

METHODS OF ENHANCING ENGINEERED IMMUNE CELL CYTOTOXICITY**CROSS REFERENCE TO RELATED APPLICATIONS**

This application claims the benefit of and priority to U.S. Provisional Application No.
5 63/600,011, filed November 16, 2023, which is hereby incorporated by reference in its entirety
for all purposes.

SEQUENCE LISTING

The instant application contains a Sequence Listing which has been submitted
electronically in XML format and is hereby incorporated by reference in its entirety. Said XML
10 copy, created on XXXX, is named STB-067WO and is XXXX bytes in size.

BACKGROUND

Cell-based therapy platforms provide promising avenues for treating a variety of
diseases. One such promising platform is CAR based therapies in the treatment of cancer,
15 including NK cell CAR-based therapies. Given their promise, improvements in cell-based
therapies are needed. An active area of exploration is engineering cell-based therapies to
produce and/or secrete effector molecules such as cytokines, a process referred to as armoring,
that enhance the cell-based therapy. For example, unarmored CAR therapies have poor efficacy
in solid tumors and armoring can impact the entire cancer immunity cycle and boost the activity
20 of CAR. However, uncontrolled or unregulated armoring strategies can have negative impacts
on treatment, such as off-target effects and toxicity in subjects. Thus, additional methods of
controlling and regulating the armoring of cell-based therapies, such as regulating production
and/or secretion of payload effector molecules, as well as optimizing cytokine supplementation
for improving in vivo survival, expansion, and efficacy of cell-based therapies are required.
25

SUMMARY

Provided herein is a method of treating a subject known or suspected of having cancer,
the method comprising: (A) administering a cell therapy to the subject, wherein the cell therapy
comprises an immunoresponsive cell engineered to express a recombinant cytokine, wherein the
30 immunoresponsive cell engineered to express the recombinant cytokine comprises an exogenous
polynucleotide encoding a controlled release cytokine, oriented from N-terminal to C-terminal,
having the formula: **S – C – MT** or **MT – C – S** wherein S comprises a secretable effector
molecule, wherein the secretable effector molecule comprises the recombinant cytokine, C

comprises a protease cleavage site, and MT comprises a cell membrane tethering domain, wherein **S – C – MT** or **MT – C – S** is configured to be expressed as a single polypeptide; and (B) administering a pharmaceutically effective amount of IL-2.

5 In some aspects, the immunoresponsive cell comprises an exogenous polynucleotide encoding an inhibitory chimeric antigen receptor (iCAR) comprising: (i) an antigen-binding domain specific for endomucin (EMCN); (ii) one or more intracellular inhibitory domains that inhibit an immune response; and (iii) one or more polypeptides selected from the group consisting of: a signal peptide, a transmembrane domain, a hinge domain, a spacer region, one or more peptide linkers, and combinations thereof.

10 In some aspects, the immunoresponsive cell comprises an exogenous polynucleotide encoding a bivalent activating chimeric antigen receptor (aCAR) comprising: (i) an antigen-binding domain specific for FLT3; (ii) an antigen-binding domain specific for CD33; (iii) one or more intracellular signaling domains that stimulate an immune response; and (iv) one or more polypeptides selected from the group consisting of: a signal peptide, a transmembrane domain, a hinge domain, a spacer region, one or more peptide linkers, and combinations thereof.

15 Provided herein is method of treating a subject known or suspected of having cancer, the method comprising: (A) administering a cell therapy to the subject, wherein the cell therapy comprises an immunoresponsive cell engineered to express a recombinant cytokine; and (B) administering a pharmaceutically effective amount of IL-2.

20 In some aspects, the immunoresponsive cell is engineered to express the recombinant cytokine is from a multicistronic expression system.

In some aspects, the administration of IL-2 comprises multiple administrations. In some aspects, the multiple administrations of IL-2 is at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, or at least 9 administrations. In some aspects, wherein IL-2 is administered within 4, within 5, within 7, or within 8 hours following the start of the administration of the cell therapy. In some aspects, IL-2 is administered within 4 to 8 hours following the administration of the cell therapy. In some aspects, IL-2 is administered within 6 to 8 hours following the administration of the cell therapy. In some aspects, IL-2 is administered within 4 hours following the administration of the cell therapy. In some aspects, IL-2 is administered within 5 hours following the administration of the cell therapy. In some aspects, IL-2 is administered within 6 hours following the administration of the cell therapy. In some aspects, IL-2 is administered within 7 hours following the administration of the cell therapy. In some aspects, IL-2 is administered within 8 hours following the administration of the cell therapy. In some aspects, IL-2 is administered between 4 to 8 hours following the administration of the cell

therapy. In some aspects, IL-2 is administered between 6 to 8 hours following the administration of the cell therapy. In some aspects, IL-2 is administered within 24 or 48 hours after the administering of the cell therapy.

In some aspects, the administration of the cell therapy comprises multiple
5 administrations. In some aspects, IL-2 is administered at least 1, at least 2, a least 3 times a week following each administration of the cell therapy. In some aspects, IL-2 is administered at least 1 time a week following administration of each dose of a cell therapy. In some aspects, IL-2 is administered at least 2 times a week following administration of each dose of a cell therapy. In some aspects, IL-2 is administered at least 3 times a week following administration of each
10 dose of a cell therapy. In some aspects, wherein the cell therapy is administered again at least on day 7 and/or day 14 following the initial administration of the cell therapy. In some aspects, the cell therapy is administered again at least on day 7 following the initial administration of the cell therapy. In some aspects, the cell therapy is administered again at least on day 7 and day 14 following the initial administration of the cell therapy. In some aspects, the cell therapy and the
15 pharmaceutically effective amount of IL-2 are administered again on at least day 7 and/or day 14 following the initial administration of the cell therapy. In some aspects, the cell therapy and the pharmaceutically effective amount of IL-2 are administered again on at least day 7 following the initial administration of the cell therapy. In some aspects, wherein the cell therapy and the pharmaceutically effective amount of IL-2 are administered again on at least day 7 and day 14
20 following the initial administration of the cell therapy. In some aspects, the pharmaceutically effective amount of of IL-2 is between 1-10 MIU, optionally wherein the pharmaceutically effective amount of a dose of 3 MIU.

In some aspects, the recombinant cytokine comprises IL-15 or a functional fragment thereof. In some aspects, the recombinant cytokine comprises crIL15.

25 In some aspects, the subject has, prior to administration of the cell therapy, been administered one or more lymphodepletion agents. In some aspects, the one or more lymphodepletion agents is administered 5 days prior to administration of the cell therapy. In some aspects, the one or more lymphodepletion agents is administered daily until administration of the cell therapy. In some aspects, the one or more lymphodepletion agents
30 comprises fludarabine, cytarabine (Ara-C), or combinations thereof. In some aspects, the one or more lymphodepletion agents are administered intravenously. In some aspects, fludarabine is administered at a dose of 30 mg/m² and cytarabine (Ara-C) is administered at a dose of 2 g/m².

In some aspects, the method of treatment comprises a reduction in tumor volume in the subject. In some aspects, the method of treatment comprises treatment of a solid tumor in the

subject. In some aspects, the method of treatment comprises inducing an immune response in the subject.

In some aspects, the immunoresponsive cell engineered to express the recombinant cytokine comprises an exogenous polynucleotide encoding a controlled release cytokine, oriented from N-terminal to C-terminal, having the formula: **S – C – MT** or **MT – C – S** wherein S comprises a secretable effector molecule, wherein the secretable effector molecule comprises the recombinant cytokine, C comprises a protease cleavage site, and MT comprises a cell membrane tethering domain, wherein **S – C – MT** or **MT – C – S** is configured to be expressed as a single polypeptide.

In some aspects, the immunoresponsive cell comprises an exogenous polynucleotide encoding an inhibitory chimeric antigen receptor (iCAR) comprising: (i) an antigen-binding domain specific for endomucin (EMCN); (ii) one or more intracellular inhibitory domains that inhibit an immune response; and (iii) one or more polypeptides selected from the group consisting of: a signal peptide, a transmembrane domain, a hinge domain, a spacer region, one or more peptide linkers, and combinations thereof.

In some aspects, the immunoresponsive cell comprises an exogenous polynucleotide encoding a bivalent activating chimeric antigen receptor (aCAR) comprising: (i) an antigen-binding domain specific for FLT3; (ii) an antigen-binding domain specific for CD33; (iii) one or more intracellular signaling domains that stimulate an immune response; and (iv) one or more polypeptides selected from the group consisting of: a signal peptide, a transmembrane domain, a hinge domain, a spacer region, one or more peptide linkers, and combinations thereof.

In some aspects, the immunoresponsive cell comprises an engineered nucleic acid comprising: (A) an exogenous polynucleotide encoding a controlled release cytokine, oriented from N-terminal to C-terminal, having the formula: **S – C – MT** or **MT – C – S** wherein S comprises a secretable effector molecule, wherein the secretable effector molecule comprises the recombinant cytokine, C comprises a protease cleavage site, and MT comprises a cell membrane tethering domain, wherein **S – C – MT** or **MT – C – S** is configured to be expressed as a single polypeptide; (B) an exogenous polynucleotide encoding an inhibitory chimeric antigen receptor (iCAR) comprising: (i) an antigen-binding domain specific for endomucin (EMCN); (ii) one or more intracellular inhibitory domains that inhibit an immune response; and (iii) one or more polypeptides selected from the group consisting of: a signal peptide, a transmembrane domain, a hinge domain, a spacer region, one or more peptide linkers, and combinations thereof; and (C) an exogenous polynucleotide encoding a bivalent activating chimeric antigen receptor (aCAR) comprising: (i) an antigen-binding domain specific for FLT3;

(ii) an antigen-binding domain specific for CD33; (iii) one or more intracellular signaling domains that stimulate an immune response; and (iv) one or more polypeptides selected from the group consisting of: a signal peptide, a transmembrane domain, a hinge domain, a spacer region, one or more peptide linkers, and combinations thereof.

- 5 In some aspects, the exogenous polynucleotides encoding each of the membrane-cleavable chimeric protein, the iCAR, and the bivalent aCAR are linked together by a polynucleotide linker encoding a 2A ribosome skipping element, optionally wherein the 2A ribosome skipping element is selected from the group consisting of: a T2A ribosome skipping element, a E2A ribosome skipping element, a P2A ribosome skipping element, a F2A ribosome skipping
10 element, ribosome skipping element fusions thereof, and combinations thereof, optionally wherein the ribosome skipping element fusion comprises an E2A/T2A ribosome skipping element, optionally wherein the E2A/T2A ribosome skipping element comprises the amino acid sequence QCTNYALLKLAGDVESNPGPGSGEGRSLLTCGDVEENPGP (SEQ ID NO: 221), optionally wherein the E2A/T2A ribosome skipping element is encoded by a
15 polynucleotide sequence comprising the sequence
CAGTGTACCAACTACGCCCTGCTGAAACTGGCCGGCGACGTGGAATCTAATCCTGG
ACCTGGATCTGGCGAGGGACGCGGGAGTCTACTGACGTGTGGAGACGTGGAGGAA
AACCTGGACCT (SEQ ID NO: 222) or the sequence
CAGTGCACAAATTATGCACTGCTGAAGCTCGCCGGGGATGTCGAGAGTAACCCAGG
20 ACCTGGAAGCGGAGAAGGTCGTGGTAGTCTACTAACGTGTGGTGATGTAGAAGAAA
ATCCTGGACCT (SEQ ID NO: 223).

- In some aspects, the membrane-cleavable chimeric protein, the iCAR, and the bivalent aCAR are encoded in order from 5' to 3': (i) membrane-cleavable chimeric protein; (ii) bivalent aCAR; and (iii) iCAR; or from 5' to 3': (i) membrane-cleavable chimeric protein; (ii) iCAR; and
25 (iii) bivalent aCAR.

- In some aspects, the secretable effector molecule comprises a signal peptide or a signal-anchor sequence, optionally wherein the signal peptide comprises a native signal peptide native to the secretable effector molecule, optionally wherein the signal peptide comprises a non-native signal peptide or the signal-anchor sequence comprises a non-native signal-anchor sequence
30 non-native to the secretable effector molecule, optionally wherein the non-native signal peptide or the non-native signal-anchor sequence is selected from the group consisting of: IgE, IL-12, IL-2, optimized IL-2, trypsinogen-2, Gaussia luciferase, CD5, human IgKVII, murine IgKVII, VSV-G, prolactin, serum albumin preprotein, azurocidin preprotein, osteonectin, CD33, IL-6, IL-8, CCL2, TIMP2, VEGFB, osteoprotegerin, serpin E1, GROalpha, CXCL12, IL-21, CD8,

NKG2D, TNFR2, GMCSF, and GM-CSFRa, optionally wherein the non-native signal peptide comprises an IgE signal peptide, optionally wherein the IgE signal peptide comprises the amino acid sequence MDWTWILFLVAAATRVHS (SEQ ID NO: 228), optionally wherein the IgE signal peptide is encoded by a polynucleotide sequence comprising the sequence

5 ATGGACTGGACTTGGATACTCTTTCTGGTCGCTGCCGCCACACGGGTGCACTCT
(SEQ ID NO: 229).

In some aspects, the protease cleavage site further comprises the N-terminal peptide linker, optionally selected from the group consisting of: SGGGSGGGGSG (SEQ ID NO: 230); GGGSGGGGSGGGSLQ (SEQ ID NO: 231); GGS (SEQ ID NO: 232); GSGGGS (SEQ ID NO: 233); GSGGSGGGS (SEQ ID NO: 234); GSGGSGGSGGGS (SEQ ID NO: 235); GSGGSGGSGGSGGGS (SEQ ID NO: 236); GGGGS (SEQ ID NO: 237); GGGSGGGS (SEQ ID NO: 238); GGGSGGGS (SEQ ID NO: 239); GGGSGGSGGGSGGGS (SEQ ID NO: 240); GGGSGGSGGGSGGGSGGGS (SEQ ID NO: 241); GGGGS (SEQ ID NO: 242); GGGGSGGGGS (SEQ ID NO: 243); GGGGSGGGSGGGGS (SEQ ID NO: 244);

15 GGGGSGGGSGGGSGGGGS (SEQ ID NO: 245); GGGGSGGGSGGGSGGGSGGGGS (SEQ ID NO: 246); GSTSGSGKPGSGEGSTKG (SEQ ID NO: 247); and EAAAKEAAAKEAAAKEAAK (SEQ ID NO: 248), optionally wherein the protease cleavage site further comprises the N-terminal peptide linker SGGGSGGGGSG (SEQ ID NO: 230), optionally wherein the protease cleavage

20 site further comprises a C-terminal peptide linker, optionally selected from the group consisting of: SGGGSGGGGSG (SEQ ID NO: 230); GGGSGGGGSGGGSLQ (SEQ ID NO: 231); GGS (SEQ ID NO: 232); GSGGGS (SEQ ID NO: 233); GSGGSGGGS (SEQ ID NO: 234); GSGGSGGSGGGS (SEQ ID NO: 235); GSGGSGGSGGSGGGS (SEQ ID NO: 236); GGGGS (SEQ ID NO: 237); GGGSGGGS (SEQ ID NO: 238); GGGSGGSGGGS (SEQ ID NO: 239);

25 GGGSGGGSGGGSGGGS (SEQ ID NO: 240); GGGSGGGSGGGSGGGSGGGS (SEQ ID NO: 241); GGGGS (SEQ ID NO: 242); GGGGSGGGGS (SEQ ID NO: 243); GGGGSGGGSGGGGS (SEQ ID NO: 244); GGGGSGGGSGGGSGGGGS (SEQ ID NO: 245); GGGGSGGGSGGGSGGGSGGGGS (SEQ ID NO: 246); GSTSGSGKPGSGEGSTKG (SEQ ID NO: 247); and EAAAKEAAAKEAAAKEAAK (SEQ

30 ID NO: 248), optionally wherein the protease cleavage site further comprises the C-terminal linker GGGSGGGGSGGGSLQ (SEQ ID NO: 231), optionally wherein the protease cleavage site comprises a Tumor Necrosis Factor- α Converting Enzyme (TACE)-specific cleavage site, optionally wherein the TACE-specific cleavage site comprises the amino acid sequence VTPEPIFSLI (SEQ ID NO: 191), optionally, wherein the TACE-specific cleavage site

comprises the amino acid sequence SGGGSGGGSGVTPEPIFSLIGGSGGGSGGGSLQ (SEQ ID NO: 250), optionally wherein the TACE-specific cleavage site is encoded by a polynucleotide sequence comprising the sequence

TCAGGCGGCGGTGGTAGTGGAGGCGGAGGCTCAGGCGTGACCCCTGAGCCTATCTT
 5 CAGCCTGATCGGCGGAGGTTCCGGAGGTGGCGGTTCCGGCGGAGGATCTCTTCAA
 (SEQ ID NO: 251).

In some aspects, the cell membrane tethering domain comprises a transmembrane domain selected from the group consisting of: PDGFR-beta, CD8, CD28, CD3zeta-chain, CD4, 4-1BB, OX40, ICOS, CTLA-4, PD-1, LAG-3, 2B4, LNGFR, NKG2D, EpoR, TNFR2, LIR1,
 10 B7-1, and BTLA, optionally wherein the cell membrane tethering domain comprises a B7-1 transmembrane domain, optionally wherein the B7-1 transmembrane domain comprises the amino acid sequence LLPSWAITLISVNGIFVICCLTYCFAPRCRERRRNERLRRESVRPV (SEQ ID NO: 204), optionally wherein the B7-1 transmembrane domain is encoded by a polynucleotide sequence comprising the sequence

15 TTGCTGCCTAGCTGGGCCATCACACTGATCTCCGTGAACGGCATCTTCGTGATCTGC
 TGCCTGACCTACTGCTTCGCCCCTAGATGCAGAGAGCGGAGAAGAAACGAGCGGCT
 GAGAAGAGAAAGCGTGCGGCCTGTG (SEQ ID NO: 252), optionally wherein the membrane-cleavable chimeric protein, oriented from N-terminal to C-terminal, has the formula **S – C – MT**, optionally wherein the membrane-cleavable chimeric protein comprises the amino
 20 acid sequence

MDWTWILFLVAAATRVHSNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAM
 KCFLLELQVISLESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEEELEEKNIKEFLQS
 FVHIVQMFINTSSGGGSGGGSGVTPEPIFSLIGGSGGGSGGGSLQLLPSWAITLISV
 NGIFVICCLTYCFAPRCRERRRNERLRRESVRPV (SEQ ID NO: 226), optionally wherein
 25 the membrane-cleavable chimeric protein is encoded by a polynucleotide sequence comprising the sequence

ATGGACTGGACTTGGATACTCTTTCTGGTCGCTGCCGCCACACGGGTGCACTCTAAT
 TGGGTCAACGTGATCAGCGACCTGAAGAAGATCGAGGACCTGATCCAGAGCATGCA
 CATCGACGCCACACTGTACACCGAGTCCGATGTGCACCCTAGCTGCAAAGTGACCG
 30 CCATGAAGTGCTTTCTGCTGGAAGTCAAGTGATCAGCCTGGAAAGCGGCGACGCC
 AGCATCCACGATACCGTGGAATCTGATCATCCTGGCCAACAACAGCCTGTCCAG
 CAACGGCAATGTGACCGAGAGCGGCTGCAAAGAGTGCGAGGAACTGGAAGAGAAG
 AACATCAAAGAGTTTCTGCAGAGCTTCGTCCACATCGTGCAGATGTTTCATCAACACC
 TCATCAGGCGGCGGTGGTAGTGGAGGCGGAGGCTCAGGCGTGACCCCTGAGCCTAT

CTTCAGCCTGATCGGCGGAGGTTCCGGAGGTGGCGGTTCCGGCGGAGGATCTCTTC
 AATTGCTGCCTAGCTGGGCCATCACACTGATCTCCGTGAACGGCATCTTCGTGATCT
 GCTGCCTGACCTACTGCTTCGCCCCTAGATGCAGAGAGCGGAGAAGAAACGAGCGG
 CTGAGAAGAGAAAGCGTGCGGCCTGTG (SEQ ID NO: 227).

- 5 In some aspects, the antigen-binding domain specific for EMCN comprises a heavy chain variable (VH) region and a light chain variable (VL) region, wherein: the EMCN-VH comprises: a heavy chain complementarity determining region 1 (CDR-H1) having the amino acid sequence of RYDMH (SEQ ID NO: 291), a heavy chain complementarity determining region 2 (CDR-H2) having the amino acid sequence of VIWGNGNTHYHSALKS (SEQ ID NO: 296), and a heavy chain complementarity determining region 3 (CDR-H3) having the amino acid sequence of RIKD (SEQ ID NO: 298), and the EMCN-VL comprises: a light chain complementarity determining region 1 (CDR-L1) having the amino acid sequence of KSSQSLVASDENTYLN (SEQ ID NO: 299), a light chain complementarity determining region 2 (CDR-L2) having the amino acid sequence of QVSKLDS (SEQ ID NO: 300), and a light chain complementarity determining region 3 (CDR-L3) having the amino acid sequence of LQGIHLPWT (SEQ ID NO: 301), and wherein the amino acid sequences of the CDR-H1, the CDR-H2, the CDR-H3, the CDR-L1, the CDR-L2, and the CDR-L3 of the reference antibody are defined based on the Kabat numbering scheme; optionally wherein the EMCN-VH and the EMCN-VL are separated by a peptide linker, optionally wherein the antigen-binding domain specific for EMCN comprises the structure VH-L-VL or VL-L-VH, wherein L is the peptide linker, optionally wherein the peptide linker comprises the amino acid sequence SGGGSGGGSGG(SEQ ID NO: 230); GGGSGGGSGGGSLQ(SEQ ID NO: 231); GGS(SEQ ID NO: 232); GGS(GSGG(SEQ ID NO: 233); GGS(GSGG(SEQ ID NO: 234); GGS(GSGGSGG(SEQ ID NO: 235); GGS(GSGGSGGSGG(SEQ ID NO: 236); GGS(GSGGSGGSGG(SEQ ID NO: 237); GGS(GSGGSGGSGG(SEQ ID NO: 238); GGS(GSGGSGGSGGSGG(SEQ ID NO: 239); GGS(GSGGSGGSGGSGGSGG(SEQ ID NO: 240); GGS(GSGGSGGSGGSGGSGGSGG(SEQ ID NO: 241); GGS(GSGGSGGSGGSGGSGGSGGSGG(SEQ ID NO: 242); GGS(GSGGSGGSGGSGGSGGSGGSGGSGG(SEQ ID NO: 243); GGS(GSGGSGGSGGSGGSGGSGGSGGSGGSGG(SEQ ID NO: 244); GGS(GSGGSGGSGGSGGSGGSGGSGGSGGSGGSGG(SEQ ID NO: 245); GGS(GSGGSGGSGGSGGSGGSGGSGGSGGSGGSGGSGG(SEQ ID NO: 246); GSTSGSGKPGSGEGSTKG(SEQ ID NO: 247); or EAAAKEAAAKEAAAKEAAK(SEQ ID NO: 248), optionally wherein the peptide linker comprises the amino acid sequence GGGGSGGGSGGGSGG (SEQ ID NO: 244), optionally wherein the peptide linker is encoded by a polynucleotide sequence comprising the sequence

GGAGGCGGAGGATCTGGTGGCGGAGGAAGTGGCGGAGGCGGTTCT (SEQ ID NO: 253).

In some aspects, the intracellular inhibitory domain comprises a LIR1 intracellular inhibitory domain, optionally wherein the LIR1 intracellular inhibitory domain comprises the amino acid sequence

LRHRRQGKHWSTSTQRKADFQHPAGAVGPEPTDRGLQWRSSPAADAQEENLYAAVKH
TQPEDGVEMDTRSPHDEDPQAVTYAEVKHSRPRREMASPPSPLSGEFLDTKDRQAEED
RQMDTEAAASEAPQDVITYAQLHSLTLRREATEPPPSQEGPSPAVPSIYATLAIH (SEQ ID NO: 285), optionally wherein the LIR1 intracellular inhibitory domain is encoded by a

polynucleotide sequence comprising the sequence

CTGCGGCACAGAAGGCAGGGCAAGCACTGGACAAGCACCCAGAGAAAGGCCGACT
TTCAGCATCCTGCTGGCGCCGTTGGACCTGAGCCTACAGATAGAGGACTGCAGTGG
CGGTCTAGCCCTGCCGCTGATGCCCAAGAGGAAAATCTTTACGCCGCCGTGAAGCA
CACCCAGCCTGAGGATGGCGTGGAAATGGACACCAGATCTCCCCACGATGAGGACC
CTCAGGCCGTGACATACGCAGAAGTGAAGCACTCCAGACCTCGGAGAGAGATGGC
AAGCCCTCCATCTCCTCTGAGCGGCGAGTTCCTGGACACCAAAGACAGACAGGCCG
AAGAGGACAGACAGATGGATACCGAAGCCGCCGTTCTGAAGCCCCACAGGATGT
GACATATGCCCAGCTGCATAGCCTGACACTGCGGAGAGAAGCCACAGAGCCTCCAC
CTTCTCAAGAAGGCCCATCTCCTGCCGTGCCTTCCATCTATGCCACTCTGGCCATTC
AC (SEQ ID NO: 286).

In some aspects, (A) the signal peptide is present in the iCAR, optionally wherein the signal peptide of the iCAR comprises a native signal peptide native or a non-native signal peptide, optionally wherein the non-native signal peptide or the non-native signal-anchor sequence is selected from the group consisting of: IgE, IL-12, IL-2, optimized IL-2, trypsinogen-2, Gaussia luciferase, CD5, human IgKVII, murine IgKVII, VSV-G, prolactin, serum albumin preprotein, azurocidin preprotein, osteonectin, CD33, IL-6, IL-8, CCL2, TIMP2, VEGFB, osteoprotegerin, serpin E1, GROalpha, CXCL12, IL-21, CD8, NKG2D, TNFR2, and GMCSF, optionally wherein the signal peptide of the iCAR comprises a CD8 signal peptide, optionally wherein the CD8 signal peptide comprises the amino acid sequence

MALPVTALLLPLALLHAARP (SEQ ID NO: 137), optionally wherein the CD8 signal peptide is encoded by a polynucleotide sequence comprising the sequence

ATGGCTCTGCCCCGTGACAGCTTTGCTGCTGCCTTTGGCACTGCTGCTGCATGCTGCT
AGACCA (SEQ ID NO: 416); and/or wherein (B) the hinge domain is present in the iCAR

comprises, optionally wherein the hinge domain of the iCAR is selected from the group consisting of a human Ig (immunoglobulin) hinge, an IgG4 hinge, an IgG2 hinge, a CD8a hinge, or an IgD hinge, a KIR2DS2 hinge, an LNGFR hinge, a LIR1 hinge, a PDGFR-beta

extracellular linker, and combinations thereof, optionally wherein the hinge domain of the iCAR comprises a LIR1 hinge, optionally wherein the LIR1 hinge comprises the amino acid sequence HPSPLELVVSGPSGGPSSPTTGPTSTSGPEDQPLTPTGSDPQSGLGRHLGV (SEQ ID NO: 417), optionally wherein the LIR1 hinge comprises is encoded by a polynucleotide sequence comprising the sequence

CACCCATCCGATCCTCTCGAGCTGGTGGTTTCTGGACCTTCTGGCGGCCCTAGCAGC
CCTACAACAGGACCTACAAGCACAAGCGGCCCTGAGGACCAACCTCTGACACCAAC
AGGCAGCGATCCTCAGTCTGGACTGGGGAGACATCTGGGCGTT (SEQ ID NO: 418);

optionally wherein the hinge domain of the iCAR comprises a CD8 hinge, optionally wherein the CD8 hinge comprises the amino acid sequence

TTTPAPRPPTPAPTIALQPLSLRPEACRPAAGGAVHTRGLDFACD (SEQ ID NO: 271),

optionally wherein the CD8 hinge comprises is encoded by a polynucleotide sequence comprising the sequence

ACAACAACACCCGCACCTCGGCCTCCAACTCCAGCTCCAACAATTGCACTGCAACC
CCTGAGTCTGAGGCCCGAGGCCTGTAGGCCAGCAGCTGGCGGAGCTGTTCACTA
GAGGCCTGGACTTTGCCTGTGAC (SEQ ID NO: 283); and/or wherein (C) the

transmembrane domain of the iCAR is present, optionally wherein the transmembrane domain of the iCAR comprises a transmembrane domain selected from the group consisting of: PDGFR-beta, CD8, CD28, CD3zeta-chain, CD4, 4-1BB, OX40, ICOS, CTLA-4, PD-1, LAG-3, 2B4, LNGFR, NKG2D, EpoR, TNFR2, B7-1, LIR1, and BTLA, optionally wherein the transmembrane domain of the iCAR comprises a LIR1 transmembrane domain, optionally

wherein the LIR1 transmembrane domain comprises the amino acid sequence

VIGILVAVILLLLLLLLLLFLI (SEQ ID NO: 259), optionally wherein the LIR1 transmembrane domain is encoded by a polynucleotide sequence comprising the sequence
GTGATCGGCATTCTGGTCGCCGTGATCCTGCTCCTGTTGCTCCTGCTGCTTCTGTTCC
TGATC (SEQ ID NO: 260).

In some aspects, the iCAR comprises the amino acid sequence:

MALPVTALLLPLALLHAARPEVQLVESGGGLVQPGGSLRLSCAASGFTFSRYDMHWV
RQAPGKGLEWVSVIWGNNGNTHYHSALKSRFTISRDNKNTLYLQMNSLRAEDTAVYY
CTLRIKDWGQGTMVTVSSGGGGSGGGSGGGSDVVMVTSPLSLPVTLGQPASISCKS
SQSLVASDENTYLNWFQQRPGQSPRRLIYQVSKLDSGVPDRFSGSGSGTDFTLKISRVEA

EDVGVYYCLQGIHLPWTFGQGTKLEIK_{Ngaa}HPSDPLELVVSGPSGGPSSPTTGPTSTSGP
EDQPLTPTGSDPQSGLGRLGVVIGILVAVILLLLLLLLLLLFLILRHRRQGKHWSTQORKA
DFQHPAGAVGPEPTDRGLQWRSSPAADAQEENLYAAVKHTQPEDGVEMDTRSPHDED
PQAVTYAEVKHSRPRREMASPPSPLSGEFLDTKDRQAEEDRQMDTEAAASEAPQDVITY
5 AQLHSLTLRREATEPPPSQEGPSPAVPSIYATLAIH (SEQ ID NO: 419), optionally wherein
the iCAR is encoded by a polynucleotide sequence comprising the sequence
ATGGCTCTGCCCCTGACAGCTTTGCTGCTGCCTTTGGCACTGCTGCTGCATGCTGCT
AGACCAGAGGTGCAGCTGGTTGAATCTGGCGGAGGACTGGTTCAGCCTGGCGGATC
TCTGAGACTGTCTTGTGCCGCCAGCGGCTTCACCTTCAGCAGATACGATATGCACTG
10 GGTCCGACAGGCCCCTGGCAAAGGACTTGAATGGGTGTCCGTGATCTGGGGCAACG
GCAACACACACTACCACAGCGCCCTGAAGTCCCGGTTCAACATCTCCAGAGACAAC
AGCAAGAACACCCTGTACCTGCAGATGAACAGCCTGAGAGCCGAGGACACCGCCGT
GTACTACTGCACCCTGAGAATCAAGGATTGGGGCCAGGGCACCATGGTCACCGTTT
CTTCTGGAGGCGGAGGATCTGGTGGCGGAGGAAGTGGCGGAGGCGGTTCTGACGTG
15 GTCATGACACAGAGCCCTCTGAGCCTGCCTGTGACACTGGGACAGCCTGCCAGCAT
CAGCTGCAAGTCTAGCCAGTCTCTGGTGGCCAGCGACGAGAACACCTACCTGAACT
GGTTCCAGCAGAGGCCCGGACAGTCTCCTAGACGGCTGATCTACCAGGTGTCCAAG
CTGGATAGCGGCGTGCCCGATAGATTTTCTGGCAGCGGCTCTGGCACCGACTTCACC
CTGAAGATCAGCAGAGTGGAAGCCGAGGACGTGGGCGTGTACTACTGTCTGCAAGG
20 CATCCATCTGCCTTGGACCTTTGGCCAGGGCACAAAGCTGGAAATCAAGAATGGCG
CTGCACACCCATCCGATCCTCTCGAGCTGGTGGTTTCTGGACCTTCTGGCGGCCCTA
GCAGCCCTACAACAGGACCTACAAGCACAAAGCGGCCCTGAGGACCAACCTCTGACA
CCAACAGGCAGCGATCCTCAGTCTGGACTGGGGAGACATCTGGGCGTTGTGATCGG
CATTCTGGTGCCTGTATCCTGCTCCTGTTGCTCCTGCTGCTTCTGTTTCTGATCCTG
25 CGGCACAGAAGGCAGGGCAAGCACTGGACAAGCACCCAGAGAAAGGCCGACTTTC
AGCATCCTGCTGGCGCCGTTGGACCTGAGCCTACAGATAGAGGACTGCAGTGGCGG
TCTAGCCCTGCCGCTGATGCCCAAGAGGAAAATCTTTACGCCGCCGTGAAGCACAC
CCAGCCTGAGGATGGCGTGGAAATGGACACCAGATCTCCCCACGATGAGGACCTC
AGGCCGTGACATACGCAGAAGTGAAGCACTCCAGACCTCGGAGAGAGATGGCAAG
30 CCCTCCATCTCCTCTGAGCGGCGAGTTCCTGGACACCAAAGACAGACAGGCCGAAG
AGGACAGACAGATGGATACCGAAGCCGCGCTTCTGAAGCCCCACAGGATGTGACA
TATGCCCAGCTGCATAGCCTGACACTGCGGAGAGAAGCCACAGAGCCTCCACCTTC
TCAAGAAGGCCCATCTCCTGCCGTGCCTTCCATCTATGCCACTCTGGCCATTAC
(SEQ ID NO: 420).

In some aspects, the antigen-binding domain specific for FLT3 comprises a heavy chain variable (VH) region and a light chain variable (VL) region, wherein: the FLT3-VH comprises: a heavy chain complementarity determining region 1 (CDR-H1) having the amino acid sequence of GGTFSSY AIS (SEQ ID NO: 360), a heavy chain complementarity determining region 2 (CDR-H2) having the amino acid sequence of GIPIFGTANYAQKFQG (SEQ ID NO: 361), and a heavy chain complementarity determining region 3 (CDR-H3) having the amino acid sequence of FALFGFREQA FDI (SEQ ID NO: 362), and the FLT3-VL comprises: a light chain complementarity determining region 1 (CDR-L1) having the amino acid sequence of RASQSISSY LN (SEQ ID NO: 363), a light chain complementarity determining region 2 (CDR-L2) having the amino acid sequence of AASSLQS (SEQ ID NO: 364), and a light chain complementarity determining region 3 (CDR-L3) having the amino acid sequence of QQSYSTPFT (SEQ ID NO: 365), and wherein the amino acid sequences of the CDR-H1, the CDR-H2, the CDR-H3, the CDR-L1, the CDR-L2, and the CDR-L3 of the reference antibody are defined based on the Kabat numbering scheme; optionally wherein: the FLT3-VH comprises the amino acid sequence

EVQLVQSGAEVKKPGSSVKV SCKASGGTFSSY AISWVRQAPGQGLEWMGGIPIFGTAN
Y AQKFQGRVTITADKSTSTAYMELSSLRSEDTAVYYCATFALFGFREQA FDIWGQGTTV
TVSS (SEQ ID NO: 315); and the FLT3-VL comprises the amino acid sequence

DIQMTQSPSSLSASVGDRVTITCRASQSISSY LN WYQQKPGKAPKLLIYAASSLQSGVPS
RFSGSGSGTDFTLTISSLQPEDLATYYCQQSYSTPFTFGPGTKVDIK (SEQ ID NO: 316),
optionally wherein the FLT3-VH, and the FLT3-VL are separated by peptide linkers.

In some aspects, the antigen-binding domain specific for CD33 comprises a heavy chain variable (VH) region and a light chain variable (VL) region, wherein: the CD33-VH comprises: a heavy chain complementarity determining region 1 (CDR-H1) having the amino acid sequence of DYNMH (SEQ ID NO: 402), a heavy chain complementarity determining region 2 (CDR-H2) having the amino acid sequence of YIYPYNGGTGYNQKFKSKA (SEQ ID NO: 403), and a heavy chain complementarity determining region 3 (CDR-H3) having the amino acid sequence of GRPAMDYWGQ (SEQ ID NO: 404), and the CD33-VL comprises: a light chain complementarity determining region 1 (CDR-L1) having the amino acid sequence of RASESVDNYGISFMN (SEQ ID NO: 405), a light chain complementarity determining region 2 (CDR-L2) having the amino acid sequence of AASNQGS (SEQ ID NO: 406), and a light chain complementarity determining region 3 (CDR-L3) having the amino acid sequence of QQSKEVPWT (SEQ ID NO: 407), and wherein the amino acid sequences of the CDR-H1, the CDR-H2, the CDR-H3, the CDR-L1, the CDR-L2, and the CDR-L3 of the reference antibody

are defined based on the Kabat numbering scheme; optionally wherein: the CD33-VH comprises the amino acid sequence

QVQLVQSGAEVKKPGSSVKVSCKASGYTFTDYNMHWVRQAPGQGLEWIGYIYPYNGG
TGYNQKFKSKATITADESTNTAYMELSSLRSEDVAVYYCARGRPAMDYWGQGTLLVTV

5 SS (SEQ ID NO: 329); and the CD33-VL comprises the amino acid sequence

DIQMTQSPSSLSASVGDRVTITCRASESDNYGISFMNWFQQKPGKAPKLLIYAASNQG
SGVPSRFSGSGSGTDFTLTISSLQPDDFATYYCQQSKEVPWTFGQGTKVEIK (SEQ ID

NO: 330), optionally wherein the CD33-VH, and the CD33-VL are separated by peptide linkers.

In some aspects, the aCAR antigen binding domains comprises the structure (FLT3-VH)
10 – L1 – (CD33-VH) – L2 – (CD33-VL) – L3 – (FLT3-VL), wherein L1, L2, and L3 are a first, a second, and a third peptide linker, respectively; optionally wherein the L1, L2, and/or L3 are each independently selected from the group consisting of: SEQ ID Nos 230 – 248, optionally wherein the L1 peptide linker is the amino acid sequence GGGGS (SEQ ID NO: 242) or GGGGSGGGGS (SEQ ID NO: 243), optionally wherein the L1 peptide linker GGGGS (SEQ
15 ID NO: 242) is encoded by a polynucleotide sequence comprising the sequence GGCGGCGGTGGCTCT (SEQ ID NO: 254) or the L1 peptide linker GGGGSGGGGS (SEQ ID NO: 243) is encoded by a polynucleotide sequence comprising the sequence GGAGGCGGAGGATCTGGTGGTGGTGGATCT (SEQ ID NO: 256), optionally wherein the L2 peptide linker is the amino acid sequence GSTSGSGKPGSGEGSTKG (SEQ ID NO: 247),
20 optionally wherein the L2 peptide linker is encoded by a polynucleotide sequence comprising the sequence GGCTCTACATCTGGCTCTGGCAAACCTGGAAGCGGCGAGGGATCTACCAAGGGC (SEQ ID NO: 249), optionally wherein the L2 peptide linker is the amino acid sequence GGGGSGGGGS (SEQ ID NO: 243), optionally wherein the L2 peptide linker is encoded by a
25 polynucleotide sequence comprising the sequence GGTGGCGGAGGAAGTGGCGGCGGAGGCTCT (SEQ ID NO: 257), optionally wherein the L3 peptide linker is the amino acid sequence GGGGS (SEQ ID NO: 242) or GGGGSGGGGS (SEQ ID NO: 243), optionally wherein the L3 peptide linker GGGGS (SEQ ID NO: 242) is encoded by a polynucleotide sequence comprising the sequence GGTGGCGGCGGATCC (SEQ
30 ID NO: 255) or the L3 peptide linker GGGGSGGGGS (SEQ ID NO: 243) is encoded by a polynucleotide sequence comprising the sequence GGCGGTGGCGGATCTGGCGGAGGTGGCAGT (SEQ ID NO: 258).

In some aspects, the aCAR intracellular signaling domains that stimulate an immune response is selected from the group consisting of: CD3-zeta, FcR gamma, FcR beta, CD3 gamma, CD3 delta, CD3 epsilon, CD5, CD22, CD79a, CD79b, CD278, FcεRI, DAP10, DAP12, CD66d, CD97, CD2, ICOS, CD27, CD154, CD8, OX40, 4-1BB, CD28, ZAP40, CD30, GITR, HVEM, DAP10, DAP12, MyD88, 2B4, CD40, PD-1, LFA-1, CD7, LIGHT, NKG2C, B7-H3, an MHC class I molecule, a TNF receptor protein, an Immunoglobulin-like protein, a cytokine receptor, an integrin, a SLAM protein, an activating NK cell receptor, BTLA, a Toll ligand receptor, CDS, ICAM-1, (CD11a/CD18), BAFFR, KIRDS2, SLAMF7, NKp80 (KLRF1), NKp44, NKp30, NKp46, CD19, CD4, IL2R beta, IL2R gamma, IL7R alpha, ITGA4, VLA1, CD49a, ITGA4, IA4, CD49D, ITGA6, VLA-6, CD49f, ITGAD, CD11d, ITGAE, CD103, ITGAL, CD11a, ITGAM, CD11b, ITGAX, CD11c, ITGB1, CD29, ITGB2, CD18, ITGB7, NKG2D, TNFR2, TRANCE/RANKL, DNAM1 (CD226), SLAMF4 (CD244, 2B4), CD84, CD96 (Tactile), CEACAM1, CRTAM, Ly9 (CD229), CD160 (BY55), PSGL1, CD100 (SEMA4D), CD69, SLAMF6 (NTB-A, Ly108), SLAM (SLAMF1, CD150, IPO-3), BLAME (SLAMF8), SELPLG (CD162), LTBR, LAT, GADS, SLP-76, PAG/Cbp, CD19a, and combinations thereof, optionally wherein the aCAR intracellular signaling domains that stimulate an immune response comprise a CD28 co-stimulatory domain and a CD3ζ signaling domain, optionally wherein the CD28 co-stimulatory domain comprises the amino acid sequence RSKRSRLHSDYMNMTPRRPGPTRKHYPYAPPRDFAAYRS (SEQ ID NO: 287), optionally wherein the CD28 co-stimulatory domain is encoded by a polynucleotide sequence comprising the sequence

AGAAGCAAGCGGAGCAGACTGCTGCACAGCGACTACATGAACATGACCCCTAGAC
GGCCCGGACCTACCAGAAAGCACTACCAGCCTTACGCTCCTCCTAGAGATTTCGCC
GCCTACCGGTCC (SEQ ID NO: 288), optionally wherein the CD3ζ signaling domain comprises the amino acid sequence

RVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDRRRGRDPEMGGKPRRKNPQEG
LYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR
(SEQ ID NO: 289), optionally wherein the CD3ζ signaling domain is encoded by a polynucleotide sequence comprising the sequence

AGAGTGAAGTTCAGCAGGAGCGCAGACGCCCCGCGTACAAGCAGGGCCAGAACC
AGCTCTATAACGAGCTCAATCTAGGACGAAGAGAGGAGTACGATGTTTTGGACAAG
AGACGTGGCCGGGACCCTGAGATGGGGGGAAAGCCGAGAAGGAAGAACCCTCAGG
AAGGCCTGTACAATGAACTGCAGAAAGATAAGATGGCGGAGGCCTACAGTGAGAT
TGGGATGAAAGGCGAGCGCCGGAGGGGCAAGGGGCACGATGGCCTTTACCAGGGT

CTCAGTACAGCCACCAAGGACACCTACGACGCCCTTCACATGCAGGCCCTGCCCCC
TCGC (SEQ ID NO: 290), optionally wherein

(A) the hinge domain of the aCAR is present, optionally wherein the hinge domain of the aCAR is selected from the group consisting of a human Ig (immunoglobulin) hinge, an IgG4 hinge, an
5 IgG2 hinge, a CD8a hinge, or an IgD hinge, a KIR2DS2 hinge, an LNGFR hinge, a LIR1 hinge, a PDGFR-beta extracellular linker, and combinations thereof, optionally wherein the hinge domain of the aCAR comprises a CD8 hinge, optionally wherein the CD8 hinge comprises the amino acid sequence

ALSNSIMYFSHFVPVFLPAKPTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGL

10 DFACD (SEQ ID NO: 272), optionally wherein the CD8 hinge comprises is encoded by a polynucleotide sequence comprising the sequence

GCCCTGAGCAACAGCATCATGTACTTCAGCCACTTCGTGCCCCGTGTTTCTGCCCCGCC
AAGCCTACAACAACCCCTGCTCCTAGACCACCTACACCAGCTCCTACAATCGCCAG
CCAGCCTCTGTCTCTGAGGCCCGAAGCTTG TAGACCAGCTGCTGGCGGAGCCGTGC

15 ATACAAGAGGACTGGATTTTGCCTGCGAC (SEQ ID NO: 284), and/or wherein (B) the transmembrane domain of the aCAR is present, optionally wherein the transmembrane domain of the aCAR is selected from the group consisting of a human Ig (immunoglobulin) hinge, an IgG4 hinge, an IgG2 hinge, a CD8a hinge, or an IgD hinge, a KIR2DS2 hinge, an LNGFR hinge, a LIR1 hinge, a PDGFR-beta extracellular linker, and combinations thereof, optionally
20 wherein the transmembrane domain of the aCAR comprises a CD8 hinge, optionally wherein the CD8 transmembrane comprises the amino acid sequence

IYIWAPLAGTCGVLLLSLVITLYCNHR (SEQ ID NO: 206), optionally wherein the CD8 transmembrane comprises is encoded by a polynucleotide sequence comprising the sequence
ATCTATATCTGGGCCCCCTCTGGCTGGCACATGCGGAGTTCTGCTGCTCAGCCTGGTC

25 ATCACCCTGTACTGCAACCACAGA (SEQ ID NO: 261); and/or wherein (C) the aCAR signal peptide of the aCAR is present, optionally wherein the signal peptide of the aCAR is selected from the group consisting of: IgE, IL-12, IL-2, optimized IL-2, trypsinogen-2, Gaussia luciferase, CD5, human IgKVII, murine IgKVII, VSV-G, prolactin, serum albumin preprotein, azurocidin preprotein, osteonectin, CD33, IL-6, IL-8, CCL2, TIMP2, VEGFB, osteoprotegerin,
30 serpin E1, GROalpha, CXCL12, IL-21, CD8, NKG2D, TNFR2, GMCSF, and GM-CSFRa, optionally wherein the signal peptide of the aCAR comprises a GM-CSFRa signal peptide, optionally wherein the GM-CSFRa signal peptide comprises the amino acid sequence

MLLLVTSLLLCELPHPAFLIP (SEQ ID NO: 423), optionally wherein the GM-CSFRa signal peptide is encoded by a polynucleotide sequence comprising the sequence

35 ATGCTGCTGCTGGTTACATCTCTGCTGCTGTGCGAGCTGCCCCATCCTGCCTTTCTGC

TTATTCCT (SEQ ID NO: 424), optionally wherein the aCAR comprises the amino acid sequence

MLLLVTSLLLCELPHPAFLLIPEVQLVQSGAEVKKPGSSVKVSCASGGTFSSYAISWVR
 QAPGQGLEWMGGIIPFGTANYAQKFQGRVTITADKSTSTAYMELSSLRSEDNAVYYCA
 5 TFALFGFREQAFDIWGGTTVTVSSGGGGSQVQLVQSGAEVKKPGSSVKVSCASGYT
 FTDYNMHWVRQAPGQGLEWIGYIYPYNGGTGYNQKFKSKATITADESTNTAYMELSSL
 RSEDNAVYYCARGRPAMDYWGQGTLLTVSSGSTSGSGKPGSGEGSTKGDQMTQSPSS
 LSASVGDRVTITCRASESDNYGISFMNWFQQKPGKAPKLLIYAASNQSGVPSRFSGS
 GSGTDFTLTISSLQPDFFATYYCQQSKEVPWTFGQGTKVEIKGGGGSDIQMTQSPSSLSA
 10 SVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGS
 TISSLPEDLATYYCQQSYSTPFTFGPGTKVDIKALSNSIMYFSHFVPVFLPAKPTTTPAP
 RPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYWAPLAGTCGVLLLSLVITL
 YCNHRRSKRSRLHSDYMNMTPRRPGPTRKHYPYAPPRDFAAYRSRVKFSRSADAPA
 YKQGQNQLYNELNLGRREEYDVLDRGRDPEMGGKPRRKNPQEGLYNELQKDKMA
 15 EAYSEIGMKGERRRGKGHDLGLYQGLSTATKDTYDALHMQALPPR (SEQ ID NO: 414),
 optionally wherein the aCAR comprises the amino acid sequence
 MLLLVTSLLLCELPHPAFLLIPEVQLVQSGAEVKKPGSSVKVSCASGGTFSSYAISWVR
 QAPGQGLEWMGGIIPFGTANYAQKFQGRVTITADKSTSTAYMELSSLRSEDNAVYYCA
 TFALFGFREQAFDIWGGTTVTVSSGGGGSGGGGSQVQLVQSGAEVKKPGSSVKVSCA
 20 ASGYTFTDYNMHWVRQAPGQGLEWIGYIYPYNGGTGYNQKFKSKATITADESTNTAY
 MELSSLRSEDNAVYYCARGRPAMDYWGQGTLLTVSSGGGGSGGGGSDIQMTQSPSSLS
 ASVGDRVTITCRASESDNYGISFMNWFQQKPGKAPKLLIYAASNQSGVPSRFSGS
 GTDFTLTISLQPDFFATYYCQQSKEVPWTFGQGTKVEIKGGGGSGGGGSDIQMTQSPS
 SLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGS
 25 DFTLTISLQPEDLATYYCQQSYSTPFTFGPGTKVDIKALSNSIMYFSHFVPVFLPAKPTT
 TPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYWAPLAGTCGVLLLS
 LVITLYCNHRRSKRSRLHSDYMNMTPRRPGPTRKHYPYAPPRDFAAYRSRVKFSRSA
 DAPAYKQGQNQLYNELNLGRREEYDVLDRGRDPEMGGKPRRKNPQEGLYNELQK
 DKMAEAYSEIGMKGERRRGKGHDLGLYQGLSTATKDTYDALHMQALPPR (SEQ ID NO:
 30 415).

In some aspects, the cell is selected from the group consisting of a T cell, a Natural Killer (NK) cell, a cytotoxic T lymphocyte (CTL), a regulatory T cell, a Natural Killer T (NKT) cell, a myeloid cell, a macrophage, a human embryonic stem cell (ESC), an ESC-derived cell, a

pluripotent stem cell, and induced pluripotent stem cell (iPSC), and an iPSC-derived cell, optionally wherein the cell is an NK cell.

In some aspects, the method comprising administering a therapeutically effective dose of any of the isolated cells disclosed herein, optionally wherein the subject has cancer, optionally
5 wherein the isolated cell is derived from the subject or is allogeneic with reference to the subject.

In some aspects, the method comprising administering to a subject having a tumor a therapeutically effective dose of any of the isolated cells disclosed herein, optionally wherein the isolated cell is derived from the subject or is allogeneic with reference to the subject.

10 In some aspects, the method of making an engineered cell, comprises transducing an isolated cell with the multicistronic expression system disclosed herein, an expression vector disclosed herein or a polynucleotide disclosed herein.

Provided herein is a method of treating a subject known or suspected of having cancer, the method comprising: (A) administering a cell therapy to the subject, wherein the cell therapy
15 comprises an immunoresponsive cell engineered to express a recombinant cytokine, wherein the immunoresponsive cell engineered to express the recombinant cytokine comprises an exogenous polynucleotide encoding a controlled release cytokine, oriented from N-terminal to C-terminal, having the formula: **S – C – MT** or **MT – C – S** wherein S comprises a secretable effector molecule, wherein the secretable effector molecule comprises the recombinant cytokine, C
20 comprises a protease cleavage site, and MT comprises a cell membrane tethering domain, wherein **S – C – MT** or **MT – C – S** is configured to be expressed as a single polypeptide; and (B) administering a pharmaceutically effective amount of an agent selected from the group consisting of: (i) cytarabine (Ara-C); (ii) decitabine; (iii) venetoclax; (iv) a combination of fludarabine and Ara-C; or (V) or a combination of decitabine and venetoclax.

25 In some aspects, the immunoresponsive cell comprises an exogenous polynucleotide encoding an inhibitory chimeric antigen receptor (iCAR) comprising: (i) an antigen-binding domain specific for endomucin (EMCN); (ii) one or more intracellular inhibitory domains that inhibit an immune response; and (iii) one or more polypeptides selected from the group consisting of: a signal peptide, a transmembrane domain, a hinge domain, a spacer region, one or
30 more peptide linkers, and combinations thereof.

In some aspects, the immunoresponsive cell comprises an exogenous polynucleotide encoding a bivalent activating chimeric antigen receptor (aCAR) comprising: (i) an antigen-binding domain specific for FLT3; (ii) an antigen-binding domain specific for CD33; (iii) one or more intracellular signaling domains that stimulate an immune response; and (iv) one or more polypeptides selected from the group consisting of: a signal peptide, a transmembrane domain, a hinge domain, a spacer region, one or more peptide linkers, and combinations thereof.

In some aspects, the agent comprises the combination of fludarabine and Ara-C. In some aspects, the fludarabine is administered at a dose of 30 mg/m² and the cytarabine (Ara-C) is administered at a dose of 2 g/m². In some aspects, the agent is administered intravenously. In some aspects, wherein the agent is administered 5 days prior to administration of the cell therapy. In some aspects, when the Ara-C is administered, the Ara-C is administered at a dose of 2 g/m². In some aspects, the agent comprises the combination of fludarabine and Ara-C, wherein the fludarabine is administered at a dose of 30 mg/m² and the Ara-C is administered at a dose of 2 g/m², and wherein the agent is administered 5 days prior to administration of the cell therapy.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 provides a schematic illustrating components and organization of the overall FLT3 OR CD33 NOT EMCN logic-gated CAR-NK cell system.

FIG. 2 shows fold change in four lots of FLT3 OR CD33 NOT EMCN logic-gated CAR-NK or donor-matched non-engineered NK live cell counts over time (days) in culture.

FIG. 3 shows fold change in four lots of FLT3 OR CD33 NOT EMCN logic-gated CAR-NK or donor-matched non-engineered NK live cell counts over time (days) in culture with media without IL-2 supplementation or low dose IL-2 (10 IU/mL) containing media.

FIG. 4 shows fold change in four lots of FLT3 OR CD33 NOT EMCN logic-gated CAR-NK or donor-matched non-engineered NK live cell counts over time (days) in culture in media without IL-2 supplementation, low dose IL-2 (10 IU/mL) containing media, or high dose IL-2 (500 IU/mL) and IL15 (10 ng/mL) containing media.

FIG. 5 provides a schematic of the experimental conditions of IL-2 treatment of FLT3 OR CD33 NOT EMCN logic-gated CAR-NK cells prior to performing a cytotoxicity assay.

FIG. 6 shows the CD33 and FLT3 expression of different AML cell lines as measured by flow cytometry and the percent cytotoxicity of non-engineered NK cells or FLT3 OR CD33 NOT EMCN logic-gated CAR-NK cells in the presence of "IL-2 4 day" treatment against an AML cell line that did not express CD33 and FLT3 (KG-1a).

FIG. 7 shows percent cytotoxicity of FLT3 OR CD33 NOT EMCN logic-gated CAR-NK cells against different AML cell lines at effector:target (E:T) ratios of 2:1, 1:1 and 0.5:1 when treated with IL-2 as depicted in **FIG. 5**.

FIG. 8 provides a schematic of the experimental conditions of IL-2 treatment of FLT3 OR
5 CD33 NOT EMCN logic-gated CAR-NK cells during a serial killing assay.

FIG. 9 shows percent cytotoxicity of FLT3 OR CD33 NOT EMCN logic-gated CAR-NK cells or donor-matched non-engineered NK cells against different AML cell lines over 3 rounds of serial killing.

FIG. 10 shows percent cytotoxicity of FLT3 OR CD33 NOT EMCN logic-gated CAR-NK cells
10 against different AML cell lines over 3 rounds of serial killing in the presence or absence of IL-2 during the serial killing assay as depicted in **FIG. 8**.

DETAILED DESCRIPTION

The present disclosure provides methods of treating subjects, such as subjects known or
15 suspected of having cancer, with a cell therapy in combination with a IL-2 cytokine therapy.

Cell therapies include immunoresponsive cells engineered to express a recombinant cytokine. Recombinant cytokines can include a controlled release cytokine. As used herein, the terms “membrane-cleavable,” “controlled-release,” and “calibrated release” are used interchangeably. In various embodiments, the controlled release protein disclosed herein is a
20 controlled release cytokine. In certain embodiments, the cytokine is a controlled-release IL-15 (crIL15) cytokine. Controlled release cytokines are described further herein (e.g., “Membrane Cleavable Chimeric Proteins”).

Recombinant cytokines can include a controlled release cytokine, oriented from N-terminal to C-terminal, having the formula: **S – C – MT** or **MT – C – S**, where S represents a
25 secretable effector molecule that includes the recombinant cytokine, C represents a protease cleavage site, and MT represents a cell membrane tethering domain, and where **S – C – MT** or **MT – C – S** is configured to be expressed as a single polypeptide.

Cell therapies can include multicistronic expression systems, *i.e.*, more than one separate polypeptide (*e.g.*, multiple chimeric proteins) can be produced from a single mRNA transcript.
30 An illustrative non-limiting multicistronic system can include an polynucleotide encoding (i) a controlled-release cytokine; (ii) a bivalent aCAR; and (iii) an iCAR. The multicistronic systems include FLT3 OR CD33 NOT EMCN logic-gated CARs and a membrane-cleavable chimeric protein, controlled-release IL-15 (crIL15).

Multicistronic expression systems can include engineered nucleic acids encoding:

(A) an exogenous polynucleotide encoding a membrane-cleavable chimeric protein, oriented from N-terminal to C-terminal, having the formula: **S – C – MT** or **MT – C – S** wherein S includes a secretable effector molecule, wherein the secretable effector molecule includes IL-15, C includes a protease cleavage site, and MT includes a cell membrane tethering domain. **S – C – MT** or **MT – C – S** is configured to be expressed as a single polypeptide;

(B) an exogenous polynucleotide encoding an inhibitory chimeric antigen receptor (iCAR) including: (i) an antigen-binding domain specific for endomucin (EMCN); (ii) one or more intracellular inhibitory domains that inhibit an immune response, and (iii) one or more polypeptides selected from the group consisting of: a signal peptide, a transmembrane domain, a hinge domain, a spacer region, one or more peptide linkers, and combinations thereof; and

(C) an exogenous polynucleotide encoding a bivalent activating chimeric antigen receptor (aCAR) including: (i) an antigen-binding domain specific for FLT3; (ii) an antigen-binding domain specific for CD33; (iii) one or more intracellular signaling domains that stimulate an immune response, and (iv) one or more polypeptides selected from the group consisting of: a signal peptide, a transmembrane domain, a hinge domain, a spacer region, one or more peptide linkers, and combinations thereof.

Engineered nucleic acids can be multicistronic through the use of various linkers, *e.g.*, a polynucleotide sequence encoding a first chimeric proteins can be linked to a nucleotide sequence encoding a second chimeric protein, such as in a first gene:linker:second gene 5' to 3' orientation. A linker can encode a 2A ribosome skipping element, such as T2A. Other 2A ribosome skipping elements include, but are not limited to, E2A, P2A, and F2A. 2A ribosome skipping elements allow production of separate polypeptides encoded by the first and second genes are produced during translation. 2A ribosome skipping elements can include fusion peptides of 2A ribosome skipping elements, including, but not limited to, an E2A/T2A ribosome skipping element. In certain embodiments, the E2A/T2A ribosome skipping element comprises the amino acid sequence of

GSGQCTNYALLKLAGDVESNPGPGSGEGRGSLTCTGCDVEENPGP (SEQ ID NO: 219). One exemplary nucleic acid encoding the E2A/T2A ribosome skipping element is GGTAGCGGCCAGTGTTACCAACTACGCCCTGCTGAAACTGGCCGGCGACGTGGAATCTAATCCTGGACCTGGATCTGGCGAGGGACGCGGGAGTCTACTGACGTGTGGAGACGTGGAGGAAAACCCTGGACCT (SEQ ID NO: 220). In certain embodiments, a nucleic acid encoding the E2A/T2A ribosome skipping element includes a sequence that is at least 90%, at

least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 220. In some embodiments, an engineered nucleic acid disclosed herein comprises an E2A/T2A ribosome skipping element. In certain

- 5 QCTNYALLKLAGDVESNPGPGSGEGRGSLLTCGDVEENPGP (SEQ ID NO: 221). One exemplary nucleic acid encoding the E2A/T2A ribosome skipping element is
CAGTGTACCAACTACGCCCTGCTGAACTGGCCGGCGACGTGGAATCTAATCCTGG
ACCTGGATCTGGCGAGGGACGCGGGAGTCTACTGACGTGTGGAGACGTGGAGGAA
AACCTGGACCT (SEQ ID NO: 222). Another exemplary nucleic acid encoding the
10 E2A/T2A ribosome skipping element is
CAGTGCACAAATTATGCACTGCTGAAGCTCGCCGGGGATGTCGAGAGTAACCCAGG
ACCTGGAAGCGGAGAAGGTCGTGGTAGTCTACTAACGTGTGGTGATGTAGAAGAAA
ATCCTGGACCT (SEQ ID NO: 223). In certain embodiments, a nucleic acid encoding the
E2A/T2A ribosome skipping element includes a sequence that is at least 90%, at least 91%, at
15 least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 222 or SEQ ID NO: 223.

- A linker can encode a cleavable linker polypeptide sequence, such as a Furin cleavage site or a TEV cleavage site, wherein following expression the cleavable linker polypeptide is cleaved such that separate polypeptides encoded by the first and second genes are produced. A
20 cleavable linker can include a polypeptide sequence, such as such a flexible linker (*e.g.*, a Gly-Ser-Gly sequence), that further promotes cleavage.

A linker can encode an Internal Ribosome Entry Site (IRES), such that separate polypeptides encoded by the first and second genes are produced during translation. A linker can encode a splice acceptor, such as a viral splice acceptor.

- 25 A linker can be a combination of linkers, such as a Furin-2A linker that can produce separate polypeptides through 2A ribosome skipping followed by further cleavage of the Furin site to allow for complete removal of 2A residues. In some embodiments, a combination of linkers can include a Furin sequence, a flexible linker, and 2A linker. Accordingly, in some embodiments, the linker is a Furin-Gly-Ser-Gly-2A fusion polypeptide. In some embodiments, a
30 linker of the present disclosure is a Furin-Gly-Ser-Gly-T2A fusion polypeptide.

In general, a multicistronic system can use any number or combination of linkers, to express any number of genes or portions thereof (*e.g.*, an engineered nucleic acid can encode a first, a second, and a third chimeric protein, each separated by linkers such that separate polypeptides encoded by the first, second, and third chimeric proteins are produced).

In general, the exogenous polynucleotides encoding each of the membrane-cleavable chimeric protein, the iCAR, and the bivalent aCAR can be encoded in any order. For example, the membrane-cleavable chimeric protein, the iCAR, and the bivalent aCAR can be encoded by the multicistronic system in order from 5' to 3': (i) membrane-cleavable chimeric protein; (ii) bivalent aCAR; and (iii) iCAR. In another example, the membrane-cleavable chimeric protein, the iCAR, and the bivalent aCAR can be encoded by the multicistronic system in order from 5' to 3': i) membrane-cleavable chimeric protein; (ii) iCAR; and (iii) bivalent aCAR.

Methods of Cell Therapy Treatment

Provided herein are methods that include delivering, or administering, to a subject (*e.g.*, a human subject) engineered cells as provided herein as a cell therapy to produce *in vivo* at least one protein of interest produced by the engineered cells (*e.g.*, a recombinant cytokine, such as controlled-release cytokines including crIL-15, and/or any of the chimeric proteins provided for herein, such as any of the multicistronic systems encoding each of (i) a membrane-cleavable chimeric protein; (ii) a bivalent aCAR; and (iii) an iCAR described herein, or the secreted effector molecules provided for herein following protease cleavage of the chimeric protein). Further provided herein are methods that include delivering, or administering, to a subject (*e.g.*, a human subject) engineered cells as provided herein to produce *in vivo* at least two proteins of interest, *e.g.*, at least two of the chimeric proteins provided for herein, such as each of (i) a membrane-cleavable chimeric protein; (ii) a bivalent aCAR; and (iii) an iCAR described herein, produced by the engineered cells.

Further provided herein are methods that include delivering, or administering, to a subject (*e.g.*, a human subject) any of the delivery vehicles described herein, such as any of the delivery vehicles described herein comprising any of the proteins of interest described herein, *e.g.*, any of the chimeric proteins provided for herein, such as each of (i) a membrane-cleavable chimeric protein; (ii) a bivalent aCAR; and (iii) an iCAR described herein. Further provided herein are methods that include delivering, or administering, to a subject (*e.g.*, a human subject) any of the delivery vehicles described herein, such as any of the delivery vehicles described herein comprising two or more proteins of, *e.g.*, at least two of the chimeric proteins provided for herein, such as each of (i) a membrane-cleavable chimeric protein; (ii) a bivalent aCAR; and (iii) an iCAR described herein.

In general, each of the cell therapies described herein are administered as part of therapy that also includes an IL-2 cytokine therapy, described in further detail herein (*e.g.*, see "Combination Therapies").

In some embodiments, the engineered cells or delivery vehicles are administered via intravenous, intraperitoneal, intratracheal, subcutaneous, intratumoral, oral, anal, intranasal (*e.g.*, packed in a delivery particle), or arterial (*e.g.*, internal carotid artery) routes. Thus, the engineered cells or delivery vehicles may be administered systemically or locally (*e.g.*, to a TME or via intratumoral administration). An engineered cell can be isolated from a subject, such as a subject known or suspected to have cancer. An engineered cell can be allogenic with reference to the subject being administered a treatment. Allogenic modified cells can be HLA-matched to the subject being administered a treatment. Delivery vehicles can be any of the lipid structure delivery systems described herein. Delivery vehicles can be any of the nanoparticles described herein.

Engineered cells or delivery vehicles can be administered alone or in combination with other treatments, either simultaneously or sequentially dependent upon the condition to be treated. For example, engineered cells or delivery vehicles can be administered in combination with one or more IMiDs described herein. FDA-approved IMiDs can be administered in their approved fashion. In another example, engineered cells or delivery vehicles can be administered in combination with a checkpoint inhibitor therapy. Exemplary checkpoint inhibitors include, but are not limited to, anti-PD-1 antibodies, anti-PD-L1 antibodies, anti-PD-L2 antibodies, anti-CTLA-4 antibodies, anti-LAG-3 antibodies, anti-TIM-3 antibodies, anti-TIGIT antibodies, anti-VISTA antibodies, anti-KIR antibodies, anti-B7-H3 antibodies, anti-B7-H4 antibodies, anti-HVEM antibodies, anti-BTLA antibodies, anti-GAL9 antibodies, anti-A2AR antibodies, anti-phosphatidylserine antibodies, anti-CD27 antibodies, anti-TNF α antibodies, anti-TREMI antibodies, and anti-TREM2 antibodies. Illustrative immune checkpoint inhibitors include pembrolizumab (anti-PD-1; MK-3475/Keytruda® - Merck), nivolumab (anti-PD-1; Opdivo® - BMS), pidilizumab (anti-PD-1 antibody; CT-011 - Teva/CureTech), AMP224 (anti-PD-1; NCI), avelumab (anti-PD-L1; Bavencio® - Pfizer), durvalumab (anti-PD-L1; MEDI4736/Imfinzi® - Medimmune/AstraZeneca), atezolizumab (anti-PD-L1; Tecentriq® - Roche/Genentech), BMS-936559 (anti-PD-L1 - BMS), tremelimumab (anti-CTLA-4; Medimmune/AstraZeneca), ipilimumab (anti-CTLA-4; Yervoy® - BMS), lirilumab (anti-KIR; BMS), monalizumab (anti-NKG2A; Innate Pharma/AstraZeneca). In other examples, engineered cells or delivery vehicles can be administered in combination with TGF β inhibitors, VEGF inhibitors, or HPGE2. In another example, engineered cells or delivery vehicles can be administered in combination with an anti-CD40 antibody.

Some methods comprise selecting a subject (or patient population) having a tumor (or cancer) and treating that subject with engineered cells or delivery vehicles that modulate tumor-mediated immunosuppressive mechanisms.

The engineered cells or delivery vehicles of the present disclosure may be used, in some instances, to treat cancer, such as ovarian cancer. Other cancers are described herein. For example, the engineered cells may be used to treat bladder tumors, brain tumors, breast tumors, cervical tumors, colorectal tumors, esophageal tumors, gliomas, kidney tumors, liver tumors, lung tumors, melanomas, ovarian tumors, pancreatic tumors, prostate tumors, skin tumors, thyroid tumors, and/or uterine tumors. The engineered cells or delivery vehicles of the present disclosure can be used to treat cancers with tumors located in the peritoneal space of a subject.

The methods provided herein also include delivering a preparation of engineered cells or delivery vehicles. A preparation, in some embodiments, is a substantially pure preparation, containing, for example, less than 5% (*e.g.*, less than 4%, 3%, 2%, or 1%) of cells other than engineered cells. A preparation may comprise 1×10^5 cells/kg to 1×10^7 cells/kg cells. Preparation of engineered cells or delivery vehicles can include pharmaceutical compositions having one or more pharmaceutically acceptable carriers. For example, preparations of engineered cells or delivery vehicles can include any of the engineered viruses, such as an engineered AAV virus, or any of the engineered viral vectors, such as AAV vector, described herein.

***In vivo* Expression**

The methods provided herein also include delivering a composition *in vivo* capable of producing the engineered cells described herein, *e.g.*, capable of delivering any of the engineered nucleic acids described herein to a cell *in vivo*. Such compositions include any of the viral-mediated delivery platforms, any of the lipid structure delivery systems, any of the nanoparticle delivery systems, any of the genomic editing systems, or any of the other engineering delivery systems described herein capable of engineering a cell *in vivo*.

The methods provided herein also include delivering a composition *in vivo* capable of producing any of the proteins of interest described herein, *e.g.*, any of the multicistronic systems encoding each of (i) a membrane-cleavable chimeric protein; (ii) a bivalent aCAR; and (iii) an iCAR described herein. The methods provided herein also include delivering a composition *in vivo* capable of producing two or more of the proteins of interest described herein. Compositions capable of *in vivo* production of proteins of interest include, but are not limited to, any of the engineered nucleic acids described herein. Compositions capable of *in vivo* production proteins of interest can be a naked mRNA or a naked plasmid.

Combination Therapies

In some embodiments, genetically modified cells of the present disclosure (*e.g.*, immunoresponsive cells) expressing one or more proteins including an antigen-binding domain

(e.g., scFv) of the present disclosure, such as a chimeric receptor of the present disclosure (e.g., an iCAR and/or an aCAR), and including a controlled-release cytokine (also referred to as a calibrated release cytokine or a membrane-cleavable cytokine herein) of the present disclosure (e.g., controlled-release crIL15) may be used in combination with other known agents and

5 therapies.

In general, each of the cell therapies described herein (e.g., any of the engineered immunoresponsive cells described herein) are administered as part of therapy that also includes an IL-2 cytokine therapy.

In some embodiments, administration of the IL-2 cytokine can comprise multiple
10 administrations. In some embodiments, the multiple administrations of IL-2 is at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, or at least 9 administrations. The IL-2 cytokine can be administered once a day or more than once a day, e.g., twice a day, three times a day, or four times a day. The IL-2 cytokine can be administered for more than one day, e.g., the cytokine is administered for 2 days, 3 days, 4 days, 5 days, 6 days, 1 week, 2 weeks, 3 weeks, or 4 weeks.
15 For example, the IL-2 cytokine is administered once a day for 7 days.

In some embodiments, the cytokine IL-2 can be administered simultaneously or concurrently with the genetically modified cells, e.g., administered on the same day. In some embodiments, the IL-2 administration is within 4, within 5, within 6, within 7, or within 8 hours following the start of the administration of the genetically modified cells. In some embodiments,
20 the IL-2 administration is within 4 hours following the start of the administration of the genetically modified cells. In some embodiments, the IL-2 administration is within 5 hours following the start of the administration of the genetically modified cells. In some embodiments, the IL-2 administration is within 6 hours following the start of the administration of the genetically modified cells. In some embodiments, the IL-2 administration is within 7 hours
25 following the start of the administration of the genetically modified cells. In some embodiments, the IL-2 administration is within 8 hours following the start of the administration of the genetically modified cells. In some embodiments, the IL-2 administration is within 4 to 8 hours following the start of the administration of the genetically modified cells. In some embodiments, the IL-2 administration is within 6 to 8 hours following the start of the administration of the
30 genetically modified cells. In some embodiments, the IL-2 administration is between 4 to 8 hours following the start of the administration of the genetically modified cells. In some embodiments, the IL-2 cytokine can be administered within 24 or 48 hours after the administering of the genetically modified cells. The
35 IL-2 cytokine may be prepared in the same pharmaceutical composition as the genetically

modified cells, or may be prepared in a separate pharmaceutical composition. Alternatively, the IL-2 cytokine can be administered shortly after administration of the genetically modified cells, e.g., 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, or 7 days after administration of the genetically modified cells. In some embodiments the administration of the genetically modified cells comprises multiple administrations. In some embodiments the administration of the genetically modified cells comprises multiple administrations and the cytokine IL-2 can be administered simultaneously or concurrently with the genetically modified cells, e.g., administered on the same day. In some embodiments where the IL-2 cytokine is administered in a dosing regimen that occurs over more than one day, the first day of the IL-2 cytokine dosing regimen can be on the same day as administration with the genetically modified cells, or the first day of the IL-2 cytokine dosing regimen can be 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, or 7 days after administration of the genetically modified cells. In one embodiment, on the first day, the genetically modified cells are administered to the subject, and on the second day, the IL-2 cytokine is administered once a day for the next 7 days. In some embodiments, the IL-2 cytokine is administered at least 1, at least 2, at least 3 times a week following each administration of the genetically modified cells. In some embodiments, the IL-2 cytokine is administered at least 1 time a week following each administration of the genetically modified cells. In some embodiments, the IL-2 cytokine is administered at least 2 times a week following each administration of the genetically modified cells. In some embodiments, the IL-2 cytokine is administered at least 3 times a week following each administration of the genetically modified cells. In some embodiments, the IL-2 cytokine is administered for a period of time after administration of the genetically modified cells, e.g., at least 2 weeks, 3 weeks, 4 weeks, 6 weeks, 8 weeks, 10 weeks, 12 weeks, 4 months, 5 months, 6 months, 7 months, 8 months, 9 months, 10 months, 11 months, or 1 year or more after administration of the genetically modified cells. In one embodiment, the IL-2 cytokine is administered after assessment of the subject's response to the genetically modified cells.

In some embodiments, the genetically modified cells are administered again on day 7 and/or day 14 following the initial administration of the genetically modified cells. In some embodiments, the genetically modified cells are administered again on day 7 following the initial administration of the genetically modified cells. In some embodiments, the genetically modified cells are administered again on day 7 and day 14 following the initial administration of the genetically modified cells. In some embodiments, the genetically modified cells and the IL-2 cytokine are administered again on day 7 and/or day 14 following the initial administration of the genetically modified cells. In some embodiments, the genetically modified cells and the IL-2 cytokine are administered again on day 7 following the initial administration of the genetically

modified cells. In some embodiments, the genetically modified cells and the IL-2 cytokine are administered again on day 7 and day 14 following the initial administration of the genetically modified cells.

In some embodiments, IL-2 cytokine is administered at a pharmaceutically effective dose. In some embodiments, the pharmaceutically effective dose of IL-2 is 3 MIU. In some embodiments, the pharmaceutically effective dose of IL-2 is at least about 1 - 10 MIU. In some embodiments, the pharmaceutically effective dose of IL-2 is at least about 1 - 9 MIU. In some embodiments, the pharmaceutically effective dose of IL-2 is at least about 1 - 8 MIU. In some embodiments, the pharmaceutically effective dose of IL-2 is at least about 1 - 7 MIU. In some embodiments, the pharmaceutically effective dose of IL-2 is at least about 1 - 6 MIU. In some embodiments, the pharmaceutically effective dose of IL-2 is at least about 1 - 5 MIU. In some embodiments, the pharmaceutically effective dose of IL-2 is at least about 1 - 4 MIU. In some embodiments, the pharmaceutically effective dose of IL-2 is at least about 1 - 3 MIU. In some embodiments, the pharmaceutically effective dose of IL-2 is at least about 1 - 2 MIU. In some embodiments, the pharmaceutically effective dose of IL-2 is at least about 2 MIU. In some embodiments, the pharmaceutically effective dose of IL-2 is at least about 3 MIU. In some embodiments, the pharmaceutically effective dose of IL-2 is at least about 4 MIU. In some embodiments, the pharmaceutically effective dose of IL-2 is at least about 5 MIU. In some embodiments, the pharmaceutically effective dose of IL-2 is at least about 6 MIU.

In some embodiments, the pharmaceutically effective dose of IL-2 is between 1 - 10 MIU. In some embodiments, the pharmaceutically effective dose of IL-2 is between 1 - 10 MIU. In some embodiments, the pharmaceutically effective dose of IL-2 is between 1 - 9 MIU. In some embodiments, the pharmaceutically effective dose of IL-2 is between 1 - 8 MIU. In some embodiments, the pharmaceutically effective dose of IL-2 is between 1 - 7 MIU. In some embodiments, the pharmaceutically effective dose of IL-2 is between 1 - 6 MIU. In some embodiments, the pharmaceutically effective dose of IL-2 is between 1 - 5 MIU. In some embodiments, the pharmaceutically effective dose of IL-2 is between 1 - 4 MIU. In some embodiments, the pharmaceutically effective dose of IL-2 is between 1 - 3 MIU. In some embodiments, the pharmaceutically effective dose of IL-2 is between 1 - 2 MIU. In some embodiments, the pharmaceutically effective dose of IL-2 is 2 MIU. In some embodiments, the pharmaceutically effective dose of IL-2 is 4 MIU. In some embodiments, the pharmaceutically effective dose of IL-2 is 5 MIU. In some embodiments, the pharmaceutically effective dose of IL-2 is 6 MIU.

In some embodiments, a combination therapy of the present disclosure comprises a genetically modified cells of the present disclosure that can be administered in combination with

one or more additional therapeutic agents, including therapeutic agents in addition to IL-2. In some embodiments, the genetically modified cell and the one or more additional therapeutic agents, including IL-2 itself, can be administered simultaneously, in the same or in separate compositions, or sequentially. For sequential administration, the genetically modified can be administered first, and the one or more additional agents can be administered second, or the order of administration can be reversed. In some embodiments, the genetically modified cells are further modified to express one or more additional therapeutic agents.

In some embodiments, a genetically modified cell of the present disclosure may be used in a treatment regimen in combination with surgery, chemotherapy, radiation, immunosuppressive agents (e.g., cyclosporin, azathioprine, methotrexate, mycophenolate, and FK506), antibodies, or other immunoablative agents (e.g., CAMPATH or anti-CD3 antibodies), cytoxin, fludarabine, cyclosporin, FK506, rapamycin, mycophenolic acid, steroids, FR901228, cytokines, irradiation, and peptide vaccines.

In some embodiments, a genetically modified cell of the present disclosure (e.g., immunoresponsive cells) may be used in combination with a lymphodepleting agent. Suitable lymphodepleting agents reduce or decrease lymphocytes, e.g., B cell lymphocytes and/or T cell lymphocytes, prior to immunotherapy. Examples of suitable lymphodepleting agents include, without limitation, fludarabine, cytarabine (Ara-C), cyclophosphamide, corticosteroids, alemtuzumab, total body irradiation (TBI), and any combination thereof. In some embodiments, the lymphodepletion agents are administered 5 days prior to administration of the genetically modified cells (e.g., any of the cell therapies described herein). In some embodiments, the lymphodepletion agents are administered daily until administration of the genetically modified cells. In some embodiments, the lymphodepletion agents are fludarabine administered at a dose of 30 mg/m² and cytarabine (Ara-C) administered at a dose of 2 g/m². In some embodiments, the lymphodepletion agents are fludarabine administered at a dose of 30 mg/m² and cytarabine (Ara-C) administered at a dose of 2 g/m² administered 5 days prior to administration of the genetically modified cells.

In some embodiments, a genetically modified cell of the present disclosure may be used in combination with a chemotherapeutic agent. Suitable chemotherapeutic agents include, without limitation, an anthracycline (e.g., doxorubicin), a vinca alkaloid (e.g., vinblastine, vincristine, vindesine, vinorelbine), an alkylating agent (e.g., cyclophosphamide, decarbazine, melphalan, ifosfamide, temozolomide), an immune cell antibody (e.g., alemtuzumab, gemtuzumab, rituximab, tositumomab), an antimetabolite (e.g., folic acid antagonists, pyrimidine analogs, purine analogs and adenosine deaminase inhibitors, such as fludarabine), an mTOR inhibitor, a TNFR glucocorticoid induced TNFR related protein (GITR) agonist, a

proteasome inhibitor (e.g., aclacinomycin A, gliotoxin or bortezomib), an immunomodulator such as thalidomide or a thalidomide derivative (e.g., lenalidomide).

Examples of general chemotherapeutic agents suitable for use in combination therapies include, without limitation, anastrozole (Arimidex®), bicalutamide (Casodex®), bleomycin sulfate (Blenoxane®), busulfan (Myleran®), busulfan injection (Busulfex®), capecitabine (Xeloda®), N4-pentoxycarbonyl-5- deoxy-5-fluorocytidine, carboplatin (Paraplatin®), carmustine (BiCNU®), chlorambucil (Leukeran®), cisplatin (Piatinol®), cladribine (Leustatin®), cyclophosphamide (Cytoxan® or Neosar®), cytarabine, cytosine arabinoside (Cytosar-U®), cytarabine liposome injection (DepoCyt®), dacarbazine (DTIC-Dome®), dactinomycin (Actinomycin D, Cosmegen), daunorubicin hydrochloride (Cerubidine®), daunorubicin citrate liposome injection (DaunoXome®), dexamethasone, docetaxel (Taxotere®), doxorubicin hydrochloride (Adriamycin®, Rubex®), etoposide (Vepesid®), fludarabine phosphate (Fludara®), 5- fluorouracil (Adrucil®, Efudex®), flutamide (Eulexin®), tezacitibine, Gemcitabine (difluorodeoxycytidine), hydroxyurea (Hydrea®), Idarubicin (Idaniycin®), ifosfamide (IFEX®), irinotecan (Camptosar®), L-asparaginase (ELSPAR®), leucovorin calcium, melphalan (Alkeran®), 6-mercaptopurine (Purinethol®), methotrexate (Folex®), mitoxantrone (Novantrone®), mylotarg, paclitaxel (Taxol®), phoenix (Yttrium90/MX-DTPA), pentostatin, polifeprosan 20 with carmustine implant (Gliadel®), tamoxifen citrate (Nolvadex®), teniposide (Vumon®), 6-thioguanine, thiotepa, tirapazamine (Tirazone®), topotecan hydrochloride for injection (Hycamptin®), vinblastine (Velban®), vincristine (Oncovin®), and vinorelbine (Navelbine®).

Examples of suitable alkylating agents include, without limitation, nitrogen mustards, ethylenimine derivatives, alkyl sulfonates, nitrosoureas and triazenes): uracil mustard (Aminouracil Mustard®, Chlorethaminacil®, Demethyldopan®, Desmethyldopan®, Haemanthamine®, Nordopan®, Uracil nitrogen mustard®, Uracilmostaza®, Uramustin®, Uramustine®), chlormethine (Mustargen®), cyclophosphamide (Cytoxan®, Neosar®, Clafen®, Endoxan®, Procytox®, Rev immune™), ifosfamide (Mitoxana®), melphalan (Alkeran®), Chlorambucil (Leukeran®), pipobroman (Amedel®, Vercyte®), triethylenemelamine (Hemel®, Hexalen®, Hexastat®), triethylenethiophosphoramine, Temozolomide (Temodar®), thiotepa (Thioplex®), busulfan (Busilvex®, Myleran®), carmustine (BiCNU®), lomustine (CeeNU®), streptozocin (Zanosar®), and Dacarbazine (DTIC-Dome®). Additional exemplary alkylating agents include, without limitation, Oxaliplatin (Eloxatin®); Temozolomide (Temodar® and Temodal®); Dactinomycin (also known as actinomycin-D, Cosmegen®); Melphalan (also known as L-PAM, L-sarcolysin, and phenylalanine mustard, Alkeran®); Altretamme (also known as hexamethylmelamine (HMM), Hexalen®); Carmustine (BiCNU®); Bendamustine

(Treanda®); Busulfan (Busulfex® and Myleran®); Carboplatin (Paraplatin®); Lomustine (also known as CCNU, CeeNU®); Cisplatin (also known as CDDP, Platinol® and Platinol®-AQ); Chlorambucil (Leukeran®); Cyclophosphamide (Cytoxan® and Neosar®); Dacarbazine (also known as DTIC, DIC and imidazole carboxamide, DTIC-Dome®); Aitretamine (also known as
5 hexamethylmelamine (HMM), Hexalen®); Ifosfamide (Ifex®); Prednumustine; Procarbazine (Matulane®); Mechlorethamine (also known as nitrogen mustard, mustine and mechlorethamine hydrochloride, Mustargen®); Streptozocin (Zanosar®); Thiotepa (also known as thiophosphoamide, TESP and TSPA, Thioplex®); Cyclophosphamide (Endoxan®, Cytoxan®, Neosar®, Procytox®, Revimmune®); and Bendamustine HCl (Treanda®).

10 Examples of suitable mTOR inhibitors include, without limitation, temsirolimus, ridaforolimus (deferolimus), AP23573, MK8669, everolimus (Afimtor® or RADOOL), rapamycin (AY22989, Sirolimus®), and XL765.

Examples of suitable immunomodulators include, without limitation, afutuzumab, pegfilgrastim (Neulasta®), lenalidomide (CC-5013, Revlimid®), thalidomide (Thalomid®),
15 actimid (CC4047), and IRX-2.

Examples of suitable anthracyclines include, without limitation, doxorubicin (Adriamycin® and Rubex®); bleomycin (lenoxane®); daunorubicin (daunorubicin hydrochloride, daunomyem, and rubidomycin hydrochloride, Cerubidine®); daunorubicin liposomal (daunorubicin citrate liposome, DaunoXome®); mitoxantrone (DHAD,
20 Novantrone®); epirubicin (Ellence™); idarubicin (Idamycin®, Idamycin PES®); mitomycin C (Mutamycin®); geldanamycin; herbimycin; ravidomycin; and desacet Iravidomycin.

Examples of suitable vinca alkaloids include, without limitation, vinorelbine tartrate (Navelbine®), Vincristine (Oncovin®), and Vindesine (Eldisine®); vinblastine (also known as vinblastine sulfate, vincalukoblastine and VLB, Alkaban-AQ® and Velban®); and vinorelbine
25 (Navelbme®).

Examples of suitable proteasome inhibitors include, without limitation, bortezomib (Velcade®); carfilzomib; marizomib (NPI-0052); ixazomib citrate (MLN-9708); delanzomib (CEP-18770); and ONX-0912.

In some embodiments, a genetically modified cell of the present disclosure is
30 administered in combination with a CD20 inhibitor, e.g., an anti-CD20 antibody, or fragment thereof. Exemplary anti-CD20 antibodies include, without limitation, rituximab, ofatumumab, ocrelizumab, veltuzumab, obinutuzumab, TRU- 015 (Trubion Pharmaceuticals), ocaratuzumab, and Prol31921.

In some embodiments, a genetically modified cell of the present disclosure is
35 administered in combination with an oncolytic virus. In some embodiments, oncolytic viruses

are capable of selectively replicating in and triggering the death of or slowing the growth of a cancer cell. In some cases, oncolytic viruses have no effect or a minimal effect on non-cancer cells. Suitable oncolytic viruses include, without limitation, an oncolytic adenovirus, oncolytic Herpes Simplex Viruses, oncolytic retrovirus, oncolytic parvovirus, oncolytic vaccinia virus, 5 oncolytic Sindbis virus, oncolytic influenza virus, or oncolytic RNA virus (e.g., oncolytic reovirus, oncolytic Newcastle Disease Virus (NDV), oncolytic measles virus, or oncolytic vesicular stomatitis virus (VSV)). In some embodiments, the oncolytic virus is a recombinant oncolytic virus.

In some embodiments, a genetically modified cell of the present disclosure is 10 administered to a subject in combination with a protein tyrosine phosphatase inhibitor, e.g., a SHP-1 inhibitor or a SHP-2 inhibitor. In one embodiment, a genetically modified cell of the present disclosure can be used in combination with a kinase inhibitor. Examples of suitable kinase inhibitors include, without limitation, CDK4 inhibitors, CDK4/6 inhibitors, BTK inhibitors, phosphatidylinositol 3 -kinase (PI3K) inhibitors, mTOR inhibitors, MNK inhibitors, 15 and anaplastic lymphoma kinase (ALK) inhibitors.

In some embodiments, a genetically modified cell of the present disclosure is administered to a subject in combination with a modulator of myeloid-derived suppressor cells (MDSCs). MDSCs accumulate in the periphery and at the tumor site of many solid tumors. These cells suppress T cell responses, thereby hindering the efficacy of chimeric receptor- 20 expressing cell therapy. Without being bound by theory, it is believed that administration of a MDSC modulator enhances the efficacy of a genetically modified cell of the present disclosure. Examples of suitable modulators of MDSCs include, without limitation, MCS110 and BLZ945.

In some embodiments, a genetically modified cell of the present disclosure is administered to a subject in combination with an agent that inhibits or reduces the activity of 25 immunosuppressive plasma cells. Immunosuppressive plasma cells have been shown to impede T cell-dependent immunogenic chemotherapy, such as oxaliplatin (Shalapour et al., Nature 2015, 521 :94- 101). In one embodiment, immunosuppressive plasma cells can express one or more of IgA, interleukin (IL)-10, and PD-L1.

In some embodiments, a genetically modified cell of the present disclosure is 30 administered to a subject in combination with an interleukin-15 (IL-15) polypeptide, an interleukin-15 receptor alpha (IL-15Ra) polypeptide, or a combination of both an IL-15 polypeptide and an IL-15Ra polypeptide. In some embodiments, a genetically modified cell of the present disclosure is further modified to express an interleukin-15 (IL-15) polypeptide, an interleukin-15 receptor alpha (IL-15Ra) polypeptide, or a combination of both an IL-15

polypeptide and an IL-15Ra polypeptide. In some embodiments, the interleukin-15 (IL-15) polypeptide expressed by the genetically modified cells is controlled-release IL-15 (crIL15).

In some embodiments, a subject having cancer (e.g., AML) is administered a genetically modified cell of the present disclosure in combination with an agent, e.g., cytotoxic or chemotherapy agent, a biologic therapy (e.g., antibody, e.g., monoclonal antibody, or cellular therapy), or an inhibitor (e.g., kinase inhibitor). In some embodiments, the subject is administered a genetically modified cell of the present disclosure in combination with a cytotoxic agent, e.g., CPX-351 (Celator Pharmaceuticals), cytarabine, daunorubicin, vosaroxin (Sunesis Pharmaceuticals), sapacitabine (Cyclacel Pharmaceuticals), idarubicin, or mitoxantrone. CPX-351 is a liposomal formulation comprising cytarabine and daunorubicin at a 5: 1 molar ratio. In some embodiments, the subject is administered a chimeric receptor-expressing cell described herein in combination with a hypomethylating agent, e.g., a DNA methyltransferase inhibitor, e.g., azacytidine or decitabine. In some embodiments, the subject is administered a genetically modified cell of the present disclosure in combination with a biologic therapy, e.g., an antibody or cellular therapy, e.g., 225Ac-lintuzumab (Actimab-A; Actinium Pharmaceuticals), IPH2102 (Innate Pharma/Bristol Myers Squibb), SGN-CD33A (Seattle Genetics), or gemtuzumab ozogamicin (Mylotarg; Pfizer). In some embodiments, the subject is administered a genetically modified cell of the present disclosure in combination with a FLT3 inhibitor, e.g., sorafenib (Bayer), midostaurin (Novartis), quizartinib (Daiichi Sankyo), crenolanib (Arog Pharmaceuticals), PLX3397 (Daiichi Sankyo), AKN-028 (Akinion Pharmaceuticals), or ASP2215 (Astellas). In some embodiments, the subject is administered a genetically modified cell of the present disclosure in combination with an isocitrate dehydrogenase (IDH) inhibitor, e.g., AG-221 (Celgene/ Agios) or AG- 120 (Agios/Celgene). In some embodiments, the subject is administered a genetically modified cell of the present disclosure in combination with a cell cycle regulator, e.g., inhibitor of polo-like kinase 1 (Plkl), e.g., volasertib (Boehringer Ingelheim); or an inhibitor of cyclin-dependent kinase 9 (Cdk9), e.g., alvocidib (Tolero Pharmaceuticals/Sanofi Aventis). In some embodiments, the subject is administered a genetically modified cell of the present disclosure in combination with a B cell receptor signaling network inhibitor, e.g., an inhibitor of B-cell lymphoma 2 (Bcl- 2), e.g., venetoclax (Abbvie/Roche); or an inhibitor of Button's tyrosine kinase (Btk), e.g., ibrutinib (Pharmacyclics/Johnson & Johnson Janssen Pharmaceutical). In some embodiments, the subject is administered a genetically modified cell of the present disclosure in combination with an inhibitor of M1 aminopeptidase; an inhibitor of histone deacetylase (HDAC), e.g., pracinostat (MEI Pharma); a multi-kinase inhibitor, e.g., rigosertib (Onconova

Therapeutics/Baxter/SymBio); or a peptidic CXCR4 inverse agonist, e.g., BL-8040 (BioLineRx).

In some embodiments, a subject can be administered an agent which enhances the activity or fitness of a genetically modified cell of the present disclosure. For example, the agent may inhibit a molecule that modulates or regulates, e.g., inhibits, T cell function. In some embodiments, the molecule that modulates or regulates T cell function is an inhibitory molecule. In some embodiments, inhibitory molecules, such as Programmed Death 1 (PD-1) can decrease the ability of the genetically modified cell to mount an immune effector response. Examples of suitable inhibitory molecules include, without limitation, PD-1, PD-L1, CTLA4, TIM3, CEACAM (e.g., CEACAM-1, CEACAM-3 and/or CEACAM-5), LAG3, VISTA, BTLA, TIGIT, LIR-1 (LILRB1), CD 160, 2B4, CD80, CD86, B7-H3 (CD276), B7-H4 (VTCN1), HVEM (TNFRSF14 or CD270), KIR, A2aR, MHC class I, MHC class II, GAL9, adenosine, and TGF beta. Inhibition of a molecule that modulates or regulates, e.g., inhibits, T cell function, e.g., by inhibition at the DNA, RNA or protein level, can optimize the performance of genetically modified cells of the present disclosure. In some embodiments, an agent, e.g., an inhibitory nucleic acid, e.g., an inhibitory nucleic acid, e.g., an inhibitory nucleic acid, e.g., a dsRNA, e.g., an siRNA or shRNA, a clustered regularly interspaced short palindromic repeats (CRISPR), a transcription-activator like effector nuclease (TALEN), or a zinc finger endonuclease (ZFN), can be used to inhibit expression of an inhibitory molecule in the genetically modified cell. In one embodiment, the inhibitor is an shRNA. In some embodiments, a genetically modified cell of the present disclosure may be further modified to express an inhibitory nucleic acid, e.g., an inhibitory nucleic acid, e.g., an inhibitory nucleic acid, e.g., a dsRNA, e.g., an siRNA or shRNA, a clustered regularly interspaced short palindromic repeats (CRISPR), a transcription-activator like effector nuclease (TALEN), or a zinc finger endonuclease (ZFN), can be used to inhibit expression of an inhibitory molecule in the genetically modified cell.

In one embodiment, the agent that modulates or regulates, e.g., inhibits, T-cell function is inhibited within a genetically modified cell of the present disclosure. In such embodiments, a dsRNA molecule that inhibits expression of a molecule that modulates or regulates, e.g., inhibits, T-cell function is linked to the nucleic acid that encodes a component, e.g., all of the components, of a chimeric receptor of the present disclosure. In one embodiment, a nucleic acid molecule that encodes a dsRNA molecule that inhibits expression of the molecule that modulates or regulates, e.g., inhibits, T-cell function is operably linked to a promoter, e.g., a HI- or a U6-derived promoter such that the dsRNA molecule that inhibits expression of the molecule that modulates or regulates, e.g., inhibits, T-cell function is expressed, e.g., is expressed within a

the genetically modified cell. In one embodiment the nucleic acid molecule that encodes a dsRNA molecule that inhibits expression of the molecule that modulates or regulates, e.g., inhibits, T-cell function is present on the same vector, e.g., a lentiviral vector, that comprises a nucleic acid molecule that encodes a component, e.g., all of the components, of the chimeric receptor. In such an embodiment the nucleic acid molecule that encodes a dsRNA molecule that inhibits expression of the molecule that modulates or regulates, e.g., inhibits, T-cell function is located on the vector, e.g., the lentiviral vector, 5'- or 3'- to the nucleic acid that encodes a component, e.g., all of the components, of the chimeric receptor. The nucleic acid molecule that encodes a dsRNA molecule that inhibits expression of the molecule that modulates or regulates, e.g., inhibits, T-cell function can be transcribed in the same or different direction as the nucleic acid that encodes a component, e.g., all of the components, of the chimeric receptor. In one embodiment the nucleic acid molecule that encodes a dsRNA molecule that inhibits expression of the molecule that modulates or regulates, e.g., inhibits, T-cell function is present on a vector other than the vector that comprises a nucleic acid molecule that encodes a component, e.g., all of the components, of the chimeric receptor. In one embodiment, the nucleic acid molecule that encodes a dsRNA molecule that inhibits expression of the molecule that modulates or regulates, e.g., inhibits, T-cell function is transiently expressed within the genetically modified cell. In one embodiment, the nucleic acid molecule that encodes a dsRNA molecule that inhibits expression of the molecule that modulates or regulates, e.g., inhibits, T-cell function is stably integrated into the genome of a genetically modified cell of the present disclosure.

In one embodiment, an agent that modulates or regulates, e.g., inhibits, T-cell function can be an antibody or antibody fragment that binds to an inhibitory molecule. For example, the agent can be an antibody or antibody fragment that binds to PD-1, PD-L1, PD-L2 or CTLA4. In one embodiment, the agent is an antibody or antibody fragment that binds to TIM3. In one embodiment, the agent is an antibody or antibody fragment that binds to LAG3.

In some embodiments, the agent which enhances the activity of the genetically modified cell is a CEACAM inhibitor (e.g., CEACAM-1, CEACAM-3, and/or CEACAM-5 inhibitor). In one embodiment, the inhibitor of CEACAM is an anti-CEACAM antibody molecule. In one embodiment, the agent which enhances activity of a genetically modified cell of the present disclosure is miR-17-92. In some embodiments, the agent which enhances the activity of the genetically modified cell is CD40L. In some embodiments, the agent which enhances the activity of the genetically modified cell is GM-CSF. In some embodiments, a genetically modified cell of the present disclosure is further modified to express an antibody or antibody fragment that binds to an inhibitory molecule of the present disclosure.

In one embodiment, the agent which enhances activity of a genetically modified cell of the present disclosure is a cytokine. Cytokines have important functions related to immunoresponsive cell expansion, differentiation, survival, and homeostats. Cytokines that can be administered to the subject receiving a genetically modified cell of the present disclosure include, without limitation, IL-4, IL-7, IL-9, IL-12, IL-15, IL-18, and IL-21, or a combination thereof, and can include cytokines in addition to the IL-2 cytokine therapies described herein. The cytokine, inclusive of IL-2, can be administered once a day or more than once a day, e.g., twice a day, three times a day, or four times a day. The cytokine can be administered for more than one day, e.g., the cytokine is administered for 2 days, 3 days, 4 days, 5 days, 6 days, 1 week, 2 weeks, 3 weeks, or 4 weeks. For example, the cytokine is administered once a day for 7 days. In some embodiments, a genetically modified cell of the present disclosure is further modified to express one or more cytokines, such as IL-4, IL-7, IL-9, IL-12, IL-15, IL-18, and IL-21, and can also include a genetically modified cell modified to express IL-2. Cells modified to express one or more cytokines can be the same cell modified to express a protein of interest described herein (e.g., a recombinant cytokine, such as conditional release cytokines including crIL-15, and/or any of the chimeric proteins provided for herein, such as any of the multicistronic systems encoding each of (i) a membrane-cleavable chimeric protein; (ii) a bivalent aCAR; and (iii) an iCAR described herein, or the secreted effector molecules provided for herein following protease cleavage of the chimeric protein). Cells modified to express one or more cytokines can be a different cell modified to express a protein of interest described herein.

In some embodiments, the cytokine can be administered simultaneously or concurrently with the genetically modified cells, e.g., administered on the same day. The cytokine may be prepared in the same pharmaceutical composition as the genetically modified cells, or may be prepared in a separate pharmaceutical composition. Alternatively, the cytokine can be administered shortly after administration of the genetically modified cells, e.g., 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, or 7 days after administration of the genetically modified cells. In some embodiments where the cytokine is administered in a dosing regimen that occurs over more than one day, the first day of the cytokine dosing regimen can be on the same day as administration with the genetically modified cells, or the first day of the cytokine dosing regimen can be 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, or 7 days after administration of the genetically modified cells. In one embodiment, on the first day, the genetically modified cells are administered to the subject, and on the second day, a cytokine is administered once a day for the next 7 days. In some embodiments, the cytokine is administered for a period of time after administration of the genetically modified cells, e.g., at least 2 weeks, 3 weeks, 4 weeks, 6 weeks, 8 weeks, 10 weeks, 12 weeks, 4 months, 5 months, 6 months, 7 months, 8 months, 9

months, 10 months, 11 months, or 1 year or more after administration of the genetically modified cells. In one embodiment, the cytokine is administered after assessment of the subject's response to the genetically modified cells.

5 **Membrane Cleavable Chimeric Proteins**

Multicistronic systems herein encode membrane-cleavable chimeric proteins having the formula **S – C – MT** or **MT – C – S**, oriented from N-terminal to C-terminal and expressed as a single polypeptide. S refers to a secretable effector molecule. C refers to a protease cleavage site. MT refers to a cell membrane tethering domain. The membrane-cleavable chimeric protein
10 is engineered such that secretion of the effector molecule can be regulated in a protease-dependent manner. Specifically, the membrane-cleavable chimeric protein is engineered such that secretion of the effector molecule can be regulated as part of a “Membrane-Cleavable” system, where incorporation of a protease cleavage site (“C”) and a cell membrane tethering domain (“MT”) allow for regulated secretion of an effector molecule in a protease-dependent
15 manner. Without wishing to be bound by theory, the components of the Membrane-Cleavable system present in the membrane-cleavable chimeric protein generally regulate secretion through the below cellular processes:

- MT: The cell membrane tethering domain contains a transmembrane domain (or a transmembrane-intracellular domain) that directs cellular-trafficking of the chimeric
20 protein such that the protein is inserted into, or otherwise associated with, a cell membrane (“tethered”)
- C: Following expression and localization of the chimeric protein into the cell membrane, the protease cleavage site directs cleavage of the chimeric protein such that the effector molecule is released (“secreted”) into the extracellular space.
25 Generally, the protease cleavage site is protease-specific, including sites engineered to be protease-specific. The protease cleavage site can be selected or engineered to achieve optimal protein expression, cell-type specific cleavage, cell-state specific cleavage, and/or cleavage and release of the payload at desired kinetics (*e.g.*, ratio of membrane-bound to secreted chimeric protein levels)

30 Membrane-cleavable chimeric proteins are also described in International PCT Application No. WO2022098922, which is hereby incorporated by reference for all purposes.

In some aspects, membrane-cleavable chimeric proteins (or engineered nucleic acids encoding the membrane-cleavable chimeric proteins) are provided for herein having a protein of

interest (*e.g.*, any of the effector molecules described herein), a protease cleavage site, and a cell membrane tethering domain.

An “effector molecule,” refers to a molecule (*e.g.*, a nucleic acid such as DNA or RNA, or a protein (polypeptide) or peptide) that binds to another molecule and modulates the biological activity of that molecule to which it binds. For example, an effector molecule may act as a ligand to increase or decrease enzymatic activity, gene expression, or cell signaling. Thus, in some embodiments, an effector molecule modulates (activates or inhibits) different immunomodulatory mechanisms. By directly binding to and modulating a molecule, an effector molecule may also indirectly modulate a second, downstream molecule.

In certain embodiments described herein (*e.g.*, in general, for all membrane-cleavable chimeric proteins described herein), an effector molecule is a secretable effector molecule (*e.g.*, referred to as “S” in the formula **S – C – MT** or **MT – C – S** for membrane-cleavable chimeric proteins described herein). Non-limiting examples of effector molecules include cytokines, chemokines, enzymes that modulate metabolite levels, growth factors, co-activation molecules, tumor microenvironment modifiers, ligands, peptides, enzymes, antibodies, antibodies or decoy molecules that modulate cytokines, homing molecules, and/or integrins.

The term “modulate” encompasses maintenance of a biological activity, inhibition (partial or complete) of a biological activity, and stimulation/activation (partial or complete) of a biological activity. The term also encompasses decreasing or increasing (*e.g.*, enhancing) a biological activity. Two different effector molecules are considered to “modulate different tumor-mediated immunosuppressive mechanisms” when one effector molecule modulates a tumor-mediated immunosuppressive mechanism (*e.g.*, stimulates T cell signaling) that is different from the tumor-mediated immunosuppressive mechanism modulated by the other effector molecule (*e.g.*, stimulates antigen presentation and/or processing).

Modulation by an effector molecule may be direct or indirect. Direct modulation occurs when an effector molecule binds to another molecule and modulates activity of that molecule. Indirect modulation occurs when an effector molecule binds to another molecule, modulates activity of that molecule, and as a result of that modulation, the activity of yet another molecule (to which the effector molecule is not bound) is modulated.

In some embodiments, modulation of a tumor-mediated immunosuppressive mechanism by at least one effector molecule results in an increase in an immunostimulatory and/or anti-tumor immune response (*e.g.*, systemically or in the tumor microenvironment) by at least 10% (*e.g.*, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, or 200%). For example, modulation of a tumor-mediated immunosuppressive mechanism may result in an increase in an immunostimulatory and/or anti-tumor immune response by at least 20%, at least 30%, at least

40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 100%. In some embodiments, modulation of a tumor-mediated immunosuppressive mechanism results in an increase in an immunostimulatory and/or anti-tumor immune response 10-20%, 10-30%, 10-40%, 10-50%, 10-60%, 10-70%, 10-80%, 10-90%, 10-100%, 10-200%, 20-30%, 20-40%, 20-50%, 20-60%, 20-70%, 20-80%, 20-90%, 20-100%, 20-200%, 50-60%, 50-70%, 50-80%, 50-90%, 50-100%, or 50-200%. It should be understood that “an increase” in an immunostimulatory and/or anti-tumor immune response, for example, systemically or in a tumor microenvironment, is relative to the immunostimulatory and/or anti-tumor immune response that would otherwise occur, in the absence of the effector molecule(s).

10 In some embodiments, modulation of a tumor-mediated immunosuppressive mechanism by at least one effector molecule results in an increase in an immunostimulatory and/or anti-tumor immune response (*e.g.*, systemically or in the tumor microenvironment) by at least 2 fold (*e.g.*, 2, 3, 4, 5, 10, 25, 20, 25, 50, or 100 fold). For example, modulation of a tumor-mediated immunosuppressive mechanism may result in an increase in an immunostimulatory and/or anti-tumor immune response by at least 3 fold, at least 5 fold, at least 10 fold, at least 20 fold, at least 50 fold, or at least 100 fold. In some embodiments, modulation of a tumor-mediated immunosuppressive mechanism results in an increase in an immunostimulatory and/or anti-tumor immune response by 2-10, 2-20, 2-30, 2-40, 2-50, 2-60, 2-70, 2-80, 2-90, or 2-100 fold.

20 Non-limiting examples of immunostimulatory and/or anti-tumor immune mechanisms include T cell signaling, activity and/or recruitment, antigen presentation and/or processing, natural killer cell-mediated cytotoxic signaling, activity and/or recruitment, dendritic cell differentiation and/or maturation, immune cell recruitment, pro-inflammatory macrophage signaling, activity and/or recruitment, stroma degradation, immunostimulatory metabolite production, stimulator of interferon genes (STING) signaling (which increases the secretion of IFN and Th1 polarization, promoting an anti-tumor immune response), and/or Type I interferon signaling. An effector molecule may stimulate at least one (one or more) of the foregoing immunostimulatory mechanisms, thus resulting in an increase in an immunostimulatory response. Changes in the foregoing immunostimulatory and/or anti-tumor immune mechanisms may be assessed, for example, using *in vitro* assays for T cell proliferation or cytotoxicity, *in vitro* antigen presentation assays, expression assays (*e.g.*, of particular markers), and/or cell secretion assays (*e.g.*, of cytokines).

30 In some embodiments, modulation of a tumor-mediated immunosuppressive mechanism by at least one effector molecule results in a decrease in an immunosuppressive response (*e.g.*, systemically or in the tumor microenvironment) by at least 10% (*e.g.*, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, or 200%). For example, modulation of a tumor-mediated

immunosuppressive mechanism may result in a decrease in an immunosuppressive response by at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 100%. In some embodiments, modulation of a tumor-mediated immunosuppressive mechanism results in a decrease in an immunosuppressive response 10-20%, 10-30%, 10-40%, 10-50%, 10-60%, 10-70%, 10-80%, 10-90%, 10-100%, 10-200%, 20-30%, 20-40%, 20-50%, 20-60%, 20-70%, 20-80%, 20-90%, 20-100%, 20-200%, 50-60%, 50-70%, 50-80%, 50-90%, 50-100%, or 50-200%. It should be understood that “a decrease” in an immunosuppressive response, for example, systemically or in a tumor microenvironment, is relative to the immunosuppressive response that would otherwise occur, in the absence of the effector molecule(s).

In some embodiments, modulation of a tumor-mediated immunosuppressive mechanism by at least one effector molecule results in a decrease in an immunosuppressive response (*e.g.*, systemically or in the tumor microenvironment) by at least 2 fold (*e.g.*, 2, 3, 4, 5, 10, 25, 50, or 100 fold). For example, modulation of a tumor-mediated immunosuppressive mechanism may result in a decrease in an immunosuppressive response by at least 3 fold, at least 5 fold, at least 10 fold, at least 20 fold, at least 50 fold, or at least 100 fold. In some embodiments, modulation of a tumor-mediated immunosuppressive mechanism results in a decrease in an immunosuppressive response by 2-10, 2-20, 2-30, 2-40, 2-50, 2-60, 2-70, 2-80, 2-90, or 2-100 fold.

Non-limiting examples of immunosuppressive mechanisms include negative costimulatory signaling, pro-apoptotic signaling of cytotoxic cells (*e.g.*, T cells and/or NK cells), T regulatory (Treg) cell signaling, tumor checkpoint molecule production/maintenance, myeloid-derived suppressor cell signaling, activity and/or recruitment, immunosuppressive factor/metabolite production, and/or vascular endothelial growth factor signaling. An effector molecule may inhibit at least one (one or more) of the foregoing immunosuppressive mechanisms, thus resulting in a decrease in an immunosuppressive response. Changes in the foregoing immunosuppressive mechanisms may be assessed, for example, by assaying for an increase in T cell proliferation and/or an increase in IFN γ production (negative co-stimulatory signaling, T_{reg} cell signaling and/or MDSC); Annexin V/PI flow staining (pro-apoptotic signaling); flow staining for expression, *e.g.*, PDL1 expression (tumor checkpoint molecule production/maintenance); ELISA, LUMINEX®, RNA via qPCR, enzymatic assays, *e.g.*, IDO tryptophan catabolism (immunosuppressive factor/metabolite production); and phosphorylation of PI3K, Akt, p38 (VEGF signaling).

In some embodiments, effector molecules function additively: the effect of two effector molecules, for example, may be equal to the sum of the effect of the two effector molecules functioning separately. In other embodiments, effector molecules function synergistically: the effect of two effector molecules, for example, may be greater than the combined function of the two effector molecules.

Effector molecules that modulate tumor-mediated immunosuppressive mechanisms and/or modify tumor microenvironments may be, for example, secreted factors (*e.g.*, cytokines, chemokines, antibodies, and/or decoy receptors that modulate extracellular mechanisms involved in the immune system), inhibitors (*e.g.*, antibodies, antibody fragments, ligand TRAP and/or small blocking peptides), intracellular factors that control cell state (*e.g.*, microRNAs and/or transcription factors that modulate the state of cells to enhance pro-inflammatory properties), factors packaged into exosomes (*e.g.*, microRNAs, cytosolic factors, and/or extracellular factors), surface displayed factors (*e.g.*, checkpoint inhibitors, TRAIL), and and/or metabolic genes (*e.g.*, enzymes that produce/modulate or degrade metabolites or amino acids).

In some embodiments, at least one of the effector molecules stimulates an immunostimulatory mechanism in the tumor microenvironment and/or inhibits an immunosuppressive mechanism in the tumor microenvironment.

In some embodiments, at least one of the effector molecules (a) stimulates T cell signaling, activity and/or recruitment, (b) stimulates antigen presentation and/or processing, (c) stimulates natural killer cell-mediated cytotoxic signaling, activity and/or recruitment, (d) stimulates dendritic cell differentiation and/or maturation, (e) stimulates immune cell recruitment, (f) stimulates pro-inflammatory macrophage signaling, activity and/or recruitment or inhibits anti-inflammatory macrophage signaling, activity and/or recruitment, (g) stimulates stroma degradation, (h) stimulates immunostimulatory metabolite production, (i) stimulates Type I interferon signaling, (j) inhibits negative costimulatory signaling, (k) inhibits pro-apoptotic signaling of anti-tumor immune cells, (l) inhibits T regulatory (T_{reg}) cell signaling, activity and/or recruitment, (m) inhibits tumor checkpoint molecules, (n) stimulates stimulator of interferon genes (STING) signaling, (o) inhibits myeloid-derived suppressor cell signaling, activity and/or recruitment, (p) degrades immunosuppressive factors/metabolites, (q) inhibits vascular endothelial growth factor signaling, and/or (r) directly kills tumor cells.

In some embodiments, effector molecules may be selected from the following non-limiting classes of molecules: cytokines, antibodies, chemokines, nucleotides, peptides, and enzymes. Non-limiting examples of the foregoing classes of effector molecules are listed in **Table 1** and specific sequences encoding exemplary effector molecules are listed in **Table 2**.

Effector molecules can be human, such as those listed in **Table 1** or **Table 2** or human

equivalents of murine effector molecules listed in **Table 1** or **Table 2**. Effector molecules can be human-derived, such as the endogenous human effector molecule or an effector molecule modified and/or optimized for function, *e.g.*, codon optimized to improve expression, modified to improve stability, or modified at its signal sequence (*see below*). Various programs and algorithms for optimizing function are known to those skilled in the art and can be selected based on the improvement desired, such as codon optimization for a specific species (*e.g.*, human, mouse, bacteria, etc.).

In some embodiments, the effector molecule comprises interleukin 12 (IL-12), for example, p35 and p40 as a dimer that is generally referred to in the art as IL12p70. In some embodiments, the first effector molecule comprises an IL12p70 fusion protein. In some embodiments, the IL12p70 fusion protein is a human IL12p70 fusion protein. In some embodiments, the human IL12p70 fusion protein comprises the sequence shown in SEQ ID NO: 203.

In some embodiments, the effector molecule comprises interleukin 15 (IL-15). In some embodiments, the effector molecule consists of IL-15 (see, *e.g.*, SEQ ID NO: 199). In some embodiments, the effector molecule comprises a fusion protein including IL-15 and an extracellular portion of IL-15 Receptor α (IL-15R α), such as the sushi domain as shown in SEQ ID NO: 201. An exemplary IL-15/IL-15R α sushi domain fusion is provided as SEQ ID NO: 202. In some embodiments, the effector molecule includes IL-15 having the amino acid sequence

NWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGLDASIH
DTVENLILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS (SEQ ID
NO: 224). In some embodiments, IL-15 is encoded by a polynucleotide sequence comprising the sequence

AATTGGGTCAACGTGATCAGCGACCTGAAGAAGATCGAGGACCTGATCCAGAGCAT
GCACATCGACGCCACACTGTACACCGAGTCCGATGTGCACCCTAGCTGCAAAGTGA
CCGCCATGAAGTGCTTTCTGCTGGAAGTCAAGTGATCAGCCTGGAAAGCGGCGAC
GCCAGCATCCACGATACCGTGGAATCTGATCATCCTGGCCAACAACAGCCTGTC
CAGCAACGGCAATGTGACCGAGAGCGGCTGCAAAGAGTGCGAGGAACTGGAAGAG
AAGAACATCAAAGAGTTTCTGCAGAGCTTCGTCCACATCGTGCAGATGTTTCATCAA
CACCTCA (SEQ ID NO: 225). In some embodiments, IL-15 is encoded by a polynucleotide sequence that includes a sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to

AATTGGGTCAACGTGATCAGCGACCTGAAGAAGATCGAGGACCTGATCCAGAGCAT
GCACATCGACGCCACACTGTACACCGAGTCCGATGTGCACCCTAGCTGCAAAGTGA

CCGCCATGAAGTGCTTTCTGCTGGAAGTCAAGTGATCAGCCTGGAAAGCGGCGAC
GCCAGCATCCACGATACCGTGGAATCTGATCATCCTGGCCAACAACAGCCTGTC
CAGCAACGGCAATGTGACCGAGAGCGGCTGCAAAGAGTGCGAGGAACTGGAAGAG
AAGAACATCAAAGAGTTTCTGCAGAGCTTCGTCCACATCGTGCAGATGTTTCATCAA

5 CACCTCA (SEQ ID NO: 225).

In some embodiments, the membrane-cleavable chimeric protein includes the amino acid sequence

MDWTWILFLVAAATRVHSNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAM
KCFLLELQVISLESGDASIHDTVENLILANNSLSSNGNVTESGCKECEEELEEKNIKEFLQS
10 FVHIVQMFINTSSGGGSGGGSGVTPPIFSLIGGSGGGSGGGSLQLLPSWAITLISV
NGIFVICCLTYCFAPRCRERRRNERLRRESVRPV (SEQ ID NO: 226). In some
embodiments, the membrane-cleavable chimeric protein is encoded by a polynucleotide
sequence comprising the sequence

ATGGACTGGACTTGGATACTCTTTCTGGTCGCTGCCGCCACACGGGTGCACTCTAAT
15 TGGGTCAACGTGATCAGCGACCTGAAGAAGATCGAGGACCTGATCCAGAGCATGCA
CATCGACGCCACACTGTACACCGAGTCCGATGTGCACCCTAGCTGCAAAGTGACCG
CCATGAAGTGCTTTCTGCTGGAAGTCAAGTGATCAGCCTGGAAAGCGGCGACGCC
AGCATCCACGATACCGTGGAATCTGATCATCCTGGCCAACAACAGCCTGTCCAG
CAACGGCAATGTGACCGAGAGCGGCTGCAAAGAGTGCGAGGAACTGGAAGAGAAG
20 AACATCAAAGAGTTTCTGCAGAGCTTCGTCCACATCGTGCAGATGTTTCATCAACACC
TCATCAGGCGGCGGTGGTAGTGAGGCGGAGGCTCAGGCGTGACCCCTGAGCCTAT
CTTCAGCCTGATCGGCGGAGGTTCCGGAGGTGGCGGTTCCGGCGGAGGATCTCTTC
AATTGCTGCCTAGCTGGGCCATCACACTGATCTCCGTGAACGGCATCTTCGTGATCT
GCTGCCTGACCTACTGCTTCGCCCCTAGATGCAGAGAGCGGAGAAGAAACGAGCGG
25 CTGAGAAGAGAAAGCGTGCGGCCTGTG (SEQ ID NO: 227). In some embodiments, the
membrane-cleavable chimeric protein is encoded by a polynucleotide sequence that includes a
sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%,
at least 96%, at least 97%, at least 98%, or at least 99% identical to

ATGGACTGGACTTGGATACTCTTTCTGGTCGCTGCCGCCACACGGGTGCACTCTAAT
30 TGGGTCAACGTGATCAGCGACCTGAAGAAGATCGAGGACCTGATCCAGAGCATGCA
CATCGACGCCACACTGTACACCGAGTCCGATGTGCACCCTAGCTGCAAAGTGACCG
CCATGAAGTGCTTTCTGCTGGAAGTCAAGTGATCAGCCTGGAAAGCGGCGACGCC
AGCATCCACGATACCGTGGAATCTGATCATCCTGGCCAACAACAGCCTGTCCAG
CAACGGCAATGTGACCGAGAGCGGCTGCAAAGAGTGCGAGGAACTGGAAGAGAAG
35 AACATCAAAGAGTTTCTGCAGAGCTTCGTCCACATCGTGCAGATGTTTCATCAACACC

TCATCAGGCGGCGGTGGTAGTGAGGCGGAGGCTCAGGCGTGACCCCTGAGCCTAT
 CTTACAGCCTGATCGGCGGAGGTTCCGGAGGTGGCGGTTCCGGCGGAGGATCTCTTC
 AATTGCTGCCTAGCTGGGCCATCACACTGATCTCCGTGAACGGCATCTTCGTGATCT
 GCTGCCTGACCTACTGCTTCGCCCCCTAGATGCAGAGAGCGGAGAAGAAACGAGCGG
 5 CTGAGAAGAGAAAGCGTGCGGCCTGTG (SEQ ID NO: 227).

Table 1. Exemplary Effector Molecules

Effector name	Category	Function
anti-CD40 or CD40 Ligand	Agonist antibody	Stimulates B-cells and antigen presenting cells.
Flt3L	Ligand agonist	Stimulates myeloid cells and antigen presenting cells
CXCL10-11 fusion	Chemokine	Attracts T-cells
TGFb blocking peptides	Antagonist peptides	Inhibit TGFb pathway, TME modifier
Adenosine deaminase (ADA)	TME modifier	Degradation of suppressive adenosine in the TME
Kyneurinase	TME modifier	Degradation of kyneurine
HPGE2	TME modifier	Degradation of PGE2
CXCL13	Chemokine	Attracts B-cells
anti PD-1/PD-L1	Agonist antibody	Remove checkpoint
anti-CTLA-4	Agonist antibody	Remove checkpoint
anti-VEGF	Antagonist antibody	Neutralizes an immunosuppressive/angiogenesis factor
anti-TNFa	Antagonist antibody	Neutralizes cytokine/pro-tumor factor
anti-IL-10	Antagonist antibody	Neutralizes immunosuppressive cytokine
anti-SDF1/CXCL12	Antagonist antibody	Neutralizes pro-tumor chemokine
(TβRII)2 trap	Capture trap	Neutralizes an immunosuppressive cytokine
CCL21	Chemokine	Attracts leukocytes/NK
CCL1	Chemokine	Attracts leukocytes/NK
CCL17	Chemokine	Attracts leukocytes/NK
CCL19	Chemokine	Attracts leukocytes/NK
CCL21	Chemokine	Attracts leukocytes/NK
CCL20	Chemokine	Attracts leukocytes/NK
CCL21a	Chemokine	Attracts leukocytes/NK
MIP1b (CCL5)	Chemokine	Attracts leukocytes/NK
CXCL10	Chemokine	Attracts leukocytes/NK
CXCL11	Chemokine	Attracts leukocytes/NK
CCL2	Chemokine	Attracts monocytes
MIP-1alpha (CCL3)	Chemokine	Attracts leukocytes/NK
XCL1	Chemokine	Attracts leukocytes/NK

Effector name	Category	Function
IFNbeta	Cytokine	T cell response, tumor cell killing
IFNgamma	Cytokine	T cell response, tumor cell killing
IL-12	Cytokine	T cells, NK cells
IL-1beta	Cytokine	T cells, NK cells
IL-15	Cytokine	Stimulates T-cells and NK
IL-2	Cytokine	Stimulates T-cells and NK
IL-21	Cytokine	Stimulates T-cells
IL-24	Cytokine	Stimulates T-cells
IL36-gamma	Cytokine	Stimulates T-cells
IL-7	Cytokine	Stimulates T-cells
IL-22	Cytokine	Stimulates T-cells
IL-18	Cytokine	Stimulates T-cells
Granzymes/Perforin	Enzyme	Direct tumor cell killing
OX86 (anti-OX40)	ligand	Stimulates T-cells
anti-TGFbeta	Neutralizing antibody	Neutralizes an Immunosuppressive cytokine
TRAIL	Receptor/ligand	Direct tumor cell killing
FASL (CD49L)	Receptor/ligand	Direct tumor cell killing
OX40-L	Receptor/Ligand	Stimulates T-cells
cGAS	secreted molecule	Stimulates antigen-presenting cells
41BBL	secreted molecule	Co-activation of T-cells
CD40L	secreted molecule	Stimulates T-cells
GM-CSF	secreted molecule	Growth factor for monocytes
STING	secreted molecule	Stimulates antigen-presenting cells
HAC-V 'microbody'_PD1	Antagonist antibody	inhibits checkpoint
yCD	Pro-drug	Converts to cytotoxic molecule upon activation
CpG/Nucleotides	Nucleotides	STING agonist

Table 2: Exemplary effector molecule sequences

IL-12 (Human) (SEQ ID NO: 56)
ATGTGCCATCAGCAGCTTGTTCATATCTTGGTTTTCACTTGTATTCTTGGCCAGCCCTTTGGTTGCG ATCTGGGAGCTCAAGAAGGATGTGTACGTTGTAGAGCTGGACTGGTACCCCGATGCTCCCGGTG AGATGGTCGTTTTGACATGTGACACTCCAGAAGAGGACGGTATTACGTGGACTCTGGACCAGTC CTCCGAAGTTCTTGGTTCTGGTAAGACTCTGACTATCCAGGTGAAAGAATTTGGGGATGCGGGAC AATACACATGCCACAAGGGAGGCGAGGTGTTGTCTCATAGTTTGCTGCTTCTCCACAAGAAAGA GGATGGAATCTGGAGCACCGACATACTCAAGGATCAAAAGGAACCCAAAAATAAGACATTTCTG CGATGTGAGGCTAAGAACTATAGTGGCCGCTTCACTTGTGGTGGCTGACTACCATCAGCACAG ATCTCACGTTTTTCAGTAAAAAGTAGTAGAGGTTCAAGTGATCCTCAAGGGGTAAACGTGCGGTGC TGCAACACTGTCTGTGAACGCGTAAGAGGAGATAATAAGGAGTACGAGTATTCCTAGAAATGC CAAGAGGACAGTGCTTGTCTGCGGCCGAGGAGTCTCTCCCAATAGAAGTGATGGTGGACGCGG TGCATAAACTGAAATATGAGAACTACACAAGCAGTTTTTTTATAAGAGATATCATCAAGCCCGA TCCGCCGAAGAATTTGCAACTTAAACCGCTTAAAAACTCACGCCAGGTGAAAGTATCCTGGGAG TATCCGGATACATGGTCAACACCACACAGCTATTTTCCCTTACCTTCTGTGTGCAGGTCCAAGG GAAGAGCAAAAGGGAGAAGAAGGACAGGGTATTCAGTATAAACTTCCGCGACGGTCATCTG CCGAAAAACGCTAGTATATCTGTACGGGCGCAGGATAGGTACTATAGTTCTTCTTGGTCTGAGT GGGCTCAGTTCCTGTCTTGGGGGAGGAAGTGGAGGAGGGTCCGGCGGTGGAAGCGGGGGAG

GGAGTCGCAACTTGGCAGTGGCTACACCAGATCCAGGCATGTTTCCATGTCTGCATCATTCCCAG
 AATCTCCTGAGAGCGGTGTCAAATATGCTCCAAAAAGCGAGACAAACACTGGAATTTTACCCGT
 GTACCAGTGAGGAGATTGATCACCAGGACATAACCAAGGACAAGACCTCAACTGTAGAAGCGT
 GTTTGCCGCTGGAGTTGACTAAGAATGAGTCCTGCCTCAATTCCAGAGAAACTTCATTCACTACT
 AACGGCAGTTGTCTTGCATCCCGGAAAACGTCCTTTATGATGGCCCTTTGCCTTAGTTCAATTTAC
 GAGGATCTTAAAAATGTATCAAGTGGAGTTTAAACCATGAATGCTAAACTTCTTATGGACCCCA
 AACGACAAATTTTTCTGGATCAGAATATGCTTGCCGTGATAGACGAACTCATGCAGGCGCTTAAT
 TTTAACTCCGAAACAGTTCCACAAAAATCTAGCCTTGAAGAACCTGATTTTTATAAAACGAAGAT
 TAAACTGTGTATCCTGCTGCATGCCTTTCGCATCCGAGCTGTCACAATCGATAGGGTTATGTCCT
 ACCTTAACGCGAGCtaG

IL-12p70 (Human; codon optimized; bold denotes signal sequence) (SEQ ID NO: 57)

ATGTGCCATCAGCAACTCGTCATCTCCTGGTTCTCCCTTGTTTCCTCGCTTCCCTCTGGT
CGCCATTTGGGAACTGAAGAAGGACGTCTACGTGGTTCGAGCTGGATTGGTACCCGGACGCCCC
GGAGAAATGGTCGTGCTGACTTGCGATACGCCAGAAGAGGACGGCATAACCTGGACCTGGATC
AGAGCTCCGAGGTGCTCGGAAGCGGAAAGACCCTGACCATTCAAGTCAAGGAGTTTCGGCGACGC
GGGCCAGTACACTTGCCACAAGGGTGGCGAAGTGTCTGTCCCACTCCCTGCTGCTGCTGCACAAG
AAAGAGGATGGAATCTGGTCCACTGACATCCTCAAGGACCAAAAAGAACCGAAGAACAAGACC
TTCTTCCGCTGCGAAGCCAAGAACTACAGCGGTTCGGTTACCTGTTGGTGGCTGACGACAATCTC
CACCGACCTGACTTTCTCCGTGAAGTCGTACGGGGATCAAGCGATCCTCAGGGCGTGACCTGTG
GAGCCGCACTCTGTCCGCGAGAGAGTCAAGGGGAGACAACAAGGAATATGAGTACTCCGTGG
AATGCCAGGAGGACAGCGCTGCCCTGCCGCGGAAGAGTCCCTGCCTATCGAGGTTCATGGTTCGA
TGCCGTGCATAAGCTGAAATACGAGAACTACACTTCCCTCCTTCTTTATCCGCGACATCATCAAGC
CTGACCCCCCAAGAACTTGCAGCTGAAGCCACTCAAGAACTCCCGCCAAGTGGAAAGTGTCTTG
GGAAATATCCAGACACTTGGAGCACCCCGCACTCATACTTCTCGCTCACTTTCTGTGTGCAAGTGC
AGGGAAAGTCCAAACGGGAGAAGAAAGACCGGGTGTTCACCGACAAAACCTCCGCCACTGTGA
TTTGTCCGAAGAACGCGTCAATCAGCGTCCCGGGCGCAGGATAGATACTACTCGTCCTCCTGGAG
CGAATGGGCCAGCGTGCCTTGTTCGGTGGCGGATCAGGCGGAGGTTTCAGGAGGAGGCTCCGGA
GGAGGTTCCCGGAACCTCCCTGTGGCAACCCCGACCTTGAATGTTCCCGTGCTACACCACTC
CCAAAACCTCCTGAGGGCTGTGTGCAACATGTTGCAGAAGGCCCGCCAGACCCTTGAGTTCTAC
CCCTGCACCTCGGAAGAAATTGATCACCAGGACATCACCAAGGACAAGACCTCGACCGTGGAAG
CCTGCCTGCCGCTGGAACCTGACCAAGAACGAATCGTGTCTGAACTCCCGCGAGACAAGCTTTAT
CACTAACGGCAGTGCCTGGCGTCGAGAAAGACCTCATTTCATGATGGCGCTCTCTTCTCCTCGA
TCTACGAAGACTGTGAAGATGTATCAGGTTCAGTTCAGAGCTTCAAGACCATGAACGCCAAGCTGCTCATGGA
CCCGAAGCGGCAGATCTTCTGGACCAGAATATGCTCGCCGTGATTGATGAACTGATGCAGGCC
CTGAATTTCAACTCCGAGACTGTGCCTCAAAAGTCCAGCCTGGAAGAACCGGACTTCTACAAGA
CCAAGATCAAGCTGTGCATCCTGTTGCACGCTTTCGCATTTCGAGCCGTGACCATTGACCGCGTG
ATGTCCTACCTGAACGCCAGT

IL-12 (Mouse) (SEQ ID NO: 58)

ATGTGTCCACAGAAGCTGACAATAAGTTGGTTTGCCATTGTCTCCTCGGTGAGCCCACTCATGGC
 AATGTGGGAACTCGAAAAGGATGTCTACGTGGTAGAAGTAGATTGGACTCCAGACGCGCCAGGG
 GAGACAGTGAATTTGACATGTGACACACCAGAAGAAGATGACATTACATGGACATCTGACCAAC
 GCCATGGCGTAATAGGGAGTGGGAAAACACTCACGATCACAGTTAAAGAGTTCTTGGATGCTGG
 TCAATATACTTGCCATAAAGGCGGCGAGACACTCAGCCACTCACATTTGCTTTTGCTAAAAAAG
 AGAATGGCATTTGGAGCACTGAAATACTTAAGAACTTTAAGAACAAAGACATTTCTCAAGTGTGA
 GGCCCCAATTACAGCGGCAGGTTACGTGCTCATGGCTGGTCCAGCGCAACATGGACCTCAAG
 TTTAACATAAAAATCTTCTTCTCTTACCTGACTCCAGAGCTGTTACTTGCGGCATGGCTTCTCTG
 AGCGCAGAAAAAGTAACGTTGGATCAAAGAGACTACGAAAAGTACTCTGTTTCTTGTCAAGAGG
 ATGTTACGTGCCGACGGCCGAAGAAACGCTTCCAATTGAACTCGCGTTGGAAGCTCGCCAACA
 AAACAAGTATGAAAACCTACAGTACAAGCTTCTTTATACGGGATATAATTAAACCCGATCCCCC
 AAGAACTTGCAAAATGAAACCACTTAAGAACAGCCAGGTGGAAGTTTCTGGGAGTATCCAGACT
 CATGGAGTACTCCTCACAGCTATTTTCTCTGAAATTTCTTTGTAAGGATACAACGGAAGAAAGAG
 AAGATGAAAGAGAGACGAGGAGGGTTGTAATCAGAAGGGAGCGTTTCTCGTGAGAAAAACGCTT
 ACCGAAGTCCAATGTAAAGGTGGCAATGTGTGCGTCCAAGCTCAGGATAGATACTATAATTCAA
 GTTGCTCCAAGTGGGCTGTGTTCCATGCCGCGTTCGGAGCGGGGGAGGTAGCGGAGGAGGTAG
 TGGGGGTGGGTTCAGGAGGAGGGAGTCGAGTTATCCCGGTGTCAGGCCCCGACGCTGCTTGAGC
 CAGAGTCGCAACCTCCTTAAGACAACAGATGACATGGTGAAAACAGCACGCGAAAAAGCTTAAA
 CACTACTCTTGTACGGCGGAGGATATTGATCACGAGGATATTACCCGAGACCAAACTAGCACTTT
 GAAAACCTGTCTGCCCCTTGAACTTCATAAAAATGAGAGCTGTCTGGCTACACGAGAGACGTCA
 AGTACGACTAGGGGCAGCTGTCTCCCGCCGCAAAAGACAAGCCTCATGATGACGCTCTGTTTGG

GTTCCATTTACGAGGACTTGAAAATGTATCAAACGGAGTTCCAGGCTATAAATGCGGCGTTGCA
GAACCATAACCATCAACAAATTATACTTGATAAAGGCATGTTGGTGGCGATTGATGAACATCATG
CAGAGTCTCAATCACAACGGGGAAAACGTTGAGACAGAAACCCCAAGTCGGTGAAAGCGGACCCA
TATCGAGTAAAAATGAAGCTCTGCATTTCTGCTTCACGCATTCAGCACTAGAGTTGTTACCATCAA
CCGGGTAAATGGGATATCTCTCCAGTGCGtaG

IL21 (Human; codon optimized; bold denotes signal sequence) (SEQ ID NO: 59)

ATGGAACGCATTGTGATCTGCCTGATGGTCATCTTCCTGGGCACCTT**AGTGCACAAGTCGA**
GCAGCCAGGGACAGGACAGGCACATGATTAGAATGCGCCAGCTCATCGATATCGTGGACCAGT
TGAAGAACTACGTGAACGACCTGGTGGCCGAGTTCTGCGGCCCCCGAAGATGTGGAAACCAA
TTGCGAATGGTCGGCATTTCCTGCTTTCAAAGGCACAGCTCAAGTCCGCTAACACCGGGAAACA
ACGAACGGATCATCAACGTGTCCATCAAAAAGCTGAAGCGGAAGCCTCCCTCCACCAACGCCGG
ACGGAGGCAGAAGCATAGGCTGACTTGCCCGTCATGCGACTCCTACGAGAAGAAGCCGCCGAA
GGAGTTCCTGGAGCGGTTCAAGTCGCTCCTGCAAAAGATGATTCATCAGCACCTGTCTCCCGGA
CTCATGGGTCGAGGATTCA

IL-12p70_T2A_IL21 (Human; codon optimized; bold denotes signal sequences) (SEQ ID NO: 60)

ATGTGCCATCAGCAACTCGTCATCTCCTGGTTCTCCCTTGTGTTCCCTCGCTTCCCTCTGGT
CGCCATTTGGGAACTGAAGAAGGACGTCTACGTGGTCGAGCTGGATTGGTACCCGGACGCCCCCT
GGAGAAATGGTCGTGCTGACTTGCGATACGCCAGAAGAGGACGGCATAACCTGGACCCTGGATC
AGAGCTCCGAGGTGCTCGGAAGCGGAAGACCCTGACCATTCAAGTCAAGGAGTTTCGGCGACGC
GGGCCAGTACACTTGCCACAAGGGTGGCGAAGTGTCTGCCACTCCCTGCTGCTGTCACAAG
AAAGAGGATGGAATCTGGTCCACTGACATCTCAAGGACCAAAAAGAACCGAAGAACAAGACC
TTCTCCGCTGCGAAGCCAAGAACTACAGCGGTTCGGTTACCTGTTGGTGGCTGACGACAATCTC
CACCGACCTGACTTTCTCCGTGAAGTCGTACGGGGATCAAGCGATCCTCAGGGCGTGACCTGTG
GAGCCGCCACTCTGTCCGCCGAGAGAGTCAGGGGAGACAACAAGGAATATGAGTACTCCGTGG
AATGCCAGGAGGACAGCGCCTGCCCTGCCGCGGAAGAGTCCCTGCCTATCGAGGTCATGGTTCGA
TGCCGTGCATAAGCTGAAATACGAGAACTACACTTCCTCCTTTTATCCGCGACATCATCAAGC
CTGACCCCCCAAGAACCTTGACGCTGAAGCCACTCAAGAAGTCCCGCCAAGTGGAAAGTGTCTTG
GGAATATCCAGACACTTGGAGCACCCCGCACTCATACTTCTCGCTCACTTTCTGTGTGCAAGTGC
AGGGAAAAGTCCAAACGGGAGAAGAAAGACCGGGTGTTCACCGACAAAACCTCCGCCACTGTGA
TTTGTTCGGAAGAACGCGTCAATCAGCGTCCGGGCGCAGGATAGATACTACTCGTCTCCTGGAG
CGAATGGGCCAGCGTGCCTTGTTCGGTGGCGGATCAGGCGGAGGTTTCAGGAGGAGGCTCCGGA
GGAGGTTCCCGGAACCTCCCTGTGGCAACCCCGACCCCTGGAATGTTCCCGTGCTACACCACTC
CCAAAACCTCCTGAGGGCTGTGTGCAACGTTTGCAGAAAGGCCCGCCAGACCCTTGAGTTCTAC
CCCTGCACCTCGGAAGAAATTTGATCACGAGGACATCACCAAGGACAAGACCTCGACCGTGGAAG
CCTGCCTGCCGCTGGAAGTGAACAAGAACGAATCGTGTCTGAACTCCCGCGAGACAAGCTTTAT
CACTAACGGCAGCTGCCTGGCGTCGAGAAAGACCTCATTCATGATGGCGCTCTGTCTTCTCTCGA
TCTACGAAGATCTGAAGATGTATCAGGTTCGAGTTCAAGACCATGAACGCCAAGCTGCTCATGGA
CCCGAAGCGGCAGATCTTCTGGACCAGAATATGCTCGCCGTGATTGATGAACTGATGCAGGCC
CTGAATTTCAACTCCGAGACTGTGCCTCAAAAGTCCAGCCTGGAAGAACCGGACTTCTACAAGA
CCAAGATCAAGTGTGTCATCTGTTGACGCTTTCGCGATTTCGAGCCGTGACCAATTGACCGGTG
ATGTCTACCTGAACGCCAGTAGACGGAACCGCGGAAGCGGAGAGGGCAGAGGCTCGCTGCTT
ACATGCGGGGACGTGGAAGAGAACCCCGGTCCGATGGAACGCATTGTGATCTGCCTGATGGT
CATCTTCCTGGGCACCTT**AGTGCACAAGTCGAGCAGCCAGGGACAGGACAGGCACATGATTA**
GAATGCGCCAGCTCATCGATATCGTGGACCAGTTGAAGA**ACTACGTGAACGACCTGGTGGCCGA**
GTTCTGCGGCCCCCGAAGATGTGGA**ACCAATTGCGAATGGTCGGCATTTCCTGCTTTCAA**
AGGCACAGCTCAAGTCCGCTAACACCGGGAACAACGAACGGATCATCAACGTGTCCATCAAAAA
GCTGAAGCGGAAGCCTCCCTCCACCAACGCCGACGAGGCAGAAAGCATAGGCTGACTTGCCCCG
TCATGCGACTCCTACGAGAAGAAGCCGCCGAAGGAGTTCTGGAGCGGTTCAAGTCGCTCCTGC
AAAAGATGATTCATCAGCACCTGTCTCCCGGACTCATGGGTCTGAGGATTCA

IL-12_2A_CCL21a (Human) (SEQ ID NO: 61)

ATGTGCCATCAGCAGCTTGTATATCTTGGTTTTCACTTGTATTCTGGCCAGCCCTTTGGTTGCG
ATCTGGGAGCTCAAGAAGGATGTGTACGTTGTAGAGCTGGACTGGTACCCCGATGCTCCCGGTG
AGATGGTTCGTTTTGACATGTGACACTCCAGAAGAGGACGGTATTACGTGGACTCTGGACAGTC
CTCCGAAGTTCTTGGTTCTGGTAAGACTCTGACTATCCAGGTGAAAGAATTTGGGGATGCGGGAC
AATACACATGCCACAAGGGAGGCGAGGTGTTGTCTCATAGTTTGCTGCTTCTCCACAAGAAAGA
GGATGGAATCTGGAGCACCGACATACTCAAGGATCAAAAGGAACCCAAAAAATAAGACATTTCTG
CGATGTGAGGCTAAGAAGTATAGTGGCCGCTTCACTTGTGGTGGCTGACTACCATCAGCACAG

ATCTCACGTTTTTCAGTAAAAAGTAGTAGAGGTTCAAGTGATCCTCAAGGGGTAACGTGCGGTGC
TGCAACACTGTCTGCTGAACGCGTAAGAGGAGATAATAAGGAGTACGAGTATTCGTAGAATGC
CAAGAGGACAGTGCTTGCTGCGGCCGAGGAGTCTCTCCCAATAGAAGTGATGGTGGACGCGG
TGCATAAACTGAAATATGAGAACTACACAAGCAGTTTTTTTATAAGAGATATCATCAAGCCCCGA
TCCGCCGAAGAATTTGCAACTTAAACCGCTTAAAAACTCACGCCAGGTTGAAGTATCCTGCGGAG
TATCCGGATACATGGTCAACACCACACAGCTATTTTTCCCTTACCTTCTGTGTGCAGGTCCAAGG
GAAGAGCAAAAAGGGAGAAGAAGGACAGGGTATTCACCTGATAAAAACCTCCGCGACGGTCATCTG
CCGAAAAAACGCTAGTATATCTGTACGGGCGCAGGATAGGTACTATAGTTCTTCTTGGTCTGAGT
GGGCTCAGTTCCGTGCTCTGGGGGAGGAAGTGGAGGAGGGTCCGGCGGTGGAAGCGGGGGAG
GGAGTCGCAACTTGCCAGTGGCTACACCAGATCCAGGCATGTTTCCATGTCTGCATCATTCCCAG
AATCTCTGAGAGCGGTGTCAAAATATGCTCCAAAAAGCGAGACAAACACTGGAATTTTACCCGT
GTACCAGTGAGGAGATTGATCACGAGGACATAACCAAGGACAAGACCTCAACTGTAGAAGCGT
GTTTCCCGCTGGAGTTGACTAAGAATGAGTCTGCTCAATTCCAGAGAACTTGCTCAGCTTACT
AACGGCAGTTGTCTTGCATCCCGGAAAAACGTCCTTTATGATGGCCCTTTGCCTTAGTTCAATTTAC
GAGGATCTTAAAAATGTATCAAGTGGAGTTTAAAACCATGAATGCTAAACTTCTTATGGACCCCA
AACGACAAATTTTTCTGGATCAGAATATGCTTGCCGTGATAGACGAACTCATGCAGGCGCTTAAT
TTAACTCCGAAACAGTTCCACAAAAATCTAGCCTTGAAGAACCTGATTTTTATAAAACGAAGAT
TAAACTGTGTATCCTGCTGCATGCCTTTCGCATCCGAGCTGTCACAATCGATAGGGTTATGTCT
ACCTTAACGCGAGCCGCGCAAGAGGGTTCCGGAGAGGGAAGGGGTAGTCTGCTCAGCTGCG
GCGATGTTGAAGAAAAATCCTGGTCCCATGGCGCAAAGTCTGGCTCTTTCACCTCTGATCCTGGTC
TTGGCCTTCGGGATTCCGAGGACCCAAGGAAGTGTGGTGGCGCCCAAGATTGTTGCCTTAAAT
ACAGCCAGCGGAAAAATACCCGCGAAAGTGGTCAGGAGTTATAGAAAACAGGAGCCTTCCCTGG
GTTGTAGTATCCCCGCCATACTTTTCTCCCGAGAAAACGGAGCCAGGCCGAACCTGTGCGCTGAC
CCTAAGGAACCTTTGGGTGCAACAACCTTATGCAACACCTGGATAAGACACCTTCTCCTCAAAAGC
CAGCTCAGGGGTGCGGAAAAGATAGAGGCGCCTCAAAAACCGGAAAAAAGGGCAAAGGTTCTA
AAGGATGTAAGCGGACTGAACGCTCTCAAACGCCTAAAGGGCCGtaG

IL-12_2A_CCL21a (Mouse) (SEQ ID NO: 62)

ATGTGTCCACAGAAGCTGACAATAAGTTGGTTTGCCATTGTCTCTCTGGTGAGCCCACTCATGGC
AATGTGGGAACTCGAAAAGGATGTCTACGTGGTAGAAGTAGATTGGACTCCAGACGCGCCAGGG
GAGACAGTGAATTTGACATGTGACACACCAGAAGAAGATGACATTACATGGACATCTGACCAAC
GCCATGGCGTAATAGGGAGTGGGAAAACACTCACGATCACAGTTAAAGAGTCTTGGATGCTGG
TCAATATACTTGCAATAAAGGCGGCGAGACACTCAGCCACTCACATTTGCTTTTGCAATAAAAAAG
AGAATGGCATTTGGAGCACTGAAATACTTAAGAACTTTAAGAACAAAGACATTTCTCAAGTGTGA
GGCCCCATAATTACAGCGGCAGGTTTACGTGCTCATGGCTGGTCCAGCGCAACATGGACCTCAAG
TTTAACATAAAAATCTTCTTCTCTCACCTGACTCCAGAGCTGTTACTTGCGGCATGGCTTCTCTG
AGCGCAGAAAAAGTAACGTTGGATCAAAGAGACTACGAAAAGTACTCTGTTTCTTGTCAAGAGG
ATGTTACGTGCCCCGACGGCCGAAGAAACGCTTCCAATTGAACTCGCGTTGGAAGCTCGCCAACA
AAACAAGTATGAAAACACTACAGTACAAGCTTCTTTATACGGGATATAATTAAACCCGATCCCCC
AAGAACTTGCAAAATGAAACCACTTAAGAACAGCCAGGTGGAAGTTTCTGGGAGTATCCAGACT
CATGGAGTACTCTCACAGCTATTTTCTCTGAAATTTCTTTGTAAGGATACAACGGAAGAAAGAG
AAGATGAAAGAGACCGAGGAGGGTTGTAATCAGAAGGGAGCGTTTCTCGTGGAGAAAACGTCT
ACCGAAGTCCAATGTAAAGGTGGCAATGTGTGCGTCCAAGCTCAGGATAGATACTATAATTCAA
GTTGCTCCAAGTGGGCTGTGTTCCATGCCGCGTTCGGAGCGGGGGAGGTAGCGGAGGAGGTAG
TGGGGGTGGGTCAGGAGGAGGGAGTCAAGTTATCCCGGTGTCAGGCCCCGCACGCTGCTTGAGC
CAGAGTCGCAACCTCCTTAAGACAACAGATGATCATGGTGAAAACAGCACGCGAAAAAGCTTAAA
CACTACTCTGTACGGCGGAGGATATTGATCACGAGGATATTACCCGAGACCAAACTAGCACTTT
GAAAACCTGTCTGCCCTTGAACCTTCATAAAAATGAGAGCTGTCTGGCTACACGAGAGACGTCA
AGTACGACTAGGGGCAGCTGTCTCCCGCCGAAAAGACAAGCCTCATGATGACGCTCTGTTTGG
GTTCCATTTACGAGGACTTGAAAATGTATCAAACGGAGTTCCAGGCTATAAATGCGGCGTTGCA
GAACCATAAACATCAACAAATTATACTTGATAAAGGCATGTTGGTGGCGATTGATGAACTCATG
CAGAGTCTCAATCAACCGGGGAAACGTTGAGACAGAAAACCCCAAGTCGGTGAGCGGACCCA
TATCGAGTAAAAATGAAGCTCTGCATTCTGCTTACGCATTACGACTAGAGTTGTTACCATCAA
CCGGGTAAATGGGATATCTCTCCAGTGCGCGGCGCAAGAGGGGTTCCGGAGAGGGAAGGGGTAG
TCTGCTCACCTGCGGCGATGTTGAAGAAAATCCTGGTCCCATGGCGCAAATGATGACCTTTCCC
TGCTGAGTCTTGCTCTGCGCTCTGCATCCCGTGGACGCAGGGGTCTGATGGGGGGGGCCAAGA
CTGTTGCCTGAAGTATTCACAAAAAAGATACCGTACTCTATTGTGAGAGGGTACAGGAAGCAA
GAACCTCCTTGGGTGCCCTATACCAGCAATTTCTTTCTCCCCACGCAAGCATTCCAAACCAGA
ACTGTGTGCGAACCCTGAGGAGGGTTGGGTACAGAACTTGATGCGAAGGCTTGACCAGCCCCCA
GCCCCTGGAAGCAGTCACCTGGGTGCAGAAAAAACAGAGGTACTTCAAAGAGCGGCAAGAAA
GGCAAAGGGAGTAAAGGATGTAAAAGAACGGAGCAGACCCAGCCTTACGAGGCTaG

CCL21a_2A_IL-12 (Mouse) (SEQ ID NO: 63)

ATGGCGCAAATGATGACCCCTTTCCCTGCTGAGTCTTGTCTCGCGCTCTGCATCCCCGTTGGACGCA
GGGGTCTGATGGGGGGGCAAGACTGTTGCCTGAAGTATTACAAAAAAGATAACCGTACTCT
ATTGTGAGAGGGTACAGGAAGCAAGAACCCTCCTTGGGTTGCCCTATACCAGCAATTCTTTCTC
CCCACGCAAGCATTCCAAACCAGAACTGTGTGCGAACCCCGAGGAGGGTTGGGTACAGAACTTG
ATGCGAAGGCTTGACCAAGCCCCAGCCCCCTGGCAAGCAGTCACCTGGGTGCAGAAAAAACAGA
GGTACTTCAAAGAGCGGCAAGAAAGGCAAGGGAGTAAAGGATGTAAAGAACGGAGCAGAC
CCAGCCTTACAGAGGCCGGCGCAAGAGGGGTTCCGGAGAGGGAAGGGGTAGTCTGCTCACCTGC
GGCGATGTTGAAGAAAATCCTGGTCCCATGTGTCCACAGAAGCTGACAATAAGTTGGTTTGCCA
TTGTCTCCTGGTGAGCCCACTCATGGCAATGTGGGAACTCGAAAAGGATGTCTACGTGGTAGA
AGTAGATTGGACTCCAGACGCGCCAGGGGAGACAGTGAATTTGACATGTGACACACCAGAAGA
AGATGACATTACATGGACATCTGACCAACGCCATGGCGTAATAGGGAGTGGGAAAAACTCACG
ATCACAGTTAAAGAGTTCTTGGATGCTGGTCAATATACTTGCCATAAAGCGCGCAGACACTCA
GCCACTCACATTTGCTTTTGCCATAAAAAAGAGAATGGCATTTGGAGCACTGAAATACTTAAGAA
CTTTAAGAACAAGACATTTCTCAAGTGTGAGGCCCTAATTACAGCGGCAGGTTACGTGCTCAT
GGCTGGTCCAGCGCAACATGGACCTCAAGTTTAACATAAAAATCTTCTCCTTTCACCTGACTCC
AGAGCTGTTACTTGCGGCATGGCTTCTCTGAGCGCAGAAAAAGTAACGTTGGATCAAAGAGACT
ACGAAAAGTACTCTGTTTCTGTCAAGAGGATGTTACGTGCCCCGACGGCCGAAGAAACGCTTCC
AATTGAAGTCTCGGTTGGAAGCTCGCCAACAAAACAAGTATGAAAACACAGTACAAGCTTCTTT
ATACGGGATATAATTAACCCCGATCCCCCAAGAACTTGCAAATGAAACCACTTAAGAACAGCC
AGGTGGAAGTTTCTGGGAGTATCCAGACTCATGGAGTACTCCTCACAGCTATTTTCTCTGAAA
TTCTTTGTAAGGATACAACGGAAGAAAGAGAAGATGAAAGAGACCGAGGAGGGTGTAAATCAG
AAGGAGCGGTTTCTCGTGGAAGAAAACGTTACCGAAGTCCAATGTAAAGGTGGGAATGTGTGCG
TCCAAGCTCAGGATAGATACTATAATTCAAGTTGTCCAAGTGGGCTGTGTTCCATGCCGCGTT
CGGAGCGGGGAGGTAGCGGAGGAGGTAGTGGGGGTGGGTGACGAGGAGGGAGTCTGAGTTATC
CCGGTGTGAGGCCCCGACGCTGCTTGAGCCAGAGTCGCAACCTCCTTAAGACAACAGATGACA
TGGTGA AAAACAGCACGCGAAAAGCTTAAACACTACTCTTGTACGGCGGAGGATATTGATCACGA
GGATATTACCCGAGACCAAACTAGCACTTTGAAAACCTGTCTGCCCTTGAACCTCATAAAAATG
AGAGCTGTCTGGCTACACGAGAGACGTCAAGTACGACTAGGGGCAGCTGTCTCCCGCCGCAAAA
GACAAGCCTCATGATGACGCTCTGTTTGGGTTCATTTACGAGGACTTGAAAATGTATCAAACGG
AGTTCCAGGCTATAAATGCGGCGTTGCAGAACCATTAACCATCAACAAATTATCTTGATAAAGG
CATGTTGGTGGCGATTGATGAACTCATGCAGAGTCTCAATCACAACGGGGAAACGTTGAGACAG
AAACCCCCAGTCGGTGAAGCGGACCCATATCGAGTAAAAATGAAGCTCTGCATTCTGCTTCACG
CATTACGACTAGAGTTGTTACCATCAACCGGGTAATGGGATATCTCTCCAGTGCGtaG

IL7 (Mouse) (SEQ ID NO: 64)

ATGTTTCATGTGTCCTTCAGGTACATATTTGGTATCCCACCCTTATATTGGTGCTCTTGCTGTA
ACCAGCTCTGAATGTATATAAAAAGACAAGGAGGGCAAAGCATACGAGTCCGTATTGATGATCT
CAATCGATGAACCTTGACAAGATGACAGGGACCGATTCTAATTGTCCAAATAACGAGCCAACTT
CTTTCGGAAACACGTGTGTGATGATACAAAAGAAGCTGCTTTCTTAACAGAGCTGCCAGAAAA
CTCAAGCAGTTCTCAAGATGAATATATCCGAGGAATTTAACGTGCATCTCCTCACAGTATCTCA
GGGAACCTCAAACCTTGTAACTGCACTTCTAAGGAGGAGAAGAATGTCAAAGAGCAGAAGAA
AAATGATGCATGTTTGTGAAACGGCTGTTGAGGGAGATCAAAACATGCTGGAATAAAATCCTC
AAGGGCTCAATTtaG

IL-15 (Human) (SEQ ID NO: 65)

ATGGAAACAGACACATTGCTGCTTTGGGTATTGTTGCTCTGGGTGCCTGGATCAACAGGAACTG
GGTAAACGTAATTTACAGATCTGAAGAAGATCGAGGACCTTATTCAATCCATGCACATCGATGCC
ACTCTCTACACCGAAAGCGACGTTACCCATCTTGCAAGGTGACCGCTATGAAATGTGAATTGTT
GGAACCTCAGGTAATTTCTCTGGAGAGCGGCGATGCCTCAATACATGACACCGTTGAAAATCTTA
TCATCCTTGCTAATGATTCACTCTCTAGTAATGGGAACGTAACAGAGAGCGGGTGTAAAGGAGTG
TGAAGAACTGGAGGAGAAAAACATTAAGGAATTTTGCAGTCATTCTGTCATATAGTGCAAATG
TTCATAAACACTTCCAGAAGAAAGCGAGGCTCTGGGGAGGGGCGAGGCTCTCTGCTGACCTGTG
GGGATGTAGAAGAGAATCCAGGTCCCATGGACCGGCTGACCAGCTCATTCTGCTTCTGATTGTG
CCAGCCTACGTGCTCTCCATCACATGTCTCCCCCAATGAGCGTCGAGCATGCTGACATCTGGGT
GAAGTCATACTCCTTGTACAGCAGAGAGAGATACATTTGTAATTCCGGATTCAAGCGCAAGGCC
GGCACCTCCTCTCTGACAGAGTGCGTCTTAACAAGCAACCAACGTAGCACATTGGACCACAC
CATCCTTGAAAGTGATACGAGAACCTAAATCTTGCGATAAGACTCATACTGTGTCACCTGTGTC
GCCCCAGAACTGCTTGGCGGACCCTCAGTATTTTGTTCACCAAGCCAAAAAGACACACTCAT
GATATCCAGAACCTCTGAGGTGACCTGTGTGCTGTAGACGTTTCCACGAAGATCTGAAGTAA

AATTCAACTGGTACGTGGATGGGGTCGAAGTCCATAACGCCAAGACTAAACCAAGGGAGGAAAC
AGTATAACTCTACTTACCGAGTAGTTTCTGTGTTGACCGTGCTGCACCAGGACTGGTTGAACGGG
AAGGAGTACAAATGCAAGGTGAGCAATAAAGCTCTGCCCCGACCAATCGAAAAGACAATATCT
AAGGCCAAGGGGAGCCACGAGAGCCCCAGGTATACACACTGCCACCTCACGCGATGAATTG
ACTAAGAACCAGGTTTCCCTGACCTGTCTTGTAAGGTTTCTACCCTTCCGACATAGCTGTTGA
GTGGGAAAGTAACGGGCAGCCAGAGAACAATTACAAGACAACCTCCACCCGTTCTTGATAGCGAT
GGATCATTTTTTCTGTATTCCAAACTCACTGTCGATAAAAGTCGCTGGCAGCAAGGCAATGTTTT
TAGCTGCTCAGTCATGCACGAAGCACTGCATAATCACTACACACAAAAAAGTTTGTCCCTTAGCC
CTGGTAAGtaG

IL-15 (Human) (SEQ ID NO: 66)

ATGTACTCAATGCAGTTGGCCTCCTGTGTAACATTGACCTTGGTCCCTCTTGGTCAACAGCAATTG
GATCGATGTACGCTACGACTTGGAGAAGATTGAGTCCCTTATACAGAGTATACACATAGATACA
ACCTTGATACACTGACAGTGACTTCCATCCAGCTGTAAAGTGACTGCAATGAACGTTTGTGTT
GGAGTTGCAAGTAATTCTGCATGAATACAGCAACATGACCCTCAATGAAACCGTTAGGAATGTC
CTTTATCTCGCAAATTCTACTCTGAGTAGCAATAAGAATGTTGCCGAAAGCGGCTGCAAGGAGT
GCGAAGAACTGGAGGAAAAAACTTTCACCGAGTTTCTCCAGAGTTTCATCAGAATTGTCCAAAT
GTTTCATTAATACAAGTAGTGGTGGTGGGAGCGGGGTGGAGGCAAGTGGGGGAGGTGGGAGCGG
AGGTGGAGGGTCCGGAGGGGGGAGCCTTCAAGGCACTACTTGTCTCCACCCGTATCCATCGAG
CACGCCGATATTCGAGTTAAAAATTATAGTGTTAATAGCAGAGAACGATACGTCTGCAACTCAG
GGTTTAAGAGAAAGGCCGGAACCTCAACTCTCATAGAATGCGTGATTAATAAGAATACTAACTG
CGCACATTGGACTACTCCAGTCTCAAGTGCATACGCGATCCATCTCTCGCTCATTACTACCAG
TACCTACAGTGTTACTCCTAAGGTGACCTCTCAGCCCGAATCACCATCTCCCAGCGCAAAAGAG
CCTGAGGCCTTTTCTCCTAAATCAGACACTGCTATGACTACAGAAACAGCCATAATGCCAGGAA
GCCGGCTGACACCATCTCAAACTACCAGCGCAGGCACAACCTGGGACTGGCTCCCACAAAAGCTC
ACGCGCACCAAGTCTCGCCGCAACAATGACATTGGAGCCTACAGCCAGCACATCTCTTAGAATC
ACAGAAATTCTCCCCACAGTAGCAAGATGACCAAGGTGGCAATTAGTACCAGCGTCCTTCTGT
AGGAGCTGGAGTTGTGATGGCATTTTTGGCATGGTATATCAAAAGCAGGtaG

IL-15 (Mouse) (SEQ ID NO: 67)

ATGAAGATCCTCAAGCCATACATGCGAAACACTAGTATTAGCTGTTACTTGTGTTTTCTGCTGAA
TAGTCATTTTTTGACTGAAGCAGGAATCCATGTATTTATACTCGGTTGTGTGCTGTAGGTCGCG
AAAGACTGAGGCTAATTGGATTGACGTGCGCTATGATCTTGAAAAAATAGAGTCCTTGATTCAA
TCAATACACATCGATACCACTCTCTACACCGACAGTGATTTCCATCCTTCTGCAAGGTAACAGC
TATGAATTGCTTCTCCTGGAGCTCCAAGTCACTTCCATGAGTACTCCAACATGACTTTGAACG
AAACTGTAAGAAACGTATTGTATCTGGCTAATAGCACCTTGTCTAGTAACAAAAATGTGGCAGA
GAGCGCTGCAAGAATGTGAAGAATTGGAAGAGAAAACATTTACAGAGTTCTGCAATCCTTT
ATTCGCATCGTCCAAATGTTTATCAATACCTCTtaG

IL-15 (Mouse) (SEQ ID NO: 68)

ATGTATTCCATGCAACTTGCCAGTTGTGTAACCCCTACTCTCGTCCCTGCTCGTTAATTCCGCTGGT
GCTAACTGGATAGATGTTTCGATACGATCTGGAAAAGATTGAGTCCCTTATCCAATCCATTATAT
AGATACCACCTTTTATACTGACAGCGACTTCCATCCTTCTTGCAAGGTGACCGCTATGAATTGTT
TCCTGCTGGAACCTCAAGTTATTCTGCATGAATACTCTAATATGACACTTAACGAGACCGTAAGA
AATGTTCTCTATCTCGCTAATAGTACTTTGAGCTCAAATAAGAACGTGGCCGAGTCTGGGTGTAA
GGAATGCGAAGAGCTGGAAGAAAAGACATTCACCGAGTTTCTCCAGTCTTTCATACGGATTGTG
CAGATGTTTATCAACACATCAGATTACAAAGACGACGATGATAAGtaG

IL-18 (Mouse) (SEQ ID NO: 69)

ATGGCAGCCATGTCTGAGGACTCTTGTGTGAACCTTTAAAGAAATGATGTTTCATAGACAATACACT
CTACTTTATACCTGAGGAGAATGGAGATTGGAATCTGACAACCTTTGGCAGGCTGCATTGCACTA
CCGCAGTTATCCGAAACATCAACGATCAGGTACTGTTGTTGATAAAAAGACAACCTGTATTTCGAG
GACATGACCGACATAGATCAGTCTGCCTCAGAGCCCCAGACTAGGCTTATCATCTATATGTACAA
GGACAGCGAAGTACGAGGCCTGGCTGTTACACTCTCAGTCAAAGACTCTAAGATGAGCACCCCTG
TCATGCAAGAACAAAATTATCAGTTTGGAGAGATGGACCCACCTGAAAACATAGATGACATTC
AGTCAGACCTCATTTTTTTTCAAAAGCGGGTACCAGGACACAACAAAATGGAATTTGAATCATC
ACTCTACGAAGGACATTTCTTGCATGCCAGAAAGAGGATGACGCATTCAAATTGATCCTGAAA
AAAAAGGACGAAAATGGTGATAAATCAGTCATGTTTACATTGACCAATCTTACCAAAGTtaG

IL-18 (Mouse) (SEQ ID NO: 70)

ATGGCTGCAATGTCTGAAGATAGCTGTGTCAACTTTAAGGAGATGATGTTTCATTGATAATACTTT GTACTTTTATACCTGAAGAAAATGGAGACCTTGAGTCAGACAACCTTCGGGAGACTGCACTGCACA ACTGCCGTTATCCGAAACATAAAATGATCAAGTATGTTCGTGGACAAAAGACAACCAAGTCTTTG AGGATATGACAGACATCGACCAATCCGCATCTGAACCTCAGACTAGGCTGATCATCTATATGTA CGCCGACTCCGAAGTAAGAGGCCCTTGCTGTGACACTTAGTGTTAAGGATAGTAAGATGAGCACA CTGTCTGTGAAGAATAAGATTATATCTTTTGAAGAGATGGACCCTCCCGAGAACATAGATGACAT CCAGAGCGACTTGATCTTCTTTCAGAAGCGAGTGCCAGGCCATAACAAGATGGAATTTGAATCA TCTCTTTATGAAGGCCATTTCCCTCGCATGTCAAAAGGAGGACGATGCCTTCAAGCTCATTCTGAA AAAAAAGACGAGAACGGTGATAAGAGCGTGATGTTCACTCTGACAAATCTGCACCAGTCAtaG
IL-18 (Human) (SEQ ID NO: 71)
ATGTATCGCATGCAACTCCTGTCTGCATTGCTCTGAGCTTGGCTTTGGTAACCAACTCATACTTC GGGAACTGGAGAGTAAACTCTCCGTAATCAGGAATCTTAATGACCAAGTATTGTTTATTGACC AGGGCAACCGCCCGTTGTTTCGAGGATATGACTGATTCTGACTGTCGGGATAACGCTCCGAGAAC TATCTTTATCATTTCAATGTACAAGGACAGCCAACCGCGGGTATGGCTGTGACAATCAGATGTCA AATGTGAGAAGATTTCCACGCTGTCTGCGAAAAACAAGATAATTTCTTTCAAAGAAATGAACCC CCCTGACAATATAAAGGATACAAAGAGTGATATCATCTTCTTTTCAGAGGTCCGTGCCCGGCCAC GATAATAAGATGCAATTTGAAAGTTCATCTTATGAGGGGTACTTTTGGCATGCGAGAAAGAAA GGGATCTCTTCAAGTTGATCCTGAAGAAGGAGGACGAATTGGGCGACCGCTCCATCATGTTAC AGTCCAGAACGAGGACtaG
IL-18 (Human) (SEQ ID NO: 72)
ATGTACCGCATGCAGCTCCTGAGTTGTATTGCCCTTTCCCTCGCTCTCGTTACCAATTCTTACTTC GGTAAGCTTGCCTCTAACTCTCTGTTATTAGGAACCTGAACGACCAAGTCTTTTTCATAGACCA AGGGAACAGACCACTGTTTGAAGATATGACGGATAGCGATTGCCGAGATAATGCCCTTAGGACG ATTTTTATCATTAGTATGTATGCGGACTCTCAACCGAGGGGGATGGCCGTTACTATAAGTGTGAA ATGCGAGAAAAATCAACGCTCAGTTGTGAGAACAAAATCATAAGTTTCAAGGAGATGAATCCA CCTGATAACATCAAAGACACTAAGTCTGATATTATTTTCCAACGAAGTGTTCCGGGACACGA TAACAAAATGCAATTTGAGAGCTCCTCATACGAGGGCTACTTCTCGCGTGTGAGAAAAGAAAGG GATTTGTTTAAAGCTTATCCTCAAGAAAGAGGACGAGTTGGGGGATCGGAGCATAATGTTTACCG TACAGAATGAGGACtaG
IL-21 (Mouse) (SEQ ID NO: 73)
ATGGAGCGGACACTCGTGTGTCTTGTCTGTAATTTTTCTCGGGACAGTCGCACACAAGTCCTCACC CCAGGGTCTTGATCGCTTCTCATACGCCTCCGACATTTGATCGACATTTGTAGAGCAGCTCAAAA TTTACGAGAATGACCTCGATCCCGAGCTTTTGAGTGCTCCCCAAGACGTTAAGGGTCATTGCGAG CAGCGAGCTTTTGCTTGCTTCCAGAAGGCCAAGTTGAAACCAAGCAACCTGGTAATAATAAGA CTTTCATCATCGACTTGGTCGCCCCAACTCCGAAGGAGGCTGCCTGCCCGGCGCGGAGGAAAAAA ACAAAAGCATATTGCAAAGTGTCTTCATGTGATTCATACGAAAAGCGGACTCCCAAAGAGTTC TTGGAAAGGTTGAAATGGCTTCTTCAGAAGATGATTCATCAACATTTGTCAtaG
IFN-beta (Human) (SEQ ID NO: 74)
ATGACCAACAAATGCCTTTTGCAAAATTGCCCTGCTTTTGTGTTTATGCACTACCGCATTGAGCAT GTCATATAACCTCCTCGGCTTCCTTCAGAGATCATCAAACCTTCAGTGTGAGAACTGCTTTGGC AACTTAATGGCAGGCTCGAATATTGTCTGAAAGATCGGATGAATTTGACATTTCCAGAAGAAAT AAAACAGCTTCAACAATTCCAGAAAGAGGACGCCGCCCTGACTATTTACGAGATGCTCCAGAAT ATCTTCGCCATTTTCCGGCAGGACAGCTCATCCACGGGGTGAATGAGACTATTGTAGAAAATCT TCTGGCTAATGTGTACCATCAAATTAATCACCTCAAACGGTGCTTGAGGAAAAACTTGAAAAG GAAGATTTACACGGGGCAAGTTGATGTCTCCCTGCACCTTAAACGATACTACGGCAGGATTCT TCATTACTTGAAGGCTAAGGAGTATAGCCATTGCGCGTGACAAATTGTACGGGTAGAAATACTG CGAAACTTTTATTTTCATCAACCGGCTCACTGGATACCTTAGAAAATtaG
IFN-beta (Mouse) (SEQ ID NO: 75)
ATGAACAATCGGTGGATACTCCACGCCGCATTTCTCTCTGCTTTAGCACGACGGCCCTGTCCAT CAACTACAAACAGCTTCAGTTGCAGGAGCGGACTAACATAAGGAAGTGCCAGGAACTGCTGGA ACAGCTTAATGGTAAAATTAATCTTACATACCGAGCTGACTTCAAAATTCCTATGGAAATGACCG AGAAGATGCAGAAATCCTACACGGCATTCGCCATCCAGGAAATGCTCCAGAACGTATTTCTCGT GTTCCGCAATAATTTCTTCTACGGGTGGGAACGAAACCATTGTTGTTAGACTGCTTGACGAAC TGCATCAGCAAACCGTGTTCCTTAAACCGTGCTTGAGGAGAAAGCAGGAGGAGCGCCTGACTTG GGAGATGTCTAGTACCGCACTTCACTTGAAATCCTACTACTGGCGCGTTCAGCGGTATCTGAAGC

TGATGAAGTATAA CT CATACGCCTGGATGGTAGTGC GCGCAGAGATCTTCAGAACTTTCTTATC ATCCGGCGACTGACCCGAACTTTCAAGAA TtaG
IFN-gamma (Human) (SEQ ID NO: 76)
ATGAAGTACACTAGCTATATATTGGCCTTCCAGCTTTCATCGTATTGGGTAGCCTCGGATGCTA TTGCCAAGACCCGTATGTCAAAGAAGCCGAAAAATCTCAAAAAGTATTTCAATGCCGGACACTCA GACGTCGCGGATAACGGTACACTGTTTCTTGGCATCCTGAAAAATTGGAAGGAAGAGAGTGACA GAAAAATAATGCAGTCACAAATAGTGTCTTTTACTTTAAGCTGTTCAAAAATTTCAAGGATGAC CAAAGTATCCAGAAGAGTGTTGAAACTATCAAAGAGGACATGAATGTGAAATTTCTTTAACAGTA ATAAGAAGAAGCGCGATGACTTCGAGAACTCACTAATTACAGCGTAACGGATCTTAACGTCCA ACGCAAGGCAATCCACGAGCTTATACAGGTAATGGCTGAGCTTAGTCCCGCAGCCAAGACAGGG AAGAGAAAAAGGTCTCAAATGCTTTTTCGGGGCCGGCGAGCTTCACAAtaG
IFN-gamma (Mouse) (SEQ ID NO: 77)
ATGAACGCTACGCATTGCATCCTCGCACTCCAATTGTTCTCATGGCTGTGT CAGGGTGT TACTG TCACGGTACTGT CATAGAAAGCCTCGAATCCCTGAATACTATTTTAACAGTAGCGGTATAGATG TAGAAGAAAAAGTCTCTCTTTCTTGACATCTGGAGGAATTGGCAAAAGGATGGAGACATGAAGAT TCTCCAATCTCAGATTATATCATTTTACTTTGAGGCTTTTGGAGGTTCTGAAGGATAACCAGGCCA TCAGCAATAATATCAGCGTAATTGAATCTCACCTTATTACAACATTTTCTCAAATTTCCAAGGCA AAGAAAGATGCTTTCATGTCTATCGCGAAATTTGAGGTGAACAATCCCTCAGGTACAAAGGCAAG CCTTTAACGAGCTGATTAGAGTTGTACATCAGTTGTTGCCCGAAAGTAGTCTTAGAAAAACGCAAA CGGAGCCGATGCTaG
IFN-alpha (Mouse) (SEQ ID NO: 78)
ATGGCAAGGTTGTGCGCTTTTCTCATGGTACTGGCTGTGCTCTCCTATTGGCCTACTTGT TCTCTG GGATGCGACTTGCCACAGACCCACAATCTGCGGAATAAGAGGGCTCTGACTCTGCTGGTGCAAA TGAGACGGCTCTCTCCACTTAGCTGTTGAAAGATAGAAAGGATTTCCGGGTTC CCCCAGGAGAA GGTGGATGCCCAGCAGATCAAGAAGGCACAGGCTATCCCCGTCCTTTCCGAGCTGACCCAGCAA ATTTTGAACATCTTTACAAGTAAGGATAGTT CAGCTGCATGGAATACCACACTTTTGGATTCTTTT TGTAACGATCTGCATCAGCAGCTGAACGATCTCCAGGGATGCCTGATGCAGCAAGTCGGCGTGC AAGAATTTCCACTCACCCAGGAGGACGCTGTGCGCTGACCCTAAGGAAC TTTGGGTGCAACAAC T GTGTACCTCCGGGAGAAAAAGCATTCACCCTGCGCTTG GGAAGTAGTCAGGGCCGAAGTATGGA GAGCCCTTAGTAGCTCCGCTAATGTACTGGGCGGTTGCGGGAAGAGAAAtaG
CCL21 (Human) (SEQ ID NO: 79)
ATGGCGCAAAGTCTGGCTCTTTCACTCCTGATCCTGGTCTTGGCCTTCGGGATTCCGAGGACCCA AGGAAGTGATGGTGGCGCCCAAGATTGTTGCCTTAAATACAGCCAGCGGAAAAATACCCGCGAAA GTGGTCAGGAGTTATAGAAAACAGGAGCCTTCCCTGGGTTGTAGTATCCCCGCCATACTTTTCCT CCCGAGAAAAACGGAGCCAGGCCGAAC TGTGCGCTGACCCTAAGGAAC TTTGGGTGCAACAAC T ATGCAACACCTGGATAAGACACCTTCTCCTCAAAAAGCCAGCTCAGGGCTGCCGAAAAGATAGAG GCGCTCAAAAACCGGAAAAAAGGGCAAAGGTTCTAAAGGATGTAAGCGGACTGAACGCTCTC AAACGCCTAAAGGGCCGtaG
CCL21a (Mouse) (SEQ ID NO: 80)
ATGGCGCAAATGATGACCTTTCCCTGCTGAGTCTTGTCTCGCGCTCTGCATCCCGTGGACGCA GGGGTCTGATGGGGGGGGCCAAAGACTGTTGCCTGAAGTATTCACAAAAAAGATACCGTACTCT ATTGTCAGAGGGTACAGGAAGCAAGAACCCTCCTTGGGTTGCCCTATACCAGCAATTTCTTCTC CCCACGCAAGCATTTCAAACCAGAACTGTGTGCGAACC CCGAGGAGGGTTGGGTACAGAACTTG ATGCGAAGGCTTGACCAGCCCCCAGCCCCTG GCAAGCAGTCACCTGGGTGCAGAAAAAACAGA GGTACTTCAAAGAGCGGCAAGAAAGGCAAAGGGAGTAAAGGATGTA AAGAACGGAGCAGAC CCAGCCTTACGAGGCTaG
Tail-less CCL21 (Human) (SEQ ID NO: 81)
ATGGCGCAAAGTCTGGCTCTTTCACTCCTGATCCTGGTCTTGGCCTTCGGGATTCCGAGGACCCA AGGAAGTGATGGTGGCGCCCAAGATTGTTGCCTTAAATACAGCCAGCGGAAAAATACCCGCGAAA GTGGTCAGGAGTTATAGAAAACAGGAGCCTTCCCTGGGTTGTAGTATCCCCGCCATACTTTTCCT CCCGAGAAAAACGGAGCCAGGCCGAAC TGTGCGCTGACCCTAAGGAAC TTTGGGTGCAACAAC T ATGCAACACCTGGATAAGACACCTTCTCCTCAAAAAGCCAGCTCAGGGCTaG
Tail-less CCL21 (Mouse) (SEQ ID NO: 82)

ATGGCGCAAATGATGACCCCTTCCCTGCTGAGTCTTGTCTCGCGCTCTGCATCCCGTGGACGCA GGGGTCTGATGGGGGGGGGCAAGACTGTTGCCCTGAAGTATTCACAAAAAAGATACCGTACTCT ATTGTCAGAGGGTACAGGAAGCAAGAACCCTCCTTGGGTGCCCCTATACCAGCAATTCTTTCTC CCCACGCAAGCATTCCAAACCAGAACTGTGTGCGAACCCTGAGGAGGGTTGGGTACAGAACTTG ATGCGAAGGCTTGACCAGCCCCCAGCCCTGGCAAGCAGTCACCTGGGtaG
CCL19 (Mouse) (SEQ ID NO: 83)
ATGGCACCCCGCTCACACCCCTTGCTTGCTTTTTCTCTGCTTGCTCTGGACCTTCCCCGCTCCT ACCCTTGAGGAGCCAATGATGCCGAGGATTGCTGCCCTGAGTGTTACACAAAGGCCAATACCAG GGAATATAGTGAAGGCATTCCGGTATCTGCTCAATGAAGATGGGTGCAGAGTCCCCGAGTTGT CTTTACAACATTGCGAGGTTACCAGCTTTGTGCTCCCCCAGACCAGCCTTGGGTAGATCGCATT TTCGCCGTTGAAGAAGAGCTCAGCAAAGAATAAGGGCAATTCCACACGGAGAGCCCCGCTC CtaG
CCL19 (Mouse) (SEQ ID NO: 84)
ATGAAATCAGCAGTCCCTTTCTTGCTCGGGATTATTTTCTGGAACAATGTGGAGTGAGGGGAAC ACTCGTAATAAGAAACGCTCGGTGCTCATGCATATCAACATCACGGGGCAGTATCCACTACAAA TCCCTGAAGGATCTGAAGCAGTTCGCCCCAAGCCCTAACTGTAACAAGACCGAAATTATCGCAA CTCTCAAAAATGGAGATCAGACTTGTCTTGACCCAGATTGAGCAAAATGTCAAGAAGCTGATGAA AGAGTGGGAAAAGAAGATTTCACAAAAAAGCAAAAACGCGGCAAGAAACATCAAAAAGA ACATGAAAAACAGGAAACCTAAGACTCCCCAGTCAAGGAGAAGATCCCCGAAGACAACCTaG
CXCL11 (Mouse) (SEQ ID NO: 85)
ATGAACAGAAAAAGTTACCGCTATAGCACTTGCTGCCATAATATGGGCCACCGCAGCTCAAGGGT TCCTGATGTTCAAGCAGGGCCGATGCCCTCTGCATTGGCCCTGGAATGAAGGCCGTGAAAATGGC CGAAATAGAAAAAGCTAGTGTATATACCCCTCTAACGGTTGCGATAAAGTCGAGGTTATAGTC ACAATGAAAGCTCATAAACGCCAACGCTGCCCTCGACCCCGGTCTAAGCAGGCTAGGCTCATAA TGCAAGCAATCGAGAAGAAAACTTTCTTAGACGGCAAAACATGtaG
CXCL10 (Mouse) (SEQ ID NO: 86)
ATGAACCCATCTGCCGCCGTTATTTCTGTCTGATACTCCTTGGGCTGAGTGGCACACAAGGCAT ACCCCTCGCCCGCAGTCCGGTGTAATTGTATACATATTGACGACGGCCCTGTTAGAATGCGGG CCATCGGTAAGCTGGAGATTATACCAGCAAGCCTTAGTTGTCCAGGGTTGAAATCATAGCAAC TATGAAAAAAACGACGAACAAAGATGTTTGAATCCCGAGAGCAAGACAATCAAAAACCTTAT GAAAGCATTTAGTCAAAAACGCTCTAAACGCGCTCCaG
CXCL10 (Human) (SEQ ID NO: 87)
ATGAATCAGACGGCAATCCCTATATGCTGCCCTTATATTCCTTACTCTCTCAGGGATACAAGGGT ACCACTTTCTCGGACTGTTGCTGCACTTGCAATTCAATATCTAACCAACCTGTAAATCCGCGGA GCCTGGAAAAATGGAGATTATACCTGCTTCTCAATTCTGCCCTCGGGTGGAATCATCGCCACT ATGAAGAAGAAAGGGCGAGAAAAGGTGTCTGAATCCAGAGTCAAAGGCAATCAAAAACCTGCTG AAAGCGGTGTCAAAGGAACGGTCCAAGAGATCACCCtaG
CXCL11-CXCL10 (Mouse) (SEQ ID NO: 88)
ATGAACAGGAAAGTAACAGCCATTGCATTGGCTGCCATCATCTGGGCCACCGCAGCACAGGGT TTCTGATGTTTAAGCAAGGGCGCTGTCTCTGTATAGGCCAGGCATGAAGGCCGTGAAGATGGC AGAGATTGAGAAGGCATCTGTGATTATCCTTCTAACGGGTGCGATAAAGTCGAAGTTATTGTGA CAATGAAGGCACACAAAACGCCAACGGTGTTTGACCCACGATCTAAACAGGCAAGATTGATTAT GCAAGCCATCGAGAAAAAGAACTTTCTCCGAAGGCAAAATATGATCCCTTTGGCTCGGACAGTG CGGTGTAAGTGTATTACATCGACGATGGGCCAGTACGGATGAGAGCAATAGGAAAGCTCGAAA TCATACCCGCTCATTGTCTTGTCCAGGGTGGAATAATCGCCACTATGAAAAAGAACGATGA ACAGAGGTGTCTCAACCCAGAGAGTAAGACTATCAAGAACCTTATGAAGGCATTGAGTCAGAAG AGGTCAAAGCGAGCACCAaG
XCL1 (Human) (SEQ ID NO: 89)
ATGAGACTTCTCATATTGGCGCTTCTCGGGATATGTTCTCTTACGGCATACATAGTTGAGGGGGT GGGATCTGAGGTTAGCGATAAACGAACCTGTGTAGTCTTACAACACAGAGGCTTCCAGTCTCCA GGATAAAAAACATACGATAACTGAGGGATCTCTCAGAGCGGTATCTTCATAACGAAGAGGGG CCTGAAGGTCTGTGCTGACCCACAAGCGACTTGGGTAAAGGACGTTGTGCGGAGCATGGACAGG

AAGAGCAATACTCGCAACAACATGATCCAAACCAACCTACGGGCACCCAACAGTCAACCAAT ACTGCGGTAACATTGACGGGGtaG
XCL1 (Mouse) (SEQ ID NO: 90)
ATGCGCCTCCTTCTGCTGACTTTTCTGGGTGTATGTTGCCTGACACCCTGGGTCTAGAAAGGAGT AGGAACCGAGGTTCTGGAAGAGTCTCATGTGTAAACCTGACAGACACAACGACTCCCCGTCCAA AAAATCAAGACCTATATAATCTGGGAGGGGGCAATGCGGGCCGTCATTTCTGTGACTAAACGAG GTCTCAAAATCTGCGCCGACCCCGAGGCTAAGTGGGTGAAGGCAGCCATTAAGACCGTGGATGG GAGAGCCAGCACCAGAAAGAACATGGCCGAAACAGTACCTACTGGCGCACAGCGGTCAACCTC AACTGCTATAACCTTGACAGGAtaG
m_sCD40L #1 (SEQ ID NO: 91)
ATGGAGACTGACACTCTGCTTCTGTGGGTGTTGCTGCTGTGGGTGCCTGGCAGTACAGGCGATAT GCAACGAGGTGACGAGGACCCCTCAAATCGCCGCCCATGTAGTCTCTGAAGCTAATAGCAACGCT GCATCCGTCTTGACAGTGGGCAAAGAAAGGCTACTATACTATGAAGTCCAACCTTGGTAATGCTTG AAAACGGCAAGCAGTTGACTGTCAAGAGAGAGGGGACTTTATTACGTCTATACCCAAGTCACATT CTGTAGCAATCGAGAACCCTCCTCACAGAGGCCCTTTATAGTGGGACTCTGGCTTAAACCAAGTA GCGGCTCTGAGCGCATACTGTTGAAAGCCGCAAACACACACAGCTCTTCCCAACTCTGCGAGCA GCAATCCGTGCATCTCGGTGGAGTATTTGAGCTTCAAGCCGGTGCCTCAGTGTGTTGTGAACGTCA CTGAGGCCTCCAGGTCATACATCGAGTTGGGTTTCAGTCTCTTCGGCTTGCTCAAGCTCtaG
m_sCD40L #2 (SEQ ID NO: 92)
ATGGAACTGATACATTGCTGCTCTGGGTTTTGCTGCTCTGGGTGCCTGGGAGTACAGGCGACAT GAGGAGGCAGTTTCGAGGATCTCGTTAAGGATATTACCCTTAATAAGGAGGAGAAGAAAGAAAA CTCTTTTGAGATGCAACGAGGGGACGAAGATCCTCAGATCGCTGCTACCGTGGTCTCTGAAGCTA ACAGCAACGCCGCTTCTGTCTCCAGTGGGCCAAGAAAGGTTATTACACCATGAAATCAAACCT TGTAATGCTTGAAAACGGGAAACAGCTTACAGTGAAGAGGGAAAGGCTTTACTACGTCTATACC CAGGTAACCTTCTGCTCAAACAGAGAACCATCAAGCCAGAGGCCATTATAGTGGGGCTCTGGC TCAAACCTTCCAGTGGCAGCGAGAGAATCTTGTGAAAGCTGCTAATACACATAGTAGTAGCCA GCTTTGCGAGCAACAGTCAGTCCACCTCGGGGGGGTGTGTTGAGTTGCAAGCAGGGGCCCTCAGTA TTCGTGAATGTCACTGAGGCTTCCCAGGTAATTCACAGGGTAGGCTTTAGTTTATTTCGGTTTGT GAAGCTTtaG
m_sCD40L #3 (SEQ ID NO: 93)
ATGCGAAGAATGCAGCTTCTGCTCCTTATTGCTCTGAGTCTCGCCCTTGTACCAACTCCGGGGA CAGAATGAAACAAATCGAGGACAAAATTGAAGAAATACTGAGTAAAATATATCACATCGAAAA CGAAATTGCACGCATTAAGAAATTGATTGGCGAACGCACCAAGTGGCGGGCTCTGGTGGCACCGGA GGTTACAGGCGGGACCGGGGGCTCTGACAAAGTCGAAGAGGAGGTTAACCTTCATGAGGACTTTG TGTTTCATCAAGAAGCTGAAACGGTGCAATAAAGGAGAAGGTTCTTTGAGCCTCCTTAATTGCGA AGAGATGCGACGACAGTTTCGAGGATCTGGTTAAGGACATTACACTTAATAAGGAAGAGAAAAA GGAGAACTCTTTGAAATGCAGCGCGGCGATGAAGATCCCCAGATAGCCGCCCATGTGCTCTCT GAGGCCAACTCTAACGCAGCATCCGTCTCCAGTGGGCTAAGAAAGGATATTATACTATGAAAA GCAATTTGGTCATGCTCGAAAACGGTAAACAGCTCACTGTGTTAAGAGAGAAGGCCTCTATTACGT ATATACTCAAGTAACTTTCTGTTCTAATAGGGAACCCTCCTCTCAAAGACCTTTTATCGTAGGAC TCTGGTTGAAACCAAGTAGCGGTAGTGAAAGGATTCTGCTCAAAGCAGCTAATACTCACTCCAG CAGTCAACTGTGCGAACAACAAAGCGTTACCTCGGGGGCGTCTTTGAACTTCAGGCAGGTGCC AGTGTGTTTCGTCAACGTAACAGAAGCATCCCAGGTAATTCATCGAGTAGGGTTTTCTAGCTTTGG TTTGTGAAGCTGtaG
anti-CD40_FGK4.5 (SEQ ID NO: 94)
ATGGAACTGATCGCCTGTTGCTCTGGGTACTTCTTCTGTGGGTGCCTGGGTCCACTGGTGACAC TGTACTTACACAATCACCCGCTTTGGCCGTTTCTCTGGTGAACGGGTCACAATTAGTTGCCGAG CTTCCGATTCTGTATCTACTCTTATGCATTGGTATCAACAAAAACCTGGTCAGCAGCCAAAATTG CTCATTTATCTTGCTAGTCACTTGGAGTCCGGCGTACCTGCTCGATTTCAGCGGTAGTGGGTCTGG CACAGATTTCACTTTGACCATAGATCCCGTGGAGGCCGATGACACTGCAACCTACTATTGCCAGC AATCCTGGAACGACCTTTGGACTTTGCGCGGCGGCACCAAGCTGGAACCTCAAGCGAGCAGATGC TGCCCCAACCGTTAGTATATTCCACCCCTCAACCGAACAACCTCGCCACAGGAGGCGCTAGTGTG GTGTGCTTATGAACAATTTCTATCCACGAGACATTAGCGTCAAGTGGAAAAATTGATGGGACAG AAAGGCGAGATGGAGTTTTGGATTCAAGTAACAGACCAGGATTCAAAGGATTCTACCTATAGCAT GAGCTCCACCTTGAGCCTGACCAAAGCTGATTATGAATCTCATAACCTGTATACTTGTGAAGTGG

TGCATAAGACTTCTAGCTCACCAGTGGTTAAATCTTTTAACCGCAACGAATGTCGGCGCAAGAG
GGGTTCCGGAGAGGGAAGGGGTAGTCTGCTCACCTGCGGCGATGTTGAAGAAAATCCTGGTCCC
ATGGACATTCGGCTCTCTTTGGTATTCTTGGTACTTTTATAAAGGGGGTGCAATGTGAAGTCCA
GCTCGTGGAAGCGGTGGGGGCTGGTTCAGCCCGGTTCGCAGCCTTAAACTTAGTTGCGCAGCA
TCCGGATTTACATTTTCTGACTATAACATGGCCTGGGTTCGACAGGCACCCAAAAAAGGGCTGG
AGTGGGTCGCAACTATCATATACGATGGTTCCTGGGACATACTATAGAGATTCAAGTGAAGGGCG
CTTTACAATAAGCAGGGACAATGCTAAGTCTACCTTGTATCTTCAGATGGACTCCCTGAGGAGCG
AAGATACAGCAACATATTATTGTGCTACAAACCGCTGGTTGCTGCTTCATTATTTGACTACTGG
GGTCAGGGCGTCATGGTAAGTGTATCAAGCGCCGAGACCACAGCCCCTTCTGTATATCCATTGGC
ACCAGGTACTGCTCTGAAATCCAACCTCAATGGTAACCTTGGATGTCTGGTTAAGGGTTATTTTC
CCGAGCCCGTCACAGTTACTTGAACCTCTGGGGCCCTTTCTAGCGGAGTCCATACCTTTCCCGCC
GTTTTCAGAGTGGTCTGTACACCTTACCTCAAGCGTCACAGTTCATCTAGCACATGGAGCTC
CCAGGCAGTAACCTTGTAAATGTGGCCCATCCAGCCTCTCAACTAAGGTAGATAAAAAAGATCGTT
CCCAGAGAATGCAATCCATGTGGATGCACCGGGTCTGAGGTTCAGCAGTGTGTTTATTTCCACC
CAAGACTAAAGATGTATTGACTATTACTCTTACACCCAAAGTAACCTGCGTGGTGGTTGATATTA
GTCAAAATGATCCCGAGGTACGGTCTCTTGGTTTATCGACGACGTGCAAGTACATACAGCTCAG
ACACACGCTCCCGAGAAACAAAGCAATTCACCTCTTAGGAGCGTGTCCGAGTTGCCAATCGTAC
ATAGGGATTGGCTTAATGGCAAGACCTTTAAGTGTAAGGTCAATTCAGGGGCATTCCCCGCACC
AATAGAGAAGAGTATAAGCAAACCCGAGGGGACACCCAGAGGTCCACAGGTCTATACAATGGC
TCCCCCAAGGAAGAGATGACCCAAAGTCAAGTCTCAATTACATGTATGGTGAAGGGCTTTTAT
CCACCCGACATATACACTGAGTGGAAGATGAATGGACAGCCCCAAGAGAATTATAAAAACTC
CCCCTACCATGGACACCGACGGGTCTTATTTCTTTATAGTAAATTGAACGTGAAAAAGGAGACC
TGGCAACAAGGCAACACTTTCACCTGCTCCGTTCTTACGAGGGCCTGCATAATCATCATACCGA
AAAGTCTCTCAGTCATTCTCCAGGTAAGtaG

CD40L_2 (Human) (SEQ ID NO: 95)

ATGGAACAGATACGTTGCTGTGTGGGTACTTCTCCTTTGGGTCCCTGGCAGCACAGGGGACGA
GAATAGTTTCGAAATGCAGAAGGGCGACCAGAACCCACAGATCGCGGCTCACGTTATATCAGAA
GCAAGTAGTAAGACCACTTCCGTACTTCAGTGGGCTGAAAAAGGATATTACACCATGTCCAACA
ATCTCGTGACACTGGAGAACGGTAAACAACCTTACGGTGAAACGACAGGGCCTCTATTACATCTA
CGCTCAGGTGACATTCTGCTCAAATAGGGAGGCTTCTAGTCAAGCGCCCTTCATCGCCAGCCTGT
GCCTCAAATCTCCGGCCGGTTTGAACGAATCCTGTTGCGAGCGGCCAATACCCATAGCTCAGCT
AAACCTTGCGGCCAGCAGAGTATTCATCTTGGTGGTGTGTTGAACCTCAGCCGGGAGCATCTGT
GTTCTGCAACGTAAACGGACCCTAGCCAAGTGTCTCATGGGACAGGTTTACATCCTTCGGACTCC
TCAAGTTGtaG

Flt3L (Human) (SEQ ID NO: 96)

ATGACAGTTCTCGCGCCAGCTTGGAGTCCCAACCATACTTGTCTTTGCTTCTGCTTCTGTCTCT
GGCCTGAGTGGGACCCAAGATTGTTCCCTTTCAACATTCCCCAATTAGTTCTGATTTTGCAGTGAA
GATTAGAGAGCTCTCAGACTATCTGCTGCAAGATTATCCTGTACAGTCGCTTCAAACCTGCAAG
ACGAAGAGCTCTGCGGTGCCTTGTGGCGGTGGTCTTGGCTCAAAGATGGATGGAGAGACTGAA
AACCGTAGCAGGCAGCAAGATGCAGGGTCTCCTGGAAAGGGTGAACACGGAAATGCCATTTTGTG
ACCAAGTGCGCGTTCCAGCCCCACCGAGTTGTCTCCGGTTTGTTCAAACGAATATATCCCGGTT
GCTCCAGGAAACCTCAGAACAACTGGTGGCTTTGAAACCCTGGATCACAAGACAAAACCTTAGT
CGGTGCCTCGAACTCCAGTGCCAACCGAGATCTTCTACACTTCCCCCCCCGTGGTCCCCGCGCCC
GTTGGAAGCAACGGCCCCAtaG

TGFb TRAP (Human) (SEQ ID NO: 97)

ATGGCCTGGAGTCTCTGTTTCTGACTCTTATAACTCACTGTGCCGGCAGTTGGGCTATACCCCT
CATGTACAGAAGTCTGTAAACAACGACATGATTGTAACCGACAATAATGGCGCAGTGAAATTCC
CACAACCTGTGTAAGTTCTGTGATGTACGGTTTAGTACATGCGACAATCAAAAAAGCTGTATGTCT
AACTGCTCTATTACATCCATATGTGAAAAACCTCAGGAGGTGTGTGTTGCCGTTTGGCGAAAAAA
TGATGAGAATATCACACTGGAGACAGTATGTATGACCTAAACTGCCATACCATGATTTCATAC
TGGAGGACGCCGCCAGTCCCTAAGTGCATTATGAAAGAGAAAAAGAAACCCGGTGAAACATTCTT
TATGTGCTCTTGTAGCTCTGACGAGTGTAAACGACAACATTATATTCAGCGAGGAGTACAATACAA
GCAACCCCGATATACCACTCAGTACAAAAAAGTGTCAACAACGATATGATTGTTACCGACAA
TAACGGAGCTGTAAAGTTTCTCAGTTGTGCAAGTTCTGCGATGTACGATTCTCTACCTGCGACA
ACCAAAAGTCATGTATGTCTAACTGTTCCATAACCTCCATCTGCGAGAAGCCCCAGGAAGTCTGC
GTCGCCGTGTGGCGGAAAAACGACGAGAATATCACTCTTGAACCGTTTGTATGATCCTAAAC
TGCCCTATCACGACTTTATTCTGGAAGATGCTGCTTCCCCCTAAGTGTATCATGAAAGAAAAGAAG

AAACCTGGGGAGACATTCTTTATGTGTTTCATGCTCCTCCGATGAGTGTAACGACAATATCATCTTCTCTGAGGAATACAACACTTCTAACCTGATtaG
Fresolimumab (Human) (SEQ ID NO: 98)
ATGGCCTGGTCCCCTCTTTTCTGACCCTCATCACACACTGTGTCAGGCTCATGGGCTGAGACCGTCTTGACCCAGTCCCCAGGAACCTTTGTCTCTGTCTCCGGTGAAGAGCTACCCCTAGTTGTCTGAGCCTCTCAGTCCCTTGGTTCTAGCTATCTCGCTTGGTACCAGCAAAAGCCAGGCCAGGCCCCACGACTGCTGATCTACGGAGCATCTTCACGGGCTCCCGGCATTCCCGATCGATTTCCGGATCTGGTAGTGGTACAGATTTACACTGACCATATCTCGCCTGGAGCCCGAGGACTTTGCTGTTTATTATTGTCAGCAGTACGCCGATTCTCTATCACTTTTGGACAGGGAACCCGCTGGAGATTAAGCGCACAGTAGCAGCTCCATCCGCTCTTATCTTTCCACCATCAGATGAACAGCTCAAGAGTGGGACCGCAAGTGTAGTATGCCTGCTGAACAATTTTTACCCTAGAGAGGCCAAAGTGCAGTGGAAGGTGGATAACGCCTCCAGAGTGGCAATAGTCAAGAAAGTGTTACTGAGCAAGATAGTAAGGACTCTACATACTCTTTGAGTTCTACTTTGACCCTGTCAAAAGCAGATTATGAAAAACATAAGGTGTATGCATGTGAAGTTACACACCAAGGGTTGTCTCTCCAGTTACAAAATCTTTTAATAGAGGAGAGTGGCCGCCGCAAAACGCGGTAGTGGAGAAGGTCGAGGCTCACTCTTGACCTGTGGCGACGTGGAAGAAAAATCCCGGTCCTATGGATTGGACTTGGAGGGTATTTTGTCTTTTGGCAGTAACACCTGGAGCTCACCCCCAAGTACAGCTCGTCCAATCTGGTGCCGAGGTTAAAAAGCCTGGAAGTTCAGTGAAGGTCTCTTGCAAGGCATCTGGATACACCTTTTCACTAACGTCATATCCTGGGTACGGCAAGCCCCAGGACAGGGACTTGAGTGGATGGGAGGGGTCATCCCCATCGTGGACATTGCTAATTACGCTCAGCGATTCAAAGGGGGGTTACTATAACTGCCGACGAGTCTACCTCAACTACCTACATGGAGTTGTCTCTCTCCGCTCCGAGGACACTGCTGTATATTACTGTGCCAGCACTCTCGGGTTGGTGTGGATGCCATGGACTATTGGGACAAGGAACCCCTGGTGACAGTTAGCTCCGCAAGCACTAAAGGCCCTTCTGTTTTTCCCTTGGCACCTTGTAGTAGGTCTACCTCTGAGTCTACAGCAGCACTTGGATGCTTGGTTAAGGACTATTTTCCCGAGCCAGTTACAGTCTCTTGGAACAGTGGTGCCCTCACAAGTGGGGTTCATACCTTCCCCGCAGTCTCCAGAGTAGTGGCCTTTACAGCCTCTCATCAGTTGTGACTGTTCCCTAGTTTCATCACTCGGTACTAAGACATATACATGTAACGTAGACCACAAGCCAAGCAACACAAAAGTAGACAAACGAGTCGAATCTAAGTATGGACCCCTTGTCCCTCTGTCTGCTCCCGAGTTCCCTTGGGGGCCCTTCCGTTTCTTGTCTTCTCCCAAGCCCAAGGATACCCTCATGATCTCACGAACCCCAAGGTAACATGTGTGGTTGTTGACGTAAGTCAGGAAGATCCCGAAGTGCAATTTAATTGGTACGTGGATGGCGTCGAAGTCCATAACGCTAAAAACAAAACCCCGAGAGGAACAATTCAATTCACATATCGGGTGGTGAAGTATTGACCGTTCTTCACCAAGATTGGCTGAACGGCAAGGAGTATAAGTGTAAAGTAAGCAACAAGGCTGCAAGTATGCAAAAAACAGATATCTAAAGCTAAGGGCCAACCAAGGGAACCAACAGTATATACATTGCCCCCTCTCAGGAAGAGATGACAAAGAATCAAGTTAGCCTGACCTGTTTGGTAAAGGGGTTCTATCCCTCAGATATAGCAGTCGAGTGGGAATCTAACGGCCAGCCCGAGAATAATTATAAAACAACCCCCCTGTGTTGGACTCAGACGGCAGCTTCTTTCTCTATTACGGCTCACGTGTGATAAGTCCCGATGGCAGGAGGGGAATGTTTTCAGCTGTAGCGTGATGCACGAAGCTCTCCACAACCACTATACACAGAAAAGTTTGTCTTTGTCCCTTGGAAAAtaG
TGFb neutralizing peptide (Human) (SEQ ID NO: 99)
ATGAGTACATCCTTTCCAGAGCTGGATCTGGAGAATTTTGAAGTATGACGACAGTGCCGAAGCCTGCTACCTCGGGGACATAGTCGCATTTCGGGACAACTCTTTTGTCTGTATTTTACGCCCTGGTGTTCATTTGGCCTGGTTGGAAATCTGTTGGTCTGACTCGCTCTCACCAATTCCCGAAAACCCAAAAGTATAACAGACATATACCTGTTGAATCTGGCACTGAGTGACCTTTTGTTCGTCGCCACCCTTCCTTTTGGACACACTACCTTATCAGTCACGAGGGGCTTCATAATGCTATGTGCAAGCTCACTACTGCCTTCTTCTTTATCGGATTCTTCGGGGGTATCTTTTTATCACAGTTATTAGCATTGACCGATACCTTGCATAGTGTCTCGCAGCCAACTCAATGAACAACCGCACCGTGCAGCATGGAGTGACTATTTCTTGGTGTGGGCCGCTGCTATACTTGTGCGCAGCCCTCAATTCATGTTTACCAAAAAGGAAAGACAAAGAGTGCCTCGGAGATTACCCTGAGGTGTTGCAAGAAATGTGGCCTGTACTTCGAAATAGCGAAGTGAATATACTCGGCTTTGCTCTTCCTCTGCTCATCATGTCATTCTGTTATTTTCGAATAATCCAAACATTGTTTCAGCTGTAAGAACCGAAAGAAAGCCCGCGCCGTACGCCTGATTCTGCTCGTTGTGTCGCCTTTTCTGTTTTGGACTCCTTACAACATAATGATATTCCTGGAGACTCTCAAAATCTATAACCTTTTTTCCCTCCTGTGATATGAAAAGGGACCTTAGATTGGCTCTCAGTGTCACTGAAACAGTAGCCTTAGCCATTGTTGTCTCAACCCTTCATATATGCAATTTGCAGGGGAAAAGTTCCGGCGGTATCTCGGACATTTGTATCGGAAGTGCTTGGCCGTGTTGTGTGGTATCCTGTCCATACCGGATTCTCTCCTGAGAGTCAACGGAGCCGCAAGATTCAATCCTGTCCAGTTTCACTCACTATACTTCAGAGGGGATGGCAGCCTTCTGCTC
Kyneurinase #1 (SEQ ID NO: 100)

ATGGAGACCGACACTTTGTTGCTGTGGGTACTTTTGTGTGGGTCCCAGGATCTACCGGGGATAT
GGAACCTCTCCTCTTGAAGTCCAGTAGACGCCGTGCGCCGCATTGCAGCCGAGTTGAATTGCG
ATCCAACAGATGAACGCGTTGCCCTGAGGCTCGACGAAGAGGATAAAATTGTCACATTTTCAGGAA
CTGCTTTTACATTCCAAAGATGAGGGATCTTCCATCCATAGATCTTAGCCTCGTGTCCGAGGATG
ACGATGCCATATATTTTCTTGGGAACAGTCTTGGGTTCAGCCAAAAATGGTACGGACATATCTC
GAAGAGGAGCTGGACAAATGGGCTAAAATGGGTGCTTACGGCCACGACGTGGGAAAACGCCCC
TGGATAGTTGGCGACGAATCTATCGTGAGTCTTATGAAAAGATATAGTTGGAGCACATGAGAAAG
AAATTGCACTGATGAATGCCCTTACTATCAATCTGCATCTCCTCTTGCTTTTATTCTTTAAGCCCA
CTCCTAAACGCCACAAAATACTTTTGGGAAGCAAAAGCCTTTCCAAGCGACCACTACGCTATTGA
GTCACAAATACAACCTCCATGGACTTGATGTGGAAAAGTCTATGCGGATGGTAAAACACGCGAA
GGCGAGGAGACCCCTCGAATGGAGGACATACTTGAGGTTCATCGAAGAAGAAGGAGATAGTATA
GCAGTTATCCTTTTCAGCGGGCTGCACCTTACACAGGTCAACTCTTTAACATTCCAGCTATTACT
AAGGAGGAGCTAAAGGATGCTTCGTGGGCTTTGACCTTGACACGCGAGTAGGAAACCATAGTA
AGCTCCGCTTGACGATTGGGGCGTTGATTTGCGCTGCTGGTGTTCATATAAGTATCTTAACTCA
GGAGCTGGTGGGTGGCAGGCGCATTCGTACACGAGAAACACGCTCATACCGTAAAGCCTGCAC
TGGTAGGGTGGTTTCGGACACGATCTCTTACCCGCTTCAATATGGATAATAAACTCCAGCTTATA
CCTGGCGCCAATGGATTTCAGGATCTCAAACTCCTCTATTTTGCTCGTTTGACGTTTGACGCGATCT
CTTGAGGTGTTCCAGCAGGCTACCATGACTGCACCTCCGCCGGAAGTCAATCCTTTTGACCGGATA
CTTGGAGTATATGCTGAAACATTATCACTCAAAAGATAACACTGAGAATAAGGGCCCCATAGTA
AACATTATCACTCCATCTCGGGCTGAAGAGCGCGGCTGCCAACTCACATTGACTTTTTCCATTCC
CAAGAAGTCAGTGTTCAAAGAGTTGGAGAAACGGGGGTTGTATGTGATAAGCGGGAGCCAGA
TGGAATCCGCGTTGCCCCAGTCCCCCTCTATAATTCTTTTACGATGTATACAAGTTTATTAGACT
GCTGACAAGTATCTTGGACTCATCTGAGCGATCT_{taG}

Kynurinase #2 (SEQ ID NO: 101)

ATGGAACCTCTCCTCTTGAAGTCCAGTAGACGCCGTGCGCCGCATTGCAGCCGAGTTGAATTG
CGATCCAACAGATGAACGCGTTGCCCTGAGGCTCGACGAAGAGGATAAAATTGTCACATTTTCAGG
AACTGCTTTTACATTCCAAAGATGAGGGATCTTCCATCCATAGATCTTAGCCTCGTGTCCGAGGA
TGACGATGCCATATATTTTCTTGGGAACAGTCTTGGGTTCAGCCAAAAATGGTACGGACATATC
TCGAAGAGGAGCTGGACAAATGGGCTAAAATGGGTGCTTACGGCCACGACGTGGGAAAACGCC
CCTGGATAGTTGGCGACGAATCTATCGTGAGTCTTATGAAAAGATATAGTTGGAGCACATGAGAA
AGAAATTGCACTGATGAATGCCCTTACTATCAATCTGCATCTCCTCTTGCTTTTATTCTTTAAGCC
CACTCCTAAACGCCACAAAATACTTTTGGGAAGCAAAAGCCTTTCCAAGCGACCACTACGCTATTG
AGTCACAAATACAACCTCCATGGACTTGATGTGGAAAAGTCTATGCGGATGGTAAAACACGCGA
AGGCGAGGAGACCCCTCGAATGGAGGACATACTTGAGGTTCATCGAAGAAGAAGGAGATAGTAT
AGCAGTTATCCTTTTCAGCGGGCTGCACCTTACACAGGTCAACTCTTTAACATTCCAGCTATTAC
TAAGGCAGGCCACGCTAAAGGATGCTTCGTGGGCTTTGACCTTGACACGCGAGTAGGAAACGTA
GAGCTCCGCTTGACGATTGGGGCGTTGATTTGCGCTGCTGGTGTTCATATAAGTATCTTAACTC
AGGAGCTGGTGGGTGGCAGGCGCATTCGTACACGAGAAACACGCTCATACCGTAAAGCCTGCA
CTGGTAGGGTGGTTTCGGACACGATCTCTTACCCGCTTCAATATGGATAATAAACTCCAGCTTAT
ACCTGGCGCCAATGGATTTCAGGATCTCAAACTCCTCTATTTTGCTCGTTTGACGTTTGACGCGATC
TCTTGAGGTGTTCCAGCAGGCTACCATGACTGCACCTCCGCCGGAAGTCAATCCTTTTGACCGGAT
ACTTGGAGTATATGCTGAAACATTATCACTCAAAAGATAACACTGAGAATAAGGGCCCCATAGT
AAACATTATCACTCCATCTCGGGCTGAAGAGCGCGGCTGCCAACTCACATTGACTTTTTCCATTCC
CCAAGAAGTCAGTGTTCAAAGAGTTGGAGAAACGGGGGTTGTATGTGATAAGCGGGAGCCAG
ATGGAATCCGCGTTGCCCCAGTCCCCCTCTATAATTCTTTTACGATGTATACAAGTTTATTAGAC
TGCTGACAAGTATCTTGGACTCATCTGAGCGATCT_{taG}

VEGF (SEQ ID NO: 102)

ATGAATTTCTTGCTGAGCTGGGTGCATTGGACACTCGCATTGTTGCTGTACTTGCACCATGCCAA
GTGGTCCCAGGCTGCACCCACTACTGAGGGCGAGCAAAAGTCTCATGAGGTGATTAAATTTATG
GACGTTTACCAACGATCATACTGTGGCCAATCGAAACCCCTCGTAGATATATTCCAGGAGTACCC
AGACGAGATCGAATACATTTTCAAGCCCTCATGTGTCCCATTGATGCGATGTGCTGGGTGCTGTA
ACGACGAAGCACTTGAATGTGTCCCCACCTCCGAGAGTAACATCACAATGCAATAATGAGAAT
CAAGCCCCACCAATCCCAACATATCGGTGAAATGTCAATTCCTTCAGCATTTCCCGCTGCGAGTGCC
GGCCTAAGAAGGACCGCACCAAAACCAGAGAACCATTGTGAACCTGTTCTGAGAGACGGAAGC
ACTTGTTCGTACAGGACCCTCAAACATGCAAGTGCAGCTGTAAGAATACCGACTCACGGTGTAA
AGCTAGGCAACTGGAGCTTAATGAAAGGACCTGCCGATGCGATAAAACCCAGGAG_{Gtaa}

GM-CSF (SEQ ID NO: 103)
ATGTGGTTGCAGAATTTGCTCTTCCTGGGGATTGTGGTCTACAGCCTCTCCGCACCTACCCGCTCT CCTATCACAGTTACAAGACCCCTGGAACATGTGGAGGCCATTAAAGAAGCATTGAATTTGTTGG ACGATATGCCCCGTACCCTGAATGAAGAAGTAGAAGTTGTTTCTAATGAGTTCAGCTTTAAAAA ATTGACCTGTGTGCAGACACGGCTTAAAAATTTTGAACAGGGACTTAGAGGAACTTTACTAAG CTGAAGGGGGCACCTTAACATGACAGCTTCTTATTATCAGACCTATTGTCCTCCAACACCTGAAAC CGACTGTGAAACACAGGTAACCACTTACGCCGATTTTATTGATTCTTTGAAAACATTCTCACC ATATACCATTTGAGTGTAAGAAGCCAGGCCAAAAAGtaG
Anti-PD1 (SEQ ID NO: 104)
ATGGAACACTGACACACTTCTTCTGTGGGTCTTGCTCCTGTGGGTCCCAGGCTCTACTGGTGACAG TCCTGATAGGCCATGGAACCCACCTACCTTTAGTCCAGCCTTGCTCGTCGTAACCGAAGGGGACA ACGCTACATTACCTGCTCTTTTAGCAATACTTCTGAGAGTTTTCATGTAGTCTGGCATCGGGAG AGTCCATCCGGACAAACAGATACTTTGGCCGCTTTTCCAGAGGATAGGTCTCAACCTGGGCAAG ACGCAAGGTTTCGAGTCACACAGCTTCCTAACGGGAGAGATTTTCACATGTCTGTAGTTCGGGCA CGCCGAAATGATTCTGGCACATATGTTTGCAGGTGTGATCTCACTTGCTCCAAAGATTCAAATAAA GGAGAGCCTTCGCGCCGAGTTGCGGGTGACTGAGCGGGAGCCCAAGTCTGCGACAAAACCCAT ACTTGTCACCCCTGTGGCGGCGGGTTCATCCGGTGCGGGTCTGGGGGGCAACCAAGAGAGCCAC AGGTATATACTCTTCCCCCAGCAGAGAAGAAATGACAAAAAACCAAGTGCCCTGACATGTCT GGTTAAAGGATTTTATCCCAGTGACATTGCTGTAGAATGGGAATCCAATGGTCAACCCGAGAAT AACTACAAAACCACTCTCCAGTATTGGACAGTGACGGTTCTTCTCTCTATTCCAACTTAC AGTGGATAAATCCCGCTGGCAGCAAGGGAATGTATTAGCTGTAGTGTATGCACGAAGCTCTT CATAACCATTATACACAGAAATCTCTTCCCTGAGCCCAGGTAAAtaG
Adenosine Deaminase (ADA) #1 (Mouse) (SEQ ID NO: 105)
ATGGAGACTGATACACTTTTGCTCTGGGTTTTGCTCTTGTTGGGTACCAGGGTCTACTGGAGATGC ACAAACCTCTGCATTCAACAAGCCTAAGGTAGAGCTTCATGTCCATTTGGACGGAGCCATAAAA CCTGAAACCATACTCTATTTCCGGCAAGAAACGGGGTATAGCACTTCCCGCTGATACCGTGGAAG AGTTGAGAAATATCATTGGCATGGACAAACCTCTTAGCCTGCCTGGCTTTCTTGCAAAGTTCGAC TACTATATGCCAGTTATAGCAGGGTGTAGAGAAGCAATAAAGCGAATCGCCTATGAGTTCGTTG AGATGAAGGCTAAAGAAGGAGTTGTTTACGTGGAAGTCCGGTACTCACCTCATCTGCTTGCTAAT AGCAAGGTGGACCCAATGCCATGGAATCAAACCTGAAGGTGATGTAACCCCTGACGATGTGGTCTG ATTTGGTCAATCAAGGTCTCCAAGAAGCGAGCAGGCTTTCCGGCATTAAAGGTAAGAAGTATATT GTGCTGTATGCGACATCAACCTTCATGGTCCCTGGAGGTCTCTGAATTGTGCAAAAAGTACAATC AAAAAACAGTGGTCGCAATGGATCTCGCTGGAGATGAGACCATAGAAGGTTCTCTCTTTTCCCC GGTCATGTGCAAGCATATGAAGGGGCTGTCAAAAATGGTATCCACCGCACCGTCCACGCAGGGG AAGTAGGGTCCCCAGAAGTAGTCAGGGAAGCCGTTGACATTTGAAAACAGAAAGAGTCGGGC ATGGCTACCAATAGAGGACGAAGCCTTGTAATCGACTTTTGAAAGAAAATATGCACCTT CGAGGTCTGTCCCTGGAGTTTATATCTCACCGGAGCATGGGACCCCAAAACAACCCACGCCGTC GTACGCTTCAAGAATGATAAGGCAAACTACAGTTTGAATACAGATGATCCACTGATATTCAAGT CAACACTTGACACTGACTACCAGATGACAAAAAAGATATGGGTTTACCGAAGAAGAGTTCAA GAGATTGAACATTAACGCAGCAAAAAGCTCCTTCTGCCAGAGGAAGAGAAAAAAGAATTGCTT GAAAGGTTGTATCGAGAATACCAA
Adenosine Deaminase (ADA) #2 (Mouse) (SEQ ID NO: 106)
ATGGCACAAACTCCAGCTTTTAATAAGCCCCAAAGTGGAACCTTCATGTTCATCTGGATGGGGCAAT TAAGCCCGAAACTATATTGTACTTTGGCAAAAAGAGGGGTATTGCCCTGCCAGCAGATACCGTT GAGGAGCTTCGCAACATCATTGGGATGGACAAGCCCCCTCTCTCTGCCAGGTTTCTCGCTAAATT CGATTATTATATGCCTGTTATTGCTGGTTGCCGGGAGGCCATCAAGAGGATAGCCTACGAGTTTG TTGAGATGAAGGCCAAAGAGGGCGTGGTGTACGTAGAGGTCAGATACAGCCCTCACCTGCTTGC CAACAGCAAGGTGGACCCAATGCCCTGGAACCAAACCGAGGGGGATGTCACTCCCGACGACGTT GTAGACCTCGTAAATCAGGGCCTTCAAGAGGGCGAGCAGGCATTTGGCATAAAAGTCCGGTCTA TACTCTGCTGTATGAGGCACCAACCCCTCTGGTCTTTGGAGGTACTTGAGTTGTGTAAGAAATAC AATCAAAAAGACTGTAGTCGCCATGGATCTTGCAGGCGATGAAACCATCGAGGGTAGCTCCTTGT TCCCTGGACATGTTGAAGCCTACGAGGGGGCCGTAAAAAATGGGATACACAGGACTGTCCACGC TGGTGAAGTCGGAAGCCCAGAGGTGGTAAGGGAGGCAGTTGACATACTCAAGACAGAGCGGGT TGGACACGGATACCACACAATTGAGGACGAGGCCCTGTATAACCGCCTCCTCAAAGAGAACATG CATTTTGAGGTGTGTCCTTGGTCCAGCTACCTGACTGGTGTCTGGGACCCTAAAACAACCTACGC CGTGGTCCGGTTCAAGAACGATAAAGCCAATTACTCTTTGAATACCGACGACCCCTCATATTCA AATCAACATTGGATACCGACTACCAAAATGACCAAAAAGGATATGGGGTTTACTGAAGAGGAGTT

CAAGAGGCTCAACATAAATGCCGCTAAATCCTCCTTTCTCCCCGAGGAAGAAAAAAGAAGCTC CTTGAGCGGCTGTATAGGGAGTATCAA
4-1BBL #1 (Mouse) (SEQ ID NO: 107)
ATGGAAACAGATACACTCTTGCTCTGGGTACTGCTTCTGTGGGTCCCCGGCTCTACTGGGGATGA AGATGATGTAATACTACTACAGAAGAACTCGCTCCCGCTCTTGTCCCCCACCCAAGGGTACCTGCG CCGTTTGGATGGCTGGCATCCCAGGACATCCAGGTACAACGGTACCCCCGGAAGAGATGGTCG GGATGGAACCTCCCGCGGAGAAGGGCGAAAAAGGGGATGCAGGGCTTCTGGGACCTAAAGGTGA AACAGGGGACGTTTGAATGACTGGTGCAGAAGGGCTCGCGGCTTTCCTGGCACCCCTGGGAGG AAAGGAGAGCCCCGAGAGCTCCAGAGAACTGAACCTCGGCCTGCACTACTATACTACTTCCC CTAATCTTGGGACCCGCGAGAACAACGCCGATCAGGTTACACCTGTAAGCCATATCGGGTGCCC CAATACTACCCAGCAAGGGAGTCCCGTGTTCGCAAAGCTTTTGGCTAAAAACCAAGCATCCCTG TGTAACACTACTCTTAATTGGCATTACACAAGACGGTGCTGGTAGCTCTTATCTTTCTCAGGGGCT GCGGTACGAAGAAGATAAGAAGGAATTGGTTGTGGATTCTCCAGGACTCTATTATGTCTTTCTCG AATTGAAGCTCAGTCCCACCTTCACAAACATGACACAAAGTCCAGGGGCTGGGTAAAGTCTGGT ACTCCAAGCAAAGCCCCAGGTTGACGATTTCGACAATTGGCACTCACCGTAGAGCTTTTCCC GCTCCATGGAAAATAAACTTGTGTGATCGGTTCATGGTCACAGCTCTTGCTGCTTAAGGCAGGGCAT CGCCTCTCAGTGGGTCTGAGAGCTTATTGTCATGGTGCACAAGATGCTTACAGGGATTGGGAATT GTCCTACCCAAACACTACAAGTTTCGGGTGTTCCTTGTCAAACCTGATAACCCATGGGAGtaG
4-1BBL #2 (Mouse) (SEQ ID NO: 108)
ATGGAAACTGATACACTCCTCCTGTGGGTCTTCTTTTGTGGGTGCCCGGATCAACCGGCGATGG CTGGATGGCAGGCATCCCAGGACACCCAGGACACAACGGTACTCCAGGTGAGACGGTCGGGAT GGGACTCCTGGGGAGAAAGGCGAGAAAGGGGACGCTGGTTTGTCTCGGTCTTAAGGGGGAAACC GGGGATGTAGGAATGACAGGGGCTGAAGGGCTCGGGGATTTCTGGGACACCAGGCAGGAAG GGTGAACCAGGGGAGGCCCTCCAGCGCACCGAGCCACGGCCAGCTCTGACCATAACAACAAGT CCAAACCTGGGCACACGCGAAAACAATGCTGACCAGGTGACTCCTGTAAGTCACATCGGATGCC CTAACACTACACAACAGGGCTCTCCTGTATTTGCAAAGCTTCTCGCAAAAAATCAAGCATCACTT TGTAATACAACCTGAACCTGGCATTCTCAGGACGGAGCAGGGTCTCTTATTGTCTCAAGGGCT CCGCTACGAAGAAGATAAAAAGGAATTGGTTGTGACAGTCCAGGTTTGTATTATGTGTTTTTGG AACTTAAGCTGTACCAACCTTCACTAACACCGGCCACAAGGTCCAAGGCTGGGTAGTCTTGT TTGCAAGCCAAACCTCAAGTGGATGATTTTGACAATCTGGCTTTGACTGTTGAGCTTTTCCATG CAGTATGGAGAATAAACTGGTTGATCGGTTCATGGTTCACAGCTCCTTCTGCTCAAGGCCGGACAT AGGCTGAGTGTGGGACTTCGGGCCTACTTGCACGGCGCCAGGACGCATACCGAGACTGGGAAC TCAGCTACCCTAACACAACCTCTTTTGGGTGTTCCTTGTCAAACCCGATAATCCTTGGGAAtaG
HPGE2 #1 (Mouse) (SEQ ID NO: 109)
ATGGAGACTGATACTTTGCTCCTGTGGGTCTTCTCCTGTGGGTTCCTGGTTCCACAGGGGATAT GCATGTCAATGGCAAGGTAGCACTCGTGACTGGGGCTGCACAGGGTATCGGGAAAGCTTTTGCC GAGGCCCTGTTGCTGCATGGCGCCAAGGTCGCTTTGGTAGATTGGAACCTGGAGGCTGGAGTTA AATGCAAAGCTGCACTCGACGAACAATTTGAGCCTCAAAAAACCTCTTTGTGCAAGTGTGACGTT GCTGACCAAAAGCAACTCAGGGACACATTCAGGAAGGTCGTAGACCATTTCGGACGCCTCGATA TACTCGTTAATAATGCCGGGTAAACAACGAAAGAAGTGGGAACAAACATTGCAAAATCAACCT GGTAAGTGTCAATTAGCGGAACCTATCTGGGTCTTGATTATATGAGCAAGCAGAACGGGGGCGAG GGCGGGATCATTATCAACATGTCAAGTCTTGCCGGATTGATGCCAGTTGCTCAGCAGCCTGTTTA CTGTGCCAGCAAGCACGGTATTATTGGGTTTACCCGGAGTGCCGCCATGGCCGCAAATCTTATGA AGAGTGGGGTAAGACTGAATGTTATCTGCCAGGTTTCGTAGATACCCCAATCCTGGAGAGCAT CGAGAAGGAGGAAAATATGGGACAATACATTGAATATAAAGATCAAATCAAGGCTATGATGAA GTTCTACGGGGTCTGCATCCATCCACAATTGCCAACGGGCTCATTAACTCTGATTGAGGACGACG CCTTGAACGGAGCTATAATGAAAATCACAGCTTCCAAAGGCATTCATTCCAAGATTATGATATA TCACCCTTGCTTGTCAAGGCTCCTCTGACAAGT
HPGE2 #2 (Mouse) (SEQ ID NO: 110)
ATGCATGTCAATGGCAAGGTAGCACTCGTGACTGGGGCTGCACAGGGTATCGGGAAAGCTTTTG CCGAGGCCCTGTTGCTGCATGGCGCCAAGGTCGCTTTGGTAGATTGGAACCTGGAGGCTGGAGTT AAATGCAAAGCTGCACTCGACGAACAATTTGAGCCTCAAAAAACCTCTTTGTGCAAGTGTGACG TTGCTGACCAAAAGCAACTCAGGGACACATTCAGGAAGGTCGTAGACCATTTCGGACGCCTCGA TATACTCGTTAATAATGCCGGGTAAACAACGAAAGAAGTGGGAACAAACATTGCAAAATCAAC CTGGTAAGTGTCAATTAGCGGAACCTATCTGGGTCTTGATTATATGAGCAAGCAGAACGGGGGCG AGGGCGGGATCATTATCAACATGTCAAGTCTTGCCGGATTGATGCCAGTTGCTCAGCAGCCTGTT

TACTGTGCCAGCAAGCACGGTATTATTGGGTTTACCCGGAGTGCCGCCATGGCCGCAAATCTTAT GAAGAGTGGGGTAAGACTGAATGTTATCTGCCCAGGTTTCGTAGATACCCCAATCCTGGAGAGC ATCGAGAAGGAGGAAAATATGGGACAATACATTGAATATAAAGATCAAATCAAGGCTATGATG AAGTTCTACGGGGTCTGCATCCATCCACAATTGCCAACGGGCTCATTAATCTGATTGAGGACGA CGCCTTGAACGGAGCTATAATGAAAATCACAGCTTCCAAAGGCATTCACCTCCAAGATTATGAT ATATCACCCCTTGCTTGTCAGGGCTCCTCTGACAAGT
Human IL-15 Polypeptide Sequence (SEQ ID NO: 199)
MVLGTIDLCSCFSAGLPKTEANWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLEL QVISLESGDASIHTVENLILANNSLSSNGNVTESGCKECEEELEEKNIKEFLQSFVHIVQMFINTS
Human IL-15 Polypeptide Sequence (SEQ ID NO: 224)
NWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLELQVISLESGDASIHTVENLIL ANNSLSSNGNVTESGCKECEEELEEKNIKEFLQSFVHIVQMFINTS
Human IL-15R α Polypeptide Sequence (SEQ ID NO: 200)
MAPRRARGCRTLGLPALLLLLLRPPATRGITCPPPMSVEHADIWVKSYSLSYRERYICNSGFKRKAG TSSLTECVLNKATNVAHWTTPSLKCIRDPAHVHQRAPPSTVTTAGVTPQPESLSPSGKEPAASSPSSN NTAATTAAIVPGSQLMPKSPSTGTTEISSHESHGTPSQTTAKNWELTASASHQPPGVYPQGHSDTT VAISTSTVLLCGLSAVSLACYLKSRQTPPLASVEMEAMEALPVTWGTSSRDEDLNCSHHL
Human IL-15R α sushi domain Polypeptide Sequence (SEQ ID NO: 201)
ITCPPPMSVEHADIWVKSYSLSYRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIR
Human IL-15/IL-15R α sushi domain Polypeptide Sequence (SEQ ID NO: 202)
MDWTWILFLVAAATRVHSNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLELQ VISLESGDASIHTVENLILANNSLSSNGNVTESGCKECEEELEEKNIKEFLQSFVHIVQMFINTSSGGSG GGSGGGSGGGSLQITCPPPMSVEHADIWVKSYSLSYRERYICNSGFKRKAGTSSLTECVLNKATN VAHWTTTPSLKCIR
IL-12 (IL-12p70) Polypeptide Sequence (SEQ ID NO: 203)
MCHQQLVISWFLVFLASPLVAIWELKKDVYVVELDWYPDAPGEMVVLTCDTPEEDGITWTLQSS EVLGSGKTLTIQVKEFGDAGQYTCHKGGEVLSHSLLLLHKKEDGIWSTDILKDQKEPKNKTFLRCEA KNYSGRFTCWWLTTISTDLTFSVKSSRGSSDPQGVTCGAATLSAERVGRDNKEYEYSVEQEDSACP AAEESLPPIEVMDAVHKLKYENYTSSFFIRDIIKPDPPKNLQLKPLKNSRQVEVSWEYPDTWSTPHSY FSLTFCVQVQGKSKREKKDRVFTDKTSATVICRKNASISVRAQDRYSSSWSEWASVPCSGGGSGGG SGGGSGGSRNLPVATPDGMFCLHHSQNLLRAVSNMLQKARQTLEFYPTSEEIDHEDITKDKTS TVEACLPLELTKNESCLNSRETSFITNGSCLASRKTSFMMALCLSSIYEDLKMYQVEFKTMNAKLLM DPKRQIFLDQNMLAVIDELMQALNFNSETVPQKSSLEEPDFYKTKIKLCILLHAFRIRAVTIDRVMSYL NAS

Secretion Signals and Signal-Anchors

- The one or more effector molecules of the chimeric proteins provided for herein can be secretable effector molecules having a secretion signal peptide (also referred to as a signal peptide or signal sequence) at the chimeric protein's N-terminus (*e.g.*, an effector molecule's N-terminus for **S – C – MT**) that direct newly synthesized proteins destined for secretion or membrane localization (also referred to as membrane insertion) to the proper protein processing pathways. For chimeric proteins having the formula **MT – C – S**, a membrane tethering domain generally has a signal-anchor sequence (*e.g.*, signal-anchor sequences of a Type II transmembrane protein) that direct newly synthesized proteins destined for membrane

localization to the proper protein processing pathways. For chimeric proteins having the formula **S – C – MT**, a membrane tethering domain having a reverse signal-anchor sequence (*e.g.*, signal-anchor sequences of certain Type III transmembrane proteins) can be used, generally without a separate secretion signal peptide, that direct newly synthesized proteins destined for

5 membrane localization to the proper protein processing pathways.

In general, for all membrane-cleavable chimeric proteins described herein, the one or more effector molecules are secretable effector molecules (referred to as “S” in the formula **S – C – MT** or **MT – C – S**). In embodiments with two or more chimeric proteins, each chimeric protein can comprise a secretion signal. In embodiments with two or more chimeric proteins,

10 each chimeric protein can comprise a secretion signal such that each effector molecule is capable of secretion from an engineered cell following cleavage of the protease cleavage site.

The secretion signal peptide operably associated with an effector molecule can be a native secretion signal peptide (*e.g.*, the secretion signal peptide generally endogenously associated with the given effector molecule). The secretion signal peptide operably associated

15 with an effector molecule can be a non-native secretion signal peptide native secretion signal peptide. Non-native secretion signal peptides can promote improved expression and function, such as maintained secretion, in particular environments, such as tumor microenvironments. Non-limiting examples of non-native secretion signal peptide are shown in **Table 3**.

A secretion signal peptide can be an IgE signal peptide. An IgE signal peptide can

20 include the amino acid sequence MDWTWILFLVAAATRVHS (SEQ ID NO: 228). An IgE signal peptide can be encoded by a polynucleotide sequence that includes the sequence ATGGACTGGACTTGGATACTCTTTCTGGTCGCTGCCGCCACACGGGTGCACTCT (SEQ ID NO: 229). An IgE signal peptide can be encoded by a polynucleotide sequence that includes a sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at

25 least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to ATGGACTGGACTTGGATACTCTTTCTGGTCGCTGCCGCCACACGGGTGCACTCT (SEQ ID NO: 229).

A secretion signal peptide of the membrane-cleavable chimeric protein (*e.g.*, IL-15) can be an IgE signal peptide. An IgE signal peptide of the membrane-cleavable chimeric protein

30 (*e.g.*, IL-15) can include the amino acid sequence MDWTWILFLVAAATRVHS (SEQ ID NO: 228). An IgE signal peptide of the membrane-cleavable chimeric protein (*e.g.*, IL-15) can be encoded by a polynucleotide sequence that includes the sequence ATGGACTGGACTTGGATACTCTTTCTGGTCGCTGCCGCCACACGGGTGCACTCT (SEQ ID NO: 229). An IgE signal peptide of the membrane-cleavable chimeric protein (*e.g.*, IL-

35 15) can be encoded by a polynucleotide sequence that includes a sequence that is at least 90%, at

least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to

ATGGACTGGACTTGGATACTCTTTCTGGTCGCTGCCGCCACACGGGTGCACTCT
(SEQ ID NO: 229).

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Table 3. Exemplary Signal Secretion Peptides

Name	Protein SEQUENCE	Source (Uniprot)	DNA SEQUENCE
<i>IL-12</i>	MCHQQLVISWFSLV FLASPLVA (SEQ ID NO: 112)	P29460	ATGTGTCACCAGCAGCTCGTTATATC CTGGTTTAGTTTGGTGTTCCTCGCTT CACCCCTGGTGGCA (SEQ ID NO: 31)
<i>IL-12 (Codon Optimized)</i>	MCHQQLVISWFSLV FLASPLVA (SEQ ID NO: 112)	-	ATGTGCCATCAGCAACTCGTCATCTC CTGGTTCTCCCTTGTTTCCTCGCTTC CCCTCTGGTCGCC (SEQ ID NO: 32)
<i>IL-2 (Optimized)</i>	MQLLSIALILALV (SEQ ID NO: 113)	-	ATGCAACTGCTGTCATGTATCGCACT CATCCTGGCGCTGGTA (SEQ ID NO: 33)
<i>IL-2 (Native)</i>	MYRMQLLSIALSL ALVTNS (SEQ ID NO: 114)	P60568	ATGTATCGGATGCAACTTTTGAGCTG CATCGCATTGTCTCTGGCGCTGGTGA CAAATTCC (SEQ ID NO: 34)
<i>Trypsinogen-2</i>	MNLLILTFVAAAV A (SEQ ID NO: 115)	P07478	ATGAATCTCTTGCTCATACTTACGTT TGTCGCTGCTGCCGTTGCG (SEQ ID NO: 35)
<i>Gaussia Luciferase</i>	MGVKVLFALICIAV AEA (SEQ ID NO: 116)	-	ATGGGCGTGAAGGTCTTGTTGCCCT TATCTGCATAGCTGTTGCGGAGGCG (SEQ ID NO: 36)
<i>CD5</i>	MPMGSQPLATLYL LGMLVASCLG (SEQ ID NO: 117)	P06127	ATGCCGATGGGGAGCCTTCAACCTTT GGCAACGCTTTATCTTCTGGGGATGT TGGTTGCTAGTTGCCTTGGG (SEQ ID NO: 37)
<i>IgKVII (mouse)</i>	METDTLLLWVLLL WVPGSTGD (SEQ ID NO: 118)		ATGGAACTGACACGTTGTTGCTGT GGGTATTGCTCTTGTTGGGTCCCAGGA TCTACGGGCGAC (SEQ ID NO: 38)
<i>IgKVII (human)</i>	MDMRVPAQLLGLL LLWLRGARC (SEQ ID NO: 119)	P01597	ATGGATATGAGGGTTCCTCGCCAGC TTTTGGGGCTGCTTTTGTGTGGCTT CGAGGGGCTCGGTGT (SEQ ID NO: 39)
<i>VSV-G</i>	MKCLLYLAFLFIGV NC (SEQ ID NO: 120)	-	ATGAAGTGTCTGTTGTACCTGGCGTT TCTGTTCAATTGGTGTAACCTGT (SEQ ID NO: 40)
<i>Prolactin</i>	MNIKGSPWKGSLLL LLVSNLLLCQSVAP (SEQ ID NO: 121)	P01236	ATGAATATCAAAGGAAGTCCGTGGA AGGGTAGTCTCCTGCTGCTCCTCGTA TCTAACCTTCTCCTTTGTCAATCCGT GGCACCC (SEQ ID NO: 41)
<i>Serum albumin preproprotein</i>	MKWVTFISLLFLFSS AYS (SEQ ID NO: 122)	P02768	ATGAAATGGGTAACATTATATCAC TTCTCTTTCTGTTTCAGCTCTGCGTATT CT (SEQ ID NO: 42)

Name	Protein SEQUENCE	Source (Uniprot)	DNA SEQUENCE
<i>Azurocidin preproprotein</i>	MTRLTVLALLAGLLASSRA (SEQ ID NO: 123)	20160	ATGACAAGGCTTACTGTTTTGGCTCTCTCGCTGGACTCTTGGCTTCCTCCGAGCA (SEQ ID NO: 43)
<i>Osteonectin (BM40)</i>	MRAWIFFLLCLAGRALA (SEQ ID NO: 124)	P09486	ATGAGGGCTTGGATTTTTTTTCTGCTCTGCCTTGCCGGTCGAGCCCTGGCG (SEQ ID NO: 44)
<i>CD33</i>	MPLLLLLPLWAGALA (SEQ ID NO: 125)	P20138	ATGCCTCTTCTGCTTTTGCTTCCTCTTTGTGGGCAGGTGCCCTCGCA (SEQ ID NO: 45)
<i>IL-6</i>	MNSFSTSAFGPVAFSLGLLLVLPAAFPAP (SEQ ID NO: 126)	P05231	ATGAACTCTTCTCAACCTCTGCGTTGGTCCGGTCGCTTTCTCCCTTGGGCTCCTGCTTGTCTTGCCAGCAGCGTTTCTGCGCCA (SEQ ID NO: 46)
<i>IL-8</i>	MTSKLAVALLAAFLISAALC (SEQ ID NO: 127)	P10145	ATGACAAGTAAACTGGCGGTAGCCTTGCTCGCGCCTTTTGTATTTCGCA GCCCTTTGT (SEQ ID NO: 47)
<i>CCL2</i>	MKVSAALLCLLLIAATHFIPQGLA (SEQ ID NO: 128)	P13500	ATGAAGGTAAGTGCAGCGTTGCTTGCCTTCTCCTCATGTCAGCGACCTTATTCTCAAGGGCTGGCC (SEQ ID NO: 48)
<i>TIMP2</i>	MGAAARTLRLALGLLLATLLRPADA (SEQ ID NO: 129)	P16035	ATGGGAGCGGCAGCTAGAACAATTCGACTTGCCCTTGGGCTCTTGCTCCTTGCAACCCTCCTTAGACCTGCCGACGCA (SEQ ID NO: 49)
<i>VEGFB</i>	MSPLLRLLLLAALLQLAPAQA (SEQ ID NO: 130)	P49765	ATGTCACCGTTGTTGCGGAGATTGCTGTTGGCCGCACCTTTGCAACTGGCTCTGCTCAAGCC (SEQ ID NO: 50)
<i>Osteoprotegerin</i>	MNLLCCALVFLDISIKWTTQ (SEQ ID NO: 131)	O00300	ATGAATAACCTGCTCTGTTGTGCGCTCGTGTTCCTGGACATTTCTATAAAATGGACAACGCAA (SEQ ID NO: 51)
<i>Serpin E1</i>	MQMSPALTCLVLGLALVFGECSA (SEQ ID NO: 132)	P05121	ATGCAAATGTCTCCTGCCCTTACCTGTCTCGTACTTGGTCTTGCGCTCGTATTGGAGAGGGATCAGCC (SEQ ID NO: 52)
<i>GROalpha</i>	MARAALSAAPSNPRLLRVALLLLLVAAGRRRAAG (SEQ ID NO: 133)	P09341	ATGGCAAGGGCTGCACTCAGTGCTGCCCGTCTAATCCCAGATTGCTTCGAGTTGCATTGCTTCTTCTGTTGCTGGTTGCAGCTGGTAGGAGAGCAGCGGGT (SEQ ID NO: 53)
<i>CXCL12</i>	MNAKVVVVVLVLVLTALCLSDG (SEQ ID NO: 134)	P48061	ATGAATGCAAAAGTCGTGGTCTGCTGCTGGTTTTGGTTCTGACGGCGTTGTGTTAGTGATGGG (SEQ ID NO: 54)
<i>IL-21 (Codon Optimized)</i>	MERIVICLMVIFLGLVHKSSS (SEQ ID NO: 135)	Q9HBE4	ATGGAACGCATTGTGATCTGCCTGATGGTCATCTTCCTGGGCACCTTAGTGCACAAGTCGAGCAGC (SEQ ID NO: 55)
<i>CD8</i>	MALPVTALLPLALLHAARP (SEQ ID NO: 136)	-	ATGGCCTTACCAAGTGACCGCCTTGCTCCTGCCGCTGGCCTTGCTGCTCCACGCCGCCAGGCCG (SEQ ID NO: 139)

Name	Protein SEQUENCE	Source (Uniprot)	DNA SEQUENCE
<i>CD8 (Codon Optimized)</i>	MALPVTALLLPLAL LLHAARP (SEQ ID NO: 137)	-	ATGGCGCTCCCGGTGACAGCACTTCT CTTGCCTCTTGCCCTGCTGTTGCATG CCGCGCGCCCA (SEQ ID NO: 140) OR ATGGCTCTGCCCCGTGACAGCTTTGCT GCTGCCTTTGGCACTGCTGCTGCATG CTGCTAGACCA (SEQ ID NO: 416)
<i>GMCSF</i>	MLLVTSLLLCELPHP AFLIP (SEQ ID NO: 138)	-	ATGTTGCTCGTGACATCCCTCTTGCT TTGTGAGTTGCCTCATCCCGCATTC TGCTCATCCCA (SEQ ID NO: 141)
<i>NKG2D</i>	PFFFCFIAVAMGIR FIIMVA (SEQ ID NO: 192)		CCCTTCTTCTTCTGTTGCTTTATCGCC GTGGCCATGGGCATCCGCTTCATCAT TATGGTGGCC (SEQ ID NO: 193)
<i>IgE</i>	MDWTWILFLVAAA TRVHS (SEQ ID NO: 228)		ATGGACTGGACTTGGATACTCTTTCT GGTCGCTGCCGCCACACGGGTGCAC TCT (SEQ ID NO: 229)
<i>GM-CSFRa</i>	MLLVTSLLLCELPHP PAFLIP (SEQ ID 423).		ATGCTGCTGCTGGTTACATCTCTGCT GCTGTGCGAGCTGCCCCATCCTGCCT TTCTGCTTATTCCT (SEQ ID NO: 424)

Protease Cleavage Site

In certain embodiments, the chimeric proteins provided for herein (*e.g.*, in general, for all membrane-cleavable chimeric proteins described herein) contain a protease cleavage site (*e.g.*, referred to as “C” in the formula **S – C – MT** or **MT – C – S** for membrane-cleavable chimeric proteins described herein). In general, the protease cleavage site can be any amino acid sequence motif capable of being cleaved by a protease. Examples of protease cleavage sites include, but are not limited to, a Type 1 transmembrane protease cleavage site, a Type II transmembrane protease cleavage site, a GPI anchored protease cleavage site, an ADAM8 protease cleavage site, an ADAM9 protease cleavage site, an ADAM10 protease cleavage site, an ADAM12 protease cleavage site, an ADAM15 protease cleavage site, an ADAM17 protease cleavage site, an ADAM19 protease cleavage site, an ADAM20 protease cleavage site, an ADAM21 protease cleavage site, an ADAM28 protease cleavage site, an ADAM30 protease cleavage site, an ADAM33 protease cleavage site, a BACE1 protease cleavage site, a BACE2 protease cleavage site, a SIP protease cleavage site, an MT1-MMP protease cleavage site, an MT3-MMP protease cleavage site, an MT5-MMP protease cleavage site, a furin protease cleavage site, a PCSK7 protease cleavage site, a matriptase protease cleavage site, a matriptase-2 protease cleavage site, an MMP9 protease cleavage site, or an NS3 protease cleavage site.

One example of a protease cleavage site is a hepatitis C virus (HCV) nonstructural protein 3 (NS3) protease cleavage site, including, but not limited to, a NS3/NS4A, a NS4A/NS4B, a NS4B/NS5A, or a NS5A/NS5B cleavage site. For a description of NS3 protease

and representative sequences of its cleavage sites for various strains of HCV, see, e.g., Hepatitis C Viruses: Genomes and Molecular Biology (S.L. Tan ed., Taylor & Francis, 2006), Chapter 6, pp. 163-206; herein incorporated by reference in its entirety. For example, the sequences of HCV NS4A/4B protease cleavage site; HCV NS5A/5B protease cleavage site; C-terminal
5 degron with NS4A/4B protease cleavage site; N-terminal degron with HCV NS5A/5B protease cleavage site are provided. Representative NS3 sequences are listed in the National Center for Biotechnology Information (NCBI) database. See, for example, NCBI entries: Accession Nos. YP_001491553, YP_001469631, YP_001469632, NP_803144, NP_671491, YP_001469634, YP_001469630, YP_001469633, ADA68311, ADA68307, AFP99000, AFP98987, ADA68322,
10 AFP99033, ADA68330, AFP99056, AFP99041, CBF60982, CBF60817, AHH29575, AIZ00747, AIZ00744, ABI36969, ABN05226, KF516075, KF516074, KF516056, AB826684, AB826683, JX171009, JX171008, JX171000, EU847455, EF154714, GU085487, JX171065, JX171063; all of which sequences (as entered by the date of filing of this application) are herein incorporated by reference.

15 Another example of a protease cleavage site is an ADAM17-specific protease (also referred to as Tumor Necrosis Factor- α Converting Enzyme [TACE]) cleavage site. An ADAM17-specific protease cleavage site can be an endogenous sequence of a substrate naturally cleaved by ADAM17. An ADAM17-specific protease cleavage site can be an engineered sequence capable of being cleaved by ADAM17. An engineered ADAM17-specific
20 protease cleavage site can be an engineered for specific desired properties including, but not limited to, optimal expression of the chimeric proteins, specificity for ADAM17, rate-of-cleavage by ADAM17, ratio of secreted and membrane-bound chimeric protein levels, and cleavage in different cell states. A protease cleavage site can be selected for specific cleavage by ADAM17. For example, certain protease cleavage sites capable of being cleaved by ADAM17
25 are also capable of cleavage by additional ADAM family proteases, such as ADAM10. Accordingly, an ADAM17-specific protease cleavage site can be selected and/or engineered such that cleavage by other proteases, such as ADAM10, is reduced or eliminated. A protease cleavage site can be selected for rate-of-cleavage by ADAM17. For example, it can be desirable to select a protease cleavage site demonstrating a specific rate-of-cleavage by ADAM17, such as
30 reduced cleavage kinetics relative to an endogenous sequence of a substrate naturally cleaved by ADAM17. In such cases, in general, a specific rate-of-cleavage can be selected to regulate the rate of processing of the chimeric protein, which in turn regulates the rate of release/secretion of the payload effector molecule. Accordingly, an ADAM17-specific protease cleavage site can be selected and/or engineered such that the sequence demonstrates a desired rate-of-cleavage by
35 ADAM17. A protease cleavage site can be selected for both specific cleavage by ADAM17 and

rate-of-cleavage by ADAM17. Exemplary ADAM17-specific protease cleavage sites, including those demonstrating particular specificity and rate-of-cleavage kinetics, are shown in **Table 4A** below with reference to the site of cleavage (P5-P1: N-terminal; P1'-P5': C-terminal). Further details of ADAM17 and ADAM10, including expression and protease cleavage sites, are described in Sharma, *et al.* (J Immunol October 15, 2017, 199 (8) 2865-2872), Pham *et al.* (Anticancer Res. 2017 Oct;37(10):5507-5513), Caescu *et al.* (Biochem J. 2009 Oct 23; 424(1): 79–88), and Tucher *et al.* (J. Proteome Res. 2014, 13, 4, 2205–2214), each herein incorporated by reference for purposes.

Table 4A – Various ADAM17 Protease Cleavage Site Sequences

P5	P4	P3	P2	P1	P1'	P2'	P3'	P4'	P5'	FULL SEQ	SEQ ID NO
P	R	A	E	A	V	K	G	G		PRAEAVKGG	179
P	R	A	E	A	L	K	G	G		PRAEALKGG	180
P	R	A	E	Y	S	K	G	G		PRAEYSKGG	181
P	R	A	E	P	I	K	G	G		PRAEPIKGG	182
P	R	A	E	A	Y	K	G	G		PRAEAYKGG	183
P	R	A	E	S	S	K	G	G		PRAESSKGG	184
P	R	A	E	F	T	K	G	G		PRAEFTKGG	185
D	E	P	H	Y	S	Q	R	R		DEPHYSQRR	187
P	P	L	G	P	I	F	N	P	G	PPLGPIFNPG	188
P	L	A	Q	A	Y	R	S	S		PLAQAYRSS	189
T	P	I	D	S	S	F	N	P	D	TPIDSSFNPD	190
V	T	P	E	P	I	F	S	L	I	VTPEPIFSLI	191

10

In some embodiments, the protease cleavage site comprises a first region having the amino acid sequence of PRAE (SEQ ID NO: 176). In some embodiments, the protease cleavage site comprises a second region having the amino acid sequence of KGG (SEQ ID NO: 177). In some embodiments, the first region is located N-terminal to the second region. In some embodiments, the protease cleavage site comprises the amino acid sequence of PRAEX₁X₂KGG (SEQ ID NO: 178), wherein X₁ is A, Y, P, S, or F, and wherein X₂ is V, L, S, I, Y, T, or A. In some embodiments, the protease cleavage site comprises the amino acid sequence of PRAEX₁X₂KGG (SEQ ID NO: 178), wherein X₁ is A, Y, P, S, or F, and wherein X₂ is V, L, S, I, Y, or T. In some embodiments, the protease cleavage site comprises the amino acid sequence of PRAEAVKGG (SEQ ID NO: 179). In some embodiments, the protease cleavage site

20

comprises the amino acid sequence of PRAEALKGG (SEQ ID NO: 180). In some embodiments, the protease cleavage site comprises the amino acid sequence of PRAEYSKGG (SEQ ID NO: 181). In some embodiments, the protease cleavage site comprises the amino acid sequence of PRAEPIKGG (SEQ ID NO: 182). In some embodiments, the protease cleavage site comprises the amino acid sequence of PRAEAYKGG (SEQ ID NO: 183). In some embodiments, the protease cleavage site comprises the amino acid sequence of PRAESSKGG (SEQ ID NO: 184). In some embodiments, the protease cleavage site comprises the amino acid sequence of PRAEFTKGG (SEQ ID NO: 185). In some embodiments, the protease cleavage site comprises the amino acid sequence of PRAEAAKGG (SEQ ID NO: 186).

10 In some embodiments, the protease cleavage site comprises the amino acid sequence of DEPHYSQRR (SEQ ID NO: 187). In some embodiments, the protease cleavage site comprises the amino acid sequence of PPLGPIFNPG (SEQ ID NO: 188). In some embodiments, the protease cleavage site comprises the amino acid sequence of PLAQAYRSS (SEQ ID NO: 189). In some embodiments, the protease cleavage site comprises the amino acid sequence of TPIDSSFNPD (SEQ ID NO: 190). In some embodiments, the protease cleavage site comprises the amino acid sequence of VTPEPIFSLI (SEQ ID NO: 191). The protease cleavage sites of SEQ ID NOs: 187, 189, and 191 are cleavable by ADAM17.

In some embodiments, the protease cleavage site can include an N-terminal peptide linker, such as SGGGSGGGGSG (SEQ ID NO: 230). In some embodiments, the protease cleavage site can include a C-terminal peptide linker, such as GGGSGGGGSGGGSLQ (SEQ ID NO: 231). In some embodiments, the protease cleavage site can include an N-terminal peptide linker and a C-terminal peptide linker, such as both table (SEQ ID NO: 230) and GGGSGGGGSGGGSLQ (SEQ ID NO: 231).

In some embodiments, the protease cleavage site can include a Tumor Necrosis Factor- α Converting Enzyme (TACE)-specific cleavage site (also referred to as ADAM17), an N-terminal peptide linker, and a C-terminal peptide linker. In some embodiments, the protease cleavage site can include the TACE-specific cleavage site VTPEPIFSLI (SEQ ID NO: 191), an N-terminal peptide linker, and a C-terminal peptide linker. In some embodiments, the protease cleavage site can include the TACE-specific cleavage site, an N-terminal peptide linker, and a C-terminal peptide linker and have the amino acid sequence SGGGSGGGGSGVTPEPIFSLIGGGSGGGGSGGGSLQ (SEQ ID NO: 250). In some embodiments, the protease cleavage site can include the TACE-specific cleavage site, an N-terminal peptide linker, and a C-terminal peptide linker encoded by a polynucleotide sequence comprising the sequence

35 TCAGGCGGCGGTGGTAGTGGAGGCGGAGGCTCAGGCGTGACCCCTGAGCCTATCTT

CAGCCTGATCGGCGGAGGTTCCGGAGGTGGCGGTTCCGGCGGAGGATCTCTTCAA
(SEQ ID NO: 251). In some embodiments, the protease cleavage site can include the TACE -
specific cleavage site, an N-terminal peptide linker, and a C-terminal peptide linker encoded by
a polynucleotide sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least
5 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to
TCAGGCGGCGGTGGTAGTGGAGGCGGAGGCTCAGGCGTGACCCCTGAGCCTATCTT
CAGCCTGATCGGCGGAGGTTCCGGAGGTGGCGGTTCCGGCGGAGGATCTCTTCAA
(SEQ ID NO: 251).

In some embodiments, the protease cleavage site comprises the amino acid sequence of
10 ITQGLAVSTISSFF (SEQ ID NO: 198), which is a cleavage site that is native to CD16 and is
cleavable by ADAM17.

The protease cleavage site can be C-terminal of the secretable effector molecule. The
protease cleavage site can be N-terminal of the secretable effector molecule. In general, for all
membrane-cleavable chimeric proteins described herein, the protease cleavage site is either: (1)
15 C-terminal of the secretable effector molecule and N-terminal of the cell membrane tethering
domain (in other words, the protease cleavage site is in between the secretable effector molecule
and the cell membrane tethering domain); or (2) N-terminal of the secretable effector molecule
and C-terminal of the cell membrane tethering domain (also between the secretable effector
molecule and the cell membrane tethering domain with domain orientation inverted). The
20 protease cleavage site can be connected to the secretable effector molecule by a polypeptide
linker, *i.e.*, a polypeptide sequence not generally considered to be part of the effector molecule
or protease cleavage site. The protease cleavage site can be connected to the cell membrane
tethering domain by a polypeptide linker, *i.e.*, a polypeptide sequence not generally considered
to be part of the cell membrane tethering domain or protease cleavage site. A polypeptide linker
25 can be any amino acid sequence that connects a first polypeptide sequence and a second
polypeptide sequence. A polypeptide linker can be a flexible linker (*e.g.*, a Gly-Ser-Gly
sequence). Examples of polypeptide linkers include, but are not limited to, GSG linkers (*e.g.*,
[GS]₄GG [SEQ ID NO: 504]), A(EAAAK)₃A (SEQ ID NO: 505), and Whitlow linkers (*e.g.*, a
“KEGS”(SEQ ID NO: 506) linker such as the amino acid sequence
30 KESGSVSSEQLAQFRSLD (SEQ ID NO: 184), an eGK linker such as the amino acid sequence
EGKSSGSGSESKST (SEQ ID NO: 185), and linkers described in more detail in Issued U.S.
Pat. No. 5,990,275 herein incorporated by reference). Additional exemplary polypeptide linkers
include SEQ ID NO: 194, SEQ ID NO: 195, SEQ ID NO: 196, and SEQ ID NO: 197. Other
polypeptide linkers may be selected based on desired properties (*e.g.*, length, flexibility, amino
35 acid composition etc.) and are known to those skilled in the art.

In the Membrane-Cleavable system, following expression and localization of the chimeric protein into the cell membrane, the protease cleavage site directs cleavage of the chimeric protein such that the effector molecule is released (“secreted”) into the extracellular space of a cell.

5 In general, a protease that cleaves the protease cleavage site is a protease specific for that specific protease cleavage site. For example, in the case of a disintegrin and metalloproteinase (“ADAM”) family protease, the protease that cleaves a specific ADAM protease cleavage site is generally limited to the ADAM protease(s) that specifically recognize the specific ADAM protease cleavage site motif. A protease cleavage site can be selected and/or engineered such
10 that cleavage by undesired proteases is reduced or eliminated. Proteases can be membrane-bound or membrane-associated. Proteases can be secreted, *e.g.*, secreted in a specific cellular environment, such as a tumor microenvironment (“TME”).

A protease that cleaves the protease cleavage site of the chimeric protein can be expressed in the same cell that expresses the chimeric protein. A protease that cleaves the
15 protease cleavage site of the chimeric protein can be endogenous to a cell expressing the chimeric protein. In other words, a cell engineered to express the chimeric protein can endogenously express the protease specific for the protease cleavage site present in the chimeric protein. Endogenous expression of the protease refers to both expression under generally homeostatic conditions (*e.g.*, a cell generally considered to be healthy), and also to differential
20 expression under non-homeostatic conditions (*e.g.*, upregulated expression in a tumor cell). The protease cleavage site can be selected based on the known proteases endogenously expressed by a desired cell population. In such cases, in general, the cleavage of the protease cleavage site (and thus release/secretion of a payload) can be restricted to only those cells of interest due to the cell-restricted protease needing to come in contact with the protease cleavage site of
25 chimeric protein expressed in the same cell. For example, and without wishing to be bound by theory, ADAM17 is believed to be restricted in its endogenous expression to NK cell and T cells. Thus, selection of an ADAM17-specific protease cleavage site may restrict the cleavage of the protease cleavage site to NK cell and T cells co-expressing the chimeric protein. In other examples, a protease cleavage site can be selected for a specific tumor-associated protease
30 known to be expressed in a particular tumor population of interest (*e.g.*, in a specific tumor cell engineered to express the chimeric protein). Protease and/or expression databases can be used to select an appropriate protease cleavage site, such as selecting a protease cleavage site cleaved by a tumor-associated proteases through consulting Oncomine (www.oncomine.org), the European Bioinformatic Institute (www.ebi.ac.uk) in particular (www.ebi.ac.uk/gxa), PMAP
35 (www.proteolysis.org), ExPASy Peptide Cutter (ca.expasy.org/tools/peptide_cutter) and

PMAP.Cut DB (cutdb.burnham.org), each of which is incorporated by reference for all purposes.

A protease that cleaves the protease cleavage site of the chimeric protein can be heterologous to a cell expressing the chimeric protein. For example, a cell engineered to express
5 the chimeric protein can also be engineered to express a protease not generally expressed by the cell that is specific for the protease cleavage site present in the chimeric protein. A cell engineered to express both the chimeric protein and the protease can be engineered to express each from separate engineered nucleic acids or from a multicistronic systems (multicistronic and multi-promoter systems are described in greater detail in the Section herein titled “Multicistronic
10 and Multiple Promoter Systems”). Heterologous proteases and their corresponding protease cleavage site can be selected as described above with reference to endogenous proteases.

A protease that cleaves the protease cleavage site of the chimeric protein can be expressed on a separate distinct cell than the cell that expresses the chimeric protein. For example, the protease can be generally expressed in a specific cellular environment, such as a
15 tumor microenvironment. In such cases, in general, the cleavage of the protease cleavage site can be restricted to only those cellular environments of interest (*e.g.*, a tumor microenvironment) due to the environment-restricted protease needing to come in contact with the protease cleavage site. In embodiments having membrane-cleavable chimeric proteins, in general, the secretion of the effector molecule can be restricted to only those cellular environments of interest (*e.g.*, a
20 tumor microenvironment) due to the environment-restricted protease needing to come in contact with the protease cleavage site. A protease that cleaves the protease cleavage site of the chimeric protein can be endogenous to the separate distinct cell. A protease that cleaves the protease cleavage site of the chimeric protein can be heterologous to the separate distinct cell. For example, the separate distinct cell can be engineered to express a protease not generally
25 expressed by the separate distinct cell.

Proteases include, but are not limited to, a Type 1 transmembrane protease, a Type II transmembrane protease, a GPI anchored protease, an ADAM8 protease, an ADAM9 protease, an ADAM10 protease, an ADAM12 protease, an ADAM15 protease, an ADAM17 protease, an ADAM19 protease, an ADAM20 protease, an ADAM21 protease, an ADAM28 protease, an
30 ADAM30 protease, an ADAM33 protease, a BACE1 protease, a BACE2 protease, a SIP protease, an MT1-MMP protease, an MT3-MMP protease, an MT5-MMP protease, a furin protease, a PCSK7 protease, a matriptase protease, a matriptase-2 protease, and an MMP9 protease. A protease can be an NS3 protease. A protease can be an ADAM17 protease.

Proteases can be tumor associated proteases, such as, a cathepsin, a cysteine protease, an
35 aspartyl protease, a serine protease, or a metalloprotease. Specific examples of tumor associated

proteases include Cathepsin B, Cathepsin L, Cathepsin S, Cathepsin D, Cathepsin E, Cathepsin A, Cathepsin G, Thrombin, Plasmin, Urokinase, Tissue Plasminogen Activator, Metalloproteinase 1 (MMP1), MMP2, MMP3, MMP4, MMP7, MMP8, MMP9, MMP10, MMP11, MMP12, MMP13, MMP14, MMP15, MMP16, MMP17, MMP20, MMP21, MMP23, 5 MMP24, MMP25, MMP26, MMP28, ADAM, ADAMTS, CD10 (CALLA), or prostate specific antigen.

Proteases can also include, but are not limited to, proteases (including proteases with exemplary cognate protease cleavage sites) described in International Application Pub. No. WO2023205614, which is hereby incorporated by reference for all purposes.

10 Protease enzymatic activity can be regulated. For example, certain proteases can be inactivated by the presence or absence of a specific agent (e.g., that binds to the protease, such as specific small molecule inhibitors). Such proteases can be referred to as a “repressible protease.” Exemplary inhibitors for certain proteases are described in International Application Pub. No. WO2023205614, which is hereby incorporated by reference for all purposes. For 15 example, an NS3 protease can be repressed by a protease inhibitor including, but not limited to, simeprevir, danoprevir, asunaprevir, ciluprevir, boceprevir, sovalprevir, paritaprevir, telaprevir, grazoprevir, glecaprevir, and voxiloprevir. In another example, protease activity can be regulated through regulating expression of the protease itself, such as engineering a cell to express a protease using an inducible promoter system (e.g., Tet On/Off systems) or cell-specific 20 promoters (promoters that can be used to express a heterologous protease are described in more detail in the Section herein titled “Promoters”). A protease can also contain a degron, such as any of the degrons described herein, and can be regulated using any of the degron systems described herein.

Protease enzymatic activity can also be regulated through selection of a specific protease 25 cleavage site. For example, a protease cleavage site can be selected and/or engineered such that the sequence demonstrates a desired rate-of-cleavage by a desired protease, such as reduced cleavage kinetics relative to an endogenous sequence of a substrate naturally cleaved by the desired protease. As another example, a protease cleavage site can be selected and/or engineered such that the sequence demonstrates a desired rate-of-cleavage in a cell-state specific manner. 30 For example, various cell states (e.g., following cellular signaling, such as immune cell activation) can influence the expression and/or localization of certain proteases. As an illustrative example, ADAM17 protein levels and localization is known to be influenced by signaling, such as through Protein kinase C (PKC) signaling pathways (e.g., activation by the PKC activator Phorbol-12-myristat-13-acetat [PMA]). Accordingly, a protease cleavage site can 35 be selected and/or engineered such that cleavage of the protease cleavage site and subsequent

release of an effector molecule is increased or decreased, as desired, depending on the protease properties (*e.g.*, expression and/or localization) in a specific cell state. As another example, a protease cleavage site (particularly in combination with a specific membrane tethering domain) can be selected and/or engineered for optimal protein expression of the chimeric protein.

5

Cell Membrane Tethering Domain

The membrane-cleavable chimeric proteins provided for herein contain a cell-membrane tethering domain (referred to as “MT” in the formula **S – C – MT** or **MT – C – S**). In general, the cell-membrane tethering domain can be any amino acid sequence motif capable of directing the chimeric protein to be localized to (*e.g.*, inserted into), or otherwise associated with, the cell membrane of the cell expressing the chimeric protein. The cell-membrane tethering domain can be a transmembrane-intracellular domain. The cell-membrane tethering domain can be a transmembrane domain. The cell-membrane tethering domain can be an integral membrane protein domain (*e.g.*, a transmembrane domain). The cell-membrane tethering domain can be derived from a Type I, Type II, or Type III transmembrane protein. The cell-membrane tethering domain can include post-translational modification tag, or motif capable of post-translational modification to modify the chimeric protein to include a post-translational modification tag, where the post-translational modification tag allows association with a cell membrane. Examples of post-translational modification tags include, but are not limited to, lipid-anchor domains (*e.g.*, a GPI lipid-anchor, a myristoylation tag, or palmitoylation tag). Examples of cell-membrane tethering domains include, but are not limited to, a transmembrane-intracellular domain and/or transmembrane domain derived from PDGFR-beta, CD8, CD28, CD3zeta-chain, CD4, 4-1BB, OX40, ICOS, CTLA-4, PD-1, LAG-3, 2B4, LNGFR, NKG2D, EpoR, TNFR2, B7-1, or BTLA. The cell membrane tethering domain can be a cell surface receptor or a cell membrane-bound portion thereof.

In some embodiments, the cell-membrane tethering domain comprises a transmembrane domain derived from a B7-1 polypeptide. In some embodiments, the B7-1 transmembrane domain comprises the sequence
LLPSWAITLISVNGIFVICCLTYCFAPRCRERRRNERLRRESVRPV (SEQ ID NO: 204). In some embodiments, the B7-1 transmembrane domain is encoded by a polynucleotide sequence having the sequence

TTGCTGCCTAGCTGGGCCATCACACTGATCTCCGTGAACGGCATCTTCGTGATCTGCTGCCTGACCTACTGCTTCGCCCCTAGATGCAGAGAGCGGAGAAGAAACGAGCGGCTGAGAAGAGAAAGCGTGCGGCCTGTG (SEQ ID NO: 252). In some embodiments, the B7-1

transmembrane domain is encoded by a polynucleotide sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to

TTGCTGCCTAGCTGGGCCATCACACTGATCTCCGTGAACGGCATCTTCGTGATCTGC
 5 TGCCTGACCTACTGCTTCGCCCCTAGATGCAGAGAGCGGAGAAGAAACGAGCGGCT
 GAGAAGAGAAAGCGTGCGGCCTGTG (SEQ ID NO: 252).

In some embodiments, the cell-membrane tethering domain of the membrane-cleavable chimeric protein (*e.g.*, IL-15) includes a transmembrane domain derived from a B7-1 polypeptide. In some embodiments, the B7-1 transmembrane domain of the membrane-
 10 cleavable chimeric protein (*e.g.*, IL-15) includes the sequence

LLPSWAITLISVNGIFVICCLTYCFAPRCRERRRNERLRRESVRPV (SEQ ID NO: 204). In some embodiments, the B7-1 transmembrane domain of the membrane-cleavable chimeric protein (*e.g.*, IL-15) is encoded by a polynucleotide sequence having the sequence
 TTGCTGCCTAGCTGGGCCATCACACTGATCTCCGTGAACGGCATCTTCGTGATCTGC
 15 TGCCTGACCTACTGCTTCGCCCCTAGATGCAGAGAGCGGAGAAGAAACGAGCGGCT
 GAGAAGAGAAAGCGTGCGGCCTGTG (SEQ ID NO: 252). In some embodiments, the B7-1 transmembrane domain of the membrane-cleavable chimeric protein (*e.g.*, IL-15) is encoded by a polynucleotide sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to
 20 TTGCTGCCTAGCTGGGCCATCACACTGATCTCCGTGAACGGCATCTTCGTGATCTGC
 TGCCTGACCTACTGCTTCGCCCCTAGATGCAGAGAGCGGAGAAGAAACGAGCGGCT
 GAGAAGAGAAAGCGTGCGGCCTGTG (SEQ ID NO: 252).

In some embodiments, the cell-membrane tethering domain comprises a transmembrane domain derived from a CD8 polypeptide. Any suitable CD8 polypeptide may be used.
 25 Exemplary CD8 polypeptides include, without limitation, NCBI Reference Nos. NP_001139345 and AAA92533.1. Examples of CD8 transmembrane domains include

IYIWAPLAGTCGVLLLSLVIT (SEQ ID NO: 205), IYIWAPLAGTCGVLLLSLVITLYCNHR (SEQ ID NO: 206), and IYIWAPLAGTCGVLLLSLVITLYCNHRN (SEQ ID NO: 207). In some embodiments, the transmembrane domain comprises the sequence

30 IYIWAPLAGTCGVLLLSLVIT (SEQ ID NO: 205). In some embodiments, the transmembrane domain comprises the sequence IYIWAPLAGTCGVLLLSLVITLYCNHR (SEQ ID NO: 206).

In some embodiments, the transmembrane domain comprises the sequence IYIWAPLAGTCGVLLLSLVITLYCNHRN (SEQ ID NO: 207). In some embodiments, the cell-membrane tethering domain comprises a hinge and transmembrane domain derived from CD8. In
 35 some embodiments, the CD8 hinge comprises the sequence

TTTPAPRPPTPAPTIALQPLSLRPEACRPAAGGAVHTRGLDFACD (SEQ ID NO: 271). In some embodiments, the CD8 hinge comprises the sequence

AAAFVPVFLPAKPTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYI WAPLAGTCGVLLLSLVITLYCNHRN (SEQ ID NO: 209).

5 In general, for all membrane-cleavable chimeric proteins described herein, the cell membrane tethering domain is either: (1) C-terminal of the protease cleavage site and N-terminal of any intracellular domain, if present (in other words, the cell membrane tethering domain is in between the protease cleavage site and, if present, an intracellular domain); or (2) N-terminal of the protease cleavage site and C-terminal of any intracellular domain, if present
10 (also between the protease cleavage site and, if present, an intracellular domain with domain orientation inverted). In embodiments featuring a degron associated with the chimeric protein, the degron domain is the terminal cytoplasmic-oriented domain, specifically relative to the cell membrane tethering (in other words, the cell membrane tethering domain is in between the protease cleavage site and the degron). The cell membrane tethering domain can be connected to
15 the protease cleavage site by a polypeptide linker, *i.e.*, a polypeptide sequence not generally considered to be part of cell membrane tethering domain or protease cleavage site. The cell membrane tethering domain can be connected to an intracellular domain, if present, by a polypeptide linker, *i.e.*, a polypeptide sequence not generally considered to be part of the cell membrane tethering domain or the intracellular domain. The cell membrane tethering domain
20 can be connected to the degron, if present, by a polypeptide linker, *i.e.*, a polypeptide sequence not generally considered to be part of the cell membrane tethering domain or degron. A polypeptide linker can be any amino acid sequence that connects a first polypeptide sequence and a second polypeptide sequence. A polypeptide linker can be a flexible linker (*e.g.*, a Gly-Ser-Gly sequence). Examples of polypeptide linkers include, but are not limited to, GSG linkers
25 (*e.g.*, [GS]₄GG [SEQ ID NO: 504]), A(EAAAK)₃A (SEQ ID NO: 505), and Whitlow linkers (*e.g.*, a “KEGS” (SEQ ID NO: 506) linker such as the amino acid sequence KESGVSSEQLAQFRSLD (SEQ ID NO: 184), an eGK linker such as the amino acid sequence EGKSSGSGSESKST (SEQ ID NO: 185), and linkers described in more detail in Issued U.S. Pat. No. 5,990,275 herein incorporated by reference). Additional polypeptide linkers include
30 SEQ ID NO: 194, SEQ ID NO: 195, SEQ ID NO: 196, and SEQ ID NO: 197. Other polypeptide linkers may be selected based on desired properties (*e.g.*, length, flexibility, amino acid composition etc.) and are known to those skilled in the art.

In general, the cell-membrane tethering domain is oriented such that the secreted effector molecule and the protease cleavage site are extracellularly exposed following insertion into, or
35 association with, the cell membrane, such that the protease cleavage site is capable of being

cleaved by its respective protease and releasing (“secreting”) the effector molecule into the extracellular space.

Degron Systems and Domains

5 In some embodiments, any of the proteins described herein can include a degron domain including, but not limited to, a protease, a transcription factor, a promoter or constituent of a promoter system (*e.g.*, an ACP), and/or any of the membrane-cleavable chimeric protein described herein. In general, the degron domain can be any amino acid sequence motif capable of directing regulated degradation, such as regulated degradation through a ubiquitin-mediated pathway. In the presence of an immunomodulatory drug (IMiD), the degron domain directs 10 ubiquitin-mediated degradation of a degron-fusion protein.

The degron domain can be a cereblon (CRBN) polypeptide substrate domain capable of binding CRBN in response to an immunomodulatory drug (IMiD) including, but not limited to, IKZF1, IKZF3, CK1a, ZFP91, GSPT1, MEIS2, GSS E4F1, ZN276, ZN517, ZN582, ZN653, 15 ZN654, ZN692, ZN787, and ZN827, or a fragment thereof that is capable of drug-inducible binding of CRBN. The CRBN polypeptide substrate domain can be a chimeric fusion product of native CRBN polypeptide sequences, such as a IKZF3/ZFP91/IKZF3 chimeric fusion product having the amino acid sequence of

FNVLMVHKRSHTGERPLQCEICGFTCRQKGNLLRHIKLHTGEKPFKCHLCNYACQRRD

20 AL (SEQ ID NO: 175). Degron domains, and in particular CRBN degron systems, are described in more detail in International Application Pub. No. WO2019/089592A1, herein incorporated by reference for all purposes. Other examples of degron domains include, but are not limited to HCV NS4 degron, PEST (two copies of residues 277-307 of human IκBα; SEQ ID NO: 161), GRR (residues 352-408 of human p105; SEQ ID NO: 162), DRR (residues 210-295 of yeast 25 Cdc34; SEQ ID NO: 163), SNS (tandem repeat of SP2 and NB (SP2-NB-SP2 of influenza A or influenza B; *e.g.*, SEQ ID NO: 164), RPB (four copies of residues 1688-1702 of yeast RPB; SEQ ID NO: 165), SPmix (tandem repeat of SP1 and SP2 (SP2-SP1-SP2-SP1-SP2 of influenza A virus M2 protein; SEQ ID NO: 166), NS2 (three copies of residues 79-93 of influenza A virus NS protein; SEQ ID NO: 167), ODC (residues 106-142 of ornithine decarboxylase; SEQ ID NO: 30 168), Nek2A, mouse ODC (residues 422–461; SEQ ID NO: 169), mouse ODC_DA (residues 422-461 of mODC including D433A and D434A point mutations), an APC/C degron, a COP1 E3 ligase binding degron motif, a CRL4-Cdt2 binding PIP degron, an actinfilin-binding degron, a KEAP1 binding degron, a KLHL2 and KLHL3 binding degron, an MDM2 binding motif, an N-degron, a hydroxyproline modification in hypoxia signaling, a phytohormone-dependent SCF-

LRR-binding degron, an SCF ubiquitin ligase binding phosphodegron, a phytohormone-dependent SCF-LRR-binding degron, a DSGxxS (SEQ ID NO: 509) phospho-dependent degron, an Siah binding motif, an SPOP SBC docking motif, or a PCNA binding PIP box.

Regulated degradation can be drug-inducible. Drugs capable of mediating/regulating degradation can be small-molecule compounds. Drugs capable of mediating/regulating degradation can include an “immunomodulatory drug” (IMiD). In general, as used herein, IMiDs refer to a class of small-molecule immunomodulatory drugs containing an imide group. Cereblon (CRBN) is known target of IMiDs and binding of an IMiD to CRBN or a CRBN polypeptide substrate domain alters the substrate specificity of the CRBN E3 ubiquitin ligase complex leading to degradation of proteins having a CRBN polypeptide substrate domain (*e.g.*, any of secretable effector molecules or other proteins of interest described herein). For degron domains having a CRBN polypeptide substrate domain, examples of imide-containing IMiDs include, but are not limited to, a thalidomide, a lenalidomide, or a pomalidomide. The IMiD can be an FDA-approved drug.

Chimeric proteins described herein can contain a degron domain (*e.g.*, referred to as “D” in the formula **S – C – MT – D** or **D – MT – C – S** for membrane-cleavable chimeric proteins described herein). In the absence of an IMiD, degron/ubiquitin-mediated degradation of the chimeric protein does not occur. Following expression and localization of the chimeric protein into the cell membrane, the protease cleavage site directs cleavage of the chimeric protein such that the effector molecule is released (“secreted”) into the extracellular space. In the presence of an immunomodulatory drug (IMiD), the degron domain directs ubiquitin-mediated degradation of the chimeric protein such that secretion of the effector molecule is reduced or eliminated. In general, for membrane-cleavable chimeric proteins fused to a degron domain, the degron domain is the terminal cytoplasmic-oriented domain, specifically relative to the cell membrane tethering domain, *e.g.*, the most C-terminal domain in the formula **S – C – MT – D** or the most N-terminal domain in the formula **D – MT – C – S**. The degron domain can be connected to the cell membrane tethering domain by a polypeptide linker, *i.e.*, a polypeptide sequence not generally considered to be part of the cell membrane tethering domain or the degron domain. A polypeptide linker can be any amino acid sequence that connects a first polypeptide sequence and a second polypeptide sequence. A polypeptide linker can be a flexible linker (*e.g.*, a Gly-Ser-Gly sequence). Examples of polypeptide linkers include, but are not limited to, GSG linkers (*e.g.*, [GS]₄GG [SEQ ID NO: 504]), A(EAAAK)₃A (SEQ ID NO: 505), and Whitlow linkers (*e.g.*, a “KEGS” (SEQ ID NO: 506) linker such as the amino acid sequence KESGVSSEQLAQFRSLD (SEQ ID NO: 184), an eGK linker such as the amino acid sequence EGKSSGSGSESKST (SEQ ID NO: 185), and linkers described in more detail in Issued U.S.

Pat. No. 5,990,275 herein incorporated by reference). Additional polypeptide linkers include SEQ ID NO: 194, SEQ ID NO: 195, SEQ ID NO: 196, and SEQ ID NO: 197. Other polypeptide linkers may be selected based on desired properties (*e.g.*, length, flexibility, amino acid composition etc.) and are known to those skilled in the art. In general, the degron is oriented in relation to the cell membrane tethering domain such that the degron is exposed to the cytosol following localization to the cell membrane such that the degron domain is capable of mediating degradation (*e.g.*, exposure to the cytosol and cytosol) and is capable of mediating ubiquitin-mediated degradation.

For degron-fusion proteins, the degron domain can be N-terminal or C-terminal of the protein of interest, *e.g.*, the effector molecule. The degron domain can be connected to the protein of interest by a polypeptide linker, *i.e.*, a polypeptide sequence not generally considered to be part of the protein of interest or the degron domain. A polypeptide linker can be any amino acid sequence that connects a first polypeptide sequence and a second polypeptide sequence. A polypeptide linker can be a flexible linker (*e.g.*, a Gly-Ser-Gly sequence). Examples of polypeptide linkers include, but are not limited to, GSG linkers (*e.g.*, [GS]₄GG [SEQ ID NO: 504]), A(EAAAK)₃A (SEQ ID NO: 505), and Whitlow linkers (*e.g.*, a “KEGS” (SEQ ID NO: 506) linker such as the amino acid sequence KESGSVSSEQLAQFRSLD (SEQ ID NO: 184), an eGK linker such as the amino acid sequence EGKSSGSGSESKST (SEQ ID NO: 185), and linkers described in more detail in Issued U.S. Pat. No. 5,990,275 herein incorporated by reference). Additional polypeptide linkers include SEQ ID NO: 194, SEQ ID NO: 195, SEQ ID NO: 196, and SEQ ID NO: 197. Other polypeptide linkers may be selected based on desired properties (*e.g.*, length, flexibility, amino acid composition etc.) and are known to those skilled in the art. A polypeptide linker can be cleavable, *e.g.*, any of the protease cleavage sites described herein.

Homing Molecules

A “tumor microenvironment” is the cellular environment in which a tumor exists, including surrounding blood vessels, immune cells, fibroblasts, bone marrow-derived inflammatory cells, lymphocytes, signaling molecules and the extracellular matrix (ECM) (see, *e.g.*, Pattabiraman, D.R. & Weinberg, R.A. *Nature Reviews Drug Discovery* 13, 497–512 (2014); Balkwill, F.R. *et al. J Cell Sci* 125, 5591-5596, 2012; and Li, H. *et al. J Cell Biochem* 101(4), 805-15, 2007).

In some embodiments, engineered nucleic acids are configured to produce at least one homing molecule. For example, in membrane-cleavable chimeric proteins described herein

containing a secreted effector molecule, the secreted effector molecule can be a homing molecule. "Homing," refers to active navigation (migration) of a cell to a target site (*e.g.*, a cell, tissue (*e.g.*, tumor), or organ). A "homing molecule" refers to a molecule that directs cells to a target site. In some embodiments, a homing molecule functions to recognize and/or initiate
5 interaction of an engineered cell to a target site. Non-limiting examples of homing molecules include CXCR1, CCR9, CXCR2, CXCR3, CXCR4, CCR2, CCR4, FPR2, VEGFR, IL6R, CXCR1, CSCR7, and PDGFR.

In some embodiments, a homing molecule is a chemokine receptor (cell surface molecule that binds to a chemokine). Chemokines are small cytokines or signaling proteins
10 secreted by cells that can induce directed chemotaxis in cells. Chemokines can be classified into four main subfamilies: CXC, CC, CX3C and XC, all of which exert biological effects by binding selectively to chemokine receptors located on the surface of target cells. In some embodiments, engineered nucleic acids are configured to produce CXCR4, a chemokine receptor which allows engineered cells to home along a chemokine gradient towards a stromal
15 cell-derived factor 1 (also known as SDF1, C-X-C motif chemokine 12, and CXCL12)-expressing cell, tissue, or tumor. Non-limiting examples of chemokine receptors that may be encoded by the engineered nucleic acids of the present disclosure include: CXC chemokine receptors (*e.g.*, CXCR1, CXCR2, CXCR3, CXCR4, CXCR5, CXCR6, and CXCR7), CC chemokine receptors (CCR1, CCR2, CCR3, CCR4, CCR5, CCR6, CCR7, CCR8, CCR9,
20 CCR10, and CCR11), CX3C chemokine receptors (*e.g.*, CX3CR1, which binds to CX3CL1), and XC chemokine receptors (*e.g.*, XCR1). In some embodiments, a chemokine receptor is a G protein-linked transmembrane receptor, or a member of the tumor necrosis factor (TNF) receptor superfamily (including but not limited to TNFRSF1A, TNFRSF1B). In some embodiments, engineered nucleic acids are configured to produce CXCL8, CXCL9, and/or CXCL10 (promote
25 T-cell recruitment), CCL3 and/or CXCL5, CCL21 (Th1 recruitment and polarization).

In some embodiments, engineered nucleic acids are configured to produce G-protein coupled receptors (GPCRs) that detect N-formylated-containing oligopeptides (including but not limited to FPR2 and FPRL1).

In some embodiments, engineered nucleic acids are configured to produce receptors that
30 detect interleukins (including but not limited to IL6R).

In some embodiments, engineered nucleic acids are configured to produce receptors that detect growth factors secreted from other cells, tissues, or tumors (including but not limited to FGFR, PDGFR, EGFR, and receptors of the VEGF family, including but not limited to VEGF-C and VEGF-D).

In some embodiments, a homing molecule is an integrin. Integrins are transmembrane receptors that facilitate cell-extracellular matrix (ECM) adhesion. Integrins are obligate heterodimers having two subunits: α (alpha) and β (beta). The α subunit of an integrin may be, without limitation: ITGA1, ITGA2, ITGA3, ITGA4, ITGA5, ITGA6, ITGA7, ITGA8, ITGA9, 5 ITGA10, ITGA11, ITGAD, ITGAE, ITGAL, ITGAM, ITGAV, ITGA2B, ITGAX. The β subunit of an integrin may be, without limitation: ITGB1, ITGB2, ITGB3, ITGB4, ITGB5, ITGB6, ITGB7, and ITGB8. Engineered nucleic acids can be configured to produce any combination of the integrin α and β subunits.

In some embodiments, a homing molecule is a matrix metalloproteinase (MMP). MMPs 10 are enzymes that cleave components of the basement membrane underlying the endothelial cell wall. Non-limiting examples of MMPs include MMP-2, MMP-9, and MMP. In some embodiments, engineered nucleic acids are configured to produce an inhibitor of a molecule (*e.g.*, protein) that inhibits MMPs. For example, engineered nucleic acids can be configured to express an inhibitor (*e.g.*, an RNAi molecule) of membrane type 1 MMP (MT1-MMP) or TIMP 15 metalloproteinase inhibitor 1 (TIMP-1).

In some embodiments, a homing molecule is a ligand that binds to selectin (*e.g.*, hematopoietic cell E-/L-selectin ligand (HCELL), Dykstran *et al.*, Stem Cells. 2016 Oct;34(10):2501-2511) on the endothelium of a target tissue, for example.

The term “homing molecule” also encompasses transcription factors that regulate the 20 production of molecules that improve/enhance homing of cells.

In some embodiments, a homing molecule includes an antibody, such as anti-integrin $\alpha 4 \beta 7$ or anti-MAdCAM.

Chimeric Antigen Receptors (CARs)

Certain aspects of the present disclosure relate to chimeric receptors that have any one of 25 the antigen-specific binding domains described herein (*e.g.*, EMCN-specific, FLT3-specific, and/or CD33-specific) and are capable of specifically binding to a protein, an antigen-derived antigen, or an antigen-derived epitope.

In some embodiments, the chimeric receptor is a chimeric antigen receptor (CAR). In general, CARs are chimeric proteins that include an antigen-binding domain and polypeptide 30 molecules that are heterologous to the antigen-binding domain, such as peptides heterologous to an antibody that an antigen-binding domain may be derived from. Polypeptide molecules that are heterologous to the antigen-binding domain can include, but are not limited to, a transmembrane domain, one or more intracellular signaling domains, a hinge domain, a spacer region, one or more peptide linkers, or combinations thereof.

In some embodiments, CARs are engineered receptors that graft or confer a specificity of interest (*e.g.*, EMCN, FLT3, or CD33) onto an immune effector cell. In certain embodiments, CARs can be used to graft the specificity of an antibody onto an immunoresponsive cell, such as a T cell. In some embodiments, CARs of the present disclosure comprise an extracellular
5 antigen-binding domain (*e.g.*, an scFv) fused to a transmembrane domain, fused to one or more intracellular signaling domains.

In some embodiments, the chimeric antigen receptor is an activating chimeric antigen receptor (aCAR and also generally referred to as CAR unless otherwise specified). In some
10 embodiments, binding of the chimeric antigen receptor to its cognate ligand is sufficient to induce activation of the immunoresponsive cell. In some embodiments, binding of the chimeric antigen receptor to its cognate ligand is sufficient to induce stimulation of the immunoresponsive cell. In some embodiments, activation of an immunoresponsive cell results in killing of target cells. In some embodiments, activation of an immunoresponsive cell results in cytokine or chemokine expression and/or secretion by the immunoresponsive cell. In some
15 embodiments, stimulation of an immunoresponsive cell results in cytokine or chemokine expression and/or secretion by the immunoresponsive cell. In some embodiments, stimulation of an immunoresponsive cell induces differentiation of the immunoresponsive cell. In some embodiments, stimulation of an immunoresponsive cell induces proliferation of the immunoresponsive cell. In some embodiments, activation and/or stimulation of the
20 immunoresponsive cell can be combinations of the above responses.

The number of ABDs in a binding molecule, such as the chimeric proteins described herein, defines the “valency” of the binding molecule. A binding molecule having a single ABD is “monovalent”. A binding molecule having a plurality of ABDs is said to be “multivalent”. A multivalent binding molecule having two ABDs is “bivalent.” A multivalent binding molecule
25 having three ABDs is “trivalent.” A multivalent binding molecule having four ABDs is “tetravalent.” In various multivalent embodiments, all of the plurality of ABDs have the same recognition specificity and can be referred to as a “monospecific multivalent” binding molecule. In other multivalent embodiments, at least two of the plurality of ABDs have different recognition specificities. Such binding molecules are multivalent and “multispecific.” In
30 multivalent embodiments in which the ABDs collectively have two recognition specificities, the binding molecule is “bispecific.” In multivalent embodiments in which the ABDs collectively have three recognition specificities, the binding molecule is “trispecific.” In multivalent embodiments in which the ABDs collectively have a plurality of recognition specificities for different epitopes present on the same antigen, the binding molecule is “multiparatopic.”

Multivalent embodiments in which the ABDs collectively recognize two epitopes on the same antigen are “biparatopic.”

In various multivalent embodiments, multivalency of the binding molecule improves the avidity of the binding molecule for a specific target. As described herein, “avidity” refers to the overall strength of interaction between two or more molecules, e.g. a multivalent binding molecule for a specific target, wherein the avidity is the cumulative strength of interaction provided by the affinities of multiple ABDs. Avidity can be measured by the same methods as those used to determine affinity, as described above. In certain embodiments, the avidity of a binding molecule for a specific target is such that the interaction is a specific binding interaction, wherein the avidity between two molecules has a K_D value below $10^{-6}M$, $10^{-7}M$, $10^{-8}M$, $10^{-9}M$, or $10^{-10}M$. In certain embodiments, the avidity of a binding molecule for a specific target has a K_D value such that the interaction is a specific binding interaction, wherein the one or more affinities of individual ABDs do not have a K_D value that qualifies as specifically binding their respective antigens or epitopes on their own. In certain embodiments, the avidity is the cumulative strength of interaction provided by the affinities of multiple ABDs for separate antigens on a shared specific target or complex, such as separate antigens found on an individual cell. In certain embodiments, the avidity is the cumulative strength of interaction provided by the affinities of multiple ABDs for separate epitopes on a shared individual antigen.

In some embodiments, an aCAR can be a bivalent-bispecific CAR. In some embodiments, an aCAR can be a bivalent-bispecific CAR expressed on a cell of the present disclosure (e.g., an immunoresponsive cell) as OR-logic gates to increase the potential targets of an activating chimeric receptor (e.g., an OR-logic gate targeting multiple tumor targets). A bivalent-bispecific aCAR can include an antigen-binding domain specific for FLT3 and an antigen-binding domain specific for CD33. A bivalent-bispecific aCAR can include i) an antigen-binding domain specific for FLT3; (ii) an antigen-binding domain specific for CD33; (iii) one or more intracellular signaling domains that stimulate an immune response, and (iv) one or more polypeptides including, but not limited to, a signal peptide, a transmembrane domain, a hinge domain, a spacer region, one or more peptide linkers, and combinations thereof.

A CAR of the present disclosure may be a first, second, or third generation CAR. “First generation” CARs comprise a single intracellular signaling domain, generally derived from a T cell receptor chain. “First generation” CARs generally have the intracellular signaling domain from the CD3-zeta ($CD3\zeta$) chain, which is the primary transmitter of signals from endogenous TCRs. “First generation” CARs can provide de novo antigen recognition and cause activation of both $CD4^+$ and $CD8^+$ T cells through their $CD3\zeta$ chain signaling domain in a single fusion

molecule, independent of HLA-mediated antigen presentation. "Second generation" CARs add a second intracellular signaling domain from one of various co-stimulatory molecules (e.g., CD28, 4-1BB, ICOS, OX40) to the cytoplasmic tail of the CAR to provide additional signals to the T cell. "Second generation" CARs provide both co-stimulation (e.g., CD28 or 4-1BB) and
5 activation (CD3 ζ). Preclinical studies have indicated that "Second Generation" CARs can improve the anti-tumor activity of immunoresponsive cell, such as a T cell. "Third generation" CARs have multiple intracellular co-stimulation signaling domains (e.g., CD28 and 4-1BB) and an intracellular activation signaling domain (CD3 ζ).

10 In some embodiments, the chimeric antigen receptor is a chimeric inhibitory receptor (iCAR). In some embodiments, the one or more chimeric inhibitory receptors bind antigens that are expressed on a non-tumor cell derived from a tissue selected from the group consisting of brain, neuronal tissue, endocrine, bone, bone marrow, immune system, endothelial tissue, muscle, lung, liver, gallbladder, pancreas, gastrointestinal tract, kidney, urinary bladder, male reproductive organs, female reproductive organs, adipose, soft tissue, and skin.

15 In some embodiments, a chimeric inhibitory receptor (e.g., an EMCN-specific chimeric inhibitory receptor) may be used, for example, with one or more activating chimeric receptors (e.g., activating chimeric TCRs or CARs, such as FLT3 and/or CD33 aCARs) expressed on a cell of the present disclosure (e.g., an immunoresponsive cell) as NOT-logic gates to control, modulate, or otherwise inhibit one or more activities of the one or more activating chimeric
20 receptors. For instance, if a healthy cell expresses both an antigen that is recognized by a tumor-targeting chimeric receptor and an antigen that is recognized by a chimeric inhibitory receptor, an immunoresponsive cell expressing the tumor antigen may bind to the healthy cell. In such a case, the inhibitory chimeric antigen will also bind its cognate ligand on the healthy cell and the inhibitory function of the chimeric inhibitory receptor will reduce, decrease, prevent, or inhibit
25 the activation of the immunoresponsive cell via the tumor-targeting chimeric receptor ("NOT-logic gating"). In some embodiments, a chimeric inhibitory receptor of the present disclosure may inhibit one or more activities of a cell of the present disclosure (e.g., an immunoresponsive cell). In some embodiments, an immunoresponsive cell may comprise one or more tumor-targeting chimeric receptors and one or more chimeric inhibitory receptors that targets an
30 antigen that is not expressed, or generally considered to be expressed, on the tumor (e.g., EMCN). Combinations of tumor-targeting chimeric receptors and chimeric inhibitory receptors in the same immunoresponsive cell may be used to reduce on-target off-tumor toxicity.

In some embodiments, the extracellular antigen-binding domain of a CAR of the present disclosure binds to one or more antigens (e.g., EMCN, FLT3, or CD33) with a dissociation

constant (K_d) of about 2×10^{-7} M or less, about 1×10^{-7} M or less, about 9×10^{-8} M or less, about 1×10^{-8} M or less, about 9×10^{-9} M or less, about 5×10^{-9} M or less, about 4×10^{-9} M or less, about 3×10^{-9} M or less, about 2×10^{-9} M or less, or about 1×10^{-9} M or less. In some embodiments, the K_d ranges from about 2×10^{-7} M to about 1×10^{-9} M.

5 Binding of the extracellular antigen-binding domain of a CAR of the present disclosure can be determined by, for example, an enzyme-linked immunosorbent assay (ELISA), a radioimmunoassay (RIA), FACS analysis, a bioassay (e.g., growth inhibition), bio-layer interferometry (e.g., Octet/FORTEBIO®), surface plasmon resonance (SPR) technology (e.g., Biacore®), or a Western Blot assay. Each of these assays generally detect the presence of
10 protein-antibody complexes of particular interest by employing a labeled reagent (e.g., an antibody or scFv) specific for the complex of interest. For example, the scFv can be radioactively labeled and used in an RIA assay. The radioactive isotope can be detected by such means as the use of a γ counter or a scintillation counter or by autoradiography. In certain
15 embodiments, the extracellular antigen-binding domain of the CAR is labeled with a fluorescent marker. Non-limiting examples of fluorescent markers include green fluorescent protein (GFP), blue fluorescent protein (e.g., EBFP, EBFP2, Azurite, and mKalamal), cyan fluorescent protein (e.g., ECFP, Cerulean, and CyPet), and yellow fluorescent protein (e.g., YFP, Citrine, Venus, and YPet). In certain embodiments, the extracellular antigen-binding domain of the CAR is
20 labeled with a secondary antibody specific for the extracellular antigen-binding domain and wherein the secondary antibody is labeled (e.g., radioactively or with a fluorescent marker).

In some embodiments, CARs of the present disclosure comprise an extracellular antigen-binding domain that binds to EMCN, FLT3, or CD33. In some embodiments, CARs of the present disclosure comprise an extracellular antigen-binding domain that binds to an EMCN protein, an EMCN-derived antigen, or an EMCN-derived epitope. In some embodiments, CARs
25 of the present disclosure comprise an extracellular antigen-binding domain that binds to a FLT3 protein, a FLT3-derived antigen, or a FLT3-derived epitope. In some embodiments. In some embodiments, CARs of the present disclosure comprise an extracellular antigen-binding domain that binds to an CD33 protein, a CD33-derived antigen, or a CD33-derived epitope. CARs of the present disclosure comprise an extracellular antigen-binding domain, a transmembrane domain,
30 and one or more intracellular signaling domains. In some embodiments, the extracellular antigen-binding domain comprises an scFv. In some embodiments, the extracellular antigen-binding domain comprises a Fab fragment, which may be crosslinked. In certain embodiments, the extracellular binding domain is a F(ab)₂ fragment.

Extracellular Antigen-binding Domain

Antigen-binding domains of the present disclosure can include any domain that binds to the antigen including, without limitation, a monoclonal antibody, a polyclonal antibody, a recombinant antibody, a bispecific antibody, a conjugated antibody, a human antibody, a humanized antibody, and a functional fragment thereof, including but not limited to a single-domain antibody (sdAb) such as a heavy chain variable domain (VH), a light chain variable domain (VL) and a variable domain (VHH) of camelid derived nanobody, and to an alternative scaffold known in the art to function as antigen-binding domain, such as a recombinant fibronectin domain, a T cell receptor (TCR), a recombinant TCR with enhanced affinity, or a fragment thereof, e.g., single chain TCR, and the like. In some instances, it is beneficial for the antigen-binding domain to be derived from the same species in which the CAR will ultimately be used in.

In some embodiments, the extracellular antigen-binding domain comprises an antibody. In certain embodiments, the antibody is a human antibody. In certain embodiments, the antibody is a chimeric antibody. In some embodiments, the extracellular antigen-binding domain comprises an antigen-binding fragment of an antibody.

In some embodiments, the extracellular antigen-binding domain comprises a F(ab) fragment. In certain embodiments, the extracellular antigen-binding domain comprises a F(ab') fragment.

In some embodiments, the extracellular antigen-binding domain comprises an scFv. In some embodiments, the extracellular antigen-binding domain comprises two single chain variable fragments (scFvs). In some embodiments, each of the two scFvs binds to a distinct epitope on the same antigen. In some embodiments, the extracellular antigen-binding domain comprises a first scFv and a second scFv. In some embodiments, the first scFv and the second scFv bind distinct epitopes on the same antigen. In certain embodiments, the scFv is a mammalian scFv. In certain embodiments, the scFv is a chimeric scFv. In certain embodiments, the scFv comprises a heavy chain variable domain (VH) and a light chain variable domain (VL).

In certain embodiments, the VH and VL are separated by a peptide linker. In certain embodiments, the peptide linker comprises any of the amino acid sequences shown in **Table 6**.

In certain embodiments, the scFv comprises the structure VH-L-VL or VL-L-VH, wherein VH is the heavy chain variable domain, L is the peptide linker, and VL is the light chain variable domain. In some embodiments, each of the one or more scFvs comprises the structure VH-L-VL or VL-L-VH, wherein VH is the heavy chain variable domain, L is the peptide linker, and VL is the light chain variable domain. When there are two or more scFv linked together,

each scFv can be linked to the next scFv with a peptide linked. In some embodiments, each of the one or more scFvs is separated by a peptide linker.

In some embodiments, a peptide linker includes the amino acid sequence GGGGSGGGSGGGGS (SEQ ID NO: 244). In some embodiments, a peptide linker between antigen binding domains of an iCAR comprises the amino acid sequence GGGGSGGGSGGGGS (SEQ ID NO: 244). In some embodiments, a peptide linker is encoded by a polynucleotide sequence comprising the sequence GGAGGCGGAGGATCTGGTGGCGGAGGAAGTGGCGGAGGCGGTTCT (SEQ ID NO: 253). In some embodiments, a nucleic acid encoding peptide linker includes a sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to GGAGGCGGAGGATCTGGTGGCGGAGGAAGTGGCGGAGGCGGTTCT (SEQ ID NO: 253).

In some embodiments, a peptide linker includes the amino acid sequence GGGGS (SEQ ID NO: 242). In some embodiments, a peptide linker between antigen binding domains of an aCAR comprises the amino acid sequence GGGGS (SEQ ID NO: 242). In some embodiments, a peptide linker is encoded by a polynucleotide sequence comprising the sequence GGCGGCGGTGGCTCT (SEQ ID NO: 254) or GGTGGCGGCGGATCC (SEQ ID NO: 255). In some embodiments, a nucleic acid encoding peptide linker includes a sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to GGCGGCGGTGGCTCT (SEQ ID NO: 254) or GGTGGCGGCGGATCC (SEQ ID NO: 255).

In some embodiments, a peptide linker includes the amino acid sequence GGGGSGGGGS (SEQ ID NO: 243). In some embodiments, a peptide linker between antigen binding domains of an aCAR comprises the amino acid sequence GGGGSGGGGS (SEQ ID NO: 243). In some embodiments, a peptide linker is encoded by a polynucleotide sequence comprising the sequence GGAGGCGGAGGATCTGGTGGTGGTGGATCT (SEQ ID NO: 256), GGTGGCGGAGGAAGTGGCGGCGGAGGCTCT (SEQ ID NO: 257), or GGCGGTGGCGGATCTGGCGGAGGTGGCAGT (SEQ ID NO: 258). In some embodiments, a nucleic acid encoding peptide linker includes a sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to GGAGGCGGAGGATCTGGTGGTGGTGGATCT (SEQ ID NO: 256), GGTGGCGGAGGAAGTGGCGGCGGAGGCTCT (SEQ ID NO: 257), or GGCGGTGGCGGATCTGGCGGAGGTGGCAGT (SEQ ID NO: 258).

- In some embodiments, a peptide linker includes the amino acid sequence GSTSGSGKPGSGEGSTKG (SEQ ID NO: 247). In some embodiments, a peptide linker between antigen binding domains of an aCAR comprises the amino acid sequence GSTSGSGKPGSGEGSTKG (SEQ ID NO: 247). In some embodiments, a peptide linker is
- 5 encoded by a polynucleotide sequence comprising the sequence GGCTCTACATCTGGCTCTGGCAAACCTGGAAGCGGCGAGGGATCTACCAAGGGC (SEQ ID NO: 249). In some embodiments, a nucleic acid encoding peptide linker includes a sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to
- 10 GGCTCTACATCTGGCTCTGGCAAACCTGGAAGCGGCGAGGGATCTACCAAGGGC (SEQ ID NO: 249).

Table 6. Peptide Linkers

Linker	Amino Acid Sequence	SEQ ID NO:
GS-linker	SGGGGSGGGGSG	230
GS-linker	GGGSGGGGSGGGSLQ	231
(G ₂ S) ₁ scFv linker	GGG	232
(G ₂ S) ₂ scFv linker	GGSGGS	233
(G ₂ S) ₃ scFv linker	GGSGGSGGS	234
(G ₂ S) ₄ scFv linker	GGSGGSGGSGGS	235
(G ₂ S) ₅ scFv linker	GGSGGSGGSGGSGGS	236
(G ₃ S) ₁ scFv linker	GGGS	237
(G ₃ S) ₂ scFv linker	GGGSGGGS	238
(G ₃ S) ₃ scFv linker	GGGSGGGSGGGS	239
(G ₃ S) ₄ scFv linker	GGGSGGGSGGGSGGGS	240
(G ₃ S) ₅ scFv linker	GGGSGGGSGGGSGGGSGGGS	241
(G ₄ S) ₁ linker	GGGGS	242
(G ₄ S) ₂ linker	GGGGSGGGGS	243
(G ₄ S) ₃ linker	GGGGSGGGSGGGGS	244
(G ₄ S) ₄ linker	GGGSGGGGSGGGSGGGGS	245
(G ₄ S) ₅ linker	GGGSGGGGSGGGSGGGSGGGGS	246
Whitlow linker [“Loop 6” linker]	GSTSGSGKPGSGEGSTKG	247
scFv linker 2	EAAAKEAAAKEAAKEAAAK	248

In some embodiments, the immune effector cell comprises a first chimeric receptor and a second chimeric receptor. The antigen-binding domain of the first chimeric receptor and the antigen-binding domain of the second chimeric receptor can be an appropriate antigen binding domain described herein or known in the art. For example, the first or second antigen-binding domain can be one or more antibodies, antigen-binding fragments of an antibody, F(ab) fragments, F(ab') fragments, single chain variable fragments (scFvs), or single-domain antibodies (sdAbs). In some embodiments, the antigen-binding domain of the first chimeric receptor and/or the second chimeric receptor comprises two single chain variable fragments (scFvs). In some embodiments, each of the two scFvs binds to a distinct epitope on the same antigen. In some embodiments, the antigen-binding domain of the first chimeric receptor can be specific for EMCN and the chimeric receptor can be specific for a second distinct antigen, such as a cancer antigen (*e.g.*, an antigen expressed on a myeloid cell, such as an AML cell).

In some embodiments, the extracellular antigen-binding domain comprises a single-domain antibody (sdAb). In certain embodiments, the sdAb is a humanized sdAb. In certain embodiments, the sdAb is a chimeric sdAb.

In some embodiments, a CAR of the present disclosure may comprise two or more antigen-binding domains, three or more antigen-binding domains, four or more antigen-binding domains, five or more antigen-binding domains, six or more antigen-binding domains, seven or more antigen-binding domains, eight or more antigen-binding domains, nine or more antigen-binding domains, or ten or more antigen-binding domains. In some embodiments, each of the two or more antigen-binding domains binds the same antigen. In some embodiments, each of the two or more antigen-binding domains binds a different epitope of the same antigen. In some embodiments, each of the two or more antigen-binding domains binds a different antigen.

In some embodiments, the CAR comprises two antigen-binding domains. In some embodiments, the two antigen-binding domains are attached to one another via a flexible linker. In some embodiments, each of the two-antigen-binding domains may be independently selected from an antibody, an antigen-binding fragment of an antibody, an scFv, a sdAb, a recombinant fibronectin domain, a T cell receptor (TCR), a recombinant TCR with enhanced affinity, and a single chain TCR. In some embodiments, the CAR comprising two antigen-binding domains is a bispecific CAR or a tandem CAR (tanCAR).

In certain embodiments, the bispecific CAR or tanCAR comprises an antigen-binding domain comprising a bispecific antibody or antibody fragment (*e.g.*, scFv). In some embodiments, within each antibody or antibody fragment (*e.g.*, scFv) of a bispecific antibody molecule, the VH can be upstream or downstream of the VL. In some embodiments, the upstream antibody or antibody fragment (*e.g.*, scFv) is arranged with its VH (VH₁) upstream of

its VL (VL₁) and the downstream antibody or antibody fragment (e.g., scFv) is arranged with its VL (VL₂) upstream of its VH (VH₂), such that the overall bispecific antibody molecule has the arrangement VH₁-VL₁-VL₂-VH₂. In other embodiments, the upstream antibody or antibody fragment (e.g., scFv) is arranged with its VL (VL₁) upstream of its VH (VH₁) and the downstream antibody or antibody fragment (e.g., scFv) is arranged with its VH (VH₂) upstream of its VL (VL₂), such that the overall bispecific antibody molecule has the arrangement VL₁-VH₁-VH₂-VL₂. In some embodiments, a linker is disposed between the two antibodies or antibody fragments (e.g., scFvs), for example, between VL₁ and VL₂ if the construct is arranged as VH₁-VL₁-VL₂-VH₂, or between VH₁ and VH₂ if the construct is arranged as VL₁-VH₁-VH₂-VL₂. The linker may be a linker as described herein, e.g., a (Gly₄-Ser)_n linker, wherein n is 1, 2, 3, 4, 5, or 6. In general, the linker between the two scFvs should be long enough to avoid mispairing between the domains of the two scFvs. In some embodiments, a linker is disposed between the VL and VH of the first scFv. In some embodiments, a linker is disposed between the VL and VH of the second scFv. In constructs that have multiple linkers, any two or more of the linkers may be the same or different. Accordingly, in some embodiments, a bispecific CAR or tanCAR comprises VLs, VHs, and may further comprise one or more linkers in an arrangement as described herein.

In an illustrative non-limiting example, a CD33/FLT3 bispecific bivalent aCAR can have antigen binding domains encoded in the following order: (FLT3-VH) – L1 – (CD33-VH) – L2 – (CD33-VL) – L3 – (FLT3-VL), wherein L1, L2, and L3 are a first, a second, and a third peptide linker, respectively. L1, L2, and L3 can be the same peptide linker. L1, L2, and L3 can be the same peptide linker encoded by the same polynucleotide sequence. L1, L2, and L3 can be the same peptide linker encoded by different polynucleotide sequences. L1 and L3 can be the same peptide linker and L2 a different peptide linker. L1 and L3 can be the same peptide linker encoded by the same polynucleotide sequence. L1 and L3 can be the same peptide linker encoded by different polynucleotide sequences. L1, L2, and L3 can each be different peptide linkers.

CAR Transmembrane Domains

In some embodiments, the transmembrane domain of a CAR of the present disclosure (e.g., the EMCN-specific, FLT3-specific, and/or CD33-specific CARs described herein) comprises a hydrophobic alpha helix that spans at least a portion of a cell membrane. It has been shown that different transmembrane domains can result in different receptor stability. After antigen recognition, receptors cluster and a signal is transmitted to the cell. In some embodiments, the transmembrane domain of a CAR of the present disclosure can comprise the

transmembrane domain of a CD8 polypeptide, a CD28 polypeptide, a CD3-zeta polypeptide, a CD4 polypeptide, a 4-1BB polypeptide, an OX40 polypeptide, an ICOS polypeptide, a CTLA-4 polypeptide, a PD-1 polypeptide, a LAG-3 polypeptide, a 2B4 polypeptide, a BTLA polypeptide, a LIR-1 (LILRB1) polypeptide, or can be a synthetic peptide, or any combination thereof.

In some embodiments, the transmembrane domain is derived from a CD8 polypeptide. Any suitable CD8 polypeptide may be used. Exemplary CD8 polypeptides include, without limitation, NCBI Reference Nos. NP_001139345 and AAA92533.1. In some embodiments, the transmembrane domain is derived from a CD28 polypeptide. Any suitable CD28 polypeptide may be used. Exemplary CD28 polypeptides include, without limitation, NCBI Reference Nos. NP_006130.1 and NP_031668.3. In some embodiments, the transmembrane domain is derived from a CD3-zeta polypeptide. Any suitable CD3-zeta polypeptide may be used. Exemplary CD3-zeta polypeptides include, without limitation, NCBI Reference Nos. NP_932170.1 and NP_001106862.1. In some embodiments, the transmembrane domain is derived from a CD4 polypeptide. Any suitable CD4 polypeptide may be used. Exemplary CD4 polypeptides include, without limitation, NCBI Reference Nos. NP_000607.1 and NP_038516.1. In some embodiments, the transmembrane domain is derived from a 4-1BB polypeptide. Any suitable 4-1BB polypeptide may be used. Exemplary 4-1BB polypeptides include, without limitation, NCBI Reference Nos. NP_001552.2 and NP_001070977.1. In some embodiments, the transmembrane domain is derived from an OX40 polypeptide. Any suitable OX40 polypeptide may be used. Exemplary OX40 polypeptides include, without limitation, NCBI Reference Nos. NP_003318.1 and NP_035789.1. In some embodiments, the transmembrane domain is derived from an ICOS polypeptide. Any suitable ICOS polypeptide may be used. Exemplary ICOS polypeptides include, without limitation, NCBI Reference Nos. NP_036224 and NP_059508. In some embodiments, the transmembrane domain is derived from a CTLA-4 polypeptide. Any suitable CTLA-4 polypeptide may be used. Exemplary CTLA-4 polypeptides include, without limitation, NCBI Reference Nos. NP_005205.2 and NP_033973.2. In some embodiments, the transmembrane domain is derived from a PD-1 polypeptide. Any suitable PD-1 polypeptide may be used. Exemplary PD-1 polypeptides include, without limitation, NCBI Reference Nos. NP_005009 and NP_032824. In some embodiments, the transmembrane domain is derived from a LAG-3 polypeptide. Any suitable LAG-3 polypeptide may be used. Exemplary LAG-3 polypeptides include, without limitation, NCBI Reference Nos. NP_002277.4 and NP_032505.1. In some embodiments, the transmembrane domain is derived from a 2B4 polypeptide. Any suitable 2B4 polypeptide may be used. Exemplary 2B4 polypeptides include, without limitation, NCBI Reference Nos. NP_057466.1 and NP_061199.2. In some embodiments, the

transmembrane domain is derived from a BTLA polypeptide. Any suitable BTLA polypeptide may be used. Exemplary BTLA polypeptides include, without limitation, NCBI Reference Nos. NP_861445.4 and NP_001032808.2. Any suitable LIR-1 (LILRB1) polypeptide may be used. Exemplary LIR-1 (LILRB1) polypeptides include, without limitation, NCBI Reference Nos.

5 NP_001075106.2 and NP_001075107.2.

In some embodiments, the transmembrane domain comprises a polypeptide comprising an amino acid sequence that is at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% homologous to the sequence of NCBI Reference No. NP_001139345, AAA92533.1,
 10 NP_006130.1, NP_031668.3, NP_932170.1, NP_001106862.1, NP_000607.1, NP_038516.1, NP_001552.2, NP_001070977.1, NP_003318.1, NP_035789.1, NP_036224, NP_059508, NP_005205.2, NP_033973.2, NP_005009, NP_032824, NP_002277.4, NP_032505.1, NP_057466.1, NP_061199.2, NP_861445.4, or NP_001032808.2, or fragments thereof. In some embodiments, the homology may be determined using standard software such as BLAST or
 15 FASTA. In some embodiments, the polypeptide may comprise one conservative amino acid substitution, up to two conservative amino acid substitutions, or up to three conservative amino acid substitutions. In some embodiments, the polypeptide can have an amino acid sequence that is a consecutive portion of NCBI Reference No. NP_001139345, AAA92533.1, NP_006130.1, NP_031668.3, NP_932170.1, NP_001106862.1, NP_000607.1, NP_038516.1, NP_001552.2,
 20 NP_001070977.1, NP_003318.1, NP_035789.1, NP_036224, NP_059508, NP_005205.2, NP_033973.2, NP_005009, NP_032824, NP_002277.4, NP_032505.1, NP_057466.1, NP_061199.2, NP_861445.4, or NP_001032808.2 that is at least 20, at least 30, at least 40, at least 50, at least 60, at least 70, at least 80, at least 90, at least 100, at least 110, at least 120, at least 130, at least 140, at least 150, at least 160, at least 170, at least 180, at least 190, at least
 25 200, at least 210, at least 220, at least 230, or at least 240 amino acids in length.

Further examples of suitable polypeptides from which a transmembrane domain may be derived include, without limitation, the transmembrane region(s) of the alpha, beta or zeta chain of the T-cell receptor, CD27, CD3 epsilon, CD45, CD5, CD9, CD16, CD22, CD33, CD37, CD64, CD80, CD86, CD134, CD137, CD154, KIRDS2, CD2, CD27, LFA-1 (CD11a, CD18),
 30 GITR, CD40, BAFFR, HVEM (LIGHTR), SLAMF7, NKp80 (KLRF1), NKp44, NKp30, NKp46, CD160, CD19, IL2R beta, IL2R gamma, IL7R α , ITGA1, VLA1, CD49a, ITGA4, IA4, CD49D, ITGA6, VLA-6, CD49f, ITGAD, CD11d, ITGAE, CD103, ITGAL, CD11a, LFA-1, ITGAM, CD11b, ITGAX, CD11c, ITGB1, CD29, ITGB2, CD18, LFA-1, ITGB7, TNFR2, DNAM1 (CD226), SLAMF4 (CD244, 2B4), CD84, CD96 (Tactile), CEACAM1, CRTAM, Ly9

(CD229), CD160 (BY55), PSGL1, CD100 (SEMA4D), SLAMF6 (NTB-A, Ly108), SLAM (SLAMF1, CD150, IPO-3), BLAME (SLAMF8), SELPLG (CD162), LTBR, PAG/Cbp, NKG2D, and NG2C.

In some embodiments, the transmembrane domain comprises the sequence

- 5 IYIWAPLAGTCGVLLLSLVIT (SEQ ID NO: 205). In some embodiments, the transmembrane domain comprises the sequence IYIWAPLAGTCGVLLLSLVITLYCNHR (SEQ ID NO: 206). In some embodiments, the transmembrane domain comprises the sequence IYIWAPLAGTCGVLLLSLVITLYCNHRN (SEQ ID NO: 207).

- 10 In some embodiments, an iCAR transmembrane domain includes a LIR1 transmembrane domain. In some embodiments, an iCAR transmembrane domain includes a LIR1 transmembrane domain having the sequence VIGILVAVILLLLLLLLLLFLI (SEQ ID NO: 259). In some embodiments, an iCAR transmembrane domain includes a LIR1 transmembrane domain encoded by a polynucleotide sequence having the sequence

- GTGATCGGCATTCTGGTCGCCGTGATCCTGCTCCTGTTGCTCCTGCTGCTTCTGTTCC
15 TGATC (SEQ ID NO: 260). In some embodiments, an iCAR transmembrane domain includes a LIR1 transmembrane domain encoded by a polynucleotide sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to
GTGATCGGCATTCTGGTCGCCGTGATCCTGCTCCTGTTGCTCCTGCTGCTTCTGTTCC
20 TGATC (SEQ ID NO: 260).

- In some embodiments, an aCAR transmembrane domain includes a CD8 transmembrane domain. In some embodiments, an aCAR transmembrane domain includes a CD8 transmembrane domain having the sequence IYIWAPLAGTCGVLLLSLVITLYCNHR (SEQ ID NO: 206). In some embodiments, an aCAR transmembrane domain includes a CD8
25 transmembrane domain encoded by a polynucleotide sequence having the sequence
ATCTATATCTGGGCCCCTCTGGCTGGCACATGCGGAGTTCTGCTGCTCAGCCTGGTC
ATCACCTGTACTGCAACCACAGA (SEQ ID NO: 261). In some embodiments, an aCAR transmembrane domain includes a CD8 transmembrane domain encoded by a polynucleotide sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%,
30 at least 96%, at least 97%, at least 98%, or at least 99% identical to
ATCTATATCTGGGCCCCTCTGGCTGGCACATGCGGAGTTCTGCTGCTCAGCCTGGTC
ATCACCTGTACTGCAACCACAGA (SEQ ID NO: 261).

Spacer Region

In some embodiments, a CAR of the present disclosure (*e.g.*, the EMCN-specific, FLT3-specific, and/or CD33-specific CARs described herein) can also comprise a spacer region that links the extracellular antigen-binding domain to the transmembrane domain. The spacer region may be flexible enough to allow the antigen-binding domain to orient in different directions to facilitate antigen recognition. In some embodiments, the spacer region may be a hinge from a human protein. For example, the hinge may be a human Ig (immunoglobulin) hinge, including without limitation an IgG4 hinge, an IgG2 hinge, a CD8a hinge, or an IgD hinge. In some embodiments, the spacer region may comprise an IgG4 hinge, an IgG2 hinge, an IgD hinge, a CD28 hinge, a KIR2DS2 hinge, an LNGFR hinge, or a PDGFR-beta extracellular linker. In some embodiments, the spacer region is localized between the antigen-binding domain and the transmembrane domain. In some embodiments, a spacer region may comprise any of the amino acid sequences listed in **Table 7**, or an amino acid sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to any of the amino acid sequences listed in **Table 7**. In some embodiments, nucleic acids encoding any of the spacer regions of the present disclosure may comprise any of the nucleic acid sequences listed in **Table 8**, or a nucleic acid sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to any of the nucleic acid sequences listed in **Table 8**.

In some embodiments, an aCAR hinge domain includes a CD8 hinge. In some embodiments, an aCAR hinge domain includes a CD8 hinge having the amino acid sequence ALSNSIMYFSHFVPVFLPAKPTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACD (SEQ ID NO: 272). In some embodiments, an aCAR hinge domain includes a CD8 hinge encoded by a polynucleotide sequence having the sequence

GCCCTGAGCAACAGCATCATGTACTTCAGCCACTTCGTGCCCCGTGTTTCTGCCCCGCCAAGCCTACAACAACCCCTGCTCCTAGACCACCTACACCAGCTCCTACAATCGCCAGCCAGCCTCTGTCTCTGAGGCCCCGAAGCTTGTAGACCAGCTGCTGGCGGAGCCGTGCATACAAGAGGACTGGATTTTGCCTGCGAC (SEQ ID NO: 284). In some embodiments, an aCAR hinge domain includes a CD8 hinge encoded by a polynucleotide sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to

GCCCTGAGCAACAGCATCATGTACTTCAGCCACTTCGTGCCCCGTGTTTCTGCCCCGCCAAGCCTACAACAACCCCTGCTCCTAGACCACCTACACCAGCTCCTACAATCGCCAGCCAGCCTCTGTCTCTGAGGCCCCGAAGCTTGTAGACCAGCTGCTGGCGGAGCCGTGCATACAAGAGGACTGGATTTTGCCTGCGAC (SEQ ID NO: 284).

In some embodiments, an iCAR hinge domain includes a CD8 hinge. In some embodiments, an iCAR hinge domain includes a CD8 hinge having the amino acid sequence TTPAPRPPTPAPTIALQPLSLRPEACRPAAGGAVHTRGLDFACD (SEQ ID NO: 271). In some embodiments, an iCAR hinge domain includes a CD8 hinge encoded by a polynucleotide sequence having the sequence

ACAACAACACCCGCACCTCGGCCTCCAACTCCAGCTCCAACAATTGCACTGCAACC
CCTGAGTCTGAGGCCCGAGGCCTGTAGGCCAGCAGCTGGCGGAGCTGTTCACTA
GAGGCCTGGACTTTGCCTGTGAC (SEQ ID NO: 283). In some embodiments, an iCAR hinge domain includes a CD8 hinge encoded by a polynucleotide sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to

ACAACAACACCCGCACCTCGGCCTCCAACTCCAGCTCCAACAATTGCACTGCAACC
CCTGAGTCTGAGGCCCGAGGCCTGTAGGCCAGCAGCTGGCGGAGCTGTTCACTA
GAGGCCTGGACTTTGCCTGTGAC (SEQ ID NO: 283).

In some embodiments, an iCAR hinge domain includes a LIR1 hinge. In some embodiments, an iCAR hinge domain includes a LIR1 hinge having the amino acid sequence HPSDPLELVVSGPSGGPSSPTTGPTSTSGPEDQPLTPTGSDPQSGLGRHLGV (SEQ ID NO: 417). In some embodiments, an iCAR hinge domain includes a LIR1 hinge encoded by a polynucleotide sequence having the sequence

CACCCATCCGATCCTCTCGAGCTGGTGGTTTCTGGACCTTCTGGCGGCCCTAGCAGC
CCTACAACAGGACCTACAAGCACAAGCGGCCCTGAGGACCAACCTCTGACACCAAC
AGGCAGCGATCCTCAGTCTGGACTGGGGAGACATCTGGGCGTT (SEQ ID NO: 418). In some embodiments, an iCAR hinge domain includes a LIR1 hinge encoded by a polynucleotide sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to

CACCCATCCGATCCTCTCGAGCTGGTGGTTTCTGGACCTTCTGGCGGCCCTAGCAGC
CCTACAACAGGACCTACAAGCACAAGCGGCCCTGAGGACCAACCTCTGACACCAAC
AGGCAGCGATCCTCAGTCTGGACTGGGGAGACATCTGGGCGTT (SEQ ID NO: 418).

Table 7. Spacer Amino Acid Sequences

Amino Acid Sequence	SEQ ID NO:	Description
AAAIEVMYPPPYLDNEKSNGTIIHVKGKHLCPSPLPGPS KP	262	CD28 hinge
ESKYGPPCPSCP	263	IgG4 minimal hinge
ESKYGPPAPSAP	264	IgG4 minimal hinge, no disulfides
ESKYGPPCPPCP	265	IgG4 S228P minimal hinge, enhanced disulfide formation
EPKSCDKTHTCP	266	IgG1 minimal hinge
AAAFVPVFLPAKPTTTPAPRPPTPAPTIASQPLSLRPEACR PAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLSLVITL YCNHRN	267	Extended CD8a hinge
ACPTGLYTHSGECKACNLGEGVAQPCGANQTVCEPCL DSVTFSDVVSATEPCKPCTECVGLQSMSAPCVEADDAVC RCAYGYYQDETTGRCEACRVCEAGSGLVFSCQDKQNTV CEECPDGTYSDEADAEC	268	LNGFR hinge
ACPTGLYTHSGECKACNLGEGVAQPCGANQTV	269	Truncated LNGFR hinge (TNFR-Cys1)
AVGQDTQEVIVVPHSLPFKV	270	PDGFR-beta extracellular linker
TTTPAPRPPTPAPTIALQPLSLRPEACRPAAGGAVHTRGL DFACD	271	Example spacer derived from CD8 hinge
ALSNSIMYFSHFVPVFLPAKPTTTPAPRPPTPAPTIASQPLS LRPEACRPAAGGAVHTRGLDFACD	272	Example spacer derived from CD8 hinge
FVPVFLPAKPTTTPAPRPPTPAPTIALQPLSLRPEACRPAAGGAVHTRGLDFACD	273	Example spacer
HPSDPLELVVSGPSGGPSSPTTGPTSTSGPEDQPLTPTGSD PQSGLGRHLGV	417	LIR1 Hinge

Table 8. Spacer Nucleic Acid Sequences

Nucleic Acid Sequence	SEQ ID NO:	Description
GCAGCAGCTATCGAGGTGATGTATCCICCGCCCTACCT GGATAATGAAAAGAGTAATGGGACTATCATTCATGTA AAAGGGAAGCATCTTTGTCCTTCTCCCCTTTTCCCCGG TCCGTCTAAACCT	274	CD28 hinge
GAAAGCAAGTACGGTCCACCTTGCCCTAGCTGTCCG	275	IgG4 minimal hinge
GAATCCAAGTACGGCCCCCAGCGCCTAGTGCCCCA	276	IgG4 minimal hinge, no disulfides
GAATCTAAATATGGCCCGCCATGCCCGCCTTGCCCCA	277	IgG4 S228P minimal hinge, enhanced disulfide formation
GAACCGAAGTCTTGATGATAAACTCATACGTGCCCCG	278	IgG1 minimal hinge

Nucleic Acid Sequence	SEQ ID NO:	Description
GCTGCTGCTTTCGTACCCGTGTTCCCTCCCTGCTAAGCC TACGACTACCCCCGCACCGAGACCACCCACGCCAGCA CCCACGATTGCTAGCCAGCCCCCTTAGTTTGCAGCCAGA AGCTTGTCGGCCTGCTGCTGGTGGCGCGGTACATACCC GCGGCCTTGATTTTGCTTGCATATATATATCTGGGCG CCTCTGGCCGGAACATGCGGGTCTCTCTCTCTTCTCT GGTTATTACTCTCTACTGTAATCACAGGAAT	279	Extended CD8a hinge
GCCTGCCCCGACCGGGCTCTACACTCATAGCGGGGAAT GTTGTAAGGCATGTAACCTTGGGTGAGGGCGTCGCACA GCCCTGCGGAGCTAACCAACAGTGTGCGAACCCCTGC CTCGATAGTGTGACGTTCTCTGATGTTGTATCAGCTAC AGAGCCTTGCAAACCATGTACTGAGTGC GTTGGACTT CAGTCAATGAGCGCTCCATGTGTGGAGGCAGATGATG CGGTCTGTCGATGTGCTTACGGATACTACCAAGACGA GACAACAGGGCGGTGCGAGGCCTGTAGAGTTTGTGAG GCGGGCTCCGGGCTGGTGTTCATGTCAAGACAAGC AAAATACGGTCTGTGAAGAGTGCCCTGATGGCACCTA CTCAGACGAAGCAGATGCAGAATGC	280	LNGFR hinge
GCCTGCCCTACAGGACTCTACACGCATAGCGGTGAGT GTTGTAAAGCATGCAACCTCGGGGAAGGTGTAGCCCA GCCATGCGGGGCTAACCAACCGTTTGC	281	Truncated LNGFR hinge (TNFR-Cys1)
GCTGTGGGCCAGGACACGCAGGAGGTCATCGTGGTG CACACTCCTTGCCCTTTAAGGTG	282	PDGFR-beta extracellular linker
ACAACAACACCCGCACCTCGGCCTCCAACCTCCAGCTC CAACAATTGCACTGCAACCCCTGAGTCTGAGGCCCGA GGCCTGTAGGCCAGCAGCTGGCGGAGCTGTTACACT AGAGGCCTGGACTTTGCCTGTGAC	283	Example spacer derived from CD8 hinge
GCCCTGAGCAACAGCATCATGTACTTCAGCCACTTCGT GCCCCGTGTTTCTGCCCGCCAAGCCTACAACAACCCCTG CTCCTAGACCACCTACACCAGCTCCTACAATCGCCAGC CAGCCTCTGTCTCTGAGGCCCGAAGCTTGTAGACCAG CTGCTGGCGGAGCCGTGCATACAAGAGGACTGGATTT TGCTTGCAC	284	Example spacer derived from CD8 hinge
CACCCATCCGATCCTCTCGAGCTGGTGGTTTCTGGACC TTCTGGCGGCCCTAGCAGCCCTACAACAGGACCTACA AGCACAAGCGGCCCTGAGGACCAACCTCTGACACCA CAGGCAGCGATCCTCAGTCTGGACTGGGGAGACATCT GGGCGTT	418	LIR1 Hinge

In some embodiments, a CAR of the present disclosure may further include a short oligopeptide or polypeptide linker that is between 2 amino acid residues and 10 amino acid residues in length, and that may form the linkage between the transmembrane domain and the cytoplasmic region of the CAR. A non-limiting example of a suitable linker is a glycine-serine doublet. In some embodiments, the linker comprises the amino acid sequence of GGCKJSGGCKJS (SEQ ID NO: 208).

In some aspects, the transmembrane domain further comprises at least a portion of an extracellular domain of the same protein.

Intracellular Signaling Domains

In some embodiments, a CAR of the present disclosure (*e.g.*, the EMCN-specific, FLT3-specific, and/or CD33-specific CARs described herein) comprises one or more cytoplasmic domains or regions. The cytoplasmic domain or region of the CAR may include an intracellular signaling domain.

Examples of suitable intracellular signaling domains that may be used in CARs of the present disclosure include, without limitation, cytoplasmic sequences of the T cell receptor (TCR) and co-receptors that act in concert to modulate signal transduction following antigen receptor engagement, as well as any derivative or variant of these sequences and any recombinant sequence that has the same functional capability.

Without wishing to be bound by theory, it is believed that signals generated through the TCR alone are insufficient for full activation of the T cell and that a secondary and/or co-stimulatory signal is thus also typically required for full activation. Accordingly, T cell activation may be mediated by two distinct classes of cytoplasmic signaling sequences, those that initiate antigen-dependent primary activation through the TCR (primary intracellular signaling domains) and those that act in an antigen-independent manner to provide a secondary or co-stimulatory signal (secondary cytoplasmic domain, *e.g.*, a co-stimulatory domain). In addition, T cell signaling and function (*e.g.*, an activating signaling cascade) can be negatively regulated by inhibitory receptors present in a T cell through intracellular inhibitory co-signaling domains.

In some embodiments, the intracellular signaling domain of a CAR of the present disclosure can include an inhibitory intracellular signaling domains. Examples of inhibitory intracellular domains that can be used include PD-1, CTLA4, TIGIT, BTLA, and LIR-1 (LILRB1), TIM3, KIR3DL1, NKG2A, LAG3, SLAP1, SLAP2, Dok-1, Dok-2, LAIR1, GRB-2, CD200R, SIRP α , HAVR, GITR, PD-L1, KIR2DL1, KIR2DL2, KIR2DL3, KIR3DL2, CD94, KLRG-1, CEACAM1, LIR2, LIR3, LIR5, SIGLEC-2, and SIGLEC-10. **Table 9** and **Table 10** provide the amino acid and nucleotide sequences, respectively, of the exemplary inhibitory intracellular signaling domains. In some embodiments, the inhibitory intracellular signaling domain includes one or more intracellular inhibitory co-signaling domains (*e.g.*, see **Table 9** and **Table 10**). In some embodiments, the one or more intracellular inhibitory co-signaling domains are linked to other domains (*e.g.*, a transmembrane domain) through a peptide linker (*e.g.*, see **Table 6**) or a spacer or hinge sequence (*e.g.*, see **Tables 7 and 8**). In some embodiments, when two or more intracellular inhibitory co-signaling domains are present, the two or more intracellular inhibitory co-signaling domains can be linked through a peptide linker (*e.g.*, see **Table 6**) or a spacer or hinge sequence (*e.g.*, see **Tables 7 and 8**). In some embodiments, the

intracellular inhibitory co-signaling domain is an inhibitory domain. In some embodiments, the one or more intracellular inhibitory co-signaling domains of a chimeric protein comprises one or more ITIM-containing protein, or fragment(s) thereof. ITIMs are conserved amino acid sequences found in cytoplasmic tails of many inhibitory immune receptors. Examples of ITIM-containing proteins include, but are not limited to, PD-1, TIGIT, BTLA, and LIR-1 (LILRB1), TIM3, KIR3DL1, NKG2A, LAG3, LAIR1, SIRP α , KIR2DL1, KIR2DL2, KIR2DL3, KIR3DL2, CD94, KLRG-1, CEACAM1, LIR2, LIR3, LIR5, SIGLEC-2, and SIGLEC-10.

Table 9 - Exemplary inhibitory intracellular signaling domain amino acid sequences

Amino Acid Sequence	SEQ ID NO:	Description
CSRAARGTIGARRTGQPLKEDPSAVPVFSDYGELEDFQWREKTPEPPVP CVPEQTEYATIVFSPGMGTSSPARRGSADGPRSAQPLRPEDGHCSWPL	435	PD-1 intracellular signaling domain
AVLSKMLKKRSPLTTGVYVKMPPTPECEKQFQPYFIPIN	436	CTLA4 intracellular signaling domain
RRHQGKQNELSDTAGREINLVDHLKSEQTEASTRQNSQVLLSETGIYD NDPDLCFRMQEGSEVYSNPCLLENKPGIVYASLNHSHVIGPNSRLARNVK EAPTEYASICVRS	437	BTLA intracellular signaling domain
LRHRRQGKHWTSTQRKADFQHPAGAVGPEPTDRGLQWRSSPAADAQE ENLYAAVKHTQPEDGVEMDTRSPHDEDPQAVTYAEVKHSRPRREMASP PSPLSGEFLDTKDRQAEEDRQMDTEAAASEAPQDVITYAQLHSLTLRREA TEPPPSQEGSPAVPSIYATLAIH	285	LIR1 intracellular signaling domain
HLWCSNKKNAAVMDQEPAGNRTANSEDSDEQDPEEVITYAQLDHCVFT QRKITRPSQRPKTPPTDTILYTELPNAKPRSKVVSCL	438	KIR3DL1 intracellular signaling domain
AVLSKMLKKRSPLTTGVGVKMPPTPECEKQFQPYFIPIN	439	CTLA4 intracellular signaling domain
KEPASPLDKCHYTKDNGQFDQSAKQLNLEAYTIEQETALISNKNKGPKR QQRKPNPPLNLDYIVGQNDM	440	NKG2A (reversed) intracellular signaling domain
LTRKKKALRIHSVEGDLRRKSAGQEEWSPSAPSPPGSCVQAEAAPAGLC GEQRGEDCAELHDYFNVLSYRSLGNCSTFTETG	441	TIGIT intracellular signaling domain
MDNQGVYISDLNLPNPKRQQRKPKGNKNSILATEQEITYAELNLQKAS QDFQGNDKTYHCKDLPSAPEK	442	NKG2A intracellular signaling domain
MEIAIKYDFKATADDELSFKRGDILKVLNEECDQNWYKAELNGKDGFI PKNYIEMKPHPWFFGKIPRAKAEMLSKQRHDGAFLIRESESAPGDFSLS VKFGNDVQHFKVLRDGAGKYFLWVVKFNSLNELVDYHRSTSISRNNQI FLRDIEQVPQPTYVQALFDFDPQEDGELGFRRGDFIHVMDNSDPNWW KGACHGQTGMFPRNYVTPVNRNV	443	GRB2 intracellular signaling domain
MDGAVMEGPLFLQSQRFGTKRWRKTWAVLYPASPHGVARLEFFDHKG SSSGGGRGSSRRDLCKVIRLAECVSVAPVTVEPPEPGATAFRDLTAQRS HLLAADAPSSAAWVQTLCRNAFPKGSWTLPNTDNPPLSALEMLENSL	444	Dok-1 intracellular signaling domain

YSPTWEGSQFWVTVQRTEAAERCGLHGSYVLRVEAERLTLLTVGAQSQ ILEPLLSWPYTLRLRYGRDKVMFSFEAGRRCPSGPGTFTFQTAQGNDIFQ AVETAIHRQKAQKGAGQGHDVLRADSHEGEVAEGKLSPSPGPQELLDS PPALYAEPLDSLRIAPCPSQDSLYSDPLDSTSAQAGEGVQRKKPLYWDL YEHAQQQLLKAKLTDPKEDPIYDEPEGLAPVPPQGLYDLPREPKDAWW CQARVKEEGYELPYNPATDDYAVPPPRSTKPLLAPKPQGPAPFPEPGTAT GSGIKSHNSALYSQVQKSGASGSWDCGLSRVGTDTKGVKSEGST		
MGDGA VKQGF LYLQQQTFGKKWRRFGASLYGGSDCALARLELQEGP EKPRRCEAARKVIRLSDCLRVAEAGGEASSPRDTSAFFLETKERLYLLAA PAAERGDWVQAICLLAFPGQRKELSGPEGKQSRPCMEENELYSSAVTVG PHKEFAVTMRPTEASERCHLRGSYTLRAGESALELWGGPEPGTQLYDW PYRFLRRFGRDKVTFSEAGRRCVSGEGNFEFETRQGNEIFLALAEAISA QKNAAPATPQPAPATIPASLPRPDSPYSRPHDSLPPSPPTTPVPAPRPRGQ EGEYAVPFDAVARSLGKNFRGILAVPPQLLADPLYDSIEETLPPRPDIH DEPEGVAALSLYDSPQEPRGEAWRRQATADRDPAQLQHVQPAGQDFSA SGWQPGTEYDNVVLKKGPK	445	Dok-2 intracellular signaling domain
PAPAERPLPNEGLDSDFLAVLSDYPSDPISPIFRERGEKLRVISDEGGWW KAISLSTGRESYIPGICVARVYHGWLFEGLGRDKAEELLQLPDTKVGSF MIRESETKKGFYSLSVRHRQVKHYRIFRLPNNWYYISPRLTFQCLEDLVN HYSEVADGLCCVLTPCLTQSTAAPAVRASSPVTLRQKTVDWRRVSRL QEDPEGTENPLGVDESLSYGLRESIASYLSLTSEDNTSFDRKKKSISLMY GGSKRKSSFFSSPPYFED	446	SLAP1 _{aa8-aa276} intracellular signaling domain
PAPAERPLPNEGLDSDFLAVLSDYPSDPISPIFRERGEKLRVISDEGGWW KAISLSTGRESYIPGICVARVYHGWLFEGLGRDKAEELLQLPDTKVGSF MIRESETKKGFYSLSVRHRQVKHYRIFRLPNNWYYISPRLTFQCLEDLVN HYSEVADGLCCVLTPCLTQSTAAPAVRASSPVTLRQKTVDWRRVSRL QEDPEGTENPLGVDESLSYGLRESIASYLSLTSEDNTSF	447	SLAP1 _{aa8-aa247} intracellular signaling domain
RKSLPSPSLSSSVQGGPVTMEAERSKATAVALGSFPAGGPAELSLRLGE PLTIVSEDGDWWTVLSEVSGREYNIPSVHVAKVSHGWLYEGLSREKAE LLLLPGNPGGAFLIRESQTRRGSYSLSVRLSRPASWDRIHYRIHCLDNG WLYISPRLTFPSLQALVDHYSELADDICLLKEPCVLQRAGPLPGKDIP VTVQRTPLNWKELDSSLLFSEAATGEESLLSEGLRESLSFYISLNDEAVSL DDA	448	SLAP2 _{aa8-aa261} intracellular signaling domain
KVNGCRKYKLNKTESTPVVEEDEMOPYASYTEKNNPLYDTTNKVKASE ALQSEVDTDLHTL	449	CD200R intracellular signaling domain
RIRQKKAQGSTSSTRLHEPEKNAREITQDNDITYADLNLPKGKKPAPQA AEPNNHTEYASIQTSPQPA SEDTLTYADLDMVHLNRTPKQPAPKPEPSFS EYASVQVPRK	450	SIRP α intracellular signaling domain
HRWCSNKKNAAVMDQESAGNRTANSEDSDEQDPQEVYTYQLNHCVFT QRKITRPSQRPKTPPTDIIVYTELPNAESRSKVVSCP	451	KIR2DL1 intracellular signaling domain
MTDSVIYSMLELPTATQAQNDYGPQQKSSSSRPSCSCLGSG	452	KLRG1 intracellular signaling domain
HRQNQIKQGPPRSKDEEQKPQQRPD LAVDVLERTADKATVNGLPKEDR ETDTSALAAGSSQEVTYAQLDHWALTQRTARAVSPQSTKPMASITYA AVARH	453	LAIR1 intracellular signaling domain
LRHRRQGKHWTSQRKADFQHPAGAVGPEPTDRGLQWRSSPAADAQE ENLYAAVKDTQPEDGVEMDTRAAASEAPQDVTYAQLHSLTLRRKATEP PPSQEREPPAEPSIYATLAIH	454	LIR2 intracellular signaling domain
RRQRHSHKRTSDQRKTD FQRPAGAAETEPKDRGLLRSSPAADVQEEN LYAAVKDTQSEDRVELDSQSPHDEDPQAVTYAPVKHSSPRREMASPPSS	455	LIR3 intracellular signaling domain

LSGEFLDTKDRQVEEDRQMDTEAAASEASQDVTYAQLHSLTLRRKATE PPPSQEGEPPEPSIYATLAIH		
QHWRQKGKHTLAQRQADFQRPPGAAEPEPKDGGGLQRRSSPAADVQGE NFCAAVKNTQPEDGVEMDTRQSPHDEDPQAVTYAKVKHSRPRREMAS PPSPLSGEFLDTKDRQAEEDRQMDTEAAASEAPQDVTYAQLHSFTLRQK ATEPPPSQEGASPAEPSVYATLAIH	456	LIR5 intracellular signaling domain
KLQRRWKRTQSQQGLQENSSGQSFFVRNKKVRRAPLSEGPSLGCYNP MMEDGISYTTLRFPEMNIPRTGDAESSEMQRPPDCDDTVTYSALHKRQ VG DYENVIPDFEDEGIHYSELIQFGVGERPQAQENVYVILKH	457	SIGLEC-2 intracellular signaling domain
KILPKRRTQTETPRPRFSRHSTILDYINVVPTAGPLAQKRNQKATPNSPRT PLPPGAPSPESKKNQKKQYQLPSFPEPKSSTQAPESQESQEELHYATLNFP GVRPRPEARMPKGTQADYAEVKFQ	458	SIGLEC-10 intracellular signaling domain

Table 10 - Exemplary inhibitory intracellular signaling domain nucleic acid sequences

Nucleic Acid Sequence	SEQ ID NO:	Description
TGTAGCAGAGCCGCCAGAGGAACAATCGGCGCCAGAAGAACAGGCC AGCCTCTGAAAGAGGACCCCTCTGCCGTTCTGTGTTTCAGCGTGGAC TATGGCGAGCTGGATTTCCAGTGGCGGGAAAAGACACCCGAGCCTCC AGTGCCTTGTGTGCCTGAGCAGACAGAGTACGCCACCATCGTGTTC CTAGCGGCATGGGCACATCTAGCCCTGCCAGAAGAGGATCTGCCGAC GGACCTAGATCTGCCAGCCTCTTAGACCTGAGGACGGCCACTGTTC TTGGCCTCTT	459	PD-1 intracellular signaling domain
TGTAGCCGAGCGGCCAGAGGCACAATCGGGGCAAGACGAACAGGAC AGCCGCTCAAAGAGGACCCAGTGCAGTCCCGTTTTCTCCGTGGAT TACGGAGAACTGGATTTCCAGTGGCGGGAGAAGACACCAGAGCCCC CGGTGCCCTGCGTGCCGAGCAGACTGAGTACGCCACGATTGTGTTT CCCTCTGGAATGGGGACTTCATCCCCGCTAGGCGCGGCTCAGCTGA TGGCCCAAGATCCGCTCAACCGTTGCGGCCAGAGGACGGGCATTGCA GTTGGCCTCTG	460	PD-1 intracellular signaling domain
GCCGTGTCTCTGAGCAAGATGCTGAAGAAGCGGAGCCCTCTGACCAC CGGCGTGACGTGAAAATGCCTCCTACCGAGCCTGAGTGCGAGAAGC AGTTCCAGCCTTACTTCATCCCCATCAAC	461	CTLA4 intracellular signaling domain
AGGAGACATCAGGGGAAGCAGAATGAACTCAGCGATACAGCAGGGC GAGAAATTAATTTGGTAGACGCGCATCTGAAGTCCGAACAGACAGA GGCTTCTACTAGACAGAACTCCCAAGTTTGTGAGTGAGACGGGGA TCTATGATAATGATCCCGATCTGTGTTTGAATGCAGGAGGGTAGT GAAGTCTACTCAAACCCGTGCCTGGAAGAAAATAAGCCCGGCATTGT TTACGCTAGTTTGAATCATTCTGTAATAGCCCCGAACCTCAGACTGG CTCGCAATGTGAAGGAGGCCCAACTGAGTATGCGTCCATTGCGTG CGGTCT	462	BTLA intracellular signaling domain
AGAAGACATCAGGGGAAGCAGAATGAACTCAGCGATACAGCAGGGC GAGAAATTAATTTGGTAGACGCGCATCTGAAGTCCGAACAGACAGA GGCTTCTACTAGACAGAACTCCCAAGTTTGTGAGTGAGACGGGGA TCTATGATAATGATCCCGATCTGTGTTTGAATGCAGGAGGGTAGT GAAGTCTACTCAAACCCGTGCCTGGAAGAAAATAAGCCCGGCATTGT TTACGCTAGTTTGAATCATTCTGTAATAGCCCCGAACCTCAGACTGG CTCGCAATGTGAAGGAGGCCCAACTGAGTATGCGTCCATTGCGTG CGGTCT	463	BTLA intracellular signaling domain
AGAAGGCACCAGGGAAAGCAGAACGAGCTGAGCGATACCGCCGGCA GAGAAATCAACCTGGTGGACGCCCACCTGAAAAGCGAGCAGACAGA GGCCAGACACAGACAGAATAGCCAGGTGCTGCTGAGCGAGACAGGC ATCTACGACAACGACCCCGACCTGTGCTTCCGGATGCAAGAGGGAAG CGAGGTGTACAGCAACCCCTGCCTGGAAGAGAACAAGCCCGGCATC	464	BTLA intracellular signaling domain

GTGTACGCTAGCCTGAACCACTCTGTGATCGGCCCCAATTCCAGACT GGCCCGGAACGTGAAAGAGGCCCCCTACAGAGTACGCCAGCATCTGC GTCAGAAGC		
TTGCGCCACAGACGGCAGGGAAAGCACTGGACTAGTACGCAGAGGA AAGCGGACTTCCAGCATCCCGCAGGAGCCGTGGGGCCTGAACCCACT GATCGCGGCCTTCAATGGAGGTCTAGCCCGCGCGCAGACGCACAAG AGGAAAACTTGTACGCAGCCGTTAAGCACACCCAACCGGAGGACGG CGTTGAGATGGATACCCGCTCCCCCTCACGATGAAGACCCCTCAAGCAG TCACCTACGCGGAAGTAAAGCATAGCCGCCCCAGACGGGAAATGGC TAGCCCGCCGTCCCCCTTAGCGGGGAATTTCTGGACACTAAAGATA GGCAGGCGGAAGAGGACCGCCAAATGGATACAGAGGCGGCGGCAA GTGAAGCACCTCAAGACGTTACTTACGCTCAACTTCACAGCCTTACC CTCAGGCGAGAAGCGACTGAACCACCCCTTCCCAAGAAGGGCCAA GCCCAGCGGTTCTTCTATCTATGCTACTCTTGCTATTAC	465	LIR1 intracellular signaling domain
CTGCGGCACAGACGGCAGGGCAAGCACTGGACAAGCACACAGAGAA AGGCCGACTTTCAGCACCCCTGCTGGTGCCGTTGGACCTGAGCCTACA GATAGAGGACTGCAGTGGCGGTCTAGCCCTGCCGCTGATGCTCAAGA GGAAAACTGTACGCCGCGGTGAAGCACACCCAACCTGAAGATGGC GTGGAATGGACACCAGATCTCCCCACGATGAGGACCCCTCAGGCCGT GACATATGCCGAAGTGAAGCACTCCCCGGCTCGGAGAGAAATGGCT AGCCCTCCAAGTCTCTGAGCGGCGAGTTCTTGACACCAAGGATAG ACAGGCCGAAGAGGACCGGCAGATGGATACAGAAGCTGCCGCATCT GAGGCCCCACAGGATGTGACTTATGCCAGCTGCACAGCCTGACACT GCGGAGAGAAGCCACAGAGCCTCCACCTTCTCAAGAGGGCCCATCTC CAGCCGTGCCTAGCATCTATGCCACACTGGCCATCCAC	466	LIR1 intracellular signaling domain
GCCGTGTCACTTAGTAAGATGCTGAAGAAGAGGTCACCACTGACGAC AGGGGTTGGAGTGAAGATGCCACCCACAGAACCCGAATGTGAGAAG CAATTCCAGCCTTATTTCAATCCAATAAAT	467	CTLA4 intracellular signaling domain
CATCTGTGGTGTTCTAATAAGAAGAATGCTGCTGTGATGGATCAAGA GCCCCGTGGTAACAGAACGGCCAAACAGTGAAGATAGCGATGAGCAG GACCCAGAAGAAGTGACCTACGCCCACTCGACCACTGTGTTTTTAC GCAGCGGAAAAATCCTCGACCCCTCTCAACGACCCAAAACGCCGCCTA CGGACACCATACTCTACACCGAACTGCCGAACGCCAAACCACGGTCC AAGGTGGTATCATGTCCG	468	KIR3DL1 intracellular signaling domain
CTGCGGCACAGAAGGCAGGGCAAGCACTGGACAAGCACCCAGAGAA AGGCCGATTTTCAGCACCCCTGCTGGCGCCGTTGGACCTGAGCCTACA GATAGAGGACTGCAGTGGCGGTCTAGCCCTGCTGCCGATGCTCAAGA GGAAAACTGTACGCCGCGGTGAAGCACACCCAACCTGAAGATGGC GTGGAATGGACACCAGATCTCCCCACGATGAGGACCCCTCAGGCCGT GACATACGCTGAAGTGAAGCACTCCCCGGCTCGGAGAGAAATGGCT AGCCCTCCAAGTCTCTGAGCGGCGAGTTCTTGACACCAAGGATAG ACAGGCCGAAGAGGACCGGCAGATGGATACAGAAGCTGCCGCCTCT GAAGCCCCACAGGATGTGACATATGCCAGCTGCATAGCCTGACACT GCGGAGAGAAGCCACAGAGCCTCCACCTTCTCAAGAGGGCCCATCTC CAGCCGTGCCTAGCATCTATGCCACACTGGCCATTAC	469	LIR1 intracellular signaling domain
AAGGAGCCTGCGTCCCCGTTGGATAAATGCCACTATACTAAGGATAA CGGTCAGTTTCGATCAGAGTGCAAAGCAACTTAACCTGGAGGCTTACA CTATAGAGCAAGAAACAGCGCTGATAAGTAATAAGAACGGTAAGCC AAAGCGACAGCAGAGGAAACCCAATCCTCCGCTTAACCTGGATAGCT ACATCGTCGGGCAAAATGACATG	470	NKG2A (reversed) intracellular signaling domain
CTGACCAGAAAGAAGAAGGCCCTGAGAATCCACAGCGTGGAAGGCG ACCTGCGGAGAAAGTCTGCCGACAGAAGAGTGGTCCCCTAGCGC TCCATCTCCACCTGGATCTTGTGTGCAGGCCGAAGCAGCTCCTGCTG GACTGTGTGGCGAACAGAGAGGGCGAAGATTGCGCCGAGCTGCACGA CTACTTCAACGTGCTGAGCTACAGAAGCCTGGGCAACTGCAGCTTCT TCACCGAGACAGGA	471	TIGIT intracellular signaling domain

ATGGACAACCAGGGCGTGATCTACAGCGACCTGAACCTGCCTCCTAA TCCTAAGCGGCAGCAGAGAAAGCCCAAGGGCAACAAGAACAGCATC CTGGCCACCGAGCAAGAGATCACCTACGCCGAGCTGAATCTGCAGA AGGCCAGCCAGGACTTCCAGGGCAACGACAAGACCTACCACTGCAA GGACCTGCCTAGCGCTCCCGAGAAG	472	NKG2A intracellular signaling domain
ATGGACAACCAGGGCGTCATCTACAGCGACCTGAACCTGCCTCCTAA TCCAAAGCGGCAGCAGCGGAAGCCCAAGGGCAACAAGAATAGCATC CTGGCCACCGAGCAAGAGATCACCTACGCCGAGCTGAATCTGCAGA AGGCCAGCCAGGATTTCCAGGGCAACGACAAGACCTACCACTGCAA GGACCTGCCTAGCGCTCCTGAGAAA	473	NKG2A intracellular signaling domain (codon optimized)
ATGGAAGCCATTGCCAAATACGACTTCAAGGCCACCGCCGACGACG AGCTGAGCTTCAAGAGAGGCGACATCCTGAAGGTGCTGAACGAGGA ATGCGACCAGAACTGGTACAAGGCCGAGCTGAACGGCAAGGACGGC TTCATCCCCAAGAACTACATCGAGATGAAGCCCCATCCATGGTTCTT CGGCAAGATCCCCAGAGCCAAGGCCGAAGAGATGCTGAGCAAGCAG AGACACGACGGCGCCTTCTGATCCGGGAATCTGAATCTGCCCTGG CGACTTCAGCCTGAGCGTGAAGTTCGGCAACGACGTGCAGCACTTCA AGGTCTCTGAGAGATGGCGCCGAAAGTACTTCTGTGGGTCTGTGAAG TTTAACAGCCTGAACGAGCTGGTGGACTACCACAGATCCACCAGCGT GTCCCGGAACCAGCAGATCTTCTGCGGGACATCGAACAGGTGCCAC AGCAGCCAACATACGTGCAGGCCCTGTTCGACTTCGACCCTCAAGAG GATGGCGAGCTGGGCTTTAGACGGGGCGATTTCATCCACGTGATGGA CAACAGCGACCCCAACTGGTGGAAGGGCGCTTGTTCATGGACAGACC GGCATGTTCCCCAGAACTACGTGACCCCTGTGAACCGGAACGTG	474	GRB2 intracellular signaling domain
ATGGATGGCGCCGTGATGGAAGGCCCTCTGTCTTCTCCAGAGCCAGAG ATTCCGGACCAAGCGGTGGCGGAAAACATGGGCGCTTCTGTACCCTG CCTCTCCTCATGGCGTGGCCCGGCTGGAATTTTCGATCACAAGGGCT CTAGCAGCGGCGGAGGCAGAGGATCTAGTAGACGGCTGGACTGCAA AGTGATCCGGCTGGCCGAGTGTGTGTCTGTGGCTCCTGTGACCGTGG AAACCCCTCCTGAACCTGGCGCCACAGCCTTCAGACTGGATACAGCC CAGAGAAGCCATCTGCTGGCCGCGGATGCTCCTTCTTCTGCTGCTTGG GTGCAGACCCTGTGCCGGAACGCTTTTCCTAAAGGCAGCTGGACACT GGCCCTACCGACAATCCTCCTAAGCTGAGCGCCCTGGAATGCTGG AAAACAGCCTGTACAGCCCCACCTGGGAGGGCTCTCAGTTTGGGTC ACCGTGCAGAGAACAGAGGCCGCGGAAAGATGTGGCCTGCACGGCT CTTATGTGCTGAGAGTGGAAGCCGAGAGACTGACCCCTGCTGACAGTG GGAGCCCAGTCTCAGATCCTGGAACCTCTGCTGAGCTGGCCCTACAC ACTGCTGAGAAGATACGGCCGGGACAAAGTGATGTTTCAGCTTCGAG GCCGGCAGAAGATGTCCTTCTGGCCCTGGCACATTACATTTTCAGAC AGCCAGGGCAACGACATCTTCCAGGCTGTGGAACCGCCATCCACA GACAGAAGGCCAGGGAAAAGCCGGCCAGGGACACGATGTTCTGAG AGCCGATTCTACGAGGGCGAAGTGGCCGAGGGAAAGCTTCTTCTC CACCTGGACCTCAAGAGCTGCTGGATAGCCCTCCTGCTCTGTATGCC GAGCCTCTGGACAGCCTGAGAATCGCCCTTGTCCAAGCCAGGACTC TCTGTACAGCGATCCCCTGGATAGCACATCTGCCAAGCTGGCGAAG GCGTGCAGAGGAAGAAGCCCTGTACTGGGATCTGTACGAGCACGCT CAGCAGCAACTGCTGAAGGCCAAGCTGACAGACCCCAAGAGGACC CCATCTACGACGAGCCTGAAGGACTTGCTCCAGTGCCACCTCAGGGC CTGTACGATCTGCCTAGAGAGCCTAAAGACGCCTGGTGGTGTGAGGC CAGAGTGAAAGAGGAAGGCTACGAGCTGCCTTACAACCCCGCCACC GATGATTATGCCGTGCCTCCTCCAAGAAGCACAAACCACTGCTGGC CCCAAGCCTCAGGGACCTGCTTTTCTGAGCCTGGAACAGCCACAG GCAGCGGCATCAAGAGCCACAATAGCGCCCTGTATAGCCAGGTGCA GAAAAGCGGCGCCAGCGGCTCTTGGGATTGTGGACTTAGCAGAGTG GGCACCGACAAGACCGGCGTGAAGTCTGAGGGAAGCACA	475	Dok-1 intracellular signaling domain

ATGGGAGATGGCGCCGTGAAGCAGGGCTTTCTGTATCTCCAGCAGCA GCAGACCTTCGGCAAGAAGTGGCGGAGATTTGGCGCCTCTCTGTACG GCGGCTCTGATTGTGCTCTGGCCCCGACTGGAAGTGAAGAGGGACCT GAGAAGCCCAGAAGATGCGAGGGCCGCCAGAAAAGTGATCCGGCTGA GCGATTGTCTGAGAGTGGCTGAAGCAGGCGGCCGAAGCCAGCTCTCCT AGAGATACCAGCGCATCTCTCCTGGAAACAAAAGAGCGGGCTGTACCT GCTGGCCGCTCCTGCTGCTGAAAGAGGCGATTGGGTCCAAGCCATCT GCCTGCTGGCTTTTCCCGGCCAGAGAAAAGAGCTGTCTGGCCCTGAG GGCAAGCAGAGCAGGCCCTTGCATGGAAGAGAACGAGCTGTACTCCA GCGCCGTGACAGTGGGCCCTCACAAAGAATTTGCCGTGACCATGAGG CCCACCGAGGCCAGCGAAAGATGTCACCTGAGAGGCAGCTACACCC TGAGAGCCGGCGAATCTGCTCTGGAACCTTTGGGGAGGACCTGAGCCT GGCACACAGCTGTACGACTGGCCCTACAGATTCTCTGCGGAGATTCCG CCGGGACAAAAGTGACCTTCAGCTTTGAGGCTGGCCGCAGATGTGTGT CCGGCGAGGGCAATTTGAGTTTCGAGACAAGACAGGGCAACGAGAT CTTCCTGGCTCTGGAAGAGGCCATCAGCGCCCAGAAAAATGCTGCC CTGCTACACCTCAGCCTCAGCCTGCTACAATCCCTGCCTCTCTGCCCA GACCTGACAGCCCTTATAGCAGACCCACGACTCTCTGCCTCCACCTT CTCCAACAACACCCGTGCCTGCTCCTAGACCTAGAGGACAAGAGGGC GAGTACGCCGTGCCTTTTGATGCCGTGGCTAGAAGCCTGGGCAAGAA CTTCAGAGGCATCTGGCTGTGCCTCCACAGCTGCTGGCTGACCTCT GTACGACAGCATCGAGGAAACCCTGCCTCCAAGACCTGACCACATCT ACGACGAGCCTGAAGGCGTGGCAGCCCTGTCTCTGTATGACTCCCT CAAGAGCCTAGAGGCGAAGCCTGGCGTAGACAGGCTACCGCCGATA GAGATCCTGCCGACTGCAACATGTGCAGCCAGCCGGCCAGGATTTT TCTGCCTCTGGATGGCAGCCAGGCACCGAGTACGATAACGTGGTGT GAAGAAGGGCCCCAAG	476	Dok-2 intracellular signaling domain
CCAGCCCCAGCGGAGCGACCGCTGCCAAACCCTGAAGGGCTCGACA GTGACTTTTTGGCTGTCTCTCCGACTATCCTAGTCCCGATATCAGCC CCCCGATATTCAGGCGCGGTGAAAACTCCGAGTCATCAGCGATGAG GGGGGTGGTGGAAAGCCATCAGCCTGAGTACCGGACGAGAGTCAT ACATTCTGGAATATGTGTAGCGAGGGTGTACCACGGTTGGCTGTTC GAGGGTCTGGGAAGAGATAAGGCCGAGGAACTTCTCCAACCTCCAG ATACAAAAGTCGGTAGTTTATGATTTCGGGAAAGTGAACTAAAAAG GGTTCTATAGCCTCTCAGTTCCGCATAGGCAGGTCAAGCATTATCG GATATTTTCGCTTGCCTAACAACTGGTACTACATAAGTCCCCGACTCAC ATTCCAATGCCTGGAGGACCTCGTGAATCACTATTGAGAAGTTGCAG ATGGCCTCTGCTGCGTACTACGACACCCTGCCTTACCCAGAGTACA GCGGCCCCGGCTGTTCCGGCATCTTCCAGCCCAGTAACACTCAGGCA AAAAACTGTGGATTGGCGCAGAGTCTCACGCCCTCAGGAAGATCCTG AGGGTACGGAAAAATCCGCTCGGTGTGGACGAGTCACTGTTCTCCTAT GGCTTGAGGGAATCTATAGCGTCTTACCTTTCCCTGACGTCTGAAGAT AATACGTCTTTCGATCGCAAAAAAATCAATATCTCTTATGTATGG CGGAAGCAAAAGGAAATCATCATTCTTTCTCTCCACCATATTTTCA AGAT	477	SLAP1 ^{aa8-aa276} intracellular signaling domain
CCAGCCCCAGCGGAGCGACCGCTGCCAAACCCTGAAGGGCTCGACA GTGACTTTTTGGCTGTCTCTCCGACTATCCTAGTCCCGATATCAGCC CCCCGATATTCAGGCGCGGTGAAAACTCCGAGTCATCAGCGATGAG GGGGGTGGTGGAAAGCCATCAGCCTGAGTACCGGACGAGAGTCAT ACATTCTGGAATATGTGTAGCGAGGGTGTACCACGGTTGGCTGTTC GAGGGTCTGGGAAGAGATAAGGCCGAGGAACTTCTCCAACCTCCAG ATACAAAAGTCGGTAGTTTATGATTTCGGGAAAGTGAACTAAAAAG GGTTCTATAGCCTCTCAGTTCCGCATAGGCAGGTCAAGCATTATCG GATATTTTCGCTTGCCTAACAACTGGTACTACATAAGTCCCCGACTCAC ATTCCAATGCCTGGAGGACCTCGTGAATCACTATTGAGAAGTTGCAG ATGGCCTCTGCTGCGTACTACGACACCCTGCCTTACCCAGAGTACA GCGGCCCCGGCTGTTCCGGCATCTTCCAGCCCAGTAACACTCAGGCA AAAAACTGTGGATTGGCGCAGAGTCTCACGCCCTCAGGAAGATCCTG AGGGTACGGAAAAATCCGCTCGGTGTGGACGAGTCACTGTTCTCCTAT	478	SLAP1 ^{aa8-aa247} intracellular signaling domain

GGCTTGAGGGAATCTATAGCGTCTTACCTTTCCTTGACGTCTGAAGAT AATACGTCTTTC		
AGAAAAATCCCTCCCCAGTCCAAGCCTGTCTAGTAGCGTTCAGGGTCA GGGCCCAGTTACTATGGAAGCGGAACGATCCAAGGCTACCGCAGTTG CTCTGGGTTTCAATCCCGGCTGGAGGGCCAGCGAACTCTCCTTGCGC CTGGGTGAGCCACTTACCATCGTTTCTGAGGACGGAGATTGGTGGAC GGTTCTTCTGAAGTATCTGGGAGAGAATACAACATACCGTCAGTTC ATGTCGCAAAAAGTTTACACGGTTGGCTTTACGAGGGACTCAGCAGG GAAAAAGCGGAGGAATTGTTGCTTTTGCCCGGCAATCCTGGCGGAGC ATTTTGTATAAGAGAGAGTCAAGACCCGGAGAGGCAGTTATTCCCTGT CAGTTAGACTCAGCAGACCTGCGAGTTGGGATAGGATTCGGCACTAC AGGATTCACTGCCTTGATAACGGCTGGCTGTATATATCTCCTCGCCTG ACATTCCCTAGCCTCCAAGCGTTGGTTGATCATTACTCTGAACTGGCA GACGATATCTGCTGCCTGCTCAAGGAACCGTGCCTCCTCAGAGGGC AGGCCCACTTCTGGCAAGGATATTCCACTTCCAGTCACGGTTCAA GAACCCCCCTCAATTGGAAGGAGCTGGATAGCTCTCCTCTTTTCCG AGGCCGCTACAGGTGAAGAATCTCTGTGTCTGAAGGATTGAGAGAG AGTCTCTCCTTCTACATCTCTCTGAATGATGAAGCAGTGTCTATTGGAC GACGCA	479	SLAP2 ^{aa8-aa261} intracellular signaling domain
AAAGTGAACGGCTGCCGGAAGTACAAGCTGAACAAGACCGAGAGCA CCCCTGTGGTGGAAGAGGACGAGATGCAGCCTTACGCCAGCTACACC GAGAAGAACAACCTCTGTACGACACCACCAACAAAGTGAAGGCCA GCGAGGCCCTCCAGAGCGAGGTTGACACAGATCTGCACACCCTG	480	CD200R intracellular signaling domain
CACCGGTGGTGCAGCAACAAGAAAAACGCCGCCGTGATGGACCAAG AGAGCGCCGAAATAGAACCGCCAACAGCGAGGATAGCGACGAGCA GGACCCTCAAGAAGTGACCTACACACAGCTGAACCACTGCGTGTTC CCCAGCGGAAGATCACCAGACCTAGCCAGCGGCCTAAGACACCACC TACCGACATCATCGTGTACACCGAGCTGCCCAACGCCGAGAGCAGAT CCAAGGTCGTGTCTCTGCTCCT	481	KIR2DL1 intracellular signaling domain
ATGACCGACAGCGTGATCTACAGCATGCTCGAGCTGCCCTACAGCCAC ACAGGCCCAGAATGATTACGGCCCTCAGCAGAAGTCCAGCTCCAGCA GACCTAGCTGTAGCTGTCTTGGCTCCGGC	482	KLRG1 intracellular signaling domain
CACCGGCAGAACAGATCAAGCAGGGCCCTCCTAGAAGCAAGGACG AGGAACAGAAAGCCTCAGCAGAGGCCTGATCTGGCCGTGGACGTGCT GGAAAGAACAGCCGATAAGGCCACCGTGAACGGCCTGCCTGAGAAG GACAGAGAGACAGACATCTGCCCTGGCCGCTGGCAGCTCCCAAG AAGTGACATACGCCAGCTGGACCACTGGGCCCTGACACAGAGAAC TGCCAGAGCTGTGTCCCTCAGAGCACCAAACTATGGCCGAGAGCA TCACCTATGCCGCCGTGGCCAGACAT	483	LAIR1 intracellular signaling domain
CTGCGGCACAGAAGGCAGGGCAAGCACTGGACAAGCACCCAGAGAA AGGCCGATTTTACGACACCCTGCTGGCGCCGTTGGACCTGAGCCTACA GATAGAGGACTGCAGTGGCGGTCTAGCCCTGCTGCCGATGCTCAAGA GGAAAACCTGTACGCCGCCGTGAAGGACACCCAACCTGAAGATGGC GTGGAATGGACACCAGAGCCGCCGATCTGAAGCCCCTCAGGATGT GACATACGCCCAGCTGCATAGCCTGACACTGAGGCGGAAAGCCACA GAGCCTCCACCTAGCCAAGAGAGAGCCTCCTGCCGAGCCTAGCAT CTATGCCACACTGGCCATTAC	484	LIR2 intracellular signaling domain
CGGAGACAGAGACACAGCAAGCACAGAACCAGCGACCAGAGAAAG ACCGACTTCCAGAGGCCTGCTGGCGCCGCTGAGACAGAGCCTAAAG ATAGAGGACTGCTGCGGAGAAGCAGCCCTGCTGCCGATGTGCAAGA GGAAAATCTGTACGCCGCCGTGAAGGACACCCAGAGCGAGGATAGA GTGGAATGGACAGCCAGTCTCCTCACGACGAAGATCCTCAGGCCGT GACATACGCCCCTGTGAAGCACAGCAGCCCTCGGAGAGAAATGGCC TCTCCACCTTCTAGCCTGAGCGGCGAGTTCTTGACACCAAGGACAG ACAGGTGGAAGAGGACCGGCAGATGGATACAGAAGCTGCCGCCCTCT GAAGCCAGCCAGGATGTGACATATGCCAGCTGCATAGCCTGACACT	485	LIR3 intracellular signaling domain

GCGGCGGAAAAGCTACAGAGCCTCCTCCATCTCAAGAGGGCGAGCCTC CAGCCGAGCCTAGCATCTATGCCACACTGGCCATTAC		
CAGCATTGGAGACAGGGAAGCACAGAACACTGGCCCAGCGGCAGG CCGATTTCCAAAGACCTCCAGGTGCCGCCGAACCTGAGCCTAAAGAT GGTGGCCTGCAGCGGAGATCTTCTCCTGCTGCCGATGTGCAGGGCGA GAATTTTTGTGCCGCCGTGAAGAACACCCAGCCTGAGGATGGCGTGG AAATGGACACCAGACAGAGCCCTCACGACGAGGATCCACAGGCCGT GACATACGCCAAAGTGAAGCACAGCCGGCCTCGGAGAGAAATGGCT AGCCCTCCAAGTCTCTGAGCGGCGAGTTCCTGGACACCAAAGACAG ACAGGCCGAAGAGGACCGGCAGATGGATACAGAAGCTGCCGCCCTCT GAAGCCCTCAGGATGTGACATATGCCAGCTGCATAGCTTCACCT GCGGCAGAAAGCCACAGAGCCTCCACCTTCTCAAGAGGGCGCTTCTC CAGCCGAGCCATCTGTGTATGCCACACTGGCCATTAC	486	LIR5 intracellular signaling domain
AAGCTGCAGCGGAGATGGAAGAGAACACAGAGCCAGCAGGGCCTGC AAGAGAATAGCAGCGGCCAGAGCTTCTTCGTGCCGAACAAGAAAGT GCGGAGAGCCCTCTGTCTGAGGGACCTCATAGCCTGGGCTGTTACA ACCCCATGATGGAAGATGGCATCAGCTACACCACACTGCGGTTCCTCC GAGATGAACATCCCTAGAACAGGCGACGCCGAGAGCAGCGAAATGC AAAGACCTCCTCTGACTGCGACGACACCGTGACATATAGCGCCCTG CACAAGAGACAAGTGGGCGACTACGAGAACGTGATCCCTGACTTCCC TGAGGACGAGGGCATCCACTACAGCGAGCTGATCCAGTTTGGCGTGG GCGAAAGACCCAGGCTCAAGAGAATGTGGACTACGTGATCCTGAA GCAC	487	SIGLEC-2 intracellular signaling domain
AAGATCCTGCCTAAGCGGCGCACCCAGACCGAGACTCCTAGACCTAG ATTACGCCGGCACAGCACCATCCTGGACTACATCAACGTGGTGCCTA CCGCCGGACCACTGGCTCAGAAGAGAAACCAGAAGGCCACACCTAA CAGCCCCAGAACACCTCTTCCACCTGGCGCACCTTCTCCAGAGAGCA AGAAGAACCAGAAGAAGCAGTACCAGCTGCCTAGCTTCCCCGAGCC TAAGAGCAGTACACAGGCCCTGAGAGCCAAGAGTCCCAAGAGGAA CTGCACTACGCCACACTGAACTTCCCGGCGTCAGACCTAGACCTGA GGCCAGAATGCCTAAGGGCACCCAGGCCGATTACGCCGAAGTGAAG TTTCAA	488	SIGLEC-10 intracellular signaling domain

In some embodiments, an iCAR includes a LIR1 intracellular inhibitory domain. In some embodiments, an iCAR includes a LIR1 intracellular inhibitory domain having the amino acid sequence

- 5 LRHRRQGKHWSTSTQRKADFQHPAGAVGPEPTDRGLQWRSSPAADAQENLYAAVKH
TQPEDGVEMDTRSPHDEDPQAVTYAEVKHSRPRREMASPPSPLSGEFLDTKDRQAEED
RQMDTEAAASEAPQDVITYAQLHSLTLRREATEPPPSQEGPSPAVPSIYATLAIH (SEQ ID
NO: 285). In some embodiments, an iCAR includes a LIR1 intracellular inhibitory domain
encoded by a polynucleotide sequence comprising the sequence
- 10 CTGCGGCACAGAAGGCAGGGCAAGCACTGGACAAGCACCCAGAGAAAGGCCGACT
TTCAGCATCCTGCTGGCGCCGTTGGACCTGAGCCTACAGATAGAGGACTGCAGTGG
CGGTCTAGCCCTGCCGCTGATGCCCAAGAGGAAAATCTTTACGCCGCCGTGAAGCA
CACCCAGCCTGAGGATGGCGTGGAATGGACACCAGATCTCCCCACGATGAGGACC
CTCAGGCCGTGACATACGCAGAAGTGAAGCACTCCAGACCTCGGAGAGAGATGGC
- 15 AAGCCCTCCATCTCCTCTGAGCGGCGAGTTCCTGGACACCAAAGACAGACAGGCCG

AAGAGGACAGACAGATGGATACCGAAGCCGCGCTTCTGAAGCCCCACAGGATGT
GACATATGCCCAGCTGCATAGCCTGACACTGCGGAGAGAAGCCACAGAGCCTCCAC
CTTCTCAAGAAGGCCCATCTCCTGCCGTGCCTTCCATCTATGCCACTCTGGCCATTC

AC (SEQ ID NO: 286). In some embodiments, an iCAR includes a LIR1 intracellular inhibitory
5 domain encoded by a polynucleotide sequence that is at least 90%, at least 91%, at least 92%, at
least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%
identical to

CTGCGGCACAGAAGGCAGGGCAAGCACTGGACAAGCACCCAGAGAAAGGCCGACT
TTCAGCATCCTGCTGGCGCCGTTGGACCTGAGCCTACAGATAGAGGACTGCAGTGG
10 CGGTCTAGCCCTGCCGCTGATGCCCAAGAGGAAAATCTTTACGCCGCGCTGAAGCA
CACCCAGCCTGAGGATGGCGTGGAAATGGACACCAGATCTCCCCACGATGAGGACC
CTCAGGCCGTGACATACGCAGAAAGTGAAGCACTCCAGACCTCGGAGAGAGATGGC
AAGCCCTCCATCTCCTCTGAGCGGCGAGTTCCTGGACACCAAAGACAGACAGGCCG
AAGAGGACAGACAGATGGATACCGAAGCCGCGCTTCTGAAGCCCCACAGGATGT
15 GACATATGCCCAGCTGCATAGCCTGACACTGCGGAGAGAAGCCACAGAGCCTCCAC
CTTCTCAAGAAGGCCCATCTCCTGCCGTGCCTTCCATCTATGCCACTCTGGCCATTC
AC (SEQ ID NO: 286).

In some embodiments, the one or more intracellular inhibitory co-signaling domains
comprise one or more non-ITIM scaffold proteins, or a fragment(s) thereof. In some
20 embodiments, the one or more non-ITIM scaffold proteins, or fragments thereof, are selected
from GRB-2, Dok-1, Dok-2, SLAP, LAG3, HAVR, GITR, and PD-L1.

The inhibitory intracellular signaling domain can further include an enzymatic inhibitory
domain. In some embodiments, the enzymatic inhibitory domain comprises an enzyme catalytic
domain. In some embodiments, the enzyme catalytic domain is derived from an enzyme selected
25 from the group consisting of: CSK, SHP-1, PTEN, CD45, CD148, PTP-MEG1, PTP-PEST, c-
CBL, CBL-b, PTPN22, LAR, PTPH1, SHIP-1, and RasGAP. Examples of enzymatic regulation
of signaling is described in more detail in Pavel Otáhal et al. (Biochim Biophys Acta. 2011
Feb;1813(2):367-76), Kosugi A., et al. (Involvement of SHP-1 tyrosine phosphatase in TCR-
mediated signaling pathways in lipid rafts, Immunity, 2001 Jun; 14(6): 669-80), and Stanford, et
30 al. (Regulation of TCR signaling by tyrosine phosphatases: from immune homeostasis to
autoimmunity, Immunology, 2012 Sep; 137(1): 1-19), each of which is incorporated herein by
reference for all purposes.

In some embodiments, the intracellular signaling domain of a CAR of the present
disclosure can comprise a primary signaling domain regulates primary activation of the TCR
35 complex either in a stimulatory way or in an inhibitory way. Primary intracellular signaling

domains that act in a stimulatory manner may contain signaling motifs which are known as immunoreceptor tyrosine-based activation motifs (ITAMs). Examples of suitable ITAM-containing primary intracellular signaling domains that that may be used in the CARs of the present disclosure include, without limitation, those of CD3-zeta, FcR gamma, FcR beta, CD3 gamma, CD3 delta, CD3 epsilon, CD5, CD22, CD79a, CD79b, CD278 (also known as "ICOS"), FcεRI, DAP10, DAP12, and CD66d.

In some embodiments, a CAR of the present disclosure comprises an intracellular signaling domain, e.g., a primary signaling domain of CD3-zeta polypeptide. A CD3-zeta polypeptide of the present disclosure may have an amino acid sequence that is at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% homologous to the sequence of NCBI Reference No. NP_932170 or NP_001106864.2, or fragments thereof. In some embodiments, the CD3-zeta polypeptide may comprise one conservative amino acid substitution, up to two conservative amino acid substitutions, or up to three conservative amino acid substitutions. In some

embodiments, the polypeptide can have an amino acid sequence that is a consecutive portion of NCBI Reference No. NP_932170 or NP_001106864.2 that is at least 20, at least 30, at least 40, at least 50, at least 60, at least 70, at least 80, at least 90, at least 100, at least 110, at least 120, at least 130, at least 140, at least 150, or at least 160, at least 170, or at least 180 amino acids in length.

In other embodiments, a primary signaling domain comprises a modified ITAM domain, e.g., a mutated ITAM domain which has altered (e.g., increased or decreased) activity as compared to the native ITAM domain. In one embodiment, a primary signaling domain comprises a modified ITAM-containing primary intracellular signaling domain, e.g., an optimized and/or truncated ITAM-containing primary intracellular signaling domain. In one

embodiment, a primary signaling domain comprises one, two, three, four or more ITAM motifs.

In some embodiments, the intracellular signaling domain of a CAR of the present disclosure can comprise the CD3-zeta signaling domain by itself or it can be combined with any other desired intracellular signaling domain(s) useful in the context of a CAR of the present disclosure. For example, the intracellular signaling domain of the CAR can comprise a CD3-zeta chain portion and a costimulatory signaling domain. The costimulatory signaling domain may refer to a portion of the CAR comprising the intracellular domain of a costimulatory molecule. A costimulatory molecule of the present disclosure is a cell surface molecule other than an antigen receptor or its ligands that may be required for an efficient response of lymphocytes to an antigen. Examples of suitable costimulatory molecules include, without limitation, CD97,

CD2, ICOS, CD27, CD154, CD8, OX40, 4-1BB, CD28, ZAP40, CD30, GITR, HVEM, DAP10, DAP12, MyD88, 2B4, CD40, PD-1, lymphocyte function-associated antigen-1 (LFA-1), CD7, LIGHT, NKG2C, B7-H3, and a ligand that specifically binds with CD83, an MHC class I molecule, a TNF receptor protein, an Immunoglobulin-like protein, a cytokine receptor, an integrin, a signaling lymphocytic activation molecule (SLAM protein), an activating NK cell receptor, BTLA, a Toll ligand receptor, CDS, ICAM-1, (CD11a/CD18), BAFFR, KIRDS2, SLAMF7, NKp80 (KLRF1), NKp44, NKp30, NKp46, CD19, CD4, IL2R beta, IL2R gamma, IL7R alpha, ITGA4, VLA1, CD49a, ITGA4, IA4, CD49D, ITGA6, VLA-6, CD49f, ITGAD, CD11d, ITGAE, CD103, ITGAL, CD11a, ITGAM, CD11b, ITGAX, CD11c, ITGB1, CD29, ITGB2, CD18, ITGB7, NKG2D, TNFR2, TRANCE/RANKL, DNAM1 (CD226), SLAMF4 (CD244, 2B4), CD84, CD96 (Tactile), CEACAM1, CRTAM, Ly9 (CD229), CD160 (BY55), PSGL1, CD100 (SEMA4D), CD69, SLAMF6 (NTB-A, Ly108), SLAM (SLAMF1, CD150, IPO-3), BLAME (SLAMF8), SELPLG (CD162), LTBR, LAT, GADS, SLP-76, PAG/Cbp, CD19a, and the like.

15 In some embodiments, aCAR intracellular signaling domains include a CD28 co-stimulatory domain. In some embodiments, aCAR intracellular signaling domains include a CD28 co-stimulatory domain having the amino acid sequence RSKRSRLHSDYMNMTPRRPGPTRKHYPYAPPRDFAAYRS (SEQ ID NO: 287). In some embodiments, aCAR intracellular signaling domains include a CD28 co-stimulatory domain encoded by a polynucleotide sequence comprising the sequence

20 AGAAGCAAGCGGAGCAGACTGCTGCACAGCGACTACATGAACATGACCCCTAGAC GGCCCGGACCTACCAGAAAGCACTACCAGCCTTACGCTCCTCCTAGAGATTTCGCC GCCTACCGGTCC (SEQ ID NO: 288). In some embodiments, aCAR intracellular signaling domains include a CD28 co-stimulatory domain encoded by a polynucleotide sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to

25 AGAAGCAAGCGGAGCAGACTGCTGCACAGCGACTACATGAACATGACCCCTAGAC GGCCCGGACCTACCAGAAAGCACTACCAGCCTTACGCTCCTCCTAGAGATTTCGCC GCCTACCGGTCC (SEQ ID NO: 288).

30 In some embodiments, aCAR intracellular signaling domains include a CD3 ζ signaling domain. In some embodiments, aCAR intracellular signaling domains include a CD3 ζ signaling domain having the amino acid sequence RVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPGEMGGKPRRKNPQEG LYNELQKDKMAEAYSEIGMKGERRRGKGDGLYQGLSTATKDTYDALHMQALPPR

35 (SEQ ID NO: 289). In some embodiments, aCAR intracellular signaling domains include a

CD3 ζ signaling domain encoded by a polynucleotide sequence having the sequence

AGAGTGAAGTTCAGCAGGAGCGCAGACGCCCCCGCGTACAAGCAGGGCCAGAACC
 AGCTCTATAACGAGCTCAATCTAGGACGAAGAGAGGAGTACGATGTTTTGGACAAG
 AGACGTGGCCGGGACCCTGAGATGGGGGGAAAGCCGAGAAGGAAGAACCCTCAGG
 5 AAGGCCTGTACAATGAACTGCAGAAAGATAAGATGGCGGAGGCCTACAGTGAGAT
 TGGGATGAAAGGCGAGCGCCGGAGGGGCAAGGGGCACGATGGCCTTTACCAGGGT
 CTCAGTACAGCCACCAAGGACACCTACGACGCCCTTCACATGCAGGCCCTGCCCCC

TCGC (SEQ ID NO: 290). In some embodiments, aCAR intracellular signaling domains include
 a CD3 ζ signaling domain encoded by a polynucleotide sequence that is at least 90%, at least
 10 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least
 98%, or at least 99% identical to

AGAGTGAAGTTCAGCAGGAGCGCAGACGCCCCCGCGTACAAGCAGGGCCAGAACC
 AGCTCTATAACGAGCTCAATCTAGGACGAAGAGAGGAGTACGATGTTTTGGACAAG
 AGACGTGGCCGGGACCCTGAGATGGGGGGAAAGCCGAGAAGGAAGAACCCTCAGG
 15 AAGGCCTGTACAATGAACTGCAGAAAGATAAGATGGCGGAGGCCTACAGTGAGAT
 TGGGATGAAAGGCGAGCGCCGGAGGGGCAAGGGGCACGATGGCCTTTACCAGGGT
 CTCAGTACAGCCACCAAGGACACCTACGACGCCCTTCACATGCAGGCCCTGCCCCC
 TCGC (SEQ ID NO: 290).

In some embodiments, aCAR intracellular signaling domains include a CD28 co-
 20 stimulatory domain and a CD3 ζ signaling domain. In some embodiments, aCAR intracellular
 signaling domains include a CD28 co-stimulatory domain having the amino acid sequence
 RSKRSRLHSDYMNMTPRRPGPTRKHYPYAPPRDFAAYRS (SEQ ID NO: 287) and a
 CD3 ζ signaling domain having the amino acid sequence
 RVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEG
 25 LYNELQKDKMAEAYSEIGMKGERRRGKGHDLGLYQGLSTATKDTYDALHMQALPPR
 (SEQ ID NO: 289).

In some embodiments, the intracellular signaling sequences within the cytoplasmic
 portion of a CAR of the present disclosure may be linked to each other in a random or specified
 order. In some embodiments, a short oligopeptide or polypeptide linker, for example, between 2
 30 amino acids and 10 amino acids (e.g., 2 amino acids, 3 amino acids, 4 amino acids, 5 amino
 acids, 6 amino acids, 7 amino acids, 8 amino acids, 9 amino acids, or 10 amino acids) in length
 may form the linkage between intracellular signaling sequences. In one embodiment, a glycine-
 serine doublet can be used as a suitable linker. In one embodiment, a single amino acid, e.g., an
 alanine or a glycine, can be used as a suitable linker.

In some embodiments, the intracellular signaling domain comprises two or more costimulatory signaling domains, e.g., two costimulatory signaling domains, three costimulatory signaling domains, four costimulatory signaling domains, five costimulatory signaling domains, six costimulatory signaling domains, seven costimulatory signaling domains, eight costimulatory signaling domains, nine costimulatory signaling domains, 10 costimulatory signaling domains, or more costimulatory signaling domains. In one embodiment, the intracellular signaling domain comprises two costimulatory signaling domains. In some embodiments, the two or more costimulatory signaling domains are separated by a linker of the present disclosure (e.g., any of the linkers described in **Table 6**). In one embodiment, the linker is a glycine residue. In another embodiment, the linker is an alanine residue.

In some embodiments, a cell of the present disclosure expresses a CAR that includes an antigen-binding domain, a transmembrane domain, a primary signaling domain, and one or more costimulatory signaling domains.

In some embodiments, the transmembrane domain is derived from the same protein as one of the one or more intracellular signaling domains. In some embodiments, the CAR is an inhibitory CAR and includes a transmembrane domain and at least one intracellular inhibitory co-signaling domain each derived from a protein selected from PD-1, CTLA4, TIGIT, BTLA, and LIR1 (LILRB1), TIM3, KIR3DL1, NKG2A, LAG3, SLAP1, SLAP2, Dok-1, Dok-2, LAIR1, GRB-2, CD200R, SIRP α , HAVR, GITR, PD-L1, KIR2DL1, KIR2DL2, KIR2DL3, KIR3DL2, CD94, KLRG-1, CEACAM1, LIR2, LIR3, LIR5, SIGLEC-2, and SIGLEC-10.

In some embodiments, the transmembrane domain is derived from a first protein and the one or more intracellular signaling domains are derived from a second protein that are distinct from the first protein.

Natural Killer Cell Receptor (NKR) CARs

In some embodiments, a CAR of the present disclosure comprises one or more components of a natural killer cell receptor (NKR), thereby forming an NKR-CAR. The NKR component may be a transmembrane domain, a hinge domain, or a cytoplasmic domain from any suitable natural killer cell receptor, including without limitation, a killer cell immunoglobulin-like receptor (KIR), such as KIR2DL1, KIR2DL2/L3, KIR2DL4, KIR2DL5A, KIR2DL5B, KIR2DS1, KIR2DS2, KIR2DS3, KIR2DS4, KIR2DS5, KIR3DL1/S1, KIR3DL2, KIR3DL3, KIR2DP1, and KIR3DPI; a natural cytotoxicity receptor (NCR), such as NKp30, NKp44, NKp46; a signaling lymphocyte activation molecule (SLAM) family of immune cell receptor, such as CD48, CD229, 2B4, CD84, NTB-A, CRACC, BLAME, and CD2F-10; an Fc receptor (FcR), such as CD16, and CD64; and an Ly49 receptor, such as LY49A and LY49C. In some embodiments, the NKR-CAR may interact with an adaptor molecule or intracellular

signaling domain, such as DAP12. Exemplary configurations and sequences of CARs comprising NKR components are described in International Patent Publication WO2014/145252, published September 18, 2014.

Immunoresponsive cells

5 Certain aspects of the present disclosure relate to a cell, such as an immunoresponsive cell, that has been genetically engineered to comprise one or more chimeric receptors of the present disclosure or one or more nucleic acids encoding such chimeric receptors, and to methods of using such cells for treating myeloid malignancies (e.g., AML).

In some embodiments, the cell is a mammalian cell. In some embodiments, the
10 mammalian cell is a primary cell. In some embodiments, the mammalian cell is a cell line. In some embodiments, the mammalian cell, a bone marrow cell, a blood cell, a skin cell, bone cell, a muscle cell, a neuronal cell, a fat cell, a liver cell, or a heart cell. In some embodiments, the cell is a stem cell. Exemplary stem cells include, without limitation embryonic stem cells (ESCs), induced pluripotent stem cells (iPSCs), adult stem cells, and tissue-specific stem cells,
15 such as hematopoietic stem cells (blood stem cells), mesenchymal stem cells (MSC), neural stem cells, epithelial stem cells, or skin stem cells. In some embodiments, the cell is a cell that is derived or differentiated from a stem cell of the present disclosure. In some embodiments, the cell is an immune cell. Immune cells of the present disclosure may be isolated or differentiated from a stem cell of the present disclosure (e.g., from an ESC or iPSC). Exemplary immune cells
20 include, without limitation, T cells (e.g., helper T cells, cytotoxic T cells, memory T cells, regulatory T cells, natural killer T cells, alpha beta T cells, and gamma delta T cells), B cells, natural killer (NK) cells, dendritic cells, myeloid cells, macrophages, and monocytes. In some embodiments, the cell is a neuronal cell. Neuronal cells of the present disclosure may be isolated or differentiated from a stem cell of the present disclosure (e.g., from an ESC or iPSC).
25 Exemplary neuronal cells include, without limitation, neural progenitor cells, neurons (e.g., sensory neurons, motor neurons, cholinergic neurons, GABAergic neurons, glutamatergic neurons, dopaminergic neurons, or serotonergic neurons), astrocytes, oligodendrocytes, and microglia.

In some embodiments, the cell is an immunoresponsive cell. Immunoresponsive cells of
30 the present disclosure may be isolated or differentiated from a stem cell of the present disclosure (e.g., from an ESC or iPSC). Exemplary immunoresponsive cells of the present disclosure include, without limitation, cells of the lymphoid lineage. The lymphoid lineage, comprising B cells, T cells, and natural killer (NK) cells, provides for the production of antibodies, regulation of the cellular immune system, detection of foreign agents in the blood, detection of cells foreign

to the host, and the like. Examples of immunoresponsive cells of the lymphoid lineage include, without limitation, T cells, Natural Killer (NK) cells, embryonic stem cells, pluripotent stem cells, and induced pluripotent stem cells (e.g., those from which lymphoid cells may be derived or differentiated). T cells can be lymphocytes that mature in the thymus and are chiefly

5 responsible for cell-mediated immunity. T cells are involved in the adaptive immune system. In some embodiments, T cells of the present disclosure can be any type of T cells, including, without limitation, T helper cells, cytotoxic T cells, memory T cells (including central memory T cells, stem-cell-like memory T cells (or stem-like memory T cells), and two types of effector memory T cells: e.g., T_{EM} cells and T_{EMRA} cells, regulatory T cells (also known as suppressor T
10 cells), natural killer T cells, mucosal associated invariant T cells, and $\gamma\delta$ T cells. Cytotoxic T cells (CTL or killer T cells) are a subset of T lymphocytes capable of inducing the death of infected somatic or tumor cells. A patient's own T cells may be genetically modified to target specific antigens through the introduction of one or more chimeric receptors, such as a chimeric TCRs or CARs.

15 Natural killer (NK) cells can be lymphocytes that are part of cell-mediated immunity and act during the innate immune response. NK cells do not require prior activation in order to perform their cytotoxic effect on target cells.

In some embodiments, an immunoresponsive cell of the present disclosure is a T cell. T cells of the present disclosure may be autologous, allogeneic, or derived in vitro from
20 engineered progenitor or stem cells.

In some embodiments, an immunoresponsive cell of the present disclosure is a universal T cell with deficient TCR- $\alpha\beta$. Methods of developing universal T cells are described in the art, for example, in Valton et al., Molecular Therapy (2015); 23 9, 1507-1518, and Torikai et al., Blood 2012 119:5697-5705.

25 In some embodiments, an immunoresponsive cell of the present disclosure is an isolated immunoresponsive cell comprising one or more chimeric receptors of the present disclosure. In some embodiments, the immunoresponsive cell comprises one or more, two or more, three or more, four or more, five or more, six or more, seven or more, eight or more, nine or more, or ten or more chimeric receptors of the present disclosure.

30 In some embodiments, an immunoresponsive cell is a T cell. In some embodiments, an immunoresponsive cell is a Natural Killer (NK) cell.

In some embodiments, an immunoresponsive cell expresses or is capable of expressing an immune receptor. Immune receptors generally are capable of inducing signal transduction or changes in protein expression in the immune receptor-expressing cell that results in the
35 modulation of an immune response upon binding to a cognate ligand (e.g., regulate, activate,

initiate, stimulate, increase, prevent, attenuate, inhibit, reduce, decrease, inhibit, or suppress an immune response). For example, when CD3 chains present in a TCR/CAR cluster in response to ligand binding, an immunoreceptor tyrosine-based activation motifs (ITAMs)-mediated signal transduction cascade is produced. Specifically, in certain embodiments, when an endogenous

5 TCR, exogenous TCR, chimeric TCR, or a CAR (specifically an activating CAR) binds their respective antigen, a formation of an immunological synapse occurs that includes clustering of many molecules near the bound receptor (e.g., CD4 or CD8, CD3 $\gamma/\delta/\epsilon/\zeta$, etc.). This clustering of membrane bound signaling molecules allows for ITAM motifs contained within the CD3 chains to become phosphorylated that in turn can initiate a T cell activation pathway and ultimately

10 activates transcription factors, such as NF- κ B and AP-1. These transcription factors are capable of inducing global gene expression of the T cell to increase IL-2 production for proliferation and expression of master regulator T cell proteins in order to initiate a T cell mediated immune response, such as cytokine production and/or T cell mediated killing.

15 **Cells Expressing Multiple Chimeric Receptors**

In some embodiments, a cell of the present disclosure (e.g., an immunoresponsive cell) comprises two or more chimeric receptors of the present disclosure. In some embodiments, the cell comprises two or more chimeric receptors, wherein one of the two or more chimeric

20 receptors is a chimeric inhibitory receptor. In some embodiments, the cell comprises three or more chimeric receptors, wherein at least one of the three or more chimeric receptors is a chimeric inhibitory receptor. In some embodiments, the cell comprises four or more chimeric receptors, wherein at least one of the four or more chimeric receptors is a chimeric inhibitory receptor. In some embodiments, the cell comprises five or more chimeric receptors, wherein at least one of the five or more chimeric receptors is a chimeric inhibitory receptor.

25 In some embodiments, each of the two or more chimeric receptors comprise a different antigen-binding domain, e.g., that binds to the same antigen or to a different antigen. In some embodiments each antigen bound by the two or more chimeric receptors are expressed on the same cell, such as a myeloid cell type (e.g., same AML cell type). In some embodiments each antigen bound by the two or more chimeric receptors is an AML-associated antigen (e.g., FLT3,

30 CD33, CD123, CLEC12A, CXCR4, EphA3, etc.).

In embodiments where a cell of the present disclosure (e.g., an immunoresponsive cell) expresses two or more distinct chimeric receptors, the antigen-binding domain of each of the different chimeric receptors may be designed such that the antigen-binding domains do not interact with one another. For example, a cell of the present disclosure (e.g., an

35 immunoresponsive cell) expressing a first chimeric receptor (e.g., an EMCN-specific chimeric

receptor) and a second chimeric receptor may comprise a first chimeric receptor that comprises an antigen-binding domain that does not form an association with the antigen-binding domain of the second chimeric receptor. For example, the antigen-binding domain of the first chimeric receptor may comprise an antibody fragment, such as an scFv, while the antigen-binding domain of the second chimeric receptor may comprise a VHH.

Without wishing to be bound by theory, it is believed that in cells having a plurality of chimeric membrane embedded receptors that each comprise an antigen-binding domain, interactions between the antigen-binding domains of each of the receptors can be undesirable, because such interactions may inhibit the ability of one or more of the antigen-binding domains to bind their cognate antigens. Accordingly, in embodiments where cells of the present disclosure (e.g., immunoresponsive cells) express two or more chimeric receptors, the chimeric receptors comprise antigen-binding domains that minimize such inhibitory interactions. In one embodiment, the antigen-binding domain of one chimeric receptor comprises an scFv and the antigen-binding domain of the second chimeric receptor comprises a single VH domain, e.g., a camelid, shark, or lamprey single VH domain, or a single VH domain derived from a human or mouse sequence.

In some embodiments, when present on the surface of a cell, binding of the antigen-binding domain of the first chimeric receptor to its cognate antigen is not substantially reduced by the presence of the second chimeric receptor. In some embodiments, binding of the antigen-binding domain of the first chimeric receptor to its cognate antigen in the presence of the second chimeric receptor is 85%, 90%, 95%, 96%, 97%, 98%, or 99% of binding of the antigen-binding domain of the first chimeric receptor to its cognate antigen in the absence of the second chimeric receptor. In some embodiments, when present on the surface of a cell, the antigen-binding domains of the first chimeric receptor and the second chimeric receptor associate with one another less than if both were scFv antigen-binding domains. In some embodiments, the antigen-binding domains of the first chimeric receptor and the second chimeric receptor associate with one another 85%, 90%, 95%, 96%, 97%, 98%, or 99% less than if both were scFv antigen-binding domains.

Chimeric Inhibitory Receptors

In some embodiments, a cell of the present disclosure (e.g., an immunoresponsive cell) comprises one or more chimeric inhibitory receptors of the present disclosure. In some embodiments, each of the one or more chimeric inhibitory receptors comprises an antigen-binding domain that binds an antigen generally expressed on normal cells (e.g., cells generally considered to be healthy) but not on tumor cells, such as AML cells. In some embodiments, a

chimeric inhibitory receptor includes an antigen-binding domain that binds EMCN (*e.g.*, an EMCN-specific antigen-binding domain having one or more of the amino acid sequences listed in **Table 11**).

5 In some embodiments, the one or more chimeric inhibitory receptors bind antigens that are expressed on a non-tumor cell derived from a tissue selected from the group consisting of brain, neuronal tissue, endocrine, bone, bone marrow, immune system, endothelial tissue, muscle, lung, liver, gallbladder, pancreas, gastrointestinal tract, kidney, urinary bladder, male reproductive organs, female reproductive organs, adipose, soft tissue, and skin.

10 In some embodiments, a chimeric inhibitory receptor (*e.g.*, an EMCN-specific chimeric inhibitory receptor) may be used, for example, with one or more activating chimeric receptors (*e.g.*, activating chimeric TCRs or CARs) expressed on a cell of the present disclosure (*e.g.*, an immunoresponsive cell) as NOT-logic gates to control, modulate, or otherwise inhibit one or more activities of the one or more activating chimeric receptors. In some embodiments, a chimeric inhibitory receptor of the present disclosure may inhibit one or more activities of a cell
15 of the present disclosure (*e.g.*, an immunoresponsive cell).

In some embodiments, a cell of the present disclosure comprises one or more chimeric inhibitory receptors of the present disclosure and further comprises a tumor-targeting chimeric receptor that binds to one or more tumor-associated antigens. In some embodiments, the one or more tumor-associated antigens include an AML-associated antigen. In some embodiments, the
20 one or more tumor-associated antigens include CD33. In some embodiments, the one or more tumor-associated antigens include FLT3. In some embodiments, the one or more tumor-associated antigens include CD33 and FLT3.

Co-stimulatory ligands

25 In some embodiments, a cell of the present disclosure (*e.g.*, an immunoresponsive cell) can further include one or more recombinant or exogenous co-stimulatory ligands. For example, the cell can be further transduced with one or more co-stimulatory ligands, such that the cell co-expresses or is induced to co-express one or more chimeric receptors of the present disclosure (*e.g.*, the EMCN-specific, FLT3-specific, and/or CD33-specific CARs described herein) and one
30 or more co-stimulatory ligands. Without wishing to be bound by theory, it is believed that the interaction between the one or more chimeric receptors and the one or more co-stimulatory ligands may provide a non-antigen-specific signal important for full activation of the cell. Examples of suitable co-stimulatory ligands include, without limitation, members of the tumor necrosis factor (TNF) superfamily, and immunoglobulin (Ig) superfamily ligands. TNF is a
35 cytokine involved in systemic inflammation and stimulates the acute phase reaction. Its primary

role is in the regulation of immune cells. Members of TNF superfamily share a number of common features. The majority of TNF superfamily members are synthesized as type II transmembrane proteins (extracellular C-terminus) containing a short cytoplasmic segment and a relatively long extracellular region. Examples of suitable TNF superfamily members include, without limitation, nerve growth factor (NGF), CD40L (CD40L)/CD 154, CD137L/4-1BBL, TNF- α , CD134L/OX40L/CD252, CD27L/CD70, Fas ligand (FasL), CD30L/CD153, tumor necrosis factor beta (TNF β)/lymphotoxin- α (LT α), lymphotoxin-beta (LT β), CD257/B cell-activating factor (BAFF)/Bly s/THANK/Tall-1, glucocorticoid-induced TNF Receptor ligand (GITRL), and TNF-related apoptosis-inducing ligand (TRAIL), LIGHT (TNFSF 14). The immunoglobulin (Ig) superfamily is a large group of cell surface and soluble proteins that are involved in the recognition, binding, or adhesion processes of cells. These proteins share structural features with immunoglobulins and possess an immunoglobulin domain (fold). Examples of suitable immunoglobulin superfamily ligands include, without limitation, CD80 and CD86, both ligands for CD28, PD-L1/(B7-H1) that ligands for PD-1. In certain embodiments, the one or more co-stimulatory ligands are selected from 4-1BBL, CD80, CD86, CD70, OX40L, CD48, TNFRSF14, PD-L1, and combinations thereof.

Chemokine receptor

In some embodiments, a cell of the present disclosure (e.g., an immunoresponsive cell) comprises one or more chimeric receptors (e.g., the EMCN-specific, FLT3-specific, and/or CD33-specific CARs described herein) and may further include one or more chemokine receptors. For example, transgenic expression of chemokine receptor CCR2b or CXCR2 in cells, such as T cells, enhances trafficking to CCL2-secreting or CXCL1-secreting solid tumors (Craddock et al, J Immunother. 2010 Oct; 33(8):780-8 and Kershaw et al. Hum Gene Ther. 2002 Nov 1; 13(16): 1971 -80). Without wishing to be bound by theory, it is believed that chemokine receptors expressed on chimeric receptor-expressing cells of the present disclosure may recognize chemokines secreted by tumors and improve targeting of the cell to the tumor, which may facilitate the infiltration of the cell to the tumor and enhance the antitumor efficacy of the cell. Chemokine receptors of the present disclosure may include a naturally occurring chemokine receptor, a recombinant chemokine receptor, or a chemokine-binding fragment thereof. Examples of suitable chemokine receptors that may expressed on a cell of the present disclosure include, without limitation, a CXC chemokine receptor, such as CXCR1, CXCR2, CXCR3, CXCR4, CXCR5, CXCR6, or CXCR7; a CC chemokine receptor, such as CCR1 , CCR2, CCR3, CCR4, CCR5, CCR6, CCR7, CCR8, CCR9, CCR10, or CCR11; a CX3C chemokine receptor, such as CX3CR1; an XC chemokine receptor, such as XCR1; and

chemokine-binding fragments thereof. In some embodiments, the chemokine receptor to be expressed on the cell is chosen based on the chemokines secreted by the tumor.

Chimeric Receptor Regulation

5 Some embodiments of the present disclosure relate to regulating one or more chimeric receptor activities of chimeric receptor-expressing cells of the present disclosure (*e.g.*, the EMCN-specific CARs described herein). There are several ways chimeric receptor activities can be regulated. In some embodiments, a regulatable chimeric receptor, wherein one or more chimeric receptor activities can be controlled, may be desirable to optimize the safety and/or

10 efficacy of the chimeric receptor therapy. For example, inducing apoptosis using a caspase fused to a dimerization domain (see, *e.g.*, Di et al., *N Engl. J. Med.* 2011 Nov. 3; 365(18): 1673-1683) can be used as a safety switch in the chimeric receptor therapy. In some embodiments, a chimeric receptor-expressing cell of the present disclosure can also express an inducible Caspase-9 (iCaspase-9) that, upon administration of a dimerizer drug, such as rimiducid (IUPAC

15 name: [(1R)-3-(3,4-dimethoxyphenyl)-1-[3-[2-[2-[[2-[3-[(1R)-3-(3,4-dimethoxyphenyl)-1-[(2S)-1-[(2S)-2-(3,4,5-trimethoxyphenyl)butanoyl]piperidine-2-carbonyl]oxypropyl]phenoxy]acetyl]amino]ethylamino]-2-oxoethoxy]phenyl]propyl] (2S)-1-[(2S)-2-(3,4,5-trimethoxyphenyl)butanoyl]piperidine-2-carboxylate), induces activation of the Caspase-9 and results in apoptosis of the cells. In some embodiments, the iCaspase-9 contains a

20 binding domain that comprises a chemical inducer of dimerization (CID) that mediates dimerization in the presence of the CID, which results in inducible and selective depletion of the chimeric receptor-expressing cells.

Alternatively, in some embodiments a chimeric receptor of the present disclosure may be regulated by utilizing a small molecule or an antibody that deactivates or otherwise inhibits

25 chimeric receptor activity. For example, an antibody may delete the chimeric receptor-expressing cells by inducing antibody dependent cell-mediated cytotoxicity (ADCC). In some embodiments, a chimeric receptor-expressing cell of the present disclosure may further express an antigen that is recognized by a molecule that is capable of inducing cell death by ADCC or complement-induced cell death. For example, a chimeric receptor-expressing cell of the present

30 disclosure may further express a receptor capable of being targeted by an antibody or antibody fragment. Examples of suitable receptors that may be targeted by an antibody or antibody fragment include, without limitation, EpCAM, VEGFR, integrins (*e.g.*, $\alpha\beta 3$, $\alpha 4$, $\alpha I\beta 3$, $\alpha 4\beta 7$, $\alpha 5\beta 1$, $\alpha\beta 3$, αv), members of the TNF receptor superfamily (*e.g.*, TRAIL-R1 and TRAIL-R2), PDGF receptor, interferon receptor, folate receptor, GPNMB, ICAM-1, HLA-DR, CEA, CA-

35 125, MUC1, TAG-72, IL-6 receptor, 5T4, GD2, GD3, CD2, CD3, CD4, CD5, CD11,

CD11a/LFA-1, CD15, CD18/ITGB2, CD19, CD20, CD22, CD23/IgE Receptor, CD25, CD28, CD30, CD33, CD38, CD40, CD41, CD44, CD51, CD52, CD62L, CD74, CD80, CD125, CD147/basigin, CD152/CTLA-4, CD154/CD40L, CD195/CCR5, CD319/SLAMF7, and EGFR, and truncated versions thereof.

5 In some embodiments, a chimeric receptor-expressing cell of the present disclosure may also express a truncated epidermal growth factor receptor (EGFR) that lacks signaling capacity but retains an epitope that is recognized by molecules capable of inducing ADCC (e.g., WO2011/056894).

10 In some embodiments, a chimeric receptor-expressing cell of the present disclosure further includes a highly expressing compact marker/suicide gene that combines target epitopes from both CD32 and CD20 antigens in the chimeric receptor-expressing cell, which binds an anti-CD20 antibody (e.g., rituximab) resulting in selective depletion of the chimeric receptor-expressing cell by ADCC. Other methods for depleting chimeric receptor-expressing cells of the present disclosure may include, without limitation, administration of a monoclonal anti-CD52
15 antibody that selectively binds and targets the chimeric receptor-expressing cell for destruction by inducing ADCC. In some embodiments, the chimeric receptor-expressing cell can be selectively targeted using a chimeric receptor ligand, such as an anti-idiotypic antibody. In some embodiments, the anti-idiotypic antibody can cause effector cell activity, such as ADCC or ADC activity. In some embodiments, the chimeric receptor ligand can be further coupled to an agent
20 that induces cell killing, such as a toxin. In some embodiments, a chimeric receptor-expressing cell of the present disclosure may further express a target protein recognized by a cell depleting agent of the present disclosure. In some embodiments, the target protein is CD20 and the cell depleting agent is an anti-CD20 antibody. In such embodiments, the cell depleting agent is administered once it is desirable to reduce or eliminate the chimeric receptor-expressing cell. In
25 some embodiments, the cell depleting agent is an anti-CD52 antibody.

In some embodiments, a regulated chimeric receptor comprises a set of polypeptides, in which the components of a chimeric receptor of the present disclosure are partitioned on separate polypeptides or members. For example, the set of polypeptides may include a dimerization switch that, when in the presence of a dimerization molecule, can couple the
30 polypeptides to one another to form a functional chimeric receptor.

Endomucin-Specific Antigen-binding Domains

The present disclosure provides antigen-binding domains (e.g., single-chain variable fragments) that bind to endomucin (EMCN), chimeric proteins including antigen-binding domains that bind to EMCN (e.g., any of the iCARs described herein), and nucleic acids

encoding such antigen-binding domains and chimeric proteins. Without wishing to be bound by theory, EMCN is a sialoglycoprotein that interferes with assembly of focal adhesion complexes and inhibits interaction between cells and extracellular matrix. EMCN-specific antigen-binding domains bind to human EMCN (*e.g.*, Uniprot Q9ULC0, herein incorporated by reference for all purposes) or an epitope fragment thereof. EMCN can be expressed on cells generally considered to be healthy, such as healthy hematopoietic stem cells (HSCs), HSPCs, healthy multipotent progenitors (MPPs), healthy lympho-myeloid primed progenitor (LMPPs), and healthy hematopoietic progenitor cells (HPCs). EMCN can be expressed on hematopoietic stem and progenitor cells (HSPCs). EMCN can be expressed on HSCs. EMCN can be expressed on MPPs. EMCN can be expressed on LMPPs. EMCN can be expressed on HPCs. EMCN-specific antibodies have been previously described, including CBFYE-0213, V.7.C7.1, L4B1, L5F12, L10B5, L3F12, L6H3, , L9H8, and L10F12, as described in Samulowitz U. et al., Am. J. Path., 2002 May, 160(5):1669-1681, herein incorporated by reference for all purposes.

The present disclosure provides an EMCN-specific antigen-binding domain including one or more of the amino acid sequences listed in **Table 11**.

Table 11 - EMCN-specific antigen-binding domains

Amino Acid Sequence	SEQ ID NO:	Description
RYDMH	291	CDR-H1 of Kabat-annotated CDR
GFTFSRY	292	CDR-H1 Version 1 of Chothia-annotated CDR
GFTLSRY	293	CDR-H1 Version 2 of Chothia-annotated CDR
GFSFSRY	294	CDR-H1 Version 3 of Chothia-annotated CDR
GFSLRY	295	CDR-H1 Version 4 of Chothia-annotated CDR
VIWGNGNTHYHSALKS	296	CDR-H2 of Kabat-annotated CDR
WGNGN	297	CDR-H2 of Chothia-annotated CDR
RIKD	298	CDR-H3 (Kabat- and Chothia-annotated CDR)
KSSQSLVASDENTYLN	299	CDR-L1 (Kabat- and Chothia-annotated CDR)
QVSKLDS	300	CDR-L2 (Kabat- and Chothia-annotated CDR)
LQGIHLPWT	301	CDR-L3(Kabat- and Chothia-annotated CDR)

Amino Acid Sequence	SEQ ID NO:	Description
EVQLVESGGGLVQPGGSLRLSCAASGFTFSRYDMHWVRQAPGKGLEWVSVIWGNGNTHYHSALKSRFTISRDN SKNTLYLQMNSLRAEDTAVYYCTLRKDWGQGTMTVSS	302	VH 3-23 Version 1
EVQLVESGGGLVQPGGSLRLSCAAS GFTLSRYDMHWVRQAPGKGLEWVSVIWGNGNTHYHSALKSRFTISRDN SKNTLYLQMNSLRAEDTAVYYCTLRKDWGQGTMTVSS	303	VH 3-23 Version 2
EVQLVESGGGLVQPGGSLRLSCAAS GFSFSRYDMHWVRQAPGKGLEWVSVIWGNGNTHYHSALKSRFTISRDN SKNTLYLQMNSLRAEDTAVYYCTLRKDWGQGTMTVSS	304	VH 3-23 Version 3
EVQLVESGGGLVQPGGSLRLSCAAS GFSLRYDMHWVRQAPGKGLEWVSVIWGNGNTHYHSALKSRFTISRDN SKNTLYLQMNSLRAEDTAVYYCTLRKDWGQGTMTVSS	305	VH 3-23 Version 4
QVQLVESGGGVVQPGRSLRLSCAASGFTFSRYDMHWVRQAPGKGLEWVAVIWGNGNTHYHSALKSRFTISRDN SKNTLYLQMNSLRAEDTAVYYCTLRKDWGQGTMTVSS	306	VH 3-33 Version 1
QVQLVESGGGVVQPGRSLRLSCAAS GFTLSRYDMHWVRQAPGKGLEWVAVIWGNGNTHYHSALKSRFTISRDN SKNTLYLQMNSLRAEDTAVYYCTLRKDWGQGTMTVSS	307	VH 3-33 Version 2
QVQLVESGGGVVQPGRSLRLSCAAS GFSFSRYDMHWVRQAPGKGLEWVAVIWGNGNTHYHSALKSRFTISRDN SKNTLYLQMNSLRAEDTAVYYCTLRKDWGQGTMTVSS	308	VH 3-33 Version 3
QVQLVESGGGVVQPGRSLRLSCAAS GFSLRYDMHWVRQAPGKGLEWVAVIWGNGNTHYHSALKSRFTISRDN SKNTLYLQMNSLRAEDTAVYYCTLRKDWGQGTMTVSS	309	VH 3-33 Version 4
DVVMTQSPLSLPVTLGQPASISCKSSQSLVASDENTYLNWFQQRPQGSPRRLIYQVSKLDSGVPDRFSGSGTDFTLKISRVEAEDVGVYYCLQGIHLPWTFGQGTKLEIK	310	VL 2-30

In some embodiments, the antigen-binding domain specific for EMCN includes a heavy chain variable (VH) region and a light chain variable (VL) region, wherein: the EMCN-VH includes a heavy chain complementarity determining region 1 (CDR-H1) having the amino acid sequence of RYDMH (SEQ ID NO: 291), a heavy chain complementarity determining region 2 (CDR-H2) having the amino acid sequence of VIWGNGNTHYHSALKS (SEQ ID NO: 296), and a heavy chain complementarity determining region 3 (CDR-H3) having the amino acid sequence of RIKD (SEQ ID NO: 298), and the EMCN-VL includes a light chain complementarity determining region 1 (CDR-L1) having the amino acid sequence of KSSQSLVASDENTYLN (SEQ ID NO: 299), a light chain complementarity determining region 2 (CDR-L2) having the amino acid sequence of QVSKLDS (SEQ ID NO: 300), and a light chain complementarity determining region 3 (CDR-L3) having the amino acid sequence of LQGIHLPWT (SEQ ID NO: 301), and where the amino acid sequences of the CDR-H1, the

CDR-H2, the CDR-H3, the CDR-L1, the CDR-L2, and the CDR-L3 of the reference antibody are defined based on the Kabat numbering scheme.

In some embodiments, the antigen-binding domain specific for EMCN includes a heavy chain variable (VH) region and a light chain variable (VL) region, wherein: the EMCN-VH

5 includes the amino acid sequence

EVQLVESGGGLVQPGGSLRLSCAASGFTFSRYDMHWVRQAPGKGLEWVSVIWGNGNT
HYHSALKSRFTISRDN SKNTLYLQMNSLRAEDTAVYYCTLRIKDWGQGTMVTVSS

(SEQ ID NO: 302); and the EMCN-VL includes the amino acid sequence

DVVMTQSPLSLPVTLGQPASISCKSSQSLVASDENTYLNWFQQRPGQSPRRLIYQVSKL
10 DSGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCLQGIHLPWTFGQGTKLEIK (SEQ ID
NO: 310).

In some embodiments, the antigen-binding domain specific for EMCN includes the amino acid sequence

EVQLVESGGGLVQPGGSLRLSCAASGFTFSRYDMHWVRQAPGKGLEWVSVIWGNGNT
15 HYHSALKSRFTISRDN SKNTLYLQMNSLRAEDTAVYYCTLRIKDWGQGTMVTVSSGGG
GSGGGGSGGGGSDVVMTQSPLSLPVTLGQPASISCKSSQSLVASDENTYLNWFQQRPG
QSPRRLIYQVSKLDSGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCLQGIHLPWTFGQ
GTKLEIK (SEQ ID NO: 311). In some embodiments, the antigen-binding domain specific for

EMCN is encoded by a polynucleotide sequence comprising the sequence

20 GAGGTGCAGCTGGTTGAATCTGGCGGAGGACTGGTTCAGCCTGGCGGATCTCTGAG
ACTGTCTTGTGCCGCCAGCGGCTTCACCTTCAGCAGATACGATATGCACTGGGTCCG
ACAGGCCCTGGCAAAGGACTTGAATGGGTGTCCGTGATCTGGGGCAACGGCAACA
CACACTACCACAGCGCCCTGAAGTCCCGGTTACCATCTCCAGAGACAACAGCAAG
AACACCCTGTACCTGCAGATGAACAGCCTGAGAGCCGAGGACACCGCCGTGTACTA
25 CTGCACCCTGAGAATCAAGGATTGGGGCCAGGGCACCATGGTCACCGTTTCTTCTG
GAGGCGGAGGATCTGGTGGCGGAGGAAGTGGCGGAGGCGGTTCTGACGTGGTCAT
GACACAGAGCCCTCTGAGCCTGCCTGTGACACTGGGACAGCCTGCCAGCATCAGCT
GCAAGTCTAGCCAGTCTCTGGTGGCCAGCGACGAGAACACCTACCTGAAGTGGTTC
CAGCAGAGGCCCGGACAGTCTCCTAGACGGCTGATCTACCAGGTGTCCAAGCTGGA
30 TAGCGGCGTGCCCGATAGATTTTCTGGCAGCGGCTCTGGCACCGACTTCACCCTGAA
GATCAGCAGAGTGGAAGCCGAGGACGTGGGCGTGTACTACTGTCTGCAAGGCATCC
ATCTGCCTTGACCTTTGGCCAGGGCACAAAGCTGGAAATCAAG (SEQ ID NO: 312).

In some embodiments, the antigen-binding domain specific for EMCN is encoded by a polynucleotide sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least

35 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to

GAGGTGCAGCTGGTTGAATCTGGCGGAGGACTGGTTCAGCCTGGCGGATCTCTGAG
ACTGTCTTGTGCCGCCAGCGGCTTCACCTTCAGCAGATACGATATGCACTGGGTCCG
ACAGGCCCTGGCAAAGGACTTGAATGGGTGTCCGTGATCTGGGGCAACGGCAACA
CACACTACCACAGCGCCCTGAAGTCCCGGTTACCATCTCCAGAGACAACAGCAAG
5 AACACCCTGTACCTGCAGATGAACAGCCTGAGAGCCGAGGACACCGCCGTGTACTA
CTGCACCCTGAGAATCAAGGATTGGGGCCAGGGCACCATGGTCACCGTTTCTTCTG
GAGGCGGAGGATCTGGTGGCGGAGGAAGTGGCGGAGGCGGTTCTGACGTGGTCAT
GACACAGAGCCCTCTGAGCCTGCCTGTGACACTGGGACAGCCTGCCAGCATCAGCT
GCAAGTCTAGCCAGTCTCTGGTGGCCAGCGACGAGAACACCTACCTGAACTGGTTC
10 CAGCAGAGGCCCGGACAGTCTCCTAGACGGCTGATCTACCAGGTGTCCAAGCTGGA
TAGCGGCGTGCCCGATAGATTTTCTGGCAGCGGCTCTGGCACCGACTTCACCCTGAA
GATCAGCAGAGTGGAAGCCGAGGACGTGGGCGTGTACTACTGTCTGCAAGGCATCC
ATCTGCCTTGGACCTTTGGCCAGGGCACAAAGCTGGAAATCAAG (SEQ ID NO: 312).

15 **FLT3 and CD33-Specific Antigen-binding Domains**

The present disclosure provides antigen-binding domains (e.g., single-chain variable fragments) that bind to FLT3 and CD33, chimeric proteins including antigen-binding domains that bind to FLT3 and CD33 (e.g., any of the aCARs described herein), and nucleic acids encoding such antigen-binding domains and chimeric proteins.

20 In some embodiments, chimeric receptors comprise one or more of the amino acid sequences listed in **Table A1** or **Table A2**. In some embodiments, activating chimeric receptors (aCARS) comprise one or more of the amino acid sequences listed in **Table A1** or **Table A2**. In some embodiments, bispecific-bivalent aCARS comprise one or more of the amino acid sequences listed in **Table A1** or **Table A2**. Table A1 provides the variable domains of an

25 antibody heavy chain or light chain. The CDRs were determined using the Kabat method and are in *bold italic* in **Table A1** for each variable heavy chain or variable light chain and shown in **Table A2**. In some embodiments, nucleic acids encoding any of the chimeric receptors of the present disclosure comprise one or more of the nucleic acid sequences listed in **Table B**.

Table A1		
Amino Acid Sequence	SEQ ID NO:	Description
EVQLVQSGAEVKKPGASVKVSCKAS GYTFTSYMH WARQAP GQGLEWMGI INPSGGSTSYAQKFQGR VTMTRDTSTSTVYMEL SSLRSEDTAVYYCARVVA AVADYWGQ GLTVSS	313	Heavy chain variable domain of anti-FLT3 antibody D4-3

Table A1		
Amino Acid Sequence	SEQ ID NO:	Description
DVVMQTQSPLSLPVTGPGEPAISCRSS QSLH SNNGYNYLDWYLQ KPGQSPQLLIY LGSN RASGVDPDRFSGSGSGTDFTLKISRVEAED VGVYYCMQSLQTPFTFGPGTKVDIK	314	Light chain variable domain of anti-FLT3 antibody D4-3
EVQLVQSGAEVKKPGSSVKVSCKASGG TFSS YAISWVRQAPG QGLEWMGG IIPIFGTANYAQKFQGR VTITADKSTSTAYMELSS LRSEDTAVYYCAT FALFGFREQA FDIWGQGTTVTVSS	315	Heavy chain variable domain of anti-FLT3 antibody NC7
DIQMTQSPSSLSASVGDRVTITCRAS QSISS YLNWYQQKPGKA PKLLIYAAS LQSG VPSRFSGSGSGTDFTLTISLQPEDLATYYC QSYSTPFT FGPGTKVDIK	316	Light chain variable domain of anti-FLT3 antibody NC7
EVQLVQSGAEVKKPGASVKVSCKASGY TF SY MHW VRQAP GQGLEWMG IINPSGG STSY AQKFQGR VTMTTRDTSTSTVYMEL SSLRSEDTAVYYCARG VGAHDA FDIWGQGTTVTVSS	317	Heavy chain variable domain of anti-FLT3 antibody EB10
DVVMQTQSPLSLPVTGPGEPAISCRSS QSLH SNNGNNYLDWYLQ KPGQSPQLLIY LGSN RASGVDPDRFSGSGSDTDFTLQISRVEAED VGVYYCM QGTH PAISFGQGTRLEIK	318	Light chain variable domain of anti-FLT3 antibody EB10
QVQLQQPGAELVKPGASLKLSCKSSGYTFTSY WMH WVRQRP GHGLEWIG EIDPS SYKDYN QKFKD KATLTVDRSSNTAYMHL SSLTSDDSAVYYCAR AITTTP DFDWGQGTTLTVSS	319	Heavy chain variable domain of anti-FLT3 antibody 4G8
DIVLTQSPATLSVTPGDSVLS CRASQ SISNNLHWYQQKSHES PRLLIKYAS Q SISGIPSRFSGSGSGTDFTLSINSVETEDFGVYFC Q QSN TWPYTGGGKLEIKR	320	Light chain variable domain of anti-FLT3 antibody 4G8
QVTLKESGPTLVKPTETLTCTLSGFSLNN ARMG VSWIRQPP GKCLEWLA HIFSNDEKSYSTSL KNRLTISKDSSKTQVVLMTN VDPVDATYYCAR IVGYGSGWYGFFD YWGQGTTLTVSS	321	Heavy chain variable domain of anti-FLT3 antibody FL_39
DIQMTQSPSSLSASVGDRVTITCRAS QGI RNDLGWYQQKPGK APKRLIYAAS TLQSG VPSRFSGSGSGTEFTLTISLQPEDFATYY CLQHNSYPLT FGCGTKVEIK	322	Light chain variable domain of anti-FLT3 antibody FL_39
QVTLKESGPVLVKPTETLTCTVSGFSLRN ARMA VSWIRQPP GKTLLEWLA HIFSNDEKSYSTSL KSRLTISKDTSKQVVLMTN MDPVDATYYCAR IVGYGSGWYG YFDYWGQGTTLTVSS	323	Heavy chain variable domain of anti-FLT3 antibody FL_16
DIQMTQSPSSVSASVGDRVTITCRAS QDI RYDLAWYQQKPGK APKRLIYAAS SLQSG VPSRFSGSGSGTEFTLTISLQPEDFATYY CLQHN FYPLTFGGGKVEIK	324	Light chain variable domain of anti-FLT3 antibody FL_16
EVQLVESGGGLVQPGGSLRLSCAAS GFTF SSYGMHWVRQAP GKGLEWVAV ISYDGS NKYYADSVKGRFTISRDN SKNTLYLQM NSLRAEDTAVYYCAN LAPWA AYWGQGTTLTVSS	325	Heavy chain variable domain of anti-FLT3 antibody ml0006
EIVLTQSPLSLPVTGPGEPAISCRSS QSLH SNNGYNYLDWYLQK PGQSPQLLIY LGSN RASGVDPDRFSGSGSGTDFTLKISRVEAEDV GVYYCM QALQTPHT FGQGTKLEIK	326	Light chain variable domain of anti-FLT3 antibody ml0006
QVQLKQSGPGLVQPSQSLITCTVSGFSLT NYGLH WVRQSPGK GLEWL GVIW SGGSTDYNA AFIS RLSISKDNSKQVFFKMNSLQ ADDTAIYYCAR KGGI YAN HYYAM DYWGQGTSTVTVSS	327	Heavy chain variable domain of anti-FLT3 antibody BV10
DIVMTQSPSSLSVSAGEKVTMSCK SSQSL NSGN QKN YMAWY QQKPGQPPKLLIY GAST RESGVDPDRFTGSGSGTDFTLTISSVQA EDLAVYYC QNDH SYPLTFGAGTKLELKR	328	Light chain variable domain of anti-FLT3 antibody BV10

Table A1		
Amino Acid Sequence	SEQ ID NO:	Description
QVQLVQSGAEVKKPGSSVKVSKASGYTFTDYNMHWVRQAPGQGLEWIGYIYPYNGGTGYNQKFKSKATITADESTNTAYMELSLRSED TAVYYCARGRPAMDYWGQGLTVTVSS	329	Heavy chain variable domain of anti-CD33 antibody lintuzumab
DIQMTQSPSSLSASVGDRTITCRASESDNYGISFMNWFQQKPGKAPKLLIYAASNQSGVPSRFSGSGSGTDFTLTISSLQPDFATYYCQQSKEVPWTFGQGTKVEIK	330	Light chain variable domain of anti-CD33 antibody lintuzumab
EVQLVQSGAEVKKPGSSVKVSKASGYTITDSNIHWVRQAPGQSLEWIGYIYPYNGGTGYNQKFKNRATLTVDNPTNTAYMELSLRSED TAFYYCVNGNPWLAYWGQGLTVTVSS	331	Heavy chain variable domain of anti-CD33 antibody gentuzumab
DIQLTQSPSTLSASVGDRTITCRASELDNYGIRFLTWFFQQKPGKAPKLLMYAASNQSGVPSRFSGSGSGTEFTLTISLQPDFATYYCQQTKVPWTFGQGTKVEVKR	332	Light chain variable domain of anti-CD33 antibody gentuzumab

Table B		
Nucleic Acid Sequence	SEQ ID NO:	Description
GAAGTGCAACTTGTTCAGAGCGGGGCAGAAGTTAAGAAGCCAGGCGCTTCGTCAAGGTGAGTTGCAAGGCAAGTGGATACACCTTTACGAGTTATTATATGCACTGGGCACGGCAGGCCCTGGTCAGGGCCTCGAATGGATGGGGATTATAAATCCTTCTGGCGGGTCAACCAGCTACGCACAAAAATTCAAGGTCGGGTGACAATGACGCGCGACACGTCAACGAGTACAGTGTATATGGAATTGCTAGCCTGAGGTCCGAGGATACTGCTGTCTATTATTGTGCTCGCGTGGTCGCTGCTGTGGCAGACTACTGGGGTCAGGGTACACTTGTGACGGTAAGCAGC	333	Heavy chain variable domain of anti-FLT3 antibody D4-3
GACGTAGTTATGACACAGTCTCCACTGTTCATTGCCAGTAACACCAGGTGAGCCCGCCTCCATCTCATGTAGATCCTCCCAATCTCCTTCATTCAAACGGGTATAATTATCTCGACTGGTATTTGCAGAAACCGGGCCAGAGCCCTCAACTGCTCATCTATTTGGGGAGCAACCGGGCCTCTGGTGTCCCTGATAGATTCTCCGGGAGTGGATCAGGTACGGATTTTACACTGAAGATCAGCAGGGTGGAAGCAGAAGATGTTGGTGTGTATTACTGTATGCAATCACTCCAGACCCCGTTACC TTTGGGCCTGGAACAAAGGTAGATATTAA	334	Light chain variable domain of anti-FLT3 antibody D4-3
GAGGTTCAACTGGTACAAAGCGGAGCCGAGGTAAAGAAACCAGGGAGTAGCGTCAAAGTGTCTGCAAAGCCTCAGGCGGCACATTCAGTAGCTATGCTATTTCATGGGTACGCCAAGCACCAGGACAGGGGTGGAGTGGATGGGCGGGATTATCCCCATCTTCGGTACGGCAAATATGCACAAAAGTTCCAGGGACGAGTCAACCATCACGGCTGATAAGTCCACCTCCACCGCCTATATGGAGCTGAGTTCCCTTCGGAGCGAGGATACTGCTGTGTATTATTGTGCCACGTTTCGCAC TGTTCGGTTTTTCGGGAGCAGGCGTTTGATATTTGGGGACAAGGCACAACG GTCACGGTCAGTTCA	335	Heavy chain variable domain of anti-FLT3 antibody NC7
GACATTCAGATGACCCAGAGTCCCTCTTCATTGAGTGCGAGCGTCCGGTGATCGGGTTACGATAACCTGTAGGGCCTCCCAAAGTATATCATCATATTTGAAC TGGTACCAACAGAAACCTGGGAAAGCGCCGAAGCTCCTTATCTATGCTGC CAGCTCTTTGCAAAGCGGTGTGCCCTCACGGTTCTCCGGTAGTGGGTCCGG GACCGACTTCACCTTTGACCATCAGCAGCCTTCAGCCAGAGGATCTTGCCAC TTATTACTGCCAGCAATCTTATAGCACACCGTTTACATTCGGTCCAGGCAC AAAGGTAGACATTAAAG	336	Light chain variable domain of anti-FLT3 antibody NC7
GAGGTACAGCTTGTGCAGAGTGGAGCAGAAGTTAAAAAACCCGGAGCTTCGTGAAGGTAAGCTGCAAGGCTCAGGATATACATTTACTAGCTACTACATGCACTGGGTCCGCCAAGCTCCGGGCCAAGGCCTTGAATGGATGGGCATCA	337	Heavy chain variable domain of

Table B		
Nucleic Acid Sequence	SEQ ID NO:	Description
TAAATCCCAGTGGAGGCTCAACGAGCTATGCACAAAAGTTCCAAGGGCGCGTTACCATGACGCGCGACACCAGCACGTCACCGTCTATATGGAACTCTCAAGTTTGCATCTGAAGATACGGCTGTCTACTATTGCGCACGAGGGGTCGGAGCGCATGACGCCTTCGACATCTGGGGACAAGGGACTACAGTAACTGTGTCAAGC		anti-FLT3 antibody EB10
GATGTTGTTATGACACAGTCTCCCTCTCTTTGCCTGTTACGCCTGGCGAGCCGCCTCTATTTCTTGTGCTAGTCAGAGCCTGCTGCATTCTAATGGAAACAACTATTTGGACTGGTACTTGCAAAAGCCGGGTCAAAGTCCC	338	Light chain variable domain of anti-FLT3 antibody EB10
CAAGTCCAACCTTCAGCAGCCAGGCGCTGAGTTGGTTAAACCGGGCGCAAGCCTCAAACCTTAGTTGCAAGTCATCCGGATATACTTTCACGTCTTATTGGATGCATTGGGTACGACAAAAGACCTGGTCACGGCCTCGAATGGATTGGCGAAA TCGACCCGTCAGACAGCTACAAGGATTACAACCAGAAATTCAAAGATAAGGCAACACTTACTGTGGATCGCTCAAGTAACACGGCTTACATGCACCTCTCTTCACTCACGTCTGACGACAGTGCGGTGTATTATTGCGCCCGCGCTATTACAACAACCCCTTTCGATTTCTGGGGCCAGGGTACTACGCTCACAGTCTCATCC	339	Heavy chain variable domain of anti-FLT3 antibody 4G8
GATATCGTCCTCACCCAATCCCCGGCTACTTTGAGTGTAACACCAGGCGACAGCGTGCTACTGTCTATGCCGAGCCTCCCAGTCAATCAGCAATAATCTGCAT TGGTATCAACAGAAATCACACGAATCCCCCGACTTTTGATAAAGTATGCGTCACAGTCCATATCAGGCATTCCAGTAGGTTTTTCAGGCAGTGGTTTCAGGTACTGACTTCACCCTCTCCATTAACTCTGTAGAAACAGAGGACTTTGGCGTCTACTTCTGTCTAGCAATCCAACACCTGGCCTTATACATTGGCGGGCGGCACTAAGCTGGAAATTAAGAGA	340	Light chain variable domain of anti-FLT3 antibody 4G8
CAAGTAACCCCTTAAAGAGTCCGGCCCCACTTTGGTTAAACCTACTGAAACA CTTACACTCACATGCACATTGTCCGGCTTTTCACTCAACAACGCAAGGATG GGTGTGTCTCGATTGCGCCAGCCCCCTGGAAAATGTTTGGAATGGCTCGCTCATATATTTAGCAACGACGAGAAAAAGTTACTCAACTTCACTCAAGAACCG CCTCACTATTAGCAAAGATTCTTCCAAAACCCAAGTAGTTCTGACAATGACGAATGTAGACCCAGTCGATACTGCAACTTACTATTGCGCACGGAATAGTCG GTTACGGGAGTGGCTGGTATGGGTTTTTCGACTATTGGGGACAGGGCACTCTTGTAACAGTAAGTAGC	341	Heavy chain variable domain of anti-FLT3 antibody FL39
GACATCCAGATGACTCAATCTCCATCTAGCCTCTCAGCGTCTGTGGGCGATCGAGTCACCATCACCTGTAGGGCTTCCCAGGGTATAAGGAATGATTTGGGCTGGTACCAGCAAAAACCGGGTAAGGCTCCGAAACGACTGATATACGCAGCTTCTACGTTGCAATCCGGGGTGCCATCCAGATTTAGTGGCAGCGGGAGCGGTACTGAGTTTACGCTGACTATCTCCTCACTTCAGCCAGAGGATTTGCGCA CGTACTATTGTCTGCAACACAACCTCCTATCCGCTGACCTTCGGGTGCGGGA CAAAGGTGGAAATTAATA	342	Light chain variable domain of anti-FLT3 antibody FL39
CAAGTGACCTTGAAGGAGTCAGGGCCAGTGTGGTAAAACCTACTGAGAC TCTACGTTGACATGCACGGTATCAGGTTTCAGCCTGAGGAACGCTCGGATGGCCGTACGTTGGATACGCCAGCCGCCAGGCCAAAACCTTGAATGGTTGG CGCACATATTCAGTAACGACGAGAAATCTTACTCTACATCCCTTAAGTCTC GCCTCACCATTCTAAAGACACATCCAAATCACAAGTGGTACTCACGATGA CAAACATGGACCTGTGACACTGCTACATATTATTGTGCTAGGATAGTGG GCTACGGTAGCGGATGGTACGGTTATTTTGATTACTGGGGACAAGGGACG CTTGTTACGGTGTCTCA	343	Heavy chain variable domain of anti-FLT3 antibody FL16
GACATTACAGATGACCCAGTCTCCGTCCAGCGTTAGCGCAAGCGTGGGGGATAGAGTCACTATTACGTGTAGAGCCAGTCAAGATATACGGTACGATCTTGC TTGGTATCAGCAAAAACCGGGAAAAGCCCCGAAGAGACTTATATATGCAGCTTCTCCTTGCAAAGCGGGGTCCCATCCCGGTTTAGTGGTAGTGGTTCCG	345	Light chain variable domain of anti-FLT3

Table B		
Nucleic Acid Sequence	SEQ ID NO:	Description
GAACAGAGTTACGCTGACTATTTTCATCACTGCAACCCGAAGATTTTGCCA CCTACTACTGCCITTAACACAATTTCTATCCTCTTACCTTCGGCGGAGGTAC TAAGGTAGAGATTAAG		antibody FL16
GAAGTACAGTTGGTTGAGAGTGGTGGAGGACTCGTTCAACCTGGCGGTAG TTTGCGACTCAGCTGCGCGGCTTCCGGTTTCACCTTCTCATCCTATGGGATG CACTGGGTGACAGACAAGCCCCCTGGAAAGGGCCTCGAATGGGTTGCTGTGAT TAGCTATGACGGCTCTAATAAATACTATGCAGATAGTGTAAGGGGAGAT TTACGATTTCTCGCGATAATAGCAAAAATACGCTGTACCTGCAAAATGGAA ACCAACAGCCTGCGAGCGGAAGATACGGCGGTTTATTACTGCGCGAATCT TGCCCCGTGGGCAGCATACTGGGGACAGGGGACGTTGGTGACGGTAAGCA GT	346	Heavy chain variable domain of anti-FLT3 antibody ml0006
GAGATTGTGCTCACCCAGTCTCCACTCAGCCTTCTGTAAACGCCCGGTGAG CCTGCCTCTATATCATGCCGAAGTTCCCAAAGCCTTCTGCACTCAAACGGC TATACTACTTGGACTGGTACCTCCAGAAGCCCCGGCCAAAGTCCTCAACTG TTGATATACCTGGGGTCCAACCGGGCATCAGGAGTACCTGATAGATTCTCA GGAAGTGGGTGAGGAACCGACTTCACGCTGAAAATTAGTCGCGTAGAGGC GGAAGATGTAGGTGTGTATTACTGTATGCAGGCGTTGCAAAACCCGCACA CTTTTGGACAGGGAACCAAACTGGAAATAAAGACCAGTAGTGGT	347	Light chain variable domain of anti-FLT3 antibody ml0006
CAGGTCCAACCTGAAACAAAGCGGTCCCGGTCTTGTCCAGCCCTCCCAATCT CTCAGTATTACTTGCAGTGTGTACAGTTTCAGCCTCACGAACTACGGTCTG CATTGGGTCCGCCAGTCTCCAGGAAAAGGCCTGGAGTGGCTCGGTGTTATC TGGAGTGGTGGAAGTACGGATTACAATGCTGCCTTTATCTCTCGGCTCAGT ATCTCCAAAGATAACTCTAAGTCCCAAGTCTTTTCAAATGAAGTCTTTG CAGGCAGATGATACGGCCATATACTATTGCGCACGCAAGGGTGGGATCTA CTATGCAAACTACTATTACGCGATGGACTACTGGGGCCAAGGCACGAGTG TTACCGTGTCAAGC	348	Heavy chain variable domain of anti-FLT3 antibody BV10
GACATAGTGATGACTCAGTCTCCGTCTCTCTTTCCGTGAGTGCGGGCGAA AAGGTTACCATGTCTCTGCAAAAGTTCACAGTCACTTCTCAATTCTGGCAAC CAAAAAAATTACATGGCATGGTATCAACAGAAACCAGGTACGCCGCAAAA GCTCCTCATATATGGTGCATCAACGCGAGAGTCAGGCGTACCTGACAGGTT TACCGGATCTGGCAGCGGTACAGACTTTACTCTTACCATATCAAGTGTGCA GGCAGAGGACTTGGCGGTATACTATTGTCAAAACGATCATAGTTACCCTCT TACATTTGGCGCGGGCACTAAACTGGAGCTGAAACGC	349	Light chain variable domain of anti-FLT3 antibody BV10
CAGGTTTCACTGGTTCACTGTGGCGCCGAAGTGAAGAAACCTGGCAGCAG CGTGAAGGTGTCTTGCAAGGCCAGCGGCTACACCTTTACCGACTACAACA TGCAGTGGGTCCGACAGGCCCTGGACAAGGACTTGAGTGGATCGGCTAC ATCTACCCCTACAATGGCGGCACCGGCTACAACCAGAAGTTCAAGAGCAA GGCCACCATCACCGCCGACGAGAGCACAAACACCGCCTACATGGAAGTGA GCAGCCTGAGAAAGCGAGGACACCGCCGTGTACTACTGTGCCAGAGGCAGA CCCCCATGGATTATTGGGGACAGGGCACCCCTGGTCAACCGTTTCTAGC	350	Heavy chain variable domain of anti-CD33 antibody lintuzumab
GATATCCAGATGACACAGAGCCCCAGCAGCCTGTCTGCCAGCGTGGGAGA TAGAGTGACCATCACCTGTAGAGCCAGCGAGAGCGTGGACAACCTACGGCA TCAGCTTCATGAACTGGTTCCAGCAGAAGCCCGCAAGGCCCTAAGCTG CTGATCTACGCCCGCAGCAATCAAGGCAGCGGAGTGCCTAGCAGATTTTC CGGCTCTGGCAGCGGCACCGATTTCACCTTGACAATCTCTAGCCTCCAGCC TGACGACTTCGCCACCTACTACTGCCAGCAGAGCAAAGAGGTGCCCTGGA CATTCGGCCAGGGCACAAAGGTGGAATCAAG	351	Light chain variable domain of anti-CD33 antibody lintuzumab
GAAGTGCAGCTGGTTTCACTGTGGCGCCGAAGTGAAGAAACCTGGCAGCAG CGTGAAGGTGTCTTGCAAGGCCAGCGGCTACACCATCACCGACAGCAACA TCCACTGGGTCCGACAGGCTCCAGGCCAGTCTCTTGAGTGGATCGGCTACA TCTACCCCTACAACGGCGGCACCGACTACAACCAGAAGTTCAAGAACCGG GCCACACTGACCGTGGACAACCCCTACCAATACCGCCTACATGGAACTGAG	352	Heavy chain variable domain of anti-CD33

Table B		
Nucleic Acid Sequence	SEQ ID NO:	Description
CAGCCTGCGGAGCGAGGACACCGCCTTTTACTACTGCGTGAACGGCAACC CCTGGCTGGCCTATTGGGGACAGGGAACACTGGTCACAGTGTCTAGC		antibody gemtuzumab
GATATTGAGCTGACACAGAGCCCCAGCACACTGTCTGCCTCTGTGGGCGAC AGAGTGACCATCACCTGTAGAGCCAGCGAGAGCCTGGACAACCTACGGCAT CAGATTTCTGACCTGGTTCCAGCAGAAGCCCGCAAGGCTCCTAAGCTGCT GATGTACGCCGCCAGCAATCAAGGCAGCGGAGTGCCTAGCAGATTTTCCG GCTCTGGCAGCGGCACAGAGTTCACCTGACAATCTTAGCCTCCAGCCTG ACGACTTCGCCACCTACTACTGCCAGCAGACCAAGAGAGGTGCCCTGGTCCT TTGGACAGGGCACCAAGGTGGAAGTGAAGCGG	353	Light chain variable domain of anti-CD33 antibody gemtuzumab

Table A2												
Ab	SEQ ID NO	CDR-H1	SEQ ID NO	CDR-H2	SEQ ID NO	CDR-H3	SEQ ID NO	CDR-L1	SEQ ID NO	CDR-L2	SEQ ID NO	CDR-L3
FLT3 D4-3	354	GYTF TSYY MH	355	IINPSGG STSYAQ KFQG	356	VVAA AVAD Y	357	RSSQS LLHSN GYN LD	358	LGSN RA	359	MQSL QTPF T
FLT3 NC7	360	GGTF SSYA IS	361	GIPIFGT ANYAQ KFQG	362	FALFG FREQA FDI	363	RASQS ISSYL N	364	AASS LQS	365	QGSY STPF T
FLT3 EB10	366	GYTF TSYY MH	367	IINPSGG STSYAQ KFQG	368	GVGA HDAF DI	369	RSSQS LLHSN GNNY LD	370	LGSN RA	371	MQG THPA IS
FLT3 4G8	372	SYW MH	373	EIDPSDS YKDYN QKFKD	374	AITTT PFDF	375	RASQS ISNNL H	376	YASQ SIS	377	QQSN TWPY T
FLT3 FL_39	378	NAR MGV S	379	HIFSNDE KSYSTS LKN	380	IVGYG SGWY GFFDY	381	RASQ GIRND LG	382	AAST LQS	383	LQHN SYPL T
FLT3 FL_16	384	NAR MAV S	385	HIFSNDE KSYSTS LKS	386	IVGYG SGWY GYFD Y	387	RASQ DIRYD LA	388	AASS LQS	389	LQHN FYPL T
FLT3 ml0006	390	GFTF SSYG	391	ISYDGS NK	392	ANLA PWAA YW	393	QSLH SNGY NY	394	LGS	395	MQA LQTP HT
FLT3 BV10	396	NYGL H	397	VIWGG STDYNA AFIS	398	KGGIY YANH YYAM DY	399	KSSQS LLNSG NQKN YMA	400	GAST RES	401	QND HSYP LT
CD33 antibody lintuzumab	402	DYN MH	403	YIYPYN GGTGYN QKFKSK A	404	GRPA MDYW GQ	405	RASES VDNY GISFM N	406	AASN QGS	407	QSK EVPW T

CD33 antibody gemtuzumab	408	GYTI TDSN	409	IYPYNG GT	410	VNGN PWLA Y	411	ESLDN YGIRF	412	AAS	413	QGTK EVPW S
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In some embodiments, an antigen-binding domain specific for CD33 includes a heavy chain variable (VH) region and a light chain variable (VL) region, wherein: the CD33-VH includes: a heavy chain complementarity determining region 1 (CDR-H1) having the amino acid sequence of DYNMH (SEQ ID NO: 402), a heavy chain complementarity determining region 2 (CDR-H2) having the amino acid sequence of YIYPYNGGTGYNQKFKSKA (SEQ ID NO: 403), and a heavy chain complementarity determining region 3 (CDR-H3) having the amino acid sequence of GRPAMDYWGQ (SEQ ID NO: 404), and the CD33-VL includes: a light chain complementarity determining region 1 (CDR-L1) having the amino acid sequence of RASESVDNYGISFMN (SEQ ID NO: 405), a light chain complementarity determining region 2 (CDR-L2) having the amino acid sequence of AASNQGS (SEQ ID NO: 406), and a light chain complementarity determining region 3 (CDR-L3) having the amino acid sequence of QQSKEVPWT (SEQ ID NO: 407), and wherein the amino acid sequences of the CDR-H1, the CDR-H2, the CDR-H3, the CDR-L1, the CDR-L2, and the CDR-L3 of the reference antibody are defined based on the Kabat numbering scheme.

In some embodiments, an antigen-binding domain specific for CD33 includes a CD33-VH having the amino acid sequence
 QVQLVQSGAEVKKPGSSVKVSCKASGYTFTDYNMHWVRQAPGGLEWIGYIYPYNGG
 TGYNQKFKSKATITADESTNTAYMELSSLRSEDVAVYYCARGRPAMDYWGQGTLVTV
 SS (SEQ ID NO: 329), and a CD33-VL having the amino acid sequence
 DIQMTQSPSSLSASVGDRVTITCRASESVDNYGISFMNWFQQKPGKAPKLLIYAASNQG
 SGVPSRFSGSGSGTDFLTISLQPDFFATYYCQQSKEVPWTFGQGTKVEIK (SEQ ID
 NO: 330).

In some embodiments, an antigen-binding domain specific for FLT3 includes a heavy chain variable (VH) region and a light chain variable (VL) region, wherein: the FLT3-VH includes a heavy chain complementarity determining region 1 (CDR-H1) having the amino acid sequence of GGTFSSY AIS (SEQ ID NO: 360), a heavy chain complementarity determining region 2 (CDR-H2) having the amino acid sequence of GIPIFGTANYAQKFQG (SEQ ID NO: 361), and a heavy chain complementarity determining region 3 (CDR-H3) having the amino acid sequence of FALFGFREQAFDI (SEQ ID NO: 362), and the FLT3-VL includes a light chain complementarity determining region 1 (CDR-L1) having the amino acid sequence of

RASQSISSYLN (SEQ ID NO: 363), a light chain complementarity determining region 2 (CDR-L2) having the amino acid sequence of AASSLQS (SEQ ID NO: 364), and a light chain complementarity determining region 3 (CDR-L3) having the amino acid sequence of QQSYSTPFT (SEQ ID NO: 365), and wherein the amino acid sequences of the CDR-H1, the CDR-H2, the CDR-H3, the CDR-L1, the CDR-L2, and the CDR-L3 of the reference antibody are defined based on the Kabat numbering scheme.

In some embodiments, an antigen-binding domain specific for FLT3 includes a FLT3-VH having the amino acid sequence
 EVQLVQSGAEVKKPGSSVKVSKASGGTFSSYAISWVRQAPGQGLEWMGGIPIFGTAN
 10 YAQKFQGRVTITADKSTSTAYMELSSLRSEDVAVYYCATFALFGFREQAFDIWGQGTTV
 TVSS (SEQ ID NO: 315), and a FLT3-VL having the amino acid sequence
 DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPS
 RFGSGSGTDFLTITSLQPEDLATYYCQQSYSTPFTFGPGTKVDIK (SEQ ID NO: 316).

In some embodiments, a bivalent-bispecific aCAR includes an antigen-binding domain specific for FLT3 and an antigen-binding domain specific for CD33.

In some embodiments, a bivalent-bispecific aCAR specific for FLT3 and CD33 includes the amino acid sequence
 MLLLVTSLLLCELPHPAFLLIPEVQLVQSGAEVKKPGSSVKVSKASGGTFSSYAISWVR
 QAPGQGLEWMGGIPIFGTANYAQKFQGRVTITADKSTSTAYMELSSLRSEDVAVYYCA
 20 TFALFGFREQAFDIWGQGTTVTVSSGGGGSQVQLVQSGAEVKKPGSSVKVSKASGYT
 FTDYNMHWVRQAPGQGLEWIGYIYPYNGGTGYNQKFKSKATITADESTNTAYMELSSL
 RSEDVAVYYCARGRPAMDYWGQGTTLTVSSGSTSGSGKPGSGEGSTKGDIQMTQSPSS
 LSASVGDRVTITCRASEVDNYGISFMNWFQQKPGKAPKLLIYAASNQSGVPSRFGSG
 GSGTDFLTITSLQPDDFATYYCQQSKEVPWTFGQGTKVEIKGGGGSQDIQMTQSPSSLSA
 25 SVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFGSGSGTDFLT
 TITSLQPEDLATYYCQQSYSTPFTFGPGTKVDIKALNSIMYFSHFVPVFLPAKPTTTPAP
 RPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITL
 YCNHRRSKRSRLHSDYMNMTPRRPGPTRKHYPYAPPRDFAAYRSRVKFSRSADAPA
 YKQGQNQLYNELNLGRREEYDVLDRRGRDPEMGGKPRRKNPQEGLYNELQKDKMA
 30 EAYSEIGMKGERRRGKGHDLGLYQGLSTATKDTYDALHMQALPPR (SEQ ID NO: 414).

In some embodiments, a bivalent-bispecific aCAR specific for FLT3 and CD33 includes the amino acid sequence
 MLLLVTSLLLCELPHPAFLLIPEVQLVQSGAEVKKPGSSVKVSKASGGTFSSYAISWVR
 QAPGQGLEWMGGIPIFGTANYAQKFQGRVTITADKSTSTAYMELSSLRSEDVAVYYCA
 35 TFALFGFREQAFDIWGQGTTVTVSSGGGGSQVQLVQSGAEVKKPGSSVKVSK

ASGYTFTDYNMHWVRQAPGQGLEWIGYIYPYNGGTGYNQKFKSKATITADESTNTAY
 MELSSLRSED TAVYYCARGRPAMDYWGQGLTVTVSSGGGGSGGGGSDIQMTQSPSSLS
 ASVGDRVTITCRASESDNYGISFMNWFQQKPGKAPKLLIYAASNQGSVPSRFSGSGS
 GTDFTLTISLQPD FATTYYCQQSKEVPWTFGQGTKVEIKGGGGSGGGGSDIQMTQSPS
 5 SLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGT
 DFTLTISLQPEDLATYYCQQSYSTPFTFGPGTKVDIKALSNSIMYFSHFVPVFLPAKPTT
 TPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLS
 LVITLYCNHRRSKRSRLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSA
 DAPAYKQGQNQLYNELNLGRREEYDVLDRRGRDPEMGGKPRRKNPQEGLYNELQK
 10 DKMAEAYSEIGMKGERRRGKGHDLGLYQGLSTATKDTYDALHMQALPPR (SEQ ID NO:
 415).

Engineered Nucleic Acids

Provided herein are multicistronic expression systems that include engineered nucleic acids encoding (i) a membrane-cleavable chimeric protein; (ii) a bivalent aCAR; and (iii) an iCAR.

In certain embodiments described herein, the multicistronic expression systems included engineered nucleic acids that encode an expression cassette containing a promoter and an exogenous polynucleotide sequence encoding each of (i) a membrane-cleavable chimeric protein; (ii) a bivalent aCAR; and (iii) an iCAR.

The promoter is operably linked to expression cassette of the multicistronic system such that the exogenous polynucleotide sequence encoding each of the membrane-cleavable chimeric protein, the bivalent aCAR, and the iCAR is configured to be expressed as a single polypeptide.

An “engineered nucleic acid” is a nucleic acid that does not occur in nature. It should be understood, however, that while an engineered nucleic acid as a whole is not naturally-
 25 occurring, it may include nucleotide sequences that occur in nature. In some embodiments, an engineered nucleic acid comprises nucleotide sequences from different organisms (*e.g.*, from different species). For example, in some embodiments, an engineered nucleic acid includes a murine nucleotide sequence, a bacterial nucleotide sequence, a human nucleotide sequence, and/or a viral nucleotide sequence. The term “engineered nucleic acids” includes recombinant
 30 nucleic acids and synthetic nucleic acids. A “recombinant nucleic acid” refers to a molecule that is constructed by joining nucleic acid molecules and, in some embodiments, can replicate in a live cell. A “synthetic nucleic acid” refers to a molecule that is amplified or chemically, or by other means, synthesized. Synthetic nucleic acids include those that are chemically modified, or otherwise modified, but can base pair with naturally- occurring nucleic acid molecules.

Modifications include, but are not limited to, one or more modified internucleotide linkages and non-natural nucleic acids. Modifications are described in further detail in U.S. Pat. No.

6,673,611 and U.S. Application Publication 2004/0019001 and, each of which is incorporated by reference in their entirety. Modified internucleotide linkages can be a phosphorodithioate or

5 phosphorothioate linkage. Non-natural nucleic acids can be a locked nucleic acid (LNA), a peptide nucleic acid (PNA), glycol nucleic acid (GNA), a phosphorodiamidate morpholino oligomer (PMO or “morpholino”), and threose nucleic acid (TNA). Non-natural nucleic acids are described in further detail in International Application WO 1998/039352, U.S. Application Pub. No. 2013/0156849, and U.S. Pat. Nos. 6,670,461; 5,539,082; 5,185,444, each herein
10 incorporated by reference in their entirety. Recombinant nucleic acids and synthetic nucleic acids also include those molecules that result from the replication of either of the foregoing. Engineered nucleic acid of the present disclosure may be encoded by a single molecule (*e.g.*, included in the same plasmid or other vector) or by multiple different molecules (*e.g.*, multiple different independently-replicating molecules). Engineered nucleic acids can be an isolated
15 nucleic acid. Isolated nucleic acids include, but are not limited to a cDNA polynucleotide, an RNA polynucleotide, an RNAi oligonucleotide (*e.g.*, siRNAs, miRNAs, antisense oligonucleotides, shRNAs, etc.), an mRNA polynucleotide, a circular plasmid, a linear DNA fragment, a vector, a minicircle, a ssDNA, a bacterial artificial chromosome (BAC), and yeast artificial chromosome (YAC), and an oligonucleotide.

20 Engineered nucleic acid of the present disclosure may be produced using standard molecular biology methods (*see, e.g.*, Green and Sambrook, Molecular Cloning, A Laboratory Manual, 2012, Cold Spring Harbor Press). In some embodiments, engineered nucleic acid constructs are produced using GIBSON ASSEMBLY® Cloning (*see, e.g.*, Gibson, D.G. *et al.* Nature Methods, 343-345, 2009; and Gibson, D.G. *et al.* Nature Methods, 901-903, 2010, each
25 of which is incorporated by reference herein). GIBSON ASSEMBLY® typically uses three enzymatic activities in a single-tube reaction: 5' exonuclease, the 3' extension activity of a DNA polymerase and DNA ligase activity. The 5' exonuclease activity chews back the 5' end sequences and exposes the complementary sequence for annealing. The polymerase activity then fills in the gaps on the annealed regions. A DNA ligase then seals the nick and covalently links
30 the DNA fragments together. The overlapping sequence of adjoining fragments is much longer than those used in Golden Gate Assembly, and therefore results in a higher percentage of correct assemblies. In some embodiments, engineered nucleic acid constructs are produced using IN-FUSION® cloning (Clontech).

Promoters

In general, in all embodiments described herein, the engineered nucleic acids encoding the membrane-cleavable chimeric protein, the bivalent aCAR, and the iCAR encode an expression cassette containing a promoter. In some embodiments, an engineered nucleic acid (e.g., an engineered nucleic acid comprising an expression cassette) comprises a promoter operably linked to a nucleotide sequence (e.g., an exogenous polynucleotide sequence) encoding at least 2 distinct proteins. For example, the engineered nucleic acid may comprise a promoter operably linked to a nucleotide sequence encoding at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 8, at least 9, or at least 10 distinct proteins. In some embodiments, an engineered nucleic acid comprises a promoter operably linked to a nucleotide sequence encoding 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more distinct proteins. In some embodiments, an engineered nucleic acid (e.g., an engineered nucleic acid comprising an expression cassette) comprises a promoter operably linked to a nucleotide sequence (e.g., an exogenous polynucleotide sequence) encoding at least 2 membrane-cleavable chimeric proteins. For example, the engineered nucleic acid may comprise a promoter operably linked to a nucleotide sequence encoding at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 8, at least 9, or at least 10 membrane-cleavable chimeric proteins. In some embodiments, an engineered nucleic acid comprises a promoter operably linked to a nucleotide sequence encoding 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more membrane-cleavable chimeric proteins.

A “promoter” refers to a control region of a nucleic acid sequence at which initiation and rate of transcription of the remainder of a nucleic acid sequence are controlled. A promoter may also contain sub-regions at which regulatory proteins and molecules may bind, such as RNA polymerase and other transcription factors. Promoters may be constitutive, inducible, repressible, tissue-specific or any combination thereof. A promoter drives expression or drives transcription of the nucleic acid sequence that it regulates. Herein, a promoter is considered to be “operably linked” when it is in a correct functional location and orientation in relation to a nucleic acid sequence it regulates to control (“drive”) transcriptional initiation and/or expression of that sequence.

A promoter may be one naturally associated with a gene or sequence, as may be obtained by isolating the 5' non-coding sequences located upstream of the coding segment of a given gene or sequence. Such a promoter can be referred to as “endogenous.” In some embodiments, a coding nucleic acid sequence may be positioned under the control of a recombinant or heterologous promoter, which refers to a promoter that is not normally associated with the encoded sequence in its natural environment. Such promoters may include promoters of other

genes; promoters isolated from any other cell; and synthetic promoters or enhancers that are not "naturally occurring" such as, for example, those that contain different elements of different transcriptional regulatory regions and/or mutations that alter expression through methods of genetic engineering that are known in the art. In addition to producing nucleic acid sequences of promoters and enhancers synthetically, sequences may be produced using recombinant cloning and/or nucleic acid amplification technology, including polymerase chain reaction (PCR) (see, *e.g.*, U.S. Pat. No. 4,683,202 and U.S. Pat. No. 5,928,906).

Promoters of an engineered nucleic acid may be "inducible promoters," which refer to promoters that are characterized by regulating (*e.g.*, initiating or activating) transcriptional activity when in the presence of, influenced by or contacted by a signal. The signal may be endogenous or a normally exogenous condition (*e.g.*, light), compound (*e.g.*, chemical or non-chemical compound) or protein (*e.g.*, cytokine) that contacts an inducible promoter in such a way as to be active in regulating transcriptional activity from the inducible promoter. Activation of transcription may involve directly acting on a promoter to drive transcription or indirectly acting on a promoter by inactivation a repressor that is preventing the promoter from driving transcription. Conversely, deactivation of transcription may involve directly acting on a promoter to prevent transcription or indirectly acting on a promoter by activating a repressor that then acts on the promoter.

A promoter is "responsive to" or "modulated by" a local tumor state (*e.g.*, inflammation or hypoxia) or signal if in the presence of that state or signal, transcription from the promoter is activated, deactivated, increased, or decreased. In some embodiments, the promoter comprises a response element. A "response element" is a short sequence of DNA within a promoter region that binds specific molecules (*e.g.*, transcription factors) that modulate (regulate) gene expression from the promoter. Response elements that may be used in accordance with the present disclosure include, without limitation, a phloretin-adjustable control element (PEACE), a zinc-finger DNA-binding domain (DBD), an interferon-gamma-activated sequence (GAS) (Decker, T. *et al. J Interferon Cytokine Res.* 1997 Mar;17(3):121-34, incorporated herein by reference), an interferon-stimulated response element (ISRE) (Han, K. J. *et al. J Biol Chem.* 2004 Apr 9;279(15):15652-61, incorporated herein by reference), a NF-kappaB response element (Wang, V. *et al. Cell Reports.* 2012; 2(4): 824-839, incorporated herein by reference), and a STAT3 response element (Zhang, D. *et al. J of Biol Chem.* 1996; 271: 9503-9509, incorporated herein by reference). Other response elements are encompassed herein. Response elements can also contain tandem repeats (*e.g.*, consecutive repeats of the same nucleotide sequence encoding the response element) to generally increase sensitivity of the response

element to its cognate binding molecule. Tandem repeats can be labeled 2X, 3X, 4X, 5X, *etc.* to denote the number of repeats present.

Non-limiting examples of responsive promoters (also referred to as “inducible promoters”) (*e.g.*, TGF-beta responsive promoters) are listed in **Table 12A**, which shows the design of the promoter and transcription factor, as well as the effect of the inducer molecule towards the transcription factor (TF) and transgene transcription (T) is shown (B, binding; D, dissociation; n.d., not determined) (A, activation; DA, deactivation; DR, derepression) (see Horner, M. & Weber, W. *FEBS Letters* 586 (2012) 20784-2096m, and references cited therein).

Non-limiting examples of components of inducible promoters include those presented in **Table 12B**.

Table 12A. Examples of Responsive Promoters

System	Promoter and operator	Transcription factor (TF)	Inducer molecule	Response to inducer	
				TF	T
Transcriptional activator-responsive promoters					
AIR	PAIR (OalcA-PhCMVmin)	AlcR	Acetaldehyde	n.d.	A
ART	PART (OARG-PhCMVmin)	ArgR-VP16	l-Arginine	B	A
BIT	PBIT3 (OBirA3-PhCMVmin)	BIT (BirA-VP16)	Biotin	B	A
Cumate – activator	PCR5 (OCuO6-PhCMVmin)	cTA (CymR-VP16)	Cumate	D	DA
Cumate – reverse activator	PCR5 (OCuO6-PhCMVmin)	rcTA (rCymR-VP16)	Cumate	B	A
E-OFF	PETR (OETR-PhCMVmin)	ET (E-VP16)	Erythromycin	D	DA
NICE-OFF	PNIC (ONIC-PhCMVmin)	NT (HdnoR-VP16)	6-Hydroxy-nicotine	D	DA
PEACE	PTtgR1 (OTtgR-PhCMVmin)	TtgA1 (TtgR-VP16)	Phloretin	D	DA
PIP-OFF	PPIR (OPIR-Phsp70min)	PIT (PIP-VP16)	Pristinamycin I	D	DA
QuoRex	PSCA (OscbR-PhCMVmin)PSPA (OpapRI-PhCMVmin)	SCA (ScbR-VP16)	SCB1	D	DA
Redox	PROP (OROP-PhCMVmin)	REDOX (REX-VP16)	NADH	D	DA

System	Promoter and operator	Transcription factor (TF)	Inducer molecule	Response to inducer	
TET-OFF	PhCMV*-1 (OtetO7-PhCMVmin)	tTA (TetR-VP16)	Tetracycline	D	DA
TET-ON	PhCMV*-1 (OtetO7-PhCMVmin)	rtTA (rtTetR-VP16)	Doxycycline	B	A
TIGR	PCTA (OrheO-PhCMVmin)	CTA (RheA-VP16)	Heat	D	DA
TraR	O7x(tra box)-PhCMVmin	p65-TraR	3-Oxo-C8-HSL	B	A
VAC-OFF	P1VanO2 (OVanO2-PhCMVmin)	VanA1 (VanR-VP16)	Vanillic acid	D	DA
Transcriptional repressor-responsive promoters					
Cumate - repressor	PCuO (PCMV5-OCuO)	CymR	Cumate	D	DR
E-ON	PETRON8 (PSV40-OETR8)	E-KRAB	Erythromycin	D	DR
NICE-ON	PNIC (PSV40-ONIC8)	NS (HdnoR-KRAB)	6-Hydroxy-nicotine	D	DR
PIP-ON	PPIRON (PSV40-OPIR3)	PIT3 (PIP-KRAB)	Pristinamycin I	D	DR
Q-ON	PSCAON8 (PSV40-OscbR8)	SCS (ScbR-KRAB)	SCB1	D	DR
TET-ON repressor-based	OtetO-PHPRT	tTS-H4 (TetR-HDAC4)	Doxycycline	D	DR
T-REX	PTetO (PhCMV-OtetO2)	TetR	Tetracycline	D	DR
UREX	PUREX8 (PSV40-OhucO8)	mUTS (KRAB-HucR)	Uric acid	D	DR
VAC-ON	PVanON8 (PhCMV-OVanO8)	VanA4 (VanR-KRAB)	Vanillic acid	D	DR
Hybrid promoters					
QuoRexPIP-ON(NOT IF gate)	OscbR8-OPIR3-PhCMVmin	SCAPIT3	SCB1Pristinamycin I	DD	DADR
QuoRexE-ON(NOT IF gate)	OscbR-OETR8-PhCMVmin	SCAE-KRAB	SCB1Erythromycin	DD	DADR
TET-OFFE-ON(NOT IF gate)	OtetO7-OETR8-PhCMVmin	tTAE-KRAB	TetracyclineErythromycin	DD	DADR
TET-OFFPIP-ONE-ON	OtetO7-OPIR3-OETR8-PhCMVmin	tTAPIT3E-KRAB	TetracyclinePristinamycin IErythromycin	DDD	DADRDR

Table 12B. Exemplary Components of Inducible Promoters

Name	DNA SEQUENCE
minimal promoter; minP	AGAGGGTATATAATGGAAGCTCGACTTCCAG (SEQ ID NO: 1)
NFkB response element protein promoter; 5x NFkB- RE	GGGAATTTCGGGGGACTTTCCGGGAATTTCCGGGGACTTTCCGGGAATT TCC (SEQ ID NO: 2)
CREB response element protein promoter; 4x CRE	CACCAGACAGTGACGTCAGCTGCCAGATCCCATGGCCGTCATACTGTG ACGTCTTTCAGACACCCCATTTGACGTCAATGGGAGAA (SEQ ID NO: 3)
NFAT response element protein promoter; 3x NFAT binding sites	GGAGGAAAACTGTTTCATACAGAAGGCGTGGAGGAAAACTGTTTCA TACAGAAGGCGTGGAGGAAAACTGTTTCATACAGAAGGCGT (SEQ ID NO: 4)
SRF response element protein promoter; 5x SRE	AGGATGTCCATATTAGGACATCTAGGATGTCCATATTAGGACATCTAGG ATGTCCATATTAGGACATCTAGGATGTCCATATTAGGACATCTAGGATG TCCATATTAGGACATCT (SEQ ID NO: 5)
SRF response element protein promoter 2; 5x SRF- RE	AGTATGTCCATATTAGGACATCTACCATGTCCATATTAGGACATCTACT ATGTCCATATTAGGACATCTTGTATGTCCATATTAGGACATCTAAATG TCCATATTAGGACATCT (SEQ ID NO: 6)
AP1 response element protein promoter; 6x AP1- RE	TGAGTCAGTGACTCAGTGAGTCAGTGACTCAGTGAGTCAGTGACTCAG (SEQ ID NO: 7)
TCF-LEF response element promoter; 8x TCF-LEF-RE	AGATCAAAGGGTTTAAGATCAAAGGGCTTAAGATCAAAGGGTATAAGA TCAAAGGGCCTAAGATCAAAGGGACTAAGATCAAAGGGTTTAAGATCA AAGGGCTTAAGATCAAAGGGCCTA (SEQ ID NO: 8)
SBEx4	GTCTAGACGTCTAGACGTCTAGACGTCTAGAC (SEQ ID NO: 9)
SMAD2/3 - CAGACA x4	CAGACACAGACACAGACACAGACA (SEQ ID NO: 10)
STAT3 binding site	Ggatccggtactcgagatctgcgatctaagtaagcttgccattccggtactgttgtaaagccac (SEQ ID NO: 11)
minCMV	taggcgtgtacggtgggagccatataagcagagctcgttagtaaccgtcagatgcctgga (SEQ ID NO: 170)
YB_TATA	TCTAGAGGGTATATAATGGGGGCCA (SEQ ID NO: 171)
minTK	Ttcgcattaaggtgacgcgtgtggcctcgaacaccgagcgaccctgcagcgaccgcctaa (SEQ ID NO: 172)

Non-limiting examples of promoters include the cytomegalovirus (CMV) promoter, the elongation factor 1-alpha (EF1a) promoter, the elongation factor (EFS) promoter, the MND
5 promoter (a synthetic promoter that contains the U3 region of a modified MoMuLV LTR with myeloproliferative sarcoma virus enhancer), the phosphoglycerate kinase (PGK) promoter, the spleen focus-forming virus (SFFV) promoter, the simian virus 40 (SV40) promoter, and the ubiquitin C (UbC) promoter (see **Table 12C**).

Table 12C. Exemplary Constitutive Promoters

Name	DNA SEQUENCE
CMV	GTTGACATTGATTATTGACTAGTTATTAATAGTAATCAATTACGGGGTTCATTAGTTCATA GCCCATATATGGAGTTCCGCGTTACATAACTTACGGTAAATGGCCCGCTGGCTGACCG CCCAACGACCCCGCCCATTTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAAT AGGGACTTTCCATTGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCCACTTGGCAG TACATCAAGTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGG CCGCCTGGCATTATGCCCAGTACATGACCTTATGGGACTTTCCTACTTGGCAGTACATC TACGTATTAGTCATCGCTATTACCATGGTGATGCGGTTTTGGCAGTACATCAATGGGCGT GGATAGCGGTTTGACTCACGGGGATTTCGAAGTCTCCACCCCATTTGACGTCAATGGGAG TTTGTTTTGGCACCAAAATCAACGGGACTTTCCAAAATGTCGTAACCACTCCGCCCATTT GACGCAAATGGGCGGTAGGCGTGACGGTGGGAGGTCTATATAAGCAGAGCTC (SEQ ID NO: 12)
EF1a	GGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACATCGCCACAGTCCCCGAGAAAGTTGGG GGGAGGGGTTCGGCAATTGAACCGGTGCCTAGAGAAGGTGGCGCGGGGTAAACTGGGAA AGTGATGCCGTGTACTGGCTCCGCCTTTTTCCCGAGGGTGGGGGAGAACCGTATATAAG TGCAGTAGTCGCCGTGAACGTTCTTTTCGCAACGGGTTTGCCGCCAGAACACAGGTAA GTGCGGTGTGTGGTTCCCGCGGGCCTGGCCTCTTTACGGGTTATGGCCCTTGGCGTCTT GAATTACTTCCACCTGGCTGCAGTACGTGATTCTTGATCCCGAGCTTCGGGTTGGAAGTG GGTGGGAGAGTTTCGAGGCCTTGCGCTTAAGGAGCCCCCTTCGCTCGTGCTTGAGTTGAG GCCTGGCCTGGGCGCTGGGGCCGCCGCTGCGAATCTGGTGGCACCTTCGCGCCTGTCT CGCTGCTTTTCGATAAGTCTCTAGCCATTTAAAAATTTTTGATGACCTGCTGCGACGCTTTTT TTCTGGCAAGATAGTCTTGTAATGCGGGCCAAGATCTGCACACTGGTATTTTCGGTTTTT TGGGCCCGCGGGCGGCGACGGGGCCCGTGCCTCCAGCGCACATGTTTCGGCGAGGCGGG GCCTGCGAGCGCGACCAACGAGAATCGGACGGGGGTAGTCTCAAGCTGGCCGGCCTGC TCTGGTGCCTGTCTCGCGCCGCCGTGTATCGCCCCGCCCCGGGCGGCAAGGCTGGCCC GGTCGGCACCAGTTGCGTGAGCGGAAGATGGCCGCTTCCCGGTCTGCTGCAGGGAGC TCAAAATGGAGGACGCGGCGCTCGGGAGAGCGGGCGGGTGAGTCACCCACACAAAGGA AAAGGGCCTTTCCGTCTCAGCCGTGCTTCATGTGACTCCACGGAGTACCGGGCGCCG TCCAGGCACCTCGATTAGTTCTCGAGCTTTTGAGTACGTGCTCTTTAGGTTGGGGGAG GGGTTTTATGCGATGGAGTTTCCCCACACTGAGTGGGTGGAGACTGAAGTTAGGCCAGC TTGGCACTTGATGTAATTCTCCTTGGAATTTGCCCTTTTTGAGTTTGGATCTTGTTTATT CTCAAGCCTCAGACAGTGGTTCAAAGTTTTTTCTTCCATTTTCAGGTGTCGTGA (SEQ ID NO: 13)
EFS	GGATCTGCGATCGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACATCGCCACAGTCCCC GAGAAGTTGGGGGGAGGGGTTCGGCAATTGAACCGGTGCCTAGAGAAGGTGGCGCGGGG TAAACTGGGAAAGTGATGTCGTGTACTGGCTCCGCCTTTTTCCCGAGGGTGGGGGAGAA CCGTATATAAGTGCAGTAGTCGCCGTGAACGTTCTTTTCGCAACGGGTTTGCCGCCAGA ACACAGCTGAAGCTTCGAGGGGCTCGCATCTCTCTTCACGCGCCCGCCGCCCTACCTG AGGCCCTTCCACGCGGTTGAGTCGCTTCTGCGCCCTCCCGCCTGCGCTGCTGCTGCTG AACTGCGTCCGCCGTCTAGGTAAAGTTTAAAGCTCAGGTGAGACCGGGCCTTTGTCCGG CGCTCCCTTGGAGCCTACCTAGACTCAGCCGGCTCTCCACGCTTTGCTGACCTGCTTG CTCAACTCTACGTCTTTGTTTCTGTTTTCTGTTCTGCGCCGTTACAGATCCAAGCTGTGACC GCGCCTAC (SEQ ID NO: 14)
MND	TTTATTTAGTCTCCAGAAAAAGGGGGGAATGAAAGACCCACCTGTAGGTTTGGCAAGC TAGGATCAAGGTTAGGAACAGAGAGACAGCAGAATATGGGCCAAACAGGATATCTGTG GTAAGCAGTTCTTGCCCGGCTCAGGGCCAAGAACAGTTGGAACAGCAGAATATGGGC CAAACAGGATATCTGTGGTAAGCAGTTCTTGCCCGGCTCAGGGCCAAGAACAGATGGT CCCCAGATGCGGTCCCGCCCTCAGCAGTTTCTAGAGAACCATCAGATGTTTCCAGGGTG CCCCAGGACCTGAAATGACCCTGTGCTTATTTGAACTAACCAATCAGTTGCTTCTCG CTCTGTTTCGCGCGCTTCTGCTCCCGAGCTCAATAAAAGAGCCCA (SEQ ID NO: 15)
PGK	GGGGTTGGGGTTGCGCCTTTTCCAAGGCAGCCCTGGGTTTTCGCGAGGGACGCGGCTGCT CTGGGCGTGGTTCCGGGAAACGCAGCGGCGCGGACCTGGGTCTCGCACATTCTTCACG TCCGTTTCGAGCGTCACCCGGATCTTCGCCGCTACCCCTTGTGGGCCCCCGGCGACGCTT CCTGCTCCGCCCTAAGTCGGGAAGGTTTCTTGCAGTTTCGCGGCGTTCGCGGACGTGACA AACGGAAGCCGCACGTCTACTAGTACCCTCGCAGACGGACAGCGCCAGGGAGCAATG GCAGCGCGCCGACCGCATGGGCTGTGGCCAATAGCGGCTGCTCAGCGGGGCGCGCCG

Name	DNA SEQUENCE
	AGAGCAGCGGCCGGGAAGGGGCGGTGCGGGAGGCGGGGTGTGGGGCGGTAGTGTGGG CCCTGTTCCTGCCCCGCGCGGTGTTCCGCATTCTGCAAGCCTCCGGAGCGCACGTCCGGCA GTCGGCTCCCTCGTTGACCGAATCACCGACCTCTCTCCCCAG (SEQ ID NO: 16)
SFFV	GTAACGCCATTTTGCAAGGCATGGAAAAATACCAAACCAAGAATAGAGAAGTTCAGAT CAAGGGCGGGTACATGAAAAATAGCTAACGTTGGGCCAAACAGGATATCTGCGGTGAGC AGTTTCGGCCCCGGCCCCGGGGCCAAGAACAGATGGTTCACCGCAGTTTCGGCCCCGGCCC GAGGCCAAGAACAGATGGTCCCCAGATATGGCCCAACCCCTCAGCAGTTTCTTAAGACCC ATCAGATGTTTCCAGGCTCCCCCAAGGACCTGAAATGACCCTGCGCCTTATTTGAATTAA CCAATCAGCCTGCTTCTCGCTTCTGTTTCGCGCGCTTCTGCTTCCCGAGCTCTATAAAAGA GCTCACAACCCCTCACTCGGCGCGCCAGTCTCCGACAGACTGAGTCGCCCCGG (SEQ ID NO: 17)
SV40	CTGTGGAATGTGTGTCAGTTAGGGTGTGGAAAGTCCCCAGGCTCCCCAGCAGGCAGAAG TATGCAAAGCATGCATCTCAATTAGTCAGCAACCAGGTGTGGAAAGTCCCCAGGCTCCC CAGCAGGCAGAAGTATGCAAAGCATGCATCTCAATTAGTCAGCAACCATAGTCCCCGCC CTAACTCCGCCCCATCCCGCCCCCTAACTCCGCCCCAGTTCCGCCCCATTCTCCGCCCCATGGC TGACTAATTTTTTTTATTTATGACAGAGGCCGAGGCCCGCTTGCCTCTGAGCTATTCCAG AAGTAGTGAGGAGGCTTTTTTGGAGGCCTAGGCTTTTGCAAAAAGCT (SEQ ID NO: 18)
UbC	GCGCCGGGTTTTGGCGCCTCCCGCGGGCGCCCCCTCCTCACGGCGAGCGCTGCCACGT CAGACGAAGGGCGCAGGAGCGTTCTGTATCCTTCCGCCCCGACGCTCAGGACAGCGGC CCGCTGCTCATAAGACTCGGCCCTAGAACCCAGTATCAGCAGAAGGACATTTTAGGAC GGGACTTGGGTGACTCTAGGGCACTGGTTTTCTTTCCAGAGAGCGGAACAGGCGAGGAA AAGTAGTCCCCTCTCGGCGATTCTGCGGAGGGATCTCCGTGGGGCGGTGAACGCCGATG ATTATATAAGGACGCGCCGGGTGTGGCACAGCTAGTTCCGTGCGAGCCGGGATTTGGGT CGCGGTTCTGTGTTGTGGATCGCTGTGATCGTCACTTGGTGAGTTGCGGGCTGCTGGGCT GGCCGGGGCTTTTCGTGGCCGCCGGGCCGCTCGGTGGGACGGAAGCGTGTGGAGAGACC GCCAAGGGCTGTAGTCTGGGTCCGCGAGCAAGGTTGCCCTGAACTGGGGGTGGGGGG AGCGCACAAAATGGCGGCTGTTCCCGAGTCTTGAATGGAAGACGCTTGTAAAGCGGGCT GTGAGGTCTGTGAAACAAGGTGGGGGGCATGGTGGGCGGCAAGAACCCAAAGGTCTTGA GGCCTTCGCTAATGCGGGAAAGCTCTTATTCGGGTGAGATGGGCTGGGGCACCATCTGG GGACCCCTGACGTGAAGTTTGTCACTGACTGGAGAAGCTCGGGTTGTGCTCTGGTTGCGG GGGCGGCAGTTATGCGGTGCCGTTGGGCAGTGCACCCGTACCTTTGGGAGCGCGCGCCT CGTCGTGTCGTGACGTCACCCGTTCTGTGGCTTATAATGCAGGGTGGGGCCACCTGCCG GTAGGTGTGCGGTAGGCTTTTCTCCGTGCGAGGACGCAGGGTTCCGGGCTAGGGTAGGC TCTCCTGAATCGACAGGCGCCGGACCTCTGGTGAGGGGAGGGATAAGTGAGGCGTCAG TTTCTTTGGTCGGTTTTATGTACCTATCTTCTTAAGTAGCTGAAGCTCCGTTTTTGAACCTA TGCGCTCGGGGTGGCGAGTGTGTTTTGTGAAGTTTTTTAGGCACCTTTTGAAATGTAAT CATTTGGGTCAATATGTAATTTTCAGTGTTAGACTAGTAAAGCTTCTGCAGGTCGACTCT AGAAAATTGTCCGCTAAATCTTGCCGTTTTTGGCTTTTTTGTAGAC (SEQ ID NO: 19)
hEF1aV1	GGCTCCGTTGCCCGTCAGTGGGCAGAGCGCACATCGCCACAGTCCCCGAGAAGTTGGG GGGAGGGGTGCGCAATTGAACCGGTGCCTAGAGAAGGTGGCGCGGGGTAAACTGGGAA AGTGATGTCTGTACTGGCTCCGCCTTTTCCCGAGGGTGGGGGAGAACCGTATATAAG TGCAGTAGTCGCCGTGAACGTTCTTTTCGCAACGGGTTGCCGCCAGAACACAGGTAA GTGCCGTGTGTGGTTCCCGCGGGCCTGGCCTCTTACGGGTTATGGCCCTTGCCTGCCCTT GAATTACTTCCACCTGGCTGCAGTACGTGATTCTTGATCCCGAGCTTCGGGTTGGAAGTG GGTGGGAGAGTTTCGAGGCCTTGCCTTAAGGAGCCCCCTTCGCCTCGTGCTTGAGTTGAG GCCTGGCCTGGGCGCTGGGGCCCGCGCTGCGAATCTGGTGGCACCTTCGCGCCTGTCT CGCTGCTTTCGATAAGTCTCTAGCCATTTAAAAATTTTGATGACCTGCTGCGACGCTTTTT TTCTGGCAAGATAGTCTTGTAATGCGGGCCAAGATCTGCACACTGGTATTTTCGGTTTTT GGGGCCGCGGGCGGCGACGGGGCCCGTGCGTCCAGCGCACATGTTCCGGCAGGCGGG GCCTGCGAGCGCGGCCACCGAGAATCGGACGGGGGTAGTCTCAAGCTGGCCGGCCTGC TCTGGTGCCTGGTCTCGCGCCGCCGTGTATCGCCCCGCCCTGGGCGGCAAGGCTGGCCC GGTCGGCACCAGTTGCGTGAGCGGAAAGATGGCCGCTTCCCGGCCCTGCTGCAGGGAGC TCAAAATGGAGGACGCGGCGCTCGGGAGAGCGGGCGGGTGAGTCACCCACACAAAGGA AAAGGGCCTTTCCGTCTCAGCCGTGCTTTCATGTGACTCCACGGAGTACCGGGCGCCG TCCAGGCACCTCGATTAGTTCTCGAGCTTTTGGAGTACGTGCTTTTAGGTTGGGGGAG GGGTTTTATGCGATGGAGTTTCCCCACACTGAGTGGGTGGAGACTGAAGTTAGGCCAGC TTGGCACTTGATGTAATCTCCTTGGAATTTGCCCTTTTTGAGTTTGGATCTTGGTTCATT

Name	DNA SEQUENCE
	CTCAAGCCTCAGACAGTGGTTCAAAGTTTTTTTCTTCCATTTTCAGGTGTCGTGA (SEQ ID NO: 20)
hCAGG	ACTAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCATAGCCCATATATGGAGTTC CGCGTTACATAACTTACGGTAAATGGCCCCGCTGGCTGACCGCCCAACGACCCCCGCCC ATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCCATTGAC GTCAATGGGTGGAGTATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTATCAT ATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCCGCTGGCATTATGC CCAGTACATGACCTTATGGGACTTTCTACTTGGCAGTACATCTACGTATTAGTCATCGC TATTACCATGGTTCGAGGTGAGCCCCACGTTCTGCTTCACTCTCCCCATCTCCCCCCCCCTC CCCACCCCCAATTTTGTATTTATTTATTTTFAATTATTTTGTGTCAGCGATGGGGGCGGG GGGGGGGGGGGGGGCGCGCGCCAGGCGGGGCGGGGCGGGGCGAGGGGCGGGGCGGGGCG GAGGCGGAGAGGTGCGGCGGCAGCCAATCAGAGCGGCGCGCTCCGAGAAAGTTTCTTTT ATGGCGAGGCGGCGGCGCGGCGGCCCTATAAAAAGCGAAGCGCGCGGCGGGCGGGG AGTCGCTGCGACGCTGCCCTTCGCCCCGTGCCCGCTCCGCCGCCGCTCGCGCCGCCCG CCCCGGCTCTGACTGACCGCTTACTCCACAGGTGAGCGGGCGGGACGGCCCTTCTCC TCCGGGCTGTAAATTAGCGCTTGGTTAATGACGGCTTGTTCCTTTCTGTGGCTGCGTGA AAGCCTTGAGGGGCTCCGGGAGGGCCCTTTGTGCGGGGGGAGCGGCTCGGGGGGTGCG TGCGTGTGTGTGTGCGTGGGGAGCGCGCGTGCCTCCGCGTCCCGCGTCCCGAGTGTGCGC GCGTGTGCGGGCGCGGCGGGGCTTTGTGCGCTCCGCAAGTGTGCGCAGGGGAGCGCG GCCGGGGGCGGTGCCCGCGGTGCGGGGGGGGCTGCGAGGGGAACAAAGGCTGCGTGC GGGGTGTGTGCGTGGGGGGGTGAGCAGGGGGTGTGGGCGCGTCCGTCGGGCTGCAACC CCCCTGACCCCCCTCCCCGAGTTGCTGAGCACGGCCCGGCTTCGGGTGCGGGGCTCC GTACGGGGCGTGGCGCGGGGCTCGCCGTGCCGGGCGGGGGGTGGCGGCAGGTGGGGGT GCCGGGCGGGGCGGGGCCCTCGGGCCGGGAGGGCTCGGGGAGGGGCGCGCGG CCCCGGAGCGCCGCGGCTGTCTGAGGCGCGGCGAGCCGACGCCATTGCCTTTATGGT AATCGTGCAGAGGGGCGCAGGGACTTCCTTTGTCCCAATCTGTGCGGAGCCGAAATCT GGGAGGCGCGCGCACCCCCCTTAGCGGGCGCGGGGCGAAGCGGTGCGGCGCCGGCA GGAAGGAAATGGGCGGGGAGGGCCCTTCGTGCGTGCCTCGCGCGCCGCTCCCTTCTCCCT CTCCAGCCTCGGGGCTGTCCGCGGGGGGACGGCTGCCTTCGGGGGGGACGGGGCAGGG CGGGGTTCGGCTTCTGGCGTGTGACCGGCGGCTCTAGAGCCTCTGCTAACCATGTTTCATG CCTTCTTCTTTTCTACAGCTCCTGGGCAACGTGCTGGTTATTGTGCTGTCTCATCATTT TGGCAAAGAATTC (SEQ ID NO: 21)
hEF1aV2	Gggcagagcgcacatgccccacagtcctcccgagaaagttggggggaggggtggcaattgaaccggtgcctagagaaggtggcgggggttaa actgggaaagtgatgtctgtactgctcgccttttcccgagggtgggggagaaccgtatataagtgcagtgtgcggtgaacgttttttcgc aacgggtttgcgcgacagacag (SEQ ID NO: 22)
hACTb	CCACTAGTTCATGTCTTATATGGACTCATCTTTGCCTATTGCGACACACACTCAATGA ACACCTACTACGCGCTGCAAAGAGCCCCGAGGCTGAGGTGCCCCACCTCACCCTC TTCCTATTTTGTGTAAAAATCCAGCTTCTTGTACCACCTCCAAGGAGGGGGAGGAGG AGGAAGGCAGGTTCTCTAGGCTGAGCCGAATGCCCTCTGTGGTCCCACGCCACTGAT CGCTGCATGCCCCACCCTGGGTACACACAGTCTGTGATTCCCGGAGCAGAACGGACCC TGCCCCACCCGCTTGTGTGCTACTCAGTGGACAGACCAAGGCAAGAAAGGGTGACAA GGACAGGGTCTTCCCAGGCTGGCTTTGAGTTCCTAGCACCGCCCCGCCCAATCCTCTG TGGCACATGGAGTCTTGGTCCCCAGAGTCCCCAGCGGCTCCAGATGGTCTGGGAGGG CAGTTCAGCTGTGGCTGCGCATAGCAGACATAACAACGGACGGTGGGCCCAGACCCAGG CTGTGTAGACCCAGCCCCCGCCCCGAGTGCCTAGGTACCCACTAACGCCCCAGGC CTGGTCTTGGCTGGGCGTGAAGTACCCTCAAAAGCAGGCAGCTCCAGGGTAAAAGGT GCCCTGCCCTGTAGAGCCCACCTTCTTCCCAGGGCTGCGGCTGGGTAGGTTTGTAGCCT TCATCACGGGCCACCTCCAGCCACTGGACCGCTGGCCCTGCCCTGTCTGGGGAGTGT GGTCTTGGGACTTCTAAGTGGCCGCAAGCCACCTGACTCCCCAACACCACACTCTACC TCTCAAGCCCAGGTCTCTCCCTAGTGACCCACCCAGCACATTTAGCTAGCTGAGCCCCAC AGCCAGAGGTCCTCAGGCCCTGCTTTCAGGGCAGTTGCTCTGAAGTCGGCAAGGGGGAG TGACTGCCTGGCCACTCCATGCCCTCCAAGAGCTCCTTCTGCAGGAGCGTACAGAACCC AGGGCCCTGGCACCCGTCAGACCCCTGGCCACCCACCTGGGCGCTCAGTGCCCAAGA GATGTCCACACCTAGGATGTCCGCGGTGGGTGGGGGGCCCCGAGAGACGGGCAGGCCG GGGGCAGGCCTGGCCATGCGGGGGCCGAACCGGGCACTGCCAGCGTGGGGCGCGGGGG CCACGGCGCGCGCCCCCAGCCCCGGGGCCAGCACCCCAAGGCGGCCAACGCCAAAAC TCTCCCTCCTCCTCTTCTCAATCTCGCTCTCGCTCTTTTTTTTTTCGCAAAAGGAGGGG AGAGGGGGTAAAAAATGCTGCACTGTGCGGCGAAGCCGGTGAGTGAGCGGCGCGGGG

Name	DNA SEQUENCE
	CCAATCAGCGTGCGCCGTTCCGAAAGTTGCGCTTTTATGGCTCGAGCGGCCGCGGCGGCG CCCTATAAAACCCAGCGGCGGACGCGCCACCACCGCGAGACCGCTCCGCCCGCG AGCACAGAGCCTCGCCTTTGCCGATCCGCCGCCCGTCCACACCCGCCGCCAGGtaagcccg cagccgacggggcagcgccgtcacggccggccgcagcgcccgccgccccttcgccgtgcagagccgcgtctgggcccagcggg gggcgcatgggggggaaccggaccgcccgtggggggcgccgggagaagcccctgggctccggagatgggggacacccacgcccagttc ggagggcgagggccgctcgggagggcgctcgggggtgcccgtctcggggcggggcaaccggcggggtctttgtctgagccgggct cttgccaatggggatcgaggggtggcgcgccgggagcccccgcaggcccggtggggctggggcgccattgcgctgcgctgtgctctt tgggcgctaactgcgtgcgctgggaattggcgctaattgcgctgcgctgggactcaaggcgctaactgcgctgcgttctggggccc gggtgccgcggcctgggctggggcgaaaggcgggctcgccggaaagggtgggggtccgcggtcccgggcgcttgcgcgcacttctgc ccgagccgctggccgcccagggtgtgcccgtgcgtgcgcgcgcccgaaccggcgctgttgaaacggcgaggcggggctggcgcc cgggttggagggggtggggcctggttctgcgcgcgcggggacgcccaccagtggttgcctttatgtaataacggcgccggcc cggtctctttgtcccaatctggcgcgccggcgccccctggcgccctaaggactcgcgcgccggaagtggccaggcgggggcgac ctcgctcacagcgcccgctat (SEQ ID NO: 23)
heIF4A1	GTTGATTTCTTCATCCCTGGCACACGTCCAGGCAGTGTGCAATCCATCTCTGTACAGG GGAAAACAAATAACATTTGAGTCCAGTGGAGACCGGGAGCAGAAGTAAAGGGAAAGTGA TAACCCCCAGAGCCCGGAAGCCTCTGGAGGCTGAGACCTCGCCCCCTTGCCTGTATAGG GCCTACGGAGCCACATGACCAAGGCACTGTGCCTCCGCACGTGTGAGAGTGCAGGGCC CCAAGATGGCTGCCAGGCCTCAGAGCCTGACTCTTCTATGTCACTTCCGTACCGGCGAG AAAGGCGGGCCCTCCAGCCAATGAGGCTGCGGGGCGGGCCTTACACCTTGATAGGCACTC GAGTTATCCAATGGTGCCTGCGGGCCGAGCGACTAGGAACTAACGTCATGCCGAGTTG CTGAGCGCCGGCAGGCGGGGCCGGGGCGGCCAAACCAATGCGATGGCCGGGGCGGAGT CGGGCGCTCTATAAGTTGTGATAGGCGGGCACTCCGCCCTAGTTTCTAAGGACCATG (SEQ ID NO: 24)
hGAPDH	AGTTCCCAACTTTCCCGCTCTCAGCCTTTGAAAGAAAGAAAGGGGAGGGGGCAGGCC GCGTGCAGTCGCGAGCGGTGCTGGGCTCCGGCTCCAATTCCCCTCTCAGTCGCTCCCA AAGTCCCTCTGTTCATCCAAGCGTGTAAGGGTCCCGTCCCTTGACTCCCTAGTGTCTG CTGCCACAGTCCAGTCTGGGAACAGCACCGATCACCTCCCATCGGGCCAATCTCAG TCCCTTCCCCCTACGTCGGGGCCACACGCTCGGTGCGTGCCAGTTGAACAGGCGG CTGCGGAAAAAAGCGGGGAGAAAGTAGGGCCCGGCTACTAGCGGTTTACGGGC GCACGTAGCTCAGGCCTCAAGACCTTGGGCTGGGACTGGCTGAGCCTGGCGGGAGGCG GGGTCCGAGTCACCGCCTGCCGCCGCGCCCCGGTTTCTATAAATTGAGCCCGCAGCCT CCCGCTTCGCTCTCTGCTCCTGTTTCGACAGTCAGCCGCATCTTCTTTTGCCTGCCAG gtgaagacggggcagagaaaacccgggaggtaggacggcctgaagcggcagggggcgccgagccggatgtgtcgcgcgctgc ggggtggggccggcgccctccgattgcaggggcgggcgaggacgtgatcgcgcgggctgggcatgaggcgctgtgtggggagg ggaggggaggcgtgggtgtcgccggggccactaggcgctcactgttctcctccgcgcagCCGAGCCACATCGCTGAG ACAC (SEQ ID NO: 25)
hGRP78	AGTGCGGTTACCAGCGGAAATGCCTCGGGGTCAGAAAGTCGAGGAGAGATAGACAGCT GCTGAACCAATGGGACCAGCGGATGGGGCGGATGTTATCTACCATTTGGTGAACGTTAGA AACGGAATAGCAGCCAATGAATCAGCTGGGGGGGCGGAGCAGTGACGTTTATTGCGGAG GGGGCCGCTTCGAATCGGCGGCGGCCAGCTTGGTGGCCTGGGCCAATGAACGGCCTCCA ACGAGCAGGGCCTTACCAATCGGCGGCCTCCACGACGGGGCTGGGGGAGGGTATATA AGCCGAGTAGGCGACGGTGAGGTGACGCCGCGCAAGACAGCACAGACAGATTGACCT ATTGGGGTGTTCGCGAGTGTGAGAGGGAAGCGCCGCGGCTGTATTTCTAGACCTGCC CTTCGCTTGGTTCGTGGCGCCTTGTGACCCCGGGCCCCCTGCCGCTGCAAGTCGGAAATT GCGCTGTGCTCCTGTGCTACGGCCTGTGGCTGGACTGCCTGTGCTGCCCAACTGGCTGG CAC (SEQ ID NO: 26)
hGRP94	TAGTTTCATCACCAACCGCCACCCCCCGCCCCCGCCATCTGAAAGGGTTCTAGGGGA TTTGCAACCTCTCTCGTGTGTTTCTTCTTTCCGAGAAGCGCCGCCACACGAGAAAGCTGG CCGCGAAAGTCGTGCTGGAATCACTTCCAACGAAACCCAGGCATAGATGGGAAAGGG TGAAGAACACGTTGCCATGGCTACCGTTTCCCCGGTCACGGAATAAACGCTCTCTAGGA TCCGGAAGTAGTTCGCGCGACCTCTCTAAAAGGATGGATGTGTTCTCTGCTTACATTC ATTGGACGTTTCCCTTAGAGGCCAAGGCCGCCAGGCAAAGGGGGCGTCCACACGCGTG AGGGGCCCCGCGAGCCATTTGATTGGAGAAAAGCTGCAAACCTGACCAATCGGAAGG AGCCACGCTTCGGGCATCGGTCACCGCACCTGGACAGCTCCGATTGGGTGGAATTCGCC CCCCCTACGAATCCTCATTTGGGTGCCGTGGGTGCGTGGTGGCGGCGGATGGTGGGTT CATGTTTCCCGTCCCCCGCCGCGAGAAGTGGGGGTGAAAAGCGGCCCGACCTGCTTGG GGTGTAGTGGGCGGACCGCGCGGCTGGAGGTGTGAGGATCCGAACCCAGGGGTGGGGG

Name	DNA SEQUENCE
	GTGGAGGCGGCTCCTGCGATCGAAGGGGACTTGAGACTCACCGGCCGCACGTC (SEQ ID NO: 27)
hHSP70	GGGCCGCCCACTCCCCCTTCTCTCAGGGTCCCTGTCCCTCCAGTGAATCCCAGAAGACTCTGGAGAGTTCTGAGCAGGGGGCGGCACTCTGGCCTCTGATTGGTCCAAGGAAGGCTGGGGGCAGGACGGGAGGCGAAAACCTGGAATATTTCCCGACCTGGCAGCCTCATCGAGCTCGGTGATTGGCTCAGAAGGGAAAAGGCGGGTCTCCGTGACGACTTATAAAAGCCCAAGGGCAAGCGGTCCGGATAACGGCTAGCCTGAGGAGCTGCTGCGACAGTCCACTACCTTTTCGAGAGTGACTCCCGTTGTCCCAAGGCTTCCCAGAGCGAACCTGTGCGGCTGCAGGCACCGGCGCGTCGAGTTTCCGGCGTCCGGAAGGACCGAGCTCTTCTCGCGGATCCAGTGTCCGTTTCCAGCCCCCAATCTCAGAGCGGAGCCGACAGAGAGCAGGGAACCC (SEQ ID NO: 28)
hKINb	GCCCCACCCCCGTCCGCGTTACAACCGGGAGGCCCGCTGGGTCTTGCACCGTCACCCCTCTCCCTGTGACCGCCACCTGATAACCCAAACAACCTTCTCGCCCTCCAGTCCCCAGCTCGCCGAGCGCTTGC GG GAGCCACCCAGCCTCAGTTTCCCCAGCCCCGGGCGGGGCGAGGGGCGATGACGTCATGCCGGCGCGCGGCATTGTGGGGCGGGGCGAGGCGGGGCGCCGGGGGAGCAACACTGAGACGCCATTTTCGGCGGCGGGAGCGGCGCAGGCGGGCCGAGCGGACTGGCTGGGTTCGGCTGGGTGCTGGTGCGAGGAGCCGCGGGGCTGTGCTCGGCGGCCAAGGGGACAGCGCGTGGGTGGCCGAGGATGCTGCGGGGCGGTAGCTCCGGCGCCCCCTCGCTGGTACTGCTGCGCCGTGCCTCACACAGCCGAGGCGGGCTCGGCGCACAGTCGCTGCTCCGCGCTCGCGCCCGGCGGCGCTCCAGGTGCTGACAGCGCGAGAGAGCGCGGCCCTCAGGAGCAACAC (SEQ ID NO: 29)
hUB1b	TTCCAGAGCTTTCGAGGAAGGTTTCTTCAACTCAAATTCATCCGCTGATAATTTTCTTATATTTTCTTAAAGAAGGAAGAGAAGCGCATAGAGGAGAAGGGAAATAATTTTTTAGGAGCCTTTCTTACGGCTATGAGGAATTTGGGGCTCAGTTGAAAAGCCTAAACTGCCTCTCGGGAGGTTGGGCGCGCGAACTACTTTCAGCGGCGCACGGAGACGGCGTCTACGTGAGGGGTGAAGTGACGCAACACTCGTTGCATAAAATTTGCGCTCCGCCAGCCCGGAGCATTTAGGGGCGGTTGGCTTTGTTGGGTGAGCTTGTTTGTGTCCCTGTGGGTGGACGTGGTTGGTGATTGGCAGGATCCTGGTATCCGCTAACAGGtactggcccacagccgtaaaagacctggggggcgtagaggggggaatgggtgaggtcaagctggaggtctctgggttgggtgggcccgtgaggggagggagggcgaggtgacgcgacacccggccttctggagagtgggccttgtgacctaaagggggcgagggcaggtggcacgcgcgcgcgcagagaaactaacagacataaccaacagcgatctcgtcgcttacttggaggaagcggaagagagtagttgtgtggtcttggaacccataatttgaatccagtagagaatggtgtccctctgtgtttcaatgggatttttactcgcgagcttgggttgggtttgtttcagtttgctaacaccgtgcttagtttggcagattggagttcggtcggggagtttgaatatccggaacagtttagtgggaaagctgtggagcgttggttaagagagcgctctgatttccgctgttgacgttgaaccttgaaatgacgaatttcttataagtgacttagccttgaataatgaggggaggttgcggaatattaacgtatttaaggcatttgaaggaatgttgaatttgaagaatttaggttaaaagcaagaaataaatgacctgaggtgacacgcttatgtttacttttaactagGTCACC (SEQ ID NO: 30)
CAG	gacattgattattgactagtatttaataagtaataaattacggggctcattagttcatagcccatatatggagttccgcgttacataacttacggtaaatggcccgcctggctgaccgcccacgacccccgccattgacgtcaataatgacgtatgttcccatagtaacgccaatagggaacttccattgacgtcaatgggtggagtatttacggtaaaactgccacttggcagtagacatcaagtgtatcatatgccaaagtacgccccctattgacgtcaatgacggtaaatggcccgcctggcattatgccagtagacatgaccttatgggactttcctacttggcagtagacatctacgtatttagtcacgtctattaccatggtcaggtgagccccacgttctgtcttactctccccatctccccccctcccccccccaattttgtatttatttatttttaattattttgtgcagcgatgggggcgggggggggggggggggcgcgcgccaggcgggggcggggagggggcgggggcgaggcgggagaggtgcggcggcagccaatcagagcggcgcgctccgaaagtcttctttatggcgaggcgggcgggcgggcgccctataaaaagcgaagcgcgcgggcg (SEQ ID NO: 173)
HLP	Tgtttgctgcttgaatgtttgccatttttaggggtggacacaggacgctgtggttctgagccagggggcgactcagatccagccagtgtagcttagccctgtttgctctccgataactggggtagcttggtaataattaccagcagcctccccgttggccctctggatccactgttaataacggagcaggacaggccctgtctcctcagcttcaggcaccaccactgacctgggacagtgaat (SEQ ID NO: 174)

The promoter can be a tissue-specific promoter. In general, a tissue-specific promoter directs transcription of a nucleic acid, (*e.g.*, the engineered nucleic acids encoding the chimeric

proteins, such as membrane-cleavable chimeric proteins having the formula **S – C – MT** or **MT – C – S**) such that expression is limited to a specific cell type, organelle, or tissue. Tissue-specific promoters include, but are not limited to, albumin (liver specific, Pinkert et al., (1987)), lymphoid specific promoters (Calame and Eaton, 1988), particular promoters of T-cell receptors (Winoto and Baltimore, (1989)) and immunoglobulins; Banerji et al., (1983); Queen and Baltimore, 1983), neuron specific promoters (e.g. the neurofilament promoter; Byrne and Ruddle, 1989), pancreas specific promoters (Edlund et al., (1985)) or mammary gland specific promoters (milk whey promoter, U.S. Pat. No. 4,873,316 and European Application Publication No. 264,166) as well as developmentally regulated promoters such as the murine hox promoters (Kessel and Gruss, Science 249:374-379 (1990)) or the α -fetoprotein promoter (Campes and Tilghman, Genes Dev. 3:537-546 (1989)), the contents of each of which are fully incorporated by reference herein. The promoter can be constitutive in the respective specific cell type, organelle, or tissue. Tissue-specific promoters and/or regulatory elements can also include promoters from the liver fatty acid binding (FAB) protein gene, specific for colon epithelial cells; the insulin gene, specific for pancreatic cells; the transphyretin, .alpha.1- antitrypsin, plasminogen activator inhibitor type 1 (PAI-I), apolipoprotein AI and LDL receptor genes, specific for liver cells; the myelin basic protein (MBP) gene, specific for oligodendrocytes; the glial fibrillary acidic protein (GFAP) gene, specific for glial cells; OPSIN, specific for targeting to the eye; and the neural-specific enolase (NSE) promoter that is specific for nerve cells.

Examples of tissue-specific promoters include, but are not limited to, the promoter for creatine kinase, which has been used to direct expression in muscle and cardiac tissue and immunoglobulin heavy or light chain promoters for expression in B cells. Other tissue specific promoters include the human smooth muscle alpha-actin promoter. Exemplary tissue-specific expression elements for the liver include but are not limited to HMG-COA reductase promoter, sterol regulatory element 1, phosphoenol pyruvate carboxy kinase (PEPCK) promoter, human C-reactive protein (CRP) promoter, human glucokinase promoter, cholesterol L 7-alpha hydroxylase (CYP-7) promoter, beta- galactosidase alpha-2,6 sialyltransferase promoter, insulin-like growth factor binding protein (IGFBP-I) promoter, aldolase B promoter, human transferrin promoter, and collagen type I promoter. Exemplary tissue-specific expression elements for the prostate include but are not limited to the prostatic acid phosphatase (PAP) promoter, prostatic secretory protein of 94 (PSP 94) promoter, prostate specific antigen complex promoter, and human glandular kallikrein gene promoter (hgt-1). Exemplary tissue- specific expression elements for gastric tissue include but are not limited to the human H+/K+-ATPase alpha subunit promoter. Exemplary tissue-specific expression elements for the pancreas include but are not limited to pancreatitis associated protein promoter (PAP), elastase 1 transcriptional enhancer, pancreas

specific amylase and elastase enhancer promoter, and pancreatic cholesterol esterase gene promoter. Exemplary tissue-specific expression elements for the endometrium include, but are not limited to, the uteroglobin promoter. Exemplary tissue-specific expression elements for adrenal cells include, but are not limited to, cholesterol side-chain cleavage (SCC) promoter.

- 5 Exemplary tissue-specific expression elements for the general nervous system include, but are not limited to, gamma-gamman enolase (neuron- specific enolase, NSE) promoter. Exemplary tissue-specific expression elements for the brain include, but are not limited to, the neurofilament heavy chain (NF-H) promoter. Exemplary tissue-specific expression elements for lymphocytes include, but are not limited to, the human CGL-1/granzyme B promoter, the
- 10 terminal deoxy transferase (TdT), lambda 5, VpreB, and lck (lymphocyte specific tyrosine protein kinase p56lck) promoter, the humans CD2 promoter and its 3 ' transcriptional enhancer, and the human NK and T cell specific activation (NKG5) promoter. Exemplary tissue-specific expression elements for the colon include, but are not limited to, pp60c-src tyrosine kinase promoter, organ-specific neoantigens (OSNs) promoter, and colon specific antigen-P promoter.
- 15 Tissue-specific expression elements for breast cells are for example, but are not limited to, the human alpha-lactalbumin promoter. Exemplary tissue-specific expression elements for the lung include, but are not limited to, the cystic fibrosis transmembrane conductance regulator (CFTR) gene promoter.

- In some embodiments, a promoter of the present disclosure is modulated by signals
- 20 within a tumor microenvironment. A tumor microenvironment is considered to modulate a promoter if, in the presence of the tumor microenvironment, the activity of the promoter is increased or decreased by at least 10%, relative to activity of the promoter in the absence of the tumor microenvironment. In some embodiments, the activity of the promoter is increased or decreased by at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at
- 25 least 80%, at least 90%, at least 100%, relative to activity of the promoter in the absence of the tumor microenvironment. For example, the activity of the promoter is increased or decreased by 10-20%, 10-30%, 10-40%, 10-50%, 10-60%, 10-70%, 10-80%, 10-90%, 10-100%, 10-200%, 20-30%, 20-40%, 20-50%, 20-60%, 20-70%, 20-80%, 20-90%, 20-100%, 20-200%, 50-60%, 50-70%, 50-80%, 50-90%, 50-100%, or 50-200%, relative to activity of the promoter in the
- 30 absence of the tumor microenvironment.

- In some embodiments, the activity of the promoter is increased or decreased by at least 2 fold (*e.g.*, 2, 3, 4, 5, 10, 25, 20, 25, 50, or 100 fold), relative to activity of the promoter in the absence of the tumor microenvironment. For example, the activity of the promoter is increased or decreased by at least 3 fold, at least 5 fold, at least 10 fold, at least 20 fold, at least 50 fold, or
- 35 at least 100 fold, relative to activity of the promoter in the absence of the tumor

microenvironment. In some embodiments, the activity of the promoter is increased or decreased by 2-10, 2-20, 2-30, 2-40, 2-50, 2-60, 2-70, 2-80, 2-90, or 2-100 fold, relative to activity of the promoter in the absence of the tumor microenvironment.

5 In some embodiments, a promoter of the present disclosure is activated under a hypoxic condition. A “hypoxic condition” is a condition where the body or a region of the body is deprived of adequate oxygen supply at the tissue level. Hypoxic conditions can cause inflammation (*e.g.*, the level of inflammatory cytokines increase under hypoxic conditions). In some embodiments, the promoter that is activated under hypoxic condition is operably linked to a nucleotide encoding a chimeric proteins that decreases the expression of activity of
10 inflammatory cytokines, thus reducing the inflammation caused by the hypoxic condition. In some embodiments, the promoter that is activated under hypoxic conditions comprises a hypoxia responsive element (HRE). A “hypoxia responsive element (HRE)” is a response element that responds to hypoxia-inducible factor (HIF). The HRE, in some embodiments, comprises a consensus motif NCGTG (where N is either A or G).

15

Activation-Conditional Control Polypeptide (ACP) Promoter Systems

In some embodiments, a synthetic promoter is a promoter system including an activation-conditional control polypeptide- (ACP-) binding domain sequence and a promoter sequence. Such a system is also referred to herein as an “ACP-responsive promoter.” In general,
20 an ACP promoter system includes a first expression cassette encoding an activation-conditional control polypeptide (ACP) and a second expression cassette encoding an ACP-responsive promoter operably linked to an exogenous polynucleotide sequence, such as the exogenous polynucleotide sequence encoding the membrane-cleavable chimeric proteins described herein or any other protein of interest (*e.g.*, a protease). In some embodiments, the first expression
25 cassette and second expression cassette are each encoded by a separate engineered nucleic acid. In other embodiments, the first expression cassette and the second expression cassette are encoded by the same engineered nucleic acid. The ACP-responsive promoter can be operably linked to a nucleotide sequence encoding a single protein of interest or multiple proteins of interest.

30 The promoters of the ACP promoter system, *e.g.*, either a promoter driving expression of the ACP or the promoter sequence of the ACP-responsive promoter, can include any of the promoter sequences described herein (*see* “Promoters” above). The ACP-responsive promoter can be derived from minP, NFkB response element, CREB response element, NFAT response element, SRF response element 1, SRF response element 2, AP1 response element, TCF-LEF

response element promoter fusion, Hypoxia responsive element, SMAD binding element, STAT3 binding site, minCMV, YB_TATA, minTK, inducer molecule responsive promoters, and tandem repeats thereof. In some embodiments, the ACP-responsive promoter includes a minimal promoter.

5 In some embodiments, the ACP-binding domain includes one or more zinc finger binding sites. In some embodiments, the ACP-responsive promoter includes a minimal promoter and the ACP-binding domain includes one or more zinc finger binding sites. The ACP-binding domain can include 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more zinc finger binding sites. In some
10 embodiments, the transcription factor is a zinc-finger-containing transcription factor. In some embodiments, the zinc-finger-containing transcription factor is a synthetic transcription factor. In some embodiments, the ACP-binding domain includes one or more zinc finger binding sites and the ACP has a DNA-binding zinc finger protein domain (ZF protein domain). In some
15 embodiments, the ACP has a DNA-binding zinc finger protein domain (ZF protein domain) and an effector domain. In some embodiments, the ACP-binding domain includes one or more zinc finger binding sites and the ACP has a DNA-binding zinc finger protein domain (ZF protein domain) and an effector domain. In some embodiments, the ZF protein domain is modular in
20 design and is composed of zinc finger arrays (ZFA). A zinc finger array comprises multiple zinc finger protein motifs that are linked together. Each zinc finger motif binds to a different nucleic acid motif. This results in a ZFA with specificity to any desired nucleic acid sequence, *e.g.*, a ZFA with desired specificity to an ACP-binding domain having a specific zinc finger binding
25 site composition and/or configuration. The ZF motifs can be directly adjacent to each other, or separated by a flexible linker sequence. In some embodiments, a ZFA is an array, string, or chain of ZF motifs arranged in tandem. A ZFA can have 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 zinc finger motifs. The ZFA can have from 1-10, 1-15, 1-2, 1-3, 1-4, 1-5, 1-6, 1-7, 1-8,
30 1-9, 2-3, 2-4, 2-5, 2-6, 2-7, 2-8, 2-9, 2-10, 3-4, 3-5 3-6, 3-7, 3-8, 3-9, 3-10, 4-5, 4-6, 4-7, 4-8, 4-9, 4-10, 5-6, 5-7, 5-8, 5-9, 5-10, or 5-15 zinc finger motifs. The ZF protein domain can have 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or more ZFAs. The ZF domain can have from 1-10, 1-15, 1-2, 1-3, 1-4, 1-5, 1-6, 1-7, 1-8, 1-9, 2-3, 2-4, 2-5, 2-6, 2-7, 2-8, 2-9, 2-10, 3-4, 3-5 3-6, 3-7,
35 3-8, 3-9, 3-10, 4-5, 4-6, 4-7, 4-8, 4-9, 4-10, 5-6, 5-7, 5-8, 5-9, 5-10, or 5-15 ZFAs. In some embodiments, the ZF protein domain comprises one to ten ZFA(s). In some embodiments, the ZF protein domain comprises at least one ZFA. In some embodiments, the ZF protein domain comprises at least two ZFAs. In some embodiments, the ZF protein domain comprises at least three ZFAs. In some embodiments, the ZF protein domain comprises at least four ZFAs. In some embodiments, the ZF protein domain comprises at least five ZFAs. In some embodiments, the ZF protein domain comprises at least ten ZFAs.

In some embodiments, the ACP is a transcriptional modulator. In some embodiments, the ACP is a transcriptional repressor. In some embodiments, the ACP is a transcriptional activator. In some embodiments, the ACP is a transcription factor. In some embodiments, the ACP comprises a DNA-binding domain and a transcriptional effector domain. In some embodiments, the DNA-binding domain comprises a tetracycline (or derivative thereof) repressor (TetR) domain. In some embodiments, the ACP is an antigen recognizing receptor of the present disclosure.

The ACP can also further include an effector domain, such as a transcriptional effector domain. For instance, a transcriptional effector domain can be the effector or activator domain of a transcription factor. Transcription factor activation domains are also known as transactivation domains, and act as scaffold domains for proteins such as transcription coregulators that act to activate or repress transcription of genes. Any suitable transcriptional effector domains can be used in the ACP including, but not limited to, a Herpes Simplex Virus Protein 16 (VP16) activation domain; an activation domain consisting of four tandem copies of VP16, a VP64 activation domain; a p65 activation domain of NFκB; an Epstein-Barr virus R transactivator (Rta) activation domain; a tripartite activator comprising the VP64, the p65, and the Rta activation domains, the tripartite activator is known as a VPR activation domain; a histone acetyltransferase (HAT) core domain of the human E1A-associated protein p300, known as a p300 HAT core activation domain; a Krüppel associated box (KRAB) repression domain; a Repressor Element Silencing Transcription Factor (REST) repression domain; a WRPW motif of the hairy-related basic helix-loop-helix repressor proteins, the motif is known as a WRPW repression domain; a DNA (cytosine-5)-methyltransferase 3B (DNMT3B) repression domain; and an HP1 alpha chromoshadow repression domain, or any combination thereof.

In some embodiments, the effector domain is a transcription effector domain selected from: a Herpes Simplex Virus Protein 16 (VP16) activation domain; an activation domain consisting of four tandem copies of VP16, a VP64 activation domain; a p65 activation domain of NFκB; an Epstein-Barr virus R transactivator (Rta) activation domain; a tripartite activator comprising the VP64, the p65, and the Rta activation domains, the tripartite activator is known as a VPR activation domain; a histone acetyltransferase (HAT) core domain of the human E1A-associated protein p300, known as a p300 HAT core activation domain; a Krüppel associated box (KRAB) repression domain; a Repressor Element Silencing Transcription Factor (REST) repression domain; a WRPW motif of the hairy-related basic helix-loop-helix repressor proteins, the motif is known as a WRPW repression domain; a DNA (cytosine-5)-methyltransferase 3B (DNMT3B) repression domain; and an HP1 alpha chromoshadow repression domain.

In some embodiments, the ACP is a small molecule (*e.g.*, drug) inducible polypeptide. For example, in some embodiments, the ACP may be induced by tetracycline (or derivative thereof), and comprises a TetR domain and a VP16 effector domain. In some embodiments, the ACP includes an estrogen receptor variant, such as ERT2, and may be regulated by tamoxifen, or a metabolite thereof (such as 4-hydroxy-tamoxifen [4-OHT], N-desmethyltamoxifen, tamoxifen-N-oxide, or endoxifen), through tamoxifen-controlled nuclear localization.

In some embodiments, the ACP is a small molecule (*e.g.*, drug) inducible polypeptide that includes a repressible protease and one or more cognate cleavage sites of the repressible protease. In some embodiments, a repressible protease is active (cleaves a cognate cleavage site) in the absence of the specific agent and is inactive (does not cleave a cognate cleavage site) in the presence of the specific agent. In some embodiments, the specific agent is a protease inhibitor. In some embodiments, the protease inhibitor specifically inhibits a given repressible protease of the present disclosure. The repressible protease can be any of the proteases described herein that is capable of inactivation by the presence or absence of a specific agent (*see* “Protease Cleavage Site” above for exemplary repressible proteases, cognate cleavage sites, and protease inhibitors).

In some embodiments, the ACP has a degron domain (*see* “Degron Systems and Domains” above for exemplary degron sequences). The degron domain can be in any order or position relative to the individual domains of the ACP. For example, the degron domain can be N-terminal of the repressible protease, C-terminal of the repressible protease, N-terminal of the ZF protein domain, C-terminal of the ZF protein domain, N-terminal of the effector domain, or C-terminal of the effector domain.

Multicistronic and Multiple Promoter Systems

In some embodiments, engineered nucleic acids (*e.g.*, an engineered nucleic acid comprising an expression cassette) are configured to produce multiple chimeric proteins. For example, nucleic acids may be configured to produce 2-20 different chimeric proteins. In some embodiments, nucleic acids are configured to produce 2-20, 2-19, 2-18, 2-17, 2-16, 2-15, 2-14, 2-13, 2-12, 2-11, 2-10, 2-9, 2-8, 2-7, 2-6, 2-5, 2-4, 2-3, 3-20, 3-19, 3-18, 3-17, 3-16, 3-15, 3-14, 3-13, 3-12, 3-11, 3-10, 3-9, 3-8, 3-7, 3-6, 3-5, 3-4, 4-20, 4-19, 4-18, 4-17, 4-16, 4-15, 4-14, 4-13, 4-12, 4-11, 4-10, 4-9, 4-8, 4-7, 4-6, 4-5, 5-20, 5-19, 5-18, 5-17, 5-16, 5-15, 5-14, 5-13, 5-12, 5-11, 5-10, 5-9, 5-8, 5-7, 5-6, 6-20, 6-19, 6-18, 6-17, 6-16, 6-15, 6-14, 6-13, 6-12, 6-11, 6-10, 6-9, 6-8, 6-7, 7-20, 7-19, 7-18, 7-17, 7-16, 7-15, 7-14, 7-13, 7-12, 7-11, 7-10, 7-9, 7-8, 8-20, 8-19, 8-18, 8-17, 8-16, 8-15, 8-14, 8-13, 8-12, 8-11, 8-10, 8-9, 9-20, 9-19, 9-18, 9-17, 9-16, 9-15, 9-14,

9-13, 9-12, 9-11, 9-10, 10-20, 10-19, 10-18, 10-17, 10-16, 10-15, 10-14, 10-13, 10-12, 10-11, 11-20, 11-19, 11-18, 11-17, 11-16, 11-15, 11-14, 11-13, 11-12, 12-20, 12-19, 12-18, 12-17, 12-16, 12-15, 12-14, 12-13, 13-20, 13-19, 13-18, 13-17, 13-16, 13-15, 13-14, 14-20, 14-19, 14-18, 14-17, 14-16, 14-15, 15-20, 15-19, 15-18, 15-17, 15-16, 16-20, 16-19, 16-18, 16-17, 17-20, 17-19, 17-18, 18-20, 18-19, or 19-20 chimeric proteins. In some embodiments, nucleic acids are configured to produce 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 chimeric proteins.

In general, the engineered nucleic acids described herein are multicistronic, *i.e.*, as described above.

Engineered nucleic acids can also use multiple promoters to express genes from multiple ORFs, *i.e.*, more than one separate mRNA transcripts can be produced from a single engineered nucleic acid. For example, a first promoter can be operably linked to a polynucleotide sequence encoding a first chimeric protein, and a second promoter can be operably linked to a polynucleotide sequence encoding a second chimeric protein. In general, any number of promoters can be used to express any number of chimeric proteins. In some embodiments, at least one of the ORFs expressed from the multiple promoters can be multicistronic.

“Linkers,” as used herein can refer to polypeptides that link a first polypeptide sequence and a second polypeptide sequence, the multicistronic linkers described above, or the additional promoters that are operably linked to additional ORFs described above.

Engineered Cells

Provided herein are engineered cells, and methods of producing the engineered cells, that produce each of (i) a membrane-cleavable chimeric protein; (ii) a bivalent aCAR; and (iii) an iCAR.. In general, engineered cells of the present disclosure may be engineered to express the chimeric proteins provided for herein, such as each of the membrane-cleavable chimeric proteins, the bivalent aCARs, and the iCARs described herein. These cells are referred to herein as “engineered cells.” These cells, which typically contain engineered nucleic acid, do not occur in nature. In some embodiments, the cells are engineered to include a nucleic acid comprising a promoter operably linked to a nucleotide sequence encoding a chimeric protein, for example, a membrane-cleavable chimeric protein. An engineered cell can comprise an engineered nucleic acid integrated into the cell’s genome. An engineered cell can comprise an engineered nucleic acid capable of expression without integrating into the cell’s genome, for example, engineered with a transient expression system such as a plasmid or mRNA.

The present disclosure also encompasses additivity and synergy between a chimeric protein(s) and the engineered cell from which they are produced. In some embodiments, cells

are engineered to produce at least two (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) chimeric proteins, for example at least two membrane-cleavable chimeric proteins. In other embodiments, cells are engineered to produce at least one chimeric proteins having an effector molecule that is not natively produced by the cells. Such an effector molecule may, for example, complement the function of effector molecules natively produced by the cells.

In some embodiments, cells are engineered to express membrane-tethered anti-CD3 and/or anti-CD28 agonist extracellular domains.

In some embodiments, a cell (*e.g.*, an immune cell or a stem cell) is engineered to produce multiple chimeric proteins. For example, cells may be engineered to produce 2-20 different chimeric proteins, such as 2-20 different membrane-cleavable chimeric proteins. In some embodiments, cells engineered to produce 2-20, 2-19, 2-18, 2-17, 2-16, 2-15, 2-14, 2-13, 2-12, 2-11, 2-10, 2-9, 2-8, 2-7, 2-6, 2-5, 2-4, 2-3, 3-20, 3-19, 3-18, 3-17, 3-16, 3-15, 3-14, 3-13, 3-12, 3-11, 3-10, 3-9, 3-8, 3-7, 3-6, 3-5, 3-4, 4-20, 4-19, 4-18, 4-17, 4-16, 4-15, 4-14, 4-13, 4-12, 4-11, 4-10, 4-9, 4-8, 4-7, 4-6, 4-5, 5-20, 5-19, 5-18, 5-17, 5-16, 5-15, 5-14, 5-13, 5-12, 5-11, 5-10, 5-9, 5-8, 5-7, 5-6, 6-20, 6-19, 6-18, 6-17, 6-16, 6-15, 6-14, 6-13, 6-12, 6-11, 6-10, 6-9, 6-8, 6-7, 7-20, 7-19, 7-18, 7-17, 7-16, 7-15, 7-14, 7-13, 7-12, 7-11, 7-10, 7-9, 7-8, 8-20, 8-19, 8-18, 8-17, 8-16, 8-15, 8-14, 8-13, 8-12, 8-11, 8-10, 8-9, 9-20, 9-19, 9-18, 9-17, 9-16, 9-15, 9-14, 9-13, 9-12, 9-11, 9-10, 10-20, 10-19, 10-18, 10-17, 10-16, 10-15, 10-14, 10-13, 10-12, 10-11, 11-20, 11-19, 11-18, 11-17, 11-16, 11-15, 11-14, 11-13, 11-12, 12-20, 12-19, 12-18, 12-17, 12-16, 12-15, 12-14, 12-13, 13-20, 13-19, 13-18, 13-17, 13-16, 13-15, 13-14, 14-20, 14-19, 14-18, 14-17, 14-16, 14-15, 15-20, 15-19, 15-18, 15-17, 15-16, 16-20, 16-19, 16-18, 16-17, 17-20, 17-19, 17-18, 18-20, 18-19, or 19-20 chimeric proteins. In some embodiments, cells are engineered to produce 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 chimeric proteins.

In some embodiments, engineered cells comprise one or more engineered nucleic acids encoding a promoter operably linked to a nucleotide sequence encoding a chimeric protein. In some embodiments, cells are engineered to include a plurality of engineered nucleic acids, *e.g.*, at least two engineered nucleic acids, each encoding a promoter operably linked to a nucleotide sequence encoding at least one (*e.g.*, 1, 2 or 3) chimeric protein. For example, cells may be engineered to comprise at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 8, at least 9, or at least 10, engineered nucleic acids, each encoding a promoter operably linked to a nucleotide sequence encoding at least one (*e.g.*, 1, 2 or 3) chimeric protein. In some embodiments, the cells are engineered to comprise 2, 3, 4, 5, 6, 7, 8, 9, 10, or more engineered nucleic acids, each encoding a promoter operably linked to a nucleotide sequence encoding at least one (*e.g.*, 1, 2 or 3) chimeric protein. Engineered cells can comprise an engineered nucleic acid encoding at least one of the linkers described above, such as polypeptides that link a first

polypeptide sequence and a second polypeptide sequence, one or more multicistronic linker described above, one or more additional promoters operably linked to additional ORFs, or a combination thereof.

In some embodiments, a cell (e.g., an immune cell or a stem cell) is engineered to express a protease. In some embodiments, a cell is engineered to express a protease heterologous to a cell. In some embodiments, a cell is engineered to express a protease heterologous to a cell expressing a chimeric protein, such as a heterologous protease that cleaves the protease cleavage site of a membrane-cleavable chimeric protein. In some embodiments, engineered cells comprise one or more engineered nucleic acids encoding a promoter operably linked to a nucleotide sequence encoding a protease, such as a heterologous protease. Protease and protease cleavage sites are described in greater detail in the Section herein titled "Protease Cleavage site."

In some embodiments, a cell (e.g., an immune cell or a stem cell) is engineered to produce at least one homing molecule. "Homing," refers to active navigation (migration) of a cell to a target site (e.g., a cell, tissue (e.g., tumor), or organ). A "homing molecule" refers to a molecule that directs cells to a target site. In some embodiments, a homing molecule functions to recognize and/or initiate interaction of an engineered cell to a target site. Non-limiting examples of homing molecules include CXCR1, CCR9, CXCR2, CXCR3, CXCR4, CCR2, CCR4, FPR2, VEGFR, IL6R, CXCR1, CSCR7, and PDGFR.

In some embodiments, a homing molecule is a chemokine receptor (cell surface molecule that binds to a chemokine). Non-limiting examples of chemokine receptors that may be produced by the engineered cells of the present disclosure include: CXC chemokine receptors (e.g., CXCR1, CXCR2, CXCR3, CXCR4, CXCR5, CXCR6, and CXCR7), CC chemokine receptors (CCR1, CCR2, CCR3, CCR4, CCR5, CCR6, CCR7, CCR8, CCR9, CCR10, and CCR11), CX3C chemokine receptors (e.g., CX3CR1, which binds to CX3CL1), and XC chemokine receptors (e.g., XCR1). In some embodiments, a chemokine receptor is a G protein-linked transmembrane receptor, or a member of the tumor necrosis factor (TNF) receptor superfamily (including but not limited to TNFRSF1A, TNFRSF1B). In some embodiments, cells are engineered to produce CXCL8, CXCL9, and/or CXCL10 (promote T-cell recruitment), CCL3 and/or CXCL5, CCL21 (Th1 recruitment and polarization). In some embodiments, cells are engineered to produce CXCR4.

In some embodiments, a cell (e.g., an immune cell or a stem cell) is engineered to produce G-protein coupled receptors (GPCRs) that detect N-formylated-containing oligopeptides (including but not limited to FPR2 and FPRL1).

In some embodiments, a cell (e.g., an immune cell or a stem cell) is engineered to produce receptors that detect interleukins (including but not limited to IL6R).

In some embodiments, a cell (e.g., an immune cell or a stem cell) is engineered to produce receptors that detect growth factors secreted from other cells, tissues, or tumors (including but not limited to FGFR, PDGFR, EGFR, and receptors of the VEGF family, including but not limited to VEGF-C and VEGF-D).

5 In some embodiments, a cell (e.g., an immune cell or a stem cell) is engineered to produce one or more integrins. Cells of the present disclosure may be engineered to produce any combination of integrin α and β subunits. The α subunit of an integrin may be, without limitation: ITGA1, ITGA2, ITGA3, ITGA4, ITGA5, ITGA6, ITGA7, ITGA8, ITGA9, ITGA10, ITGA11, ITGAD, ITGAE, ITGAL, ITGAM, ITGAV, ITGA2B, ITGAX. The β subunit of an
10 integrin may be, without limitation: ITGB1, ITGB2, ITGB3, ITGB4, ITGB5, ITGB6, ITGB7, and ITGB8.

In some embodiments, a cell (e.g., an immune cell or a stem cell) is engineered to produce one or more matrix metalloproteinases (MMP). Non-limiting examples of MMPs include MMP-2, MMP-9, and MMP. In some embodiments, cells are engineered to produce an
15 inhibitor of a molecule (e.g., protein) that inhibits MMPs. For example, cells may be engineered to express an inhibitor (e.g., an RNAi molecule) of membrane type 1 MMP (MT1-MMP) or TIMP metalloproteinase inhibitor 1 (TIMP-1).

In some embodiments, a cell (e.g., an immune cell or a stem cell) is engineered to produce a ligand that binds to selectin (e.g., hematopoietic cell E-/L-selectin ligand (HCELL),
20 Dykstran *et al.*, Stem Cells. 2016 Oct;34(10):2501-2511) on the endothelium of a target tissue, for example.

The term “homing molecule” also encompasses transcription factors that regulate the production of molecules that improve/enhance homing of cells.

Also provided herein are engineered cells that are engineered to produce multiple
25 chimeric proteins, at least two of which include effector molecules that modulate different tumor-mediated immunosuppressive mechanisms. In some embodiments, at least one (e.g., 1, 2, 3, 4, 5, or more) chimeric protein includes an effector molecule that stimulates at least one immunostimulatory mechanism in the tumor microenvironment, or inhibits at least one immunosuppressive mechanism in the tumor microenvironment. In some embodiments, at least
30 one (e.g., 1, 2, 3, 4, 5, or more) chimeric protein includes an effector molecule that inhibits at least one immunosuppressive mechanism in the tumor microenvironment, and at least one chimeric protein (e.g., 1, 2, 3, 4, 5, or more) inhibits at least one immunosuppressive mechanism in the tumor microenvironment. In yet other embodiments, at least two (e.g., 2, 3, 4, 5, or more) chimeric proteins includes an effector molecule that stimulate at least one immunostimulatory
35 mechanism in the tumor microenvironment. In still other embodiments, at least two (e.g., 1, 2, 3,

4, 5, or more) chimeric proteins includes an effector molecule that inhibit at least one immunosuppressive mechanism in the tumor microenvironment.

In some embodiments, a cell (e.g., an immune cell or a stem cell) is engineered to produce at least one chimeric protein including an effector molecule that stimulates T cell signaling, activity and/or recruitment. In some embodiments, a cell is engineered to produce at least one chimeric protein that includes an effector molecule that stimulates antigen presentation and/or processing. In some embodiments, a cell is engineered to produce at least one chimeric protein that includes an effector molecule that stimulates natural killer cell-mediated cytotoxic signaling, activity and/or recruitment. In some embodiments, a cell is engineered to produce at least one chimeric protein that includes an effector molecule that stimulates dendritic cell differentiation and/or maturation. In some embodiments, a cell is engineered to produce at least one chimeric protein that includes an effector molecule that stimulates immune cell recruitment. In some embodiments, a cell is engineered to produce at least one chimeric protein includes an effector molecule that that stimulates M1 macrophage signaling, activity and/or recruitment. In some embodiments, a cell is engineered to produce at least one chimeric protein that includes an effector molecule that stimulates Th1 polarization. In some embodiments, a cell is engineered to produce at least one chimeric protein that includes an effector molecule that stimulates stroma degradation. In some embodiments, a cell is engineered to produce at least one chimeric protein that includes an effector molecule that stimulates immunostimulatory metabolite production. In some embodiments, a cell is engineered to produce at least one chimeric protein that includes an effector molecule that stimulates Type I interferon signaling. In some embodiments, a cell is engineered to produce at least one chimeric protein that includes an effector molecule that inhibits negative costimulatory signaling. In some embodiments, a cell is engineered to produce at least one chimeric protein that includes an effector molecule that inhibits pro-apoptotic signaling (e.g., via TRAIL) of anti-tumor immune cells. In some embodiments, a cell is engineered to produce at least one chimeric protein that includes an effector molecule that inhibits T regulatory (T_{reg}) cell signaling, activity and/or recruitment. In some embodiments, a cell is engineered to produce at least one chimeric protein that includes an effector molecule that inhibits tumor checkpoint molecules. In some embodiments, a cell is engineered to produce at least one chimeric protein that includes an effector molecule that activates stimulator of interferon genes (STING) signaling. In some embodiments, a cell is engineered to produce at least one chimeric protein that includes an effector molecule that inhibits myeloid-derived suppressor cell signaling, activity and/or recruitment. In some embodiments, a cell is engineered to produce at least one chimeric protein that includes an effector molecule that degrades immunosuppressive factors/metabolites. In some embodiments, a cell is engineered to produce

at least one chimeric protein that includes an effector molecule that inhibits vascular endothelial growth factor signaling. In some embodiments, a cell is engineered to produce at least one chimeric protein that includes an effector molecule that directly kills tumor cells (*e.g.*, granzyme, perforin, oncolytic viruses, cytolytic peptides and enzymes, anti-tumor antibodies, *e.g.*, that trigger ADCC).

In some embodiments, at least one chimeric protein including an effector molecule that: stimulates T cell signaling, activity and/or recruitment, stimulates antigen presentation and/or processing, stimulates natural killer cell-mediated cytotoxic signaling, activity and/or recruitment, stimulates dendritic cell differentiation and/or maturation, stimulates immune cell recruitment, stimulates macrophage signaling, stimulates stroma degradation, stimulates immunostimulatory metabolite production, or stimulates Type I interferon signaling; and at least one chimeric protein including an effector molecule that inhibits negative costimulatory signaling, inhibits pro-apoptotic signaling of anti-tumor immune cells, inhibits T regulatory (Treg) cell signaling, activity and/or recruitment, inhibits tumor checkpoint molecules, activates stimulator of interferon genes (STING) signaling, inhibits myeloid-derived suppressor cell signaling, activity and/or recruitment, degrades immunosuppressive factors/metabolites, inhibits vascular endothelial growth factor signaling, or directly kills tumor cells.

In some embodiments, a cell (*e.g.*, an immune cell or a stem cell) is engineered to produce at least one chimeric protein including an effector molecule selected from IL-12, IFN- β , IFN- γ , IL-2, IL-15, IL-7, IL-36 γ , IL-18, IL-1 β , OX40-ligand, and CD40L; and/or at least a checkpoint inhibitor. Illustrative immune checkpoint molecules that can be targeted for blocking or inhibition include, but are not limited to, CTLA-4, 4-1BB (CD137), 4-1BBL (CD137L), PDL1, PDL2, PD1, B7-H3, B7-H4, BTLA, HVEM, TIM3, GAL9, LAG3, TIM3, B7H3, B7H4, VISTA, KIR, 2B4 (belongs to the CD2 family of molecules and is expressed on all NK, $\square\square\square\square$, and memory CD8+ ($\square\square$) T cells), CD160 (also referred to as BY55), and CGEN-15049.

Immune checkpoint inhibitors include antibodies, or antigen binding fragments thereof, or other binding proteins, that bind to and block or inhibit the activity of one or more of CTLA-4, PDL1, PDL2, PD1, B7-H3, B7-H4, BTLA, HVEM, TIM3, GAL9, LAG3, TIM3, B7H3, B7H4, VISTA, KIR, 2B4, CD160, and CGEN-15049. Exemplary checkpoint inhibitors include, but are not limited to, anti-PD-1 antibodies, anti-PD-L1 antibodies, anti-PD-L2 antibodies, anti-CTLA-4 antibodies, anti-LAG-3 antibodies, anti-TIM-3 antibodies, anti-TIGIT antibodies, anti-VISTA antibodies, anti-KIR antibodies, anti-B7-H3 antibodies, anti-B7-H4 antibodies, anti-HVEM antibodies, anti-BTLA antibodies, anti-GAL9 antibodies, anti-A2AR antibodies, anti-phosphatidylserine antibodies, anti-CD27 antibodies, anti-TNF α antibodies, anti-TREMI antibodies, and anti-TREM2 antibodies. Illustrative immune checkpoint inhibitors include

pembrolizumab (anti-PD-1; MK-3475/Keytruda® - Merck), nivolumab (anti-PD-1; Opdivo® - BMS), pidilizumab (anti-PD-1 antibody; CT-011 – Teva/CureTech), AMP224 (anti-PD-1; NCI), avelumab (anti-PD-L1; Bavencio® - Pfizer), durvalumab (anti-PD-L1; MEDI4736/Imfinzi® - Medimmune/AstraZeneca), atezolizumab (anti-PD-L1; Tecentriq® - Roche/Genentech), BMS-
 5 936559 (anti-PD-L1 - BMS), tremelimumab (anti-CTLA-4; Medimmune/AstraZeneca), ipilimumab (anti-CTLA-4; Yervoy® - BMS), lirilumab (anti-KIR; BMS), monalizumab (anti-NKG2A; Innate Pharma/AstraZeneca).

In some embodiments, a cell (e.g., an immune cell or a stem cell) is engineered to produce at least one chimeric protein including an effector molecule selected from IL-12, IFN-β,
 10 IFN-γ, IL-2, IL-15, IL-7, IL-36γ, IL-18, IL-1β, OX40-ligand, and CD40L; and/or at least one checkpoint inhibitor selected from anti-PD-1 antibodies, anti-PD-L1 antibodies, anti-CTLA-4 antibodies, and anti-IL-35 antibodies; and/or at least one chimeric protein including an effector molecule selected from MIP1α (CCL3), MIP1β (CCL5), and CCL21; and/or at least one chimeric protein including an effector molecule selected from CpG oligodeoxynucleotides;
 15 and/or at least one chimeric protein including an effector molecule selected from microbial peptides.

In some embodiments, a cell (e.g., an immune cell or a stem cell) is engineered to produce IFN-β and at least one chimeric protein including an effector molecule selected from cytokines, antibodies, chemokines, nucleotides, peptides, enzymes, and stimulators of interferon
 20 genes (STINGs). In some embodiments, a cell is engineered to produce IFN-β and at least one cytokine or receptor/ligand (e.g., IL-12, IFN-γ, IL-2, IL-15, IL-7, IL-36γ, IL-18, IL-1β, OX40-ligand, and/or CD40L).

In some embodiments, a cell (e.g., an immune cell or a stem cell) is engineered to produce at least one membrane-cleavable chimeric protein where the secretable effector
 25 molecule (e.g., “S” in the formula **S – C – MT** or **MT – C – S**) is a cytokine, a chemokine, a homing molecule, a growth factor, a co-activation molecule, a tumor microenvironment modifier, a ligand, an antibody, a peptide, or an enzyme. In some embodiments, a cell is engineered to produce at least one membrane-cleavable chimeric protein where the secretable effector molecule is a cytokine. In some embodiments, a cell is engineered to produce at least
 30 one membrane-cleavable chimeric protein where the secretable effector molecule is a chemokine. In some embodiments, a cell is engineered to produce at least one membrane-cleavable chimeric protein where the secretable effector molecule is a homing molecule. In some embodiments, a cell is engineered to produce at least one membrane-cleavable chimeric protein where the secretable effector molecule is a growth factor. In some embodiments, a cell is
 35 engineered to produce at least one membrane-cleavable chimeric protein where the secretable

effector molecule is a co-activation molecule. In some embodiments, a cell is engineered to produce at least one membrane-cleavable chimeric protein where the secretable effector molecule is a tumor microenvironment modifier. In some embodiments, a cell is engineered to produce at least one membrane-cleavable chimeric protein where the secretable effector molecule is a ligand. In some embodiments, a cell is engineered to produce at least one membrane-cleavable chimeric protein where the secretable effector molecule is an antibody. In some embodiments, a cell is engineered to produce at least one membrane-cleavable chimeric protein where the secretable effector molecule is a peptide. In some embodiments, a cell is engineered to produce at least one membrane-cleavable chimeric protein where the secretable effector molecule is an enzyme.

In some embodiments, a cell is engineered to produce at least one membrane-cleavable chimeric protein where the secretable effector molecule (*e.g.*, “S” in the formula **S – C – MT** or **MT – C – S**) is IL-1-beta, IL-2, IL-4, IL-6, IL-7, IL-10, IL-12, an IL-12p70 fusion protein, IL-15, IL17A, IL18, IL21, IL22, Type I interferons, Interferon-gamma, or TNF-alpha. In some embodiments, a cell is engineered to produce at least one membrane-cleavable chimeric protein where the secretable effector molecule is CCL21a, CXCL10, CXCL11, CXCL13, a CXCL10-CXCL11 fusion protein, CCL19, CXCL9, or XCL1. In some embodiments, a cell is engineered to produce at least one membrane-cleavable chimeric protein where the secretable effector molecule is anti-integrin alpha4,beta7, anti-MAdCAM, SDF1, or MMP-2. In some embodiments, a cell is engineered to produce at least one membrane-cleavable chimeric protein where the secretable effector molecule is 4-1BBL or CD40L. In some embodiments, a cell is engineered to produce at least one membrane-cleavable chimeric protein where the secretable effector molecule is adenosine deaminase, a TGFbeta inhibitor, an immune checkpoint inhibitor, a VEGF inhibitor, or HPGE2. In some embodiments, a cell is engineered to produce at least one membrane-cleavable chimeric protein where the secretable effector molecule is an anti-TGFbeta peptide, an anti-TGFbeta antibody, a TGFb-TRAP, or combinations thereof. In some embodiments, a cell is engineered to produce at least one membrane-cleavable chimeric protein where the secretable effector molecule is an anti-VEGF antibody, an anti-VEGF peptide, or a combination thereof. In some embodiments, a cell is engineered to produce at least one membrane-cleavable chimeric protein where the secretable effector molecule is an anti-PD-1 antibody, an anti-PD-L1 antibody, an anti-PD-L2 antibody, an anti-CTLA-4 antibody, an anti-LAG-3 antibody, an anti-TIM-3 antibody, an anti-TIGIT antibody, an anti-VISTA antibody, an anti-KIR antibody, an anti-B7-H3 antibody, an anti-B7-H4 antibody, an anti-HVEM antibody, an anti-BTLA antibody, an anti-GAL9 antibody, an anti-A2AR antibody, an anti-

phosphatidylserine antibody, an anti-CD27 antibody, an anti-TNF α antibody, an anti-TREMI antibody, or an anti-TREM2 antibody.

In some embodiments, a cell is engineered to produce at least one membrane-cleavable chimeric protein where the secretable effector molecule (*e.g.*, “S” in the formula **S – C – MT** or **MT – C – S**) comprises IL-15. In some embodiments, a cell is engineered to produce at least one membrane-cleavable chimeric protein where the secretable effector molecule is a fusion of IL-15 and the sushi domain of IL-15R α . In some embodiments, a cell is engineered to produce at least one membrane-cleavable chimeric protein where the secretable effector consists of IL-15. In some embodiments, a cell is engineered to produce at least one membrane-cleavable chimeric protein where the secretable effector molecule is IL-15 and the cell is further engineered to produce one or more additional effector molecules. In some embodiments, a cell is engineered to produce at least one membrane-cleavable chimeric protein where the secretable effector molecule is IL-15 and the cell is further engineered to produce one or more additional secretable effector molecules. In some embodiments, a cell is engineered to produce at least one membrane-cleavable chimeric protein where the secretable effector molecule is IL-15 and the cell is further engineered to produce a cytokine, a chemokine, a homing molecule, a growth factor, a co-activation molecule, a tumor microenvironment modifier, a ligand, an antibody, a polynucleotide, a peptide, or an enzyme. In some embodiments, a cell is engineered to produce at least one membrane-cleavable chimeric protein where the secretable effector molecule is IL-15 and the cell is further engineered to produce IL-12, IFN- γ , IL-2, IL-7, IL-36 γ , IL-18, IL-1 β , OX40-ligand, or CD40L. In some embodiments, a cell is engineered to produce at least one membrane-cleavable chimeric protein where the secretable effector molecule is IL-15 and the cell is further engineered to produce IL-12. In some embodiments, a cell is engineered to produce at least one membrane-cleavable chimeric protein where the secretable effector molecule is IL-15 and the cell is further engineered to produce IFN- γ . In some embodiments, a cell is engineered to produce at least one membrane-cleavable chimeric protein where the secretable effector molecule is IL-15 and the cell is further engineered to produce IL-2. In some embodiments, a cell is engineered to produce at least one membrane-cleavable chimeric protein where the secretable effector molecule is IL-15 and the cell is further engineered to produce IL-7. In some embodiments, a cell is engineered to produce at least one membrane-cleavable chimeric protein where the secretable effector molecule is IL-15 and the cell is further engineered to produce IL-36 γ . In some embodiments, a cell is engineered to produce at least one membrane-cleavable chimeric protein where the secretable effector molecule is IL-15 and the cell is further engineered to produce IL-18. In some embodiments, a cell is engineered to

produce at least one membrane-cleavable chimeric protein where the secretable effector molecule is IL-15 and the cell is further engineered to produce IL-1 β . In some embodiments, a cell is engineered to produce at least one membrane-cleavable chimeric protein where the secretable effector molecule is IL-15 and the cell is further engineered to produce OX40-ligand.

- 5 In some embodiments, a cell is engineered to produce at least one membrane-cleavable chimeric protein where the secretable effector molecule is IL-15 and the cell is further engineered to produce CD40L.

10 In some embodiments, a cell is engineered to produce at least one membrane-cleavable chimeric protein and the cell is further engineered to produce one or more additional membrane-cleavable chimeric proteins.

- 15 In some embodiments, a cell is engineered to produce at least one membrane-cleavable chimeric protein where the secretable effector molecule (*e.g.*, “S” in the formula **S – C – MT** or **MT – C – S**) is IL-15 and the cell is further engineered to produce one or more additional membrane-cleavable chimeric proteins. In some embodiments, a cell is engineered to produce at least one membrane-cleavable chimeric protein where the secretable effector molecule is IL-15 and the cell is further engineered an additional membrane-cleavable chimeric protein where the additional secretable effector molecule is produce a cytokine, a chemokine, a homing molecule, a growth factor, a co-activation molecule, a tumor microenvironment modifier, a ligand, an antibody, a polynucleotide, a peptide, or an enzyme. In some embodiments, a cell is engineered to produce at least one membrane-cleavable chimeric protein where the secretable effector molecule is IL-15 and the cell is further engineered an additional membrane-cleavable chimeric protein where the additional secretable effector molecule is IL-12, IFN- γ , IL-2, IL-7, IL-36 γ , IL-18, IL-1 β , OX40-ligand, or CD40L. In some embodiments, a cell is engineered to produce at least one membrane-cleavable chimeric protein where the secretable effector molecule is IL-15 and the cell is further engineered an additional membrane-cleavable chimeric protein where the additional secretable effector molecule is IL-12. In some embodiments, a cell is engineered to produce at least one membrane-cleavable chimeric protein where the secretable effector molecule is IL-15 and the cell is further engineered an additional membrane-cleavable chimeric protein where the additional secretable effector molecule is IFN- γ . In some embodiments, a cell is engineered to produce at least one membrane-cleavable chimeric protein where the secretable effector molecule is IL-15 and the cell is further engineered an additional membrane-cleavable chimeric protein where the additional secretable effector molecule is IL-2. In some embodiments, a cell is engineered to produce at least one membrane-cleavable chimeric protein where the secretable effector molecule is IL-15 and the cell is further engineered an additional membrane-cleavable chimeric protein where the additional secretable effector molecule is IL-7.
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In some embodiments, a cell is engineered to produce at least one membrane-cleavable chimeric protein where the secretable effector molecule is IL-15 and the cell is further engineered an additional membrane-cleavable chimeric protein where the additional secretable effector molecule is IL-36 γ . In some embodiments, a cell is engineered to produce at least one

5 membrane-cleavable chimeric protein where the secretable effector molecule is IL-15 and the cell is further engineered an additional membrane-cleavable chimeric protein where the additional secretable effector molecule is IL-18. In some embodiments, a cell is engineered to produce at least one membrane-cleavable chimeric protein where the secretable effector molecule is IL-15 and the cell is further engineered an additional membrane-cleavable chimeric

10 protein where the additional secretable effector molecule is IL-1 β . In some embodiments, a cell is engineered to produce at least one membrane-cleavable chimeric protein where the secretable effector molecule is IL-15 and the cell is further engineered an additional membrane-cleavable chimeric protein where the additional secretable effector molecule is OX40-ligand. In some embodiments, a cell is engineered to produce at least one membrane-cleavable chimeric protein

15 where the secretable effector molecule is IL-15 and the cell is further engineered an additional membrane-cleavable chimeric protein where the additional secretable effector molecule is CD40L.

A cell can also be further engineered to express additional proteins in addition to the chimeric proteins (*e.g.*, the membrane-cleavable chimeric proteins having the formula **S – C –**

20 **MT** or **MT – C – S** described herein), proteins of interest, or effector molecules described herein. A cell can be further engineered to express one or more antigen recognizing receptors. Examples of antigens that may be targeted by one or more antigen recognizing receptors include, but are not limited to, 5T4, ADAM9, AFP, AXL, B7-H3, B7-H4, B7-H6, BCMA, C4.4, CA6, Cadherin 3, Cadherin 6, CCR4, CD19, CD20, CD22, CD123, CD133, CD138, CD142,

25 CD166, CD25, CD30, CD33, CD352, CD37, CD38, CD44, CD56, CD66e, CD70, CD71, CD74, CD79b, CD80, CEA, CEACAM5, Claudin18.2, cMet, CSPG4, CTLA, DLK1, DLL3, DR5, EGFR, ENPP3, EpCAM, EphA2, Ephrin A4, ETBR, FGFR2, FGFR3, FLT3, FR α , FRb, GCC, GD2, GFR α 4, gpA33, GPC2, GPC3, gpNBM, GPRC5, HER2, IL-13R, IL-13Ra, IL-13Ra2, IL-8, IL-15, IL1RAP, Integrin α V, KIT, L1CAM, LAMP1, Lewis Y, LeY, LIV-1,

30 LRRC, LY6E, MCSP, Mesothelin, MUC1, MUC16, MUC1C, NaPi2B, Nectin 4, NKG2D, NOTCH3, NY ESO 1, Ovarin, P-cadherin, pan-Erb2, PSCA, PSMA, PTK7, ROR1, S Aures, SCT, SLAMF7, SLITRK6, SSTR2, STEAP1, Survivin, TDGF1, TIM1, TROP2, and WT1.

An antigen recognizing receptor can include an antigen-binding domain, such as an antibody, an antigen-binding fragment of an antibody, a F(ab) fragment, a F(ab') fragment, a

35 single chain variable fragment (scFv), or a single-domain antibody (sdAb). An antigen

recognizing receptors can include an scFv. An scFv can include a heavy chain variable domain (VH) and a light chain variable domain (VL), which can be separated by a peptide linker. For example, an scFv can include the structure VH-L-VL or VL-L-VH, wherein VH is the heavy chain variable domain, L is the peptide linker, and VL is the light chain variable domain.

5 An antigen recognizing receptor can be a chimeric antigen receptor (CAR). A CAR can have one or more intracellular signaling domains, such as a CD3zeta-chain intracellular signaling domain, a CD97 intracellular signaling domain, a CD11a-CD18 intracellular signaling domain, a CD2 intracellular signaling domain, an ICOS intracellular signaling domain, a CD27 intracellular signaling domain, a CD154 intracellular signaling domain, a CD8 intracellular signaling domain, an OX40 intracellular signaling domain, a 4-1BB intracellular signaling domain, a CD28 intracellular signaling domain, a ZAP40 intracellular signaling domain, a CD30 intracellular signaling domain, a GITR intracellular signaling domain, an HVEM intracellular signaling domain, a DAP10 intracellular signaling domain, a DAP12 intracellular signaling domain, a MyD88 intracellular signaling domain, fragments thereof, combinations thereof, or combinations of fragments thereof. A CAR can have a transmembrane domain, such as a CD8 transmembrane domain, a CD28 transmembrane domain, a CD3zeta-chain transmembrane domain, a CD4 transmembrane domain, a 4-1BB transmembrane domain, an OX40 transmembrane domain, an ICOS transmembrane domain, a CTLA-4 transmembrane domain, a PD-1 transmembrane domain, a LAG-3 transmembrane domain, a 2B4 transmembrane domain, a BTLA transmembrane domain, fragments thereof, combinations thereof, or combinations of fragments thereof. A CAR can have a spacer region between the antigen-binding domain and the transmembrane domain.

An antigen recognizing receptor can be a T cell receptor (TCR).

25 **Engineered Cell Types**

Also provided herein are engineered cells. Cells can be engineered to comprise any of the engineered nucleic acids described herein (*e.g.*, any of the multicistronic systems encoding each of the membrane-cleavable chimeric proteins, the bivalent aCARs, and the iCARs described herein). Cells can be engineered to possess any of the features of any of the engineered cells described herein. In a particular aspect, provided herein are cells engineered to produce each of (i) a membrane-cleavable chimeric protein; (ii) a bivalent aCAR; and (iii) an iCAR. In a particular aspect, provided herein are cells engineered to produce two or more chimeric proteins. In a particular aspect, provided herein are cells engineered to produce two or more of the chimeric proteins described herein, where each chimeric protein is a different protein of interest

or effector molecule. In a particular aspect, provided herein are cells engineered to produce any of the chimeric proteins described herein and engineered to separately produce a different effector molecule or protein of interest (*e.g.*, a homing molecule, antigen receptor, etc.).

The engineered cells can be an immune cell, including but not limited to, a T cell, a CD8+ T cell, a CD4+ T cell, a gamma-delta ($\gamma\delta$) T cell, a cytotoxic T lymphocyte (CTL), a regulatory T cell, a viral-specific T cell, a Natural Killer T (NKT) cell, a Natural Killer (NK) cell, a B cell, a tumor-infiltrating lymphocyte (TIL), an innate lymphoid cell, a mast cell, an eosinophil, a basophil, a neutrophil, a myeloid cell, a macrophage, a monocyte, or a dendritic cell. The engineered cells can be a T cell. The engineered cells can be an NK cell. Cells engineered to express a chimeric antigen receptor (CAR) are also referred to as CAR cells. For example, T cells engineered to express a CAR are also referred to as CAR-T cells and likewise NK cells engineered to express a CAR are also referred to as CAR-NK cells.

The engineered cells can be a stem cell, including but not limited to, a human embryonic stem cell (ESC), an ESC-derived cell, a pluripotent stem cell, a mesenchymal stromal cell (MSC), an induced pluripotent stem cell (iPSC), or an iPSC-derived cell.

The engineered cells can be tumor-derived cells. Examples of tumor cells include, but are not limited to, a bladder tumor cell, a brain tumor cell, a breast tumor cell, a cervical tumor cell, a colorectal tumor cell, an esophageal tumor cell, a glioma cell, a kidney tumor cell, a liver tumor cell, a lung tumor cell, a melanoma cell, an ovarian tumor cell, a pancreatic tumor cell, a prostate tumor cell, a skin tumor cell, a thyroid tumor cell, and a uterine tumor cell.

A cell can be engineered to produce the chimeric proteins using methods known to those skilled in the art. For example, cells can be transduced to engineer the tumor. In an embodiment, the cell is transduced using a virus.

In a particular embodiment, the cell is transduced using an oncolytic virus. Examples of oncolytic viruses include, but are not limited to, an oncolytic herpes simplex virus, an oncolytic adenovirus, an oncolytic measles virus, an oncolytic influenza virus, an oncolytic Indiana vesiculovirus, an oncolytic Newcastle disease virus, an oncolytic vaccinia virus, an oncolytic poliovirus, an oncolytic myxoma virus, an oncolytic reovirus, an oncolytic mumps virus, an oncolytic Maraba virus, an oncolytic rabies virus, an oncolytic rotavirus, an oncolytic hepatitis virus, an oncolytic rubella virus, an oncolytic dengue virus, an oncolytic chikungunya virus, an oncolytic respiratory syncytial virus, an oncolytic lymphocytic choriomeningitis virus, an oncolytic morbillivirus, an oncolytic lentivirus, an oncolytic replicating retrovirus, an oncolytic rhabdovirus, an oncolytic Seneca Valley virus, an oncolytic sindbis virus, and any variant or derivative thereof.

The virus, including any of the oncolytic viruses described herein, can be a recombinant virus that encodes one more transgenes encoding one or more chimeric proteins, such as any of the engineered nucleic acids described herein. The virus, including any of the oncolytic viruses described herein, can be a recombinant virus that encodes one more transgenes encoding one or more of the two or more chimeric proteins, such as any of the engineered nucleic acids described herein.

Also provided herein are engineered erythrocytes. Erythrocytes can be engineered to comprise any of the engineered nucleic acids described herein. Erythrocytes can be engineered to possess any of the features of any of the engineered cells described herein. In a particular aspect, provided herein are erythrocytes engineered to produce one or more of the chimeric proteins described herein. In a particular aspect, provided herein are erythrocytes engineered to produce two or more of the chimeric proteins described herein.

Also provided herein are engineered platelet cells. Platelet cells can be engineered to comprise any of the engineered nucleic acids described herein. Platelet cells can be engineered to possess any of the features of any of the engineered cells described herein. In a particular aspect, provided herein are platelet cells engineered to produce one or more of the chimeric proteins described herein. In a particular aspect, provided herein are platelet cells engineered to produce two or more of the chimeric proteins described herein.

Also provided herein are engineered bacterial cells. Bacterial cells can be engineered to comprise any of the engineered nucleic acids described herein. Bacterial cells can be engineered to possess any of the features of any of the engineered cells described herein. In a particular aspect, provided herein are bacterial cells engineered to produce two or more of the chimeric proteins described herein. Bacterial cells can be engineered to produce one or more mammalian-derived chimeric proteins. Bacterial cells can be engineered to produce two or more mammalian-derived chimeric proteins. Examples of bacterial cells include, but are not limited to, *Clostridium beijerinckii*, *Clostridium sporogenes*, *Clostridium novyi*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Listeria monocytogenes*, *Salmonella typhimurium*, and *Salmonella choleraesuis*.

An engineered cell can be a human cell. An engineered cell can be a human primary cell. An engineered primary cell can be a tumor infiltrating primary cell. An engineered primary cell can be a primary T cell. An engineered primary cell can be a hematopoietic stem cell (HSC). An engineered primary cell can be a natural killer (NK) cell. An engineered primary cell can be any somatic cell. An engineered primary cell can be a MSC. Human cells (*e.g.*, immune cells) can be engineered to comprise any of the engineered nucleic acids described herein. Human cells (*e.g.*, immune cells) can be engineered to possess any of the features of any of the engineered cells

described herein. In a particular aspect, provided herein are human cells (*e.g.*, immune cells) engineered to produce one or more of the chimeric proteins described herein. In a particular aspect, provided herein are human cells (*e.g.*, immune cells) engineered to produce two or more of the chimeric proteins described herein.

5 An engineered cell can be isolated from a subject (autologous), such as a subject known or suspected to have cancer. Cell isolation methods are known to those skilled in the art and include, but are not limited to, sorting techniques based on cell-surface marker expression, such as FACS sorting, positive isolation techniques, and negative isolation, magnetic isolation, and combinations thereof. An engineered cell can be allogenic with reference to the subject being
10 administered a treatment. Allogenic modified cells can be HLA-matched to the subject being administered a treatment. An engineered cell can be a cultured cell, such as an *ex vivo* cultured cell. An engineered cell can be an *ex vivo* cultured cell, such as a primary cell isolated from a subject. A cultured cell can be cultured with one or more cytokines.

Also provided herein are methods that include culturing the engineered cells of the
15 present disclosure. Methods of culturing the engineered cells described herein are known. One skilled in the art will recognize that culturing conditions will depend on the particular engineered cell of interest. One skilled in the art will recognize that culturing conditions will depend on the specific downstream use of the engineered cell, for example, specific culturing conditions for subsequent administration of the engineered cell to a subject.

20

Methods of Engineering Cells

Also provided herein are compositions and methods for engineering cells to produce one or more proteins of interest or effector molecules (*e.g.*, the multicistronic expression systems that encode each of (i) a membrane-cleavable chimeric protein; (ii) a bivalent aCAR; and (iii) an
25 iCAR described herein).

In general, cells are engineered to produce proteins of interest or effector molecules through introduction (*i.e.*, delivery) of polynucleotides encoding the one or more proteins of interest or effector molecules, *e.g.*, the chimeric proteins described herein including the protein of interest or effector molecule, into the cell's cytosol and/or nucleus. For example, the
30 polynucleotides encoding the one or more chimeric proteins can be any of the multicistronic expression systems that encode each of (i) a membrane-cleavable chimeric protein; (ii) a bivalent aCAR; and (iii) an iCAR described herein. Delivery methods include, but are not limited to, viral-mediated delivery, lipid-mediated transfection, nanoparticle delivery, electroporation, sonication, and cell membrane deformation by physical means. One skilled in

the art will appreciate the choice of delivery method can depend on the specific cell type to be engineered.

Viral-Mediated Delivery

5 Viral vector-based delivery platforms can be used to engineer cells. In general, a viral vector-based delivery platform engineers a cell through introducing (*i.e.*, delivering) into a host cell. For example, a viral vector-based delivery platform can engineer a cell through introducing any of the engineered nucleic acids described herein (*e.g.*, any of the multicistronic systems encoding each of each of (i) a membrane-cleavable chimeric protein; (ii) a bivalent aCAR; and
10 (iii) an iCAR described herein, and/or any of the expression cassettes described herein containing a promoter and an exogenous polynucleotide sequence encoding the chimeric proteins, oriented from N-terminal to C-terminal). A viral vector-based delivery platform can be a nucleic acid, and as such, an engineered nucleic acid can also encompass an engineered virally-derived nucleic acid. Such engineered virally-derived nucleic acids can also be referred
15 to as recombinant viruses or engineered viruses.

 A viral vector-based delivery platform can encode more than one engineered nucleic acid, gene, or transgene within the same nucleic acid. For example, an engineered virally-derived nucleic acid, *e.g.*, a recombinant virus or an engineered virus, can encode one or more transgenes, including, but not limited to, any of the engineered nucleic acids described herein
20 that encode one or more of the chimeric proteins described herein. The one or more transgenes encoding the one or more chimeric proteins can be configured to express the one or more chimeric proteins and/or other protein of interest. A viral vector-based delivery platform can encode one or more genes in addition to the one or more transgenes (*e.g.*, transgenes encoding the one or more chimeric proteins and/or other protein of interest), such as viral genes needed
25 for viral infectivity and/or viral production (*e.g.*, capsid proteins, envelope proteins, viral polymerases, viral transcriptases, *etc.*), referred to as cis-acting elements or genes.

 A viral vector-based delivery platform can comprise more than one viral vector, such as separate viral vectors encoding the engineered nucleic acids, genes, or transgenes described herein, and referred to as trans-acting elements or genes. For example, a helper-dependent viral
30 vector-based delivery platform can provide additional genes needed for viral infectivity and/or viral production on one or more additional separate vectors in addition to the vector encoding the one or more chimeric proteins and/or other protein of interest. One viral vector can deliver more than one engineered nucleic acids, such as one vector that delivers engineered nucleic acids that are configured to produce two or more chimeric proteins and/or other protein of

interest. More than one viral vector can deliver more than one engineered nucleic acids, such as more than one vector that delivers one or more engineered nucleic acid configured to produce one or more chimeric proteins and/or other protein of interest. The number of viral vectors used can depend on the packaging capacity of the above mentioned viral vector-based vaccine

5 platforms, and one skilled in the art can select the appropriate number of viral vectors.

In general, any of the viral vector-based systems can be used for the *in vitro* production of molecules, such as the chimeric proteins, effector molecules, and/or other protein of interest described herein, or used *in vivo* and *ex vivo* gene therapy procedures, *e.g.*, for *in vivo* delivery of the engineered nucleic acids encoding one or more chimeric proteins and/or other protein of
10 interest. The selection of an appropriate viral vector-based system will depend on a variety of factors, such as cargo/payload size, immunogenicity of the viral system, target cell of interest, gene expression strength and timing, and other factors appreciated by one skilled in the art.

Viral vector-based delivery platforms can be RNA-based viruses or DNA-based viruses. Exemplary viral vector-based delivery platforms include, but are not limited to, a herpes simplex
15 virus, a adenovirus, a measles virus, an influenza virus, a Indiana vesiculovirus, a Newcastle disease virus, a vaccinia virus, a poliovirus, a myxoma virus, a reovirus, a mumps virus, a Maraba virus, a rabies virus, a rotavirus, a hepatitis virus, a rubella virus, a dengue virus, a chikungunya virus, a respiratory syncytial virus, a lymphocytic choriomeningitis virus, a morbillivirus, a lentivirus, a replicating retrovirus, a rhabdovirus, a Seneca Valley virus, a
20 sindbis virus, and any variant or derivative thereof. Other exemplary viral vector-based delivery platforms are described in the art, such as vaccinia, fowlpox, self-replicating alphavirus, marabavirus, adenovirus (*See, e.g.,* Tatsis *et al.*, Adenoviruses, *Molecular Therapy* (2004) 10, 616—629), or lentivirus, including but not limited to second, third or hybrid second/third generation lentivirus and recombinant lentivirus of any generation designed to target specific
25 cell types or receptors (*See, e.g.,* Hu *et al.*, Immunization Delivered by Lentiviral Vectors for Cancer and Infectious Diseases, *Immunol Rev.* (2011) 239(1): 45-61, Sakuman *et al.*, Lentiviral vectors: basic to translational, *Biochem J.* (2012) 443(3):603-18, Cooper *et al.*, Rescue of splicing-mediated intron loss maximizes expression in lentiviral vectors containing the human ubiquitin C promoter, *Nucl. Acids Res.* (2015) 43 (1): 682-690, Zufferey *et al.*, Self-Inactivating
30 Lentivirus Vector for Safe and Efficient *In vivo* Gene Delivery, *J. Virol.* (1998) 72 (12): 9873-9880).

The sequences may be preceded with one or more sequences targeting a subcellular compartment. Upon introduction (*i.e.* delivery) into a host cell, infected cells (*i.e.*, an engineered
35 cell) can express the chimeric proteins and/or other protein of interest. Vaccinia vectors and methods useful in immunization protocols are described in, *e.g.*, U.S. Pat. No. 4,722,848.

Another vector is BCG (Bacille Calmette Guerin). BCG vectors are described in Stover *et al.* (Nature 351:456-460 (1991)). A wide variety of other vectors useful for the introduction (*i.e.*, delivery) of engineered nucleic acids, *e.g.*, Salmonella typhi vectors, and the like will be apparent to those skilled in the art from the description herein.

5 The viral vector-based delivery platforms can be a virus that targets a cell, herein referred to as an oncolytic virus. Examples of oncolytic viruses include, but are not limited to, an oncolytic herpes simplex virus, an oncolytic adenovirus, an oncolytic measles virus, an oncolytic influenza virus, an oncolytic Indiana vesiculovirus, an oncolytic Newcastle disease virus, an oncolytic vaccinia virus, an oncolytic poliovirus, an oncolytic myxoma virus, an oncolytic
10 reovirus, an oncolytic mumps virus, an oncolytic Maraba virus, an oncolytic rabies virus, an oncolytic rotavirus, an oncolytic hepatitis virus, an oncolytic rubella virus, an oncolytic dengue virus, an oncolytic chikungunya virus, an oncolytic respiratory syncytial virus, an oncolytic lymphocytic choriomeningitis virus, an oncolytic morbillivirus, an oncolytic lentivirus, an oncolytic replicating retrovirus, an oncolytic rhabdovirus, an oncolytic Seneca Valley virus, an
15 oncolytic sindbis virus, and any variant or derivative thereof. Any of the oncolytic viruses described herein can be a recombinant oncolytic virus comprising one more transgenes (*e.g.*, an engineered nucleic acid) encoding one or more chimeric proteins and/or other protein of interest. The transgenes encoding the one or more chimeric proteins and/or other protein of interest can be configured to express the chimeric proteins and/or other protein of interest.

20 The viral vector-based delivery platform can be retrovirus-based. In general, retroviral vectors are comprised of cis-acting long terminal repeats with packaging capacity for up to 6-10 kb of foreign sequence. The minimum cis-acting LTRs are sufficient for replication and packaging of the vectors, which are then used to integrate the one or more engineered nucleic acids (*e.g.*, transgenes encoding the one or more chimeric proteins and/or other protein of
25 interest) into the target cell to provide permanent transgene expression. Retroviral-based delivery systems include, but are not limited to, those based upon murine leukemia virus (MuLV), gibbon ape leukemia virus (GaLV), Simian Immuno deficiency vims (SIV), human immuno deficiency vims (HIV), and combinations thereof (see, *e.g.*, Buchscher *et al.*, J. Virol. 66:2731-2739 (1992); Johann *et al.*, J. Virol. 66:1635-1640 (1992); Sommerfelt *et al.*, Virol.
30 176:58-59 (1990); Wilson *et al.*, J. Virol. 63:2374-2378 (1989); Miller *et al.*, J. Virol. 65:2220-2224 (1991); PCT/US94/05700). Other retroviral systems include the Phoenix retrovirus system.

 The viral vector-based delivery platform can be lentivirus-based. In general, lentiviral vectors are retroviral vectors that are able to transduce or infect non-dividing cells and typically produce high viral titers. Lentiviral-based delivery platforms can be HIV-based, such as
35 ViraPower systems (ThermoFisher) or pLenti systems (Cell Biolabs). Lentiviral-based delivery

platforms can be SIV, or FIV-based. Other exemplary lentivirus-based delivery platforms are described in more detail in U.S. Pat. Nos. 7,311,907; 7,262,049; 7,250,299; 7,226,780; 7,220,578; 7,211,247; 7,160,721; 7,078,031; 7,070,993; 7,056,699; 6,955,919, each herein incorporated by reference for all purposes.

5 The viral vector-based delivery platform can be adenovirus-based. In general, adenoviral based vectors are capable of very high transduction efficiency in many cell types, do not require cell division, achieve high titer and levels of expression, and can be produced in large quantities in a relatively simple system. In general, adenoviruses can be used for transient expression of a transgene within an infected cell since adenoviruses do not typically integrate into a host's
10 genome. Adenovirus-based delivery platforms are described in more detail in Li *et al.*, Invest Ophthalmol Vis Sci 35:2543-2549, 1994; Borrás *et al.*, Gene Ther 6:515-524, 1999; Li and Davidson, PNAS 92:7700-7704, 1995; Sakamoto *et al.*, Hum Gene Ther 5:1088-1097, 1999; WO 94/12649, WO 93/03769; WO 93/19191; WO 94/28938; WO 95/11984 and WO 95/00655, each herein incorporated by reference for all purposes. Other exemplary adenovirus-based delivery
15 platforms are described in more detail in U.S. Pat. Nos. 5,585,362; 6,083,716; 7,371,570; 7,348,178; 7,323,177; 7,319,033; 7,318,919; and 7,306,793 and International Patent Application WO96/13597, each herein incorporated by reference for all purposes.

 The viral vector-based delivery platform can be adeno-associated virus (AAV)-based. Adeno-associated virus ("AAV") vectors may be used to transduce cells with engineered nucleic
20 acids (*e.g.*, any of the engineered nucleic acids described herein). AAV systems can be used for the *in vitro* production of proteins of interest, such as the chimeric proteins described herein and/or effector molecules, or used *in vivo* and *ex vivo* gene therapy procedures, *e.g.*, for *in vivo* delivery of the engineered nucleic acids encoding one or more chimeric proteins and/or other protein of interest (see, *e.g.*, West *et al.*, Virology 160:38-47 (1987); U.S. Pat. Nos. 4,797,368;
25 5,436,146; 6,632,670; 6,642,051; 7,078,387; 7,314,912; 6,498,244; 7,906,111; US patent publications US 2003-0138772, US 2007/0036760, and US 2009/0197338; Gao, *et al.*, J. Virol., 78(12):6381-6388 (June 2004); Gao, *et al.*, Proc Natl Acad Sci USA, 100(10):6081-6086 (May 13, 2003); and International Patent applications WO 2010/138263 and WO 93/24641; Kotin, Human Gene Therapy 5:793-801 (1994); Muzyczka, J. Clin. Invest. 94:1351 (1994), each herein
30 incorporated by reference for all purposes). Exemplary methods for constructing recombinant AAV vectors are described in more detail in U.S. Pat. No. 5,173,414; Tratschin *et al.*, Mol. Cell. Biol. 5:3251-3260 (1985); Tratschin, *et al.*, Mol. Cell. Biol. 4:2072-2081 (1984); Hermonat & Muzyczka, PNAS 81:6466-6470 (1984); and Samuiski *et al.*, J. Virol. 63:3822-3828 (1989), each herein incorporated by reference for all purposes. In general, an AAV-based vector
35 comprises a capsid protein having an amino acid sequence corresponding to any one of AAV1,

AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV.Rh10, AAV11 and variants thereof. In particular examples, an AAV-based vector has a capsid protein having an amino acid sequence corresponding to AAV2. In particular examples, an AAV-based vector has a capsid protein having an amino acid sequence corresponding to AAV8.

- 5 AAV vectors can be engineered to have any of the exogenous polynucleotide sequences encoding the membrane-cleavable chimeric proteins described herein having the formula: **S – C – MT** or **MT – C – S**.

The viral vector-based delivery platform can be a virus-like particle (VLP) platform. In general, VLPs are constructed by producing viral structural proteins and purifying resulting viral particles. Then, following purification, a cargo/payload (*e.g.*, any of the engineered nucleic acids described herein) is encapsulated within the purified particle *ex vivo*. Accordingly, production of VLPs maintains separation of the nucleic acids encoding viral structural proteins and the nucleic acids encoding the cargo/payload. The viral structural proteins used in VLP production can be produced in a variety of expression systems, including mammalian, yeast, insect, bacterial, or *in vivo* translation expression systems. The purified viral particles can be denatured and reformed in the presence of the desired cargo to produce VLPs using methods known to those skilled in the art. Production of VLPs are described in more detail in Seow *et al.* (Mol Ther. 2009 May; 17(5): 767–777), herein incorporated by reference for all purposes.

The viral vector-based delivery platform can be engineered to target (*i.e.*, infect) a range of cells, target a narrow subset of cells, or target a specific cell. In general, the envelope protein chosen for the viral vector-based delivery platform will determine the viral tropism. The virus used in the viral vector-based delivery platform can be pseudotyped to target a specific cell of interest. The viral vector-based delivery platform can be pantropic and infect a range of cells. For example, pantropic viral vector-based delivery platforms can include the VSV-G envelope. The viral vector-based delivery platform can be amphotropic and infect mammalian cells. Accordingly, one skilled in the art can select the appropriate tropism, pseudotype, and/or envelope protein for targeting a desired cell type.

Other Engineering Delivery Systems

30 Various additional means to introduce engineered nucleic acids (*e.g.*, any of the engineered nucleic acids described herein) into a cell or other target recipient entity are known to those skilled in the art, such as lipid-mediated delivery systems, nanoparticle/nanomaterial delivery systems, genomic editing systems, electroporation, sonication, gene gun, hydrodynamic injection, and cell membrane deformation by physical means, including exemplary versions of

those systems described in International Application Pub. No. WO2023205614, which is hereby incorporated by reference for all purposes.

Delivery Vehicles

5 Also provided herein are compositions for delivering a cargo/payload (a “delivery vehicle”).

 The cargo can comprise nucleic acids (*e.g.*, any of the engineered nucleic acids described herein, such as any of the multicistronic systems encoding each of (i) a membrane-cleavable chimeric protein; (ii) a bivalent aCAR; and (iii) an iCAR described herein), as described above.

10 The cargo can comprise proteins, carbohydrates, lipids, small molecules, and/or combinations thereof. The cargo can be any of the chimeric proteins provided for herein (*e.g.*, any of the multicistronic systems encoding each of (i) a membrane-cleavable chimeric protein; (ii) a bivalent aCAR; and (iii) an iCAR described herein). The cargo can be a combination of the chimeric proteins described herein, *e.g.*, two or more of the chimeric proteins described herein.

15 The cargo can be a combination of the chimeric protein described herein and another cargo of interest, such as another protein, carbohydrate, lipid, small molecule, and/or combination thereof.

 The delivery vehicle can comprise any composition suitable for delivering a cargo. The delivery vehicle can comprise any composition suitable for delivering a protein (*e.g.*, any of the chimeric proteins described herein). The delivery vehicle can be any of the lipid structure delivery systems described herein. For example, a delivery vehicle can be a lipid-based structure including, but not limited to, a lipid-based nanoparticle, a liposome, a micelle, an exosome, a vesicle, an extracellular vesicle, a cell, or a tissue. The delivery vehicle can be any of the nanoparticles described herein, such as nanoparticles comprising lipids (as previously

20 described), inorganic nanomaterials, and other polymeric materials.

 The delivery vehicle can be capable of delivering the cargo to a cell, such as delivering any of the chimeric proteins described herein to a cell. The delivery vehicle can be capable of delivering the cargo to a cell, such as delivering any of the chimeric proteins described herein to a cell. The delivery vehicle can be configured to target a specific cell, such as configured with a re-directing antibody to target a specific cell. The delivery vehicle can be capable of delivering

30 the cargo to a cell *in vivo*.

 The delivery vehicle can be capable of delivering the cargo to a tissue or tissue environment (*e.g.*, a tumor microenvironment), such as delivering any of the chimeric proteins described herein to a tissue or tissue environment *in vivo*. Delivering a cargo can include

secreting the cargo, such as secreting any of the chimeric proteins described herein.

Accordingly, the delivery vehicle can be capable of secreting the cargo, such as secreting any of the chimeric proteins described herein. The delivery vehicle can be capable of secreting the cargo to a tissue or tissue environment (*e.g.*, a tumor microenvironment), such as secreting any of the chimeric proteins described herein into a tissue or tissue environment. The delivery vehicle can be configured to target a specific tissue or tissue environment (*e.g.*, a tumor microenvironment), such as configured with a re-directing antibody to target a specific tissue or tissue environment.

10 Enumerated Embodiments

Embodiment 1 A method of treating a subject known or suspected of having cancer, the method comprising: administering a cell therapy to the subject, wherein the cell therapy comprises an immunoresponsive cell engineered to express a recombinant cytokine, wherein the immunoresponsive cell engineered to express the recombinant cytokine comprises an exogenous polynucleotide encoding a controlled release cytokine, oriented from N-terminal to C-terminal, having the formula: **S – C – MT** or **MT – C – S**, wherein S comprises a secretable effector molecule, wherein the secretable effector molecule comprises the recombinant cytokine, C comprises a protease cleavage site, and MT comprises a cell membrane tethering domain, wherein **S – C – MT** or **MT – C – S** is configured to be expressed as a single polypeptide; and administering a pharmaceutically effective amount of IL-2.

Embodiment 2 The method of embodiment 1, wherein the immunoresponsive cell comprises an exogenous polynucleotide encoding an inhibitory chimeric antigen receptor (iCAR) comprising: an antigen-binding domain specific for endomucin (EMCN); one or more intracellular inhibitory domains that inhibit an immune response; and one or more polypeptides selected from the group consisting of: a signal peptide, a transmembrane domain, a hinge domain, a spacer region, one or more peptide linkers, and combinations thereof.

Embodiment 3 The method of any one of embodiments 1-2, wherein the immunoresponsive cell comprises an exogenous polynucleotide encoding a bivalent activating chimeric antigen receptor (aCAR) comprising: an antigen-binding domain specific for FLT3; an antigen-binding domain specific for CD33; one or more intracellular signaling domains that stimulate an immune response; and one or more polypeptides selected from the group consisting of: a signal peptide, a transmembrane domain, a hinge domain, a spacer region, one or more peptide linkers, and combinations thereof.

Embodiment 4 A method of treating a subject known or suspected of having cancer, the method comprising: administering a cell therapy to the subject, wherein the cell therapy

comprises an immunoresponsive cell engineered to express a recombinant cytokine; and administering a pharmaceutically effective amount of IL-2.

- Embodiment 5** The method of any one of embodiments 1-4, wherein the immunoresponsive cell is engineered to express the recombinant cytokine is from a multicistronic expression system.
- Embodiment 6** The method of any one of embodiments 1-5, wherein the administration of IL-2 comprises multiple administrations.
- Embodiment 7** The method of embodiment 6, wherein the multiple administrations of IL-2 is at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, or at least 9 administrations.
- Embodiment 8** The method of any one of embodiments 1-7, wherein IL-2 is administered within 4, within 5, within 7, or within 8 hours following the start of the administration of the cell therapy.
- Embodiment 9** The method of embodiment 1-8, wherein IL-2 is administered within 4 to 8 hours following the administration of the cell therapy.
- Embodiment 10** The method of embodiment 1-8, wherein IL-2 is administered within 6 to 8 hours following the administration of the cell therapy.
- Embodiment 11** The method of embodiment 1-8, wherein IL-2 is administered within 4 hours following the administration of the cell therapy.
- Embodiment 12** The method of embodiment 1-8, wherein IL-2 is administered within 5 hours following the administration of the cell therapy.
- Embodiment 13** The method of embodiment 1-8, wherein IL-2 is administered within 6 hours following the administration of the cell therapy.
- Embodiment 14** The method of embodiment 1-8, wherein IL-2 is administered within 7 hours following the administration of the cell therapy.
- Embodiment 15** The method of embodiment 1-8, wherein IL-2 is administered within 8 hours following the administration of the cell therapy.
- Embodiment 16** The method of embodiment 1-8, wherein IL-2 is administered between 4 to 8 hours following the administration of the cell therapy.
- Embodiment 17** The method of embodiment 1-8, wherein IL-2 is administered between 6 to 8 hours following the administration of the cell therapy.
- Embodiment 18** The method of any one of embodiments 1-17, wherein IL-2 is administered within 24 or 48 hours after the administering of the cell therapy.
- Embodiment 19** The method of any one of embodiments 1-18, wherein the administration of the cell therapy comprises multiple administrations.

- Embodiment 20** The method of any one of embodiments 1-19, wherein IL-2 is administered at least 1, at least 2, a least 3 times a week following each administration of the cell therapy.
- 5 **Embodiment 21** The method of any one of embodiments 1-19, wherein IL-2 is administered at least 1 time a week following administration of each dose of a cell therapy.
- Embodiment 22** The method of any one of embodiments 1-19, wherein IL-2 is administered at least 2 times a week following administration of each dose of a cell therapy.
- Embodiment 23** The method of any one of embodiments 1-19, wherein IL-2 is administered at least 3 times a week following administration of each dose of a cell therapy.
- 10 **Embodiment 24** The method of any one of embodiments 1-23, wherein the cell therapy is administered again at least on day 7 and/or day 14 following the initial administration of the cell therapy.
- Embodiment 25** The method of any one of embodiments 1-23, wherein the cell therapy is administered again at least on day 7 following the initial administration of the cell therapy.
- 15 **Embodiment 26** The method of any one of embodiments 1-23, wherein the cell therapy is administered again at least on day 7 and day 14 following the initial administration of the cell therapy.
- Embodiment 27** The method of any one of embodiments 1-23, wherein the cell therapy and the pharmaceutically effective amount of IL-2 are administered again at least on day 7 and/or day 14 following the initial administration of the cell therapy.
- 20 **Embodiment 28** The method of any one of embodiments 1-19, wherein the cell therapy and the pharmaceutically effective amount of IL-2 are administered again at least on day 7 following the initial administration of the cell therapy
- Embodiment 29** The method of any one of embodiments 1-19, wherein the cell therapy and the pharmaceutically effective amount of IL-2 are administered again at least on day 7 and day 14 following the initial administration of the cell therapy.
- 25 **Embodiment 30** The method of any one of embodiments 1-29, wherein the pharmaceutically effective amount of IL-2 is between 1-10 MIU, optionally wherein the pharmaceutically effective amount of IL-2 is a dose of 3 MIU.
- 30 **Embodiment 31** The method of any one of embodiments 1-30, wherein the recombinant cytokine comprises IL-15 or a functional fragment thereof.
- Embodiment 32** The method of any one of embodiments 1-31, wherein the recombinant cytokine comprises crIL15.

Embodiment 33 The method of any one of embodiments 1-32, wherein the subject has, prior to administration of the cell therapy, been administered one or more lymphodepletion agents.

Embodiment 34 The method of embodiment 33, wherein the one or more lymphodepletion agents is administered 5 days prior to administration of the cell therapy.

Embodiment 35 The method of embodiments 33-34, wherein the one or more lymphodepletion agents is administered daily until administration of the cell therapy.

Embodiment 36 The method of embodiments 33-35, wherein the one or more lymphodepletion agents comprises fludarabine, cytarabine (Ara-C), or combinations thereof.

Embodiment 37 The method of embodiments 33-36, wherein the one or more lymphodepletion agents are administered intravenously.

Embodiment 38 The method of embodiments 33-37, wherein fludarabine is administered at a dose of 30 mg/m² and cytarabine (Ara-C) is administered at a dose of 2 g/m².

Embodiment 39 The method of any one of embodiments 1-38, wherein the method of treatment comprises a reduction in tumor volume in the subject.

Embodiment 40 The method of any one of embodiments 1-39, wherein the method of treatment comprises treatment of a solid tumor in the subject.

Embodiment 41 The method of any one of embodiments 1-40, wherein the method of treatment comprises inducing an immune response in the subject.

Embodiment 42 The method of any one of embodiments 1-41, wherein the immunoresponsive cell engineered to express the recombinant cytokine comprises an exogenous polynucleotide encoding a controlled release cytokine, oriented from N-terminal to C-terminal, having the formula:

S – C – MT or MT – C – S

wherein

S comprises a secretable effector molecule, wherein the secretable effector molecule comprises the recombinant cytokine,

C comprises a protease cleavage site, and

MT comprises a cell membrane tethering domain,

wherein **S – C – MT or MT – C – S** is configured to be expressed as a single polypeptide.

Embodiment 43 The method of any one of embodiments 1-42, wherein the immunoresponsive cell comprises an exogenous polynucleotide encoding an inhibitory chimeric antigen receptor (iCAR) comprising: an antigen-binding domain specific for endomucin (EMCN); one or more intracellular inhibitory domains that inhibit an immune response; and one

or more polypeptides selected from the group consisting of: a signal peptide, a transmembrane domain, a hinge domain, a spacer region, one or more peptide linkers, and combinations thereof.

Embodiment 44 The method of any one of embodiments 1-43, wherein the immunoresponsive cell comprises an exogenous polynucleotide encoding a bivalent activating chimeric antigen receptor (aCAR) comprising: an antigen-binding domain specific for FLT3; an antigen-binding domain specific for CD33; one or more intracellular signaling domains that stimulate an immune response; and one or more polypeptides selected from the group consisting of: a signal peptide, a transmembrane domain, a hinge domain, a spacer region, one or more peptide linkers, and combinations thereof.

10 **Embodiment 45** The method of any one of embodiments 1-44, wherein the immunoresponsive cell comprises an engineered nucleic acid comprising: an exogenous polynucleotide encoding a controlled release cytokine, oriented from N-terminal to C-terminal, having the formula: **S – C – MT** or **MT – C – S**, wherein **S** comprises a secretable effector molecule, wherein the secretable effector molecule comprises the recombinant cytokine, **C** comprises a protease cleavage site, and **MT** comprises a cell membrane tethering domain, wherein **S – C – MT** or **MT – C – S** is configured to be expressed as a single polypeptide; an exogenous polynucleotide encoding an inhibitory chimeric antigen receptor (iCAR) comprising: an antigen-binding domain specific for endomucin (EMCN); one or more intracellular inhibitory domains that inhibit an immune response; and one or more polypeptides selected from the group consisting of: a signal peptide, a transmembrane domain, a hinge domain, a spacer region, one or more peptide linkers, and combinations thereof; and an exogenous polynucleotide encoding a bivalent activating chimeric antigen receptor (aCAR) comprising: an antigen-binding domain specific for FLT3; an antigen-binding domain specific for CD33; one or more intracellular signaling domains that stimulate an immune response; and one or more polypeptides selected from the group consisting of: a signal peptide, a transmembrane domain, a hinge domain, a spacer region, one or more peptide linkers, and combinations thereof.

Embodiment 46 The multicistronic expression system of any one of embodiments 1-45, wherein the exogenous polynucleotides encoding each of the membrane-cleavable chimeric protein, the iCAR, and the bivalent aCAR are linked together by a polynucleotide linker encoding a 2A ribosome skipping element, optionally wherein the 2A ribosome skipping element is selected from the group consisting of: a T2A ribosome skipping element, a E2A ribosome skipping element, a P2A ribosome skipping element, a F2A ribosome skipping element, ribosome skipping element fusions thereof, and combinations thereof, optionally wherein the ribosome skipping element fusion comprises an E2A/T2A ribosome skipping element, optionally wherein the E2A/T2A ribosome skipping element comprises the amino acid sequence QCTNYALLKLAGDVESNPGPGSGEGRGSLLTCGDVEENPGP (SEQ ID NO: 221), optionally wherein the E2A/T2A ribosome skipping element is encoded by a polynucleotide sequence comprising the sequence

CAGTGTACCAACTACGCCCTGCTGAAACTGGCCGGCGACGTGGAATCTAATCCTGG
ACCTGGATCTGGCGAGGGACGCGGGAGTCTACTGACGTGTGGAGACGTGGAGGAA
AACCCTGGACCT (SEQ ID NO: 222) or the sequence

CAGTGCACAAATTATGCACTGCTGAAGCTCGCCGGGGATGTGCGAGAGTAACCCAGG
ACCTGGAAGCGGAGAAGGTCGTGGTAGTCTACTAACGTGTGGTGATGTAGAAGAAA
ATCCTGGACCT (SEQ ID NO: 223).

Embodiment 47 The multicistronic expression system of any one of embodiments 1-46, wherein the membrane-cleavable chimeric protein, the iCAR, and the bivalent aCAR are encoded in order from 5' to 3': (i) membrane-cleavable chimeric protein; (ii) bivalent aCAR; and (iii) iCAR; or from 5' to 3': (i) membrane-cleavable chimeric protein; (ii) iCAR; and (iii) bivalent aCAR.

Embodiment 48 The multicistronic expression system of any one of claims 1-47, wherein the secretable effector molecule comprises a signal peptide or a signal-anchor sequence, optionally wherein the signal peptide comprises a native signal peptide native to the secretable effector molecule, optionally wherein the signal peptide comprises a non-native signal peptide or the signal-anchor sequence comprises a non-native signal-anchor sequence non-native to the secretable effector molecule, optionally wherein the non-native signal peptide or the non-native signal-anchor sequence is selected from the group consisting of: IgE, IL-12, IL-2, optimized IL-2, trypsinogen-2, Gaussia luciferase, CD5, human IgKVII, murine IgKVII, VSV-G, prolactin, serum albumin preprotein, azurocidin preprotein, osteonectin, CD33, IL-6, IL-8, CCL2, TIMP2, VEGFB, osteoprotegerin, serpin E1, GROalpha, CXCL12, IL-21, CD8, NKG2D, TNFR2, GM-CSF, and GM-CSFRa, optionally wherein the non-native signal peptide comprises an IgE signal peptide, optionally wherein the IgE signal peptide comprises the amino acid sequence

MDWTWILFLVAAATRVHS (SEQ ID NO: 228), optionally wherein the IgE signal peptide is encoded by a polynucleotide sequence comprising the sequence

ATGGACTGGACTTGGATACTCTTTCTGGTCGCTGCCGCCACACGGGTGCACTCT
(SEQ ID NO: 229).

- 5 **Embodiment 49** The multicistronic expression system of any one of embodiments 1-48, wherein the protease cleavage site further comprises the N-terminal peptide linker, optionally selected from the group consisting of: SGGGSGGGGSG (SEQ ID NO: 230); GGGSGGGGSGGGSLQ (SEQ ID NO: 231); GGS (SEQ ID NO: 232); GGS (SEQ ID NO: 233); GGS (SEQ ID NO: 234); GGS (SEQ ID NO: 235);
- 10 GGS (SEQ ID NO: 236); GGS (SEQ ID NO: 237); GGS (SEQ ID NO: 238); GGS (SEQ ID NO: 239); GGS (SEQ ID NO: 240); GGS (SEQ ID NO: 241); GGS (SEQ ID NO: 242); GGS (SEQ ID NO: 243); GGS (SEQ ID NO: 244); GGS (SEQ ID NO: 245);
- 15 GGS (SEQ ID NO: 246); GSTSGSGKPGSGEGSTKG (SEQ ID NO: 247); and EAAAKEAAAKEAAAKEAAK (SEQ ID NO: 248), optionally wherein the protease cleavage site further comprises the N-terminal peptide linker SGGGSGGGGSG (SEQ ID NO: 230), optionally wherein the protease cleavage site further comprises a C-terminal peptide linker, optionally selected from the group consisting of: SGGGSGGGGSG (SEQ ID NO: 230); GGS (SEQ ID NO: 231);
- 20 GGS (SEQ ID NO: 232); GGS (SEQ ID NO: 233); GGS (SEQ ID NO: 234); GGS (SEQ ID NO: 235); GGS (SEQ ID NO: 236); GGS (SEQ ID NO: 237); GGS (SEQ ID NO: 238); GGS (SEQ ID NO: 239); GGS (SEQ ID NO: 240); GGS (SEQ ID NO: 241);
- 25 GGS (SEQ ID NO: 242); GGS (SEQ ID NO: 243); GGS (SEQ ID NO: 244); GGS (SEQ ID NO: 245); GGS (SEQ ID NO: 246); GSTSGSGKPGSGEGSTKG (SEQ ID NO: 247); and EAAAKEAAAKEAAAKEAAK (SEQ ID NO: 248), optionally wherein the protease cleavage site further comprises the C-terminal linker GGS (SEQ ID NO: 231), optionally wherein the protease cleavage site comprises a Tumor Necrosis Factor- α Converting Enzyme (TACE)-specific cleavage site, optionally wherein the TACE-specific cleavage site comprises the amino acid sequence VTPEIFSLI (SEQ ID NO: 191), optionally, wherein the TACE-specific cleavage site comprises the amino acid sequence SGGGSGGGGSGVTPEIFSLIGGGSGGGGSGGGSLQ
- 30 (SEQ ID NO: 250), optionally wherein the TACE-specific cleavage site is encoded by a
- 35

polynucleotide sequence comprising the sequence

TCAGGCGGCGGTGGTAGTGGAGGCGGAGGCTCAGGCGTGACCCCTGAGCCTATCTT
CAGCCTGATCGGCGGAGGTTCCGGAGGTGGCGGTTCCGGCGGAGGATCTCTTCAA
(SEQ ID NO: 251).

- 5 **Embodiment 50** The multicistronic expression system of any one of embodiments 1-49, wherein the cell membrane tethering domain comprises a transmembrane domain selected from the group consisting of: PDGFR-beta, CD8, CD28, CD3zeta-chain, CD4, 4-1BB, OX40, ICOS, CTLA-4, PD-1, LAG-3, 2B4, LNGFR, NKG2D, EpoR, TNFR2, LIR1, B7-1, and BTLA, optionally wherein the cell membrane tethering domain comprises a B7-1 transmembrane domain, optionally wherein the B7-1 transmembrane domain comprises the amino acid sequence

LLPSWAITLISVNGIFVICCLTYCFAPRCRERRRNERLRRESVRPV (SEQ ID NO: 204), optionally wherein the B7-1 transmembrane domain is encoded by a polynucleotide sequence comprising the sequence

- 10 TTGCTGCCTAGCTGGGCCATCACACTGATCTCCGTGAACGGCATCTTCGTGATCTGC
15 TGCCTGACCTACTGCTTCGCCCCCTAGATGCAGAGAGCGGAGAAGAAACGAGCGGCT
GAGAAGAGAAAGCGTGCGGCCTGTG (SEQ ID NO: 252), optionally wherein the

membrane-cleavable chimeric protein, oriented from N-terminal to C-terminal, has the formula **S – C – MT**, optionally wherein the membrane-cleavable chimeric protein comprises the amino acid sequence

- 20 MDWTWILFLVAAATRVHSNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAM
KCFLELQVISLES GDASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQS
FVHIVQMFINTSSGGGSGGGSGVTPPEIFSLIGGSGGGSGGGSLQLLPSWAITLISV
NGIFVICCLTYCFAPRCRERRRNERLRRESVRPV (SEQ ID NO: 226), optionally wherein
the membrane-cleavable chimeric protein is encoded by a polynucleotide sequence comprising
25 the sequence

- ATGGACTGGACTTGGATACTCTTTCTGGTCGCTGCCGCCACACGGGTGCACTCTAAT
TGGGTCAACGTGATCAGCGACCTGAAGAAGATCGAGGACCTGATCCAGAGCATGCA
CATCGACGCCACACTGTACACCGAGTCCGATGTGCACCCTAGCTGCAAAGTGACCG
CCATGAAGTGCTTTCTGCTGGAAGTGAAGTGATCAGCCTGGAAAGCGGCGACGCC
30 AGCATCCACGATACCGTGAAAATCTGATCATCCTGGCCAACAACAGCCTGTCCAG
CAACGGCAATGTGACCGAGAGCGGCTGCAAAGAGTGCGAGGAACTGGAAGAGAAG
AACATCAAAGAGTTTCTGCAGAGCTTCGTCCACATCGTGCAGATGTTTCATCAACACC
TCATCAGGCGGCGGTGGTAGTGGAGGCGGAGGCTCAGGCGTGACCCCTGAGCCTAT
CTTCAGCCTGATCGGCGGAGGTTCCGGAGGTGGCGGTTCCGGCGGAGGATCTCTTC
35 AATTGCTGCCTAGCTGGGCCATCACACTGATCTCCGTGAACGGCATCTTCGTGATCT

GCTGCCTGACCTACTGCTTCGCCCCCTAGATGCAGAGAGCGGAGAAGAAACGAGCGG
CTGAGAAGAGAAAGCGTGCGGCCTGTG (SEQ ID NO: 227).

Embodiment 51 The multicistronic expression system of any one of embodiments 1-50, wherein the antigen-binding domain specific for EMCN comprises a heavy chain variable (VH) region and a light chain variable (VL) region, wherein: the EMCN-VH comprises: a heavy chain complementarity determining region 1 (CDR-H1) having the amino acid sequence of RYDMH (SEQ ID NO: 291), a heavy chain complementarity determining region 2 (CDR-H2) having the amino acid sequence of VIWNGNTHYHSALKS (SEQ ID NO: 296), and a heavy chain complementarity determining region 3 (CDR-H3) having the amino acid sequence of RIKD (SEQ ID NO: 298), and the EMCN-VL comprises: a light chain complementarity determining region 1 (CDR-L1) having the amino acid sequence of KSSQSLVASDENTYLN (SEQ ID NO: 299), a light chain complementarity determining region 2 (CDR-L2) having the amino acid sequence of QVSKLDS (SEQ ID NO: 300), and a light chain complementarity determining region 3 (CDR-L3) having the amino acid sequence of LQGIHLPWT (SEQ ID NO: 301), and wherein the amino acid sequences of the CDR-H1, the CDR-H2, the CDR-H3, the CDR-L1, the CDR-L2, and the CDR-L3 of the reference antibody are defined based on the Kabat numbering scheme; optionally wherein the EMCN-VH and the EMCN-VL are separated by a peptide linker, optionally wherein the antigen-binding domain specific for EMCN comprises the structure VH-L-VL or VL-L-VH, wherein L is the peptide linker, optionally wherein the peptide linker comprises the amino acid sequence SGGGSGGGGSG (SEQ ID NO: 230); GGGSGGGGSGGGSLQ (SEQ ID NO: 231); GGS (SEQ ID NO: 232); GGS (SEQ ID NO: 233); GGS (SEQ ID NO: 234); GGS (SEQ ID NO: 235); GGS (SEQ ID NO: 236); GGS (SEQ ID NO: 237); GGS (SEQ ID NO: 238); GGS (SEQ ID NO: 239); GGS (SEQ ID NO: 240); GGS (SEQ ID NO: 241); GGS (SEQ ID NO: 242); GGS (SEQ ID NO: 243); GGS (SEQ ID NO: 244); GGS (SEQ ID NO: 245); GGS (SEQ ID NO: 246); GSTSGSGKPGSGEGSTKG (SEQ ID NO: 247); or EAAAKEAAAKEAAAKEAAK (SEQ ID NO: 248), optionally wherein the peptide linker comprises the amino acid sequence GGGGSGGGGSGGGG (SEQ ID NO: 244), optionally wherein the peptide linker is encoded by a polynucleotide sequence comprising the sequence GGAGGCGGAGGATCTGGTGGCGGAGGAAGTGGCGGAGGCGGTTCT (SEQ ID NO: 253).

Embodiment 52 The multicistronic expression system of any one of embodiments 1-51, wherein the intracellular inhibitory domain comprises a LIR1 intracellular inhibitory domain, optionally wherein the LIR1 intracellular inhibitory domain comprises the amino acid sequence LRHRRQGKHWSTSTQRKADFQHPAGAVGPEPTDRGLQWRSSPAADAQEENLYAAVKH

5 TQPEDGVEMDTRSPHDEDPQAVTYAEVKHSRPRREMASPPSPLSGEFLDTKDRQAEED
RQMDTEAAASEAPQDVITYAQLHSLTLRREATEPPPSQEGPSPAVPSIYATLAIH (SEQ ID NO: 285), optionally wherein the LIR1 intracellular inhibitory domain is encoded by a polynucleotide sequence comprising the sequence

CTGCGGCACAGAAGGCAGGGCAAGCACTGGACAAGCACCCAGAGAAAGGCCGACT

10 TTCAGCATCCTGCTGGCGCCGTTGGACCTGAGCCTACAGATAGAGGACTGCAGTGG
CGGTCTAGCCCTGCCGCTGATGCCCAAGAGGAAAATCTTTACGCCGCCGCTGAAGCA
CACCCAGCCTGAGGATGGCGTGGAAATGGACACCAGATCTCCCCACGATGAGGACC
CTCAGGCCGTGACATACGCAGAAGTGAAGCACTCCAGACCTCGGAGAGAGATGGC
AAGCCCTCCATCTCCTCTGAGCGGCGAGTTCCTGGACACCAAAGACAGACAGGCCG

15 AAGAGGACAGACAGATGGATACCGAAGCCGCCGCTTCTGAAGCCCCACAGGATGT
GACATATGCCCAGCTGCATAGCCTGACACTGCGGAGAGAAGCCACAGAGCCTCCAC
CTTCTCAAGAAGGCCCATCTCCTGCCGTGCCTTCCATCTATGCCACTCTGGCCATTC
AC (SEQ ID NO: 286).

Embodiment 53 The multicistronic expression system of any one of embodiments 1-52, wherein (A) the signal peptide is present in the iCAR, optionally wherein the signal peptide of the iCAR comprises a native signal peptide native or a non-native signal peptide, optionally wherein the non-native signal peptide or the non-native signal-anchor sequence is selected from the group consisting of: IgE, IL-12, IL-2, optimized IL-2, trypsinogen-2, Gaussia luciferase, CD5, human IgKVII, murine IgKVII, VSV-G, prolactin, serum albumin preprotein, azurocidin

20 preprotein, osteonectin, CD33, IL-6, IL-8, CCL2, TIMP2, VEGFB, osteoprotegerin, serpin E1, GROalpha, CXCL12, IL-21, CD8, NKG2D, TNFR2, and GMCSF, optionally wherein the signal peptide of the iCAR comprises a CD8 signal peptide, optionally wherein the CD8 signal peptide comprises the amino acid sequence MALPVTALLLPLALLLHAARP (SEQ ID NO: 137), optionally wherein the CD8 signal peptide is encoded by a polynucleotide sequence comprising

25 the sequence

30 ATGGCTCTGCCCCGTGACAGCTTTGCTGCTGCCTTTGGCACTGCTGCTGCATGCTGCT
AGACCA (SEQ ID NO: 416); and/or wherein (B) the hinge domain is present in the iCAR comprises, optionally wherein the hinge domain of the iCAR is selected from the group consisting of a human Ig (immunoglobulin) hinge, an IgG4 hinge, an IgG2 hinge, a CD8a hinge,

35 or an IgD hinge, a KIR2DS2 hinge, an LNGFR hinge, a LIR1 hinge, a PDGFR-beta

extracellular linker, and combinations thereof, optionally wherein the hinge domain of the iCAR comprises a LIR1 hinge, optionally wherein the LIR1 hinge comprises the amino acid sequence HPSDPLELVVSGPSGGPSSPTTGPTSTSGPEDQPLTPTGSDPQSGGLGRHLGV (SEQ ID NO: 417), optionally wherein the LIR1 hinge comprises is encoded by a polynucleotide sequence

5 comprising the sequence

CACCCATCCGATCCTCTCGAGCTGGTGGTTTCTGGACCTTCTGGCGGCCCTAGCAGC
CCTACAACAGGACCTACAAGCACAAGCGGCCCTGAGGACCAACCTCTGACACCAAC
AGGCAGCGATCCTCAGTCTGGACTGGGGAGACATCTGGGCGTT (SEQ ID NO: 418);

optionally wherein the hinge domain of the iCAR comprises a CD8 hinge, optionally wherein
10 the CD8 hinge comprises the amino acid sequence

TTTTAPRPPTPAPTIALQPLSLRPEACRPAAGGAVHTRGLDFACD (SEQ ID NO: 271),

optionally wherein the CD8 hinge comprises is encoded by a polynucleotide sequence
comprising the sequence

ACAACAACACCCGCACCTCGGCCTCCAACCTCCAGCTCCAACAATTGCACTGCAACC
15 CCTGAGTCTGAGGCCCGAGGCCTGTAGGCCAGCAGCTGGCGGAGCTGTTACACTA
GAGGCCTGGACTTTGCCTGTGAC (SEQ ID NO: 283); and/or wherein (C) the

transmembrane domain of the iCAR is present, optionally wherein the transmembrane domain
of the iCAR comprises a transmembrane domain selected from the group consisting of: PDGFR-
beta, CD8, CD28, CD3zeta-chain, CD4, 4-1BB, OX40, ICOS, CTLA-4, PD-1, LAG-3, 2B4,

20 LNGFR, NKG2D, EpoR, TNFR2, B7-1, LIR1, and BTLA, optionally wherein the

transmembrane domain of the iCAR comprises a LIR1 transmembrane domain, optionally
wherein the LIR1 transmembrane domain comprises the amino acid sequence

VIGILVAVILLLLLLLLLLFLI (SEQ ID NO: 259), optionally wherein the LIR1 transmembrane
domain is encoded by a polynucleotide sequence comprising the sequence

25 GTGATCGGCATTCTGGTCGCCGTGATCCTGCTCCTGTTGCTCCTGCTGCTTCTGTTCC
TGATC (SEQ ID NO: 260).

Embodiment 54 The multicistronic expression system of any one of embodiments 1-53,
wherein the iCAR comprises the amino acid sequence:

MALPVTALLLPLALLLHAARPEVQLVESGGGLVQPGGSLRLSCAASGFTFSRYDMHWV
30 RQAPGKGLEWVSVIWGNGNTHYHSALKSRFTISRDNKNTLYLQMNSLRAEDTAVYY
CTLRIKDWGQGTMVTVSSGGGGSGGGGSGGGGSDVVMQSPSLPVTLGQPASISCKS
SQLVASDENTYLNWFQQRPGQSPRRLIYQVSKLDSGVPDRFSGSGSGTDFTLKISRVEA
EDVGVYYCLQGIHLPWTFGQGTKLEIK_{Ngaa}HPSDPLELVVSGPSGGPSSPTTGPTSTSGP
EDQPLTPTGSDPQSGGLGRHLGVVIGILVAVILLLLLLLLLLFLILRHRRQGKHWSTQRKA
35 DFQHPAGAVGPEPTDRGLQWRSSPAADAQEENLYAAVKHTQPEDGVEMDTRSPHDED

PQAVTYAEVKHSRPRREMASPPSPLSGEFLDTKDRQAEEDRQMDTEAAASEAPQDVTY
AQLHSLTLRREATEPPPSQEGPSPAVPSIYATLAIH (SEQ ID NO: 419), optionally wherein
the iCAR is encoded by a polynucleotide sequence comprising the sequence

ATGGCTCTGCCCCGTGACAGCTTTGCTGCTGCCTTTGGCACTGCTGCTGCATGCTGCT
5 AGACCAGAGGTGCAGCTGGTTGAATCTGGCGGAGGACTGGTTCAGCCTGGCGGATC
TCTGAGACTGTCTTGTGCCGCCAGCGGCTTCACCTTCAGCAGATACGATATGCACTG
GGTCCGACAGGCCCTGGCAAAGGACTTGAATGGGTGTCCGTGATCTGGGGCAACG
GCAACACACACTACCACAGCGCCCTGAAGTCCCGGTTCAACATCTCCAGAGACAAC
AGCAAGAACACCCTGTACCTGCAGATGAACAGCCTGAGAGCCGAGGACACCGCCGT
10 GTACTACTGCACCCTGAGAATCAAGGATTGGGGCCAGGGCACCATGGTCACCGTTT
CTTCTGGAGGCGGAGGATCTGGTGGCGGAGGAAGTGGCGGAGGCGGTTCTGACGTG
GTCATGACACAGAGCCCTCTGAGCCTGCCTGTGACACTGGGACAGCCTGCCAGCAT
CAGCTGCAAGTCTAGCCAGTCTCTGGTGGCCAGCGACGAGAACACCTACCTGAACT
GGTTCCAGCAGAGGCCCGGACAGTCTCCTAGACGGCTGATCTACCAGGTGTCCAAG
15 CTGGATAGCGGCGTGCCCGATAGATTTTCTGGCAGCGGCTCTGGCACCGACTTCACC
CTGAAGATCAGCAGAGTGGAAGCCGAGGACGTGGGCGTGTACTACTGTCTGCAAGG
CATCCATCTGCCTTGGACCTTTGGCCAGGGCACAAAGCTGGAAATCAAGAATGGCG
CTGCACACCCATCCGATCCTCTCGAGCTGGTGGTTTCTGGACCTTCTGGCGGCCCTA
GCAGCCCTACAACAGGACCTACAAGCACAAGCGGCCCTGAGGACCAACCTCTGACA
20 CCAACAGGCAGCGATCCTCAGTCTGGACTGGGGAGACATCTGGGCGTTGTGATCGG
CATTCTGGTTCGCCGTGATCCTGCTCCTGTTGCTCCTGCTGCTTCTGTTCTGATCCTG
CGGCACAGAAGGCAGGGCAAGCACTGGACAAGCACCCAGAGAAAGGCCGACTTTC
AGCATCCTGCTGGCGCCGTTGGACCTGAGCCTACAGATAGAGGACTGCAGTGGCGG
TCTAGCCCTGCCGCTGATGCCCAAGAGGAAAATCTTTACGCCGCCGTGAAGCACAC
25 CCAGCCTGAGGATGGCGTGGAAATGGACACCAGATCTCCCCACGATGAGGACCCTC
AGGCCGTGACATACGCAGAAGTGAAGCACTCCAGACCTCGGAGAGAGATGGCAAG
CCCTCCATCTCCTCTGAGCGGCGAGTTCCTGGACACCAAAGACAGACAGGCCGAAG
AGGACAGACAGATGGATACCGAAGCCGCCGCTTCTGAAGCCCCACAGGATGTGACA
TATGCCCAGCTGCATAGCCTGACACTGCGGAGAGAAGCCACAGAGCCTCCACCTTC
30 TCAAGAAGGCCCATCTCCTGCCGTGCCTTCCATCTATGCCACTCTGGCCATTAC
(SEQ ID NO: 420).

Embodiment 55 The multicistronic expression system of any one of embodiments 1-54,
wherein the antigen-binding domain specific for FLT3 comprises a heavy chain variable (VH)
region and a light chain variable (VL) region, wherein: the FLT3-VH comprises: a heavy chain
35 complementarity determining region 1 (CDR-H1) having the amino acid sequence of

GGTFSSY AIS (SEQ ID NO: 360), a heavy chain complementarity determining region 2 (CDR-H2) having the amino acid sequence of GIPIFGTANYAQKFQG (SEQ ID NO: 361), and a heavy chain complementarity determining region 3 (CDR-H3) having the amino acid sequence of FALFGFREQA FDI (SEQ ID NO: 362), and the FLT3-VL comprises: a light chain complementarity determining region 1 (CDR-L1) having the amino acid sequence of RASQSISSYLN (SEQ ID NO: 363), a light chain complementarity determining region 2 (CDR-L2) having the amino acid sequence of AASSLQS (SEQ ID NO: 364), and a light chain complementarity determining region 3 (CDR-L3) having the amino acid sequence of QQSYSTPFT (SEQ ID NO: 365), and wherein the amino acid sequences of the CDR-H1, the CDR-H2, the CDR-H3, the CDR-L1, the CDR-L2, and the CDR-L3 of the reference antibody are defined based on the Kabat numbering scheme; optionally wherein: the FLT3-VH comprises the amino acid sequence

EVQLVQSGAEVKKPGSSVKV SCKASGGTFSSY AISWVRQAPGQGLEWMGGIPIFGTAN
YAQKFQGRVTITADKSTSTAYMELSSLRSED TAVYYCATFALFGFREQA FDIWGQGTTV
TVSS (SEQ ID NO: 315); and the FLT3-VL comprises the amino acid sequence

DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPS
RFSGSGSGTDFLTITSLQPEDLATYYCQQSYSTPFTFGPGTKVDIK (SEQ ID NO: 316),
optionally wherein the FLT3-VH, and the FLT3-VL are separated by peptide linkers.

Embodiment 56 The multicistronic expression system of any one of embodiments 1-55, wherein the antigen-binding domain specific for CD33 comprises a heavy chain variable (VH) region and a light chain variable (VL) region, wherein: the CD33-VH comprises: a heavy chain complementarity determining region 1 (CDR-H1) having the amino acid sequence of DYNMH (SEQ ID NO: 402), a heavy chain complementarity determining region 2 (CDR-H2) having the amino acid sequence of YIYPYNGGTGYNQKFKSKA (SEQ ID NO: 403), and a heavy chain complementarity determining region 3 (CDR-H3) having the amino acid sequence of GRPAMDYWGQ (SEQ ID NO: 404), and the CD33-VL comprises a light chain complementarity determining region 1 (CDR-L1) having the amino acid sequence of RASESVDNYGISFMN (SEQ ID NO: 405), a light chain complementarity determining region 2 (CDR-L2) having the amino acid sequence of AASNQGS (SEQ ID NO: 406), and a light chain complementarity determining region 3 (CDR-L3) having the amino acid sequence of QQSKEVPWT (SEQ ID NO: 407), and wherein the amino acid sequences of the CDR-H1, the CDR-H2, the CDR-H3, the CDR-L1, the CDR-L2, and the CDR-L3 of the reference antibody are defined based on the Kabat numbering scheme; optionally wherein: the CD33-VH comprises the amino acid sequence

QVQLVQSGAEVKKPGSSVKV SCKASGYTFTDYNMHWVRQAPGQGLEWIGYIYPYNGG

TGYNQKFKSKATITADESTNTAYMELSSLRSEDNAVYYCARGRPAMDYWGQGLTVTV
SS (SEQ ID NO: 329); and the CD33-VL comprises the amino acid sequence

DIQMTQSPSSLSASVGDRVTITCRASESVDNYGISFMNWFQQKPGKAPKLLIYAASNQG
SGVPSRFSGSGSGTDFTLTISLQPDDEATYYCQQSKEVPWTFGQGTKVEIK (SEQ ID

5 NO: 330), optionally wherein the CD33-VH, and the CD33-VL are separated by peptide linkers.

Embodiment 57 The multicistronic expression system of any one of embodiments 1-56,
wherein the aCAR antigen binding domains comprises the structure (FLT3-VH) – L1 – (CD33-
VH) – L2 – (CD33-VL) – L3 – (FLT3-VL), wherein L1, L2, and L3 are a first, a second, and a
third peptide linker, respectively; optionally wherein the L1, L2, and/or L3 are each

10 independently selected from the group consisting of: SEQ ID Nos 230 – 248, optionally wherein
the L1 peptide linker is the amino acid sequence GGGGS (SEQ ID NO: 242) or

GGGSGGGGS (SEQ ID NO: 243), optionally wherein the L1 peptide linker GGGGS (SEQ
ID NO: 242) is encoded by a polynucleotide sequence comprising the sequence

GGCGGCGGTGGCTCT (SEQ ID NO: 254) or the L1 peptide linker GGGSGGGGS (SEQ ID

15 NO: 243) is encoded by a polynucleotide sequence comprising the sequence

GGAGGCGGAGGATCTGGTGGTGGTGGATCT (SEQ ID NO: 256), optionally wherein the

L2 peptide linker is the amino acid sequence GSTSGSGKPGSGEGSTKG (SEQ ID NO: 247),

optionally wherein the L2 peptide linker is encoded by a polynucleotide sequence comprising
the sequence

20 GGCTCTACATCTGGCTCTGGCAAACCTGGAAGCGGCGAGGGATCTACCAAGGGC

(SEQ ID NO: 249), optionally wherein the L2 peptide linker is the amino acid sequence

GGGSGGGGS (SEQ ID NO: 243), optionally wherein the L2 peptide linker is encoded by a
polynucleotide sequence comprising the sequence

GGTGGCGGAGGAAGTGGCGGCGGAGGCTCT (SEQ ID NO: 257), optionally wherein the

25 L3 peptide linker is the amino acid sequence GGGGS (SEQ ID NO: 242) or GGGSGGGGS

(SEQ ID NO: 243), optionally wherein the L3 peptide linker GGGGS (SEQ ID NO: 242) is

encoded by a polynucleotide sequence comprising the sequence GGTGGCGGCGGATCC (SEQ

ID NO: 255) or the L3 peptide linker GGGSGGGGS (SEQ ID NO: 243) is encoded by a

polynucleotide sequence comprising the sequence

30 GGCGGTGGCGGATCTGGCGGAGGTGGCAGT (SEQ ID NO: 258).

Embodiment 58 The multicistronic expression system of any one of embodiments 1-57,
wherein the aCAR intracellular signaling domains that stimulate an immune response is selected
from the group consisting of: CD3-zeta, FcR gamma, FcR beta, CD3 gamma, CD3 delta, CD3
epsilon, CD5, CD22, CD79a, CD79b, CD278, FcεRI, DAP10, DAP12, CD66d, CD97, CD2,

ICOS, CD27, CD154, CD8, OX40, 4-1BB, CD28, ZAP40, CD30, GITR, HVEM, DAP10, DAP12, MyD88, 2B4, CD40, PD-1, LFA-1, CD7, LIGHT, NKG2C, B7-H3, an MHC class I molecule, a TNF receptor protein, an Immunoglobulin-like protein, a cytokine receptor, an integrin, a SLAM protein, an activating NK cell receptor, BTLA, a Toll ligand receptor, CDS,

5 ICAM-1, (CD11a/CD18), BAFFR, KIRDS2, SLAMF7, NKp80 (KLRF1), NKp44, NKp30, NKp46, CD19, CD4, IL2R beta, IL2R gamma, IL7R alpha, ITGA4, VLA1, CD49a, ITGA4, IA4, CD49D, ITGA6, VLA-6, CD49f, ITGAD, CD11d, ITGAE, CD103, ITGAL, CD11a, ITGAM, CD11b, ITGAX, CD11c, ITGB1, CD29, ITGB2, CD18, ITGB7, NKG2D, TNFR2, TRANCE/RANKL, DNAM1 (CD226), SLAMF4 (CD244, 2B4), CD84, CD96 (Tactile),

10 CEACAM1, CRTAM, Ly9 (CD229), CD160 (BY55), PSGL1, CD100 (SEMA4D), CD69, SLAMF6 (NTB-A, Ly108), SLAM (SLAMF1, CD150, IPO-3), BLAME (SLAMF8), SELPLG (CD162), LTBR, LAT, GADS, SLP-76, PAG/Cbp, CD19a, and combinations thereof, optionally wherein the aCAR intracellular signaling domains that stimulate an immune response comprise a CD28 co-stimulatory domain and a CD3 ζ signaling domain, optionally wherein the

15 CD28 co-stimulatory domain comprises the amino acid sequence
RSKRSRLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRS (SEQ ID NO: 287), optionally wherein the CD28 co-stimulatory domain is encoded by a polynucleotide sequence comprising the sequence
AGAAGCAAGCGGAGCAGACTGCTGCACAGCGACTACATGAACATGACCCCTAGAC

20 GGCCCGGACCTACCAGAAAGCACTACCAGCCTTACGCTCCTCCTAGAGATTTCGCC GCCTACCGGTCC (SEQ ID NO: 288), optionally wherein the CD3 ζ signaling domain comprises the amino acid sequence
RVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDRRRGRDPEMGGKPRRKNPQEG
LYNELQKDKMAEAYSEIGMKGERRRGKGDGLYQGLSTATKDTYDALHMQALPPR

25 (SEQ ID NO: 289), optionally wherein the CD3 ζ signaling domain is encoded by a polynucleotide sequence comprising the sequence
AGAGTGAAGTTCAGCAGGAGCGCAGACGCCCCCGCGTACAAGCAGGGCCAGAACC
AGCTCTATAACGAGCTCAATCTAGGACGAAGAGAGGAGTACGATGTTTTGGACAAG
AGACGTGGCCGGGACCCTGAGATGGGGGGAAAGCCGAGAAGGAAGAACCCTCAGG

30 AAGGCCTGTACAATGAACTGCAGAAAGATAAGATGGCGGAGGCCTACAGTGAGAT
TGGGATGAAAGGCGAGCGCCGGAGGGGCAAGGGGCACGATGGCCTTTACCAGGGT
CTCAGTACAGCCACCAAGGACACCTACGACGCCCTTCACATGCAGGCCCTGCCCCC
TCGC (SEQ ID NO: 290), optionally wherein

(A) the hinge domain of the aCAR is present, optionally wherein the hinge domain of the aCAR

35 is selected from the group consisting of a human Ig (immunoglobulin) hinge, an IgG4 hinge, an

IgG2 hinge, a CD8a hinge, or an IgD hinge, a KIR2DS2 hinge, an LNGFR hinge, a LIR1 hinge, a PDGFR-beta extracellular linker, and combinations thereof, optionally wherein the hinge domain of the aCAR comprises a CD8 hinge, optionally wherein the CD8 hinge comprises the amino acid sequence

- 5 ALSNSIMYFSHFVPVFLPAKPTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGL
DFACD (SEQ ID NO: 272), optionally wherein the CD8 hinge comprises is encoded by a polynucleotide sequence comprising the sequence
GCCCTGAGCAACAGCATCATGTACTTCAGCCACTTCGTGCCCCGTGTTTCTGCCCCGCC
AAGCCTACAACAACCCCTGCTCCTAGACCACCTACACCAGCTCCTACAATCGCCAG
10 CCAGCCTCTGTCTCTGAGGCCCCGAAGCTTGTAGACCAGCTGCTGGCGGAGCCGTGC
ATACAAGAGGACTGGATTTTGCCTGCGAC (SEQ ID NO: 284), and/or wherein

(B) the transmembrane domain of the aCAR is present, optionally wherein the transmembrane domain of the aCAR is selected from the group consisting of a human Ig (immunoglobulin)

- 15 hinge, an IgG4 hinge, an IgG2 hinge, a CD8a hinge, or an IgD hinge, a KIR2DS2 hinge, an LNGFR hinge, a LIR1 hinge, a PDGFR-beta extracellular linker, and combinations thereof, optionally wherein the transmembrane domain of the aCAR comprises a CD8 hinge, optionally wherein the CD8 transmembrane comprises the amino acid sequence
IYIWAPLAGTCGVLLLSLVITLYCNHR (SEQ ID NO: 206), optionally wherein the CD8
20 transmembrane comprises is encoded by a polynucleotide sequence comprising the sequence
ATCTATATCTGGGCCCCCTCTGGCTGGCACATGCGGAGTTCTGCTGCTCAGCCTGGTC
ATCACCTGTACTGCAACCACAGA (SEQ ID NO: 261); and/or wherein

(C) the aCAR signal peptide of the aCAR is present, optionally wherein the signal peptide of the aCAR is selected from the group consisting of: IgE, IL-12, IL-2, optimized IL-2, trypsinogen-2, Gaussia luciferase, CD5, human IgKVII, murine IgKVII, VSV-G, prolactin, serum albumin preprotein, azurocidin preprotein, osteonectin, CD33, IL-6, IL-8, CCL2, TIMP2, VEGFB, osteoprotegerin, serpin E1, GROalpha, CXCL12, IL-21, CD8, NKG2D, TNFR2, GMCSF, and GM-CSFRa, optionally wherein the signal peptide of the aCAR comprises a GM-CSFRa signal peptide, optionally wherein the GM-CSFRa signal peptide comprises the amino acid sequence

- 30 MLLLVTSLLLCELPHPAFLIP (SEQ ID NO: 423), optionally wherein the GM-CSFRa signal peptide is encoded by a polynucleotide sequence comprising the sequence
ATGCTGCTGCTGGTTACATCTCTGCTGCTGTGCGAGCTGCCCCATCCTGCCTTTCTGC
TTATTCCT (SEQ ID NO: 424), optionally wherein the aCAR comprises the amino acid
35 sequence

MLLLVTSLLLCELPHPAFLLIPEVQLVQSGAEVKKPGSSVKVSCASGGTFSSYAISWVR
 QAPGQGLEWMGGIIPFGTANYAQKFQGRVTITADKSTSTAYMELSSLRSEDNAVYYCA
 TFALFGFREQAFDIWGGTTVTVSSGGGGSQVQLVQSGAEVKKPGSSVKVSCASGYT
 FTDYNMHWVRQAPGQGLEWIGYIYPYNGGTGYNQKFKSKATITADESTNTAYMELSSL
 5 RSEDNAVYYCARGRPAMDYWGQGTTLTVSSGSTSGSGKPGSGEGSTKGDIQMTQSPSS
 LSASVGDRVTITCRASESDNYGISFMNWFQQKPGKAPKLLIYAASNQSGVPSRFSGS
 GSGTDFTLTISSLQPDDEFATYYCQQSKEVPWTFGQGTKVEIKGGGGSDIQMTQSPSSLSA
 SVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTL
 TISSLQPEDLATYYCQQSYSTPFTFGPGTKVDIKALSNSIMYFSHFVPVFLPAKPTTTPAP
 10 RPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYWAPLAGTCGVLLLSLVITL
 YCNHRRSKRSRLHSDYMNMTPRRPGPTRKHYPYAPPRDFAAYRSRVKFSRSADAPA
 YKQGQNQLYNELNLGRREEYDVLDRRGRDPEMGGKPRRKNPQEGLYNELQKDKMA
 EAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR (SEQ ID NO: 414),
 optionally wherein the aCAR comprises the amino acid sequence
 15 MLLLVTSLLLCELPHPAFLLIPEVQLVQSGAEVKKPGSSVKVSCASGGTFSSYAISWVR
 QAPGQGLEWMGGIIPFGTANYAQKFQGRVTITADKSTSTAYMELSSLRSEDNAVYYCA
 TFALFGFREQAFDIWGGTTVTVSSGGGGSQVQLVQSGAEVKKPGSSVKVSCA
 ASGYTFTDYNMHWVRQAPGQGLEWIGYIYPYNGGTGYNQKFKSKATITADESTNTAY
 MELSSLRSEDNAVYYCARGRPAMDYWGQGTTLTVSSGGGGSGGGGSDIQMTQSPSSLS
 20 ASVGDRVTITCRASESDNYGISFMNWFQQKPGKAPKLLIYAASNQSGVPSRFSGSGS
 GTDFTLTISLQPDDEFATYYCQQSKEVPWTFGQGTKVEIKGGGGSGGGGSDIQMTQSPS
 SLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGT
 DFTLTISLQPEDLATYYCQQSYSTPFTFGPGTKVDIKALSNSIMYFSHFVPVFLPAKPTT
 TPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYWAPLAGTCGVLLLS
 25 LVITLYCNHRRSKRSRLHSDYMNMTPRRPGPTRKHYPYAPPRDFAAYRSRVKFSRSA
 DAPAYKQGQNQLYNELNLGRREEYDVLDRRGRDPEMGGKPRRKNPQEGLYNELQK
 DKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR (SEQ ID NO:
 415).

Embodiment 59 An expression vector comprising the multicistronic expression system of
 30 any one of embodiments 1-58.

Embodiment 60 An isolated cell comprising the multicistronic expression system of any
 one of embodiments 1-59 or the expression vector of embodiment 59, optionally wherein the
 cell comprises an immune cell, optionally wherein the cell is selected from the group consisting
 of a T cell, a Natural Killer (NK) cell, a cytotoxic T lymphocyte (CTL), a regulatory T cell, a
 35 Natural Killer T (NKT) cell, a myeloid cell, a macrophage, a human embryonic stem cell (ESC),

an ESC-derived cell, a pluripotent stem cell, and induced pluripotent stem cell (iPSC), and an iPSC-derived cell, optionally wherein the cell is an NK cell.

Embodiment 61 A method of treating a subject in need thereof, the method comprising administering a therapeutically effective dose of any of the isolated cells of embodiment 60, optionally wherein the subject has cancer, optionally wherein the isolated cell is derived from the subject or is allogeneic with reference to the subject.

Embodiment 62 A method of stimulating a cell-mediated immune response to a tumor cell in a subject, the method comprising administering to a subject having a tumor a therapeutically effective dose of any of the isolated cells of embodiment 60, optionally wherein the isolated cell is derived from the subject or is allogeneic with reference to the subject.

Embodiment 63 A method of making an engineered cell, comprising transducing an isolated cell with the multicistronic expression system of any one of embodiments 1-58 or the expression vector of embodiment 59.

Embodiment 64 A method of treating a subject known or suspected of having cancer, the method comprising: administering a cell therapy to the subject, wherein the cell therapy comprises an immunoresponsive cell engineered to express a recombinant cytokine, wherein the immunoresponsive cell engineered to express the recombinant cytokine comprises an exogenous polynucleotide encoding a controlled release cytokine, oriented from N-terminal to C-terminal, having the formula: **S – C – MT** or **MT – C – S**, wherein S comprises a secretable effector molecule, wherein the secretable effector molecule comprises the recombinant cytokine, C comprises a protease cleavage site, and MT comprises a cell membrane tethering domain, wherein **S – C – MT** or **MT – C – S** is configured to be expressed as a single polypeptide; and administering a pharmaceutically effective amount of an agent selected from the group consisting of: cytarabine (Ara-C); decitabine; venetoclax; a combination of fludarabine and Ara-C; or a combination of decitabine and venetoclax.

Embodiment 65 The method of embodiment 64, wherein the immunoresponsive cell comprises an exogenous polynucleotide encoding an inhibitory chimeric antigen receptor (iCAR) comprising: an antigen-binding domain specific for endomucin (EMCN); one or more intracellular inhibitory domains that inhibit an immune response; and one or more polypeptides selected from the group consisting of: a signal peptide, a transmembrane domain, a hinge domain, a spacer region, one or more peptide linkers, and combinations thereof.

Embodiment 66 The method of any one of embodiments 64 or 65, wherein the immunoresponsive cell comprises an exogenous polynucleotide encoding a bivalent activating chimeric antigen receptor (aCAR) comprising an antigen-binding domain specific for FLT3; an antigen-binding domain specific for CD33; one or more intracellular signaling domains that

stimulate an immune response; and one or more polypeptides selected from the group consisting of: a signal peptide, a transmembrane domain, a hinge domain, a spacer region, one or more peptide linkers, and combinations thereof.

5 **Embodiment 67** The method of any one of embodiments 64-66, wherein the agent comprises the combination of fludarabine and Ara-C.

Embodiment 68 The method of embodiment 67, wherein the fludarabine is administered at a dose of 30 mg/m² and the cytarabine (Ara-C) is administered at a dose of 2 g/m².

Embodiment 69 The method of any one of embodiments 64-68, wherein the agent is administered intravenously.

10 **Embodiment 70** The method of any one of embodiments 64-69, wherein the agent is administered 5 days prior to administration of the cell therapy.

Embodiment 71 The method of any one of embodiments 64-70, wherein when the Ara-C is administered, the Ara-C is administered at a dose of 2 g/m².

15 **Embodiment 72** The method of any one of embodiments 64-66, wherein the agent comprises the combination of fludarabine and Ara-C, wherein the fludarabine is administered at a dose of 30 mg/m² and the Ara-C is administered at a dose of 2 g/m², and wherein the agent is administered 5 days prior to administration of the cell therapy.

EXAMPLES

20 Below are examples of specific embodiments for carrying out the present invention. The examples are offered for illustrative purposes only, and are not intended to limit the scope of the present invention in any way. For example, the experiments described and performed below demonstrate the general utility of engineering cells to secrete payloads (*e.g.*, effector molecules) and delivering those cells to induce an immunogenic response against tumors.

25 Efforts have been made to ensure accuracy with respect to numbers used (*e.g.*, amounts, temperatures, etc.), but some experimental error and deviation should, of course, be allowed for.

Example 1 – Clinical Assessment of FLT3 OR CD33 NOT EMCN logic-gated CAR-NK cell therapy combine with IL2 administration

30 Below is a description of a clinical protocol for a Phase 1, multicenter, open label study of a selective off-the-shelf FLT3 OR CD33 NOT EMCN logic-gated CAR-NK cell therapy engineered to express controlled-release IL15 (crIL15) in subjects with CD33 and/or FLT3 expressing malignancies. Some participants in the study will receive recombinant human IL2 in

addition to the cell therapy to assess the efficacy of the cell therapy in the presence of the cytokine.

Study Design

The study is made up of 4 parts that have different procedures: screening, study treatment, post-treatment follow up, and long-term follow up.

Screening

The study doctor collects information on subject's medical condition(s) and performs tests to determine if inclusion criteria are met. Subjects participate in a screening period that begins at the time a subject signs the informed consent and lasts for up to 28 days, but it may be shorter.

The following information is collected and tests are performed:

- Date of birth, age, gender, and ethnic origins/race (demographic data).
- Past and present medical conditions including significant family medical history.
- Detailed cancer history, including diagnosis and prior treatments.
- Current or recently taken medicines. This includes any non-prescription medicines, vaccines, vitamins, food supplements, and natural/herbal remedies.
- Questions about how cancer and/or symptoms affect the subject's daily life, quality of life, and work productivity.
- Height and weight
- Vital signs (temperature, blood pressure, breathing rate, heart rate)
- Physical exam
- Blood and urine tests to measure subject's health (within 14 days of first FLT3 OR CD33 NOT EMCN logic-gated CAR-NK cell therapy dose). This includes testing for HIV and hepatitis (B and C).
- Bone marrow sample (within 14 days of first FLT3 OR CD33 NOT EMCN logic-gated CAR-NK cell therapy dose and before the start of lymphodepletion (LD)).
- Pregnancy test if the subject is female and can become pregnant. The result must be negative to participate in the study.
- Tests to measure subject's heart function:
 - o Electrocardiogram (ECG): This test is performed three times within 5 minutes of each other as a routine analysis to ensure the reading is correct.
 - o Echocardiogram (ECHO) or Multigated acquisition (MUGA)

Subjects that meet all criteria to take part in the study, proceed to the study treatment phase. For subjects that do not qualify, study participation is over, and the doctor discusses other treatment options.

Study Treatment

Other cancer treatments are stopped before the subject's first dose of the FLT3 OR CD33 NOT EMCN logic-gated CAR-NK cell therapy. Part of the screening determines whether the subject is able to stay off cancer treatment before joining the study. Cancer treatments are stopped at

5 certain times depending on the type of previous cancer treatment the subject was getting.

- First, a 5-day course of conditioning chemotherapy is administered to prepare the subject to receive cell therapy.

- The conditioning therapy is a 5-day lymphodepletion with fludarabine and cytarabine (ara-C). Administration of fludarabine and cytarabine begins 7 days prior to the first dose of the

10 cell therapy. Each day of lymphodepletion, fludarabine and cytarabine are given by a slow infusion through a vein (intravenous or IV) for 30-60 minutes. Fludarabine is given first, followed 4 hours later by cytarabine. The standard dose is 30 mg/m² of fludarabine and 2 g/m² of cytarabine, but the doctor may change this dose based on the subject's medical status.

- Next, the subject receives the FLT3 OR CD33 NOT EMCN logic-gated CAR-NK cell

15 therapy. The cell therapy is given once a week for 3 weeks (on Days 0, 7, 14). Some (but not all) participants receive recombinant human IL2 (rhIL2) 6-8 hours after each cell therapy dose.

- The cell therapy dose levels that are being evaluated in this study are: 500 million cells, 1 billion cells, and 1.5 billion cells per dose. These doses are all measured as the number of CAR-positive cells, but the total number of cells the subject receives may be higher because some of

20 the NK cells may not be CAR-positive. If the subject weighs less than 50kg, the actual dose received is adjusted based on weight and is a little less than 500 million, 1 billion, or 1.5 billion cells.

- The first dose level tested in the study is 1 billion cells. A group of participants is receiving this dose. After this first group of participants finishes one treatment cycle (they must

25 receive in total 3 doses of 1 billion cells given on Days 0, 7 and 14), the study sponsor and the study doctors reviews the study data and chooses which dose level to evaluate next.

- The second dose level evaluated by the study may be higher (1.5 billion cells) or lower (500 million cells) depending on if the previous subjects have side effects. A new group of participants receives this second dose level. After the second group finishes a treatment cycle,

30 the study sponsor and study doctors review the data again and select the recommended dose going forward. There is a possibility the study sponsor and study doctors could choose to evaluate an in-between dose if study data indicates the need to do so.

- Subjects assigned to the study group also receiving rhIL2 are receiving an ultra-low dose of rhIL2 (3 MIU) approximately 6 to 8 hours after each cell therapy infusion (days 0, 7, and 14

35 of each treatment cycle). The rhIL2 is given by a small needle by subcutaneous injection. If the

subject experiences side effects from rhIL2, the study doctor may choose to skip further doses of rhIL2 and continue with chemotherapy and the cell therapy alone.

- The treatment cycle may be repeated up to 3 times. The subject's doctor decides based on side effects the subject may be experiencing and how the subject's cancer responds to the cell therapy.

Example 2 – In Vitro Persistence of FLT3 OR CD33 NOT EMCN Logic-Gated CAR-NK Cells in the Presence of IL-2 supplementation

The in vitro persistence of FLT3 OR CD33 NOT EMCN logic-gated CAR-NK cells was assessed in comparison with non-engineered (non-transduced) NK cells cultured from the same donors in the presence or absence of an exogenous cytokine, IL-2. FLT3 OR CD33 NOT EMCN logic-gated CAR-NK cells were manufactured using enriched NK cells from the peripheral blood of healthy adult donors (PBNK cells). Each lot of FLT3 OR CD33 NOT EMCN logic-gated CAR-NK cells started with the co-culture of PBNK cells and irradiated activating cells (XK562-mIL21). The NK cells were transduced with a tricistronic γ -retroviral vector to express a CD33/FLT3 bivalent activating CAR (aCAR) that recognizes and activates the killing of cells expressing CD33 and/or FLT3, an endomucin (EMCN) inhibitory CAR (iCAR) that binds and inhibits the killing of cells expressing EMCN. The same vector also encodes the controlled release IL15 (crIL15). These cells are also referred to as “engineered cells” in the Examples. A schematic illustration of the components of the engineered NK cell system is shown in **FIG. 1**.

All the components were encoded in constructs in a multicistronic system where each of the components were encoded in a single engineered nucleic acid capable of transcription as a single mRNA transcript and separated by 2A ribosome skipping elements. Following transcription, each of the encoded proteins would be translated as distinct peptides.

The cells were then transferred into a closed system and expanded until the target number of NK cells was reached (~14 days). The harvest cell suspension was volume reduced, pooled, washed, and formulated and the CAR NK cells were dispensed into vials, frozen, and stored in vapor phase liquid nitrogen (LN2) until their use in assays to determine persistence. The percentage of cells in each lot that stain positively for IL15 as determined by flow cytometry is shown in **Table 13**.

Table 13 Summary of FLT3 OR CD33 NOT EMCN Logic-Gated CAR-NK Cell Lot Parameters

Engineered cell Lot (TL) number	NK donor	% crIL15 ⁺
Lot 1	1	1.50
Lot 2	2	4.95
Lot 3	3	16.50
Lot 4	4	34.60

Note: % crIL15⁺ represents the percentage of cells that stain positively for IL15 by flow cytometry at the time of the assay. This metric does not account for cells that may express crIL15 which is then released and not detected.

Methods

Cell Counting Assay of Thawed NK Cells to Evaluate Persistence

- Four lots of FLT3 OR CD33 NOT EMCN logic-gated CAR-NK cells were thawed into NK MACS compete medium (per 500 ml: NK MACS Basal Medium, 465 mL; NK MACS Supplement, 5 mL; Human AB Serum, 25 mL; Penicillin/Streptomycin, 5 mL).
- 5e10 cells were plated per well in a 48-well non-tissue culture treated plate in NK MACS complete medium with no cytokines or “low dose” cytokine conditions (10 IU/mL wild type recombinant human IL-2 (rhIL-2)).
- Partial media exchanges were preformed twice per week.
- Two lots of NK cells were used to test “high dose” cytokine conditions and were cultured in NK MACS complete medium supplemented with 500 IU/mL rhIL-2 and 10 ng/mL recombinant human IL15 (rhIL15).
- Under high dose cytokine conditions media exchanges were performed twice per week.
- During media changes for conditions with cytokines, fresh cytokines were added at the specified concentrations.
- As the sampling of each well for cell counting necessarily agitates and mixes the whole well, and a portion of cells must be removed for counting, such procedures were minimized throughout the experiment. Instead, NK cells were plated in triplicate for each planned time point at the start of the experiment, and three wells for each culture condition were collected, counted, and discarded at each time point. The exceptions to this were the high dose cytokine growth conditions, for which a smaller number of wells were plated; in these cases, samples were taken from a subset of cells on a rotational basis, to maximize the time between which the same well would be sampled.

- Cells were counted by mixing 50 µL of cells from each sampled well with an equal volume of acridine orange/propidium iodide (AOPI) and counted by an automated cell counter. For cells cultured under high dose cytokine conditions only 30 µL of cells were used.
- 5 • **Table 14** summarizes the culture conditions and cell counting schedule.

Table 14 Culture Conditions

Test condition	Cytokine content	Lots tested	Media change schedule	Cell count schedule
No IL-2 supplementation	None	All	2X/week	2X/week
Low dose cytokine	rhIL2, 10 IU/mL	All	2X/week	2X/week
High dose cytokine	rhIL2, 500 IU/mL rhIL15, 10 ng/mL	Lot 3, Lot 4, and non-engineered controls	1X/week	2X/week in first 2 weeks, 1X/week thereafter

Abbreviations: 1X, once; 2X, twice; IU, international unit; rhIL15, recombinant human interleukin-15; rhIL-2, recombinant human interleukin-2

- The cell count at each time point was calculated using the following formula:
- 10 Total live cell number = (Live cell concentration) * (Remaining volume in well)
- The fold change in cell count at each time point was calculated using the baseline count established at day 0 for each lot of FLT3 OR CD33 NOT EMCN logic-gated CAR-NK cells. This analysis was performed using the following formula:

$$\text{Fold change in total live cell number} = \frac{\text{Total live cell number}}{\text{Baseline total live cell number}}$$

- 15
- The duration of FLT3 OR CD33 NOT EMCN logic-gated CAR-NK cells in vitro persistence under conditions without IL-2 supplementation was defined as the time point when (1) the difference in mean fold change between FLT3 OR CD33 NOT EMCN logic-gated CAR-NK cells and non-engineered NK cells was not statistically significant, and (2) the fold change in total live cell number dropped below 0.1, indicating less than 10% of seeded cells were remaining in the counted wells. The threshold of 0.1 was arbitrarily chosen to indicate a low number of remaining cells.
- 20
- Statistical software was used for statistical analysis and data representation. Means and standard error were calculated for numerical parameters. Statistical differences for each time point were calculated with t-tests using Welch’s correction.
- 25

Results

In Vitro Persistence of FLT3 OR CD33 NOT EMCN logic-gated CAR-NK cells in the absence of IL-2 supplementation

FLT3 OR CD33 NOT EMCN logic-gated CAR-NK cells demonstrated longer in vitro persistence than their donor-matched non-engineered NK cell controls. Three out of four lots demonstrated in vitro persistence of between 32-39 days during in vitro culture without IL-2 supplementation (**Table 15, FIG. 2**), while cells from Lot 2 only persisted for 7 days for reasons that are not fully explained but are potentially due to sub-optimal thawing and recovery from freezing. Lots 3 and 4 persisted for 39 and 35 days respectively, while Lot 1 persisted for 32 days.

As shown in **FIG. 2**, FLT3 OR CD33 NOT EMCN logic-gated CAR-NK cell cultures were characterized by an initial decrease in cell number at the first time point taken, followed by a period of recovery and eventual decline in cell count. For the three lots that showed in vitro persistence, the initial decrease in cell count was followed by an increase over the next 7-10 days, before cell numbers steadily decreased again until live cells were no longer detected. The increase in cell number was higher for Lot 3 and Lot 4 compared to the other lots, reaching a maximum of about 0.75-fold above what was originally seeded for Lot 3, and a maximum of about 0.2-fold above that seeded for Lot 4.

As shown in **FIG. 2**, without the supplementation of IL-2, non-engineered NK cell counts from matched lots declined sharply and rapidly, with live cells being no longer detectable by day 7 in all NK lots tested. This suggested that the non-engineered NK cells were unable to survive without cytokine support.

Table 15 - In Vitro Persistence Duration of FLT3 OR CD33 NOT EMCN Logic-Gated CAR-NK Cells Under Conditions Without IL-2 Supplementation

Lot (TL) Number	In Vitro Persistence of Engineered Cells (Days)
Lot 1	32
Lot 2	7
Lot 3	39
Lot 4	35

In Vitro Persistence of FLT3 OR CD33 NOT EMCN Logic-Gated CAR-NK Cells***Supplemented with Low Dose IL-2***

In vitro persistence was further analyzed for FLT3 OR CD33 NOT EMCN logic-gated CAR-NK cells and non-engineered NK cells in the presence of low dose cytokine (10 IU/mL IL-2). The addition of 10 IU/mL IL-2 was observed to enhance in vitro persistence of all non-engineered NK cells tested, as live cells were still detected in all IL-2-supplemented cultures at day 7, while non-engineered NK cells with no cytokines added had no detectable cells by this time point (**FIG. 3**).

The addition of 10 IU/mL IL-2 was observed to enhance FLT3 OR CD33 NOT EMCN logic-gated CAR-NK cell persistence in the following ways (**FIG. 3**):

- Lot 1: The initial drop in cell count was reduced with the addition of IL-2. About 50% of originally seeded cells (approximately the peak cell count observed without IL-2) were maintained in cultures for 18 days before cell numbers started to decline.
- Lot 2: A small portion of cells persisted for 11 days before starting to decline.
- Comparatively, in the absence of IL-2, TL14607 FLT3 OR CD33 NOT EMCN logic-gated CAR-NK cells persisted for 7 days.
- Lot 3 and Lot 4: The peak of NK cell expansion at days 10-14 was slightly higher with the addition of 10 IU/mL IL-2 compared to the condition lacking IL-2 supplementation.

Overall, these data demonstrated that even in the presence of the recombinantly expressed crIL15 cytokine, supplementation with low dose IL-2 supports the enhanced in vitro persistence of FLT3 OR CD33 NOT EMCN logic-gated CAR-NK cells, resulting in increased initial cell recovery, increased duration of cell survival, and increased cell expansion among various donors. These effects were limited and did not result in extended persistence of FLT3 OR CD33 NOT EMCN logic-gated CAR-NK cells in vitro.

In Vitro Persistence of FLT3 OR CD33 NOT EMCN logic-gated CAR-NK cells Supplemented with High Dose IL-2

FLT3 OR CD33 NOT EMCN logic-gated CAR-NK cell Lots 3 and 4, and their non-engineered NK controls, were also cultured under a high dose cytokine condition (500 IU/mL IL2 and 10 ng/mL IL15). The addition of high dose cytokines induced FLT3 OR CD33 NOT EMCN logic-gated CAR-NK cells and non-engineered NK cells to proliferate, reaching cell numbers between 3-5 fold of what was originally seeded (**FIG. 4**). NK cell numbers then decreased steadily after the proliferative peak starting at the 14-day mark for FLT3 OR CD33 NOT EMCN logic-gated CAR-NK cells and between 7-10 days for non-engineered NK cells.

The fold change in cell counts did not increase again, despite weekly cytokine replenishment. This suggested that although the high dose cytokine condition initially provided a powerful stimulus for NK cell proliferation, this effect was not permanent and NK cells would not persist indefinitely even in the presence of cytokines.

5

Example 3 – Effect of IL2 Treatment on FLT3 OR CD33 NOT EMCN logic-gated CAR-NK Cytotoxicity Against AML Cell Lines

Assessed was the in vitro cytotoxic activity against AML target cells of FLT3 OR CD33 NOT EMCN logic-gated CAR-NK cells in the presence of the cytokine IL2.

10

Methods

- The impact of different IL-2 treatments on FLT3 OR CD33 NOT EMCN logic-gated CAR-NK cell cytotoxicity was tested using the AML target cell lines MOLM-13, MV4-11, HL-60, THP-1, and Kasumi-1 that express CD33 and FLT3 (FIG. X).
- Thawed FLT3 OR CD33 NOT EMCN logic-gated CAR-NK cells were exposed to three different treatments relating to IL-2 (**FIG. 5**):
 1. “No IL-2” (IL-2 not added for duration of culture),
 2. “IL-2, 4 days” (IL-2 added during 4-day post-thaw culture period but not during cytotoxicity assay),
 3. “IL-2, 5 days” (IL-2 added during 4-day post-thaw culture period and also during cytotoxicity assay).
- The impact of “IL-2, 4 days” treatment on FLT3 OR CD33 NOT EMCN logic-gated CAR-NK cell cytotoxicity was tested using the AML target cell line KG-1a that does not express CD33 and FLT3 (**FIG. 6**).
- NK cell-mediated cytotoxicity against target cell lines was assessed using a flow cytometry-based cytotoxicity assay. Briefly, target cell lines were stained with a fluorescent labeling reagent and co-cultured with NK cells. At the end of the culture period, cells were stained with a fluorescent viability dye before being analyzed on a flow cytometer.
- NK cells were washed in RPMI-1640 + 10% FBS (assay medium) and plated in 96-well U-bottom plates. Target cell lines were stained with a labeling reagent according to vendor’s instructions and resuspended in assay medium. Co-cultures were set up with 200 μ L assay medium per well, containing 5×10^4 AML target cells mixed with NK cell numbers determined by the E:T ratio specified (e.g., 1×10^5 NK cells for E:T = 2:1; 5×10^4

30

NK cells for E:T = 1:1). To establish a baseline cytotoxicity measurement, AML target cells were cultured without NK cells.

- After overnight incubation (16 to 20 hours), cells were stained with a viability dye. In the case of cells stained with a fixable viability dye, cells were fixed after staining with 4% paraformaldehyde in DPBS for 20 minutes at room temperature, before being resuspended in FACS buffer. Samples were analyzed by flow cytometry, and the percentage of target cell events in a fixed volume (60 μ L) was recorded (total between 5,000 to 25,000 events). During flow cytometry analysis, AML target cells were distinguished from NK cells by high staining with the labeling reagent.
- Cytotoxicity was calculated by normalizing the viability of co-cultured AML target cells to their viability at baseline. The following formula was used to calculate percent NK-mediated cytotoxicity:

$$\left(1 - \frac{\% \text{ of live AML Target cells in NK co-culture}}{\% \text{ of live AML Target cells at baseline}}\right) * 100\%$$

- Statistical software was used for graphical data representation and statistical analysis. Means and standard deviations were calculated and plotted where indicated. Two-way ANOVA with Dunnett's T3 multiple comparisons test was used to calculate statistical significance between FLT3 OR CD33 NOT EMCN logic-gated CAR-NK and non-engineered NK cells at each E:T ratio.

Results

KG-1a cells that do not express CD33 and FLT3 displayed no cytotoxicity in the presence of the "IL-2, 4 days" condition whether the KG-1a cells were cultured with non-engineered NK cells or FLT3 OR CD33 NOT EMCN logic-gated CAR-NK cells indicating AML cells were not killed by the presence of IL-2 alone (**FIG. 6**). In general, FLT3 OR CD33 NOT EMCN logic-gated CAR-NK cells cultured without IL-2 had high cytotoxicity at 2:1 E:T ratio ranging from nearly 80-100% against HL-60, THP-1 and Kasumi-1 to 50-65% against MOLM-13 and MV4-11 (**FIG. 7**). With decreasing E:T ratio, the cytotoxicity generally decreased, although there was robust (50% or higher) cytotoxicity observed against HL-60, THP-1, and Kasumi-1.

Addition of IL-2 (for a period of 4 or 5 days) improved cytotoxicity against MOLM-13 at E:T ratios of 2:1 and 1:1, and against HL-60 at an E:T ratio of 0.5:1. Any additive improvement in cytotoxicity with addition of IL-2 was not assessable in other conditions, which demonstrated similar cytotoxicity across different IL-2 culture durations. This included results from the cell

lines where high cytotoxicity was observed (HL-60, THP-1 and Kasumi-1), which may have confounded the effects of IL-2.

There was no difference in cytotoxicity between the two IL-2 culture durations tested (IL-2, 4 days and IL-2, 5 days).

5 ***Example 4 – FLT3 OR CD33 NOT EMCN Logic-Gated CAR-NK Cell Serial Killing Against AML Target Cell Lines***

The serial killing activity of FLT3 OR CD33 NOT EMCN logic-gated CAR-NK cells was assessed in comparison to non-engineered NK cells against AML target cell lines and effect of IL-2 on the serial killing activity of FLT3 OR CD33 NOT EMCN logic-gated CAR-NK cells
10 against AML target cell lines.

Methods

- FLT3 OR CD33 NOT EMCN logic-gated CAR-NK cells and non-engineered NK cells were evaluated for serial killing against AML target cell lines using a flow-based cytotoxicity assay, using serial rechallenge of NK cells to fresh AML target cells every
15 24 hours. The serial killing assay was initiated with an effector to target ratio of 2:1, followed by replenishing the same number of target cells in subsequent rechallenges. To capture variability due to different batches and NK donors, a total of 6 lots of FLT3 OR CD33 NOT EMCN logic-gated CAR-NK cells and donor-matched non-engineered NK controls from 5 different donors were assessed for in vitro serial killing.
- The different lots of NK cells were tested as follows:
 - For MV4-11 and MOLM-13 serial killing assays, all 6 lots of NK cells were tested.
 - For THP-1, HL-60, and Kasumi-1 serial killing assays, 3 lots of NK cells were tested.
 - For assays involving IL-2 in the killing assay, 3 lots of NK cells were
25 tested for all target cell lines.
- A schematic of the serial killing assay with or without IL-2 is provided in **FIG 8**.
- FLT3 OR CD33 NOT EMCN logic-gated CAR-NK cells and non-engineered NK cells were thawed with NK MACS complete medium containing 20% human AB serum, then
30 cultured for 3-4 days at 37°C in the same media containing 100 IU/mL of interleukin-2 (IL-2). NK cells were then washed in filtered RPMI-1640 + 10% FBS (assay medium) and plated in 96-well U-bottom plates. NK cells were plated in replicate wells sufficient

for three sample collections in either duplicate or triplicate wells, at 1×10^5 NK cells per well.

- 5 • AML target cell lines were stained with a fluorescent labeling reagent according to manufacturer's instructions and resuspended in assay medium. 5×10^4 AML target cells were then plated into each designated well in assay medium, for a final E:T ratio of 2:1. To establish a baseline cytotoxicity measurement, some wells contained AML target cells that were cultured in the absence of NK cells.
- 10 • For the "No IL-2 in killing assay" condition, co-cultures were set up in assay medium with no additional IL-2 added. For the "With IL-2 in killing assay" condition, co-cultures were set up in assay medium with IL-2 added at a final concentration of 100 IU/mL.
- 15 • Cells were cultured for a total duration of 72 hours in a 37°C / 5% CO_2 incubator. At 24, 48, and 72 hours after the start of the experiment, at least 2 wells per condition were collected for cytotoxicity assessment by flow cytometry. To the remaining wells at each collection timepoint, another 5×10^4 fresh fluorescently-labeled AML cells were added to each well (rechallenge). Cells were collected for cytotoxicity testing 24 hours after each rechallenge.
- 20 • For the "No IL-2 in killing assay" condition, co-cultures were supplemented with fresh assay medium at each rechallenge. For the "With IL-2 in killing assay" condition, assay medium was supplemented with fresh IL-2 at each rechallenge for a final concentration of 100 IU/mL.
- 25 • At each cytotoxicity assessment time point (24, 48, and 72 hours from the start of the assay), cell co-cultures were centrifuged, resuspended in media containing 5 nM of a viability dye, and transferred to a new 96-well plate. In the case of cells stained with the fixable viability dye, cells were fixed after staining with 4% paraformaldehyde in DPBS for 20 minutes at room temperature, before being resuspended in FACS buffer.
- 30 • Co-cultures were analyzed by flow cytometry, with AML target cells distinguished by the fluorescent labeling reagent staining and dead cells identified by viability staining.
- The following formula was used to calculate NK-mediated cytotoxicity:

 - $\% \text{ Cytotoxicity} = \left[1 - \left(\frac{\% \text{ of live target cells from co-culture}}{\% \text{ of live target cells in target only well}} \right) \right] \times 100\%$
- Statistical software was used for graphical data presentation and statistical analysis. Means and standard deviations were calculated for numerical parameters. Two-way ANOVA and Dunnett's T3 multiple comparisons test were used to determine statistical

significance between non-engineered NK- and FLT3 OR CD33 NOT EMCN logic-gated CAR-NK cell-mediated serial killing.

Results

The serial killing activity of FLT3 OR CD33 NOT EMCN logic-gated CAR-NK cells was first compared to that of non-engineered NK cells against the 5 different AML cell lines. Cytotoxicity against target cell lines was observed in all 3 rounds of killing for both FLT3 OR CD33 NOT EMCN logic-gated CAR-NK cells and non-engineered NK cells (**FIG. 9**). In general, cytotoxicity was observed to decrease over each subsequent round of killing.

As shown in **FIG. 9**, FLT3 OR CD33 NOT EMCN logic-gated CAR-NK cells demonstrated significantly increased serial killing ability over three rounds compared to donor-matched non-engineered NK cells. An increase in cytotoxicity for FLT3 OR CD33 NOT EMCN logic-gated CAR-NK cells in at least one round of serial killing was observed in all 5 AML target cell lines tested. All statistical significance values are listed in **Table 16**. FLT3 OR CD33 NOT EMCN logic-gated CAR-NK cell cytotoxicity was increased compared to non-engineered NK cells against MV4-11 and MOLM-13 target cells in the 2nd and 3rd rounds of target cell re-challenge, and against THP-1, Kasumi-1, and HL-60 in the 3rd round of re-challenge.

FLT3 OR CD33 NOT EMCN logic-gated CAR-NK cell serial killing was further assessed on all AML target cell lines in the presence or absence of IL-2. As shown in **FIG. 10**, IL-2 enhanced FLT3 OR CD33 NOT EMCN logic-gated CAR-NK cells serial killing for three (MV4-11, MOLM-13, and HL-60) out of the five AML target cell lines tested. All statistical significance values are listed in **Table 17**.

Table 16 **Engineered vs Non-Engineered NK Killing Assay P-Values**

Target	Comparison	P-value
MV4-11	Non-engineered vs engineered: Round 1	ns
MV4-11	Non-engineered vs engineered: Round 2	<0.0001
MV4-11	Non-engineered vs engineered: Round 3	0.0062
MOLM-13	Non-engineered vs engineered: Round 1	0.0479
MOLM-13	Non-engineered vs engineered: Round 2	<0.0001
MOLM-13	Non-engineered vs engineered: Round 3	0.0007
THP-1	Non-engineered vs engineered: Round 1	ns
THP-1	Non-engineered vs engineered: Round 2	ns
THP-1	Non-engineered vs engineered: Round 3	0.0041
HL-60	Non-engineered vs engineered: Round 1	ns
HL-60	Non-engineered vs engineered: Round 2	ns
HL-60	Non-engineered vs engineered: Round 3	0.0002
Kasumi-1	Non-engineered vs engineered: Round 1	ns
Kasumi-1	Non-engineered vs engineered: Round 2	ns
Kasumi-1	Non-engineered vs engineered: Round 3	0.0043

Abbreviations: ns, not significant. Engineered = FLT3 OR CD33 NOT EMCN logic-gated CAR-NK cells.

Table 17 **Serial Killing in the Presence or Absence of IL2, P-Values**

Target	Comparison	P-value
MV4-11	No IL2 vs With IL2: Round 1	ns
MV4-11	No IL2 vs With IL2: Round 2	ns
MV4-11	No IL2 vs With IL2: Round 3	0.0094
MOLM-13	No IL2 vs With IL2: Round 1	ns
MOLM-13	No IL2 vs With IL2: Round 2	0.0384
MOLM-13	No IL2 vs With IL2: Round 3	<0.0001
THP-1	No IL2 vs With IL2: Round 1	ns
THP-1	No IL2 vs With IL2: Round 2	ns
THP-1	No IL2 vs With IL2: Round 3	ns
HL-60	No IL2 vs With IL2: Round 1	ns
HL-60	No IL2 vs With IL2: Round 2	0.0208
HL-60	No IL2 vs With IL2: Round 3	ns
Kasumi-1	No IL2 vs With IL2: Round 1	ns
Kasumi-1	No IL2 vs With IL2: Round 2	ns
Kasumi-1	No IL2 vs With IL2: Round 3	ns

Abbreviations: ns, not significant.

FLT3 OR CD33 NOT EMCN logic-gated CAR-NK cells recombinantly expressing crIL15 exhibited serial killing even in the absence of IL-2 supplementation during the killing assay; the lowest level of cytotoxicity observed for all conditions was between 40-50%. In 3 conditions, supplementation with IL2 showed significantly greater cytotoxicity compared to no IL-2 supplementation, even in the presence of recombinantly expressed crIL15 (see the 3rd re-challenge with MOLM-13 and MV4-11, and the 2nd re-challenge with HL-60). In other conditions tested, the average cytotoxicity of FLT3 OR CD33 NOT EMCN logic-gated CAR-NK cells without IL-2 was at least 70%, limiting the ability to assess additional cytotoxic benefit due to supplementation with IL-2.

An additional condition showing IL-2 benefit was the second re-challenge of MOLM-13; this was the only condition with over 70% cytotoxicity in the absence of IL-2, which showed further increased killing with IL-2. The remaining FLT3 OR CD33 NOT EMCN logic-gated CAR-NK cells test conditions had cytotoxicity of 70% or greater in the absence of IL-2 and did not show significant benefit upon IL-2 addition.

Other Sequences

Various sequences that can be used include those sequences listed below, as well as additional exemplary sequences described in International Application Pub. No. WO2023205614, which is hereby incorporated by reference for all purposes.

[illegible]

[illegible]

[illegible]

[illegible]

[illegible]

[illegible]

[illegible]

[illegible]

Interpretations

All references, patents and patent applications disclosed herein are incorporated by reference with respect to the subject matter for which each is cited, which in some cases may
5 encompass the entirety of the document.

The indefinite articles “a” and “an,” as used herein in the specification and in the claims, unless clearly indicated to the contrary, should be understood to mean “at least one.”

It should also be understood that, unless clearly indicated to the contrary, in any methods claimed herein that include more than one step or act, the order of the steps or acts of the method
10 is not necessarily limited to the order in which the steps or acts of the method are recited.

In the claims, as well as in the specification above, all transitional phrases such as “comprising,” “including,” “carrying,” “having,” “containing,” “involving,” “holding,” “composed of,” and the like are to be understood to be open-ended, *i.e.*, to mean including but not limited to. Only the transitional phrases “consisting of” and “consisting essentially of” shall
15 be closed or semi-closed transitional phrases, respectively, as set forth in the United States Patent Office Manual of Patent Examining Procedures, Section 2111.03.

CLAIMS

What is claimed is:

1. A method of treating a subject known or suspected of having cancer, the method comprising:

(A) administering a cell therapy to the subject, wherein the cell therapy comprises an immunoresponsive cell engineered to express a recombinant cytokine, optionally wherein the immunoresponsive cell engineered to express the recombinant cytokine comprises an exogenous polynucleotide encoding a controlled release cytokine, oriented from N-terminal to C-terminal, having the formula:

$$\mathbf{S - C - MT \text{ or } MT - C - S}$$

wherein

S comprises a secretable effector molecule, wherein the secretable effector molecule comprises the recombinant cytokine,

C comprises a protease cleavage site, and

MT comprises a cell membrane tethering domain,

wherein $\mathbf{S - C - MT}$ or $\mathbf{MT - C - S}$ is configured to be expressed as a single polypeptide; and

(B) administering a pharmaceutically effective amount of IL-2,

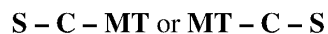
optionally wherein the immunoresponsive cell is engineered to express the recombinant cytokine is from a multicistronic expression system, optionally wherein the recombinant cytokine comprises IL-15 or a functional fragment thereof, optionally wherein the recombinant cytokine comprises crIL15.
2. The method of claim 1, wherein the immunoresponsive cell comprises
 - a. an exogenous polynucleotide encoding an inhibitory chimeric antigen receptor (iCAR) comprising: (i) an antigen-binding domain specific for endomucin (EMCN); (ii) one or more intracellular inhibitory domains that inhibit an immune response; and (iii) one or more polypeptides selected from the group consisting of: a signal peptide, a transmembrane domain, a hinge domain, a spacer region, one or more peptide linkers, and combinations thereof, and/or
 - b. an exogenous polynucleotide encoding a bivalent activating chimeric antigen receptor (aCAR) comprising: (i) an antigen-binding domain specific for FLT3;

- (ii) an antigen-binding domain specific for CD33; (iii) one or more intracellular signaling domains that stimulate an immune response; and (iv) one or more polypeptides selected from the group consisting of: a signal peptide, a transmembrane domain, a hinge domain, a spacer region, one or more peptide linkers, and combinations thereof.
3. The method of any one of claims 1-2, wherein the administration of IL-2 comprises multiple administrations, optionally wherein the multiple administrations of IL-2 is at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, or at least 9 administrations, optionally wherein IL-2 is administered within 4, within 5, within 7, or within 8 hours following the start of the administration of the cell therapy, optionally wherein IL-2 is administered within 4 to 8 hours following the administration of the cell therapy, optionally wherein IL-2 is administered within 6 to 8 hours following the administration of the cell therapy, optionally wherein IL-2 is administered within 4 hour, 5 hours, 6 hours, 7 hours or 8 hours, following the administration of the cell therapy, optionally wherein IL-2 is administered between 4 to 8 hours following the administration of the cell therapy, optionally wherein IL-2 is administered between 6 to 8 hours following the administration of the cell therapy, optionally wherein IL-2 is administered within 24 or 48 hours after the administering of the cell therapy, optionally wherein the pharmaceutically effective amount of IL-2 is between 1-10 MIU, optionally wherein the pharmaceutically effective amount of IL-2 is a dose of 3 MIU.
4. The method of any one of claims 1-3, wherein the administration of the cell therapy comprises multiple administrations, optionally wherein IL-2 is administered at least 1, at least 2, at least 3 times a week following each administration of the cell therapy, optionally wherein the cell therapy is administered again at least on day 7 and/or day 14 following the initial administration of the cell therapy, optionally wherein the cell therapy is administered again at least on day 7 following the initial administration of the cell therapy, optionally wherein the cell therapy is administered again at least on day 7 and day 14 following the initial administration of the cell therapy, optionally wherein the cell therapy and the pharmaceutically effective amount of IL-2 are administered again at least on day 7 and/or day 14 following the initial administration of the cell therapy, optionally wherein the cell therapy and the pharmaceutically effective amount of IL-2 are administered again at least on day 7 following the initial administration of the cell therapy, optionally wherein the cell therapy and the pharmaceutically effective amount of IL-2 are administered again at least on day 7 and day 14 following the initial

administration of the cell therapy, optionally wherein the pharmaceutically effective amount of IL-2 is between 1-10 MIU, optionally wherein the pharmaceutically effective amount of IL-2 is a dose of 3 MIU.

5. The method of any one of claims 1-4, wherein the subject has, prior to administration of the cell therapy, been administered one or more lymphodepletion agents, optionally wherein the one or more lymphodepletion agents is administered 5 days prior to administration of the cell therapy, optionally wherein the one or more lymphodepletion agents is administered daily until administration of the cell therapy, optionally wherein the one or more lymphodepletion agents comprises fludarabine, cytarabine (Ara-C), or combinations thereof, optionally wherein the one or more lymphodepletion agents are administered intravenously, optionally wherein fludarabine is administered at a dose of 30 mg/m² and cytarabine (Ara-C) is administered at a dose of 2 g/m².
6. The method of any one of claims 1-5, wherein the method of treatment comprises
 - i) a reduction in tumor volume in the subject;
 - ii) treatment of a solid tumor in the subject; and/or
 - iii) inducing an immune response in the subject.
7. The method of any one of claims 1-6, wherein the immunoresponsive cell comprises an engineered nucleic acid comprising at least one selected from the group consisting of:

- (A) an exogenous polynucleotide encoding a controlled release cytokine, oriented from N-terminal to C-terminal, having the formula:



wherein

S comprises a secretable effector molecule, wherein the secretable effector molecule comprises the recombinant cytokine,

C comprises a protease cleavage site, and

MT comprises a cell membrane tethering domain,

wherein **S - C - MT** or **MT - C - S** is configured to be expressed as a single polypeptide;

- (B) an exogenous polynucleotide encoding an inhibitory chimeric antigen receptor (iCAR) comprising:
 - (i) an antigen-binding domain specific for endomucin (EMCN);

- (ii) one or more intracellular inhibitory domains that inhibit an immune response; and
 - (iii) one or more polypeptides selected from the group consisting of: a signal peptide, a transmembrane domain, a hinge domain, a spacer region, one or more peptide linkers, and combinations thereof; and
- (C) an exogenous polynucleotide encoding a bivalent activating chimeric antigen receptor (aCAR) comprising:
- (i) an antigen-binding domain specific for FLT3;
 - (ii) an antigen-binding domain specific for CD33;
 - (iii) one or more intracellular signaling domains that stimulate an immune response; and
 - (iv) one or more polypeptides selected from the group consisting of: a signal peptide, a transmembrane domain, a hinge domain, a spacer region, one or more peptide linkers, and combinations thereof;

optionally wherein the membrane-cleavable chimeric protein, the iCAR, and the bivalent aCAR are encoded in order from 5' to 3': (i) membrane-cleavable chimeric protein; (ii) bivalent aCAR; and (iii) iCAR; or from 5' to 3': (i) membrane-cleavable chimeric protein; (ii) iCAR; and (iii) bivalent aCAR.

8. The multicistronic expression system of any one of claims 1-7, wherein the exogenous polynucleotides encoding each of the membrane-cleavable chimeric protein, the iCAR, and the bivalent aCAR are linked together by a polynucleotide linker encoding a 2A ribosome skipping element, optionally wherein the 2A ribosome skipping element is selected from the group consisting of: a T2A ribosome skipping element, a E2A ribosome skipping element, a P2A ribosome skipping element, a F2A ribosome skipping element, ribosome skipping element fusions thereof, and combinations thereof, optionally wherein the ribosome skipping element fusion comprises an E2A/T2A ribosome skipping element, optionally wherein the E2A/T2A ribosome skipping element comprises the amino acid sequence QCTNYALLKLAGDVESNPGPGSGEGRGSLLTCGDVEENPGP (SEQ ID NO: 221), optionally wherein the E2A/T2A ribosome skipping element is encoded by a polynucleotide sequence comprising the sequence
CAGTGTACCAACTACGCCCTGCTGAAACTGGCCGGCGACGTGGAATCTAATC
CTGGACCTGGATCTGGCGAGGGACGCGGGAGTCTACTGACGTGTGGAGACGT
GGAGGAAAACCCTGGACCT (SEQ ID NO: 222) or the sequence
CAGTGCACAAATTATGCACTGCTGAAGCTCGCCGGGGATGTTCGAGAGTAACC
CAGGACCTGGAAGCGGAGAAGGTCGTGGTAGTCTACTAACGTGTGGTGATGT
AGAAGAAAATCCTGGACCT (SEQ ID NO: 223).
9. The multicistronic expression system of any one of claims 1-8, wherein the protease cleavage site further comprises the N-terminal peptide linker, optionally selected from the group consisting of: SGGGSGGGGSG (SEQ ID NO: 230); GGGSGGGSGGGSLQ (SEQ ID NO: 231); GGS (SEQ ID NO: 232); GSGGS (SEQ ID NO: 233); GSGGSGGS (SEQ ID NO: 234); GSGGSGGSGGS (SEQ ID NO: 235); GSGGSGGSGGSGGS (SEQ ID NO: 236); GGGS (SEQ ID NO: 237); GGGSGGGS (SEQ ID NO: 238); GGGSGGSGGGS (SEQ ID NO: 239); GGGSGGSGGGSGGGS (SEQ ID NO: 240); GGGSGGSGGGSGGSGGGS (SEQ ID NO: 241); GGGGS (SEQ ID NO: 242); GGGGSGGGGS (SEQ ID NO: 243); GGGGSGGGSGGGGS (SEQ ID NO: 244); GGGGSGGGSGGGSGGGGS (SEQ ID NO: 245); GGGGSGGGSGGGSGGGSGGGGS (SEQ ID NO: 246); GSTSGSGKPGSGEGSTKG (SEQ ID NO: 247); and EAAAKEAAAKEAAAKEAAK (SEQ ID NO: 248), optionally wherein the protease cleavage site further comprises the N-terminal peptide linker SGGGSGGGGSG (SEQ ID NO: 230), optionally wherein the protease cleavage site further comprises a C-terminal peptide linker, optionally selected

from the group consisting of: SGGGSGGGGSG (SEQ ID NO: 230); GGGSGGGSGGGSLQ (SEQ ID NO: 231); GGS (SEQ ID NO: 232); GGS GGS (SEQ ID NO: 233); GGS GGS GGS (SEQ ID NO: 234); GGS GGS GGS GGS (SEQ ID NO: 235); GGS GGS GGS GGS GGS (SEQ ID NO: 236); GGS (SEQ ID NO: 237); GGS GGS GGS (SEQ ID NO: 238); GGS GGS GGS GGS (SEQ ID NO: 239); GGS GGS GGS GGS GGS GGS (SEQ ID NO: 240); GGS GGS GGS GGS GGS GGS GGS (SEQ ID NO: 241); GGS GGS (SEQ ID NO: 242); GGS GGS GGS GGS (SEQ ID NO: 243); GGS GGS GGS GGS GGS GGS (SEQ ID NO: 244); GGS GGS GGS GGS GGS GGS GGS GGS (SEQ ID NO: 245); GGS GGS GGS GGS GGS GGS GGS GGS GGS (SEQ ID NO: 246); GSTSGSGKPGSGEGSTKG (SEQ ID NO: 247); and EAAAKEAAAKEAAAKEAAK (SEQ ID NO: 248), optionally wherein the protease cleavage site further comprises the C-terminal linker GGGSGGGSGGGSLQ (SEQ ID NO: 231), optionally wherein the protease cleavage site comprises a Tumor Necrosis Factor- α Converting Enzyme (TACE)-specific cleavage site, optionally wherein the TACE-specific cleavage site comprises the amino acid sequence VTPEIFSLI (SEQ ID NO: 191), optionally, wherein the TACE-specific cleavage site comprises the amino acid sequence SGGGSGGGSGVTPEIFSLIGGGSGGGSGGGSLQ (SEQ ID NO: 250), optionally wherein the TACE-specific cleavage site is encoded by a polynucleotide sequence comprising the sequence TCAGGCGGCGGTGGTAGTGGAGGCGGAGGCTCAGGCGTGACCCCTGAGCCTA TCTTCAGCCTGATCGGCGGAGGTTCCGGAGGTGGCGGTTCGGCGGAGGATC TCTTCAA (SEQ ID NO: 251).

10. The multicistronic expression system of any one of claims 1-9, wherein the cell membrane tethering domain comprises a transmembrane domain selected from the group consisting of: PDGFR-beta, CD8, CD28, CD3zeta-chain, CD4, 4-1BB, OX40, ICOS, CTLA-4, PD-1, LAG-3, 2B4, LNGFR, NKG2D, EpoR, TNFR2, LIR1, B7-1, and BTLA, optionally wherein the cell membrane tethering domain comprises a B7-1 transmembrane domain, optionally wherein the B7-1 transmembrane domain comprises the amino acid sequence LLPSWAITLISVNGIFVICCLTYCFAPRCRERRRNERLRRESVRPV (SEQ ID NO: 204), optionally wherein the B7-1 transmembrane domain is encoded by a polynucleotide sequence comprising the sequence TTGCTGCCTAGCTGGGCCATCACACTGATCTCCGTGAACGGCATCTTCGTGAT CTGCTGCCTGACCTACTGCTTCGCCCTAGATGCAGAGAGCGGAGAAGAAAC

GAGCGGCTGAGAAGAGAAAGCGTGCGGCCTGTG (SEQ ID NO: 252), optionally wherein the membrane-cleavable chimeric protein, oriented from N-terminal to C-terminal, has the formula **S – C – MT**, optionally wherein the membrane-cleavable chimeric protein comprises the amino acid sequence

MDWTWILFLVAAATRVHSNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCK
VTAMKCFLELQVISLESGDASIHDVTENLIILANNSLSSNGNVTESGCKECEEELEE
KNIKEFLQSFVHIVQMFINTSSGGGSGGGGSGVTPEPIFSLIGGGSGGGGSGGGSL
QLLPWAITLISVNGIFVICCLTYCFAPRCRERRRNERLRRESVRPV (SEQ ID NO:

226), optionally wherein the membrane-cleavable chimeric protein is encoded by a polynucleotide sequence comprising the sequence

ATGGACTGGACTTGGATACTCTTTCTGGTCGCTGCCGCCACACGGGTGCACTC
TAATTGGGTCAACGTGATCAGCGACCTGAAGAAGATCGAGGACCTGATCCAG
AGCATGCACATCGACGCCACACTGTACACCGAGTCCGATGTGCACCCTAGCT
GCAAAGTGACCGCCATGAAGTGCTTTCTGCTGGAAGTCAAGTGATCAGCCT
GGAAAGCGGCGACGCCAGCATCCACGATACCGTGGAATACTGATCATCCTG
GCCAACAACAGCCTGTCCAGCAACGGCAATGTGACCGAGAGCGGCTGCAAAG
AGTGCGAGGAACTGGAAGAGAAGAACATCAAAGAGTTTCTGCAGAGCTTCGT
CCACATCGTGAGATGTTTCATCAACACCTCATCAGGCGGCGGTGGTAGTGGA
GGCGGAGGCTCAGGCGTGACCCCTGAGCCTATCTTCAGCCTGATCGGCGGAG
GTTCCGGAGGTGGCGGTTCCGGCGGAGGATCTCTTCAATTGCTGCCTAGCTGG
GCCATCACACTGATCTCCGTGAACGGCATCTTCGTGATCTGCTGCCTGACCTA
CTGCTTCGCCCTAGATGCAGAGAGCGGAGAAGAAACGAGCGGCTGAGAAG
AGAAAGCGTGCGGCCTGTG (SEQ ID NO: 227).

11. The multicistronic expression system of any one of claims 1-10, wherein the antigen-binding domain specific for EMCN comprises a heavy chain variable (VH) region and a light chain variable (VL) region, wherein:

the EMCN-VH comprises:

a heavy chain complementarity determining region 1 (CDR-H1) having the amino acid sequence of RYDMH (SEQ ID NO: 291),

a heavy chain complementarity determining region 2 (CDR-H2) having the amino acid sequence of VIWGNGNTHYHSALKS (SEQ ID NO: 296), and

a heavy chain complementarity determining region 3 (CDR-H3) having the amino acid sequence of RIKD (SEQ ID NO: 298), and

KDRQAEEDRQMDTEAAASEAPQDVITYAQLHSLTLRREATEPPPSQEGPSPAVPSI
 YATLAIH (SEQ ID NO: 285), optionally wherein the LIR1 intracellular inhibitory
 domain is encoded by a polynucleotide sequence comprising the sequence
 CTGCGGCACAGAAGGCAGGGCAAGCACTGGACAAGCACCCAGAGAAAGGCC
 GACTTTCAGCATCCTGCTGGCGCCGTTGGACCTGAGCCTACAGATAGAGGAC
 TGCAGTGGCGGTCTAGCCCTGCCGCTGATGCCCAAGAGGAAAATCTTTACGC
 CGCCGTGAAGCACACCCAGCCTGAGGATGGCGTGGAAATGGACACCAGATC
 TCCCCACGATGAGGACCCTCAGGCCGTGACATACGCAGAAGTGAAGCACTCC
 AGACCTCGGAGAGAGATGGCAAGCCCTCCATCTCCTCTGAGCGGCGAGTTCC
 TGGACACCAAAGACAGACAGGCCGAAGAGGACAGACAGATGGATACCGAA
 GCCGCCGCTTCTGAAGCCCCACAGGATGTGACATATGCCCAGCTGCATAGCC
 TGACACTGCGGAGAGAAGCCACAGAGCCTCCACCTTCTCAAGAAGGCCCATC
 TCCTGCCGTGCCTTCCATCTATGCCACTCTGGCCATTAC (SEQ ID NO: 286),
 optionally wherein (A) the signal peptide is present in the iCAR, optionally wherein the
 signal peptide of the iCAR comprises a native signal peptide native or a non-native
 signal peptide, optionally wherein the non-native signal peptide or the non-native signal-
 anchor sequence is selected from the group consisting of: IgE, IL-12, IL-2, optimized IL-
 2, trypsinogen-2, Gaussia luciferase, CD5, human IgKVII, murine IgKVII, VSV-G,
 prolactin, serum albumin preprotein, azurocidin preprotein, osteonectin, CD33, IL-6, IL-
 8, CCL2, TIMP2, VEGFB, osteoprotegerin, serpin E1, GROalpha, CXCL12, IL-21,
 CD8, NKG2D, TNFR2, and GMCSF, optionally wherein the signal peptide of the iCAR
 comprises a CD8 signal peptide, optionally wherein the CD8 signal peptide comprises
 the amino acid sequence MALPVTALLLPLALLLHAARP (SEQ ID NO: 137),
 optionally wherein the CD8 signal peptide is encoded by a polynucleotide sequence
 comprising the sequence
 ATGGCTCTGCCCCGTGACAGCTTTGCTGCTGCCTTTGGCACTGCTGCTGCATGC
 TGCTAGACCA (SEQ ID NO: 416); and/or wherein (B) the hinge domain is present in
 the iCAR comprises, optionally wherein the hinge domain of the iCAR is selected from
 the group consisting of a human Ig (immunoglobulin) hinge, an IgG4 hinge, an IgG2
 hinge, a CD8a hinge, or an IgD hinge, a KIR2DS2 hinge, an LNGFR hinge, a LIR1
 hinge, a PDGFR-beta extracellular linker, and combinations thereof, optionally wherein
 the hinge domain of the iCAR comprises a LIR1 hinge, optionally wherein the LIR1
 hinge comprises the amino acid sequence
 HPSDPLELVVSGPSGGPSSPTTGPTSTSGPEDQPLTPTGSDPQSGGLGRHLGV (SEQ

ID NO: 417), optionally wherein the LIR1 hinge comprises is encoded by a polynucleotide sequence comprising the sequence
CACCCATCCGATCCTCTCGAGCTGGTGGTTTCTGGACCTTCTGGCGGCCCTAG
CAGCCCTACAACAGGACCTACAAGCACAAGCGGCCCTGAGGACCAACCTCT
GACACCAACAGGCAGCGATCCTCAGTCTGGACTGGGGAGACATCTGGGCGTT
(SEQ ID NO: 418); optionally wherein the hinge domain of the iCAR comprises a CD8 hinge, optionally wherein the CD8 hinge comprises the amino acid sequence
TTTPAPRPPTPAPTIALQPLSLRPEACRPAAGGAVHTRGLDFACD (SEQ ID NO: 271), optionally wherein the CD8 hinge comprises is encoded by a polynucleotide sequence comprising the sequence
ACAACAACACCCGCACCTCGGCCTCCAACTCCAGCTCCAACAATTGCACTGC
AACCCCTGAGTCTGAGGCCCGAGGCCTGTAGGCCAGCAGCTGGCGGAGCTGT
TCACACTAGAGGCCTGGACTTTGCCTGTGAC (SEQ ID NO: 283); and/or wherein (C) the transmembrane domain of the iCAR is present, optionally wherein the transmembrane domain of the iCAR comprises a transmembrane domain selected from the group consisting of: PDGFR-beta, CD8, CD28, CD3zeta-chain, CD4, 4-1BB, OX40, ICOS, CTLA-4, PD-1, LAG-3, 2B4, LNGFR, NKG2D, EpoR, TNFR2, B7-1, LIR1, and BTLA, optionally wherein the transmembrane domain of the iCAR comprises a LIR1 transmembrane domain, optionally wherein the LIR1 transmembrane domain comprises the amino acid sequence VIGILVAVILLLLLLLLLLFLI (SEQ ID NO: 259), optionally wherein the LIR1 transmembrane domain is encoded by a polynucleotide sequence comprising the sequence
GTGATCGGCATTCTGGTCGCCGTGATCCTGCTCCTGTTGCTCCTGCTGCTTCT
GTTCTGATC (SEQ ID NO: 260), optionally wherein the iCAR comprises the amino acid sequence of
MALPVTALLPLALLLHAARPEVQLVESGGGLVQPGGSLRLSCAASGFTFSRYD
MHWVRQAPGKGLEWVSVIWGNNGNTHYHSALKSRFTISRDNKNTLYLQMNSL
RAEDTAVYYCTLRKDWGQGTMTVTSSGGGSGGGGSGGGGSDVVMQSP
LPVTLGQPASISCKSSQSLVASDENTYLNWFQQRPGQSPRRLIYQVSKLDSGVPD
RFSGSGSGTDFTLKISRVEAEDVGVYYCLQGIHLPWTFGQGTKLEIK_{Ngaa}HPSPD
LELVVSGPSGGPSSPTTGPTSTSGPEDQPLTPTGSDPQSGGLGRHLGVVIGILVAVIL
LLLLLLLLFLILRHRRQGKHWTSTQRKADFQHPAGAVGPEPTDRGLQWRSSPAA
DAQEENLYAAVKHTQPEDGVEMDTRSPHDEDPQAVTYAEVKHSRPRREMASPP
SPLSGEFLDTKDRQAEEDRQMDTEAAASEAPQDVITYAQLHSLTLRREATEPPPS

QEGPSPAVPSIYATLAIH (SEQ ID NO: 419), optionally wherein the iCAR is encoded by a polynucleotide sequence comprising the sequence of

ATGGCTCTGCCCCGTGACAGCTTTGCTGCTGCCTTTGGCACTGCTGCTGCATGC
TGCTAGACCAGAGGTGCAGCTGGTTGAATCTGGCGGAGGACTGGTTCAGCCT
GGCGGATCTCTGAGACTGTCTTGTGCCGCCAGCGGCTTCACCTTCAGCAGAT
ACGATATGCACTGGGTCCGACAGGCCCCCTGGCAAAGGACTTGAATGGGTGTC
CGTGATCTGGGGCAACGGCAACACACACTACCACAGCGCCCTGAAGTCCCG
GTTACACCATCTCCAGAGACAACAGCAAGAACACCCTGTACCTGCAGATGAAC
AGCCTGAGAGCCGAGGACACCGCCGTGTACTACTGCACCCTGAGAATCAAG
GATTGGGGGCCAGGGCACCATGGTCACCGTTTCTTCTGGAGGCGGAGGATCTG
GTGGCGGAGGAAGTGGCGGAGGCGGTTCTGACGTGGTCATGACACAGAGCC
CTCTGAGCCTGCCTGTGACACTGGGACAGCCTGCCAGCATCAGCTGCAAGTC
TAGCCAGTCTCTGGTGGCCAGCGACGAGAACACCTACCTGAACTGGTTCCAG
CAGAGGCCCCGACAGTCTCCTAGACGGCTGATCTACCAGGTGTCCAAGCTGG
ATAGCGGCGTGCCCGATAGATTTTCTGGCAGCGGCTCTGGCACCGACTTCAC
CCTGAAGATCAGCAGAGTGGAAGCCGAGGACGTGGGCGTGTACTACTGTCT
GCAAGGCATCCATCTGCCTTGGACCTTTGGCCAGGGCACAAAGCTGGAAATC
AAGAATGGCGCTGCACACCCATCCGATCCTCTCGAGCTGGTGGTTTCTGGAC
CTTCTGGCGGCCCTAGCAGCCCTACAACAGGACCTACAAGCACAAAGCGGCCC
TGAGGACCAACCTCTGACACCAACAGGCAGCGATCCTCAGTCTGGACTGGGG
AGACATCTGGGCGTTGTGATCGGCATTCTGGTCGCCGTGATCCTGCTCCTGTT
GCTCCTGCTGCTTCTGTTTCTGATCCTGCGGCACAGAAGGCAGGGCAAGCAC
TGGACAAGCACCCAGAGAAAGGCCGACTTTCAGCATCCTGCTGGCGCCGTTG
GACCTGAGCCTACAGATAGAGGACTGCAGTGGCGGTCTAGCCCTGCCGCTGA
TGCCCAAGAGGAAAATCTTTACGCCGCCGTGAAGCACACCCAGCCTGAGGAT
GGCGTGGAATGGACACCAGATCTCCCCACGATGAGGACCCTCAGGCCGTG
ACATACGCAGAAGTGAAGCACTCCAGACCTCGGAGAGAGATGGCAAGCCCT
CCATCTCCTCTGAGCGGCGAGTTCCTGGACACCAAAGACAGACAGGCCGAA
GAGGACAGACAGATGGATACCGAAGCCGCCGCTTCTGAAGCCCCACAGGAT
GTGACATATGCCAGCTGCATAGCCTGACACTGCGGAGAGAAGCCACAGAG
CCTCCACCTTCTCAAGAAGGCCCATCTCCTGCCGTGCCTTCCATCTATGCCAC
TCTGGCCATTAC (SEQ ID NO: 420).

12. The multicistronic expression system of any one of claims 1-11, wherein the antigen-binding domain specific for FLT3 comprises a heavy chain variable (VH) region and a light chain variable (VL) region, wherein:
- the FLT3-VH comprises:
- a heavy chain complementarity determining region 1 (CDR-H1) having the amino acid sequence of GGTFSSYAIS (SEQ ID NO: 360),
 - a heavy chain complementarity determining region 2 (CDR-H2) having the amino acid sequence of GIPIFGTANYAQKFQG (SEQ ID NO: 361), and
 - a heavy chain complementarity determining region 3 (CDR-H3) having the amino acid sequence of FALFGFREQAQFDI (SEQ ID NO: 362), and
- the FLT3-VL comprises:
- a light chain complementarity determining region 1 (CDR-L1) having the amino acid sequence of RASQSISSYLN (SEQ ID NO: 363),
 - a light chain complementarity determining region 2 (CDR-L2) having the amino acid sequence of AASSLQS (SEQ ID NO: 364), and
 - a light chain complementarity determining region 3 (CDR-L3) having the amino acid sequence of QQSYSTPFT (SEQ ID NO: 365), and
- wherein the amino acid sequences of the CDR-H1, the CDR-H2, the CDR-H3, the CDR-L1, the CDR-L2, and the CDR-L3 of the reference antibody are defined based on the Kabat numbering scheme; optionally wherein:
- the FLT3-VH comprises the amino acid sequence
- EVQLVQSGAEVKKPGSSVKVSKASGGTFSSYAISWVRQAPGQGLEWMGGIPI
FGTANYAQKFQGRVTITADKSTSTAYMELSSLRSEDTAVYYCATFALFGFREQA
FDIWGQGTTTVTVSS (SEQ ID NO: 315); and
- the FLT3-VL comprises the amino acid sequence
- DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQS
GVPSRFGSGSGTDFLTISLQPEDLATYYCQQSYSTPFTFGPGTKVDIK (SEQ
ID NO: 316), optionally wherein the FLT3-VH, and the FLT3-VL are separated by peptide linkers.
13. The multicistronic expression system of any one of claims 1-12, wherein the antigen-binding domain specific for CD33 comprises a heavy chain variable (VH) region and a light chain variable (VL) region, wherein:

the CD33-VH comprises:

a heavy chain complementarity determining region 1 (CDR-H1) having the amino acid sequence of DYNMH (SEQ ID NO: 402),

a heavy chain complementarity determining region 2 (CDR-H2) having the amino acid sequence of YIYPYNGGTGYNQKFKSKA (SEQ ID NO: 403), and

a heavy chain complementarity determining region 3 (CDR-H3) having the amino acid sequence of GRPAMDYWGQ (SEQ ID NO: 404), and

the CD33-VL comprises:

a light chain complementarity determining region 1 (CDR-L1) having the amino acid sequence of RASESVDNYGISFMN (SEQ ID NO: 405),

a light chain complementarity determining region 2 (CDR-L2) having the amino acid sequence of AASNQGS (SEQ ID NO: 406), and

a light chain complementarity determining region 3 (CDR-L3) having the amino acid sequence of QQSKEVPWT (SEQ ID NO: 407), and

wherein the amino acid sequences of the CDR-H1, the CDR-H2, the CDR-H3, the CDR-L1, the CDR-L2, and the CDR-L3 of the reference antibody are defined based on the Kabat numbering scheme; optionally wherein:

the CD33-VH comprises the amino acid sequence

QVQLVQSGAEVKKPGSSVKVSKASGYTFTDYNMHWVRQAPGQGLEWIGYIY
PYNGGTGYNQKFKSKATITADESTNTAYMELSSLRSEDTAVYYCARGRPAMDY
WGQGTLVTVSS (SEQ ID NO: 329); and

the CD33-VL comprises the amino acid sequence

DIQMTQSPSSLSASVGDRVTITCRASESVDNYGISFMNWFQKPGKAPKLLIYAA
SNQGSGVPSRFSGSGSGTDFLTITSLQPDDFATYYCQQSKEVPWTFGQGTKVEI
K (SEQ ID NO: 330), optionally wherein the CD33-VH, and the CD33-VL are separated by peptide linkers.

14. The multicistronic expression system of any one of claims 1-13, wherein the aCAR antigen binding domains comprises the structure (FLT3-VH) – L1 – (CD33-VH) – L2 – (CD33-VL) – L3 – (FLT3-VL), wherein L1, L2, and L3 are a first, a second, and a third peptide linker, respectively; optionally wherein the L1, L2, and/or L3 are each independently selected from the group consisting of: SEQ ID Nos 230 – 248, optionally wherein the L1 peptide linker is the amino acid sequence GGGGS (SEQ ID NO: 242) or

GGGSGGGGS (SEQ ID NO: 243), optionally wherein the L1 peptide linker GGGGS (SEQ ID NO: 242) is encoded by a polynucleotide sequence comprising the sequence GGCGGCGGTGGCTCT (SEQ ID NO: 254) or the L1 peptide linker GGGSGGGGS (SEQ ID NO: 243) is encoded by a polynucleotide sequence comprising the sequence GGAGGCGGAGGATCTGGTGGTGGTGGATCT (SEQ ID NO: 256), optionally wherein the L2 peptide linker is the amino acid sequence GSTSGSGKPGSGEGSTKG (SEQ ID NO: 247), optionally wherein the L2 peptide linker is encoded by a polynucleotide sequence comprising the sequence GGCTCTACATCTGGCTCTGGCAAACCTGGAAGCGGCGAGGGATCTACCAAGG GC (SEQ ID NO: 249), optionally wherein the L2 peptide linker is the amino acid sequence GGGSGGGGS (SEQ ID NO: 243), optionally wherein the L2 peptide linker is encoded by a polynucleotide sequence comprising the sequence GGTGGCGGAGGAAGTGGCGGCGGAGGCTCT (SEQ ID NO: 257), optionally wherein the L3 peptide linker is the amino acid sequence GGGGS (SEQ ID NO: 242) or GGGSGGGGS (SEQ ID NO: 243), optionally wherein the L3 peptide linker GGGGS (SEQ ID NO: 242) is encoded by a polynucleotide sequence comprising the sequence GGTGGCGGCGGATCC (SEQ ID NO: 255) or the L3 peptide linker GGGSGGGGS (SEQ ID NO: 243) is encoded by a polynucleotide sequence comprising the sequence GGCGGTGGCGGATCTGGCGGAGGTGGCAGT (SEQ ID NO: 258), optionally wherein the aCAR intracellular signaling domains that stimulate an immune response is selected from the group consisting of: CD3-zeta, FcR gamma, FcR beta, CD3 gamma, CD3 delta, CD3 epsilon, CD5, CD22, CD79a, CD79b, CD278, FcεRI, DAP10, DAP12, CD66d, CD97, CD2, ICOS, CD27, CD154, CD8, OX40, 4-1BB, CD28, ZAP40, CD30, GITR, HVEM, DAP10, DAP12, MyD88, 2B4, CD40, PD-1, LFA-1, CD7, LIGHT, NKG2C, B7-H3, an MHC class I molecule, a TNF receptor protein, an Immunoglobulin-like protein, a cytokine receptor, an integrin, a SLAM protein, an activating NK cell receptor, BTLA, a Toll ligand receptor, CDS, ICAM-1, (CD11a/CD18), BAFFR, KIRDS2, SLAMF7, NKp80 (KLRF1), NKp44, NKp30, NKp46, CD19, CD4, IL2R beta, IL2R gamma, IL7R alpha, ITGA4, VLA1, CD49a, ITGA4, IA4, CD49D, ITGA6, VLA-6, CD49f, ITGAD, CD11d, ITGAE, CD103, ITGAL, CD11a, ITGAM, CD11b, ITGAX, CD11c, ITGB1, CD29, ITGB2, CD18, ITGB7, NKG2D, TNFR2, TRANCE/RANKL, DNAM1 (CD226), SLAMF4 (CD244, 2B4), CD84, CD96 (Tactile), CEACAM1, CRTAM, Ly9 (CD229), CD160 (BY55), PSGL1, CD100 (SEMA4D), CD69, SLAMF6 (NTB-A, Ly108), SLAM (SLAMF1, CD150, IPO-3), BLAME (SLAMF8), SELPLG

(CD162), LTBR, LAT, GADS, SLP-76, PAG/Cbp, CD19a, and combinations thereof, optionally wherein the aCAR intracellular signaling domains that stimulate an immune response comprise a CD28 co-stimulatory domain and a CD3 ζ signaling domain, optionally wherein the CD28 co-stimulatory domain comprises the amino acid sequence RSKRSRLHSDYMNMTPRRPGPTRKHYPYAPPRDFAAYRS (SEQ ID NO: 287), optionally wherein the CD28 co-stimulatory domain is encoded by a polynucleotide sequence comprising the sequence

AGAAGCAAGCGGAGCAGACTGCTGCACAGCGACTACATGAACATGACCCCT
AGACGGCCCCGGACCTACCAGAAAGCACTACCAGCCTTACGCTCCTCCTAGAG
ATTCGCCCGCCTACCGGTCC (SEQ ID NO: 288), optionally wherein the CD3 ζ signaling domain comprises the amino acid sequence

RVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRK
NPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDAL
HMQALPPR (SEQ ID NO: 289), optionally wherein the CD3 ζ signaling domain is encoded by a polynucleotide sequence comprising the sequence

AGAGTGAAGTTCAGCAGGAGCGCAGACGCCCCCGCGTACAAGCAGGGCCAG
AACCAGCTCTATAACGAGCTCAATCTAGGACGAAGAGAGGAGTACGATGTTT
TGGACAAGAGACGTGGCCGGGACCCTGAGATGGGGGGAAAGCCGAGAAGG
AAGAACCCTCAGGAAGGCCTGTACAATGAACTGCAGAAAGATAAGATGGCG
GAGGCCTACAGTGAGATTGGGATGAAAGGCGAGCGCCGGAGGGGCAAGGG
GCACGATGGCCTTTACCAGGGTCTCAGTACAGCCACCAAGGACACCTACGAC
GCCCTTCACATGCAGGCCCTGCCCCCTCGC (SEQ ID NO: 290), optionally
wherein

(A) the hinge domain of the aCAR is present, optionally wherein the hinge domain of the aCAR is selected from the group consisting of a human Ig (immunoglobulin) hinge, an IgG4 hinge, an IgG2 hinge, a CD8a hinge, or an IgD hinge, a KIR2DS2 hinge, an LNGFR hinge, a LIR1 hinge, a PDGFR-beta extracellular linker, and combinations thereof, optionally wherein the hinge domain of the aCAR comprises a CD8 hinge, optionally wherein the CD8 hinge comprises the amino acid sequence

ALSNSIMYFSHFVPVFLPAKPTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAV
HTRGLDFACD (SEQ ID NO: 272), optionally wherein the CD8 hinge comprises is encoded by a polynucleotide sequence comprising the sequence
GCCCTGAGCAACAGCATCATGTACTTCAGCCACTTCGTGCCCCGTGTTTCTGCC
CGCCAAGCCTACAACAACCCCTGCTCCTAGACCACCTACACCAGCTCCTACA

ATCGCCAGCCAGCCTCTGTCTCTGAGGCCCGAAGCTTGTAGACCAGCTGCTG
GCGGAGCCGTGCATACAAGAGGACTGGATTTGCCTGCGAC (SEQ ID NO:
284), and/or wherein

(B) the transmembrane domain of the aCAR is present, optionally wherein the transmembrane domain of the aCAR is selected from the group consisting of a human Ig (immunoglobulin) hinge, an IgG4 hinge, an IgG2 hinge, a CD8a hinge, or an IgD hinge, a KIR2DS2 hinge, an LNGFR hinge, a LIR1 hinge, a PDGFR-beta extracellular linker, and combinations thereof, optionally wherein the transmembrane domain of the aCAR comprises a CD8 hinge, optionally wherein the CD8 transmembrane comprises the amino acid sequence IYIWAPLAGTCGVLLLSLVITLYCNHR (SEQ ID NO: 206), optionally wherein the CD8 transmembrane comprises is encoded by a polynucleotide sequence comprising the sequence

ATCTATATCTGGGCCCCCTCTGGCTGGCACATGCGGAGTTCTGCTGCTCAGCCT
GGTCATCACCCCTGTACTGCAACCACAGA (SEQ ID NO: 261); and/or wherein

(C) the aCAR signal peptide of the aCAR is present, optionally wherein the signal peptide of the aCAR is selected from the group consisting of: IgE, IL-12, IL-2, optimized IL-2, trypsinogen-2, Gaussia luciferase, CD5, human IgKVII, murine IgKVII, VSV-G, prolactin, serum albumin preprotein, azurocidin preprotein, osteonectin, CD33, IL-6, IL-8, CCL2, TIMP2, VEGFB, osteoprotegerin, serpin E1, GROalpha, CXCL12, IL-21, CD8, NKG2D, TNFR2, GMCSF, and GM-CSFRa, optionally wherein the signal peptide of the aCAR comprises a GM-CSFRa signal peptide, optionally wherein the GM-CSFRa signal peptide comprises the amino acid sequence MLLLVTSLLLCELPHPAFLIP (SEQ ID NO: 423), optionally wherein the GM-CSFRa signal peptide is encoded by a polynucleotide sequence comprising the sequence

ATGCTGCTGCTGGTTACATCTCTGCTGCTGTGCGAGCTGCCCCATCCTGCCTT
TCTGCTTATTCCT (SEQ ID NO: 424), optionally wherein the aCAR comprises the amino acid sequence

MLLLVTSLLLCELPHPAFLIPEVQLVQSGAEVKKPGSSVKVSKASGGTFSSYAI
SWVRQAPGQGLEWMGGIPIFGTANYAQKFQGRVTITADKSTSTAYMELSSLRS
EDTAVYYCATFALFGFREQAFDIWGQGTTVTVSSGGGGSQVQLVQSGAEVKKP
GSSVKVSKASGYTFTDYNMHWVRQAPGQGLEWIGYIYPYNGGTGYNQKFKS
KATITADESTNTAYMELSSLRSEDTAVYYCARGRPAMDYWGQGTTLTVSSGST

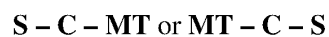
SGSGKPGSGEGSTKGDIQMTQSPSSLSASVGDRVITITCRASESVDNYGISFMNWF
 QQKPGKAPKLLIYAASNQGSVPSRFSGSGSGTDFTLTISLQPDDEFATYYCQQS
 KEVPWTFGQGTKVEIKGGGGSDIQMTQSPSSLSASVGDRVITITCRASQSISSYLN
 WYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISLQPEDLATYYCQ
 QSYSTPFTFGPGTKVDIKALSNSIMYFSHFVPVFLPAKPTTTPAPRPPTPAPTASQ
 PLSLRPEACRPAAGGAVHTRGLDFACDIYWAPLAGTCGVLLLSLVITLYCNHRR
 SKRSRLLHSDYMNMTPRRPGPTRKHYPYAPPRDFAAYRSRVKFSRSADAPAY
 KQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKD
 KMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR (SEQ
 ID NO: 414), optionally wherein the aCAR comprises the amino acid sequence
 MLLLVTSLLLCELPHPAFLLIPEVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAI
 SWVRQAPGQGLEWMGGIPIFGTANYAQKFQGRVTITADKSTSTAYMELSSLRS
 EDTAVYYCATFALFGFREQAFDIWGQGTITVTVSSGGGGSGGGGSQVQLVQSGA
 EVKKPGSSVKVSCKASGYTFTDYNMHVVRQAPGQGLEWIGYIYPYNGGTGYN
 QKFKSKATITADESTNTAYMELSSLRSEDTAVYYCARGRPAMDYWGQGLTVTV
 SSGGGGSGGGGSDIQMTQSPSSLSASVGDRVITITCRASESVDNYGISFMNWFQQ
 KPGKAPKLLIYAASNQGSVPSRFSGSGSGTDFTLTISLQPDDEFATYYCQQSKE
 VPWTFGQGTKVEIKGGGGSGGGGSDIQMTQSPSSLSASVGDRVITITCRASQSISS
 YLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISLQPEDLATY
 YCQQSYSTPFTFGPGTKVDIKALSNSIMYFSHFVPVFLPAKPTTTPAPRPPTPAPT
 ASQPLSLRPEACRPAAGGAVHTRGLDFACDIYWAPLAGTCGVLLLSLVITLYCN
 HRRSKRSRLLHSDYMNMTPRRPGPTRKHYPYAPPRDFAAYRSRVKFSRSADAP
 AYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQ
 KDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR
 (SEQ ID NO: 415).

15. An expression vector comprising the multicistronic expression system of any one of claims 1-14.
16. An isolated cell comprising the multicistronic expression system of any one of claims 1-15 or the expression vector of claim 59, optionally wherein the cell comprises an immune cell, optionally wherein the cell is selected from the group consisting of a T cell, a Natural Killer (NK) cell, a cytotoxic T lymphocyte (CTL), a regulatory T cell, a Natural Killer T (NKT) cell, a myeloid cell, a macrophage, a human embryonic stem cell

(ESC), an ESC-derived cell, a pluripotent stem cell, and induced pluripotent stem cell (iPSC), and an iPSC-derived cell, optionally wherein the cell is an NK cell.

17. A method of treating a subject in need thereof or stimulating a cell-mediated immune response to a tumor cell in a subject, the method comprising administering a therapeutically effective dose of any of the isolated cells of claim 16, optionally wherein the subject has cancer, optionally wherein the isolated cell is derived from the subject or is allogeneic with reference to the subject.
18. A method of treating a subject known or suspected of having cancer, the method comprising:

(A) administering a cell therapy to the subject, wherein the cell therapy comprises an immunoresponsive cell engineered to express a recombinant cytokine, wherein the immunoresponsive cell engineered to express the recombinant cytokine comprises an exogenous polynucleotide encoding a controlled release cytokine, oriented from N-terminal to C-terminal, having the formula:



wherein

S comprises a secretable effector molecule, wherein the secretable effector molecule comprises the recombinant cytokine,

C comprises a protease cleavage site, and

MT comprises a cell membrane tethering domain,

wherein $\mathbf{S - C - MT}$ or $\mathbf{MT - C - S}$ is configured to be expressed as a single polypeptide; and

(B) administering a pharmaceutically effective amount of an agent selected from the group consisting of:

- (i) cytarabine (Ara-C);
- (ii) decitabine;
- (iii) venetoclax;
- (iv) a combination of fludarabine and Ara-C; or
- (V) or a combination of decitabine and venetoclax;

optionally wherein the immunoresponsive cell comprises an exogenous polynucleotide encoding an inhibitory chimeric antigen receptor (iCAR) and/or an a bivalent activating chimeric antigen receptor (aCAR); optionally wherein wherein the iCAR comprises: (i) an antigen-binding domain specific for endomucin (EMCN); (ii) one or more intracellular inhibitory domains that inhibit an immune response; and (iii) one or more polypeptides selected from the group consisting of: a signal peptide, a transmembrane domain, a hinge domain, a spacer region, one or more peptide linkers, and combinations thereof; optionally wherein b) wherein the aCAR comprises (i) an antigen-binding domain specific for FLT3; (ii) an antigen-binding domain specific for CD33; (iii) one or more intracellular signaling domains that stimulate an immune response; and (iv) one or more polypeptides selected from the group consisting of: a signal peptide, a transmembrane domain, a hinge domain, a spacer region, one or more peptide linkers, and combinations thereof, optionally wherein the agent comprises the combination of fludarabine and Ara-C, optionally wherein the fludarabine is administered at a dose of 30 mg/m² and the cytarabine (Ara-C) is administered at a dose of 2 g/m², optionally wherein when the Ara-C is administered, the Ara-C is administered at a dose of 2 g/m², optionally wherein the agent is administered intravenously, optionally wherein the agent is administered 5 days prior to administration of the cell therapy.