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(54) Title: SINGLE-DOMAIN ANTIBODIES AGAINST CLL1 AND CONSTRUCTS THEREOF

(57) Abstract: Provided are single-domain antibodies targeting CLL1 and constructs thereof, including chimeric receptors, immune effector cell engagers and immunoconjugates. Further provided are engineered immune effector cells (such as T cells) comprising an anti-CLL1 chimeric receptor and optionally a second chimeric receptor targeting a second antigen or epitope. Pharmaceutical compositions, kits and methods of treating cancer are also provided.

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SINGLE-DOMAIN ANTIBODIES AGAINST CLL1 AND CONSTRUCTS THEREOF

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority benefits of International Patent Application No. PCT/CN2018/104883 filed September 10, 2018, and International Patent Application No. PCT/CN2018/104882 filed September 10, 2018, the contents of which are incorporated herein by reference in their entirety.

SUBMISSION OF SEQUENCE LISTING ON ASCII TEXT FILE

[0002] The contents of the following submission on ASCII text file are incorporated herein by reference in their entirety: a computer readable form (CRF) of the Sequence Listing (file name: 761422801100SEQLISTING.txt, date recorded: September 7, 2019, size: 217 KB).

FIELD OF THE PRESENT APPLICATION

[0003] The present invention relates to single-domain antibodies, chimeric receptors and engineered immune cells that target CLL1, and methods of use thereof.

BACKGROUND OF THE PRESENT APPLICATION

[0004] With the development of tumor immunotherapy and clinical technology, chimeric antigen receptor T cell (CAR-T) immunotherapy is now one of the most promising tumor immunotherapy approaches. Generally, a chimeric antigen receptor (CAR) comprises an extracellular domain, a transmembrane domain and an intracellular signaling domain. The extracellular domain may comprise a single chain variable fragment (scFv) targeting an identified tumor antigen. CARs can be expressed on the surface of T cells using gene transfection techniques. Upon binding to the target tumor antigen, the CARs can activate the T cells to launch specific anti-tumor response in an antigen-dependent manner without being limited by the availability of major histocompatibility complexes (MHC) specific to the target tumor antigen.

[0005] Single-domain antibodies (sdAbs) are different from conventional 4-chain antibodies by having a single monomeric antibody variable domain. For example, camelids and sharks produce sdAbs named heavy chain-only antibodies (HcAbs), which naturally lack light chains. The antigen-binding fragment in each arm of the camelid heavy-chain only antibodies has a single heavy chain variable domain (V_HH), which can have high affinity to an antigen without the aid of a light chain. Camelid V_HH is known as the smallest functional antigen-binding fragment with a molecular weight of approximately 15 kD.

[0006] Acute myeloid leukemia (AML) is a cancer of the myeloid line of blood cells, characterized by the rapid growth of immature blood cells ("blasts") that build up in the bone marrow and blood and

interfere with normal blood cells. AML may spread to other organs, such as the liver, spleen, and brain. Clinical symptoms of AML include feeling tired, shortness of breath, easy bruising and bleeding, and increased risk of infection. Without treatment, AML progresses rapidly and is typically fatal within weeks or months. AML has several subtypes for which treatments and outcomes may vary. Typically, AML is initially treated with chemotherapy, sometimes along with a targeted therapy drug. Patients may then go on to receive a stem cell transplant, additional chemotherapy, surgery, or radiation therapy. AML most commonly occurs in older adults, some of whom are not healthy enough to receive intensive chemotherapy and thus have poor clinical outcome. Although current therapies for AML often lead to remissions, almost all patients eventually relapse. There is a need for an effective immunotherapeutic agent to treat AML.

[0007] The disclosures of all publications, patents, patent applications and published patent applications referred to herein are hereby incorporated herein by reference in their entirety.

BRIEF SUMMARY OF THE PRESENT APPLICATION

[0008] The present application provides anti-CLL1 single-domain antibodies (sdAb) and constructs thereof, including chimeric receptors, immune effector cell engagers, and immunoconjugates, engineered immune cells, and methods of use thereof in cancer immunotherapy.

[0009] One aspect of the present application provides an anti-CLL1 construct comprising an single domain antibody ("sdAb") moiety that specifically binds to CLL1, wherein the sdAb moiety (*e.g.*, V_HH) comprises a CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 4, 11, 18, 25, 32, 39, 46, 53, 60, 67, 74, 81, 88, 151, 158 and 165, or a variant thereof comprising up to about 3 amino acid substitutions in the CDR1; a CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 6, 13, 20, 27, 34, 41, 48, 55, 62, 69, 76, 83, 90, 153, 160, and 167, or a variant thereof comprising up to about 3 amino acid substitutions in the CDR2; and a CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 8, 15, 22, 29, 36, 43, 50, 57, 64, 71, 78, 85, 92, 155, 162, and 169, or a variant thereof comprising up to about 3 amino acid substitutions in the CDR3. In some embodiments, the sdAb moiety comprises: (1) a CDR1 comprising the amino acid sequence of SEQ ID NO: 4, a CDR2 comprising the amino acid sequence of SEQ ID NO: 6, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 8, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; (2) a CDR1 comprising the amino acid sequence of SEQ ID NO: 11, a CDR2 comprising the amino acid sequence of SEQ ID NO: 13, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 15, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; (3) a CDR1 comprising the amino acid sequence of SEQ ID NO: 18, a CDR2 comprising the amino acid sequence of SEQ ID NO: 20, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 22, or a variant thereof

comprising up to about 5 amino acid substitutions in the CDRs; (4) a CDR1 comprising the amino acid sequence of SEQ ID NO: 25, a CDR2 comprising the amino acid sequence of SEQ ID NO: 27, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 29, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; (5) a CDR1 comprising the amino acid sequence of SEQ ID NO: 32, a CDR2 comprising the amino acid sequence of SEQ ID NO: 34, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 36, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; (6) a CDR1 comprising the amino acid sequence of SEQ ID NO: 39, a CDR2 comprising the amino acid sequence of SEQ ID NO: 41, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 43, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; (7) a CDR1 comprising the amino acid sequence of SEQ ID NO: 46, a CDR2 comprising the amino acid sequence of SEQ ID NO: 48, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 50, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; (8) a CDR1 comprising the amino acid sequence of SEQ ID NO: 53, a CDR2 comprising the amino acid sequence of SEQ ID NO: 55, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 57, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; (9) a CDR1 comprising the amino acid sequence of SEQ ID NO: 60, a CDR2 comprising the amino acid sequence of SEQ ID NO: 62, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 64, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; (10) a CDR1 comprising the amino acid sequence of SEQ ID NO: 67, a CDR2 comprising the amino acid sequence of SEQ ID NO: 69, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 71, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; (11) a CDR1 comprising the amino acid sequence of SEQ ID NO: 74, a CDR2 comprising the amino acid sequence of SEQ ID NO: 76, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 78, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; (12) a CDR1 comprising the amino acid sequence of SEQ ID NO: 81, a CDR2 comprising the amino acid sequence of SEQ ID NO: 83, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 85, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; (13) a CDR1 comprising the amino acid sequence of SEQ ID NO: 88, a CDR2 comprising the amino acid sequence of SEQ ID NO: 90, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 92, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; (14) a CDR1 comprising the amino acid sequence of SEQ ID NO: 151, a CDR2 comprising the amino acid sequence of SEQ ID NO: 153, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 155, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; (15) a CDR1 comprising the amino acid sequence of SEQ ID NO: 158, a CDR2 comprising the amino acid sequence of SEQ ID NO: 160, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 162, or a variant thereof comprising up to about 5 amino acid

substitutions in the CDRs; or (16) a CDR1 comprising the amino acid sequence of SEQ ID NO: 165, a CDR2 comprising the amino acid sequence of SEQ ID NO: 167, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 169, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs.

[0010] In some embodiments, there is provided an anti-CLL1 construct comprising an sdAb moiety that specifically binds to CLL1, wherein the sdAb moiety comprises a CDR1, a CDR2, and a CDR3 of an sdAb comprising the amino acid sequence of any one of SEQ ID NOs: 94-106 and 171-173.

[0011] In some embodiments according to any one of the anti-CLL1 constructs described herein, the sdAb moiety comprises an amino acid sequence having at least about 95% (*e.g.*, about 96%, 97%, 98%, 99% or 100%) sequence identity to the amino acid sequence of any one of SEQ ID NOs: 94-106 and 171-173. In some embodiments, the sdAb moiety comprises the amino acid sequence of any one of SEQ ID NOs: 94-106 and 171-173.

[0012] Also provided are CLL1 epitopes that any one of the anti-CLL1 sdAb moieties described above specifically bind to, and anti-CLL1 antibodies (such as anti-CLL1 sdAbs) that compete with any one of the anti-CLL1 sdAb moieties described above.

[0013] In some embodiments according to any one of the anti-CLL1 constructs described above, the sdAb moiety is a camelid antibody. In some embodiments, the sdAb moiety is a chimeric antibody. In some embodiments, the sdAb moiety is humanized. In some embodiments, the sdAb moiety is a V_HH fragment.

[0014] In some embodiments, the anti-CLL1 construct is a chimeric receptor (also referred herein as "anti-CLL1 chimeric receptor") comprising an extracellular domain comprising the sdAb moiety (*e.g.*, V_HH), a transmembrane domain, and an intracellular signaling domain comprising a primary intracellular signaling sequence of an immune effector cell (*e.g.*, T cell). In some embodiments, the primary intracellular signaling sequence is derived from CD3ζ, FcRγ, FcRβ, CD3γ, CD3δ, CD3ε, CD5, CD22, CD79a, CD79b, or CD66d. In some embodiments, the primary intracellular signaling sequence is a CD3ζ intracellular signaling sequence. In some embodiments, the intracellular signaling domain further comprises an intracellular co-stimulatory sequence. In some embodiments, the intracellular co-stimulatory sequence is derived from a co-stimulatory molecule selected from the group consisting of CD27, CD28, 4-1BB, OX40, CD40, PD-1, LFA-1, ICOS, CD2, CD7, LIGHT, NKG2C, B7-H3, TNFRSF9, TNFRSF4, TNFRSF8, CD40LG, ITGB2, KLRC2, TNFRSF18, TNFRSF14, HAVCR1, LGALS9, DAP10, DAP12, CD83, ligands of CD83 and combinations thereof. In some embodiments, the intracellular co-stimulatory sequence is derived from CD28 or 4-1BB. In some embodiments, the extracellular domain of the anti-CLL1 construct further comprises a second binding moiety that specifically binds to a second antigen or epitope. In some embodiments, the second binding moiety is an sdAb (*e.g.*, V_HH) or an scFv. In some

embodiments, the second binding moiety is an extracellular domain of a receptor. In some embodiments, the second binding moiety specifically binds to CD33, CD123 or an NKG2D ligand. In some embodiments, the second binding moiety is an anti-CD33 sdAb or an anti-CD123 sdAb. In some embodiments, the second binding moiety is an extracellular domain (ECD) of NKG2D.

[0015] In some embodiments, the anti-CLL1 construct is a chimeric receptor (also referred herein as "anti-CLL1 chimeric receptor") comprising an extracellular domain comprising the sdAb moiety (*e.g.*, V_HH), a transmembrane domain, and an intracellular signaling domain comprising an intracellular co-stimulatory sequence. In some embodiments, the intracellular co-stimulatory sequence is derived from a co-stimulatory molecule selected from the group consisting of CD27, CD28, 4-1BB, OX40, CD40, PD-1, LFA-1, ICOS, CD2, CD7, LIGHT, NKG2C, B7-H3, TNFRSF9, TNFRSF4, TNFRSF8, CD40LG, ITGB2, KLRC2, TNFRSF18, TNFRSF14, HAVCR1, LGALS9, DAP10, DAP12, CD83, ligands of CD83 and combinations thereof. In some embodiments, the intracellular co-stimulatory sequence is derived from CD28 or 4-1BB. In some embodiments, the extracellular domain of the anti-CLL1 construct further comprises a second binding moiety that specifically binds to a second antigen or epitope. In some embodiments, the second binding moiety is an sdAb or an scFv. In some embodiments, the second binding moiety is an extracellular domain of a receptor. In some embodiments, the second binding moiety specifically binds to CD33, CD123 or an NKG2D ligand. In some embodiments, the second binding moiety is an sdAb or scFv that specifically binds to CD33 or CD123. In some embodiments, the second binding moiety is an ECD of NKG2D.

[0016] One aspect of the present application provides an anti-CLL1 chimeric receptor comprising an extracellular domain comprising an anti-CLL1 sdAb moiety, a transmembrane domain, and an intracellular signaling domain, wherein the anti-CLL1 sdAb moiety comprises: a CDR1 comprising the amino acid sequence of SEQ ID NO: 39, a CDR2 comprising the amino acid sequence of SEQ ID NO: 41, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 43. In some embodiments, the intracellular signaling domain comprises a primary intracellular signaling sequence of an immune effector cell (*e.g.*, T cell). In some embodiments, the primary intracellular signaling sequence is derived from CD3ζ, FcRγ, FcRβ, CD3γ, CD3δ, CD3ε, CD5, CD22, CD79a, CD79b, or CD66d. In some embodiments, the primary intracellular signaling sequence is a CD3ζ intracellular signaling sequence. In some embodiments, the intracellular signaling domain further comprises an intracellular co-stimulatory sequence. In some embodiments, the intracellular co-stimulatory sequence is derived from a co-stimulatory molecule selected from the group consisting of CD27, CD28, 4-1BB, OX40, CD40, PD-1, LFA-1, ICOS, CD2, CD7, LIGHT, NKG2C, B7-H3, TNFRSF9, TNFRSF4, TNFRSF8, CD40LG, ITGB2, KLRC2, TNFRSF18, TNFRSF14, HAVCR1, LGALS9, DAP10, DAP12, CD83, ligands of

CD83 and combinations thereof. In some embodiments, the intracellular co-stimulatory sequence is derived from CD28 or 4-1BB.

[0017] One aspect of the present application provides a multispecific chimeric receptor comprising an extracellular domain comprising an anti-CLL1 sdAb moiety and an anti-CD33 sdAb moiety, a transmembrane domain, and an intracellular signaling domain, wherein the anti-CLL1 sdAb moiety comprises: a CDR1 comprising the amino acid sequence of SEQ ID NO: 39, a CDR2 comprising the amino acid sequence of SEQ ID NO: 41, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 43, and wherein the anti-CD33 sdAb moiety comprises: a CDR1 comprising the amino acid sequence of SEQ ID NO: 198, a CDR2 comprising the amino acid sequence of SEQ ID NO: 200, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 202. In some embodiments, the intracellular signaling domain comprises a primary intracellular signaling sequence of an immune effector cell (*e.g.*, T cell). In some embodiments, the primary intracellular signaling sequence is derived from CD3ζ, FcRγ, FcRβ, CD3γ, CD3δ, CD3ε, CD5, CD22, CD79a, CD79b, or CD66d. In some embodiments, the primary intracellular signaling sequence is a CD3ζ intracellular signaling sequence. In some embodiments, the intracellular signaling domain further comprises an intracellular co-stimulatory sequence. In some embodiments, the intracellular co-stimulatory sequence is derived from a co-stimulatory molecule selected from the group consisting of CD27, CD28, 4-1BB, OX40, CD40, PD-1, LFA-1, ICOS, CD2, CD7, LIGHT, NKG2C, B7-H3, TNFRSF9, TNFRSF4, TNFRSF8, CD40LG, ITGB2, KLRC2, TNFRSF18, TNFRSF14, HAVCR1, LGALS9, DAP10, DAP12, CD83, ligands of CD83 and combinations thereof. In some embodiments, the intracellular co-stimulatory sequence is derived from CD28 or 4-1BB.

[0018] One aspect of the present application provides an anti-CLL1 chimeric receptor comprising the amino acid sequence of any one of SEQ ID NOs: 120-132, 177-179, 181, 184-195, and 229-230.

[0019] One aspect of the present application provides an engineered immune cell comprising any one of the anti-CLL1 chimeric receptors or multispecific chimeric receptors described above, or a nucleic acid encoding the anti-CLL1 chimeric receptor, or the multispecific chimeric receptor. In some embodiments, the engineered immune cell further comprises a second chimeric receptor. In some embodiments, the second chimeric receptor comprises an extracellular domain comprising a second binding moiety that specifically binds to a second antigen or epitope, a transmembrane domain, and an intracellular signaling domain comprising a primary intracellular signaling sequence of an immune effector cell (*e.g.*, T cell). In some embodiments, the primary intracellular signaling sequence is derived from CD3ζ, FcRγ, FcRβ, CD3γ, CD3δ, CD3ε, CD5, CD22, CD79a, CD79b, or CD66d. In some embodiments, the primary intracellular signaling sequence is a CD3ζ intracellular signaling sequence. In some embodiments, the intracellular signaling domain further comprises an intracellular co-stimulatory sequence. In some

embodiments, the second chimeric receptor comprises an extracellular domain comprising a second binding moiety that specifically binds to a second antigen or epitope, a transmembrane domain, and an intracellular signaling domain comprising an intracellular co-stimulatory sequence. In some embodiments, the intracellular co-stimulatory sequence is derived from a co-stimulatory molecule selected from the group consisting of CD27, CD28, 4-1BB, OX40, CD40, PD-1, LFA-1, ICOS, CD2, CD7, LIGHT, NKG2C, B7-H3, TNFRSF9, TNFRSF4, TNFRSF8, CD40LG, ITGB2, KLRC2, TNFRSF18, TNFRSF14, HAVCR1, LGALS9, DAP10, DAP12, CD83, ligands of CD83 and combinations thereof. In some embodiments, the intracellular co-stimulatory sequence is derived from CD28 or 4-1BB. In some embodiments, the second binding moiety is an extracellular domain of a receptor. In some embodiments, the second binding moiety specifically binds to CD33, CD123 or an NKG2D ligand. In some embodiments, the second binding moiety is an sdAb or scFv that specifically binds to CD33 or CD123. In some embodiments, the second binding moiety is an ECD of NKG2D.

[0020] One aspect of the present application provides a dual chimeric receptor system comprising: (a) a first chimeric receptor comprising an extracellular domain comprising an anti-CLL1 sdAb, a transmembrane, and an intracellular signaling domain, wherein the anti-CLL1 sdAb comprises: (1) a CDR1 comprising the amino acid sequence of SEQ ID NO: 4, a CDR2 comprising the amino acid sequence of SEQ ID NO: 6, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 8; or (2) a CDR1 comprising the amino acid sequence of SEQ ID NO: 18, a CDR2 comprising the amino acid sequence of SEQ ID NO: 20, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 22; and (b) a second chimeric receptor comprising an extracellular domain comprising an anti-CD33 sdAb, a transmembrane domain, and an intracellular signaling domain, wherein the anti-CD33 sdAb comprises: (1) a CDR1 comprising the amino acid sequence of SEQ ID NO: 205, a CDR2 comprising the amino acid sequence of SEQ ID NO: 207, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 209; (2) a CDR1 comprising the amino acid sequence of SEQ ID NO: 212, a CDR2 comprising the amino acid sequence of SEQ ID NO: 214, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 216; or (3) a CDR1 comprising the amino acid sequence of SEQ ID NO: 219, a CDR2 comprising the amino acid sequence of SEQ ID NO: 221, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 223. In some embodiments, the intracellular signaling domain of the first chimeric receptor and/or the second chimeric receptor comprises a primary intracellular signaling sequence of an immune effector cell (*e.g.*, T cell). In some embodiments, the primary intracellular signaling sequence is derived from CD3ζ, FcRγ, FcRβ, CD3γ, CD3δ, CD3ε, CD5, CD22, CD79a, CD79b, or CD66d. In some embodiments, the primary intracellular signaling sequence is a CD3ζ intracellular signaling sequence. In some embodiments, the intracellular signaling domain of the first chimeric receptor and/or the second chimeric receptor further comprises an intracellular co-stimulatory sequence. In some embodiments, the intracellular co-stimulatory

sequence is derived from a co-stimulatory molecule selected from the group consisting of CD27, CD28, 4-1BB, OX40, CD40, PD-1, LFA-1, ICOS, CD2, CD7, LIGHT, NKG2C, B7-H3, TNFRSF9, TNFRSF4, TNFRSF8, CD40LG, ITGB2, KLRC2, TNFRSF18, TNFRSF14, HAVCR1, LGALS9, DAP10, DAP12, CD83, ligands of CD83 and combinations thereof. In some embodiments, the intracellular co-stimulatory sequence is derived from CD28 or 4-1BB. In some embodiments, the intracellular co-stimulatory sequence of the first chimeric receptor comprises an intracellular co-stimulatory sequence derived from CD28. In some embodiments, the intracellular co-stimulatory sequence of the second chimeric receptor comprises an intracellular co-stimulatory sequence derived from 4-1BB.

[0021] Also provided is a dual chimeric receptor construct comprising the amino acid sequence of any one of SEQ ID NOs: 234-236.

[0022] In some embodiments, there is provided an engineered immune cell comprising any one of the dual chimeric receptor systems or dual chimeric receptor constructs described above.

[0023] In some embodiments according to any one of the engineered immune cells described above, the immune cell is a T cell, an NK cell, a peripheral blood mononuclear cell (PBMC), a hematopoietic stem cell, a pluripotent stem cell, or an embryonic stem cell. In some embodiments, the immune cell is a T cell, such as a cytotoxic T cell, a helper T cell, a natural killer T cell, or a γδT cell. In some embodiments, the engineered immune cell expresses a safety-switch antigen or epitope, such as CD52, EGFR, CD20 or an epitope thereof.

[0024] In some embodiments, the anti-CLL1 construct is a monospecific molecule. In some embodiments, the anti-CLL1 construct is a multispecific molecule, such as a bispecific molecule. In some embodiments, the anti-CLL1 construct is a secreted molecule. In some embodiments, the anti-CLL1 construct comprises the sdAb moiety (*e.g.*, V_HH) linked to a second binding moiety that specifically binds to a second antigen or epitope. In some embodiments, the second binding moiety is an sdAb or an scFv. In some embodiments, the sdAb moiety is linked to the second binding moiety via a peptide linker.

[0025] In some embodiments, the anti-CLL1 construct is an immune effector cell engager, wherein the second binding moiety specifically binds to an antigen on the surface of an immune cell. In some embodiments, the anti-CLL1 construct comprises a second binding moiety that specifically binds to an antigen on the surface of a T cell. In some embodiments, the second binding moiety specifically binds to an antigen is selected from the group consisting of CD3γ, CD3δ, CD3ε, CD3ζ, CD28, OX40, GITR, 4-1BB, CD27, CD40L, and HVEM.

[0026] In some embodiments, the anti-CLL1 construct is an immunoconjugate comprising the sdAb moiety and an effector molecule. In some embodiments, the effector molecule is a therapeutic agent selected from the group consisting of a drug, a toxin, a radioisotope, a protein, a peptide, and a nucleic

acid. In some embodiments, the effector molecule is a drug or a toxin. In some embodiments, the effector molecule is a label.

[0027] One aspect of the present application provides an isolated nucleic acid comprising a nucleic acid sequence encoding any one of the anti-CLL1 constructs (including anti-CLL1 sdAbs, anti-CLL1 chimeric receptors, multispecific chimeric receptors, dual chimeric receptor systems, immune effector cell engagers and anti-CLL1 immunoconjugates) described above. In some embodiments, the nucleic acid sequence is selected from the group consisting of SEQ ID NOs: 107-119 and 174-176.

[0028] In some embodiments, there is provided an isolated nucleic acid comprising a nucleic acid sequence encoding any one of the anti-CLL1 chimeric receptors described above. In some embodiments, the isolated nucleic acid comprises a first nucleic acid sequence encoding the anti-CLL1 chimeric receptor, and a second nucleic acid sequence encoding a second chimeric receptor (*e.g.*, anti-CD33, anti-CD123, or NKG2D chimeric receptor), wherein the second nucleic acid sequence is operably linked to the first nucleic acid sequence via a third nucleic acid sequence encoding a self-cleaving peptide, such as a T2A, P2A, or F2A peptide. In some embodiments, the isolated nucleic acid further comprises a nucleic acid sequence encoding a safety-switch antigen or epitope, such as CD52, CD20, EGFR or an epitope thereof.

[0029] One aspect of the present application provides a vector comprising any one of the isolated nucleic acids described above. In some embodiments, the vector is an expression vector. In some embodiments, the vector is a viral vector, such as a lentiviral vector. In some embodiments, the vector is a non-viral vector.

[0030] One aspect of the present application provides a pharmaceutical composition comprising any one of the anti-CLL1 chimeric receptors described above, or any one of the engineered immune cells described above, and a pharmaceutically acceptable carrier. Further provided is a method of treating a disease (such as cancer) in an individual, comprising administering to the individual an effective amount of any one of the pharmaceutical compositions described above. In some embodiments, the engineered immune cell is autologous. In some embodiments, the engineered immune cell is allogenic. In some embodiments, the disease is cancer. In some embodiments, the cancer is a liquid cancer. In some embodiments, the cancer is acute myeloid leukemia (AML). In some embodiments, the cancer is chronic myelogenous leukemia (CML). In some embodiments, the cancer is myelodysplastic syndromes (MDS). In some embodiments, wherein the immune cell expresses a safety-switch antigen or epitope, the method further comprises subsequently administering an effective amount of antibody that specifically binds to the safety-switch antigen or epitope.

[0031] One aspect of the present application provides a pharmaceutical composition comprising any one of the anti-CLL1 constructs described above and a pharmaceutically acceptable carrier. In some

embodiments, there is provided a method of treating a disease (such as cancer) in an individual, comprising administering to the individual an effective amount of the pharmaceutical composition. In some embodiments, there is provided a method of treating a cancer in an individual, comprising administering to the individual an effective amount of the anti-CLL1 construct according to any one of the anti-CLL1 constructs described above. In some embodiments, the cancer is acute myeloid leukemia (AML). In some embodiments, the cancer is chronic myelogenous leukemia (CML). In some embodiments, the cancer is myelodysplastic syndromes (MDS).

[0032] Also provided are methods of use, kits, and articles of manufacture comprising any one of the anti-CLL1 sdAbs, chimeric receptors, immune effector cell engagers, immunoconjugates, engineered immune cells, isolated nucleic acids, or vectors described above.

BRIEF DESCRIPTION OF THE DRAWINGS

[0033] FIG. 1 depicts immune response of pre-immune serum and post-immune serum after final boost against human CLL1.

[0034] FIG. 2 depicts immune response of 4-chain antibodies (IgG1) and heavy-chain only antibodies (IgG2 and IgG3) in post-immune serum after boosts against human CLL1. Corresponding immunoglobulin fragments isolated from pre-immune serum were used as controls.

[0035] FIG. 3 depicts a schematic diagram of the chimeric antigen receptor (CAR) construct for screening.

[0036] FIG. 4 depicts results of a representative *in vitro* cytotoxicity assay showing cytolytic activity of CAR-T cells derived from various sdAbs against acute myeloid leukemia cell line THP-1. Un-transduced T cells (UnT) were used as the negative control, and CLL1 BM CAR-T cells were used as positive control.

[0037] FIGs. 5A-5D depict cytokine release in an *in vitro* cytotoxicity assay. FIG. 5A show levels of IFN-γ secreted by co-culture of anti-CLL1 CAR-T cells with THP-1 cells. FIG. 5B shows levels of IFN-γ secreted by anti-CLL1 CAR-T cells incubated alone. FIG. 5C shows levels of TNF-α secreted by co-culture of anti-CLL1 CAR-T cells with THP-1 cells. FIG. 5D shows levels of TNF-α secreted by anti-CLL1 CAR-T cells incubated alone.

[0038] FIGs. 6A-6C depict T cell populations in long-term co-cultures of anti-CLL1 CAR-T cells with THP-1 cells. FIG. 6A shows representative results by FACS analysis. FIG. 6B shows calculated T cell proliferation rates. FIG. 6C shows total T cell counts in the same co-cultures.

[0039] FIG. 7 depicts result of a representative *in vivo* efficacy assay showing anti-tumor ability of selected CAR-T cells in a U937-Luc xenograft mouse model. Un-transduced T cells ("UnT") were used as the negative control, and CLL1 BM CAR-T cells were used as positive control.

[0040] FIGs. 8A-8E depict schematic diagrams of CAR constructs, including regular CAR (FIG. 8A), Tandem CAR (FIG. 8B), Dual CARs (FIG. 8C), and Split CARs (FIGs. 8D-8E).

[0041] FIG. 8A shows an exemplary anti-CLL1 CAR comprising a CLL1 binding domain, a transmembrane domain, a CD28 or 4-1BB intracellular co-stimulatory sequence, and an intracellular signaling domain comprising a CD3ζ intracellular signaling sequence.

[0042] FIG. 8B shows an exemplary tandem CAR comprising an extracellular domain comprising a CLL1 binding domain and a second antigen binding domain, a transmembrane domain, and an intracellular signaling domain comprising a CD28 or 4-1BB intracellular co-stimulatory sequence and a CD3ζ intracellular signaling sequence.

[0043] FIG. 8C shows a dual CAR system comprising: (a) a first chimeric receptor comprising a CLL1 binding domain, a transmembrane domain, a CD28 or 4-1BB intracellular co-stimulatory sequence, and an intracellular signaling domain comprising a CD3ζ intracellular signaling sequence; and (b) a second chimeric receptor comprising a CD33 binding domain or an extracellular domain (ECD) of NKG2D ("NKG2D ECD"), a transmembrane domain, and an intracellular signaling domain comprising a CD28 or 4-1BB intracellular co-stimulatory sequence and a CD3ζ intracellular signaling sequence.

[0044] FIG. 8D shows a split CAR system comprising: (a) a first chimeric receptor comprising a CLL1 binding domain, a transmembrane domain, and an intracellular signaling domain comprising a CD3ζ intracellular signaling sequence; and (b) a second chimeric receptor comprising a CD33 binding domain or NKG2D ECD, a transmembrane domain, and an intracellular signaling domain comprising a CD28 or 4-1BB intracellular co-stimulatory sequence.

[0045] FIG. 8E shows a split CAR system comprising: (a) a first chimeric receptor comprising a CD33 binding domain (or a CD123 binding domain, or NKG2D ECD), a transmembrane domain, and an intracellular signaling domain comprising a CD3ζ intracellular signaling sequence; and (b) a second chimeric receptor comprising a CLL1 binding domain, a transmembrane domain, and an intracellular signaling domain comprising a CD28 or 4-1BB intracellular co-stimulatory sequence.

[0046] In FIGs. 8A-8E, the CLL1 binding domain may be any one of the anti-CLL1 sdAbs described herein. The second antigen binding domain may be NKG2D ECD or a CD33 binding domain (*e.g.*, anti-CD33 sdAb), or a CD123 binding domain (*e.g.*, anti-CD123 sdAb).

[0047] FIGs. 9A-9B depict results of a representative *in vitro* cytotoxicity assay showing cytolytic activity of AS82658-28z CAR-T cells against acute myeloid leukemia cell lines THP-1 (FIG. 9A) and MOLM-13 (FIG. 9B). Un-transduced T cells (UnT) were used as negative control, and CLL1 BM CAR-T cells were used as positive control.

[0048] FIGs. 10A-10B depict the potency of AS82658-28z CAR-T cells in inhibiting tumor cell growth in long-term co-cultures with U937 cells. FIG. 10A shows the cytotoxicity of CAR-T cells at various time points (3, 5, 7, 10, 12, 14, 17, 19, and 21 days after co-culture) as determined by FACS analysis. FIG. 10B shows T cell proliferation rates at various time points (3, 5, 7, 10, and 12 days after co-culture).

[0049] FIGs. 11A-11B depict cytokine release in long-term co-cultures assays. FIG. 11A shows levels of GM-CSF secreted by AS82658-28z CAR-T cells at various time points (3, 5, 7, 10, and 12 days after co-culture). FIG. 11B shows levels of IFN-γ released by AS82658-28z CAR-T cells at various time points (3, 5, 7, 10, and 12 days after co-culture).

[0050] FIGs. 12A-12B depicts *in vitro* toxicity of AS82658-28z CAR-T cells against hematopoietic stem cells (HSC) as determined by CFU assays. UnT cells were used as negative control, and CLL1 BM CAR-T cells were used as positive control.

[0051] FIG. 13 depicts *in vivo* efficacy of AS82658-28z CAR-T cells in a U937-Luc xenograft mouse model. UnT cells were used as negative control, and CLL1 BM CAR-T cells were used as positive control.

[0052] FIG. 14 depicts *in vitro* cytotoxicity of CLL1/CD33 tandem CAR-T cells against acute myeloid leukemia cell line THP-1. UnT cells were used as negative control, and AS82658-28z and AS49264 CAR-T cells (anti-CD33 CAR) were used as positive control.

[0053] FIG. 15A-15B depict the potency of CLL1/CD33 tandem CAR-T cells in inhibiting tumor cell growth in long-term co-cultures with U937 cells. FIG. 15A shows cytotoxicity of CAR-T cells at various time points (2, 5, 7, 9, and 12 days after co-culture) as determined by FACS analysis. FIG. 15B shows T cell proliferation rates at various time points (2, 5, 7, and 9 days after co-culture).

[0054] FIGs. 16A-16B depict cytokine release in long-term co-cultures assays. FIG. 16A shows levels of GM-CSF secreted by CLL1/CD33 tandem CAR-T cells at various time points (3, 6, and 9 days after co-culture). FIG. 16B shows levels of IFN-γ released by CLL1/CD33 tandem CAR-T cells at various time points (2, 5, 7, and 9 days after co-culture).

[0055] FIGs. 17A-17C depict *in vitro* cytotoxicity of CLL1/CD33 dual CAR-T cells against acute myeloid leukemia cell line THP-1. UnT cells were used as negative control.

[0056] FIG. 18A-18D depict *in vivo* efficacy of CLL1/CD33 dual CAR-T and single-target CAR-T cells in HL-60-Luc xenograft model. FIG. 18A and 18C show the schematics of *in vivo* efficacy studies. FIG. 18B and 18D shows the curve of tumor growth in the HL-60-Luc xenograft mouse model after CAR-T cells treatment. UnT cells were used as negative control.

DETAILED DESCRIPTION OF THE PRESENT APPLICATION

[0057] The present application provides anti-CLL1 single-domain antibodies (sdAbs) and constructs thereof, such as chimeric receptors, immune effector cell engagers, and immunoconjugates. Multivalent and multispecific chimeric receptors, dual chimeric receptor systems, and split chimeric receptor systems are also provided. The anti-CLL1 sdAbs, chimeric receptors, and engineered immune cells expressing the chimeric receptors described herein are useful agents for cancer treatment.

[0058] Accordingly, one aspect of the present application provides an anti-CLL1 construct comprising a single domain antibody ("sdAb") moiety that specifically binds to CLL1 (*e.g.*, the extracellular domain of CLL1).

[0059] In another aspect, there is provided an anti-CLL1 chimeric receptor comprising an extracellular domain comprising an anti-CLL1 sdAb (*e.g.*, V_HH), a transmembrane domain, and an intracellular signaling domain (*e.g.*, an intracellular co-stimulatory sequence and/or a primary intracellular signaling sequence of an immune effector cell, *e.g.*, a CD3ζ intracellular signaling sequence).

[0060] In another aspect, there is provided a multispecific chimeric receptor comprising an extracellular domain comprising an anti-CLL1 sdAb (*e.g.*, V_HH) and a second antigen binding domain that specifically binds to a second antigen or epitope (*e.g.*, an anti-CD33 sdAb, an anti-CD123 sdAb, or an extracellular domain of NKG2D), a transmembrane domain, and an intracellular signaling domain comprising an intracellular co-stimulatory sequence and a primary intracellular signaling sequence of an immune effector cell (*e.g.*, a CD3ζ intracellular signaling sequence).

[0061] In another aspect, there is provide a split chimeric receptor system comprising a first chimeric receptor comprising an anti-CLL1 sdAb (*e.g.*, V_HH), a transmembrane domain, and an intracellular signaling domain comprising a primary intracellular signaling sequence of an immune effector cell (*e.g.*, a CD3ζ intracellular signaling sequence); and a second chimeric receptor comprising a second antigen binding domain that specifically binds to a second antigen or epitope (*e.g.*, an anti-CD33 sdAb, an anti-CD123 sdAb, or an extracellular domain of NKG2D), a transmembrane domain, and an intracellular signaling domain comprising an intracellular co-stimulatory sequence.

[0062] In another aspect, there is provide a split chimeric receptor system comprising a first chimeric receptor comprising an anti-CLL1 sdAb (*e.g.*, V_HH), a transmembrane domain, and an intracellular co-stimulatory sequence; and a second chimeric receptor comprising a second antigen binding domain that specifically binds to a second antigen or epitope (*e.g.*, an anti-CD33 sdAb, an anti-CD123 sdAb, or an extracellular domain of NKG2D), a transmembrane domain, and an intracellular signaling domain comprising a primary intracellular signaling sequence of an immune effector cell (*e.g.*, a CD3ζ intracellular signaling sequence).

[0063] Nucleic acids encoding the anti-CLL1 constructs, engineered immune cells (such as T cells) comprising the chimeric receptors or chimeric receptor systems, pharmaceutical compositions, kits, articles of manufacture and methods of treatment are also described herein.

I. Definitions

[0064] The term "antigen" refers to any molecule capable of inducing an immune response in a host cell, or any molecule capable of binding to an antigen-specific receptor.

[0065] The term "antibody" or "antibody moiety" includes monoclonal antibodies (including full length 4-chain antibodies or full length heavy-chain only antibodies which have an immunoglobulin Fc region), antibody compositions with polyepitopic specificity, multispecific antibodies (*e.g.*, bispecific antibodies, diabodies, and single-chain molecules), as well as antibody fragments (*e.g.*, Fab, $F(ab')_2$, and Fv). The term "immunoglobulin" (Ig) is used interchangeably with "antibody" herein. Antibodies contemplated herein include single-domain antibodies, such as heavy chain only antibodies.

[0066] The basic 4-chain antibody unit is a heterotetrameric glycoprotein composed of two identical light (L) chains and two identical heavy (H) chains. An IgM antibody consists of 5 of the basic heterotetramer units along with an additional polypeptide called a J chain, and contains 10 antigen binding sites, while IgA antibodies comprise from 2-5 of the basic 4-chain units which can polymerize to form polyvalent assemblages in combination with the J chain. In the case of IgGs, the 4-chain unit is generally about 150,000 Daltons. Each L chain is linked to an H chain by one covalent disulfide bond, while the two H chains are linked to each other by one or more disulfide bonds depending on the H chain isotype. Each H and L chain also has regularly spaced intrachain disulfide bridges. Each H chain has at the N-terminus, a variable domain (V_H) followed by three constant domains (C_H) for each of the α and γ chains and four C_H domains for μ and ε isotypes. Each L chain has at the N-terminus, a variable domain (V_L) followed by a constant domain at its other end. The V_L is aligned with the V_H and the C_L is aligned with the first constant domain of the heavy chain (C_H1). Particular amino acid residues are believed to form an interface between the light chain and heavy chain variable domains. The pairing of a V_H and V_L together forms a single antigen-binding site. For the structure and properties of the different classes of antibodies, see *e.g.*, *Basic and Clinical Immunology,* 8th Edition, Daniel P. Sties, Abba I. Terr and Tristram G. Parsolw (eds), Appleton & Lange, Norwalk, Conn., 1994, page 71 and Chapter 6. The L chain from any vertebrate species can be assigned to one of two clearly distinct types, called kappa and lambda, based on the amino acid sequences of their constant domains. Depending on the amino acid sequence of the constant domain of their heavy chains (C_H), immunoglobulins can be assigned to different classes or isotypes. There are five classes of immunoglobulins: IgA, IgD, IgE, IgG and IgM, having heavy chains designated α, δ, ε, γ and μ, respectively. The γ and α classes are further divided into

subclasses on the basis of relatively minor differences in the C_H sequence and function, *e.g.*, humans express the following subclasses: IgG1, IgG2A, IgG2B, IgG3, IgG4, IgA1 and IgA2.

[0067] The term "heavy chain-only antibody" or "HCAb" refers to a functional antibody, which comprises heavy chains, but lacks the light chains usually found in 4-chain antibodies. Camelid animals (such as camels, llamas, or alpacas) are known to produce HCAbs.

[0068] The term "single-domain antibody," "single-domain antibody moiety," "sdAb" or "sdAb moiety" refers to a single antigen-binding polypeptide having three complementary determining regions (CDRs), including full-length antibodies (*e.g.*, HCAbs) and antigen-binding fragments thereof (*e.g.*, V_HH). The sdAb alone is capable of binding to the antigen without pairing with a corresponding CDR-containing polypeptide. In some cases, single-domain antibodies are engineered from camelid HCAbs, and their heavy chain variable domains are referred herein as "V_HHs". Some V_HHs may also be known as Nanobodies. Camelid sdAb is one of the smallest known antigen-binding antibody fragments (see, *e.g.*, Hamers-Casterman *et al.*, Nature 363:446-8 (1993); Greenberg *et al.*, Nature 374:168-73 (1995); Hassanzadeh-Ghassabeh *et al.*, Nanomedicine (Lond), 8:1013-26 (2013)). A basic V_HH has the following structure from the N-terminus to the C-terminus: FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4, in which FR1 to FR4 refer to framework regions 1 to 4, respectively, and in which CDR1 to CDR3 refer to the complementarity determining regions 1 to 3.

[0069] An "isolated" antibody is one that has been identified, separated and/or recovered from a component of its production environment (*e.g.*, natural or recombinant). Preferably, the isolated polypeptide is free of association with all other components from its production environment. Contaminant components of its production environment, such as that resulting from recombinant transfected cells, are materials that would typically interfere with research, diagnostic or therapeutic uses for the antibody, and may include enzymes, hormones, and other proteinaceous or non-proteinaceous solutes. In preferred embodiments, the polypeptide will be purified: (1) to greater than 95% by weight of antibody as determined by, for example, the Lowry method, and in some embodiments, to greater than 99% by weight; (1) to a degree sufficient to obtain at least 15 residues of N-terminal or internal amino acid sequence by use of a spinning cup sequenator, or (3) to homogeneity by SDS-PAGE under non-reducing or reducing conditions using Coomassie blue or, preferably, silver stain. Isolated antibody includes the antibody in situ within recombinant cells since at least one component of the antibody's natural environment will not be present. Ordinarily, however, an isolated polypeptide or antibody will be prepared by at least one purification step.

[0070] The "variable region" or "variable domain" of an antibody refers to the amino-terminal domains of the heavy or light chain of the antibody. The variable domains of the heavy chain and light chain may be referred to as "V_H" and "V_L", respectively. These domains are generally the most variable

parts of the antibody (relative to other antibodies of the same class) and contain the antigen binding sites. Heavy-chain only antibodies from the Camelid species have a single heavy chain variable region, which is referred to as "V_HH". V_HH is thus a special type of V_H.

[0071] The term "variable" refers to the fact that certain segments of the variable domains differ extensively in sequence among antibodies. The V domain mediates antigen binding and defines the specificity of a particular antibody for its particular antigen. However, the variability is not evenly distributed across the entire span of the variable domains. Instead, it is concentrated in three segments called hypervariable regions (HVRs) both in the light-chain and the heavy chain variable domains. The more highly conserved portions of variable domains are called the framework regions (FR). The variable domains of native heavy and light chains each comprise four FR regions, largely adopting a beta-sheet configuration, connected by three HVRs, which form loops connecting, and in some cases forming part of, the beta-sheet structure. The HVRs in each chain are held together in close proximity by the FR regions and, with the HVRs from the other chain, contribute to the formation of the antigen binding site of antibodies (see Kabat *et al.*, *Sequences of Immunological Interest*, Fifth Edition, National Institute of Health, Bethesda, Md. (1991)). The constant domains are not involved directly in the binding of antibody to an antigen, but exhibit various effector functions, such as participation of the antibody in antibody-dependent cellular toxicity.

[0072] The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, *i.e.*, the individual antibodies comprising the population are identical except for possible naturally occurring mutations and/or post-translation modifications (*e.g.*, isomerizations, amidations) that may be present in minor amounts. Monoclonal antibodies are highly specific, being directed against a single antigenic site. In contrast to polyclonal antibody preparations which typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody is directed against a single determinant on the antigen. In addition to their specificity, the monoclonal antibodies are advantageous in that they are synthesized by the hybridoma culture or recombinantly, uncontaminated by other immunoglobulins. The modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the present application may be made by a variety of techniques, including, for example, the hybridoma method (*e.g.*, Kohler and Milstein., *Nature,* 256:495-97 (1975); Hongo *et al.*, *Hybridoma,* 14 (3): 253-260 (1995), Harlow *et al.*, *Antibodies: A Laboratory Manual*, (Cold Spring Harbor Laboratory Press, 2nd ed. 1988); Hammerling *et al.*, in: *Monoclonal Antibodies and T-Cell Hybridomas* 563-681 (Elsevier, N.Y., 1981)), recombinant DNA methods (see, *e.g.*, U.S. Pat. No. 4,816,567), phage-display technologies (see, *e.g.*,

Clackson *et al.*, *Nature,* 352: 624-628 (1991); Marks *et al.*, *J. Mol. Biol.* 222: 581-597 (1992); Sidhu *et al.*, *J. Mol. Biol.* 338(2): 299-310 (2004); Lee *et al.*, *J. Mol. Biol.* 340(5): 1073-1093 (2004); Fellouse, *Proc. Natl. Acad. Sci. USA* 101(34): 12467-12472 (2004); and Lee *et al.*, *J. Immunol. Methods* 284(1-2): 119-132 (2004), and technologies for producing human or human-like antibodies in animals that have parts or all of the human immunoglobulin loci or genes encoding human immunoglobulin sequences (see, *e.g.*, WO 1998/24893; WO 1996/34096; WO 1996/33735; WO 1991/10741; Jakobovits *et al.*, *Proc. Natl. Acad. Sci. USA* 90: 2551 (1993); Jakobovits *et al.*, *Nature* 362: 255-258 (1993); Bruggemann *et al.*, *Year in Immunol.* 7:33 (1993); U.S. Pat. Nos. 5,545,807; 5,545,806; 5,569,825; 5,625,126; 5,633,425; and 5,661,016; Marks *et al.*, *Bio/Technology* 10: 779-783 (1992); Lonberg *et al.*, *Nature* 368: 856-859 (1994); Morrison, *Nature* 368: 812-813 (1994); Fishwild *et al.*, *Nature Biotechnol.* 14: 845-851 (1996); Neuberger, *Nature Biotechnol.* 14: 826 (1996); and Lonberg and Huszar, *Intern. Rev. Immunol.* 13: 65-93 (1995).

[0073] The terms “full-length antibody,” “intact antibody” or “whole antibody” are used interchangeably to refer to an antibody in its substantially intact form, as opposed to an antibody fragment. Specifically, full-length 4-chain antibodies include those with heavy and light chains including an Fc region. Full-length heavy-chain only antibodies include the heavy chain (such as V_HH) and an Fc region. The constant domains may be native sequence constant domains (*e.g.*, human native sequence constant domains) or amino acid sequence variants thereof. In some cases, the intact antibody may have one or more effector functions.

[0074] An “antibody fragment” comprises a portion of an intact antibody, preferably the antigen binding and/or the variable region of the intact antibody. Examples of antibody fragments include Fab, Fab′, $F(ab')_2$ and Fv fragments; diabodies; linear antibodies (see U.S. Pat. No. 5,641,870, Example 2; Zapata *et al.*, *Protein Eng.* 8(10): 1057-1062 [1995]); single-chain antibody molecules; single-domain antibodies (such as V_HH), and multispecific antibodies formed from antibody fragments. Papain digestion of antibodies produced two identical antigen-binding fragments, called “Fab” fragments, and a residual “Fc” fragment, a designation reflecting the ability to crystallize readily. The Fab fragment consists of an entire L chain along with the variable region domain of the H chain (V_H), and the first constant domain of one heavy chain (C_H1). Each Fab fragment is monovalent with respect to antigen binding, *i.e.*, it has a single antigen-binding site. Pepsin treatment of an antibody yields a single large $F(ab')_2$ fragment which roughly corresponds to two disulfide linked Fab fragments having different antigen-binding activity and is still capable of cross-linking antigen. Fab′ fragments differ from Fab fragments by having a few additional residues at the carboxy terminus of the C_H1 domain including one or more cysteines from the antibody hinge region. Fab′-SH is the designation herein for Fab′ in which the cysteine residue(s) of the constant domains bear a free thiol group. $F(ab')_2$ antibody fragments originally were produced as pairs of

Fab′ fragments which have hinge cysteines between them. Other chemical couplings of antibody fragments are also known.

[0075] The Fc fragment comprises the carboxy-terminal portions of both H chains held together by disulfides. The effector functions of antibodies are determined by sequences in the Fc region, the region which is also recognized by Fc receptors (FcR) found on certain types of cells.

[0076] "Fv" is the minimum antibody fragment which contains a complete antigen-recognition and -binding site. This fragment consists of a dimer of one heavy- and one light-chain variable region domain in tight, non-covalent association. From the folding of these two domains emanate six hypervariable loops (3 loops each from the H and L chain) that contribute the amino acid residues for antigen binding and confer antigen binding specificity to the antibody. However, even a single variable domain (or half of an Fv comprising only three HVRs specific for an antigen) has the ability to recognize and bind antigen, although at a lower affinity than the entire binding site.

[0077] "Single-chain Fv" also abbreviated as "sFv" or "scFv" are antibody fragments that comprise the V_H and V_L antibody domains connected into a single polypeptide chain. Preferably, the sFv polypeptide further comprises a polypeptide linker between the V_H and V_L domains which enables the sFv to form the desired structure for antigen binding. For a review of the sFv, see Pluckthun in *The Pharmacology of Monoclonal Antibodies*, vol. 113, Rosenburg and Moore eds., Springer-Verlag, New York, pp. 269-315 (1994).

[0078] "Functional fragments" of the antibodies described herein comprise a portion of an intact antibody, generally including the antigen binding or variable region of the intact antibody or the Fc region of an antibody which retains or has modified FcR binding capability. Examples of antibody fragments include linear antibody, single-chain antibody molecules and multispecific antibodies formed from antibody fragments.

[0079] The term "diabodies" refers to small antibody fragments prepared by constructing sFv fragments (see preceding paragraph) with short linkers (about 5-10) residues) between the V_H and V_L domains such that inter-chain but not intra-chain pairing of the V domains is achieved, thereby resulting in a bivalent fragment, *i.e.*, a fragment having two antigen-binding sites. Bispecific diabodies are heterodimers of two "crossover" sFv fragments in which the V_H and V_L domains of the two antibodies are present on different polypeptide chains. Diabodies are described in greater detail in, for example, EP 404,097; WO 93/11161; Hollinger *et al.*, *Proc. Natl. Acad. Sci. USA* 90: 6444-6448 (1993).

[0080] The monoclonal antibodies herein specifically include "chimeric" antibodies (immunoglobulins) in which a portion of the heavy and/or light chain is identical with or homologous to corresponding sequences in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is(are) identical with or homologous to

corresponding sequences in antibodies derived from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they exhibit the desired biological activity (U.S. Pat. No. 4,816,567; Morrison *et al.*, *Proc. Natl. Acad. Sci. USA,* 81:6851-6855 (1984)). Chimeric antibodies of interest herein include PRIMATTZFD® antibodies wherein the antigen-binding region of the antibody is derived from an antibody produced by, *e.g.*, immunizing macaque monkeys with an antigen of interest. As used herein, "humanized antibody" is used a subset of "chimeric antibodies."

[0081] "Humanized" forms of non-human (*e.g.*, camelid) antibodies are chimeric antibodies that contain minimal sequence derived from non-human immunoglobulin. In some embodiments, a humanized antibody is a human immunoglobulin (recipient antibody) in which residues from an HVR (hereinafter defined) of the recipient are replaced by residues from an HVR of a non-human species (donor antibody) such as mouse, rat, rabbit or non-human primate having the desired specificity, affinity, and/or capacity. In some instances, framework ("FR") residues of the human immunoglobulin are replaced by corresponding non-human residues. Furthermore, humanized antibodies may comprise residues that are not found in the recipient antibody or in the donor antibody. These modifications may be made to further refine antibody performance, such as binding affinity. In general, a humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the hypervariable loops correspond to those of a non-human immunoglobulin sequence, and all or substantially all of the FR regions are those of a human immunoglobulin sequence, although the FR regions may include one or more individual FR residue substitutions that improve antibody performance, such as binding affinity, isomerization, immunogenicity, etc. The number of these amino acid substitutions in the FR is typically no more than 6 in the H chain, and in the L chain, no more than 3. The humanized antibody optionally will also comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. For further details, see, *e.g.*, Jones *et al.*, *Nature* 321:522-525 (1986); Riechmann *et al.*, *Nature* 332:323-329 (1988); and Presta, *Curr. Op. Struct. Biol.* 2:593-596 (1992). See also, for example, Vaswani and Hamilton, *Ann. Allergy, Asthma & Immunol.* 1:105-115 (1998); Harris, *Biochem. Soc. Transactions* 23:1035-1038 (1995); Hurle and Gross, *Curr. Op. Biotech.* 5:428-433 (1994); and U.S. Pat. Nos. 6,982,321 and 7,087,409.

[0082] A "human antibody" is an antibody that possesses an amino-acid sequence corresponding to that of an antibody produced by a human and/or has been made using any of the techniques for making human antibodies as disclosed herein. This definition of a human antibody specifically excludes a humanized antibody comprising non-human antigen-binding residues. Human antibodies can be produced using various techniques known in the art, including phage-display libraries. Hoogenboom and Winter, *J. Mol. Biol.,* 227:381 (1991); Marks *et al.*, *J. Mol. Biol.,* 222:581 (1991). Also available for the preparation of human monoclonal antibodies are methods described in Cole *et al.*, *Monoclonal Antibodies and*

Cancer Therapy, Alan R. Liss, p. 77 (1985); Boerner *et al.*, *J. Immunol.,* 147(1):86-95 (1991). See also van Dijk and van de Winkel, *Curr. Opin. Pharmacol.,* 5: 368-74 (2001). Human antibodies can be prepared by administering the antigen to a transgenic animal that has been modified to produce such antibodies in response to antigenic challenge, but whose endogenous loci have been disabled, *e.g.*, immunized xenomice (see, *e.g.*, U.S. Pat. Nos. 6,075,181 and 6,150,584 regarding XENOMOUSE™ technology). See also, for example, Li *et al.*, *Proc. Natl. Acad. Sci. USA,* 103:3557-3562 (2006) regarding human antibodies generated via a human B-cell hybridoma technology.

[0083] The term "hypervariable region," "HVR," or "HV," when used herein refers to the regions of an antibody variable domain which are hypervariable in sequence and/or form structurally defined loops. Generally, sdAbs comprise three HVRs (or CDRs): HVR1 (or CDR1), HVR2 (or CDR2), and HVR3 (or CDR3). HVR3 displays the most diversity of the three HVRs, and is believed to play a unique role in conferring fine specificity to antibodies. See, *e.g.*, Hamers-Casterman *et al.*, *Nature* 363:446-448 (1993); Sheriff *et al.*, *Nature Struct. Biol.* 3:733-736 (1996).

[0084] The term "Complementarity Determining Region" or "CDR" are used to refer to hypervariable regions as defined by the Kabat system. See Kabat *et al.*, *Sequences of Proteins of Immunological Interest,* 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991)

[0085] A number of HVR delineations are in use and are encompassed herein. The Kabat Complementarity Determining Regions (CDRs) are based on sequence variability and are the most commonly used (Kabat *et al.*, *Sequences of Proteins of Immunological Interest,* 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991)). Chothia refers instead to the location of the structural loops (Chothia and Lesk, *J. Mol. Biol.* 196:901-917 (1987)). The AbM HVRs represent a compromise between the Kabat HVRs and Chothia structural loops, and are used by Oxford Molecular's AbM antibody modeling software. The "contact" HVRs are based on an analysis of the available complex crystal structures. The residues from each of these HVRs are noted below in Table 1.

Table 1. HVR delineations.

Loop	Kabat	AbM	Chothia	Contact
L1	L24-L34	L24-L34	L26-L32	L30-L36
L2	L50-L56	L50-L56	L50-L52	L46-L55
L3	L89-L97	L89-L97	L91-L96	L89-L96
H1	H31-H35B	H26-H35B	H26-H32	H30-H35B
		(Kabat Numbering)		
H1	H31-H35	H26-H35	H26-H32	H30-H35
		(Chothia Numbering)		
H2	H50-H65	H50-H58	H53-H55	H47-H58
H3	H95-H102	H95-H102	H96-H101	H93-H101

[0086] HVRs may comprise "extended HVRs" as follows: 24-36 or 24-34 (L1), 46-56 or 50-56 (L2) and 89-97 or 89-96 (L3) in the V_L and 26-35 (H1), 50-65 or 49-65 (H2) and 93-102, 94-102, or 95-102 (H3) in the V_H. The variable domain residues are numbered according to Kabat *et al.*, supra, for each of these definitions.

[0087] The amino acid residues of a sdAb (such as V_HH) are numbered according to the general numbering for V_H domains given by Kabat et al. ("Sequence of proteins of immunological interest", US Public Health Services, NIH Bethesda, Md., Publication No. 91), as applied to V_HH domains from Camelids in the article of Riechmann and Muyldermans, J. Immunol. Methods 2000 Jun. 23; 240 (1-2): 185-195. According to this numbering, FR1 of a V_HH comprises the amino acid residues at positions 1-30, CDR1 of a V_HH comprises the amino acid residues at positions 31-35, FR2 of a V_HH comprises the amino acids at positions 36-49, CDR2 of a V_HH comprises the amino acid residues at positions 50-65, FR3 of a V_HH comprises the amino acid residues at positions 66-94, CDR3 of a V_HH comprises the amino acid residues at positions 95-102, and FR4 of a V_HH comprises the amino acid residues at positions 103-113. In this respect, it should be noted that—as is well known in the art for V_H domains and for V_HH domains—the total number of amino acid residues in each of the CDRs may vary and may not correspond to the total number of amino acid residues indicated by the Kabat numbering (that is, one or more positions according to the Kabat numbering may not be occupied in the actual sequence, or the actual sequence may contain more amino acid residues than the number allowed for by the Kabat numbering).

[0088] The expression "variable-domain residue-numbering as in Kabat" or "amino-acid-position numbering as in Kabat," and variations thereof, refers to the numbering system used for heavy-chain variable domains or light-chain variable domains of the compilation of antibodies in Kabat *et al.*, supra. Using this numbering system, the actual linear amino acid sequence may contain fewer or additional amino acids corresponding to a shortening of, or insertion into, a FR or HVR of the variable domain. For example, a heavy-chain variable domain may include a single amino acid insert (residue 52a according to Kabat) after residue 52 of H2 and inserted residues (*e.g.* residues 82a, 82b, and 82c, etc. according to Kabat) after heavy-chain FR residue 82. The Kabat numbering of residues may be determined for a given antibody by alignment at regions of homology of the sequence of the antibody with a "standard" Kabat numbered sequence.

[0089] Unless indicated otherwise herein, the numbering of the residues in an immunoglobulin heavy chain is that of the EU index as in Kabat *et al.*, supra. The "EU index as in Kabat" refers to the residue numbering of the human IgG1 EU antibody.

[0090] "Framework" or "FR" residues are those variable-domain residues other than the HVR residues as herein defined.

[0091] A "human consensus framework" or "acceptor human framework" is a framework that represents the most commonly occurring amino acid residues in a selection of human immunoglobulin V_L or V_H framework sequences. Generally, the selection of human immunoglobulin V_L or V_H sequences is from a subgroup of variable domain sequences. Generally, the subgroup of sequences is a subgroup as in Kabat *et al.*, *Sequences of Proteins of Immunological Interest,* 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991). Examples include for the V_L, the subgroup may be subgroup kappa I, kappa II, kappa III or kappa IV as in Kabat *et al.*, supra. Additionally, for the VH, the subgroup may be subgroup I, subgroup II, or subgroup III as in Kabat *et al.* Alternatively, a human consensus framework can be derived from the above in which particular residues, such as when a human framework residue is selected based on its homology to the donor framework by aligning the donor framework sequence with a collection of various human framework sequences. An acceptor human framework "derived from" a human immunoglobulin framework or a human consensus framework may comprise the same amino acid sequence thereof, or it may contain pre-existing amino acid sequence changes. In some embodiments, the number of pre-existing amino acid changes are 10 or less, 9 or less, 8 or less, 7 or less, 6 or less, 5 or less, 4 or less, 3 or less, or 2 or less.

[0092] An "amino-acid modification" at a specified position, *e.g.* of the Fc region, refers to the substitution or deletion of the specified residue, or the insertion of at least one amino acid residue adjacent the specified residue. Insertion "adjacent" to a specified residue means insertion within one to two residues thereof. The insertion may be N-terminal or C-terminal to the specified residue. The preferred amino acid modification herein is a substitution.

[0093] An "affinity-matured" antibody is one with one or more alterations in one or more HVRs thereof that result in an improvement in the affinity of the antibody for antigen, compared to a parent antibody that does not possess those alteration(s). In some embodiments, an affinity-matured antibody has nanomolar or even picomolar affinities for the target antigen. Affinity-matured antibodies are produced by procedures known in the art. For example, Marks *et al.*, *Bio/Technology* 10:779-783 (1992) describes affinity maturation by V_H- and V_L -domain shuffling. Random mutagenesis of HVR and/or framework residues is described by, for example: Barbas *et al. Proc Nat. Acad. Sci. USA* 91:3809-3813 (1994); Schier *et al. Gene* 169:147-155 (1995); Yelton *et al. J. Immunol.* 155:1994-2004 (1995); Jackson *et al.*, *J. Immunol.* 154(7):3310-9 (1995); and Hawkins *et al*, *J. Mol. Biol.* 226:889-896 (1992).

[0094] As use herein, the term "specifically binds," "specifically recognizes," or is "specific for" refers to measurable and reproducible interactions such as binding between a target and an antigen binding protein (such as a chimeric receptor or an sdAb), which is determinative of the presence of the target in the presence of a heterogeneous population of molecules including biological molecules. For example, an antigen binding protein that specifically binds a target (which can be an epitope) is an antigen

binding protein that binds this target with greater affinity, avidity, more readily, and/or with greater duration than it binds other targets. In some embodiments, the extent of binding of an antigen binding protein to an unrelated target is less than about 10% of the binding of the antigen binding protein to the target as measured, *e.g.*, by a radioimmunoassay (RIA). In some embodiments, an antigen binding protein that specifically binds a target has a dissociation constant (Kd) of ≦1 μM, ≦100 nM, ≦10 nM, ≦1 nM, or ≦0.1 nM. In some embodiments, an antigen binding protein specifically binds an epitope on a protein that is conserved among the protein from different species. In some embodiments, specific binding can include, but does not require exclusive binding.

[0095] The term “specificity” refers to selective recognition of an antigen binding protein (such as a chimeric receptor or an antibody construct) for a particular epitope of an antigen. Natural antibodies, for example, are monospecific. The term "multispecific" as used herein denotes that an antigen binding protein has two or more antigen-binding sites of which at least two bind different antigens or epitopes. "Bispecific" as used herein denotes that an antigen binding protein has two different antigen-binding specificities. The term "monospecific" as used herein denotes an antigen binding protein that has one or more binding sites each of which bind the same antigen or epitope.

[0096] The term "valent" as used herein denotes the presence of a specified number of binding sites in an antigen binding protein. A natural antibody for example or a full length antibody has two binding sites and is bivalent. As such, the terms "trivalent", "tetravalent", "pentavalent" and "hexavalent" denote the presence of two binding site, three binding sites, four binding sites, five binding sites, and six binding sites, respectively, in an antigen binding protein.

[0097] “Antibody effector functions” refer to those biological activities attributable to the Fc region (a native sequence Fc region or amino acid sequence variant Fc region) of an antibody, and vary with the antibody isotype. Examples of antibody effector functions include: C1q binding and complement dependent cytotoxicity; Fc receptor binding; antibody—dependent cell-mediated cytotoxicity (ADCC); phagocytosis; down regulation of cell surface receptors (*e.g.*, B cell receptors); and B cell activation. “Reduced or minimized” antibody effector function means that which is reduced by at least 50% (alternatively 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%) from the wild type or unmodified antibody. The determination of antibody effector function is readily determinable and measurable by one of ordinary skill in the art. In a preferred embodiment, the antibody effector functions of complement binding, complement dependent cytotoxicity and antibody dependent cytotoxicity are affected. In some embodiments, effector function is eliminated through a mutation in the constant region that eliminated glycosylation, *e.g.*, “effector-less mutation.” In one aspect, the effector-less mutation is an N297A or DANA mutation (D265A+N297A) in the C_H2 region. Shields *et al.*, *J. Biol. Chem.* 276 (9): 6591-6604 (2001). Alternatively, additional mutations resulting in reduced or eliminated effector function

include: K322A and L234A/L235A (LALA). Alternatively, effector function can be reduced or eliminated through production techniques, such as expression in host cells that do not glycosylate (*e.g.*, *E. coli.*) or in which result in an altered glycosylation pattern that is ineffective or less effective at promoting effector function (*e.g.*, Shinkawa *et al.*, *J. Biol. Chem.* 278(5): 3466-3473 (2003).

[0098] "Antibody-dependent cell-mediated cytotoxicity" or ADCC refers to a form of cytotoxicity in which secreted Ig bound onto Fc receptors (FcRs) present on certain cytotoxic cells (*e.g.*, natural killer (NK) cells, neutrophils and macrophages) enable these cytotoxic effector cells to bind specifically to an antigen-bearing target cell and subsequently kill the target cell with cytotoxins. The antibodies "arm" the cytotoxic cells and are required for killing of the target cell by this mechanism. The primary cells for mediating ADCC, NK cells, express FcγRIII only, whereas monocytes express FcγRI, FcγRII and FcγRIII. Fc expression on hematopoietic cells is summarized in Table 3 on page 464 of Ravetch and Kinet, *Annu. Rev. Immunol.* 9: 457-92 (1991). To assess ADCC activity of a molecule of interest, an *in vitro* ADCC assay, such as that described in U.S. Pat. No. 5,500,362 or 5,821,337 may be performed. Useful effector cells for such assays include peripheral blood mononuclear cells (PBMC) and natural killer (NK) cells. Alternatively, or additionally, ADCC activity of the molecule of interest may be assessed *in vivo*, *e.g.*, in an animal model such as that disclosed in Clynes *et al.*, *PNAS USA* 95:652-656 (1998).

[0099] The term "Fc region" herein is used to define a C-terminal region of an immunoglobulin heavy chain, including native-sequence Fc regions and variant Fc regions. Although the boundaries of the Fc region of an immunoglobulin heavy chain might vary, the human IgG heavy-chain Fc region is usually defined to stretch from an amino acid residue at position Cys226, or from Pro230, to the carboxyl-terminus thereof. The C-terminal lysine (residue 447 according to the EU numbering system) of the Fc region may be removed, for example, during production or purification of the antibody, or by recombinantly engineering the nucleic acid encoding a heavy chain of the antibody. Accordingly, a composition of intact antibodies may comprise antibody populations with all K447 residues removed, antibody populations with no K447 residues removed, and antibody populations having a mixture of antibodies with and without the K447 residue. Suitable native-sequence Fc regions for use in the antibodies described herein include human IgG1, IgG2 (IgG2A, IgG2B), IgG3 and IgG4.

[0100] "Binding affinity" generally refers to the strength of the sum total of non-covalent interactions between a single binding site of a molecule (*e.g.*, an antibody or a CAR) and its binding partner (*e.g.*, an antigen). Unless indicated otherwise, as used herein, "binding affinity" refers to intrinsic binding affinity that reflects a 1:1 interaction between members of a binding pair (*e.g.*, antibody and antigen, or CAR and antigen). The affinity of a molecule X for its partner Y can generally be represented by the dissociation constant (Kd). Affinity can be measured by common methods known in the art, including those described

herein. Low-affinity antibodies generally bind antigen slowly and tend to dissociate readily, whereas high-affinity antibodies generally bind antigen faster and tend to remain bound longer. A variety of methods of measuring binding affinity are known in the art, any of which can be used for purposes of the present application. Specific illustrative and exemplary embodiments for measuring binding affinity are described in the following.

[0101] A “blocking” antibody or an “antagonist” antibody is one that inhibits or reduces a biological activity of the antigen it binds. In some embodiments, blocking antibodies or antagonist antibodies substantially or completely inhibit the biological activity of the antigen.

[0102] “Percent (%) amino acid sequence identity” and “homology” with respect to a peptide, polypeptide or antibody sequence are defined as the percentage of amino acid residues in a candidate sequence that are identical with the amino acid residues in the specific peptide or polypeptide sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Alignment for purposes of determining percent amino acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN or MEGALIGN™ (DNASTAR) software. Those skilled in the art can determine appropriate parameters for measuring alignment, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared.

[0103] “Chimeric receptor,” “chimeric antigen receptor” or "CAR" as used herein refers to genetically engineered receptors, which can be used to graft one or more antigen specificity onto immune cells, such as T cells. Some chimeric receptors are also known as “artificial T-cell receptors,” “chimeric T cell receptors,” or “chimeric immune receptors.” In some embodiments, the chimeric receptor comprises an extracellular domain specific for one or more antigens (such as tumor antigens) or epitopes, a transmembrane domain, and an intracellular signaling domain of a T cell and/or co-stimulatory receptors. “CAR-T” refers to a T cell that expresses a CAR. “Anti-CLL1 CAR” refers to a CAR having an extracellular binding domain specific for CLL1.

[0104] An “isolated” nucleic acid molecule encoding a chimeric receptor or an anti-CLL1 construct described herein is a nucleic acid molecule that is identified and separated from at least one contaminant nucleic acid molecule with which it is ordinarily associated in the environment in which it was produced. Preferably, the isolated nucleic acid is free of association with all components associated with the production environment. The isolated nucleic acid molecules encoding the polypeptides and antibodies herein is in a form other than in the form or setting in which it is found in nature. Isolated nucleic acid molecules therefore are distinguished from nucleic acid encoding the polypeptides and antibodies herein existing naturally in cells.

[0105] The term “control sequences” refers to DNA sequences necessary for the expression of an operably linked coding sequence in a particular host organism. The control sequences that are suitable for prokaryotes, for example, include a promoter, optionally an operator sequence, and a ribosome binding site. Eukaryotic cells are known to utilize promoters, polyadenylation signals, and enhancers.

[0106] Nucleic acid is “operably linked” when it is placed into a functional relationship with another nucleic acid sequence. For example, DNA for a presequence or secretory leader is operably linked to DNA for a polypeptide if it is expressed as a preprotein that participates in the secretion of the polypeptide; a promoter or enhancer is operably linked to a coding sequence if it affects the transcription of the sequence; or a ribosome binding site is operably linked to a coding sequence if it is positioned so as to facilitate translation. Generally, “operably linked” means that the DNA sequences being linked are contiguous, and, in the case of a secretory leader, contiguous and in reading phase. However, enhancers do not have to be contiguous. Linking is accomplished by ligation at convenient restriction sites. If such sites do not exist, the synthetic oligonucleotide adaptors or linkers are used in accordance with conventional practice.

[0107] The term “vector,” as used herein, refers to a nucleic acid molecule capable of propagating another nucleic acid to which it is linked. The term includes the vector as a self-replicating nucleic acid structure as well as the vector incorporated into the genome of a host cell into which it has been introduced. Certain vectors are capable of directing the expression of nucleic acids to which they are operatively linked. Such vectors are referred to herein as “expression vectors.”

[0108] As used herein, the term “autologous” is meant to refer to any material derived from the same individual to whom it is later to be re-introduced into the individual.

[0109] “Allogeneic” refers to a graft derived from a different individual of the same species.

[0110] The term “transfected” or “transformed” or “transduced” as used herein refers to a process by which exogenous nucleic acid is transferred or introduced into the host cell. A “transfected” or “transformed” or “transduced” cell is one which has been transfected, transformed or transduced with exogenous nucleic acid. The cell includes the primary subject cell and its progeny.

[0111] As used herein, the expressions “cell”, “cell line”, and “cell culture” are used interchangeably and all such designations include progeny. Thus, the words “transfectants” and “transfected cells” include the primary subject cell and cultures derived there from without regard for the number of transfers. It is also understood that all progeny may not be precisely identical in DNA content, due to deliberate or inadvertent mutations. Variant progeny that have the same function or biological activity as screened for in the originally transformed cell are included.

[0112] The terms “host cell,” “host cell line,” and “host cell culture” are used interchangeably and refer to cells into which exogenous nucleic acid has been introduced, including the progeny of such cells.

Host cells include "transformants" and "transformed cells," which include the primary transformed cell and progeny derived therefrom without regard to the number of passages. Progeny may not be completely identical in nucleic acid content to a parent cell, but may contain mutations. Mutant progeny that have the same function or biological activity as screened or selected for in the originally transformed cell are included herein.

[0113] As used herein, "treatment" or "treating" is an approach for obtaining beneficial or desired results including clinical results. For purposes of this invention, beneficial or desired clinical results include, but are not limited to, one or more of the following: alleviating one or more symptoms resulting from the disease, diminishing the extent of the disease, stabilizing the disease (*e.g.*, preventing or delaying the worsening of the disease), preventing or delaying the spread (*e.g.*, metastasis) of the disease, preventing or delaying the recurrence of the disease, delay or slowing the progression of the disease, ameliorating the disease state, providing a remission (partial or total) of the disease, decreasing the dose of one or more other medications required to treat the disease, delaying the progression of the disease, increasing the quality of life, and/or prolonging survival. Also encompassed by "treatment" is a reduction of pathological consequence(s) of cancer. The methods of the present application contemplate any one or more of these aspects of treatment.

[0114] As used herein, an "individual" or a "subject" refers to a mammal, including, but not limited to, human, bovine, horse, feline, canine, rodent, or primate. In some embodiments, the individual is a human.

[0115] The term "effective amount" used herein refers to an amount of an agent, such as an anti-CLL1 construct, an engineered immune cell, or a pharmaceutical composition thereof, sufficient to treat a specified disorder, condition or disease such as ameliorate, palliate, lessen, and/or delay one or more of its symptoms. In reference to cancer, an effective amount comprises an amount sufficient to cause a tumor to shrink and/or to decrease the growth rate of the tumor (such as to suppress tumor growth) or to prevent or delay other unwanted cell proliferation. In some embodiments, an effective amount is an amount sufficient to delay development. In some embodiments, an effective amount is an amount sufficient to prevent or delay recurrence. An effective amount can be administered in one or more administrations. The effective amount of the drug or composition may: (i) reduce the number of cancer cells; (ii) reduce tumor size; (iii) inhibit, retard, slow to some extent and preferably stop cancer cell infiltration into peripheral organs; (iv) inhibit (*i.e.*, slow to some extent and preferably stop) tumor metastasis; (v) inhibit tumor growth; (vi) prevent or delay occurrence and/or recurrence of tumor; and/or (vii) relieve to some extent one or more of the symptoms associated with the cancer.

[0116] "Adjuvant setting" refers to a clinical setting in which an individual has had a history of cancer, and generally (but not necessarily) been responsive to therapy, which includes, but is not limited to, surgery (*e.g.*, surgery resection), radiotherapy, and chemotherapy. However, because of their history of

cancer, these individuals are considered at risk of development of the disease. Treatment or administration in the “adjuvant setting” refers to a subsequent mode of treatment. The degree of risk (*e.g.*, when an individual in the adjuvant setting is considered as “high risk” or “low risk”) depends upon several factors, most usually the extent of disease when first treated.

[0117] “Neoadjuvant setting” refers to a clinical setting in which the method is carried out before the primary/definitive therapy.

[0118] As used herein, “delaying” the development of cancer means to defer, hinder, slow, retard, stabilize, and/or postpone development of the disease. This delay can be of varying lengths of time, depending on the history of the disease and/or individual being treated. As is evident to one skilled in the art, a sufficient or significant delay can, in effect, encompass prevention, in that the individual does not develop the disease. A method that “delays” development of cancer is a method that reduces probability of disease development in a given time frame and/or reduces the extent of the disease in a given time frame, when compared to not using the method. Such comparisons are typically based on clinical studies, using a statistically significant number of individuals. Cancer development can be detectable using standard methods, including, but not limited to, computerized axial tomography (CAT Scan), Magnetic Resonance Imaging (MRI), abdominal ultrasound, clotting tests, arteriography, or biopsy. Development may also refer to cancer progression that may be initially undetectable and includes occurrence, recurrence, and onset.

[0119] The term “pharmaceutical formulation” refers to a preparation that is in such form as to permit the biological activity of the active ingredient to be effective, and that contains no additional components that are unacceptably toxic to a subject to which the formulation would be administered. Such formulations are sterile. A “sterile” formulation is aseptic or free from all living microorganisms and their spores.

[0120] It is understood that embodiments of the present application described herein include “consisting” and/or “consisting essentially of” embodiments.

[0121] Reference to "about" a value or parameter herein includes (and describes) variations that are directed to that value or parameter *per se*. For example, description referring to "about X" includes description of "X".

[0122] As used herein, reference to "not" a value or parameter generally means and describes "other than" a value or parameter. For example, the method is not used to treat cancer of type X means the method is used to treat cancer of types other than X.

[0123] The term “about X-Y” used herein has the same meaning as “about X to about Y.”

[0124] As used herein and in the appended claims, the singular forms “a,” “an” and “the” include plural referents unless the context clearly dictates otherwise.

II. Anti-CLL1 Constructs

[0125] In one aspect, the present application provides an anti-CLL1 constructs comprising an anti-CLL1 sdAb moiety. Any one of the anti-CLL1 sdAbs described herein or antigen-binding fragments thereof (*e.g.*, V_HH) may be used in the anti-CLL1 construct. Anti-CLL1 sdAbs are described in Section "A. Anti-CLL1 single-domain antibodies" below.

[0126] In some embodiments, there is provided an anti-CLL1 construct comprising an sdAb moiety that specifically binds to CLL1, wherein the sdAb moiety comprises a CDR1 comprising the amino acid sequence of SEQ ID NO: 4, a CDR2 comprising the amino acid sequence of SEQ ID NO: 6, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 8, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs. In some embodiments, the sdAb moiety comprises the amino acid sequence of SEQ ID NO: 94, or a variant thereof comprising an amino acid sequence having at least about 95% sequence identity to the amino acid sequence of SED ID NO: 94.

[0127] In some embodiments, there is provided an anti-CLL1 construct comprising an sdAb moiety that specifically binds to CLL1, wherein the sdAb moiety comprises a CDR1 comprising the amino acid sequence of SEQ ID NO: 11, a CDR2 comprising the amino acid sequence of SEQ ID NO: 13, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 15, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs. In some embodiments, the sdAb moiety comprises the amino acid sequence of SEQ ID NO: 95, or a variant thereof comprising an amino acid sequence having at least about 95% sequence identity to the amino acid sequence of SED ID NO: 95.

[0128] In some embodiments, there is provided an anti-CLL1 construct comprising an sdAb moiety that specifically binds to CLL1, wherein the sdAb moiety comprises a CDR1 comprising the amino acid sequence of SEQ ID NO: 18, a CDR2 comprising the amino acid sequence of SEQ ID NO: 20, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 22, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs. In some embodiments, the sdAb moiety comprises the amino acid sequence of SEQ ID NO: 96, or a variant thereof comprising an amino acid sequence having at least about 95% sequence identity to the amino acid sequence of SED ID NO: 96.

[0129] In some embodiments, there is provided an anti-CLL1 construct comprising an sdAb moiety that specifically binds to CLL1, wherein the sdAb moiety comprises a CDR1 comprising the amino acid sequence of SEQ ID NO: 25, a CDR2 comprising the amino acid sequence of SEQ ID NO: 27, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 29, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs. In some embodiments, the sdAb moiety comprises the amino acid sequence of SEQ ID NO: 97, or a variant thereof comprising an amino acid sequence having at least about 95% sequence identity to the amino acid sequence of SED ID NO: 97.

[0130] In some embodiments, there is provided an anti-CLL1 construct comprising an sdAb moiety that specifically binds to CLL1, wherein the sdAb moiety comprises a CDR1 comprising the amino acid sequence of SEQ ID NO: 32, a CDR2 comprising the amino acid sequence of SEQ ID NO: 34, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 36, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs. In some embodiments, the sdAb moiety comprises the amino acid sequence of SEQ ID NO: 98, or a variant thereof comprising an amino acid sequence having at least about 95% sequence identity to the amino acid sequence of SED ID NO: 98.

[0131] In some embodiments, there is provided an anti-CLL1 construct comprising an sdAb a sdAb moiety that specifically binds to CLL1, wherein the sdAb moiety comprises a CDR1 comprising the amino acid sequence of SEQ ID NO: 39, a CDR2 comprising the amino acid sequence of SEQ ID NO: 41, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 43, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs. In some embodiments, the sdAb moiety comprises the amino acid sequence of SEQ ID NO: 99, or a variant thereof comprising an amino acid sequence having at least about 95% sequence identity to the amino acid sequence of SED ID NO: 99.

[0132] In some embodiments, there is provided an anti-CLL1 construct comprising an sdAb moiety that specifically binds to CLL1, wherein the sdAb moiety comprises a CDR1 comprising the amino acid sequence of SEQ ID NO: 46, a CDR2 comprising the amino acid sequence of SEQ ID NO: 48, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 50, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs. In some embodiments, the sdAb moiety comprises the amino acid sequence of SEQ ID NO: 100, or a variant thereof comprising an amino acid sequence having at least about 95% sequence identity to the amino acid sequence of SED ID NO: 100.

[0133] In some embodiments, there is provided an anti-CLL1 construct comprising an sdAb moiety that specifically binds to CLL1, wherein the sdAb moiety comprises a CDR1 comprising the amino acid sequence of SEQ ID NO: 53, a CDR2 comprising the amino acid sequence of SEQ ID NO: 55, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 57, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs. In some embodiments, the sdAb moiety comprises the amino acid sequence of SEQ ID NO: 101, or a variant thereof comprising an amino acid sequence having at least about 95% sequence identity to the amino acid sequence of SED ID NO: 101.

[0134] In some embodiments, there is provided an anti-CLL1 construct comprising an sdAb moiety that specifically binds to CLL1, wherein the sdAb moiety comprises a CDR1 comprising the amino acid sequence of SEQ ID NO: 60, a CDR2 comprising the amino acid sequence of SEQ ID NO: 62, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 64, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs. In some embodiments, the sdAb moiety comprises the

amino acid sequence of SEQ ID NO: 102, or a variant thereof comprising an amino acid sequence having at least about 95% sequence identity to the amino acid sequence of SED ID NO: 102.

[0135] In some embodiments, there is provided an anti-CLL1 construct comprising an sdAb moiety that specifically binds to CLL1, wherein the sdAb moiety comprises a CDR1 comprising the amino acid sequence of SEQ ID NO: 67, a CDR2 comprising the amino acid sequence of SEQ ID NO: 69, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 71, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs. In some embodiments, the sdAb moiety comprises the amino acid sequence of SEQ ID NO: 103, or a variant thereof comprising an amino acid sequence having at least about 95% sequence identity to the amino acid sequence of SED ID NO: 103.

[0136] In some embodiments, there is provided an anti-CLL1 construct comprising an sdAb moiety that specifically binds to CLL1, wherein the sdAb moiety comprises a CDR1 comprising the amino acid sequence of SEQ ID NO: 74, a CDR2 comprising the amino acid sequence of SEQ ID NO: 76, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 78, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs. In some embodiments, the sdAb moiety comprises the amino acid sequence of SEQ ID NO: 104, or a variant thereof comprising an amino acid sequence having at least about 95% sequence identity to the amino acid sequence of SED ID NO: 104.

[0137] In some embodiments, there is provided an anti-CLL1 construct comprising an sdAb moiety that specifically binds to CLL1, wherein the sdAb moiety comprises a CDR1 comprising the amino acid sequence of SEQ ID NO: 81, a CDR2 comprising the amino acid sequence of SEQ ID NO: 83, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 85, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs. In some embodiments, the sdAb moiety comprises the amino acid sequence of SEQ ID NO: 105, or a variant thereof comprising an amino acid sequence having at least about 95% sequence identity to the amino acid sequence of SED ID NO: 105.

[0138] In some embodiments, there is provided an anti-CLL1 construct comprising an sdAb moiety that specifically binds to CLL1, wherein the sdAb moiety comprises a CDR1 comprising the amino acid sequence of SEQ ID NO: 88, a CDR2 comprising the amino acid sequence of SEQ ID NO: 90, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 92, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs. In some embodiments, the sdAb moiety comprises the amino acid sequence of SEQ ID NO: 106, or a variant thereof comprising an amino acid sequence having at least about 95% sequence identity to the amino acid sequence of SED ID NO: 106.

[0139] In some embodiments, there is provided an anti-CLL1 construct comprising an sdAb moiety that specifically binds to CLL1, wherein the sdAb moiety comprises a CDR1 comprising the amino acid sequence of SEQ ID NO: 151, a CDR2 comprising the amino acid sequence of SEQ ID NO: 153, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 155, or a variant thereof comprising up to

about 5 amino acid substitutions in the CDRs. In some embodiments, the sdAb moiety comprises the amino acid sequence of SEQ ID NO: 171, or a variant thereof comprising an amino acid sequence having at least about 95% sequence identity to the amino acid sequence of SED ID NO: 174.

[0140] In some embodiments, there is provided an anti-CLL1 construct comprising an sdAb moiety that specifically binds to CLL1, wherein the sdAb moiety comprises a CDR1 comprising the amino acid sequence of SEQ ID NO: 158, a CDR2 comprising the amino acid sequence of SEQ ID NO: 160, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 162, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs. In some embodiments, the sdAb moiety comprises the amino acid sequence of SEQ ID NO: 172, or a variant thereof comprising an amino acid sequence having at least about 95% sequence identity to the amino acid sequence of SED ID NO: 175.

[0141] In some embodiments, there is provided an anti-CLL1 construct comprising an sdAb moiety that specifically binds to CLL1, wherein the sdAb moiety comprises a CDR1 comprising the amino acid sequence of SEQ ID NO: 165, a CDR2 comprising the amino acid sequence of SEQ ID NO: 167, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 169, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs. In some embodiments, the sdAb moiety comprises the amino acid sequence of SEQ ID NO: 173, or a variant thereof comprising an amino acid sequence having at least about 95% sequence identity to the amino acid sequence of SED ID NO: 176.

[0142] In some embodiments, the anti-CLL1 construct is a transmembrane molecule. In some embodiments, the anti-CLL1 construct is a secreted molecule.

[0143] In some embodiments, the anti-CLL1 construct is a monoclonal antibody comprising any one of the anti-CLL1 sdAbs described herein, including a camelid, chimeric, humanized or human antibody. In some embodiments, the anti-CLL1 construct is an antibody fragment, *e.g.*, a V_HH fragment. In some embodiments, the anti-CLL1 construct is a full-length heavy-chain only antibody comprising an Fc region of any antibody class or isotype, such as IgG1 or IgG4. In some embodiments, the Fc region has reduced or minimized effector function.

[0144] In some embodiments, the anti-CLL1 construct is a chimeric receptor comprising an extracellular domain comprising any one of the anti-CLL1 sdAbs described herein, a transmembrane domain, and an intracellular signaling domain. In some embodiments, the intracellular signaling domain comprises a primary intracellular signaling sequence of an immune cell (*e.g.*, a CD3ζ intracellular signaling sequence). In some embodiments, the intracellular signaling domain comprises an intracellular co-stimulatory sequence. In some embodiments, the intracellular signaling domain comprises both a primary intracellular signaling sequence of an immune cell (*e.g.*, a CD3ζ intracellular signaling sequence) and an intracellular co-stimulatory sequence. Anti-CLL1 chimeric receptors and chimeric receptor

systems are further described in Section "B. Chimeric receptors". Engineered immune cells comprising the anti-CLL1 chimeric receptors or chimeric receptor systems are described in Section IV.

[0145] In some embodiments, the anti-CLL1 construct is a monospecific molecule. In some embodiments, the anti-CLL1 construct is a multispecific molecule. In some embodiments, the anti-CLL1 construct is a bispecific molecule.

[0146] In some embodiments, the anti-CLL1 construct is a multispecific antigen binding protein comprising an anti-CLL1 sdAb (such as any one of the anti-CLL1 sdAbs described herein) and a second binding moiety that specifically binds to a second antigen or epitope. In some embodiments, the second binding moiety is an sdAb or an scFv. In some embodiments, the second binding moiety specifically binds to a different epitope on CLL1. In some embodiments, the second binding moiety specifically binds to a second antigen, such as a tumor antigen, or an antigen on the surface of an immune cell. In some embodiments, the anti-CLL1 sdAb is linked to the second binding moiety via a peptide linker.

[0147] In some embodiments, the anti-CLL1 construct is an immune effector cell engager comprising any one of the anti-CLL1 sdAbs described herein and a second binding moiety that specifically binds to an antigen on the surface of an immune cell, such as T cell. In some embodiments, the second binding moiety specifically binds to an antigen selected from the group consisting of CD3γ, CD3δ, CD3ε, CD3ζ, CD28, OX40, GITR, 4-1BB, CD27, CD40L, and HVEM. Immune effector cell engagers are further described in Section "C. Immune effector cell engagers" below.

[0148] In some embodiments, the anti-CLL1 construct is an immunoconjugate comprising any one of the anti-CLL1 sdAbs described herein and an effector molecule. In some embodiments, the effector molecule is a therapeutic agent selected from the group consisting of a drug, a toxin, a radioisotope, a protein, a peptide, and a nucleic acid. In some embodiments, the effector molecule is a drug or a toxin. In some embodiments, the effector molecule is a label. Immunoconjugates are further described in Section "D. Immunoconjugates" below.

A. Anti-CLL1 single-domain antibodies

[0149] One aspect of the present application provides isolated single-domain antibodies (referred herein as "anti-CLL1 sdAbs") that specifically bind to CLL1. In some embodiments, the anti-CLL1 sdAb modulates CLL1 activity. In some embodiments, the anti-CLL1 sdAb is an antagonist antibody. Further provided are antigen-binding fragments (*e.g.*, V_HH) derived from any one of the anti-CLL1 sdAbs described herein, and constructs comprising any one of the anti-CLL1 sdAbs described herein. Exemplary anti-CLL1 sdAbs are listed in Table 2 below. The anti-CLL1 constructs described herein comprise one or more anti-CLL1 sdAb moieties.

Table 2. Exemplary anti-CLL1 sdAbs.

sdAb/ SEQ ID (AA/NA)	FR1/ SEQ ID NO	CDR1/ SEQ ID	FR2/ SEQ ID	CDR2/ SEQ ID	FR3/ SEQ ID NO	CDR3/ SEQ ID	FR4/ SEQ ID
AS82472 94/107	QVQLVESGGDLVRPGGSLRLSCAAS 3	GFTFSIYDMN 4	WVRQAPGKGLEWVA 5	GISGNGYSTSYAESVKG 6	RFTISRDNAKNTVYLQLSSLKFEDTAMYYCVR 7	DAERWDENDLRR 8	KGQGTQVTVSS 9
AS82480 95/108	EVQLVESGGGSVQAGGSLRLSCAAS 10	GVTYSSACMG 11	WFRQAPGKGREVVA 12	VLYAGGSTTHYASSVKE 13	RFTISQDNAKNTVYLQMNSLKPEDTAVYYCAA 14	ALGDRSSCEWRY 15	WGQGTQVTVSS 16
AS82494 96/109	QVQLVESGGGLVQPGGSLRLSCAAS 17	GFTFSVYDMN 18	WFRQAPGKGLEWVS 19	GITGNGYTTSYADSVKG 20	RFTISRDNAKNTLYLQLNSLKSEDTAMYYCAK 21	ETN 22	RGQGTQVTVSS 23
AS82505 97/110	QVQLAESGGGLVQPGGSLRLSCVAS 24	GFTFSSYDMS 25	WVRQAPGKGVEWVS 26	TINSGGGSTYYAESAKG 27	RFTISRDNAKNTLYLQLNSLKTEDTAMYYCVK 28	GFPDDDGPGELSREYNY 29	WGQGTQVTVSS 30
AS82544 98/111	EVQLVESGGALVQPGGSLRLSCTAS 31	GFLFRVYDMN 32	WVRQAPGKGVEWIV 33	GITNNGYTTAYADSVKG 34	RFTISRDNTENTLFLQMNSLKPEDTAMYYCQT 35	DNGRV 36	RGQGTQVTVSS 37
AS82658 99/112	QVQLVESGGGSVQAGGALSLSCAAS 38	GYTVRIDYMG 39	WYRQTPGKGREPVA 40	TIASNGGTAYADSVEG 41	RFTISQDNAKNSVYLQMNTLKPGDTAMYYCAA 42	GTWPTLTY 43	FGQGTQVTVSS 44
AS82718 100/113	QVQLAESGGGLVQTGGSLRLSCTAS 45	GLNFGLYAMG 46	WFRQAPGKEREGVS 47	CINGGGGITVYSDFVKS 48	RFTISRDNAKNTLYLQMNSLKPDDTATYYCAA 49	DRSPFGSCSSDWSRSSDWSRMAEKFGY 50	WGQGTQVTVSS 51
AS83180 101/114	QVQLVESGGGSVQAGGSLRLSCVVS 52	AATNCRYIA 53	WYRQAPGKAREFVS 54	TLGSDGNTNYADSVKG 55	RFTISQGNIKNMAYLEMNSLKPEDTGMYYCGT 56	RCQIGDDWRSSD 57	WAQGTQVTVSS 58
AS83183 102/115	QVHLVESGGGSVQSGGSLRLSCAAS 59	GYAYRSYCMG 60	WFRQAPGKVLEGVA 61	AIESDGTTTYADSVMG 62	RFTISQDNAKNALYLQMNSLKPEDTAMYHCAA 63	VKGSCDSASSDTPSY 64	WGQGTQVTVSS 65
AS83309 103/116	EVQLVESGGDLVRPGGSLRLSCAAS 66	GFTFSIYDMN 67	WVRQAPGKGLEWVA 68	GISGNGYSTSYAESVKG 69	RFTISKDNAKNTVYLQLSSLKFEDTAMYYCVR 70	GGEKWDENDLRR 71	KGQGTQVTVSS 72
AS83431 104/117	QVRLVESGGGSVQSGGSLRLSCAAS 73	GYARSSTCLG 74	WFRQAPGKEVEGVA 75	IIGRDGSTGYADSVKG 76	RFTISQDNAKNTLYLHMDSLKPEDTAMYYCAA 77	VEGGCEVSEGTGEQQLAY 78	WGQGTQVTVSS 79
AS83478 105/118	QVHLMESGGGLVQPGESLRLSCAAS 80	GFIFANYEMS 81	WVRQAPGKVLEWVS 82	GINSRGNATYYADSVKG 83	RFTISRDNAEHTLYLQMNSLKPEDTAMYHCVV 84	GGMTTDQGSPDFY 85	WGQGTQVTVSS 86
AS83791	QVKLVESGGGLVQPGGSLRLSCVAS	GFAFSSADMS	WVRQAPGKGVEAVS	VINRDGASTYYADSVKG	RFTISRDNAKSTLYLQMNSLKPEDTAMYHCAV	VPENEYESGSYNY	WGQGTQVTVSS

106/119	87	88	89	90	91	92	93
AS83010 171/174	QVQLVESGGGLVQPGGSLRLSCVAS 150	GFFFSAYDMN 151	WFRQAPGKGLEWVS 152	GITGNGYTTAYADSVKG 153	RFTISRDNAKNTLYLQLNSLKSEDTAMYYCTE 154	GDN 155	RGQGTQVTVSS 156
AS83457 172/175	QVQLVESGGGLVQPGGSLRLSCAAS 157	GFFFSIYDMN 158	WFRQAPGKGLEWVS 159	GITGNGYTTAYADSVKG 160	RFTISRDNAKNTLYLQLNSLKSEDTAMYYCAQ 161	GSN 162	RGRGTQVTVSS 163
AS83591 173/176	QVQLVESGGGLVQPGGSLRLSCAAS 164	GFLFSIYDMN 165	WVRQAPGKGVEWIA 166	GITNNEHTTAYADSVKG 167	RFTISRDNTKNTLFLQMNSLKPEDTAMYYCQR 168	DDGQV 169	RGQGTQVTVSS 170

SEQ ID NO: 94 (AS82472 sdAb amino acid sequence; CDRs are underlined)
QVQLVESGGDLVRPGGSLRLSCAASGFTFSIYDMNWVRQAPGKGLEWVAGISGNGYSTSYAESVKGRFTIS
RDNAKNTVYLQLSSLKFEDTAMYYCVRDAERWDENDLRRKGQGTQVTVSS

SEQ ID NO: 95 (AS82480 sdAb amino acid sequence; CDRs are underlined)
EVQLVESGGGSVQAGGSLRLSCAASGVTYSSACMGWFRQAPGKGREVVAVLYAGGSTTHYASSVKERFTI
SQDNAKNTVYLQMNSLKPEDTAVYYCAAALGDRSSCEWRYWGQGTQVTVSS

SEQ ID NO: 96 (AS82494 sdAb amino acid sequence; CDRs are underlined)
QVQLVESGGGLVQPGGSLRLSCAASGFTFSVYDMNWFRQAPGKGLEWVSGITGNGYTTSYADSVKGRFTI
SRDNAKNTLYLQLNSLKSEDTAMYYCAKETNRGQGTQVTVSS

SEQ ID NO: 97 (AS82505 sdAb amino acid sequence; CDRs are underlined)
QVQLAESGGGLVQPGGSLRLSCVASGFTFSSYDMSWVRQAPGKGVEWVSTINSGGGSTYYAESAKGRFTI
SRDNAKNTLYLQLNSLKTEDTAMYYCVKGFPDDDGPGELSREYNYWGQGTQVTVSS

SEQ ID NO: 98 (AS82544 sdAb amino acid sequence; CDRs are underlined)
EVQLVESGGALVQPGGSLRLSCTASGFLFRVYDMNWVRQAPGKGVEWIVGITNNGYTTAYADSVKGRFTI
SRDNTENTLFLQMNSLKPEDTAMYYCQTDNGRVRGQGTQVTVSS

SEQ ID NO: 99 (AS82658 sdAb amino acid sequence; CDRs are underlined)
QVQLVESGGGSVQAGGALSLSCAASGYTVRIDYMGWYRQTPGKGREPVATIASNGGTAYADSVEGRFTIS
QDNAKNSVYLQMNTLKPGDTAMYYCAAGTWPTLTYFGQGTQVTVSS

SEQ ID NO: 100 (AS82718 sdAb amino acid sequence; CDRs are underlined)
QVQLAESGGGLVQTGGSLRLSCTASGLNFGLYAMGWFRQAPGKEREGVSCINGGGGITVYSDFVKSRFTIS
RDNAKNTLYLQMNSLKPDDTATYYCAADRSPFGSCSSDWSRSSDWSRMAEKFGYWGQGTQVTVSS

SEQ ID NO: 101 (AS83180 sdAb amino acid sequence; CDRs are underlined)
QVQLVESGGGSVQAGGSLRLSCVVSAATNCRYIAWYRQAPGKAREFVSTLGSDGNTNYADSVKGRFTISQ
GNIKNMAYLEMNSLKPEDTGMYYCGTRCQIGDDWRSSDWAQGTQVTVSS

SEQ ID NO: 102 (AS83183 sdAb amino acid sequence; CDRs are underlined)
QVHLVESGGGSVQSGGSLRLSCAASGYAYRSYCMGWFRQAPGKVLEGVAAIESDGTTTYADSVMGRFTIS
QDNAKNALYLQMNSLKPEDTAMYHCAAVKGSCDSASSDTPSYWGQGTQVTVSS

SEQ ID NO: 103 (AS83309 sdAb amino acid sequence; CDRs are underlined)
EVQLVESGGDLVRPGGSLRLSCAASGFTFSIYDMNWVRQAPGKGLEWVAGISGNGYSTSYAESVKGRFTIS
KDNAKNTVYLQLSSLKFEDTAMYYCVRGGEKWDENDLRRKGQGTQVTVSS
SEQ ID NO: 104 (AS83431 sdAb amino acid sequence; CDRs are underlined)
QVRLVESGGGSVQSGGSLRLSCAASGYARSSTCLGWFRQAPGKEVEGVAIIGRDGSTGYADSVKGRFTISQ
DNAKNTLYLHMDSLKPEDTAMYYCAAVEGGCEVSEGTGEQQLAYWGQGTQVTVSS

SEQ ID NO: 105 (AS83478 sdAb amino acid sequence; CDRs are underlined)
QVHLMESGGGLVQPGESLRLSCAASGFIFANYEMSWVRQAPGKVLEWVSGINSRGNATYYADSVKGRFTI
SRDNAEHTLYLQMNSLKPEDTAMYHCVVGGMTTDQGSPDFYWGQGTQVTVSS

SEQ ID NO: 106 (AS83591 sdAb amino acid sequence; CDRs are underlined)
QVQLVESGGGLVQPGGSLRLSCAASGFLFSIYDMNWVRQAPGKGVEWIAGITNNEHTTAYADSVKGRFTIS
RDNTKNTLFLQMNSLKPEDTAMYYCQRDDGQVRGQGTQVTVSS

SEQ ID NO: 171 (AS83010 sdAb amino acid sequence; CDRs are underlined)
QVQLVESGGGLVQPGGSLRLSCVASGFFFSAYDMNWFRQAPGKGLEWVSGITGNGYTTAYADSVKGRFTI
SRDNAKNTLYLQLNSLKSEDTAMYYCTEGDNRGQGTQVTVSS

SEQ ID NO: 172 (AS83457 sdAb amino acid sequence; CDRs are underlined)
QVQLVESGGGLVQPGGSLRLSCAASGFFFSIYDMNWFRQAPGKGLEWVSGITGNGYTTAYADSVKGRFTIS
RDNAKNTLYLQLNSLKSEDTAMYYCAQGSNRGRGTQVTVSS

SEQ ID NO: 173 (AS83591 sdAb amino acid sequence; CDRs are underlined)
QVQLVESGGGLVQPGGSLRLSCAASGFLFSIYDMNWVRQAPGKGVEWIAGITNNEHTTAYADSVKGRFTIS
RDNTKNTLFLQMNSLKPEDTAMYYCQRDDGQVRGQGTQVTVSS

SEQ ID NO: 107 (AS82472 sdAb nucleic acid sequence)
CAGGTGCAGCTGGTGGAGTCTGGGGGAGACTTGGTGCGGCCTGGGGGGTCTCTGAGACTCTCCTGTGC
AGCCTCTGGATTCACCTTCAGTATCTATGACATGAACTGGGTCCGCCAGGCTCCAGGGAAGGGACTCG
AGTGGGTCGCAGGTATTAGTGGTAATGGTTACAGTACAAGCTATGCAGAGTCCGTGAAGGGCCGATTC
ACCATCTCCAGAGACAACGCCAAGAACACAGTGTATCTACAATTGAGCAGCCTGAAATTTGAAGACAC
GGCCATGTATTACTGTGTAAGAGATGCGGAGAGGTGGGACGAGAATGACCTGCGACGGAAGGGCCAG
GGGACCCAGGTCACCGTCTCCTCA

SEQ ID NO: 108 (AS82480 sdAb nucleic acid sequence)
GAGGTGCAACTGGTGGAGTCTGGGGGAGGCTCGGTGCAGGCTGGAGGGTCTCTGAGACTCTCCTGTGC
AGCCTCTGGCGTCACGTACAGTAGTGCCTGCATGGGCTGGTTCCGCCAGGCTCCAGGAAAGGGGCGCG
AGGTGGTCGCGGTTCTTTATGCAGGTGGTAGTACCACACACTATGCCAGCTCCGTGAAGGAGCGATTC
ACCATCTCCCAAGACAACGCCAAGAACACGGTATATCTGCAGATGAACAGCCTGAAACCTGAGGACA
CTGCCGTTTACTACTGTGCGGCAGCTTTGGGTGATCGTTCAAGTTGCGAGTGGAGATACTGGGGCCAG
GGGACCCAGGTCACCGTCTCCTCA

SEQ ID NO: 109 (AS82494 sdAb nucleic acid sequence)
CAGGTTCAGCTGGTGGAGTCTGGGGGAGGCTTGGTGCAGCCTGGGGGGTCTCTGAGACTCTCCTGTGC
AGCCTCTGGATTCACCTTCAGTGTGTATGACATGAACTGGTTCCGCCAGGCTCCAGGGAAGGGACTCG
AGTGGGTCTCAGGTATTACTGGGAATGGTTATACAACATCCTACGCAGACTCCGTGAAGGGCCGATTC
ACCATCTCCAGAGACAACGCCAAGAACACGCTGTATCTGCAATTGAACAGCCTGAAAAGTGAGGACA
CGGCCATGTATTACTGTGCAAAGGAGACTAATAGGGGCCAGGGGACCCAGGTCACCGTCTCCTCA

SEQ ID NO: 110 (AS82505 sdAb nucleic acid sequence)
CAGGTGCAGCTGGCGGAGTCTGGGGGAGGCTTGGTGCAGCCTGGGGGGTCTCTGAGACTCTCCTGTGT
AGCCTCTGGATTCACCTTCAGTAGCTATGACATGAGCTGGGTCCGCCAGGCTCCAGGGAAGGGAGTCG
AGTGGGTCTCAACTATTAATAGTGGTGGTGGTAGTACATACTATGCAGAGTCCGCGAAGGGCCGATTT
ACCATCTCCAGAGACAACGCCAAGAACACGCTGTATCTGCAATTGAACAGCCTGAAAACTGAGGACA
CGGCCATGTATTACTGTGTAAAAGGGTTTCCGGACGACGATGGACCGGGGGAGTTAAGTAGAGAGTAT
AATTACTGGGGCCAGGGGACCCAGGTCACCGTCTCCTCA

SEQ ID NO: 111 (AS82544 sdAb nucleic acid sequence)
GAGGTGCAGCTGGTGGAGTCTGGGGGAGCCTTGGTGCAGCCTGGGGGGTCTCTGAGACTCTCCTGTAC
AGCCTCTGGATTTTTATTCCGTGTGTACGACATGAACTGGGTCCGCCAGGCTCCAGGGAAGGGCGTCG
AGTGGATTGTAGGTATCACAAATAATGGTTATACCACAGCCTATGCAGACTCCGTGAAGGGCCGATTC
ACCATCTCCAGAGACAACACCGAAAACACCCTGTTTCTGCAAATGAACAGCCTGAAACCTGAGGACAC

GGCCATGTATTACTGTCAGACAGATAACGGTCGTGTGCGGGGCCAGGGGACCCAGGTCACCGTCTCCT
CA

SEQ ID NO: 112 (AS82658 sdAb nucleic acid sequence)
CAGGTTCAGCTGGTGGAGTCTGGGGGAGGCTCGGTGCAGGCTGGAGGGGCTCTGAGCCTCTCCTGCGC
AGCCTCTGGATACACCGTCAGAATCGACTACATGGGCTGGTACCGCCAGACTCCAGGGAAGGGCCGC
GAGCCGGTCGCAACTATTGCCTCTAATGGTGGAACAGCCTATGCCGACTCCGTGGAGGGCCGATTTAC
CATCTCCCAAGACAACGCCAAGAACTCGGTGTATCTGCAAATGAATACCCTGAAACCTGGGGACACTG
CCATGTACTACTGTGCGGCGGGTACCTGGCCTACCTTGACTTACTTCGGCCAGGGGACCCAGGTCACC
GTCTCCTCA

SEQ ID NO: 113 (AS82718 sdAb nucleic acid sequence)
CGGTGCAGCTGGTGGAATCTGGGGGAGGCTCGGTGCAGGCTGGAGGGTCTCTGAGACTCTCCTGTGTA
GTCTCTGCAGCCACCAACTGTAGATACATTGCCTGGTACCGCCAGGCTCCAGGGAAGGCCCGCGAGTT
CGTCTCAACTCTTGGTAGTGATGGTAACACAAACTACGCAGACTCCGTGAAGGGCCGATTCACTATCT
CCCAAGGTAATATCAAGAACATGGCGTATCTGGAGATGAACAGCCTGAAACCTGAGGACACGGGCAT
GTACTACTGCGGCACAAGGTGTCAAATTGGGGATGACTGGCGATCGAGCGACTGGGCCCAGGGGTGA
AACCTGACGACACGGCCACGTATTACTGTGCGGCAGACAGAAGTCCGTTTGGCTCATGCTCAAGCGAT
TGGTCGCGCTCAAGCGATTGGTCGCGAATGGCGGAGAAGTTTGGTTATTGGGGCCAGGGGACCCAGGT
CACCGTCTCCTCA

SEQ ID NO: 114 (AS83180 sdAb nucleic acid sequence)
CAGGTGCAGCTGGTGGAATCTGGGGGAGGCTCGGTGCAGGCTGGAGGGTCTCTGAGACTCTCCTGTGT
AGTCTCTGCAGCCACCAACTGTAGATACATTGCCTGGTACCGCCAGGCTCCAGGGAAGGCCCGCGAGT
TCGTCTCAACTCTTGGTAGTGATGGTAACACAAACTACGCAGACTCCGTGAAGGGCCGATTCACTATC
TCCCAAGGTAATATCAAGAACATGGCGTATCTGGAGATGAACAGCCTGAAACCTGAGGACACGGGCA
TGTACTACTGCGGCACAAGGTGTCAAATTGGGGATGACTGGCGATCGAGCGACTGGGCCCAGGGGAC
CCAGGTCACCGTCTCCTCA

SEQ ID NO: 115 (AS83183 sdAb nucleic acid sequence)
CAGGTGCACCTGGTGGAGTCTGGGGGAGGCTCGGTGCAGTCTGGAGGGTCTCTGAGACTCTCCTGTGC
AGCCTCTGGATACGCCTACCGTAGCTACTGTATGGGCTGGTTCCGCCAGGCTCCAGGGAAGGTCCTCG
AGGGGGTCGCAGCTATTGAGAGTGATGGTACTACAACCTACGCAGACTCCGTGATGGGCCGATTCACC
ATCTCCCAAGACAACGCCAAGAATGCGCTCTATCTGCAAATGAACAGCCTGAAACCTGAGGACACGG
CCATGTATCACTGTGCGGCTGTCAAAGGGTCGTGCGATTCAGCGTCTTCCGACACCCCTAGTTACTGGG
GCCAGGGGACCCAGGTCACCGTCTCCTCA

SEQ ID NO: 116 (AS83309 sdAb nucleic acid sequence)
GAGGTGCAACTGGTGGAGTCTGGGGGAGACTTGGTGCGGCCTGGGGGGTCTCTGAGACTCTCCTGTGC
AGCCTCTGGATTCACCTTCAGTATTTATGACATGAACTGGGTCCGCCAGGCTCCAGGGAAGGGACTCG
AGTGGGTCGCAGGTATTAGTGGTAATGGTTACAGTACAAGCTATGCAGAGTCCGTGAAGGGCCGATTC
ACCATCTCCAAAGACAACGCCAAGAACACAGTGTATCTACAATTGAGCAGCCTGAAATTTGAAGACAC
GGCCATGTATTACTGTGTAAGAGGTGGGGAGAAGTGGGACGAAAATGACCTGCGACGGAAGGGCCAG
GGGACCCAGGTCACCGTCTCCTCA

SEQ ID NO: 117 (AS83431 sdAb nucleic acid sequence)
CAGGTGAGGTTAGTGGAGTCTGGGGGAGGCTCGGTGCAGTCTGGAGGGTCTCTGAGACTCTCCTGTGC
AGCCTCTGGATATGCCCGCAGTAGTACTTGTTTGGGATGGTTCCGCCAGGCTCCAGGGAAGGAGGTCG
AGGGGGTCGCAATTATTGGTAGGGATGGCAGTACGGGGTATGCAGACTCCGTGAAGGGCCGATTCAC
CATCTCCCAAGACAACGCCAAGAACACGCTGTATCTACATATGGACAGCCTGAAACCTGAGGACACG
GCTATGTATTACTGTGCGGCAGTTGAGGGCGGTTGTGAGGTGTCAGAAGGTACGGGGGAACAGCAGCT
TGCTTACTGGGGCCAGGGGACCCAGGTCACCGTCTCCTCA

SEQ ID NO: 118(AS83478 sdAb nucleic acid sequence)

CAGGTGCACCTGATGGAGTCTGGGGGAGGCTTGGTGCAGCCTGGGGAGTCTCTGAGACTCTCCTGTGC
CGCCTCTGGATTCATATTCGCTAACTACGAGATGAGCTGGGTCCGCCAGGCTCCAGGGAAGGTGCTCG
AGTGGGTCTCAGGAATTAATAGCAGAGGTAATGCGACATACTATGCAGACTCCGTGAAGGGCCGATTC
ACCATCTCCAGAGACAACGCCGAGCACACGCTGTACCTCCAAATGAACAGCCTGAAACCTGAGGACA
CGGCCATGTATCACTGTGTGGTAGGGGGTATGACCACTGATCAGGGCTCGCCAGATTTCTACTGGGGC
CAGGGGACCCAGGTCACCGTCTCCTCA

SEQ ID NO: 119 (AS83791 sdAb nucleic acid sequence)
CAGGTGAAGTTGGTGGAGTCTGGGGGAGGCTTGGTGCAGCCTGGGGGGTCTCTGAGACTCTCCTGTGT
AGCCTCTGGATTCGCATTCAGTAGTGCCGACATGAGCTGGGTCCGCCAGGCTCCAGGGAAGGGGGTCG
AAGCGGTCTCAGTTATTAATCGTGATGGTGCGAGCACATACTATGCGGACTCCGTGAAGGGCCGATTC
ACCATCTCCAGAGACAACGCCAAGAGCACGCTGTATCTGCAAATGAACAGCCTGAAACCTGAGGACA
CGGCCATGTATCACTGTGCGGTAGTCCCGGAAAACGAATATGAAAGTGGATCGTATAACTACTGGGGC
CAGGGGACCCAGGTCACCGTCTCCTCA

SEQ ID NO: 174 (AS83010 sdAb nucleic acid sequence)
CAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTGCAGCCTGGGGGGTCTCTGAGACTCTCCTGTGT
AGCCTCTGGATTCTTCTTCAGTGCGTATGACATGAACTGGTTCCGCCAGGCTCCAGGGAAGGGACTCG
AGTGGGTCTCAGGTATTACTGGGAATGGTTATACGACCGCCTACGCAGACTCCGTGAAGGGCCGATTC
ACCATCTCCAGAGACAACGCCAAGAACACGCTGTATCTGCAATTGAACAGCCTGAAAAGTGAGGACA
CGGCCATGTATTACTGTACAGAGGGAGATAATAGGGGCCAGGGGACCCAGGTCACCGTCTCCTCA

SEQ ID NO: 175 (AS83457 sdAb nucleic acid sequence)
CAGGTTCAGCTGGTGGAGTCTGGGGGAGGCTTGGTGCAGCCTGGGGGGTCTCTGAGACTCTCCTGTGC
AGCCTCTGGATTCTTTTTCAGTATTTATGACATGAACTGGTTCCGCCAGGCTCCAGGGAAGGGACTCGA
GTGGGTCTCAGGTATTACTGGGAATGGTTATACGACCGCCTACGCAGACTCCGTGAAGGGCCGATTCA
CCATCTCCAGAGACAACGCCAAGAACACGCTGTATCTGCAATTGAACAGCCTGAAAAGTGAGGACAC
GGCCATGTATTACTGTGCACAGGGATCTAATAGGGGCCGGGGGACCCAGGTCACCGTCTCCTCA

SEQ ID NO: 176 (AS83591 sdAb nucleic acid sequence)
CAGGTTCAGCTGGTGGAGTCTGGGGGAGGCTTGGTGCAGCCTGGGGGGTCTCTGAGACTCTCCTGTGC
AGCCTCTGGATTTTTATTCAGTATTTACGACATGAATTGGGTCCGCCAGGCTCCAGGGAAGGGCGTCG
AGTGGATCGCAGGTATTACAAATAATGAGCATACCACAGCCTATGCAGACTCCGTGAAGGGCCGATTC
ACCATCTCCAGAGACAACACCAAAAACACCCTGTTTCTGCAAATGAACAGCCTGAAACCTGAGGACAC
GGCCATGTATTACTGTCAGAGAGATGACGGACAAGTGCGGGGCCAGGGGACCCAGGTCACCGTCTCCT
CA

[0150] C-type lectin-like molecule-1 (CLL1), also known as CLEC12A, C-type lectin domain family 12 member A, DCAL-2, MICL, and CD371, is a type II transmembrane glycoprotein that functions as an inhibitory receptor. The expression of CLL1 is restricted in myeloid lineage cells, as well as in the majority of AML blasts. In particular, CLL1 is selectively present on leukemic stem cells in acute myeloid leukemia (AML), but absent in normal hematopoietic stem cells. CLL1 can be a suitable tumor antigen target for immunotherapeutic agents against AML. *See*, e.g., Wang J. *et al.* (2018) *J. Hematol. Oncol.,* 11:7; Zhao X. *et al.*, (2010), *Haematologica*, 95(1): 71-78; and Lu H. *et al.* (2014) *Angew Chem. Int. Ed. Engl.* 53(37): 9841-9845.

[0151] In some embodiments, the anti-CLL1 sdAb specifically binds to human CLL1. In some embodiments, the anti-CLL1 sdAb specifically binds to cynomolgus monkey CLL1. In some embodiments, the anti-CLL1 sdAb specifically binds to the extracellular domain of CLL1. In some

embodiments, the anti-CLL1 sdAb specifically binds to the amino acid sequence of SEQ ID NO: 1 or 2. In some embodiments, the anti-CLL1 sdAb specifically recognizes an epitope within human CLL1. In some embodiments, the anti-CLL1 sdAb cross-reacts with CLL1 from species other than human. In some embodiments, the anti-CLL1 sdAb is completely specific for human CLL1 and does not exhibit species or other types of non-human cross-reactivity.

[0152] In some embodiments, the anti-CLL1 sdAb cross-reacts with at least one allelic variant of the CLL1 protein (or fragments thereof). In some embodiments, the allelic variant has up to about 30 (such as about any of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, or 30) amino acid substitutions (such as a conservative substitution) when compared to the naturally occurring CLL1 (or fragments thereof). In some embodiments, the anti-CLL1 sdAb does not cross-react with any allelic variant of the CLL1 protein (or fragments thereof).

[0153] In some embodiments, there is provided an anti-CLL1 sdAb comprising one, two, or all three CDRs of the amino acid sequence of SEQ ID NO: 94. In some embodiments, there is provided an anti-CLL1 sdAb comprising one, two, or all three CDRs of the amino acid sequence of SEQ ID NO: 95. In some embodiments, there is provided an anti-CLL1 sdAb comprising one, two, or all three CDRs of the amino acid sequence of SEQ ID NO: 96. In some embodiments, there is provided an anti-CLL1 sdAb comprising one, two, or all three CDRs of the amino acid sequence of SEQ ID NO: 97. In some embodiments, there is provided an anti-CLL1 sdAb comprising one, two, or all three CDRs of the amino acid sequence of SEQ ID NO: 98. In some embodiments, there is provided an anti-CLL1 sdAb comprising one, two, or all three CDRs of the amino acid sequence of SEQ ID NO: 99. In some embodiments, there is provided an anti-CLL1 sdAb comprising one, two, or all three CDRs of the amino acid sequence of SEQ ID NO: 100. In some embodiments, there is provided an anti-CLL1 sdAb comprising one, two, or all three CDRs of the amino acid sequence of SEQ ID NO: 101. In some embodiments, there is provided an anti-CLL1 sdAb comprising one, two, or all three CDRs of the amino acid sequence of SEQ ID NO: 102. In some embodiments, there is provided an anti-CLL1 sdAb comprising one, two, or all three CDRs of the amino acid sequence of SEQ ID NO: 103. In some embodiments, there is provided an anti-CLL1 sdAb comprising one, two, or all three CDRs of the amino acid sequence of SEQ ID NO: 104. In some embodiments, there is provided an anti-CLL1 sdAb comprising one, two, or all three CDRs of the amino acid sequence of SEQ ID NO: 105. In some embodiments, there is provided an anti-CLL1 sdAb comprising one, two, or all three CDRs of the amino acid sequence of SEQ ID NO: 106. In some embodiments, there is provided an anti-CLL1 sdAb comprising one, two, or all three CDRs of the amino acid sequence of SEQ ID NO: 171. In some embodiments, there is provided an anti-CLL1 sdAb comprising one, two, or all three CDRs of the amino

acid sequence of SEQ ID NO: 172. In some embodiments, there is provided an anti-CLL1 sdAb comprising one, two, or all three CDRs of the amino acid sequence of SEQ ID NO: 173.

[0154] In some embodiments, there is provided an anti-CLL1 sdAb comprising at least one, at least two, or all three CDRs selected from: (a) a CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 4, 11, 18, 25, 32, 39, 46, 53, 60, 67, 74, 81, 88, 151, 158 and 165; (b) a CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 6, 13, 20, 27, 34, 41, 48, 55, 62, 69, 76, 83, 90, 153, 160, and 167; and (c) a CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 8, 15, 22, 29, 36, 43, 50, 57, 64, 71, 78, 85, 92, 155, 162, and 169.

[0155] In some embodiments, there is provided an anti-CLL1 sdAb comprising: (a) a CDR1 having at least about any one of 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of any one of SEQ ID NOs: 4, 11, 18, 25, 32, 39, 46, 53, 60, 67, 74, 81, 88, 151, 158 and 165; (b) a CDR2 having at least about any one of 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of any one of SEQ ID NOs: 6, 13, 20, 27, 34, 41, 48, 55, 62, 69, 76, 83, 90, 153, 160, and 167; and (c) a CDR3 having at least about any one of 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of any one of SEQ ID NOs: 8, 15, 22, 29, 36, 43, 50, 57, 64, 71, 78, 85, 92, 155, 162, and 169. In some embodiments, a CDR having at least about any one of 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity contains substitutions (*e.g.*, conservative substitutions), insertions, or deletions relative to the reference sequence, but the anti-CLL1 sdAb comprising that sequence retains the ability to bind to CLL1. In some embodiments, there is provided an anti-CLL1 sdAb comprising: (a) a CDR1 having about any one of 1, 2, or 3 amino acid substitutions (*e.g.*, conservative substitutions), insertions, or deletions to the amino acid sequence of any one of SEQ ID NOs: 4, 11, 18, 25, 32, 39, 46, 53, 60, 67, 74, 81, 88, 151, 158 and 165; (b) a CDR2 having about any one of 1, 2, or 3 amino acid substitutions (*e.g.*, conservative substitutions), insertions, or deletions to the amino acid sequence of any one of SEQ ID NOs: 6, 13, 20, 27, 34, 41, 48, 55, 62, 69, 76, 83, 90, 153, 160, and 167; and (c) a CDR3 having about any one of 1, 2, or 3 amino acid substitutions (*e.g.*, conservative substitutions), insertions, or deletions to the amino acid sequence of any one of SEQ ID NOs: 8, 15, 22, 29, 36, 43, 50, 57, 64, 71, 78, 85, 92, 155, 162, and 169.

[0156] In some embodiments, there is provided an anti-CLL1 sdAb comprising: (a) a CDR1 comprising the amino acid sequence of SEQ ID NO: 4, or a variant thereof comprising up to about 3 (*e.g.*, 1, 2, or 3) amino acid substitutions in the CDR1; (b) a CDR2 comprising the amino acid sequence of SEQ ID NO: 6, or a variant thereof comprising up to about 3 (*e.g.*, 1, 2, or 3) amino acid substitutions in the CDR2; and (c) a CDR3 comprising the amino acid sequence of SEQ ID NO: 8, or a variant thereof

comprising up to about 3 (*e.g.*, 1, 2, or 3) amino acid substitutions in the CDR3. In some embodiments, there is provided an anti-CLL1 sdAb comprising: (a) a CDR1 comprising the amino acid sequence of SEQ ID NO: 4; (b) a CDR2 comprising the amino acid sequence of SEQ ID NO: 6; and (c) a CDR3 comprising the amino acid sequence of SEQ ID NO: 8, or a variant thereof comprising up to about 5 (*e.g.*, 1, 2, 3, 4, or 5) amino acid substitutions in the CDRs. In some embodiments, there is provided an anti-CLL1 sdAb comprising: (a) a CDR1 comprising the amino acid sequence of SEQ ID NO: 4; (b) a CDR2 comprising the amino acid sequence of SEQ ID NO: 6; and (c) a CDR3 comprising the amino acid sequence of SEQ ID NO: 8. In some embodiments, there is provided a polypeptide comprising the amino acid sequences of SEQ ID NOs: 4, 6 and 8.

[0157] In some embodiments, there is provided an anti-CLL1 sdAb comprising: (a) a CDR1 comprising the amino acid sequence of SEQ ID NO: 11, or a variant thereof comprising up to about 3 (*e.g.*, 1, 2, or 3) amino acid substitutions in the CDR1; (b) a CDR2 comprising the amino acid sequence of SEQ ID NO: 13, or a variant thereof comprising up to about 3 (*e.g.*, 1, 2, or 3) amino acid substitutions in the CDR2; and (c) a CDR3 comprising the amino acid sequence of SEQ ID NO: 15, or a variant thereof comprising up to about 3 (*e.g.*, 1, 2, or 3) amino acid substitutions in the CDR3. In some embodiments, there is provided an anti-CLL1 sdAb comprising: (a) a CDR1 comprising the amino acid sequence of SEQ ID NO: 11; (b) a CDR2 comprising the amino acid sequence of SEQ ID NO: 13; and (c) a CDR3 comprising the amino acid sequence of SEQ ID NO: 15, or a variant thereof comprising up to about 5 (*e.g.*, 1, 2, 3, 4, or 5) amino acid substitutions in the CDRs. In some embodiments, there is provided an anti-CLL1 sdAb comprising: (a) a CDR1 comprising the amino acid sequence of SEQ ID NO: 11; (b) a CDR2 comprising the amino acid sequence of SEQ ID NO: 13; and (c) a CDR3 comprising the amino acid sequence of SEQ ID NO: 15. In some embodiments, there is provided a polypeptide comprising the amino acid sequences of SEQ ID NOs: 11, 13 and 15.

[0158] In some embodiments, there is provided an anti-CLL1 sdAb comprising: (a) a CDR1 comprising the amino acid sequence of SEQ ID NO: 18, or a variant thereof comprising up to about 3 (*e.g.*, 1, 2, or 3) amino acid substitutions in the CDR1; (b) a CDR2 comprising the amino acid sequence of SEQ ID NO: 20, or a variant thereof comprising up to about 3 (*e.g.*, 1, 2, or 3) amino acid substitutions in the CDR2; and (c) a CDR3 comprising the amino acid sequence of SEQ ID NO: 22, or a variant thereof comprising up to about 3 (*e.g.*, 1, 2, or 3) amino acid substitutions in the CDR3. In some embodiments, there is provided an anti-CLL1 sdAb comprising: (a) a CDR1 comprising the amino acid sequence of SEQ ID NO: 18; (b) a CDR2 comprising the amino acid sequence of SEQ ID NO: 20; and (c) a CDR3 comprising the amino acid sequence of SEQ ID NO: 22, or a variant thereof comprising up to about 5 (*e.g.*, 1, 2, 3, 4, or 5) amino acid substitutions in the CDRs. In some embodiments, there is provided an anti-CLL1 sdAb comprising: (a) a CDR1 comprising the amino acid sequence of SEQ ID

NO: 18; (b) a CDR2 comprising the amino acid sequence of SEQ ID NO: 20; and (c) a CDR3 comprising the amino acid sequence of SEQ ID NO: 22. In some embodiments, there is provided a polypeptide comprising the amino acid sequences of SEQ ID NOs: 18, 20 and 22.

[0159] In some embodiments, there is provided an anti-CLL1 sdAb comprising: (a) a CDR1 comprising the amino acid sequence of SEQ ID NO: 25, or a variant thereof comprising up to about 3 (*e.g.*, 1, 2, or 3) amino acid substitutions in the CDR1; (b) a CDR2 comprising the amino acid sequence of SEQ ID NO: 27, or a variant thereof comprising up to about 3 (*e.g.*, 1, 2, or 3) amino acid substitutions in the CDR2; and (c) a CDR3 comprising the amino acid sequence of SEQ ID NO: 29, or a variant thereof comprising up to about 3 (*e.g.*, 1, 2, or 3) amino acid substitutions in the CDR3. In some embodiments, there is provided an anti-CLL1 sdAb comprising: (a) a CDR1 comprising the amino acid sequence of SEQ ID NO: 25; (b) a CDR2 comprising the amino acid sequence of SEQ ID NO: 27; and (c) a CDR3 comprising the amino acid sequence of SEQ ID NO: 29, or a variant thereof comprising up to about 5 (*e.g.*, 1, 2, 3, 4, or 5) amino acid substitutions in the CDRs. In some embodiments, there is provided an anti-CLL1 sdAb comprising: (a) a CDR1 comprising the amino acid sequence of SEQ ID NO: 25; (b) a CDR2 comprising the amino acid sequence of SEQ ID NO: 27; and (c) a CDR3 comprising the amino acid sequence of SEQ ID NO: 29. In some embodiments, there is provided a polypeptide comprising the amino acid sequences of SEQ ID NOs: 25, 27 and 29.

[0160] In some embodiments, there is provided an anti-CLL1 sdAb comprising: (a) a CDR1 comprising the amino acid sequence of SEQ ID NO: 32, or a variant thereof comprising up to about 3 (*e.g.*, 1, 2, or 3) amino acid substitutions in the CDR1; (b) a CDR2 comprising the amino acid sequence of SEQ ID NO: 34, or a variant thereof comprising up to about 3 (*e.g.*, 1, 2, or 3) amino acid substitutions in the CDR2; and (c) a CDR3 comprising the amino acid sequence of SEQ ID NO: 36, or a variant thereof comprising up to about 3 (*e.g.*, 1, 2, or 3) amino acid substitutions in the CDR3. In some embodiments, there is provided an anti-CLL1 sdAb comprising: (a) a CDR1 comprising the amino acid sequence of SEQ ID NO: 32; (b) a CDR2 comprising the amino acid sequence of SEQ ID NO: 34; and (c) a CDR3 comprising the amino acid sequence of SEQ ID NO: 36, or a variant thereof comprising up to about 5 (*e.g.*, 1, 2, 3, 4, or 5) amino acid substitutions in the CDRs. In some embodiments, there is provided an anti-CLL1 sdAb comprising: (a) a CDR1 comprising the amino acid sequence of SEQ ID NO: 32; (b) a CDR2 comprising the amino acid sequence of SEQ ID NO: 34; and (c) a CDR3 comprising the amino acid sequence of SEQ ID NO: 36. In some embodiments, there is provided a polypeptide comprising the amino acid sequences of SEQ ID NOs: 32, 34 and 36.

[0161] In some embodiments, there is provided an anti-CLL1 sdAb comprising: (a) a CDR1 comprising the amino acid sequence of SEQ ID NO: 39, or a variant thereof comprising up to about 3 (*e.g.*, 1, 2, or 3) amino acid substitutions in the CDR1; (b) a CDR2 comprising the amino acid sequence

of SEQ ID NO: 41, or a variant thereof comprising up to about 3 (*e.g.*, 1, 2, or 3) amino acid substitutions in the CDR2; and (c) a CDR3 comprising the amino acid sequence of SEQ ID NO: 43, or a variant thereof comprising up to about 3 (*e.g.*, 1, 2, or 3) amino acid substitutions in the CDR3. In some embodiments, there is provided an anti-CLL1 sdAb comprising: (a) a CDR1 comprising the amino acid sequence of SEQ ID NO: 39; (b) a CDR2 comprising the amino acid sequence of SEQ ID NO: 41; and (c) a CDR3 comprising the amino acid sequence of SEQ ID NO: 43, or a variant thereof comprising up to about 5 (*e.g.*, 1, 2, 3, 4, or 5) amino acid substitutions in the CDRs. In some embodiments, there is provided an anti-CLL1 sdAb comprising: (a) a CDR1 comprising the amino acid sequence of SEQ ID NO: 39; (b) a CDR2 comprising the amino acid sequence of SEQ ID NO: 41; and (c) a CDR3 comprising the amino acid sequence of SEQ ID NO: 43. In some embodiments, there is provided a polypeptide comprising the amino acid sequences of SEQ ID NOs: 39, 41 and 43.

[0162] In some embodiments, there is provided an anti-CLL1 sdAb comprising: (a) a CDR1 comprising the amino acid sequence of SEQ ID NO: 46, or a variant thereof comprising up to about 3 (*e.g.*, 1, 2, or 3) amino acid substitutions in the CDR1; (b) a CDR2 comprising the amino acid sequence of SEQ ID NO: 48, or a variant thereof comprising up to about 3 (*e.g.*, 1, 2, or 3) amino acid substitutions in the CDR2; and (c) a CDR3 comprising the amino acid sequence of SEQ ID NO: 50, or a variant thereof comprising up to about 3 (*e.g.*, 1, 2, or 3) amino acid substitutions in the CDR3. In some embodiments, there is provided an anti-CLL1 sdAb comprising: (a) a CDR1 comprising the amino acid sequence of SEQ ID NO: 46; (b) a CDR2 comprising the amino acid sequence of SEQ ID NO: 48; and (c) a CDR3 comprising the amino acid sequence of SEQ ID NO: 50, or a variant thereof comprising up to about 5 (*e.g.*, 1, 2, 3, 4, or 5) amino acid substitutions in the CDRs. In some embodiments, there is provided an anti-CLL1 sdAb comprising: (a) a CDR1 comprising the amino acid sequence of SEQ ID NO: 46; (b) a CDR2 comprising the amino acid sequence of SEQ ID NO: 48; and (c) a CDR3 comprising the amino acid sequence of SEQ ID NO: 50. In some embodiments, there is provided a polypeptide comprising the amino acid sequences of SEQ ID NOs: 46, 48 and 50.

[0163] In some embodiments, there is provided an anti-CLL1 sdAb comprising: (a) a CDR1 comprising the amino acid sequence of SEQ ID NO: 53, or a variant thereof comprising up to about 3 (*e.g.*, 1, 2, or 3) amino acid substitutions in the CDR1; (b) a CDR2 comprising the amino acid sequence of SEQ ID NO: 55, or a variant thereof comprising up to about 3 (*e.g.*, 1, 2, or 3) amino acid substitutions in the CDR2; and (c) a CDR3 comprising the amino acid sequence of SEQ ID NO: 57, or a variant thereof comprising up to about 3 (*e.g.*, 1, 2, or 3) amino acid substitutions in the CDR3. In some embodiments, there is provided an anti-CLL1 sdAb comprising: (a) a CDR1 comprising the amino acid sequence of SEQ ID NO: 53; (b) a CDR2 comprising the amino acid sequence of SEQ ID NO: 55; and (c) a CDR3 comprising the amino acid sequence of SEQ ID NO: 57, or a variant thereof comprising up to

about 5 (*e.g.*, 1, 2, 3, 4, or 5) amino acid substitutions in the CDRs. In some embodiments, there is provided an anti-CLL1 sdAb comprising: (a) a CDR1 comprising the amino acid sequence of SEQ ID NO: 53; (b) a CDR2 comprising the amino acid sequence of SEQ ID NO: 55; and (c) a CDR3 comprising the amino acid sequence of SEQ ID NO: 57. In some embodiments, there is provided a polypeptide comprising the amino acid sequences of SEQ ID NOs: 53, 55 and 57.

[0164] In some embodiments, there is provided an anti-CLL1 sdAb comprising: (a) a CDR1 comprising the amino acid sequence of SEQ ID NO: 60, or a variant thereof comprising up to about 3 (*e.g.*, 1, 2, or 3) amino acid substitutions in the CDR1; (b) a CDR2 comprising the amino acid sequence of SEQ ID NO: 62, or a variant thereof comprising up to about 3 (*e.g.*, 1, 2, or 3) amino acid substitutions in the CDR2; and (c) a CDR3 comprising the amino acid sequence of SEQ ID NO: 64, or a variant thereof comprising up to about 3 (*e.g.*, 1, 2, or 3) amino acid substitutions in the CDR3. In some embodiments, there is provided an anti-CLL1 sdAb comprising: (a) a CDR1 comprising the amino acid sequence of SEQ ID NO: 60; (b) a CDR2 comprising the amino acid sequence of SEQ ID NO: 62; and (c) a CDR3 comprising the amino acid sequence of SEQ ID NO: 64, or a variant thereof comprising up to about 5 (*e.g.*, 1, 2, 3, 4, or 5) amino acid substitutions in the CDRs. In some embodiments, there is provided an anti-CLL1 sdAb comprising: (a) a CDR1 comprising the amino acid sequence of SEQ ID NO: 60; (b) a CDR2 comprising the amino acid sequence of SEQ ID NO: 62; and (c) a CDR3 comprising the amino acid sequence of SEQ ID NO: 64. In some embodiments, there is provided a polypeptide comprising the amino acid sequences of SEQ ID NOs: 60, 62 and 64.

[0165] In some embodiments, there is provided an anti-CLL1 sdAb comprising: (a) a CDR1 comprising the amino acid sequence of SEQ ID NO: 67, or a variant thereof comprising up to about 3 (*e.g.*, 1, 2, or 3) amino acid substitutions in the CDR1; (b) a CDR2 comprising the amino acid sequence of SEQ ID NO: 69, or a variant thereof comprising up to about 3 (*e.g.*, 1, 2, or 3) amino acid substitutions in the CDR2; and (c) a CDR3 comprising the amino acid sequence of SEQ ID NO: 71, or a variant thereof comprising up to about 3 (*e.g.*, 1, 2, or 3) amino acid substitutions in the CDR3. In some embodiments, there is provided an anti-CLL1 sdAb comprising: (a) a CDR1 comprising the amino acid sequence of SEQ ID NO: 67; (b) a CDR2 comprising the amino acid sequence of SEQ ID NO: 69; and (c) a CDR3 comprising the amino acid sequence of SEQ ID NO: 71, or a variant thereof comprising up to about 5 (*e.g.*, 1, 2, 3, 4, or 5) amino acid substitutions in the CDRs. In some embodiments, there is provided an anti-CLL1 sdAb comprising: (a) a CDR1 comprising the amino acid sequence of SEQ ID NO: 67; (b) a CDR2 comprising the amino acid sequence of SEQ ID NO: 69; and (c) a CDR3 comprising the amino acid sequence of SEQ ID NO: 71. In some embodiments, there is provided a polypeptide comprising the amino acid sequences of SEQ ID NOs: 67, 69 and 71.

[0166] In some embodiments, there is provided an anti-CLL1 sdAb comprising: (a) a CDR1 comprising the amino acid sequence of SEQ ID NO: 74, or a variant thereof comprising up to about 3 (*e.g.*, 1, 2, or 3) amino acid substitutions in the CDR1; (b) a CDR2 comprising the amino acid sequence of SEQ ID NO: 76, or a variant thereof comprising up to about 3 (*e.g.*, 1, 2, or 3) amino acid substitutions in the CDR2; and (c) a CDR3 comprising the amino acid sequence of SEQ ID NO: 78, or a variant thereof comprising up to about 3 (*e.g.*, 1, 2, or 3) amino acid substitutions in the CDR3. In some embodiments, there is provided an anti-CLL1 sdAb comprising: (a) a CDR1 comprising the amino acid sequence of SEQ ID NO: 74; (b) a CDR2 comprising the amino acid sequence of SEQ ID NO: 76; and (c) a CDR3 comprising the amino acid sequence of SEQ ID NO: 78, or a variant thereof comprising up to about 5 (*e.g.*, 1, 2, 3, 4, or 5) amino acid substitutions in the CDRs. In some embodiments, there is provided an anti-CLL1 sdAb comprising: (a) a CDR1 comprising the amino acid sequence of SEQ ID NO: 74; (b) a CDR2 comprising the amino acid sequence of SEQ ID NO: 76; and (c) a CDR3 comprising the amino acid sequence of SEQ ID NO: 78. In some embodiments, there is provided a polypeptide comprising the amino acid sequences of SEQ ID NOs: 74, 76 and 78.

[0167] In some embodiments, there is provided an anti-CLL1 sdAb comprising: (a) a CDR1 comprising the amino acid sequence of SEQ ID NO: 81, or a variant thereof comprising up to about 3 (*e.g.*, 1, 2, or 3) amino acid substitutions in the CDR1; (b) a CDR2 comprising the amino acid sequence of SEQ ID NO: 83, or a variant thereof comprising up to about 3 (*e.g.*, 1, 2, or 3) amino acid substitutions in the CDR2; and (c) a CDR3 comprising the amino acid sequence of SEQ ID NO: 85, or a variant thereof comprising up to about 3 (*e.g.*, 1, 2, or 3) amino acid substitutions in the CDR3. In some embodiments, there is provided an anti-CLL1 sdAb comprising: (a) a CDR1 comprising the amino acid sequence of SEQ ID NO: 81; (b) a CDR2 comprising the amino acid sequence of SEQ ID NO: 83; and (c) a CDR3 comprising the amino acid sequence of SEQ ID NO: 85, or a variant thereof comprising up to about 5 (*e.g.*, 1, 2, 3, 4, or 5) amino acid substitutions in the CDRs. In some embodiments, there is provided an anti-CLL1 sdAb comprising: (a) a CDR1 comprising the amino acid sequence of SEQ ID NO: 81; (b) a CDR2 comprising the amino acid sequence of SEQ ID NO: 83; and (c) a CDR3 comprising the amino acid sequence of SEQ ID NO: 85. In some embodiments, there is provided a polypeptide comprising the amino acid sequences of SEQ ID NOs: 81, 83 and 85.

[0168] In some embodiments, there is provided an anti-CLL1 sdAb comprising: (a) a CDR1 comprising the amino acid sequence of SEQ ID NO: 88, or a variant thereof comprising up to about 3 (*e.g.*, 1, 2, or 3) amino acid substitutions in the CDR1; (b) a CDR2 comprising the amino acid sequence of SEQ ID NO: 90, or a variant thereof comprising up to about 3 (*e.g.*, 1, 2, or 3) amino acid substitutions in the CDR2; and (c) a CDR3 comprising the amino acid sequence of SEQ ID NO: 92, or a variant thereof comprising up to about 3 (*e.g.*, 1, 2, or 3) amino acid substitutions in the CDR3. In some

embodiments, there is provided an anti-CLL1 sdAb comprising: (a) a CDR1 comprising the amino acid sequence of SEQ ID NO: 88; (b) a CDR2 comprising the amino acid sequence of SEQ ID NO: 90; and (c) a CDR3 comprising the amino acid sequence of SEQ ID NO: 92, or a variant thereof comprising up to about 5 (*e.g.*, 1, 2, 3, 4, or 5) amino acid substitutions in the CDRs. In some embodiments, there is provided an anti-CLL1 sdAb comprising: (a) a CDR1 comprising the amino acid sequence of SEQ ID NO: 88; (b) a CDR2 comprising the amino acid sequence of SEQ ID NO: 90; and (c) a CDR3 comprising the amino acid sequence of SEQ ID NO: 92. In some embodiments, there is provided a polypeptide comprising the amino acid sequences of SEQ ID NOs: 88, 90 and 92.

[0169] In some embodiments, there is provided an anti-CLL1 sdAb comprising: (a) a CDR1 comprising the amino acid sequence of SEQ ID NO: 151, or a variant thereof comprising up to about 3 (*e.g.*, 1, 2, or 3) amino acid substitutions in the CDR1; (b) a CDR2 comprising the amino acid sequence of SEQ ID NO: 153, or a variant thereof comprising up to about 3 (*e.g.*, 1, 2, or 3) amino acid substitutions in the CDR2; and (c) a CDR3 comprising the amino acid sequence of SEQ ID NO: 155, or a variant thereof comprising up to about 3 (*e.g.*, 1, 2, or 3) amino acid substitutions in the CDR3. In some embodiments, there is provided an anti-CLL1 sdAb comprising: (a) a CDR1 comprising the amino acid sequence of SEQ ID NO: 151; (b) a CDR2 comprising the amino acid sequence of SEQ ID NO: 153; and (c) a CDR3 comprising the amino acid sequence of SEQ ID NO: 155, or a variant thereof comprising up to about 5 (*e.g.*, 1, 2, 3, 4, or 5) amino acid substitutions in the CDRs. In some embodiments, there is provided an anti-CLL1 sdAb comprising: (a) a CDR1 comprising the amino acid sequence of SEQ ID NO: 151; (b) a CDR2 comprising the amino acid sequence of SEQ ID NO: 153; and (c) a CDR3 comprising the amino acid sequence of SEQ ID NO: 155. In some embodiments, there is provided a polypeptide comprising the amino acid sequences of SEQ ID NOs: 151, 153 and 155.

[0170] In some embodiments, there is provided an anti-CLL1 sdAb comprising: (a) a CDR1 comprising the amino acid sequence of SEQ ID NO: 158, or a variant thereof comprising up to about 3 (*e.g.*, 1, 2, or 3) amino acid substitutions in the CDR1; (b) a CDR2 comprising the amino acid sequence of SEQ ID NO: 160, or a variant thereof comprising up to about 3 (*e.g.*, 1, 2, or 3) amino acid substitutions in the CDR2; and (c) a CDR3 comprising the amino acid sequence of SEQ ID NO: 162, or a variant thereof comprising up to about 3 (*e.g.*, 1, 2, or 3) amino acid substitutions in the CDR3. In some embodiments, there is provided an anti-CLL1 sdAb comprising: (a) a CDR1 comprising the amino acid sequence of SEQ ID NO: 158; (b) a CDR2 comprising the amino acid sequence of SEQ ID NO: 160; and (c) a CDR3 comprising the amino acid sequence of SEQ ID NO: 162, or a variant thereof comprising up to about 5 (*e.g.*, 1, 2, 3, 4, or 5) amino acid substitutions in the CDRs. In some embodiments, there is provided an anti-CLL1 sdAb comprising: (a) a CDR1 comprising the amino acid sequence of SEQ ID NO: 158; (b) a CDR2 comprising the amino acid sequence of SEQ ID NO: 160; and (c) a CDR3

comprising the amino acid sequence of SEQ ID NO: 162. In some embodiments, there is provided a polypeptide comprising the amino acid sequences of SEQ ID NOs: 158, 160 and 162.

[0171] In some embodiments, there is provided an anti-CLL1 sdAb comprising: (a) a CDR1 comprising the amino acid sequence of SEQ ID NO: 165, or a variant thereof comprising up to about 3 (*e.g.*, 1, 2, or 3) amino acid substitutions in the CDR1; (b) a CDR2 comprising the amino acid sequence of SEQ ID NO: 167, or a variant thereof comprising up to about 3 (*e.g.*, 1, 2, or 3) amino acid substitutions in the CDR2; and (c) a CDR3 comprising the amino acid sequence of SEQ ID NO: 169, or a variant thereof comprising up to about 3 (*e.g.*, 1, 2, or 3) amino acid substitutions in the CDR3. In some embodiments, there is provided an anti-CLL1 sdAb comprising: (a) a CDR1 comprising the amino acid sequence of SEQ ID NO: 165; (b) a CDR2 comprising the amino acid sequence of SEQ ID NO: 167; and (c) a CDR3 comprising the amino acid sequence of SEQ ID NO: 169, or a variant thereof comprising up to about 5 (*e.g.*, 1, 2, 3, 4, or 5) amino acid substitutions in the CDRs. In some embodiments, there is provided an anti-CLL1 sdAb comprising: (a) a CDR1 comprising the amino acid sequence of SEQ ID NO: 165; (b) a CDR2 comprising the amino acid sequence of SEQ ID NO: 167; and (c) a CDR3 comprising the amino acid sequence of SEQ ID NO: 169. In some embodiments, there is provided a polypeptide comprising the amino acid sequences of SEQ ID NOs: 165, 167 and 169.

[0172] In some embodiments, the anti-CLL1 sdAb, including any of the embodiments described above (*i.e.*, anti-CLL1 sdAb comprising specific CDR1, CDR2, and/or CDR3) comprises a V_HH domain having at least about any one of 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of any one of SEQ ID NOs: 94-106 and 171-173. In some embodiments, a V_HH sequence having at least about any one of 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity contains substitutions (*e.g.*, conservative substitutions), insertions, or deletions relative to the reference sequence, but the anti-CLL1 sdAb comprising that sequence retains the ability to bind to CLL1. In some embodiments, a total of 1 to 10 amino acids have been substituted, inserted and/or deleted in the amino acid sequence of any one of SEQ ID NOs: 94-106 and 171-173. In some embodiments, substitutions, insertions, or deletions occur in regions outside the CDRs (i.e., in the FRs). In some embodiments, the anti-CLL1 sdAb comprises the amino acid sequence of any one of SEQ ID NOs: 94-106 and 171-173, optionally including post-translational modifications of that sequence.

[0173] In some embodiments, there is provided an isolated anti-CLL1 sdAb comprising the amino acid sequence SEQ ID NO: 94. In some embodiments, there is provided a polypeptide comprising the amino acid sequence of SEQ ID NO: 94. In some embodiments, there is provided an isolated anti-CLL1 sdAb comprising the amino acid sequence SEQ ID NO: 95. In some embodiments, there is provided a polypeptide comprising the amino acid sequence of SEQ ID NO: 95. In some embodiments, there is

provided an isolated anti-CLL1 sdAb comprising the amino acid sequence SEQ ID NO: 96. In some embodiments, there is provided a polypeptide comprising the amino acid sequence of SEQ ID NO: 96. In some embodiments, there is provided an isolated anti-CLL1 sdAb comprising the amino acid sequence SEQ ID NO: 97. In some embodiments, there is provided a polypeptide comprising the amino acid sequence of SEQ ID NO: 97. In some embodiments, there is provided an isolated anti-CLL1 sdAb comprising the amino acid sequence SEQ ID NO: 98. In some embodiments, there is provided a polypeptide comprising the amino acid sequence of SEQ ID NO: 98. In some embodiments, there is provided an isolated anti-CLL1 sdAb comprising the amino acid sequence SEQ ID NO: 99. In some embodiments, there is provided a polypeptide comprising the amino acid sequence of SEQ ID NO: 99. In some embodiments, there is provided an isolated anti-CLL1 sdAb comprising the amino acid sequence SEQ ID NO: 100. In some embodiments, there is provided a polypeptide comprising the amino acid sequence of SEQ ID NO: 100. In some embodiments, there is provided an isolated anti-CLL1 sdAb comprising the amino acid sequence SEQ ID NO: 101. In some embodiments, there is provided a polypeptide comprising the amino acid sequence of SEQ ID NO: 101. In some embodiments, there is provided an isolated anti-CLL1 sdAb comprising the amino acid sequence SEQ ID NO: 102. In some embodiments, there is provided a polypeptide comprising the amino acid sequence of SEQ ID NO: 102. In some embodiments, there is provided an isolated anti-CLL1 sdAb comprising the amino acid sequence SEQ ID NO: 103. In some embodiments, there is provided a polypeptide comprising the amino acid sequence of SEQ ID NO: 103. In some embodiments, there is provided an isolated anti-CLL1 sdAb comprising the amino acid sequence SEQ ID NO: 104. In some embodiments, there is provided a polypeptide comprising the amino acid sequence of SEQ ID NO: 104. In some embodiments, there is provided an isolated anti-CLL1 sdAb comprising the amino acid sequence SEQ ID NO: 105. In some embodiments, there is provided a polypeptide comprising the amino acid sequence of SEQ ID NO: 105. In some embodiments, there is provided an isolated anti-CLL1 sdAb comprising the amino acid sequence SEQ ID NO: 106. In some embodiments, there is provided a polypeptide comprising the amino acid sequence of SEQ ID NO: 106. In some embodiments, there is provided a polypeptide comprising the amino acid sequence of SEQ ID NO: 171. In some embodiments, there is provided a polypeptide comprising the amino acid sequence of SEQ ID NO: 172. In some embodiments, there is provided a polypeptide comprising the amino acid sequence of SEQ ID NO: 173.

[0174] In some embodiments, functional epitopes can be mapped by combinatorial alanine scanning. In this process, a combinatorial alanine-scanning strategy can be used to identify amino acids in the CLL1 protein that are necessary for interaction with anti-CLL1 sdAbs. In some embodiments, the epitope is conformational and crystal structure of anti-CLL1 sdAb bound to CLL1 may be employed to identify the epitopes.

[0175] In some embodiments, the present application provides antibodies (*e.g.*, sdAbs) which compete with any one of the anti-CLL1 sdAbs described herein for binding to CLL1. In some embodiments, the present application provides antibodies (*e.g.*, sdAbs) which compete with any one of the anti-CLL1 sdAbs provided herein for binding to an epitope on the CLL1. In some embodiments, an anti-CLL1 antibody (*e.g.*, sdAb) is provided that binds to the same epitope as an anti-CLL1 sdAb comprising the amino acid sequence of any one of SEQ ID NOs: 94-106 and 171-173. In some embodiments, an anti-CLL1 antibody (*e.g.*, sdAbs) is provided that specifically binds to CLL1 competitively with an anti-CLL1 sdAb comprising the amino acid sequence of any one of SEQ ID NOs: 94-106 and 171-173.

[0176] In some embodiments, competition assays may be used to identify a monoclonal antibody that competes with an anti-CLL1 sdAb described herein for binding to CLL1. Competition assays can be used to determine whether two antibodies bind the same epitope by recognizing identical or sterically overlapping epitopes or one antibody competitively inhibits binding of another antibody to the antigen. In certain embodiments, such a competing antibody binds to the same epitope that is bound by an antibody described herein. Exemplary competition assays include, but are not limited to, routine assays such as those provided in Harlow and Lane (1988) Antibodies: A Laboratory Manual ch.14 (Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.). Detailed exemplary methods for mapping an epitope to which an antibody binds are provided in Morris (1996) "Epitope Mapping Protocols," in Methods in Molecular Biology vol. 66 (Humana Press, Totowa, N.J.). In some embodiments, two antibodies are said to bind to the same epitope if each blocks binding of the other by 50% or more. In some embodiments, the antibody that competes with an anti-CLL1 sdAb described herein is a camelid, chimeric, humanized or human antibody. In some embodiments, the present application provides an antibody that competes with a camelid, chimeric, humanized, or human anti-CLL1 sdAb as described herein.

B. Chimeric receptors and chimeric receptor systems

[0177] One aspect of the present application provides a chimeric receptor comprising an extracellular domain comprising one or more anti-CLL1 sdAbs (*e.g.*, V_HHs), a transmembrane domain, and an intracellular signaling domain. Also provided is a chimeric receptor system comprising: (a) a first chimeric receptor comprising an extracellular domain comprising an anti-CLL1 sdAb (*e.g.*, V_HH), a transmembrane domain, and an intracellular signaling domain; and (b) a second chimeric receptor comprising an extracellular domain comprising a binding moiety that specifically binds to a second antigen or epitope. Any one of the anti-CLL1 sdAbs described in Section A can be used in the chimeric receptors or chimeric receptor systems described herein. Exemplary structures of chimeric receptors and chimeric receptor systems are shown in FIGs. 8A-8E.

[0178] In some embodiments, there is provided a chimeric receptor targeting CLL1 (also referred herein as "anti-CLL1 chimeric receptor" or "anti-CLL1 CAR") comprising: (a) an extracellular domain comprising an anti-CLL1 sdAb (such as any one of the anti-CLL1 sdAbs described herein); (b) a transmembrane domain; and (c) an intracellular signaling domain. In some embodiments, the anti-CLL1 sdAb comprises any one of the following: (1) a CDR1 comprising the amino acid sequence of SEQ ID NO: 4, a CDR2 comprising the amino acid sequence of SEQ ID NO: 6, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 8, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; (2) a CDR1 comprising the amino acid sequence of SEQ ID NO: 11, a CDR2 comprising the amino acid sequence of SEQ ID NO: 13, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 15, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; (3) a CDR1 comprising the amino acid sequence of SEQ ID NO: 18, a CDR2 comprising the amino acid sequence of SEQ ID NO: 20, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 22, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; (4) a CDR1 comprising the amino acid sequence of SEQ ID NO: 25, a CDR2 comprising the amino acid sequence of SEQ ID NO: 27, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 29, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; (5) a CDR1 comprising the amino acid sequence of SEQ ID NO: 32, a CDR2 comprising the amino acid sequence of SEQ ID NO: 34, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 36, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; (6) a CDR1 comprising the amino acid sequence of SEQ ID NO: 39, a CDR2 comprising the amino acid sequence of SEQ ID NO: 41, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 43, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; (7) a CDR1 comprising the amino acid sequence of SEQ ID NO: 46, a CDR2 comprising the amino acid sequence of SEQ ID NO: 48, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 50, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; (8) a CDR1 comprising the amino acid sequence of SEQ ID NO: 53, a CDR2 comprising the amino acid sequence of SEQ ID NO: 55, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 57, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; (9) a CDR1 comprising the amino acid sequence of SEQ ID NO: 60, a CDR2 comprising the amino acid sequence of SEQ ID NO: 62, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 64, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; (10) a CDR1 comprising the amino acid sequence of SEQ ID NO: 67, a CDR2 comprising the amino acid sequence of SEQ ID NO: 69, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 71, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; (11) a CDR1 comprising the amino acid sequence of SEQ ID NO: 74, a CDR2 comprising the amino acid sequence of SEQ ID NO: 76, and a CDR3 comprising the

amino acid sequence of SEQ ID NO: 78, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; (12) a CDR1 comprising the amino acid sequence of SEQ ID NO: 81, a CDR2 comprising the amino acid sequence of SEQ ID NO: 83, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 85, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; (13) a CDR1 comprising the amino acid sequence of SEQ ID NO: 88, a CDR2 comprising the amino acid sequence of SEQ ID NO: 90, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 92, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; (14) a CDR1 comprising the amino acid sequence of SEQ ID NO: 151, a CDR2 comprising the amino acid sequence of SEQ ID NO: 153, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 155, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; (15) a CDR1 comprising the amino acid sequence of SEQ ID NO: 158, a CDR2 comprising the amino acid sequence of SEQ ID NO: 160, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 162, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; or (16) a CDR1 comprising the amino acid sequence of SEQ ID NO: 165, a CDR2 comprising the amino acid sequence of SEQ ID NO: 167, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 169, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs. In some embodiments, the anti-CLL1 sdAb moiety comprises a V_HH domain comprising the amino acid sequence of any one of SEQ ID NOs: 94-106 and 171-173, or a variant thereof comprising an amino acid sequence having at least about 95% sequence identity to the amino acid sequence of any one of SEQ ID NOs: 94-106 and 171-173. In some embodiments, the anti-CLL1 sdAb moiety is camelid, chimeric, human, or humanized. In some embodiments, the intracellular signaling domain comprises a primary intracellular signaling sequence of an immune effector cell (such as T cell). In some embodiments, the primary intracellular signaling sequence is derived from CD3ζ, FcRγ, FcRβ, CD3γ, CD3δ, CD3ε, CD5, CD22, CD79a, CD79b, or CD66d. In some embodiments, the primary intracellular signaling sequence is derived from CD3ζ (*i.e.*, "a CD3ζ intracellular signaling sequence"). In some embodiments, the intracellular signaling domain comprises an intracellular co-stimulatory sequence. In some embodiments, the intracellular co-stimulatory sequence is derived from a co-stimulatory molecule selected from the group consisting of CD27, CD28, 4-1BB, OX40, CD40, PD-1, LFA-1, ICOS, CD2, CD7, LIGHT, NKG2C, B7-H3, TNFRSF9, TNFRSF4, TNFRSF8, CD40LG, ITGB2, KLRC2, TNFRSF18, TNFRSF14, HAVCR1, LGALS9, DAP10, DAP12, CD83, ligands of CD83 and combinations thereof. In some embodiments, the intracellular co-stimulatory sequence is derived from CD28 or 4-1BB. In some embodiments, the intracellular signaling domain comprises both a primary intracellular signaling sequence (*e.g.*, a CD3ζ intracellular signaling sequence) and an intracellular co-stimulatory sequence. In some embodiments, the intracellular signaling domain comprises a primary intracellular signaling sequence but does not comprise an intracellular co-stimulatory

sequence. In some embodiments, the intracellular signaling domain comprises an intracellular co-stimulatory sequence but does not comprise a primary intracellular signaling sequence. In some embodiments, the anti-CLL1 chimeric receptor further comprises a hinge domain (*e.g.*, a CD8 hinge domain) located between the C-terminus of the extracellular domain and the N-terminus of the transmembrane domain. In some embodiments, the anti-CLL1 chimeric receptor further comprises a signal peptide (such as a CD8 signal peptide). In some embodiments, the anti-CLL1 chimeric receptor comprises a polypeptide comprising from the N-terminus to the C-terminus: a CD8 signal peptide, the anti-CLL1 sdAb moiety, a CD8 hinge domain, a CD8 transmembrane domain, and an intracellular signaling domain comprising an intracellular co-stimulatory sequence derived from 4-1BB or CD28 and a CD3ζ intracellular signaling sequence. In some embodiments, the anti-CLL1 chimeric receptor comprises a polypeptide comprising from the N-terminus to the C-terminus: a CD8 signal peptide, the anti-CLL1 sdAb moiety, a CD8 hinge domain, a CD8 transmembrane domain, and an intracellular signaling domain comprising a CD3ζ intracellular signaling sequence. In some embodiments, the anti-CLL1 chimeric receptor comprises a polypeptide comprising from the N-terminus to the C-terminus: a CD8 signal peptide, the anti-CLL1 sdAb moiety, a CD8 hinge domain, a CD8 transmembrane domain, and an intracellular signaling domain comprising an intracellular co-stimulatory sequence derived from 4-1BB or CD28.

[0179] In some embodiments, there is provided an anti-CLL1 chimeric receptor comprising a polypeptide having at least about any one of 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of any one of SEQ ID NOs: 120-132, 177-179, 181 and 229-230. In some embodiments, there is provided an anti-CLL1 chimeric receptor comprising a polypeptide comprising the amino acid sequence of any one of SEQ ID NOs: 120-132, 177-179, 181 and 229-230. Also provided is a polypeptide comprising the amino acid sequence of any one of SEQ ID NOs: 120-132, 177-179, 181 and 229-230.

[0180] Exemplary anti-CLL1 chimeric receptors are shown in Table 3 below.

Table 3. Exemplary anti-CLL1 Chimeric Receptors.

CAR SEQ ID NO.	SP	Extra-cellular. sdAb	Hinge	TM	Intracellular signaling	
					Co-stimulatory	Primary signaling
120	CD8	AS82472	CD8	CD8	4-1BB	CD3ζ
121	CD8	AS82480	CD8	CD8	4-1BB	CD3ζ
122	CD8	AS82494	CD8	CD8	4-1BB	CD3ζ
123	CD8	AS82505	CD8	CD8	4-1BB	CD3ζ
124	CD8	AS82544	CD8	CD8	4-1BB	CD3ζ
125	CD8	AS82658	CD8	CD8	4-1BB	CD3ζ
126	CD8	AS82718	CD8	CD8	4-1BB	CD3ζ
127	CD8	AS83180	CD8	CD8	4-1BB	CD3ζ

128	CD8	AS83183	CD8	CD8	4-1BB	CD3ζ
129	CD8	AS83309	CD8	CD8	4-1BB	CD3ζ
130	CD8	AS83431	CD8	CD8	4-1BB	CD3ζ
131	CD8	AS83478	CD8	CD8	4-1BB	CD3ζ
132	CD8	AS83791	CD8	CD8	4-1BB	CD3ζ
177	CD8	AS83010	CD8	CD8	4-1BB	CD3ζ
178	CD8	AS83457	CD8	CD8	4-1BB	CD3ζ
179	CD8	AS83591	CD8	CD8	4-1BB	CD3ζ
181	CD8	AS82658	CD28	CD28	CD28	CD3ζ
229	CD8	AS82472	CD28	CD28	CD28	CD3ζ
230	CD8	AS82494	CD28	CD28	CD28	CD3ζ

[0181] In some embodiments, there is provided a chimeric receptor targeting CLL1 comprising: (a) an extracellular domain comprising an anti-CLL1 sdAb comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 39, a CDR2 comprising the amino acid sequence of SEQ ID NO: 41, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 43, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; (b) a transmembrane domain; and (c) an intracellular signaling domain. In some embodiments, the anti-CLL1 sdAb moiety comprises a V_HH domain comprising the amino acid sequence of SEQ ID NO: 99, or a variant thereof comprising an amino acid sequence having at least about 95% sequence identity to the amino acid sequence of SEQ ID NO: 99. In some embodiments, the primary intracellular signaling sequence is derived from CD3ζ. In some embodiments, the intracellular signaling domain comprises an intracellular co-stimulatory sequence. In some embodiments, the anti-CLL1 chimeric receptor further comprises a hinge domain (*e.g.*, a CD8 hinge domain) located between the C-terminus of the extracellular domain and the N-terminus of the transmembrane domain. In some embodiments, the anti-CLL1 chimeric receptor further comprises a signal peptide (such as a CD8 signal peptide). In some embodiments, the anti-CLL1 chimeric receptor comprises a polypeptide comprising from the N-terminus to the C-terminus: a CD8 signal peptide, the anti-CLL1 sdAb moiety, a CD8 hinge domain, a CD8 transmembrane domain, and an intracellular signaling domain comprising an intracellular co-stimulatory sequence derived from 4-1BB and a CD3ζ intracellular signaling sequence. In some embodiments, the anti-CLL1 chimeric receptor comprises a polypeptide comprising from the N-terminus to the C-terminus: a CD8 signal peptide, the anti-CLL1 sdAb moiety, a CD28 hinge domain, a CD28 transmembrane domain, and an intracellular signaling domain comprising an intracellular co-stimulatory sequence derived from CD28 and a CD3ζ intracellular signaling sequence. In some embodiments, the anti-CLL1 chimeric receptor comprises a polypeptide comprising the amino acid sequence of SEQ ID NO: 125 or 181, or a variant thereof comprising an amino acid sequence having at least about 95% sequence identity to the amino acid sequence of SEQ ID NO: 125 or 181.

[0182] In some embodiments, there is provided a chimeric receptor targeting CLL1 comprising: (a) an extracellular domain comprising an anti-CLL1 sdAb comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 4, a CDR2 comprising the amino acid sequence of SEQ ID NO: 6, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 8, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; (b) a transmembrane domain; and (c) an intracellular signaling domain. In some embodiments, the anti-CLL1 sdAb moiety comprises a V_HH domain comprising the amino acid sequence of SEQ ID NO: 94, or a variant thereof comprising an amino acid sequence having at least about 95% sequence identity to the amino acid sequence of SEQ ID NO: 94. In some embodiments, the primary intracellular signaling sequence is derived from CD3ζ. In some embodiments, the intracellular signaling domain comprises an intracellular co-stimulatory sequence. In some embodiments, the anti-CLL1 chimeric receptor further comprises a hinge domain (*e.g.*, a CD8 hinge domain) located between the C-terminus of the extracellular domain and the N-terminus of the transmembrane domain. In some embodiments, the anti-CLL1 chimeric receptor further comprises a signal peptide (such as a CD8 signal peptide). In some embodiments, the anti-CLL1 chimeric receptor comprises a polypeptide comprising from the N-terminus to the C-terminus: a CD8 signal peptide, the anti-CLL1 sdAb moiety, a CD8 hinge domain, a CD8 transmembrane domain, and an intracellular signaling domain comprising an intracellular co-stimulatory sequence derived from 4-1BB and a CD3ζ intracellular signaling sequence. In some embodiments, the anti-CLL1 chimeric receptor comprises a polypeptide comprising from the N-terminus to the C-terminus: a CD8 signal peptide, the anti-CLL1 sdAb moiety, a CD28 hinge domain, a CD28 transmembrane domain, and an intracellular signaling domain comprising an intracellular co-stimulatory sequence derived from CD28 and a CD3ζ intracellular signaling sequence. In some embodiments, the anti-CLL1 chimeric receptor comprises a polypeptide comprising the amino acid sequence of SEQ ID NO: 120 or 229, or a variant thereof comprising an amino acid sequence having at least about 95% sequence identity to the amino acid sequence of SEQ ID NO: 120 or 229.

[0183] In some embodiments, there is provided a chimeric receptor targeting CLL1 comprising: (a) an extracellular domain comprising an anti-CLL1 sdAb comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 18, a CDR2 comprising the amino acid sequence of SEQ ID NO: 20, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 22, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; (b) a transmembrane domain; and (c) an intracellular signaling domain. In some embodiments, the anti-CLL1 sdAb moiety comprises a V_HH domain comprising the amino acid sequence of SEQ ID NO: 96, or a variant thereof comprising an amino acid sequence having at least about 95% sequence identity to the amino acid sequence of SEQ ID NO: 96. In some embodiments, the primary intracellular signaling sequence is derived from CD3ζ. In some

embodiments, the intracellular signaling domain comprises an intracellular co-stimulatory sequence. In some embodiments, the anti-CLL1 chimeric receptor further comprises a hinge domain (*e.g.*, a CD8 hinge domain) located between the C-terminus of the extracellular domain and the N-terminus of the transmembrane domain. In some embodiments, the anti-CLL1 chimeric receptor further comprises a signal peptide (such as a CD8 signal peptide). In some embodiments, the anti-CLL1 chimeric receptor comprises a polypeptide comprising from the N-terminus to the C-terminus: a CD8 signal peptide, the anti-CLL1 sdAb moiety, a CD8 hinge domain, a CD8 transmembrane domain, and an intracellular signaling domain comprising an intracellular co-stimulatory sequence derived from 4-1BB and a CD3ζ intracellular signaling sequence. In some embodiments, the anti-CLL1 chimeric receptor comprises a polypeptide comprising from the N-terminus to the C-terminus: a CD8 signal peptide, the anti-CLL1 sdAb moiety, a CD28 hinge domain, a CD28 transmembrane domain, and an intracellular signaling domain comprising an intracellular co-stimulatory sequence derived from CD28 and a CD3ζ intracellular signaling sequence. In some embodiments, the anti-CLL1 chimeric receptor comprises a polypeptide comprising the amino acid sequence of SEQ ID NO: 122 or 230, or a variant thereof comprising an amino acid sequence having at least about 95% sequence identity to the amino acid sequence of SEQ ID NO: 122 or 230.

Multivalent chimeric receptors

[0184] The present application also provides multivalent anti-CLL1 chimeric receptors that have two or more (such as about any one of 2, 3, 4, 5, 6, or more) binding moieties that specifically bind to CLL1. In some embodiments, one or more of the binding moieties are antigen binding fragments. In some embodiments, one or more of the binding moieties comprise sdAbs. In some embodiments, one or more of the binding moieties are derived from camelid antibodies. In some embodiments, one or more of the binding moieties are derived from a four-chain antibody. In some embodiments, one or more of the binding moieties are scFvs. In some embodiments, one or more of the binding moieties are derived from human antibodies. In some embodiments, one or more of the binding moieties are extracellular domains of receptors, polypeptide ligands or other non-antibody polypeptides that specifically bind to CLL1. In some embodiments, the multivalent chimeric receptor is monospecific, *i.e.*, the multivalent chimeric receptor only targets CLL1, and comprises two or more binding sites for CLL1. In some embodiments, the multivalent chimeric receptor is multispecific, *i.e.*, the multivalent chimeric receptor targets more than one antigen or epitope. The binding moieties specific for the same antigen may bind to the same epitope of the antigen (*i.e.,* "mono-epitope chimeric receptor") or bind to different epitopes (*i.e.*, "multi-epitope chimeric receptor" such as bi-epitope chimeric receptor or tri-epitope chimeric receptor) of the antigen. The binding sites specific for the same antigen may comprise the same or different sdAbs.

[0185] In some embodiments, the present application provides a multivalent (such as bivalent, trivalent, or of higher number of valencies) chimeric receptor comprising: (a) an extracellular domain comprising a plurality (such as at least about any one of 2, 3, 4, 5, 6, or more) of binding moieties specifically binding to CLL1; (b) a transmembrane domain; and (c) an intracellular signaling domain.

[0186] In some embodiments, the present application provides a multivalent (such as bivalent, trivalent, or of higher number of valencies) chimeric receptor comprising: (a) an extracellular domain comprising a plurality (such as at least about any one of 2, 3, 4, 5, 6, or more) of anti-CLL1 sdAb moieties; (b) a transmembrane domain; and (c) an intracellular signaling domain.

[0187] In some embodiments, the present application provides a multivalent (such as bivalent, trivalent, or of higher number of valencies) chimeric receptor comprising: (a) an extracellular domain comprising an sdAb moiety specifically binding to a first epitope of CLL1, and a second binding moiety (*e.g.*, sdAb or scFv) specifically binding to a second epitope of CLL1; (b) a transmembrane domain; and (c) an intracellular signaling domain, wherein the first epitope and the second epitope are different. In some embodiments, the first anti-CLL1 sdAb is located at the N-terminus of the second CLL1 binding moiety (*e.g.*, the second anti-CLL1 sdAb). In some embodiments, the first anti-CLL1 sdAb is located at the C-terminus of the second CLL1 binding moiety (*e.g.*, the second anti-CLL1 sdAb). In some embodiments, the multivalent chimeric receptor specifically binds to two different epitopes on CLL1. In some embodiments, the multivalent chimeric receptor specifically binds to three or more different epitopes on CLL1.

[0188] In some embodiments, the binding moieties, such as sdAbs (including the plurality of sdAbs, or the first sdAb and/or the second sdAb) are camelid, chimeric, human, or humanized. In some embodiments, the binding moieties or sdAbs are fused to each other via peptide bonds or peptide linkers. In some embodiments, each peptide linker is no more than about 50 (such as no more than about any one of 35, 25, 20, 15, 10, or 5) amino acids long. In some embodiments, the transmembrane domain is selected from the group consisting of CD8α, CD4, CD28, 4-1BB, CD80, CD86, CD152 and PD1. In some embodiments, the intracellular signaling domain comprises a primary intracellular signaling sequence of an immune effector cell (such as T cell). In some embodiments, the primary intracellular signaling sequence is derived from CD3ζ, FcRγ, FcRβ, CD3γ, CD3δ, CD3ε, CD5, CD22, CD79a, CD79b, or CD66d. In some embodiments, the primary intracellular signaling sequence is derived from CD3ζ. In some embodiments, the intracellular signaling domain comprises an intracellular co-stimulatory sequence. In some embodiments, the intracellular co-stimulatory sequence is derived from a co-stimulatory molecule selected from the group consisting of CD27, CD28, 4-1BB, OX40, CD40, PD-1, LFA-1, ICOS, CD2, CD7, LIGHT, NKG2C, B7-H3, TNFRSF9, TNFRSF4, TNFRSF8, CD40LG, ITGB2, KLRC2, TNFRSF18, TNFRSF14, HAVCR1, LGALS9, DAP10, DAP12, CD83, ligands of CD83 and

combinations thereof. In some embodiments, the intracellular co-stimulatory sequence is derived from CD28 or 4-1BB. In some embodiments, the multivalent chimeric receptor further comprises a hinge domain (such as a CD8 hinge domain) located between the C-terminus of the extracellular domain and the N-terminus of the transmembrane domain. In some embodiments, the multivalent chimeric receptor further comprises a signal peptide (such as a CD8 signal peptide). In some embodiments, the anti-CLL1 chimeric receptor comprises a polypeptide comprising from the N-terminus to the C-terminus: a CD8 signal peptide, the extracellular domain, a CD8 hinge domain, a CD8 transmembrane domain, and an intracellular signaling domain comprising an intracellular co-stimulatory sequence derived from 4-1BB or CD28 and a CD3ζ intracellular signaling sequence. In some embodiments, the multivalent chimeric receptor is monospecific. In some embodiments, the multivalent chimeric receptor is multispecific, such as bispecific.

[0189] The multivalent chimeric receptors describe herein may be specially suitable for targeting multimeric antigens via synergistic binding by the different antigen binding sites, or for enhancing binding affinity or avidity to the antigen. Any of the anti-CLL1 sdAbs described herein may be used in the extracellular domain of the multivalent chimeric receptors described herein.

Multispecific chimeric receptor

[0190] The present application further provides multispecific chimeric receptors targeting two or more (such as about any one of 2, 3, 4, 5, 6, or more) different antigens. In some embodiments, the multispecific chimeric receptor has one antigen binding site for each antigen. In some embodiments, the multispecific chimeric receptor has more than two binding sites for at least one antigen. Each antigen binding site may comprise an sdAb. In some embodiments, the multispecific chimeric receptor is a bispecific chimeric receptor. In some embodiments, the multispecific chimeric receptor is a trispecific chimeric receptor.

[0191] In some embodiments, there is provided a multispecific (such as bispecific) chimeric receptor comprising: (a) an extracellular domain comprising an anti-CLL1 sdAb (such as any one of the anti-CLL1 sdAbs described herein) and a second binding domain that specifically binds to a second antigen or epitope; (b) a transmembrane domain; and (c) an intracellular signaling domain.

[0192] In some embodiments, there is provided a multispecific (such as bispecific) chimeric receptor comprising: (a) an extracellular domain comprising an anti-CLL1 sdAb (such as any one of the anti-CLL1 sdAbs described herein) and a second sdAb that specifically binds to a second antigen; (b) a transmembrane domain; and (c) an intracellular signaling domain.

[0193] In some embodiments, there is provided a multispecific (such as bispecific) chimeric receptor comprising: (a) an extracellular domain comprising an anti-CLL1 sdAb (such as any one of the anti-CLL1

sdAbs described herein) and an scFv that specifically binds to a second antigen; (b) a transmembrane domain; and (c) an intracellular signaling domain.

[0194] In some embodiments, there is provided a multispecific (such as bispecific) chimeric receptor comprising: (a) an extracellular domain comprising an anti-CLL1 sdAb (such as any one of the anti-CLL1 sdAbs described herein) and an extracellular domain of a receptor that specifically binds to a second antigen; (b) a transmembrane domain; and (c) an intracellular signaling domain.

[0195] In some embodiments, the second antigen is selected from the group consisting of NKG2D ligands, CD33, WT1, CS1, CD123, Folate Receptor β, FLT3R, B7H6, TIM3, MUC1, c-kit, CD44v6, Lewis-Y, CD99, CD27 and CD70. In some embodiments, the anti-CLL1 sdAb and/or the second binding moiety (including second sdAb, or scFv) is camelid, chimeric, human, or humanized. In some embodiments, the anti-CLL1 sdAb and the second binding moiety (including second sdAb, scFv, or an extracellular domain of a receptor) are fused to each other via a peptide bond or a peptide linker. In some embodiments, the peptide linker is no more than about 50 (such as no more than about any one of 35, 25, 20, 15, 10, or 5) amino acids long. In some embodiments, the transmembrane domain is selected from the group consisting of CD8α, CD4, CD28, 4-1BB, CD80, CD86, CD152 and PD1. In some embodiments, the intracellular signaling domain comprises a primary intracellular signaling sequence of an immune effector cell (such as T cell). In some embodiments, the primary intracellular signaling sequence is derived from CD3ζ, FcRγ, FcRβ, CD3γ, CD3δ, CD3ε, CD5, CD22, CD79a, CD79b, or CD66d. In some embodiments, the primary intracellular signaling sequence is derived from CD3ζ. In some embodiments, the intracellular signaling domain comprises an intracellular co-stimulatory sequence. In some embodiments, the intracellular co-stimulatory sequence is derived from a co-stimulatory molecule selected from the group consisting of CD27, CD28, 4-1BB, OX40, CD40, PD-1, LFA-1, ICOS, CD2, CD7, LIGHT, NKG2C, B7-H3, TNFRSF9, TNFRSF4, TNFRSF8, CD40LG, ITGB2, KLRC2, TNFRSF18, TNFRSF14, HAVCR1, LGALS9, DAP10, DAP12, CD83, ligands of CD83 and combinations thereof. In some embodiments, the intracellular co-stimulatory sequence is derived from CD28 or 4-1BB. In some embodiments, the multispecific chimeric receptor further comprises a hinge domain (such as a CD8 hinge domain) located between the C-terminus of the extracellular domain and the N-terminus of the transmembrane domain. In some embodiments, the multispecific chimeric receptor further comprises a signal peptide (such as a CD8 signal peptide). In some embodiments, the multispecific chimeric receptor comprises a polypeptide comprising from the N-terminus to the C-terminus: a CD8 signal peptide, the extracellular domain, a CD8 hinge domain, a CD8 transmembrane domain, and an intracellular signaling domain comprising an intracellular co-stimulatory sequence derived from 4-1BB or CD28 and a CD3ζ intracellular signaling sequence.

[0196] In some embodiments, there is provided a multispecific (*e.g.*, bispecific) chimeric receptor comprising: (a) an extracellular domain comprising an anti-CLL1 sdAb (such as any one of the anti-CLL1 sdAbs described herein) and an anti-CD33 sdAb or scFv; (b) a transmembrane domain, and (c) an intracellular domain comprising an intracellular co-stimulatory sequence derived from 4-1BB or CD28 and a CD3ζ intracellular signaling sequence.

[0197] In some embodiments, there is provided a multispecific (*e.g.*, bispecific) chimeric receptor comprising: (a) an extracellular domain comprising an anti-CLL1 sdAb and an anti-CD33 sdAb; (b) a transmembrane domain (*e.g.*, a CD28 transmembrane domain), and (c) an intracellular domain (*e.g.*, an intracellular domain comprising an intracellular co-stimulatory sequence derived from CD28 and a CD3ζ intracellular signaling sequence), wherein the anti-CLL1 sdAb comprises: a CDR1 comprising the amino acid sequence of SEQ ID NO: 39, a CDR2 comprising the amino acid sequence of SEQ ID NO: 41, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 43, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; and wherein the anti-CD33 sdAb comprises: a CDR1 comprising the amino acid sequence of SEQ ID NO: 198, a CDR2 comprising the amino acid sequence of SEQ ID NO: 200, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 202, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs. In some embodiments, the anti-CLL1 sdAb is at the N-terminus of the anti-CD33 sdAb. In some embodiments, the anti-CLL1 sdAb is at the C-terminus of the anti-CD33 sdAb. In some embodiments, the anti-CLL1 sdAb is fused to the anti-CD33 sdAb via a peptide linker. In some embodiments, the peptide linker comprises the amino acid sequence of any one of SEQ ID Nos: 142-147 and 182-183. In some embodiments, the anti-CLL1 sdAb moiety comprises a V_HH domain comprising the amino acid sequence of SEQ ID NO: 99, or a variant thereof comprising an amino acid sequence having at least about 95% sequence identity to the amino acid sequence of SEQ ID NO: 99. In some embodiments, the anti-CD33 sdAb moiety comprises a V_HH domain comprising the amino acid sequence of SEQ ID NO: 225, or a variant thereof comprising an amino acid sequence having at least about 95% sequence identity to the amino acid sequence of SEQ ID NO: 225. In some embodiments, the multispecific chimeric receptor comprises a polypeptide comprising from the N-terminus to the C-terminus: a CD8 signal peptide, the extracellular domain, a CD28 hinge domain, a CD28 transmembrane domain, and an intracellular signaling domain comprising an intracellular co-stimulatory sequence derived from CD28 and a CD3ζ intracellular signaling sequence.

[0198] In some embodiments, there is provided a multispecific (*e.g.*, bispecific) chimeric receptor comprising: (a) an extracellular domain comprising an anti-CLL1 sdAb and an anti-CD33 sdAb, wherein the C-terminus of the anti-CLL1 sdAb is fused to the N-terminus of the anti-CD33 sdAb via a peptide linker; (b) a transmembrane domain (*e.g.*, a CD28 transmembrane domain), and (c) an intracellular domain (*e.g.*, an intracellular domain comprising an intracellular co-stimulatory sequence derived from

CD28 and a CD3ζ intracellular signaling sequence), wherein the anti-CLL1 sdAb comprises: a CDR1 comprising the amino acid sequence of SEQ ID NO: 39, a CDR2 comprising the amino acid sequence of SEQ ID NO: 41, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 43; and wherein the anti-CD33 sdAb comprises: a CDR1 comprising the amino acid sequence of SEQ ID NO: 198, a CDR2 comprising the amino acid sequence of SEQ ID NO: 200, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 202. In some embodiments, the peptide linker comprises the amino acid sequence of SEQ ID NO: 147. In some embodiemnts, the peptide linker comprises the amino acid sequence of SEQ ID NO: 143. In some embodiemnts, the peptide linker comprises the amino acid sequence of SEQ ID NO: 183. In some embodiments, the anti-CLL1 sdAb moiety comprises a V_HH domain comprising the amino acid sequence of SEQ ID NO: 99, or a variant thereof comprising an amino acid sequence having at least about 95% sequence identity to the amino acid sequence of SEQ ID NO: 99. In some embodiments, the anti-CD33 sdAb moiety comprises a V_HH domain comprising the amino acid sequence of SEQ ID NO: 225, or a variant thereof comprising an amino acid sequence having at least about 95% sequence identity to the amino acid sequence of SEQ ID NO: 225. In some embodiments, the multispecific chimeric receptor comprises a polypeptide comprising from the N-terminus to the C-terminus: a CD8 signal peptide, the extracellular domain, a CD28 hinge domain, a CD28 transmembrane domain, and an intracellular signaling domain comprising an intracellular co-stimulatory sequence derived from CD28 and a CD3ζ intracellular signaling sequence. In some embodiments, the multispecific chimeric receptor comprises an amino acid sequence having at least about any one of 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO: 184. In some embodiments, the multispecific chimeric receptor comprises an amino acid sequence having at least about any one of 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO: 185. In some embodiments, the multispecific chimeric receptor comprises an amino acid sequence having at least about any one of 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO: 188.

[0199] In some embodiments, there is provided a multispecific (*e.g.*, bispecific) chimeric receptor comprising a polypeptide having at least about any one of 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of any one of SEQ ID NOs: 184-195. In some embodiments, there is provided a multispecific (*e.g.*, bispecific) chimeric receptor comprising a polypeptide comprising the amino acid sequence of any one of SEQ ID NOs: 184-195. Also provided is a polypeptide comprising the amino acid sequence of any one of SEQ ID NOs: 184-195.

[0200] Exemplary anti-CLL1 tandem chimeric receptors are shown in Table 4 below.

Table 4. Exemplary anti-CLL1 sdAb Tandem Chimeric Receptors.

CAR SEQ ID NO.	SP	Extra-cellular.1	Linker	Extra-cellular.2	Hinge	TM	Intracellular signaling	
							Co-stimulatory	Primary signaling
184	CD8α	AS82658	Linker-1	AS49264	CD28	CD28	CD28	CD3ζ
185	CD8α	AS82658	Linker-2	AS49264	CD28	CD28	CD28	CD3ζ
186	CD8α	AS82658	Linker-3	AS49264	CD28	CD28	CD28	CD3ζ
187	CD8α	AS82658	Linker-4	AS49264	CD28	CD28	CD28	CD3ζ
188	CD8α	AS82658	Linker-5	AS49264	CD28	CD28	CD28	CD3ζ
189	CD8α	AS82658	Linker-6	AS49264	CD28	CD28	CD28	CD3ζ
190	CD8α	AS49264	Linker-1	AS82658	CD28	CD28	CD28	CD3ζ
191	CD8α	AS49264	Linker-2	AS82658	CD28	CD28	CD28	CD3ζ
192	CD8α	AS49264	Linker-3	AS82658	CD28	CD28	CD28	CD3ζ
193	CD8α	AS49264	Linker-4	AS82658	CD28	CD28	CD28	CD3ζ
194	CD8α	AS49264	Linker-5	AS82658	CD28	CD28	CD28	CD3ζ
195	CD8α	AS49264	Linker-6	AS82658	CD28	CD28	CD28	CD3ζ

[0201] Any suitable anti-CD33 sdAb or scFv may be used for the multispecific chimeric receptor targeting CLL1 and CD33 described herein. Exemplary anti-CD33 sdAbs have been described, for example, *see*, PCT/CN2018/104882. Sequences of exemplary anti-CD33 sdAbs are shown in Table 5 below.

Table 5. Exemplary anti-CD33 sdAbs.

sdAb/ SEQ ID (AA)	FR1/ SEQ ID NO	CDR1/ SEQ ID	FR2/ SEQ ID	CDR2/ SEQ ID	FR3/ SEQ ID NO	CDR3/ SEQ ID	FR4/ SEQ ID
AS49264 225	EVQLVESGGGSVQAGGSLRLSCAAS 197	GYTYSINCMG 198	WFRQAPGKEREGVA 199	VISTGGGRTDYRDSVKG 200	RFTISQDNAKNTVYLQMNSLKPEDTAMYYCAG 201	KTTYPGYGCGLGRSAYNY 202	WGQGTQVTVSS 203
AS49814 226	QIQLVESGGGSVQAGGSLRLSCVAS 204	GYIGGHYYMG 205	WFRQAPGKEREGVA 206	AIDIDSDGRTRYAGSVQG 207	RFTISQDNAKNTLHLQMSSLKPEDTGMYYCAV 208	GVGWVPARLTPQAVSY 209	WGKGTLVTVSS 210
AS50073 227	QVQLVESGGGLVQAGGSLRLSCTAS 211	GFTFDNYVMG 212	WFRQAPGKEREGVS 213	CIGWSGGSTYYADSVKG 214	RFTISRDNAKNTLYLQMNSLKPEDTAMYYCAA 215	DQGKCSLGSAGADDMDY 216	WGRGTLVTVSS 217
AS67190 228	QVQLVESGGGLVQAGGSLRLSCAAS 218	GNVFRFNIMG 219	WYRQAPGNQRELVA 220	SIDDGGDRSYADSVEG 221	RFTISRENGKKIMYLQMNSLKPEDTAVYYCAA 222	GLGTYLNGRVSMATNY 223	WGQGTQVTVSS 224

SEQ ID NO: 225 (AS49264 sdAb amino acid sequence; CDRs are underlined)
EVQLVESGGGSVQAGGSLRLSCAAS<u>GYTYSINCMG</u>WFRQAPGKEREGVA<u>VISTGGGRTDYRDSVKG</u>RFTISQDNAKNTVYLQMNSLKPEDTAMYYCAG<u>KTTYPGYGCGLGRSAYNY</u>WGQGTQVTVSS

SEQ ID NO: 226 (AS49814 sdAb amino acid sequence; CDRs are underlined)

QIQLVESGGGSVQAGGSLRLSCVASGYIGGHYYMGWFRQAPGKEREGVAAIDIDSDGRTRYAGSVQGRFTI
SQDNAKNTLHLQMSSLKPEDTGMYYCAVGVGWVPARLTPQAVSYWGKGTLVTVSS

SEQ ID NO: 227 (AS50073 sdAb amino acid sequence; CDRs are underlined)
QVQLVESGGGLVQAGGSLRLSCTASGFTFDNYVMGWFRQAPGKEREGVSCIGWSGGSTYYADSVKGRFTI
SRDNAKNTLYLQMNSLKPEDTAMYYCAADQGKCSLGSAGADDMDYWGRGTLVTVSS

SEQ ID NO: 228 (AS67190 sdAb amino acid sequence; CDRs are underlined)
QVQLVESGGGLVQAGGSLRLSCAASGNVFRFNIMGWYRQAPGNQRELVASIDDGGDRSYADSVEGRFTIS
RENGKKIMYLQMNSLKPEDTAVYYCAAGLGTYLNGRVSMATNYWGQGTQVTVSS

[0202] In some embodiments, there is provided a multispecific (*e.g.*, bispecific) chimeric receptor comprising: (a) an extracellular domain comprising an anti-CLL1 sdAb (such as any one of the anti-CLL1 sdAbs described herein) and an anti-CD123 sdAb or scFv; (b) a transmembrane domain, and (c) an intracellular domain comprising an intracellular co-stimulatory sequence derived from 4-1BB or CD28 and a CD3ζ intracellular signaling sequence.

[0203] In some embodiments, there is provided a multispecific (*e.g.*, bispecific) chimeric receptor comprising: (a) an extracellular domain comprising an anti-CLL1 sdAb (such as any one of the anti-CLL1 sdAbs described herein) and an extracellular domain of NKG2D; (b) a transmembrane domain, and (c) an intracellular domain comprising an intracellular co-stimulatory sequence derived from 4-1BB or CD28 and a CD3ζ intracellular signaling sequence.

Chimeric receptor systems

[0204] The present application further provides chimeric receptor systems comprising two or more chimeric receptors, including dual chimeric receptor systems and split chimeric receptors.

[0205] In some embodiments, there is provided a dual chimeric receptor system comprising: (a) a first chimeric receptor comprising an extracellular domain comprising an anti-CLL1 sdAb (such as any one of the anti-CLL1 sdAbs described herein), a transmembrane domain, and an intracellular signaling domain comprising a primary intracellular signaling sequence of an immune cell (*e.g.*, T cell) and an intracellular co-stimulatory sequence; (b) a second chimeric receptor comprising an extracellular domain comprising a second binding moiety (*e.g.*, sdAb, scFv, or an extracellular domain of a receptor) that specifically binds to a second antigen or epitope, a transmembrane domain, and an intracellular signaling domain comprising a primary intracellular signaling sequence of an immune cell (*e.g.*, T cell) and an intracellular co-stimulatory sequence. In some embodiments, the second antigen is selected from the group consisting of NKG2D ligands, CD33, WT1, CS1, CD123, Folate Receptor β, FLT3R, B7H6, TIM3, MUC1, c-kit, CD44v6, Lewis-Y, CD99, CD27 and CD70. In some embodiments, the transmembrane domain is selected from the group consisting of CD8α, CD4, CD28, 4-1BB, CD80, CD86, CD152 and PD1. In some embodiments, the primary intracellular signaling sequence is derived from CD3ζ, FcRγ, FcRβ,

CD3γ, CD3δ, CD3ε, CD5, CD22, CD79a, CD79b, or CD66d. In some embodiments, the primary intracellular signaling sequence is derived from CD3ζ. In some embodiments, the intracellular co-stimulatory sequence is derived from a co-stimulatory molecule selected from the group consisting of CD27, CD28, 4-1BB, OX40, CD40, PD-1, LFA-1, ICOS, CD2, CD7, LIGHT, NKG2C, B7-H3, TNFRSF9, TNFRSF4, TNFRSF8, CD40LG, ITGB2, KLRC2, TNFRSF18, TNFRSF14, HAVCR1, LGALS9, DAP10, DAP12, CD83, ligands of CD83 and combinations thereof. In some embodiments, the intracellular co-stimulatory sequence is derived from CD28 or 4-1BB. In some embodiments, the first chimeric receptor and/or the second chimeric receptor further comprises a hinge domain (such as a CD8 hinge domain) located between the C-terminus of the extracellular domain and the N-terminus of the transmembrane domain. In some embodiments, the first chimeric receptor and/or the second chimeric receptor further comprises a signal peptide (such as a CD8 signal peptide). In some embodiments, the first chimeric receptor and the second chimeric receptor each comprises a polypeptide comprising from the N-terminus to the C-terminus: a CD8 signal peptide, the extracellular domain, a CD8 hinge domain, a CD8 transmembrane domain, and an intracellular signaling domain comprising an intracellular co-stimulatory sequence derived from 4-1BB or CD28 and a CD3ζ intracellular signaling sequence.

[0206] In some embodiments, there is provided a split chimeric receptor system comprising: (a) a first chimeric receptor comprising an extracellular domain comprising an anti-CLL1 sdAB (such as any one of the anti-CLL1 sdAbs described herein), a transmembrane domain, and an intracellular signaling domain comprising a primary intracellular signaling sequence of an immune cell (*e.g.*, T cell), wherein optionally the intracellular signaling domain does not comprise an intracellular co-stimulatory sequence; (b) a second chimeric receptor comprising an extracellular domain comprising a second binding moiety (*e.g.*, sdAb, scFv, or an extracellular domain of a receptor) that specifically binds to a second antigen or epitope, a transmembrane domain, and an intracellular signaling domain comprising an intracellular co-stimulatory sequence, wherein optionally the intracellular signaling domain does not comprise a primary intracellular signaling sequence. In some embodiments, the second antigen is selected from the group consisting of NKG2D ligands, CD33, WT1, CS1, CD123, Folate Receptor β, FLT3R, B7H6, TIM3, MUC1, c-kit, CD44v6, Lewis-Y, CD99, CD27 and CD70. In some embodiments, the transmembrane domain is selected from the group consisting of CD8α, CD4, CD28, 4-1BB, CD80, CD86, CD152 and PD1. In some embodiments, the primary intracellular signaling sequence is derived from CD3ζ, FcRγ, FcRβ, CD3γ, CD3δ, CD3ε, CD5, CD22, CD79a, CD79b, or CD66d. In some embodiments, the primary intracellular signaling sequence is derived from CD3ζ. In some embodiments, the intracellular co-stimulatory sequence is derived from a co-stimulatory molecule selected from the group consisting of CD27, CD28, 4-1BB, OX40, CD40, PD-1, LFA-1, ICOS, CD2, CD7, LIGHT, NKG2C, B7-H3, TNFRSF9, TNFRSF4, TNFRSF8, CD40LG, ITGB2, KLRC2, TNFRSF18, TNFRSF14, HAVCR1,

LGALS9, DAP10, DAP12, CD83, ligands of CD83 and combinations thereof. In some embodiments, the intracellular co-stimulatory sequence is derived from CD28 or 4-1BB. In some embodiments, the first chimeric receptor and/or the second chimeric receptor further comprises a hinge domain (such as a CD8 hinge domain) located between the C-terminus of the extracellular domain and the N-terminus of the transmembrane domain. In some embodiments, the first chimeric receptor and/or the second chimeric receptor further comprises a signal peptide (such as a CD8 signal peptide). In some embodiments, the first chimeric receptor comprises a polypeptide comprising from the N-terminus to the C-terminus: a CD8 signal peptide, the anti-CLL1 sdAb, a CD8 hinge domain, a CD8 transmembrane domain, and an intracellular signaling domain comprising a CD3ζ intracellular signaling sequence. In some embodiments, the second chimeric receptor comprises a polypeptide comprising from the N-terminus to the C-terminus: a CD8 signal peptide, the second binding domain, a CD8 hinge domain, a CD8 transmembrane domain, and an intracellular signaling domain comprising an intracellular co-stimulatory sequence derived from 4-1BB or CD28.

[0207] In some embodiments, there is provided a split chimeric receptor system comprising: (a) a first chimeric receptor comprising an extracellular domain comprising an anti-CLL1 sdAb (such as any one of the anti-CLL1 sdAbs described herein), a transmembrane domain, and an intracellular domain comprising an intracellular signaling domain comprising an intracellular co-stimulatory sequence, wherein optionally the intracellular signaling domain does not comprise a primary intracellular signaling sequence; (b) a second chimeric receptor comprising an extracellular domain comprising a second binding moiety (*e.g.*, sdAb, scFv, or an extracellular domain of a receptor) that specifically binds to a second antigen or epitope, a transmembrane domain, and an intracellular signaling domain comprising a primary intracellular signaling sequence of an immune cell (*e.g.*, T cell), wherein optionally the intracellular signaling domain does not comprise an intracellular co-stimulatory sequence. In some embodiments, the second antigen is selected from the group consisting of NKG2D ligands, CD33, WT1, CS1, CD123, Folate Receptor β, FLT3R, B7H6, TIM3, MUC1, c-kit, CD44v6, Lewis-Y, CD99, CD27 and CD70. In some embodiments, the transmembrane domain is selected from the group consisting of CD8α, CD4, CD28, 4-1BB, CD80, CD86, CD152 and PD1. In some embodiments, the primary intracellular signaling sequence is derived from CD3ζ, FcRγ, FcRβ, CD3γ, CD3δ, CD3ε, CD5, CD22, CD79a, CD79b, or CD66d. In some embodiments, the primary intracellular signaling sequence is derived from CD3ζ. In some embodiments, the intracellular co-stimulatory sequence is derived from a co-stimulatory molecule selected from the group consisting of CD27, CD28, 4-1BB, OX40, CD40, PD-1, LFA-1, ICOS, CD2, CD7, LIGHT, NKG2C, B7-H3, TNFRSF9, TNFRSF4, TNFRSF8, CD40LG, ITGB2, KLRC2, TNFRSF18, TNFRSF14, HAVCR1, LGALS9, DAP10, DAP12, CD83, ligands of CD83 and combinations thereof. In some embodiments, the intracellular co-stimulatory sequence is derived from

CD28 or 4-1BB. In some embodiments, the first chimeric receptor and/or the second chimeric receptor further comprises a hinge domain (such as a CD8 hinge domain) located between the C-terminus of the extracellular domain and the N-terminus of the transmembrane domain. In some embodiments, the first chimeric receptor and/or the second chimeric receptor further comprises a signal peptide (such as a CD8 signal peptide). In some embodiments, the first chimeric receptor comprises a polypeptide comprising from the N-terminus to the C-terminus: a CD8 signal peptide, the anti-CLL1 sdAb, a CD8 hinge domain, a CD8 transmembrane domain, and an intracellular signaling domain comprising an intracellular co-stimulatory sequence derived from 4-1BB or CD28. In some embodiments, the second chimeric receptor comprises a polypeptide comprising from the N-terminus to the C-terminus: a CD8 signal peptide, the second binding domain, a CD8 hinge domain, a CD8 transmembrane domain, and an intracellular signaling domain comprising a CD3ζ intracellular signaling sequence.

[0208] In some embodiments, there is provided a dual chimeric receptor system comprising: (a) a first chimeric receptor comprising an extracellular domain comprising an anti-CLL1 sdAb (such as any one of the anti-CLL1 sdAbs described herein), a transmembrane domain, and an intracellular signaling domain comprising an intracellular co-stimulatory sequence derived from 4-1BB or CD28 and a CD3ζ intracellular signaling sequence; and (b) a second chimeric receptor comprising an extracellular domain comprising an anti-CD33 sdAb or scFv, a transmembrane domain, and an intracellular signaling domain (*e.g.*, an intracellular signaling domain comprising an intracellular co-stimulatory sequence derived from 4-1BB or CD28 and a CD3ζ intracellular signaling sequence). In some embodiments, the transmembrane domain is selected from the group consisting of CD8α, CD4, CD28, 4-1BB, CD80, CD86, CD152 and PD1. In some embodiments, the primary intracellular signaling sequence is derived from CD3ζ. In some embodiments, the intracellular co-stimulatory sequence is derived from a co-stimulatory molecule selected from the group consisting of CD27, CD28, 4-1BB, OX40, CD30, CD40, CD3, LFA-1, ICOS, CD2, CD7, LIGHT, NKG2C, B7-H3, Ligands of CD83 and combinations thereof. In some embodiments, the first chimeric receptor and/or the second chimeric receptor further comprises a hinge domain (such as a CD8 hinge domain) located between the C-terminus of the extracellular domain and the N-terminus of the transmembrane domain. In some embodiments, the first chimeric receptor and/or the second chimeric receptor further comprises a signal peptide (such as a CD8 signal peptide).

[0209] Any suitable anti-CD33 sdAb or scFv may be used for the dual chimeric receptor systems targeting CLL1 and CD33 described herein. Exemplary anti-CD33 sdAbs have been described, for example, *see*, PCT/CN2018/104882. Sequences of exemplary anti-CD33 sdAbs are shown in Table 5.

[0210] In some embodiments, there is provided a dual chimeric receptor system comprising: (a) a first chimeric receptor comprising an extracellular domain comprising an anti-CLL1 sdAb, a transmembrane domain (*e.g.*, a CD28 transmembrane domain), and an intracellular signaling domain (*e.g.*, an intracellular

signaling domain comprising an intracellular co-stimulatory sequence derived from CD28 and a CD3ζ intracellular signaling sequence), wherein the anti-CLL1 sdAb comprises: (1) a CDR1 comprising the amino acid sequence of SEQ ID NO: 4, a CDR2 comprising the amino acid sequence of SEQ ID NO: 6, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 8, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; or (2) a CDR1 comprising the amino acid sequence of SEQ ID NO: 18, a CDR2 comprising the amino acid sequence of SEQ ID NO: 20, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 22, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; and (b) a second chimeric receptor comprising an extracellular domain comprising an anti-CD33 sdAb, a transmembrane domain (*e.g.*, CD8 transmembrane domain), and an intracellular signaling domain (*e.g.*, an intracellular signaling domain comprising an intracellular co-stimulatory sequence derived from 4-1BB and a CD3ζ intracellular signaling sequence), wherein the anti-CD33 sdAb comprises: (1) a CDR1 comprising the amino acid sequence of SEQ ID NO: 205, a CDR2 comprising the amino acid sequence of SEQ ID NO: 207, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 209, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; (2) a CDR1 comprising the amino acid sequence of SEQ ID NO: 212, a CDR2 comprising the amino acid sequence of SEQ ID NO: 214, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 216, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; or (3) a CDR1 comprising the amino acid sequence of SEQ ID NO: 219, a CDR2 comprising the amino acid sequence of SEQ ID NO: 221, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 223, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs. In some embodiments, the anti-CLL1 sdAb moiety comprises a V_HH domain comprising the amino acid sequence of SEQ ID NO: 94, or a variant thereof comprising an amino acid sequence having at least about 95% sequence identity to the amino acid sequence of SEQ ID NO: 94. In some embodiments, the anti-CLL1 sdAb moiety comprises a V_HH domain comprising the amino acid sequence of SEQ ID NO: 96, or a variant thereof comprising an amino acid sequence having at least about 95% sequence identity to the amino acid sequence of SEQ ID NO: 96. In some embodiments, the anti-CD33 sdAb moiety comprises a V_HH domain comprising the amino acid sequence of SEQ ID NO: 226, or a variant thereof comprising an amino acid sequence having at least about 95% sequence identity to the amino acid sequence of SEQ ID NO: 226. In some embodiments, the anti-CD33 sdAb moiety comprises a V_HH domain comprising the amino acid sequence of SEQ ID NO: 227, or a variant thereof comprising an amino acid sequence having at least about 95% sequence identity to the amino acid sequence of SEQ ID NO: 227. In some embodiments, the anti-CD33 sdAb moiety comprises a V_HH domain comprising the amino acid sequence of SEQ ID NO: 228, or a variant thereof comprising an amino acid sequence having at least about 95% sequence identity to the amino acid sequence of SEQ ID NO: 228. In some embodiments, the first

chimeric receptor comprises a polypeptide comprising from the N-terminus to the C-terminus: a CD8 signal peptide, the extracellular domain, a CD28 hinge domain, a CD28 transmembrane domain, and an intracellular signaling domain comprising an intracellular co-stimulatory sequence derived from CD28 and a CD3ζ intracellular signaling sequence. In some embodiments, the second chimeric receptor comprises a polypeptide comprising from the N-terminus to the C-terminus: a CD8 signal peptide, the extracellular domain, a CD8 hinge domain, a CD8 transmembrane domain, and an intracellular signaling domain comprising an intracellular co-stimulatory sequence derived from 4-1BB and a CD3ζ intracellular signaling sequence.

[0211] In some embodiments, there is provided a dual chimeric receptor system comprising: (a) a first chimeric receptor comprising an extracellular domain comprising an anti-CLL1 sdAb, a transmembrane domain (*e.g.*, a CD28 transmembrane domain), and an intracellular signaling domain (*e.g.*, an intracellular signaling domain comprising an intracellular co-stimulatory sequence derived from CD28 and a CD3ζ intracellular signaling sequence), wherein the anti-CLL1 sdAb comprises: a CDR1 comprising the amino acid sequence of SEQ ID NO: 4, a CDR2 comprising the amino acid sequence of SEQ ID NO: 6, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 8; and (b) a second chimeric receptor comprising an extracellular domain comprising an anti-CD33 sdAb, a transmembrane domain (*e.g.*, CD8 transmembrane domain), and an intracellular signaling domain (*e.g.*, an intracellular signaling domain comprising an intracellular co-stimulatory sequence derived from 4-1BB and a CD3ζ intracellular signaling sequence), wherein the anti-CD33 sdAb comprises: a CDR1 comprising the amino acid sequence of SEQ ID NO: 205, a CDR2 comprising the amino acid sequence of SEQ ID NO: 207, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 209. In some embodiments, the anti-CLL1 sdAb moiety comprises a V_HH domain comprising the amino acid sequence of SEQ ID NO: 94, or a variant thereof comprising an amino acid sequence having at least about 95% sequence identity to the amino acid sequence of SEQ ID NO: 94. In some embodiments, the anti-CD33 sdAb moiety comprises a V_HH domain comprising the amino acid sequence of SEQ ID NO: 226, or a variant thereof comprising an amino acid sequence having at least about 95% sequence identity to the amino acid sequence of SEQ ID NO: 226. In some embodiments, the first chimeric receptor comprises a polypeptide comprising from the N-terminus to the C-terminus: a CD8 signal peptide, the extracellular domain, a CD28 hinge domain, a CD28 transmembrane domain, and an intracellular signaling domain comprising an intracellular co-stimulatory sequence derived from CD28 and a CD3ζ intracellular signaling sequence. In some embodiments, the second chimeric receptor comprises a polypeptide comprising from the N-terminus to the C-terminus: a CD8 signal peptide, the extracellular domain, a CD8 hinge domain, a CD8 transmembrane domain, and an intracellular signaling domain comprising an intracellular co-stimulatory sequence derived from 4-1BB and a CD3ζ intracellular signaling sequence.

[0212] In some embodiments, there is provided a dual chimeric receptor system comprising: (a) a first chimeric receptor comprising an extracellular domain comprising an anti-CLL1 sdAb, a transmembrane domain (*e.g.*, a CD28 transmembrane domain), and an intracellular signaling domain (*e.g.*, an intracellular signaling domain comprising an intracellular co-stimulatory sequence derived from CD28 and a CD3ζ intracellular signaling sequence), wherein the anti-CLL1 sdAb comprises: a CDR1 comprising the amino acid sequence of SEQ ID NO: 4, a CDR2 comprising the amino acid sequence of SEQ ID NO: 6, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 8; and (b) a second chimeric receptor comprising an extracellular domain comprising an anti-CD33 sdAb, a transmembrane domain (*e.g.*, CD8 transmembrane domain), and an intracellular signaling domain (*e.g.*, an intracellular signaling domain comprising an intracellular co-stimulatory sequence derived from 4-1BB and a CD3ζ intracellular signaling sequence), wherein the anti-CD33 sdAb comprises: a CDR1 comprising the amino acid sequence of SEQ ID NO: 212, a CDR2 comprising the amino acid sequence of SEQ ID NO: 214, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 216. In some embodiments, the anti-CLL1 sdAb moiety comprises a V_HH domain comprising the amino acid sequence of SEQ ID NO: 94, or a variant thereof comprising an amino acid sequence having at least about 95% sequence identity to the amino acid sequence of SEQ ID NO: 94. In some embodiments, the anti-CD33 sdAb moiety comprises a V_HH domain comprising the amino acid sequence of SEQ ID NO: 227, or a variant thereof comprising an amino acid sequence having at least about 95% sequence identity to the amino acid sequence of SEQ ID NO: 227. In some embodiments, the first chimeric receptor comprises a polypeptide comprising from the N-terminus to the C-terminus: a CD8 signal peptide, the extracellular domain, a CD28 hinge domain, a CD28 transmembrane domain, and an intracellular signaling domain comprising an intracellular co-stimulatory sequence derived from CD28 and a CD3ζ intracellular signaling sequence. In some embodiments, the second chimeric receptor comprises a polypeptide comprising from the N-terminus to the C-terminus: a CD8 signal peptide, the extracellular domain, a CD8 hinge domain, a CD8 transmembrane domain, and an intracellular signaling domain comprising an intracellular co-stimulatory sequence derived from 4-1BB and a CD3ζ intracellular signaling sequence.

[0213] In some embodiments, there is provided a dual chimeric receptor system comprising: (a) a first chimeric receptor comprising an extracellular domain comprising an anti-CLL1 sdAb, a transmembrane domain (*e.g.*, a CD28 transmembrane domain), and an intracellular signaling domain (*e.g.*, an intracellular signaling domain comprising an intracellular co-stimulatory sequence derived from CD28 and a CD3ζ intracellular signaling sequence), wherein the anti-CLL1 sdAb comprises: a CDR1 comprising the amino acid sequence of SEQ ID NO: 18, a CDR2 comprising the amino acid sequence of SEQ ID NO: 20, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 22; and (b) a second chimeric receptor comprising an extracellular domain comprising an anti-CD33 sdAb, a transmembrane domain (*e.g.*, CD8

transmembrane domain), and an intracellular signaling domain (*e.g.*, an intracellular signaling domain comprising an intracellular co-stimulatory sequence derived from 4-1BB and a CD3ζ intracellular signaling sequence), wherein the anti-CD33 sdAb comprises: a CDR1 comprising the amino acid sequence of SEQ ID NO: 219, a CDR2 comprising the amino acid sequence of SEQ ID NO: 221, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 22. In some embodiments, the anti-CLL1 sdAb moiety comprises a V_HH domain comprising the amino acid sequence of SEQ ID NO: 96, or a variant thereof comprising an amino acid sequence having at least about 95% sequence identity to the amino acid sequence of SEQ ID NO: 96. In some embodiments, the anti-CD33 sdAb moiety comprises a V_HH domain comprising the amino acid sequence of SEQ ID NO: 228, or a variant thereof comprising an amino acid sequence having at least about 95% sequence identity to the amino acid sequence of SEQ ID NO: 228. In some embodiments, the first chimeric receptor comprises a polypeptide comprising from the N-terminus to the C-terminus: a CD8 signal peptide, the extracellular domain, a CD28 hinge domain, a CD28 transmembrane domain, and an intracellular signaling domain comprising an intracellular co-stimulatory sequence derived from CD28 and a CD3ζ intracellular signaling sequence. In some embodiments, the second chimeric receptor comprises a polypeptide comprising from the N-terminus to the C-terminus: a CD8 signal peptide, the extracellular domain, a CD8 hinge domain, a CD8 transmembrane domain, and an intracellular signaling domain comprising an intracellular co-stimulatory sequence derived from 4-1BB and a CD3ζ intracellular signaling sequence.

[0214] In some embodiments, there is provided a dual chimeric receptor system comprising: (a) a first chimeric receptor comprising a polypeptide having at least about any one of 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO: 229 or 230; and (b) a second chimeric receptor comprising a polypeptide having at least about any one of 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO: 231, 232 or 233. In some embodiments, there is provided a dual chimeric receptor system comprising: (a) a first chimeric receptor comprising a polypeptide comprising the amino acid sequence of SEQ ID NO: 229 or 230; and (b) a second chimeric receptor comprising a polypeptide comprising the amino acid sequence of SEQ ID NO: 231, 232 or 233. In some embodiments, there is provided a dual chimeric receptor system comprising: (a) a first chimeric receptor comprising a polypeptide comprising the amino acid sequence of SEQ ID NO: 229; and (b) a second chimeric receptor comprising a polypeptide comprising the amino acid sequence of SEQ ID NO: 231. In some embodiments, there is provided a dual chimeric receptor system comprising: (a) a first chimeric receptor comprising a polypeptide comprising the amino acid sequence of SEQ ID NO: 229; and (b) a second chimeric receptor comprising a polypeptide comprising the amino acid sequence of SEQ ID NO: 232. In some embodiments, there is provided a dual chimeric receptor system

comprising: (a) a first chimeric receptor comprising a polypeptide comprising the amino acid sequence of SEQ ID NO: 230; and (b) a second chimeric receptor comprising a polypeptide comprising the amino acid sequence of SEQ ID NO: 233.

[0215] In some embodiments, there is provided a dual chimeric receptor construct comprising a first polypeptide comprising any one of the anti-CLL1 chimeric receptor described herein and a second polypeptide comprising any one of the anti-CD33 chimeric receptor described herein, wherein the first polypeptide and the second polypeptide are fused to each other via a self-cleaving peptide (*e.g.*, P2A peptide). In some embodiments, the dual chimeric receptor construct comprises an amino acid sequence having at least about any one of 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO: 234. In some embodiments, the dual chimeric receptor construct comprises an amino acid sequence having at least about any one of 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO: 235. In some embodiments, the dual chimeric receptor construct comprises an amino acid sequence having at least about any one of 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO: 236. In some embodiments, the dual chimeric receptor construct comprises the amino acid sequence of SEQ ID NO: 234, 235 or 236. Further provided are nucleic acid(s) encoding any one of the dual chimeric receptor constructs described herein. Also provided is a polypeptide comprising the amino acid sequence of any one of SEQ ID NOs: 234-236.

[0216] Exemplary anti-CLL1 dual chimeric receptor systems are shown in Table 6 below.

Table 6. Exemplary anti-CLL1 sdAb Dual Chimeric Receptors.

Construct	SEQ ID NO.	CAR1 (CLL1)	CAR1 SEQ ID NO.	Linker	CAR2 (CD33)	CAR2 SEQ ID NO.
Dual 1	234	AS82472-28z CAR	229	P2A	AS49814 CAR	231
Dual 2	235	AS82472-28z CAR	229	P2A	AS50073 CAR	232
Dual 3	236	AS82494-28z CAR	230	P2A	AS67190 CAR	233

[0217] In some embodiments, there is provided a dual chimeric receptor system comprising: (a) a first chimeric receptor comprising an extracellular domain comprising an anti-CLL1 sdAb (such as any one of the anti-CLL1 sdAbs described herein), a transmembrane domain, and an intracellular signaling domain comprising an intracellular co-stimulatory sequence derived from 4-1BB or CD28 and a CD3ζ intracellular signaling sequence; and (b) a second chimeric receptor comprising an extracellular domain comprising an anti-CD123 sdAb or scFv, a transmembrane domain, and an intracellular signaling domain

comprising an intracellular co-stimulatory sequence derived from 4-1BB or CD28 and a CD3ζ intracellular signaling sequence.

[0218] In some embodiments, there is provided a dual chimeric receptor system comprising: (a) a first chimeric receptor comprising an extracellular domain comprising an anti-CLL1 sdAb (such as any one of the anti-CLL1 sdAbs described herein), a transmembrane domain, and an intracellular signaling domain comprising an intracellular co-stimulatory sequence derived from 4-1BB or CD28 and a CD3ζ intracellular signaling sequence; and (b) a second chimeric receptor comprising an extracellular domain comprising an extracellular domain of NKG2D, a transmembrane domain, and an intracellular signaling domain comprising an intracellular co-stimulatory sequence derived from 4-1BB or CD28 and a CD3ζ intracellular signaling sequence.

[0219] In some embodiments, there is provided a split chimeric receptor system comprising: (a) a first chimeric receptor comprising an extracellular domain comprising an anti-CLL1 sdAb (such as any one of the anti-CLL1 sdAbs described herein), a transmembrane domain, and an intracellular signaling domain comprising a CD3ζ intracellular signaling sequence; and (b) a second chimeric receptor comprising an extracellular domain comprising an anti-CD33 sdAb or scFv, a transmembrane domain, and an intracellular signaling domain comprising an intracellular co-stimulatory sequence derived from 4-1BB or CD28.

[0220] In some embodiments, there is provided a split chimeric receptor system comprising: (a) a first chimeric receptor comprising an extracellular domain comprising an anti-CLL1 sdAb (such as any one of the anti-CLL1 sdAbs described herein), a transmembrane domain, and an intracellular signaling domain comprising a CD3ζ intracellular signaling sequence; and (b) a second chimeric receptor comprising an extracellular domain comprising an anti-CD123 sdAb or scFv, a transmembrane domain, and an intracellular signaling domain comprising an intracellular co-stimulatory sequence derived from 4-1BB or CD28.

[0221] In some embodiments, there is provided a split chimeric receptor system comprising: (a) a first chimeric receptor comprising an extracellular domain comprising an anti-CLL1 sdAb (such as any one of the anti-CLL1 sdAbs described herein), a transmembrane domain, and an intracellular signaling domain comprising a CD3ζ intracellular signaling sequence; and (b) a second chimeric receptor comprising an extracellular domain comprising an extracellular domain of NKG2D, a transmembrane domain, and an intracellular signaling domain comprising an intracellular co-stimulatory sequence derived from 4-1BB or CD28.

[0222] In some embodiments, there is provided a split chimeric receptor system comprising: (a) a first chimeric receptor comprising an extracellular domain comprising an anti-CLL1 sdAb (such as any one of

the anti-CLL1 sdAbs described herein), a transmembrane domain, and an intracellular signaling domain comprising an intracellular co-stimulatory sequence derived from 4-1BB or CD28; and (b) a second chimeric receptor comprising an extracellular domain comprising an anti-CD33 sdAb or scFv, a transmembrane domain, and an intracellular signaling domain comprising a CD3ζ intracellular signaling sequence.

[0223] In some embodiments, there is provided a split chimeric receptor system comprising: (a) a first chimeric receptor comprising an extracellular domain comprising an anti-CLL1 sdAb (such as any one of the anti-CLL1 sdAbs described herein), a transmembrane domain, and an intracellular signaling domain comprising an intracellular co-stimulatory sequence derived from 4-1BB or CD28; and (b) a second chimeric receptor comprising an extracellular domain comprising an anti-CD123 sdAb or scFv, a transmembrane domain, and an intracellular signaling domain comprising a CD3ζ intracellular signaling sequence.

[0224] In some embodiments, there is provided a split chimeric receptor system comprising: (a) a first chimeric receptor comprising an extracellular domain comprising an anti-CLL1 sdAb (such as any one of the anti-CLL1 sdAbs described herein), a transmembrane domain, and an intracellular signaling domain comprising an intracellular co-stimulatory sequence derived from 4-1BB or CD28; and (b) a second chimeric receptor comprising an extracellular domain comprising an extracellular domain of NKG2D, a transmembrane domain, and an intracellular signaling domain comprising a CD3ζ intracellular signaling sequence.

Extracellular domain

[0225] The extracellular domain of the chimeric receptors described herein comprises one or more (such as any one of 1, 2, 3, 4, 5, 6 or more) binding moieties, such as sdAbs. In some embodiments, the one or more binding moieties are antibodies or antigen-binding fragments thereof. In some embodiments, the one or more binding moieties are derived from four-chain antibodies. In some embodiments, the one or more binding moieties are derived from camelid antibodies. In some embodiments, the one or more binding moieties are derived from human antibodies. In some embodiments, the one or more binding moieties are non-antibody binding proteins, such as extracellular domains of receptors, polypeptide ligands or engineered proteins that bind to an antigen. The binding moieties can be fused to each other directly via peptide bonds, or via peptide linkers.

[0226] In some embodiments, the extracellular domain comprises a second binding moiety. The second binding moiety specifically binds to a cell surface molecule. The second binding moiety may be chosen to recognize an antigen that acts as a cell surface marker on target cells associated with a special disease state. The antigens targeted by the second binding moiety may be directly or indirectly involved in the diseases.

In some embodiments, the antigen is a tumor antigen. In some embodiments, the tumor antigen is associated with an acute myeloid leukemia (AML). In some embodiments, the tumor antigen is associated with chronic myelogenous leukemia (CML). In some embodiments, the tumor antigen is associated with myelodysplastic syndromes (MDS).

[0227] Tumor antigens are proteins that are produced by tumor cells that can elicit an immune response, particularly T-cell mediated immune responses. The selection of the targeted antigen of the invention will depend on the particular type of cancer to be treated. Exemplary tumor antigens include, for example, a glioma-associated antigen, carcinoembryonic antigen (CEA), β-human chorionic gonadotropin, alphafetoprotein (AFP), lectin-reactive AFP, thyroglobulin, RAGE-1, MN-CAIX, human telomerase reverse transcriptase, RU1, RU2 (AS), intestinal carboxyl esterase, mut hsp70-2, M-CSF, prostase, prostate-specific antigen (PSA), PAP, NY-ESO-1, LAGE-la, p53, prostein, PSMA, HER2/neu, survivin and telomerase, prostate-carcinoma tumor antigen-1 (PCTA-1), MAGE, ELF2M, neutrophil elastase, ephrinB2, CD22, insulin growth factor (IGF)-I, IGF-II, IGF-I receptor and mesothelin.

[0228] In some embodiments, the tumor antigen is a tumor-specific antigen (TSA) or a tumor-associated antigen (TAA). A TSA is unique to tumor cells and does not occur on other cells in the body. A TAA associated antigen is not unique to a tumor cell, and instead is also expressed on a normal cell under conditions that fail to induce a state of immunologic tolerance to the antigen. The expression of the antigen on the tumor may occur under conditions that enable the immune system to respond to the antigen. TAAs may be antigens that are expressed on normal cells during fetal development, when the immune system is immature, and unable to respond or they may be antigens that are normally present at extremely low levels on normal cells, but which are expressed at much higher levels on tumor cells.

[0229] Non-limiting examples of TSA or TAA antigens include the following: Differentiation antigens such as MART-1/MelanA (MART-I), gp 100 (Pmel 17), tyrosinase, TRP-1, TRP-2 and tumor-specific multilineage antigens such as MAGE-1, MAGE-3, BAGE, GAGE-1, GAGE-2, pl5; overexpressed embryonic antigens such as CEA; overexpressed oncogenes and mutated tumor-suppressor genes such as p53, Ras, HER2/neu; unique tumor antigens resulting from chromosomal translocations; such as BCR-ABL, E2A-PRL, H4-RET, IGH-IGK, MYL-RAR; and viral antigens, such as the Epstein Barr virus antigens EBVA and the human papillomavirus (HPV) antigens E6 and E7. Other large, protein-based antigens include TSP-180, MAGE-4, MAGE-5, MAGE-6, RAGE, NY-ESO, pl85erbB2, pl80erbB-3, c-met, nm-23HI, PSA, TAG-72, CA 19-9, CA 72-4, CAM 17.1, NuMa, K-ras, beta-Catenin, CDK4, Mum-1, p 15, p 16, 43-9F, 5T4, 791Tgp72, alpha-fetoprotein, beta-HCG, BCA225, BTAA, CA 125, CA 15-3\CA 27.29\BCAA, CA 195, CA 242, CA-50, CAM43, CD68\P1, CO-029, FGF-5, G250, Ga733\EpCAM, HTgp-175, M344, MA-50, MG7-Ag, MOV18, NB/70K, NY-CO- 1, RCAS 1, SDCCAG16, TA-90\Mac-2 binding protein\cyclophilin C-associated protein, TAAL6, TAG72, TLP, and TPS.

[0230] The second binding moiety can be of any suitable format. In some embodiments, the second binding moiety is derived from an antibody, such as a four-chain antibody, or a single-domain antibody, such as heavy-chain only antibody. In some embodiments, the second binding moiety is an antibody fragment, such as a Fab, Fv, scFv, or V_HH. In some embodiments, the second binding moiety is an antibody fragment that specifically binds to an antigen selected from the group consisting of NKG2D ligands, CD33, WT1, CS1, CD123, Folate Receptor β, FLT3R, B7H6, TIM3, MUC1, c-kit, CD44v6, Lewis-Y, CD99, CD27 and CD70.

[0231] In some embodiments, the second binding moiety is a CD33-binding domain. In some embodiments, the CD33-binding domain is an antibody fragment (*e.g.*, an scFv or a V_HH) of an anti-CD33 antibody. In some embodiments, the CD33-binding domain is an scFv derived from hP67.6. In some embodiments, the CD33-binding domain is an anti-CD33 sdAb.

[0232] CD33, also known as Siglec-3 (sialic acid binding Ig-like lectin 3), gp67, or p67, is a transmembrane receptor expressed on cells of myeloid lineage. CD33 is the target of gemtuzumab ozogamicin (MYLOTARG®), an antibody-drug conjugate, which has been approved for treatment of patients with acute myeloid leukemia.

[0233] In some embodiments, the second binding moiety is a CD123-binding domain. In some embodiments, the CD123-binding domain is an antibody fragment (*e.g.*, an scFv or a V_HH) of an anti-CD123 antibody. In some embodiments, the CD123-binding domain is a ligand of CD123, or an IL-3 domain. In some embodiments, the IL-3 domain is derived from human IL-3, such as full-length or a functional fragment of human IL-3.

[0234] IL-3 (interleukin-3) gene is mapped on chromosome 5, encoding a protein 152 amino acids long. IL-3 is a cytokine, capable of supporting a broad range of cellular activities such as cell growth, differentiation and apoptosis. IL-13 acts by binding to the interleukin-3 receptor (IL-3R), also known as CD123 antigen. IL-3R is a heterodimeric receptor, comprising a ligand specific alpha subunit and a signal transducing beta subunit, shared by the receptors for IL-3, colony stimulating factor 2 (CSF2/GM-CSF), and interleukin 5 (IL5). Activation of the IL-3R results in the phosphorylation of the βc chain, recruitment of SH2-containing adaptor molecules such as Vav1, and downstream signal transduction via Jak2/STAT5 and the Ras/MAPK pathway.

[0235] IL-3R is a 75 kD glycoprotein and becomes 43 kD when hydrolyzed by N-glycosidase. IL-3R has three extracellular domains which are responsible for specific binding to IL-3, a transmembrane domain, and a short intercellular domain which is indispensable for intracellular signaling (Sato et al. 1993). IL-3R is a heterodimeric receptor with low affinity and high specificity for IL-3. Upon binding to IL-3, the IL-3R is activated and promotes cell proliferation and survival (Liu et al. 2015).

[0236] CD123 is overexpressed on AML blasts (*i.e.*, myelobasts). AML blasts and leukemia stem cells (LSCs) in 75 to 89% of AML patients express CD123. In sharp contrast, there is low or undetectable expression of CD123 on normal hematopoietic stem cells (HSCs) (Frankel et al. 2014; Jordan et al. 2000). Apart from AML, CD123 is also overexpressed in a variety of hematologic malignancies, including B cell lineage acute lymphoblastic leukemia, chronic myeloid leukemia, plasmacytoid dendritic cell neoplasm, and hairy cell leukemia (Munoz et al. 2001). This expression profile makes CD123 a valuable biomarker in clinical diagnosis, prognosis and intervention of the diseases. Currently, early phase clinical trials have demonstrated that CD123-targeting therapies are safe and without major adverse effects on hematopoiesis. The anti-leukemic activities of CD123-targeting therapies in humans are still being investigated.

[0237] In some embodiments, the second binding moiety is a ligand, or a ligand binding domain of a receptor, such as an extracellular domain of a receptor. In some embodiments, the second binding moiety is a ligand or ligand binding domain derived from a molecule selected from the group consisting of NKG2A, NKG2C, NKG2F, IL-3, IL-13, LLT1, AICL, DNAM-1, and NKp80. In some embodiments, the second binding moiety is an extracellular domain of NKG2D. In some embodiments, the second binding moiety comprises the amino acid sequence of SEQ ID NO: 149.

[0238] SEQ ID NO: 149 NKG2D-L binding domain

FNQEVQIPLTESYCGPCPKNWICYKNNCYQFFDESKNWYESQASCMSQNASLLKVYSKEDQDLL
KLVKSYHWMGLVHIPTNGSWQWEDGSILSPNLLTIIEMQKGDCALYASSFKGYIENCSTPNTYIC
MQRTV

[0239] NKG2D is a unique member of the NKG2 family, which are C-type lectin receptors that stimulate or inhibit cytotoxic activity of NK cells. NKG2D is a type II transmembrane-anchored glycoprotein, expressed primarily on the surface of NK cells and $CD8^+$ T cells (*e.g.*, αβ T cells and γδ T cells). It is highly conserved across multiple species, with 70% sequence identity shared between the human and murine receptors. Unlike the other NKG2 receptors that heterodimerize with CD94 and bind to nonclassical MHC glycoproteins class I, NKG2D forms homodimers and bind to cellular stress-inducible molecules. Accumulating evidence indicates that NKG2D plays a crucial role in immunosurveillance against stressed or abnormal cells, such as autologous tumor cells and virus-infected cells.

[0240] A variety of NKG2D ligands have been identified in humans, including MIC molecules (MHC class I chain-related proteins A and B, or MICA and MICB) encoded by genes in the MHC family, and ULBP molecules (UL16-binding proteins, also known as RAET1 proteins) which are clustered on human chromosome 6 (Bahram et al. 2005). All NKG2D ligands are homologous to MHC class I molecules and exhibit considerable allelic variation. Although NKG2D ligand RNAs are broadly expressed on all tissues and organs of the body, NKG2D ligands are generally absent from the surface of normal adult cells (Le Bert and Gasser 2014). However, the expression of NKG2D ligands is induced or upregulated primarily in

tissues of epithelial origin in response to cellular stress, including heat shock, DNA damage, and stalled DNA replication. Presence of NKG2D ligands on a cell flags the cell for NK cell targeting and potential elimination (Le Bert and Gasser 2014). Interestingly, high activity of DNA repair pathways in transformed cells across a variety of hematologic and solid tumors lead to expression of NKG2D ligands, which renders these cells susceptible to NK-mediated lysis (Sentman et al. 2006).

[0241] NKG2D is encoded by KLRK1 gene. NKG2D is a transmembrane receptor protein comprising three domains: cytoplasmic domain (residues 1-51 of human NKG2D), transmembrane domain (residues 52-72 of human NKG2D), and extracellular domain (residues 73-216 of human NKG2D). The extracellular domain of NKG2D contains a C-type lectin domain (residues 98-213 of human NKG2D).

Transmembrane domain

[0242] The chimeric receptors of the present application comprise a transmembrane domain that can be directly or indirectly fused to the extracellular domain. The transmembrane domain may be derived either from a natural or from a synthetic source. As used herein, a "transmembrane domain" refers to any protein structure that is thermodynamically stable in a cell membrane, preferably a eukaryotic cell membrane. Transmembrane domains compatible for use in the chimeric receptors described herein may be obtained from a naturally occurring protein. Alternatively, it can be a synthetic, non-naturally occurring protein segment, *e.g.*, a hydrophobic protein segment that is thermodynamically stable in a cell membrane.

[0243] Transmembrane domains are classified based on the three dimensional structure of the transmembrane domain. For example, transmembrane domains may form an alpha helix, a complex of more than one alpha helix, a beta-barrel, or any other stable structure capable of spanning the phospholipid bilayer of a cell. Furthermore, transmembrane domains may also or alternatively be classified based on the transmembrane domain topology, including the number of passes that the transmembrane domain makes across the membrane and the orientation of the protein. For example, single-pass membrane proteins cross the cell membrane once, and multi-pass membrane proteins cross the cell membrane at least twice (*e.g.*, 2, 3, 4, 5, 6, 7 or more times). Membrane proteins may be defined as Type I, Type II or Type III depending upon the topology of their termini and membrane-passing segment(s) relative to the inside and outside of the cell. Type I membrane proteins have a single membrane-spanning region and are oriented such that the N-terminus of the protein is present on the extracellular side of the lipid bilayer of the cell and the C-terminus of the protein is present on the cytoplasmic side. Type II membrane proteins also have a single membrane-spanning region but are oriented such that the C-terminus of the protein is present on the extracellular side of the lipid bilayer of the cell and the N-terminus of the protein is present on the cytoplasmic side. Type III membrane proteins

have multiple membrane- spanning segments and may be further sub-classified based on the number of transmembrane segments and the location of N- and C-termini.

[0244] In some embodiments, the transmembrane domain of the CAR described herein is derived from a Type I single-pass membrane protein. In some embodiments, transmembrane domains from multi-pass membrane proteins may also be compatible for use in the CARs described herein. Multi-pass membrane proteins may comprise a complex (at least 2, 3, 4, 5, 6, 7 or more) alpha helices or a beta sheet structure. Preferably, the N-terminus and the C-terminus of a multi-pass membrane protein are present on opposing sides of the lipid bilayer, *e.g.*, the N-terminus of the protein is present on the cytoplasmic side of the lipid bilayer and the C-terminus of the protein is present on the extracellular side.

[0245] In some embodiments, the transmembrane domain of the chimeric receptor comprises a transmembrane domain chosen from the transmembrane domain of an alpha, beta or zeta chain of a T-cell receptor, CD28, CD3 epsilon, CD45, CD4, CD5, CD8, CD9, CD16, CD22, CLL1, CD37, CD64, CD80, CD86, CD134, 4-1BB, CD154, KIRDS2, OX40, CD2, CD27, LFA-1 (CD11a, CD18), ICOS (CD278), 4-1BB (4-1BB), GITR, CD40, BAFFR, HVEM (LIGHTR), SLAMF7, NKp80 (KLRF1), CD160, CD19, IL-2R beta, IL-2R gamma, IL-7R a, 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, CRT AM, Ly9 (CD229), CD160 (BY55), PSGL1, CDIOO (SEMA4D), SLAMF6 (NTB-A, Lyl08), SLAM (SLAMF1, CD150, IPO-3), BLAME (SLAMF8), SELPLG (CD162), LTBR, PAG/Cbp, NKp44, NKp30, NKp46, NKG2D, and/or NKG2C. In some embodiments, the transmembrane domain is derived from a molecule selected from the group consisting of CD8α, CD4, CD28, 4-1BB, CD80, CD86, CD152 and PD1.

[0246] In some embodiments, the transmembrane domain is derived from CD8. In some embodiments, the transmembrane domain is a transmembrane domain of CD8α comprising the amino acid sequence of IYIWAPLAGTCGVLLLSLVITLYC (SEQ ID NO: 133). In some embodiments, the transmembrane domain is derived from CD28. In some embodiments, the transmembrane domain is a transmembrane domain of CD28 comprising the amino acid sequence of FWVLVVVGGVLACYSLLVTVAFIIFWV (SEQ ID NO: 134).

[0247] Transmembrane domains for use in the chimeric receptors described herein can also comprise at least a portion of a synthetic, non-naturally occurring protein segment. In some embodiments, the transmembrane domain is a synthetic, non-naturally occurring alpha helix or beta sheet. In some embodiments, the protein segment is at least approximately 20 amino acids, *e.g.*, at least 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, or more amino acids. Examples of synthetic transmembrane domains

are known in the art, for example in U.S. Patent No.7,052,906 B1 and PCT Publication No. WO 2000/032776 A2, the relevant disclosures of which are incorporated by reference herein.

[0248] The transmembrane domain may comprise a transmembrane region and a cytoplasmic region located at the C-terminal side of the transmembrane domain. The cytoplasmic region of the transmembrane domain may comprise three or more amino acids and, in some embodiments, helps to orient the transmembrane domain in the lipid bilayer. In some embodiments, one or more cysteine residues are present in the transmembrane region of the transmembrane domain. In some embodiments, one or more cysteine residues are present in the cytoplasmic region of the transmembrane domain. In some embodiments, the cytoplasmic region of the transmembrane domain comprises positively charged amino acids. In some embodiments, the cytoplasmic region of the transmembrane domain comprises the amino acids arginine, serine, and lysine.

[0249] In some embodiments, the transmembrane region of the transmembrane domain comprises hydrophobic amino acid residues. In some embodiments, the transmembrane domain of the chimeric receptor comprises an artificial hydrophobic sequence. For example, a triplet of phenylalanine, tryptophan and valine may be present at the C terminus of the transmembrane domain. In some embodiments, the transmembrane region comprises mostly hydrophobic amino acid residues, such as alanine, leucine, isoleucine, methionine, phenylalanine, tryptophan, or valine. In some embodiments, the transmembrane region is hydrophobic. In some embodiments, the transmembrane region comprises a poly-leucine-alanine sequence. The hydropathy, or hydrophobic or hydrophilic characteristics of a protein or protein segment, can be assessed by any method known in the art, for example the Kyte and Doolittle hydropathy analysis.

Intracellular signaling domain

[0250] The chimeric receptors of the present application comprise an intracellular signaling domain. The intracellular signaling domain of a single chimeric receptor or the intracellular signaling domains of two chimeric receptors in a chimeric receptor system is responsible for activation of at least one of the normal effector functions of the immune effector cell expressing the chimeric receptor(s). The term “effector function” refers to a specialized function of a cell. Effector function of a T cell, for example, may be cytolytic activity or helper activity including the secretion of cytokines. Thus the term “cytoplasmic signaling domain” refers to the portion of a protein which transduces the effector function signal and directs the cell to perform a specialized function. While usually the entire cytoplasmic signaling domain can be employed, in many cases it is not necessary to use the entire chain. To the extent that a truncated portion of the cytoplasmic signaling domain is used, such truncated portion may be used in place of the intact chain as long as it transduces the effector function signal. The term cytoplasmic

signaling domain is thus meant to include any truncated portion of the cytoplasmic signaling domain sufficient to transduce the effector function signal.

[0251] In some embodiments, the intracellular signaling domain comprises a primary intracellular signaling sequence of an immune effector cell. In some embodiments, the chimeric receptor comprises an intracellular signaling domain consisting essentially of a primary intracellular signaling sequence of an immune effector cell. "Primary intracellular signaling sequence" refers to cytoplasmic signaling sequence that acts in a stimulatory manner to induce immune effector functions. In some embodiments, the primary intracellular signaling sequence contains a signaling motif known as immunoreceptor tyrosine-based activation motif, or ITAM. An "ITAM," as used herein, is a conserved protein motif that is generally present in the tail portion of signaling molecules expressed in many immune cells. The motif may comprises two repeats of the amino acid sequence YxxL/I separated by 6-8 amino acids, wherein each x is independently any amino acid, producing the conserved motif YxxL/Ix(6-8)YxxL/I. ITAMs within signaling molecules are important for signal transduction within the cell, which is mediated at least in part by phosphorylation of tyrosine residues in the ITAM following activation of the signaling molecule. ITAMs may also function as docking sites for other proteins involved in signaling pathways. Exemplary ITAM-containing primary cytoplasmic signaling sequences include those derived from CD3ζ, FcR gamma(FCER1G), FcR beta (Fc Epsilon Rib), CD3 gamma, CD3 delta, CD3 epsilon, CD5, CD22, CD79a, CD79b, and CD66d.

[0252] In some embodiments, the primary intracellular signaling sequence is derived from CD3ζ, *i.e.*, the primary intracellular signaling sequence is a CD3ζ intracellular signaling sequence. In some embodiments, the intracellular signaling domain consists of the cytoplasmic signaling domain of CD3ζ. In some embodiments, the primary intracellular signaling sequence is a cytoplasmic signaling domain of wildtype CD3ζ. In some embodiments, the primary intracellular signaling sequence is a functional mutant of the cytoplasmic signaling domain of CD3ζ containing one or more mutations, such as Q65K. In some embodiments, the CD3ζ intracellular signaling sequence comprises the amino acid sequence of SEQ ID NO: 135.

[0253] SEQ ID NO: 135 CD3ζ intracellular signaling sequence

RVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNEL
QKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

[0254] In some embodiments, the intracellular signaling domain comprises an intracellular co-stimulatory sequence. In some embodiments, the intracellular signaling domain consists of an intracellular co-stimulatory sequence. In some embodiments, the intracellular signaling domain does not comprise a primary intracellular signaling sequence of an immune effector cell (*e.g.*, T cell). In some embodiments,

the intracellular signaling domain comprises both a primary intracellular signaling sequence of an immune effector cell (*e.g.*, T cell) and an intracellular co-stimulatory sequence. In some embodiments, the intracellular signaling domain does not comprise an intracellular co-stimulatory sequence. In some embodiments, the first chimeric receptor comprises an intracellular signaling domain comprising a primary intracellular signaling sequence of an immune effector cell (*e.g.*, T cell), and the second chimeric receptor comprises an intracellular signaling domain comprising an intracellular co-stimulatory sequence. In some embodiments, the first chimeric receptor comprises an intracellular signaling domain comprising an intracellular co-stimulatory sequence, and the second chimeric receptor comprises an intracellular signaling domain comprising a primary intracellular signaling sequence of an immune effector cell (*e.g.*, T cell).

[0255] Many immune effector cells require co-stimulation, in addition to stimulation of an antigen-specific signal, to promote cell proliferation, differentiation and survival, as well as to activate effector functions of the cell. In some embodiments, the chimeric receptor comprises at least one intracellular co-stimulatory sequence. The term “intracellular co-stimulatory sequence,” as used herein, refers to at least a portion of a protein that mediates signal transduction within a cell to induce an immune response such as an effector function. The intracellular co-stimulatory sequence of the chimeric receptor described herein can be a cytoplasmic signaling domain from a co-stimulatory protein, which transduces a signal and modulates responses mediated by immune cells, such as T cells, NK cells, macrophages, neutrophils, or eosinophils. “Intracellular co-stimulatory sequence” can be the cytoplasmic portion of a co-stimulatory molecule. The term "co-stimulatory molecule" refers to a cognate binding partner on an immune cell (such as T cell) that specifically binds with a co-stimulatory ligand, thereby mediating a co-stimulatory response by the immune cell, such as, but not limited to, proliferation and survival.

[0256] In some embodiments, the intracellular signaling domain comprises a single intracellular co-stimulatory sequence. In some embodiments, the intracellular signaling domain comprises two or more (such as about any of 2, 3, 4, or more) intracellular co-stimulatory sequences. In some embodiments, the intracellular signaling domain comprises two or more of the same intracellular co-stimulatory sequences, for example, two copies of the intracellular co-stimulatory sequence of CD28. In some embodiments, the intracellular signaling domain comprises two or more intracellular co-stimulatory sequences from different co-stimulatory proteins, such as any two or more co-stimulatory proteins described herein. In some embodiments, the intracellular signaling domain comprises a primary intracellular signaling sequence (such as cytoplasmic signaling domain of CD3ζ) and one or more intracellular co-stimulatory sequences. In some embodiments, the one or more intracellular co-stimulatory sequences and the primary intracellular signaling sequence (such as cytoplasmic signaling domain of CD3ζ) are fused to each other via optional peptide linkers. The primary intracellular signaling sequence, and the one or more

intracellular co-stimulatory sequences may be arranged in any suitable order. In some embodiments, the one or more intracellular co-stimulatory sequences are located between the transmembrane domain and the primary intracellular signaling sequence (such as cytoplasmic signaling domain of CD3ζ). Multiple intracellular co-stimulatory sequences may provide additive or synergistic stimulatory effects.

[0257] Activation of an intracellular co-stimulatory sequence in a host cell (*e.g.*, an immune cell) may induce the cell to increase or decrease the production and secretion of cytokines, phagocytic properties, proliferation, differentiation, survival, and/or cytotoxicity. The intracellular co-stimulatory sequence of any co-stimulatory molecule may be compatible for use in the chimeric receptors described herein. The type(s) of intracellular co-stimulatory sequence is selected based on factors such as the type of the immune effector cells in which the effector molecules would be expressed (*e.g.*, T cells, NK cells, macrophages, neutrophils, or eosinophils) and the desired immune effector function (*e.g.*, ADCC effect). Examples of intracellular co-stimulatory sequences for use in the chimeric receptors can be the cytoplasmic signaling domain of co-stimulatory proteins, including, without limitation, members of the B7/CD28 family (*e.g.*, B7-1/CD80, B7-2/CD86, B7-H1/PD-L1, B7-H2, B7-H3, B7-H4, B7-H6, B7-H7, BTLA/CD272, CD28, CTLA-4, Gi24/VISTA/B7-H5, ICOS/CD278, PD- 1, PD-L2/B7-DC, and PDCD6); members of the TNF superfamily (*e.g.*,4- 1BB/TNFSF9/4-1BB, 4-1BB Ligand/TNFSF9, BAFF/BLyS/TNFSF13B, BAFF R/TNFRSF13C, CD27/TNFRSF7, CD27 Ligand/TNFSF7, CD30/TNFRSF8, CD30 Ligand/TNFSF8, CD40/TNFRSF5, CD40/TNFSF5, CD40 Ligand/TNFSF5, DR3/TNFRSF25, GITR/TNFRSF18, GITR Ligand/TNFSF18, HVEM/TNFRSF14, LIGHT/TNFSF14, Lymphotoxin-alpha/TNF-beta, OX40/TNFRSF4, OX40 Ligand/TNFSF4, RELT/TNFRSF19L, TACI/TNFRSF13B, TL1A/TNFSF15, TNF-alpha, and TNF RII/TNFRSF1B); members of the SLAM family (*e.g.*, 2B4/CD244/SLAMF4, BLAME/SLAMF8, CD2, CD2F-10/SLAMF9, CD48/SLAMF2, CD58/LFA-3, CD84/SLAMF5, CD229/SLAMF3, CRACC/SLAMF7, NTB-A/SLAMF6, and SLAM/CD150); and any other co-stimulatory molecules, such as CD2, CD7, CD53, CD82/Kai-1, CD90/Thy1, CD96, CD160, CD200, CD300a/LMIR1, HLA Class I, HLA- DR, Ikaros, Integrin alpha 4/CD49d, Integrin alpha 4 beta 1, Integrin alpha 4 beta 7/LPAM-1, LAG-3, TCL1A, TCL1B, CRTAM, DAP12, Dectin-1/CLEC7A, DPPIV/CD26, EphB6, TIM-1/KIM-1/HAVCR, TIM-4, TSLP, TSLP R, lymphocyte function associated antigen-1 (LFA-1), and NKG2C.

[0258] In some embodiments, the one or more intracellular co-stimulatory sequences are selected from the group consisting of CD27, CD28, 4-1BB, OX40, CD30, CD40, CD3, lymphocyte function-associated antigen-1(LFA-1), CD2, CD7, LIGHT, NKG2C, B7-H3 and ligands that specially bind to CD83.

[0259] In some embodiments, the intracellular signaling domain in the chimeric receptor of the present application comprises an intracellular co-stimulatory sequence derived from CD28. In some embodiments, the intracellular signaling domain comprises a cytoplasmic signaling domain of CD3ζ and

an intracellular co-stimulatory sequence of CD28. In some embodiments, the intracellular signaling domain comprises an intracellular co-stimulatory sequence of CD28 comprising the amino acid sequence of RSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAP PRDFAAYRS (SEQ ID NO: 136).

[0260] In some embodiments, the intracellular signaling domain in the CAR of the present application comprises an intracellular co-stimulatory sequence derived from 4-1BB (*i.e.*, CD137). In some embodiments, the intracellular signaling domain comprises a cytoplasmic signaling domain of CD3ζ and an intracellular co-stimulatory sequence of 4-1BB. In some embodiments, the intracellular signaling domain comprises an intracellular co-stimulatory sequence of 4-1BB comprising the amino acid sequence of KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEE EEGGCEL (SEQ ID NO: 137).

[0261] In some embodiments, the intracellular signaling domain in the CAR of the present application comprises an intracellular co-stimulatory sequence derived from ICOS. In some embodiments, the intracellular signaling domain comprises a cytoplasmic signaling domain of CD3ζ and an intracellular co-stimulatory sequence of ICOS. In some embodiments, the intracellular signaling domain comprises an intracellular co-stimulatory sequence of ICOS comprising the amino acid sequence of CWLTKKKYSSSVHDPNGEYMFMRAVNTAKKSR LTDVTL (SEQ ID NO: 138).

[0262] In some embodiments, the intracellular signaling domain in the CAR of the present application comprises an intracellular co-stimulatory sequence of CD28 and an intracellular co-stimulatory sequence of 4-1BB. In some embodiments, the intracellular signaling domain comprises a cytoplasmic signaling domain of CD3ζ, an intracellular co-stimulatory sequence of CD28, and an intracellular co-stimulatory sequence of 4-1BB.

[0263] In some embodiments, the intracellular signaling domain comprises a polypeptide comprising a cytoplasmic signaling domain of CD3ζ. In some embodiments, the intracellular signaling domain comprises a polypeptide comprising an intracellular co-stimulatory sequence of CD28. In some embodiments, the intracellular signaling domain comprises a polypeptide comprising an intracellular co-stimulatory sequence of 4-1BB. In some embodiments, the intracellular signaling domain comprises a polypeptide comprising from the N-terminus to the C-terminus: an intracellular co-stimulatory sequence of 4-1BB, and a cytoplasmic signaling domain of CD3ζ. In some embodiments, the intracellular signaling domain comprises a polypeptide comprising from the N-terminus to the C-terminus: an intracellular co-stimulatory sequence of CD28, an intracellular co-stimulatory sequence of 4-1BB, and a cytoplasmic signaling domain of CD3ζ.

[0264] Also within the scope of the present disclosure are variants of any of the intracellular co-stimulatory sequences described herein, such that the intracellular co-stimulatory sequence is capable of modulating the immune response of the immune cell. In some embodiments, the intracellular co-

stimulatory sequences comprises up to 10 amino acid residue variations (*e.g.*, 1, 2, 3, 4, 5, or 8) as compared to a wild-type counterpart. Such intracellular co-stimulatory sequences comprising one or more amino acid variations may be referred to as variants. Mutation of amino acid residues of the intracellular co-stimulatory sequence may result in an increase in signaling transduction and enhanced stimulation of immune responses relative to intracellular co-stimulatory sequences that do not comprise the mutation. Mutation of amino acid residues of the intracellular co-stimulatory sequence may result in a decrease in signaling transduction and reduced stimulation of immune responses relative to intracellular co-stimulatory sequences that do not comprise the mutation.

Hinge region

[0265] The chimeric receptors of the present application may comprise a hinge domain that is located between the extracellular domain and the transmembrane domain. A hinge domain is an amino acid segment that is generally found between two domains of a protein and may allow for flexibility of the protein and movement of one or both of the domains relative to one another. Any amino acid sequence that provides such flexibility and movement of the extracellular domain relative to the transmembrane domain of the effector molecule can be used.

[0266] The hinge domain may contain about 10-100 amino acids, *e.g.*, about any one of 15-75 amino acids, 20-50 amino acids, or 30-60 amino acids. In some embodiments, the hinge domain may be at least about any one of 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 35, 40, 45, 50, 55, 60, 65, 70, or 75 amino acids in length.

[0267] In some embodiments, the hinge domain is a hinge domain of a naturally occurring protein. Hinge domains of any protein known in the art to comprise a hinge domain are compatible for use in the chimeric receptors described herein. In some embodiments, the hinge domain is at least a portion of a hinge domain of a naturally occurring protein and confers flexibility to the chimeric receptor. In some embodiments, the hinge domain is derived from CD8, such as CD8α. In some embodiments, the hinge domain is a portion of the hinge domain of CD8α, *e.g.*, a fragment containing at least 15 (*e.g.*, 20, 25, 30, 35, or 40) consecutive amino acids of the hinge domain of CD8α. In some embodiments, the hinge domain of CD8α comprises the amino acid sequence of TTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACD (SEQ ID NO: 139). In some embodiments, the hinge domain is derived from CD28. In some embodiments, the hinge domain of CD28 comprises the amino acid sequence of IEVMYPPPYLDNEKSNGTIIHVKGKHLCPSPLFPGPSKP (SEQ ID NO: 140).

[0268] Hinge domains of antibodies, such as an IgG, IgA, IgM, IgE, or IgD antibodies, are also compatible for use in the chimeric receptor systems described herein. In some embodiments, the hinge

domain is the hinge domain that joins the constant domains CH1 and CH2 of an antibody. In some embodiments, the hinge domain is of an antibody and comprises the hinge domain of the antibody and one or more constant regions of the antibody. In some embodiments, the hinge domain comprises the hinge domain of an antibody and the CH3 constant region of the antibody. In some embodiments, the hinge domain comprises the hinge domain of an antibody and the CH2 and CH3 constant regions of the antibody. In some embodiments, the antibody is an IgG, IgA, IgM, IgE, or IgD antibody. In some embodiments, the antibody is an IgG antibody. In some embodiments, the antibody is an IgG1, IgG2, IgG3, or IgG4 antibody. In some embodiments, the hinge region comprises the hinge region and the CH2 and CH3 constant regions of an IgG1 antibody. In some embodiments, the hinge region comprises the hinge region and the CH3 constant region of an IgG1 antibody.

[0269] Non-naturally occurring peptides may also be used as hinge domains for the chimeric receptors described herein. In some embodiments, the hinge domain between the C-terminus of the extracellular ligand-binding domain of an Fc receptor and the N- terminus of the transmembrane domain is a peptide linker, such as a (GxS)n linker, wherein x and n, independently can be an integer between 3 and 12, including 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or more. In some embodiments, the peptide linker comprises the amino acid sequence of GGGGSGGGGSGGGGS (SEQ ID NO: 141).

Signal peptide

[0270] The chimeric receptors of the present application may comprise a signal peptide (also known as a signal sequence) at the N-terminus of the polypeptide. In general, signal peptides are peptide sequences that target a polypeptide to the desired site in a cell. In some embodiments, the signal peptide targets the effector molecule to the secretory pathway of the cell and will allow for integration and anchoring of the effector molecule into the lipid bilayer. Signal peptides including signal sequences of naturally occurring proteins or synthetic, non-naturally occurring signal sequences may be compatible for use in the chimeric receptors described herein. In some embodiments, the signal peptide is derived from a molecule selected from the group consisting of CD8, GM-CSF receptor α, and IgG1 heavy chain. In some embodiments, the signal peptide is derived from CD8, such as CD8α. In some embodiments, the signal peptide of CD8α comprises the amino acid sequence of MALPVTALLLPLALLLHAARP (SEQ ID NO: 142).

C. Immune effector cell engagers

[0271] One aspect of the present application provides an immune effector cell engager comprising: (a) a target cell binding domain comprising an anti-CLL1 sdAb (such as any one of the anti-CLL1 sdAbs described herein), and (b) an immune effector cell binding domain comprising an antigen-binding fragment that specifically binds to an antigen on an immune effector cell. In some embodiments, the anti-CLL1 sdAb

comprises any one of the following: (1) a CDR1 comprising the amino acid sequence of SEQ ID NO: 4, a CDR2 comprising the amino acid sequence of SEQ ID NO: 6, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 8, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; (2) a CDR1 comprising the amino acid sequence of SEQ ID NO: 11, a CDR2 comprising the amino acid sequence of SEQ ID NO: 13, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 15, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; (3) a CDR1 comprising the amino acid sequence of SEQ ID NO: 18, a CDR2 comprising the amino acid sequence of SEQ ID NO: 20, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 22, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; (4) a CDR1 comprising the amino acid sequence of SEQ ID NO: 25, a CDR2 comprising the amino acid sequence of SEQ ID NO: 27, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 29, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; (5) a CDR1 comprising the amino acid sequence of SEQ ID NO: 32, a CDR2 comprising the amino acid sequence of SEQ ID NO: 34, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 36, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; (6) a CDR1 comprising the amino acid sequence of SEQ ID NO: 39, a CDR2 comprising the amino acid sequence of SEQ ID NO: 41, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 43, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; (7) a CDR1 comprising the amino acid sequence of SEQ ID NO: 46, a CDR2 comprising the amino acid sequence of SEQ ID NO: 48, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 50, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; (8) a CDR1 comprising the amino acid sequence of SEQ ID NO: 53, a CDR2 comprising the amino acid sequence of SEQ ID NO: 55, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 57, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; (9) a CDR1 comprising the amino acid sequence of SEQ ID NO: 60, a CDR2 comprising the amino acid sequence of SEQ ID NO: 62, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 64, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; (10) a CDR1 comprising the amino acid sequence of SEQ ID NO: 67, a CDR2 comprising the amino acid sequence of SEQ ID NO: 69, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 71, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; (11) a CDR1 comprising the amino acid sequence of SEQ ID NO: 74, a CDR2 comprising the amino acid sequence of SEQ ID NO: 76, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 78, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; (12) a CDR1 comprising the amino acid sequence of SEQ ID NO: 81, a CDR2 comprising the amino acid sequence of SEQ ID NO: 83, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 85, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; (13) a CDR1 comprising the amino acid sequence of SEQ ID NO: 88, a CDR2

comprising the amino acid sequence of SEQ ID NO: 90, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 92, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; (14) a CDR1 comprising the amino acid sequence of SEQ ID NO: 151, a CDR2 comprising the amino acid sequence of SEQ ID NO: 153, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 155, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; (15) a CDR1 comprising the amino acid sequence of SEQ ID NO: 158, a CDR2 comprising the amino acid sequence of SEQ ID NO: 160, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 162, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; or (16) a CDR1 comprising the amino acid sequence of SEQ ID NO: 165, a CDR2 comprising the amino acid sequence of SEQ ID NO: 167, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 169, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs. In some embodiments, the anti-CLL1 sdAb comprises a V_HH domain comprising the amino acid sequence of any one of SEQ ID NOs: 94-106 and 171-173, or a variant thereof comprising an amino acid sequence having at least about 95% sequence identity to the amino acid sequence of any one of SEQ ID NOs: 94-106 and 171-173. In some embodiments, the antigen-binding fragment in the immune effector cell binding domain is a Fab, scFv, or sdAb. In some embodiments, the target cell binding domain is fused to the N-terminus of the immune effector cell binding domain. In some embodiments, the target cell binding domain is fused to the C-terminus of the immune effector cell binding domain. In some embodiments, the target cell binding domain is fused to the immune effector cell binding domain via a peptide linker. In some embodiments, the immune effector cell binding domain specifically binds to an antigen selected from the group consisting of CD3γ, CD3δ, CD3ε, CD3ζ, CD28, OX40, GITR, CD137, CD27, CD40L, and HVEM.

[0272] In some embodiments, there is provided a T cell engager comprising: (a) a target cell binding domain comprising an anti-CLL1 sdAb (such as any one of the anti-CLL1 sdAbs described herein), and (b) a T cell binding domain comprising an antigen-binding fragment that specifically binds to an antigen on a T cell. In some embodiments, the anti-CLL1 sdAb comprises any one of the following: (1) a CDR1 comprising the amino acid sequence of SEQ ID NO: 4, a CDR2 comprising the amino acid sequence of SEQ ID NO: 6, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 8, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; (2) a CDR1 comprising the amino acid sequence of SEQ ID NO: 11, a CDR2 comprising the amino acid sequence of SEQ ID NO: 13, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 15, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; (3) a CDR1 comprising the amino acid sequence of SEQ ID NO: 18, a CDR2 comprising the amino acid sequence of SEQ ID NO: 20, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 22, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; (4) a CDR1 comprising the amino acid sequence of SEQ ID NO: 25, a CDR2 comprising the amino

acid sequence of SEQ ID NO: 27, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 29, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; (5) a CDR1 comprising the amino acid sequence of SEQ ID NO: 32, a CDR2 comprising the amino acid sequence of SEQ ID NO: 34, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 36, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; (6) a CDR1 comprising the amino acid sequence of SEQ ID NO: 39, a CDR2 comprising the amino acid sequence of SEQ ID NO: 41, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 43, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; (7) a CDR1 comprising the amino acid sequence of SEQ ID NO: 46, a CDR2 comprising the amino acid sequence of SEQ ID NO: 48, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 50, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; (8) a CDR1 comprising the amino acid sequence of SEQ ID NO: 53, a CDR2 comprising the amino acid sequence of SEQ ID NO: 55, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 57, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; (9) a CDR1 comprising the amino acid sequence of SEQ ID NO: 60, a CDR2 comprising the amino acid sequence of SEQ ID NO: 62, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 64, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; (10) a CDR1 comprising the amino acid sequence of SEQ ID NO: 67, a CDR2 comprising the amino acid sequence of SEQ ID NO: 69, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 71, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; (11) a CDR1 comprising the amino acid sequence of SEQ ID NO: 74, a CDR2 comprising the amino acid sequence of SEQ ID NO: 76, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 78, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; (12) a CDR1 comprising the amino acid sequence of SEQ ID NO: 81, a CDR2 comprising the amino acid sequence of SEQ ID NO: 83, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 85, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; (13) a CDR1 comprising the amino acid sequence of SEQ ID NO: 88, a CDR2 comprising the amino acid sequence of SEQ ID NO: 90, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 92, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; (14) a CDR1 comprising the amino acid sequence of SEQ ID NO: 151, a CDR2 comprising the amino acid sequence of SEQ ID NO: 153, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 155, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; (15) a CDR1 comprising the amino acid sequence of SEQ ID NO: 158, a CDR2 comprising the amino acid sequence of SEQ ID NO: 160, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 162, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; or (16) a CDR1 comprising the amino acid sequence of SEQ ID NO: 165, a CDR2 comprising the amino acid sequence of SEQ ID NO: 167, and a CDR3 comprising the amino acid sequence of SEQ ID

NO: 169, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs. In some embodiments, the anti-CLL1 sdAb comprises a V_HH domain comprising the amino acid sequence of any one of SEQ ID NOs: 94-106 and 171-173, or a variant thereof comprising an amino acid sequence having at least about 95% sequence identity to the amino acid sequence of any one of SEQ ID NOs: 94-106 and 171-173. In some embodiments, the antigen-binding fragment in the T cell binding domain is a Fab, scFv, or sdAb. In some embodiments, the target cell binding domain is fused to the N-terminus of the T cell binding domain. In some embodiments, the target cell binding domain is fused to the C-terminus of T cell binding domain. In some embodiments, the target cell binding domain is fused to the T cell binding domain via a peptide linker. In some embodiments, the immune effector cell binding domain specifically binds to an antigen selected from the group consisting of CD3γ, CD3δ, CD3ε, CD3ζ, CD28, OX40, GITR, CD137, CD27, CD40L, and HVEM. In some embodiments, the immune effector cell binding domain comprises an antigen-binding fragment that specifically binds to CD3, such as CD3ε.

Target cell binding domain

[0273] The immune effector cell engagers described herein comprise a target cell binding domain comprising an anti-CLL1 sdAb. In some embodiments, the target cell binding domain consists of an anti-CLL1 sdAb. In some embodiments, the target cell binding domain comprises an anti-CLL1 sdAb and one or more antigen-binding fragments derived from single-domain antibodies or four-chain antibodies that specifically bind to an antigen on a target cell. In some embodiments, the target cell is a tumor cell or a myeloid cell.

[0274] In some embodiments, the target cell binding domain has two or more (such as about any one of 2, 3, 4, 5, 6, or more) antigen binding fragments such as single-domain antibodies. In some embodiments, the multivalent target cell binding domain targets CLL1 only, and comprises two or more antigen binding fragments for CLL1. In some embodiments, the multivalent target cell binding domain targets more than one antigen, and the multivalent target cell binding domain comprises two or more antigen binding fragments for at least one antigen. The antigen binding fragments specific for the same antigen may bind to the same epitope of the antigen or bind to different epitopes of the antigen. The antigen binding fragments specific for the same antigen may comprise the same or different single-domain antibodies.

[0275] In some embodiments, the target cell binding domain comprises a plurality of anti-CLL1 sdAbs. In some embodiments, the plurality of the anti-CLL1 sdAb is fused to each other via peptide bonds or peptide linkers. In some embodiments, each peptide linker is no more than about 50 (such as no more than about any one of 35, 25, 20, 15, 10, or 5) amino acids long.

[0276] In some embodiments, the target cell binding domain can specifically bind to two or more (such as about any one of 2, 3, 4, 5, 6, or more) different antigens. In some embodiments, the multispecific target

cell binding domain has one antigen binding fragments for each antigen. In some embodiments, the multispecific target cell binding domain has more than two antigen binding fragments for at least one antigen. Each antigen binding fragment may comprise a single-domain antibody.

[0277] Depending on the desired antigens to be targeted, the target cell binding domain can be engineered to include the appropriate single-domain antibodies that are specific to the desired antigens. In some embodiments, the target cell binding domain comprises an anti-CLL1 sdAb and an anti-CD33 sdAb. The antigen binding fragments (such as sdAbs) can be arranged in any suitable order. For example, a first sdAb is fused to the N-terminus or the C-terminus of a second sdAb. A suitable peptide linker may be placed between different sdAbs to avoid steric hindrance between the sdAbs.

Immune effector cell binding domain

[0278] The immune effector cell engagers described herein comprise an immune effector cell binding domain. The immune effector cell binding domain comprises an antigen-binding fragment that specifically binds to an antigen on an immune effector cell. Immune effector cells include, but are not limited to, T cells and NK cells.

[0279] In some embodiments, the immune effector cell binding domain specifically binds to CD3, such as human CD3. "CD3" is known in the art as a multi-protein complex of six chains (see, Abbas and Lichtman, 2003; Janeway *et al.*, p172 and 178, 1999). In mammals, the complex comprises a CD3 gamma chain, a CD3 delta chain, two CD3 epsilon chains, and a homodimer of CD3 zeta chains. CD3 as used herein may be from various animal species, including human, primate, mouse, rat, or other mammals. In some embodiments, the immune effector cell binding domain comprises an antigen-binding fragment that specifically binds to an individual CD3 chain, such as CD3 gamma chain, CD3 delta chain, or CD3 epsilon chain. In some embodiments, the antigen-binding fragment specifically binds to a complex formed from two or more individual CD3 chains (*e.g.*, a complex of more than one CD3 epsilon chains, a complex of a CD3 gamma and CD3 epsilon chain, a complex of a CD3 delta and CD3 epsilon chain). In some embodiments, the antigen-binding fragment specifically binds to a CD3 epsilon chain.

[0280] The antigen-binding fragment targeting CD3 can be of any suitable antigen-binding fragments, including but not limited to Fab, scFv, and sdAb (*e.g.*, V_HH). In some embodiments, the antigen-binding fragment is murine, camelid, chimeric, human or humanized. The antigen-binding fragment can be designed based on any known CD3 antibodies in the art, including, but not limited to, SP34 mouse monoclonal antibody, (see, for example, Pressano, S. The EMBO J. 4:337-344, 1985; Alarcon, B. EMBO J. 10:903-912, 1991; Salmeron A. et al., J. Immunol. 147:3047-52, 1991; Yoshino N. et al., Exp. Anim 49:97-110, 2000; Conrad M L. et al., Cytometry 71A:925-33, 2007; and Yang et al., J. Immunol. 137:1097-1100: 1986), Cris-7 monoclonal antibody (Reinherz, E. L. et al. (eds.), Leukocyte typing II, Springer Verlag, New York,

(1986)), BC3 monoclonal antibody (Anasetti et al. (1990) J. Exp. Med. 172:1691), OKT3 (Ortho multicenter Transplant Study Group (1985) N. Engl. J. Med. 313:337) and derivatives thereof such as OKT3 ala-ala (Herold et al. (2003) J. Clin. Invest. 11:409), visilizumab (Carpenter et al. (2002) Blood 99:2712), 145-2C11 monoclonal antibody (Hirsch et al. (1988) J. Immunol. 140: 3766), UCHT-1 (Beverley, P C and Callard, R. E. (1981) Eur. J. Immunol. 11: 329-334), anti-CD3 sdAbs (such as 60E11 and 117G03) described in WO2016180982, and CD3 binding molecules described in WO2004/106380; WO2004/106381; WO2010/037838; WO2008/119567; WO2007/042261; WO2010/0150918; the contents of each of the references are incorporated herein by reference in their entireties. In some embodiments, the anti-CD3 antigen-binding fragment is derived from OKT3, L2K, UCHT1, 60E11 or 117G03. In some embodiments, the anti-CD3 antigen-binding fragment is an scFv derived from OKT3, L2K or UCHT. In some embodiments, the anti-CD3 antigen-binding fragment is a V_HH derived from 60E11 or 117G03. In some embodiments, the anti-CD3 antigen-binding fragment is derived from an antibody that binds to the same epitope as OKT3, L2K, UCHT1, 60E11 or 117G03. In some embodiments, the anti-CD3 antigen-binding fragment is derived from an antibody that specifically binds to CD3 competitively with OKT3, L2K, UCHT1, 60E11 or 117G03.

Signal peptide

[0281] The immune effector cell engagers of the present application may comprise a signal peptide (also known as a signal sequence) at the N-terminus of the polypeptide. In general, signal peptides are peptide sequences that target a polypeptide to the desired site in a cell. In some embodiments, the signal peptide targets the immune effector cell engager to the secretory pathway of the cell and will allow secretion of the immune effector cell engager into the cell culture media. Signal peptides including signal sequences of naturally occurring proteins or synthetic, non-naturally occurring signal sequences. In some embodiments, the signal peptide is derived from a human albumin signal peptide. In some embodiments, the signal peptide is derived from a human azurocidin secretion signal.

Peptide linkers

[0282] The target cell binding domain and the immune effector cell binding domain may be fused to each other via a peptide linker. In some embodiments, the target cell binding domain and the immune effector cell binding domain are directly fused to each other without any peptide linker.

[0283] In some embodiments, the various antigen-binding fragments (such as sdAbs) in the multispecific or multivalent target cell binding domain are fused to each other via peptide linker(s). In some embodiments, the antigen-binding fragments (such as sdAbs) are directly fused to each other without any peptide linkers. The peptide linkers connecting different antigen-binding fragments (such as sdAbs) may be the same or different.

[0284] Each peptide linker in an immune effector cell engager may have the same or different length and/or sequence depending on the structural and/or functional features of the antigen-binding fragments (such as sdAbs) and/or the various domains. Each peptide linker may be selected and optimized independently. The length, the degree of flexibility and/or other properties of the peptide linker(s) used in the immune effector cell engagers may have some influence on properties, including but not limited to the affinity, specificity or avidity for one or more particular antigens or epitopes. For example, longer peptide linkers may be selected to ensure that two adjacent domains do not sterically interfere with one another. For example, in a multivalent or multispecific target cell binding domain that comprises sdAbs directed against a multimeric antigen, the length and flexibility of the peptide linkers are preferably such that it allows each antigen-binding fragment (such as sdAb) to bind to the antigenic determinant on each of the subunits of the multimer.

[0285] In some embodiment, a peptide linker comprises flexible residues (such as glycine and serine) so that the adjacent domains are free to move relative to each other. For example, a $(GGGGS)_3$ linker (SEQ ID NO: 141) can be a suitable peptide linker between the target cell binding domain and the immune effector cell binding domain. In some embodiments, the peptide linker is no more than about 50 (such as no more than about any one of 35, 25, 20, 15, 10, or 5) amino acids long.

[0286] The peptide linker can be of any suitable length. In some embodiments, the peptide linker is at least about any of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 50, 75, 100 or more amino acids long. In some embodiments, the peptide linker is no more than about any of 100, 75, 50, 40, 35, 30, 25, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5 or fewer amino acids long. In some embodiments, the length of the peptide linker is any of about 1 amino acid to about 10 amino acids, about 1 amino acids to about 20 amino acids, about 1 amino acid to about 30 amino acids, about 5 amino acids to about 15 amino acids, about 10 amino acids to about 25 amino acids, about 5 amino acids to about 30 amino acids, about 10 amino acids to about 30 amino acids long, about 30 amino acids to about 50 amino acids, about 50 amino acids to about 100 amino acids, or about 1 amino acid to about 100 amino acids.

[0287] The peptide linker may have a naturally occurring sequence, or a non-naturally occurring sequence. For example, a sequence derived from the hinge region of heavy chain only antibodies may be used as the linker. *See,* for example, WO1996/34103. In some embodiments, the peptide linker is a flexible linker. Exemplary flexible linkers include glycine polymers $(G)_n$, glycine-serine polymers (including, for example, $(GS)_n$, $(GSGGS)_n$, $(GGGS)_n$, and $(GGGGS)_n$, where n is an integer of at least one), glycine-alanine polymers, alanine-serine polymers, and other flexible linkers known in the art. In some embodiments, the peptide linker comprises the amino acid sequence GGGGS (SEQ ID NO: 143), $(GGGGS)_2$ (SEQ ID NO: 144), $(GGGGS)_3$ (SEQ ID NO: 141), $(GGGGS)_4$ (SEQ ID NO: 182), $(GGGGS)_5$ (SEQ ID NO: 183),

$(GGGS)_2$ (SEQ ID NO: 145), $(GGGS)_4$ (SEQ ID NO: 146), or GSTSGSGKPGSGEGSTKG (SEQ ID NO: 147).

D. Immunoconjugates

[0288] In one aspect, the present application provides immunoconjugates comprising an anti-CLL1 sdAb (such as any one of the anti-CLL1 sdAbs described herein) and an effector molecule. Exemplary effector molecules include, but are not limited to, a drug, a toxin, a radioisotope, a protein, a peptide, a nucleic acid, and a label.

[0289] In some embodiments, there is provided an immunoconjugate comprising an anti-CLL1 sdAb (such as any one of the anti-CLL1 sdAbs described herein) conjugated to one or more cytotoxic agents, such as chemotherapeutic agents or drugs, growth inhibitory agents, toxins (*e.g.*, protein toxins, enzymatically active toxins of bacterial, fungal, plant, or animal origin, or fragments thereof), or radioactive isotopes.

[0290] In some embodiments, an immunoconjugate is an antibody-drug conjugate (ADC) in which an anti-CLL1 sdAb is conjugated to one or more drugs, including but not limited to a maytansinoid (*see* U.S. Patent Nos. 5,208,020, 5,416,064 and European Patent EP 0 425 235 B1); an auristatin such as monomethylauristatin drug moieties DE and DF (MMAE and MMAF) (*see* U.S. Patent Nos. 5,635,483 and 5,780,588, and 7,498,298); a dolastatin; a calicheamicin or derivative thereof (see U.S. Patent Nos. 5,712,374, 5,714,586, 5,739,116, 5,767,285, 5,770,701, 5,770,710, 5,773,001, and 5,877,296; Hinman et al., *Cancer Res.* 53:3336-3342 (1993); and Lode et al., *Cancer Res.* 58:2925-2928 (1998)); an anthracycline such as daunomycin or doxorubicin (*see* Kratz et al., *Current Med. Chem.* 13:477-523 (2006); Jeffrey et al., *Bioorganic & Med. Chem. Letters* 16:358-362 (2006); Torgov et al., *Bioconj. Chem.* 16:717-721 (2005); Nagy et al., *Proc. Natl. Acad. Sci. USA* 97:829-834 (2000); Dubowchik et al., *Bioorg. & Med. Chem. Letters* 12:1529-1532 (2002); King et al., *J. Med. Chem.* 45:4336-4343 (2002); and U.S. Patent No. 6,630,579); methotrexate; vindesine; a taxane such as docetaxel, paclitaxel, larotaxel, tesetaxel, and ortataxel; a trichothecene; and CC1065.

[0291] In some embodiments, an immunoconjugate comprises an anti-CLL1 sdAb as described herein conjugated to an enzymatically active toxin or fragment thereof, including but not limited to diphtheria A chain, nonbinding active fragments of diphtheria toxin, exotoxin A chain (from Pseudomonas aeruginosa), ricin A chain, abrin A chain, modeccin A chain, alpha-sarcin, Aleurites fordii proteins, dianthin proteins, Phytolaca americana proteins (PAPI, PAPII, and PAP-S), momordica charantia inhibitor, curcin, crotin, sapaonaria officinalis inhibitor, gelonin, mitogellin, restrictocin, phenomycin, enomycin, and the tricothecenes.

[0292] In some embodiments, an immunoconjugate comprises an anti-CLL1 sdAb as described herein conjugated to a radioactive atom to form a radioconjugate. A variety of radioactive isotopes are available for the production of radioconjugates. Examples include At^{211}, I^{131}, I^{125}, Y^{90}, Re^{186}, Re^{188}, Sm^{153}, Bi^{212}, P^{32}, Pb^{212} and radioactive isotopes of Lu. When the radioconjugate is used for detection, it may comprise a radioactive atom for scintigraphic studies, for example tc99m or I123, or a spin label for nuclear magnetic resonance (NMR) imaging (also known as magnetic resonance imaging, "MRI"), such as iodine-123, iodine-131, indium-111, fluorine-19, carbon-13, nitrogen-15, oxygen-17, gadolinium, manganese or iron.

[0293] Conjugates of an antibody and cytotoxic agent may be made using a variety of bifunctional protein coupling agents such as N-succinimidyl-3-(2-pyridyldithio) propionate (SPDP), succinimidyl-4-(N-maleimidomethyl) cyclohexane-1-carboxylate (SMCC), iminothiolane (IT), bifunctional derivatives of imidoesters (such as dimethyl adipimidate HCl), active esters (such as disuccinimidyl suberate), aldehydes (such as glutaraldehyde), bis-azido compounds (such as bis (p-azidobenzoyl) hexanediamine), bis-diazonium derivatives (such as bis-(p-diazoniumbenzoyl)-ethylenediamine), diisocyanates (such as toluene 2,6-diisocyanate), and bis-active fluorine compounds (such as 1,5-difluoro-2,4-dinitrobenzene). For example, a ricin immunotoxin can be prepared as described in Vitetta et al., *Science* 238:1098 (1987). Carbon-14-labeled 1-isothiocyanatobenzyl-3-methyldiethylene triaminepentaacetic acid (MX-DTPA) is an exemplary chelating agent for conjugation of radionucleotide to the antibody. See WO94/11026. The linker may be a "cleavable linker" facilitating release of a cytotoxic drug in the cell. For example, an acid-labile linker, peptidase-sensitive linker, photolabile linker, dimethyl linker or disulfide-containing linker (Chari et al., *Cancer Res.* 52:127-131 (1992); U.S. Patent No. 5,208,020) may be used.

[0294] The immunoconjugates or ADCs herein expressly contemplate, but are not limited to such conjugates prepared with cross-linker reagents including, but not limited to, BMPS, EMCS, GMBS, HBVS, LC-SMCC, MBS, MPBH, SBAP, SIA, SIAB, SMCC, SMPB, SMPH, sulfo-EMCS, sulfo-GMBS, sulfo-KMUS, sulfo-MBS, sulfo-SIAB, sulfo-SMCC, and sulfo-SMPB, and SVSB (succinimidyl-(4-vinylsulfone)benzoate) which are commercially available (*e.g.*, from Pierce Biotechnology, Inc., Rockford, IL., U.S.A).

[0295] In some embodiments, any of the anti-CLL1 sdAbs provided herein is useful for detecting the presence of CLL1 in a biological sample. The term "detecting" as used herein encompasses quantitative or qualitative detection. In certain embodiments, a biological sample is blood, serum or other liquid samples of biological origin. In some embodiments, a biological sample comprises a cell or tissue.

[0296] In some embodiments, the present application provides an immunoconjugate comprising an anti-CLL1 sdAb (such as any one of the anti-CLL1 sdAbs described herein) and a label. In some embodiments, the label is conjugated to the anti-CLL1 sdAb. In some embodiments, there is provided a

method of detecting CLL1 in a cell, comprising contacting the cell with the immunoconjugate. In some embodiments, a method of detecting the presence of CLL1 in a biological sample is provided. In some embodiments, the method comprises detecting the presence of CLL1 protein in a biological sample. In some embodiments, the CLL1 is human CLL1. In some embodiments, the method comprises contacting the biological sample with the immunoconjugate under conditions permissive for binding of the anti-CLL1 sdAb to CLL1, and detecting signal from the label. Such method may be an *in vitro* or *in vivo* method. In some embodiments, there is provided a method of diagnosing a disease associated with CLL1 expression (*e.g.*, acute myeloid leukemia) in an individual, comprising administering to the individual the immunoconjugate, and detecting the label in the individual. In some embodiments, the immunoconjugate is used to select subjects eligible for therapy with any of the anti-CLL1 therapeutic agents described herein (*e.g.*, anti-CLL1 sdAb, chimeric receptor, immune effector cell engager, and engineered immune cell), wherein CLL1 is a biomarker for selection of patients.

[0297] In some embodiments, labeled anti-CLL1 sdAbs are provided. Labels include, but are not limited to, labels or moieties that are detected directly (such as fluorescent, chromophoric, electron-dense, chemiluminescent, and radioactive labels), as well as moieties, such as enzymes or ligands, that are detected indirectly, *e.g.*, through an enzymatic reaction or molecular interaction. Exemplary labels include, but are not limited to, the radioisotopes ^{32}P, ^{14}C, ^{125}I, ^{3}H, and ^{131}I, fluorophores such as rare earth chelates or fluorescein and its derivatives, rhodamine and its derivatives, dansyl, umbelliferone, luceriferases, *e.g.*, firefly luciferase and bacterial luciferase (U.S. Patent No. 4,737,456), luciferin, 2,3-dihydrophthalazinediones, horseradish peroxidase (HRP), alkaline phosphatase, β-galactosidase, glucoamylase, lysozyme, saccharide oxidases, *e.g.*, glucose oxidase, galactose oxidase, and glucose-6-phosphate dehydrogenase, heterocyclic oxidases such as uricase and xanthine oxidase, coupled with an enzyme that employs hydrogen peroxide to oxidize a dye precursor such as HRP, lactoperoxidase, or microperoxidase, biotin/avidin, spin labels, bacteriophage labels, stable free radicals, and the like.

E. Features of antibody moieties

[0298] In some embodiments, any antibody moiety in the anti-CLL1 constructs described herein may incorporate any of the features, singly or in combination, as described in sections 1-7 below.

1. Antibody Affinity

[0299] In some embodiments, an antibody moiety provided herein has a dissociation constant (Kd) of ≤ 1μM, ≤ 100 nM, ≤ 10 nM, ≤ 1 nM, ≤ 0.1 nM, ≤ 0.01 nM, or ≤ 0.001 nM (*e.g.* 10^{-8} M or less, *e.g.* from 10^{-8} M to 10^{-13} M, *e.g.*, from 10^{-9} M to 10^{-13} M).

[0300] In some embodiments, Kd is measured by a radiolabeled antigen binding assay (RIA) performed with the Fab version or V_HH fragment of an antibody of interest and its antigen as described by

the following assay. For example, solution binding affinity of Fabs for antigen is measured by equilibrating Fab with a minimal concentration of (^{125}I)-labeled antigen in the presence of a titration series of unlabeled antigen, then capturing bound antigen with an anti-Fab antibody-coated plate (see, *e.g.*, Chen et al., *J. Mol. Biol.* 293:865-881(1999)).

[0301] In some embodiments, Kd is measured using surface plasmon resonance assays using a BIACORE®-2000 or a BIACORE®-3000 (BIAcore, Inc., Piscataway, NJ) at 25°C with immobilized antigen CM5 chips at ~10 response units (RU). Briefly, carboxymethylated dextran biosensor chips (CM5, BIACORE, Inc.) are activated with *N*-ethyl-*N'*- (3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC) and *N*-hydroxysuccinimide (NHS) according to the supplier's instructions. Antigen is diluted with 10 mM sodium acetate, pH 4.8, to 5 μg/ml (~0.2 μM) before injection at a flow rate of 5 μl/minute to achieve approximately 10 response units (RU) of coupled protein. Following the injection of antigen, 1 M ethanolamine is injected to block unreacted groups. For kinetics measurements, two-fold serial dilutions of Fab or V_HH of the antibody of interest (0.78 nM to 500 nM) are injected in PBS with 0.05% polysorbate 20 (TWEEN-20™) surfactant (PBST) at 25°C at a flow rate of approximately 25 μl/min. Association rates (k_{on}) and dissociation rates (k_{off}) are calculated using a simple one-to-one Langmuir binding model (BIACORE® Evaluation Software version 3.2) by simultaneously fitting the association and dissociation sensorgrams. The equilibrium dissociation constant (Kd) is calculated as the ratio k_{off}/k_{on}. See, *e.g.*, Chen et al., *J. Mol. Biol.* 293:865-881 (1999). If the on-rate exceeds 10^6 M^{-1} s^{-1} by the surface plasmon resonance assay above, then the on-rate can be determined by using a fluorescent quenching technique that measures the increase or decrease in fluorescence emission intensity (excitation = 295 nm; emission = 340 nm, 16 nm band-pass) at 25°C of a 20 nM anti-antigen antibody moiety in PBS, pH 7.2, in the presence of increasing concentrations of antigen as measured in a spectrometer, such as a stop-flow equipped spectrophometer (Aviv Instruments) or a 8000-series SLM-AMINCO™ spectrophotometer (ThermoSpectronic) with a stirred cuvette.

2. Antibody Fragments

[0302] In some embodiments, an antibody moiety provided herein is an antibody fragment. Antibody fragments include, but are not limited to, Fab, Fab', Fab'-SH, $F(ab')_2$, Fv, and scFv fragments, V_HH, and other fragments described below. For a review of certain antibody fragments, see Hudson et al. *Nat. Med.* 9:129-134 (2003). For a review of scFv fragments, see, *e.g.*, Pluckthün, in *The Pharmacology of Monoclonal Antibodies*, vol. 113, Rosenburg and Moore eds., (Springer-Verlag, New York), pp. 269-315 (1994); see also WO 93/16185; and U.S. Patent Nos. 5,571,894 and 5,587,458. For discussion of Fab and $F(ab')_2$ fragments comprising salvage receptor binding epitope residues and having increased in vivo half-life, see U.S. Patent No. 5,869,046.

[0303] Diabodies are antibody fragments with two antigen-binding sites that may be bivalent or bispecific. See, for example, EP 404,097; WO 1993/01161; Hudson et al., *Nat. Med.* 9:129-134 (2003); and Hollinger et al., *Proc. Natl. Acad. Sci. USA* 90: 6444-6448 (1993). Triabodies and tetrabodies are also described in Hudson et al., *Nat. Med.* 9:129-134 (2003).

[0304] Antibody fragments can be made by various techniques, including but not limited to proteolytic digestion of an intact antibody as well as production by recombinant host cells (*e.g. E. coli* or phage), as described herein.

3. Chimeric and Humanized Antibodies

[0305] In some embodiments, an antibody moiety provided herein is a chimeric antibody. Certain chimeric antibodies are described, *e.g.*, in U.S. Patent No. 4,816,567; and Morrison et al., *Proc. Natl. Acad. Sci. USA*, 81:6851-6855 (1984)). In one example, a chimeric antibody comprises a non-human variable region (*e.g.*, a variable region derived from a camelid species, such as llama) and a human constant region. In a further example, a chimeric antibody is a "class switched" antibody in which the class or subclass has been changed from that of the parent antibody. Chimeric antibodies include antigen-binding fragments thereof.

[0306] In some embodiments, a chimeric antibody is a humanized antibody. Typically, a non-human antibody is humanized to reduce immunogenicity to humans, while retaining the specificity and affinity of the parental non-human antibody. Generally, a humanized antibody comprises one or more variable domains in which HVRs, *e.g.*, CDRs, (or portions thereof) are derived from a non-human antibody, and FRs (or portions thereof) are derived from human antibody sequences. A humanized antibody optionally will also comprise at least a portion of a human constant region. In some embodiments, some FR residues in a humanized antibody are substituted with corresponding residues from a non-human antibody (*e.g.*, the antibody from which the HVR residues are derived), *e.g.*, to restore or improve antibody specificity or affinity.

[0307] Humanized antibodies and methods of making them are reviewed, *e.g.*, in Almagro and Fransson, *Front. Biosci.* 13:1619-1633 (2008), and are further described, *e.g.*, in Riechmann et al., *Nature* 332:323-329 (1988); Queen et al., *Proc. Nat'l Acad. Sci. USA* 86:10029-10033 (1989); US Patent Nos. 5, 821,337, 7,527,791, 6,982,321, and 7,087,409; Kashmiri *et al.*, *Methods* 36:25-34 (2005) (describing SDR (a-CDR) grafting); Padlan, *Mol. Immunol.* 28:489-498 (1991) (describing "resurfacing"); Dall'Acqua et al., *Methods* 36:43-60 (2005) (describing "FR shuffling"); and Osbourn et al., *Methods* 36:61-68 (2005) and Klimka et al., *Br. J. Cancer*, 83:252-260 (2000) (describing the "guided selection" approach to FR shuffling).

[0308] Human framework regions that may be used for humanization include but are not limited to: framework regions selected using the "best-fit" method (*see, e.g.*, Sims et al. *J. Immunol.* 151:2296

(1993)); framework regions derived from the consensus sequence of human antibodies of a particular subgroup of light or heavy chain variable regions (see, *e.g.*, Carter et al. *Proc. Natl. Acad. Sci. USA*, 89:4285 (1992); and Presta et al. *J. Immunol.*, 151:2623 (1993)); human mature (somatically mutated) framework regions or human germline framework regions (see, *e.g.*, Almagro and Fransson, *Front. Biosci.* 13:1619-1633 (2008)); and framework regions derived from screening FR libraries (see, *e.g.*, Baca et al., *J. Biol. Chem.* 272:10678-10684 (1997) and Rosok et al., *J. Biol. Chem.* 271:22611-22618 (1996)).

[0309] In some embodiments, the sdAbs are modified, such as humanized, without diminishing the native affinity of the domain for antigen and while reducing its immunogenicity with respect to a heterologous species. For example, the amino acid residues of the antibody variable domain (V_HH) of an llama antibody can be determined, and one or more of the Camelid amino acids, for example, in the framework regions, are replaced by their human counterpart as found in the human consensus sequence, without that polypeptide losing its typical character, *i.e.* the humanization does not significantly affect the antigen binding capacity of the resulting polypeptide. Humanization of Camelid sdAbs requires the introduction and mutagenesis of a limited amount of amino acids in a single polypeptide chain. This is in contrast to humanization of scFv, Fab', (Fab')2 and IgG, which requires the introduction of amino acid changes in two chains, the light and the heavy chain and the preservation of the assembly of both chains.

[0310] Single-domain antibodies comprising a V_HH domain can be humanized to have human-like sequences. In some embodiments, the FR regions of the V_HH domain used herein comprise at least about any one of 50%, 60%, 70%, 80%, 90%, 95% or more of amino acid sequence homology to human V_H framework regions. One exemplary class of humanized V_HH domains is characterized in that the V_HHs carry an amino acid from the group consisting of glycine, alanine, valine, leucine, isoleucine, proline, phenylalanine, tyrosine, tryptophan, methionine, serine, threonine, asparagine, or glutamine at position 45, such as, for example, L45 and a tryptophan at position 103, according to the Kabat numbering. As such, polypeptides belonging to this class show a high amino acid sequence homology to human V_H framework regions and said polypeptides might be administered to a human directly without expectation of an unwanted immune response therefrom, and without the burden of further humanization.

[0311] Another exemplary class of humanized Camelid sdAbs has been described in WO 03/035694 and contains hydrophobic FR2 residues typically found in conventional antibodies of human origin or from other species, but compensating this loss in hydrophilicity by the charged arginine residue on position 103 that substitutes the conserved tryptophan residue present in V_H from double-chain antibodies. As such, peptides belonging to these two classes show a high amino acid sequence homology to human V_H framework regions and said peptides might be administered to a human directly without expectation of an unwanted immune response therefrom, and without the burden of further humanization.

4. Human Antibodies

[0312] In some embodiments, an antibody moiety provided herein is a human antibody. Human antibodies can be produced using various techniques known in the art. Human antibodies are described generally in van Dijk and van de Winkel, *Curr. Opin. Pharmacol.* 5: 368-74 (2001) and Lonberg, *Curr. Opin. Immunol.* 20:450-459 (2008). Transgenic mice or rats capable of producing fully human sdAbs are known in the art. See, *e.g.*, US20090307787A1, U.S. Pat. No. 8,754,287, US20150289489A1, US20100122358A1, and WO2004049794.

[0313] Human antibodies may be prepared by administering an immunogen to a transgenic animal that has been modified to produce intact human antibodies or intact antibodies with human variable regions in response to antigenic challenge. Such animals typically contain all or a portion of the human immunoglobulin loci, which replace the endogenous immunoglobulin loci, or which are present extrachromosomally or integrated randomly into the animal's chromosomes. In such transgenic mice, the endogenous immunoglobulin loci have generally been inactivated. For review of methods for obtaining human antibodies from transgenic animals, see Lonberg, *Nat. Biotech.* 23:1117-1125 (2005). *See also, e.g.*, U.S. Patent Nos. 6,075,181 and 6,150,584 describing XENOMOUSE™ technology; U.S. Patent No. 5,770,429 describing HUMAB® technology; U.S. Patent No. 7,041,870 describing K-M MOUSE® technology, and U.S. Patent Application Publication No. US 2007/0061900, describing VELOCIMOUSE® technology). Human variable regions from intact antibodies generated by such animals may be further modified, *e.g.*, by combining with a different human constant region.

[0314] Human antibodies can also be made by hybridoma-based methods. Human myeloma and mouse-human heteromyeloma cell lines for the production of human monoclonal antibodies have been described. (*See, e.g.*, Kozbor *J. Immunol.*, 133: 3001 (1984); Brodeur et al., *Monoclonal Antibody Production Techniques and Applications*, pp. 51-63 (Marcel Dekker, Inc., New York, 1987); and Boerner et al., *J. Immunol.*, 147: 86 (1991).) Human antibodies generated via human B-cell hybridoma technology are also described in Li et al., *Proc. Natl. Acad. Sci. USA*, 103:3557-3562 (2006). Additional methods include those described, for example, in U.S. Patent No. 7,189,826 (describing production of monoclonal human IgM antibodies from hybridoma cell lines) and Ni, *Xiandai Mianyixue*, 26(4):265-268 (2006) (describing human-human hybridomas). Human hybridoma technology (Trioma technology) is also described in Vollmers and Brandlein, *Histology and Histopathology*, 20(3):927-937 (2005) and Vollmers and Brandlein, *Methods and Findings in Experimental and Clinical Pharmacology*, 27(3):185-91 (2005).

[0315] Human antibodies may also be generated by isolating Fv clone variable domain sequences selected from human-derived phage display libraries. Such variable domain sequences may then be

combined with a desired human constant domain. Techniques for selecting human antibodies from antibody libraries are described below.

[0316] One technique for obtaining V_HH sequences directed against a particular antigen or target involves suitably immunizing a transgenic mammal that is capable of expressing heavy chain antibodies (i.e. so as to raise an immune response and/or heavy chain antibodies directed against said antigen or target), obtaining a suitable biological sample from said transgenic mammal that contains (nucleic acid sequences encoding) said V_HH sequences (such as a blood sample, serum sample or sample of B-cells), and then generating V_HH sequences directed against said antigen or target, starting from said sample, using any suitable technique known per se (such as any of the methods described herein or a hybridoma technique). For example, for this purpose, the heavy chain antibody-expressing mice and the further methods and techniques described in WO 02/085945, WO 04/049794 and WO 06/008548 and Janssens et al., Proc. Natl. Acad. Sci. USA. 2006 Oct. 10; 103(41):15130-5 can be used. For example, such heavy chain antibody expressing mice can express heavy chain antibodies with any suitable (single) variable domain, such as (single) variable domains from natural sources (*e.g.* human (single) variable domains, Camelid (single) variable domains or shark (single) variable domains), as well as for example synthetic or semi-synthetic (single) variable domains.

5. Library-Derived Antibodies

[0317] Antibody moieties of the present application may be isolated by screening combinatorial libraries for antibodies with the desired activity or activities. For example, a variety of methods are known in the art for generating phage display libraries and screening such libraries for antibodies possessing the desired binding characteristics. Such methods are reviewed, *e.g.*, in Hoogenboom et al. in *Methods in Molecular Biology* 178:1-37 (O'Brien et al., ed., Human Press, Totowa, NJ, 2001) and further described, *e.g.*, in the McCafferty et al., *Nature* 348:552-554; Clackson et al., *Nature* 352: 624-628 (1991); Marks et al., *J. Mol. Biol.* 222: 581-597 (1992); Marks and Bradbury, in *Methods in Molecular Biology* 248:161-175 (Lo, ed., Human Press, Totowa, NJ, 2003); Sidhu et al., *J. Mol. Biol.* 338(2): 299-310 (2004); Lee et al., *J. Mol. Biol.* 340(5): 1073-1093 (2004); Fellouse, *Proc. Natl. Acad. Sci. USA* 101(34): 12467-12472 (2004); and Lee et al., *J. Immunol. Methods* 284(1-2): 119-132(2004). Methods for constructing sdAb libraries have been described, for example, see U.S. Pat. NO. 7371849.

[0318] In certain phage display methods, repertoires of V_H and V_L genes are separately cloned by polymerase chain reaction (PCR) and recombined randomly in phage libraries, which can then be screened for antigen-binding phage as described in Winter et al., *Ann. Rev. Immunol.*, 12: 433-455 (1994). Phage typically display antibody fragments, either as single-chain Fv (scFv) fragments or as Fab fragments. Libraries from immunized sources provide high-affinity antibodies to the immunogen without the requirement of constructing hybridomas. Alternatively, the naive repertoire can be cloned (*e.g.*, from

human) to provide a single source of antibodies to a wide range of non-self and also self-antigens without any immunization as described by Griffiths et al., *EMBO J,* 12: 725-734 (1993). Finally, naive libraries can also be made synthetically by cloning unrearranged V-gene segments from stem cells, and using PCR primers containing random sequence to encode the highly variable CDR3 regions and to accomplish rearrangement *in vitro*, as described by Hoogenboom and Winter, *J. Mol. Biol.*, 227: 381-388 (1992). Patent publications describing human antibody phage libraries include, for example: US Patent No. 5,750,373, and US Patent Publication Nos. 2005/0079574, 2005/0119455, 2005/0266000, 2007/0117126, 2007/0160598, 2007/0237764, 2007/0292936, and 2009/0002360.

[0319] Antibodies or antibody fragments isolated from human antibody libraries are considered human antibodies or human antibody fragments herein.

6. Multispecific Antibodies

[0320] In some embodiments, an antibody moiety provided herein is a multispecific antibody, *e.g.* a bispecific antibody. Bispecific antibodies can be prepared as full length antibodies or antibody fragments. Techniques for making multispecific antibodies include, but are not limited to, recombinant co-expression of two immunoglobulin heavy chain-light chain pairs having different specificities (see Milstein and Cuello, *Nature* 305: 537 (1983)), WO 93/08829, and Traunecker et al., *EMBO J.* 10: 3655 (1991)), and "knob-in-hole" engineering (*see, e.g.*, U.S. Patent No. 5,731,168). Multi-specific antibodies may also be made by engineering electrostatic steering effects for making antibody Fc-heterodimeric molecules (WO 2009/089004A1); cross-linking two or more antibodies or fragments (*see, e.g.*, US Patent No. 4,676,980, and Brennan et al., *Science*, 229: 81 (1985)); using leucine zippers to produce bi-specific antibodies (*see, e.g.*, Kostelny et al., *J. Immunol.*, 148(5):1547-1553 (1992)); using "diabody" technology for making bispecific antibody fragments (see, *e.g.*, Hollinger et al., *Proc. Natl. Acad. Sci. USA*, 90:6444-6448 (1993)); and using single-chain Fv (sFv) dimers (*see, e.g.*, Gruber et al., *J. Immunol.*, 152:5368 (1994)); and preparing trispecific antibodies as described, *e.g.*, in Tutt et al. *J. Immunol.* 147: 60 (1991); and creating polypeptides comprising tandem single-domain antibodies (*see,* e.g., U.S. Patent Application No. 20110028695; and Conrath et al. J. Biol. Chem., 2001; 276(10):7346-50). Engineered antibodies with three or more functional antigen binding sites, including "Octopus antibodies," are also included herein (*see, e.g.,* US 2006/0025576A1).

7. Antibody Variants

[0321] In some embodiments, amino acid sequence variants of the antibody moieties provided herein are contemplated. For example, it may be desirable to improve the binding affinity and/or other biological properties of the antibody moiety. Amino acid sequence variants of an antibody moiety may be prepared by introducing appropriate modifications into the nucleic acid sequence encoding the antibody moiety, or by peptide synthesis. Such modifications include, for example, deletions from,

and/or insertions into and/or substitutions of residues within the amino acid sequences of the antibody moiety. Any combination of deletion, insertion, and substitution can be made to arrive at the final construct, provided that the final construct possesses the desired characteristics, *e.g.*, antigen-binding.

a) Substitution, Insertion, and Deletion Variants

[0322] In some embodiments, antibody variants having one or more amino acid substitutions are provided. Sites of interest for substitutional mutagenesis include the HVRs and FRs. Conservative substitutions are shown in Table 7 under the heading of "Preferred substitutions." More substantial changes are provided in Table 7 under the heading of "exemplary substitutions," and as further described below in reference to amino acid side chain classes. Amino acid substitutions may be introduced into an antibody of interest and the products screened for a desired activity, *e.g.*, retained/improved antigen binding, decreased immunogenicity, or improved ADCC or CDC.

TABLE 7. Amino Acid Substitutions

Original Residue	Exemplary Substitutions	Preferred Substitutions
Ala (A)	Val; Leu; Ile	Val
Arg (R)	Lys; Gln; Asn	Lys
Asn (N)	Gln; His; Asp, Lys; Arg	Gln
Asp (D)	Glu; Asn	Glu
Cys (C)	Ser; Ala	Ser
Gln (Q)	Asn; Glu	Asn
Glu (E)	Asp; Gln	Asp
Gly (G)	Ala	Ala
His (H)	Asn; Gln; Lys; Arg	Arg
Ile (I)	Leu; Val; Met; Ala; Phe; Norleucine	Leu
Leu (L)	Norleucine; Ile; Val; Met; Ala; Phe	Ile
Lys (K)	Arg; Gln; Asn	Arg
Met (M)	Leu; Phe; Ile	Leu
Phe (F)	Trp; Leu; Val; Ile; Ala; Tyr	Tyr
Pro (P)	Ala	Ala
Ser (S)	Thr	Thr
Thr (T)	Val; Ser	Ser
Trp (W)	Tyr; Phe	Tyr

Original Residue	Exemplary Substitutions	Preferred Substitutions
Tyr (Y)	Trp; Phe; Thr; Ser	Phe
Val (V)	Ile; Leu; Met; Phe; Ala; Norleucine	Leu

[0323] Amino acids may be grouped according to common side-chain properties:

(1) hydrophobic: Norleucine, Met, Ala, Val, Leu, Ile;

(2) neutral hydrophilic: Cys, Ser, Thr, Asn, Gln;

(3) acidic: Asp, Glu;

(4) basic: His, Lys, Arg;

(5) residues that influence chain orientation: Gly, Pro;

(6) aromatic: Trp, Tyr, Phe.

[0324] Non-conservative substitutions will entail exchanging a member of one of these classes for another class.

[0325] One type of substitutional variant involves substituting one or more hypervariable region residues of a parent antibody (*e.g.*, a humanized or human antibody). Generally, the resulting variant(s) selected for further study will have modifications (*e.g.,* improvements) in certain biological properties (*e.g.*, increased affinity, reduced immunogenicity) relative to the parent antibody and/or will have substantially retained certain biological properties of the parent antibody. An exemplary substitutional variant is an affinity matured antibody, which may be conveniently generated, *e.g.*, using phage display-based affinity maturation techniques such as those described herein. Briefly, one or more HVR residues are mutated and the variant antibodies displayed on phage and screened for a particular biological activity (*e.g.* binding affinity).

[0326] Alterations (*e.g.*, substitutions) may be made in HVRs, *e.g.*, to improve antibody affinity. Such alterations may be made in HVR "hotspots," *i.e.*, residues encoded by codons that undergo mutation at high frequency during the somatic maturation process (*see, e.g.*, Chowdhury, *Methods Mol. Biol.* 207:179-196 (2008)), and/or SDRs (a-CDRs), with the resulting variant V_H or V_L being tested for binding affinity. Affinity maturation by constructing and reselecting from secondary libraries has been described, *e.g.*, in Hoogenboom et al. in *Methods in Molecular Biology* 178:1-37 (O'Brien et al., ed., Human Press, Totowa, NJ, (2001).) In some embodiments of affinity maturation, diversity is introduced into the variable genes chosen for maturation by any of a variety of methods (*e.g.*, error-prone PCR, chain shuffling, or oligonucleotide-directed mutagenesis). A secondary library is then created. The library is then screened to identify any antibody variants with the desired affinity. Another method to introduce diversity involves HVR-directed approaches, in which several HVR residues (*e.g.*, 4-6 residues at a time)

are randomized. HVR residues involved in antigen binding may be specifically identified, *e.g.*, using alanine scanning mutagenesis or modeling. CDR-H3 and CDR-L3 in particular are often targeted.

[0327] In some embodiments, substitutions, insertions, or deletions may occur within one or more HVRs so long as such alterations do not substantially reduce the ability of the antibody moiety to bind antigen. For example, conservative alterations (*e.g.*, conservative substitutions as provided herein) that do not substantially reduce binding affinity may be made in HVRs. Such alterations may be outside of HVR "hotspots" or CDRs. In some embodiments of the variant V_HH sequences provided above, each HVR either is unaltered, or contains no more than one, two or three amino acid substitutions.

[0328] A useful method for identification of residues or regions of an antibody moiety that may be targeted for mutagenesis is called "alanine scanning mutagenesis" as described by Cunningham and Wells (1989) *Science*, 244:1081-1085. In this method, a residue or group of target residues (*e.g.*, charged residues such as Arg, Asp, His, Lys, and Glu) are identified and replaced by a neutral or negatively charged amino acid (*e.g.*, alanine or polyalanine) to determine whether the interaction of the antibody moiety with antigen is affected. Further substitutions may be introduced at the amino acid locations demonstrating functional sensitivity to the initial substitutions. Alternatively, or additionally, a crystal structure of an antigen-antibody complex to identify contact points between the antibody and antigen. Such contact residues and neighboring residues may be targeted or eliminated as candidates for substitution. Variants may be screened to determine whether they contain the desired properties.

[0329] Amino acid sequence insertions include amino- and/or carboxyl-terminal fusions ranging in length from one residue to polypeptides containing a hundred or more residues, as well as intrasequence insertions of single or multiple amino acid residues. Examples of terminal insertions include an antibody with an N-terminal methionyl residue. Other insertional variants of the antibody molecule include the fusion to the N- or C-terminus of the antibody to an enzyme (*e.g.,* for ADEPT) or a polypeptide which increases the serum half-life of the antibody.

b) Glycosylation variants

[0330] In some embodiments, an antibody moiety provided herein is altered to increase or decrease the extent to which the antibody moiety is glycosylated. Addition or deletion of glycosylation sites to an antibody moiety may be conveniently accomplished by altering the amino acid sequence such that one or more glycosylation sites is created or removed.

[0331] Where the antibody moiety comprises an Fc region, the carbohydrate attached thereto may be altered. Native antibodies produced by mammalian cells typically comprise a branched, biantennary oligosaccharide that is generally attached by an N-linkage to Asn297 of the CH2 domain of the Fc region. *See, e.g.*, Wright et al. *TIBTECH* 15:26-32 (1997). The oligosaccharide may include various carbohydrates, *e.g.*, mannose, N-acetyl glucosamine (GlcNAc), galactose, and sialic acid, as well as a

fucose attached to a GlcNAc in the "stem" of the biantennary oligosaccharide structure. In some embodiments, modifications of the oligosaccharide in an antibody of the present application may be made in order to create antibody variants with certain improved properties.

[0332] In some embodiments, antibody variants are provided having a carbohydrate structure that lacks fucose attached (directly or indirectly) to an Fc region. For example, the amount of fucose in such antibody may be from 1% to 80%, from 1% to 65%, from 5% to 65% or from 20% to 40%. The amount of fucose is determined by calculating the average amount of fucose within the sugar chain at Asn297, relative to the sum of all glycostructures attached to Asn 297 (*e.g.*, complex, hybrid and high mannose structures) as measured by MALDI-TOF mass spectrometry, as described in WO 2008/077546, for example. Asn297 refers to the asparagine residue located at about position 297 in the Fc region (EU numbering of Fc region residues); however, Asn297 may also be located about ± 3 amino acids upstream or downstream of position 297, *i.e.,* between positions 294 and 300, due to minor sequence variations in antibodies. Such fucosylation variants may have improved ADCC function. *See, e.g.,* US Patent Publication Nos. US 2003/0157108 (Presta, L.); US 2004/0093621 (Kyowa Hakko Kogyo Co., Ltd). Examples of publications related to "defucosylated" or "fucose-deficient" antibody variants include: US 2003/0157108; WO 2000/61739; WO 2001/29246; US 2003/0115614; US 2002/0164328; US 2004/0093621; US 2004/0132140; US 2004/0110704; US 2004/0110282; US 2004/0109865; WO 2003/085119; WO 2003/084570; WO 2005/035586; WO 2005/035778; WO2005/053742; WO2002/031140; Okazaki et al. *J. Mol. Biol.* 336:1239-1249 (2004); Yamane-Ohnuki et al. *Biotech. Bioeng.* 87: 614 (2004). Examples of cell lines capable of producing defucosylated antibodies include Lec13 CHO cells deficient in protein fucosylation (Ripka et al. *Arch. Biochem. Biophys.* 249:533-545 (1986); US Patent Application No. US 2003/0157108 A1, Presta, L; and WO 2004/056312 A1, Adams *et al.*, especially at Example 11), and knockout cell lines, such as alpha-1,6-fucosyltransferase gene, *FUT8,* knockout CHO cells (*see, e.g.*, Yamane-Ohnuki et al. *Biotech. Bioeng.* 87: 614 (2004); Kanda, Y. et al., *Biotechnol. Bioeng.*, 94(4):680-688 (2006); and WO2003/085107).

[0333] Antibody variants are further provided with bisected oligosaccharides, *e.g.*, in which a biantennary oligosaccharide attached to the Fc region of the antibody is bisected by GlcNAc. Such antibody variants may have reduced fucosylation and/or improved ADCC function. Examples of such antibody variants are described, *e.g.*, in WO 2003/011878 (Jean-Mairet et al.); US Patent No. 6,602,684 (Umana et al.); and US 2005/0123546 (Umana *et al.*). Antibody variants with at least one galactose residue in the oligosaccharide attached to the Fc region are also provided. Such antibody variants may have improved CDC function. Such antibody variants are described, *e.g.*, in WO 1997/30087 (Patel et al.); WO 1998/58964 (Raju, S.); and WO 1999/22764 (Raju, S.).

c) Fc region variants

[0334] In some embodiments, one or more amino acid modifications may be introduced into the Fc region of an antibody provided herein, thereby generating an Fc region variant. The Fc region variant may comprise a human Fc region sequence (*e.g.*, a human IgG1, IgG2, IgG3 or IgG4 Fc region) comprising an amino acid modification (*e.g.* a substitution) at one or more amino acid positions.

[0335] In some embodiments, the present application contemplates an antibody variant that possesses some but not all effector functions, which make it a desirable candidate for applications in which the half-life of the antibody *in vivo* is important yet certain effector functions (such as complement and ADCC) are unnecessary or deleterious. *In vitro* and/or *in vivo* cytotoxicity assays can be conducted to confirm the reduction/depletion of CDC and/or ADCC activities. For example, Fc receptor (FcR) binding assays can be conducted to ensure that the antibody lacks FcγR binding (hence likely lacking ADCC activity), but retains FcRn binding ability. The primary cells for mediating ADCC, NK cells, express Fc(RIII only, whereas monocytes express Fc(RI, Fc(RII and Fc(RIII. FcR expression on hematopoietic cells is summarized in Table 3 on page 464 of Ravetch and Kinet, *Annu. Rev. Immunol.* 9:457-492 (1991). Non-limiting examples of *in vitro* assays to assess ADCC activity of a molecule of interest is described in U.S. Patent No. 5,500,362 (*see, e.g.* Hellstrom, I. et al. *Proc. Nat'l Acad. Sci. USA* 83:7059-7063 (1986)) and Hellstrom, I et al., *Proc. Nat'l Acad. Sci. USA* 82:1499-1502 (1985); 5,821,337 (*see* Bruggemann, M. et al., *J. Exp. Med.* 166:1351-1361 (1987)). Alternatively, non-radioactive assays methods may be employed (*see*, for example, ACTI™ non-radioactive cytotoxicity assay for flow cytometry (CellTechnology, Inc. Mountain View, CA; and CYTOTOX 96® non-radioactive cytotoxicity assay (Promega, Madison, WI). Useful effector cells for such assays include peripheral blood mononuclear cells (PBMC) and Natural Killer (NK) cells. Alternatively, or additionally, ADCC activity of the molecule of interest may be assessed *in vivo*, *e.g.*, in an animal model such as that disclosed in Clynes et al. *Proc. Nat'l Acad. Sci. USA* 95:652-656 (1998). C1q binding assays may also be carried out to confirm that the antibody is unable to bind C1q and hence lacks CDC activity. *See, e.g.*, C1q and C3c binding ELISA in WO 2006/029879 and WO 2005/100402. To assess complement activation, a CDC assay may be performed (*see*, for example, Gazzano-Santoro *et al.*, *J. Immunol. Methods* 202:163 (1996); Cragg, M.S. et al., *Blood* 101:1045-1052 (2003); and Cragg, M.S. and M.J. Glennie, *Blood* 103:2738-2743 (2004)). FcRn binding and *in vivo* clearance/half-life determinations can also be performed using methods known in the art (*see, e.g.*, Petkova, S.B. et al., *Int'l. Immunol.* 18(12):1759-1769 (2006)).

[0336] Antibodies with reduced effector function include those with substitution of one or more of Fc region residues 238, 265, 269, 270, 297, 327 and 329 (U.S. Patent No. 6,737,056). Such Fc mutants include Fc mutants with substitutions at two or more of amino acid positions 265, 269, 270, 297 and 327,

including the so-called "DANA" Fc mutant with substitution of residues 265 and 297 to alanine (US Patent No. 7,332,581).

[0337] Certain antibody variants with improved or diminished binding to FcRs are described. (*See, e.g.*, U.S. Patent No. 6,737,056; WO 2004/056312, and Shields et al., *J. Biol. Chem.* 9(2): 6591-6604 (2001).)

[0338] In some embodiments, an antibody variant comprises an Fc region with one or more amino acid substitutions which improve ADCC, *e.g.*, substitutions at positions 298, 333, and/or 334 of the Fc region (EU numbering of residues).

[0339] In some embodiments, alterations are made in the Fc region that result in altered (*i.e.*, either improved or diminished) C1q binding and/or Complement Dependent Cytotoxicity (CDC), *e.g.*, as described in US Patent No. 6,194,551, WO 99/51642, and Idusogie et al. *J. Immunol.* 164: 4178-4184 (2000).

[0340] Antibodies with increased half-lives and improved binding to the neonatal Fc receptor (FcRn), which is responsible for the transfer of maternal IgGs to the fetus (Guyer et al., *J. Immunol.* 117:587 (1976) and Kim et al., *J. Immunol.* 24:249 (1994)), are described in US2005/0014934A1 (Hinton et al.). Those antibodies comprise an Fc region with one or more substitutions therein which improve binding of the Fc region to FcRn. Such Fc variants include those with substitutions at one or more of Fc region residues: 238, 256, 265, 272, 286, 303, 305, 307, 311, 312, 317, 340, 356, 360, 362, 376, 378, 380, 382, 413, 424 or 434, *e.g.*, substitution of Fc region residue 434 (US Patent No. 7,371,826).

[0341] See also Duncan & Winter, *Nature* 322:738-40 (1988); U.S. Patent No. 5,648,260; U.S. Patent No. 5,624,821; and WO 94/29351 concerning other examples of Fc region variants.

d) Cysteine engineered antibody variants

[0342] In some embodiments, it may be desirable to create cysteine engineered antibodies, *e.g.*, "thioMAbs," in which one or more residues of an antibody moiety are substituted with cysteine residues. In particular embodiments, the substituted residues occur at accessible sites of the antibody moiety. By substituting those residues with cysteine, reactive thiol groups are thereby positioned at accessible sites of the antibody moiety and may be used to conjugate the antibody to other moieties, such as drug moieties or linker-drug moieties, to create an immunoconjugate, as described further herein. In some embodiments, any one or more of the following residues may be substituted with cysteine: A118 (EU numbering) of the heavy chain; and S400 (EU numbering) of the heavy chain Fc region. Cysteine engineered antibodies may be generated as described, *e.g.*, in U.S. Patent No. 7,521,541.

e) Antibody Derivatives

[0343] In some embodiments, an antibody moiety provided herein may be further modified to contain additional nonproteinaceous moieties that are known in the art and readily available. The moieties

suitable for derivatization of the antibody moiety include but are not limited to water soluble polymers. Non-limiting examples of water soluble polymers include, but are not limited to, polyethylene glycol (PEG), copolymers of ethylene glycol/propylene glycol, carboxymethylcellulose, dextran, polyvinyl alcohol, polyvinyl pyrrolidone, poly-1, 3-dioxolane, poly-1,3,6-trioxane, ethylene/maleic anhydride copolymer, polyaminoacids (either homopolymers or random copolymers), and dextran or poly(n-vinyl pyrrolidone)polyethylene glycol, propropylene glycol homopolymers, prolypropylene oxide/ethylene oxide co-polymers, polyoxyethylated polyols (*e.g.*, glycerol), polyvinyl alcohol, and mixtures thereof. Polyethylene glycol propionaldehyde may have advantages in manufacturing due to its stability in water. The polymer may be of any molecular weight, and may be branched or unbranched. The number of polymers attached to the antibody moiety may vary, and if more than one polymer are attached, they can be the same or different molecules. In general, the number and/or type of polymers used for derivatization can be determined based on considerations including, but not limited to, the particular properties or functions of the antibody moiety to be improved, whether the antibody moiety derivative will be used in a therapy under defined conditions, *etc.*

III. Methods of preparing anti-CLL1 constructs

[0344] The anti-CLL1 constructs, including anti-CLL1 sdAbs, immune effector cell engagers, and antibody moieties of the immunoconjugates as described herein may be prepared using any methods known in the art or as described herein. Compositions and methods of preparing anti-CLL1 chimeric receptors and chimeric receptor systems are described in Section IV.

[0345] Methods of preparing sdAbs have been described. See, for example, Els Pardon et al, *Nature Protocol*, 2014; 9(3): 674. Single-domain antibodies (such as V_HHs) may be obtained using methods known in the art such as by immunizing a *Camelid* species (such as camel or llama) and obtaining hybridomas therefrom, or by cloning a library of sdAbs using molecular biology techniques known in the art and subsequent selection by ELISA with individual clones of unselected libraries or by using phage display.

[0346] For recombinant production of the sdAbs, the nucleic acids encoding the sdAbs are isolated and inserted into a replicable vector for further cloning (amplification of the DNA) or for expression. DNA encoding the sdAb is readily isolated and sequenced using conventional procedures (*e.g.*, by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of the antibody). Many vectors are available. The choice of vector depends in part on the host cell to be used. Generally, preferred host cells are of either prokaryotic or eukaryotic (generally mammalian) origin.

Nucleic acids and vectors

[0347] Nucleic acid molecules comprising polynucleotides that encode one or more chains of any one of the anti-CLL1 constructs described herein are provided.

[0348] In some embodiments, there is provided an isolated nucleic acid encoding any one of the anti-CLL1 sdAbs described herein. In some embodiments, an isolated nucleic acid encoding an anti-CLL1 sdAb is provided wherein the nucleic acid comprises a sequence having at least about any one of 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the nucleic acid sequence of any one of SEQ ID NOs: 107-119 and 174-176. In some embodiments, there is provided an isolated nucleic acid comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 107-119 and 174-176.

[0349] Nucleic acid molecules may be constructed using recombinant DNA techniques conventional in the art. In some embodiments, a nucleic acid molecule is an expression vector that is suitable for expression in a selected host cell.

[0350] Vectors comprising polynucleotides that encode any one of the anti-CLL1 constructs described herein are provided. Such vectors include, but are not limited to, DNA vectors, phage vectors, viral vectors, retroviral vectors, *etc.* In some embodiments, the vector is an expression vector.

Host Cells

[0351] In some embodiments, the anti-CLL1 construct may be expressed in prokaryotic cells, such as bacterial cells; or in eukaryotic cells, such as fungal cells (such as yeast), plant cells, insect cells, and mammalian cells. Such expression may be carried out, for example, according to procedures known in the art. Exemplary eukaryotic cells that may be used to express polypeptides include, but are not limited to, COS cells, including COS 7 cells; 293 cells, including 293-6E cells; CHO cells, including CHO-S, DG44. Lec13 CHO cells, and FUT8 CHO cells; PER.C6® cells (Crucell); and NSO cells. In some embodiments, the anti-CLL1 construct may be expressed in yeast. See, *e.g.*, U.S. Publication No. US 2006/0270045 A1. In some embodiments, a particular eukaryotic host cell is selected based on its ability to make desired post-translational modifications to the anti-CLL1 construct. For example, in some embodiments, CHO cells produce polypeptides that have a higher level of sialylation than the same polypeptide produced in 293 cells.

[0352] Introduction of one or more nucleic acids into a desired host cell may be accomplished by any method, including but not limited to, calcium phosphate transfection, DEAE-dextran mediated transfection, cationic lipid-mediated transfection, electroporation, transduction, infection, *etc.* Nonlimiting exemplary methods are described, *e.g.*, in Sambrook *et al.*, Molecular Cloning, A Laboratory

Manual, 3rd ed. Cold Spring Harbor Laboratory Press (2001). Nucleic acids may be transiently or stably transfected in the desired host cells, according to any suitable method.

[0353] The invention also provides host cells comprising any of the polynucleotides or vectors described herein. In some embodiments, the invention provides a host cell comprising an anti-CLL1 construct. Any host cells capable of over-expressing heterologous DNAs can be used for the purpose of isolating the genes encoding the antibody, polypeptide or protein of interest. Non-limiting examples of mammalian host cells include but not limited to COS, HeLa, and CHO cells. See also PCT Publication No. WO 87/04462. Suitable non-mammalian host cells include prokaryotes (such as *E. coli* or *B. subtillis*) and yeast (such as *S. cerevisae*, *S. pombe*; or *K. lactis*).

Expression and purification

[0354] In some embodiments, a method of making an anti-CLL1 construct is provided, wherein the method comprises culturing a host cell comprising a nucleic acid encoding the anti-CLL1 construct under conditions suitable for expression of the anti-CLL1 construct, and optionally recovering the anti-CLL1 construct from the host cell (or host cell culture medium).

[0355] The anti-CLL1 construct may be purified by any suitable method. Such methods include, but are not limited to, the use of affinity matrices or hydrophobic interaction chromatography. Suitable affinity ligands include the ROR1 ECD and ligands that bind antibody constant regions. For example, a Protein A, Protein G, Protein A/G, or an antibody affinity column may be used to bind the constant region and to purify an anti-CLL1 construct comprising a constant region. Hydrophobic interactive chromatography, for example, a butyl or phenyl column, may also suitable for purifying some polypeptides such as antibodies. Ion exchange chromatography (*e.g.* anion exchange chromatography and/or cation exchange chromatography) may also suitable for purifying some polypeptides such as antibodies. Mixed-mode chromatography (*e.g.* reversed phase/anion exchange, reversed phase/cation exchange, hydrophilic interaction/anion exchange, hydrophilic interaction/cation exchange, *etc.*) may also suitable for purifying some polypeptides such as antibodies. Many methods of purifying polypeptides are known in the art.

[0356] In some embodiments, an anti-CLL1 construct is produced in a cell-free system. Nonlimiting exemplary cell-free systems are described, *e.g.*, in Sitaraman *et al.*, *Methods Mol. Biol.* 498: 229-44 (2009); Spirin, *Trends Biotechnol.* 22: 538-45 (2004); Endo *et al.*, *Biotechnol. Adv.* 21: 695-713 (2003).

[0357] Also provided are anti-CLL1 constructs prepared by any one of the methods described herein. In some embodiments, the anti-CLL1 construct is prepared in a host cell. In some embodiments, the anti-CLL1 construct is prepared in a cell-free system. In some embodiments, the anti-CLL1 construct is purified. In some embodiments, the present application provides a cell culture media comprising an anti-

CLL1 construct. In some embodiments, the present application provides a host cell culture fluid comprising an anti-CLL1 construct.

IV. Engineered immune cells

[0358] One aspect of the present application provides host cells (such as immune cells) comprising any one of the anti-CLL1 chimeric receptors or chimeric receptor systems as described herein.

[0359] Thus, in some embodiments, there is provided an engineered immune cell (such as T cell) comprising an anti-CLL1 chimeric receptor comprising: (a) an extracellular domain comprising an anti-CLL1 sdAb (such as any one of the anti-CLL1 sdAbs described herein); (b) a transmembrane domain; and (c) an intracellular signaling domain. In some embodiments, the intracellular signaling domain comprises a primary intracellular signaling sequence of an immune effector cell (such as T cell). In some embodiments, the primary intracellular signaling sequence is derived from CD3ζ, FcRγ, FcRβ, CD3γ, CD3δ, CD3ε, CD5, CD22, CD79a, CD79b, or CD66d. In some embodiments, the primary intracellular signaling sequence is derived from CD3ζ. In some embodiments, the intracellular signaling domain comprises an intracellular co-stimulatory sequence. In some embodiments, the intracellular co-stimulatory sequence is derived from a co-stimulatory molecule selected from the group consisting of CD27, CD28, 4-1BB, OX40, CD40, PD-1, LFA-1, ICOS, CD2, CD7, LIGHT, NKG2C, B7-H3, TNFRSF9, TNFRSF4, TNFRSF8, CD40LG, ITGB2, KLRC2, TNFRSF18, TNFRSF14, HAVCR1, LGALS9, DAP10, DAP12, CD83, ligands of CD83 and combinations thereof. In some embodiments, the engineered immune cell expresses a safety-switch antigen or epitope, such as CD52, EGFR, or CD20.

[0360] In some embodiments, there is provided an engineered immune cell (such as T cell) comprising an anti-CLL1 chimeric receptor comprising: (a) an extracellular domain comprising an anti-CLL1 sdAb (such as any one of the anti-CLL1 sdAbs described herein); (b) a transmembrane domain; and (c) an intracellular signaling domain comprising a CD3ζ intracellular signaling sequence and an intracellular co-stimulatory sequence derived from CD28 or 4-1BB. In some embodiments, the engineered immune cell expresses a safety-switch antigen or epitope, such as CD52, EGFR, or CD20.

[0361] In some embodiments, there is provided an engineered immune cell (such as T cell) comprising: (a) a first chimeric receptor comprising an extracellular domain comprising an anti-CLL1 sdAb (such as any one of the anti-CLL1 sdAbs described herein), a transmembrane domain, and an intracellular signaling domain comprising a primary intracellular signaling sequence of an immune cell (*e.g.*, T cell) and an intracellular co-stimulatory sequence; (b) a second chimeric receptor comprising an extracellular domain comprising a second binding moiety (*e.g.*, sdAb, scFv, or an extracellular domain of a receptor) that specifically binds to a second antigen or epitope, a transmembrane domain, and an intracellular signaling domain comprising a primary intracellular signaling sequence of an immune cell

(*e.g.*, T cell) and an intracellular co-stimulatory sequence. In some embodiments, the intracellular signaling domain of each of the first chimeric receptor and the second chimeric receptor comprises a CD3ζ intracellular signaling sequence and an intracellular co-stimulatory sequence derived from CD28 or 4-1BB. In some embodiments, the second binding moiety is an anti-CD33 or anti-CD123 sdAb or scFv. In some embodiments, the second binding moiety is an extracellular domain of NKG2D. In some embodiments, the engineered immune cell expresses a safety-switch antigen or epitope, such as CD52, EGFR, or CD20.

[0362] In some embodiments, there is provided an engineered immune cell (such as T cell) comprising: (a) a first chimeric receptor comprising an extracellular domain comprising an anti-CLL1 sdAb (such as any one of the anti-CLL1 sdAbs described herein), a transmembrane domain, and an intracellular signaling domain comprising a primary intracellular signaling sequence of an immune cell (*e.g.*, T cell); (b) a second chimeric receptor comprising an extracellular domain comprising a second binding moiety (*e.g.*, sdAb, scFv, or an extracellular domain of a receptor) that specifically binds to a second antigen or epitope, a transmembrane domain, and an intracellular signaling domain comprising an intracellular co-stimulatory sequence. In some embodiments, the intracellular signaling domain of the first chimeric receptor comprises a CD3ζ intracellular signaling sequence. In some embodiments, the intracellular signaling domain of the second chimeric receptor comprises an intracellular co-stimulatory sequence derived from CD28 or 4-1BB. In some embodiments, the second binding moiety is an anti-CD33 or anti-CD123 sdAb or scFv. In some embodiments, the second binding moiety is an extracellular domain of NKG2D. In some embodiments, the engineered immune cell expresses a safety-switch antigen or epitope, such as CD52, EGFR, or CD20.

[0363] In some embodiments, there is provided an engineered immune cell (such as T cell) comprising: (a) a first chimeric receptor comprising an extracellular domain comprising an anti-CLL1 sdAb (such as any one of the anti-CLL1 sdAbs described herein), a transmembrane domain, and an intracellular domain comprising an intracellular signaling domain comprising an intracellular co-stimulatory sequence; (b) a second chimeric receptor comprising an extracellular domain comprising a second binding moiety (*e.g.*, sdAb, scFv, or an extracellular domain of a receptor) that specifically binds to a second antigen or epitope, a transmembrane domain, and an intracellular signaling domain comprising a primary intracellular signaling sequence of an immune cell (*e.g.*, T cell). In some embodiments, the intracellular signaling domain of the first chimeric receptor comprises an intracellular co-stimulatory sequence derived from CD28 or 4-1BB. In some embodiments, the intracellular signaling domain of the second chimeric receptor comprises a CD3ζ intracellular signaling sequence. In some embodiments, the second binding moiety is an anti-CD33 or anti-CD123 sdAb or scFv. In some

embodiments, the second binding moiety is an extracellular domain of NKG2D. In some embodiments, the engineered immune cell expresses a safety-switch antigen or epitope, such as CD52, EGFR, or CD20.

[0364] In some embodiments according to any one of the engineered immune cells described above, the anti-CLL1 sdAb comprises any one of the following: (1) a CDR1 comprising the amino acid sequence of SEQ ID NO: 4, a CDR2 comprising the amino acid sequence of SEQ ID NO: 6, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 8, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; (2) a CDR1 comprising the amino acid sequence of SEQ ID NO: 11, a CDR2 comprising the amino acid sequence of SEQ ID NO: 13, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 15, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; (3) a CDR1 comprising the amino acid sequence of SEQ ID NO: 18, a CDR2 comprising the amino acid sequence of SEQ ID NO: 20, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 22, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; (4) a CDR1 comprising the amino acid sequence of SEQ ID NO: 25, a CDR2 comprising the amino acid sequence of SEQ ID NO: 27, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 29, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; (5) a CDR1 comprising the amino acid sequence of SEQ ID NO: 32, a CDR2 comprising the amino acid sequence of SEQ ID NO: 34, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 36, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; (6) a CDR1 comprising the amino acid sequence of SEQ ID NO: 39, a CDR2 comprising the amino acid sequence of SEQ ID NO: 41, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 43, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; (7) a CDR1 comprising the amino acid sequence of SEQ ID NO: 46, a CDR2 comprising the amino acid sequence of SEQ ID NO: 48, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 50, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; (8) a CDR1 comprising the amino acid sequence of SEQ ID NO: 53, a CDR2 comprising the amino acid sequence of SEQ ID NO: 55, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 57, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; (9) a CDR1 comprising the amino acid sequence of SEQ ID NO: 60, a CDR2 comprising the amino acid sequence of SEQ ID NO: 62, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 64, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; (10) a CDR1 comprising the amino acid sequence of SEQ ID NO: 67, a CDR2 comprising the amino acid sequence of SEQ ID NO: 69, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 71, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; (11) a CDR1 comprising the amino acid sequence of SEQ ID NO: 74, a CDR2 comprising the amino acid sequence of SEQ ID NO: 76, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 78, or a variant thereof comprising up to about 5 amino acid

substitutions in the CDRs; (12) a CDR1 comprising the amino acid sequence of SEQ ID NO: 81, a CDR2 comprising the amino acid sequence of SEQ ID NO: 83, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 85, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; (13) a CDR1 comprising the amino acid sequence of SEQ ID NO: 88, a CDR2 comprising the amino acid sequence of SEQ ID NO: 90, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 92, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; (14) a CDR1 comprising the amino acid sequence of SEQ ID NO: 151, a CDR2 comprising the amino acid sequence of SEQ ID NO: 153, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 155, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; (15) a CDR1 comprising the amino acid sequence of SEQ ID NO: 158, a CDR2 comprising the amino acid sequence of SEQ ID NO: 160, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 162, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; or (16) a CDR1 comprising the amino acid sequence of SEQ ID NO: 165, a CDR2 comprising the amino acid sequence of SEQ ID NO: 167, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 169, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs. In some embodiments, the anti-CLL1 sdAb comprises a V_HH domain comprising the amino acid sequence of any one of SEQ ID NOs: 94-106 and 171-173, or a variant thereof comprising an amino acid sequence having at least about 95% sequence identity to the amino acid sequence of any one of SEQ ID NOs: 94-106 and 171-173. In some embodiments, the chimeric receptor further comprises a hinge domain (*e.g.*, a CD8 hinge domain) located between the C-terminus of the extracellular domain and the N-terminus of the transmembrane domain. In some embodiments, the chimeric receptor further comprises a signal peptide (such as a CD8 signal peptide).

[0365] The engineered immune cell may further express one or more therapeutic proteins and/or immunomodulators, such as immune checkpoint inhibitors. See, for example, International Patent Application NOs. PCT/CN2016/073489 and PCT/CN2016/087855, which are incorporated herein by reference in their entirety.

Nucleic acids and vectors

[0366] In some embodiments, there is provided an isolated nucleic acid encoding any of the anti-CLL1 chimeric receptors or chimeric receptor systems provided herein. In some embodiments, there is provided a nucleic acid comprising a first polynucleotide encoding a first chimeric receptor comprising: an extracellular domain comprising an anti-CLL1 sdAb, a transmembrane domain, and an intracellular signaling domain; and a second polynucleotide encoding a second chimeric receptor comprising: an extracellular domain comprising a second binding moiety that specifically binds to a second antigen or epitope, a transmembrane domain, and an intracellular signaling domain. In some embodiments, the first polynucleotide is operably linked to a first promoter, and the second polynucleotide is operably linked to

a second promoter. In some embodiments, the first polynucleotide and the second polynucleotide are linked to the same promoter. In some embodiments, the first polynucleotide and the second polynucleotide are operably linked to each other via a third polynucleotide encoding a self-cleaving peptide, such as T2A, P2A, or F2A. In some embodiments, the self-cleaving peptide is P2A. In some embodiments, the self-cleaving peptide comprises the amino acid sequence of GSGATNFSLLKQAGDVEENPGP (SEQ ID NO: 148). In some embodiments, the isolated nucleic acid is a DNA. In some embodiments, the isolated nucleic acid is an RNA.

[0367] In some embodiments, the present application provides vectors for cloning and expressing any one of the anti-CLL1 chimeric receptors or chimeric receptor systems described herein. In some embodiments, the vector is suitable for replication and integration in eukaryotic cells, such as mammalian cells. In some embodiments, the vector is a viral vector. Examples of viral vectors include, but are not limited to, adenoviral vectors, adeno-associated virus vectors, lentiviral vector, retroviral vectors, vaccinia vector, herpes simplex viral vector, and derivatives thereof. Viral vector technology is well known in the art and is described, for example, in Sambrook *et al.* (2001, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, New York), and in other virology and molecular biology manuals.

[0368] A number of viral based systems have been developed for gene transfer into mammalian cells. For example, retroviruses provide a convenient platform for gene delivery systems. The heterologous nucleic acid can be inserted into a vector and packaged in retroviral particles using techniques known in the art. The recombinant virus can then be isolated and delivered to the engineered mammalian cell *in vitro* or ex vivo. A number of retroviral systems are known in the art. In some embodiments, adenovirus vectors are used. A number of adenovirus vectors are known in the art. In some embodiments, lentivirus vectors are used. In some embodiments, self-inactivating lentiviral vectors are used. For example, self-inactivating lentiviral vectors carrying chimeric receptors can be packaged with protocols known in the art. The resulting lentiviral vectors can be used to transduce a mammalian cell (such as primary human T cells) using methods known in the art. Vectors derived from retroviruses such as lentivirus are suitable tools to achieve long-term gene transfer, because they allow long-term, stable integration of a transgene and its propagation in progeny cells. Lentiviral vectors also have low immunogenicity, and can transduce non-proliferating cells.

[0369] In some embodiments, the vector is a non-viral vector. In some embodiments, the vector is a transposon, such as a Sleeping Beauty (SB) transposon system, or a PiggyBac transposon system. In some embodiments, the vector is a polymer-based non-viral vector, including for example, poly(lactic-co-glycolic acid) (PLGA) and poly lactic acid (PLA), poly(ethylene imine) (PEI), and dendrimers. In some embodiments, the vector is a cationic-lipid based non-viral vector, such as cationic liposome, lipid nanoemulsion, and solid lipid nanoparticle (SLN). In some embodiments, the vector is a peptide-based

gene non-viral vector, such as poly-L-lysine. Any of the known non-viral vectors suitable for genome editing can be used for introducing the chimeric receptor-encoding nucleic acids to the engineered immune cells. *See*, for example, Yin H. et al. *Nature Rev. Genetics* (2014) 15:521-555; Aronovich EL *et al.* "The Sleeping Beauty transposon system: a non-viral vector for gene therapy." *Hum. Mol. Genet.* (2011) R1: R14-20; and Zhao S. *et al.* "PiggyBac transposon vectors: the tools of the human gene editing." *Transl. Lung Cancer Res.* (2016) 5(1): 120-125, which are incorporated herein by reference. In some embodiments, any one or more of the nucleic acids encoding a chimeric receptor or chimeric receptor system is introduced to the engineered immune cells by a physical method, including, but not limited to electroporation, sonoporation, photoporation, magnetofection, hydroporation.

[0370] In some embodiments, the vector comprises any one of the nucleic acids encoding an anti-CLL1 constructs as described herein. The nucleic acid can be cloned into the vector using any known molecular cloning methods in the art, including, for example, using restriction endonuclease sites and one or more selectable markers. In some embodiments, the nucleic acid is operably linked to a promoter. Varieties of promoters have been explored for gene expression in mammalian cells, and any of the promoters known in the art may be used in the present invention. Promoters may be roughly categorized as constitutive promoters or regulated promoters, such as inducible promoters.

[0371] In some embodiments, the nucleic acid encoding the chimeric receptor is operably linked to a constitutive promoter. Constitutive promoters allow heterologous genes (also referred to as transgenes) to be expressed constitutively in the host cells. Exemplary constitutive promoters contemplated herein include, but are not limited to, Cytomegalovirus (CMV) promoters, human elongation factors-1alpha (hEF1α), ubiquitin C promoter (UbiC), phosphoglycerokinase promoter (PGK), simian virus 40 early promoter (SV40), and chicken β-Actin promoter coupled with CMV early enhancer (CAGG). The efficiencies of such constitutive promoters on driving transgene expression have been widely compared in a huge number of studies. For example, Michael C. Milone *et al* compared the efficiencies of CMV, hEF1α, UbiC and PGK to drive chimeric receptor expression in primary human T cells, and concluded that hEF1α promoter not only induced the highest level of transgene expression, but was also optimally maintained in the CD4 and CD8 human T cells (Molecular Therapy, 17(8): 1453-1464 (2009)). In some embodiments, the nucleic acid encoding the chimeric receptor is operably linked to a hEF1α promoter.

[0372] In some embodiments, the nucleic acid encoding the chimeric receptor is operably linked to an inducible promoter. Inducible promoters belong to the category of regulated promoters. The inducible promoter can be induced by one or more conditions, such as a physical condition, microenvironment of the engineered immune cell, or the physiological state of the engineered immune cell, an inducer (*i.e.*, an inducing agent), or a combination thereof. In some embodiments, the inducing condition does not induce the expression of endogenous genes in the engineered mammalian cell, and/or in the subject that receives

the pharmaceutical composition. In some embodiments, the inducing condition is selected from the group consisting of: inducer, irradiation (such as ionizing radiation, light), temperature (such as heat), redox state, tumor environment, and the activation state of the engineered mammalian cell.

[0373] In some embodiments, the vector also contains a selectable marker gene or a reporter gene to select cells expressing the chimeric receptor from the population of host cells transfected through lentiviral vectors. Both selectable markers and reporter genes may be flanked by appropriate regulatory sequences to enable expression in the host cells. For example, the vector may contain transcription and translation terminators, initiation sequences, and promoters useful for regulation of the expression of the nucleic acid sequences.

[0374] In some embodiments, the vector comprises one or more nucleic acids encoding chimeric receptors. In some embodiments, the vector comprises a nucleic acid comprising a first nucleic acid sequence encoding a first chimeric receptor and a second nucleic acid sequence encoding a second chimeric receptor, wherein the first nucleic acid is operably linked to the second nucleic acid via a third nucleic acid sequence encoding a self-cleaving peptide. In some embodiments, the self-cleaving peptide is selected from the group consisting of T2A, P2A and F2A. In some embodiments, the self-cleaving peptide is P2A. In some embodiments, the self-cleaving peptide comprises the amino acid sequence of SEQ ID NO: 148. In some embodiments, the vector further comprises a nucleic acid encoding a safety-switch antigen or epitope. In some embodiments, the safety-switch antigen or epitope is derived from CD52, EGFR or CD20.

Immune cells

[0375] In some embodiments, the engineered immune cells are immune effector cells. "Immune effector cells" are immune cells that can perform immune effector functions. In some embodiments, the immune effector cells express at least FcγRIII and perform ADCC effector function. Examples of immune effector cells which mediate ADCC include peripheral blood mononuclear cells (PBMC), natural killer (NK) cells, monocytes, cytotoxic T cells, neutrophils, and eosinophils.

[0376] In some embodiments, the immune cells are T cells, such as cytotoxic T cell and/or helper T cell. In some embodiments, the T cells are $CD4^+/CD8^-$, $CD4^-/CD8^+$, $CD4^+/CD8^+$, $CD4^-/CD8^-$, or combinations thereof. In some embodiments, the T cells produce IL-2, TFN, and/or TNF upon expressing the CAR and binding to the target cells, such as $CLL1^+$ tumor cells. In some embodiments, the CD8+ T cells lyse antigen-specific target cells upon expressing the CAR and binding to the target cells. In some embodiments, the immune cells are γδ T cells.

[0377] In some embodiments, the immune cells are NK cells. In other embodiments, the immune cells can be derived from established cell lines, for example, NK-92 cells.

[0378] In some embodiments, the immune cells are natural killer T cells.

[0379] In some embodiments, the immune cells are differentiated from a stem cell, such as a hematopoietic stem cell, a pluripotent stem cell, an iPS, or an embryonic stem cell.

[0380] The engineered immune cells are prepared by introducing the CARs into the immune cells, such as T cells. In some embodiments, the CAR is introduced to the immune cells by transfecting any one of the isolated nucleic acids or any one of the vectors described in Section III. In some embodiments, the CAR is introduced to the immune cells by inserting proteins into the cell membrane while passing cells through a microfluidic system, such as CELL SQUEEZE® (*see*, for example, U.S. Patent Application Publication No. 20140287509).

[0381] Methods of introducing vectors or isolated nucleic acids into a mammalian cell are known in the art. The vectors described can be transferred into an immune cell by physical, chemical, or biological methods.

[0382] Physical methods for introducing the vector into an immune cell include calcium phosphate precipitation, lipofection, particle bombardment, microinjection, electroporation, and the like. Methods for producing cells comprising vectors and/or exogenous nucleic acids are well-known in the art. *See*, for example, Sambrook *et al.* (2001) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, New York. In some embodiments, the vector is introduced into the cell by electroporation.

[0383] Biological methods for introducing the vector into an immune cell include the use of DNA and RNA vectors. Viral vectors have become the most widely used method for inserting genes into mammalian, *e.g.*, human cells.

[0384] Chemical means for introducing the vector into an immune cell include colloidal dispersion systems, such as macromolecule complexes, nanocapsules, microspheres, beads, and lipid-based systems including oil-in-water emulsions, micelles, mixed micelles, and liposomes. An exemplary colloidal system for use as a delivery vehicle *in vitro* is a liposome (*e.g.*, an artificial membrane vesicle).

[0385] In some embodiments, RNA molecules encoding any one of the chimeric receptors or chimeric receptor systems described herein may be prepared by a conventional method (*e.g.*, *in vitro* transcription) and then introduced into the immune cells via known methods such as mRNA electroporation. See, *e.g.*, Rabinovich *et al.*, Human Gene Therapy 17:1027-1035.

[0386] In some embodiments, the transduced or transfected immune cell is propagated ex vivo after introduction of the vector or isolated nucleic acid. In some embodiments, the transduced or transfected immune cell is cultured to propagate for at least about any of 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 10 days, 12 days, or 14 days. In some embodiments, the transduced or transfected immune cell is further evaluated or screened to select the engineered mammalian cell.

[0387] Reporter genes may be used for identifying potentially transfected cells and for evaluating the functionality of regulatory sequences. In general, a reporter gene is a gene that is not present in or

expressed by the recipient organism or tissue and that encodes a polypeptide whose expression is manifested by some easily detectable property, *e.g.*, enzymatic activity. Expression of the reporter gene is assayed at a suitable time after the DNA has been introduced into the recipient cells. Suitable reporter genes may include genes encoding luciferase, beta-galactosidase, chloramphenicol acetyl transferase, secreted alkaline phosphatase, or the green fluorescent protein gene (*e.g.*, Ui-Tei *et al.* FEBS Letters 479: 79-82 (2000)). Suitable expression systems are well known and may be prepared using known techniques or obtained commercially.

[0388] Other methods to confirm the presence of the nucleic acid encoding the chimeric receptors or chimeric receptor systems in the engineered immune cell, include, for example, molecular biological assays well known to those of skill in the art, such as Southern and Northern blotting, RT-PCR and PCR; biochemical assays, such as detecting the presence or absence of a particular peptide, *e.g.*, by immunological methods (such as ELISAs and Western blots).

1. Sources of immune cells

[0389] Prior to expansion and genetic modification of the immune cells, a source of immune cells (*e.g.*, T cells) is obtained from an individual. Immune cells (*e.g.*, T cells) can be obtained from a number of sources, including peripheral blood mononuclear cells, bone marrow, lymph node tissue, cord blood, thymus tissue, tissue from a site of infection, ascites, pleural effusion, spleen tissue, and tumors. In some embodiments, any number of immune cell (*e.g.*, T cell) lines available in the art, may be used. In some embodiments, immune cells (*e.g.*, T cells) can be obtained from a unit of blood collected from a subject using any number of techniques known to the skilled artisan, such as FICOLL™ separation. In some embodiments, cells from the circulating blood of an individual are obtained by apheresis. The apheresis product typically contains lymphocytes, including T cells, monocytes, granulocytes, B cells, other nucleated white blood cells, red blood cells, and platelets. In some embodiments, the cells collected by apheresis may be washed to remove the plasma fraction and to place the cells in an appropriate buffer or media for subsequent processing steps. In some embodiments, the cells are washed with phosphate buffered saline (PBS). In some embodiments, the wash solution lacks calcium and may lack magnesium or may lack many if not all divalent cations. Again, surprisingly, initial activation steps in the absence of calcium lead to magnified activation. As those of ordinary skill in the art would readily appreciate a washing step may be accomplished by methods known to those in the art, such as by using a semi-automated “flow-through” centrifuge (for example, the Cobe 2991 cell processor, the Baxter CytoMate, or the Haemonetics Cell Saver 5) according to the manufacturer's instructions. After washing, the cells may be resuspended in a variety of biocompatible buffers, such as, for example, Ca^{2+}-free, Mg^{2+}-free PBS, PlasmaLyte A, or other saline solution with or without buffer. Alternatively, the undesirable components of the apheresis sample may be removed and the cells directly resuspended in culture media.

[0390] In some embodiments, T cells are isolated from peripheral blood lymphocytes by lysing the red blood cells and depleting the monocytes, for example, by centrifugation through a PERCOLL™ gradient or by counterflow centrifugal elutriation. A specific subpopulation of T cells, such as $CD3^+$, $CD28^+$, $CD4^+$, $CD8^+$, $CD45RA^+$, and $CD45RO^+$T cells, can be further isolated by positive or negative selection techniques. For example, in some embodiments, T cells are isolated by incubation with anti-CD3/anti-CD28-conjugated beads, such as DYNABEADS® M-450 CD3/CD28 T, for a time period sufficient for positive selection of the desired T cells. In some embodiments, the time period is about 30 minutes. In a further embodiment, the time period ranges from 30 minutes to 36 hours or longer and all integer values there between. In a further embodiment, the time period is at least 1, 2, 3, 4, 5, or 6 hours. In some embodiments, the time period is 10 to 24 hours. In some embodiments, the incubation time period is 24 hours. For isolation of T cells from patients with leukemia, use of longer incubation times, such as 24 hours, can increase cell yield. Longer incubation times may be used to isolate T cells in any situation where there are few T cells as compared to other cell types, such in isolating tumor infiltrating lymphocytes (TIL) from tumor tissue or from immune-compromised individuals. Further, use of longer incubation times can increase the efficiency of capture of $CD8^+$ T cells. Thus, by simply shortening or lengthening the time T cells are allowed to bind to the CD3/CD28 beads and/or by increasing or decreasing the ratio of beads to T cells (as described further herein), subpopulations of T cells can be preferentially selected for or against at culture initiation or at other time points during the process. Additionally, by increasing or decreasing the ratio of anti-CD3 and/or anti-CD28 antibodies on the beads or other surface, subpopulations of T cells can be preferentially selected for or against at culture initiation or at other desired time points. The skilled artisan would recognize that multiple rounds of selection can also be used. In some embodiments, it may be desirable to perform the selection procedure and use the "unselected" cells in the activation and expansion process. "Unselected" cells can also be subjected to further rounds of selection.

[0391] Enrichment of an immune cell (*e.g.*, T cell) population by negative selection can be accomplished with a combination of antibodies directed to surface markers unique to the negatively selected cells. One method is cell sorting and/or selection via negative magnetic immunoadherence or flow cytometry that uses a cocktail of monoclonal antibodies directed to cell surface markers present on the cells negatively selected. For example, to enrich for $CD4^+$ cells by negative selection, a monoclonal antibody cocktail typically includes antibodies to CD14, CD20, CD11b, CD16, HLA-DR, and CD8. In certain embodiments, it may be desirable to enrich for or positively select for regulatory T cells which typically express $CD4^+$, $CD25^+$, CD62Lhi, $GITR^+$, and $FoxP3^+$. Alternatively, in certain embodiments, T regulatory cells are depleted by anti-C25 conjugated beads or other similar method of selection.

[0392] For isolation of a desired population of cells by positive or negative selection, the concentration of cells and surface (*e.g.*, particles such as beads) can be varied. In certain embodiments, it may be desirable to significantly decrease the volume in which beads and cells are mixed together (i.e., increase the concentration of cells), to ensure maximum contact of cells and beads. For example, in one embodiment, a concentration of 2 billion cells/ml is used. In one embodiment, a concentration of 1 billion cells/ml is used. In a further embodiment, greater than 100 million cells/ml is used. In a further embodiment, a concentration of cells of 10, 15, 20, 25, 30, 35, 40, 45, or 50 million cells/ml is used. In yet another embodiment, a concentration of cells from 75, 80, 85, 90, 95, or 100 million cells/ml is used. In further embodiments, concentrations of 125 or 150 million cells/ml can be used. Using high concentrations can result in increased cell yield, cell activation, and cell expansion. Further, use of high cell concentrations allows more efficient capture of cells that may weakly express target antigens of interest, such as $CD28^-$negative T cells, or from samples where there are many tumor cells present (i.e., leukemic blood, tumor tissue, etc.). Such populations of cells may have therapeutic value and would be desirable to obtain. For example, using high concentration of cells allows more efficient selection of $CD8^+$ T cells that normally have weaker CD28 expression.

[0393] In some embodiments, it may be desirable to use lower concentrations of cells. By significantly diluting the mixture of T cells and surface (*e.g.*, particles such as beads), interactions between the particles and cells is minimized. This selects for cells that express high amounts of desired antigens to be bound to the particles. For example, $CD4^+$ T cells express higher levels of CD28 and are more efficiently captured than $CD8^+$ T cells in dilute concentrations. In some embodiments, the concentration of cells used is 5×10^6/ml. In some embodiments, the concentration used can be from about 1×10^5/ml to 1×10^6/ml, and any integer value in between.

[0394] In some embodiments, the cells may be incubated on a rotator for varying lengths of time at varying speeds at either 2-10ºC, or at room temperature.

[0395] Immune cells (*e.g.*, T cells) for stimulation can also be frozen after a washing step. Without being bound by theory, the freeze and subsequent thaw step provides a more uniform product by removing granulocytes and to some extent monocytes in the cell population. After the washing step that removes plasma and platelets, the cells may be suspended in a freezing solution. While many freezing solutions and parameters are known in the art and will be useful in this context, one method involves using PBS containing 20% DMSO and 8% human serum albumin, or culture media containing 10% Dextran 40 and 5% Dextrose, 20% Human Serum Albumin and 7.5% DMSO, or 31.25% Plasmalyte-A, 31.25% Dextrose 5%, 0.45% NaCl, 10% Dextran 40 and 5% Dextrose, 20% Human Serum Albumin, and 7.5% DMSO or other suitable cell freezing media containing for example, Hespan and PlasmaLyte A, the cells then are frozen to −80ºC at a rate of 1° per minute and stored in the vapor phase of a liquid nitrogen

storage tank. Other methods of controlled freezing may be used as well as uncontrolled freezing immediately at −20°C or in liquid nitrogen.

[0396] In some embodiments, cryopreserved cells are thawed and washed as described herein and allowed to rest for one hour at room temperature prior to activation.

[0397] Also contemplated in the present application is the collection of blood samples or apheresis product from a subject at a time period prior to when the expanded cells as described herein might be needed. As such, the source of the cells to be expanded can be collected at any time point necessary, and desired cells, such as T cells, isolated and frozen for later use in immunotherapy for any number of diseases or conditions that would benefit from immunotherapy, such as those described herein. In one embodiment a blood sample or an apheresis is taken from a generally healthy subject. In certain embodiments, a blood sample or an apheresis is taken from a generally healthy subject who is at risk of developing a disease, but who has not yet developed a disease, and the cells of interest are isolated and frozen for later use. In certain embodiments, the immune cells (*e.g.*, T cells) may be expanded, frozen, and used at a later time. In certain embodiments, samples are collected from a patient shortly after diagnosis of a particular disease as described herein but prior to any treatments. In some embodiments, the cells are isolated for a patient and frozen for later use in conjunction with (*e.g.*, before, simultaneously or following) bone marrow or stem cell transplantation, T cell ablative therapy using either chemotherapy agents such as, fludarabine, external-beam radiation therapy (XRT), cyclophosphamide, or antibodies such as OKT3 or CAMPATH. In another embodiment, the cells are isolated prior to and can be frozen for later use for treatment following B-cell ablative therapy such as agents that react with CD20, *e.g.*, Rituxan.

[0398] In some embodiments, immune cells (*e.g.*, T cells) are obtained from a patient directly following treatment. In this regard, it has been observed that following certain cancer treatments, in particular treatments with drugs that damage the immune system, shortly after treatment during the period when patients would normally be recovering from the treatment, the quality of immune cells (*e.g.*, T cells) obtained may be optimal or improved for their ability to expand ex vivo. Likewise, following ex vivo manipulation using the methods described herein, these cells may be in a preferred state for enhanced engraftment and in vivo expansion. Thus, it is contemplated within the context of the present invention to collect blood cells, including T cells, dendritic cells, or other cells of the hematopoietic lineage, during this recovery phase. Further, in certain embodiments, mobilization (for example, mobilization with GM-CSF) and conditioning regimens can be used to create a condition in a subject wherein repopulation, recirculation, regeneration, and/or expansion of particular cell types is favored, especially during a defined window of time following therapy. Illustrative cell types include T cells, B cells, dendritic cells, and other cells of the immune system.

2. Activation and Expansion of immune cells

[0399] Whether prior to or after genetic modification of the immune cells (*e.g.*, T cells) with the chimeric receptors or chimeric receptor systems described herein, the immune cells (*e.g.*, T cells) can be activated and expanded generally using methods as described, for example, in U.S. Pat. Nos. 6,352,694; 6,534,055; 6,905,680; 6,692,964; 5,858,358; 6,887,466; 6,905,681; 7,144,575; 7,067,318; 7,172,869; 7,232,566; 7,175,843; 5,883,223; 6,905,874; 6,797,514; 6,867,041; and U.S. Patent Application Publication No. 20060121005.

[0400] Generally, T cells can be expanded by contact with a surface having attached thereto an agent that stimulates a CD3/TCR complex associated signal and a ligand that stimulates a co-stimulatory molecule on the surface of the T cells. In particular, T cell populations may be stimulated as described herein, such as by contact with an anti-CD3 antibody, or antigen-binding fragment thereof, or an anti-CD2 antibody immobilized on a surface, or by contact with a protein kinase C activator (*e.g.*, bryostatin) in conjunction with a calcium ionophore. For co-stimulation of an accessory molecule on the surface of the T cells, a ligand that binds the accessory molecule is used. For example, a population of T cells can be contacted with an anti-CD3 antibody and an anti-CD28 antibody, under conditions appropriate for stimulating proliferation of the T cells. To stimulate proliferation of either $CD4^+$ T cells or $CD8^+$ T cells, an anti-CD3 antibody and an anti-CD28 antibody. Examples of an anti-CD28 antibody include 9.3, B-T3, XR-CD28 (Diaclone, Besancon, France) can be used as can other methods commonly known in the art (Berg et al., Transplant Proc. 30(8):3975-3977, 1998; Haanen et al., J. Exp. Med. 190(9):13191328, 1999; Garland et al., J. Immunol Meth. 227(1-2):53-63, 1999).

[0401] In some embodiments, the primary stimulatory signal and the co-stimulatory signal for the T cell may be provided by different protocols. For example, the agents providing each signal may be in solution or coupled to a surface. When coupled to a surface, the agents may be coupled to the same surface (i.e., in “cis” formation) or to separate surfaces (i.e., in “trans” formation). Alternatively, one agent may be coupled to a surface and the other agent in solution. In one embodiment, the agent providing the co-stimulatory signal is bound to a cell surface and the agent providing the primary activation signal is in solution or coupled to a surface. In certain embodiments, both agents can be in solution. In another embodiment, the agents may be in soluble form, and then cross-linked to a surface, such as a cell expressing Fc receptors or an antibody or other binding agent which will bind to the agents. In this regard, see for example, U.S. Patent Application Publication Nos. 20040101519 and 20060034810 for artificial antigen presenting cells (aAPCs) that are contemplated for use in activating and expanding T cells in the present invention.

[0402] In some embodiments, the T cells, are combined with agent-coated beads, the beads and the cells are subsequently separated, and then the cells are cultured. In an alternative embodiment, prior to

culture, the agent-coated beads and cells are not separated but are cultured together. In a further embodiment, the beads and cells are first concentrated by application of a force, such as a magnetic force, resulting in increased ligation of cell surface markers, thereby inducing cell stimulation.

[0403] By way of example, cell surface proteins may be ligated by allowing paramagnetic beads to which anti-CD3 and anti-CD28 are attached to contact the T cells. In one embodiment the cells (for example, 10^4 to 10^9 T cells) and beads (for example, DYNABEADS® M-450 CD3/CD28 T paramagnetic beads at a ratio of 1:1) are combined in a buffer, preferably PBS (without divalent cations such as, calcium and magnesium). Again, those of ordinary skill in the art can readily appreciate any cell concentration may be used. For example, the target cell may be very rare in the sample and comprise only 0.01% of the sample or the entire sample (i.e., 100%) may comprise the target cell of interest. Accordingly, any cell number is within the context of the present invention. In certain embodiments, it may be desirable to significantly decrease the volume in which particles and cells are mixed together (i.e., increase the concentration of cells), to ensure maximum contact of cells and particles. For example, in one embodiment, a concentration of about 2 billion cells/ml is used. In another embodiment, greater than 100 million cells/ml is used. In a further embodiment, a concentration of cells of 10, 15, 20, 25, 30, 35, 40, 45, or 50 million cells/ml is used. In yet another embodiment, a concentration of cells from 75, 80, 85, 90, 95, or 100 million cells/ml is used. In further embodiments, concentrations of 125 or 150 million cells/ml can be used. Using high concentrations can result in increased cell yield, cell activation, and cell expansion. Further, use of high cell concentrations allows more efficient capture of cells that may weakly express target antigens of interest, such as CD28-negative T cells. Such populations of cells may have therapeutic value and would be desirable to obtain in certain embodiments. For example, using high concentration of cells allows more efficient selection of CD8+ T cells that normally have weaker CD28 expression.

[0404] In some embodiments, the mixture may be cultured for several hours (about 3 hours) to about 14 days or any hourly integer value in between. In another embodiment, the mixture may be cultured for 21 days. In one embodiment of the invention the beads and the immune cells (*e.g.*, T cells) are cultured together for about eight days. In another embodiment, the beads and immune cells (*e.g.*, T cells) are cultured together for 2-3 days. Several cycles of stimulation may also be desired such that culture time of immune cells (*e.g.*, T cells) can be 60 days or more. Conditions appropriate for immune cell (*e.g.*, T cell) culture include an appropriate media (*e.g.*, Minimal Essential Media or RPMI Media 1640 or, X-vivo 15, (Lonza)) that may contain factors necessary for proliferation and viability, including serum (*e.g.*, fetal bovine or human serum), interleukin-2 (IL-2), insulin, IFN-γ, IL-4, IL-7, GM-CSF, IL-10, IL-12, IL-15, TGFβ, and TNF-α or any other additives for the growth of cells known to the skilled artisan. Other additives for the growth of cells include, but are not limited to, surfactant, plasmanate, and reducing agents such as N-acetyl-cysteine and 2-mercaptoethanol. Media can include RPMI 1640, AIM-V,

DMEM, MEM, α-MEM, F-12, X-Vivo 15, and X-Vivo 20, Optimizer, with added amino acids, sodium pyruvate, and vitamins, either serum-free or supplemented with an appropriate amount of serum (or plasma) or a defined set of hormones, and/or an amount of cytokine(s) sufficient for the growth and expansion of immune cells (*e.g.*, T cells). Antibiotics, *e.g.*, penicillin and streptomycin, are included only in experimental cultures, not in cultures of cells that are to be infused into a subject. The target cells are maintained under conditions necessary to support growth, for example, an appropriate temperature (*e.g.*, 37 °C) and atmosphere (*e.g.*, air plus 5% CO_2). Immune cells (*e.g.*, T cells) that have been exposed to varied stimulation times may exhibit different characteristics. For example, typical blood or apheresed peripheral blood mononuclear cell products have a helper T cell population (TH, $CD4^+$) that is greater than the cytotoxic or suppressor T cell population (TC, CD8). Ex vivo expansion of T cells by stimulating CD3 and CD28 receptors produces a population of T cells that prior to about days 8-9 consists predominately of TH cells, while after about days 8-9, the population of T cells comprises an increasingly greater population of TC cells. Accordingly, depending on the purpose of treatment, infusing a subject with a T cell population comprising predominately of TH cells may be advantageous. Similarly, if an antigen-specific subset of TC cells has been isolated it may be beneficial to expand this subset to a greater degree.

[0405] Further, in addition to CD4 and CD8 markers, other phenotypic markers vary significantly, but in large part, reproducibly during the course of the cell expansion process. Thus, such reproducibility enables the ability to tailor an activated T cell product for specific purposes.

V. Pharmaceutical compositions

[0406] Further provided by the present application are pharmaceutical compositions comprising any one of the anti-CLL1 constructs (including anti-CLL1 sdAbs, chimeric receptors, immune effector cell engagers, and immunoconjugates), or any one of the engineered immune cells comprising any one of the anti-CLL1 chimeric receptors or chimeric receptor systems as described herein, and a pharmaceutically acceptable carrier. Pharmaceutical compositions can be prepared by mixing an anti-CLL1 construct, or a plurality of engineered immune cells having the desired degree of purity with optional pharmaceutically acceptable carriers, excipients or stabilizers (Remington's Pharmaceutical Sciences 16th edition, Osol, A. Ed. (1980)), in the form of lyophilized formulations or aqueous solutions.

[0407] Acceptable carriers, excipients, or stabilizers are nontoxic to recipients at the dosages and concentrations employed, and include buffers, antioxidants including ascorbic acid, methionine, Vitamin E, sodium metabisulfite; preservatives, isotonicifiers, stabilizers, metal complexes (*e.g.* Zn-protein complexes); chelating agents such as EDTA and/or non-ionic surfactants.

[0408] In order for the pharmaceutical compositions to be used for in vivo administration, they must be sterile. The pharmaceutical composition may be rendered sterile by filtration through sterile filtration membranes. The pharmaceutical compositions herein generally are placed into a container having a sterile access port, for example, an intravenous solution bag or vial having a stopper pierceable by a hypodermic injection needle.

[0409] The route of administration is in accordance with known and accepted methods, such as by single or multiple bolus or infusion over a long period of time in a suitable manner, *e.g.*, injection or infusion by subcutaneous, intravenous, intraperitoneal, intramuscular, intraarterial, intralesional or intraarticular routes, topical administration, inhalation or by sustained release or extended-release means.

[0410] The pharmaceutical compositions described herein may also contain more than one active compound or agent as necessary for the particular indication being treated, preferably those with complementary activities that do not adversely affect each other. Alternatively, or in addition, the composition may comprise a cytotoxic agent, chemotherapeutic agent, cytokine, immunosuppressive agent, or growth inhibitory agent. Such molecules are suitably present in combination in amounts that are effective for the purpose intended.

[0411] The active ingredients may also be entrapped in microcapsules prepared, for example, by coascervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or gelatin-microcapsules and poly-(methylmethacylate) microcapsules, respectively, in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles and nanocapsules) or in macroemulsions. Such techniques are disclosed in *Remington's Pharmaceutical Sciences* 18th edition.

VI. Methods of treatment

[0412] One aspect of the present application provides methods of treating a disease (such as cancer) in an individual, comprising administering to the individual an effective amount of any one of the anti-CLL1 constructs described herein. In some embodiments, the present application provides methods and compositions for use in cell immunotherapy. In some embodiments, the cell immunotherapy is for treating cancer, including but not limited to hematological malignancies and solid tumors. Any of the anti-CLL1 sdAbs, immune effector cell engagers, chimeric receptors, immunoconjugates, and engineered immune cells (such as CAR-T cells) described herein may be used in the method of treating cancer. Exemplary cancer types include, but are not limited to, acute myeloid leukemia (AML), chronic myelogenous leukemia (CML), and myelodysplastic syndrome (MDS). In some embodiments, the methods and compositions described herein may be used for treating other diseases that are associated with CLL1.

[0413] In some embodiments, there is provided a method of treating a disease (such as cancer, *e.g.*, AML, CML or MDS) in an individual (such as a human individual), comprising administering to the individual an effective amount of a pharmaceutical composition comprising an anti-CLL1 construct comprising an sdAb moiety that specifically binds to CLL1, wherein the sdAb moiety (*e.g.*, V_HH) comprises: (1) a CDR1 comprising the amino acid sequence of SEQ ID NO: 4, a CDR2 comprising the amino acid sequence of SEQ ID NO: 6, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 8, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; (2) a CDR1 comprising the amino acid sequence of SEQ ID NO: 11, a CDR2 comprising the amino acid sequence of SEQ ID NO: 13, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 15, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; (3) a CDR1 comprising the amino acid sequence of SEQ ID NO: 18, a CDR2 comprising the amino acid sequence of SEQ ID NO: 20, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 22, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; (4) a CDR1 comprising the amino acid sequence of SEQ ID NO: 25, a CDR2 comprising the amino acid sequence of SEQ ID NO: 27, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 29, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; (5) a CDR1 comprising the amino acid sequence of SEQ ID NO: 32, a CDR2 comprising the amino acid sequence of SEQ ID NO: 34, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 36, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; (6) a CDR1 comprising the amino acid sequence of SEQ ID NO: 39, a CDR2 comprising the amino acid sequence of SEQ ID NO: 41, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 43, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; (7) a CDR1 comprising the amino acid sequence of SEQ ID NO: 46, a CDR2 comprising the amino acid sequence of SEQ ID NO: 48, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 50, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; (8) a CDR1 comprising the amino acid sequence of SEQ ID NO: 53, a CDR2 comprising the amino acid sequence of SEQ ID NO: 55, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 57, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; (9) a CDR1 comprising the amino acid sequence of SEQ ID NO: 60, a CDR2 comprising the amino acid sequence of SEQ ID NO: 62, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 64, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; (10) a CDR1 comprising the amino acid sequence of SEQ ID NO: 67, a CDR2 comprising the amino acid sequence of SEQ ID NO: 69, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 71, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; (11) a CDR1 comprising the amino acid sequence of SEQ ID NO: 74, a CDR2 comprising the amino acid sequence of SEQ ID NO: 76, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 78, or a

variant thereof comprising up to about 5 amino acid substitutions in the CDRs; (12) a CDR1 comprising the amino acid sequence of SEQ ID NO: 81, a CDR2 comprising the amino acid sequence of SEQ ID NO: 83, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 85, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; (13) a CDR1 comprising the amino acid sequence of SEQ ID NO: 88, a CDR2 comprising the amino acid sequence of SEQ ID NO: 90, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 92, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; (14) a CDR1 comprising the amino acid sequence of SEQ ID NO: 151, a CDR2 comprising the amino acid sequence of SEQ ID NO: 153, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 155, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; (15) a CDR1 comprising the amino acid sequence of SEQ ID NO: 158, a CDR2 comprising the amino acid sequence of SEQ ID NO: 160, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 162, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; or (16) a CDR1 comprising the amino acid sequence of SEQ ID NO: 165, a CDR2 comprising the amino acid sequence of SEQ ID NO: 167, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 169, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs. In some embodiments, the anti-CLL1 sdAb comprises a V_HH domain comprising the amino acid sequence of any one of SEQ ID NOs: 94-106 and 171-173, or a variant thereof comprising an amino acid sequence having at least about 95% sequence identity to the amino acid sequence of any one of SEQ ID NOs: 94-106 and 171-173. In some embodiments, the anti-CLL1 construct is a heavy-chain only antibody. In some embodiments, the anti-CLL1 construct is a multispecific antibody, such as a bispecific antibody. In some embodiments, the anti-CLL1 construct is an immunoconjugate.

[0414] In some embodiments, there is provided a method of treating a disease (such as cancer, *e.g.*, AML, CML or MDS) in an individual (such as a human individual), comprising administering to the individual an effective amount of a pharmaceutical composition comprising an immune effector cell engager comprising: (a) a target cell binding domain comprising an anti-CLL1 sdAb (such as any one of the anti-CLL1 sdAbs described herein), and (b) an immune effector cell binding domain comprising an antigen-binding fragment that specifically binds to an antigen on an immune effector cell. In some embodiments, the anti-CLL1 sdAb comprises any one of the following: (1) a CDR1 comprising the amino acid sequence of SEQ ID NO: 4, a CDR2 comprising the amino acid sequence of SEQ ID NO: 6, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 8, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; (2) a CDR1 comprising the amino acid sequence of SEQ ID NO: 11, a CDR2 comprising the amino acid sequence of SEQ ID NO: 13, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 15, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; (3) a CDR1 comprising the amino acid sequence of SEQ ID NO: 18, a CDR2 comprising

the amino acid sequence of SEQ ID NO: 20, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 22, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; (4) a CDR1 comprising the amino acid sequence of SEQ ID NO: 25, a CDR2 comprising the amino acid sequence of SEQ ID NO: 27, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 29, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; (5) a CDR1 comprising the amino acid sequence of SEQ ID NO: 32, a CDR2 comprising the amino acid sequence of SEQ ID NO: 34, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 36, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; (6) a CDR1 comprising the amino acid sequence of SEQ ID NO: 39, a CDR2 comprising the amino acid sequence of SEQ ID NO: 41, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 43, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; (7) a CDR1 comprising the amino acid sequence of SEQ ID NO: 46, a CDR2 comprising the amino acid sequence of SEQ ID NO: 48, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 50, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; (8) a CDR1 comprising the amino acid sequence of SEQ ID NO: 53, a CDR2 comprising the amino acid sequence of SEQ ID NO: 55, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 57, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; (9) a CDR1 comprising the amino acid sequence of SEQ ID NO: 60, a CDR2 comprising the amino acid sequence of SEQ ID NO: 62, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 64, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; (10) a CDR1 comprising the amino acid sequence of SEQ ID NO: 67, a CDR2 comprising the amino acid sequence of SEQ ID NO: 69, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 71, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; (11) a CDR1 comprising the amino acid sequence of SEQ ID NO: 74, a CDR2 comprising the amino acid sequence of SEQ ID NO: 76, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 78, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; (12) a CDR1 comprising the amino acid sequence of SEQ ID NO: 81, a CDR2 comprising the amino acid sequence of SEQ ID NO: 83, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 85, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; (13) a CDR1 comprising the amino acid sequence of SEQ ID NO: 88, a CDR2 comprising the amino acid sequence of SEQ ID NO: 90, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 92, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; (14) a CDR1 comprising the amino acid sequence of SEQ ID NO: 151, a CDR2 comprising the amino acid sequence of SEQ ID NO: 153, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 155, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; (15) a CDR1 comprising the amino acid sequence of SEQ ID NO: 158, a CDR2 comprising the amino acid sequence of SEQ ID NO: 160, and a

CDR3 comprising the amino acid sequence of SEQ ID NO: 162, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; or (16) a CDR1 comprising the amino acid sequence of SEQ ID NO: 165, a CDR2 comprising the amino acid sequence of SEQ ID NO: 167, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 169, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs. In some embodiments, the anti-CLL1 sdAb comprises a V_HH domain comprising the amino acid sequence of any one of SEQ ID NOs: 94-106 and 171-173, or a variant thereof comprising an amino acid sequence having at least about 95% sequence identity to the amino acid sequence of any one of SEQ ID NOs: 94-106 and 171-173. In some embodiments, the antigen-binding fragment in the immune effector cell binding domain is a Fab, scFv, or sdAb. In some embodiments, the target cell binding domain is fused to the N-terminus of the immune effector cell binding domain. In some embodiments, the target cell binding domain is fused to the C-terminus of the immune effector cell binding domain. In some embodiments, the target cell binding domain is fused to the immune effector cell binding domain via a peptide linker. In some embodiments, the immune effector cell is T cell. In some embodiments, the immune effector cell binding domain specifically binds to an antigen selected from the group consisting of CD3γ, CD3δ, CD3ε, CD3ζ, CD28, OX40, GITR, CD137, CD27, CD40L, and HVEM.

[0415] In some embodiments, there is provided a method of treating a disease (such as cancer, *e.g.*, AML, CML or MDS) in an individual (such as a human individual), comprising administering to the individual an effective amount of a pharmaceutical composition comprising an engineered immune cell (*e.g.*, T cell) comprising: an anti-CLL1 chimeric receptor comprising: (a) an extracellular domain comprising an anti-CLL1 sdAb (such as any one of the anti-CLL1 sdAbs described herein); (b) a transmembrane domain; and (c) an intracellular signaling domain. In some embodiments, the intracellular signaling domain comprises a primary intracellular signaling sequence of an immune effector cell (such as T cell). In some embodiments, the primary intracellular signaling sequence is derived from CD3ζ, FcRγ, FcRβ, CD3γ, CD3δ, CD3ε, CD5, CD22, CD79a, CD79b, or CD66d. In some embodiments, the primary intracellular signaling sequence is derived from CD3ζ. In some embodiments, the intracellular signaling domain comprises an intracellular co-stimulatory sequence. In some embodiments, the intracellular co-stimulatory sequence is derived from a co-stimulatory molecule selected from the group consisting of CD27, CD28, 4-1BB, OX40, CD40, PD-1, LFA-1, ICOS, CD2, CD7, LIGHT, NKG2C, B7-H3, TNFRSF9, TNFRSF4, TNFRSF8, CD40LG, ITGB2, KLRC2, TNFRSF18, TNFRSF14, HAVCR1, LGALS9, DAP10, DAP12, CD83, ligands of CD83 and combinations thereof. In some embodiments, the intracellular co-stimulatory sequence is derived from CD28 or 4-1BB. In some embodiments, the anti-CLL1 chimeric receptor comprises: (a) an extracellular domain comprising an anti-CLL1 sdAb (such as any one of the anti-CLL1 sdAbs described herein); (b) a transmembrane domain; and (c) an intracellular

signaling domain comprising a CD3ζ intracellular signaling sequence and an intracellular co-stimulatory sequence derived from CD28 or 4-1BB. In some embodiments, the anti-CLL1 sdAb comprises any one of the following: (1) a CDR1 comprising the amino acid sequence of SEQ ID NO: 4, a CDR2 comprising the amino acid sequence of SEQ ID NO: 6, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 8, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; (2) a CDR1 comprising the amino acid sequence of SEQ ID NO: 11, a CDR2 comprising the amino acid sequence of SEQ ID NO: 13, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 15, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; (3) a CDR1 comprising the amino acid sequence of SEQ ID NO: 18, a CDR2 comprising the amino acid sequence of SEQ ID NO: 20, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 22, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; (4) a CDR1 comprising the amino acid sequence of SEQ ID NO: 25, a CDR2 comprising the amino acid sequence of SEQ ID NO: 27, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 29, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; (5) a CDR1 comprising the amino acid sequence of SEQ ID NO: 32, a CDR2 comprising the amino acid sequence of SEQ ID NO: 34, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 36, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; (6) a CDR1 comprising the amino acid sequence of SEQ ID NO: 39, a CDR2 comprising the amino acid sequence of SEQ ID NO: 41, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 43, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; (7) a CDR1 comprising the amino acid sequence of SEQ ID NO: 46, a CDR2 comprising the amino acid sequence of SEQ ID NO: 48, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 50, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; (8) a CDR1 comprising the amino acid sequence of SEQ ID NO: 53, a CDR2 comprising the amino acid sequence of SEQ ID NO: 55, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 57, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; (9) a CDR1 comprising the amino acid sequence of SEQ ID NO: 60, a CDR2 comprising the amino acid sequence of SEQ ID NO: 62, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 64, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; (10) a CDR1 comprising the amino acid sequence of SEQ ID NO: 67, a CDR2 comprising the amino acid sequence of SEQ ID NO: 69, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 71, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; (11) a CDR1 comprising the amino acid sequence of SEQ ID NO: 74, a CDR2 comprising the amino acid sequence of SEQ ID NO: 76, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 78, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; (12) a CDR1 comprising the amino acid sequence of SEQ ID NO: 81, a CDR2 comprising the amino acid sequence of SEQ ID

NO: 83, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 85, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; (13) a CDR1 comprising the amino acid sequence of SEQ ID NO: 88, a CDR2 comprising the amino acid sequence of SEQ ID NO: 90, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 92, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; (14) a CDR1 comprising the amino acid sequence of SEQ ID NO: 151, a CDR2 comprising the amino acid sequence of SEQ ID NO: 153, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 155, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; (15) a CDR1 comprising the amino acid sequence of SEQ ID NO: 158, a CDR2 comprising the amino acid sequence of SEQ ID NO: 160, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 162, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; or (16) a CDR1 comprising the amino acid sequence of SEQ ID NO: 165, a CDR2 comprising the amino acid sequence of SEQ ID NO: 167, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 169, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs. In some embodiments, the anti-CLL1 sdAb comprises a V_HH domain comprising the amino acid sequence of any one of SEQ ID NOs: 94-106 and 171-173, or a variant thereof comprising an amino acid sequence having at least about 95% sequence identity to the amino acid sequence of any one of SEQ ID NOs: 94-106 and 171-173. In some embodiments, the anti-CLL1 chimeric receptor comprises the amino acid sequence of any one of SEQ ID NOs: 120-132, 177-179, 181 and 229-230, or a variant thereof comprising an amino acid sequence having at least about 95% sequence identity to the amino acid sequence of any one of SEQ ID NOs: 120-132, 177-179, and 181 and 229-230. In some embodiments, the anti-CLL1 chimeric receptor comprises the amino acid sequence of SEQ ID NO: 125 or 181. In some embodiments, the engineered immune effector cell expresses a safety-switch antigen or epitope, such as CD52, EGFR, or CD20. In some embodiments, the method further comprises subsequently administering an effective amount of a therapeutic antibody specifically binding to the safety-switch antigen or epitope.

[0416] In some embodiments, there is provided a method of treating a disease (such as cancer, *e.g.*, AML, CML or MDS) in an individual (such as a human individual), comprising administering to the individual an effective amount of a pharmaceutical composition comprising an engineered immune cell (*e.g.*, T cell) comprising a multispecific (*e.g.*, bispecific) chimeric receptor comprising: (a) an extracellular domain comprising an anti-CLL1 sdAb (such as any one of the anti-CLL1 sdAbs described herein) and a second sdAb that specifically binds to a second antigen or epitope (*e.g.*, sdAb, scFv, or an extracellular domain of a receptor); (b) a transmembrane domain; and (c) an intracellular signaling domain. In some embodiments, the intracellular signaling domain comprises a CD3ζ intracellular signaling sequence and an intracellular co-stimulatory sequence derived from CD28 or 4-1BB. In some embodiments, the second binding moiety is an anti-CD33 or anti-CD123 sdAb or scFv. In some

embodiments, the second binding moiety is an extracellular domain of NKG2D. In some embodiments, the anti-CLL1 sdAb comprises any one of the following: (1) a CDR1 comprising the amino acid sequence of SEQ ID NO: 4, a CDR2 comprising the amino acid sequence of SEQ ID NO: 6, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 8, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; (2) a CDR1 comprising the amino acid sequence of SEQ ID NO: 11, a CDR2 comprising the amino acid sequence of SEQ ID NO: 13, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 15, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; (3) a CDR1 comprising the amino acid sequence of SEQ ID NO: 18, a CDR2 comprising the amino acid sequence of SEQ ID NO: 20, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 22, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; (4) a CDR1 comprising the amino acid sequence of SEQ ID NO: 25, a CDR2 comprising the amino acid sequence of SEQ ID NO: 27, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 29, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; (5) a CDR1 comprising the amino acid sequence of SEQ ID NO: 32, a CDR2 comprising the amino acid sequence of SEQ ID NO: 34, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 36, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; (6) a CDR1 comprising the amino acid sequence of SEQ ID NO: 39, a CDR2 comprising the amino acid sequence of SEQ ID NO: 41, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 43, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; (7) a CDR1 comprising the amino acid sequence of SEQ ID NO: 46, a CDR2 comprising the amino acid sequence of SEQ ID NO: 48, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 50, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; (8) a CDR1 comprising the amino acid sequence of SEQ ID NO: 53, a CDR2 comprising the amino acid sequence of SEQ ID NO: 55, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 57, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; (9) a CDR1 comprising the amino acid sequence of SEQ ID NO: 60, a CDR2 comprising the amino acid sequence of SEQ ID NO: 62, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 64, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; (10) a CDR1 comprising the amino acid sequence of SEQ ID NO: 67, a CDR2 comprising the amino acid sequence of SEQ ID NO: 69, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 71, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; (11) a CDR1 comprising the amino acid sequence of SEQ ID NO: 74, a CDR2 comprising the amino acid sequence of SEQ ID NO: 76, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 78, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; (12) a CDR1 comprising the amino acid sequence of SEQ ID NO: 81, a CDR2 comprising the amino acid sequence of SEQ ID NO: 83, and a CDR3 comprising the amino acid sequence

of SEQ ID NO: 85, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; (13) a CDR1 comprising the amino acid sequence of SEQ ID NO: 88, a CDR2 comprising the amino acid sequence of SEQ ID NO: 90, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 92, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; (14) a CDR1 comprising the amino acid sequence of SEQ ID NO: 151, a CDR2 comprising the amino acid sequence of SEQ ID NO: 153, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 155, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; (15) a CDR1 comprising the amino acid sequence of SEQ ID NO: 158, a CDR2 comprising the amino acid sequence of SEQ ID NO: 160, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 162, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; or (16) a CDR1 comprising the amino acid sequence of SEQ ID NO: 165, a CDR2 comprising the amino acid sequence of SEQ ID NO: 167, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 169, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs. In some embodiments, the anti-CLL1 sdAb comprises a V_HH domain comprising the amino acid sequence of any one of SEQ ID NOs: 94-106 and 171-173, or a variant thereof comprising an amino acid sequence having at least about 95% sequence identity to the amino acid sequence of any one of SEQ ID NOs: 94-106 and 171-173. In some embodiments, the multispecific chimeric receptor comprising a polypeptide comprising the amino acid sequence of any one of SEQ ID NOs: 184-195, or a variant thereof comprising an amino acid sequence having at least about 95% sequence identity to the amino acid sequence of any one of SEQ ID NOs: 184-195. In some embodiments, the multispecific chimeric receptor comprises the amino acid sequence of SEQ ID NO: 184, 185 or 188. In some embodiments, the engineered immune effector cell expresses a safety-switch antigen or epitope, such as CD52, EGFR, or CD20. In some embodiments, the method further comprises subsequently administering an effective amount of a therapeutic antibody specifically binding to the safety-switch antigen or epitope.

[0417] In some embodiments, there is provided a method of treating a disease (such as cancer, *e.g.*, AML, CML or MDS) in an individual (such as a human individual), comprising administering to the individual an effective amount of a pharmaceutical composition comprising an engineered immune cell (*e.g.*, T cell) comprising: (a) a first chimeric receptor comprising an extracellular domain comprising an anti-CLL1 sdAb (such as any one of the anti-CLL1 sdAbs described herein), a transmembrane domain, and an intracellular signaling domain comprising a primary intracellular signaling sequence of an immune cell (*e.g.*, a CD3ζ intracellular signaling sequence); (b) a second chimeric receptor comprising an extracellular domain comprising a second binding moiety (*e.g.*, sdAb, scFv, or an extracellular domain of a receptor) that specifically binds to a second antigen or epitope, a transmembrane domain, and an intracellular signaling domain comprising an intracellular co-stimulatory sequence (*e.g.*, an intracellular

co-stimulatory sequence derived from CD28 or 4-1BB). In some embodiments, there is provided a method of treating a disease (such as cancer, *e.g.*, AML, CML or MDS) in an individual (such as a human individual), comprising administering to the individual an effective amount of a pharmaceutical composition comprising an engineered immune cell (*e.g.*, T cell) comprising: (a) a first chimeric receptor comprising an extracellular domain comprising an anti-CLL1 sdAb (such as any one of the anti-CLL1 sdAbs described herein), a transmembrane domain, and an intracellular domain comprising an intracellular signaling domain comprising an intracellular co-stimulatory sequence (*e.g.*, an intracellular co-stimulatory sequence derived from CD28 or 4-1BB); (b) a second chimeric receptor comprising an extracellular domain comprising a second binding moiety (*e.g.*, sdAb, scFv, or an extracellular domain of a receptor) that specifically binds to a second antigen or epitope, a transmembrane domain, and an intracellular signaling domain comprising a primary intracellular signaling sequence of an immune cell (*e.g.*, a CD3ζ intracellular signaling sequence). In some embodiments, the second binding moiety is an anti-CD33 or anti-CD123 sdAb or scFv. In some embodiments, the second binding moiety is an extracellular domain of NKG2D. In some embodiments, the anti-CLL1 sdAb comprises any one of the following: (1) a CDR1 comprising the amino acid sequence of SEQ ID NO: 4, a CDR2 comprising the amino acid sequence of SEQ ID NO: 6, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 8, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; (2) a CDR1 comprising the amino acid sequence of SEQ ID NO: 11, a CDR2 comprising the amino acid sequence of SEQ ID NO: 13, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 15, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; (3) a CDR1 comprising the amino acid sequence of SEQ ID NO: 18, a CDR2 comprising the amino acid sequence of SEQ ID NO: 20, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 22, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; (4) a CDR1 comprising the amino acid sequence of SEQ ID NO: 25, a CDR2 comprising the amino acid sequence of SEQ ID NO: 27, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 29, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; (5) a CDR1 comprising the amino acid sequence of SEQ ID NO: 32, a CDR2 comprising the amino acid sequence of SEQ ID NO: 34, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 36, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; (6) a CDR1 comprising the amino acid sequence of SEQ ID NO: 39, a CDR2 comprising the amino acid sequence of SEQ ID NO: 41, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 43, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; (7) a CDR1 comprising the amino acid sequence of SEQ ID NO: 46, a CDR2 comprising the amino acid sequence of SEQ ID NO: 48, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 50, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; (8) a CDR1 comprising the amino acid

sequence of SEQ ID NO: 53, a CDR2 comprising the amino acid sequence of SEQ ID NO: 55, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 57, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; (9) a CDR1 comprising the amino acid sequence of SEQ ID NO: 60, a CDR2 comprising the amino acid sequence of SEQ ID NO: 62, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 64, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; (10) a CDR1 comprising the amino acid sequence of SEQ ID NO: 67, a CDR2 comprising the amino acid sequence of SEQ ID NO: 69, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 71, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; (11) a CDR1 comprising the amino acid sequence of SEQ ID NO: 74, a CDR2 comprising the amino acid sequence of SEQ ID NO: 76, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 78, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; (12) a CDR1 comprising the amino acid sequence of SEQ ID NO: 81, a CDR2 comprising the amino acid sequence of SEQ ID NO: 83, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 85, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; (13) a CDR1 comprising the amino acid sequence of SEQ ID NO: 88, a CDR2 comprising the amino acid sequence of SEQ ID NO: 90, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 92, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; (14) a CDR1 comprising the amino acid sequence of SEQ ID NO: 151, a CDR2 comprising the amino acid sequence of SEQ ID NO: 153, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 155, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; (15) a CDR1 comprising the amino acid sequence of SEQ ID NO: 158, a CDR2 comprising the amino acid sequence of SEQ ID NO: 160, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 162, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; or (16) a CDR1 comprising the amino acid sequence of SEQ ID NO: 165, a CDR2 comprising the amino acid sequence of SEQ ID NO: 167, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 169, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs. In some embodiments, the anti-CLL1 sdAb comprises a V_HH domain comprising the amino acid sequence of any one of SEQ ID NOs: 94-106 and 171-173, or a variant thereof comprising an amino acid sequence having at least about 95% sequence identity to the amino acid sequence of any one of SEQ ID NOs: 94-106 and 171-173. In some embodiemnts, the engineered immune cell comprises a dual chimeric receptor construct comprising the first chimeric receptor fused to the second chimeric receptor via a self-cleaving peptide (*e.g.*, P2A peptide). In some embodiments, the dual chimeric receptor construct comprises the amino acid sequence of any one of SEQ ID NOs: 234-236, or a variant thereof comprising an amino acid sequence having at least about 95% sequence identity to the amino acid sequence of any one of SEQ ID NOs: 234-236. In some embodiments, the engineered immune effector cell expresses a safety-switch antigen or

epitope, such as CD52, EGFR, or CD20. In some embodiments, the method further comprises subsequently administering an effective amount of a therapeutic antibody specifically binding to the safety-switch antigen or epitope.

[0418] The methods described herein are suitable for treating various cancers, including both solid cancer and liquid cancer. The methods are applicable to cancers of all stages, including early stage, advanced stage and metastatic cancer. The methods described herein may be used as a first therapy, second therapy, third therapy, or combination therapy with other types of cancer therapies known in the art, such as chemotherapy, surgery, radiation, gene therapy, immunotherapy, bone marrow transplantation, stem cell transplantation, targeted therapy, cryotherapy, ultrasound therapy, photodynamic therapy, radio-frequency ablation or the like, in an adjuvant setting or a neoadjuvant setting. In some embodiments, the cancer is acute myeloid leukemia (AML), chronic myelogenous leukemia (CML) or myelodysplastic syndromes (MDS).

[0419] Administration of the anti-CLL1 constructs or pharmaceutical compositions thereof may be carried out in any convenient manner, including by injection, ingestion, transfusion, implantation or transplantation. The pharmaceutical compositions may be administered to a patient transarterially, subcutaneously, intradermally, intratumorally, intranodally, intramedullary, intramuscularly, intravenously, or intraperitoneally. In some embodiments, the pharmaceutical composition is administered systemically. In some embodiments, the pharmaceutical composition is administered to an individual by infusion, such as intravenous infusion. Infusion techniques for immunotherapy are known in the art (see, *e.g.*, Rosenberg *et al.*, New Eng. J. of Med. 319: 1676 (1988)). In some embodiments, the pharmaceutical composition is administered to an individual by intradermal or subcutaneous injection. In some embodiments, the compositions are administered by intravenous injection. In some embodiments, the compositions are injected directly into a tumor, or a lymph node. In some embodiments, the pharmaceutical composition is administered locally to a site of tumor, such as directly into tumor cells, or to a tissue having tumor cells.

[0420] Dosages and desired drug concentration of pharmaceutical compositions of the present application may vary depending on the particular use envisioned. The determination of the appropriate dosage or route of administration is well within the skill of an ordinary artisan. Animal experiments provide reliable guidance for the determination of effective doses for human therapy. Interspecies scaling of effective doses can be performed following the principles laid down by Mordenti, J. and Chappell, W. "The Use of Interspecies Scaling in Toxicokinetics," In *Toxicokinetics and New Drug Development*, Yacobi *et al.*, Eds, Pergamon Press, New York 1989, pp. 42-46. It is within the scope of the present application that different formulations will be effective for different treatments and different disorders,

and that administration intended to treat a specific organ or tissue may necessitate delivery in a manner different from that to another organ or tissue.

[0421] In some embodiments, wherein the pharmaceutical composition comprises any one of the anti-CLL1 constructs described herein, the pharmaceutical composition is administered at a dosage of about 10 ng/kg up to about 100 mg/kg of body weight of the individual or more per day, for example, at about 1 mg/kg/day to 10 mg/kg/day, depending upon the route of administration.

[0422] In some embodiments, wherein the pharmaceutical composition comprises any one of the engineered immune cells described herein, the pharmaceutical composition is administered at a dosage of at least about any of 10^4, 10^5, 10^6, 10^7, 10^8, or 10^9 cells/kg of body weight of the individual. In some embodiments, the pharmaceutical composition is administered at a dosage of any of about 10^4 to about 10^5, about 10^5 to about 10^6, about 10^6 to about 10^7, about 10^7 to about10^8, about 10^8 to about 10^9, about 10^4 to about 10^9, about 10^4 to about 10^6, about 10^6 to about 10^8, or about 10^5 to about 10^7 cells/kg of body weight of the individual. In some embodiments, the pharmaceutical composition is administered at a dose of at least about any $1x10^5$, $2x10^5$, $3x10^5$, $4x10^5$, $5x10^5$, $6x10^5$, $7x10^5$, $8x10^5$, $9x10^5$, $1x10^6$, $2x10^6$, $3x10^6$, $4x10^6$, $5x10^6$, $6x10^6$, $7x10^6$, $8x10^6$, $9x10^6$, $1x10^7$ cells/kg or more.

[0423] In some embodiments, the pharmaceutical composition is administered for a single time. In some embodiments, the pharmaceutical composition is administered for multiple times (such as any of 2, 3, 4, 5, 6, or more times). In some embodiments, the pharmaceutical composition is administered once per week to once per year. In some embodiments, the interval between administrations is about 1 week to a year. The optimal dosage and treatment regime for a particular patient can readily be determined by one skilled in the art of medicine by monitoring the patient for signs of disease and adjusting the treatment accordingly.

[0424] Moreover, dosages may be administered by one or more separate administrations, or by continuous infusion. In some embodiments, the pharmaceutical composition is administered in split doses, such as about any one of 2, 3, 4, 5, or more doses. In some embodiments, the split doses are administered over about a week. In some embodiments, the dose is equally split. In some embodiments, the split doses are about 20%, about 30% and about 50% of the total dose. In some embodiments, the interval between consecutive split doses is about 1 day, 2 days, 3 days or longer. For repeated administrations over several days or longer, depending on the condition, the treatment is sustained until a desired suppression of disease symptoms occurs. However, other dosage regimens may be useful. The progress of this therapy is easily monitored by conventional techniques and assays.

[0425] In some embodiments, the amount of the pharmaceutical composition is effective to cause an objective clinical response in the individual. In some embodiments, the amount of the pharmaceutical composition is effective to cause disease remission (partial or complete) in the individual. In some

embodiments, the amount of the pharmaceutical composition is effective to prevent relapse or disease progression of the cancer in the individual. In some embodiments, the amount of the pharmaceutical composition is effective to prolong survival (such as disease free survival) in the individual. In some embodiments, the pharmaceutical composition is effective to improve quality of life in the individual.

[0426] In some embodiments, the amount of the pharmaceutical composition is effective to inhibit growth or reducing the size of a solid or lymphatic tumor. In some embodiments, the size of the solid or lymphatic tumor is reduced for at least about 10% (including for example at least about any of 20%, 30%, 40%, 60%, 70%, 80%, 90%, or 100%). In some embodiments, a method of inhibiting growth or reducing the size of a solid or lymphatic tumor in an individual is provided.

[0427] In some embodiments, the amount of the pharmaceutical composition is effective to inhibit tumor metastasis in the individual. In some embodiments, at least about 10% (including for example at least about any of 20%, 30%, 40%, 60%, 70%, 80%, 90%, or 100%) metastasis is inhibited. In some embodiments, a method of inhibiting metastasis to lymph node is provided. In some embodiments, a method of inhibiting metastasis to the lung is provided. In some embodiments, a method of inhibiting metastasis to the liver is provided. Metastasis can be assessed by any known methods in the art, such as by blood tests, bone scans, x-ray scans, CT scans, PET scans, and biopsy.

VII. Kits and articles of manufacture

[0428] Further provided are kits, unit dosages, and articles of manufacture comprising any one of the anti-CLL1 sdAbs, anti-CLL1 constructs (such as chimeric receptors, immune effector cell engagers, and immunoconjugates), or engineered immune cells described herein. In some embodiments, a kit is provided which contains any one of the pharmaceutical compositions described herein. In some embodiments, the kit further comprises instructions for its use.

[0429] The kits of the present application are in suitable packaging. Suitable packaging includes, but is not limited to, vials, bottles, jars, flexible packaging (*e.g.*, sealed Mylar or plastic bags), and the like. Kits may optionally provide additional components such as buffers and interpretative information. The present application thus also provides articles of manufacture, which include vials (such as sealed vials), bottles, jars, flexible packaging, and the like.

[0430] The articles of manufacture may comprise a container and a label or package insert on or associated with the container. Suitable containers include, for example, bottles, vials, syringes, *etc.* The containers may be formed from a variety of materials such as glass or plastic. Generally, the container holds a composition which is effective for treating a disease or disorder (such as cancer) described herein, and may have a sterile access port (for example the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). The label or package insert indicates that

the composition is used for treating a particular disease or condition in an individual. The label or package insert will further comprise instructions for administering the composition to the individual. The label may indicate directions for reconstitution and/or use. The container holding the pharmaceutical composition may be a multi-use vial, which allows for repeat administrations (*e.g.* from 2-6 administrations) of the reconstituted formulation. Package insert refers to instructions customarily included in commercial packages of therapeutic products that contain information about the indications, usage, dosage, administration, contraindications and/or warnings concerning the use of such therapeutic products. Additionally, the article of manufacture may further comprise a second container comprising a pharmaceutically-acceptable buffer, such as bacteriostatic water for injection (BWFI), phosphate-buffered saline, Ringer's solution and dextrose solution. It may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, and syringes.

[0431] The kits or articles of manufacture may include multiple unit doses of the pharmaceutical composition and instructions for use, packaged in quantities sufficient for storage and use in pharmacies, for example, hospital pharmacies and compounding pharmacies.

EXAMPLES

[0432] The examples below are intended to be purely exemplary of the invention and should therefore not be considered to limit the invention in any way. The following examples and detailed description are offered by way of illustration and not by way of limitation.

Example 1: Generation of anti-CLL1 sdAbs

Preparation of recombinant extracellular domains of CLL1 proteins

[0433] The extracellular protein domains (ECD) of CLL1 proteins from human or cynomolgus monkey were prepared by transient expression in human embryonic kidney HEK293 cells transfected with plasmids encoding the respective amino acid sequences in Table 8.

[0434] Each expression plasmid was complexed with 293FECTIN™ (Life Technologies) and added to suspension-cultured 293-F cells (derived from HEK293 cells). Eight days post-transfection, the culture supernatants were collected and the corresponding soluble protein was purified by IMAC (GE Healthcare) to produce a protein batch.

Table 8. Extracellular domains of CLL1 proteins

Protein	Description	Sequence
huCLL1	human CLL1 ECD (65-265)	HVTLKIEMKKMNKLQNISEELQRNISLQLMSNMNISNKIRNLSTTLQTIAT KLCRELYSKEQEHKCKPCPRRWIWHKDSCYFLSDDVQTWQESKMACAA QNASLLKINNKNALEFIKSQSRSYDYWLGLSPEEDSTRGMRVDNIINSSA

		WVIRNAPDLNNMYCGYINRLYVQYYHCTYKKRMICEKMANPVQLGST YFREA (SEQ ID NO: 1)
cynoCLL 1	cynomolgus monkey CLL1 ECD (65-265)	HITLKTAMKKMNKLQNINEELQRNVSLQLMSNMNSSNKIRNLSTTLQTI ATRLCRELYSKEQEHKCKPCPRRWIWHKDSCYFLSDDVRTWQESRMAC AAQNASLLKINNKNALEFIKSQSTSYPYWLGLSPEKDYSYGTSVDDIINSS AWVTRNASDLNNMFCGYINRIYVHYDYCIYRKKMICEKMANPVQLGFI HFREA (SEQ ID NO: 2)

Immunization

[0435] Camels were immunized with each recombinant ECD of CLL1 under all current animal welfare regulations. For immunization, the antigen was formulated as an emulsion with CFA (Complete Freund's adjuvant; primary immunization) or IFA (incomplete Freund's adjuvant; boost immunizations). The antigen was administered subcutaneously at the neck. Each animal received 6 injections of the emulsion, containing 200 μg of CLL1-His protein in CFA emulsion and 5 subsequent injections of CLL1-His protein in IFA emulsion at two-week intervals. At different time points during immunization, 10 ml blood samples were collected from the animal and sera were prepared. The induction of an antigen specific humoral immune response was verified using the serum samples using an ELISA-based assay with immobilized CLL1-His protein. As shown in FIGs. 1-2, the post-immune serum and immunoglobulins (including both 4-chain antibodies and heavy-chain only antibodies) specifically bind to the CLL1-His protein in a concentration-dependent manner. Four days after the last immunization, a blood sample of 180 ml was collected. Peripheral blood lymphocytes (PBLs), as the genetic source of the camel HCAbs, were isolated from the blood sample using a Ficoll-Paque gradient (Amersham Biosciences), yielding 2.5×10^8 PBLs.

Library construction

[0436] RNA extracted from PBLs was used as starting material for RT-PCR to amplify sdAb encoding gene fragments. These fragments were cloned into an in-house phagemid vector. The vector encoded a C-terminal His-Tag that was in-frame with each sdAb coding sequence. The library size was about 3×10^9. Phage libraries were prepared according to standard protocols and stored after filter-sterilization at 4°C for further use.

Selections and high-throughput screening

[0437] The phage libraries were screened by solid panning as well as cell-based panning. Only a single round of selection was performed for each of the two panning conditions. Each selection output was analyzed to determine an enrichment factor (*i.e.*, number of phages present in the eluate relative to control), diversity and percentage of CLL1 positive clones based on ELISA results. Based on these parameters, the best clones were chosen for further analysis. To this end, the output from each selection

was re-cloned as a pool into a soluble expression vector for high-throughput screening. The expression vector encodes a C-terminal His-tag that is in-frame with each sdAb coding sequence. Colonies were picked and grown in 96-deep-well plates (1 mL volume/well) and induced by adding IPTG and 0.1% Triton for sdAb expression in the supernatant.

[0438] The supernatants were first screened for their ability to bind to the corresponding CLL1 protein using an ELISA assay. The positive binders were sequenced and unique clones were selected for further characterization.

[0439] The unique clones were grown in 2YT medium and induced by IPTG for sdAb expression in the supernatant. The supernatants of unique binders were analyzed for their ability to bind to a CLL1-expressing HKE293/huCLL-1 cell line and cancer cell line AML193 using a FACS assay. Affinities of selected binders in supernatants to recombinant human and cynomolgus monkey CLL1 proteins were determined by surface plasmon resonance (SPR) on a BIACORE® T200 instrument. The dissociation phase was used to calculate the k_d values for each sdAb. Binding data of selected anti-CLL1 sdAbs are shown in Table 9.

Table 9. Binding properties of selected sdAbs

sdAb	Human CLL1			Cyno CLL1			AML193	HKE293/ huCLL-1
	k_a	k_d	K_D	k_a	k_d	K_D		
AS82472	1.5E+06	2.1E-02	1.4E-08	2.5E+06	1.0E-01	4.2E-08	+/-	+/-
AS82480	1.1E+06	1.6E-03	1.5E-09	2.3E+06	1.1E-01	4.7E-08	++	+
AS82494	5.4E+06	1.1E-01	2.1E-08	1.2E+06	1.2E-01	9.8E-08	+/-	+/-
AS82505	4.1E+05	3.4E-04	8.3E-10	1.1E+06	6.4E-04	5.9E-10	+	-
AS82544	7.9E+05	6.8E-03	8.6E-09	1.8E+06	7.0E-02	3.9E-08	+	+
AS82658	5.4E+05	8.4E-04	1.6E-09	1.3E+06	2.5E-03	1.9E-09	++	++
AS82718	2.1E+06	5.0E-02	2.4E-08	4.6E+05	1.2E-03	2.6E-09	+	+
AS83180	2.5E+05	7.7E-04	3.0E-09	8.5E+05	2.1E-03	2.4E-09	+	+
AS83183	9.9E+04	1.0E-04	1.0E-09	8.6E+05	2.2E-04	2.6E-10	+/-	+/-
AS83309	8.5E+05	6.2E-03	7.3E-09	1.2E+06	7.2E-04	6.3E-10	+/-	+/-
AS83431	4.8E+05	1.8E-04	3.7E-10	8.8E+05	2.1E-04	2.4E-10	+	-
AS83478	3.8E+05	7.7E-04	2.0E-09	8.3E+05	1.2E-03	1.4E-09	+/-	-
AS83791	4.6E+05	2.0E-04	4.4E-10	9.5E+05	2.0E-04	2.2E-10	+/-	-
AS83010	1.2E+06	1.0E-02	8.4E-09	1.4E+06	4.4E-03	3.2E-09	+/-	+/-
AS83457	1.2E+06	3.8E-02	3.1E-08	9.1E+05	1.7E-02	1.9E-08	+/-	+/-
AS83591	1.2E+06	3.8E-02	3.1E-08	9.1E+05	1.7E-02	1.9E-08	+/-	+/-

Example 2: Generation and screening of anti-CLL1 CAR-T

Generation of CAR constructs

[0440] Each isolated sdAb (SEQ ID NOs: 94-106 and 171-173) was cloned into a lentiviral expression vector with the intracellular co-stimulatory sequence of 4-1BB and intracellular domain of CD3ζ as shown in FIG. 3 and Table 3. A positive control CAR having an anti-CLL1 scFv domain (CLL1 BM

CAR) was also constructed. The CAR constructs (SEQ ID NOs: 120-132 and 180) were cloned into an expression vector with an EF1α promoter for expression. Sequences of the CAR constructs are shown below.

[0441] SEQ ID NO: 120 (AS82472 CAR)

MALPVTALLLPLALLLHAARPQVQLVESGGDLVRPGGSLRLSCAASGFTFSIYDMNWVRQAPGK
GLEWVAGISGNGYSTSYAESVKGRFTISRDNAKNTVYLQLSSLKFEDTAMYYCVRDAERWDEN
DLRRKGQGTQVTVSSTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAP
LAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFS
RSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKM
AEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

[0442] SEQ ID NO: 121 (AS82480 CAR)

MALPVTALLLPLALLLHAARPEVQLVESGGGSVQAGGSLRLSCAASGVTYSSACMGWFRQAPG
KGREVVAVLYAGGSTTHYASSVKERFTISQDNAKNTVYLQMNSLKPEDTAVYYCAAALGDRSS
CEWRYWGQGTQVTVSSTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIW
APLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVK
FSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKD
KMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

[0443] SEQ ID NO: 122 (AS82494 CAR)

MALPVTALLLPLALLLHAARPQVQLVESGGGLVQPGGSLRLSCAASGFTFSVYDMNWFRQAPG
KGLEWVSGITGNGYTTSYADSVKGRFTISRDNAKNTLYLQLNSLKSEDTAMYYCAKETNRGQG
TQVTVSSTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVL
LLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAY
KQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIG
MKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

[0444] SEQ ID NO: 123 (AS82505 CAR)

MALPVTALLLPLALLLHAARPQVQLAESGGGLVQPGGSLRLSCVASGFTFSSYDMSWVRQAPG
KGVEWVSTINSGGGSTYYAESAKGRFTISRDNAKNTLYLQLNSLKTEDTAMYYCVKGFPDDDG
PGELSREYNYWGQGTQVTVSSTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFAC
DIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCE
LRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNE
LQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

[0445] SEQ ID NO: 124 (AS82544 CAR)

MALPVTALLLPLALLLHAARPEVQLVESGGALVQPGGSLRLSCTASGFLFRVYDMNWVRQAPG
KGVEWIVGITNNGYTTAYADSVKGRFTISRDNTENTLFLQMNSLKPEDTAMYYCQTDNGRVRG
QGTQVTVSSTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCG
VLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPA
YKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEI
GMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

[0446] SEQ ID NO: 125 (AS82658 CAR)

MALPVTALLLPLALLLHAARPQVQLVESGGGSVQAGGALSLSCAASGYTVRIDYMGWYRQTPG
KGREPVATIASNGGTAYADSVEGRFTISQDNAKNSVYLQMNTLKPGDTAMYYCAAGTWPTLTY

FGQGTQVTVSSTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGT
CGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSAD
APAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEA
YSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

[0447] SEQ ID NO: 126 (AS82718 CAR)

MALPVTALLLPLALLLHAARPQVQLAESGGGLVQTGGSLRLSCTASGLNFGLYAMGWFRQAPG
KEREGVSCINGGGGITVYSDFVKSRFTISRDNAKNTLYLQMNSLKPDDTATYYCAADRSPFGSCS
SDWSRSSDWSRMAEKFGYWGQGTQVTVSSTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAV
HTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCR
FPEEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRK
NPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

[0448] SEQ ID NO: 127 (AS83180 CAR)

MALPVTALLLPLALLLHAARPQVQLVESGGGSVQAGGSLRLSCVVSAATNCRYIAWYRQAPGK
AREFVSTLGSDGNTNYADSVKGRFTISQGNIKNMAYLEMNSLKPEDTGMYYCGTRCQIGDDWR
SSDWAQGTQVTVSSTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPL
AGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRS
ADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMA
EAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

[0449] SEQ ID NO: 128 (AS83183 CAR)

MALPVTALLLPLALLLHAARPQVHLVESGGGSVQSGGSLRLSCAASGYAYRSYCMGWFRQAPG
KVLEGVAAIESDGTTTYADSVMGRFTISQDNAKNALYLQMNSLKPEDTAMYHCAAVKGSCDSA
SSDTPSYWGQGTQVTVSSTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYI
WAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRV
KFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQK
DKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

[0450] SEQ ID NO: 129 (AS83309 CAR)

MALPVTALLLPLALLLHAARPEVQLVESGGDLVRPGGSLRLSCAASGFTFSIYDMNWVRQAPGK
GLEWVAGISGNGYSTSYAESVKGRFTISKDNAKNTVYLQLSSLKFEDTAMYYCVRGGEKWDEN
DLRRKGQGTQVTVSSTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAP
LAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFS
RSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKM
AEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

[0451] SEQ ID NO: 130 (AS83431 CAR)

MALPVTALLLPLALLLHAARPQVRLVESGGGSVQSGGSLRLSCAASGYARSSTCLGWFRQAPGK
EVEGVAIIGRDGSTGYADSVKGRFTISQDNAKNTLYLHMDSLKPEDTAMYYCAAVEGGCEVSE
GTGEQQLAYWGQGTQVTVSSTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACD
IYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCEL
RVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNEL
QKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

[0452] SEQ ID NO: 131 (AS83478 CAR)

MALPVTALLLPLALLLHAARPQVHLMESGGGLVQPGESLRLSCAASGFIFANYEMSWVRQAPG
KVLEWVSGINSRGNATYYADSVKGRFTISRDNAEHTLYLQMNSLKPEDTAMYHCVVGGMTTD

QGSPDFYWGQGTQVTVSSTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYI
WAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRV
KFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQK
DKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

[0453] SEQ ID NO: 132 (AS83791 CAR)

MALPVTALLLPLALLLHAARPQVKLVESGGGLVQPGGSLRLSCVASGFAFSSADMSWVRQAPG
KGVEAVSVINRDGASTYYADSVKGRFTISRDNAKSTLYLQMNSLKPEDTAMYHCAVVPENEYE
SGSYNYWGQGTQVTVSSTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYI
WAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRV
KFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQK
DKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

[0454] SEQ ID NO: 177 (AS83010 CAR)

MALPVTALLLPLALLLHAARPQVQLVESGGGLVQPGGSLRLSCVASGFFFSAYDMNWFRQAPG
KGLEWVSGITGNGYTTAYADSVKGRFTISRDNAKNTLYLQLNSLKSEDTAMYYCTEGDNRGQG
TQVTVSSTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVL
LLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAY
KQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIG
MKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

[0455] SEQ ID NO: 178 (AS83457 CAR)

MALPVTALLLPLALLLHAARPQVQLVESGGGLVQPGGSLRLSCAASGFFFSIYDMNWFRQAPGK
GLEWVSGITGNGYTTAYADSVKGRFTISRDNAKNTLYLQLNSLKSEDTAMYYCAQGSNRGRGT
QVTVSSTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLL
LSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYK
QGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGM
KGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

[0456] SEQ ID NO: 179 (AS83591 CAR)

MALPVTALLLPLALLLHAARPQVQLVESGGGLVQPGGSLRLSCAASGFLFSIYDMNWVRQAPGK
GVEWIAGITNNEHTTAYADSVKGRFTISRDNTKNTLFLQMNSLKPEDTAMYYCQRDDGQVRGQ
GTQVTVSSTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGV
LLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAY
KQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIG
MKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

[0457] SEQ ID NO: 180 (CLL1 BM CAR)

MALPVTALLLPLALLLHAARPDIQLTQSPSSLSASVGDRVSFTCQASQDINNFLNWYQQKPGKAP
KLLIYDASNLETGVPSRFSGSGSGTDFTFTISSLQPEDIATYYCQQYGNLPFTFGGGTKVEIKRGGG
GSGGGGSGGGGSQVQLQESGPGLVKPSETLSLTCTVSGGSISSYYWSWIRQPPGKGLEWIGYIYY
SGSTNYNPSLKSRVTISVDTSKNQFSLKLSSVTAADTAVYYCVSLVYCGGDCYSGFDYWGQGTL
VTVSSAAALDNEKSNGTIIHVKGKHLCPSPLFPGPSKPFWVLVVWGGVLACYSLLVTVAFIIFWV
RSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYN

ELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGK
GHDGLYQGLSTATKDTYDALHMQALPPR

Preparation of lentivirus

[0458] The lentivirus packaging plasmid mixture including pCMV-ΔR-8.47 and pMD2.G (Addgene, Cat#12259) was pre-mixed with each vector PLLV-hEF1α-CLL1 comprising a CAR construct at a pre-optimized ratio with polyethylenimine. The mixture was then added to the HEK293 cells. Supernatants from the cells were collected after overnight incubation. The virus-containing supernatants were filtered through a 0.45 μm PES filter, followed by ultra-centrifugation to pellet the lentivirus. The virus pellets were rinsed with pre-chilled PBS. The virus was aliquoted and stored at -80°C immediately. The virus titer was determined by measurement of transduction efficiency to supT1 cell line using a flow cytometry assay.

Collection and transduction of T lymphocytes

[0459] Leukocytes were collected from healthy donors by apheresis. Peripheral blood mononuclear cells (PBMCs) were isolated using FICOLL-PAQUE™ PLUS Media (GE Healthcare, Cat#17-5442-02) according to manufacturer's protocol. Human T cells were purified from PMBCs using a Pan-T cell isolation kit (Miltenyi, Cat#130-096-535), following manufacturer's protocol. The purified T cells were subsequently pre-activated for 48 hours with a human T cell activation/expansion kit (Miltenyi, Cat#130-091-441) according to manufacturer's protocol in which anti-CD3/CD28 MACSiBead particles were added at a bead-to-cell ratio of 1:2. The pre-activated T cells were transduced with each lentivirus stock in the presence of 7 μg/ml polybrene. The transduced cells were then transferred to the cell culture incubator for transgene expression under suitable conditions.

***In vitro* cytotoxicity assay**

[0460] For quick evaluation of anti-tumor activities of CAR-T cells *in vitro*, LDH (lactate dehydrogenase) assay for cytotoxicity was performed. On day 6 or day 11 after transduction, transduced T cells were harvested and co-incubated with target cells ($CLL1^+$ AML cell line THP-1) at an E/T ratio (**E**ffector: CAR-T/**T**arget: THP-1) of 5: 1 or 1:1 for 20 hours. Un-transduced T cells ("UnT") from the same batch were used as a negative control. The assay was performed following the manufacturer's manual (Roche, 11644793001). The cytotoxicity was calculated by the equation below ($[LDH]_{E+T}$: the LDH released from E/T co-incubation, $[LDH]_E$: the LDH released from Effector only, $[LDH]_{max}$: the LDH released from target cells treated with Triton X-100, $[LDH]_{min}$: the LDH released from untreated target cells):

$$\text{Cytotoxicity } \% = \frac{[LDH]_{E+T} - [LDH]_E - [LDH]_{min}}{[LDH]_{max} - [LDH]_{min}} \times 100$$

[0461] As shown in FIG. 4, all anti-CLL1 CAR-T cells showed stronger cytotoxicity against THP-1 cells than CLL1 BM CAR-T cells.

IFN-γ and TNF-α Secretion detected by HTRF

[0462] Another measure of effector T-cell activation and proliferation is the production of effector cytokines such as IFN- γ and TNF- α. Supernatant from the *in vitro* cytotoxicity assay were collected to assess CAR-induced cytokine release. HTRF assays for IFN-γ (Cisbio, Cat# 62HIFNGPEH) and TNFα (Cisbio, Cat# 62HTNFAPEH) were performed according to the manufacturer's manual.

[0463] The corresponding cytokine release results are shown in FIGs. 5A-5D. All anti-CLL1 CAR-T cells exhibited potent killing activity against THP-1 cells, and released IFN-γ and TNF-α in response to THP-1 cells.

Long-term co-culture assay

[0464] To evaluate the long-term killing efficacy of CAR-T cells, we performed long-term co-culture assays, which mimic the dynamic killing process *in vivo*. Tumor cell lines (*e.g.*, THP-1) were labeled with CFSE (SIGMA-ALDRICH, Cat# 21888-25MG-F). Transduced or non-transduced T cells ($2x10^5$/well) were co-cultured with tumor cell lines (*e.g.*, CFSE-THP-1 cells, $2x10^5$ or $4x10^5$/well) at an E:T ratio of 1:1 or 1:2 in 24-well plates, in the absence of exogenous cytokines (IL-2). Part of the cells were harvested and stained for CD3 after 2 or 3 days' co-culture. Tumor cells were identified by $CFSE^+$ signal. For serial co-culture assays, the remaining T cells were then re-challenged with fresh CFSE-THP-1 cells at the same E:T ratio. Co-cultures were carried on until tumor cells outgrew. The T cell proliferation rate at each time point is calculated by dividing the number of T cells at the time point by the number of T cells at a previous time point.

[0465] Representative result of long-term co-culture assay by FACS detection was shown in FIG. 6A. Calculated T cell proliferation rate and total T cell count from the same experiment were shown in FIG. 6B and FIG. 6C, respectively. The data indicate that CAR-T derived from sdAb AS83478 proliferated and persisted better than CLL1 BM CAR and the rest clones tested.

Colony forming unit (CFU) assay

[0466] The potential cytotoxicity of anti-CLL1 CAR-T cells on normal leukemic progenitor cells *in vitro* is assessed with CFU assay. $CD34^+$ cells (HemaCare, Cat#:CB34C-2) immunomagnetically isolated from cord blood (CB) are co-cultured with either test CAR-T cells, unT cells or media alone (untreated) for 6 hours at an E:T (T cell : CB cells) ratio of 10:1. A total number of 5,000 mixed cells per well are then plated in MethoCult H4034 Optimum medium (STEMCELL Technologies), and cultured for 5-7 days before

counting the colony forming units. BCMA and untransduced T cells are used as negative controls. Data are presented as mean±SEM of colony numbers in triplicated petri dishes for each sample.

***In vivo* efficacy evaluation in mouse xenograft model**

[0467] U937-Luc were cultured, resuspended in $HBSS^{-/-}$ and injected intravenously at 2x 10^6 cells per mouse. Bioluminescent imaging (BLI) was conducted weekly or biweekly post tumor inoculation to monitor model development. The animals were randomized based on the BLI photon numbers and animal body weight. After randomization, a single dose of CAR-T cells (AS82494 CAR-T, AS82658 CAR-T, AS83183 CAR-T, AS83431 CAR-T, AS83478 CAR-T and positive control CLL1 BM CAR-T) or UnT cells were infused intravenously. Weekly BLI imaging was performed to record tumor growth.

[0468] As shown in FIG. 7, mice treated wtih AS83183, AS83431 or AS82658 CAR-T cells were tumor free (BLI around 10^6) after 2-3 weeks post infusion, while mice treated with UnT or vehicle exhibited rapid tumor progression and had to be euthanized before the end of the experiment. However, mice treated with AS83183 and AS83431 CAR-T cells died after becoming tumor free because of uncontrolled CAR-T cell expansion. These results show that our anti-CLL1 sdAb CAR-T cells were more potent than CLL1 BM CAR.

[0469] FACS analysis of peripheral blood samples is conducted to determine the persistence of CAR-T cells. At the endpoint of the study, FACS analysis of peripheral blood, spleen and bone marrow samples, *etc.*, is performed.

***In vivo* toxicity evaluation in mouse model with humanized immune system**

[0470] To reconstitute human immune system (HIS) in mouse, NCG mice are infused with cord blood-derived $CD34^+$ cells (HemaCare, Cat#:CB34C-2). Reconstitution is confirmed by flow cytometry analysis of peripheral blood samples from various human immune subsets. To prepare CAR-T cells, human T cells are isolated from spleens of HIS mice, expanded and transduced with the candidate CAR expressing lentivirus, all as described above. To study toxicity of CAR-T cells, HIS mice are infused with 2 x10^6 CAR-T cells derived from the same $CD34^+$ cell donor. Animal health status and body weight are monitored twice a week and human immune cell subsets in mouse peripheral blood are detected by flow cytometry weekly. At indicated time points and the conclusion of the study, animals are euthanized and organs such as spleen and bone marrow are harvested and analyzed for the presence of human immune cells and hematopoietic stem cells using FACS.

***In vivo* toxicity evaluation in non-human primate (NHP) model**

[0471] To prepare CAR-T cells, T cells derived from *Cynomolgus macaques* are isolated, expanded and transduced with the candidate CAR-expressing lentivirus. To study short-term toxicity of the CAR-T,

animals are pre-treated with cyclophosphamide and fludarabine before an infusion of autologous CAR-T cells. After infusion, recipient animals are monitored daily for clinical signs and symptoms of cytokine release syndrome (CRS) and neurotoxicity. Persistence and population change in the CAR-T cells are assessed by flow cytometry analysis of peripheral blood samples. CRS-related cytokine levels are assessed by an ELISA or Meso Scale Discovery assay. At indicated time points and the conclusion of the study, animals are euthanized and organs such as spleen and bone marrow are harvested and analyzed using FACS.

Example 3: Generation and evaluation of additional anti-CLL1 CAR constructs

Generation of CAR constructs

[0472] Leading anti-CLL1 sdAbs from Example 2 are used as CLL1-binding domain to construct additional CARs and CAR systems as shown in FIGs. 8A-8E.

[0473] For example, "regular CARs" as shown in FIG. 8A having a monospecific extracellular domain, and an intracellular signaling domain comprising both an intracellular co-stimulatory sequence and a CD3ζ intracellular signaling sequence can respond and kill CLL1 expressing cells. "Tandem CARs" as shown in FIG. 8B are constructed by fusing two binding domains specifically recognizing different targets via a peptide linker to form the extracellular domain in a single CAR molecule. Tandem CARs can respond to cells expressing either one of the two target molecules, such as CLL1, CD33 and NKG2D ligands. Tandem CARs are expected to bind to target cells expressing both target molecules simultaneously with higher affinity and specificity, resulting in improved response at a low dosage. "Dual CARs" as shown in FIG. 8C are constructed by expressing two fully functional CARs against distinct targets. Dual CARs can respond to cells expressing either CLL1 or any one of CD33, CD123 or NKG2D ligands. "Split CARs" as shown in FIGs. 8D-8E have complete response to cells expressing both CLL1 and either one of CD33, CD123 or NKG2D ligands, but have only marginal response to cells expressing only one of CLL1, CD33, CD123 or NKG2D ligands, which may result in a better safety profile.

Safety switch

[0474] To limit potential toxicity by the CAR-T cells, certain safety switches may be included in the CAR constructs. For example, the CAR-T cells are engineered to further express full-length antigen or certain peptide epitope sequences of CD20, EGFR or CD52. To deplete CAR-T cells, antibodies against the engineered antigen or epitope can be administered, thereby eliminating the CAR-T cells via antibody-dependent cell-mediated cytotoxicity.

***In vivo* antibody-mediated CAR-T cell elimination**

[0475] To assess completeness and to identify optimal timing of antibody-mediated CAR-T cell elimination, mice engrafted with HL-60, MV4-11, THP-1 or U937 (n=5 to 8 mice per cohort; time point=week 0) are administered 1 dose of saline, untransduced T cells (unT) or $1.0x10^6$ CAR-T cells intravenously at indicated time point. Animals are then administered by intraperitoneal injection (i.p.) 1 dose of 1 or 5 mg/kg alemtuzumab (humanized anti-CD52 antibody) at indicated time points post-CAR-T administration. Animals are assessed weekly by BLI and FACS quantification of human AML and CAR-T cells, as described above. Murine bone marrow and spleens are harvested for quantification of human AML and T-cells by FACS. Histopathologic and immunohistochemical analyses of murine tissues are performed in some studies to assess completeness of T-cell ablation with alemtuzumab.

[0476] Additional cohorts of mice engrafted with HL-60, MV4-11, THP-1 or U937 (n=5 per treatment) are treated with saline, unT, or CAR-T-CD20 cells ($1.0x10^6$ cells per mouse) as described above. Animals are subsequently treated with the anti-CD20 antibody rituximab (Roche) at a dose of 1 mg/kg intraperitoneally at 4 weeks following CAR-T-CD20 to eliminate T cells. Animals are assessed by BLI and FACS to quantify leukemia burden and CAR-T cells.

[0477] For cetuximab-mediated T cell depletion, additional cohorts of mice engrafted with HL-60, MV4-11, THP-1 or U937 (n=5 per treatment) are treated with saline, unT, or CAR-T-EGFR cells ($1.0x 10^6$ cells per mouse) as described above. Animals are subsequently treated with the anti-EGFR antibody cetuximab (Bristol-Myers Squibb) at a dose of 1 mg/kg intraperitoneally at 4 weeks following CAR-T-CD20 to eliminate T cells. Animals are assessed by BLI and FACS to quantify leukemia burden and CAR-T cells.

Evaluation of CAR constructs

The CAR constructs, with or without safety switch, are tested for their *in vitro* cytotoxicity against AML cell lines, *in vivo* efficacy in xenograft AML animal models, *in vitro* hematopoietic toxicity with a CFU assay, *in vivo* elimination by corresponding antibodies and *in vivo* toxicity with HIS mice and NHP studies.

Example 4: Generation and evaluation of AS82658-28z CAR construct

Generation of CAR construct

[0478] AS82658 sdAb was cloned into a lentiviral expression vector with the intracellular co-stimulatory sequence of CD28 and intracellular domain of CD3ζ as shown in FIG. 8A. The CAR construct (AS82658-28z) was cloned into an expression vector with an EF1α promoter for expression. The sequence of AS82658-28z is shown below.

[0479] SEQ ID NO: 181 (AS82658-28z CAR):

MALPVTALLLPLALLLHAARPQVQLVESGGGSVQAGGALSLSCAASGYTVRIDYMGWYRQTPG
KGREPVATIASNGGTAYADSVEGRFTISQDNAKNSVYLQMNTLKPGDTAMYYCAAGTWPTLTY
FGQGTQVTVSSIEVMYPPPYLDNEKSNGTIIHVKGKHLCPSPLFPGPSKPFWVLVVVGGVLACYS
LLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADAP
AYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSE
IGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

Prepartion of CAR-T cells

[0480] Lentiviruses encoding AS82658-28z CAR and CLL1 BM CAR (positive control) were prepared as described in Example 2. T lymphocytes were collected and transduced with the lentiviruses according to the protocol in Example 2.

***In vitro* cytotoxicity assay**

[0481] The anti-tumor activities of AS82658-28z CAR-T cells were assessed using the *in vitro* LDH (lactate dehydrogenase) assay as described in Example 2. As shown in FIGs. 9A-9B, *in vitro* cytotoxicity of AS82658-28z against both THP-1 (with moderate expression of CLL1) and MOLM-13 cells (with low expression of CLL1) was comparable to that of CLL1 BM CAR.

Long-term co-culture assay

[0482] The long-term killing efficacy of CAR-T cells against tumor cells was assessed using long-term co-culture assays. AML tumor cell lines (*e.g.*, U937) were labeled with CFSE (SIGMA-ALDRICH, Cat# 21888-25MG-F). Transduced or non-transduced T cells ($1x10^5$/well) were co-cultured with tumor cell lines (*e.g.*, CFSE-U937 cells, $4x10^5$ well) at an E:T ratio of 1:4 in 24-well plates, in the absence of exogenous cytokines (*e.g.*, IL-2). Fractions of the cells were harvested and stained for CD3 after 2 or 3 days' co-culture. Tumor cells were identified by the $CFSE^+$ signal. For serial co-culture assays, the remaining T cells were then re-challenged with fresh CFSE-U937 cells at the same E:T ratio. Co-cultures were carried on until tumor cells predominated in the co-cultures. The T cell proliferation rate at each time point is calculated by dividing the number of T cells at the time point by the number of T cells at a previous time point.

[0483] The killing efficacy of CAR-T cells in the repeated tumor stimulation assay is shown in FIG. 10A. The positive control CLL1 BM CAR-T cells were exhausted after 5 rounds of tumor stimulation, while AS82658-28z CAR-T cells persisteded until 9 rounds of tumor stimulation. Also, AS8265-28z CAR-T cells proliferated faster than CLL1 BM CAR *in vitro* (FIG. 10B). These results indicate that AS82658-28z CAR-T cells have more potent anti-tumor activity than CLL1 BM CAR-T cells *in vitro*.

IFN-γ and GM-CSF Secretion detected by HTRF

[0484] Another measure of effector T-cell activation and proliferation is the production of effector cytokines such as IFN- γ and GM-CSF. Supernatants from the long-term co-culture assays were collected to assess CAR-induced cytokine release. HTRF assays for IFN-γ (Cisbio, Cat# 62HIFNGPEH) and GM-CSF (Cisbio, Cat# 62HGMCSFPEG) were performed according to the manufacturer's manual.

[0485] Results of a representative cytokines release assay are shown in FIGs. 11A-11B. AS82658-28z CAR-T cells secreted higher levels of IFN-γ and GM-CSF than CLL1 BM CAR-T cells in co-cultures with U937 cells at later time points (5, 7, 10 and 12 days after co-culture started).

Colony forming unit (CFU) assay

[0486] The potential toxicity of anti-CLL1 CAR-T cells against normal hematopoietic stem cells (HSCs) is assessed with an *in vitro* CFU assay. $CD34^+$ cells (HemaCare, Cat#:CB34C-2) immunomagnetically isolated from cord blood (CB) were co-cultured with either test CAR-T cells, unT cells or media alone (untreated) for 6 hours at an E:T (T cell : CB cells) ratio of 10:1. A total number of 5,000 mixed cells per well were then plated in MethoCult H4034 Optimum medium (STEMCELL Technologies), and cultured for 5-7 days before counting the colony forming units. Untransduced T cells were used as negative control. CLL1 BM CAR-T cells were used as positive control. Data is presented as mean±SEM of colony numbers in triplicated petri dishes for each sample.

[0487] As shown in FIG. 12A, HSCs pre-incubated with UnT cells did not exhibit abnormal development compared to the vehicle control, as the colony numbers of the UnT group is the same as that of the medium group. Compared to UnT, the development of HSCs pre-incubated with AS82658-28z CAR-T cells or CLL1 BM CAR-T cells was both inhibited as fewer colonies formed in these groups than in the UnT group (FIG. 12B). There was no significant difference between AS82658-28z CAR and CLL1 BM CAR groups. These data indicates that anti-CLL1 CAR-T therapy might affect hematopoietic functions, and HSC transplantation may be needed for AML treatment.

***In vivo* efficacy evaluation in mouse xenograft model**

[0488] The *in vivo* efficacy of AS82658-28z CAR-T cells was evaluated in a U937-Luc xenograft mouse model as described in Example 2.

[0489] As shown in FIG. 13, mice treated with AS82658-28z CAR-T cells or CLL1 BM CAR-T cells were tumor free (BLI around 10^6) after 2-3 weeks post injection, while mice treated with UnT or vehicle exhibited rapid tumor progression and had to be euthanized before the end of the experiment. The anti-tumor activity of AS82658-28z CAR-T cells was comparable to that of CLL1 BM *in vivo*.

Example 5: Generation and evaluation of anti-CLL1/CD33 tandem CAR constructs

Generation of CAR constructs

[0490] Exemplary tandem CARs as shown in FIG. 8B were constructed by fusing two binding domains specifically recognizing different targets (CLL1 and CD33) via a peptide linker to form the extracellular domain in a single CAR molecule. Anti- CLL1/CD33 tandem CARs were cloned into a lentiviral expression vector with the intracellular co-stimulatory sequence of CD28 and intracellular domain of CD3ζ as shown in FIG. 8B and Table 4. AS49264 CAR, comprising the anti-CD33 sdAb domain as the extracellular domain and an intracellular co-stimulatory sequence of CD28 and an intracellular domain of CD3ζ, was also constructed. The CAR constructs were cloned into an expression vector with an EF1α promoter for expression. The sequences of exemplary tandem CARs are shown below.

[0491] SEQ ID NO: 184 (Tan1):

MALPVTALLLPLALLLHAARPQVQLVESGGGSVQAGGALSLSCAASGYTVRIDYMGWYRQTPG
KGREPVATIASNGGTAYADSVEGRFTISQDNAKNSVYLQMNTLKPGDTAMYYCAAGTWPTLTY
FGQGTQVTVSSGGGGSEVQLVESGGGSVQAGGSLRLSCAASGYTYSINCMGWFRQAPGKEREG
VAVISTGGGRTDYRDSVKGRFTISQDNAKNTVYLQMNSLKPEDTAMYYCAGKTTYPGYGCGLG
RSAYNYWGQGTQVTVSSIEVMYPPPYLDNEKSNGTIIHVKGKHLCPSPLFPGPSKPFWVLVVVG
GVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRVKF
SRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDK
MAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

[0492] SEQ ID NO: 185 (Tan2):

MALPVTALLLPLALLLHAARPQVQLVESGGGSVQAGGALSLSCAASGYTVRIDYMGWYRQTPG
KGREPVATIASNGGTAYADSVEGRFTISQDNAKNSVYLQMNTLKPGDTAMYYCAAGTWPTLTY
FGQGTQVTVSSGGGGSGGGGSEVQLVESGGGSVQAGGSLRLSCAASGYTYSINCMGWFRQAPG
KEREGVAVISTGGGRTDYRDSVKGRFTISQDNAKNTVYLQMNSLKPEDTAMYYCAGKTTYPGY
GCGLGRSAYNYWGQGTQVTVSSIEVMYPPPYLDNEKSNGTIIHVKGKHLCPSPLFPGPSKPFWVL
VVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRS
RVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNEL
QKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

[0493] SEQ ID NO: 186 (Tan3):

MALPVTALLLPLALLLHAARPQVQLVESGGGSVQAGGALSLSCAASGYTVRIDYMGWYRQTPG
KGREPVATIASNGGTAYADSVEGRFTISQDNAKNSVYLQMNTLKPGDTAMYYCAAGTWPTLTY
FGQGTQVTVSSGGGGSGGGGSGGGGSEVQLVESGGGSVQAGGSLRLSCAASGYTYSINCMGWF
RQAPGKEREGVAVISTGGGRTDYRDSVKGRFTISQDNAKNTVYLQMNSLKPEDTAMYYCAGKT
TYPGYGCGLGRSAYNYWGQGTQVTVSSIEVMYPPPYLDNEKSNGTIIHVKGKHLCPSPLFPGPSK
PFWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDF
AAYRSRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEG
LYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

[0494] SEQ ID NO: 187 (Tan4):

MALPVTALLLPLALLLHAARPQVQLVESGGGSVQAGGALSLSCAASGYTVRIDYMGWYRQTPG
KGREPVATIASNGGTAYADSVEGRFTISQDNAKNSVYLQMNTLKPGDTAMYYCAAGTWPTLTY
FGQGTQVTVSSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGSVQAGGSLRLSCAASGYTYSIN
CMGWFRQAPGKEREGVAVISTGGGRTDYRDSVKGRFTISQDNAKNTVYLQMNSLKPEDTAMY
YCAGKTTYPGYGCGLGRSAYNYWGQGTQVTVSSIEVMYPPPYLDNEKSNGTIIHVKGKHLCPSP
LFPGPSKPFWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQP
YAPPRDFAAYRSRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRR
KNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

[0495] SEQ ID NO: 188 (Tan5):

MALPVTALLLPLALLLHAARPQVQLVESGGGSVQAGGALSLSCAASGYTVRIDYMGWYRQTPG
KGREPVATIASNGGTAYADSVEGRFTISQDNAKNSVYLQMNTLKPGDTAMYYCAAGTWPTLTY
FGQGTQVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGSVQAGGSLRLSCAASGY
TYSINCMGWFRQAPGKEREGVAVISTGGGRTDYRDSVKGRFTISQDNAKNTVYLQMNSLKPED
TAMYYCAGKTTYPGYGCGLGRSAYNYWGQGTQVTVSSIEVMYPPPYLDNEKSNGTIIHVKGKH
LCPSPLFPGPSKPFWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRK
HYQPYAPPRDFAAYRSRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMG
GKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQA
LPPR

[0496] SEQ ID NO: 189 (Tan6):

MALPVTALLLPLALLLHAARPQVQLVESGGGSVQAGGALSLSCAASGYTVRIDYMGWYRQTPG
KGREPVATIASNGGTAYADSVEGRFTISQDNAKNSVYLQMNTLKPGDTAMYYCAAGTWPTLTY
FGQGTQVTVSSGSTSGSGKPGSGEGSTKGEVQLVESGGGSVQAGGSLRLSCAASGYTYSINCMG
WFRQAPGKEREGVAVISTGGGRTDYRDSVKGRFTISQDNAKNTVYLQMNSLKPEDTAMYYCAG
KTTYPGYGCGLGRSAYNYWGQGTQVTVSSIEVMYPPPYLDNEKSNGTIIHVKGKHLCPSPLFPGP
SKPFWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPR
DFAAYRSRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQ
EGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

[0497] SEQ ID NO: 190 (Tan7):

MALPVTALLLPLALLLHAARPEVQLVESGGGSVQAGGSLRLSCAASGYTYSINCMGWFRQAPG
KEREGVAVISTGGGRTDYRDSVKGRFTISQDNAKNTVYLQMNSLKPEDTAMYYCAGKTTYPGY
GCGLGRSAYNYWGQGTQVTVSSGGGGSQVQLVESGGGSVQAGGALSLSCAASGYTVRIDYMG
WYRQTPGKGREPVATIASNGGTAYADSVEGRFTISQDNAKNSVYLQMNTLKPGDTAMYYCAA
GTWPTLTYFGQGTQVTVSSIEVMYPPPYLDNEKSNGTIIHVKGKHLCPSPLFPGPSKPFWVLVVV
GGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRVK
FSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKD
KMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

[0498] SEQ ID NO: 191 (Tan8):

MALPVTALLLPLALLLHAARPEVQLVESGGGSVQAGGSLRLSCAASGYTYSINCMGWFRQAPG
KEREGVAVISTGGGRTDYRDSVKGRFTISQDNAKNTVYLQMNSLKPEDTAMYYCAGKTTYPGY
GCGLGRSAYNYWGQGTQVTVSSGGGGSGGGGSQVQLVESGGGSVQAGGALSLSCAASGYTVR
IDYMGWYRQTPGKGREPVATIASNGGTAYADSVEGRFTISQDNAKNSVYLQMNTLKPGDTAMY
YCAAGTWPTLTYFGQGTQVTVSSIEVMYPPPYLDNEKSNGTIIHVKGKHLCPSPLFPGPSKPFWV
LVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYR
SRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNEL
QKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

[0499] SEQ ID NO: 192 (Tan9):

MALPVTALLLPLALLLHAARPEVQLVESGGGSVQAGGSLRLSCAASGYTYSINCMGWFRQAPG
KEREGVAVISTGGGRTDYRDSVKGRFTISQDNAKNTVYLQMNSLKPEDTAMYYCAGKTTYPGY
GCGLGRSAYNYWGQGTQVTVSSGGGGSGGGGSGGGGSQVQLVESGGGSVQAGGALSLSCAAS
GYTVRIDYMGWYRQTPGKGREPVATIASNGGTAYADSVEGRFTISQDNAKNSVYLQMNTLKPG
DTAMYYCAAGTWPTLTYFGQGTQVTVSSIEVMYPPPYLDNEKSNGTIIHVKGKHLCPSPLFPGPS
KPFWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRD
FAAYRSRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQE
GLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

[0500] SEQ ID NO: 193 (Tan10):

MALPVTALLLPLALLLHAARPEVQLVESGGGSVQAGGSLRLSCAASGYTYSINCMGWFRQAPG
KEREGVAVISTGGGRTDYRDSVKGRFTISQDNAKNTVYLQMNSLKPEDTAMYYCAGKTTYPGY
GCGLGRSAYNYWGQGTQVTVSSGGGGSGGGGSGGGGSGGGGSQVQLVESGGGSVQAGGALSL
SCAASGYTVRIDYMGWYRQTPGKGREPVATIASNGGTAYADSVEGRFTISQDNAKNSVYLQMN
TLKPGDTAMYYCAAGTWPTLTYFGQGTQVTVSSIEVMYPPPYLDNEKSNGTIIHVKGKHLCPSP
LFPGPSKPFWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQP
YAPPRDFAAYRSRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRR
KNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

[0501] SEQ ID NO: 194 (Tan11):

MALPVTALLLPLALLLHAARPEVQLVESGGGSVQAGGSLRLSCAASGYTYSINCMGWFRQAPG
KEREGVAVISTGGGRTDYRDSVKGRFTISQDNAKNTVYLQMNSLKPEDTAMYYCAGKTTYPGY
GCGLGRSAYNYWGQGTQVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSQVQLVESGGGSVQA
GGALSLSCAASGYTVRIDYMGWYRQTPGKGREPVATIASNGGTAYADSVEGRFTISQDNAKNSV
YLQMNTLKPGDTAMYYCAAGTWPTLTYFGQGTQVTVSSIEVMYPPPYLDNEKSNGTIIHVKGK
HLCPSPLFPGPSKPFWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTR
KHYQPYAPPRDFAAYRSRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEM
GGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQ
ALPPR

[0502] SEQ ID NO: 195 (Tan12):

MALPVTALLLPLALLLHAARPEVQLVESGGGSVQAGGSLRLSCAASGYTYSINCMGWFRQAPG
KEREGVAVISTGGGRTDYRDSVKGRFTISQDNAKNTVYLQMNSLKPEDTAMYYCAGKTTYPGY
GCGLGRSAYNYWGQGTQVTVSSGSTSGSGKPGSGEGSTKGQVQLVESGGGSVQAGGALSLSCA
ASGYTVRIDYMGWYRQTPGKGREPVATIASNGGTAYADSVEGRFTISQDNAKNSVYLQMNTLK
PGDTAMYYCAAGTWPTLTYFGQGTQVTVSSIEVMYPPPYLDNEKSNGTIIHVKGKHLCPSPLFPG
PSKPFWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPP
RDFAAYRSRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNP
QEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

[0503] SEQ ID NO: 196 (AS49264 CAR):

MALPVTALLLPLALLLHAARPEVQLVESGGGSVQAGGSLRLSCAASGYTYSINCMGWFRQAPG
KEREGVAVISTGGGRTDYRDSVKGRFTISQDNAKNTVYLQMNSLKPEDTAMYYCAGKTTYPGY
GCGLGRSAYNYWGQGTQVTVSSTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFA
CDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGC
ELRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYN
ELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

Prepartion of CAR-T cells

[0504] Lentiviruses encoding the tandem CARs (Tan 1-Tan 12), AS82658-28z CAR, and AS49264 CAR (positive control) were prepared as described in Example 2. T lymphocytes were collected and transduced with the lentiviruses according to the protocol in Example 2.

***In vitro* cytotoxicity assay**

[0505] The anti-tumor activities of tandem CAR-T cells were assessed using the *in vitro* LDH (lactate dehydrogenase) assay as described in Example 2.

[0506] As shown in FIG. 14, *in vitro* cytotoxicity of tandem CARs against THP-1 was higher than both single-target CAR-T cells (AS82658-28z or AS49264 CAR-T), which indicates that tandem CARs against two targets (*e.g.*, CLL1 and CD33) are more efficacious than single-target CARs for tumor elimination.

Long-term co-culture assay

[0507] The long-term killing efficacy of CAR-T cells against tumor cells was assessed using long-term co-culture assays as described in Example 2.

[0508] The killing efficacy of various tandem CAR-T cells in the repeated tumor stimulation assay is shown in FIG. 15A. Anti-CLL1 single-target CAR-T cells (AS82658-28z CAR-T) and ani-CD33 single-target CAR-T cells (AS49264 CAR-T) were exhausted after 3 rounds of tumor stimulation, while most tandem CAR-T cells persisteded until 4 or 5 rounds of tumor stimulation. Also, tandem CAR-T cells proliferated faster than AS82658-28z or AS49264 CAR-T cells *in vitro* (FIG. 15B). These results further demonstrate that CLL1/CD33 tandem CAR-T cells have more potent anti-tumor activity than both single-target CAR-T cells *in vitro*.

IFN-γ and GM-CSF Secretion detected by HTRF

[0509] Cytokine release (IFN-γ and GM-CSF) by tandem CAR-T cells in long-term co-cultures with tumor cells was assessed as described in Example 3.

[0510] Results of a representative cytokines release assay are shown in FIGs. 16A-16B. Comparable levels of cytokines were released by tandem CAR-T cells as single-target CAR-T cells (AS82658-28z and AS49264 CAR-T) in response to U937 cells *in vitro*.

Example 6: Generation and evaluation of anti-CLL1/CD33 dual CAR constructs

Generation of CAR constructs

[0511] Exemplary dual CARs as shown in FIG. 8C were constructed by expressing two fully functional CARs against CLL1 and CD33 respectively. The dual CAR constructs as shown in Table 6

were cloned into an expression vector with an EF1α promoter for expression. Sequences of the anti-CD33 sdAbs, AS49814, AS50073 and AS67190 are shown in Table 5. The sequences of exemplary dual CAR constructs and individual CARs are shown below.

[0512] SEQ ID NO: 234 (Dual1):

MALPVTALLLPLALLLHAARPQVQLVESGGDLVRPGGSLRLSCAASGFTFSIYDMNWVRQAPGK
GLEWVAGISGNGYSTSYAESVKGRFTISRDNAKNTVYLQLSSLKFEDTAMYYCVRDAERWDEN
DLRRKGQGTQVTVSSIEVMYPPPYLDNEKSNGTIIHVKGKHLCPSPLFPGPSKPFWVLVVVGGVL
ACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSA
DAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAE
AYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPRGSGATNFSLLKQAGDVEENP
GPMALPVTALLLPLALLLHAARPQIQLVESGGGSVQAGGSLRLSCVASGYIGGHYYMGWFRQAP
GKEREGVAAIDIDSDGRTRYAGSVQGRFTISQDNAKNTLHLQMSSLKPEDTGMYYCAVGVGWV
PARLTPQAVSYWGKGTLVTVSSTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFA
CDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGC
ELRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYN
ELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

[0513] SEQ ID NO: 235 (Dual2):

MALPVTALLLPLALLLHAARPQVQLVESGGDLVRPGGSLRLSCAASGFTFSIYDMNWVRQAPGK
GLEWVAGISGNGYSTSYAESVKGRFTISRDNAKNTVYLQLSSLKFEDTAMYYCVRDAERWDEN
DLRRKGQGTQVTVSSIEVMYPPPYLDNEKSNGTIIHVKGKHLCPSPLFPGPSKPFWVLVVVGGVL
ACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSA
DAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAE
AYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPRGSGATNFSLLKQAGDVEENP
GPMALPVTALLLPLALLLHAARPQVQLVESGGGLVQAGGSLRLSCTASGFTFDNYVMGWFRQA
PGKEREGVSCIGWSGGSTYYADSVKGRFTISRDNAKNTLYLQMNSLKPEDTAMYYCAADQGKC
SLGSAGADDMDYWGRGTLVTVSSTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDF
ACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGG
CELRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLY
NELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

[0514] SEQ ID NO: 236 (Dual3):

MALPVTALLLPLALLLHAARPQVQLVESGGGLVQPGGSLRLSCAASGFTFSVYDMNWFRQAPG
KGLEWVSGITGNGYTTSYADSVKGRFTISRDNAKNTLYLQLNSLKSEDTAMYYCAKETNRGQG
TQVTVSSIEVMYPPPYLDNEKSNGTIIHVKGKHLCPSPLFPGPSKPFWVLVVVGGVLACYSLLVT
VAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADAPAYKQ
GQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMK
GERRRGKGHDGLYQGLSTATKDTYDALHMQALPPRGSGATNFSLLKQAGDVEENPGPMALPVT
ALLLPLALLLHAARPQVQLVESGGGLVQAGGSLRLSCAASGNVFRFNIMGWYRQAPGNQRELV
ASIDDGGDRSYADSVEGRFTISRENGKKIMYLQMNSLKPEDTAVYYCAAGLGTYLNGRVSMAT
NYWGQGTQVTVSSTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPL
AGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRS
ADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMA
EAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

[0515] SEQ ID NO: 229 (AS82472-28z CAR):

MALPVTALLLPLALLLHAARPQVQLVESGGDLVRPGGSLRLSCAASGFTFSIYDMNWVRQAPGK
GLEWVAGISGNGYSTSYAESVKGRFTISRDNAKNTVYLQLSSLKFEDTAMYYCVRDAERWDEN
DLRRKGQGTQVTVSSIEVMYPPPYLDNEKSNGTIIHVKGKHLCPSPLFPGPSKPFWVLVVVGGVL
ACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSA
DAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAE
AYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

[0516] SEQ ID NO: 230 (AS82494-28z CAR):

MALPVTALLLPLALLLHAARPQVQLVESGGGLVQPGGSLRLSCAASGFTFSVYDMNWFRQAPG
KGLEWVSGITGNGYTTSYADSVKGRFTISRDNAKNTLYLQLNSLKSEDTAMYYCAKETNRGQG
TQVTVSSIEVMYPPPYLDNEKSNGTIIHVKGKHLCPSPLFPGPSKPFWVLVVVGGVLACYSLLVT
VAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADAPAYKQ
GQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMK
GERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

[0517] SEQ ID NO: 231 (AS49814 CAR):

MALPVTALLLPLALLLHAARPQIQLVESGGGSVQAGGSLRLSCVASGYIGGHYYMGWFRQAPG
KEREGVAAIDIDSDGRTRYAGSVQGRFTISQDNAKNTLHLQMSSLKPEDTGMYYCAVGVGWVP
ARLTPQAVSYWGKGTLVTVSSTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFAC
DIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCE
LRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNE
LQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

[0518] SEQ ID NO: 232 (AS50073 CAR):

MALPVTALLLPLALLLHAARPQVQLVESGGGLVQAGGSLRLSCTASGFTFDNYVMGWFRQAPG
KEREGVSCIGWSGGSTYYADSVKGRFTISRDNAKNTLYLQMNSLKPEDTAMYYCAADQGKCSL
GSAGADDMDYWGRGTLVTVSSTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFA
CDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGC
ELRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYN
ELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

[0519] SEQ ID NO: 233 (AS67190 CAR):

MALPVTALLLPLALLLHAARPQVQLVESGGGLVQAGGSLRLSCAASGNVFRFNIMGWYRQAPG
NQRELVASIDDGGDRSYADSVEGRFTISRENGKKIMYLQMNSLKPEDTAVYYCAAGLGTYLNG
RVSMATNYWGQGTQVTVSSTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDI
YIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCEL
RVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNEL
QKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

Prepartion of CAR-T cells

[0520] Lentiviruses encoding the dual CAR constructs (Dual 1-Dual 3) as well as the individual CARs contained therein were prepared as described in Example 2. T lymphocytes were collected and transduced with the lentiviruses according to the protocol in Example 2.

***In vitro* cytotoxicity assay**

[0521] The anti-tumor activities of dual CAR-T cells were assessed using the *in vitro* LDH (lactate dehydrogenase) assay as described in Example 2.

[0522] As shown in FIGs. 17A-17C, *in vitro* cytotoxicity of dual CAR-T cells against THP-1 was stronger than anti-CLL1 CAR-T cells, and was comparable to that of anti-CD33 CAR-T cells. These data indicates that dual CAR-T cells against two distinct targets (e.g., CLL1 and CD33) are more efficacious than single-target anti-CLL1 CAR-T cells for tumor elimination.

***In vivo* efficacy evaluation in mouse xenograft model**

[0523] HL-60-Luc (a human leukemia cell line) were cultured, resuspended in $HBSS^{-/-}$ and injected intravenously at 1x 10^7 cells per mouse. Bioluminescent imaging (BLI) was conducted weekly or biweekly post tumor inoculation to monitor model development. The animals were randomized based on the BLI photon numbers and animal body weights. After randomization, a single dose of CAR-T cells or UnT cells were infused intravenously. Weekly BLI imaging was performed to record tumor growth.

[0524] As shown in FIGs. 18B and 18D, mice treated with dual CAR-T cells (Dual1, Dual2 and Dual3) were tumor free (BLI around 10^6) after 3-4 weeks post injection, while mice treated wtih UnT or vehicle exhibited rapid tumor progression and had to be euthanized before the end of the experiment. Tumor growth in mice treated with dual CAR-T cells was significant slower than that in mice treated with single-target CAR T-cells (AS82472 CAR, AS49814 CAR and AS67190 CAR). Combined with the results from Example 5, these data demonstrates that dual target CAR-T cells (tandem CARs or dual CARs) are more efficactious as tumor therapy than single-target CAR-T cells.

CLAIMS

What is claimed is:

1. An anti-CLL1 construct comprising a single domain antibody ("sdAb") moiety that specifically binds to CLL1, wherein the sdAb moiety comprises a CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 4, 11, 18, 25, 32, 39, 46, 53, 60, 67, 74, 81, 88, 151, 158 and 165, or a variant thereof comprising up to about 3 amino acid substitutions in the CDR1; a CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 6, 13, 20, 27, 34, 41, 48, 55, 62, 69, 76, 83, 90, 153, 160, and 167, or a variant thereof comprising up to about 3 amino acid substitutions in the CDR2; and a CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 8, 15, 22, 29, 36, 43, 50, 57, 64, 71, 78, 85, 92, 155, 162, and 169, or a variant thereof comprising up to about 3 amino acid substitutions in the CDR3.
2. The anti-CLL1 construct of claim 1, wherein the sdAb moiety comprises:
(1) a CDR1 comprising the amino acid sequence of SEQ ID NO: 4, a CDR2 comprising the amino acid sequence of SEQ ID NO: 6, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 8, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs;
(2) a CDR1 comprising the amino acid sequence of SEQ ID NO: 11, a CDR2 comprising the amino acid sequence of SEQ ID NO: 13, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 15, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs;
(3) a CDR1 comprising the amino acid sequence of SEQ ID NO: 18, a CDR2 comprising the amino acid sequence of SEQ ID NO: 20, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 22, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs;
(4) a CDR1 comprising the amino acid sequence of SEQ ID NO: 25, a CDR2 comprising the amino acid sequence of SEQ ID NO: 27, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 29, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs;
(5) a CDR1 comprising the amino acid sequence of SEQ ID NO: 32, a CDR2 comprising the amino acid sequence of SEQ ID NO: 34, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 36, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs;
(6) a CDR1 comprising the amino acid sequence of SEQ ID NO: 39, a CDR2 comprising the amino acid sequence of SEQ ID NO: 41, and a CDR3 comprising the amino acid sequence of

SEQ ID NO: 43, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs;

(7) a CDR1 comprising the amino acid sequence of SEQ ID NO: 46, a CDR2 comprising the amino acid sequence of SEQ ID NO: 48, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 50, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs;

(8) a CDR1 comprising the amino acid sequence of SEQ ID NO: 53, a CDR2 comprising the amino acid sequence of SEQ ID NO: 55, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 57, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs;

(9) a CDR1 comprising the amino acid sequence of SEQ ID NO: 60, a CDR2 comprising the amino acid sequence of SEQ ID NO: 62, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 64, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs;

(10) a CDR1 comprising the amino acid sequence of SEQ ID NO: 67, a CDR2 comprising the amino acid sequence of SEQ ID NO: 69, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 71, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs;

(11) a CDR1 comprising the amino acid sequence of SEQ ID NO: 74, a CDR2 comprising the amino acid sequence of SEQ ID NO: 76, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 78, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs;

(12) a CDR1 comprising the amino acid sequence of SEQ ID NO: 81, a CDR2 comprising the amino acid sequence of SEQ ID NO: 83, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 85, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs;

(13) a CDR1 comprising the amino acid sequence of SEQ ID NO: 88, a CDR2 comprising the amino acid sequence of SEQ ID NO: 90, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 92, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs;

(14) a CDR1 comprising the amino acid sequence of SEQ ID NO: 151, a CDR2 comprising the amino acid sequence of SEQ ID NO: 153, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 155, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs;

(15) a CDR1 comprising the amino acid sequence of SEQ ID NO: 158, a CDR2 comprising the amino acid sequence of SEQ ID NO: 160, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 162, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs; or

(16) a CDR1 comprising the amino acid sequence of SEQ ID NO: 165, a CDR2 comprising the amino acid sequence of SEQ ID NO: 167, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 169, or a variant thereof comprising up to about 5 amino acid substitutions in the CDRs.

3. The anti-CLL1 construct of claim 1, wherein the sdAb moiety comprises:

(1) a CDR1 comprising the amino acid sequence of SEQ ID NO: 4, a CDR2 comprising the amino acid sequence of SEQ ID NO: 6, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 8;

(2) a CDR1 comprising the amino acid sequence of SEQ ID NO: 11, a CDR2 comprising the amino acid sequence of SEQ ID NO: 13, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 15;

(3) a CDR1 comprising the amino acid sequence of SEQ ID NO: 18, a CDR2 comprising the amino acid sequence of SEQ ID NO: 20, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 22;

(4) a CDR1 comprising the amino acid sequence of SEQ ID NO: 25, a CDR2 comprising the amino acid sequence of SEQ ID NO: 27, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 29;

(5) a CDR1 comprising the amino acid sequence of SEQ ID NO: 32, a CDR2 comprising the amino acid sequence of SEQ ID NO: 34, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 36;

(6) a CDR1 comprising the amino acid sequence of SEQ ID NO: 39, a CDR2 comprising the amino acid sequence of SEQ ID NO: 41, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 43;

(7) a CDR1 comprising the amino acid sequence of SEQ ID NO: 46, a CDR2 comprising the amino acid sequence of SEQ ID NO: 48, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 50;

(8) a CDR1 comprising the amino acid sequence of SEQ ID NO: 53, a CDR2 comprising the amino acid sequence of SEQ ID NO: 55, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 57;

(9) a CDR1 comprising the amino acid sequence of SEQ ID NO: 60, a CDR2 comprising the amino acid sequence of SEQ ID NO: 62, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 64;

(10) a CDR1 comprising the amino acid sequence of SEQ ID NO: 67, a CDR2 comprising the amino acid sequence of SEQ ID NO: 69, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 71;

(11) a CDR1 comprising the amino acid sequence of SEQ ID NO: 74, a CDR2 comprising the amino acid sequence of SEQ ID NO: 76, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 78;

(12) a CDR1 comprising the amino acid sequence of SEQ ID NO: 81, a CDR2 comprising the amino acid sequence of SEQ ID NO: 83, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 85;

(13) a CDR1 comprising the amino acid sequence of SEQ ID NO: 88, a CDR2 comprising the amino acid sequence of SEQ ID NO: 90, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 92;

(14) a CDR1 comprising the amino acid sequence of SEQ ID NO: 151, a CDR2 comprising the amino acid sequence of SEQ ID NO: 153, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 155;

(15) a CDR1 comprising the amino acid sequence of SEQ ID NO: 158, a CDR2 comprising the amino acid sequence of SEQ ID NO: 160, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 162; or

(16) a CDR1 comprising the amino acid sequence of SEQ ID NO: 165, a CDR2 comprising the amino acid sequence of SEQ ID NO: 167, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 169.

4. An anti-CLL1 construct comprising an sdAb moiety that specifically binds to CLL1, wherein the sdAb moiety comprises a CDR1, a CDR2, and a CDR3 of an sdAb comprising the amino acid sequence of any one of SEQ ID NOs: 94-106 and 171-173.
5. The anti-CLL1 construct of any one of claims 1-4, wherein the sdAb moiety comprises an amino acid sequence having at least about 95% sequence identity to the amino acid sequence of any one of SEQ ID NOs: 94-106 and 171-173.
6. The anti-CLL1 construct of claim 5, wherein the sdAb moiety comprises the amino acid sequence of any one of SEQ ID NOs: 94-106 and 171-173.
7. The anti-CLL1 construct of any one of claims 1-6, wherein the anti-CLL1 construct comprises an extracellular domain comprising the sdAb moiety, a transmembrane domain, and an intracellular

signaling domain comprising a primary intracellular signaling sequence of an immune effector cell.

8. The anti-CLL1 construct of claim 7, wherein the primary intracellular signaling sequence is derived from CD3ζ, FcRγ, FcRβ, CD3γ, CD3δ, CD3ε, CD5, CD22, CD79a, CD79b, or CD66d.
9. The anti-CLL1 construct of claim 7 or 8, wherein the intracellular signaling domain further comprises an intracellular co-stimulatory sequence.
10. The anti-CLL1 construct of claim 7 or 8, wherein the intracellular signaling domain does not comprise an intracellular co-stimulatory sequence.
11. The anti-CLL1 construct of any one of claims 1-6, wherein the anti-CLL1 construct comprises an extracellular domain comprising the sdAb moiety, a transmembrane domain, and an intracellular signaling domain comprising an intracellular co-stimulatory sequence.
12. The anti-CLL1 construct of claim 9 or 11, wherein the intracellular co-stimulatory sequence is derived from a co-stimulatory molecule selected from the group consisting of CD27, CD28, 4-1BB, OX40, CD40, PD-1, LFA-1, ICOS, CD2, CD7, LIGHT, NKG2C, B7-H3, TNFRSF9, TNFRSF4, TNFRSF8, CD40LG, ITGB2, KLRC2, TNFRSF18, TNFRSF14, HAVCR1, LGALS9, DAP10, DAP12, CD83, ligands of CD83 and combinations thereof.
13. An anti-CLL1 construct comprising an extracellular domain comprising an anti-CLL1 sdAb moiety, a transmembrane domain, and an intracellular signaling domain, wherein the anti-CLL1 sdAb moiety comprises: a CDR1 comprising the amino acid sequence of SEQ ID NO: 39, a CDR2 comprising the amino acid sequence of SEQ ID NO: 41, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 43.
14. The anti-CLL1 construct of any one of claims 7-13, wherein the extracellular domain of the anti-CLL1 construct further comprises a second binding moiety that specifically binds to a second antigen or epitope.
15. The anti-CLL1 construct of claim 14, wherein the second binding moiety is an sdAb, an scFv, or an extracellular domain of a receptor.
16. The anti-CLL1 construct of claim 15, wherein the second binding moiety is an sdAb or scFv that specifically binds to CD33 or CD123.
17. The anti-CLL1 construct of claim 15, wherein the second binding moiety is an extracellular domain of NKG2D.
18. An anti-CLL1 construct comprising an extracellular domain comprising an anti-CLL1 sdAb moiety and an anti-CD33 sdAb moiety, a transmembrane domain, and an intracellular signaling domain, wherein the anti-CLL1 sdAb moiety comprises: a CDR1 comprising the amino acid sequence of SEQ ID NO: 39, a CDR2 comprising the amino acid sequence of SEQ ID NO: 41,

and a CDR3 comprising the amino acid sequence of SEQ ID NO: 43, and wherein the anti-CD33 sdAb moiety comprises: a CDR1 comprising the amino acid sequence of SEQ ID NO: 198, a CDR2 comprising the amino acid sequence of SEQ ID NO: 200, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 202.

19. An anti-CLL1 construct comprising the amino acid sequence of any one of SEQ ID NOs: 120-132, 177-179, 181, 184-195, 229-230 and 234-236.
20. A nucleic acid encoding the anti-CLL1 construct of any one of claims 1-19.
21. An engineered immune cell comprising the anti-CLL1 construct of any one of claims 1-19, or the nucleic acid of claim 20.
22. The engineered immune cell of claim 21, further comprising a second chimeric receptor.
23. The engineered immune cell of claim 22, wherein the second chimeric receptor comprises an extracellular domain comprising a second binding moiety that specifically binds to a second antigen or epitope, a transmembrane domain, and an intracellular signaling domain comprising a primary intracellular signaling sequence of an immune effector cell.
24. The engineered immune cell of claim 23, wherein the intracellular signaling domain further comprises an intracellular co-stimulatory sequence.
25. The engineered immune cell of claim 22, wherein the second chimeric receptor comprises an extracellular domain comprising a second binding moiety that specifically binds to a second antigen or epitope, a transmembrane domain, and an intracellular signaling domain comprising an intracellular co-stimulatory sequence.
26. The engineered immune cell of any one of claims 23-25, wherein the second binding moiety is an sdAb, an scFv, or an extracellular domain of a receptor.
27. The engineered immune cell of claim 26, wherein the second binding moiety is an sdAb or scFv that specifically binds to CD33 or CD123.
28. The engineered immune cell of claim 26, wherein the second binding moiety is an extracellular domain of NKG2D.
29. An engineered immune cell comprising: (a) a first chimeric receptor comprising an extracellular domain comprising an anti-CLL1 sdAb, a transmembrane, and an intracellular signaling domain, wherein the anti-CLL1 sdAb comprises: (1) a CDR1 comprising the amino acid sequence of SEQ ID NO: 4, a CDR2 comprising the amino acid sequence of SEQ ID NO: 6, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 8; or (2) a CDR1 comprising the amino acid sequence of SEQ ID NO: 18, a CDR2 comprising the amino acid sequence of SEQ ID NO: 20, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 22; and (b) a second chimeric receptor comprising an extracellular domain comprising an anti-CD33 sdAb, a transmembrane

domain, and an intracellular signaling domain, wherein the anti-CD33 sdAb comprises: (1) a CDR1 comprising the amino acid sequence of SEQ ID NO: 205, a CDR2 comprising the amino acid sequence of SEQ ID NO: 207, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 209; (2) a CDR1 comprising the amino acid sequence of SEQ ID NO: 212, a CDR2 comprising the amino acid sequence of SEQ ID NO: 214, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 216; or (3) a CDR1 comprising the amino acid sequence of SEQ ID NO: 219, a CDR2 comprising the amino acid sequence of SEQ ID NO: 221, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 223.

30. The engineered immune cell of any one of claims 21-29, wherein the immune cell is a T cell.
31. The engineered immune cell of claim 30, wherein the immune cell is selected from the group consisting of a cytotoxic T cell, a helper T cell, a natural killer T cell, and a γδT cell.
32. The engineered immune cell of any one of claims 21-31, wherein the engineered immune cell expresses a safety-switch antigen or epitope.
33. The engineered immune cell of claim 32, wherein the safety-switch antigen or epitope is derived from CD52, EGFR or CD20.
34. The anti-CLL1 construct of any one of claims 1-6, wherein the anti-CLL1 construct is a monospecific molecule.
35. The anti-CLL1 construct of any one of claims 1-6, wherein the anti-CLL1 construct is a multispecific molecule.
36. The anti-CLL1 construct of claim 35, wherein the anti-CLL1 construct comprises the sdAb moiety linked to a second binding moiety that specifically binds to a second antigen or epitope.
37. The anti-CLL1 construct of claim 36, wherein the second binding moiety is an sdAb or an scFv.
38. The anti-CLL1 construct of claim 36 or 37, wherein the sdAb moiety is linked to the second binding moiety via a peptide linker.
39. The anti-CLL1 construct of any one of claims 36-38, wherein the second binding moiety specifically binds to an antigen on the surface of a T cell.
40. The anti-CLL1 construct of claim 39, wherein the second binding moiety specifically binds to an antigen selected from the group consisting of CD3γ, CD3δ, CD3ε, CD3ζ, CD28, OX40, GITR, 4-1BB, CD27, CD40L, and HVEM.
41. The anti-CLL1 construct of any one of claims 1-6, wherein the anti-CLL1 construct is an immunoconjugate comprising the sdAb moiety and an effector molecule.
42. The anti-CLL1 construct of claim 41, wherein the effector molecule is a therapeutic agent selected from the group consisting of a drug, a toxin, a radioisotope, a protein, a peptide, and a nucleic acid.

43. The anti-CLL1 construct of claim 41, wherein the effector molecule is a drug or a toxin.
44. The anti-CLL1 construct of claim 41, wherein the effector molecule is a label.
45. A pharmaceutical composition comprising the anti-CLL1 construct of any one of claims 1-19 and 34-44 or the engineered immune cell of any one of claims 21-33.
46. A method of treating an individual having a cancer, comprising administering to the individual an effective amount of the anti-CLL1 construct of any one of claims 1-19 and 34-44 or the engineered immune cell of any one of claims 21-33.
47. The method of claim 46, wherein the cancer is acute myeloid leukemia (AML), chronic myeloid leukemia (CML) and myelodysplastic syndrome (MDS).

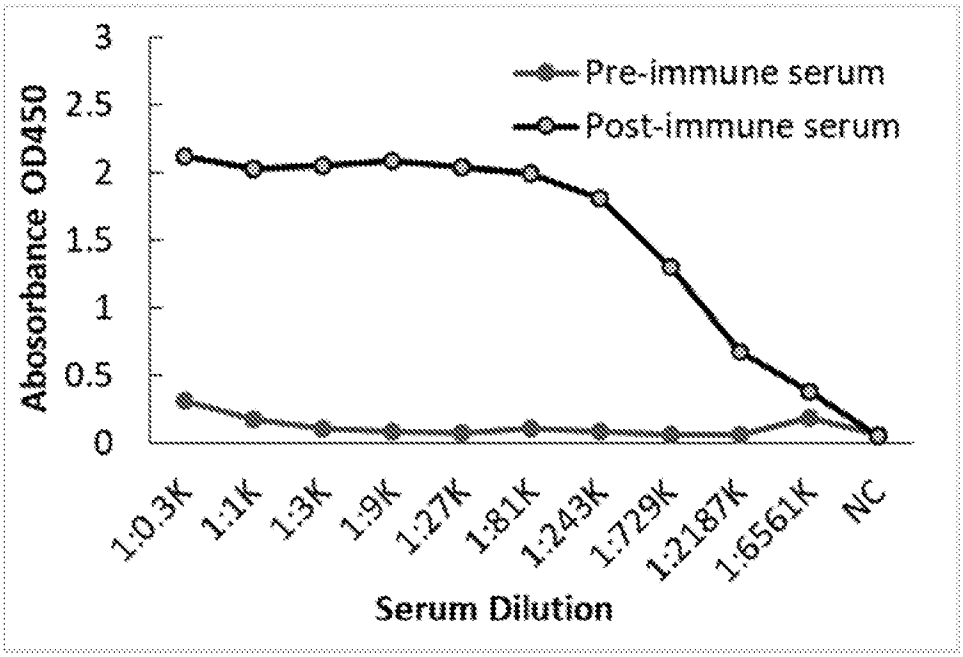

Pre-immune serum
Post-immune serum
Abosorbance OD450
3
2.5
2
1.5
1
0.5
0
1:0.3K
1:1K
1:3K
1:9K
1:27K
1:81K
1:243K
1:729K
1:2187K
1:6561K
NC
Serum Dilution

FIG. 1

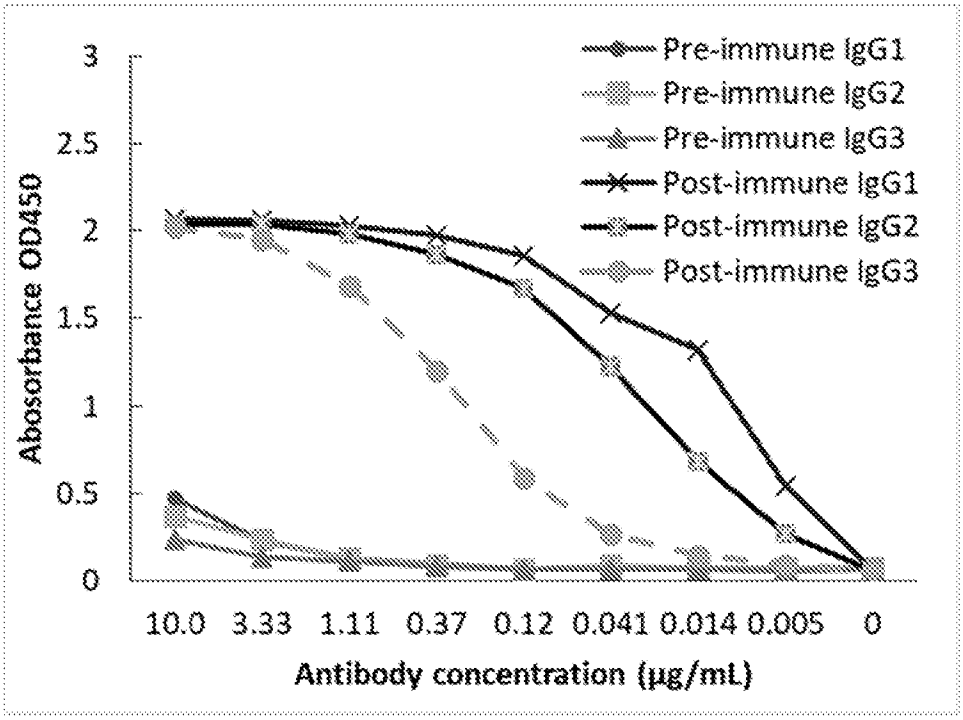
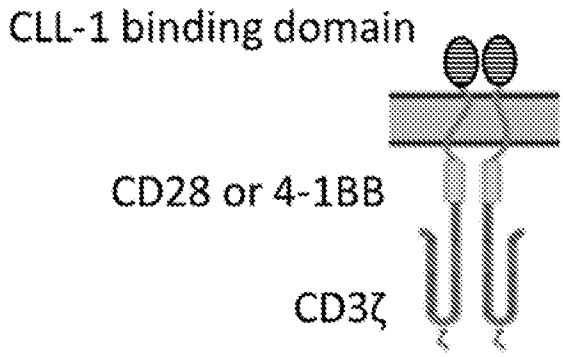

Pre-immune IgG1
Pre-immune IgG2
Pre-immune IgG3
Post-immune IgG1
Post-immune IgG2
Post-immune IgG3
Abosorbance OD450
3
2.5
2
1.5
1
0.5
0
10.0
3.33
1.11
0.37
0.12
0.041
0.014
0.005
0
Antibody concentration (μg/mL)

FIG. 2



SP	CLL1-sdAb	CD8 Hinge	CD8 TM	4-1BB Costimulatory domain	CD3zeta Intracellular domain

FIG. 3

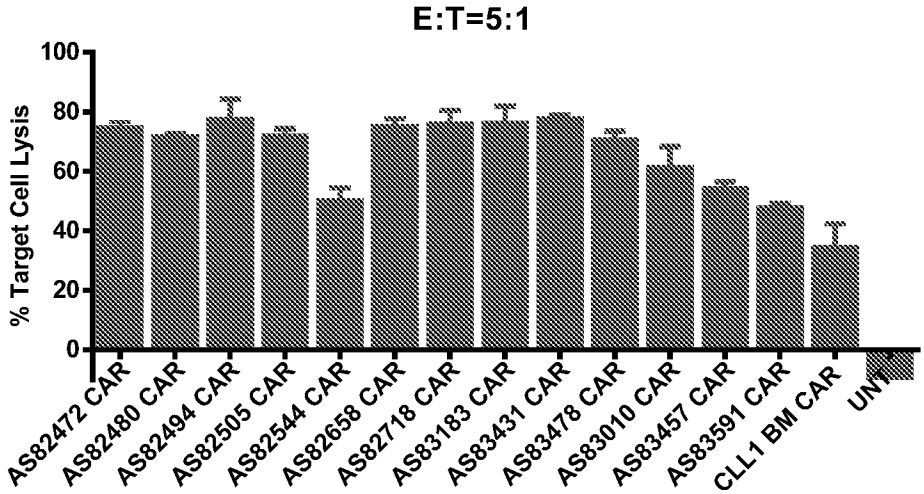


FIG. 4

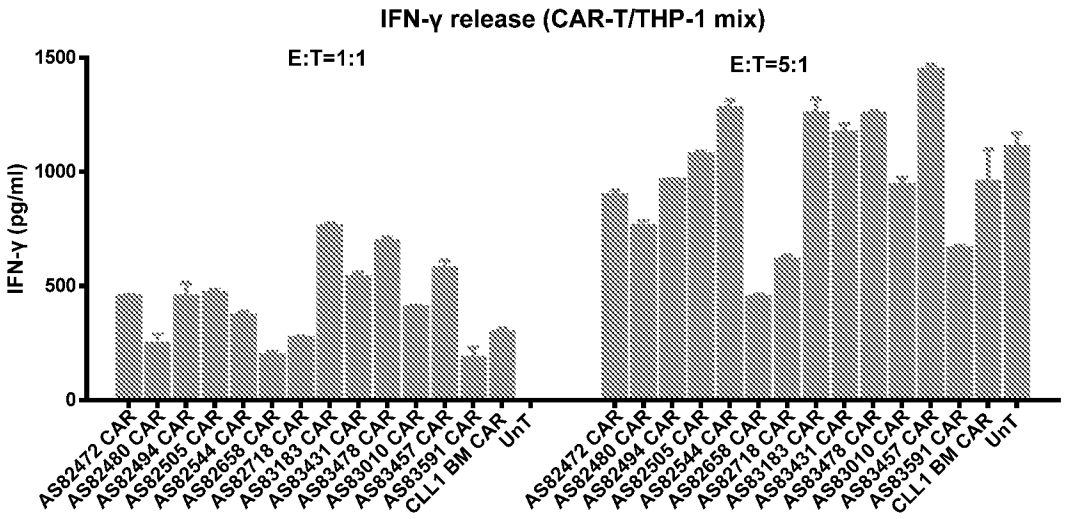

IFN-γ release (CAR-T/THP-1 mix)
E:T=1:1
E:T=5:1
IFN-γ (pg/ml)
1500
1000
500
0
AS82472 CAR
AS82480 CAR
AS82494 CAR
AS82505 CAR
AS82544 CAR
AS82658 CAR
AS82718 CAR
AS83183 CAR
AS83431 CAR
AS83478 CAR
AS83010 CAR
AS83457 CAR
AS83591 CAR
CLL1 BM CAR
UnT

FIG. 5A

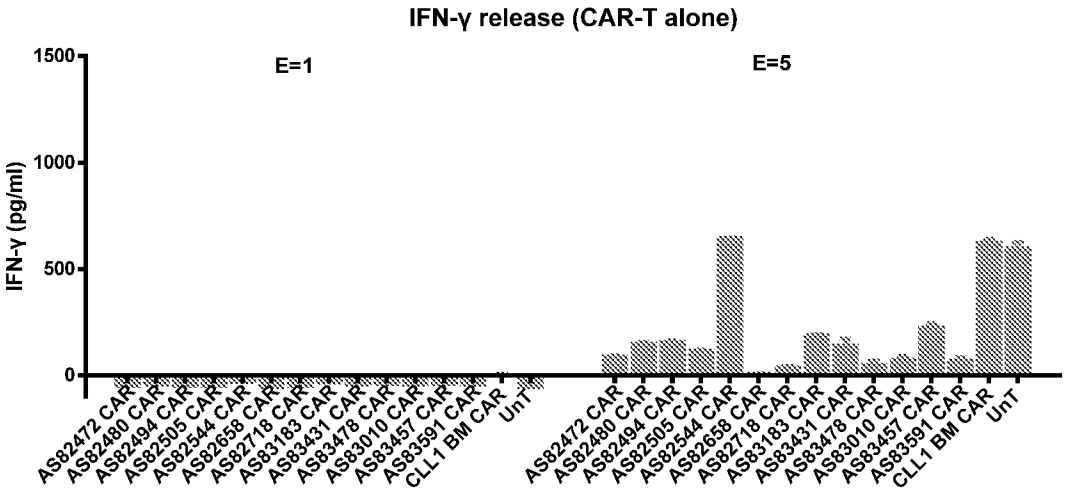

IFN-γ release (CAR-T alone)
E=1
E=5
IFN-γ (pg/ml)
1500
1000
500
0
AS82472 CAR
AS82480 CAR
AS82494 CAR
AS82505 CAR
AS82544 CAR
AS82658 CAR
AS82718 CAR
AS83183 CAR
AS83431 CAR
AS83478 CAR
AS83010 CAR
AS83457 CAR
AS83591 CAR
CLL1 BM CAR
UnT

FIG. 5B

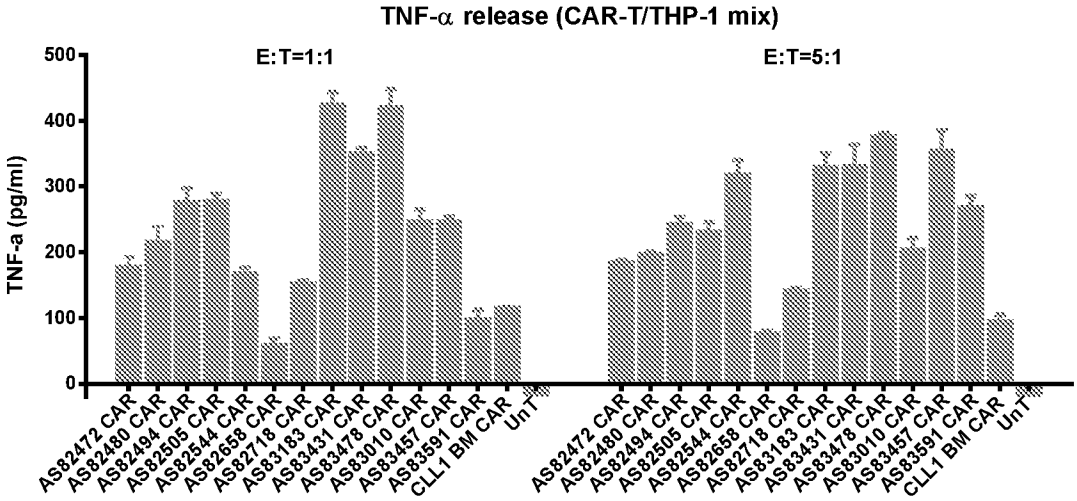

TNF-α release (CAR-T/THP-1 mix)
E:T=1:1
E:T=5:1
TNF-a (pg/ml)
500
400
300
200
100
0
AS82472 CAR
AS82480 CAR
AS82494 CAR
AS82505 CAR
AS82544 CAR
AS82658 CAR
AS82718 CAR
AS83183 CAR
AS83431 CAR
AS83478 CAR
AS83010 CAR
AS83457 CAR
AS83591 CAR
CLL1 BM CAR
UnT

FIG. 5C

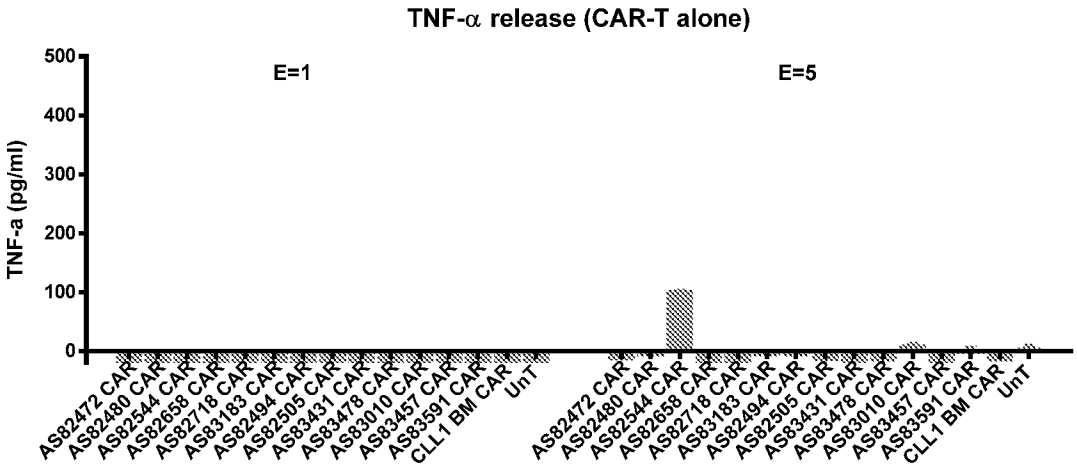
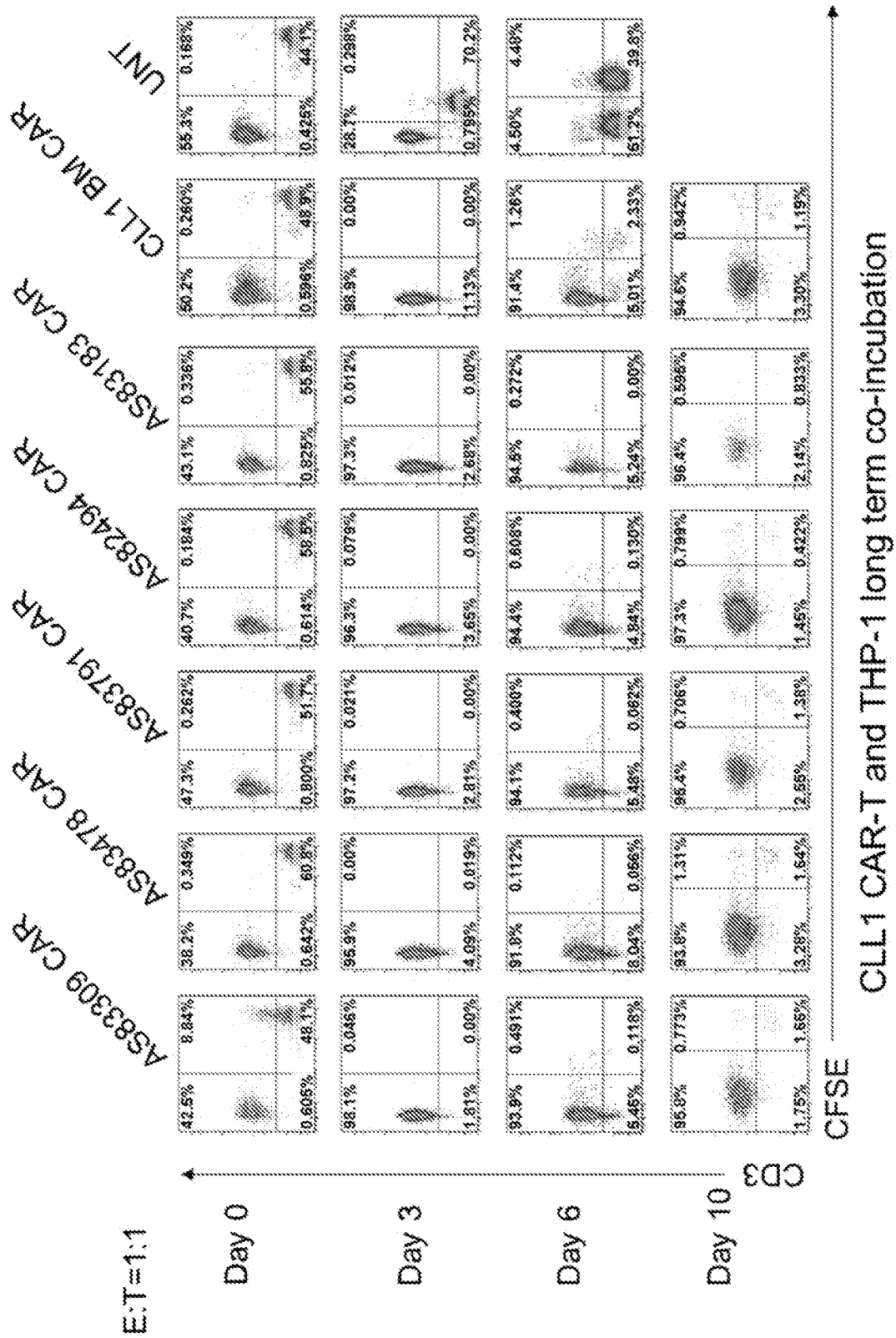

TNF-α release (CAR-T alone)
E=1
E=5
TNF-a (pg/ml)
500
400
300
200
100
0
AS82472 CAR
AS82480 CAR
AS82544 CAR
AS82658 CAR
AS82718 CAR
AS83183 CAR
AS82494 CAR
AS82505 CAR
AS83431 CAR
AS83478 CAR
AS83010 CAR
AS83457 CAR
AS83591 CAR
CLL1 BM CAR
UnT

FIG. 5D



CLL1 CAR-T and THP-1 long term co-incubation

FIG. 6A

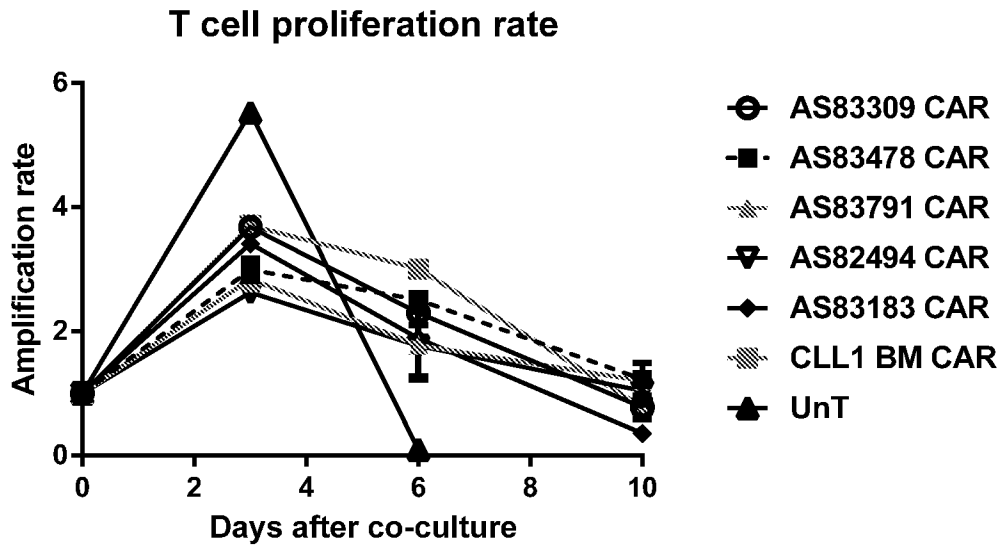

T cell proliferation rate
Amplification rate
0
2
4
6
0
2
4
6
8
10
Days after co-culture
AS83309 CAR
AS83478 CAR
AS83791 CAR
AS82494 CAR
AS83183 CAR
CLL1 BM CAR
UnT

FIG. 6B

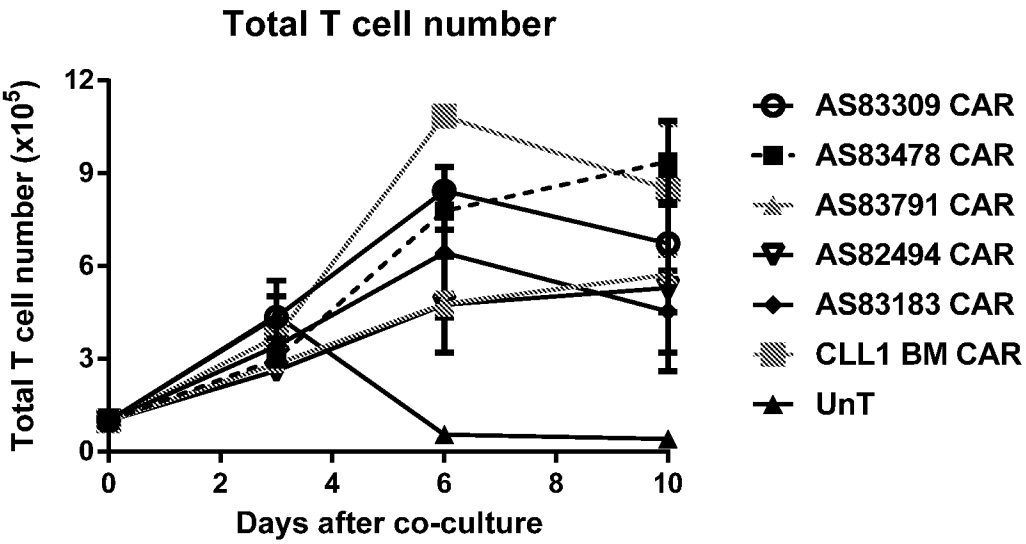

Total T cell number
Total T cell number (x10^5)
0
3
6
9
12
0
2
4
6
8
10
Days after co-culture
AS83309 CAR
AS83478 CAR
AS83791 CAR
AS82494 CAR
AS83183 CAR
CLL1 BM CAR
UnT

FIG. 6C

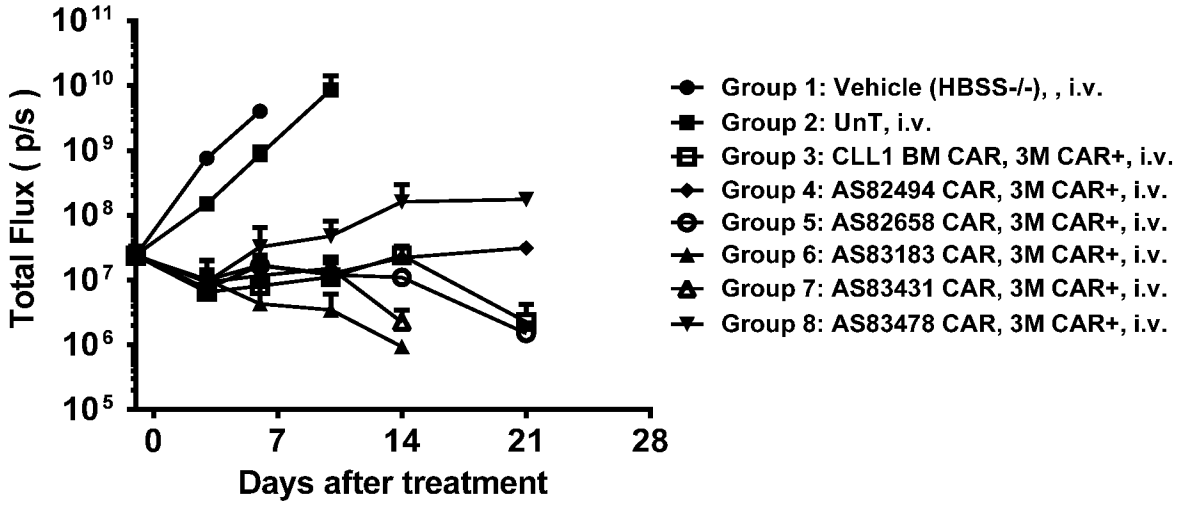

Total Flux (p/s)
10^{11}
10^{10}
10^{9}
10^{8}
10^{7}
10^{6}
10^{5}
0
7
14
21
28
Days after treatment
Group 1: Vehicle (HBSS-/-), , i.v.
Group 2: UnT, i.v.
Group 3: CLL1 BM CAR, 3M CAR+, i.v.
Group 4: AS82494 CAR, 3M CAR+, i.v.
Group 5: AS82658 CAR, 3M CAR+, i.v.
Group 6: AS83183 CAR, 3M CAR+, i.v.
Group 7: AS83431 CAR, 3M CAR+, i.v.
Group 8: AS83478 CAR, 3M CAR+, i.v.

FIG. 7

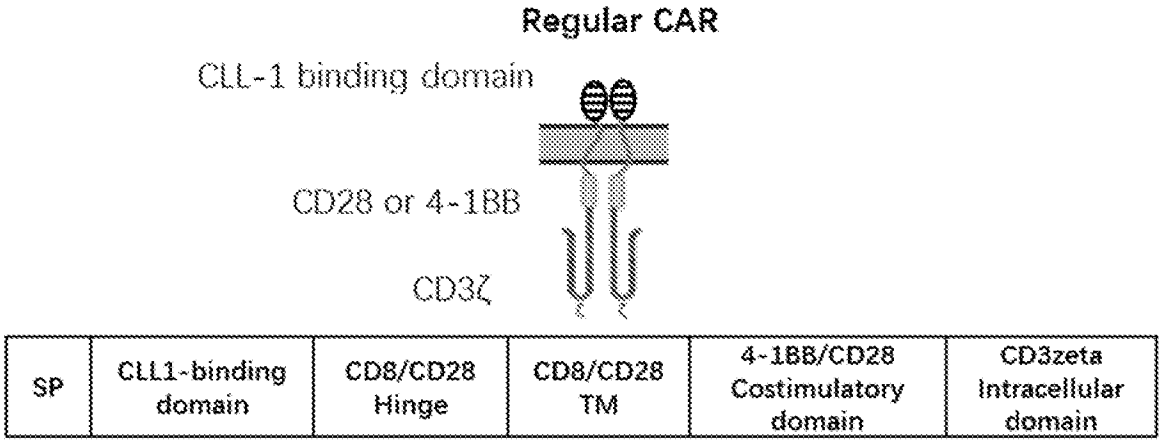
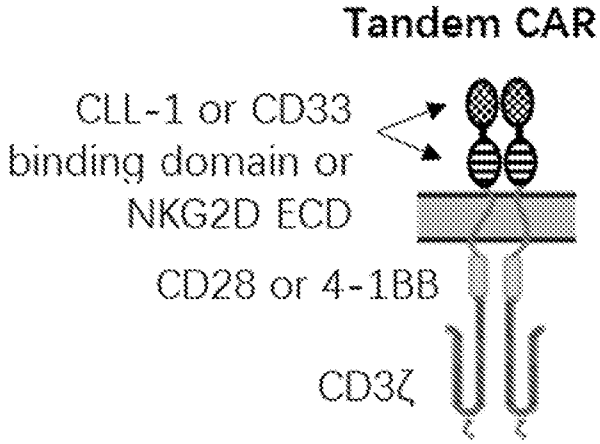

Regular CAR
CLL-1 binding domain
CD28 or 4-1BB
CD3ζ
SP
CLL1-binding domain
CD8/CD28 Hinge
CD8/CD28 TM
4-1BB/CD28 Costimulatory domain
CD3zeta Intracellular domain

FIG. 8A



SP	CLL1- or CD33-binding domain or NKG2D ECD	Linker	CLL1- or CD33-binding domain or NKG2D ECD	CD8 /CD28 Hinge	CD8 /CD28 TM	4-1BB/CD28 Costimulatory domain	CD3zeta intracellular domain

FIG. 8B

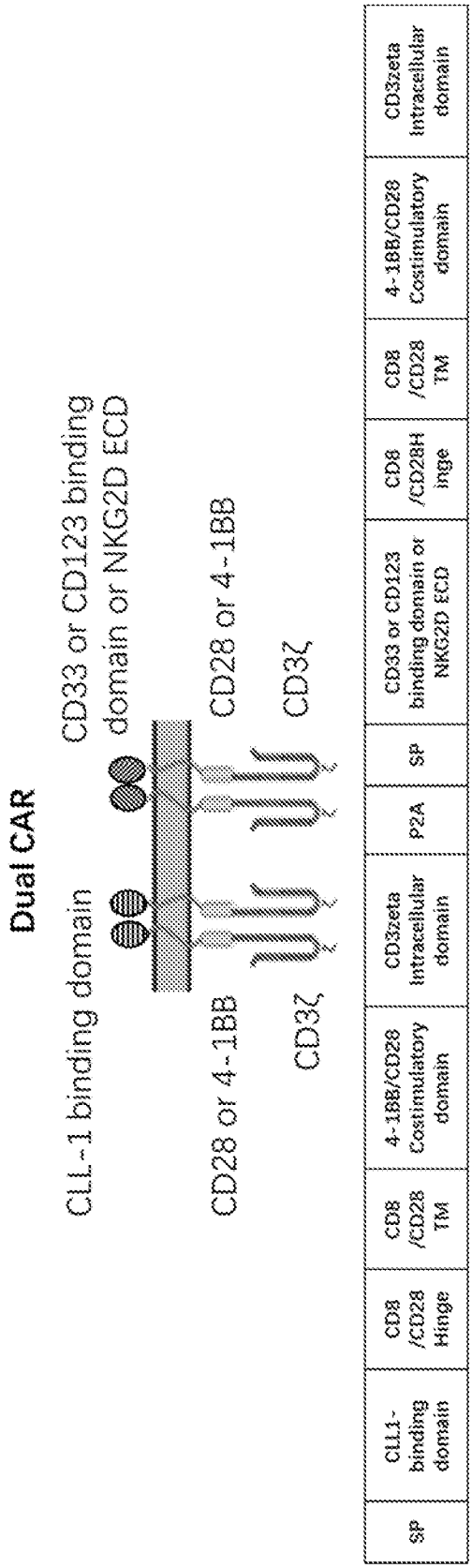

Dual CAR
CLL-1 binding domain
CD33 or CD123 binding domain or NKG2D ECD
CD28 or 4-1BB
CD3ζ
CD28 or 4-1BB
CD3ζ
SP
CLL1-binding domain
CD8 /CD28 Hinge
CD8 /CD28 TM
4-1BB/CD28 Costimulatory domain
CD3zeta intracellular domain
P2A
SP
CD33 or CD123 binding domain or NKG2D ECD
CD8 /CD28H inge
CD8 /CD28 TM
4-1BB/CD28 Costimulatory domain
CD3zeta intracellular domain

FIG. 8C

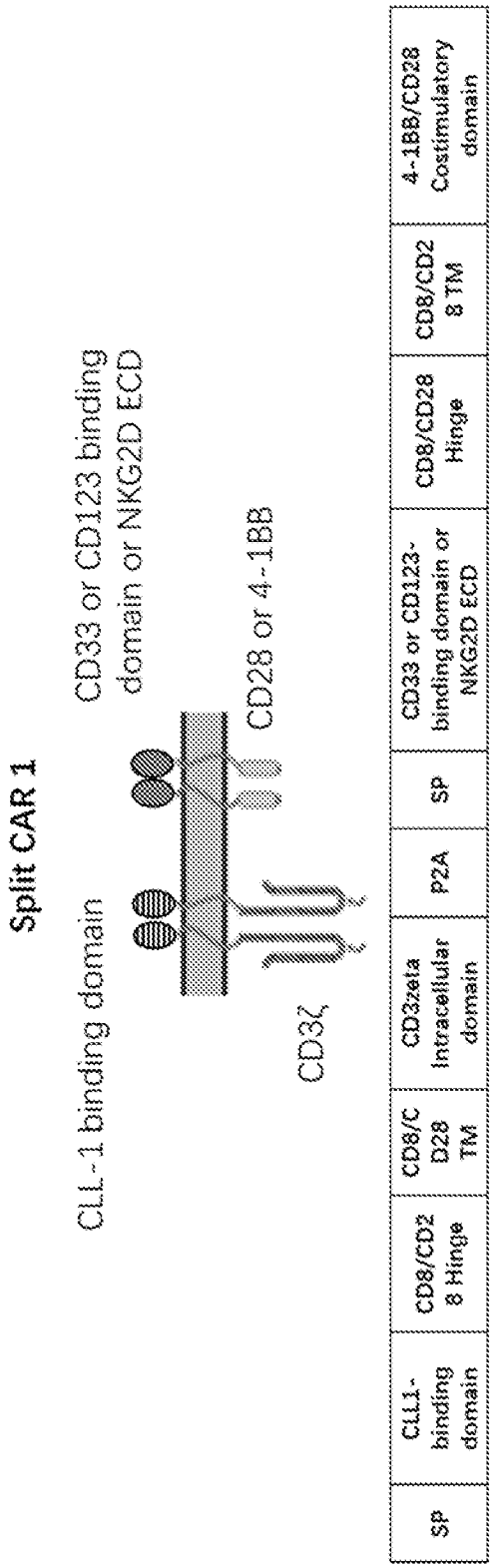

Split CAR 1
CLL-1 binding domain
CD33 or CD123 binding domain or NKG2D ECD
CD28 or 4-1BB
CD3ζ
SP
CLL1-binding domain
CD8/CD28 Hinge
CD8/CD28 TM
CD3zeta Intracellular domain
P2A
SP
CD33 or CD123-binding domain or NKG2D ECD
CD8/CD28 Hinge
CD8/CD28 TM
4-1BB/CD28 Costimulatory domain

FIG. 8D

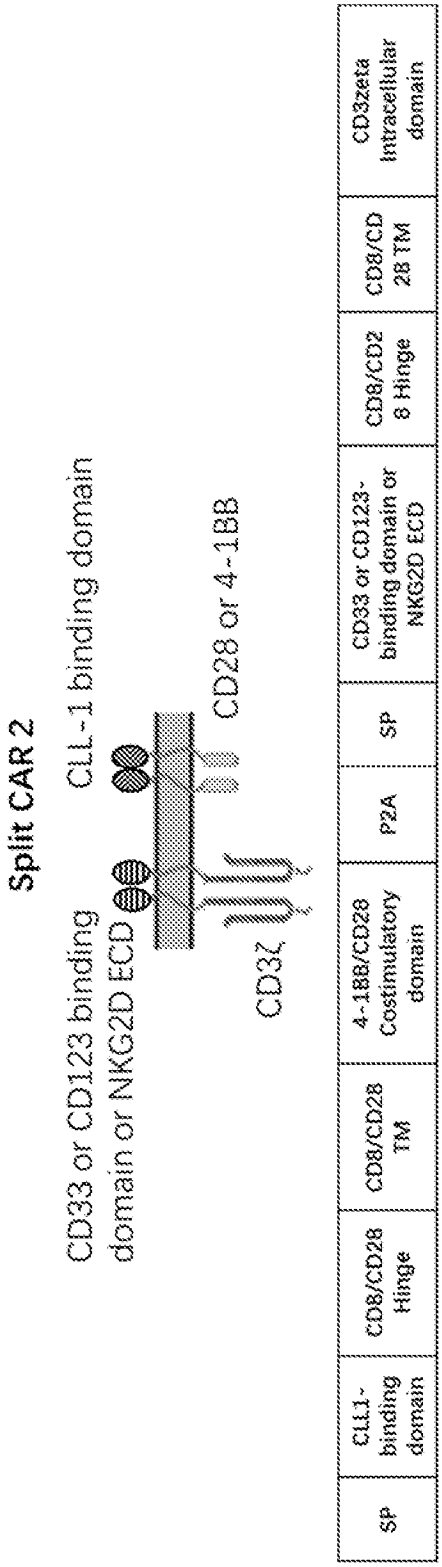

Split CAR 2
CD33 or CD123 binding domain or NKG2D ECD
CLL-1 binding domain
CD28 or 4-1BB
CD3ζ
SP
CLL1-binding domain
CD8/CD28 Hinge
CD8/CD28 TM
4-1BB/CD28 Costimulatory domain
P2A
SP
CD33 or CD123-binding domain or NKG2D ECD
CD8/CD28 Hinge
CD8/CD28 TM
CD3zeta Intracellular domain

FIG. 8E

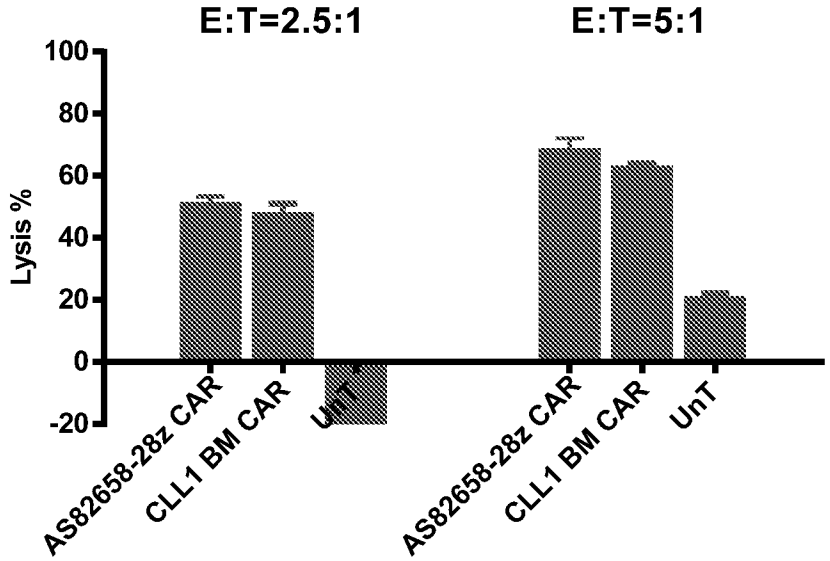

E:T=2.5:1
E:T=5:1
Lysis %
100
80
60
40
20
0
-20
AS82658-28z CAR
CLL1 BM CAR
UnT
AS82658-28z CAR
CLL1 BM CAR
UnT

FIG. 9A

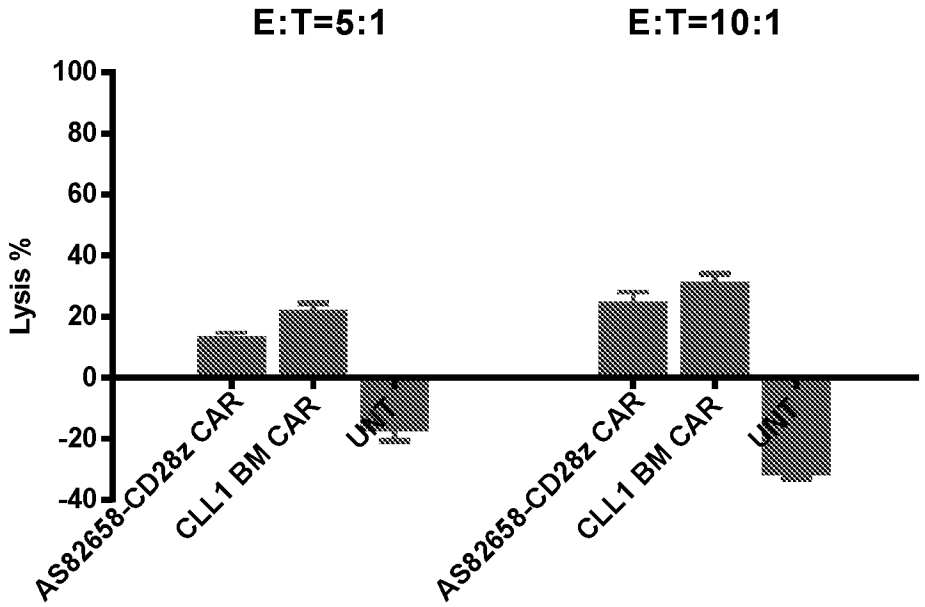

E:T=5:1
E:T=10:1
Lysis %
100
80
60
40
20
0
-20
-40
AS82658-CD28z CAR
CLL1 BM CAR
UnT
AS82658-CD28z CAR
CLL1 BM CAR
UnT

FIG. 9B

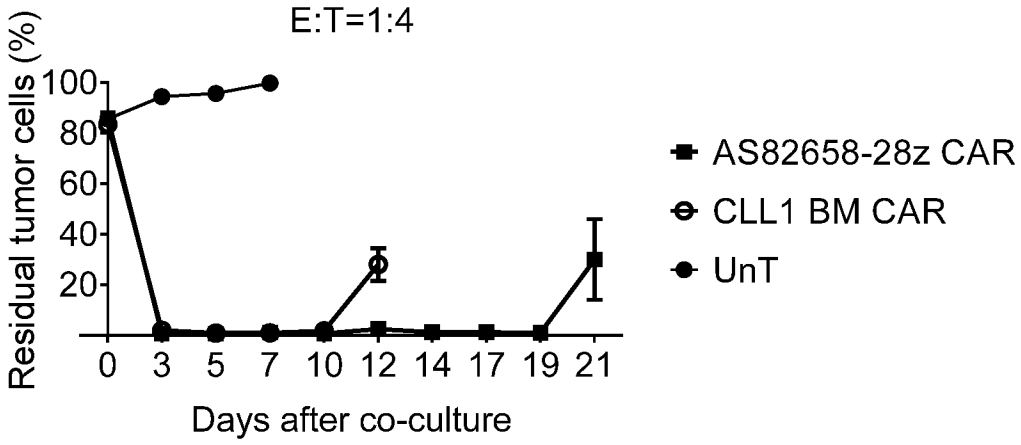


FIG. 10A

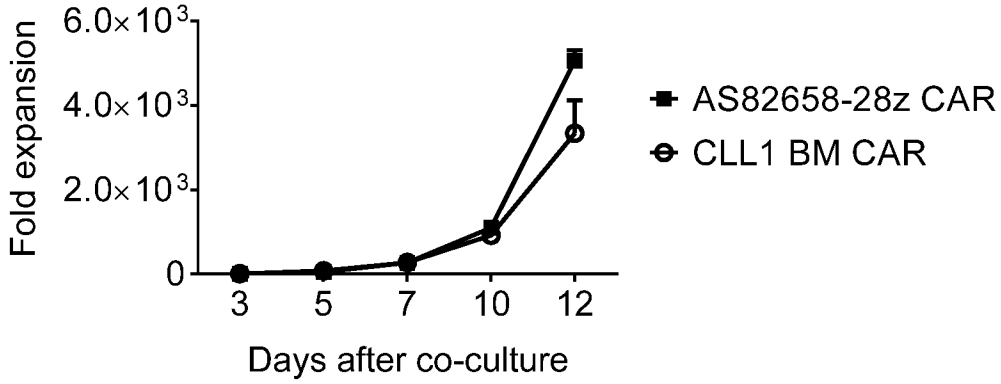


FIG. 10B

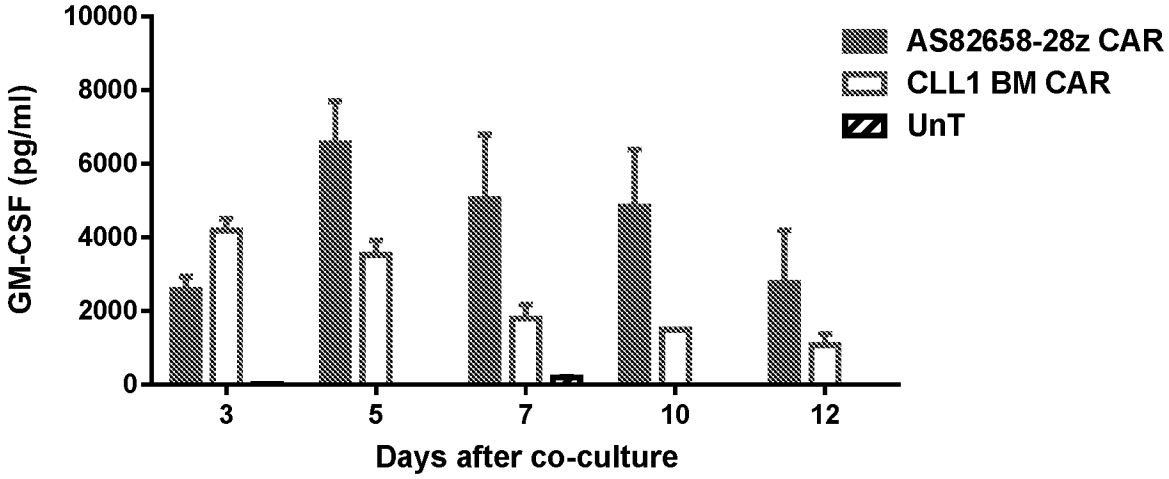


FIG. 11A

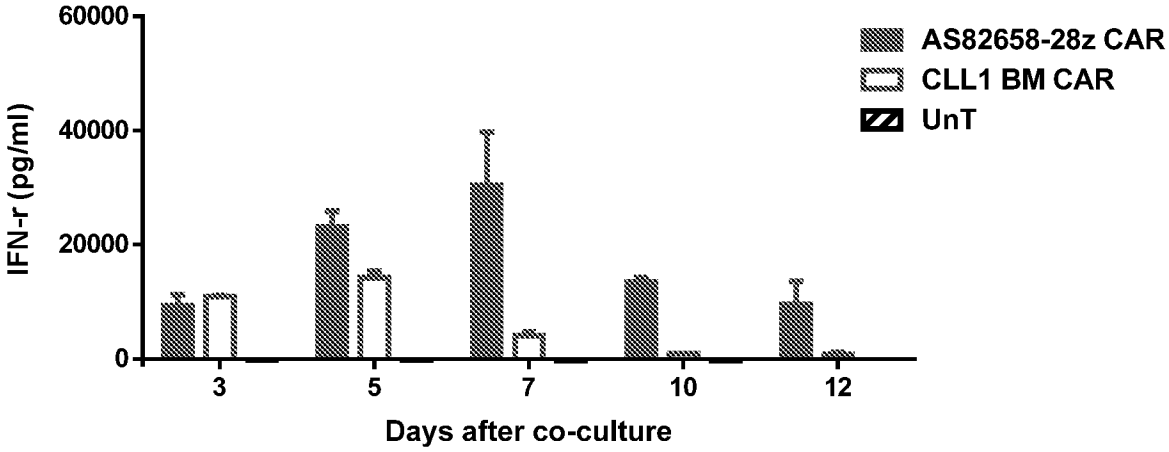


FIG. 11B

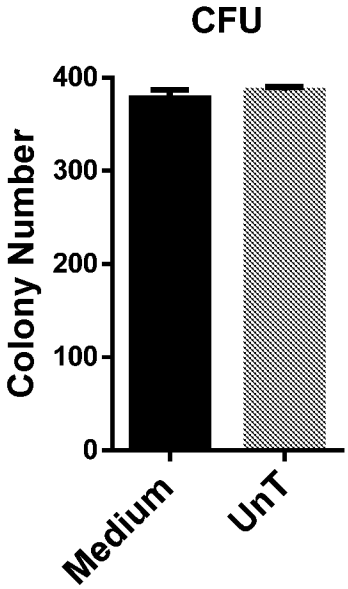


FIG. 12A

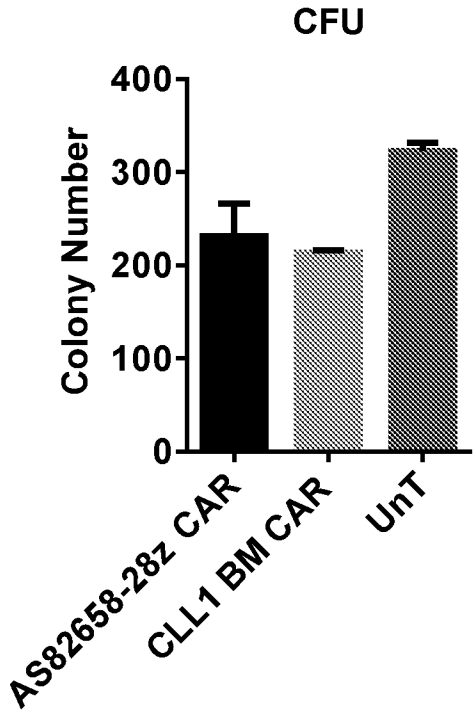


FIG. 12B

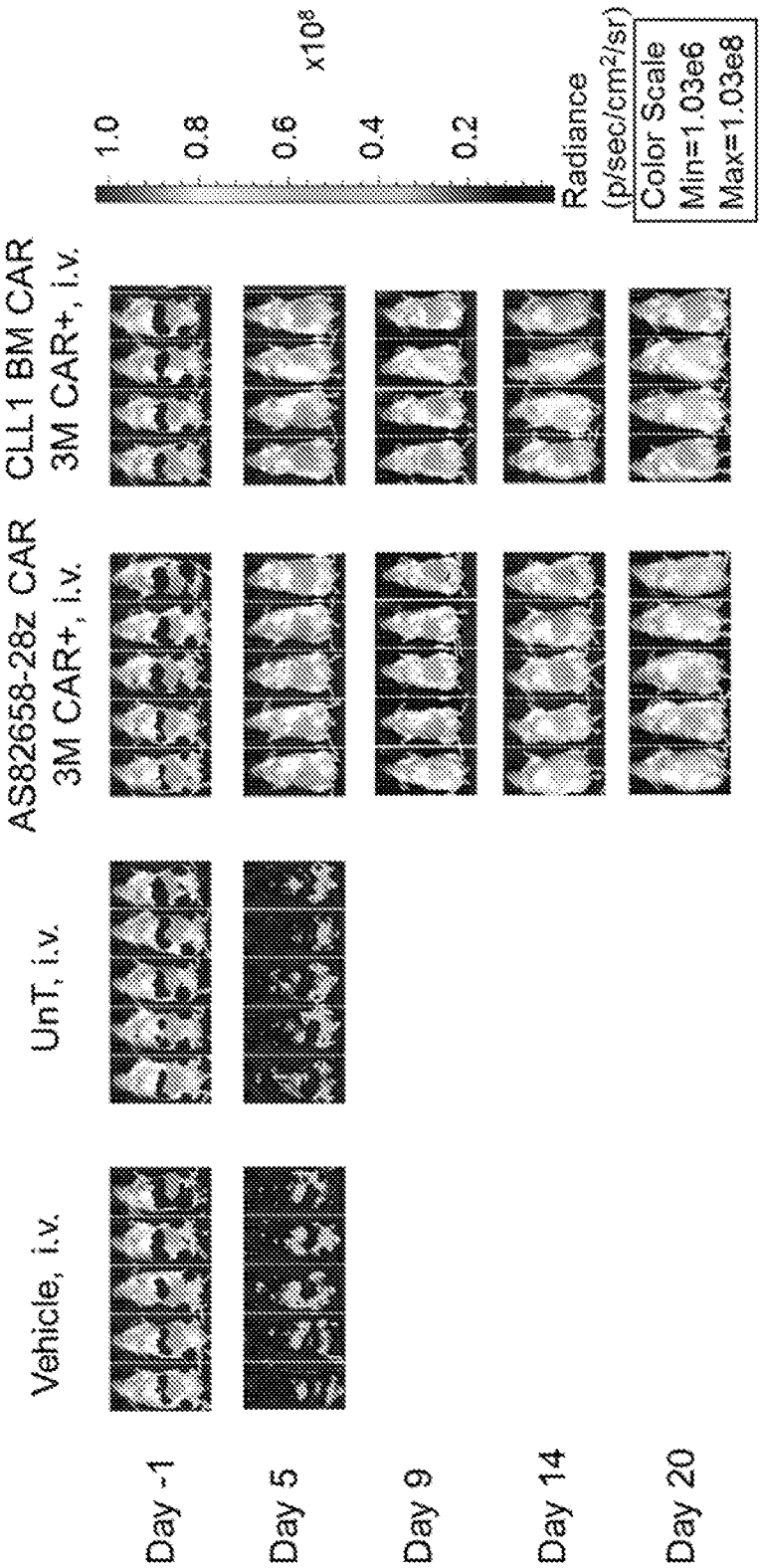

Vehicle, i.v.
UnT, i.v.
AS82658-28z CAR 3M CAR+, i.v.
CLL1 BM CAR 3M CAR+, i.v.
Day -1
Day 5
Day 9
Day 14
Day 20
1.0
0.8
0.6
0.4
0.2
x10^8
Radiance (p/sec/cm²/sr)
Color Scale
Min=1.03e6
Max=1.03e8

FIG. 13

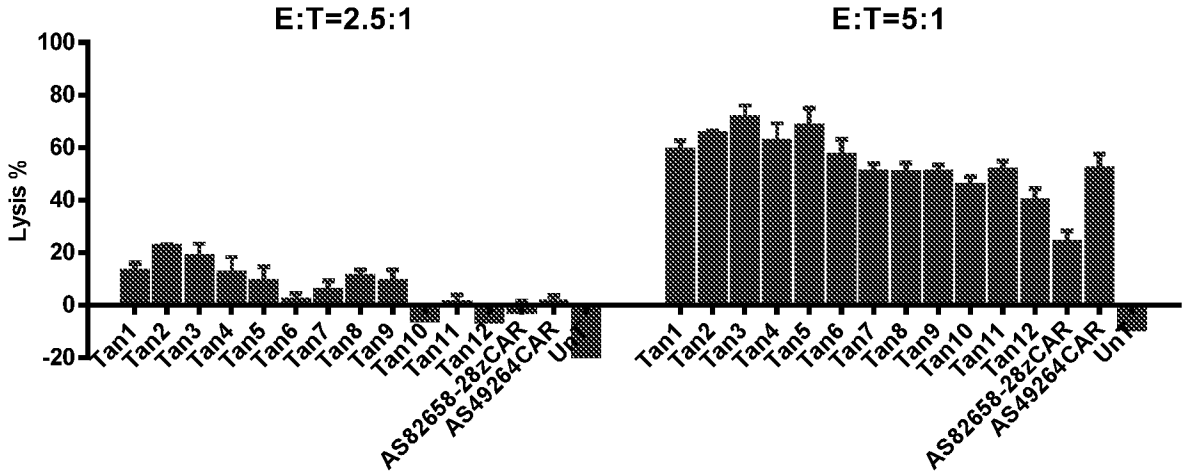


FIG. 14

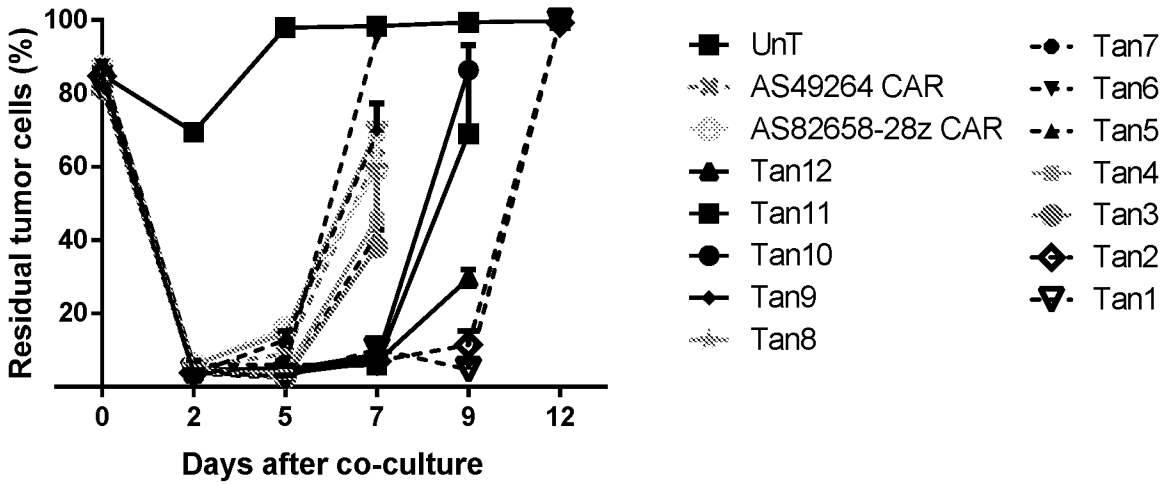


FIG. 15A

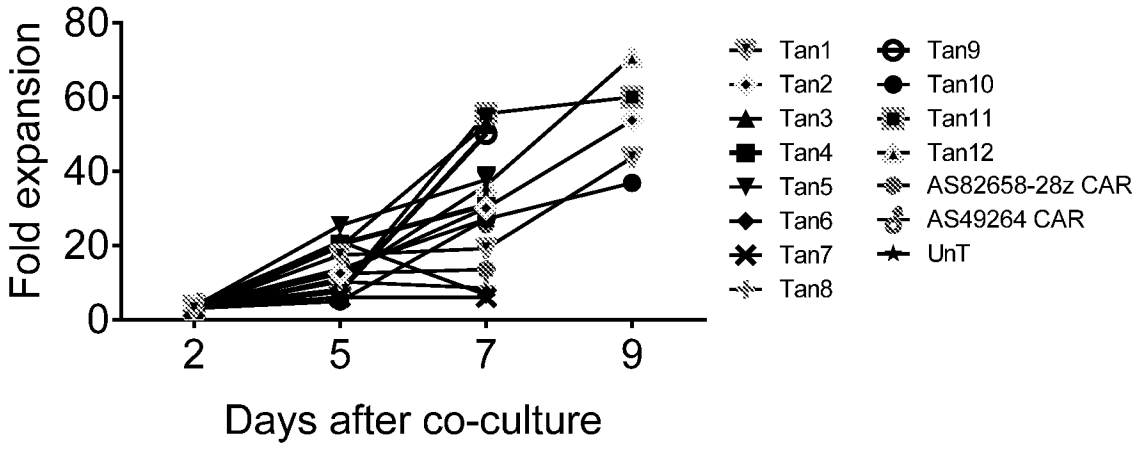


FIG. 15B

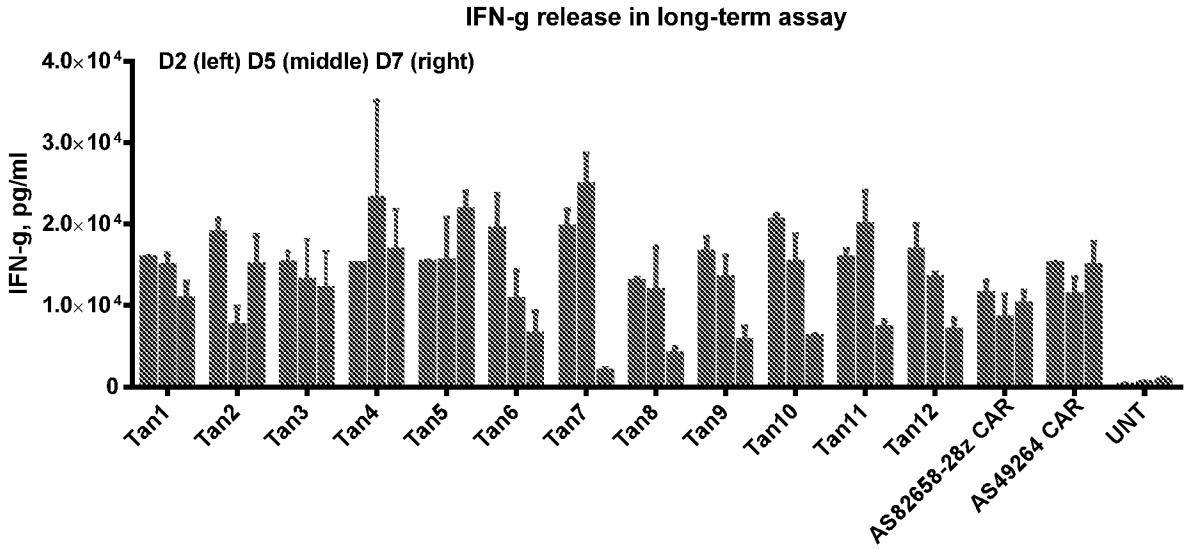


FIG. 16A

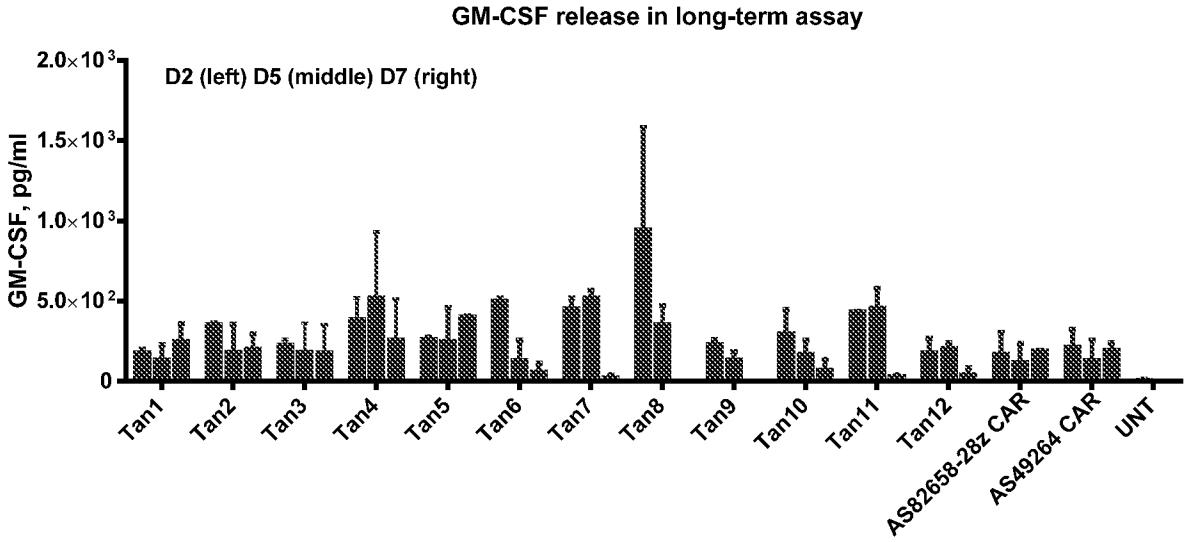


FIG. 16B

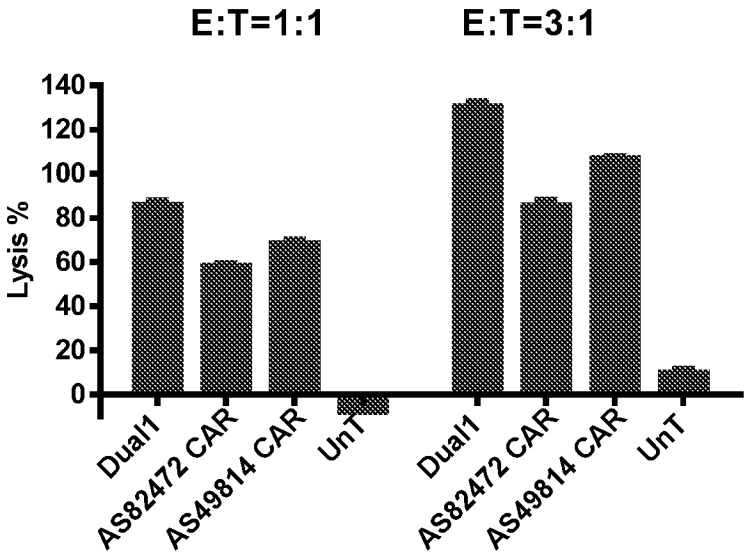


FIG. 17A

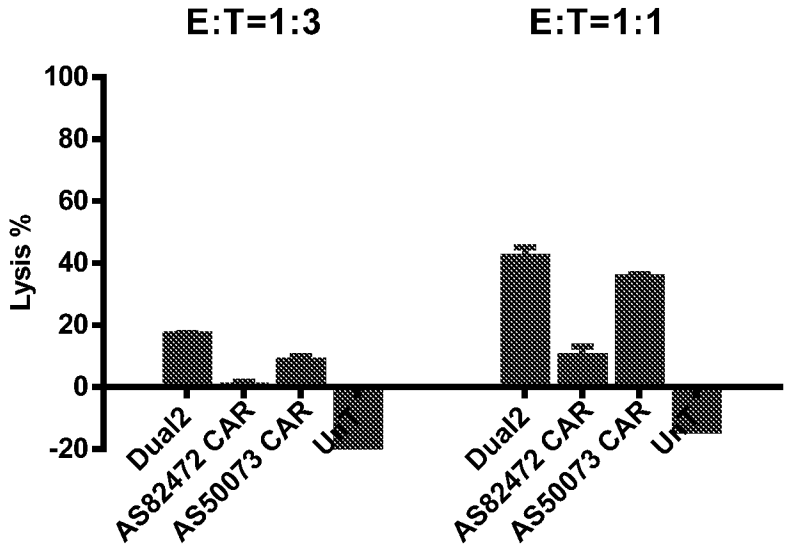


FIG. 17B

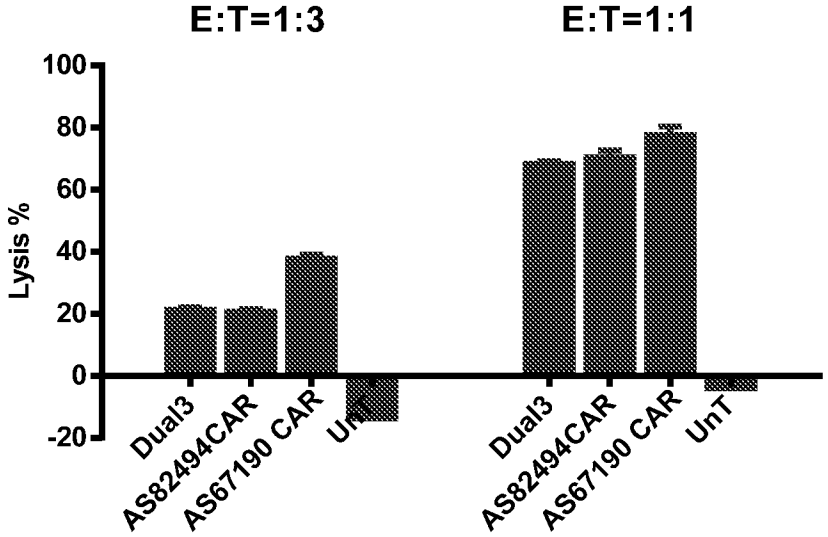


FIG. 17C

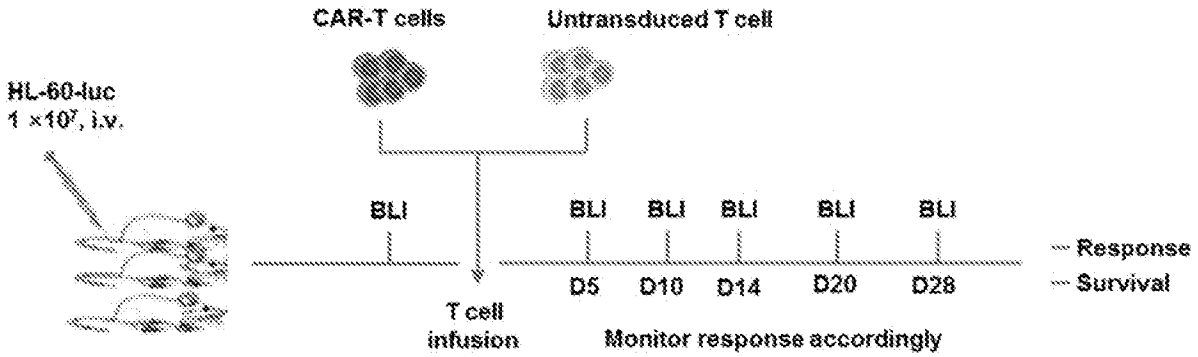


FIG. 18A

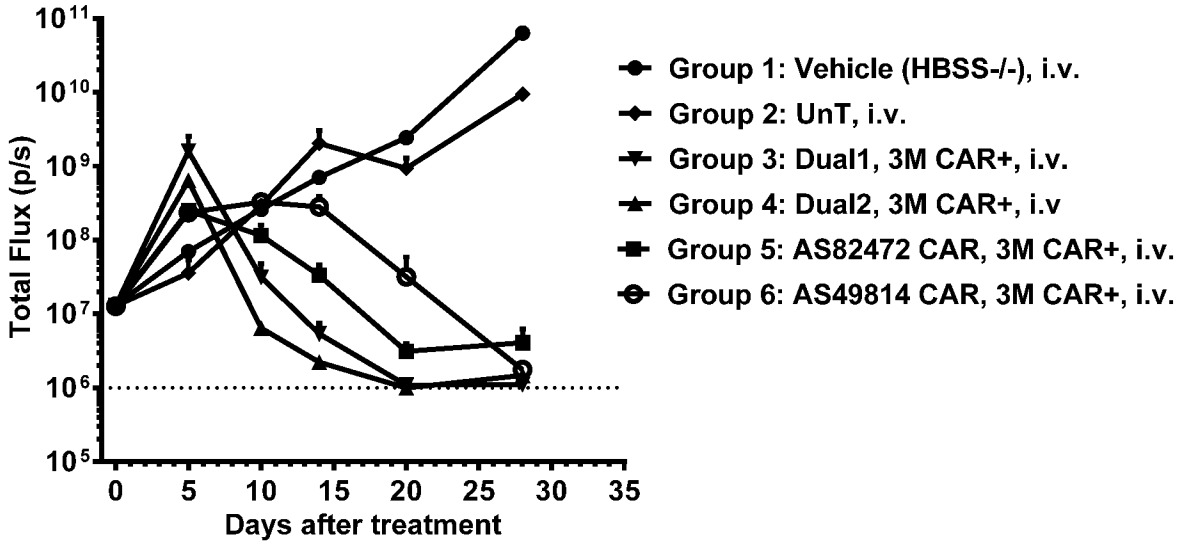


FIG. 18B

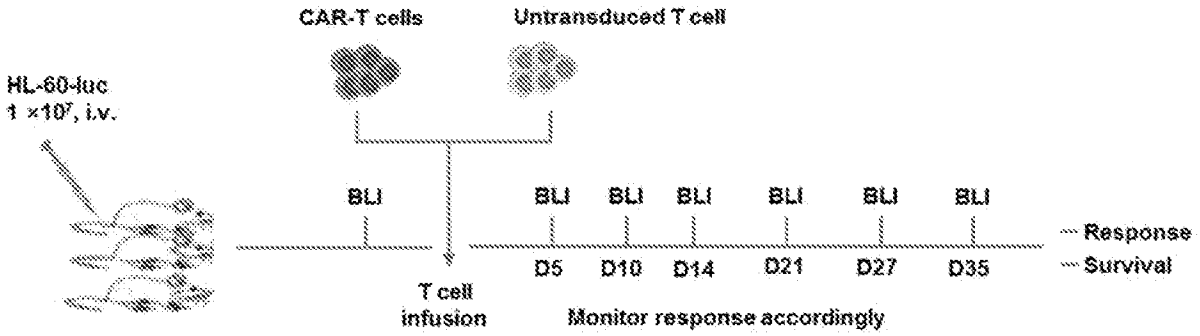


FIG. 18C

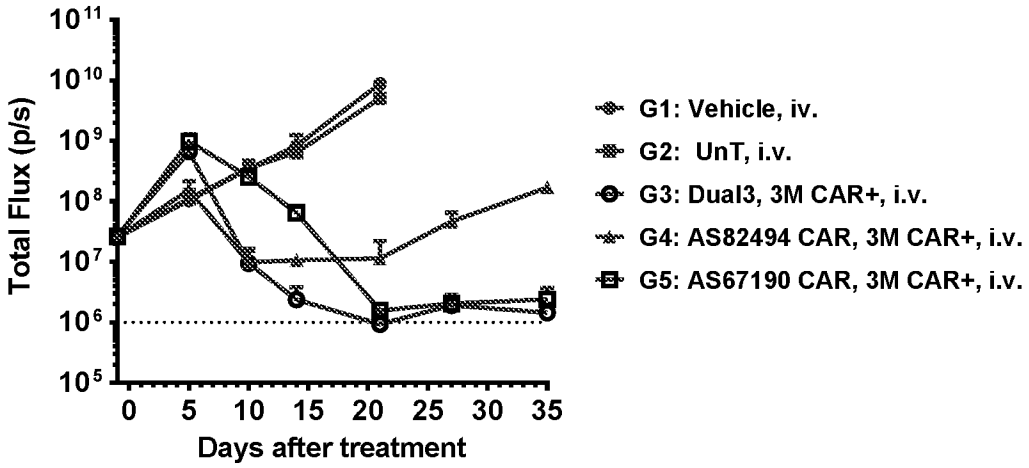

Total Flux (p/s)
10^11
10^10
10^9
10^8
10^7
10^6
10^5
0
5
10
15
20
25
30
35
Days after treatment
G1: Vehicle, iv.
G2: UnT, i.v.
G3: Dual3, 3M CAR+, i.v.
G4: AS82494 CAR, 3M CAR+, i.v.
G5: AS67190 CAR, 3M CAR+, i.v.

FIG. 18D

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CN2019/105056

A. CLASSIFICATION OF SUBJECT MATTER

C07K 16/28(2006.01)i; C12N 5/10(2006.01)i; A61K 39/395(2006.01)i

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

C07K C12N A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CNABS,DWPI,SIPOABS,CNTXT,EPTXT,USTXT,WOTXT,PUBMED,CNKI,NCBI,EBI,STN:CLL1, sdAb, single domain antibody, monoclonal antibody,CDR,HVR,heavy chain,SEQ ID NOs:4, 11, 18, 25, 32, 39, 46, 53, 60, 67, 74, 81, 88, 151, 158, 165,6, 13, 20, 27, 34, 41, 48, 55, 62, 69, 76, 83, 90, 153, 160, 167,8, 15, 22, 29, 36, 43, 50, 57, 64, 71, 78, 85, 92, 155, 162,169.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 2016368994 A1 (GENENTEC,INC.) 22 December 2016 (2016-12-22) the whole document	1-47
A	US 2018086831 A1 (UCL BUSINESS PLC.) 29 March 2018 (2018-03-29) the whole document	1-47
A	WO 2017132279 A1 (GENENTEC,INC.ET AL.) 03 August 2017 (2017-08-03) the whole document	1-47
A	CN 107001465 A (GENENTEC,INC.) 01 August 2017 (2017-08-01) the whole document	1-47
A	WO 2016168766 A1 (THE CALIFORNIA INSTITUTE FOR BIOMEDICAL RESEARCH) 20 October 2016 (2016-10-20) the whole document	1-47
A	WO 2017125897 A1 (NOVARTIS AG) 27 July 2017 (2017-07-27) the whole document	1-47

☐ Further documents are listed in the continuation of Box C. ☑ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"D" document cited by the applicant in the international application
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
27 November 2019	**17 December 2019**
Name and mailing address of the ISA/CN **National Intellectual Property Administration, PRC** **6, Xitucheng Rd., Jimen Bridge, Haidian District, Beijing** **100088** **China** Facsimile No. **(86-10)62019451**	Authorized officer **GUAN,Bing** Telephone No. **(86-10)53961945**

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CN2019/105056

Box No. II **Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☑ Claims Nos.: **46-47**
because they relate to subject matter not required to be searched by this Authority, namely:

[1] The subject matter of claims 46-47 relates to the treatment of CLL1-related disease, and does not warrant an international search according to the criteria set out in PCT Rule 39.1(iv).A international search is still carried out on the basis of the use of the pharmaceutical composition for manufacturingof medicament for the treatment of CLL1-related disease.

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/CN2019/105056

Patent document cited in search report			Publication date (day/month/year)	Patent family member(s)			Publication date (day/month/year)
US	2016368994	A1	22 December 2016	WO	2016205200	A1	22 December 2016
				EP	3310378	A1	25 April 2018
				CN	107847568	A	27 March 2018
				TW	201718647	A	01 June 2017
				JP	2018522541	A	16 August 2018
				None			
US	2018086831	A1	29 March 2018	CA	2937157	A1	25 January 2018
WO	2017132279	A1	03 August 2017	EP	3408671	A1	05 December 2018
				CN	109073635	A	21 December 2018
				WO	2017132279	A8	28 June 2018
				JP	2019504321	A	14 February 2019
CN	107001465	A	01 August 2017	PE	06702017	A1	06 June 2017
				WO	2016040868	A1	17 March 2016
				AU	2015314744	A1	02 March 2017
				BR	112017004802	A2	12 December 2017
				TW	201625690	A	16 July 2016
				US	2019248903	A1	15 August 2019
				CR	20170095	A	19 July 2017
				US	10266597	B2	23 April 2019
				CL	2017000590	A1	06 October 2017
				US	2018009894	A1	11 January 2018
				KR	20170066421	A	14 June 2017
				MX	2017003022	A	12 May 2017
				SG	11201701627P	A	30 March 2017
				EP	3191520	A1	19 July 2017
				AR	101846	A1	18 January 2017
				SG	10201804931Q	A	30 July 2018
				US	10227412	B2	12 March 2019
				US	2016075787	A1	17 March 2016
				CA	2958479	A1	17 March 2016
				JP	2017534256	A	24 November 2017
				US	2018044421	A1	15 February 2018
				US	2018044420	A1	15 February 2018
				US	9751946	B2	05 September 2017
				EA	201790569	A1	31 August 2017
				US	10239947	B2	26 March 2019
WO	2016168766	A1	20 October 2016	WO	2016168769	A1	20 October 2016
				US	2018100026	A1	12 April 2018
WO	2017125897	A1	27 July 2017	EP	3405492	A1	28 November 2018