

CHIMERIC RECEPTORS AND METHODS OF USE THEREOF

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Application Nos. 63/151,483 filed February 19, 2021, and 63/092,736 filed October 16, 2020, each of which is hereby incorporated in its entirety by reference for all purposes.

SEQUENCE LISTING

[0002] The instant application contains a Sequence Listing which has been submitted via EFS-Web and is hereby incorporated by reference in its entirety. Said ASCII copy, created on ~~Month-XX~~November 29, 20XX2021, is named STB-022WO_XXXXXUS_sequencelistingSL.txt, and is 169,498X,XXX,XXX bytes in size.

BACKGROUND

[0003] Chimeric antigen receptor (CAR) based adoptive cell therapies used to redirect the specificity and function of immunoresponsive cells, such as T cells, have shown efficacy in patients with lymphoid malignancies (Pule et al., *Nat. Med.* (14):1264-1270 (2008); Maude et al., *N Engl J Med.* (371):1507-17 (2014); Brentjens et al., *Sci Transl Med.* (5):177ra38 (2013)). CAR T cells have been shown to induce complete remission in patients with CD19-expressing malignancies for whom chemotherapies have led to drug resistance and tumor progression. The success of CD19 CAR therapy provides optimism for treating other hematological malignancies, such as acute myeloid leukemia (AML). Acute myeloid leukemia is the most common acute leukemia in adults. AML is a cancer of the myeloid line of blood cells and is characterized by the rapid growth of abnormal cells that build up in the bone marrow and blood and interfere with normal blood cells. Sometimes, AML can spread to the brain, skin, or gums. The standard chemotherapy treatments for AML have not changed substantially over the past 40 years (Pulte et al., 2008), and overall survival remains very poor.

[0004] One challenge to developing CAR therapy for AML is the lack of suitable targets. The ability to identify appropriate CAR targets is important to effectively targeting and treating the tumor without damaging normal cells that express the same target antigen. Thus, there remains a need for CAR-T cell-based AML therapies that target AML cells without targeting normal cells or tissues.

SUMMARY

[0005] In one aspect, ~~p~~ Provided for herein is a chimeric protein comprising an antigen-binding domain specific for endomucin (EMCN) and a heterologous molecule or moiety, wherein the antigen-binding domain comprises a heavy chain variable (VH) region and a light chain variable (VL) region, wherein: (a) the VH comprises: a heavy chain complementarity determining region 1 (CDR-H1) having the amino acid sequence of RYDMH (SEQ ID NO: 102), a heavy chain complementarity determining region 2 (CDR-H2) having the amino acid sequence of VIWGNGNTHYHSALKS (SEQ ID NO: 103), and a heavy chain complementarity determining region 3 (CDR-H3) having the amino acid sequence of RIKD (SEQ ID NO: 4), and the VL comprises: a light chain complementarity determining region 1 (CDR-L1) having the amino acid sequence of KSSQSLVASDENTYLN (SEQ ID NO: 10), a light chain complementarity determining region 2 (CDR-L2) having the amino acid sequence of QVSKLDS (SEQ ID NO: 11), and a light chain complementarity determining region 3 (CDR-L3) having the amino acid sequence of LQGIHLPWT (SEQ ID NO: 12), 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; (b) the VH comprises: a heavy chain complementarity determining region 1 (CDR-H1) having the amino acid sequence of GFSLSR Y (SEQ ID NO: 2), a heavy chain complementarity determining region 2 (CDR-H2) having the amino acid sequence of WGNGN (SEQ ID NO: 3), and a heavy chain complementarity determining region 3 (CDR-H3) having the amino acid sequence of RIKD (SEQ ID NO: 4), and the VL comprises: a light chain complementarity determining region 1 (CDR-L1) having the amino acid sequence of KSSQSLVASDENTYLN (SEQ ID NO: 10), a light chain complementarity determining region 2 (CDR-L2) having the amino acid sequence of QVSKLDS (SEQ ID NO: 11), and a light chain complementarity determining region 3 (CDR-L3) having the amino acid sequence of LQGIHLPWT (SEQ ID NO: 12), 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 Chothia numbering scheme; or (c) the VH comprises: a heavy chain complementarity determining region 1 (CDR-H1) contained within the VH region amino acid sequence of SEQ ID NO: 1, a heavy chain complementarity determining region 2 (CDR-H2) contained within the VH region amino acid sequence of SEQ ID NO: 1, and a heavy chain complementarity determining region 3 (CDR-H3) contained within the VH region amino acid sequence of SEQ ID NO: 1, and the VL comprises: a light chain complementarity determining region 1 (CDR-L1) are contained

within the VL region amino acid sequence of SEQ ID NO: 9, a light chain complementarity determining region 2 (CDR-L2) are contained within the VL region amino acid sequence of SEQ ID NO: 9, and a light chain complementarity determining region 3 (CDR-L3) are contained within the VL region amino acid sequence of SEQ ID NO: 9, and optionally 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 or Chothia numbering scheme.

[0006] In some [aspect examples](#), the antigen-binding domain comprises a heavy chain variable (VH) region and a light chain variable (VL) region, wherein the VH comprises: a heavy chain complementarity determining region 1 (CDR-H1) having the amino acid sequence of RYDMH (SEQ ID NO: 102), a heavy chain complementarity determining region 2 (CDR-H2) having the amino acid sequence of VIWGNGNTHYHSALKS (SEQ ID NO: 103), and a heavy chain complementarity determining region 3 (CDR-H3) having the amino acid sequence of RIKD (SEQ ID NO: 4), and the VL comprises: a light chain complementarity determining region 1 (CDR-L1) having the amino acid sequence of KSSQSLVASDENTYLN (SEQ ID NO: 10), a light chain complementarity determining region 2 (CDR-L2) having the amino acid sequence of QVSKLDS (SEQ ID NO: 11), and a light chain complementarity determining region 3 (CDR-L3) having the amino acid sequence of LQGIHLPWT (SEQ ID NO: 12), 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.

[0007] In some [aspect examples](#), the antigen-binding domain comprises a heavy chain variable (VH) region and a light chain variable (VL) region, wherein the VH comprises: a heavy chain complementarity determining region 1 (CDR-H1) having the amino acid sequence of GFSLSRY (SEQ ID NO: 2), a heavy chain complementarity determining region 2 (CDR-H2) having the amino acid sequence of WGNGN (SEQ ID NO: 3), and a heavy chain complementarity determining region 3 (CDR-H3) having the amino acid sequence of RIKD (SEQ ID NO: 4), and the VL comprises: a light chain complementarity determining region 1 (CDR-L1) having the amino acid sequence of KSSQSLVASDENTYLN (SEQ ID NO: 10), a light chain complementarity determining region 2 (CDR-L2) having the amino acid sequence of QVSKLDS (SEQ ID NO: 11), and a light chain complementarity determining region 3 (CDR-L3) having the amino acid sequence of LQGIHLPWT (SEQ ID NO: 12), 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 Chothia numbering scheme.

[0008] In some [aspectexamples](#), the antigen-binding domain comprises a heavy chain variable (VH) region and a light chain variable (VL) region, wherein the VH comprises: a heavy chain complementarity determining region 1 (CDR-H1) contained within the VH region amino acid sequence of SEQ ID NO: 1, a heavy chain complementarity determining region 2 (CDR-H2) contained within the VH region amino acid sequence of SEQ ID NO: 1, and a heavy chain complementarity determining region 3 (CDR-H3) contained within the VH region amino acid sequence of SEQ ID NO: 1, and the VL comprises: a light chain complementarity determining region 1 (CDR-L1) are contained within the VL region amino acid sequence of SEQ ID NO: 9, a light chain complementarity determining region 2 (CDR-L2) are contained within the VL region amino acid sequence of SEQ ID NO: 9, and a light chain complementarity determining region 3 (CDR-L3) are contained within the VL region amino acid sequence of SEQ ID NO: 9, and optionally 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 or Chothia numbering scheme.

[0009] In some [aspectexample](#), the VH region comprises the amino acid sequence of SEQ ID NO: 1. In some [aspectexamples](#), the VL region comprises the amino acid sequence of SEQ ID NO: 9.

[0010] In some [aspectexample](#), the antigen-binding domain comprises a single chain variable fragment (scFv). In some [aspectexamples](#), the VH and VL of the scFv are separated by a peptide linker. In some [aspectexamples](#), the antigen-binding domain 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 [aspectexamples](#), the scFv comprises an amino acid sequence selected from the group consisting of: SEQ ID Nos: 17-22.

[0011] In some [aspectexample](#), the chimeric protein is a chimeric antigen receptor (CAR), and wherein the heterologous molecule or moiety comprises a polypeptide selected from the group consisting of: a transmembrane domain, one or more intracellular signaling domains, a hinge domain, a spacer region, one or more peptide linkers, and combinations thereof.

[0012] In some [aspectexample](#), the CAR is an inhibitory CAR comprising one or more intracellular inhibitory domains that inhibit an immune response. In some [aspectexamples](#), the intracellular inhibitory domain comprises an enzymatic inhibitory domain or an intracellular inhibitory co-signaling domain.

[0013] [In another aspect,](#) ~~a~~Also provided herein is an engineered nucleic acid encoding the chimeric protein as previously described.

[0014] In another aspect, aAlso provided herein is an expression vector comprising an engineered nucleic acid as previously described.

[0015] In another aspect, the present disclosure provides an isolated cell comprising the engineered nucleic acid as described herein or the expression vector as described herein. Also provided herein is an isolated cell comprising an engineered nucleic acid as previously described.

[0016] In another aspect, aAlso provided herein is a population of engineered cells expressing an engineered nucleic acid or an expression vector as previously described.

[0017] In some aspectexamples, the isolated cell or population of cells further comprises one or more tumor-targeting chimeric receptors expressed on the cell surface. In some aspectexamples, the one or more tumor-targeting chimeric receptors is a chimeric antigen receptor (CAR) or an engineered T cell receptor.

[0018] In some aspectexamples, the cell or population of cells is selected from the group consisting of: a T cell, a CD8⁺ T cell, a CD4⁺ T cell, a 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, a dendritic cell, an erythrocyte, a platelet cell, 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), and an iPSC-derived cell.

[0019] In another aspect, aAlso provided herein is a pharmaceutical composition comprising an effective amount of a cell or population of engineered cells of as previously described and a pharmaceutically acceptable carrier, pharmaceutically acceptable excipient, or a combination thereof.

[0020] In yet another aspect, the present disclosure provides the cells as described herein or the composition as described herein for use in therapy. Also provided herein is 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 cells as previously described or a composition of as previously described.

[0021] In a further aspect, the present disclosure provides a use of the cells as described herein or the composition as described herein in the manufacture of a medicament for treating a subject having a tumor. Also provided herein is a method of treating a subject having a tumor, the method comprising administering a therapeutically effective dose of any of the cells as previously described or the composition of as previously described.

[0022] Also provided for herein is a chimeric protein comprising an antigen-binding domain specific for endomucin (EMCN) and a heterologous molecule or moiety, wherein the antigen-binding domain comprises a heavy chain variable (VH) region and a light chain variable (VL) region, and wherein the VH comprises a heavy chain complementarity determining region 3 (CDR-H3) having the amino acid sequence of RIKD (SEQ ID NO:4).

[0023] Also provided for herein is a chimeric protein comprising an antigen-binding domain specific for endomucin (EMCN) and a heterologous molecule or moiety, wherein the antigen-binding domain comprises a heavy chain variable (VH) region and a light chain variable (VL) region, wherein the VH comprises a heavy chain complementarity determining region 1 (CDR-H1), a heavy chain complementarity determining region 2 (CDR-H2), and a heavy chain complementarity determining region 3 (CDR-H3), and wherein the amino acid sequences of CDR-H1, CDR-H2, and CDR-H3 are contained within the VH region amino acid sequence of SEQ ID NO:1.

[0024] Also provided for herein is a chimeric protein comprising an antigen-binding domain specific for endomucin (EMCN) and a heterologous molecule or moiety, wherein the antigen-binding domain comprises a heavy chain variable (VH) region and a light chain variable (VL) region, and wherein the VH comprises: (a) a heavy chain complementarity determining region 1 (CDR-H1) having the amino acid sequence of GFSLSRYS (SEQ ID NO:2), a heavy chain complementarity determining region 2 (CDR-H2) having the amino acid sequence of WGNGN (SEQ ID NO:3), and a heavy chain complementarity determining region 3 (CDR-H3) having the amino acid sequence of RIKD (SEQ ID NO:4), or (b) a heavy chain complementarity determining region 1 (CDR-H1) having the amino acid sequence of RYDMH (SEQ ID NO: 102), a heavy chain complementarity determining region 2 (CDR-H2) having the amino acid sequence of VIWGNGNTHYHSALKS (SEQ ID NO: 103), and a heavy chain complementarity determining region 3 (CDR-H3) having the amino acid sequence of RIKD (SEQ ID NO: 4).

[0025] In some [aspectexamples](#), the VL comprises a light chain complementarity determining region 1 (CDR-L1), a light chain complementarity determining region 2 (CDR-L2), and a light chain complementarity determining region 3 (CDR-L3), wherein the amino acid sequences of CDR-L1, CDR-L2, and CDR-L3 are contained within the VL region amino acid sequence of SEQ ID NO:9. In some [aspectexamples](#), the amino acid sequences of the CDR-L1, the CDR-L2, and the CDR-L3 of the reference antibody are defined based on the Kabat numbering scheme. In some [aspectexamples](#), the amino acid sequences of the CDR-L1, the CDR-L2, and the CDR-L3 of the reference antibody are defined based on the Chothia numbering scheme. In some [aspectexamples](#), the VL comprises: a light chain complementarity determining region 1 (CDR-L1) having the amino acid sequence of KSSQSLVASDENTYLN (SEQ ID NO:10), a light chain complementarity determining region 2 (CDR-L2) having the amino acid sequence of QVSKLDS (SEQ ID NO:11), and a light chain complementarity determining region 3 (CDR-L3) having the amino acid sequence of LQGIHLPWT (SEQ ID NO:12).

[0026] Also provided for herein is a chimeric protein comprising an antigen-binding domain specific for endomucin (EMCN) and a heterologous molecule or moiety, wherein the antigen-binding domain comprises a heavy chain variable (VH) region and a light chain variable (VL) region, wherein the VL comprises a light chain complementarity determining region 1 (CDR-L1), a light chain complementarity determining region 2 (CDR-L2), and a light chain complementarity determining region 3 (CDR-L3), and wherein the amino acid sequences of CDR-L1, CDR-L2, and CDR-L3 are contained within the VL region amino acid sequence of SEQ ID NO:9. In some [aspectexamples](#), the amino acid sequences of the CDR-L1, the CDR-L2, and the CDR-L3 of the reference antibody are defined based on the Kabat numbering scheme. In some [aspectexamples](#), the amino acid sequences of the CDR-L1, the CDR-L2, and the CDR-L3 of the reference antibody are defined based on the Chothia numbering scheme.

[0027] Also provided for herein is a chimeric protein comprising an antigen-binding domain specific for endomucin (EMCN) and a heterologous molecule or moiety, wherein the antigen-binding domain comprises a heavy chain variable (VH) region and a light chain variable (VL) region, and wherein the VL comprises: a light chain complementarity determining region 1 (CDR-L1) having the amino acid sequence of KSSQSLVASDENTYLN (SEQ ID NO:10), a light chain complementarity determining region 2 (CDR-L2) having the amino acid sequence of QVSKLDS (SEQ ID NO:11), and a light chain complementarity determining region 3 (CDR-L3) having the amino acid sequence of LQGIHLPWT (SEQ ID NO:12).

[0028] In some [aspectexamples](#), the VH comprises a heavy chain complementarity determining region 1 (CDR-H1), a heavy chain complementarity determining region 2 (CDR-H2), and a heavy chain complementarity determining region 3 (CDR-H3), wherein the amino acid sequences of CDR-H1, CDR-H2, and CDR-H3 are contained within the VH region amino acid sequence of SEQ ID NO:1. In some [aspectexamples](#), the VH comprises: a heavy chain complementarity determining region 1 (CDR-H1) having the amino acid sequence of GFSLSRYS (SEQ ID NO:2), a heavy chain complementarity determining region 2 (CDR-H2) having the amino acid sequence of WGNGN (SEQ ID NO:3), and a heavy chain complementarity determining region 3 (CDR-H3) having the amino acid sequence of RIKD (SEQ ID NO:4). In some [aspectexamples](#), the VH comprises: a heavy chain complementarity determining region 1 (CDR-H1) having the amino acid sequence of RYDMH (SEQ ID NO: 102), a heavy chain complementarity determining region 2 (CDR-H2) having the amino acid sequence of VIWGNGNTHYHSALKS (SEQ ID NO: 103), and a heavy chain complementarity determining region 3 (CDR-H3) having the amino acid sequence of RIKD (SEQ ID NO: 4).

[0029] Also provided for herein is a chimeric protein comprising an antigen-binding domain specific for endomucin (EMCN) and a heterologous molecule or moiety, wherein the antigen-binding domain comprises a heavy chain variable (VH) region and a light chain variable (VL) region, wherein the VH comprises: (a) a heavy chain complementarity determining region 1 (CDR-H1) having the amino acid sequence of GFSLSRY (SEQ ID NO:2), a heavy chain complementarity determining region 2 (CDR-H2) having the amino acid sequence of WGNGN (SEQ ID NO:3), and a heavy chain complementarity determining region 3 (CDR-H3) having the amino acid sequence of RIKD (SEQ ID NO:4), or (b) a heavy chain complementarity determining region 1 (CDR-H1) having the amino acid sequence of RYDMH (SEQ ID NO: 102), a heavy chain complementarity determining region 2 (CDR-H2) having the amino acid sequence of VIWGNGNTHYHSALKS (SEQ ID NO: 103), and a heavy chain complementarity determining region 3 (CDR-H3) having the amino acid sequence of RIKD (SEQ ID NO: 4), and wherein the VL comprises: a light chain complementarity determining region 1 (CDR-L1) having the amino acid sequence of KSSQSLVASDENTYLN (SEQ ID NO:10), a light chain complementarity determining region 2 (CDR-L2) having the amino acid sequence of QVSKLDS (SEQ ID NO:11), and a light chain complementarity determining region 3 (CDR-L3) having the amino acid sequence of LQGIHLPWT (SEQ ID NO:12).

[0030] In some [aspectexamples](#), the VH region comprises an amino acid sequence with 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% identity to the amino acid sequence of SEQ ID NO:1. In some [aspectexamples](#), the VH region comprises the amino acid sequence of SEQ ID NO:1.

[0031] In some [aspectexample](#), the VL region comprises an amino acid sequence with 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% identity to the amino acid sequence of SEQ ID NO:9. In some [aspectexamples](#), the VL region comprises the amino acid sequence of SEQ ID NO:9.

[0032] Also provided for herein is a chimeric protein comprising an antigen-binding domain specific for endomucin (EMCN) and a heterologous molecule or moiety, wherein the antigen-binding domain comprises a heavy chain variable (VH) region and a light chain variable (VL) region, and wherein the VH comprises an amino acid sequence with 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% identity to the amino acid sequence of SEQ ID NO:1. In some [aspectexamples](#), the VH region comprises the amino acid sequence of SEQ ID NO:1. In some [aspectexamples](#), the VL region comprises an amino acid sequence with 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% identity to the amino acid sequence of SEQ ID NO:9. In some aspectexamples, the VL region comprises the amino acid sequence of SEQ ID NO:9.

[0033] Also provided for herein is a chimeric protein comprising an antigen-binding domain specific for endomucin (EMCN) and a heterologous molecule or moiety, wherein the antigen-binding domain comprises an antibody or antigen-binding fragment thereof, wherein the antibody or antigen-binding fragment thereof comprises a heavy chain variable (VH) region and a light chain variable (VL) region, and wherein the VL comprises an amino acid sequence with 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% identity to the amino acid sequence of SEQ ID NO:9. In some aspectexamples, the VL region comprises the amino acid sequence of SEQ ID NO:9. In some aspectexamples, the VH region comprises the amino acid sequence of SEQ ID NO:1.

[0034] Also provided for herein is a chimeric protein comprising an antigen-binding domain specific for endomucin (EMCN) and a heterologous molecule or moiety, wherein the antigen-binding domain competes with a reference antibody or antigen-binding fragment thereof for binding to EMCN, wherein the reference antibody or antigen-binding fragment thereof comprises a heavy chain variable (VH) region and a light chain variable (VL) region, wherein the VH comprises: a heavy chain complementarity determining region 1 (CDR-H1) having the amino acid sequence of GFSLSRY (SEQ ID NO:2), a heavy chain complementarity determining region 2 (CDR-H2) having the amino acid sequence of WGNGN (SEQ ID NO:3), and a heavy chain complementarity determining region 3 (CDR-H3) having the amino acid sequence of RIKD (SEQ ID NO:4), and wherein the VL comprises: a light chain complementarity determining region 1 (CDR-L1) having the amino acid sequence of KSSQSLVASDENTYLN (SEQ ID NO:10), a light chain complementarity determining region 2 (CDR-L2) having the amino acid sequence of QVSKLDS (SEQ ID NO:11), and a light chain complementarity determining region 3 (CDR-L3) having the amino acid sequence of LQGIHLPWT (SEQ ID NO:12).

[0035] Also provided for herein is a chimeric protein comprising an antigen-binding domain specific for endomucin (EMCN) and a heterologous molecule or moiety, wherein the antigen-binding domain binds essentially the same EMCN epitope as a reference antibody or antigen-binding fragment thereof, wherein the reference antibody or antigen-binding fragment thereof comprises a heavy chain variable (VH) region and a light chain variable (VL) region, wherein the VH comprises: a heavy chain complementarity determining region 1 (CDR-H1) having

the amino acid sequence of GFSLSR Y (SEQ ID NO:2), a heavy chain complementarity determining region 2 (CDR-H2) having the amino acid sequence of WGNGN (SEQ ID NO:3), and a heavy chain complementarity determining region 3 (CDR-H3) having the amino acid sequence of RIKD (SEQ ID NO:4), and wherein the VL comprises: a light chain complementarity determining region 1 (CDR-L1) having the amino acid sequence of KSSQSLVASDENTYLN (SEQ ID NO:10), a light chain complementarity determining region 2 (CDR-L2) having the amino acid sequence of QVSKLDS (SEQ ID NO:11), and a light chain complementarity determining region 3 (CDR-L3) having the amino acid sequence of LQGIHLPWT (SEQ ID NO:12).

[0036] Also provided for herein is a chimeric protein comprising an antigen-binding domain specific for endomucin (EMCN) and a heterologous molecule or moiety, wherein the antigen-binding domain binds an epitope of human EMCN that is the same as the EMCN epitope bound by a reference antibody or antigen-binding fragment thereof, wherein the reference antibody or antigen-binding fragment thereof comprises a heavy chain variable (VH) region and a light chain variable (VL) region, wherein the VH comprises: a heavy chain complementarity determining region 1 (CDR-H1) having the amino acid sequence of GFSLSR Y (SEQ ID NO:2), a heavy chain complementarity determining region 2 (CDR-H2) having the amino acid sequence of WGNGN (SEQ ID NO:3), and a heavy chain complementarity determining region 3 (CDR-H3) having the amino acid sequence of RIKD (SEQ ID NO:4), and wherein the VL comprises: a light chain complementarity determining region 1 (CDR-L1) having the amino acid sequence of KSSQSLVASDENTYLN (SEQ ID NO:10), a light chain complementarity determining region 2 (CDR-L2) having the amino acid sequence of QVSKLDS (SEQ ID NO:11), and a light chain complementarity determining region 3 (CDR-L3) having the amino acid sequence of LQGIHLPWT (SEQ ID NO:12). In some [aspectexamples](#), the VH region of the reference antibody or antigen-binding fragment thereof comprises the amino acid sequence of SEQ ID NO:1. In some [aspectexamples](#), the VL region of the reference antibody or antigen-binding fragment thereof comprises the amino acid sequence of SEQ ID NO:9.

[0037] In some [aspectexamples](#), the antigen-binding domain comprises a F(ab) fragment, a F(ab') fragment, or a single chain variable fragment (scFv). In some [aspectexamples](#), the antibody or antigen-binding fragment thereof comprises a single chain variable fragment (scFv).

[0038] In some [aspectexamples](#), the VH and VL of the scFv are separated by a peptide linker. In some [aspectexamples](#), the antigen-binding domain 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 [aspectexamples](#), In some [aspectexamples](#), the peptide linker comprises an amino acid sequence selected from the group consisting of: SEQ ID Nos:23-39.

[0039] In some [aspectexamples](#), the scFv comprises an amino acid sequence selected from the group consisting of: SEQ ID Nos:17-22.

[0040] In some [aspectexamples](#), the chimeric protein is an antibody-drug conjugate, and wherein the heterologous molecule or moiety comprises a therapeutic agent.

[0041] In some [aspectexamples](#), the chimeric protein is a chimeric antigen receptor (CAR), and wherein the heterologous molecule or moiety comprises a polypeptide selected from the group consisting of: a transmembrane domain, one or more intracellular signaling domains, a hinge domain, a spacer region, one or more peptide linkers, and combinations thereof. In some [aspectexamples](#), the CAR comprises a transmembrane domain. In some [aspectexamples](#), the CAR comprises one or more intracellular signaling domains. In some [aspectexamples](#), the CAR is an activating CAR comprising one or more intracellular signaling domains that stimulate an immune response. In some [aspectexamples](#), the CAR is an inhibitory CAR comprising one or more intracellular inhibitory domains that inhibit an immune response. In some [aspectexamples](#), the intracellular inhibitory domain comprises an enzymatic inhibitory domain. In some [aspectexamples](#), the intracellular inhibitory domain comprises an intracellular inhibitory co-signaling domain. In some [aspectexamples](#), the CAR comprises a spacer region between the antigen-binding domain and the transmembrane domain. In some [aspectexamples](#), the spacer region has an amino acid sequence selected from the group consisting of SEQ ID NOs:40-48.

[0042] Also provided herein is a single chain variable fragment (scFv) specific for endomucin (EMCN), comprising a heavy chain variable (VH) region and a light chain variable (VL) region, wherein the VH comprises a heavy chain complementarity determining region 3 (CDR-H3) having the amino acid sequence of RIKD (SEQ ID NO: 4).

[0043] Also provided herein is a single chain variable fragment (scFv) specific for endomucin (EMCN), wherein the antigen-binding domain comprises a heavy chain variable (VH) region and a light chain variable (VL) region, wherein the VH comprises a heavy chain complementarity determining region 1 (CDR-H1), a heavy chain complementarity determining region 2 (CDR-H2), and a heavy chain complementarity determining region 3 (CDR-H3), and wherein the amino acid sequences of CDR-H1, CDR-H2, and CDR-H3 are contained within the VH region amino acid sequence of SEQ ID NO: 1. In some [aspectexamples](#), the amino acid sequences of the CDR-H1, the CDR-H2, and the CDR-H3 of the reference antibody are defined based on the Kabat numbering scheme. In some [aspectexamples](#), the amino acid

sequences of the CDR-H1, the CDR-H2, and the CDR-H3 of the reference antibody are defined based on the Chothia numbering scheme.

[0044] Also provided herein is a single chain variable fragment (scFv) specific for endomucin (EMCN), wherein the antigen-binding domain comprises a heavy chain variable (VH) region and a light chain variable (VL) region, and wherein the VH comprises: (a) a heavy chain complementarity determining region 1 (CDR-H1) having the amino acid sequence of RYDMH (SEQ ID NO: 102), a heavy chain complementarity determining region 2 (CDR-H2) having the amino acid sequence of VIWGNGNTHYHSALKS (SEQ ID NO: 103), and a heavy chain complementarity determining region 3 (CDR-H3) having the amino acid sequence of RIKD (SEQ ID NO: 4), 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; or (b) a heavy chain complementarity determining region 1 (CDR-H1) having the amino acid sequence of GFSLSRV (SEQ ID NO: 2), a heavy chain complementarity determining region 2 (CDR-H2) having the amino acid sequence of WGNGN (SEQ ID NO: 3), and a heavy chain complementarity determining region 3 (CDR-H3) having the amino acid sequence of RIKD (SEQ ID NO: 4), 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 Chothia numbering scheme.

[0045] In some [aspectexamples](#), the VL of the scFv comprises a light chain complementarity determining region 1 (CDR-L1), a light chain complementarity determining region 2 (CDR-L2), and a light chain complementarity determining region 3 (CDR-L3), wherein the amino acid sequences of CDR-L1, CDR-L2, and CDR-L3 are contained within the VL region amino acid sequence of SEQ ID NO: 9.

[0046] In some [aspectexamples](#), the VL of the scFv comprises: a light chain complementarity determining region 1 (CDR-L1) having the amino acid sequence of KSSQSLVASDENTYLN (SEQ ID NO: 10), a light chain complementarity determining region 2 (CDR-L2) having the amino acid sequence of QVSKLDS (SEQ ID NO: 11), and a light chain complementarity determining region 3 (CDR-L3) having the amino acid sequence of LQGIHLPWT (SEQ ID NO: 12).

[0047] Also provided herein is a single chain variable fragment (scFv) specific for endomucin (EMCN), wherein the antigen-binding domain comprises a heavy chain variable (VH) region and a light chain variable (VL) region, wherein the VL comprises a light chain complementarity determining region 1 (CDR-L1), a light chain complementarity determining

region 2 (CDR-L2), and a light chain complementarity determining region 3 (CDR-L3), and wherein the amino acid sequences of CDR-L1, CDR-L2, and CDR-L3 are contained within the VL region amino acid sequence of SEQ ID NO: 9. In some [aspectexamples](#), 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 [aspectexamples](#), 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 Chothia numbering scheme.

[0048] Also provided herein is a single chain variable fragment (scFv) specific for endomucin (EMCN), wherein the antigen-binding domain comprises a heavy chain variable (VH) region and a light chain variable (VL) region, and wherein the VL comprises: a light chain complementarity determining region 1 (CDR-L1) having the amino acid sequence of KSSQSLVASDENTYLN (SEQ ID NO: 10), a light chain complementarity determining region 2 (CDR-L2) having the amino acid sequence of QVSKLDS (SEQ ID NO: 11), and a light chain complementarity determining region 3 (CDR-L3) having the amino acid sequence of LQGIHLPWT (SEQ ID NO: 12).

[0049] In some [aspectexamples](#), the VH of the scFv comprises a heavy chain complementarity determining region 1 (CDR-H1), a heavy chain complementarity determining region 2 (CDR-H2), and a heavy chain complementarity determining region 3 (CDR-H3), wherein the amino acid sequences of CDR-H1, CDR-H2, and CDR-H3 are contained within the VH region amino acid sequence of SEQ ID NO: 1. In some [aspectexamples](#), the amino acid sequences of the CDR-H1, the CDR-H2, and the CDR-H3 of the reference antibody are defined based on the Kabat numbering scheme. In some [aspectexamples](#), the amino acid sequences of the CDR-H1, the CDR-H2, and the CDR-H3 of the reference antibody are defined based on the Chothia numbering scheme.

[0050] In some [aspectexample](#), the VH of the scFv comprises: a heavy chain complementarity determining region 1 (CDR-H1) having the amino acid sequence of GFSLSRY (SEQ ID NO: 2), a heavy chain complementarity determining region 2 (CDR-H2) having the amino acid sequence of WGNGN (SEQ ID NO: 3), and a heavy chain complementarity determining region 3 (CDR-H3) having the amino acid sequence of RIKD (SEQ ID NO: 4).

[0051] In some [aspectexamples](#), the VH of the scFv comprises: or a heavy chain complementarity determining region 1 (CDR-H1) having the amino acid sequence of RYDMH (SEQ ID NO: 102), a heavy chain complementarity determining region 2 (CDR-H2) having the amino acid

sequence of VIWGNGNTHYHSALKS (SEQ ID NO: 103), and a heavy chain complementarity determining region 3 (CDR-H3) having the amino acid sequence of RIKD (SEQ ID NO: 4).

[0052] Also provided herein is a single chain variable fragment (scFV) specific for endomucin (EMCN), wherein the antigen-binding domain comprises a heavy chain variable (VH) region and a light chain variable (VL) region, wherein the VH comprises: (a) a heavy chain complementarity determining region 1 (CDR-H1) having the amino acid sequence of GFSLSRV (SEQ ID NO: 2), a heavy chain complementarity determining region 2 (CDR-H2) having the amino acid sequence of WGNGN (SEQ ID NO: 3), and a heavy chain complementarity determining region 3 (CDR-H3) having the amino acid sequence of RIKD (SEQ ID NO: 4), or (b) a heavy chain complementarity determining region 1 (CDR-H1) having the amino acid sequence of RYDMH (SEQ ID NO: 102), a heavy chain complementarity determining region 2 (CDR-H2) having the amino acid sequence of VIWGNGNTHYHSALKS (SEQ ID NO: 103), and a heavy chain complementarity determining region 3 (CDR-H3) having the amino acid sequence of RIKD (SEQ ID NO: 4), and wherein the VL comprises: a light chain complementarity determining region 1 (CDR-L1) having the amino acid sequence of KSSQSLVASDENTYLN (SEQ ID NO: 10), a light chain complementarity determining region 2 (CDR-L2) having the amino acid sequence of QVSKLDS (SEQ ID NO: 11), and a light chain complementarity determining region 3 (CDR-L3) having the amino acid sequence of LQGIHLPWT (SEQ ID NO: 12).

[0053] In some [aspectexamples](#), the VH of the scFv comprises an amino acid sequence with 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% identity to the amino acid sequence of SEQ ID NO: 1.

[0054] In some [aspectexamples](#), the VH of the scFv comprises the amino acid sequence of SEQ ID NO: 1.

[0055] In some [aspectexamples](#), the VL of the scFv comprises an amino acid sequence with 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% identity to the amino acid sequence of SEQ ID NO: 9.

[0056] In some [aspectexamples](#), the VL of the scFv comprises the amino acid sequence of SEQ ID NO: 9.

[0057] Also provided herein is a single chain variable fragment (scFV) specific for endomucin (EMCN),

wherein the antigen-binding domain comprises a heavy chain variable (VH) region and a light chain variable (VL) region, and

wherein the VH comprises an amino acid sequence with 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% identity to the amino acid sequence of SEQ ID NO: 1.

[0058] In some [aspectexamples](#), the VH region of the scFv comprises the amino acid sequence of SEQ ID NO: 1.

[0059] In some [aspectexamples](#), the VL region of the scFv comprises an amino acid sequence with 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% identity to the amino acid sequence of SEQ ID NO: 9.

[0060] In some [aspectexamples](#), the VL region of the scFv comprises the amino acid sequence of SEQ ID NO: 9.

[0061] Also provided herein is a single chain variable fragment (scFV) comprising an antigen-binding domain specific for endomucin (EMCN), wherein the antigen-binding domain comprises an antibody or antigen-binding fragment thereof, wherein the antibody or antigen-binding fragment thereof comprises a heavy chain variable (VH) region and a light chain variable (VL) region, and wherein the VL comprises an amino acid sequence with 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% identity to the amino acid sequence of SEQ ID NO: 9.

[0062] In some [aspectexamples](#), the VH region of the scFv comprises the amino acid sequence of SEQ ID NO: 1.

[0063] Also provided herein is a single chain variable fragment (scFV) comprising an antigen-binding domain specific for endomucin (EMCN), wherein the antigen-binding domain competes with a reference antibody or antigen-binding fragment thereof for binding to EMCN, wherein the reference antibody or antigen-binding fragment thereof comprises a heavy chain variable (VH) region and a light chain variable (VL) region, wherein the VH comprises: a heavy chain complementarity determining region 1 (CDR-H1) having the amino acid sequence of GFSLSRV (SEQ ID NO: 2), a heavy chain complementarity determining region 2 (CDR-H2) having the amino acid sequence of WGNGN (SEQ ID NO: 3), and a heavy chain complementarity determining region 3 (CDR-H3) having the amino acid sequence of RIKD (SEQ ID NO: 4), and wherein the VL comprises: a light chain complementarity determining region 1 (CDR-L1) having the amino acid sequence of

KSSQSLVASDENTYLN (SEQ ID NO: 10), a light chain complementarity determining region 2 (CDR-L2) having the amino acid sequence of QVSKLDS (SEQ ID NO: 11), and a light chain complementarity determining region 3 (CDR-L3) having the amino acid sequence of LQGIHLPWT (SEQ ID NO: 12), 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 Chothia annotation and numbering scheme.

[0064] Also provided herein is a single chain variable fragment (scFV) comprising an antigen-binding domain specific for endomucin (EMCN), wherein the antigen-binding domain binds essentially the same EMCN epitope as a reference antibody or antigen-binding fragment thereof, wherein the reference antibody or antigen-binding fragment thereof comprises a heavy chain variable (VH) region and a light chain variable (VL) region, wherein the VH comprises: a heavy chain complementarity determining region 1 (CDR-H1) having the amino acid sequence of GFSLSRYS (SEQ ID NO: 2), a heavy chain complementarity determining region 2 (CDR-H2) having the amino acid sequence of WGNGN (SEQ ID NO: 3), and a heavy chain complementarity determining region 3 (CDR-H3) having the amino acid sequence of RIKD (SEQ ID NO: 4), and wherein the VL comprises: a light chain complementarity determining region 1 (CDR-L1) having the amino acid sequence of KSSQSLVASDENTYLN (SEQ ID NO: 10), a light chain complementarity determining region 2 (CDR-L2) having the amino acid sequence of QVSKLDS (SEQ ID NO: 11), and a light chain complementarity determining region 3 (CDR-L3) having the amino acid sequence of LQGIHLPWT (SEQ ID NO: 12), 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 Chothia annotation and numbering scheme.

[0065] In some aspect examples, an scFv as previously described comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 17-22.

[0066] Also provided herein is a single chain variable fragment (scFV) comprising an antigen-binding domain specific for endomucin (EMCN), wherein the antigen-binding domain binds an epitope of human EMCN that is the same as the EMCN epitope bound by a reference antibody or antigen-binding fragment thereof, wherein the reference antibody or antigen-binding fragment thereof comprises a heavy chain variable (VH) region and a light chain variable (VL) region, wherein the VH comprises: a heavy chain complementarity determining region 1 (CDR-H1) having the amino acid sequence of GFSLSRYS (SEQ ID NO: 2), a heavy chain

complementarity determining region 2 (CDR-H2) having the amino acid sequence of WGNGN (SEQ ID NO: 3), and a heavy chain complementarity determining region 3 (CDR-H3) having the amino acid sequence of RIKD (SEQ ID NO: 4), and wherein the VL comprises: a light chain complementarity determining region 1 (CDR-L1) having the amino acid sequence of KSSQSLVASDENTYLN (SEQ ID NO: 10), a light chain complementarity determining region 2 (CDR-L2) having the amino acid sequence of QVSKLDS (SEQ ID NO: 11), and a light chain complementarity determining region 3 (CDR-L3) having the amino acid sequence of LQGIHLPWT (SEQ ID NO: 12), 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 Chothia annotation and numbering scheme.

[0067] In some aspect examples, the VH region of the reference antibody or antigen-binding fragment thereof comprises the amino acid sequence of SEQ ID NO: 1.

[0068] In some aspect examples, the VL region of the reference antibody or antigen-binding fragment thereof comprises the amino acid sequence of SEQ ID NO: 9.

[0069] Also provided for herein is a composition comprising any of the chimeric proteins or scFvs provided herein and a pharmaceutically acceptable carrier, pharmaceutically acceptable excipient, or a combination thereof.

[0070] Also provided for herein is an engineered nucleic acid encoding any of the chimeric proteins or scFvs provided herein. Also provided for herein is an expression vector comprising any of the engineered nucleic acids provided herein.

[0071] Also provided for herein is a composition comprising any of the engineered nucleic acids provided herein or any of the expression vectors provided herein, and a pharmaceutically acceptable carrier, pharmaceutically acceptable excipient, or a combination thereof.

[0072] Also provided for herein is a method of making an engineered cell, comprising transducing an isolated cells with any of the engineered nucleic acids provided herein or any of the expression vectors provided herein.

[0073] Also provided for herein is an engineered cell produced by any of the methods provided herein.

[0074] Also provided for herein is an isolated cell comprising any of the engineered nucleic acids provided herein, any of the expression vectors provided herein, or any of the compositions provided herein.

[0075] Also provided for herein is a population of engineered cells expressing any of the engineered nucleic acids provided herein, any of the expression vectors provided herein.

[0076] Also provided for herein is an isolated cell or population of cells comprising any of the chimeric proteins or scFvs provided herein.

[0077] Also provided for herein is a population of engineered cells expressing any of the chimeric proteins provided herein. In some [aspectexamples](#), the chimeric protein is recombinantly expressed. In some [aspectexamples](#), the chimeric protein is expressed from a vector or a selected locus from the genome of the cell. In some [aspectexamples](#), the cell or population of cells further comprises one or more tumor-targeting chimeric receptors expressed on the cell surface. In some [aspectexamples](#), each of the one or more tumor-targeting chimeric receptors is a chimeric antigen receptors (CAR) or an engineered T cell receptor. In some [aspectexamples](#), the cell or population of cells is selected from the group consisting of: a T cell, a CD8⁺ T cell, a CD4⁺ T cell, a 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, a dendritic cell, an erythrocyte, a platelet cell, 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), and an iPSC-derived cell. In some [aspectexamples](#), the cell is autologous. In some [aspectexamples](#), the cell is allogeneic.

[0078] Also provided for herein is a pharmaceutical composition comprising an effective amount of any of the cells or population of engineered cells provided herein and a pharmaceutically acceptable carrier, pharmaceutically acceptable excipient, or a combination thereof.

[0079] Also provided for herein is a pharmaceutical composition comprising an effective amount of genetically modified cells expressing any of the chimeric proteins provided herein and a pharmaceutically acceptable carrier, pharmaceutically acceptable excipient, or a combination thereof. In some [aspectexamples](#), the pharmaceutical composition is for treating and/or preventing a tumor.

[0080] Also provided for herein is a method of treating a subject in need thereof, the method comprising administering a therapeutically effective dose of any of the compositions provided herein, or any of the cells provided herein.

[0081] Also provided for herein is 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 compositions provided herein, or any of the cells provided herein.

[0082] Also provided for herein is a method of treating a subject having a tumor, the method comprising administering a therapeutically effective dose of any of the compositions provided herein, or any of the cells provided herein.

[0083] In some [aspectexamples](#), the method includes administering a composition comprising an immunocompetent cell or population of cells expressing an inhibitory as described herein, and the cell or population of cells further express one or more tumor-targeting chimeric receptors. In some [aspectexamples](#), the method results in reduced off-target effects as compared to a method of administering an equivalent composition including a cell or population of cells comprising the one or more tumor-targeting chimeric receptors but lacking the inhibitory CAR.

[0084] Also provided for herein is a kit for treating and/or preventing a tumor, comprising any of the chimeric proteins provided herein. In some [aspectexamples](#), the kit further comprises written instructions for using the chimeric protein for producing one or more antigen-specific cells for treating and/or preventing a tumor in a subject.

[0085] Also provided for herein is a kit for treating and/or preventing a tumor, comprising any of the cells or population of cells provided herein. In some [aspectexamples](#), the kit further comprises written instructions for using the cell for treating and/or preventing a tumor in a subject.

[0086] Also provided for herein is a kit for treating and/or preventing a tumor, comprising any of the isolated nucleic acids provided herein. In some [aspectexamples](#), the kit further comprises written instructions for using the nucleic acid for producing one or more antigen-specific cells for treating and/or preventing a tumor in a subject.

[0087] Also provided for herein is a kit for treating and/or preventing a tumor, comprising any of the vectors provided herein. In some [aspectexamples](#), the kit further comprises written instructions for using the vector for producing one or more antigen-specific cells for treating and/or preventing a tumor in a subject.

[0088] Also provided for herein is a kit for treating and/or preventing a tumor, comprising any of the compositions provided herein. In some [aspectexamples](#), the kit further comprises written instructions for using the composition for treating and/or preventing a tumor in a subject.

BRIEF DESCRIPTION OF THE DRAWINGS

[0100] The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee.

[0101] These and other features, aspects, and advantages of the present disclosure will become better understood with regard to the following description, and accompanying drawings.

[0102] **FIG. 1.** Sequencing results for the light-chain variable regions of Antibody-1 (Ab1) using Chothia naming scheme. **FIG. 1 discloses SEQ ID NO: 9.**

[0103] **FIG. 2.** Sequencing results for the heavy-chain variable regions of Antibody-1 (Ab1) using Chothia naming scheme. **FIG.2 discloses SEQ ID NO: 1.**

[0104] **FIG. 3A.** Gating strategy for establishing EMCN expression baseline.

[0105] **FIG. 3B.** No Virus transduction control cell lines showing baseline EMCN expression.

[0106] **FIG. 3C.** Transduced cell lines showing EMCN expression on Day 3 following transduction.

[0107] **FIG. 4A.** Transduced cell lines showing EMCN expression on Day 24 post-transduction following 21 days of drug selection with 1µg/mL Puromycin.

[0108] **FIG. 4B.** Transduced cell lines showing EMCN expression on Day 24 post-transduction following 21 days of drug selection with 0.5µg/mL Puromycin.

[0109] **FIG. 5.** Gating strategy for establishing EMCN expression baseline for analysis of cells engineered to express EMCN.

[0110] **FIG. 6A.** Gating strategy for establishing the CAR expression baseline was established using a No Virus transduction control.

[0111] **FIG. 6B.** CAR expression of transduced cells for CAR constructs SB00819, SB01052, SB02405, and SB02406.

[0112] **FIG. 6C.** CAR expression of transduced cells for CAR constructs SB02407, SB02408, SB02409, and SB02410.

[0113] **FIG. 7A.** Percent killing (normalized to a No Virus transduction T cell control) following co-incubation of FLT3-specific or CD33-specific CAR T cells with parental Molm13 (top panel) or SEM (bottom panel).

[0114] **FIG. 7B.** Percent killing (normalized to a No Virus transduction T cell control) following co-incubation of FLT3-specific or CD33-specific CAR T cells with Molm13 (top panel) or SEM (bottom panel) target cells engineered to express EMCN.

RNA may also include introns to the extent that the nucleotide sequence encoding the protein may in some version contain an intron(s).

[0147] As used herein, the term "ligand" refers to a molecule that binds to a receptor. In particular, the ligand binds a receptor on another cell, allowing for cell-to-cell recognition and/or interaction.

[0148] The terms "effective amount" and "therapeutically effective amount" are used interchangeably herein, and refer to an amount of a compound, formulation, material, or composition, as described herein effective to achieve a particular biological result. In some embodiments, an "effective amount" or a "therapeutically effective amount" is an amount sufficient to arrest, ameliorate, or inhibit the continued proliferation, growth, or metastasis of a disease or disorder of interest, e.g., a myeloid disorder.

[0149] As used herein, the term "immunoresponsive cell" refers to a cell that functions in an immune response (e.g., an immune effector response) or a progenitor, or progeny thereof. Examples of immune effector cells include, without limitation, alpha/beta T cells, gamma/delta T cells, B cells, natural killer (NK) cells, natural killer T (NKT) cells, mast cells, and myeloid-derived phagocytes.

[0150] As used herein, the term "immune effector response" or "immune effector function" refers to a function or response, e.g., of an immunoresponsive cell, that enhances or promotes an immune attack of a target cell. For example, an immune effector function or response may refer to a property of a T cell or NK cell that promotes killing or the inhibition of growth or proliferation, of a target cell. In the case of a T cell, primary stimulation and co-stimulation are examples of immune effector function or response.

[0151] As used herein, the term "flexible polypeptide linker" or "linker" refers to a peptide linker that consists of amino acids such as glycine and/or serine residues used alone or in combination, to link variable heavy and variable light chain regions together. In one embodiment, the flexible polypeptide linker is a Gly/Ser linker and comprises the amino acid sequence (Gly-Gly-Gly-Gly-Ser)_n ([SEQ ID NO: 107](#)) or (Gly-Gly-Gly-Ser)_n ([SEQ ID NO: 108](#)), where n is a positive integer equal to or greater than 1. For example, n=1, n=2, n=3, n=4, n=5, n=6, n=7, n=8, n=9, or n=10. In some embodiments, the flexible polypeptide linkers include, but are not limited to, Gly₄Ser ([SEQ ID NO: 33](#)) or (Gly₄Ser)₃ ([SEQ ID NO: 35](#)). In other embodiments, the linkers include multiple repeats of (Gly₂Ser) ([SEQ ID NO: 23](#)), (GlySer) or (Gly₃Ser) ([SEQ ID NO: 28](#)). In some embodiments, the flexible polypeptide linkers include a Whitlow linker (e.g., GSTSGSGKPGSGEGSTKG [SEQ ID NO:38]). Also

included within the scope of the present disclosure are linkers described, for example, in WO2012/138475.

[0188] 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).

[0189] 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. [\(SEQ ID NO: 109\)](#). 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.

[0190] In some embodiments, chimeric receptors comprise a bivalent CAR. In some embodiments, the bivalent CAR is an EMCN bivalent CAR. In some embodiments, the bivalent EMCN CAR comprises one or more of the anti-EMCN sequences shown in Table A. In some embodiments, the ABDs of the bivalent EMCN CAR each comprises the same ABD.

Table 3 – Spacer Nucleic Acid Sequences

Nucleic Acid Sequence	SEQ ID NO:	Description
GCAGCAGCTATCGAGGTGATGTATCCTCCGCCCTACC TGGATAATGAAAAGAGTAATGGGACTATCATTCATGT AAAAGGGAAGCATCTTTGTCCTTCTCCCCTTTTCCCCG GTCCGTCTAAACCT	49	CD28 hinge
GAAAGCAAGTACGGTCCACCTTGCCCTAGCTGTCCG	50	IgG4 minimal hinge
GAATCCAAGTACGGCCCCCAGCGCCTAGTGCCCCA	51	IgG4 minimal hinge, no disulfides
GAATCTAAATATGGCCCGCCATGCCCGCCTTGCCCCA	52	IgG4 S228P minimal hinge, enhanced disulfide formation
GAACCGAAGTCTTGTGATAAACTCATACGTGCCCG	53	IgG1 minimal hinge
GCTGCTGCTTTCGTACCCGTGTTCCCTCCCTGCTAAGCC TACGACTACCCCGCACCAGAGACCACCCACGCCAGC ACCCACGATTGCTAGCCAGCCCCTTAGTTTGCGACCA GAAGCTTGTGCGCCTGCTGCTGGTGGCGCGGTACATA CCCGCGGCCTTGATTTTGCTTGCGATATATATATCTGG GCGCCTCTGGCCGGAACATGCGGGGTCTCTCCTCTTT CTCTGGTTATTACTCTCTACTGTAATCACAGGAAT	~~56~~ 54	Extended CD8a hinge
GCCTGCCCCGACCGGGCTCTACACTCATAGCGGGGAAT GTTGTAAGGCATGTAACCTGGGTGAGGGCGTGCGACA GCCCTGCGGAGCTAACCACAGTGTGCGAACCCCTG CCTCGATAGTGTGACGTTCTCTGATGTTGTATCAGCTA CAGAGCCTTGCAAACCATGTACTGAGTGCGTTGGACT TCAGTCAATGAGCGCTCCATGTGTGGAGGCAGATGAT GCGGTCTGTGATGTGCTTACGGATACTACCAAGACG AGACAACAGGGCGGTGCGAGGCCTGTAGAGTTTGTG AGGCGGGCTCCGGGCTGGTGTTCATGTCAAGACAA GCAAAATACGGTCTGTGAAGAGTGCCCTGATGGCACC TACTCAGACGAAGCAGATGCAGAATGC	55	LNGFR hinge
GCCTGCCCTACAGGACTCTACACGCATAGCGGTGAGT GTTGTAAAGCATGCAACCTCGGGGAAGGTGTAGCCC AGCCATGCGGGGCTAACCACCGTTTGC	56	Truncated LNGFR hinge (TNFR-Cys1)
GCTGTGGGCCAGGACACGCAGGAGGTCATCGTGGTG CCACACTCCTTGCCCTTTAAGGTG	57	PDGFR-beta extracellular linker

[0199] In some embodiments, the spacer region comprises the sequence shown in SEQ ID NO:40. In some embodiments, the spacer region comprises the sequence shown in SEQ ID NO:41. In some embodiments, the spacer region comprises the sequence shown in SEQ ID NO:42. In some embodiments, the spacer region comprises the sequence shown in SEQ ID NO:43. In some embodiments, the spacer region comprises the sequence shown in SEQ ID NO:44. In some embodiments, the spacer region comprises the sequence shown in SEQ ID NO:45. In some embodiments, the spacer region comprises the sequence shown in SEQ ID NO:46. In some embodiments, the spacer region comprises the sequence shown in SEQ ID NO:47. In some embodiments, the spacer region comprises the sequence shown in SEQ ID NO:48. In some embodiments, the spacer region comprises the sequence shown in SEQ ID NO:49. In some embodiments, the spacer region comprises the sequence

cytotoxicity against the target cells was assessed by flow cytometry (analysis performed using FlowJo software) and presented as percent killing normalized to a No Virus transduction T cell control.

Results

[0347] CAR T cells specific for FLT3, CD33, or various constructs for EMCN were generated. Each receptor included a cytoplasmic signaling domain such that binding to a target antigen should stimulate an immune response, such as cytokine production and/or target cell killing. For EMCN-specific CAR constructs, different VH and VL orientations together with various scFv linkers (G4S [\(SEQ ID NO: 33\)](#), (G4S)₃ [\(SEQ ID NO: 35\)](#), and Whitlow) were also assessed.

[0348] Following lentiviral transduction of primary human T cells, the engineered cells were assessed for CAR expression. The gating strategy for establishing the CAR expression baseline was established using a No Virus transduction control (**FIG. 6A**). As shown in **FIG. 6B** and **FIG. 6C**, CAR expression was observed on between 61-91% of transduced cells.

[0349] The various CAR T cells were then assessed for functional activity. Each of the CAR T cells was co-incubated with Molm13 target cells, SEM target cells, Molm13 target cells engineered to express EMCN, or SEM target cells engineered to express EMCN. Parental and engineered Molm13 and SEM cell lines natively express FLT3 and CD33, as demonstrated above (*see* **FIG. 5C** and **FIG. 5D**). Co-incubation of FLT3-specific or CD33-specific CAR T cells with parental Molm13 (**FIG. 7A**; top panel) or SEM (**FIG. 7A**; bottom panel) target cells led to target cell killing (second and third columns, respectively), while co-incubation with EMCN-specific CAR T cells did not demonstrate detectable levels of target cell killing (columns 4-9). In contrast, co-incubation of CAR T cells with Molm13 (**FIG. 7B**; top panel) or SEM (**FIG. 7B**; bottom panel) target cells engineered to express EMCN led to target cell killing for both FLT3-specific or CD33-specific CAR T cells (second and third columns, respectively), as well as EMCN-specific CAR T cells (columns 4-9). Thus, CAR T cells engineered with an EMCN-specific CAR demonstrated target-specific functional activity for all constructs tested.

was added to dilute the virus. The cells were then cultured as normal. Day 4 post-transduction, CAR expression was assessed via antibody staining and flow cytometry.

[0352] For functional assays, T cells and target cells are co-cultured on day 9 after transduction (ET ratio: 1:1, 96-well plate, 200ul total medium volume). To distinguish target cells and T cells, target cells are stained with CellTrace Violet dye.

[0353] For cytokine production assays, supernatant is collected after a 5 or 18-hour co-culture and stored at -80 degree for evaluation by Luminex assay.

[0354] For cytotoxicity assays, cells are collected after an 18-hour co-incubation and stained with Sytox Red cell viability dye to distinguish live/dead target cells. Cytotoxicity is assessed by flow cytometry (FlowJo) and presented as percent killing normalized to a No Virus transduction T cell control.

Results

[0355] CAR T cells specific for EMCN and FLT3 or specific for EMCN and CD33 are generated. FLT3 and CD33 CARs include a cytoplasmic signaling domain such that binding to a target antigen should stimulate an immune response, such as cytokine production and/or target cell killing. EMCN CARs include an inhibitory cytoplasmic domain such that binding to a target antigen should inhibit an immune response, such as that stimulated by the FLT3-specific or CD33-specific CARs binding to their respective targets. Such a system is referred to as a “NOT gate.” For EMCN-specific CAR constructs, different VH and VL orientations together with various scFv linkers (G4S [\(SEQ ID NO: 33\)](#), (G4S)₃ [\(SEQ ID NO: 35\)](#), and Whitlow) are also assessed.

[0356] Following lentiviral transduction of primary human T cells, the engineered cells are assessed and confirmed for CAR co-expression. The various CAR T cells are then assessed for functional activity. Co-incubation of CAR T cells with parental Molm13 or SEM target cells leads to target cell killing. In contrast, co-incubation of CAR T cells with Molm13 or SEM target cells engineered to express EMCN leads to reduced or eliminated target cell killing relative to killing of parental target cells. Thus, CAR T cells engineered with an EMCN-specific CAR with an inhibitory cytoplasmic domain demonstrates the ability to reduce or minimize killing of EMCN-expressing cells establishing an effective NOT gate system to avoid undesired killing of EMCN-positive cells.

95	Anti-EMCN- CD8 hinge- LIR5 TM- LIR5 iCAR (CD8 SS bold)	MALPVTALLLPLALLLHAARPQVQLKESGPGLVQPSQTLSTCTVSGF SLSRYDMHWVRQPPGQGLEWMGVIWGNNGNTHYHSALKSRLSISRDTSK SQVFLKMNSLQTEDTAIFYCTLRIKDWPGTMVTVSSGGGGSGGGGGSG GGGSDIVMTQTPPSLSVALGQSVSISCKSSQSLVASDENTYLNWLLQSPG RSPKRLIYQVSKLDSGVPDRFSGSGSEKDFTLKISRVEAEDLGVYYCLQG IHLPWTFGGGTKELEKKGKIPNPLLGLDSTNGAATTTAPRPPTPAPTIAL QPLSLRPEACRPAAGGAVHTRGLDFACDVLIGVLVVSILLLSLLLFLLLQ HWRQKGHRTLAAQRQADFQRPPGAAEPEPKDGGGLQRRSSPAADVQGENF CAAVKNTQPEDGVEMDTRQSPHDEDPQAVTYAKVKHSRPRREMASPPS PLSGEFLDTKDRQAEEDRQMDTEAAASEAPQDVTYAQLHSFTLRQKAT EPPPSQEGASPAEPSVYATLAIH
96	Anti-EMCN- CD8 hinge- SIGLEC-2 TM- SIGLEC- 2 iCAR (CD8 SS bold)	MALPVTALLLPLALLLHAARPQVQLKESGPGLVQPSQTLSTCTVSGF SLSRYDMHWVRQPPGQGLEWMGVIWGNNGNTHYHSALKSRLSISRDTSK SQVFLKMNSLQTEDTAIFYCTLRIKDWPGTMVTVSSGGGGSGGGGGSG GGGSDIVMTQTPPSLSVALGQSVSISCKSSQSLVASDENTYLNWLLQSPG RSPKRLIYQVSKLDSGVPDRFSGSGSEKDFTLKISRVEAEDLGVYYCLQG IHLPWTFGGGTKELEKKGKIPNPLLGLDSTNGAATTTAPRPPTPAPTIAL QPLSLRPEACRPAAGGAVHTRGLDFACDVAVGLGSCLAAILLAICGLKLQ RRWKRTQSQQGLQENSSGQSFVRNKKVRRAPLSEGPLSLGCYNPMME DGISYTTLRFPENIPRTGDAESSEMQRPPDCDDTVTYSALHKRQVGD YENVIPDFPEDEGIHYSELIQFGVGERPQAQENVYVILKH
97	Anti-EMCN- CD8 hinge- SIGLEC-10 TM- SIGLEC- 10 iCAR (CD8 SS bold)	MALPVTALLLPLALLLHAARPQVQLKESGPGLVQPSQTLSTCTVSGF SLSRYDMHWVRQPPGQGLEWMGVIWGNNGNTHYHSALKSRLSISRDTSK SQVFLKMNSLQTEDTAIFYCTLRIKDWPGTMVTVSSGGGGSGGGGGSG GGGSDIVMTQTPPSLSVALGQSVSISCKSSQSLVASDENTYLNWLLQSPG RSPKRLIYQVSKLDSGVPDRFSGSGSEKDFTLKISRVEAEDLGVYYCLQG IHLPWTFGGGTKELEKKGKIPNPLLGLDSTNGAATTTAPRPPTPAPTIAL QPLSLRPEACRPAAGGAVHTRGLDFACDGAFLGIGITALLFLCLALIMKI LPKRRTQTETPRPRFSRHSTILDYINVVPTAGPLAQKRNQKATPNSPRTPL PPGAPSPESKKNQKKQYQLPSFPEPKSSTQAPESQESQEELHYATLNFPG VRPRPEARMPKGTQADYAEVKFQ
98	Anti-EMCN scFv nucleotide sequence with (G4S) ³ (SEQ ID NO: 35) linker	CAGGTGCAGCTGAAAGAGTCTGGACCTGGACTGGTGCAGCCCAGCCA AACACTGAGCCTGACCTGTACCGTGTCGGCTTCAGCCTGAGCAGAT ACGACATGCACTGGGTCCGACAGCCTCCAGGACAAGGCTTGGAATGG ATGGGCGTGATCTGGGGCAACGGCAACACACACTATCACAGCGCCCT GAAGTCCCGGCTGAGCATCAGCAGAGATAACCAGCAAGAGCCAGGTG TTCCTGAAGATGAACAGCCTGCAGACCGAGGACACCGCCATCTATTT CTGCACCCTGCGGATCAAGGATTGGGGCCCTGGCACAATGGTCACCG TTTCTAGCGGAGGCGGAGGATCTGGTGGCGGAGGAAGTGGCGGAGG CGGTTCTGATATCGTGATGACCCAGACACCTCCTAGCCTGTCTGTGGC TCTGGGCCAGTCTGTGTCCATCAGCTGCAAGAGCAGCCAGAGCCTGG TGGCCTCCGACGAGAACACCTACCTGAATTGGCTGCTGCAGAGCCCC GGCAGAAGCCCCAAGAGACTGATCTACCAGGTGTCCAAGCTGGACA GCGGCGTGCCCGATAGATTTTCTGGCAGCGGCAGCGAGAAGGACTTC ACCCTGAAGATCTCCAGAGTGGAAGCCGAGGACCTGGGCGTGTACTA CTGTCTGCAAGGCATCCATCTGCCTTGGACCTTTGGAGGCGGCACAA AGCTGGAAGTGAAG
99	anti-FLT3 scFv	EVQLVQSGAEVKKPGSSVKVSKASGGTFSSYAISWVRQAPGQGLEWM GGIPIFGTANYAQKFQGRVTITADKSTSTAYMELSSLRSEDVAVYYCAT FALFGFREQAFDIWGGQTTVTVSSGGGGSGGGGSGGGGSDIQMTQSPSS LSASVGDRTITCRASQSISSYLNWYQQKPGKAPKLLIYAASLQSGVPS RFSGSGSGTDFLTISLQPEDLATYYCQQSYSTPFTFGPGTKVDIK
100	anti-FLT3- CD28/ CD3 ζ aCAR (IgK signal sequence bold; AGGS-Flag italic)	METDTLLLWVLLLWVPGSTGAGGSDYKDDDDKGGSEVQLVQSGAEV KKPGSSVKVSKASGGTFSSYAISWVRQAPGQGLEWMGGIPIFGTANY AQKFQGRVTITADKSTSTAYMELSSLRSEDVAVYYCATFALFGFREQA FDIWGGQTTVTVSSGGGGSGGGGSGGGGSDIQMTQSPSSLSASVGDRTI TCRASQSISSYLNWYQQKPGKAPKLLIYAASLQSGVPSRFSGSGSGTDF LTISLQPEDLATYYCQQSYSTPFTFGPGTKVDIKTTTPAPRPPTPAPTIA LQPLSLRPEACRPAAGGAVHTRGLDFACDFWVLVVVGGLVACYLLVT VAFIIFWVRSKRSLHSDYMNMTPRRPGPTRKHYPYAPPRDFAAYRS

NK cell treatments, including (Group A) No NK cells (PBS control), (Group B) NK cells expressing aFLT3 aCAR, or (Group C) NK cells expressing both aFLT3 aCAR and aEMCN iCAR. Peripheral blood are collected from these mice weekly and prepared for flow cytometry analysis with red blood cell lysis and staining with antibodies and viability dyes to discriminate NALM6 cells that do or do not express the safety antigen. Although NK cells in Group B express the aCAR and reduce target cell number, both NK cells in Groups A and B do not have the iCAR or recognize the safety antigen, so in both groups the % of target cells that express EMCN remain at 50% as initially injected. However, NK cells in Group C recognize the safety antigen on the EMCN+ NALM6 subpopulation, reducomg the NK cell-mediated killing of the target cells. The results demonstrate successful NOT gate circuit function based on an elevation in the % of target cells in peripheral blood that are EMCN+ as compared to the two control groups.

INCORPORATION BY REFERENCE

[0367] All publications, patents, patent applications and other documents cited in this application are hereby incorporated by reference in their entirety for all purposes to the same extent as if each individual publication, patent, patent application or other document were individually indicated to be incorporated by reference for all purposes.

EQUIVALENTS

[0368] While various specific embodiments have been illustrated and described, the above specification is not restrictive. It will be appreciated that various changes can be made without departing from the spirit and scope of the present disclosure(s). Many variations will become apparent to those skilled in the art upon review of this specification.

OTHER SEQUENCES

[0369] Other sequences related to the present disclosure are presented below:

Sequence	SEQ ID NO:	Description
GGAGGCGGAGGATCTGGTGGCGGAGGAAGTGGCGGAGG CGGTTCT	64	(G4S) ₃ (SEQ ID NO: 35) linker nucleic acid
GGTGGTGGTGGCAGTGGTGGCGGTGGCTCAGGTGGCGGC GGATCAGGCGGTGGTGGTTCTGGCGGCGGTGGATCT	65	(G4S) ₅ (SEQ ID NO: 37) linker nucleic acid

CLAIMS

What is claimed is:

1. A chimeric protein comprising an antigen-binding domain specific for endomucin (EMCN) and a heterologous molecule or moiety, wherein the antigen-binding domain comprises a heavy chain variable (VH) region and a light chain variable (VL) region, wherein

(a) the VH comprises:

a heavy chain complementarity determining region 1 (CDR-H1) having the amino acid sequence of RYDMH (SEQ ID NO: 102),

a heavy chain complementarity determining region 2 (CDR-H2) having the amino acid sequence of VIWGNGNTHYHSALKS (SEQ ID NO: 103), and

a heavy chain complementarity determining region 3 (CDR-H3) having the amino acid sequence of RIKD (SEQ ID NO: 4), and

the VL comprises:

a light chain complementarity determining region 1 (CDR-L1) having the amino acid sequence of KSSQSLVASDENTYLN (SEQ ID NO: 10),

a light chain complementarity determining region 2 (CDR-L2) having the amino acid sequence of QVSKLDS (SEQ ID NO: 11), and

a light chain complementarity determining region 3 (CDR-L3) having the amino acid sequence of LQGIHLPWT (SEQ ID NO: 12), 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; or

(b) the VH comprises:

a heavy chain complementarity determining region 1 (CDR-H1) having the amino acid sequence of GFSLSRY (SEQ ID NO: 2), a heavy chain complementarity determining region 2 (CDR-H2) having the amino acid sequence of WGNGN (SEQ ID NO: 3), and a heavy chain complementarity determining region 3 (CDR-H3) having the amino acid sequence of RIKD (SEQ ID NO: 4), and

the VL comprises:

a light chain complementarity determining region 1 (CDR-L1) having the amino acid sequence of KSSQSLVASDENTYLN (SEQ ID NO: 10), a light chain complementarity determining region 2 (CDR-L2) having the amino acid sequence of QVSKLDS (SEQ ID NO: 11), and a light chain complementarity determining region 3 (CDR-L3) having the amino acid sequence of LQGIHLPWT (SEQ ID NO: 12), 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 Chothia numbering scheme; or

(c) the VH comprises:

a heavy chain complementarity determining region 1 (CDR-H1) contained within the VH region amino acid sequence of SEQ ID NO: 1, a heavy chain complementarity determining region 2 (CDR-H2) contained within the VH region amino acid sequence of SEQ ID NO: 1, and a heavy chain complementarity determining region 3 (CDR-H3) contained within the VH region amino acid sequence of SEQ ID NO: 1, and

the VL comprises:

a light chain complementarity determining region 1 (CDR-L1) are contained within the VL region amino acid sequence of SEQ ID NO: 9, a light chain complementarity determining region 2 (CDR-L2) are contained within the VL region amino acid sequence of SEQ ID NO: 9, and a light chain complementarity determining region 3 (CDR-L3) are contained within the VL region amino acid sequence of SEQ ID NO: 9, and

optionally 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 or Chothia numbering scheme.

2. The chimeric protein of claim 1, wherein the VH region comprises the amino acid sequence of SEQ ID NO: 1.

3. The chimeric protein of claim 1 or claim 2, wherein the VL region comprises the amino acid sequence of SEQ ID NO: 9.

4. The chimeric protein of any one of claims 1-3, wherein the antigen-binding domain comprises a single chain variable fragment (scFv).
5. The chimeric protein of claim 4, wherein the VH and VL of the scFv are separated by a peptide linker.
6. The chimeric protein of claim 5, wherein the antigen-binding domain 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.
7. The chimeric protein of any one of claims 4-6, wherein the scFv comprises an amino acid sequence selected from the group consisting of: SEQ ID Nos: 17-22.
8. The chimeric protein of any one of claims 1-7, wherein the chimeric protein is a chimeric antigen receptor (CAR), and wherein the heterologous molecule or moiety comprises a polypeptide selected from the group consisting of: a transmembrane domain, one or more intracellular signaling domains, a hinge domain, a spacer region, one or more peptide linkers, and combinations thereof.
9. The chimeric protein of claim 8, wherein the CAR is an inhibitory CAR comprising one or more intracellular inhibitory domains that inhibit an immune response.
10. The chimeric protein of claim 9, wherein the intracellular inhibitory domain comprises an enzymatic inhibitory domain or an intracellular inhibitory co-signaling domain.
11. An engineered nucleic acid encoding the chimeric protein of any one of claims 1-10.
12. An expression vector comprising the engineered nucleic acid of claim 11.
13. An isolated cell comprising the engineered nucleic acid of claim 11 or the expression vector of claim 12.
14. A population of engineered cells expressing the engineered nucleic acid of claim 11 or the expression vector of claim 12.

15. The cell or population of cells of claim 13 or claim 14, wherein the cell or population of cells further comprises one or more tumor-targeting chimeric receptors expressed on the cell surface.

16. The cell or population of cells of claim 15, wherein each of the one or more tumor-targeting chimeric receptors is a chimeric antigen receptor (CAR) or an engineered T cell receptor.

17. The cell or population of cells of any one of claims 13-16, wherein the cell or population of cells is selected from the group consisting of: a T cell, a CD8⁺ T cell, a CD4⁺ T cell, a 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, a dendritic cell, an erythrocyte, a platelet cell, 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), and an iPSC-derived cell.

18. A pharmaceutical composition comprising an effective amount of the cell or population of engineered cells of any one of claims 13-17 and a pharmaceutically acceptable carrier, pharmaceutically acceptable excipient, or a combination thereof.

19. ~~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~~ The cells of any one of claims 13-17 or the composition of claim 18 for use in therapy.

20. ~~A method of treating a subject having a tumor, the method comprising administering a therapeutically effective dose of any~~ Use of the cells of any one of claims 13-17 or the composition of claim 18 in the manufacture of a medicament for treating a subject having a tumor.