neutral charge DNA binding peptide with or without a NLS sequence, a TREX1 enzyme or any combination thereof.

[055] In certain embodiments of this method of expressing a CAR, the conditions suitable for integration of the CAR or a sequence encoding the CAR of the disclosure comprise at least one of a buffer and one or more supplemental factor(s). In certain embodiments, a transposon or vector of the disclosure comprise the CAR or a sequence encoding the CAR of the disclosure. In certain embodiments, the buffer may comprise PBS, HBSS, OptiMEM, BTXpress, Amaxa Nucleofector, Human T cell nucleofection buffer or any combination thereof. In certain embodiments, the one or more supplemental factor(s) may comprise (a) a recombinant human cytokine, a chemokine, an interleukin or any combination thereof; (b) a salt, a mineral, a metabolite or any combination thereof; (c) a cell medium; (d) an inhibitor of cellular DNA sensing, metabolism, differentiation, signal transduction, one or more apoptotic pathway(s) or combinations thereof; and (e) a reagent that modifies or stabilizes one or more

nucleic acids. The recombinant human cytokine, the chemokine, the interleukin or any combination thereof may comprise IL2, IL7, IL12, IL15, IL21, IL1, IL3, IL4, IL5, IL6, IL8, CXCL8, IL9, IL10, IL11, IL13, IL14, IL16, IL17, IL18, IL19, IL20, IL22, IL23, IL25, IL26, IL27, IL28, IL29, IL30, IL31, IL32, IL33, IL35, IL36, GM-CSF, IFN-gamma, IL-1 alpha/IL-1F1, IL-1 beta/IL-1F2, IL-12 p70, IL-12/IL-35 p35, IL-13, IL-17/IL-17A, IL-17A/F Heterodimer, IL-17F, IL-18/IL-1F4, IL-23, IL-24, IL-32, IL-32 beta, IL-32 gamma, IL-33, LAP (TGF-beta 1), Lymphotoxin-alpha/TNF-beta, TGF-beta, TNF-alpha, TRANCE/TNFSF11/RANK L or any combination thereof. The salt, the mineral, the metabolite or any combination thereof may comprise HEPES, Nicotinamide, Heparin, Sodium Pyruvate, L-Glutamine, MEM Non-Essential Amino Acid Solution, Ascorbic Acid, Nucleosides, FBS/FCS, Human serum, serum-substitute, anti-biotics, pH adjusters, Earle's Salts, 2-Mercaptoethanol, Human transferrin, Recombinant human insulin, Human serum albumin, Nucleofector PLUS Supplement, KCL, MgCl₂, Na₂HPO₄, NaH₂PO₄, Sodium lactobionate, Mannitol, Sodium succinate, Sodium Chloride, CNa, Glucose, Ca(NO₃)₂, Tris/HCl, K₂HPO₄, KH₂PO₄, Polyethylenimine, Poly-ethylene-glycol, Poloxamer 188, Poloxamer 181, Poloxamer 407, Poly-vinylpyrrolidone, Pop313, Crown-5, or any combination thereof. The cell medium may comprise PBS, HBSS, OptiMEM, DMEM, RPMI 1640, AIM-V, X-VIVO 15, CellGro DC Medium, CTS OpTimizer T Cell Expansion SFM, TexMACS Medium, PRIME-XV T Cell Expansion Medium, ImmunoCult-XF T Cell Expansion Medium or any combination thereof. The inhibitor of cellular DNA sensing, metabolism, differentiation, signal transduction, one or more apoptotic pathway(s) or combinations thereof comprise inhibitors of TLR9, MyD88, IRAK, TRAF6, TRAF3, IRF-7, NF-KB, Type 1 Interferons, pro-inflammatory cytokines, cGAS, STING, Sec5, TBK1, IRF-3, RNA pol III, RIG-1, IPS-1, FADD, RIP1, TRAF3, AIM2, ASC, Caspase1, Pro-IL1B, PI3K, Akt, Wnt3A, inhibitors of glycogen synthase kinase-3 β (GSK-3 β) (e.g. TWS119), Bafilomycin, Chloroquine, Quinacrine, AC-YVAD-CMK, Z-VAD-FMK, Z-IETD-FMK or any combination thereof. The reagent that modifies or stabilizes one or more nucleic acids comprises a pH modifier, a DNA-binding protein, a lipid, a phospholipid, CaPO₄, a net neutral charge DNA binding peptide with or without a NLS sequence, a TREX1 enzyme or any combination thereof.

[056] In certain embodiments of this method of expressing a CAR, the expansion and selection steps occur sequentially. The expansion may occur prior to selection. The expansion may occur following selection, and, optionally, a further (i.e. second) selection may occur following expansion.

[057] In certain embodiments of this method of expressing a CAR, the expansion and selection steps may occur simultaneously.

[058] In certain embodiments of this method of expressing a CAR, the expansion may comprise contacting at least one cell of the modified cell population with an antigen to stimulate the at least one cell through the CAR, thereby generating an expanded cell population. The antigen may be presented on the surface of a substrate. The substrate may have any form, including, but not limited to a surface, a well, a bead or a plurality thereof, and a matrix. The substrate may further comprise a paramagnetic or magnetic component. In certain embodiments of this method of expressing a CAR, the antigen may be presented on the surface of a substrate, wherein the substrate is a magnetic bead, and wherein a magnet may be used to remove or separate the magnetic beads from the modified and expanded cell population. The antigen may be presented on the surface of a cell or an artificial antigen presenting cell. Artificial antigen presenting cells of the disclosure may include, but are not limited to, tumor cells and stem cells.

[059] In certain embodiments of this method of expressing a CAR, wherein the transposon or vector comprises a selection gene and wherein the selection step comprises contacting at least one cell of the modified cell population with a compound to which the selection gene confers resistance, thereby identifying a cell expressing the selection gene as surviving the selection and identifying a cell failing to express the selection gene as failing to survive the selection step.

[060] In certain embodiments of this method of expressing a CAR, the expansion and/or selection steps may proceed for a period of 10 to 14 days, inclusive of the endpoints.

[061] The disclosure provides a composition comprising the modified, expanded and selected cell population of the methods of the disclosure.

[062] The disclosure provides a method of treating cancer in a subject in need thereof, comprising administering to the subject a composition of the disclosure, wherein the CAR specifically binds to an antigen on a tumor cell. In certain embodiments, the tumor cell may be a malignant tumor cell. In certain embodiments, comprising administering to the subject the composition comprising a modified cell or cell population of the disclosure, the cell or cell population may be autologous. In certain embodiments, comprising administering to the subject the composition comprising a modified cell or cell population of the disclosure, the cell or cell population may be allogeneic.

[063] The disclosure provides a method of treating an autoimmune condition in a subject in need thereof, comprising administering to the subject a composition of the disclosure,

wherein the CAR specifically binds to an antigen on an autoimmune cell of the subject. In certain embodiments, the autoimmune cell may be a lymphocyte that specifically binds to a self-antigen on a target cell of the subject. In certain embodiments, the autoimmune cell may be a B lymphocyte (i.e. a B cell). In certain embodiments, the autoimmune cell may be a T lymphocyte (i.e. a T cell). In certain embodiments, comprising administering to the subject the composition comprising a modified cell or cell population of the disclosure, the cell or cell population may be autologous. In certain embodiments, comprising administering to the subject the composition comprising a modified cell or cell population of the disclosure, the cell or cell population may be allogeneic.

[064] The disclosure provides a method of treating an infection in a subject in need thereof, comprising administering to the subject a composition of the disclosure, wherein the CAR specifically binds to an antigen on a cell comprising an infectious agent, a cell in communication with an infectious agent or a cell exposed to an infection agent. In some embodiments, a cell in communication with an infectious agent may be in air communication (e.g. the infectious agent is airborne or inhaled) or fluid communication (e.g. the infectious agent is carried in an aqueous or a biological fluid) with the infectious agent. The infectious agent causing the infection of the host cell may be a bacterium, a virus, a yeast, or a microbe. The infectious agent may induce in the cell or the cell's host organism (the subject), exemplary conditions including, but not limited to, a viral infection, an immunodeficiency condition, an inflammatory condition and a proliferative disorder. In certain embodiments, the infection causes tuberculosis, microencephaly, neurodegeneration or malaria. In certain embodiments, the infection causes microencephaly in a fetus of the subject. In certain embodiments, including those wherein the infection causes microencephaly in a fetus of the subject, the infectious agent is a virus and wherein the virus is a Zika virus. In certain embodiments, the immunodeficiency condition is acquired immune deficiency syndrome (AIDS). In certain embodiments, the proliferative disorder is a cancer. In certain embodiments, the cancer is cervical cancer and wherein the infectious agent is a human papilloma virus (HPV). In certain embodiments, comprising administering to the subject the composition comprising a modified cell or cell population of the disclosure, the cell or cell population may be autologous. In certain embodiments, comprising administering to the subject the composition comprising a modified cell or cell population of the disclosure, the cell or cell population may be allogeneic.

[065] The disclosure provides a method of treating a mast cell disease in a subject in need thereof, comprising administering to the subject a composition of the disclosure, wherein the

CAR specifically binds to an antigen on a mast cell. In certain embodiments, the CAR specifically binds to an antigen on a mast cell of the subject. In certain embodiments, the mast cell disease may include, but is not limited to, disorders associated with an excessive proliferation of mast cells, disorders associated with mast cells having abnormal activity, and disorders associated with both abnormal numbers of mast cells and abnormal mast cell activity. Exemplary disorders associated with an excessive proliferation of mast cells include, but are not limited to, mastocytosis, cutaneous mastocytosis (e.g., urticaria pigmentosa or maculopapular cutaneous mastocytosis), systemic mastocytosis (including mast cell leukaemia), and localized mast cell proliferations. Exemplary disorders associated with mast cells having abnormal activity, include, but are not limited to, mast cell activation syndrome (MCAS) or mast cell activation disorder (MCAD), allergic disease (including anaphylaxis), asthma, inflammatory disease (including autoimmune related inflammation of, for example, joint tissues, arthritis, etc.), or any combination thereof. In certain embodiments, comprising administering to the subject the composition comprising a modified cell or cell population of the disclosure, the cell or cell population may be autologous. In certain embodiments, comprising administering to the subject the composition comprising a modified cell or cell population of the disclosure, the cell or cell population may be allogeneic.

The disclosure provides a method of treating a degenerative disease in a subject in need thereof, comprising administering to the subject a composition of the disclosure, wherein the CAR specifically binds to an antigen on a deleterious cell or an aged cell. In certain embodiments, the CAR specifically binds to an antigen on a deleterious cell or an aged cell of the subject. In certain embodiments, the degenerative disease may include, but is not limited to, a neurodegenerative disorder, a metabolic disorder, a vascular disorder and aging.

Exemplary neurodegenerative disorders include, but are not limited to, disorders associated with a loss of a function or efficacy of one or more of a neuron, a glial cell or a microglia. Exemplary neurodegenerative disorders include, but are not limited to, disorders associated with an accumulation of one or more of a signaling molecule, a protein, or a prion that interferes with a function or decreases an efficacy of one or more of a neuron, a glial cell or a microglia. Exemplary metabolic disorders include, but are not limited to, disorders associated with mitochondrial disorders, interruptions of the electron transport chain, interruptions of cellular production of ATP, a loss of a function or a decreased efficacy of one or more mitochondria of one or more of a neuron, a glial cell or a microglia. Exemplary metabolic disorders include, but are not limited to, disorders associated with a loss of circulating blood flow or a decreased blood flow to a neuron, a glial cell or a microglia (e.g. a stroke); a

transient or permanent state of hypoxia in a neuron, a glial cell or a microglia (for example, sufficient to release free radicals in a cell); a loss of circulating CNS or a decreased CNS flow to a neuron, a glial cell or a microglia during a sleep state of the subject sufficient to decrease efficacy of removal of a waste product of a neuron, a glial cell or a microglia during that sleep state. Exemplary aging disorders include, but are not limited to, disorders associated with an increased shortened or shortened telomeres on one or more chromosomes of a neuron, a glial cell or a microglia; a loss of a function or a decreased efficacy of telomerase in a neuron, a glial cell or a microglia; or a loss of a function or a decreased efficacy of a DNA repair mechanism in a neuron, a glial cell or a microglia. In certain embodiments, the deleterious cell or the aged cell interferes with a function or decreases an efficacy of another cell in a network comprising the deleterious cell or the aged cell and the targeted removal of the deleterious cell or the aged cell improves or restores a function or increases an efficacy of the network. In certain embodiments, the deleterious cell or the aged cell may transform the function or efficacy of a second cell and the targeted removal of the deleterious cell or the aged cell prevents the transformation of the second cell. In certain embodiments, the degenerative disease is a neurodegenerative disorder and the deleterious cell or the aged cell is a stem cell, an immune cell, a neuron, a glia or a microglia. In certain embodiments, the degenerative disease is a metabolic disorder and the deleterious cell or the aged cell is a stem cell, a somatic cell, a neuron, a glia or a microglia. In certain embodiments, the degenerative disease is a vascular disorder and the deleterious cell or the aged cell is a stem cell, a somatic cell, an immune cell, an endothelial cell, a neuron, a glia or a microglia. In certain embodiments, the degenerative disease is aging and the deleterious cell or the aged cell is an oocyte, a sperm, a stem cell, a somatic cell, an immune cell, an endothelial cell, a neuron, a glia or a microglia. In certain embodiments, comprising administering to the subject the composition comprising a modified cell or cell population of the disclosure, the cell or cell population may be autologous. In certain embodiments, comprising administering to the subject the composition comprising a modified cell or cell population of the disclosure, the cell or cell population may be allogeneic.

[066] The disclosure provides a method of modifying a cell therapy in a subject in need thereof, comprising administering to the subject a composition comprising a cell comprising a transposon or vector of the composition comprising an inducible proapoptotic polypeptide, wherein apoptosis may be selectively induced in the cell by contacting the cell with an induction agent. In certain embodiments, the cell is autologous. In certain embodiments, the cell is allogeneic. In certain embodiments of this method, the cell therapy is an adoptive cell

therapy. In certain embodiments of this method, modifying the cell therapy comprises a termination of the cell therapy. In certain embodiments of this method, modifying the cell therapy comprises a depletion of a portion of the cells provided in the cell therapy. In certain embodiments, the method further comprises the step of administering an inhibitor of the induction agent to inhibit modification of the cell therapy, thereby restoring the function and/or efficacy of the cell therapy.

[067] Methods of modifying a cell therapy of the disclosure may be used to terminate or dampen a therapy in response to, for example, a sign of recovery or a sign of decreasing disease severity/progression, a sign of disease remission/cessation, and/or the occurrence of an adverse event. Cell therapies of the disclosure may be resumed by inhibiting the induction agent should a sign or symptom of the disease reappear or increase in severity and/or an adverse event is resolved.

BRIEF DESCRIPTION OF THE DRAWINGS

[068] Figure 1 is a schematic diagram depicting a piggyBac CARTyrin construct of the disclosure of 7676 base pairs that includes a transposon comprising a CARTyrin (comprising a CD8a signal peptide, a Centyrin, a CD8a hinge sequence, and a transmembrane sequence, and a CD3z costimulatory domain).

[069] Figure 2 is a schematic diagram of the amino acid sequence of a P-BCMA -101 construct of the disclosure.

[070] Figure 3A-B is a schematic diagram of the nucleic acid sequence of a P-BCMA -101 construct of the disclosure.

[071] Figure 4 is a schematic diagram depicting the construction of a CARTyrin of the disclosure and a table contrasting characteristics of Centyrins and antibodies.

[072] Figure 5A is a series of cell sorting plots depicting CARTyrin expression following electroporation with 5 µg of CARTyrin mRNA.

[073] Figure 5B, left panel, is a series of cell sorting plots depicting CARTyrin function following challenge with the control K562 cell line and the BCMA expressing H929 cell line and, right panel, a graph showing a quantification of the plots of the left panel (with the addition of data from challenge with the BCMA-expressing line U266).

[074] Figure 5C is a graphs depicting CARTyrin activity as a function of amount of mRNA used during electroporation of T-cells.

[075] Figure 6 is a schematic diagram depicting an in vivo tumor challenge study timeline using the A08 CARTyrin in mice.

[076] Figure 7 is a pair of graphs showing a complete (100%) survival of A08 CARTyrin-treated mice. Tumor burden was assessed by presence of M-protein. There was no detectable M-protein in protected animals.

[077] Figure 8 is a schematic diagram depicting an exemplary inducible truncated caspase 9 polypeptide of the disclosure.

[078] Figure 9 is a series of flow cytometry plots depicting the abundance of cells moving from an area of live cells (the gated lower right quadrant) to an area populated by apoptotic cells (the upper left quadrant) as a function of increasing dosage of the induction agent (AP1903) in cells modified to express a therapeutic agent (a CARTyrin) alone or in combination with an inducible caspase polypeptide of the disclosure (encoded by an iC9 construct (also known as a “safety switch”) introduced into cells by a piggyBac (PB) transposase) at day 12 post nucleofection.

[079] Figure 10 is a series of flow cytometry plots depicting the abundance of cells moving from an area of live cells (the gated lower right quadrant) to an area populated by apoptotic cells (the upper left quadrant) as a function of increasing dosage of the induction agent (AP1903) in cells modified to express a therapeutic agent (a CARTyrin) alone or in combination with an inducible caspase polypeptide of the disclosure (encoded by an iC9 construct (also known as a “safety switch”) introduced into cells by a piggyBac (PB) transposase) at day 19 post nucleofection.

[080] Figure 11 is a pair of graphs depicting a quantification of the aggregated results shown either in Figure 9 (left graph) or Figure 10 (right graph). Specifically, these graphs show the impact of the iC9 safety switch on the percent cell viability as a function of the concentration of the induction agent (AP1903) of the iC9 switch for each modified cell type at either day 12 (Figure 9 and left graph) or day 19 (Figure 10 and right graph).

DETAILED DESCRIPTION

[081] The disclosure provides chimeric antigen receptors comprising at least one Centyrin. Chimeric antigen receptors of the disclosure may comprise more than one Centyrin. For example, a bi-specific CAR may comprise two Centyrins that specifically bind two distinct antigens.

[082] Centyrins of the disclosure specifically bind to an antigen. Chimeric antigen receptors of the disclosure comprising one or more Centyrins that specifically bind an antigen may be used to direct the specificity of a cell, (e.g. a cytotoxic immune cell) towards the specific antigen.

[083] Centyrins of the disclosure may comprise a consensus sequence comprising LPAPKNLVVSEVTEDSLRLSWTAPDAAFDSEFLIQYQESEKVGGEAINLTVPGSERSYDL TGLKPGTEYTVSIYGVKGGHRSNPLSAEFTT (SEQ ID NO: 1).

[084] Chimeric antigen receptors of the disclosure may comprise a signal peptide of human CD2, CD3 δ , CD3 ϵ , CD3 γ , CD3 ζ , CD4, CD8 α , CD19, CD28, 4-1BB or GM-CSFR. A hinge/spacer domain of the disclosure may comprise a hinge/spacer/stalk of human CD8 α , IgG4, and/or CD4. An intracellular domain or endodomain of the disclosure may comprise an intracellular signaling domain of human CD3 ζ and may further comprise human 4-1BB, CD28, CD40, ICOS, MyD88, OX-40 intracellular segment, or any combination thereof. Exemplary transmembrane domains include, but are not limited to a human CD2, CD3 δ , CD3 ϵ , CD3 γ , CD3 ζ , CD4, CD8 α , CD19, CD28, 4-1BB or GM-CSFR transmembrane domain.

[085] The disclosure provides genetically modified cells, such as T cells, NK cells, hematopoietic progenitor cells, peripheral blood (PB) derived T cells (including T cells from G-CSF-mobilized peripheral blood), umbilical cord blood (UCB) derived T cells rendered specific for one or more antigens by introducing to these cells a CAR and/or CARTyrin of the disclosure. Cells of the disclosure may be modified by electrotransfer of a transposon encoding a CAR or CARTyrin of the disclosure and a plasmid comprising a sequence encoding a transposase of the disclosure (preferably, the sequence encoding a transposase of the disclosure is an mRNA sequence).

[086] Transposons of the disclosure be episomally maintained or integrated into the genome of the recombinant/modified cell. The transposon may be part of a two component piggyBac system that utilizes a transposon and transposase for enhanced non-viral gene transfer. In certain embodiments of this method, the transposon is a plasmid DNA transposon with a sequence encoding the chimeric antigen receptor flanked by two cis-regulatory insulator elements. In certain embodiments, the transposon is a piggyBac transposon. In certain embodiments, and, in particular, those embodiments wherein the transposon is a piggyBac transposon, the transposase is a piggyBacTM or a Super piggyBacTM (SPB) transposase.

[087] In certain embodiments of the methods of the disclosure, the transposon is a plasmid DNA transposon with a sequence encoding the antigen receptor flanked by two cis-regulatory insulator elements. In certain embodiments, the transposon is a piggyBac transposon. In certain embodiments, and, in particular, those embodiments wherein the transposon is a piggyBac transposon, the transposase is a piggyBacTM or a Super piggyBacTM (SPB) transposase. In certain embodiments, and, in particular, those embodiments wherein the

transposase is a Super piggyBac™ (SPB) transposase, the sequence encoding the transposase is an mRNA sequence.

[088] In certain embodiments of the methods of the disclosure, the transposase enzyme is a piggyBac™ (PB) transposase enzyme. The piggyBac (PB) transposase enzyme may comprise or consist of an amino acid sequence at least 75%, 80%, 85%, 90%, 95%, 99% or any percentage in between identical to:

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1  MGSSLDDEHI  LSALLQSDDE  LVGEDSDSEI  SDHVSEDDVQ  SDTEEFIDE  VHEVQPTSSG
61  SEILDEQNVI  EQPGSSLASN  RILTLPQRTI  RGKNKHCWST  SKSTRRSRVS  ALNIVRSQRG
121 PTRMCRNIYD  PLLCFKLFFT  DEIISEIVKW  TNAEISLKRR  ESMTGATFRD  TNEDEIYAFF
181 GILVMTAVRK  DNHMSTDDL  DRSLSMVYVS  VMSRDRFDFL  IRCLRMDDKS  IRPTLRENDV
241 FTPVRKIWDL  FIHQCIQNYT  PGAHLTIDEQ  LLGFRGRCPF  RMYIPNKPSK  YGIKILMMCD
301 SGYKYMINGM  PYLGRGTQTN  GVPLGEYYVK  ELSKPVHGSC  RNITCDNWFT  SIPLAKNLLQ
361 EPYKLTIVGT  VRSNKREIPE  VLKNSRSPV  GTSMFCFDGP  LTLVSYKPKP  AKMVYLLSSC
421 DEDASINEST  GKPQMVMYYN  QTKGGVDTLD  QMCSVMTCR  KTNRWPMALL  YGMINIACIN
481 SFIIYSHNVS  SKGEKVQSRK  KFMRNLYMSL  TSSFMRKRLE  APTLKRYLRD  NISNILPNEV
541 PGTSDDSTEE  PVMKKRTYCT  YCPSKIRRKA  NASCKKCKKV  ICREHNIDMC  QSCF (SEQ ID NO:
12) .

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[089] In certain embodiments of the methods of the disclosure, the transposase enzyme is a piggyBac™ (PB) transposase enzyme that comprises or consists of an amino acid sequence having an amino acid substitution at one or more of positions 30, 165, 282, or 538 of the sequence:

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1  MGSSLDDEHI  LSALLQSDDE  LVGEDSDSEI  SDHVSEDDVQ  SDTEEFIDE  VHEVQPTSSG
61  SEILDEQNVI  EQPGSSLASN  RILTLPQRTI  RGKNKHCWST  SKSTRRSRVS  ALNIVRSQRG
121 PTRMCRNIYD  PLLCFKLFFT  DEIISEIVKW  TNAEISLKRR  ESMTGATFRD  TNEDEIYAFF
181 GILVMTAVRK  DNHMSTDDL  DRSLSMVYVS  VMSRDRFDFL  IRCLRMDDKS  IRPTLRENDV
241 FTPVRKIWDL  FIHQCIQNYT  PGAHLTIDEQ  LLGFRGRCPF  RMYIPNKPSK  YGIKILMMCD
301 SGYKYMINGM  PYLGRGTQTN  GVPLGEYYVK  ELSKPVHGSC  RNITCDNWFT  SIPLAKNLLQ
361 EPYKLTIVGT  VRSNKREIPE  VLKNSRSPV  GTSMFCFDGP  LTLVSYKPKP  AKMVYLLSSC
421 DEDASINEST  GKPQMVMYYN  QTKGGVDTLD  QMCSVMTCR  KTNRWPMALL  YGMINIACIN
481 SFIIYSHNVS  SKGEKVQSRK  KFMRNLYMSL  TSSFMRKRLE  APTLKRYLRD  NISNILPNEV
541 PGTSDDSTEE  PVMKKRTYCT  YCPSKIRRKA  NASCKKCKKV  ICREHNIDMC  QSCF (SEQ ID NO:
12) .

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[090] In certain embodiments, the transposase enzyme is a piggyBac™ (PB) transposase enzyme that comprises or consists of an amino acid sequence having an amino acid substitution at two or more of positions 30, 165, 282, or 538 of the sequence of SEQ ID NO: 12. In certain embodiments, the transposase enzyme is a piggyBac™ (PB) transposase enzyme that comprises or consists of an amino acid sequence having an amino acid

substitution at three or more of positions 30, 165, 282, or 538 of the sequence of SEQ ID NO: 12. In certain embodiments, the transposase enzyme is a piggyBac™ (PB) transposase enzyme that comprises or consists of an amino acid sequence having an amino acid substitution at each of the following positions 30, 165, 282, and 538 of the sequence of SEQ ID NO: 12. In certain embodiments, the amino acid substitution at position 30 of the sequence of SEQ ID NO: 12 is a substitution of a valine (V) for an isoleucine (I). In certain embodiments, the amino acid substitution at position 165 of the sequence of SEQ ID NO: 12 is a substitution of a serine (S) for a glycine (G). In certain embodiments, the amino acid substitution at position 282 of the sequence of SEQ ID NO: 12 is a substitution of a valine (V) for a methionine (M). In certain embodiments, the amino acid substitution at position 538 of the sequence of SEQ ID NO: 12 is a substitution of a lysine (K) for an asparagine (N).

[091] In certain embodiments of the methods of the disclosure, the transposase enzyme is a Super piggyBac™ (sPBo) transposase enzyme. In certain embodiments, the Super piggyBac™ (sPBo) transposase enzymes of the disclosure may comprise or consist of the amino acid sequence of the sequence of SEQ ID NO: 12 wherein the amino acid substitution at position 30 is a substitution of a valine (V) for an isoleucine (I), the amino acid substitution at position 165 is a substitution of a serine (S) for a glycine (G), the amino acid substitution at position 282 is a substitution of a valine (V) for a methionine (M), and the amino acid substitution at position 538 is a substitution of a lysine (K) for an asparagine (N). In certain embodiments, the Super piggyBac™ (sPBo) transposase enzyme may comprise or consist of an amino acid sequence at least 75%, 80%, 85%, 90%, 95%, 99% or any percentage in between identical to:

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1  MGSSLDDEHI  LSALLQSDDE  LVGEDSDSEV  SDHVSDDVQ  SDTEEFIDE  VHEVQPTSSG
61  SEILDEQNVI  EQPGSSLASN  RILTLPQRTI  RGKNKHCWST  SKSTRSRVS  ALNIVRSQRG
121 PTRMCRNIYD  PLLCFKLFFT  DEIISEIVKW  TNAEISLKRR  ESMTSATFRD  TNEDEIYAFF
181 GILVMTAVRK  DNHMSTDDL  DRSLSMVYVS  VMSRDRFDFL  IRCLRMDDKS  IRPTLRENDV
241 FTPVRKIIDL  FIHQCIQNYT  PGAHLTIDEQ  LLGFRGRCPF  RVYIPNKPSK  YGIKILMMCD
301 SGTKYMINGM  PYLGRGTQTN  GVPLGEYVVK  ELSKPVHGSC  RNITCDNWFT  SIPLAKNLLQ
361 EPYKLTIYGT  VRSNKREIPE  VLKNSRSRPV  GTSMFCFDGP  LTLVSYKPKP  AKMVYLLSSC
421 DEDASINEST  GKPQMVMYYN  QTKGGVDTLD  QMCSVMTCR  KTNRWPMALL  YGMINIACIN
481 SFIIYSHNVS  SKGEKVQSRK  KFMRNLYMSL  TSSFMRKRLE  APTLKRYLRD  NISNILPKEV
541 PGTSDDSTEE  PVMKKRTYCT  YCPSKIRRKA  NASCKKCKKV  ICREHNIDMC  QSCF (SEQ ID NO:
2) .

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[092] In certain embodiments of the methods of the disclosure, including those embodiments wherein the transposase comprises the above-described mutations at positions 30, 165, 282 and/or 538, the piggyBac™ or Super piggyBac™ transposase enzyme may

further comprise an amino acid substitution at one or more of positions 3, 46, 82, 103, 119, 125, 177, 180, 185, 187, 200, 207, 209, 226, 235, 240, 241, 243, 258, 296, 298, 311, 315, 319, 327, 328, 340, 421, 436, 456, 470, 486, 503, 552, 570 and 591 of the sequence of SEQ ID NO: 12 or SEQ ID NO: 2. In certain embodiments, including those embodiments wherein the transposase comprises the above-described mutations at positions 30, 165, 282 and/or 538, the piggyBac™ or Super piggyBac™ transposase enzyme may further comprise an amino acid substitution at one or more of positions 46, 119, 125, 177, 180, 185, 187, 200, 207, 209, 226, 235, 240, 241, 243, 296, 298, 311, 315, 319, 327, 328, 340, 421, 436, 456, 470, 485, 503, 552 and 570. In certain embodiments, the amino acid substitution at position 3 of SEQ ID NO: 12 or SEQ ID NO: 2 is a substitution of an asparagine (N) for a serine (S). In certain embodiments, the amino acid substitution at position 46 of SEQ ID NO: 12 or SEQ ID NO: 2 is a substitution of a serine (S) for an alanine (A). In certain embodiments, the amino acid substitution at position 46 of SEQ ID NO: 12 or SEQ ID NO: 2 is a substitution of a threonine (T) for an alanine (A). In certain embodiments, the amino acid substitution at position 82 of SEQ ID NO: 12 or SEQ ID NO: 2 is a substitution of a tryptophan (W) for an isoleucine (I). In certain embodiments, the amino acid substitution at position 103 of SEQ ID NO: 12 or SEQ ID NO: 2 is a substitution of a proline (P) for a serine (S). In certain embodiments, the amino acid substitution at position 119 of SEQ ID NO: 12 or SEQ ID NO: 2 is a substitution of a proline (P) for an arginine (R). In certain embodiments, the amino acid substitution at position 125 of SEQ ID NO: 12 or SEQ ID NO: 2 is a substitution of an alanine (A) a cysteine (C). In certain embodiments, the amino acid substitution at position 125 of SEQ ID NO: 12 or SEQ ID NO: 2 is a substitution of a leucine (L) for a cysteine (C). In certain embodiments, the amino acid substitution at position 177 of SEQ ID NO: 12 or SEQ ID NO: 2 is a substitution of a lysine (K) for a tyrosine (Y). In certain embodiments, the amino acid substitution at position 177 of SEQ ID NO: 12 or SEQ ID NO: 2 is a substitution of a histidine (H) for a tyrosine (Y). In certain embodiments, the amino acid substitution at position 180 of SEQ ID NO: 12 or SEQ ID NO: 2 is a substitution of a leucine (L) for a phenylalanine (F). In certain embodiments, the amino acid substitution at position 180 of SEQ ID NO: 12 or SEQ ID NO: 2 is a substitution of an isoleucine (I) for a phenylalanine (F). In certain embodiments, the amino acid substitution at position 180 of SEQ ID NO: 12 or SEQ ID NO: 2 is a substitution of a valine (V) for a phenylalanine (F). In certain embodiments, the amino acid substitution at position 185 of SEQ ID NO: 12 or SEQ ID NO: 2 is a substitution of a leucine (L) for a methionine (M). In certain embodiments, the amino acid substitution at position 187 of SEQ ID NO: 12 or SEQ ID NO: 2 is a substitution of a

glycine (G) for an alanine (A). In certain embodiments, the amino acid substitution at position 200 of SEQ ID NO: 12 or SEQ ID NO: 2 is a substitution of a tryptophan (W) for a phenylalanine (F). In certain embodiments, the amino acid substitution at position 207 of SEQ ID NO: 12 or SEQ ID NO: 2 is a substitution of a proline (P) for a valine (V). In certain embodiments, the amino acid substitution at position 209 of SEQ ID NO: 12 or SEQ ID NO: 2 is a substitution of a phenylalanine (F) for a valine (V). In certain embodiments, the amino acid substitution at position 226 of SEQ ID NO: 12 or SEQ ID NO: 2 is a substitution of a phenylalanine (F) for a methionine (M). In certain embodiments, the amino acid substitution at position 235 of SEQ ID NO: 12 or SEQ ID NO: 2 is a substitution of an arginine (R) for a leucine (L). In certain embodiments, the amino acid substitution at position 240 of SEQ ID NO: 12 or SEQ ID NO: 12 is a substitution of a lysine (K) for a valine (V). In certain embodiments, the amino acid substitution at position 241 of SEQ ID NO: 12 or SEQ ID NO: 2 is a substitution of a leucine (L) for a phenylalanine (F). In certain embodiments, the amino acid substitution at position 243 of SEQ ID NO: 12 or SEQ ID NO: 2 is a substitution of a lysine (K) for a proline (P). In certain embodiments, the amino acid substitution at position 258 of SEQ ID NO: 12 or SEQ ID NO: 2 is a substitution of a serine (S) for an asparagine (N). In certain embodiments, the amino acid substitution at position 296 of SEQ ID NO: 12 or SEQ ID NO: 2 is a substitution of a tryptophan (W) for a leucine (L). In certain embodiments, the amino acid substitution at position 296 of SEQ ID NO: 12 or SEQ ID NO: 2 is a substitution of a tyrosine (Y) for a leucine (L). In certain embodiments, the amino acid substitution at position 296 of SEQ ID NO: 12 or SEQ ID NO: 2 is a substitution of a phenylalanine (F) for a leucine (L). In certain embodiments, the amino acid substitution at position 298 of SEQ ID NO: 12 or SEQ ID NO: 2 is a substitution of a leucine (L) for a methionine (M). In certain embodiments, the amino acid substitution at position 298 of SEQ ID NO: 12 or SEQ ID NO: 2 is a substitution of an alanine (A) for a methionine (M). In certain embodiments, the amino acid substitution at position 298 of SEQ ID NO: 12 or SEQ ID NO: 2 is a substitution of a valine (V) for a methionine (M). In certain embodiments, the amino acid substitution at position 311 of SEQ ID NO: 12 or SEQ ID NO: 2 is a substitution of an isoleucine (I) for a proline (P). In certain embodiments, the amino acid substitution at position 311 of SEQ ID NO: 12 or SEQ ID NO: 2 is a substitution of a valine for a proline (P). In certain embodiments, the amino acid substitution at position 315 of SEQ ID NO: 12 or SEQ ID NO: 2 is a substitution of a lysine (K) for an arginine (R). In certain embodiments, the amino acid substitution at position 319 of SEQ ID NO: 12 or SEQ ID NO: 2 is a substitution of a glycine (G) for a threonine (T). In certain embodiments, the amino acid

substitution at position 327 of SEQ ID NO: 12 or SEQ ID NO: 2 is a substitution of an arginine (R) for a tyrosine (Y). In certain embodiments, the amino acid substitution at position 328 of SEQ ID NO: 12 or SEQ ID NO: 2 is a substitution of a valine (V) for a tyrosine (Y). In certain embodiments, the amino acid substitution at position 340 of SEQ ID NO: 12 or SEQ ID NO: 2 is a substitution of a glycine (G) for a cysteine (C). In certain embodiments, the amino acid substitution at position 340 of SEQ ID NO: 12 or SEQ ID NO: 2 is a substitution of a leucine (L) for a cysteine (C). In certain embodiments, the amino acid substitution at position 421 of SEQ ID NO: 12 or SEQ ID NO: 2 is a substitution of a histidine (H) for the aspartic acid (D). In certain embodiments, the amino acid substitution at position 436 of SEQ ID NO: 12 or SEQ ID NO: 2 is a substitution of an isoleucine (I) for a valine (V). In certain embodiments, the amino acid substitution at position 456 of SEQ ID NO: 12 or SEQ ID NO: 2 is a substitution of a tyrosine (Y) for a methionine (M). In certain embodiments, the amino acid substitution at position 470 of SEQ ID NO: 12 or SEQ ID NO: 2 is a substitution of a phenylalanine (F) for a leucine (L). In certain embodiments, the amino acid substitution at position 485 of SEQ ID NO: 12 or SEQ ID NO: 2 is a substitution of a lysine (K) for a serine (S). In certain embodiments, the amino acid substitution at position 503 of SEQ ID NO: 12 or SEQ ID NO: 2 is a substitution of a leucine (L) for a methionine (M). In certain embodiments, the amino acid substitution at position 503 of SEQ ID NO: 12 or SEQ ID NO: 2 is a substitution of an isoleucine (I) for a methionine (M). In certain embodiments, the amino acid substitution at position 552 of SEQ ID NO: 12 or SEQ ID NO: 2 is a substitution of a lysine (K) for a valine (V). In certain embodiments, the amino acid substitution at position 570 of SEQ ID NO: 12 or SEQ ID NO: 2 is a substitution of a threonine (T) for an alanine (A). In certain embodiments, the amino acid substitution at position 591 of SEQ ID NO: 12 or SEQ ID NO: 2 is a substitution of a proline (P) for a glutamine (Q). In certain embodiments, the amino acid substitution at position 591 of SEQ ID NO: 12 or SEQ ID NO: 2 is a substitution of an arginine (R) for a glutamine (Q).

[093] In certain embodiments of the methods of the disclosure, including those embodiments wherein the transposase comprises the above-described mutations at positions 30, 165, 282 and/or 538, the piggyBac™ transposase enzyme may comprise or the Super piggyBac™ transposase enzyme may further comprise an amino acid substitution at one or more of positions 103, 194, 372, 375, 450, 509 and 570 of the sequence of SEQ ID NO: 12 or SEQ ID NO: 2. In certain embodiments of the methods of the disclosure, including those embodiments wherein the transposase comprises the above-described mutations at positions 30, 165, 282 and/or 538, the piggyBac™ transposase enzyme may comprise or the Super

piggyBacTM transposase enzyme may further comprise an amino acid substitution at two, three, four, five, six or more of positions 103, 194, 372, 375, 450, 509 and 570 of the sequence of SEQ ID NO: 12 or SEQ ID NO: 2. In certain embodiments, including those embodiments wherein the transposase comprises the above-described mutations at positions 30, 165, 282 and/or 538, the piggyBacTM transposase enzyme may comprise or the Super piggyBacTM transposase enzyme may further comprise an amino acid substitution at positions 103, 194, 372, 375, 450, 509 and 570 of the sequence of SEQ ID NO: 12 or SEQ ID NO: 2. In certain embodiments, the amino acid substitution at position 103 of SEQ ID NO: 12 or SEQ ID NO: 2 is a substitution of a proline (P) for a serine (S). In certain embodiments, the amino acid substitution at position 194 of SEQ ID NO: 12 or SEQ ID NO: 2 is a substitution of a valine (V) for a methionine (M). In certain embodiments, the amino acid substitution at position 372 of SEQ ID NO: 12 or SEQ ID NO: 2 is a substitution of an alanine (A) for an arginine (R). In certain embodiments, the amino acid substitution at position 375 of SEQ ID NO: 12 or SEQ ID NO: 2 is a substitution of an alanine (A) for a lysine (K). In certain embodiments, the amino acid substitution at position 450 of SEQ ID NO: 12 or SEQ ID NO: 2 is a substitution of an asparagine (N) for an aspartic acid (D). In certain embodiments, the amino acid substitution at position 509 of SEQ ID NO: 12 or SEQ ID NO: 2 is a substitution of a glycine (G) for a serine (S). In certain embodiments, the amino acid substitution at position 570 of SEQ ID NO: 12 or SEQ ID NO: 2 is a substitution of a serine (S) for an asparagine (N). In certain embodiments, the piggyBacTM transposase enzyme may comprise a substitution of a valine (V) for a methionine (M) at position 194 of SEQ ID NO: 12. In certain embodiments, including those embodiments wherein the piggyBacTM transposase enzyme may comprise a substitution of a valine (V) for a methionine (M) at position 194 of SEQ ID NO: 12, the piggyBacTM transposase enzyme may further comprise an amino acid substitution at positions 372, 375 and 450 of the sequence of SEQ ID NO: 12 or SEQ ID NO: 2. In certain embodiments, the piggyBacTM transposase enzyme may comprise a substitution of a valine (V) for a methionine (M) at position 194 of SEQ ID NO: 12, a substitution of an alanine (A) for an arginine (R) at position 372 of SEQ ID NO: 12, and a substitution of an alanine (A) for a lysine (K) at position 375 of SEQ ID NO: 12. In certain embodiments, the piggyBacTM transposase enzyme may comprise a substitution of a valine (V) for a methionine (M) at position 194 of SEQ ID NO: 12, a substitution of an alanine (A) for an arginine (R) at position 372 of SEQ ID NO: 12, a substitution of an alanine (A) for a lysine (K) at position 375 of SEQ ID NO: 12 and a substitution of an asparagine (N) for an aspartic acid (D) at position 450 of SEQ ID NO: 12. ***Scaffold Proteins***

[094] Protein scaffolds of the disclosure may be derived from a fibronectin type III (FN3) repeat protein, encoding or complementary nucleic acids, vectors, host cells, compositions, combinations, formulations, devices, and methods of making and using them. In a preferred embodiment, the protein scaffold is comprised of a consensus sequence of multiple FN3 domains from human Tenascin-C (hereinafter “Tenascin”). In a further preferred embodiment, the protein scaffold of the present invention is a consensus sequence of 15 FN3 domains. The protein scaffolds of the disclosure can be designed to bind various molecules, for example, a cellular target protein. In a preferred embodiment, the protein scaffolds of the disclosure can be designed to bind an epitope of a wild type and/or variant form of an antigen.

[095] Protein scaffolds of the disclosure may include additional molecules or moieties, for example, the Fc region of an antibody, albumin binding domain, or other moiety influencing half-life. In further embodiments, the protein scaffolds of the disclosure may be bound to a nucleic acid molecule that may encode the protein scaffold.

[096] The disclosure provides at least one method for expressing at least one protein scaffold based on a consensus sequence of multiple FN3 domains, in a host cell, comprising culturing a host cell as described herein under conditions wherein at least one protein scaffold is expressed in detectable and/or recoverable amounts.

[097] The disclosure provides at least one composition comprising (a) a protein scaffold based on a consensus sequence of multiple FN3 domains and/or encoding nucleic acid as described herein; and (b) a suitable and/or pharmaceutically acceptable carrier or diluent.

[098] The disclosure provides a method of generating libraries of a protein scaffold based on a fibronectin type III (FN3) repeat protein, preferably, a consensus sequence of multiple FN3 domains and, more preferably, a consensus sequence of multiple FN3 domains from human Tenascin. The library is formed by making successive generations of scaffolds by altering (by mutation) the amino acids or the number of amino acids in the molecules in particular positions in portions of the scaffold, e.g., loop regions. Libraries can be generated by altering the amino acid composition of a single loop or the simultaneous alteration of multiple loops or additional positions of the scaffold molecule. The loops that are altered can be lengthened or shortened accordingly. Such libraries can be generated to include all possible amino acids at each position, or a designed subset of amino acids. The library members can be used for screening by display, such as in vitro or CIS display (DNA, RNA, ribosome display, etc.), yeast, bacterial, and phage display.

[0099] Protein scaffolds of the disclosure provide enhanced biophysical properties, such as stability under reducing conditions and solubility at high concentrations; they may be expressed and folded in prokaryotic systems, such as *E. coli*, in eukaryotic systems, such as yeast, and in in vitro transcription/translation systems, such as the rabbit reticulocyte lysate system.

[0100] The disclosure provides a method of generating a scaffold molecule that binds to a particular target by panning the scaffold library of the invention with the target and detecting binders. In other related aspects, the disclosure comprises screening methods that may be used to generate or affinity mature protein scaffolds with the desired activity, e.g., capable of binding to target proteins with a certain affinity. Affinity maturation can be accomplished by iterative rounds of mutagenesis and selection using systems, such as phage display or in vitro display. Mutagenesis during this process may be the result of site directed mutagenesis to specific scaffold residues, random mutagenesis due to error-prone PCR, DNA shuffling, and/or a combination of these techniques.

[0101] The disclosure provides an isolated, recombinant and/or synthetic protein scaffold based on a consensus sequence of fibronectin type III (FN3) repeat protein, including, without limitation, mammalian-derived scaffold, as well as compositions and encoding nucleic acid molecules comprising at least one polynucleotide encoding protein scaffold based on the consensus FN3 sequence. The disclosure further includes, but is not limited to, methods of making and using such nucleic acids and protein scaffolds, including diagnostic and therapeutic compositions, methods and devices.

[0102] The protein scaffolds of the disclosure offer advantages over conventional therapeutics, such as ability to administer locally, orally, or cross the blood-brain barrier, ability to express in *E. Coli* allowing for increased expression of protein as a function of resources versus mammalian cell expression ability to be engineered into bispecific or tandem molecules that bind to multiple targets or multiple epitopes of the same target, ability to be conjugated to drugs, polymers, and probes, ability to be formulated to high concentrations, and the ability of such molecules to effectively penetrate diseased tissues and tumors.

[0103] Moreover, the protein scaffolds possess many of the properties of antibodies in relation to their fold that mimics the variable region of an antibody. This orientation enables the FN3 loops to be exposed similar to antibody complementarity determining regions (CDRs). They should be able to bind to cellular targets and the loops can be altered, e.g., affinity matured, to improve certain binding or related properties.

[0104] Three of the six loops of the protein scaffold of the disclosure correspond topologically to the complementarity determining regions (CDRs 1-3), i.e., antigen-binding regions, of an antibody, while the remaining three loops are surface exposed in a manner similar to antibody CDRs. These loops span at or about residues 13-16, 22-28, 38-43, 51-54, 60-64, and 75-81 of SEQ ID NO: 13. Preferably, the loop regions at or about residues 22-28, 51-54, and 75-81 are altered for binding specificity and affinity. One or more of these loop regions are randomized with other loop regions and/or other strands maintaining their sequence as backbone portions to populate a library and potent binders can be selected from the library having high affinity for a particular protein target. One or more of the loop regions can interact with a target protein similar to an antibody CDR interaction with the protein.

[0105] Scaffolds of the disclosure may comprise an antibody mimetic.

[0106] The term “antibody mimetic” is intended to describe an organic compound that specifically binds a target sequence and has a structure distinct from a naturally-occurring antibody. Antibody mimetics may comprise a protein, a nucleic acid, or a small molecule. The target sequence to which an antibody mimetic of the disclosure specifically binds may be an antigen. Antibody mimetics may provide superior properties over antibodies including, but not limited to, superior solubility, tissue penetration, stability towards heat and enzymes (e.g. resistance to enzymatic degradation), and lower production costs. Exemplary antibody mimetics include, but are not limited to, an affibody, an affilin, an affimer, an affitin, an alphabody, an anticalin, and avimer (also known as avidity multimer), a DARPin (Designed Ankyrin Repeat Protein), a Fynomer, a Kunitz domain peptide, and a monobody.

[0107] Affibody molecules of the disclosure comprise a protein scaffold comprising or consisting of one or more alpha helix without any disulfide bridges. Preferably, affibody molecules of the disclosure comprise or consist of three alpha helices. For example, an affibody molecule of the disclosure may comprise an immunoglobulin binding domain. An affibody molecule of the disclosure may comprise the Z domain of protein A.

[0108] Affilin molecules of the disclosure comprise a protein scaffold produced by modification of exposed amino acids of, for example, either gamma-B crystallin or ubiquitin. Affilin molecules functionally mimic an antibody's affinity to antigen, but do not structurally mimic an antibody. In any protein scaffold used to make an affilin, those amino acids that are accessible to solvent or possible binding partners in a properly-folded protein molecule are considered exposed amino acids. Any one or more of these exposed amino acids may be modified to specifically bind to a target sequence or antigen.

[0109] Affimer molecules of the disclosure comprise a protein scaffold comprising a highly stable protein engineered to display peptide loops that provide a high affinity binding site for a specific target sequence. Exemplary affimer molecules of the disclosure comprise a protein scaffold based upon a cystatin protein or tertiary structure thereof. Exemplary affimer molecules of the disclosure may share a common tertiary structure of comprising an alpha-helix lying on top of an anti-parallel beta-sheet.

[0110] Affitin molecules of the disclosure comprise an artificial protein scaffold, the structure of which may be derived, for example, from a DNA binding protein (e.g. the DNA binding protein Sac7d). Affitins of the disclosure selectively bind a target sequence, which may be the entirety or part of an antigen. Exemplary affitins of the disclosure are manufactured by randomizing one or more amino acid sequences on the binding surface of a DNA binding protein and subjecting the resultant protein to ribosome display and selection. Target sequences of affitins of the disclosure may be found, for example, in the genome or on the surface of a peptide, protein, virus, or bacteria. In certain embodiments of the disclosure, an affitin molecule may be used as a specific inhibitor of an enzyme. Affitin molecules of the disclosure may include heat-resistant proteins or derivatives thereof.

[0111] Alphabody molecules of the disclosure may also be referred to as Cell-Penetrating Alphabodies (CPAB). Alphabody molecules of the disclosure comprise small proteins (typically of less than 10 kDa) that bind to a variety of target sequences (including antigens). Alphabody molecules are capable of reaching and binding to intracellular target sequences. Structurally, alphabody molecules of the disclosure comprise an artificial sequence forming single chain alpha helix (similar to naturally occurring coiled-coil structures). Alphabody molecules of the disclosure may comprise a protein scaffold comprising one or more amino acids that are modified to specifically bind target proteins. Regardless of the binding specificity of the molecule, alphabody molecules of the disclosure maintain correct folding and thermostability.

[0112] Anticalin molecules of the disclosure comprise artificial proteins that bind to target sequences or sites in either proteins or small molecules. Anticalin molecules of the disclosure may comprise an artificial protein derived from a human lipocalin. Anticalin molecules of the disclosure may be used in place of, for example, monoclonal antibodies or fragments thereof. Anticalin molecules may demonstrate superior tissue penetration and thermostability than monoclonal antibodies or fragments thereof. Exemplary anticalin molecules of the disclosure may comprise about 180 amino acids, having a mass of approximately 20 kDa. Structurally, anticalin molecules of the disclosure comprise a barrel structure comprising

antiparallel beta-strands pairwise connected by loops and an attached alpha helix. In preferred embodiments, anticalin molecules of the disclosure comprise a barrel structure comprising eight antiparallel beta-strands pairwise connected by loops and an attached alpha helix.

[0113] Avimer molecules of the disclosure comprise an artificial protein that specifically binds to a target sequence (which may also be an antigen). Avimers of the disclosure may recognize multiple binding sites within the same target or within distinct targets. When an avimer of the disclosure recognize more than one target, the avimer mimics function of a bi-specific antibody. The artificial protein avimer may comprise two or more peptide sequences of approximately 30-35 amino acids each. These peptides may be connected via one or more linker peptides. Amino acid sequences of one or more of the peptides of the avimer may be derived from an A domain of a membrane receptor. Avimers have a rigid structure that may optionally comprise disulfide bonds and/or calcium. Avimers of the disclosure may demonstrate greater heat stability compared to an antibody.

[0114] DARPins (Designed Ankyrin Repeat Proteins) of the disclosure comprise genetically-engineered, recombinant, or chimeric proteins having high specificity and high affinity for a target sequence. In certain embodiments, DARPins of the disclosure are derived from ankyrin proteins and, optionally, comprise at least three repeat motifs (also referred to as repetitive structural units) of the ankyrin protein. Ankyrin proteins mediate high-affinity protein-protein interactions. DARPins of the disclosure comprise a large target interaction surface.

[0115] Fynomers of the disclosure comprise small binding proteins (about 7 kDa) derived from the human Fyn SH3 domain and engineered to bind to target sequences and molecules with equal affinity and equal specificity as an antibody.

[0116] Kunitz domain peptides of the disclosure comprise a protein scaffold comprising a Kunitz domain. Kunitz domains comprise an active site for inhibiting protease activity. Structurally, Kunitz domains of the disclosure comprise a disulfide-rich alpha+beta fold. This structure is exemplified by the bovine pancreatic trypsin inhibitor. Kunitz domain peptides recognize specific protein structures and serve as competitive protease inhibitors. Kunitz domains of the disclosure may comprise Ecallantide (derived from a human lipoprotein-associated coagulation inhibitor (LACI)).

[0117] Monobodies of the disclosure are small proteins (comprising about 94 amino acids and having a mass of about 10 kDa) comparable in size to a single chain antibody. These genetically engineered proteins specifically bind target sequences including antigens. Monobodies of the disclosure may specifically target one or more distinct proteins or target

sequences. In preferred embodiments, monobodies of the disclosure comprise a protein scaffold mimicking the structure of human fibronectin, and more preferably, mimicking the structure of the tenth extracellular type III domain of fibronectin. The tenth extracellular type III domain of fibronectin, as well as a monobody mimetic thereof, contains seven beta sheets forming a barrel and three exposed loops on each side corresponding to the three complementarity determining regions (CDRs) of an antibody. In contrast to the structure of the variable domain of an antibody, a monobody lacks any binding site for metal ions as well as a central disulfide bond. Multispecific monobodies may be optimized by modifying the loops BC and FG. Monobodies of the disclosure may comprise an adnectin.

[0118] Such a method can comprise administering an effective amount of a composition or a pharmaceutical composition comprising at least one scaffold protein to a cell, tissue, organ, animal or patient in need of such modulation, treatment, alleviation, prevention, or reduction in symptoms, effects or mechanisms. The effective amount can comprise an amount of about 0.001 to 500 mg/kg per single (e.g., bolus), multiple or continuous administration, or to achieve a serum concentration of 0.01-5000 µg/ml serum concentration per single, multiple, or continuous administration, or any effective range or value therein, as done and determined using known methods, as described herein or known in the relevant arts.

Production and Generation of Scaffold Proteins

[0119] At least one scaffold protein of the disclosure can be optionally produced by a cell line, a mixed cell line, an immortalized cell or clonal population of immortalized cells, as well known in the art. See, e.g., Ausubel, et al., ed., *Current Protocols in Molecular Biology*, John Wiley & Sons, Inc., NY, N.Y. (1987-2001); Sambrook, et al., *Molecular Cloning: A Laboratory Manual*, 2nd Edition, Cold Spring Harbor, N.Y. (1989); Harlow and Lane, *Antibodies, a Laboratory Manual*, Cold Spring Harbor, N.Y. (1989); Colligan, et al., eds., *Current Protocols in Immunology*, John Wiley & Sons, Inc., NY (1994-2001); Colligan et al., *Current Protocols in Protein Science*, John Wiley & Sons, NY, N.Y., (1997-2001).

[0120] Amino acids from a scaffold protein can be altered, added and/or deleted to reduce immunogenicity or reduce, enhance or modify binding, affinity, on-rate, off-rate, avidity, specificity, half-life, stability, solubility or any other suitable characteristic, as known in the art.

[0121] Optionally, scaffold proteins can be engineered with retention of high affinity for the antigen and other favorable biological properties. To achieve this goal, the scaffold proteins can be optionally prepared by a process of analysis of the parental sequences and various conceptual engineered products using three-dimensional models of the parental and

engineered sequences. Three-dimensional models are commonly available and are familiar to those skilled in the art. Computer programs are available which illustrate and display probable three-dimensional conformational structures of selected candidate sequences and can measure possible immunogenicity (e.g., Immunofilter program of Xencor, Inc. of Monrovia, Calif.). Inspection of these displays permits analysis of the likely role of the residues in the functioning of the candidate sequence, i.e., the analysis of residues that influence the ability of the candidate scaffold protein to bind its antigen. In this way, residues can be selected and combined from the parent and reference sequences so that the desired characteristic, such as affinity for the target antigen(s), is achieved. Alternatively, or in addition to, the above procedures, other suitable methods of engineering can be used.

Screening of Scaffold Proteins

[0122] Screening protein scaffolds for specific binding to similar proteins or fragments can be conveniently achieved using nucleotide (DNA or RNA display) or peptide display libraries, for example, in vitro display. This method involves the screening of large collections of peptides for individual members having the desired function or structure. The displayed nucleotide or peptide sequences can be from 3 to 5000 or more nucleotides or amino acids in length, frequently from 5-100 amino acids long, and often from about 8 to 25 amino acids long. In addition to direct chemical synthetic methods for generating peptide libraries, several recombinant DNA methods have been described. One type involves the display of a peptide sequence on the surface of a bacteriophage or cell. Each bacteriophage or cell contains the nucleotide sequence encoding the particular displayed peptide sequence. Such methods are described in PCT Patent Publication Nos. 91/17271, 91/18980, 91/19818, and 93/08278.

[0123] Other systems for generating libraries of peptides have aspects of both in vitro chemical synthesis and recombinant methods. See, PCT Patent Publication Nos. 92/05258, 92/14843, and 96/19256. See also, U.S. Pat. Nos. 5,658,754; and 5,643,768. Peptide display libraries, vector, and screening kits are commercially available from such suppliers as Invitrogen (Carlsbad, Calif.), and Cambridge Antibody Technologies (Cambridgeshire, UK). See, e.g., U.S. Pat. Nos. 4,704,692, 4,939,666, 4,946,778, 5,260,203, 5,455,030, 5,518,889, 5,534,621, 5,656,730, 5,763,733, 5,767,260, 5,856,456, assigned to Enzon; 5,223,409, 5,403,484, 5,571,698, 5,837,500, assigned to Dyax, 5,427,908, 5,580,717, assigned to Affymax; 5,885,793, assigned to Cambridge Antibody Technologies; 5,750,373, assigned to Genentech, 5,618,920, 5,595,898, 5,576,195, 5,698,435, 5,693,493, 5,698,417, assigned to Xoma, Colligan, supra; Ausubel, supra; or Sambrook, supra.

[0124] The protein scaffolds of the disclosure can bind human or other mammalian proteins with a wide range of affinities (KD). In a preferred embodiment, at least one protein scaffold of the present invention can optionally bind to a target protein with high affinity, for example, with a KD equal to or less than about 10^{-7} M, such as but not limited to, 0.1-9.9 (or any range or value therein) $\times 10^{-8}$, 10^{-9} , 10^{-10} , 10^{-11} , 10^{-12} , 10^{-13} , 10^{-14} , 10^{-15} or any range or value therein, as determined by surface plasmon resonance or the Kinexa method, as practiced by those of skill in the art.

[0125] The affinity or avidity of a protein scaffold for an antigen can be determined experimentally using any suitable method. (See, for example, Berzofsky, et al., "Antibody-Antigen Interactions," In Fundamental Immunology, Paul, W. E., Ed., Raven Press: New York, N.Y. (1984); Kuby, Janis Immunology, W.H. Freeman and Company: New York, N.Y. (1992); and methods described herein). The measured affinity of a particular protein scaffold-antigen interaction can vary if measured under different conditions (e.g., salt concentration, pH). Thus, measurements of affinity and other antigen-binding parameters (e.g., KD, Kon, Koff) are preferably made with standardized solutions of protein scaffold and antigen, and a standardized buffer, such as the buffer described herein.

[0126] Competitive assays can be performed with the protein scaffold of the disclosure in order to determine what proteins, antibodies, and other antagonists compete for binding to a target protein with the protein scaffold of the present invention and/or share the epitope region. These assays as readily known to those of ordinary skill in the art evaluate competition between antagonists or ligands for a limited number of binding sites on a protein. The protein and/or antibody is immobilized or insolubilized before or after the competition and the sample bound to the target protein is separated from the unbound sample, for example, by decanting (where the protein/antibody was preinsolubilized) or by centrifuging (where the protein/antibody was precipitated after the competitive reaction). Also, the competitive binding may be determined by whether function is altered by the binding or lack of binding of the protein scaffold to the target protein, e.g., whether the protein scaffold molecule inhibits or potentiates the enzymatic activity of, for example, a label. ELISA and other functional assays may be used, as well known in the art.

Nucleic Acid Molecules

[0127] Nucleic acid molecules of the disclosure encoding protein scaffolds can be in the form of RNA, such as mRNA, hnRNA, tRNA or any other form, or in the form of DNA, including, but not limited to, cDNA and genomic DNA obtained by cloning or produced synthetically, or any combinations thereof. The DNA can be triple-stranded, double-stranded

or single-stranded, or any combination thereof. Any portion of at least one strand of the DNA or RNA can be the coding strand, also known as the sense strand, or it can be the non-coding strand, also referred to as the anti-sense strand.

[0128] Isolated nucleic acid molecules of the disclosure can include nucleic acid molecules comprising an open reading frame (ORF), optionally, with one or more introns, e.g., but not limited to, at least one specified portion of at least one protein scaffold; nucleic acid molecules comprising the coding sequence for a protein scaffold or loop region that binds to the target protein; and nucleic acid molecules which comprise a nucleotide sequence substantially different from those described above but which, due to the degeneracy of the genetic code, still encode the protein scaffold as described herein and/or as known in the art. Of course, the genetic code is well known in the art. Thus, it would be routine for one skilled in the art to generate such degenerate nucleic acid variants that code for specific protein scaffolds of the present invention. See, e.g., Ausubel, et al., supra, and such nucleic acid variants are included in the present invention.

[0129] As indicated herein, nucleic acid molecules of the disclosure which comprise a nucleic acid encoding a protein scaffold can include, but are not limited to, those encoding the amino acid sequence of a protein scaffold fragment, by itself; the coding sequence for the entire protein scaffold or a portion thereof; the coding sequence for a protein scaffold, fragment or portion, as well as additional sequences, such as the coding sequence of at least one signal leader or fusion peptide, with or without the aforementioned additional coding sequences, such as at least one intron, together with additional, non-coding sequences, including but not limited to, non-coding 5' and 3' sequences, such as the transcribed, non-translated sequences that play a role in transcription, mRNA processing, including splicing and polyadenylation signals (for example, ribosome binding and stability of mRNA); an additional coding sequence that codes for additional amino acids, such as those that provide additional functionalities. Thus, the sequence encoding a protein scaffold can be fused to a marker sequence, such as a sequence encoding a peptide that facilitates purification of the fused protein scaffold comprising a protein scaffold fragment or portion.

Polynucleotides Selectively Hybridizing to a Polynucleotide as Described Herein

[0130] The disclosure provides isolated nucleic acids that hybridize under selective hybridization conditions to a polynucleotide disclosed herein. Thus, the polynucleotides of this embodiment can be used for isolating, detecting, and/or quantifying nucleic acids comprising such polynucleotides. For example, polynucleotides of the present invention can be used to identify, isolate, or amplify partial or full-length clones in a deposited library. In

some embodiments, the polynucleotides are genomic or cDNA sequences isolated, or otherwise complementary to, a cDNA from a human or mammalian nucleic acid library.

[0131] Preferably, the cDNA library comprises at least 80% full-length sequences, preferably, at least 85% or 90% full-length sequences, and, more preferably, at least 95% full-length sequences. The cDNA libraries can be normalized to increase the representation of rare sequences. Low or moderate stringency hybridization conditions are typically, but not exclusively, employed with sequences having a reduced sequence identity relative to complementary sequences. Moderate and high stringency conditions can optionally be employed for sequences of greater identity. Low stringency conditions allow selective hybridization of sequences having about 70% sequence identity and can be employed to identify orthologous or paralogous sequences.

[0132] Optionally, polynucleotides of this invention will encode at least a portion of a protein scaffold encoded by the polynucleotides described herein. The polynucleotides of this invention embrace nucleic acid sequences that can be employed for selective hybridization to a polynucleotide encoding a protein scaffold of the present invention. See, e.g., Ausubel, *supra*; Colligan, *supra*, each entirely incorporated herein by reference.

Construction of Nucleic Acids

[0133] The isolated nucleic acids of the disclosure can be made using (a) recombinant methods, (b) synthetic techniques, (c) purification techniques, and/or (d) combinations thereof, as well-known in the art.

[0134] The nucleic acids can conveniently comprise sequences in addition to a polynucleotide of the present invention. For example, a multi-cloning site comprising one or more endonuclease restriction sites can be inserted into the nucleic acid to aid in isolation of the polynucleotide. Also, translatable sequences can be inserted to aid in the isolation of the translated polynucleotide of the disclosure. For example, a hexa-histidine marker sequence provides a convenient means to purify the proteins of the disclosure. The nucleic acid of the disclosure, excluding the coding sequence, is optionally a vector, adapter, or linker for cloning and/or expression of a polynucleotide of the disclosure.

[0135] Additional sequences can be added to such cloning and/or expression sequences to optimize their function in cloning and/or expression, to aid in isolation of the polynucleotide, or to improve the introduction of the polynucleotide into a cell. Use of cloning vectors, expression vectors, adapters, and linkers is well known in the art. (See, e.g., Ausubel, *supra*; or Sambrook, *supra*).

Recombinant Methods for Constructing Nucleic Acids

[0136] The isolated nucleic acid compositions of this disclosure, such as RNA, cDNA, genomic DNA, or any combination thereof, can be obtained from biological sources using any number of cloning methodologies known to those of skill in the art. In some embodiments, oligonucleotide probes that selectively hybridize, under stringent conditions, to the polynucleotides of the present invention are used to identify the desired sequence in a cDNA or genomic DNA library. The isolation of RNA, and construction of cDNA and genomic libraries are well known to those of ordinary skill in the art. (See, e.g., Ausubel, *supra*; or Sambrook, *supra*).

Nucleic Acid Screening and Isolation Methods

[0137] A cDNA or genomic library can be screened using a probe based upon the sequence of a polynucleotide of the disclosure. Probes can be used to hybridize with genomic DNA or cDNA sequences to isolate homologous genes in the same or different organisms. Those of skill in the art will appreciate that various degrees of stringency of hybridization can be employed in the assay; and either the hybridization or the wash medium can be stringent. As the conditions for hybridization become more stringent, there must be a greater degree of complementarity between the probe and the target for duplex formation to occur. The degree of stringency can be controlled by one or more of temperature, ionic strength, pH and the presence of a partially denaturing solvent, such as formamide. For example, the stringency of hybridization is conveniently varied by changing the polarity of the reactant solution through, for example, manipulation of the concentration of formamide within the range of 0% to 50%. The degree of complementarity (sequence identity) required for detectable binding will vary in accordance with the stringency of the hybridization medium and/or wash medium. The degree of complementarity will optimally be 100%, or 70-100%, or any range or value therein. However, it should be understood that minor sequence variations in the probes and primers can be compensated for by reducing the stringency of the hybridization and/or wash medium.

[0138] Methods of amplification of RNA or DNA are well known in the art and can be used according to the disclosure without undue experimentation, based on the teaching and guidance presented herein.

[0139] Known methods of DNA or RNA amplification include, but are not limited to, polymerase chain reaction (PCR) and related amplification processes (see, e.g., U.S. Pat. Nos. 4,683,195, 4,683,202, 4,800,159, 4,965,188, to Mullis, et al.; 4,795,699 and 4,921,794 to Tabor, et al; 5,142,033 to Innis; 5,122,464 to Wilson, et al.; 5,091,310 to Innis; 5,066,584 to Gyllensten, et al; 4,889,818 to Gelfand, et al; 4,994,370 to Silver, et al; 4,766,067 to Biswas;

4,656,134 to Ringold) and RNA mediated amplification that uses anti-sense RNA to the target sequence as a template for double-stranded DNA synthesis (U.S. Pat. No. 5,130,238 to Malek, et al, with the tradename NASBA), the entire contents of which references are incorporated herein by reference. (See, e.g., Ausubel, supra; or Sambrook, supra.)

[0140] For instance, polymerase chain reaction (PCR) technology can be used to amplify the sequences of polynucleotides of the disclosure and related genes directly from genomic DNA or cDNA libraries. PCR and other in vitro amplification methods can also be useful, for example, to clone nucleic acid sequences that code for proteins to be expressed, to make nucleic acids to use as probes for detecting the presence of the desired mRNA in samples, for nucleic acid sequencing, or for other purposes. Examples of techniques sufficient to direct persons of skill through in vitro amplification methods are found in Berger, supra, Sambrook, supra, and Ausubel, supra, as well as Mullis, et al., U.S. Pat. No. 4,683,202 (1987); and Innis, et al., PCR Protocols A Guide to Methods and Applications, Eds., Academic Press Inc., San Diego, Calif. (1990). Commercially available kits for genomic PCR amplification are known in the art. See, e.g., Advantage-GC Genomic PCR Kit (Clontech). Additionally, e.g., the T4 gene 32 protein (Boehringer Mannheim) can be used to improve yield of long PCR products.

Synthetic Methods for Constructing Nucleic Acids

[0141] The isolated nucleic acids of the disclosure can also be prepared by direct chemical synthesis by known methods (see, e.g., Ausubel, et al., supra). Chemical synthesis generally produces a single-stranded oligonucleotide, which can be converted into double-stranded DNA by hybridization with a complementary sequence, or by polymerization with a DNA polymerase using the single strand as a template. One of skill in the art will recognize that while chemical synthesis of DNA can be limited to sequences of about 100 or more bases, longer sequences can be obtained by the ligation of shorter sequences.

Recombinant Expression Cassettes

[0142] The disclosure further provides recombinant expression cassettes comprising a nucleic acid of the disclosure. A nucleic acid sequence of the disclosure, for example, a cDNA or a genomic sequence encoding a protein scaffold of the disclosure, can be used to construct a recombinant expression cassette that can be introduced into at least one desired host cell. A recombinant expression cassette will typically comprise a polynucleotide of the disclosure operably linked to transcriptional initiation regulatory sequences that will direct the transcription of the polynucleotide in the intended host cell. Both heterologous and non-heterologous (i.e., endogenous) promoters can be employed to direct expression of the nucleic acids of the disclosure.

[0143] In some embodiments, isolated nucleic acids that serve as promoter, enhancer, or other elements can be introduced in the appropriate position (upstream, downstream or in the intron) of a non-heterologous form of a polynucleotide of the disclosure so as to up or down regulate expression of a polynucleotide of the disclosure. For example, endogenous promoters can be altered in vivo or in vitro by mutation, deletion and/or substitution.

Vectors and Host Cells

[0144] The disclosure also relates to vectors that include isolated nucleic acid molecules of the disclosure, host cells that are genetically engineered with the recombinant vectors, and the production of at least one protein scaffold by recombinant techniques, as is well known in the art. See, e.g., Sambrook, et al., supra; Ausubel, et al., supra, each entirely incorporated herein by reference.

[0145] For example, the PB-EF1a vector may be used. The vector comprises the following nucleotide sequence:

tgtacatagattaaccctagaagataatcatattgtgacgtacgttaaagataatcatgcgtaaaattgacgcatgtgtttatcggtctgtat
tatcgaggttatttattaattgaatagatattaagttttattatattacacttacataactaataaaattcaacaacaattatttatgtttattt
atttataaaaaaaaaaactcaaaatttcttctataaagtaacaaaacttttatgaataacctgcagccccgggggatgcagagggac
agccccccccaaagccccaggatgtaattacgtccctccccgctagggggcagcagcgagcccgccggggctccgctccg
gtccggcgctcccccgcatccccgagccggcagcgtgcggggacagccgggcacggggaaggtggcacgggatcgcttctct
ctgaacgcttctcgctgctctttgagcctgcagacacctggggggatacggggaaaagttgactgtgctttcgatcgaacctaggaca
gttagctttgcaaagatggataaagttttaaacagagaggaatctttgcagctaattggacctttaggtcttgaaggagtgggaattggc
tccggtgcccgtcagtgggcagagcgacatcgccacagtccccgagaagttggggggaggggtcggcaattgaaccgggtgcct
agagaaggtggcgcggggttaaactgggaaagtgtgctgtactggctccgccttttcccgagggtgggggagaaccgtatataa
gtgcagtagtcgcccgtgaacgttctttcgcaacgggtttccgcccagaacacaggttaagtccgtgtgtgttcccgcgggcctggc
ctctttacgggttatggccttgcgtgccttgaattacttccacctggctgcagtacgtgattcttgatcccgagcttcgggttgaagtgg
gtgggagagttcagggccttgcgttaaggagccccctgcctcgtgcttgagttgaggcctggcctgggcgtggggccgcccgt
gcgaatctggtggcaccttcgcgcctgtctcgtgctttcgataagtctctagccattttaaattttgatgacctgctgcgacgtttttct
ggcaagatagctttaaagtcgggccaagatctgcacactggtatttcggttttggggccgcgggcgacggggcccgctgcgt
ccagcgacatgttcggcgaggcgggcctgcgagcgggccaccgagaatcggacggggtagtctcaagctggccggcctgc
tctggtgcttggcctcgcgcccgtgtatgccccgccctggggcggaaggctggccggctggcaccagttgcgtgagcggaa
agatggccgcttcccgccctgctgcagggagctcaaaatggaggacggcgctcgggagagcggggcggtgagtcaccaca
caaaggaaaaggccttccgtcctcagccgtcgttcatgtgactccacggagtaccggcgccgtccaggcacctcgattagtct
cgagcttttgagtagctgctttaggttggggggaggggtttatgcgatggagttccccacactgagtggtggagactgaagtta
ggccagcttggcacttgatgtaattctccttggaaattgccctttttagtttggatcttggttcattctcaagcctcagacagtggttcaaagt
tttttctccatttcaggtgtcgtgagaattctaatacgaactcactatagggtgtgctgtctcatctttggcaaagattggccaccaagctt

gtctgcaggagggctgacgcctctagacgggcccgcctccggatccacgggtaccgatcacatatgcctttaattaaactagtt
 ctatagtgtcacctaaattcccttttagtgagggttaatggccgtagggccgccaatgggtccagacatgataagatacattgatgagtt
 tggacaaaccacaactagaatgcagtgaaaaaatgctttatttgtgaaatttgtgatgctattgctttatttgaaccattataagctgcaat
 aaacaagttaacaacaacaattgcattcattttatgtttcaggttcagggggagggtgtgggagggtttttcggactctaggacctgcgcagtg
 cgcttggcgtaatcatggtcatagctgtttcctgtttccccgtatccccccagggtgtctgcaggctcaaagagcagcgagaagcggttca
 gaggaagcgatcccgtgccacctccccgtgccgggctgtcccccacgctgccggctcggggatgcggggggaggcgccgga
 ccggagcggagccccgggcggtcgtcgtgccccctagcgggggaggagacgttaattacatccctgggggctttgggggggggc
 tgtccctctcaccgggtggagctccagcttttctgaattggggccccccctcaggggtatcgatgatatctataacaagaaaatatat
 atataataagttatcacgtaagtagaacatgaataacaatataattatcgatgagttaaatcttaaaagtcacgtaaaagataatcatgcg
 tcattttgactcacgcggtcgttatagttcaaaatcagtgacacttaccgcattgacaagcacgcctcacgggagctccaagcggcgact
 gagatgtcctaaatgcacagcgacggattcgctatttagaaagagagagcaatattcaagaatgcagtcgtaattttacgcagact
 atctttctagggttaattctagctagccttaagggcgctatttgcgttgcgtcactgcccgtttccagtcgggaaacctgtcgtgccagc
 tgcattaatgaatcggccaacgcgcggggagaggcggtttgcgtattgggcgctcttccgcttctcgtcactgactcgtcgcgtcgc
 gtcgttccggtgcggcgagcggatcagctcactcaaaggcggttaatacgggtatccacagaatcaggggataacgcaggaaagaa
 catgacaaaaatcccttaacgtgagtttctgctccactgagcgtcagaccccgtagaaaagatcaaaggatcttcttgagatcctttttct
 gcgcglaatctgctgcttgaacaaaaaaaccaccgctaccagcgggtggtttgttgcggatcaagagctaccaactcttttccgaa
 ggtaactggcttcagcagagcgcagataccaatactgttcttctagtgtagccgtagttagggccaccacttcaagaactctgtagcacc
 gcctacatacctcgtcgtgtaactctgttaccagtggctgctgccagtgggcgataagtcgtgtcttaccgggttgactcaagacgata
 gttaccggataaggcgagcggctgggctgaacggggggttctgtgcacacagcccagcttgagcgaacgacctacaccgaactg
 agatacctacagcgtgagctatgagaagcgccacgcttcccgaaggagaaaggcgacaggtatccggttaagcggcagggtcg
 gaacaggagagcgcacgaggagcttccagggggaaacgcctggtatctttatagtcctgtcgggttccggacctctgacttgagcg
 tctgattttgtgatgctcgtcagggggggcgagcctatggaaaaacgccagcaacgcggccttttacggttccctggccttttctggtcc
 ttttctcacatgagattatcaaaaaggatcttcacctagatccttttaaaataaaaatgaagtttaaatcaatctaaagtatatatgagtaaa
 ctggtctgacagtcagaagaactcgtcaagaaggcgatagaaggcgatgcgtcgaatcgggagcggcgataccgtaaagcac
 gaggaagcggctcagcccattcgccccaagctcttcagcaatatcacgggtagccaacgctatgtcctgatagcgggtccgccacacc
 cagccggccacagtcgatgaatccagaaaagcggccattttccacctgatattcggcaagcaggcatcgccatgggtcacgacgag
 atcctcgcgctcgggcatgctgccttgagcctggcgaacagttcggtggcgagcccctgatgctcttcgtccagatcatcctgat
 cgacaagaccggttccatccgagtagctcgtcgtcgtatgcgatgttctgcttgggtggtcgaatgggcaggtagccggatcaagcgt
 atgcagccgcccattgcatcagccatgatggatactttctcggcaggagcaaggtgagatgacaggagatcctgccccggcacttc
 gcccaatagcagccagtccttccccgttcagtgacaacgtcgagcacagctgcgcaaggaaacgcccgtcgtggccagccacgata
 gccgcgtcgcctcgtcttgagttcattcagggcaccggacaggtcggcttgcacaaaagaaccgggcccctgcgctgacagcc
 ggaacacggcgcatcagagcagccgattgtctgtgtgccagtcataggcaatagcctctccaccaagcggcgccggagaacct
 gcgtgcaatccatcttgttaatcataatattattgaagcatttatcaggggtcgtctcgtccccgtctcctcccaatgcattgcaatattggc

cattagccatattattcattgggtatatagcataaatcaatattggctattggccattgcatacgttgatctatatcataata (SEQ ID NO: 40)

[0146] The polynucleotides can optionally be joined to a vector containing a selectable marker for propagation in a host. Generally, a plasmid vector is introduced in a precipitate, such as a calcium phosphate precipitate, or in a complex with a charged lipid. If the vector is a virus, it can be packaged in vitro using an appropriate packaging cell line and then transduced into host cells.

[0147] The DNA insert should be operatively linked to an appropriate promoter. The expression constructs will further contain sites for transcription initiation, termination and, in the transcribed region, a ribosome binding site for translation. The coding portion of the mature transcripts expressed by the constructs will preferably include a translation initiating at the beginning and a termination codon (e.g., UAA, UGA or UAG) appropriately positioned at the end of the mRNA to be translated, with UAA and UAG preferred for mammalian or eukaryotic cell expression.

[0148] Expression vectors will preferably but optionally include at least one selectable marker. Such markers include, e.g., but are not limited to, ampicillin, zeocin (*Sh bla* gene), puromycin (*pac* gene), hygromycin B (*hygB* gene), G418/Geneticin (*neo* gene), mycophenolic acid, or glutamine synthetase (GS, U.S. Pat. Nos. 5,122,464; 5,770,359; 5,827,739), blasticidin (*bsd* gene), resistance genes for eukaryotic cell culture as well as ampicillin, zeocin (*Sh bla* gene), puromycin (*pac* gene), hygromycin B (*hygB* gene), G418/Geneticin (*neo* gene), kanamycin, spectinomycin, streptomycin, carbenicillin, bleomycin, erythromycin, polymyxin B, or tetracycline resistance genes for culturing in *E. coli* and other bacteria or prokaryotics (the above patents are entirely incorporated hereby by reference). Appropriate culture mediums and conditions for the above-described host cells are known in the art. Suitable vectors will be readily apparent to the skilled artisan. Introduction of a vector construct into a host cell can be effected by calcium phosphate transfection, DEAE-dextran mediated transfection, cationic lipid-mediated transfection, electroporation, transduction, infection or other known methods. Such methods are described in the art, such as Sambrook, supra, Chapters 1-4 and 16-18; Ausubel, supra, Chapters 1, 9, 13, 15, 16.

[0149] Expression vectors will preferably but optionally include at least one selectable cell surface marker for isolation of cells modified by the compositions and methods of the disclosure. Selectable cell surface markers of the disclosure comprise surface proteins, glycoproteins, or group of proteins that distinguish a cell or subset of cells from another defined subset of cells. Preferably the selectable cell surface marker distinguishes those cells

modified by a composition or method of the disclosure from those cells that are not modified by a composition or method of the disclosure. Such cell surface markers include, e.g., but are not limited to, “cluster of designation” or “classification determinant” proteins (often abbreviated as “CD”) such as a truncated or full length form of CD19, CD271, CD34, CD22, CD20, CD33, CD52, or any combination thereof. Cell surface markers further include the suicide gene marker RQR8 (Philip B et al. Blood. 2014 Aug 21; 124(8):1277-87).

[0150] Expression vectors will preferably but optionally include at least one selectable drug resistance marker for isolation of cells modified by the compositions and methods of the disclosure. Selectable drug resistance markers of the disclosure may comprise wild-type or mutant Neo, DHFR, TYMS, FRANCEF, RAD51C, GCS, MDR1, ALDH1, NKX2.2, or any combination thereof.

[0151] At least one protein scaffold of the disclosure can be expressed in a modified form, such as a fusion protein, and can include not only secretion signals, but also additional heterologous functional regions. For instance, a region of additional amino acids, particularly charged amino acids, can be added to the N-terminus of a protein scaffold to improve stability and persistence in the host cell, during purification, or during subsequent handling and storage. Also, peptide moieties can be added to a protein scaffold of the disclosure to facilitate purification. Such regions can be removed prior to final preparation of a protein scaffold or at least one fragment thereof. Such methods are described in many standard laboratory manuals, such as Sambrook, *supra*, Chapters 17.29-17.42 and 18.1-18.74; Ausubel, *supra*, Chapters 16, 17 and 18.

[0152] Those of ordinary skill in the art are knowledgeable in the numerous expression systems available for expression of a nucleic acid encoding a protein of the disclosure. Alternatively, nucleic acids of the disclosure can be expressed in a host cell by turning on (by manipulation) in a host cell that contains endogenous DNA encoding a protein scaffold of the disclosure. Such methods are well known in the art, e.g., as described in U.S. Pat. Nos. 5,580,734, 5,641,670, 5,733,746, and 5,733,761, entirely incorporated herein by reference.

[0153] Illustrative of cell cultures useful for the production of the protein scaffolds, specified portions or variants thereof, are bacterial, yeast, and mammalian cells as known in the art. Mammalian cell systems often will be in the form of monolayers of cells although mammalian cell suspensions or bioreactors can also be used. A number of suitable host cell lines capable of expressing intact glycosylated proteins have been developed in the art, and include the COS-1 (e.g., ATCC CRL 1650), COS-7 (e.g., ATCC CRL-1651), HEK293, BHK21 (e.g., ATCC CRL-10), CHO (e.g., ATCC CRL 1610) and BSC-1 (e.g., ATCC CRL-

26) cell lines, Cos-7 cells, CHO cells, hep G2 cells, P3X63Ag8.653, SP2/0-Ag14, 293 cells, HeLa cells and the like, which are readily available from, for example, American Type Culture Collection, Manassas, Va. (www.atcc.org). Preferred host cells include cells of lymphoid origin, such as myeloma and lymphoma cells. Particularly preferred host cells are P3X63Ag8.653 cells (ATCC Accession Number CRL-1580) and SP2/0-Ag14 cells (ATCC Accession Number CRL-1851). In a particularly preferred embodiment, the recombinant cell is a P3X63Ab8.653 or an SP2/0-Ag14 cell.

[0154] Expression vectors for these cells can include one or more of the following expression control sequences, such as, but not limited to, an origin of replication; a promoter (e.g., late or early SV40 promoters, the CMV promoter (U.S. Pat. Nos. 5,168,062; 5,385,839), an HSV tk promoter, a pgk (phosphoglycerate kinase) promoter, an EF-1 alpha promoter (U.S. Pat. No. 5,266,491), at least one human promoter; an enhancer, and/or processing information sites, such as ribosome binding sites, RNA splice sites, polyadenylation sites (e.g., an SV40 large T Ag poly A addition site), and transcriptional terminator sequences. See, e.g., Ausubel et al., *supra*; Sambrook, et al., *supra*. Other cells useful for production of nucleic acids or proteins of the present invention are known and/or available, for instance, from the American Type Culture Collection Catalogue of Cell Lines and Hybridomas (www.atcc.org) or other known or commercial sources.

[0155] When eukaryotic host cells are employed, polyadenylation or transcription terminator sequences are typically incorporated into the vector. An example of a terminator sequence is the polyadenylation sequence from the bovine growth hormone gene. Sequences for accurate splicing of the transcript can also be included. An example of a splicing sequence is the VP1 intron from SV40 (Sprague, et al., *J. Virol.* 45:773-781 (1983)). Additionally, gene sequences to control replication in the host cell can be incorporated into the vector, as known in the art.

Purification of a Protein Scaffold

[0156] A protein scaffold can be recovered and purified from recombinant cell cultures by well-known methods including, but not limited to, protein A purification, ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. High performance liquid chromatography ("HPLC") can also be employed for purification. See, e.g., Colligan, *Current Protocols in Immunology*, or *Current Protocols in Protein Science*,

John Wiley & Sons, NY, N.Y., (1997-2001), e.g., Chapters 1, 4, 6, 8, 9, 10, each entirely incorporated herein by reference.

[0157] Protein scaffolds of the disclosure include naturally purified products, products of chemical synthetic procedures, and products produced by recombinant techniques from a prokaryotic or eukaryotic host, including, for example, *E. coli*, yeast, higher plant, insect and mammalian cells. Depending upon the host employed in a recombinant production procedure, the protein scaffold of the disclosure can be glycosylated or can be non-glycosylated. Such methods are described in many standard laboratory manuals, such as Sambrook, *supra*, Sections 17.37-17.42; Ausubel, *supra*, Chapters 10, 12, 13, 16, 18 and 20, Colligan, *Protein Science*, *supra*, Chapters 12-14, all entirely incorporated herein by reference.

Amino Acid Codes

[0158] The amino acids that make up protein scaffolds of the disclosure are often abbreviated. The amino acid designations can be indicated by designating the amino acid by its single letter code, its three letter code, name, or three nucleotide codon(s) as is well understood in the art (see Alberts, B., et al., *Molecular Biology of The Cell*, Third Ed., Garland Publishing, Inc., New York, 1994). A protein scaffold of the disclosure can include one or more amino acid substitutions, deletions or additions, either from natural mutations or human manipulation, as specified herein. Amino acids in a protein scaffold of the disclosure that are essential for function can be identified by methods known in the art, such as site-directed mutagenesis or alanine-scanning mutagenesis (e.g., Ausubel, *supra*, Chapters 8, 15; Cunningham and Wells, *Science* 244:1081-1085 (1989)). The latter procedure introduces single alanine mutations at every residue in the molecule. The resulting mutant molecules are then tested for biological activity, such as, but not limited to, at least one neutralizing activity. Sites that are critical for protein scaffold binding can also be identified by structural analysis, such as crystallization, nuclear magnetic resonance or photoaffinity labeling (Smith, et al., *J. Mol. Biol.* 224:899-904 (1992) and de Vos, et al., *Science* 255:306-312 (1992)).

[0159] As those of skill will appreciate, the invention includes at least one biologically active protein scaffold of the disclosure. Biologically active protein scaffolds have a specific activity at least 20%, 30%, or 40%, and, preferably, at least 50%, 60%, or 70%, and, most preferably, at least 80%, 90%, or 95%-99% or more of the specific activity of the native (non-synthetic), endogenous or related and known protein scaffold. Methods of assaying and quantifying measures of enzymatic activity and substrate specificity are well known to those of skill in the art.

[0160] In another aspect, the disclosure relates to protein scaffolds and fragments, as described herein, which are modified by the covalent attachment of an organic moiety. Such modification can produce a protein scaffold fragment with improved pharmacokinetic properties (e.g., increased in vivo serum half-life). The organic moiety can be a linear or branched hydrophilic polymeric group, fatty acid group, or fatty acid ester group. In particular embodiments, the hydrophilic polymeric group can have a molecular weight of about 800 to about 120,000 Daltons and can be a polyalkane glycol (e.g., polyethylene glycol (PEG), polypropylene glycol (PPG)), carbohydrate polymer, amino acid polymer or polyvinyl pyrrolidone, and the fatty acid or fatty acid ester group can comprise from about eight to about forty carbon atoms.

[0161] The modified protein scaffolds and fragments of the disclosure can comprise one or more organic moieties that are covalently bonded, directly or indirectly, to the antibody. Each organic moiety that is bonded to a protein scaffold or fragment of the disclosure can independently be a hydrophilic polymeric group, a fatty acid group or a fatty acid ester group. As used herein, the term “fatty acid” encompasses mono-carboxylic acids and di-carboxylic acids. A “hydrophilic polymeric group,” as the term is used herein, refers to an organic polymer that is more soluble in water than in octane. For example, polylysine is more soluble in water than in octane. Thus, a protein scaffold modified by the covalent attachment of polylysine is encompassed by the disclosure. Hydrophilic polymers suitable for modifying protein scaffolds of the disclosure can be linear or branched and include, for example, polyalkane glycols (e.g., PEG, monomethoxy-polyethylene glycol (mPEG), PPG and the like), carbohydrates (e.g., dextran, cellulose, oligosaccharides, polysaccharides and the like), polymers of hydrophilic amino acids (e.g., polylysine, polyarginine, polyaspartate and the like), polyalkane oxides (e.g., polyethylene oxide, polypropylene oxide and the like) and polyvinyl pyrrolidone. Preferably, the hydrophilic polymer that modifies the protein scaffold of the disclosure has a molecular weight of about 800 to about 150,000 Daltons as a separate molecular entity. For example, PEG5000 and PEG 20,000, wherein the subscript is the average molecular weight of the polymer in Daltons, can be used. The hydrophilic polymeric group can be substituted with one to about six alkyl, fatty acid or fatty acid ester groups. Hydrophilic polymers that are substituted with a fatty acid or fatty acid ester group can be prepared by employing suitable methods. For example, a polymer comprising an amine group can be coupled to a carboxylate of the fatty acid or fatty acid ester, and an activated carboxylate (e.g., activated with N,N-carbonyl diimidazole) on a fatty acid or fatty acid ester can be coupled to a hydroxyl group on a polymer.

[0162] Fatty acids and fatty acid esters suitable for modifying protein scaffolds of the disclosure can be saturated or can contain one or more units of unsaturation. Fatty acids that are suitable for modifying protein scaffolds of the disclosure include, for example, n-dodecanoate (C12, laurate), n-tetradecanoate (C14, myristate), n-octadecanoate (C18, stearate), n-eicosanoate (C20, arachidate), n-docosanoate (C22, behenate), n-triacontanoate (C30), n-tetracontanoate (C40), cis- Δ^9 -octadecanoate (C18, oleate), all cis- $\Delta^{5,8,11,14}$ -eicosatetraenoate (C20, arachidonate), octanedioic acid, tetradecanedioic acid, octadecanedioic acid, docosanedioic acid, and the like. Suitable fatty acid esters include mono-esters of dicarboxylic acids that comprise a linear or branched lower alkyl group. The lower alkyl group can comprise from one to about twelve, preferably, one to about six, carbon atoms.

[0163] The modified protein scaffolds and fragments can be prepared using suitable methods, such as by reaction with one or more modifying agents. A “modifying agent” as the term is used herein, refers to a suitable organic group (e.g., hydrophilic polymer, a fatty acid, a fatty acid ester) that comprises an activating group. An “activating group” is a chemical moiety or functional group that can, under appropriate conditions, react with a second chemical group thereby forming a covalent bond between the modifying agent and the second chemical group. For example, amine-reactive activating groups include electrophilic groups, such as tosylate, mesylate, halo (chloro, bromo, fluoro, iodo), N-hydroxysuccinimidyl esters (NHS), and the like. Activating groups that can react with thiols include, for example, maleimide, iodoacetyl, acryloyl, pyridyl disulfides, 5-thiol-2-nitrobenzoic acid thiol (TNB-thiol), and the like. An aldehyde functional group can be coupled to amine- or hydrazide-containing molecules, and an azide group can react with a trivalent phosphorous group to form phosphoramidate or phosphorimide linkages. Suitable methods to introduce activating groups into molecules are known in the art (see for example, Hermanson, G. T., *Bioconjugate Techniques*, Academic Press: San Diego, Calif. (1996)). An activating group can be bonded directly to the organic group (e.g., hydrophilic polymer, fatty acid, fatty acid ester), or through a linker moiety, for example, a divalent C1-C12 group wherein one or more carbon atoms can be replaced by a heteroatom, such as oxygen, nitrogen or sulfur. Suitable linker moieties include, for example, tetraethylene glycol, $-(CH_2)_3-$, $-NH-(CH_2)_6-NH-$, $-(CH_2)_2-NH-$ and $-CH_2-O-CH_2-CH_2-O-CH_2-CH_2-O-CH_2-NH-$. Modifying agents that comprise a linker moiety can be produced, for example, by reacting a mono-Boc-alkyldiamine (e.g., mono-Boc-ethylenediamine, mono-Boc-diaminohexane) with a fatty acid in the presence of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) to

form an amide bond between the free amine and the fatty acid carboxylate. The Boc protecting group can be removed from the product by treatment with trifluoroacetic acid (TFA) to expose a primary amine that can be coupled to another carboxylate, as described, or can be reacted with maleic anhydride and the resulting product cyclized to produce an activated maleimido derivative of the fatty acid. (See, for example, Thompson, et al., WO 92/16221, the entire teachings of which are incorporated herein by reference.)

[0164] The modified protein scaffolds of the disclosure can be produced by reacting a protein scaffold or fragment with a modifying agent. For example, the organic moieties can be bonded to the protein scaffold in a non-site specific manner by employing an amine-reactive modifying agent, for example, an NHS ester of PEG. Modified protein scaffolds and fragments comprising an organic moiety that is bonded to specific sites of a protein scaffold of the disclosure can be prepared using suitable methods, such as reverse proteolysis (Fisch et al., *Bioconjugate Chem.*, 3:147-153 (1992); Werlen et al., *Bioconjugate Chem.*, 5:411-417 (1994); Kumaran et al., *Protein Sci.* 6(10):2233-2241 (1997); Itoh et al., *Bioorg. Chem.*, 24(1): 59-68 (1996); Capellas et al., *Biotechnol. Bioeng.*, 56(4):456-463 (1997)), and the methods described in Hermanson, G. T., *Bioconjugate Techniques*, Academic Press: San Diego, Calif. (1996).

Protein Scaffold Compositions Comprising Further Therapeutically Active Ingredients

[0165] Protein scaffold compounds, compositions or combinations of the present disclosure can further comprise at least one of any suitable auxiliary, such as, but not limited to, diluent, binder, stabilizer, buffers, salts, lipophilic solvents, preservative, adjuvant or the like. Pharmaceutically acceptable auxiliaries are preferred. Non-limiting examples of, and methods of preparing such sterile solutions are well known in the art, such as, but limited to, Gennaro, Ed., *Remington's Pharmaceutical Sciences*, 18th Edition, Mack Publishing Co. (Easton, Pa.) 1990. Pharmaceutically acceptable carriers can be routinely selected that are suitable for the mode of administration, solubility and/or stability of the protein scaffold, fragment or variant composition as well known in the art or as described herein.

[0166] Pharmaceutical excipients and additives useful in the present composition include, but are not limited to, proteins, peptides, amino acids, lipids, and carbohydrates (e.g., sugars, including monosaccharides, di-, tri-, tetra-, and oligosaccharides; derivatized sugars, such as alditols, aldonic acids, esterified sugars and the like; and polysaccharides or sugar polymers), which can be present singly or in combination, comprising alone or in combination 1-99.99% by weight or volume. Exemplary protein excipients include serum albumin, such as human serum albumin (HSA), recombinant human albumin (rHA), gelatin, casein, and the like.

Representative amino acid/protein components, which can also function in a buffering capacity, include alanine, glycine, arginine, betaine, histidine, glutamic acid, aspartic acid, cysteine, lysine, leucine, isoleucine, valine, methionine, phenylalanine, aspartame, and the like. One preferred amino acid is glycine.

[0167] Carbohydrate excipients suitable for use in the invention include, for example, monosaccharides, such as fructose, maltose, galactose, glucose, D-mannose, sorbose, and the like; disaccharides, such as lactose, sucrose, trehalose, cellobiose, and the like; polysaccharides, such as raffinose, melezitose, maltodextrins, dextrans, starches, and the like; and alditols, such as mannitol, xylitol, maltitol, lactitol, xylitol sorbitol (glucitol), myoinositol and the like. Preferred carbohydrate excipients for use in the present invention are mannitol, trehalose, and raffinose.

[0168] Protein scaffold compositions can also include a buffer or a pH-adjusting agent; typically, the buffer is a salt prepared from an organic acid or base. Representative buffers include organic acid salts, such as salts of citric acid, ascorbic acid, gluconic acid, carbonic acid, tartaric acid, succinic acid, acetic acid, or phthalic acid; Tris, tromethamine hydrochloride, or phosphate buffers. Preferred buffers for use in the present compositions are organic acid salts, such as citrate.

[0169] Additionally, protein scaffold compositions of the invention can include polymeric excipients/additives, such as polyvinylpyrrolidones, ficolls (a polymeric sugar), dextrans (e.g., cyclodextrins, such as 2-hydroxypropyl- β -cyclodextrin), polyethylene glycols, flavoring agents, antimicrobial agents, sweeteners, antioxidants, antistatic agents, surfactants (e.g., polysorbates, such as "TWEEN 20" and "TWEEN 80"), lipids (e.g., phospholipids, fatty acids), steroids (e.g., cholesterol), and chelating agents (e.g., EDTA).

[0170] These and additional known pharmaceutical excipients and/or additives suitable for use in the protein scaffold, portion or variant compositions according to the invention are known in the art, e.g., as listed in "Remington: The Science & Practice of Pharmacy", 19th ed., Williams & Williams, (1995), and in the "Physician's Desk Reference", 52nd ed., Medical Economics, Montvale, N.J. (1998), the disclosures of which are entirely incorporated herein by reference. Preferred carrier or excipient materials are carbohydrates (e.g., saccharides and alditols) and buffers (e.g., citrate) or polymeric agents. An exemplary carrier molecule is the mucopolysaccharide, hyaluronic acid, which may be useful for intraarticular delivery.

T Cell Isolation from a Leukapheresis Product

[0171] A leukapheresis product or blood may be collected from a subject at clinical site using a closed system and standard methods (e.g., a COBE Spectra Apheresis System). Preferably, the product is collected according to standard hospital or institutional Leukapheresis procedures in standard Leukapheresis collection bags. For example, in preferred embodiments of the methods of the disclosure, no additional anticoagulants or blood additives (heparin, etc.) are included beyond those normally used during leukapheresis.

[0172] Alternatively, white blood cells (WBC)/Peripheral Blood Mononuclear Cells (PBMC) (using Biosafe Sepax 2 (Closed/Automated)) or T cells (using CliniMACS® Prodigy (Closed/Automated)) may be isolated directly from whole blood. However, in certain subjects (e.g. those diagnosed and/or treated for cancer), the WBC/PBMC yield may be significantly lower when isolated from whole blood than when isolated by leukapheresis.

[0173] Either the leukapheresis procedure and/or the direct cell isolation procedure may be used for any subject of the disclosure.

[0174] The leukapheresis product, blood, WBC/PBMC composition and/or T-cell composition should be packed in insulated containers and should be kept at controlled room temperature (+19°C to +25°C) according to standard hospital or institutional blood collection procedures approved for use with the clinical protocol. The leukapheresis product, blood, WBC/PBMC composition and/or T-cell composition should not be refrigerated.

[0175] The cell concentration leukapheresis product, blood, WBC/PBMC composition and/or T-cell composition should not exceed 0.2×10^9 cells per mL during transportation. Intense mixing of the leukapheresis product, blood, WBC/PBMC composition and/or T-cell composition should be avoided.

[0176] If the leukapheresis product, blood, WBC/PBMC composition and/or T-cell composition has to be stored, e.g. overnight, it should be kept at controlled room temperature (same as above). During storage, the concentration of the leukapheresis product, blood, WBC/PBMC composition and/or T-cell composition should never exceed 0.2×10^9 cell per mL.

[0177] Preferably, cells of the leukapheresis product, blood, WBC/PBMC composition and/or T-cell composition should be stored in autologous plasma. In certain embodiments, if the cell concentration of the leukapheresis product, blood, WBC/PBMC composition and/or T-cell composition is higher than 0.2×10^9 cell per mL, the product should be diluted with autologous plasma.

[0178] Preferably, the leukapheresis product, blood, WBC/PBMC composition and/or T-cell composition should not be older than 24 hours when starting the labeling and separation

procedure. The leukapheresis product, blood, WBC/PBMC composition and/or T-cell composition may be processed and/or prepared for cell labeling using a closed and/or automated system (e.g., CliniMACS Prodigy).

[0179] An automated system may perform additional buffy coat isolation, possibly by ficoliation, and/or washing of the cellular product (e.g., the leukapheresis product, blood, WBC/PBMC composition and/or T cell composition).

[0180] A closed and/or automated system may be used to prepare and label cells for T-Cell isolation (from, for example, the leukapheresis product, blood, WBC/PBMC composition and/or T cell composition).

[0181] Although WBC/PBMCs may be nucleofected directly (which is easier and saves additional steps), the methods of the disclosure may include first isolating T cells prior to nucleofection. The easier strategy of directly nucleofecting PBMC requires selective expansion of CAR+ cells that is mediated via CAR signaling, which by itself is proving to be an inferior expansion method that directly reduces the *in vivo* efficiency of the product by rendering T cells functionally exhausted. The product may be a heterogeneous composition of CAR+ cells including T cells, NK cells, NKT cells, monocytes, or any combination thereof, which increases the variability in product from patient to patient and makes dosing and CRS management more difficult. Since T cells are thought to be the primary effectors in tumor suppression and killing, T cell isolation for the manufacture of an autologous product may result in significant benefits over the other more heterogeneous composition.

[0182] T cells may be isolated directly, by enrichment of labeled cells or depletion of labeled cells in a one-way labeling procedure or, indirectly, in a two-step labeling procedure.

According to certain enrichment strategies of the disclosure, T cells may be collected in a Cell Collection Bag and the non-labeled cells (non-target cells) in a Negative Fraction Bag. In contrast to an enrichment strategy of the disclosure, the non-labeled cells (target cells) are collected in a Cell Collection Bag and the labeled cells (non-target cells) are collected in a Negative Fraction Bag or in the Non-Target Cell Bag, respectively. Selection reagents may include, but are not limited to, antibody-coated beads. Antibody-coated beads may either be removed prior to a modification and/or an expansion step, or, retained on the cells prior to a modification and/or an expansion step. One or more of the following non-limiting examples of cellular markers may be used to isolate T-cells: CD3, CD4, CD8, CD25, anti-biotin, CD1c, CD3/CD19, CD3/CD56, CD14, CD19, CD34, CD45RA, CD56, CD62L, CD133, CD137, CD271, CD304, IFN-gamma, TCR alpha/beta, and/or any combination thereof. Methods for the isolation of T-cells may include one or more reagents that specifically bind and/or

detectably-label one or more of the following non-limiting examples of cellular markers may be used to isolate T-cells: CD3, CD4, CD8, CD25, anti-biotin, CD1c, CD3/CD19, CD3/CD56, CD14, CD19, CD34, CD45RA, CD56, CD62L, CD133, CD137, CD271, CD304, IFN-gamma, TCR alpha/beta, and/or any combination thereof. These reagents may or may not be “Good Manufacturing Practices” (“GMP”) grade. Reagents may include, but are not limited to, Thermo DynaBeads and Miltenyi CliniMACS products. Methods of isolating T-cells of the disclosure may include multiple iterations of labeling and/or isolation steps. At any point in the methods of isolating T-cells of the disclosure, unwanted cells and/or unwanted cell types may be depleted from a T cell product composition of the disclosure by positively or negatively selecting for the unwanted cells and/or unwanted cell types. A T cell product composition of the disclosure may contain additional cell types that may express CD4, CD8, and/or another T cell marker(s).

[0183] Methods of the disclosure for nucleofection of T cells may eliminate the step of T cell isolation by, for example, a process for nucleofection of T cells in a population or composition of WBC/PBMCs that, following nucleofection, includes an isolation step or a selective expansion step via TCR signaling.

[0184] Certain cell populations may be depleted by positive or negative selection before or after T cell enrichment and/or sorting. Examples of cell compositions that may be depleted from a cell product composition may include myeloid cells, CD25+ regulatory T cells (T Regs), dendritic cells, macrophages, red blood cells, mast cells, gamma-delta T cells, natural killer (NK) cells, a Natural Killer (NK)-like cell (e.g. a Cytokine Induced Killer (CIK) cell), induced natural killer (iNK) T cells, NK T cells, B cells, or any combination thereof.

[0185] T cell product compositions of the disclosure may include CD4+ and CD8+ T-Cells. CD4+ and CD8+ T-Cells may be isolated into separate collection bags during an isolation or selection procedure. CD4+ T cells and CD8+ T cells may be further treated separately, or treated after reconstitution (combination into the same composition) at a particular ratio.

[0186] The particular ratio at which CD4+ T cells and CD8+ T cells may be reconstituted may depend upon the type and efficacy of expansion technology used, cell medium, and/or growth conditions utilized for expansion of T-cell product compositions. Examples of possible CD4+: CD8+ ratios include, but are not limited to, 50%:50%, 60%:40%, 40%:60%, 75%:25% and 25%:75%.

[0187] CD8+ T cells exhibit a potent capacity for tumor cell killing, while CD4+ T cells provide many of the cytokines required to support CD8+ T cell proliferative capacity and function. Because T cells isolated from normal donors are predominantly CD4+, the T-cell

product compositions are artificially adjusted in vitro with respect to the CD4+:CD8+ ratio to improve upon the ratio of CD4+ T cells to CD8+ T cells that would otherwise be present in vivo. An optimized ratio may also be used for the ex vivo expansion of the autologous T-cell product composition. In view of the artificially adjusted CD4+:CD8+ ratio of the T-cell product composition, it is important to note that the product compositions of the disclosure may be significantly different and provide significantly greater advantage than any naturally-occurring population of T-cells.

[0188] Preferred methods for T cell isolation may include a negative selection strategy for yielding untouched pan T cell, meaning that the resultant T-cell composition includes T-cells that have not been manipulated and that contain a naturally-occurring variety/ratio of T-cells.

[0189] Reagents that may be used for positive or negative selection include, but are not limited to, magnetic cell separation beads. Magnetic cell separation beads may or may not be removed or depleted from selected populations of CD4+ T cells, CD8+ T cells, or a mixed population of both CD4+ and CD8+ T cells before performing the next step in a T-cell isolation method of the disclosure.

[0190] T cell compositions and T cell product compositions may be prepared for cryopreservation, storage in standard T Cell Culture Medium, and/or genetic modification.

[0191] T cell compositions, T cell product compositions, unstimulated T cell compositions, resting T cell compositions or any portion thereof may be cryopreserved using a standard cryopreservation method optimized for storing and recovering human cells with high recovery, viability, phenotype, and/or functional capacity. Commercially-available cryopreservation media and/or protocols may be used. Cryopreservation methods of the disclosure may include a DMSO free cryopreservant (e.g. CryoSOfree™ DMSO-free Cryopreservation Medium) reduce freezing-related toxicity.

[0192] T cell compositions, T cell product compositions, unstimulated T cell compositions, resting T cell compositions or any portion thereof may be stored in a culture medium. T cell culture media of the disclosure may be optimized for cell storage, cell genetic modification, cell phenotype and/or cell expansion. T cell culture media of the disclosure may include one or more antibiotics. Because the inclusion of an antibiotic within a cell culture media may decrease transfection efficiency and/or cell yield following genetic modification via nucleofection, the specific antibiotics (or combinations thereof) and their respective concentration(s) may be altered for optimal transfection efficiency and/or cell yield following genetic modification via nucleofection.

[0193] T cell culture media of the disclosure may include serum, and, moreover, the serum composition and concentration may be altered for optimal cell outcomes. Human AB serum is preferred over FBS/FCS for culture of T cells because, although contemplated for use in T cell culture media of the disclosure, FBS/FCS may introduce xeno-proteins. Serum may be isolated from the blood of the subject for whom the T-cell composition in culture is intended for administration, thus, a T cell culture medium of the disclosure may comprise autologous serum. Serum-free media or serum-substitute may also be used in T-cell culture media of the disclosure. In certain embodiments of the T-cell culture media and methods of the disclosure, serum-free media or serum-substitute may provide advantages over supplementing the medium with xeno-serum, including, but not limited to, healthier cells that have greater viability, nucleofect with higher efficiency, exhibit greater viability post-nucleofection, display a more desirable cell phenotype, and/or greater/faster expansion upon addition of expansion technologies.

[0194] T cell culture media may include a commercially-available cell growth media. Exemplary commercially-available cell growth media include, but are not limited to, PBS, HBSS, OptiMEM, DMEM, RPMI 1640, AIM-V, X-VIVO 15, CellGro DC Medium, CTS OpTimizer T Cell Expansion SFM, TexMACS Medium, PRIME-XV T Cell Expansion Medium, ImmunoCult-XF T Cell Expansion Medium, or any combination thereof.

[0195] T cell compositions, T cell product compositions, unstimulated T cell compositions, resting T cell compositions or any portion thereof may be prepared for genetic modification. Preparation of T cell compositions, T cell product compositions, unstimulated T cell compositions, resting T cell compositions or any portion thereof for genetic modification may include cell washing and/or resuspension in a desired nucleofection buffer. Cryopreserved T-cell compositions may be thawed and prepared for genetic modification by nucleofection. Cryopreserved cells may be thawed according to standard or known protocols. Thawing and preparation of cryopreserved cells may be optimized to yield cells that have greater viability, nucleofect with higher efficiency, exhibit greater viability post-nucleofection, display a more desirable cell phenotype, and/or greater/faster expansion upon addition of expansion technologies. For example, Grifols Albutein (25% human albumin) may be used in the thawing and/or preparation process.

Genetic modification of an autologous T cell product composition

[0196] T cell compositions, T cell product compositions, unstimulated T cell compositions, resting T cell compositions or any portion thereof may be genetically modified using, for example, a nucleofection strategy such as electroporation. The total number of cells to be

nucleofected, the total volume of the nucleofection reaction, and the precise timing of the preparation of the sample may be optimized to yield cells that have greater viability, nucleofect with higher efficiency, exhibit greater viability post-nucleofection, display a more desirable cell phenotype, and/or greater/faster expansion upon addition of expansion technologies.

[0197] Nucleofection and/or electroporation may be accomplished using, for example, Lonza Amaxa, MaxCyte PulseAgile, Harvard Apparatus BTX, and/or Invitrogen Neon. Non-metal electrode systems, including, but not limited to, plastic polymer electrodes, may be preferred for nucleofection.

[0198] Prior to genetic modification by nucleofection, T cell compositions, T cell product compositions, unstimulated T cell compositions, resting T cell compositions or any portion thereof may be resuspended in a nucleofection buffer. Nucleofection buffers of the disclosure include commercially-available nucleofection buffers. Nucleofection buffers of the disclosure may be optimized to yield cells that have greater viability, nucleofect with higher efficiency, exhibit greater viability post-nucleofection, display a more desirable cell phenotype, and/or greater/faster expansion upon addition of expansion technologies.

Nucleofection buffers of the disclosure may include, but are not limited to, PBS, HBSS, OptiMEM, BTXpress, Amaxa Nucleofector, Human T cell nucleofection buffer and any combination thereof. Nucleofection buffers of the disclosure may comprise one or more supplemental factors to yield cells that have greater viability, nucleofect with higher efficiency, exhibit greater viability post-nucleofection, display a more desirable cell phenotype, and/or greater/faster expansion upon addition of expansion technologies.

Exemplary supplemental factors include, but are not limited to, recombinant human cytokines, chemokines, interleukins and any combination thereof. Exemplary cytokines, chemokines, and interleukins include, but are not limited to, IL2, IL7, IL12, IL15, IL21, IL1, IL3, IL4, IL5, IL6, IL8, CXCL8, IL9, IL10, IL11, IL13, IL14, IL16, IL17, IL18, IL19, IL20, IL22, IL23, IL25, IL26, IL27, IL28, IL29, IL30, IL31, IL32, IL33, IL35, IL36, GM-CSF, IFN-gamma, IL-1 alpha/IL-1F1, IL-1 beta/IL-1F2, IL-12 p70, IL-12/IL-35 p35, IL-13, IL-17/IL-17A, IL-17A/F Heterodimer, IL-17F, IL-18/IL-1F4, IL-23, IL-24, IL-32, IL-32 beta, IL-32 gamma, IL-33, LAP (TGF-beta 1), Lymphotoxin-alpha/TNF-beta, TGF-beta, TNF-alpha, TRANCE/TNFSF11/RANK L and any combination thereof. Exemplary supplemental factors include, but are not limited to, salts, minerals, metabolites or any combination thereof. Exemplary salts, minerals, and metabolites include, but are not limited to, HEPES, Nicotinamide, Heparin, Sodium Pyruvate, L-Glutamine, MEM Non-Essential Amino Acid

Solution, Ascorbic Acid, Nucleosides, FBS/FCS, Human serum, serum-substitute, antibiotics, pH adjusters, Earle's Salts, 2-Mercaptoethanol, Human transferrin, Recombinant human insulin, Human serum albumin, Nucleofector PLUS Supplement, KCL, MgCl₂, Na₂HPO₄, NaH₂PO₄, Sodium lactobionate, Mannitol, Sodium succinate, Sodium Chloride, CINa, Glucose, Ca(NO₃)₂, Tris/HCl, K₂HPO₄, KH₂PO₄, Polyethylenimine, Poly-ethylene-glycol, Poloxamer 188, Poloxamer 181, Poloxamer 407, Poly-vinylpyrrolidone, Pop313, Crown-5, and any combination thereof. Exemplary supplemental factors include, but are not limited to, media such as PBS, HBSS, OptiMEM, DMEM, RPMI 1640, AIM-V, X-VIVO 15, CellGro DC Medium, CTS OpTimizer T Cell Expansion SFM, TexMACS Medium, PRIME-XV T Cell Expansion Medium, ImmunoCult-XF T Cell Expansion Medium and any combination thereof. Exemplary supplemental factors include, but are not limited to, inhibitors of cellular DNA sensing, metabolism, differentiation, signal transduction, the apoptotic pathway and combinations thereof. Exemplary inhibitors include, but are not limited to, inhibitors of TLR9, MyD88, IRAK, TRAF6, TRAF3, IRF-7, NF-KB, Type 1 Interferons, pro-inflammatory cytokines, cGAS, STING, Sec5, TBK1, IRF-3, RNA pol III, RIG-1, IPS-1, FADD, RIP1, TRAF3, AIM2, ASC, Caspase1, Pro-IL1B, PI3K, Akt, Wnt3A, inhibitors of glycogen synthase kinase-3β (GSK-3 β) (e.g. TWS119), Bafilomycin, Chloroquine, Quinacrine, AC-YVAD-CMK, Z-VAD-FMK, Z-IETD-FMK and any combination thereof. Exemplary supplemental factors include, but are not limited to, reagents that modify or stabilize one or more nucleic acids in a way to enhance cellular delivery, enhance nuclear delivery or transport, enhance the facilitated transport of nucleic acid into the nucleus, enhance degradation of epi-chromosomal nucleic acid, and/or decrease DNA-mediated toxicity. Exemplary reagents that modify or stabilize one or more nucleic acids include, but are not limited to, pH modifiers, DNA-binding proteins, lipids, phospholipids, CaPO₄, net neutral charge DNA binding peptides with or without NLS sequences, TREX1 enzyme, and any combination thereof.

[0199] Transposition reagents, including a transposon and a transposase, may be added to a nucleofection reaction of the disclosure prior to, simultaneously with, or after an addition of cells to a nucleofection buffer (optionally, contained within a nucleofection reaction vial or cuvette). Transposons of the disclosure may comprise plasmid DNA, linearized plasmid DNA, a PCR product, DOGGYBONE™ DNA, an mRNA template, a single or double-stranded DNA, a protein-nucleic acid combination or any combination thereof. Transposons of the disclosure may comprise one or more sequences that encode one or more TTAA site(s), one or more inverted terminal repeat(s) (ITRs), one or more long terminal repeat(s)

(LTRs), one or more insulator(s), one or more promotor(s), one or more full-length or truncated gene(s), one or more polyA signal(s), one or more self-cleaving 2A peptide cleavage site(s), one or more internal ribosome entry site(s) (IRES), one or more enhancer(s), one or more regulator(s), one or more replication origin(s), and any combination thereof.

[0200] Transposons of the disclosure may comprise one or more sequences that encode one or more full-length or truncated gene(s). Full-length and/or truncated gene(s) introduced by transposons of the disclosure may encode one or more of a signal peptide, a Centyrin, a single chain variable fragment (scFv), a hinge, a transmembrane domain, a costimulatory domain, a chimeric antigen receptor (CAR), a chimeric T-cell receptor (CAR-T), a CARTyrin (a CAR-T comprising a Centyrin), a receptor, a ligand, a cytokine, a drug resistance gene, a tumor antigen, an allo or auto antigen, an enzyme, a protein, a peptide, a poly-peptide, a fluorescent protein, a mutein or any combination thereof.

[0201] Transposons of the disclosure may be prepared in water, TAE, TBE, PBS, HBSS, media, a supplemental factor of the disclosure or any combination thereof.

[0202] Transposons of the disclosure may be designed to optimize clinical safety and/or improve manufacturability. As a non-limiting example, transposons of the disclosure may be designed to optimize clinical safety and/or improve manufacturability by eliminating unnecessary sequences or regions and/or including a non-antibiotic selection marker. Transposons of the disclosure may or may not be GMP grade.

[0203] Transposase enzymes of the disclosure may be encoded by one or more sequences of plasmid DNA, mRNA, protein, protein-nucleic acid combination or any combination thereof.

[0204] Transposase enzymes of the disclosure may be prepared in water, TAE, TBE, PBS, HBSS, media, a supplemental factor of the disclosure or any combination thereof.

Transposase enzymes of the disclosure or the sequences/constructs encoding or delivering them may or may not be GMP grade.

[0205] Transposons and transposase enzymes of the disclosure may be delivered to a cell by any means.

[0206] Although compositions and methods of the disclosure include delivery of a transposon and/or transposase of the disclosure to a cell by plasmid DNA (pDNA), the use of a plasmid for delivery may allow the transposon and/or transposase to be integrated into the chromosomal DNA of the cell, which may lead to continued transposase expression. Accordingly, transposon and/or transposase enzymes of the disclosure may be delivered to a cell as either mRNA or protein to remove any possibility for chromosomal integration.

[0207] Transposons and transposases of the disclosure may be pre-incubated alone or in combination with one another prior to the introduction of the transposon and/or transposase into a nucleofection reaction. The absolute amounts of each of the transposon and the transposase, as well as the relative amounts, e.g., a ratio of transposon to transposase may be optimized.

[0208] Following preparation of nucleofection reaction, optionally, in a vial or cuvette, the reaction may be loaded into a nucleofector apparatus and activated for delivery of an electric pulse according to the manufacturer's protocol. Electric pulse conditions used for delivery of a transposon and/or a transposase of the disclosure (or a sequence encoding a transposon and/or a transposase of the disclosure) to a cell may be optimized for yielding cells with enhanced viability, higher nucleofection efficiency, greater viability post-nucleofection, desirable cell phenotype, and/or greater/faster expansion upon addition of expansion technologies. When using Amaxa nucleofector technology, each of the various nucleofection programs for the Amaxa 2B or 4D nucleofector are contemplated.

[0209] Following a nucleofection reaction of the disclosure, cells may be gently added to a cell medium. For example, when T cells undergo the nucleofection reaction, the T cells may be added to a T cell medium. Post-nucleofection cell media of the disclosure may comprise any one or more commercially-available media. Post-nucleofection cell media of the disclosure (including post-nucleofection T cell media of the disclosure) may be optimized to yield cells with greater viability, higher nucleofection efficiency, exhibit greater viability post-nucleofection, display a more desirable cell phenotype, and/or greater/faster expansion upon addition of expansion technologies. Post-nucleofection cell media of the disclosure (including post-nucleofection T cell media of the disclosure) may comprise PBS, HBSS, OptiMEM, DMEM, RPMI 1640, AIM-V, X-VIVO 15, CellGro DC Medium, CTS OpTimizer T Cell Expansion SFM, TexMACS Medium, PRIME-XV T Cell Expansion Medium, ImmunoCult-XF T Cell Expansion Medium and any combination thereof. Post-nucleofection cell media of the disclosure (including post-nucleofection T cell media of the disclosure) may comprise one or more supplemental factors of the disclosure to enhance viability, nucleofection efficiency, viability post-nucleofection, cell phenotype, and/or greater/faster expansion upon addition of expansion technologies. Exemplary supplemental factors include, but are not limited to, recombinant human cytokines, chemokines, interleukins and any combination thereof. Exemplary cytokines, chemokines, and interleukins include, but are not limited to, IL2, IL7, IL12, IL15, IL21, IL1, IL3, IL4, IL5, IL6, IL8, CXCL8, IL9, IL10, IL11, IL13, IL14, IL16, IL17, IL18, IL19, IL20, IL22, IL23,

IL25, IL26, IL27, IL28, IL29, IL30, IL31, IL32, IL33, IL35, IL36, GM-CSF, IFN-gamma, IL-1 alpha/IL-1F1, IL-1 beta/IL-1F2, IL-12 p70, IL-12/IL-35 p35, IL-13, IL-17/IL-17A, IL-17A/F Heterodimer, IL-17F, IL-18/IL-1F4, IL-23, IL-24, IL-32, IL-32 beta, IL-32 gamma, IL-33, LAP (TGF-beta 1), Lymphotoxin-alpha/TNF-beta, TGF-beta, TNF-alpha, TRANCE/TNFSF11/RANK L and any combination thereof. Exemplary supplemental factors include, but are not limited to, salts, minerals, metabolites or any combination thereof. Exemplary salts, minerals, and metabolites include, but are not limited to, HEPES, Nicotinamide, Heparin, Sodium Pyruvate, L-Glutamine, MEM Non-Essential Amino Acid Solution, Ascorbic Acid, Nucleosides, FBS/FCS, Human serum, serum-substitute, antibiotics, pH adjusters, Earle's Salts, 2-Mercaptoethanol, Human transferrin, Recombinant human insulin, Human serum albumin, Nucleofector PLUS Supplement, KCL, MgCl₂, Na₂HPO₄, NaH₂PO₄, Sodium lactobionate, Mannitol, Sodium succinate, Sodium Chloride, CINa, Glucose, Ca(NO₃)₂, Tris/HCl, K₂HPO₄, KH₂PO₄, Polyethylenimine, Poly-ethylene-glycol, Poloxamer 188, Poloxamer 181, Poloxamer 407, Poly-vinylpyrrolidone, Pop313, Crown-5, and any combination thereof. Exemplary supplemental factors include, but are not limited to, media such as PBS, HBSS, OptiMEM, DMEM, RPMI 1640, AIM-V, X-VIVO 15, CellGro DC Medium, CTS OpTimizer T Cell Expansion SFM, TexMACS Medium, PRIME-XV T Cell Expansion Medium, ImmunoCult-XF T Cell Expansion Medium and any combination thereof. Exemplary supplemental factors include, but are not limited to, inhibitors of cellular DNA sensing, metabolism, differentiation, signal transduction, the apoptotic pathway and combinations thereof. Exemplary inhibitors include, but are not limited to, inhibitors of TLR9, MyD88, IRAK, TRAF6, TRAF3, IRF-7, NF-KB, Type 1 Interferons, pro-inflammatory cytokines, cGAS, STING, Sec5, TBK1, IRF-3, RNA pol III, RIG-1, IPS-1, FADD, RIP1, TRAF3, AIM2, ASC, Caspase1, Pro-IL1B, PI3K, Akt, Wnt3A, inhibitors of glycogen synthase kinase-3 β (GSK-3 β) (e.g. TWS119), Bafilomycin, Chloroquine, Quinacrine, AC-YVAD-CMK, Z-VAD-FMK, Z-IETD-FMK and any combination thereof. Exemplary supplemental factors include, but are not limited to, reagents that modify or stabilize one or more nucleic acids in a way to enhance cellular delivery, enhance nuclear delivery or transport, enhance the facilitated transport of nucleic acid into the nucleus, enhance degradation of epi-chromosomal nucleic acid, and/or decrease DNA-mediated toxicity. Exemplary reagents that modify or stabilize one or more nucleic acids include, but are not limited to, pH modifiers, DNA-binding proteins, lipids, phospholipids, CaPO₄, net neutral charge DNA binding peptides with or without NLS sequences, TREX1 enzyme, and any combination thereof.

[0210] Post-nucleofection cell media of the disclosure (including post-nucleofection T cell media of the disclosure) may be used at room temperature or pre-warmed to, for example to between 32°C to 37°C, inclusive of the endpoints. Post-nucleofection cell media of the disclosure (including post-nucleofection T cell media of the disclosure) may be pre-warmed to any temperature that maintains or enhances cell viability and/or expression of a transposon or portion thereof of the disclosure.

[0211] Post-nucleofection cell media of the disclosure (including post-nucleofection T cell media of the disclosure) may be contained in tissue culture flasks or dishes, G-Rex flasks, Bioreactor or cell culture bags, or any other standard receptacle. Post-nucleofection cell cultures of the disclosure (including post-nucleofection T cell cultures of the disclosure) may be kept still, or, alternatively, they may be perturbed (e.g. rocked, swirled, or shaken).

[0212] Post-nucleofection cell cultures may comprise genetically-modified cells. Post-nucleofection T cell cultures may comprise genetically-modified T cells. Genetically modified cells of the disclosure may be either rested for a defined period of time or stimulated for expansion by, for example, the addition of a T Cell Expander technology. In certain embodiments, genetically modified cells of the disclosure may be either rested for a defined period of time or immediately stimulated for expansion by, for example, the addition of a T Cell Expander technology. Genetically modified cells of the disclosure may be rested to allow them sufficient time to acclimate, time for transposition to occur, and/or time for positive or negative selection, resulting in cells with enhanced viability, higher nucleofection efficiency, greater viability post-nucleofection, desirable cell phenotype, and/or greater/faster expansion upon addition of expansion technologies. Genetically modified cells of the disclosure may be rested, for example, for 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or more hours. In certain embodiments, genetically modified cells of the disclosure may be rested, for example, for an overnight. In certain aspects, an overnight is about 12 hours. Genetically modified cells of the disclosure may be rested, for example, for 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or more days.

[0213] Genetically modified cells of the disclosure may be selected following a nucleofection reaction and prior to addition of an expander technology. For optimal selection of genetically-modified cells, the cells may be allowed to rest in a post-nucleofection cell medium for at least 2-14 days to facilitate identification of modified cells (e.g., differentiation of modified from non-modified cells).

[0214] As early as 24-hours post-nucleofection, expression of a CAR/CARTyrin and selection marker of the disclosure may be detectable in modified T cells upon successful

nucleofection of a transposon of the disclosure. Due to epi-chromosomal expression of the transposon, expression of a selection marker alone may not differentiate modified T cells (those cells in which the transposon has been successfully integrated) from unmodified T cells (those cells in which the transposon was not successfully integrated). When epi-chromosomal expression of the transposon obscures the detection of modified cells by the selection marker, the nucleofected cells (both modified and unmodified cells) may be rested for a period of time (e.g. 2-14 days) to allow the cells to cease expression or lose all epi-chromosomal transposon expression. Following this extended resting period, only modified T cells should remain positive for expression of selection marker. The length of this extended resting period may be optimized for each nucleofection reaction and selection process. When epi-chromosomal expression of the transposon obscures the detection of modified cells by the selection marker, selection may be performed without this extended resting period, however, an additional selection step may be included at a later time point (e.g. either during or after the expansion stage).

[0215] Selection of genetically modified cells of the disclosure may be performed by any means. In certain embodiments of the methods of the disclosure, selection of genetically modified cells of the disclosure may be performed by isolating cells expressing a specific selection marker. Selection markers of the disclosure may be encoded by one or more sequences in the transposon. Selection markers of the disclosure may be expressed by the modified cell as a result of successful transposition (i.e., not encoded by one or more sequences in the transposon). In certain embodiments, genetically modified cells of the disclosure contain a selection marker that confers resistance to a deleterious compound of the post-nucleofection cell medium. The deleterious compound may comprise, for example, an antibiotic or a drug that, absent the resistance conferred by the selection marker to the modified cells, would result in cell death. Exemplary selection markers include, but are not limited to, wild type (WT) or mutant forms of one or more of the following genes: neo, DHFR, TYMS, ALDH, MDR1, MGMT, FANCF, RAD51C, GCS, and NKX2.2. Exemplary selection markers include, but are not limited to, a surface-expressed selection marker or surface-expressed tag may be targeted by Ab-coated magnetic bead technology or column selection, respectively. A cleavable tag such as those used in protein purification may be added to a selection marker of the disclosure for efficient column selection, washing, and elution. In certain embodiments, selection markers of the disclosure are not expressed by the modified cells (including modified T cells) naturally and, therefore, may be useful in the physical isolation of modified cells (by, for example, cell sorting techniques). Exemplary

selection markers of the disclosure are not expressed by the modified cells (including modified T cells) naturally include, but are not limited to, full-length, mutated, or truncated forms of CD271, CD19 CD52, CD34, RQR8, CD22, CD20, CD33 and any combination thereof.

[0216] Genetically modified cells of the disclosure may be selective expanded following a nucleofection reaction. In certain embodiments, modified T cells comprising a CAR/CARTyrin may be selectively expanded by CAR/CARTyrin stimulation. Modified T cells comprising a CAR/CARTyrin may be stimulated by contact with a target-covered reagent (e.g. a tumor line or a normal cell line expressing a target or expander beads covered in a target). Alternatively, modified T cells comprising a CAR/CARTyrin may be stimulated by contact with an irradiated tumor cell, an irradiated allogeneic normal cell, an irradiated autologous PBMC. To minimize contamination of cell product compositions of the disclosure with a target-expressing cell used for stimulation, for example, when the cell product composition may be administered directly to a subject, the stimulation may be performed using expander beads coated with CAR/CARTyrin target protein. Selective expansion of modified T cells comprising a CAR/CARTyrin by CAR/CARTyrin stimulation may be optimized to avoid functionally-exhausting the modified T-cells.

[0217] Selected genetically-modified cells of the disclosure may be cryopreserved, rested for a defined period of time, or stimulated for expansion by the addition of a Cell Expander technology. Selected genetically-modified cells of the disclosure may be cryopreserved, rested for a defined period of time, or immediately stimulated for expansion by the addition of a Cell Expander technology. When the selected genetically-modified cells are T cells, the T cells may be stimulated for expansion by the addition of a T-Cell Expander technology. Selected genetically modified cells of the disclosure may be rested, for example, for 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or more hours. In certain embodiments, selected genetically modified cells of the disclosure may be rested, for example, for an overnight. In certain aspects, an overnight is about 12 hours. Selected genetically modified cells of the disclosure may be rested, for example, for 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or more days. Selected genetically modified cells of the disclosure may be rested for any period of time resulting in cells with enhanced viability, higher nucleofection efficiency, greater viability post-nucleofection, desirable cell phenotype, and/or greater/faster expansion upon addition of expansion technologies.

[0218] Selected genetically-modified cells (including selected genetically-modified T cells of the disclosure) may be cryopreserved using any standard cryopreservation method, which

may be optimized for storing and/or recovering human cells with high recovery, viability, phenotype, and/or functional capacity. Cryopreservation methods of the disclosure may include commercially-available cryopreservation media and/or protocols.

[0219] A transposition efficiency of selected genetically-modified cells (including selected genetically-modified T cells of the disclosure) may be assessed by any means. For example, prior to the application of an expander technology, expression of the transposon by selected genetically-modified cells (including selected genetically-modified T cells of the disclosure) may be measured by fluorescence-activated cell sorting (FACS). Determination of a transposition efficiency of selected genetically-modified cells (including selected genetically-modified T cells of the disclosure) may include determining a percentage of selected cells expressing the transposon (e.g. a CAR). Alternatively, or in addition, a purity of T cells, a Mean Fluorescence Intensity (MFI) of the transposon expression (e.g. CAR expression), an ability of a CAR (delivered in the transposon) to mediate degranulation and/or killing of a target cell expressing the CAR ligand, and/or a phenotype of selected genetically-modified cells (including selected genetically-modified T cells of the disclosure) may be assessed by any means.

[0220] Cell product compositions of the disclosure may be released for administration to a subject upon meeting certain release criteria. Exemplary release criteria may include, but are not limited to, a particular percentage of modified, selected and/or expanded T cells expressing detectable levels of a CAR on the cell surface.

Genetic modification of an autologous T cell product composition

[0221] Genetically-modified cells (including genetically-modified T cells) of the disclosure may be expanded using an expander technology. Expander technologies of the disclosure may comprise a commercially-available expander technology. Exemplary expander technologies of the disclosure include stimulation a genetically-modified T cell of the disclosure via the TCR. While all means for stimulation of a genetically-modified T cell of the disclosure are contemplated, stimulation a genetically-modified T cell of the disclosure via the TCR is a preferred method, yielding a product with a superior level of killing capacity.

[0222] To stimulate a genetically-modified T cell of the disclosure via the TCR, Thermo Expander DynaBeads may be used at a 3:1 bead to T cell ratio. If the expander beads are not biodegradable, the beads may be removed from the expander composition. For example, the beads may be removed from the expander composition after about 5 days. To stimulate a genetically-modified T cell of the disclosure via the TCR, a Miltenyi T Cell

Activation/Expansion Reagent may be used. To stimulate a genetically-modified T cell of the disclosure via the TCR, StemCell Technologies' ImmunoCult Human CD3/CD28 or CD3/CD28/CD2 T Cell Activator Reagent may be used. This technology may be preferred since the soluble tetrameric antibody complexes would degrade after a period and would not require removal from the process.

[0223] Artificial antigen presenting cells (APCs) may be engineered to co-express the target antigen and may be used to stimulate a cell or T-cell of the disclosure through a TCR and/or CAR of the disclosure. Artificial APCs may comprise or may be derived from a tumor cell line (including, for example, the immortalized myelogenous leukemia line K562) and may be engineered to co-express multiple costimulatory molecules or technologies (such as CD28, 4-1BBL, CD64, mbIL-21, mbIL-15, CAR target molecule, etc.). When artificial APCs of the disclosure are combined with costimulatory molecules, conditions may be optimized to prevent the development or emergence of an undesirable phenotype and functional capacity, namely terminally-differentiated effector T cells.

[0224] Irradiated PBMCs (auto or allo) may express some target antigens, such as CD19, and may be used to stimulate a cell or T-cell of the disclosure through a TCR and/or CAR of the disclosure. Alternatively, or in addition, irradiated tumor cells may express some target antigens and may be used to stimulate a cell or T-cell of the disclosure through a TCR and/or CAR of the disclosure.

[0225] Plate-bound and/or soluble anti-CD3, anti-CD2 and/or anti-CD28 stimulate may be used to stimulate a cell or T-cell of the disclosure through a TCR and/or CAR of the disclosure.

[0226] Antigen-coated beads may display target protein and may be used to stimulate a cell or T-cell of the disclosure through a TCR and/or CAR of the disclosure. Alternatively, or in addition, expander beads coated with a CAR/CARTyrin target protein may be used to stimulate a cell or T-cell of the disclosure through a TCR and/or CAR of the disclosure.

[0227] Expansion methods drawn to stimulation of a cell or T-cell of the disclosure through the TCR or CAR/CARTyrin and via surface-expressed CD2, CD3, CD28, 4-1BB, and/or other markers on genetically-modified T cells.

[0228] An expansion technology may be applied to a cell of the disclosure immediately post-nucleofection until approximately 24 hours post-nucleofection. While various cell media may be used during an expansion procedure, a desirable T Cell Expansion Media of the disclosure may yield cells with, for example, greater viability, cell phenotype, total expansion, or greater capacity for in vivo persistence, engraftment, and/or CAR-mediated killing. Cell media of the

disclosure may be optimized to improve/enhance expansion, phenotype, and function of genetically-modified cells of the disclosure. A preferred phenotype of expanded T cells may include a mixture of T stem cell memory, T central, and T effector memory cells. Expander Dynabeads may yield mainly central memory T cells which may lead to superior performance in the clinic.

[0229] Exemplary T cell expansion media of the disclosure may include, in part or in total, PBS, HBSS, OptiMEM, DMEM, RPMI 1640, AIM-V, X-VIVO 15, CellGro DC Medium, CTS OpTimizer T Cell Expansion SFM, TexMACS Medium, PRIME-XV T Cell Expansion Medium, ImmunoCult-XF T Cell Expansion Medium, or any combination thereof. T cell expansion media of the disclosure may further include one or more supplemental factors. Supplemental factors that may be included in a T cell expansion media of the disclosure enhance viability, cell phenotype, total expansion, or increase capacity for in vivo persistence, engraftment, and/or CAR-mediated killing. Supplemental factors that may be included in a T cell expansion media of the disclosure include, but are not limited to, recombinant human cytokines, chemokines, and/or interleukins such as IL2, IL7, IL12, IL15, IL21, IL1, IL3, IL4, IL5, IL6, IL8, CXCL8, IL9, IL10, IL11, IL13, IL14, IL16, IL17, IL18, IL19, IL20, IL22, IL23, IL25, IL26, IL27, IL28, IL29, IL30, IL31, IL32, IL33, IL35, IL36, GM-CSF, IFN-gamma, IL-1 alpha/IL-1F1, IL-1 beta/IL-1F2, IL-12 p70, IL-12/IL-35 p35, IL-13, IL-17/IL-17A, IL-17A/F Heterodimer, IL-17F, IL-18/IL-1F4, IL-23, IL-24, IL-32, IL-32 beta, IL-32 gamma, IL-33, LAP (TGF-beta 1), Lymphotoxin-alpha/TNF-beta, TGF-beta, TNF-alpha, TRANCE/TNFSF11/RANK L, or any combination thereof. Supplemental factors that may be included in a T cell expansion media of the disclosure include, but are not limited to, salts, minerals, and/or metabolites such as HEPES, Nicotinamide, Heparin, Sodium Pyruvate, L-Glutamine, MEM Non-Essential Amino Acid Solution, Ascorbic Acid, Nucleosides, FBS/FCS, Human serum, serum-substitute, anti-biotics, pH adjusters, Earle's Salts, 2-Mercaptoethanol, Human transferrin, Recombinant human insulin, Human serum albumin, Nucleofector PLUS Supplement, KCL, MgCl₂, Na₂HPO₄, NaH₂PO₄, Sodium lactobionate, Mannitol, Sodium succinate, Sodium Chloride, CINa, Glucose, Ca(NO₃)₂, Tris/HCl, K₂HPO₄, KH₂PO₄, Polyethylenimine, Poly-ethylene-glycol, Poloxamer 188, Poloxamer 181, Poloxamer 407, Poly-vinylpyrrolidone, Pop313, Crown-5 or any combination thereof. Supplemental factors that may be included in a T cell expansion media of the disclosure include, but are not limited to, inhibitors of cellular DNA sensing, metabolism, differentiation, signal transduction, and/or the apoptotic pathway such as inhibitors of TLR9, MyD88, IRAK, TRAF6, TRAF3, IRF-7, NF-KB, Type 1 Interferons,

pro-inflammatory cytokines, cGAS, STING, Sec5, TBK1, IRF-3, RNA pol III, RIG-1, IPS-1, FADD, RIP1, TRAF3, AIM2, ASC, Caspase1, Pro-IL1B, PI3K, Akt, Wnt3A, inhibitors of glycogen synthase kinase-3 β (GSK-3 β) (e.g. TWS119), Bafilomycin, Chloroquine, Quinacrine, AC-YVAD-CMK, Z-VAD-FMK, Z-IETD-FMK, or any combination thereof.

[0230] Supplemental factors that may be included in a T cell expansion media of the disclosure include, but are not limited to, reagents that modify or stabilize nucleic acids in a way to enhance cellular delivery, enhance nuclear delivery or transport, enhance the facilitated transport of nucleic acid into the nucleus, enhance degradation of epi-chromosomal nucleic acid, and/or decrease DNA-mediated toxicity, such as pH modifiers, DNA-binding proteins, lipids, phospholipids, CaPO₄, net neutral charge DNA binding peptides with or without NLS sequences, TREX1 enzyme, or any combination thereof.

[0231] Genetically-modified cells of the disclosure may be selected during the expansion process by the use of selectable drugs or compounds. For example, in certain embodiments, when a transposon of the disclosure may encode a selection marker that confers to genetically-modified cells resistance to a drug added to the culture medium, selection may occur during the expansion process and may require approximately 1-14 days of culture for selection to occur. Examples of drug resistance genes that may be used as selection markers encoded by a transposon of the disclosure, include, but are not limited to, wild type (WT) or mutant forms of the genes neo, DHFR, TYMS, ALDH, MDR1, MGMT, FANCF, RAD51C, GCS, NKX2.2, or any combination thereof. Examples of corresponding drugs or compounds that may be added to the culture medium to which a selection marker may confer resistance include, but are not limited to, G418, Puromycin, Ampicillin, Kanamycin, Methotrexate, Mephalan, Temozolomide, Vincristine, Etoposide, Doxorubicin, Bendamustine, Fludarabine, Aredia (Pamidronate Disodium), Becenun (Carmustine), BiCNU (Carmustine), Bortezomib, Carfilzomib, Carmubris (Carmustine), Carmustine, Clafen (Cyclophosphamide), Cyclophosphamide, Cytosan (Cyclophosphamide), Daratumumab, Darzalex (Daratumumab), Doxil (Doxorubicin Hydrochloride Liposome), Doxorubicin Hydrochloride Liposome, Dox-SL (Doxorubicin Hydrochloride Liposome), Elotuzumab, Empliciti (Elotuzumab), Evacet (Doxorubicin Hydrochloride Liposome), Farydak (Panobinostat), Ixazomib Citrate, Kyprolis (Carfilzomib), Lenalidomide, LipoDox (Doxorubicin Hydrochloride Liposome), Mozobil (Plerixafor), Neosar (Cyclophosphamide), Ninlaro (Ixazomib Citrate), Pamidronate Disodium, Panobinostat, Plerixafor, Pomalidomide, Pomalyst (Pomalidomide), Revlimid (Lenalidomide), Synovir (Thalidomide), Thalidomide, Thalomid (Thalidomide), Velcade (Bortezomib), Zoledronic Acid, Zometa (Zoledronic Acid), or any combination thereof.

[0232] A T-Cell Expansion process of the disclosure may occur in a cell culture bag in a WAVE Bioreactor, a G-Rex flask, or in any other suitable container and/or reactor.

[0233] A cell or T-cell culture of the disclosure may be kept steady, rocked, swirled, or shaken.

[0234] A cell or T-cell expansion process of the disclosure may optimize certain conditions, including, but not limited to culture duration, cell concentration, schedule for T cell medium addition/removal, cell size, total cell number, cell phenotype, purity of cell population, percentage of genetically-modified cells in growing cell population, use and composition of supplements, the addition/removal of expander technologies, or any combination thereof.

[0235] A cell or T-cell expansion process of the disclosure may continue until a predefined endpoint prior to formulation of the resultant expanded cell population. For example, a cell or T-cell expansion process of the disclosure may continue for a predetermined amount of time: at least, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24 hours; at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30 days; at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 weeks; at least 1, 2, 3, 4, 5, 6, months, or at least 1 year. A cell or T-cell expansion process of the disclosure may continue until the resultant culture reaches a predetermined overall cell density: 1, 10, 100, 1000, 104, 105, 106, 107, 108, 109, 1010 cells per volume (μ l, ml, L) or any density in between. A cell or T-cell expansion process of the disclosure may continue until the genetically-modified cells of a resultant culture demonstrate a predetermined level of expression of a transposon of the disclosure: 1%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% or any percentage in between of a threshold level of expression (a minimum, maximum or mean level of expression indicating the resultant genetically-modified cells are clinically-efficacious). A cell or T-cell expansion process of the disclosure may continue until the proportion of genetically-modified cells of a resultant culture to the proportion of unmodified cells reaches a predetermined threshold: at least 1:10, 1:9, 1:8, 1:7, 1:6, 1:5, 1:4, 1:3, 1:2, 1:1, 2:1, 2:1, 4:1, 5:1, 6:1, 7:1, 8:1, 9:1 10:1 or any ratio in between.

Analysis of genetically-modified autologous T cells for release

[0236] A percentage of genetically-modified cells may be assessed during or after an expansion process of the disclosure. Cellular expression of a transposon by a genetically-modified cell of the disclosure may be measured by fluorescence-activated cell sorting (FACS). For example, FACS may be used to determine a percentage of cells or T cells expressing a CAR of the disclosure. Alternatively, or in addition, a purity of genetically-modified cells or T cells, the Mean Fluorescence Intensity (MFI) of a CAR expressed by a

genetically-modified cell or T cell of the disclosure, an ability of the CAR to mediate degranulation and/or killing of a target cell expressing the CAR ligand, and/or a phenotype of CAR+ T cells may be assessed.

[0237] Compositions of the disclosure intended for administration to a subject may be required to meet one or more “release criteria” that indicate that the composition is safe and efficacious for formulation as a pharmaceutical product and/or administration to a subject. Release criteria may include a requirement that a composition of the disclosure (e.g. a T-cell product of the disclosure) comprises a particular percentage of T cells expressing detectable levels of a CAR of the disclosure on their cell surface.

[0238] The expansion process should be continued until a specific criterion has been met (e.g. achieving a certain total number of cells, achieving a particular population of memory cells, achieving a population of a specific size).

[0239] Certain criterion signal a point at which the expansion process should end. For example, cells should be formulated, reactivated, or cryopreserved once they reach a cell size of 300fL (otherwise, cells reaching a size above this threshold may start to die).

Cryopreservation immediately once a population of cells reaches an average cell size of less than 300 fL may yield better cell recovery upon thawing and culture because the cells haven’t yet reached a fully quiescent state prior to cryopreservation (a fully quiescent size is approximately 180 fL). Prior to expansion, T cells of the disclosure may have a cell size of about 180 fL, but may more than quadruple their cell size to approximately 900 fL at 3 days post-expansion. Over the next 6-12 days, the population of T-cells will slowly decrease cell size to full quiescence at 180 fL.

[0240] A process for preparing a cell population for formulation may include, but is not limited to the steps of, concentrating the cells of the cell population, washing the cells, and/or further selection of the cells via drug resistance or magnetic bead sorting against a particular surface-expressed marker. A process for preparing a cell population for formulation may further include a sorting step to ensure the safety and purity of the final product. For example, if a tumor cell from a patient has been used to stimulate a genetically-modified T-cell of the disclosure or that have been genetically-modified in order to stimulate a genetically-modified T-cell of the disclosure that is being prepared for formulation, it is critical that no tumor cells from the patient are included in the final product.

Cell product infusion and/or cryopreservation for infusion

[0241] A pharmaceutical formulation of the disclosure may be distributed into bags for infusion, cryopreservation, and/or storage.

[0242] A pharmaceutical formulation of the disclosure may be cryopreserved using a standard protocol and, optionally, an infusible cryopreservation medium. For example, a DMSO free cryopreservant (e.g. CryoSOfree™ DMSO-free Cryopreservation Medium) may be used to reduce freezing-related toxicity. A cryopreserved pharmaceutical formulation of the disclosure may be stored for infusion to a patient at a later date. An effective treatment may require multiple administrations of a pharmaceutical formulation of the disclosure and, therefore, pharmaceutical formulations may be packaged in pre-aliquoted “doses” that may be stored frozen but separated for thawing of individual doses.

[0243] A pharmaceutical formulation of the disclosure may be stored at room temperature. An effective treatment may require multiple administrations of a pharmaceutical formulation of the disclosure and, therefore, pharmaceutical formulations may be packaged in pre-aliquoted “doses” that may be stored together but separated for administration of individual doses.

[0244] A pharmaceutical formulation of the disclosure may be archived for subsequent re-expansion and/or selection for generation of additional doses to the same patient in the case of an allogenic therapy who may need an administration at a future date following, for example, a remission and relapse of a condition.

Formulations

[0245] As noted above, the disclosure provides for stable formulations, which preferably comprise a phosphate buffer with saline or a chosen salt, as well as preserved solutions and formulations containing a preservative as well as multi-use preserved formulations suitable for pharmaceutical or veterinary use, comprising at least one protein scaffold in a pharmaceutically acceptable formulation. Preserved formulations contain at least one known preservative or optionally selected from the group consisting of at least one phenol, m-cresol, p-cresol, o-cresol, chlorocresol, benzyl alcohol, phenylmercuric nitrite, phenoxyethanol, formaldehyde, chlorobutanol, magnesium chloride (e.g., hexahydrate), alkylparaben (methyl, ethyl, propyl, butyl and the like), benzalkonium chloride, benzethonium chloride, sodium dehydroacetate and thimerosal, polymers, or mixtures thereof in an aqueous diluent. Any suitable concentration or mixture can be used as known in the art, such as about 0.0015%, or any range, value, or fraction therein. Non-limiting examples include, no preservative, about 0.1-2% m-cresol (e.g., 0.2, 0.3, 0.4, 0.5, 0.9, 1.0%), about 0.1-3% benzyl alcohol (e.g., 0.5, 0.9, 1.1, 1.5, 1.9, 2.0, 2.5%), about 0.001-0.5% thimerosal (e.g., 0.005, 0.01), about 0.001-2.0% phenol (e.g., 0.05, 0.25, 0.28, 0.5, 0.9, 1.0%), 0.0005-1.0% alkylparaben(s) (e.g.,

0.00075, 0.0009, 0.001, 0.002, 0.005, 0.0075, 0.009, 0.01, 0.02, 0.05, 0.075, 0.09, 0.1, 0.2, 0.3, 0.5, 0.75, 0.9, 1.0%), and the like.

[0246] As noted above, the invention provides an article of manufacture, comprising packaging material and at least one vial comprising a solution of at least one protein scaffold with the prescribed buffers and/or preservatives, optionally in an aqueous diluent, wherein said packaging material comprises a label that indicates that such solution can be held over a period of 1, 2, 3, 4, 5, 6, 9, 12, 18, 20, 24, 30, 36, 40, 48, 54, 60, 66, 72 hours or greater. The invention further comprises an article of manufacture, comprising packaging material, a first vial comprising lyophilized at least one protein scaffold, and a second vial comprising an aqueous diluent of prescribed buffer or preservative, wherein said packaging material comprises a label that instructs a patient to reconstitute the at least one protein scaffold in the aqueous diluent to form a solution that can be held over a period of twenty-four hours or greater.

[0247] The at least one protein scaffold used in accordance with the present invention can be produced by recombinant means, including from mammalian cell or transgenic preparations, or can be purified from other biological sources, as described herein or as known in the art.

[0248] The range of at least one protein scaffold in the product of the present invention includes amounts yielding upon reconstitution, if in a wet/dry system, concentrations from about 1.0 µg/ml to about 1000 mg/ml, although lower and higher concentrations are operable and are dependent on the intended delivery vehicle, e.g., solution formulations will differ from transdermal patch, pulmonary, transmucosal, or osmotic or micro pump methods.

[0249] Preferably, the aqueous diluent optionally further comprises a pharmaceutically acceptable preservative. Preferred preservatives include those selected from the group consisting of phenol, m-cresol, p-cresol, o-cresol, chlorocresol, benzyl alcohol, alkylparaben (methyl, ethyl, propyl, butyl and the like), benzalkonium chloride, benzethonium chloride, sodium dehydroacetate and thimerosal, or mixtures thereof. The concentration of preservative used in the formulation is a concentration sufficient to yield an anti-microbial effect. Such concentrations are dependent on the preservative selected and are readily determined by the skilled artisan.

[0250] Other excipients, e.g., isotonicity agents, buffers, antioxidants, and preservative enhancers, can be optionally and preferably added to the diluent. An isotonicity agent, such as glycerin, is commonly used at known concentrations. A physiologically tolerated buffer is preferably added to provide improved pH control. The formulations can cover a wide range

of pHs, such as from about pH 4 to about pH 10, and preferred ranges from about pH 5 to about pH 9, and a most preferred range of about 6.0 to about 8.0. Preferably, the formulations of the present invention have a pH between about 6.8 and about 7.8. Preferred buffers include phosphate buffers, most preferably, sodium phosphate, particularly, phosphate buffered saline (PBS).

[0251] Other additives, such as a pharmaceutically acceptable solubilizers like Tween 20 (polyoxyethylene (20) sorbitan monolaurate), Tween 40 (polyoxyethylene (20) sorbitan monopalmitate), Tween 80 (polyoxyethylene (20) sorbitan monooleate), Pluronic F68 (polyoxyethylene polyoxypropylene block copolymers), and PEG (polyethylene glycol) or non-ionic surfactants, such as polysorbate 20 or 80 or poloxamer 184 or 188, Pluronic® polyols, other block co-polymers, and chelators, such as EDTA and EGTA, can optionally be added to the formulations or compositions to reduce aggregation. These additives are particularly useful if a pump or plastic container is used to administer the formulation. The presence of pharmaceutically acceptable surfactant mitigates the propensity for the protein to aggregate.

[0252] The formulations of the present invention can be prepared by a process which comprises mixing at least one protein scaffold and a preservative selected from the group consisting of phenol, m-cresol, p-cresol, o-cresol, chlorocresol, benzyl alcohol, alkylparaben, (methyl, ethyl, propyl, butyl and the like), benzalkonium chloride, benzethonium chloride, sodium dehydroacetate and thimerosal or mixtures thereof in an aqueous diluent. Mixing the at least one protein scaffold and preservative in an aqueous diluent is carried out using conventional dissolution and mixing procedures. To prepare a suitable formulation, for example, a measured amount of at least one protein scaffold in buffered solution is combined with the desired preservative in a buffered solution in quantities sufficient to provide the protein and preservative at the desired concentrations. Variations of this process would be recognized by one of ordinary skill in the art. For example, the order the components are added, whether additional additives are used, the temperature and pH at which the formulation is prepared, are all factors that can be optimized for the concentration and means of administration used.

[0253] The claimed formulations can be provided to patients as clear solutions or as dual vials comprising a vial of lyophilized at least one protein scaffold that is reconstituted with a second vial containing water, a preservative and/or excipients, preferably, a phosphate buffer and/or saline and a chosen salt, in an aqueous diluent. Either a single solution vial or dual vial requiring reconstitution can be reused multiple times and can suffice for a single or multiple

cycles of patient treatment and thus can provide a more convenient treatment regimen than currently available.

[0254] The present claimed articles of manufacture are useful for administration over a period ranging from immediate to twenty-four hours or greater. Accordingly, the presently claimed articles of manufacture offer significant advantages to the patient. Formulations of the invention can optionally be safely stored at temperatures of from about 2° C. to about 40° C. and retain the biological activity of the protein for extended periods of time, thus allowing a package label indicating that the solution can be held and/or used over a period of 6, 12, 18, 24, 36, 48, 72, or 96 hours or greater. If preserved diluent is used, such label can include use up to 1-12 months, one-half, one and a half, and/or two years.

[0255] The solutions of at least one protein scaffold of the invention can be prepared by a process that comprises mixing at least one protein scaffold in an aqueous diluent. Mixing is carried out using conventional dissolution and mixing procedures. To prepare a suitable diluent, for example, a measured amount of at least one protein scaffold in water or buffer is combined in quantities sufficient to provide the protein and, optionally, a preservative or buffer at the desired concentrations. Variations of this process would be recognized by one of ordinary skill in the art. For example, the order the components are added, whether additional additives are used, the temperature and pH at which the formulation is prepared, are all factors that can be optimized for the concentration and means of administration used.

[0256] The claimed products can be provided to patients as clear solutions or as dual vials comprising a vial of lyophilized at least one protein scaffold that is reconstituted with a second vial containing the aqueous diluent. Either a single solution vial or dual vial requiring reconstitution can be reused multiple times and can suffice for a single or multiple cycles of patient treatment and thus provides a more convenient treatment regimen than currently available.

[0257] The claimed products can be provided indirectly to patients by providing to pharmacies, clinics, or other such institutions and facilities, clear solutions or dual vials comprising a vial of lyophilized at least one protein scaffold that is reconstituted with a second vial containing the aqueous diluent. The clear solution in this case can be up to one liter or even larger in size, providing a large reservoir from which smaller portions of the at least one protein scaffold solution can be retrieved one or multiple times for transfer into smaller vials and provided by the pharmacy or clinic to their customers and/or patients.

[0258] Recognized devices comprising single vial systems include pen-injector devices for delivery of a solution, such as BD Pens, BD Autojector®, Humaject®, NovoPen®, B-

D®Pen, AutoPen®, and OptiPen®, GenotropinPen®, Genotronorm Pen®, Humatro Pen®, Reco-Pen®, Roferon Pen®, Biojector®, Iject®, J-tip Needle-Free Injector®, Intraject®, Medi-Ject®, e.g., as made or developed by Becton Dickinson (Franklin Lakes, N.J., www.bectondickenson.com), Disetronic (Burgdorf, Switzerland, www.disetronic.com), Bioject, Portland, Oreg. (www.bioject.com); National Medical Products, Weston Medical (Peterborough, UK, www.weston-medical.com), Medi-Ject Corp (Minneapolis, Minn., www.mediject.com), and similarly suitable devices. Recognized devices comprising a dual vial system include those pen-injector systems for reconstituting a lyophilized drug in a cartridge for delivery of the reconstituted solution, such as the HumatroPen®. Examples of other devices suitable include pre-filled syringes, auto-injectors, needle free injectors and needle free IV infusion sets.

[0259] The products presently claimed include packaging material. The packaging material provides, in addition to the information required by the regulatory agencies, the conditions under which the product can be used. The packaging material of the present invention provides instructions to the patient to reconstitute at least one protein scaffold in the aqueous diluent to form a solution and to use the solution over a period of 2-24 hours or greater for the two vial, wet/dry, product. For the single vial, solution product, the label indicates that such solution can be used over a period of 2-24 hours or greater. The presently claimed products are useful for human pharmaceutical product use.

[0260] The formulations of the present invention can be prepared by a process that comprises mixing at least one protein scaffold and a selected buffer, preferably, a phosphate buffer containing saline or a chosen salt. Mixing at least one protein scaffold and buffer in an aqueous diluent is carried out using conventional dissolution and mixing procedures. To prepare a suitable formulation, for example, a measured amount of at least one protein scaffold in water or buffer is combined with the desired buffering agent in water in quantities sufficient to provide the protein and buffer at the desired concentrations. Variations of this process would be recognized by one of ordinary skill in the art. For example, the order the components are added, whether additional additives are used, the temperature and pH at which the formulation is prepared, are all factors that can be optimized for the concentration and means of administration used.

[0261] The claimed stable or preserved formulations can be provided to patients as clear solutions or as dual vials comprising a vial of lyophilized protein scaffold that is reconstituted with a second vial containing a preservative or buffer and excipients in an aqueous diluent. Either a single solution vial or dual vial requiring reconstitution can be reused multiple times

and can suffice for a single or multiple cycles of patient treatment and thus provides a more convenient treatment regimen than currently available.

[0262] Other formulations or methods of stabilizing the protein scaffold may result in other than a clear solution of lyophilized powder comprising the protein scaffold. Among non-clear solutions are formulations comprising particulate suspensions, said particulates being a composition containing the protein scaffold in a structure of variable dimension and known variously as a microsphere, microparticle, nanoparticle, nanosphere, or liposome. Such relatively homogenous, essentially spherical, particulate formulations containing an active agent can be formed by contacting an aqueous phase containing the active agent and a polymer and a nonaqueous phase followed by evaporation of the nonaqueous phase to cause the coalescence of particles from the aqueous phase as taught in U.S. Pat. No. 4,589,330.

Porous microparticles can be prepared using a first phase containing active agent and a polymer dispersed in a continuous solvent and removing said solvent from the suspension by freeze-drying or dilution-extraction-precipitation as taught in U.S. Pat. No. 4,818,542.

Preferred polymers for such preparations are natural or synthetic copolymers or polymers selected from the group consisting of gelatin agar, starch, arabinogalactan, albumin, collagen, polyglycolic acid, polylactic acid, glycolide-L(-) lactide poly(epsilon-caprolactone, poly(epsilon-caprolactone-CO-lactic acid), poly(epsilon-caprolactone-CO-glycolic acid), poly(beta-hydroxy butyric acid), polyethylene oxide, polyethylene, poly(alkyl-2-cyanoacrylate), poly(hydroxyethyl methacrylate), polyamides, poly(amino acids), poly(2-hydroxyethyl DL-aspartamide), poly(ester urea), poly(L-phenylalanine/ethylene glycol/1,6-diisocyanatohexane) and poly(methyl methacrylate). Particularly preferred polymers are polyesters, such as polyglycolic acid, polylactic acid, glycolide-L(-) lactide poly(epsilon-caprolactone, poly(epsilon-caprolactone-CO-lactic acid), and poly(epsilon-caprolactone-CO-glycolic acid). Solvents useful for dissolving the polymer and/or the active include: water, hexafluoroisopropanol, methylenechloride, tetrahydrofuran, hexane, benzene, or hexafluoroacetone sesquihydrate. The process of dispersing the active containing phase with a second phase may include pressure forcing said first phase through an orifice in a nozzle to affect droplet formation.

[0263] Dry powder formulations may result from processes other than lyophilization, such as by spray drying or solvent extraction by evaporation or by precipitation of a crystalline composition followed by one or more steps to remove aqueous or nonaqueous solvent. Preparation of a spray-dried protein scaffold preparation is taught in U.S. Pat. No. 6,019,968. The protein scaffold-based dry powder compositions may be produced by spray drying

solutions or slurries of the protein scaffold and, optionally, excipients, in a solvent under conditions to provide a respirable dry powder. Solvents may include polar compounds, such as water and ethanol, which may be readily dried. Protein scaffold stability may be enhanced by performing the spray drying procedures in the absence of oxygen, such as under a nitrogen blanket or by using nitrogen as the drying gas. Another relatively dry formulation is a dispersion of a plurality of perforated microstructures dispersed in a suspension medium that typically comprises a hydrofluoroalkane propellant as taught in WO 9916419. The stabilized dispersions may be administered to the lung of a patient using a metered dose inhaler. Equipment useful in the commercial manufacture of spray dried medicaments are manufactured by Buchi Ltd. or Niro Corp.

[0264] At least one protein scaffold in either the stable or preserved formulations or solutions described herein, can be administered to a patient in accordance with the present invention via a variety of delivery methods including SC or IM injection; transdermal, pulmonary, transmucosal, implant, osmotic pump, cartridge, micro pump, or other means appreciated by the skilled artisan, as well-known in the art.

Therapeutic Applications

[0265] The present invention also provides a method for modulating or treating a disease, in a cell, tissue, organ, animal, or patient, as known in the art or as described herein, using at least one protein scaffold of the present invention, e.g., administering or contacting the cell, tissue, organ, animal, or patient with a therapeutic effective amount of protein scaffold. The present invention also provides a method for modulating or treating a disease, in a cell, tissue, organ, animal, or patient including, but not limited to, a malignant disease.

[0266] The present invention also provides a method for modulating or treating at least one malignant disease in a cell, tissue, organ, animal or patient, including, but not limited to, at least one of: leukemia, acute leukemia, acute lymphoblastic leukemia (ALL), acute lymphocytic leukemia, B-cell, T-cell or FAB ALL, acute myeloid leukemia (AML), acute myelogenous leukemia, chronic myelocytic leukemia (CML), chronic lymphocytic leukemia (CLL), hairy cell leukemia, myelodysplastic syndrome (MDS), a lymphoma, Hodgkin's disease, a malignant lymphoma, non-Hodgkin's lymphoma, Burkitt's lymphoma, multiple myeloma, Kaposi's sarcoma, colorectal carcinoma, pancreatic carcinoma, nasopharyngeal carcinoma, malignant histiocytosis, paraneoplastic syndrome/hypercalcemia of malignancy, solid tumors, bladder cancer, breast cancer, colorectal cancer, endometrial cancer, head cancer, neck cancer, hereditary nonpolyposis cancer, Hodgkin's lymphoma, liver cancer, lung cancer, non-small cell lung cancer, ovarian cancer, pancreatic cancer, prostate cancer, renal

cell carcinoma, testicular cancer, adenocarcinomas, sarcomas, malignant melanoma, hemangioma, metastatic disease, cancer related bone resorption, cancer related bone pain, and the like.

[0267] Any method of the present invention can comprise administering an effective amount of a composition or pharmaceutical composition comprising at least one protein scaffold to a cell, tissue, organ, animal or patient in need of such modulation, treatment or therapy. Such a method can optionally further comprise co-administration or combination therapy for treating such diseases or disorders, wherein the administering of said at least one protein scaffold, specified portion or variant thereof, further comprises administering, before concurrently, and/or after, at least one selected from at least one of an alkylating agent, an a mitotic inhibitor, and a radiopharmaceutical. Suitable dosages are well known in the art. See, e.g., Wells et al., eds., *Pharmacotherapy Handbook*, 2nd Edition, Appleton and Lange, Stamford, Conn. (2000); *PDR Pharmacopoeia*, Tarascon Pocket Pharmacopoeia 2000, Deluxe Edition, Tarascon Publishing, Loma Linda, Calif. (2000); *Nursing 2001 Handbook of Drugs*, 21st edition, Springhouse Corp., Springhouse, Pa., 2001; *Health Professional's Drug Guide 2001*, ed., Shannon, Wilson, Stang, Prentice-Hall, Inc, Upper Saddle River, N.J. each of which references are entirely incorporated herein by reference.

[0268] Preferred doses can optionally include about 0.1-99 and/or 100-500 mg/kg/administration, or any range, value or fraction thereof, or to achieve a serum concentration of about 0.1-5000 µg/ml serum concentration per single or multiple administration, or any range, value or fraction thereof. A preferred dosage range for the protein scaffold of the present invention is from about 1 mg/kg, up to about 3, about 6 or about 12 mg/kg of body weight of the patient.

[0269] Alternatively, the dosage administered can vary depending upon known factors, such as the pharmacodynamic characteristics of the particular agent, and its mode and route of administration; age, health, and weight of the recipient; nature and extent of symptoms, kind of concurrent treatment, frequency of treatment, and the effect desired. Usually a dosage of active ingredient can be about 0.1 to 100 milligrams per kilogram of body weight. Ordinarily 0.1 to 50, and preferably, 0.1 to 10 milligrams per kilogram per administration or in sustained release form is effective to obtain desired results.

[0270] As a non-limiting example, treatment of humans or animals can be provided as a one-time or periodic dosage of at least one protein scaffold of the present invention about 0.1 to 100 mg/kg or any range, value or fraction thereof per day, on at least one of day 1-40, or,

alternatively or additionally, at least one of week 1-52, or, alternatively or additionally, at least one of 1-20 years, or any combination thereof, using single, infusion or repeated doses.

[0271] Dosage forms (composition) suitable for internal administration generally contain from about 0.001 milligram to about 500 milligrams of active ingredient per unit or container. In these pharmaceutical compositions the active ingredient will ordinarily be present in an amount of about 0.5-99.999% by weight based on the total weight of the composition.

[0272] For parenteral administration, the protein scaffold can be formulated as a solution, suspension, emulsion, particle, powder, or lyophilized powder in association, or separately provided, with a pharmaceutically acceptable parenteral vehicle. Examples of such vehicles are water, saline, Ringer's solution, dextrose solution, and about 1-10% human serum albumin. Liposomes and nonaqueous vehicles, such as fixed oils, can also be used. The vehicle or lyophilized powder can contain additives that maintain isotonicity (e.g., sodium chloride, mannitol) and chemical stability (e.g., buffers and preservatives). The formulation is sterilized by known or suitable techniques.

[0273] Suitable pharmaceutical carriers are described in the most recent edition of Remington's Pharmaceutical Sciences, A. Osol, a standard reference text in this field.

Alternative Administration

[0274] Many known and developed modes can be used according to the present invention for administering pharmaceutically effective amounts of at least one protein scaffold according to the present invention. While pulmonary administration is used in the following description, other modes of administration can be used according to the present invention with suitable results. Protein scaffolds of the present invention can be delivered in a carrier, as a solution, emulsion, colloid, or suspension, or as a dry powder, using any of a variety of devices and methods suitable for administration by inhalation or other modes described here within or known in the art.

Parenteral Formulations and Administration

[0275] Formulations for parenteral administration can contain as common excipients sterile water or saline, polyalkylene glycols, such as polyethylene glycol, oils of vegetable origin, hydrogenated naphthalenes and the like. Aqueous or oily suspensions for injection can be prepared by using an appropriate emulsifier or humidifier and a suspending agent, according to known methods. Agents for injection can be a non-toxic, non-orally administrable diluting agent, such as aqueous solution, a sterile injectable solution or suspension in a solvent. As the usable vehicle or solvent, water, Ringer's solution, isotonic saline, etc. are allowed; as an ordinary solvent or suspending solvent, sterile involatile oil can be used. For these purposes,

any kind of involatile oil and fatty acid can be used, including natural or synthetic or semisynthetic fatty oils or fatty acids; natural or synthetic or semisynthetic mono- or di- or tri-glycerides. Parental administration is known in the art and includes, but is not limited to, conventional means of injections, a gas pressured needle-less injection device as described in U.S. Pat. No. 5,851,198, and a laser perforator device as described in U.S. Pat. No. 5,839,446 entirely incorporated herein by reference.

Alternative Delivery

[0276] The invention further relates to the administration of at least one protein scaffold by parenteral, subcutaneous, intramuscular, intravenous, intrarticular, intrabronchial, intraabdominal, intracapsular, intracartilaginous, intracavitary, intracelial, intracerebellar, intracerebroventricular, intracolic, intracervical, intragastric, intrahepatic, intramyocardial, intraosteal, intrapelvic, intrapericardiac, intraperitoneal, intrapleural, intraprostatic, intrapulmonary, intrarectal, intrarenal, intraretinal, intraspinal, intrasynovial, intrathoracic, intrauterine, intravesical, intralesional, bolus, vaginal, rectal, buccal, sublingual, intranasal, or transdermal means. At least one protein scaffold composition can be prepared for use for parenteral (subcutaneous, intramuscular or intravenous) or any other administration particularly in the form of liquid solutions or suspensions; for use in vaginal or rectal administration particularly in semisolid forms, such as, but not limited to, creams and suppositories; for buccal, or sublingual administration, such as, but not limited to, in the form of tablets or capsules; or intranasally, such as, but not limited to, the form of powders, nasal drops or aerosols or certain agents; or transdermally, such as not limited to a gel, ointment, lotion, suspension or patch delivery system with chemical enhancers such as dimethyl sulfoxide to either modify the skin structure or to increase the drug concentration in the transdermal patch (Junginger, et al. In "Drug Permeation Enhancement," Hsieh, D. S., Eds., pp. 59-90 (Marcel Dekker, Inc. New York 1994, entirely incorporated herein by reference), or with oxidizing agents that enable the application of formulations containing proteins and peptides onto the skin (WO 98/53847), or applications of electric fields to create transient transport pathways, such as electroporation, or to increase the mobility of charged drugs through the skin, such as iontophoresis, or application of ultrasound, such as sonophoresis (U.S. Pat. Nos. 4,309,989 and 4,767,402) (the above publications and patents being entirely incorporated herein by reference).

Pulmonary/Nasal Administration

[0277] For pulmonary administration, preferably, at least one protein scaffold composition is delivered in a particle size effective for reaching the lower airways of the lung or sinuses.

According to the invention, at least one protein scaffold can be delivered by any of a variety of inhalation or nasal devices known in the art for administration of a therapeutic agent by inhalation. These devices capable of depositing aerosolized formulations in the sinus cavity or alveoli of a patient include metered dose inhalers, nebulizers, dry powder generators, sprayers, and the like. Other devices suitable for directing the pulmonary or nasal administration of protein scaffolds are also known in the art. All such devices can use formulations suitable for the administration for the dispensing of protein scaffold in an aerosol. Such aerosols can be comprised of either solutions (both aqueous and nonaqueous) or solid particles.

[0278] Metered dose inhalers like the Ventolin metered dose inhaler, typically use a propellant gas and require actuation during inspiration (See, e.g., WO 94/16970, WO 98/35888). Dry powder inhalers like Turbuhaler™ (Astra), Rotahaler® (Glaxo), Diskus® (Glaxo), Spiros™ inhaler (Dura), devices marketed by Inhale Therapeutics, and the Spinhaler® powder inhaler (Fisons), use breath-actuation of a mixed powder (U.S. Pat. No. 4,668,218 Astra, EP 237507 Astra, WO 97/25086 Glaxo, WO 94/08552 Dura, U.S. Pat. No. 5,458,135 Inhale, WO 94/06498 Fisons, entirely incorporated herein by reference). Nebulizers like AERx™ Aradigm, the Ultravent® nebulizer (Mallinckrodt), and the Acorn II® nebulizer (Marquest Medical Products) (U.S. Pat. No. 5,404,871 Aradigm, WO 97/22376), the above references entirely incorporated herein by reference, produce aerosols from solutions, while metered dose inhalers, dry powder inhalers, etc. generate small particle aerosols. These specific examples of commercially available inhalation devices are intended to be a representative of specific devices suitable for the practice of this invention, and are not intended as limiting the scope of the invention.

[0279] Preferably, a composition comprising at least one protein scaffold is delivered by a dry powder inhaler or a sprayer. There are several desirable features of an inhalation device for administering at least one protein scaffold of the present invention. For example, delivery by the inhalation device is advantageously reliable, reproducible, and accurate. The inhalation device can optionally deliver small dry particles, e.g., less than about 10 µm, preferably about 1-5 µm, for good respirability.

Administration of Protein Scaffold Compositions as a Spray

[0280] A spray including protein scaffold composition can be produced by forcing a suspension or solution of at least one protein scaffold through a nozzle under pressure. The nozzle size and configuration, the applied pressure, and the liquid feed rate can be chosen to achieve the desired output and particle size. An electrospray can be produced, for example,

by an electric field in connection with a capillary or nozzle feed. Advantageously, particles of at least one protein scaffold composition delivered by a sprayer have a particle size less than about 10 μm , preferably, in the range of about 1 μm to about 5 μm , and, most preferably, about 2 μm to about 3 μm .

[0281] Formulations of at least one protein scaffold composition suitable for use with a sprayer typically include protein scaffold composition in an aqueous solution at a concentration of about 0.1 mg to about 100 mg of at least one protein scaffold composition per ml of solution or mg/gm, or any range, value, or fraction therein. The formulation can include agents, such as an excipient, a buffer, an isotonicity agent, a preservative, a surfactant, and, preferably, zinc. The formulation can also include an excipient or agent for stabilization of the protein scaffold composition, such as a buffer, a reducing agent, a bulk protein, or a carbohydrate. Bulk proteins useful in formulating protein scaffold compositions include albumin, protamine, or the like. Typical carbohydrates useful in formulating protein scaffold compositions include sucrose, mannitol, lactose, trehalose, glucose, or the like. The protein scaffold composition formulation can also include a surfactant, which can reduce or prevent surface-induced aggregation of the protein scaffold composition caused by atomization of the solution in forming an aerosol. Various conventional surfactants can be employed, such as polyoxyethylene fatty acid esters and alcohols, and polyoxyethylene sorbitol fatty acid esters. Amounts will generally range between 0.001 and 14% by weight of the formulation. Especially preferred surfactants for purposes of this invention are polyoxyethylene sorbitan monooleate, polysorbate 80, polysorbate 20, or the like. Additional agents known in the art for formulation of a protein, such as protein scaffolds, or specified portions or variants, can also be included in the formulation.

Administration of Protein Scaffold Compositions by a Nebulizer

[0282] Protein scaffold compositions of the invention can be administered by a nebulizer, such as jet nebulizer or an ultrasonic nebulizer. Typically, in a jet nebulizer, a compressed air source is used to create a high-velocity air jet through an orifice. As the gas expands beyond the nozzle, a low-pressure region is created, which draws a solution of protein scaffold composition through a capillary tube connected to a liquid reservoir. The liquid stream from the capillary tube is sheared into unstable filaments and droplets as it exits the tube, creating the aerosol. A range of configurations, flow rates, and baffle types can be employed to achieve the desired performance characteristics from a given jet nebulizer. In an ultrasonic nebulizer, high-frequency electrical energy is used to create vibrational, mechanical energy, typically employing a piezoelectric transducer. This energy is transmitted to the formulation

of protein scaffold composition either directly or through a coupling fluid, creating an aerosol including the protein scaffold composition. Advantageously, particles of protein scaffold composition delivered by a nebulizer have a particle size less than about 10 μm , preferably, in the range of about 1 μm to about 5 μm , and, most preferably, about 2 μm to about 3 μm .

[0283] Formulations of at least one protein scaffold suitable for use with a nebulizer, either jet or ultrasonic, typically include a concentration of about 0.1 mg to about 100 mg of at least one protein scaffold per ml of solution. The formulation can include agents, such as an excipient, a buffer, an isotonicity agent, a preservative, a surfactant, and, preferably, zinc. The formulation can also include an excipient or agent for stabilization of the at least one protein scaffold composition, such as a buffer, a reducing agent, a bulk protein, or a carbohydrate. Bulk proteins useful in formulating at least one protein scaffold compositions include albumin, protamine, or the like. Typical carbohydrates useful in formulating at least one protein scaffold include sucrose, mannitol, lactose, trehalose, glucose, or the like. The at least one protein scaffold formulation can also include a surfactant, which can reduce or prevent surface-induced aggregation of the at least one protein scaffold caused by atomization of the solution in forming an aerosol. Various conventional surfactants can be employed, such as polyoxyethylene fatty acid esters and alcohols, and polyoxyethylene sorbital fatty acid esters. Amounts will generally range between about 0.001 and 4% by weight of the formulation. Especially preferred surfactants for purposes of this invention are polyoxyethylene sorbitan mono-oleate, polysorbate 80, polysorbate 20, or the like. Additional agents known in the art for formulation of a protein, such as protein scaffold, can also be included in the formulation.

Administration of Protein Scaffold Compositions by a Metered Dose Inhaler

[0284] In a metered dose inhaler (MDI), a propellant, at least one protein scaffold, and any excipients or other additives are contained in a canister as a mixture including a liquefied compressed gas. Actuation of the metering valve releases the mixture as an aerosol, preferably containing particles in the size range of less than about 10 μm , preferably, about 1 μm to about 5 μm , and, most preferably, about 2 μm to about 3 μm . The desired aerosol particle size can be obtained by employing a formulation of protein scaffold composition produced by various methods known to those of skill in the art, including jet-milling, spray drying, critical point condensation, or the like. Preferred metered dose inhalers include those manufactured by 3M or Glaxo and employing a hydrofluorocarbon propellant. Formulations of at least one protein scaffold for use with a metered-dose inhaler device will generally include a finely divided powder containing at least one protein scaffold as a suspension in a

non-aqueous medium, for example, suspended in a propellant with the aid of a surfactant. The propellant can be any conventional material employed for this purpose, such as chlorofluorocarbon, a hydrochlorofluorocarbon, a hydrofluorocarbon, or a hydrocarbon, including trichlorofluoromethane, dichlorodifluoromethane, dichlorotetrafluoroethanol and 1,1,1,2-tetrafluoroethane, HFA-134a (hydrofluoroalkane-134a), HFA-227 (hydrofluoroalkane-227), or the like. Preferably, the propellant is a hydrofluorocarbon. The surfactant can be chosen to stabilize the at least one protein scaffold as a suspension in the propellant, to protect the active agent against chemical degradation, and the like. Suitable surfactants include sorbitan trioleate, soya lecithin, oleic acid, or the like. In some cases, solution aerosols are preferred using solvents, such as ethanol. Additional agents known in the art for formulation of a protein can also be included in the formulation. One of ordinary skill in the art will recognize that the methods of the current invention can be achieved by pulmonary administration of at least one protein scaffold composition via devices not described herein.

Oral Formulations and Administration

[0285] Formulations for oral administration rely on the co-administration of adjuvants (e.g., resorcinols and nonionic surfactants, such as polyoxyethylene oleyl ether and n-hexadecylpolyethylene ether) to increase artificially the permeability of the intestinal walls, as well as the co-administration of enzymatic inhibitors (e.g., pancreatic trypsin inhibitors, diisopropylfluorophosphate (DFF) and trasylol) to inhibit enzymatic degradation.

Formulations for delivery of hydrophilic agents including proteins and protein scaffolds and a combination of at least two surfactants intended for oral, buccal, mucosal, nasal, pulmonary, vaginal transmembrane, or rectal administration are taught in U.S. Pat. No. 6,309,663. The active constituent compound of the solid-type dosage form for oral administration can be mixed with at least one additive, including sucrose, lactose, cellulose, mannitol, trehalose, raffinose, maltitol, dextran, starches, agar, arginates, chitins, chitosans, pectins, gum tragacanth, gum arabic, gelatin, collagen, casein, albumin, synthetic or semisynthetic polymer, and glyceride. These dosage forms can also contain other type(s) of additives, e.g., inactive diluting agent, lubricant, such as magnesium stearate, paraben, preserving agent, such as sorbic acid, ascorbic acid, .alpha.-tocopherol, antioxidant such as cysteine, disintegrator, binder, thickener, buffering agent, sweetening agent, flavoring agent, perfuming agent, etc.

[0286] Tablets and pills can be further processed into enteric-coated preparations. The liquid preparations for oral administration include emulsion, syrup, elixir, suspension and

solution preparations allowable for medical use. These preparations can contain inactive diluting agents ordinarily used in said field, e.g., water. Liposomes have also been described as drug delivery systems for insulin and heparin (U.S. Pat. No. 4,239,754). More recently, microspheres of artificial polymers of mixed amino acids (proteinoids) have been used to deliver pharmaceuticals (U.S. Pat. No. 4,925,673). Furthermore, carrier compounds described in U.S. Pat. No. 5,879,681 and U.S. Pat. No. 5,871,753 and used to deliver biologically active agents orally are known in the art.

Mucosal Formulations and Administration

[0287] A formulation for orally administering a bioactive agent encapsulated in one or more biocompatible polymer or copolymer excipients, preferably, a biodegradable polymer or copolymer, affording microcapsules which due to the proper size of the resultant microcapsules results in the agent reaching and being taken up by the folliculi lymphatic aggregati, otherwise known as the “Peyer's patch,” or “GALT” of the animal without loss of effectiveness due to the agent having passed through the gastrointestinal tract. Similar folliculi lymphatic aggregati can be found in the bronchei tubes (BALT) and the large intestine. The above-described tissues are referred to in general as mucosally associated lymphoreticular tissues (MALT). For absorption through mucosal surfaces, compositions and methods of administering at least one protein scaffold include an emulsion comprising a plurality of submicron particles, a mucoadhesive macromolecule, a bioactive peptide, and an aqueous continuous phase, which promotes absorption through mucosal surfaces by achieving mucoadhesion of the emulsion particles (U.S. Pat. No. 5,514,670). Mucous surfaces suitable for application of the emulsions of the present invention can include corneal, conjunctival, buccal, sublingual, nasal, vaginal, pulmonary, stomachic, intestinal, and rectal routes of administration. Formulations for vaginal or rectal administration, e.g., suppositories, can contain as excipients, for example, polyalkyleneglycols, vaseline, cocoa butter, and the like. Formulations for intranasal administration can be solid and contain as excipients, for example, lactose or can be aqueous or oily solutions of nasal drops. For buccal administration, excipients include sugars, calcium stearate, magnesium stearate, pregelatinated starch, and the like (U.S. Pat. No. 5,849,695).

Transdermal Formulations and Administration

[0288] For transdermal administration, the at least one protein scaffold is encapsulated in a delivery device, such as a liposome or polymeric nanoparticles, microparticle, microcapsule, or microspheres (referred to collectively as microparticles unless otherwise stated). A number of suitable devices are known, including microparticles made of synthetic polymers, such as

polyhydroxy acids, such as polylactic acid, polyglycolic acid and copolymers thereof, polyorthoesters, polyanhydrides, and polyphosphazenes, and natural polymers, such as collagen, polyamino acids, albumin and other proteins, alginate and other polysaccharides, and combinations thereof (U.S. Pat. No. 5,814,599).

Prolonged Administration and Formulations

[0289] It can be desirable to deliver the compounds of the present invention to the subject over prolonged periods of time, for example, for periods of one week to one year from a single administration. Various slow release, depot or implant dosage forms can be utilized. For example, a dosage form can contain a pharmaceutically acceptable non-toxic salt of the compounds that has a low degree of solubility in body fluids, for example, (a) an acid addition salt with a polybasic acid, such as phosphoric acid, sulfuric acid, citric acid, tartaric acid, tannic acid, pamoic acid, alginic acid, polyglutamic acid, naphthalene mono- or di-sulfonic acids, polygalacturonic acid, and the like; (b) a salt with a polyvalent metal cation, such as zinc, calcium, bismuth, barium, magnesium, aluminum, copper, cobalt, nickel, cadmium and the like, or with an organic cation formed from e.g., N,N'-dibenzyl-ethylenediamine or ethylenediamine; or (c) combinations of (a) and (b), e.g., a zinc tannate salt. Additionally, the compounds of the present invention or, preferably, a relatively insoluble salt, such as those just described, can be formulated in a gel, for example, an aluminum monostearate gel with, e.g., sesame oil, suitable for injection. Particularly preferred salts are zinc salts, zinc tannate salts, pamoate salts, and the like. Another type of slow release depot formulation for injection would contain the compound or salt dispersed for encapsulation in a slow degrading, non-toxic, non-antigenic polymer, such as a polylactic acid/polyglycolic acid polymer for example as described in U.S. Pat. No. 3,773,919. The compounds or, preferably, relatively insoluble salts, such as those described above, can also be formulated in cholesterol matrix silastic pellets, particularly for use in animals. Additional slow release, depot or implant formulations, e.g., gas or liquid liposomes, are known in the literature (U.S. Pat. No. 5,770,222 and "Sustained and Controlled Release Drug Delivery Systems", J. R. Robinson ed., Marcel Dekker, Inc., N.Y., 1978).

Infusion of Modified Cells as Adoptive Cell Therapy

[0290] The disclosure provides modified cells that express one or more CARs and/or CARTyrins of the disclosure that have been selected and/or expanded for administration to a subject in need thereof. Modified cells of the disclosure may be formulated for storage at any temperature including room temperature and body temperature. Modified cells of the disclosure may be formulated for cryopreservation and subsequent thawing. Modified cells of

the disclosure may be formulated in a pharmaceutically acceptable carrier for direct administration to a subject from sterile packaging. Modified cells of the disclosure may be formulated in a pharmaceutically acceptable carrier with an indicator of cell viability and/or CAR/CARTyrin expression level to ensure a minimal level of cell function and CAR/CARTyrin expression. Modified cells of the disclosure may be formulated in a pharmaceutically acceptable carrier at a prescribed density with one or more reagents to inhibit further expansion and/or prevent cell death.

Inducible Proapoptotic Polypeptides

[0291] Inducible proapoptotic polypeptides of the disclosure are superior to existing inducible polypeptides because the inducible proapoptotic polypeptides of the disclosure are far less immunogenic. While inducible proapoptotic polypeptides of the disclosure are recombinant polypeptides, and, therefore, non-naturally occurring, the sequences that are recombined to produce the inducible proapoptotic polypeptides of the disclosure do not comprise non-human sequences that the host human immune system could recognize as “non-self” and, consequently, induce an immune response in the subject receiving an inducible proapoptotic polypeptide of the disclosure, a cell comprising the inducible proapoptotic polypeptide or a composition comprising the inducible proapoptotic polypeptide or the cell comprising the inducible proapoptotic polypeptide.

[0292] The disclosure provides inducible proapoptotic polypeptides comprising a ligand binding region, a linker, and a proapoptotic peptide, wherein the inducible proapoptotic polypeptide does not comprise a non-human sequence. In certain embodiments, the non-human sequence comprises a restriction site. In certain embodiments, the proapoptotic peptide is a caspase polypeptide. In certain embodiments, the caspase polypeptide is a caspase 9 polypeptide. In certain embodiments, the caspase 9 polypeptide is a truncated caspase 9 polypeptide. Inducible proapoptotic polypeptides of the disclosure may be non-naturally occurring.

[0293] Caspase polypeptides of the disclosure include, but are not limited to, caspase 1, caspase 2, caspase 3, caspase 4, caspase 5, caspase 6, caspase 7, caspase 8, caspase 9, caspase 10, caspase 11, caspase 12, and caspase 14. Caspase polypeptides of the disclosure include, but are not limited to, those caspase polypeptides associated with apoptosis including caspase 2, caspase 3, caspase 6, caspase 7, caspase 8, caspase 9, and caspase 10. Caspase polypeptides of the disclosure include, but are not limited to, those caspase polypeptides that initiate apoptosis, including caspase 2, caspase 8, caspase 9, and caspase 10. Caspase

polypeptides of the disclosure include, but are not limited to, those caspase polypeptides that execute apoptosis, including caspase 3, caspase 6, and caspase 7.

[0294] Caspase polypeptides of the disclosure may be encoded by an amino acid or a nucleic acid sequence having one or more modifications compared to a wild type amino acid or a nucleic acid sequence. The nucleic acid sequence encoding a caspase polypeptide of the disclosure may be codon optimized. The one or more modifications to an amino acid and/or nucleic acid sequence of a caspase polypeptide of the disclosure may increase an interaction, a cross-linking, a cross-activation, or an activation of the caspase polypeptide of the disclosure compared to a wild type amino acid or a nucleic acid sequence. Alternatively, or in addition, the one or more modifications to an amino acid and/or nucleic acid sequence of a caspase polypeptide of the disclosure may decrease the immunogenicity of the caspase polypeptide of the disclosure compared to a wild type amino acid or a nucleic acid sequence.

[0295] Caspase polypeptides of the disclosure may be truncated compared to a wild type caspase polypeptide. For example, a caspase polypeptide may be truncated to eliminate a sequence encoding a Caspase Activation and Recruitment Domain (CARD) to eliminate or minimize the possibility of activating a local inflammatory response in addition to initiating apoptosis in the cell comprising an inducible caspase polypeptide of the disclosure. The nucleic acid sequence encoding a caspase polypeptide of the disclosure may be spliced to form a variant amino acid sequence of the caspase polypeptide of the disclosure compared to a wild type caspase polypeptide. Caspase polypeptides of the disclosure may be encoded by recombinant and/or chimeric sequences. Recombinant and/or chimeric caspase polypeptides of the disclosure may include sequences from one or more different caspase polypeptides. Alternatively, or in addition, recombinant and/or chimeric caspase polypeptides of the disclosure may include sequences from one or more species (e.g. a human sequence and a non-human sequence). Caspase polypeptides of the disclosure may be non-naturally occurring.

[0296] The ligand binding region of an inducible proapoptotic polypeptide of the disclosure may include any polypeptide sequence that facilitates or promotes the dimerization of a first inducible proapoptotic polypeptide of the disclosure with a second inducible proapoptotic polypeptide of the disclosure, the dimerization of which activates or induces cross-linking of the proapoptotic polypeptides and initiation of apoptosis in the cell.

[0297] The ligand-binding (“dimerization”) region may comprise any polypeptide or functional domain thereof that will allow for induction using a natural or unnatural ligand (i.e. and induction agent), for example, an unnatural synthetic ligand. The ligand-binding

region may be internal or external to the cellular membrane, depending upon the nature of the inducible proapoptotic polypeptide and the choice of ligand (i.e. induction agent). A wide variety of ligand-binding polypeptides and functional domains thereof, including receptors, are known. Ligand-binding regions of the disclosure may include one or more sequences from a receptor. Of particular interest are ligand-binding regions for which ligands (for example, small organic ligands) are known or may be readily produced. These ligand-binding regions or receptors may include, but are not limited to, the FKBP and cyclophilin receptors, the steroid receptors, the tetracycline receptor, and the like, as well as “unnatural” receptors, which can be obtained from antibodies, particularly the heavy or light chain subunit, mutated sequences thereof, random amino acid sequences obtained by stochastic procedures, combinatorial syntheses, and the like. In certain embodiments, the ligand-binding region is selected from the group consisting of a FKBP ligand-binding region, a cyclophilin receptor ligand-binding region, a steroid receptor ligand-binding region, a cyclophilin receptors ligand-binding region, and a tetracycline receptor ligand-binding region.

[0298] The ligand-binding regions comprising one or more receptor domain(s) may be at least about 50 amino acids, and fewer than about 350 amino acids, usually fewer than 200 amino acids, either as the natural domain or truncated active portion thereof. The binding region may, for example, be small (< 25 kDa, to allow efficient transfection in viral vectors), monomeric, nonimmunogenic, have synthetically accessible, cell permeable, nontoxic ligands that can be configured for dimerization.

[0299] The ligand-binding regions comprising one or more receptor domain(s) may be intracellular or extracellular depending upon the design of the inducible proapoptotic polypeptide and the availability of an appropriate ligand (i.e. induction agent). For hydrophobic ligands, the binding region can be on either side of the membrane, but for hydrophilic ligands, particularly protein ligands, the binding region will usually be external to the cell membrane, unless there is a transport system for internalizing the ligand in a form in which it is available for binding. For an intracellular receptor, the inducible proapoptotic polypeptide or a transposon or vector comprising the inducible proapoptotic polypeptide may encode a signal peptide and transmembrane domain 5' or 3' of the receptor domain sequence or may have a lipid attachment signal sequence 5' of the receptor domain sequence. Where the receptor domain is between the signal peptide and the transmembrane domain, the receptor domain will be extracellular.

[0300] Antibodies and antibody subunits, e.g., heavy or light chain, particularly fragments, more particularly all or part of the variable region, or fusions of heavy and light chain to

create high-affinity binding, can be used as a ligand binding region of the disclosure. Antibodies that are contemplated include ones that are an ectopically expressed human product, such as an extracellular domain that would not trigger an immune response and generally not expressed in the periphery (i.e., outside the CNS/brain area). Such examples, include, but are not limited to low affinity nerve growth factor receptor (LNGFR), and embryonic surface proteins (i.e., carcinoembryonic antigen). Yet further, antibodies can be prepared against haptenic molecules, which are physiologically acceptable, and the individual antibody subunits screened for binding affinity. The cDNA encoding the subunits can be isolated and modified by deletion of the constant region, portions of the variable region, mutagenesis of the variable region, or the like, to obtain a binding protein domain that has the appropriate affinity for the ligand. In this way, almost any physiologically acceptable haptenic compound can be employed as the ligand or to provide an epitope for the ligand. Instead of antibody units, natural receptors can be employed, where the binding region or domain is known and there is a useful or known ligand for binding.

[0301] For multimerizing the receptor, the ligand for the ligand-binding region/receptor domains of the inducible proapoptotic polypeptides may be multimeric in the sense that the ligand can have at least two binding sites, with each of the binding sites capable of binding to a ligand receptor region (i.e. a ligand having a first binding site capable of binding the ligand-binding region of a first inducible proapoptotic polypeptide and a second binding site capable of binding the ligand-binding region of a second inducible proapoptotic polypeptide, wherein the ligand-binding regions of the first and the second inducible proapoptotic polypeptides are either identical or distinct). Thus, as used herein, the term “multimeric ligand binding region” refers to a ligand-binding region of an inducible proapoptotic polypeptide of the disclosure that binds to a multimeric ligand. Multimeric ligands of the disclosure include dimeric ligands. A dimeric ligand of the disclosure may have two binding sites capable of binding to the ligand receptor domain. In certain embodiments, multimeric ligands of the disclosure are a dimer or higher order oligomer, usually not greater than about tetrameric, of small synthetic organic molecules, the individual molecules typically being at least about 150 Da and less than about 5 kDa, usually less than about 3 kDa. A variety of pairs of synthetic ligands and receptors can be employed. For example, in embodiments involving natural receptors, dimeric FK506 can be used with an FKBP12 receptor, dimerized cyclosporin A can be used with the cyclophilin receptor, dimerized estrogen with an estrogen receptor, dimerized glucocorticoids with a glucocorticoid receptor, dimerized tetracycline with the tetracycline receptor, dimerized vitamin D with the vitamin D receptor, and the like. Alternatively higher

orders of the ligands, e.g., trimeric can be used. For embodiments involving unnatural receptors, e.g., antibody subunits, modified antibody subunits, single chain antibodies comprised of heavy and light chain variable regions in tandem, separated by a flexible linker, or modified receptors, and mutated sequences thereof, and the like, any of a large variety of compounds can be used. A significant characteristic of the units comprising a multimeric ligand of the disclosure is that each binding site is able to bind the receptor with high affinity, and preferably, that they are able to be dimerized chemically. Also, methods are available to balance the hydrophobicity/hydrophilicity of the ligands so that they are able to dissolve in serum at functional levels, yet diffuse across plasma membranes for most applications.

[0302] Activation of inducible proapoptotic polypeptides of the disclosure may be accomplished through, for example, chemically induced dimerization (CID) mediated by an induction agent to produce a conditionally controlled protein or polypeptide. Proapoptotic polypeptides of the disclosure not only inducible, but the induction of these polypeptides is also reversible, due to the degradation of the labile dimerizing agent or administration of a monomeric competitive inhibitor.

[0303] In certain embodiments, the ligand binding region comprises a FK506 binding protein 12 (FKBP12) polypeptide. In certain embodiments, the ligand binding region comprises a FKBP12 polypeptide having a substitution of valine (V) for phenylalanine (F) at position 36 (F36V). In certain embodiments, in which the ligand binding region comprises a FKBP12 polypeptide having a substitution of valine (V) for phenylalanine (F) at position 36 (F36V), the induction agent may comprise AP1903, a synthetic drug (CAS Index Name: 2-Piperidinecarboxylic acid, 1-[(2S)-1-oxo-2-(3,4,5-trimethoxyphenyl)butyl]-, 1,2-ethanediylbis[imino(2-oxo-2,1-ethanediyl)oxy-3,1-phenylene[(1R)-3-(3,4-dimethoxyphenyl)propylidene]]ester, [2S-[1(R*),2R*[S*[S*[1(R*),2R*]]]]-(9CI) CAS Registry Number: 195514-63-7; Molecular Formula: C₇₈H₉₈N₄O₂₀; Molecular Weight: 1411.65)). In certain embodiments, in which the ligand binding region comprises a FKBP12 polypeptide having a substitution of valine (V) for phenylalanine (F) at position 36 (F36V), the induction agent may comprise AP20187 (CAS Registry Number: 195514-80-8 and Molecular Formula: C₈₂H₁₀₇N₅O₂₀). In certain embodiments, the induction agent is an AP20187 analog, such as, for example, AP1510. As used herein, the induction agents AP20187, AP1903 and AP1510 may be used interchangeably.

[0304] AP1903 API is manufactured by Alphora Research Inc. and AP1903 Drug Product for Injection is made by Formatech Inc. It is formulated as a 5 mg/mL solution of AP1903 in a 25% solution of the non-ionic solubilizer Solutol HS 15 (250 mg/mL, BASF). At room

temperature, this formulation is a clear, slightly yellow solution. Upon refrigeration, this formulation undergoes a reversible phase transition, resulting in a milky solution. This phase transition is reversed upon re-warming to room temperature. The fill is 2.33 mL in a 3 mL glass vial (approximately 10 mg AP1903 for Injection total per vial). Upon determining a need to administer AP1903, patients may be, for example, administered a single fixed dose of AP1903 for Injection (0.4 mg/kg) via IV infusion over 2 hours, using a non-DEHP, non-ethylene oxide sterilized infusion set. The dose of AP1903 is calculated individually for all patients, and is not be recalculated unless body weight fluctuates by $\geq 10\%$. The calculated dose is diluted in 100 mL in 0.9% normal saline before infusion. In a previous Phase I study of AP1903, 24 healthy volunteers were treated with single doses of AP1903 for Injection at dose levels of 0.01, 0.05, 0.1, 0.5 and 1.0 mg/kg infused IV over 2 hours. AP1903 plasma levels were directly proportional to dose, with mean C_{max} values ranging from approximately 10-1275 ng/mL over the 0.01-1.0 mg/kg dose range. Following the initial infusion period, blood concentrations demonstrated a rapid distribution phase, with plasma levels reduced to approximately 18, 7, and 1% of maximal concentration at 0.5, 2 and 10 hours post-dose, respectively. AP1903 for Injection was shown to be safe and well tolerated at all dose levels and demonstrated a favorable pharmacokinetic profile. Iuliucci J D, et al., J Clin Pharmacol. 41: 870-9, 2001.

[0305] The fixed dose of AP1903 for injection used, for example, may be 0.4 mg/kg intravenously infused over 2 hours. The amount of AP1903 needed in vitro for effective signaling of cells is 10-100 nM (1600 Da MW). This equates to 16-160 µg/L or ~0.016-1.6 µg/kg (1.6-160 µg/kg). Doses up to 1 mg/kg were well-tolerated in the Phase I study of AP1903 described above. Therefore, 0.4 mg/kg may be a safe and effective dose of AP1903 for this Phase I study in combination with the therapeutic cells.

[0306] The amino acid and/or nucleic acid sequence encoding ligand binding of the disclosure may contain sequence one or more modifications compared to a wild type amino acid or nucleic acid sequence. For example, the amino acid and/or nucleic acid sequence encoding ligand binding region of the disclosure may be a codon-optimized sequence. The one or more modifications may increase the binding affinity of a ligand (e.g. an induction agent) for the ligand binding region of the disclosure compared to a wild type polypeptide. Alternatively, or in addition, the one or more modifications may decrease the immunogenicity of the ligand binding region of the disclosure compared to a wild type polypeptide. Ligand binding regions of the disclosure and/or induction agents of the disclosure may be non-naturally occurring.

[0307] Inducible proapoptotic polypeptides of the disclosure comprise a ligand binding region, a linker and a proapoptotic peptide, wherein the inducible proapoptotic polypeptide does not comprise a non-human sequence. In certain embodiments, the non-human sequence comprises a restriction site. The linker may comprise any organic or inorganic material that permits, upon dimerization of the ligand binding region, interaction, cross-linking, cross-activation, or activation of the proapoptotic polypeptides such that the interaction or activation of the proapoptotic polypeptides initiates apoptosis in the cell. In certain embodiments, the linker is a polypeptide. In certain embodiments, the linker is a polypeptide comprising a G/S rich amino acid sequence (a “GS” linker). In certain embodiments, the linker is a polypeptide comprising the amino acid sequence GGGGS (SEQ ID NO: 25). In preferred embodiments, the linker is a polypeptide and the nucleic acid encoding the polypeptide does not contain a restriction site for a restriction endonuclease. Linkers of the disclosure may be non-naturally occurring.

[0308] Inducible proapoptotic polypeptides of the disclosure may be expressed in a cell under the transcriptional regulation of any promoter capable of initiating and/or regulating the expression of an inducible proapoptotic polypeptide of the disclosure in that cell. The term “promoter” as used herein refers to a promoter that acts as the initial binding site for RNA polymerase to transcribe a gene. For example, inducible proapoptotic polypeptides of the disclosure may be expressed in a mammalian cell under the transcriptional regulation of any promoter capable of initiating and/or regulating the expression of an inducible proapoptotic polypeptide of the disclosure in a mammalian cell, including, but not limited to native, endogenous, exogenous, and heterologous promoters. Preferred mammalian cells include human cells. Thus, inducible proapoptotic polypeptides of the disclosure may be expressed in a human cell under the transcriptional regulation of any promoter capable of initiating and/or regulating the expression of an inducible proapoptotic polypeptide of the disclosure in a human cell, including, but not limited to, a human promoter or a viral promoter. Exemplary promoters for expression in human cells include, but are not limited to, a human cytomegalovirus (CMV) immediate early gene promoter, a SV40 early promoter, a Rous sarcoma virus long terminal repeat, β -actin promoter, a rat insulin promoter and a glyceraldehyde-3-phosphate dehydrogenase promoter, each of which may be used to obtain high-level expression of an inducible proapoptotic polypeptide of the disclosure. The use of other viral or mammalian cellular or bacterial phage promoters which are well known in the art to achieve expression of an inducible proapoptotic polypeptide of the disclosure is contemplated as well, provided that the levels of expression are sufficient for initiating

apoptosis in a cell. By employing a promoter with well-known properties, the level and pattern of expression of the protein of interest following transfection or transformation can be optimized.

[0309] Selection of a promoter that is regulated in response to specific physiologic or synthetic signals can permit inducible expression of the inducible proapoptotic polypeptide of the disclosure. The ecdysone system (Invitrogen, Carlsbad, Calif.) is one such system. This system is designed to allow regulated expression of a gene of interest in mammalian cells. It consists of a tightly regulated expression mechanism that allows virtually no basal level expression of a transgene, but over 200-fold inducibility. The system is based on the heterodimeric ecdysone receptor of *Drosophila*, and when ecdysone or an analog such as muristerone A binds to the receptor, the receptor activates a promoter to turn on expression of the downstream transgene high levels of mRNA transcripts are attained. In this system, both monomers of the heterodimeric receptor are constitutively expressed from one vector, whereas the ecdysone-responsive promoter, which drives expression of the gene of interest, is on another plasmid. Engineering of this type of system into a vector of interest may therefore be useful. Another inducible system that may be useful is the Tet-Off™ or Tet-On™ system (Clontech, Palo Alto, Calif.) originally developed by Gossen and Bujard (Gossen and Bujard, Proc. Natl. Acad. Sci. USA, 89:5547-5551, 1992; Gossen et al., Science, 268:1766-1769, 1995). This system also allows high levels of gene expression to be regulated in response to tetracycline or tetracycline derivatives such as doxycycline. In the Tet-On™ system, gene expression is turned on in the presence of doxycycline, whereas in the Tet-Off™ system, gene expression is turned on in the absence of doxycycline. These systems are based on two regulatory elements derived from the tetracycline resistance operon of *E. coli*: the tetracycline operator sequence (to which the tetracycline repressor binds) and the tetracycline repressor protein. The gene of interest is cloned into a plasmid behind a promoter that has tetracycline-responsive elements present in it. A second plasmid contains a regulatory element called the tetracycline-controlled transactivator, which is composed, in the Tet-Off™ system, of the VP16 domain from the herpes simplex virus and the wild-type tetracycline repressor. Thus in the absence of doxycycline, transcription is constitutively on. In the Tet-On™ system, the tetracycline repressor is not wild type and in the presence of doxycycline activates transcription. For gene therapy vector production, the Tet-Off™ system may be used so that the producer cells could be grown in the presence of tetracycline or doxycycline and prevent expression of a potentially toxic transgene, but when the vector is introduced to the patient, the gene expression would be constitutively on.

[0310] In some circumstances, it is desirable to regulate expression of a transgene in a gene therapy vector. For example, different viral promoters with varying strengths of activity are utilized depending on the level of expression desired. In mammalian cells, the CMV immediate early promoter is often used to provide strong transcriptional activation. The CMV promoter is reviewed in Donnelly, J. J., et al., 1997. *Annu. Rev. Immunol.* 15:617-48. Modified versions of the CMV promoter that are less potent have also been used when reduced levels of expression of the transgene are desired. When expression of a transgene in hematopoietic cells is desired, retroviral promoters such as the LTRs from MLV or MMTV are often used. Other viral promoters that are used depending on the desired effect include SV40, RSV LTR, HIV-1 and HIV-2 LTR, adenovirus promoters such as from the E1A, E2A, or MLP region, AAV LTR, HSV-TK, and avian sarcoma virus.

[0311] In other examples, promoters may be selected that are developmentally regulated and are active in particular differentiated cells. Thus, for example, a promoter may not be active in a pluripotent stem cell, but, for example, where the pluripotent stem cell differentiates into a more mature cell, the promoter may then be activated.

[0312] Similarly tissue specific promoters are used to effect transcription in specific tissues or cells so as to reduce potential toxicity or undesirable effects to non-targeted tissues. These promoters may result in reduced expression compared to a stronger promoter such as the CMV promoter, but may also result in more limited expression, and immunogenicity (Bojak, A., et al., 2002. *Vaccine* 20:1975-79; Cazeaux, N., et al., 2002. *Vaccine* 20:3322-31). For example, tissue specific promoters such as the PSA associated promoter or prostate-specific glandular kallikrein, or the muscle creatine kinase gene may be used where appropriate.

[0313] Examples of tissue specific or differentiation specific promoters include, but are not limited to, the following: B29 (B cells); CD14 (monocytic cells); CD43 (leukocytes and platelets); CD45 (hematopoietic cells); CD68 (macrophages); desmin (muscle); elastase-1 (pancreatic acinar cells); endoglin (endothelial cells); fibronectin (differentiating cells, healing tissues); and Flt-1 (endothelial cells); GFAP (astrocytes).

[0314] In certain indications, it is desirable to activate transcription at specific times after administration of the gene therapy vector. This is done with such promoters as those that are hormone or cytokine regulatable. Cytokine and inflammatory protein responsive promoters that can be used include K and T kininogen (Kageyama et al., (1987) *J. Biol. Chem.*, 262, 2345-2351), c-fos, TNF-alpha, C-reactive protein (Arcone, et al., (1988) *Nucl. Acids Res.*, 16(8), 3195-3207), haptoglobin (Oliviero et al., (1987) *EMBO J.*, 6, 1905-1912), serum amyloid A2, C/EBP alpha, IL-1, IL-6 (Poli and Cortese, (1989) *Proc. Nat'l Acad. Sci. USA*,

86, 8202-8206), Complement C3 (Wilson et al., (1990) Mol. Cell. Biol., 6181-6191), IL-8, alpha-1 acid glycoprotein (Prowse and Baumann, (1988) Mol Cell Biol, 8, 42-51), alpha-1 antitrypsin, lipoprotein lipase (Zechner et al., Mol. Cell. Biol., 2394-2401, 1988), angiotensinogen (Ron, et al., (1991) Mol. Cell. Biol., 2887-2895), fibrinogen, c-jun (inducible by phorbol esters, TNF-alpha, UV radiation, retinoic acid, and hydrogen peroxide), collagenase (induced by phorbol esters and retinoic acid), metallothionein (heavy metal and glucocorticoid inducible), Stromelysin (inducible by phorbol ester, interleukin-1 and EGF), alpha-2 macroglobulin and alpha-1 anti-chymotrypsin. Other promoters include, for example, SV40, MMTV, Human Immunodeficiency Virus (MV), Moloney virus, ALV, Epstein Barr virus, Rous Sarcoma virus, human actin, myosin, hemoglobin, and creatine.

[0315] It is envisioned that any of the above promoters alone or in combination with another can be useful depending on the action desired. Promoters, and other regulatory elements, are selected such that they are functional in the desired cells or tissue. In addition, this list of promoters should not be construed to be exhaustive or limiting; other promoters that are used in conjunction with the promoters and methods disclosed herein.

EXAMPLES

Example 1: Characterization of P-BCMA-101 (a/k/a anti-BCMA CARTyrin (A08))

[0316] Expression of CARTyrins of the disclosure was evaluated following mRNA electroporation of a sequence encoding a CARTyrin into T cells. Functionality of CARTyrin-expressing T cells was measured by degranulation against tumor lines. Characterization further assays correlations with functionality.

[0317] Figure 4 depicts the structure of the A08 anti-BCMA CARTyrin.

[0318] Figures 5-8 demonstrate the in vitro and in vivo characterization of P-BCMA-101 (encoding the A08 anti-BCMA CARTyrin).

[0319] In vitro evaluation of the A08 CARTyrin demonstrated high levels of surface expression following lentiviral transduction of human primary T cells and strong cytotoxic function (e.g. proliferation) against BCMA+ tumor cells (see Figures 5A-C). Following this strong performance in vitro, the ability of the A08 CARTyrin to function in vivo was evaluated.

[0320] Figure 6 depicts the treatment schedule for an in vivo study in mice using the A08 CARTyrin. Results of this study show that 100% of mice treated with P-BCMA-101 (encoding the A08 CARTyrin) survived to day 21 (see Figure 7). This complete survival of treated animals at day 21 was accompanied by a showing of zero tumor burden (as assessed

by M-protein abundance, which was not detectable in these animals at day 21) (see Figure 7). Figure 8 provides a series of photographs further illustrating tumor burden in control animal as well as those treated with P-BCMA-101. Animals expressing the A08 CARTyrin demonstrate a reduce tumor burden compared to controls.

Example 2: Expression and Function of piggyBac integrated iC9 safety switch into human pan T-cells

[0321] Human pan T-cells were nucleofected using an Amaxa 4D nucleofector with one of four piggyBac transposons. Modified T cells receiving the “mock” condition were nucleofected with an empty piggyBac transposon. Modified T cells received either a piggyBac transposon containing a therapeutic agent alone (a sequence encoding a CARTyrin) or a piggyBac transposon containing an integrated iC9 sequence and a therapeutic agent (a sequence encoding a CARTyrin).

[0322] Figure 8 provides a schematic diagram of the iC9 safety switch, which contains a ligand binding region, a linker, and a truncated caspase 9 polypeptide. Specifically, the iC9 polypeptide contains a ligand binding region comprising a FK506 binding protein 12 (FKBP12) polypeptide including a substitution of valine (V) for phenylalanine (F) at position 36 (F36V). The FKBP12 polypeptide of the iC9 polypeptide is encoded by an amino acid sequence comprising

GVQVETISPGDGRTPKRGQTCVVHYTGMLLEDGKKVDSSRDRNKPFFKFMLGKQEVI
RGWEEGVAQMSVGQRAKLISPDIAYGATGHPGIIPPHATLVFDVELLKLE (SEQ ID
NO: 23). The FKBP12 polypeptide of the iC9 polypeptide is encoded by a nucleic acid
sequence comprising

GGGGTCCAGGTCGAGACTATTTACACAGGGGATGGGCGAACATTTCCAAAAAGG
GGCCAGACTTGCCTCGTGCATTACACCGGGATGCTGGAGGACGGGAAGAAAGTG
GACAGCTCCAGGGATCGCAACAAGCCCTTCAAGTTCATGCTGGGAAAGCAGGAA
GTGATCCGAGGATGGGAGGAAGGCGTGGCACAGATGTCAGTCGGCCAGCGGGCC
AAACTGACCATTAGCCCTGACTACGCTTATGGAGCAACAGGCCACCCAGGGATC
ATTCCCCCTCATGCCACCCTGGTCTTCGAT GTGGAAGCTGCTGAAGCTGGAG (SEQ
ID NO: 24). The linker region of the iC9 polypeptide is encoded by an amino acid

comprising GGGGS (SEQ ID NO: 25) and a nucleic acid sequence comprising
GGAGGAGGAGGATCC (SEQ ID NO: 26). The nucleic acid sequence encoding the linker
region of the iC9 polypeptide is encoded by an amino acid comprising

GFGDVGALESLRGNADLAYISLMEPCGHCLIINNPNFCRESGLRTRTGSNIDCEKLRR
RFSSLHFMVEVKGDLTAKKMVLALLELAQQDHGALDCCVVVILSHGCQASHLQFPG

AVYGTGDCPVSVVEKIVNIFNGTSCPSLGGKPKLFFIQACGGEQKDHGFEVASTSPEDE
SPGSNPEPDATPFQEGLRTFDQLDAISSLPTPSDIFVSYSTFPGFVSWRDPKSGSWYVE
TLDDIFEQWAHSEDLQSLLLRVANAVSVKGIYKQMPGCNFLRKKLFFKTS (SEQ ID

NO: 27). The nucleic acid sequence encoding the linker region of the iC9 polypeptide is encoded by a nucleic acid sequence comprising

TTTGGGGACGTGGGGGCCCTGGAGTCTCTGCGAGGAAATGCCGATCTGGCTTACA
TCCTGAGCATGGAACCCTGCGGCCACTGTCTGATCATTACAATGTGAACTTCTG
CAGAGAAAGCGGACTGCGAACACGGACTGGCTCCAATATTGACTGTGAGAAGCT
GCGGAGAAGGTTCTCTAGTCTGCACTTTATGGTCGAAGTGAAAGGGGATCTGACC
GCCAAGAAAATGGTGCTGGCCCTGCTGGAGCTGGCTCAGCAGGACCATGGAGCT
CTGGATTGCTGCGTGGTCGTGATCCTGTCCCACGGGTGCCAGGCTTCTCATCTGC
AGTTCCCCGGAGCAGTGTACGGAACAGACGGCTGTCCTGTCAGCGTGGAGAAGA
TCGTCAACATCTTCAACGGCACTTCTTGCCCTAGTCTGGGGGGAAAGCCAAAAC
GTTCTTTATCCAGGCCTGTGGCGGGGAACAGAAAGATCACGGCTTCGAGGTGGC
CAGCACCAGCCCTGAGGACGAATCACCAGGGAGCAACCCTGAACCAGATGCAAC
TCCATTCCAGGAGGGACTGAGGACCTTTGACCAGCTGGATGCTATCTCAAGCCTG
CCCACTCCTAGTGACATTTTCGTGTCTTACAGTACCTTCCCAGGCTTTGTCTCATG
GCGCGATCCCAAGTCAGGGAGCTGGTACGTGGAGACACTGGACGACATCTTTGA
ACAGTGGGCCCATTACAGAGGACCTGCAGAGCCTGCTGCTGCGAGTGGCAAACGC
TGTCTCTGTGAAGGGCATCTACAAACAGATGCCCGGGTGCTTCAATTTTCTGAGA
AAGAACTGTTCTTTAAGACTTCC (SEQ ID NO: 28).

[0323] To test the iC9 safety switch, each of the four modified T cells were incubated for 24 hours with 0, 0.1 nM, 1 nM, 10 nM, 100 nM or 1000 nM AP1903 (an induction agent for AP1903). Viability was assessed by flow cytometry using 7-aminoactinomycin D (7-AAD), a fluorescent intercalator, as a marker for cells undergoing apoptosis.

[0324] Cell viability was assessed at day 12 (see Figure 9). The data demonstrate a shift of cell populations from the lower right to the upper left quadrants with increasing concentration of the induction agent in cells containing the iC9 construct; however, this effect is not observed in cells lacking the iC9 construct (those receiving only the CARTyrin), in which cells are evenly distributed among these two areas regardless of the concentration of the induction agent. Moreover, cell viability was assessed at day 19 (see Figure 10). The data reveal the same trend as shown in Figure 9 (day 12 post-nucleofection); however, the population shift to the upper left quadrant is more pronounced at this later time point (day 19 post-nucleofection).

[0325] A quantification of the aggregated results was performed and is provided in Figure 11, showing the significant impact of the iC9 safety switch on the percent cell viability as a function of the concentration of the induction agent (AP1903) of the iC9 switch for each modified cell type at either day 12 (Figure 9 and left graph) or day 19 (Figure 10 and right graph). The presence of the iC9 safety switch induces apoptosis in a significant majority of cells by day 12 and the effect is even more dramatic by day 19.

[0326] The results of this study show that the iC9 safety switch is extremely effective at eliminating active cells upon contact with an induction agent (e.g. AP1903) because AP1903 induces apoptosis at even the lowest concentrations of the study (0.1 nM). Furthermore, the iC9 safety switch may be functionally expressed as part of a tricistronic vector.

INCORPORATION BY REFERENCE

[0327] Every document cited herein, including any cross referenced or related patent or application is hereby incorporated herein by reference in its entirety unless expressly excluded or otherwise limited. The citation of any document is not an admission that it is prior art with respect to any invention disclosed or claimed herein or that it alone, or in any combination with any other reference or references, teaches, suggests or discloses any such invention. Further, to the extent that any meaning or definition of a term in this document conflicts with any meaning or definition of the same term in a document incorporated by reference, the meaning or definition assigned to that term in this document shall govern.

OTHER EMBODIMENTS

[0328] While particular embodiments of the disclosure have been illustrated and described, various other changes and modifications can be made without departing from the spirit and scope of the disclosure. The scope of the appended claims includes all such changes and modifications that are within the scope of this disclosure.

CLAIMS

What is claimed is:

1. A chimeric antigen receptor (CAR) comprising:
(a) an ectodomain comprising an antigen recognition region, wherein the antigen recognition region comprises at least one Centyrin;
(b) a transmembrane domain, and
(c) an endodomain comprising at least one costimulatory domain.
2. The CAR of claim 1, wherein the ectodomain of (a) further comprises a signal peptide.
3. The CAR of claim 1 or 2, wherein the ectodomain of (a) further comprises a hinge between the antigen recognition region and the transmembrane domain.
4. The CAR of claim 2 or 3, wherein the signal peptide comprises a sequence encoding a human CD2, CD3 δ , CD3 ϵ , CD3 γ , CD3 ζ , CD4, CD8 α , CD19, CD28, 4-1BB or GM-CSFR signal peptide.
5. The CAR of claim 2 or 3, wherein the signal peptide comprises a sequence encoding a human CD8 α signal peptide.
6. The CAR of claim 5, wherein the signal peptide comprises an amino acid sequence comprising MALPVTALLLPLALLLHAARP (SEQ ID NO: 3).
7. The CAR of claim 5 or 6, wherein the signal peptide is encoded by a nucleic acid sequence comprising atggcactgccagtcaccgccctgctgctgcctctggctctgctgctgcacgcagctagacca (SEQ ID NO: 45).
8. The CAR of any one of the preceding claims, wherein the transmembrane domain comprises a sequence encoding a human CD2, CD3 δ , CD3 ϵ , CD3 γ , CD3 ζ , CD4, CD8 α , CD19, CD28, 4-1BB or GM-CSFR transmembrane domain.

9. The CAR of any one of the preceding claims, wherein the transmembrane domain comprises a sequence encoding a human CD8 α transmembrane domain.
10. The CAR of claim 9, wherein the transmembrane domain comprises an amino acid sequence comprising IYIWAPLAGTCGVLLLSLVITLYC (SEQ ID NO: 4).
11. The CAR of claim 9 or 10, wherein the transmembrane domain is encoded by a nucleic acid sequence comprising
atctacatttgggcaccactggccgggacctgtggagtgtgctgctgctgagcctggatcacactgtactgc (SEQ ID NO: 5).
12. The CAR of any one of the preceding claims, wherein the endodomain comprises a human CD3 ζ endodomain.
13. The CAR of any one of the preceding claims, wherein the at least one costimulatory domain comprises a human 4-1BB, CD28, CD40, ICOS, MyD88, OX-40 intracellular segment, or any combination thereof.
14. The CAR of any one of the preceding claims, wherein the at least one costimulatory domain comprises a human CD28 and/or a 4-1BB costimulatory domain.
15. The CAR of claim 13 or 14, wherein the CD28 costimulatory domain comprises an amino acid sequence comprising
RVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQE
GLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPP
R (SEQ ID NO: 6).
16. The CAR of claim 15, wherein the CD28 costimulatory domain is encoded by a nucleic acid sequence comprising
cgcgatgaagttagtcgatcagcagatgccccagcttacaacaggacagaccagctgtataacgagctgaatctgggccgccga
gaggaatatgacgtgctggataagcggagaggacgcgacccccgaatgggaggcaagccaggcgcaaaaacctcaggaagg
cctgtataacgagctgcagaaggacaaaatggcagaagcctattctgagatcggcatgaagggggagcgacggagaggcaaagg
gcacgatgggctgtaccagggactgagcaccgccacaaggacacctatgatgctctgcatatgcaggcactgcctccaagg
(SEQ ID NO: 7).

17. The CAR of claim 13 or 14, wherein the 4-1BB costimulatory domain comprises an amino acid sequence comprising

KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCEL (SEQ ID NO: 8).

18. The CAR of claim 17, wherein the 4-1BB costimulatory domain is encoded by a nucleic acid sequence comprising

aagagaggcaggaagaaactgctgtatatatttcaaacagcccttcacgcgccctgcagactaccaggaggaagacgggtgctcc
gtcgattccctgaggaagaggaaggcgggtgtgagctg (SEQ ID NO: 9).

19. The CAR of any one of claims 14-18, wherein the 4-1BB costimulatory domain is located between the transmembrane domain and the CD28 costimulatory domain.

20. The CAR of any one of claims 2-19, wherein the hinge comprises a sequence derived from a human CD8 α , IgG4, and/or CD4 sequence.

21. The CAR of any one of claims 2-19, wherein the hinge comprises a sequence derived from a human CD8 α sequence.

22. The CAR of claim 20 or 21, wherein the hinge comprises an amino acid sequence comprising TTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACD (SEQ ID NO: 10).

23. The CAR of claim 22, wherein the hinge is encoded by a nucleic acid sequence comprising

actaccacaccagcacctagaccaccaactccagctccaaccatcgcgagtcagcccctgagctctgagacctgaggcctgcaggcc
agctgcaggaggagctgtgcaccaggggcctggacttcgctgcgac (SEQ ID NO: 11).

24. The CAR of any one of the preceding claims, wherein the at least one Centyrin comprises a protein scaffold, wherein the scaffold is capable of specifically binding an antigen.

25. The CAR of any one of the preceding claims, wherein the at least one Centyrin comprises a protein scaffold comprising a consensus sequence of at least one fibronectin type III (FN3) domain, wherein the scaffold is capable of specifically binding an antigen.

26. The CAR of claim 25, wherein the at least one fibronectin type III (FN3) domain is derived from a human protein.
27. The CAR of claim 26, wherein the human protein is Tenascin-C.
28. The CAR of any one of claims 25-27, wherein the consensus sequence comprises LPAPKNLVVSEVTEDSLRLSWTAPDAAFDSFLIQYQSEKVGGEAINLTVPGSERSYDL TGLKPGTEYTVSIYGVKGGHRSNPLSAEFTT (SEQ ID NO: 1).
29. The CAR of any one of claims 25-27, wherein the consensus sequence comprises MLPAPKNLVVSEVTEDSLRLSWTAPDAAFDSFLIQYQSEKVGGEAINLTVPGSERSYD LTGLKPGTEYTVSIYGVKGGHRSNPLSAEFTT (SEQ ID NO: 13).
30. The CAR of claim 29, wherein the consensus sequence is encoded by a nucleic acid sequence comprising
 atgtgcctgcaccaaagaacctggtggtgtctcatgtgacagaggatagtgccagactgtcatggactgctcccgacgcagccttcg
 atagttttatcatcgtgtaccgggagaaacatcgaaaccggcgaggccattgtcctgacagtgccagggtccgaacgctcttatgacctg
 acagatctgaagcccgaactgagtactatgtgcagatcgccggcgctcaaaggaggcaatatcagcttccctctgtccgcaatcttcac
 caca (SEQ ID NO: 14).
31. The CAR of any one of claims 25-30, wherein the consensus sequence is modified at one or more positions within
- (a) a A-B loop comprising or consisting of the amino acid residues TEDS (SEQ ID NO: 15) at positions 13-16 of the consensus sequence;
 - (b) a B-C loop comprising or consisting of the amino acid residues TAPDAAF (SEQ ID NO: 16) at positions 22-28 of the consensus sequence;
 - (c) a C-D loop comprising or consisting of the amino acid residues SEKVGE (SEQ ID NO: 17) at positions 38-43 of the consensus sequence;
 - (d) a D-E loop comprising or consisting of the amino acid residues GSER (SEQ ID NO: 18) at positions 51-54 of the consensus sequence;
 - (e) a E-F loop comprising or consisting of the amino acid residues GLKPG (SEQ ID NO: 19) at positions 60-64 of the consensus sequence;

(f) a F-G loop comprising or consisting of the amino acid residues KGGHRSN (SEQ ID NO: 20) at positions 75-81 of the consensus sequence; or

(g) any combination of (a)-(f).

32. The CAR of any one of claims 25-31, comprising a consensus sequence of at least 5 fibronectin type III (FN3) domains.

33. The CAR of any one of claims 25-31, comprising a consensus sequence of at least 10 fibronectin type III (FN3) domains.

34. The CAR of any one of claims 25-31, comprising a consensus sequence of at least 15 fibronectin type III (FN3) domains.

35. The CAR of any one of claims 24-34, wherein the scaffold binds an antigen with at least one affinity selected from a K_D of less than or equal to $10^{-9}M$, less than or equal to $10^{-10}M$, less than or equal to $10^{-11}M$, less than or equal to $10^{-12}M$, less than or equal to $10^{-13}M$, less than or equal to $10^{-14}M$, and less than or equal to $10^{-15}M$.

36. The CAR of claim 35, wherein the K_D is determined by surface plasmon resonance.

37. A composition comprising the CAR of any one of the preceding claims and at least one pharmaceutically acceptable carrier.

38. A transposon comprising the CAR of any one of the preceding claims.

39. The transposon of claim 38, wherein the transposon further comprises a selection gene.

40. The transposon of claim 39, wherein the selection gene encodes a gene product essential for cell viability and survival.

41. The transposon of claim 39, wherein the selection gene encodes a gene product essential for cell viability and survival when challenged by selective cell culture conditions.

42. The transposon of claim 41, wherein the selective cell culture conditions comprise a compound harmful to cell viability or survival and wherein the gene product confers resistance to the compound.
43. The transposon of claim 39, wherein the selection gene comprises *neo*, DHFR (Dihydrofolate Reductase), TYMS (Thymidylate Synthetase), MGMT (O(6)-methylguanine-DNA methyltransferase), multidrug resistance gene (MDR1), ALDH1 (Aldehyde dehydrogenase 1 family, member A1), FRNCF, RAD51C (RAD51 Paralog C), GCS (glucosylceramide synthase), NKX2.2 (NK2 Homeobox 2) or any combination thereof.
44. The transposon of any one of claims 38 to 43, wherein the transposon comprises an inducible caspase polypeptide comprising
- (a) a ligand binding region,
 - (b) a linker, and
 - (c) a truncated caspase 9 polypeptide,
- wherein the inducible caspase polypeptide does not comprise a non-human sequence.
45. The transposon of claim 44, wherein the non-human sequence is a restriction site.
46. The transposon of claim 44 or 45, wherein the ligand binding region inducible caspase polypeptide comprises a FK506 binding protein 12 (FKBP12) polypeptide.
47. The transposon of claim 46, wherein the amino acid sequence of the FK506 binding protein 12 (FKBP12) polypeptide comprises a modification at position 36 of the sequence.
48. The transposon of claim 47, wherein the modification is a substitution of valine (V) for phenylalanine (F) at position 36 (F36V).
49. The transposon of any one of claims 46-48, wherein the FKBP12 polypeptide is encoded by an amino acid sequence comprising
GVQVETISPGDGRTFPKRGQTCVVHYTGMLDGGKVDSSRDRNKPFFKMLGKQEVI
RGWEEGVAQMSVGQRAKLTIISPDYAYGATGHPGIIPPHATLVFDVELLKLE (SEQ ID
NO: 23).

50. The transposon of claim 49, wherein the FKBP12 polypeptide is encoded by a nucleic acid sequence comprising

GGGGTCCAGGTCGAGACTATTTACACAGGGGATGGGCGAACATTTCCAAAAAGG
GGCCAGACTTGCGTCGTGCATTACACCGGGATGCTGGAGGACGGGAAGAAAGTG
GACAGCTCCAGGGATCGCAACAAGCCCTTCAAGTTCATGCTGGGAAAGCAGGAA
GTGATCCGAGGATGGGAGGAAGGCGTGGCACAGATGTCAGTCGGCCAGCGGGCC
AAACTGACCATTAGCCCTGACTACGCTTATGGAGCAACAGGCCACCCAGGGATC
ATTCCCCCTCATGCCACCCTGGTCTTCGAT GTGGAAGTCTGAAGCTGGAG (SEQ
ID NO: 24).

51. The transposon of any one of claims 44-50, wherein the linker region of the inducible proapoptotic polypeptide is encoded by an amino acid comprising GGGGS (SEQ ID NO: 25).

52. The transposon of claim 51, wherein the linker region of the inducible proapoptotic polypeptide is encoded by a nucleic acid sequence comprising GGAGGAGGAGGATCC (SEQ ID NO: 26).

53. The transposon of any one of claims 44-52, wherein the truncated caspase 9 polypeptide of the inducible proapoptotic polypeptide is encoded by an amino acid sequence that does not comprise an arginine (R) at position 87 of the sequence.

54. The transposon of any one of claims 44-53, wherein the truncated caspase 9 polypeptide of the inducible proapoptotic polypeptide is encoded by an amino acid sequence that does not comprise an alanine (A) at position 282 the sequence.

55. The transposon of any one of claims 44-54, wherein the truncated caspase 9 polypeptide of the inducible proapoptotic polypeptide is encoded by an amino acid comprising

GFGDVGALESLRGNADLAYISLMEPCGHCLIINNPNFCRESGLRTRTGSNIDCEKLRR
RFSSLHFMVEVKGDLTAKKMVLALLELAQQDHGALDCCVVVILSHGCQASHLQFPG
AVYGTGDCPVSVVEKIVNIFNGTSCPSLGGKPKLFFIQACGGEQKDHGFEVASTSPEDE
SPGSNPEPDATPFQEGLRTFDQLDAISSLPTPSDIFVSYSTFPGFVSWRDPKSGSWYVE

TLDDIFEQWAHSEDLQSLLLRVANAVSVKGIYKQMPGCNFLRKKLFFKTS (SEQ ID NO: 27).

56. The transposon of claim 55, wherein the truncated caspase 9 polypeptide of the inducible proapoptotic polypeptide is encoded by a nucleic acid sequence comprising
TTTGGGGACGTGGGGGCCCTGGAGTCTCTGCGAGGAAATGCCGATCTGGCTTACA
TCCTGAGCATGGAACCCTGCGGCCACTGTCTGATCATTAAACAATGTGAACTTCTG
CAGAGAAAGCGGACTGCGAACACGGACTGGCTCCAATATTGACTGTGAGAAGCT
GCGGAGAAGGTTCTCTAGTCTGCACTTTATGGTCGAAGTGAAAGGGGATCTGACC
GCCAAGAAAATGGTGCTGGCCCTGCTGGAGCTGGCTCAGCAGGACCATGGAGCT
CTGGATTGCTGCGTGGTCGTGATCCTGTCCCACGGGTGCCAGGCTTCTCATCTGC
AGTTCCCCGGAGCAGTGTACGGAACAGACGGCTGTCCTGTCAGCGTGGAGAAGA
TCGTCAACATCTTCAACGGCACTTCTTGCCCTAGTCTGGGGGGAAAGCCAAAAC
GTTCTTTATCCAGGCCTGTGGCGGGGAACAGAAAGATCACGGCTTCGAGGTGGC
CAGCACCAGCCCTGAGGACGAATCACCAGGGAGCAACCCTGAACCAGATGCAAC
TCCATTCCAGGAGGGACTGAGGACCTTTGACCAGCTGGATGCTATCTCAAGCCTG
CCCACTCCTAGTGACATTTTCGTGTCTTACAGTACCTTCCCAGGCTTTGTCTCATG
GCGCGATCCCAAGTCAGGGAGCTGGTACGTGGAGACACTGGACGACATCTTTGA
ACAGTGGGCCCATTACAGAGGACCTGCAGAGCCTGCTGCTGCGAGTGGCAAACGC
TGTCTCTGTGAAGGGCATCTACAAACAGATGCCCGGGTGCTTCAATTTTCTGAGA
AAGAACTGTTCTTTAAGACTTCC (SEQ ID NO: 28).

57. The transposon of any one of claims 44-56, wherein of the inducible proapoptotic polypeptide is encoded by an amino acid sequence comprising
GVQVETISPGDGRTFPKRGQTCVVHYTGMLLEDGKKVDSSRDRNKPFFKMLGKQEVI
RGWEEGVAQMSVGQRAKLTISPDYAYGATGHPGIIPPHATLVFDVELLKLEGGGGGS
GFGDVGALESLRGNADLAYISLMEPCGHCLIINNPNFCRESGLRTRTGSNIDCEKLRR
RFSSLHFMVEVKGDLTAKKMVLALLELAQQDHGALDCCVVVILSHGCQASHLQFPG
AVYGTGDCPVSVEKIVNIFNGTSCPSLGGKPKLFFIQACGGEQKDHGFEVASTPEDE
SPGSNPEPDATPFQEGLRTFDQLDAISSLPTPSDIFVSYSTFPGFVSWRDPKSGSWYVE
TLDDIFEQWAHSEDLQSLLLRVANAVSVKGIYKQMPGCNFLRKKLFFKTS (SEQ ID NO: 29).

58. The transposon of claim 57, wherein of the inducible proapoptotic polypeptide is encoded by a nucleic acid sequence comprising

GGGGTCCAGGTCGAGACTATTTACACAGGGGATGGGCGAACATTTCCAAAAAGG
GGCCAGACTTGCGTCGTGCATTACACCGGGATGCTGGAGGACGGGAAGAAAGTG
GACAGCTCCAGGGATCGCAACAAGCCCTTCAAGTTCATGCTGGGAAAGCAGGAA
GTGATCCGAGGATGGGAGGAAGGCGTGGCACAGATGTCAGTCGGCCAGCGGGCC
AAACTGACCATTAGCCCTGACTACGCTTATGGAGCAACAGGCCACCCAGGGATC
ATTCCCCCTCATGCCACCCTGGTCTTCGATGTGGAAGTCTGTAAGCTGGAGGGAG
GAGGAGGATCCGAATTTGGGGACGTGGGGGCCCTGGAGTCTCTGCGAGGAAATG
CCGATCTGGCTTACATCCTGAGCATGGAACCCTGCGGCCACTGTCTGATCATTAA
CAATGTGAACTTCTGCAGAGAAAGCGGACTGCGAACACGGACTGGCTCCAATAT
TGACTGTGAGAAGCTGCGGAGAAGGTTCTCTAGTCTGCACTTTATGGTCGAAGTG
AAAGGGGATCTGACCGCCAAGAAAATGGTGCTGGCCCTGCTGGAGCTGGCTCAG
CAGGACCATGGAGCTCTGGATTGCTGCGTGGTCGTGATCCTGTCCCACGGGTGCC
AGGCTTCTCATCTGCAGTTCCCCGGAGCAGTGTACGGAACAGACGGCTGTCCTGT
CAGCGTGGAGAAGATCGTCAACATCTTCAACGGCACTTCTTGCCCTAGTCTGGGG
GGAAAGCCAAAAGTGTCTTTATCCAGGCCTGTGGCGGGGAACAGAAAGATCAC
GGCTTCGAGGTGGCCAGCACCAGCCCTGAGGACGAATCACCAGGGAGCAACCCT
GAACCAGATGCAACTCCATTCCAGGAGGGACTGAGGACCTTTGACCAGCTGGAT
GCTATCTCAAGCCTGCCCCACTCCTAGTGACATTTTCGTGTCTTACAGTACCTTCCC
AGGCTTTGTCTCATGGCGCGATCCCAAGTCAGGGAGCTGGTACGTGGAGACACT
GGACGACATCTTTGAACAGTGGGCCCATTGAGAGGACCTGCAGAGCCTGCTGCT
GCGAGTGGCAAACGCTGTCTCTGTGAAGGGCATCTACAAACAGATGCCCGGGTG
CTTCAATTTTCTGAGAAAGAAAGTGTCTTTAAGACTTCC (SEQ ID NO: 30).

59. The transposon of any one of claims 38 to 58, wherein the transposon comprises at least one self-cleaving peptide.

60. The transposon of any one of claims 39 to 58, wherein the transposon comprises at least one self-cleaving peptide and wherein a self-cleaving peptide is located between the CAR and the selection gene.

61. The transposon of any one of claims 44 to 60, wherein the transposon comprises at least one self-cleaving peptide and wherein a self-cleaving peptide is located between the CAR and the inducible proapoptotic polypeptide.
62. The transposon of any one of claims 44 to 61, wherein the transposon comprises at least two self-cleaving peptides and wherein a first self-cleaving peptide is located upstream of the inducible proapoptotic polypeptide and a second self-cleaving peptide is located downstream of the inducible proapoptotic polypeptide.
63. The transposon of any one of claims 38 to 62, wherein the transposon comprises at least one self-cleaving peptide and wherein a first self-cleaving peptide is located upstream of the CAR and a second self-cleaving peptide is located downstream of the CAR.
64. The transposon of any one of claims 59 to 63, wherein the at least one self-cleaving peptide comprises T2A peptide, GSG-T2A peptide, an E2A peptide, a GSG-E2A peptide, an F2A peptide, a GSG-F2A peptide, a P2A peptide, or a GSG-P2A peptide.
65. The transposon of claim 64, wherein the T2A peptide comprises an amino acid sequence comprising EGRGSLTCTGDVEENPGP (SEQ ID NO: 31).
66. The transposon of claim 64, wherein the GSG-T2A peptide comprises an amino acid sequence comprising GSGEGRGSLTCTGDVEENPGP (SEQ ID NO: 32).
67. The transposon of claim 64, wherein the E2A peptide comprises an amino acid sequence comprising QCTNYALLKLAGDVESNPGP (SEQ ID NO: 34).
68. The transposon of claim 64, wherein the GSG-E2A peptide comprises an amino acid sequence comprising GSGQCTNYALLKLAGDVESNPGP (SEQ ID NO: 35).
69. The transposon of claim 64, wherein the F2A peptide comprises an amino acid sequence comprising VKQTLNFDLLKLAGDVESNPGP (SEQ ID NO: 36).
70. The transposon of claim 64, wherein the GSG-F2A peptide comprises an amino acid sequence comprising GSGVKQTLNFDLLKLAGDVESNPGP (SEQ ID NO: 37).

71. The transposon of claim 64, wherein the P2A peptide comprises an amino acid sequence comprising ATNFSLLKQAGDVEENPGP (SEQ ID NO: 38).
72. The transposon of claim 64, wherein the GSG-P2A peptide comprises an amino acid sequence comprising GSGATNFSLLKQAGDVEENPGP (SEQ ID NO: 39).
73. The transposon of any one of claims 38-72, wherein the transposon is a piggyBac transposon.
74. A composition comprising the transposon of any one of claims 38 to 73.
75. The composition of claim 74, further comprising a plasmid comprising a sequence encoding a transposase enzyme.
76. The composition of claim 75, wherein the sequence encoding a transposase enzyme is an mRNA sequence.
77. The composition of any one of claims 74 to 76, wherein the transposase is a piggyBac transposase.
78. The composition of claim 77, wherein the piggyBac transposase comprises an amino acid sequence comprising SEQ ID NO: 12.
79. The composition of claim 77 or 78, wherein the piggyBac transposase is a hyperactive variant and wherein the hyperactive variant comprises an amino acid substitution at one or more of positions 30, 165, 282 and 538 of SEQ ID NO: 12.
80. The composition of claim 79, wherein the amino acid substitution at position 30 of SEQ ID NO: 12 is a substitution of a valine (V) for an isoleucine (I) (I30V).
81. The composition of claim 79, wherein the amino acid substitution at position 165 of SEQ ID NO: 12 is a substitution of a serine (S) for a glycine (G) (G165S).

82. The composition of claim 79, wherein the amino acid substitution at position 282 of SEQ ID NO: 12 is a substitution of a valine (V) for a methionine (M) (M282V).

83. The composition of claim 79, wherein the amino acid substitution at position 538 of SEQ ID NO: 12 is a substitution of a lysine (K) for an asparagine (N) (N538K).

84. The composition of any one of claims 77 to 83, wherein the transposase is a Super piggyBac (sPBo) transposase.

85. The composition of claim 84, wherein the Super piggyBac (sPBo) transposase comprises an amino acid sequence comprising SEQ ID NO: 2.

86. A vector comprising the CAR of any one of claims 1-36.

87. The vector of claim 86, wherein the vector is a viral vector.

88. The vector of claim 87, wherein the viral vector comprises a sequence isolated or derived from a retrovirus, a lentivirus, an adenovirus, an adeno-associated virus or any combination thereof.

89. The vector of claim 87 or 88, wherein the viral vector comprises a sequence isolated or derived from an adeno-associated virus.

90. The vector of any one of claims 87 to 89, wherein the viral vector is a recombinant vector.

91. The vector of claim 86, wherein the vector is a nanoparticle vector.

92. The vector of claim 91, wherein the nanoparticle vector comprises a nucleic acid, an amino acid, a polymers, a micelle, lipid, an organic molecule, an inorganic molecule or any combination thereof.

93. The vector of any one of claims 86 to 92, wherein the vector further comprises a selection gene.

94. The vector of claim 93, wherein the selection gene encodes a gene product essential for cell viability and survival.
95. The vector of claim 93, wherein the selection gene encodes a gene product essential for cell viability and survival when challenged by selective cell culture conditions.
96. The vector of claim 95, wherein the selective cell culture conditions comprise a compound harmful to cell viability or survival and wherein the gene product confers resistance to the compound.
97. The vector of any one of claims 93 to 96, wherein the selection gene comprises *neo*, DHFR (Dihydrofolate Reductase), TYMS (Thymidylate Synthetase), MGMT (O(6)-methylguanine-DNA methyltransferase), multidrug resistance gene (MDR1), ALDH1 (Aldehyde dehydrogenase 1 family, member A1), FRNCF, RAD51C (RAD51 Paralog C), GCS (glucosylceramide synthase), NKX2.2 (NK2 Homeobox 2) or any combination thereof.
98. The vector of any one of claims 86 to 97, wherein the vector comprises an inducible caspase polypeptide comprising
- (a) a ligand binding region,
 - (b) a linker, and
 - (c) a truncated caspase 9 polypeptide,
- wherein the inducible caspase polypeptide does not comprise a non-human sequence.
99. The vector of claim 98, wherein the non-human sequence is a restriction site.
100. The vector of claim 98 or 99, wherein the ligand binding region inducible caspase polypeptide comprises a FK506 binding protein 12 (FKBP12) polypeptide.
101. The vector of claim 100, wherein the amino acid sequence of the FK506 binding protein 12 (FKBP12) polypeptide comprises a modification at position 36 of the sequence.
102. The vector of claim 101, wherein the modification is a substitution of valine (V) for phenylalanine (F) at position 36 (F36V).

103. The vector of any one of claims 100 to 102, wherein the FKBP12 polypeptide is encoded by an amino acid sequence comprising

GVQVETISPGDGRTPKRGQTCVVHYTGMLLEDGKKVDSSRDRNKPFKFMLGKQEVI
RGWEEGVAQMSVGQRAKLTISPDYAYGATGHPGIIPPHATLVFDVELLKLE (SEQ ID
NO: 23).

104. The vector of claim 103, wherein the FKBP12 polypeptide is encoded by a nucleic acid sequence comprising

GGGGTCCAGGTCGAGACTATTTACACAGGGGATGGGCGAACATTTCCAAAAAGG
GGCCAGACTTGCGTCGTGCATTACACCGGGATGCTGGAGGACGGGAAGAAAGTG
GACAGCTCCAGGGATCGCAACAAGCCCTTCAAGTTCATGCTGGGAAAGCAGGAA
GTGATCCGAGGATGGGAGGAAGGCGTGGCACAGATGTCAGTCGGCCAGCGGGCC
AAACTGACCATTAGCCCTGACTACGCTTATGGAGCAACAGGCCACCCAGGGATC
ATTCCCCCTCATGCCACCCTGGTCTTCGAT GTGGAAGCTGCTGAAGCTGGAG (SEQ
ID NO: 24).

105. The vector of any one of claims 98 to 104, wherein the linker region of the inducible proapoptotic polypeptide is encoded by an amino acid comprising GGGGS (SEQ ID NO: 25).

106. The vector of claim 105, wherein the linker region of the inducible proapoptotic polypeptide is encoded by a nucleic acid sequence comprising GGAGGAGGAGGATCC (SEQ ID NO: 26).

107. The vector of any one of claims 98-106, wherein the truncated caspase 9 polypeptide of the inducible proapoptotic polypeptide is encoded by an amino acid sequence that does not comprise an arginine (R) at position 87 of the sequence.

108. The vector of any one of claims 98-107, wherein the truncated caspase 9 polypeptide of the inducible proapoptotic polypeptide is encoded by an amino acid sequence that does not comprise an alanine (A) at position 282 the sequence.

109. The vector of any one of claims 98-108, wherein the truncated caspase 9 polypeptide of the inducible proapoptotic polypeptide is encoded by an amino acid comprising
 GFGDVGALESRLGNADLAYISLMEPCGHCLIINNPNFCRESGLRTRTGSNIDCEKLRR
 RFSSLHFMVEVKGDLTAKKMVLALLELAQQDHGALDCCVVVILSHGCQASHLQFPG
 AVYGTGDCPVSVVEKIVNIFNGTSCPSLGGKPKLFFIQACGGEQKDHGFEVASTSPEDE
 SPGSNPEPDATPFQEGLRTFDQLDAISSLPSTDFVSYSTFPGFVSWRDPKSGSWYVE
 TLDDIFEQWAHSEDLQSLLLRVANAVSVKGIYKQMPGCNFLRKKLFFKTS (SEQ ID
 NO: 27).

110. The vector of claim 109, wherein the truncated caspase 9 polypeptide of the inducible proapoptotic polypeptide is encoded by a nucleic acid sequence comprising
 TTTGGGGACGTGGGGGCCCTGGAGTCTCTGCGAGGAAATGCCGATCTGGCTTACA
 TCCTGAGCATGGAACCCTGCGGCCACTGTCTGATCATTACAATGTGAACCTTCTG
 CAGAGAAAGCGGACTGCGAACACGGACTGGCTCCAATATTGACTGTGAGAAGCT
 GCGGAGAAGGTTCTCTAGTCTGCACTTTATGGTCGAAGTGAAAGGGGATCTGACC
 GCCAAGAAAATGGTGCTGGCCCTGCTGGAGCTGGCTCAGCAGGACCATGGAGCT
 CTGGATTGCTGCGTGGTCGTGATCCTGTCCACGGGTGCCAGGCTTCTCATCTGC
 AGTTCCCCGGAGCAGTGTACGGAACAGACGGCTGTCTGTGAGCGTGGAGAAGA
 TCGTCAACATCTTCAACGGCACTTCTTGCCCTAGTCTGGGGGGAAAGCCAAAAC
 GTTCTTTATCCAGGCCTGTGGCGGGGAACAGAAAGATCACGGCTTCGAGGTGGC
 CAGCACCAGCCCTGAGGACGAATCACCAGGGAGCAACCCTGAACCAGATGCAAC
 TCCATTCCAGGAGGGACTGAGGACCTTTGACCAGCTGGATGCTATCTCAAGCCTG
 CCCACTCCTAGTGACATTTTCGTGTCTTACAGTACCTTCCCAGGCTTTGTCTCATG
 GCGCGATCCCAAGTCAGGGAGCTGGTACGTGGAGACACTGGACGACATCTTTGA
 ACAGTGGGCCCATTGAGAGGACCTGCAGAGCCTGCTGCTGCGAGTGGCAAACGC
 TGTCTCTGTGAAGGGCATCTACAAACAGATGCCCGGGTGCTTCAATTTTCTGAGA
 AAGAACTGTTCTTTAAGACTTCC (SEQ ID NO: 28).

111. The vector of any one of claims 98-110, wherein of the inducible proapoptotic polypeptide is encoded by an amino acid sequence comprising
 GVQVETISPGDGRTPKRGQTCVVHYTGMLDGGKVDSSRDRNKPFFKMLGKQEVI
 RGWEEGVAQMSVGQRAKLTISSPDYAYGATGHPGIIPPHATLVFDVELLKLEGGGGGS
 GFGDVGALESRLGNADLAYISLMEPCGHCLIINNPNFCRESGLRTRTGSNIDCEKLRR
 RFSSLHFMVEVKGDLTAKKMVLALLELAQQDHGALDCCVVVILSHGCQASHLQFPG

AVYGTGDCPVSV EKIVNIFNGTSCPSLGGKPKLFFIQACGGEQKDHGFEVASTSPEDE
SPGSNPEPDATPFQEGLRTFDQLDAISSLPTPSDIFVSYSTFPGFVSWRDPKSGSWYVE
TLDDIFEQWAHSEDLQSLLLRVANAVSVKGIYKQMPGCNFLRKKLFFKTS (SEQ ID
NO: 29).

112. The vector of claim 111, wherein of the inducible proapoptotic polypeptide is encoded by a nucleic acid sequence comprising

GGGGTCCAGGTCGAGACTATTTACACAGGGGATGGGCGAACATTTCCAAAAAGG
GGCCAGACTTGCGTCGTGCATTACACCGGGATGCTGGAGGACGGGAAGAAAGTG
GACAGCTCCAGGGATCGCAACAAGCCCTTCAAGTTCATGCTGGGAAAGCAGGAA
GTGATCCGAGGATGGGAGGAAGGCGTGGCACAGATGTCAGTCGGCCAGCGGGCC
AAACTGACCATTAGCCCTGACTACGCTTATGGAGCAACAGGCCACCCAGGGATC
ATTCCCCCTCATGCCACCCTGGTCTTCGATGTGGAAGCTGCTGAAGCTGGAGGGAG
GAGGAGGATCCGAATTTGGGGACGTGGGGGCCCTGGAGTCTCTGCGAGGAAATG
CCGATCTGGCTTACATCCTGAGCATGGAACCCTGCGGCCACTGTCTGATCATTAA
CAATGTGAACTTCTGCAGAGAAAGCGGACTGCGAACACGGACTGGCTCCAATAT
TGACTGTGAGAAGCTGCGGAGAAGGTTCTCTAGTCTGCACTTTATGGTCGAAGTG
AAAGGGGATCTGACCGCCAAGAAAATGGTGCTGGCCCTGCTGGAGCTGGCTCAG
CAGGACCATGGAGCTCTGGATTGCTGCGTGGTCGTGATCCTGTCCCACGGGTGCC
AGGCTTCTCATCTGCAGTTCCCCGGAGCAGTGTACGGAACAGACGGCTGTCTGT
CAGCGTGGAGAAGATCGTCAACATCTTCAACGGCACTTCTTGCCCTAGTCTGGGG
GGAAAGCCAAAAGTGTCTTTATCCAGGCCTGTGGCGGGGAACAGAAAGATCAC
GGCTTCGAGGTGGCCAGCACCAGCCCTGAGGACGAATCACCAGGGAGCAACCCT
GAACCAGATGCAACTCCATTCCAGGAGGGACTGAGGACCTTTGACCAGCTGGAT
GCTATCTCAAGCCTGCCCCACTCCTAGTGACATTTTCGTGTCTTACAGTACCTTCCC
AGGCTTTGTCTCATGGCGCGATCCCAAGTCAGGGAGCTGGTACGTGGAGACACT
GGACGACATCTTTGAACAGTGGGCCCATTGAGAGGACCTGCAGAGCCTGCTGCT
GCGAGTGGCAAACGCTGTCTCTGTGAAGGGCATCTACAAACAGATGCCCGGGTG
CTTCAATTTTCTGAGAAAGAAAGTGTCTTTAAGACTTCC (SEQ ID NO: 30).

113. The vector of any one of claims 86 to 112, wherein the vector comprises at least one self-cleaving peptide.

114. The vector of any one of claims 86 to 112, wherein the vector comprises at least one self-cleaving peptide and wherein a self-cleaving peptide is located between the CAR and a selection gene.

115. The vector of any one of claims 98 to 114, wherein the transposon comprises at least one self-cleaving peptide and wherein a self-cleaving peptide is located between the CAR and the inducible proapoptotic polypeptide.

116. The vector of any one of claims 98 to 115, wherein the transposon comprises at least two self-cleaving peptides and wherein a first self-cleaving peptide is located upstream of the inducible proapoptotic polypeptide and a second self-cleaving peptide is located downstream of the inducible proapoptotic polypeptide.

117. The vector of any one of claims 86-116, wherein the vector comprises at least one self-cleaving peptide and wherein a first self-cleaving peptide is located upstream of the CAR and a second self-cleaving peptide is located downstream of the CAR.

118. The vector of any one of claims 113-117, wherein the at least one self-cleaving peptide comprises aT2A peptide, GSG-T2A peptide, an E2A peptide, a GSG-E2A peptide, an F2A peptide, a GSG-F2A peptide, a P2A peptide, or a GSG-P2A peptide.

119. The vector of claim 118, wherein the T2A peptide comprises an amino acid sequence comprising EGRGSLTTCGDVEENPGP (SEQ ID NO: 31).

120. The vector of claim 118, wherein the GSG-T2A peptide comprises an amino acid sequence comprising GSGEGRGSLTTCGDVEENPGP (SEQ ID NO: 32).

121. The vector of claim 118, wherein the E2A peptide comprises an amino acid sequence comprising QCTNYALLKLAGDVESNPGP (SEQ ID NO: 34).

122. The vector of claim 118, wherein the GSG-E2A peptide comprises an amino acid sequence comprising GSGQCTNYALLKLAGDVESNPGP (SEQ ID NO: 35).

123. The vector of claim 118, wherein the F2A peptide comprises an amino acid sequence comprising VKQTLNFDLLKLAGDVESNPGP (SEQ ID NO: 36).
124. The vector of claim 118, wherein the GSG-F2A peptide comprises an amino acid sequence comprising GSGVKQTLNFDLLKLAGDVESNPGP (SEQ ID NO: 37).
125. The vector of claim 118, wherein the P2A peptide comprises an amino acid sequence comprising ATNFSLLKQAGDVEENPGP (SEQ ID NO: 38).
126. The vector of claim 118, wherein the GSG-P2A peptide comprises an amino acid sequence comprising GSGATNFSLLKQAGDVEENPGP (SEQ ID NO: 39).
127. A composition comprising the vector of any one of claims 86 to 126.
128. A cell comprising the CAR of any one of claims 1-36.
129. A cell comprising the transposon or transposase of any one of claims 38 to 85.
130. A cell comprising the vector of any one of claims 86 to 126.
131. The cell of any one of claims 128 to 130, wherein the cell expresses the CAR on the cell surface.
132. The cell of any one of claims 128 to 131, wherein the cell is an immune cell.
133. The cell of claim 132, wherein the immune cell is a T-cell, a Natural Killer (NK) cell, a Natural Killer (NK)-like cell, a hematopoietic progenitor cell, a peripheral blood (PB) derived T cell or an umbilical cord blood (UCB) derived T-cell.
134. The cell of claim 132, wherein the immune cell is a T-cell.
135. The cell of any one of claims 128 to 131, wherein the cell is an artificial antigen presenting cell.

136. The cell of any one of claims 128 to 131, wherein the cell is a tumor cell.
137. The cell of any one of claims 118 to 136, wherein the cell is autologous.
138. The cell of any one of claims 118 to 136, wherein the cell is allogeneic.
139. A composition comprising the cell of any one of claims 128-138.
140. A method for expressing a chimeric antigen receptor (CAR) on the surface of a cell, comprising:
- (a) obtaining a cell population;
 - (b) contacting the cell population to a composition comprising a CAR according to any one of claims 1-36 or a sequence encoding the CAR, under conditions sufficient to transfer the CAR across a cell membrane of at least one cell in the cell population, thereby generating a modified cell population;
 - (c) culturing the modified cell population under conditions suitable for integration of the CAR;
 - (d) expanding and/or selecting at least one cell from the modified cell population that express the CAR on the cell surface.
141. The method of claim 140, wherein the cell population comprises leukocytes.
142. The method of claim 141, wherein the cell population comprises CD4⁺ and CD8⁺ leukocytes in an optimized ratio.
143. The method of claim 142, wherein the optimized ratio of CD4⁺ to CD8⁺ leukocytes does not naturally occur *in vivo*.
144. The method of claim 140, wherein a transposon or vector comprises the CAR or the sequence encoding the CAR.
145. The method of claim 140, wherein a transposon of any one of claims 38 to 73 comprises the CAR or the sequence encoding the CAR.

146. The method of claim 144 or 145, wherein the transposon comprises a piggyBac transposon.
147. The method of claim 146, further comprising a composition comprising a plasmid comprising a sequence encoding a transposase enzyme.
148. The method of claim 147, wherein the sequence encoding a transposase enzyme is an mRNA sequence.
149. The method of any one of claims 147 or 148, wherein the transposase is a piggyBac transposase.
150. The method of claim 149, wherein the piggyBac transposase comprises an amino acid sequence comprising SEQ ID NO: 12.
151. The method of claim 149 or 150, wherein the piggyBac transposase is a hyperactive variant and wherein the hyperactive variant comprises an amino acid substitution at one or more of positions 30, 165, 282 and 538 of SEQ ID NO: 12.
152. The method of claim 151, wherein the amino acid substitution at position 30 of SEQ ID NO: 12 is a substitution of a valine (V) for an isoleucine (I) (I30V).
153. The method of claim 151, wherein the amino acid substitution at position 165 of SEQ ID NO: 12 is a substitution of a serine (S) for a glycine (G) (G165S).
154. The method of claim 151, wherein the amino acid substitution at position 282 of SEQ ID NO: 12 is a substitution of a valine (V) for a methionine (M) (M282V).
155. The method of claim 151, wherein the amino acid substitution at position 538 of SEQ ID NO: 12 is a substitution of a lysine (K) for an asparagine (N) (N538K).
156. The method of any one of claims 149-155, wherein the transposase is a Super piggyBac (sPBo) transposase.

157. The method of claim 156, wherein the Super piggyBac (sPBo) transposase comprises an amino acid sequence comprising SEQ ID NO: 2.

158. The method of claim 140, wherein a vector of any one of claims 76 to 116 comprises the CAR or the sequence encoding the CAR.

159. The method of claim 140, 144, 145, or 158, wherein the conditions sufficient to transfer the sequence encoding the CAR across a cell membrane of at least one cell in the cell population comprise nucleofection.

160. The method of any one of claims 140 to 158, wherein the conditions sufficient to transfer the sequence encoding the CAR across a cell membrane of at least one cell in the cell population of (b) comprise at least one of an application of one or more pulses of electricity at a specified voltage, a buffer, and one or more supplemental factor(s).

161. The method of claim 160, wherein the buffer comprises PBS, HBSS, OptiMEM, BTXpress, Amaxa Nucleofector, Human T cell nucleofection buffer or any combination thereof.

162. The method of claim 160 or 161, wherein the one or more supplemental factor(s) comprise

(a) a recombinant human cytokine, a chemokine, an interleukin or any combination thereof;

(b) a salt, a mineral, a metabolite or any combination thereof;

(c) a cell medium;

(d) an inhibitor of cellular DNA sensing, metabolism, differentiation, signal transduction, one or more apoptotic pathway(s) or combinations thereof; and

(e) a reagent that modifies or stabilizes one or more nucleic acids.

163. The method of claim 162, wherein the recombinant human cytokine, the chemokine, the interleukin or any combination thereof comprise IL2, IL7, IL12, IL15, IL21, IL1, IL3, IL4, IL5, IL6, IL8, CXCL8, IL9, IL10, IL11, IL13, IL14, IL16, IL17, IL18, IL19, IL20, IL22, IL23, IL25, IL26, IL27, IL28, IL29, IL30, IL31, IL32, IL33, IL35, IL36, GM-CSF, IFN-gamma, IL-1 alpha/IL-1F1, IL-1 beta/IL-1F2, IL-12 p70, IL-12/IL-35 p35, IL-13, IL-17/IL-

17A, IL-17A/F Heterodimer, IL-17F, IL-18/IL-1F4, IL-23, IL-24, IL-32, IL-32 beta, IL-32 gamma, IL-33, LAP (TGF-beta 1), Lymphotoxin-alpha/TNF-beta, TGF-beta, TNF-alpha, TRANCE/TNFSF11/RANK L or any combination thereof.

164. The method of claim 162, wherein the salt, the mineral, the metabolite or any combination thereof comprise HEPES, Nicotinamide, Heparin, Sodium Pyruvate, L-Glutamine, MEM Non-Essential Amino Acid Solution, Ascorbic Acid, Nucleosides, FBS/FCS, Human serum, serum-substitute, anti-biotics, pH adjusters, Earle's Salts, 2-Mercaptoethanol, Human transferrin, Recombinant human insulin, Human serum albumin, Nucleofector PLUS Supplement, KCL, MgCl₂, Na₂HPO₄, NaH₂PO₄, Sodium lactobionate, Mannitol, Sodium succinate, Sodium Chloride, CINA, Glucose, Ca(NO₃)₂, Tris/HCl, K₂HPO₄, KH₂PO₄, Polyethylenimine, Poly-ethylene-glycol, Poloxamer 188, Poloxamer 181, Poloxamer 407, Poly-vinylpyrrolidone, Pop313, Crown-5, or any combination thereof.

165. The method of claim 162, wherein the cell medium comprises PBS, HBSS, OptiMEM, DMEM, RPMI 1640, AIM-V, X-VIVO 15, CellGro DC Medium, CTS OpTimizer T Cell Expansion SFM, TexMACS Medium, PRIME-XV T Cell Expansion Medium, ImmunoCult-XF T Cell Expansion Medium or any combination thereof.

166. The method of claim 162, wherein the inhibitor of cellular DNA sensing, metabolism, differentiation, signal transduction, one or more apoptotic pathway(s) or combinations thereof comprise inhibitors of TLR9, MyD88, IRAK, TRAF6, TRAF3, IRF-7, NF-KB, Type 1 Interferons, pro-inflammatory cytokines, cGAS, STING, Sec5, TBK1, IRF-3, RNA pol III, RIG-1, IPS-1, FADD, RIP1, TRAF3, AIM2, ASC, Caspase1, Pro-IL1B, PI3K, Akt, Wnt3A, glycogen synthase kinase-3 β (GSK-3 β), TWS119, Bafilomycin, Chloroquine, Quinacrine, AC-YVAD-CMK, Z-VAD-FMK, Z-IETD-FMK or any combination thereof.

167. The method of claim 162, wherein the reagent that modifies or stabilizes one or more nucleic acids comprises a pH modifier, a DNA-binding protein, a lipid, a phospholipid, CaPO₄, a net neutral charge DNA binding peptide with or without a NLS sequence, a TREX1 enzyme or any combination thereof.

168. The method of any one of claims 140 to 158, wherein the conditions suitable for integration of the CAR or the sequence encoding the CAR comprise at least one of a buffer and one or more supplemental factor(s).

169. The method of claim 168, wherein the buffer comprises PBS, HBSS, OptiMEM, BTXpress, Amaxa Nucleofector, Human T cell nucleofection buffer or any combination thereof.

170. The method of claim 168 or 169, wherein the one or more supplemental factor(s) comprise

(a) a recombinant human cytokine, a chemokine, an interleukin or any combination thereof;

(b) a salt, a mineral, a metabolite or any combination thereof;

(c) a cell medium;

(d) an inhibitor of cellular DNA sensing, metabolism, differentiation, signal transduction, one or more apoptotic pathway(s) or combinations thereof; and

(e) a reagent that modifies or stabilizes one or more nucleic acids.

171. The method of claim 170, wherein the recombinant human cytokine, the chemokine, the interleukin or any combination thereof comprise IL2, IL7, IL12, IL15, IL21, IL1, IL3, IL4, IL5, IL6, IL8, CXCL8, IL9, IL10, IL11, IL13, IL14, IL16, IL17, IL18, IL19, IL20, IL22, IL23, IL25, IL26, IL27, IL28, IL29, IL30, IL31, IL32, IL33, IL35, IL36, GM-CSF, IFN-gamma, IL-1 alpha/IL-1F1, IL-1 beta/IL-1F2, IL-12 p70, IL-12/IL-35 p35, IL-13, IL-17/IL-17A, IL-17A/F Heterodimer, IL-17F, IL-18/IL-1F4, IL-23, IL-24, IL-32, IL-32 beta, IL-32 gamma, IL-33, LAP (TGF-beta 1), Lymphotoxin-alpha/TNF-beta, TGF-beta, TNF-alpha, TRANCE/TNFSF11/RANK L or any combination thereof.

172. The method of claim 170, wherein the salt, the mineral, the metabolite or any combination thereof comprise HEPES, Nicotinamide, Heparin, Sodium Pyruvate, L-Glutamine, MEM Non-Essential Amino Acid Solution, Ascorbic Acid, Nucleosides, FBS/FCS, Human serum, serum-substitute, anti-biotics, pH adjusters, Earle's Salts, 2-Mercaptoethanol, Human transferrin, Recombinant human insulin, Human serum albumin, Nucleofector PLUS Supplement, KCL, MgCl₂, Na₂HPO₄, NaH₂PO₄, Sodium lactobionate, Mannitol, Sodium succinate, Sodium Chloride, CINA, Glucose, Ca(NO₃)₂, Tris/HCl,

K₂HPO₄, KH₂PO₄, Polyethylenimine, Poly-ethylene-glycol, Poloxamer 188, Poloxamer 181, Poloxamer 407, Poly-vinylpyrrolidone, Pop313, Crown-5, or any combination thereof.

173. The method of claim 170, wherein the cell medium comprises PBS, HBSS, OptiMEM, DMEM, RPMI 1640, AIM-V, X-VIVO 15, CellGro DC Medium, CTS OpTimizer T Cell Expansion SFM, TexMACS Medium, PRIME-XV T Cell Expansion Medium, ImmunoCult-XF T Cell Expansion Medium or any combination thereof.

174. The method of claim 170, wherein the inhibitor of cellular DNA sensing, metabolism, differentiation, signal transduction, one or more apoptotic pathway(s) or combinations thereof comprise inhibitors of TLR9, MyD88, IRAK, TRAF6, TRAF3, IRF-7, NF-KB, Type 1 Interferons, pro-inflammatory cytokines, cGAS, STING, Sec5, TBK1, IRF-3, RNA pol III, RIG-1, IPS-1, FADD, RIP1, TRAF3, AIM2, ASC, Caspase1, Pro-IL1B, PI3K, Akt, Wnt3A, glycogen synthase kinase-3 β (GSK-3 β), TWS119, Bafilomycin, Chloroquine, Quinacrine, AC-YVAD-CMK, Z-VAD-FMK, Z-IETD-FMK or any combination thereof.

175. The method of claim 170, wherein the reagent that modifies or stabilizes one or more nucleic acids comprises a pH modifier, a DNA-binding protein, a lipid, a phospholipid, CaPO₄, a net neutral charge DNA binding peptide with or without a NLS sequence, a TREX1 enzyme or any combination thereof.

176. The method of any one of claims 140 to 175, wherein the expansion and selection of (d) occur sequentially.

177. The method of claim 176, wherein the expansion occurs prior to selection.

178. The method of claim 176, wherein the expansion occurs following selection.

179. The method of claim 178, wherein a further selection occurs following expansion.

180. The method of any one of claims 140 to 175, wherein the expansion and selection of (d) occur simultaneously.

181. The method of any one of claims 140 to 180, wherein the expansion comprises contacting at least one cell of the modified cell population with an antigen to stimulate the at least one cell through the CAR.

182. The method of claim 181, wherein the antigen is presented on the surface of a substrate.

183. The method of claim 182, wherein the substrate is a bead or a plurality of beads.

184. The method of claim 183, wherein the bead or plurality of beads is/are separated from the modified cell population following expansion.

185. The method of claim 181, wherein the antigen is presented on the surface of a cell.

186. The method of claim 185, wherein the antigen is presented on the surface of an artificial antigen presenting cell.

187. The method of any one of claims 140 to 186, wherein the transposon or vector comprises a selection gene and wherein the selection step comprises contacting at least one cell of the modified cell population with a compound to which the selection gene confers resistance, thereby identifying a cell expressing the selection gene as surviving the selection and identifying a cell failing to express the selection gene as failing to survive the selection step.

188. The method of any one of claims 140 to 187, wherein the expansion and selection steps proceed for a period of 10 to 14 days, inclusive of the endpoints.

189. A composition comprising the expanded and selected cell population of any one of claims 140 to 188.

190. A method of treating cancer in a subject in need thereof, comprising administering to the subject the composition of any one of claims 37, 74-85, 127, 139 or 189, wherein the CAR specifically binds to an antigen on a tumor cell.

191. The method of claim 190, wherein the tumor cell is a malignant tumor cell.

192. The method of claim 190 or 191, comprising administering to the subject the composition of claim 139 or 185, wherein the cell or cell population is autologous.
193. The method of claim 190 or 191, comprising administering to the subject the composition of claim 139 or 185, wherein the cell or cell population is allogeneic.
194. A method of treating an autoimmune condition in a subject in need thereof, comprising administering to the subject the composition of any one of claims 37, 74-85, 127, 139 or 189, wherein the CAR specifically binds to an antigen on an autoimmune cell of the subject.
195. The method of claim 194, wherein the autoimmune cell is a lymphocyte that specifically binds to a self-antigen on a target cell of the subject.
196. The method of claim 194 or 195, wherein the autoimmune cell is a B lymphocyte.
197. The method of claim 194 or 195, wherein the autoimmune cell is a T lymphocyte.
198. The method of any one of claims 194 to 197, comprising administering to the subject the composition of claim 139 or 185, wherein the cell or cell population is autologous.
199. The method of any one of claims 194 to 197, comprising administering to the subject the composition of claim 139 or 185, wherein the cell or cell population is allogeneic.
200. A method of treating or preventing an infection in a subject in need thereof, comprising administering to the subject the composition of any one of claims 37, 74-85, 127, 139 or 189, wherein the CAR specifically binds to an antigen on a cell comprising an infectious agent, a cell in communication with an infectious agent or a cell exposed to an infectious agent.
201. The method of claim 200, wherein the infectious agent is a bacterium, a virus, a yeast or a microbe.

202. The method of claim 200 or 201, wherein the infectious agent may induce one or more of an infection, an immunodeficiency condition, an inflammatory condition, and a proliferative disorder.
203. The method of claim 202, wherein the infection causes tuberculosis, microencephaly, neurodegeneration or malaria.
204. The method of claim 202 or 203, wherein the infection causes microencephaly in a fetus of the subject.
205. The method of claim 204, wherein the infectious agent is a virus and wherein the virus is a Zika virus.
206. The method of claim 202, wherein the immunodeficiency condition is acquired immune deficiency syndrome (AIDS).
207. The method of claim 202, wherein the proliferative disorder is a cancer.
208. The method of claim 207, wherein the cancer is cervical cancer and wherein the infectious agent is a human papilloma virus (HPV).
209. The method of any one of claims 200 to 208, comprising administering to the subject the composition of claim 139 or 185, wherein the cell or cell population is autologous.
210. The method of any one of claims 200 to 208, comprising administering to the subject the composition of claim 139 or 185, wherein the cell or cell population is allogeneic.
211. A method of treating a mast cell disease in a subject in need thereof, comprising administering to the subject the composition of any one of claims 37, 74-85, 127, 139 or 189, wherein the CAR specifically binds to an antigen on a mast cell.
212. The method of claim 211, wherein the mast cell disease is a disorder associated with an excessive proliferation of mast cells.

213. The method of claim 212, wherein the mast cell disease is mastocytosis.
214. The method of claim 213, wherein the mast cell disease is a disorder associated with an abnormal activity of a mast cell.
215. The method of claim 214, wherein the mast cell disease is mast cell activation syndrome (MCAS), an allergic disease, asthma or an inflammatory disease.
216. The method of any one of claims 211 to 216, comprising administering to the subject the composition of claim 139 or 185, wherein the cell or cell population is autologous.
217. The method of any one of claims 211 to 216, comprising administering to the subject the composition of claim 139 or 185, wherein the cell or cell population is allogeneic.
218. A method of treating a degenerative disease in a subject in need thereof, comprising administering to the subject the composition of any one of claims 37, 74-85, 127, 139 or 189, wherein the CAR specifically binds to an antigen on a deleterious cell or an aged cell.
219. The method of claim 218, wherein the degenerative disease is a neurodegenerative disorder, a metabolic disorder, a vascular disorder or aging.
220. The method of claim 218 or 219, wherein the degenerative disease is a neurodegenerative disorder and wherein the deleterious cell or the aged cell is a stem cell, an immune cell, a neuron, a glia or a microglia.
221. The method of claim 218 or 219, wherein the degenerative disease is a metabolic disorder and wherein the deleterious cell or the aged cell is a stem cell, a somatic cell, a neuron, a glia or a microglia.
222. The method of claim 218 or 219, wherein the degenerative disease is a vascular disorder and wherein the deleterious cell or the aged cell is a stem cell, a somatic cell, an immune cell, an endothelial cell, a neuron, a glia or a microglia.

223. The method of claim 218 or 219, wherein the degenerative disease is aging and wherein the deleterious cell or the aged cell is an oocyte, a sperm, a stem cell, a somatic cell, an immune cell, an endothelial cell, a neuron, a glia or a microglia.
224. The method of any one of claims 218 to 223, comprising administering to the subject the composition of claim 139 or 185, wherein the cell or cell population is autologous.
225. The method of any one of claims 218 to 223, comprising administering to the subject the composition of claim 139 or 185, wherein the cell or cell population is allogeneic.
226. A method of modifying a cell therapy in a subject in need thereof, comprising administering to the subject a composition comprising a cell comprising a transposon of any one of claims 38 to 73, wherein apoptosis may be selectively induced in the cell by contacting the cell with an induction agent.
227. A method of modifying a cell therapy in a subject in need thereof, comprising administering to the subject a composition comprising a cell comprising a vector of any one of claims 86 to 126, wherein apoptosis may be selectively induced in the cell by contacting the cell with an induction agent.
228. The method of claim 226 or 227, wherein the cell is autologous.
229. The method of claim 226 or 227, wherein the cell is allogeneic.
230. The method of claim any one of claims 226 to 229, wherein the cell therapy is an adoptive cell therapy.
231. The method of claim any one of claims 226 to 230, wherein the modifying is a termination of the cell therapy.
232. The method of claim any one of claims 226 to 230, wherein the modifying is a depletion of a portion of the cells provided in the cell therapy.

233. The method of claim any one of claims 226 to 232, further comprising the step of administering an inhibitor of the induction agent to inhibit modification of the cell therapy, thereby restoring the function and/or efficacy of the cell therapy.

FIGURE 2

P-BCMA-101

SIGNAL PEPTIDE: MALPVTALLLLPLALLLHAARP (SEQ ID NO: 3)

CENTYRIN (A08):

MLPAPKNLVVSRITEDSARLSWTAPDAAFDSFPIRYIETLIWGEAIWLDVPGSERSYDLTGLKPGTEYAVVITGVKGGRF
SSPLVASFTT (SEQ ID NO: 41)

HINGE (CD8A): TTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACD (SEQ ID NO: 10)

TRANSMEMBRANE (CD8A): IYIWAFLAGTCGVLLLSLVITLYC (SEQ ID NO: 4)

COSTIMULATORY DOMAIN #1 (4-1BB): KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEGGCEL (SEQ ID NO: 8)

COSTIMULATORY DOMAIN #2 (CD28):

RVKFSRSADAPAYKQGQNQLYNELNLRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGM
KGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR (SEQ ID NO: 6)

P-BCMA-101 CONSTRUCT:

MALPVTALLLLPLALLLHAARMLPAPKNLVVSRITEDSARLSWTAPDAAFDSFPIRYIETLIWGEAIWLDVPGSERSYDL
TGLKPGTEYAVVITGVKGGRFSSPLVASFTTTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWA
PLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEGGCELRVKFSRSADAPAYKQGQN
QLYNELNLRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGL
STATKDTYDALHMQALPPR (SEQ ID NO: 42)

FIGURE 3A

P-BCMA-101

SIGNAL PEPTIDE: MALPVTALLPLALLHARP (SEQ ID NO: 3)

CENTYRIN (A08):

MILPAPKNLVVSRITEDSARLSWTAPDAAFDSFPIRYIETLIWGEAIWLDVPGSERSYDLTGLKPGTEYAVVITGVKGGR
FSSPLVASFTT (SEQ ID NO: 43)

HINGE (CD8A): TTPAPRPPTPTAPTIASQPLSLRPEACRPAAAGGAVHTRGLDFACD (SEQ ID NO: 10)

TRANSMEMBRANE (CD8A): IYIWAPLAGTCGVLLLSLVITLYC (SEQ ID NO: 4)

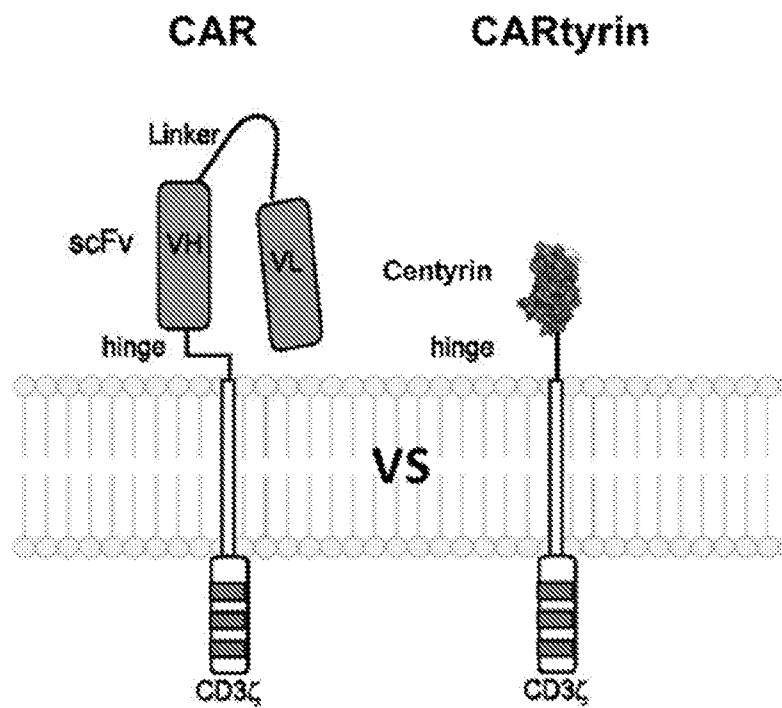
COSTIMULATORY DOMAIN #1 (4-1BB): KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEGGCEL (SEQ ID
NO: 8)

COSTIMULATORY DOMAIN #2 (CD28):

RVKFSRSADAPAYKQGQNQLYNELNLRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQDKDMAEAYSEIG
MKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR (SEQ ID NO: 6)

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FIGURE 4



		scFv	Centyrin
	Size:	~260 aa	~90 aa
	Components:	Heavy & Light Chain Variable Regions, Flexible Linker	Fibronectin type III domain
	Derived:	Mainly Rodent Abs	Human
	Stability:	Not as stable as original IgG format	Stable

FIGURE 5A

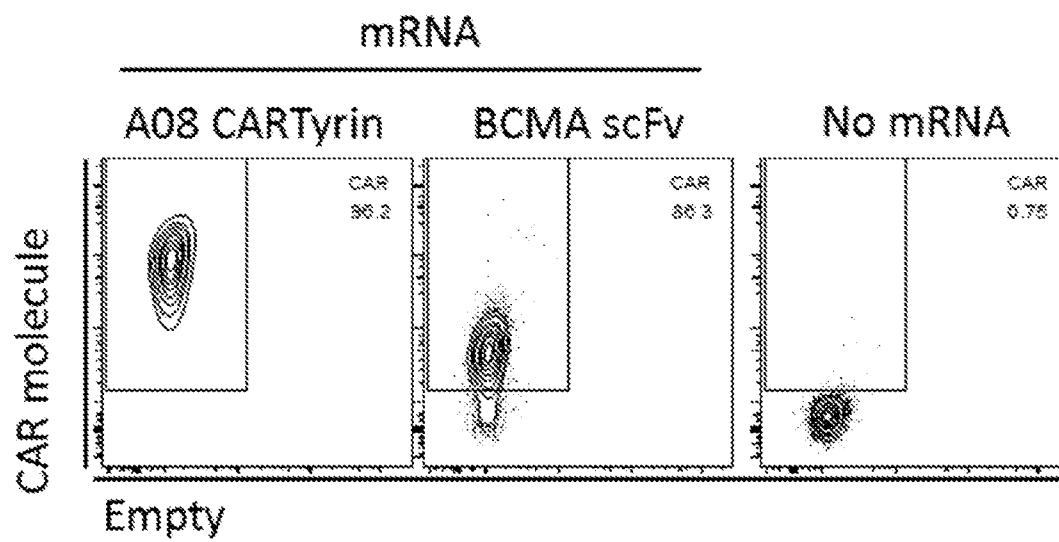


FIGURE 5B

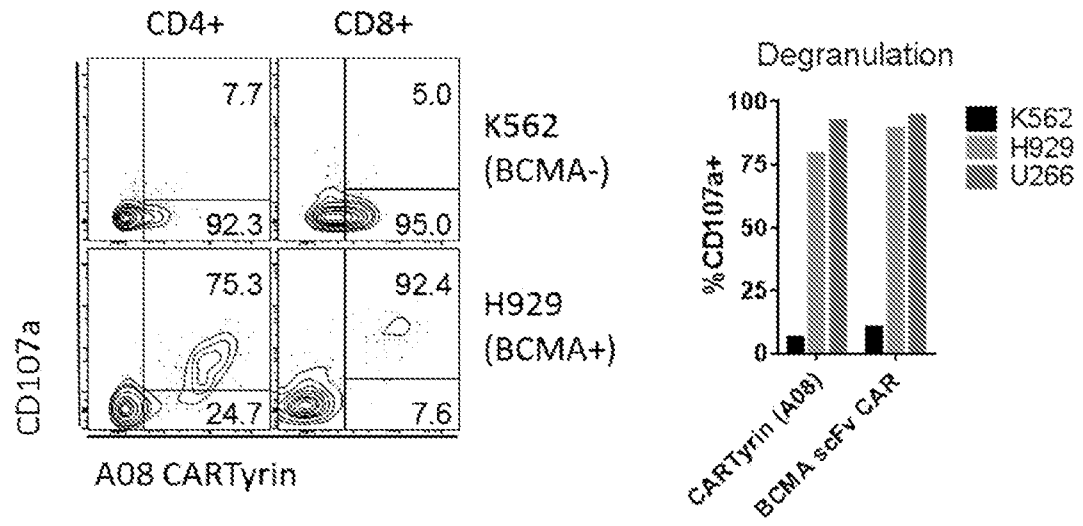


FIGURE 5C

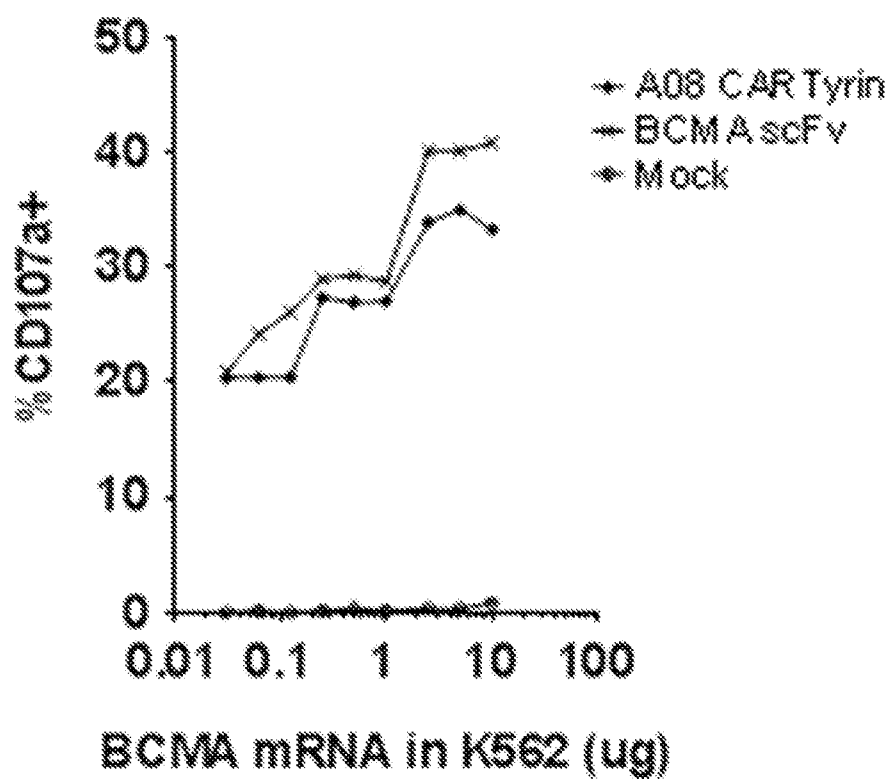


FIGURE 6

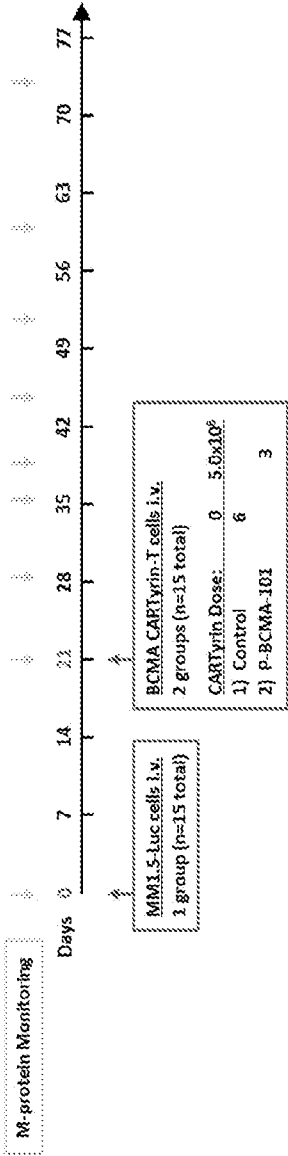


FIGURE 7

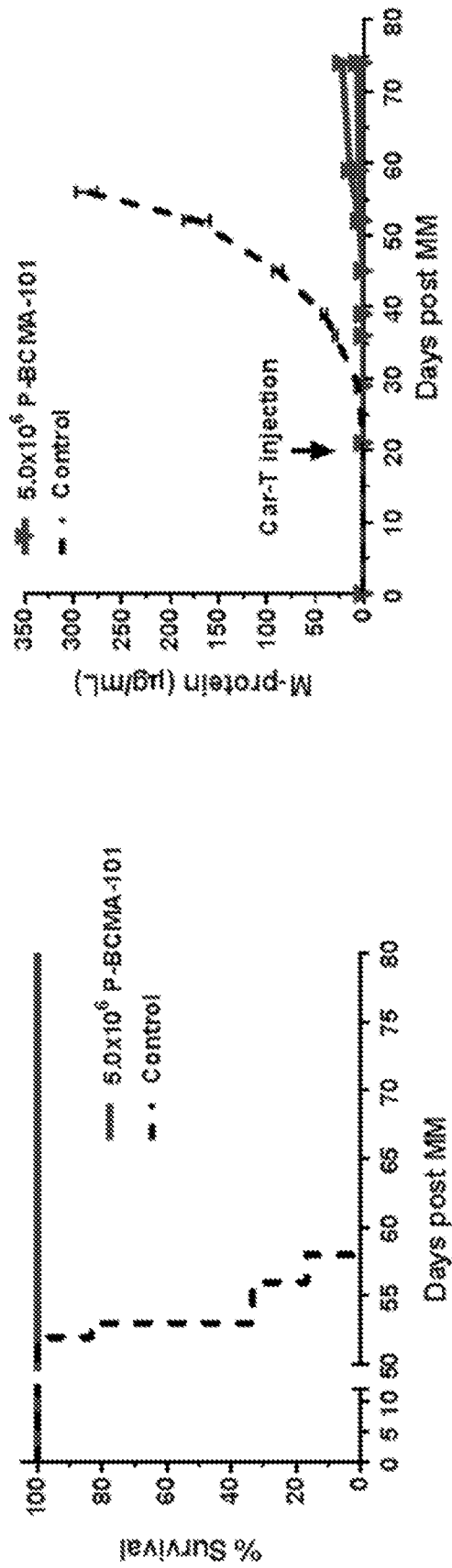


FIGURE 8

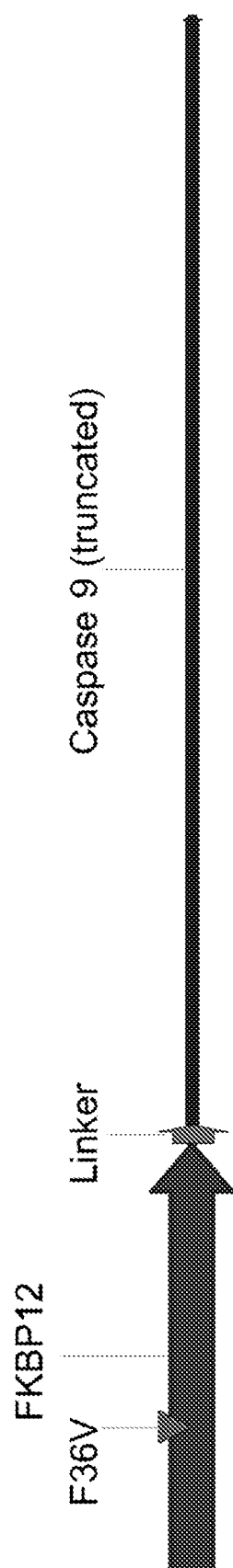


FIGURE 9

iC9/AP1903 Safety Switch Day 12

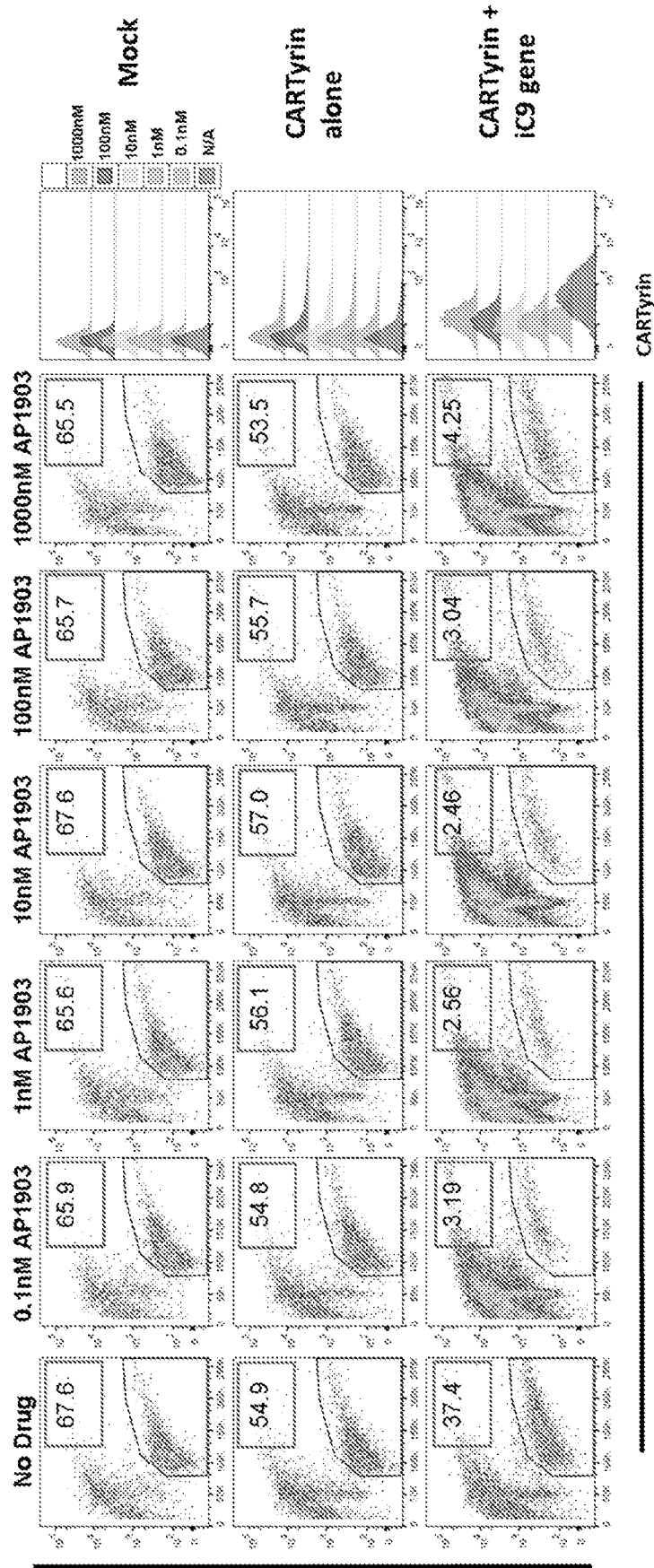


FIGURE 10
iC9/AP1903 Safety Switch Day 19

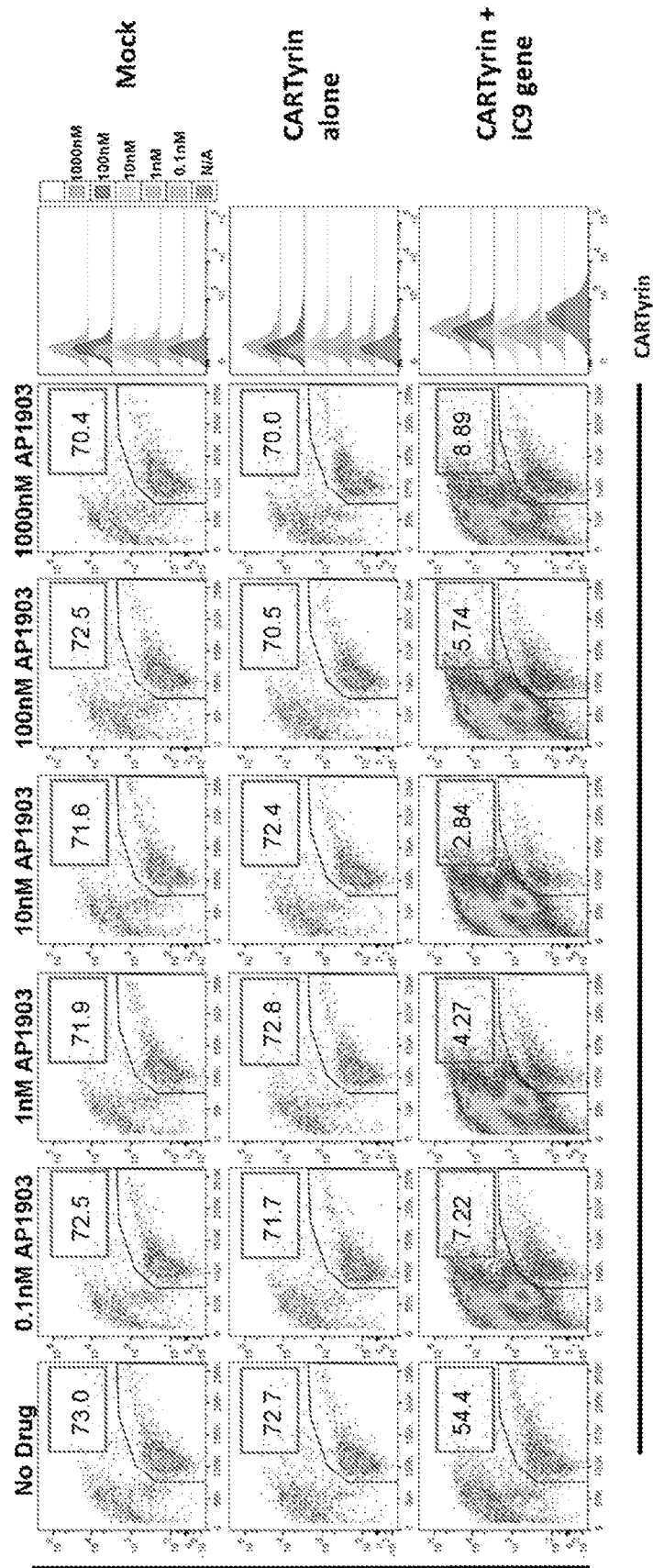
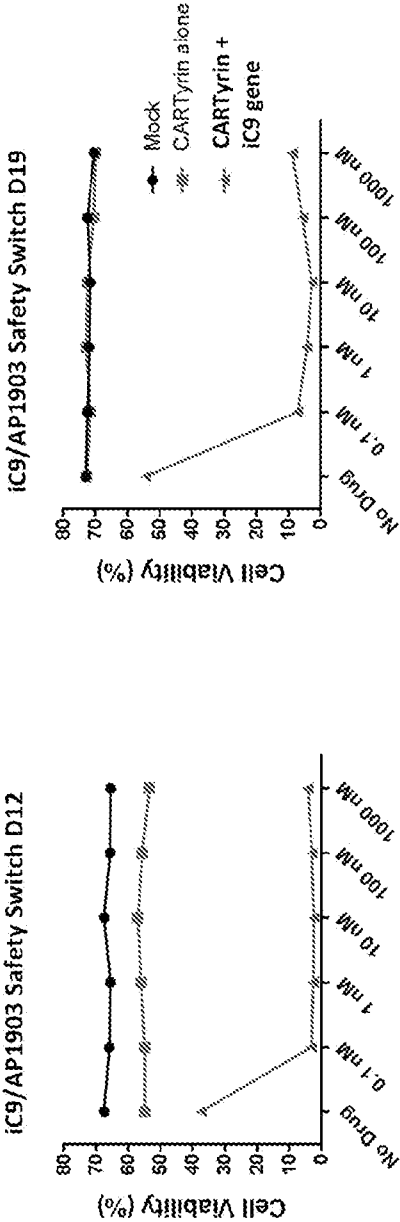


FIGURE 11

ic9/AP1903 Safety Switch Summary



>95% of the CARTyrin⁺ T cells expressing the ic9 safety switch were eliminated within 24 hours

