

immune effector cells, engineered utilizing the vectors described herein, and methods of treating a subject using these pharmaceutical compositions. The recombinant vectors disclosed herein are particularly useful in modifying immune effector cells (*e.g.*, T cells) for use in adoptive cell therapy.

[0005] Accordingly, in one aspect, the instant disclosure provides a recombinant vector comprising a polycistronic expression cassette, wherein said polycistronic expression cassette comprises a transcriptional regulatory element operably linked to a polynucleotide that comprises, from 5' to 3': a first polynucleotide sequence that encodes a chimeric antigen receptor (CAR) that comprises an extracellular antigen-binding domain that specifically binds to CD19, a transmembrane domain, and a cytoplasmic domain; a second polynucleotide sequence that comprises an F2A element; a third polynucleotide sequence that encodes a fusion protein that comprises IL-15, or a functional fragment or functional variant thereof, and IL-15R α , or a functional fragment or functional variant thereof; a fourth polynucleotide sequence that comprises a T2A element; and a fifth polynucleotide sequence that encodes a marker protein.

[0006] In some [embodimentexamples](#), said F2A element comprises a polynucleotide sequence that encodes the amino acid sequence of SEQ ID NO: 137, or the amino acid sequence of SEQ ID NO: 137, comprising 1, 2, or 3 amino acid modifications. In some [embodimentexamples](#), said F2A element comprises a polynucleotide sequence at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the polynucleotide sequence of SEQ ID NO: 141. In some [embodimentexamples](#), said F2A element comprises a polynucleotide sequence that encodes the amino acid sequence of SEQ ID NO: 138, or the amino acid sequence of SEQ ID NO: 138, comprising 1, 2, or 3 amino acid modifications. In some [embodimentexamples](#), said F2A element comprises a polynucleotide sequence at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the polynucleotide sequence of SEQ ID NO: 142.

[0007] In some [embodimentexamples](#), said T2A element comprises a polynucleotide sequence that encodes the amino acid sequence of SEQ ID NO: 139, or the amino acid sequence of SEQ ID NO: 139, comprising 1, 2, or 3 amino acid modifications. In some [embodimentexamples](#), said T2A element comprises a polynucleotide sequence at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the polynucleotide sequence SEQ ID NO: 143. In some [embodimentexamples](#), said T2A element comprises a polynucleotide sequence that encodes the amino acid sequence of SEQ ID NO: 140 or 182, or the amino acid sequence of SEQ ID NO: 140 or 182, comprising 1, 2, or 3 amino acid modifications. In some [embodimentexamples](#), said T2A element

comprises a polynucleotide sequence at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the polynucleotide sequence of SEQ ID NO: 144, 145, or 165.

[0008] In some [embodimentexamples](#), said antigen-binding domain comprises: a heavy chain variable region (VH) comprising complementarity determining regions VH CDR1, VH CDR2, and VH CDR3; and a light chain variable region (VL) comprising complementarity determining regions VL CDR1, VL CDR2, and VL CDR3. In some [embodimentexamples](#), said antigen-binding domain comprises an scFv that comprises said VH and said VL operably linked via a first peptide linker.

[0009] In some [embodimentexamples](#), said VH comprises the VH CDR1, VH CDR2, and VH CDR3 amino acid sequences set forth in SEQ ID NO: 2. In some [embodimentexamples](#), said VH CDR1 comprises the amino acid sequence of SEQ ID NO: 6; or the amino acid sequence of SEQ ID NO: 6, comprising 1, 2, or 3 amino acid modifications; said VH CDR2 comprises the amino acid sequence of SEQ ID NO: 7; or the amino acid sequence of SEQ ID NO: 7, comprising 1, 2, or 3 amino acid modifications; and said VH CDR3 comprises the amino acid sequence of SEQ ID NO: 8; or the amino acid sequence of SEQ ID NO: 8, comprising 1, 2, or 3 amino acid modifications.

[0010] In some [embodimentexamples](#), said VL comprises the VL CDR1, VL CDR2, and VL CDR3 amino acid sequences set forth in SEQ ID NO: 1. In some [embodimentexamples](#), said VL CDR1 comprises the amino acid sequence of SEQ ID NO: 3; or the amino acid sequence of SEQ ID NO: 3, comprising 1, 2, or 3 amino acid modifications; said VL CDR2 comprises the amino acid sequence of SEQ ID NO: 4; or the amino acid sequence of SEQ ID NO: 4, comprising 1, 2, or 3 amino acid modifications; and said VL CDR3 comprises the amino acid sequence of SEQ ID NO: 5; or the amino acid sequence of SEQ ID NO: 5, comprising 1, 2, or 3 amino acid modifications.

[0011] In some [embodimentexamples](#), said VH comprises an amino acid sequence at least 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 2. In some [embodimentexamples](#), said VH is encoded by a polynucleotide sequence at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the polynucleotide sequence of SEQ ID NO: 20.

[0012] In some [embodimentexamples](#), said VL comprises an amino acid sequence at least 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 1. In some [embodimentexamples](#), said VL is encoded by a polynucleotide sequence at least 75%, 80%, 85%, 90%,

95%, 96%, 97%, 98%, 99%, or 100% identical to the polynucleotide sequence of SEQ ID NO: 19.

[0013] In some [embodimentexamples](#), said first peptide linker comprises the amino acid sequence of SEQ ID NO: 9 or SEQ ID NO: 17, or the amino acid sequence of SEQ ID NO: 9 or 17, comprising 1, 2, or 3 amino acid modifications. In some [embodimentexamples](#), said first peptide linker is encoded by a polynucleotide sequence at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the polynucleotide sequence of SEQ ID NO: 27 or SEQ ID NO: 35. In some [embodimentexamples](#), said first peptide linker is encoded by a polynucleotide sequence at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the polynucleotide sequence of SEQ ID NO: 27.

[0014] In some [embodimentexamples](#), said CAR further comprises a hinge region positioned between said antigen-binding domain and said transmembrane domain of said CAR. In some [embodimentexamples](#), said hinge region comprises the amino acid sequence of SEQ ID NO: 37, 38, or 39, or the amino acid sequence of SEQ ID NO: 37, 38, or 39, comprising 1, 2, or 3 amino acid modifications. In some [embodimentexamples](#), said hinge region is encoded by a polynucleotide sequence at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the polynucleotide sequence of SEQ ID NO: 40, 41, or 42.

[0015] In some [embodimentexamples](#), said transmembrane domain of said CAR comprises the amino acid sequence of SEQ ID NO: 43, 44, or 45, or the amino acid sequence of SEQ ID NO: 43, 44, or 45, comprising 1, 2, or 3 amino acid modifications. In some [embodimentexamples](#), said transmembrane domain of said CAR is encoded by a polynucleotide sequence at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the polynucleotide sequence of SEQ ID NO: 49, 50, 51, or 52.

[0016] In some [embodimentexamples](#), said hinge region and said transmembrane domain together comprise the amino acid sequence of SEQ ID NO: 46, 47, or 48, or the amino acid sequence of SEQ ID NO: 46, 47, or 48, comprising 1, 2, or 3 amino acid modifications. In some [embodimentexamples](#), said hinge region and said transmembrane domain together are encoded by a polynucleotide sequence at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the polynucleotide sequence of SEQ ID NO: 53, 54, 55, or 56.

[0017] In some [embodimentexamples](#), said cytoplasmic domain comprises a primary signaling domain of human CD3 ζ , or a functional fragment or functional variant thereof. In some [embodimentexamples](#), said cytoplasmic domain comprises an amino acid sequence at least 95%, 96%,

97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 60. In some [embodimentexamples](#), said cytoplasmic domain is encoded by a polynucleotide sequence at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the polynucleotide sequence of SEQ ID NO: 67 or 68.

[0018] In some [embodimentexamples](#), said cytoplasmic domain comprises a co-stimulatory domain, or functional fragment or variant thereof, of a protein selected from the group consisting of CD28, 4-1BB, OX40, CD2, CD7, CD27, CD30, CD40, CDS, ICAM-1, LFA-1, B7-H3, and ICOS. In some [embodimentexamples](#), said protein is CD28 or 4-1BB.

[0019] In some [embodimentexamples](#), said protein is CD28. In some [embodimentexamples](#), said cytoplasmic domain comprises the amino acid sequence of SEQ ID NO: 57 or 58, or the amino acid sequence of SEQ ID NO: 57 or 58, comprising 1, 2, or 3 amino acid modifications. In some [embodimentexamples](#), said cytoplasmic domain is encoded by a polynucleotide sequence at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the polynucleotide sequence of SEQ ID NO: 64 or 65.

[0020] In some [embodimentexamples](#), said protein is 4-1BB. In some [embodimentexamples](#), said cytoplasmic domain comprises the amino acid sequence of SEQ ID NO: 59, or the amino acid sequence of SEQ ID NO: 59, comprising 1, 2, or 3 amino acid modifications. In some [embodimentexamples](#), said cytoplasmic domain is encoded by a polynucleotide sequence at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the polynucleotide sequence of SEQ ID NO: 66.

[0021] In some [embodimentexamples](#), said cytoplasmic domain comprises the amino acid sequence of SEQ ID NO: 61, 62, or 63, or the amino acid sequence of SEQ ID NO: 61, 62, or 63, comprising 1, 2, or 3 amino acid modifications. In some [embodimentexamples](#), said cytoplasmic domain is encoded by a polynucleotide sequence at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the polynucleotide sequence of SEQ ID NO: 69, 70, or 71.

[0022] In some [embodimentexamples](#), said CAR comprises an amino acid sequence at least at least 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 72, 74, 76, 77, 78, 79, 80, or 81. In some [embodimentexamples](#), said CAR is encoded by a polynucleotide sequence at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the polynucleotide sequence of SEQ ID NO: 82, 83, 86, 87, 90, 91, 92, 93, 94, or 95.

[0023] In some [embodimentexamples](#), said IL-15, or said functional fragment or functional variant thereof, is operably linked to said IL-15R α , or said functional fragment or functional variant

thereof, via a second peptide linker. In some [embodimentexamples](#), said fusion protein comprises an amino acid sequence at least 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 119, 121, or 180. In some [embodimentexamples](#), said fusion protein is encoded by a polynucleotide sequence at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the polynucleotide sequence of SEQ ID NO: 126, 127, 130, 131, or 181.

[0024] In some [embodimentexamples](#), said marker protein comprises: domain III of HER1, or a functional fragment or functional variant thereof; an N-terminal portion of domain IV of HER1; and a transmembrane domain of CD28, or a functional fragment or functional variant thereof.

[0025] In some [embodimentexamples](#), said domain III of HER1, or a functional fragment or functional variant thereof, comprises an amino acid sequence at least 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 98. In some [embodimentexamples](#), said domain III of HER1, or a functional fragment or functional variant thereof, is encoded by a polynucleotide sequence at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the polynucleotide sequence of SEQ ID NO: 110 or 164.

[0026] In some [embodimentexamples](#), said N-terminal portion of domain IV of HER1 comprises amino acids 1-40, 1-39, 1-38, 1-37, 1-36, 1-35, 1-34, 1-33, 1-32, 1-31, 1-30, 1-29, 1-28, 1-27, 1-26, 1-25, 1-24, 1-23, 1-22, 1-21, 1-20, 1-19, 1-18, 1-17, 1-16, 1-15, 1-14, 1-13, 1-12, 1-11, or 1-10 of SEQ ID NO: 99. In some [embodimentexamples](#), said N-terminal portion of domain IV of HER1 comprises amino acids 1-21 of SEQ ID NO: 99. In some [embodimentexamples](#), said N-terminal portion of domain IV of HER1 comprises the amino acid sequence of SEQ ID NO: 100, or the amino acid sequence of SEQ ID NO: 100, comprising 1, 2, or 3 amino acid modifications. In some [embodimentexamples](#), said N-terminal portion of domain IV of HER1 is encoded by a polynucleotide sequence at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the polynucleotide sequence of SEQ ID NO: 112.

[0027] In some [embodimentexamples](#), said transmembrane region of CD28 comprises the amino acid sequence of SEQ ID NO: 101, or the amino acid sequence of SEQ ID NO: 101, comprising 1, 2, or 3 amino acid modifications. In some [embodimentexamples](#), said transmembrane region of CD28 is encoded by a polynucleotide sequence at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the polynucleotide sequence of SEQ ID NO: 113.

[0028] In some [embodimentexamples](#), said marker protein comprises an amino acid sequence at least 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 96, 97, 166, or 167. In some [embodimentexamples](#), said marker protein is encoded by a polynucleotide

sequence at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the polynucleotide sequence of SEQ ID NO: 107, 108, 109, 162, 173, or 174.

[0029] In some [embodimentexamples](#), said regulatory element comprises a promoter. In some [embodimentexamples](#), said promoter is a human elongation factor 1-alpha (hEF-1 α) hybrid promoter. In some [embodimentexamples](#), said promoter comprises a polynucleotide sequence at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the polynucleotide sequence of SEQ ID NO: 146.

[0030] In some [embodimentexamples](#), said vector further comprises a polyA sequence 3' of said fifth polynucleotide sequence. In some [embodimentexamples](#), said polyA sequence comprises a polynucleotide sequence at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the polynucleotide sequence of SEQ ID NO: 148.

[0031] In another [aspectexample](#), the instant disclosure provides a recombinant vector comprising a polycistronic expression cassette, wherein said polycistronic expression cassette comprises a transcriptional regulatory element operably linked to a polynucleotide that comprises, from 5' to 3': a first polynucleotide sequence that encodes a CAR that comprises an amino acid sequence at least 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 72 or 74; a second polynucleotide sequence that comprises an F2A element; a third polynucleotide sequence that encodes a fusion protein that comprises an amino acid sequence at least 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 119, 121, or 180; a fourth polynucleotide sequence that comprises a T2A element; and a fifth polynucleotide sequence that encodes a marker protein that comprises an amino acid sequence at least 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 96 or 97.

[0032] In some [embodimentexamples](#), said F2A element comprises a polynucleotide sequence that encodes the amino acid sequence of SEQ ID NO: 137, or the amino acid sequence of SEQ ID NO: 137, comprising 1, 2, or 3 amino acid modifications. In some [embodimentexamples](#), said F2A element comprises a polynucleotide sequence at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the polynucleotide sequence of SEQ ID NO: 141. In some [embodimentexamples](#), said F2A element comprises a polynucleotide sequence that encodes the amino acid sequence of SEQ ID NO: 138, or the amino acid sequence of SEQ ID NO: 138, comprising 1, 2, or 3 amino acid modifications. In some [embodimentexamples](#), said F2A element comprises a

polynucleotide sequence at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the polynucleotide sequence of SEQ ID NO: 142.

[0033] In some [embodimentexamples](#), said T2A element comprises a polynucleotide sequence that encodes the amino acid sequence of SEQ ID NO: 139, or the amino acid sequence of SEQ ID NO: 139, comprising 1, 2, or 3 amino acid modifications. In some [embodimentexamples](#), said T2A element comprises a polynucleotide sequence at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the polynucleotide sequence SEQ ID NO: 143. In some [embodimentexamples](#), said T2A element comprises a polynucleotide sequence that encodes the amino acid sequence of SEQ ID NO: 140 or 182, or the amino acid sequence of SEQ ID NO: 140 or 182, comprising 1, 2, or 3 amino acid modifications. In some [embodimentexamples](#), said T2A element comprises a polynucleotide sequence at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the polynucleotide sequence of SEQ ID NO: 144, 145, or 165.

[0034] In another [aspectexample](#), the instant disclosure provides a recombinant vector comprising a polycistronic expression cassette, wherein said polycistronic expression cassette comprises a transcriptional regulatory element operably linked to a polynucleotide that comprises, from 5' to 3': a first polynucleotide sequence at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the polynucleotide sequence of SEQ ID NO: 82, 83, 86, or 87; a second polynucleotide sequence that comprises an F2A element; a third polynucleotide sequence at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the polynucleotide sequence of SEQ ID NO: 126, 127, 130, 131, or 181; a fourth polynucleotide sequence that comprises a T2A element; and a fifth polynucleotide sequence at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the polynucleotide sequence of SEQ ID NO: 107, 108, 109, or 162.

[0035] In some [embodimentexamples](#), said F2A element comprises a polynucleotide sequence that encodes the amino acid sequence of SEQ ID NO: 137, or the amino acid sequence of SEQ ID NO: 137, comprising 1, 2, or 3 amino acid modifications. In some [embodimentexamples](#), said F2A element comprises a polynucleotide sequence at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the polynucleotide sequence of SEQ ID NO: 141. In some [embodimentexamples](#), said F2A element comprises a polynucleotide sequence that encodes the amino acid sequence of SEQ ID NO: 138, or the amino acid sequence of SEQ ID NO: 138, comprising 1, 2, or 3 amino acid modifications. In some [embodimentexamples](#), said F2A element comprises a

polynucleotide sequence at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the polynucleotide sequence of SEQ ID NO: 142.

[0036] In some [embodimentexamples](#), said T2A element comprises a polynucleotide sequence that encodes the amino acid sequence of SEQ ID NO: 139, or the amino acid sequence of SEQ ID NO: 139, comprising 1, 2, or 3 amino acid modifications. In some [embodimentexamples](#), said T2A element comprises a polynucleotide sequence at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the polynucleotide sequence SEQ ID NO: 143. In some [embodimentexamples](#), said T2A element comprises a polynucleotide sequence that encodes the amino acid sequence of SEQ ID NO: 140 or 182, or the amino acid sequence of SEQ ID NO: 140 or 182, comprising 1, 2, or 3 amino acid modifications. In some [embodimentexamples](#), said T2A element comprises a polynucleotide sequence at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the polynucleotide sequence of SEQ ID NO: 144, 145, or 165.

[0037] In some [embodimentexamples](#) of the recombinant vectors described herein, the vector further comprises a Left inverted terminal repeat (ITR) and a Right ITR, wherein said Left ITR and said Right ITR flank said polycistronic expression cassette. In some [embodimentexamples](#), the recombinant vector comprises, from 5' to 3': said Left ITR; said transcriptional regulatory element; said first polynucleotide sequence; said second polynucleotide sequence; said third polynucleotide sequence; said fourth polynucleotide sequence; said fifth polynucleotide sequence; and said Right ITR.

[0038] In another [aspectexample](#), the instant disclosure provides a recombinant vector comprising a polycistronic expression cassette, wherein said polycistronic expression cassette comprises a transcriptional regulatory element operably linked to a polynucleotide sequence at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the polynucleotide sequence of SEQ ID NO: 149. In another [aspectexample](#), the instant disclosure provides a recombinant vector comprising a polycistronic expression cassette, wherein said polycistronic expression cassette comprises a transcriptional regulatory element operably linked to a polynucleotide that encodes an amino acid sequence at least 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 152. In one aspect, the instant disclosure provides a recombinant vector comprising a polycistronic expression cassette, wherein said polycistronic expression cassette comprises (a) a transcriptional regulatory element operably linked to a polynucleotide sequence at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the polynucleotide sequence of SEQ ID NO: 149; or (b) a transcriptional regulatory element operably linked to a polynucleotide that encodes an amino acid sequence at least 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 152.

[0039] In some [embodimentexamples](#), any of the recombinant vectors described herein further comprise a Left inverted terminal repeat (ITR) and a Right ITR, wherein said Left ITR and said Right ITR flank said polycistronic expression cassette. In some [embodimentexamples](#), said Left ITR and said Right ITR are ITRs of a DNA transposon selected from the group consisting of a Sleeping

Beauty transposon, a piggyBac transposon, TcBuster transposon, and a Tol2 transposon. In some [embodimentexamples](#), said DNA transposon is said Sleeping Beauty transposon.

[0040] In some [embodimentexamples](#) of the recombinant vectors described herein, said vector is a non-viral vector. In some [embodimentexamples](#), said non-viral vector is a plasmid. In some [embodimentexamples](#) of the recombinant vectors described herein, said vector is a viral vector. In some [embodimentexamples](#) of the recombinant vectors described herein, said vector is a polynucleotide.

[0041] In another [aspectexample](#), the instant disclosure provides a polynucleotide encoding an amino acid sequence at least 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 152.

[0042] In another [aspectexample](#), the instant disclosure provides a population of cells that comprise the vector as described herein. In some [embodimentexamples](#), said vector is integrated into the genome of said population of cells. [In one aspect, the instant disclosure provides a population of recombinant cells that comprise the vector as described herein.](#)

[0043] In another [aspectexample](#), the instant disclosure provides a population of cells that comprise a polynucleotide encoding an amino acid sequence at least 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 152. In some [embodimentexamples](#), said polynucleotide is integrated into the genome of said population of cells.

[0044] In another [aspectexample](#), the instant disclosure provides a population of cells that comprise a polypeptide comprising an amino acid sequence encoded by a polynucleotide encoding an amino acid sequence at least 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 152.

[0045] In some [embodimentexamples](#) of the populations of cells described herein, the cells comprise a CAR comprising the amino acid sequence of SEQ ID NO: 72, 73, 74, 75, 76, 77, 78, 79, 80, or 81; a fusion protein comprising the amino acid sequence of SEQ ID NO: 119, 120, 121, 122, 180, or 183; and a marker protein comprising the amino acid sequence of SEQ ID NO: 96, 97, 166, or 167. In some [embodimentexamples](#) of the populations of cells described herein, the cells comprise a CAR comprising the amino acid sequence of SEQ ID NO: 74; a fusion protein comprising the amino acid sequence of SEQ ID NO: 121; and a marker protein comprising the amino acid sequence of SEQ ID NO: 97. In some [embodimentexamples](#) of the populations of cells described herein, the cells comprise a CAR comprising the amino acid sequence of SEQ ID NO: 75; a fusion protein comprising the amino acid sequence of SEQ ID NO: 122; and a marker protein comprising the amino acid sequence of SEQ ID NO: 97.

[0046] In some embodimentexamples of the populations of cells described herein, the cells are immune effector cells. In some embodimentexamples, said immune effector cells are selected from the group consisting of T cells, natural killer (NK) cells, B cells, mast cells, and myeloid-derived phagocytes. In some embodimentexamples, said immune effector cells are T cells. In some embodimentexamples, the population of T cells comprise alpha/beta T cells, gamma/delta T cells, or natural killer T (NK-T) cells. In some embodimentexamples, the population of T cells comprise CD4⁺ T cells, CD8⁺ T cells, or both CD4⁺ T cells and CD8⁺ T cells.

[0047] In some embodimentexamples of the populations of cells described herein, the cells are *ex vivo*. In some embodimentexamples of the populations of cells described herein, the cells are human.

[0047a] In one aspect, the instant disclosure provides a method of producing a population of engineered cells, comprising: a. introducing into a population of cells the recombinant vector described herein, and a DNA transposase or a polynucleotide encoding a DNA transposase; and b. culturing said population of cells under conditions wherein said transposase integrates the polycistronic expression cassette into the genome of said population of cells, thereby producing the population of engineered cells.

[0048] In another aspectexample, the instant disclosure provides a method of producing a population of engineered cells, comprising: introducing into a population of cells a recombinant vector comprising a Left ITR and a Right ITR, wherein said Left ITR and said Right ITR flank said polycistronic expression cassette and culturing said population of cells under conditions wherein said transposase integrates the polycistronic expression cassette into the genome of said population of cells, thereby producing the population of engineered cells. In some embodimentexamples, the recombinant vector comprises, from 5' to 3': said Left ITR; said transcriptional regulatory element; said first polynucleotide sequence; said second polynucleotide sequence; said third polynucleotide sequence; said fourth polynucleotide sequence; said fifth polynucleotide sequence; and said Right ITR.

[0049] In some embodimentexamples, said Left ITR and said Right ITR are ITRs of a DNA transposon selected from the group consisting of a Sleeping Beauty transposon, a piggyBac transposon, a TcBuster transposon, and a Tol2 transposon. In some embodimentexamples, said DNA transposon is said Sleeping Beauty transposon. In some embodimentexamples, said transposase is a Sleeping Beauty transposase. In some embodimentexamples, said Sleeping Beauty transposase is selected from the group consisting of SB11, SB100X, hSB110, and hSB81. In some embodimentexamples, said Sleeping Beauty transposase is SB11. In some embodimentexamples, said SB11 comprises an amino acid sequence at least 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 160. In some embodimentexamples, said SB11 is encoded by a polynucleotide sequence at least at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the polynucleotide sequence of SEQ ID NO: 161. In some embodimentexamples, said polynucleotide encoding said DNA transposase is a DNA vector or an RNA vector.

[0050] In some embodimentexamples, said Left ITR comprises a polynucleotide sequence at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the polynucleotide sequence of SEQ ID NO: 155 or 156; and said Right ITR comprises a polynucleotide sequence at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the polynucleotide sequence of SEQ ID NO: 157, 159, or 184.

[0051] In some embodimentexamples, said recombinant vector, and said DNA transposase or polynucleotide encoding said DNA transposase, are introduced to said population of cells using electro-transfer, calcium phosphate precipitation, lipofection, particle bombardment, microinjection, mechanical deformation by passage through a microfluidic device, or a colloidal dispersion system. In some embodimentexamples, said recombinant vector, and said DNA transposase or polynucleotide encoding said DNA transposase, are introduced to said population of cells using electro-transfer. In some embodimentexamples, said method is completed in less than two days. In some embodimentexamples, said method is completed in 1-2 days. In some embodimentexamples, said method is completed in more than two days.

[0052] In some embodimentexamples, said population of cells is cryopreserved and thawed before introduction of said recombinant vector and said DNA transposase or polynucleotide encoding said DNA transposase. In some embodimentexample, said population of cells is rested before introduction of said recombinant vector and said DNA transposase or polynucleotide encoding said DNA transposase. In some embodimentexamples, said population of cells comprises human *ex vivo* cells. In some embodimentexamples, said population of cells is not activated *ex vivo*. In some embodimentexamples, said population of cells comprises T cells.

[0052a] In one aspect, the instant disclosure provides use of (a) the population of cells described herein, or (b) the population of engineered cells described herein in the manufacture of a medicament for treating cancer or autoimmune disease or disorder in a subject in need thereof.

[0053] In another aspectexample, the instant disclosure provides a method of treating cancer in a subject in need thereof comprising administering to the subject a therapeutically effective amount of a population of cells described herein, thereby treating the cancer.

[0054] In another aspectexample, the instant disclosure provides a method of treating cancer in a subject in need thereof comprising administering to the subject a therapeutically effective amount of a population of engineered cells produced by a method of producing a population of engineered cells described herein, thereby treating the cancer.

[0055] In another aspectexample, the instant disclosure provides a method of treating an autoimmune disease or disorder in a subject in need thereof comprising administering to the subject a therapeutically effective amount of a population of cells described herein, thereby treating the autoimmune disease or disorder.

[0056] In another aspectexample, the instant disclosure provides a method of treating an autoimmune disease or disorder in a subject in need thereof comprising administering to the subject a therapeutically effective amount of a population of engineered cells produced by a method of producing a population of engineered cells described herein, thereby treating the autoimmune disease or disorder.

[0057] In some embodimentexamples, any of the polynucleotide sequences described herein (e.g., polynucleotide sequences set forth in Tables 1-7, 10, 11, and 13) may be followed by a stop codon (e.g., TAA, TAG, or TGA) at the 3' end, with or without an intervening polynucleotide sequence.

4. BRIEF DESCRIPTION OF THE DRAWINGS

[0058] **FIG. 1A** is a schematic of the CD19-specific CAR CD19CAR, which incorporates, from N terminus to C terminus, an N-terminal signal sequence; anti-human CD19 VL; peptide linker; anti-human CD19 VH; human CD8 α hinge domain; human CD8 α transmembrane (TM) domain; human CD28 cytoplasmic domain; and human CD3 ζ cytoplasmic domain. **FIG. 1B** is a schematic of the membrane-bound IL-15/IL-15R α fusion protein mbIL15, which incorporates, from N terminus to C terminus, an N-terminal signal sequence; human IL-15; linker peptide; and human IL-15R α . **FIG. 1C** is a schematic of the marker protein HER1t, which incorporates, from N terminus to C terminus, an N-terminal signal sequence; domain III of human HER1; truncated domain IV of human HER1; peptide linker; and human CD28 TM domain.

[0059] **FIG. 2** is a schematic diagram depicting double transposition (dTp) and single transposition (sTp) approaches using an SB11 transposon/transposase system to generate CAR-T cells expressing CD19CAR, mbIL15, and HER1t.

[0060] **FIGS. 3A-3E** are graphs showing percent cell viability (**FIG. 3A**), CD3 frequency (**FIG. 3B**), CD19CAR expression (**FIG. 3C**), mbIL15 expression (**FIG. 3D**), and HER1t expression (**FIG. 3E**) on Day 1 post-electroporation of T cell-enriched cryopreserved cell product from three separate donors with no plasmid (Negative Control), 1:1 combination of Plasmids DP1 and DP2 (dTp Control), or Plasmids A-F.

[0061] **FIGS. 4A-4F** are sets of 2-parameter flow plots showing transgene co-expression as assessed on Day 1 and at the end of AaPC stimulation cycles ("Stims") 1, 2, 3, and 4 for dTp Control-modified T cells from Donor A. Percent cells are shown in each quadrant. Specifically, **FIG. 4A** is a set of flow plots showing CD19CAR expression vs. CD3 expression. **FIG. 4B** is a set of flow plots showing HER1t expression vs. CD3 expression. **FIG. 4C** is a set of flow plots