CD20 BINDING MOLECULES AND USES THEREOF

CROSS REFERENCE

[0001] This application claims benefit of priority of International Patent Application No. PCT/CN2020/102463 filed on July 16, 2020, the content of which is incorporated herein by reference in its entirety.

SEQUENCE LISTING

[0002] This application incorporates by reference a Sequence Listing submitted with this application as a text file, entitled 14651-026-228_SEQ_LISTING.txt, created on July 9, 2021, and is 236,191 bytes in size.

1. FIELD

[0003] The present disclosure relates to anti-CD20 single domain antibodies, chimeric antigen receptors, engineered immune effector cells, and methods of use thereof. The present disclosure further relates to activation and expansion of cells for therapeutic uses, especially to chimeric antigen receptor-based T cell immunotherapies.

2. BACKGROUND

[0004] CD20 is a surface antigen expressed at certain stages of B-cell differentiation. Targeting the CD20-positive B cells with therapeutic monoclonal antibodies (mAbs) has been an effectual strategy in the treatment of hematologic malignancies such as non-Hodgkin's lymphoma (NHL) and chronic lymphocytic leukemia (CLL). Initial success with Rituximab (RTX) has encouraged the creation and development of more effective CD20 based therapeutics. However, treatment with conventional mAbs has not been adequate to overcome the problems such as refractory/relapsed diseases (Shanehbandi *et al.*, *Current Cancer Drug Targets*, 17(5): 423-444 (2007)).

[0005] Chimeric antigen receptor T (CAR-T) cell therapy is an emerging and effective cancer immunotherapy. Especially in hematological malignancies, CAR-T cells have achieved exciting results. Early clinical studies of CD20 CAR-T cells have been conducted, which indicate that CD20 CAR-T cells have encouraging therapeutic potential in B-cell NHLs (Brudno and Kochenderfer, *Nature Reviews Clinical Oncology*, 15: 31-46 (2018)). However, the application of CAR-T cells is obviously hampered by adverse effects, such as cytokines release syndrome and on-target off-tumor toxicity (Yu *et al.*, *Molecular Cancer*, 18 (1): 125 (2019)).

[0006] Thus, improved CD20-binding molecules and engineered CD20-targeting cells are needed. For example, there is a need to develop stable and small-sized CD20 binding molecules for use in more effective or efficient CAR-T therapies.

3. SUMMARY

[0007] In one aspect, provided herein is an anti-CD20 single domain antibody (sdAb) comprising:

CDR2 comprising the amino acid sequence of SEQ ID NO: 34; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 16; (xviii) a CDR1 comprising the amino acid sequence of SEQ ID NO: 22; a CDR2 comprising the amino acid sequence of SEQ ID NO: 35; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 19; (xix) a CDR1 comprising the amino acid sequence of SEQ ID NO: 20 or 197; a CDR2 comprising the amino acid sequence of SEQ ID NO: 36; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 16; (xx) a CDR1 comprising the amino acid sequence of SEQ ID NO: 20 or 197; a CDR2 comprising the amino acid sequence of SEQ ID NO: 37; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 16; (xxi) a CDR1 comprising the amino acid sequence of SEQ ID NO: 20 or 197; a CDR2 comprising the amino acid sequence of SEQ ID NO: 38; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 16; (xxii) a CDR1 comprising the amino acid sequence of SEQ ID NO: 4; a CDR2 comprising the amino acid sequence of SEQ ID NO: 33; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 39; (xxiii) a CDR1 comprising the amino acid sequence of SEQ ID NO: 4; a CDR2 comprising the amino acid sequence of SEQ ID NO: 33; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 40; (xxiv) a CDR1 comprising the amino acid sequence of SEQ ID NO: 165 or 199; a CDR2 comprising the amino acid sequence of SEQ ID NO: 166; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 167; (xxv) a CDR1 comprising the amino acid sequence of SEQ ID NO: 168; a CDR2 comprising the amino acid sequence of SEQ ID NO: 169; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 170; (xxvi) a CDR1 comprising the amino acid sequence of SEQ ID NO: 171 or 200; a CDR2 comprising the amino acid sequence of SEQ ID NO: 172; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 173; (xxvii) a CDR1 comprising the amino acid sequence of SEQ ID NO: 174; a CDR2 comprising the amino acid sequence of SEQ ID NO: 175; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 176; (xxviii) a CDR1 comprising the amino acid sequence of SEQ ID NO: 177 or 197; a CDR2 comprising the amino acid sequence of SEQ ID NO: 178; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 179; or (xxix) a CDR1 comprising the amino acid sequence of SEQ ID NO: 180; a CDR2 comprising the amino acid sequence of SEQ ID NO: 181; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 182.

[0008] In another example~~aspect~~, provided herein is an anti-CD20 single domain antibody (sdAb) comprising: (i) a CDR1, a CDR2, and a CDR3 having the amino acid sequences of the CDR1, CDR2, and CDR3, respectively, as set forth in SEQ ID NO: 41; (ii) a CDR1, a CDR2, and a CDR3 having the amino acid sequences of the CDR1, CDR2, and CDR3, respectively, as set forth in SEQ ID NO: 42; (iii) a CDR1, a CDR2, and a CDR3 having the amino acid sequences of the CDR1, CDR2, and CDR3, respectively, as set forth in SEQ ID NO: 43; (iv) a CDR1, a CDR2, and a CDR3 having the amino acid sequences of the CDR1, CDR2, and CDR3,

respectively, as set forth in SEQ ID NO: 44; (v) a CDR1, a CDR2, and a CDR3 having the amino acid sequences of the CDR1, CDR2, and CDR3, respectively, as set forth in SEQ ID NO: 45; (vi) a CDR1, a CDR2, and a CDR3 having the amino acid sequences of the CDR1, CDR2, and CDR3, respectively, as set forth in SEQ ID NO: 46; (vii) a CDR1, a CDR2, and a CDR3 having the amino acid sequences of the CDR1, CDR2, and CDR3, respectively, as set forth in SEQ ID NO: 47; (viii) a CDR1, a CDR2, and a CDR3 having the amino acid sequences of the CDR1, CDR2, and CDR3, respectively, as set forth in SEQ ID NO: 48; (ix) a CDR1, a CDR2, and a CDR3 having the amino acid sequences of the CDR1, CDR2, and CDR3, respectively, as set forth in SEQ ID NO: 49; (x) a CDR1, a CDR2, and a CDR3 having the amino acid sequences of the CDR1, CDR2, and CDR3, respectively, as set forth in SEQ ID NO: 50; (xi) a CDR1, a CDR2, and a CDR3 having the amino acid sequences of the CDR1, CDR2, and CDR3, respectively, as set forth in SEQ ID NO: 51; (xii) a CDR1, a CDR2, and a CDR3 having the amino acid sequences of the CDR1, CDR2, and CDR3, respectively, as set forth in SEQ ID NO: 52; (xiii) a CDR1, a CDR2, and a CDR3 having the amino acid sequences of the CDR1, CDR2, and CDR3, respectively, as set forth in SEQ ID NO: 53; (xiv) a CDR1, a CDR2, and a CDR3 having the amino acid sequences of the CDR1, CDR2, and CDR3, respectively, as set forth in SEQ ID NO: 54; (xv) a CDR1, a CDR2, and a CDR3 having the amino acid sequences of the CDR1, CDR2, and CDR3, respectively, as set forth in SEQ ID NO: 55; (xvi) a CDR1, a CDR2, and a CDR3 having the amino acid sequences of the CDR1, CDR2, and CDR3, respectively, as set forth in SEQ ID NO: 56; (xvii) a CDR1, a CDR2, and a CDR3 having the amino acid sequences of the CDR1, CDR2, and CDR3, respectively, as set forth in SEQ ID NO: 57; (xviii) a CDR1, a CDR2, and a CDR3 having the amino acid sequences of the CDR1, CDR2, and CDR3, respectively, as set forth in SEQ ID NO: 58; (xix) a CDR1, a CDR2, and a CDR3 having the amino acid sequences of the CDR1, CDR2, and CDR3, respectively, as set forth in SEQ ID NO: 59; (xx) a CDR1, a CDR2, and a CDR3 having the amino acid sequences of the CDR1, CDR2, and CDR3, respectively, as set forth in SEQ ID NO: 60; (xxi) a CDR1, a CDR2, and a CDR3 having the amino acid sequences of the CDR1, CDR2, and CDR3, respectively, as set forth in SEQ ID NO: 61; (xxii) a CDR1, a CDR2, and a CDR3 having the amino acid sequences of the CDR1, CDR2, and CDR3, respectively, as set forth in SEQ ID NO: 62; or (xxiii) a CDR1, a CDR2, and a CDR3 having the amino acid sequences of the CDR1, CDR2, and CDR3, respectively, as set forth in SEQ ID NO: 63; (xxiv) a CDR1, a CDR2, and a CDR3 having the amino acid sequences of the CDR1, CDR2, and CDR3, respectively, as set forth in SEQ ID NO: 183; (xxv) a CDR1, a CDR2, and a CDR3 having the amino acid sequences of the CDR1, CDR2, and CDR3, respectively, as set forth in SEQ ID NO: 184; or (xxvi) a CDR1, a CDR2, and a CDR3 having the amino acid sequences of the CDR1, CDR2, and CDR3, respectively, as set forth in SEQ ID NO: 185. In one aspect, provided herein is an anti-CD20 single domain antibody (sdAb) comprising: (i) a CDR1, a CDR2, and a CDR3 having the amino acid sequences of the CDR1, CDR2, and CDR3, respectively, as set forth in SEQ ID NO: 41; (ii) a CDR1, a CDR2, and a CDR3 having the amino acid sequences of the CDR1, CDR2, and CDR3, respectively, as set forth in SEQ ID NO: 42; (iii) a CDR1, a CDR2, and a CDR3 having the amino acid

sequences of the CDR1, CDR2, and CDR3, respectively, as set forth in SEQ ID NO: 43; (iv) a CDR1, a CDR2, and a CDR3 having the amino acid sequences of the CDR1, CDR2, and CDR3, respectively, as set forth in SEQ ID NO: 44; (v) a CDR1, a CDR2, and a CDR3 having the amino acid sequences of the CDR1, CDR2, and CDR3, respectively, as set forth in SEQ ID NO: 45; (vi) a CDR1, a CDR2, and a CDR3 having the amino acid sequences of the CDR1, CDR2, and CDR3, respectively, as set forth in SEQ ID NO: 46; (vii) a CDR1, a CDR2, and a CDR3 having the amino acid sequences of the CDR1, CDR2, and CDR3, respectively, as set forth in SEQ ID NO: 47; (viii) a CDR1, a CDR2, and a CDR3 having the amino acid sequences of the CDR1, CDR2, and CDR3, respectively, as set forth in SEQ ID NO: 48; (ix) a CDR1, a CDR2, and a CDR3 having the amino acid sequences of the CDR1, CDR2, and CDR3, respectively, as set forth in SEQ ID NO: 49; (x) a CDR1, a CDR2, and a CDR3 having the amino acid sequences of the CDR1, CDR2, and CDR3, respectively, as set forth in SEQ ID NO: 50; (xi) a CDR1, a CDR2, and a CDR3 having the amino acid sequences of the CDR1, CDR2, and CDR3, respectively, as set forth in SEQ ID NO: 51; (xii) a CDR1, a CDR2, and a CDR3 having the amino acid sequences of the CDR1, CDR2, and CDR3, respectively, as set forth in SEQ ID NO: 52; (xiii) a CDR1, a CDR2, and a CDR3 having the amino acid sequences of the CDR1, CDR2, and CDR3, respectively, as set forth in SEQ ID NO: 53; (xiv) a CDR1, a CDR2, and a CDR3 having the amino acid sequences of the CDR1, CDR2, and CDR3, respectively, as set forth in SEQ ID NO: 54; (xv) a CDR1, a CDR2, and a CDR3 having the amino acid sequences of the CDR1, CDR2, and CDR3, respectively, as set forth in SEQ ID NO: 55; (xvi) a CDR1, a CDR2, and a CDR3 having the amino acid sequences of the CDR1, CDR2, and CDR3, respectively, as set forth in SEQ ID NO: 56; (xvii) a CDR1, a CDR2, and a CDR3 having the amino acid sequences of the CDR1, CDR2, and CDR3, respectively, as set forth in SEQ ID NO: 57; (xviii) a CDR1, a CDR2, and a CDR3 having the amino acid sequences of the CDR1, CDR2, and CDR3, respectively, as set forth in SEQ ID NO: 58; (xix) a CDR1, a CDR2, and a CDR3 having the amino acid sequences of the CDR1, CDR2, and CDR3, respectively, as set forth in SEQ ID NO: 59; (xx) a CDR1, a CDR2, and a CDR3 having the amino acid sequences of the CDR1, CDR2, and CDR3, respectively, as set forth in SEQ ID NO: 60; (xxi) a CDR1, a CDR2, and a CDR3 having the amino acid sequences of the CDR1, CDR2, and CDR3, respectively, as set forth in SEQ ID NO: 61; (xxii) a CDR1, a CDR2, and a CDR3 having the amino acid sequences of the CDR1, CDR2, and CDR3, respectively, as set forth in SEQ ID NO: 62; (xxiii) a CDR1, a CDR2, and a CDR3 having the amino acid sequences of the CDR1, CDR2, and CDR3, respectively, as set forth in SEQ ID NO: 63; (xxiv) a CDR1, a CDR2, and a CDR3 having the amino acid sequences of the CDR1, CDR2, and CDR3, respectively, as set forth in SEQ ID NO: 183; (xxv) a CDR1, a CDR2, and a CDR3 having the amino acid sequences of the CDR1, CDR2, and CDR3, respectively, as set forth in SEQ ID NO: 184; or (xxvi) a CDR1, a CDR2, and a CDR3 having the amino acid sequences of the CDR1, CDR2, and CDR3, respectively, as set forth in SEQ ID NO: 185.

[0009] In some ~~embodiment~~examples, the CDR1, CDR2 or CDR3 are determined according to the Kabat numbering scheme, the IMGT numbering scheme, the AbM numbering scheme, the Chothia numbering scheme, the Contact numbering scheme, or a combination thereof.

[0010] In some ~~embodiment~~examples, the anti-CD20 sdAb provided herein further comprises one or more FR regions as set forth in SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 43, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 46, SEQ ID NO: 47, SEQ ID NO: 48, SEQ ID NO: 49, SEQ ID NO: 50, SEQ ID NO: 51, SEQ ID NO: 52, SEQ ID NO: 53, SEQ ID NO: 54, SEQ ID NO: 55, SEQ ID NO: 56, SEQ ID NO: 57, SEQ ID NO: 58, SEQ ID NO: 59, SEQ ID NO: 60, SEQ ID NO: 61, SEQ ID NO: 62, SEQ ID NO: 63, SEQ ID NO: 183, SEQ ID NO: 184 and/or SEQ ID NO: 185.

[0011] In some ~~embodiment~~examples, the anti-CD20 sdAb provided herein comprises the amino acid sequence of SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 43, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 46, SEQ ID NO: 47, SEQ ID NO: 48, SEQ ID NO: 49, SEQ ID NO: 50, SEQ ID NO: 51, SEQ ID NO: 52, SEQ ID NO: 53, SEQ ID NO: 54, SEQ ID NO: 55, SEQ ID NO: 56, SEQ ID NO: 57, SEQ ID NO: 58, SEQ ID NO: 59, SEQ ID NO: 60, SEQ ID NO: 61, SEQ ID NO: 62, SEQ ID NO: 63, SEQ ID NO: 183, SEQ ID NO: 184 or SEQ ID NO: 185.

[0012] In other ~~embodiment~~examples, the anti-CD20 sdAb provided herein comprises or consists of an amino acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or more sequence identity with the sequence of SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 43, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 46, SEQ ID NO: 47, SEQ ID NO: 48, SEQ ID NO: 49, SEQ ID NO: 50, SEQ ID NO: 51, SEQ ID NO: 52, SEQ ID NO: 53, SEQ ID NO: 54, SEQ ID NO: 55, SEQ ID NO: 56, SEQ ID NO: 57, SEQ ID NO: 58, SEQ ID NO: 59, SEQ ID NO: 60, SEQ ID NO: 61, SEQ ID NO: 62, SEQ ID NO: 63, SEQ ID NO: 183, SEQ ID NO: 184 or SEQ ID NO: 185.

[0013] In some ~~embodiment~~examples, the anti-CD20 sdAb is a camelid sdAb. In some ~~embodiment~~examples, the anti-CD20 sdAb is a humanized sdAb. In some ~~embodiment~~examples, the anti-CD20 sdAb is a VHH domain. In some ~~embodiment~~examples, the anti-CD20 sdAb is a humanized VHH domain.

[0014] In some ~~embodiment~~examples, the anti-CD20 sdAb is genetically fused or chemically conjugated to an agent.

[0015] In another aspect, provided herein is a chimeric antigen receptor (CAR), comprising: (a) an extracellular antigen binding domain comprising the anti-CD20 sdAb provided herein; (b) a transmembrane domain; and (c) an intracellular signaling domain. In some ~~embodiment~~examples, the extracellular antigen binding domain further comprises one or more additional antigen binding domain(s). In some ~~embodiment~~examples, the extracellular antigen binding domain further comprises one additional antigen binding domain. In other ~~embodiment~~examples, the extracellular antigen binding domain further comprises two additional antigen binding domains. In some ~~embodiment~~examples, the

one or more additional antigen binding domain(s) bind to one or more antigen(s) selected from a group consisting of CD20, CD19, CD22, CD33, CD38, BCMA, CS1, ROR1, GPC3, CD123, IL-13R, CD138, c-Met, EGFRvIII, GD-2, NY-ESO-1, MAGE A3, and glycolipid F77. In some ~~embodiment~~examples, the CD20 CAR is monospecific. In some ~~embodiment~~examples, the CD20 CAR is monovalent. In some ~~embodiment~~examples, the CD20 CAR is multivalent. In some ~~embodiment~~examples, the extracellular antigen binding domain comprises at least two anti-CD20 sdAbs provided herein.

[0016] In some ~~embodiment~~examples, provided herein is a multivalent (such as bivalent and trivalent) CAR comprising a polypeptide comprising: (a) an extracellular antigen binding domain comprising two or more single domain antibodies (sdAbs) specifically binding to CD20; (b) a transmembrane domain; and (c) an intracellular signaling domain. In some ~~embodiment~~examples, the extracellular antigen binding domain comprises two single domain antibodies (sdAbs) specifically binding to CD20 provided herein. In other ~~embodiment~~examples, the extracellular antigen binding domain comprises three single domain antibodies (sdAbs) specifically binding to CD20 provided herein.

[0017] In some ~~embodiment~~examples according to any one of the CARs described above, the transmembrane domain is derived from a molecule selected from a group consisting of CD8α, CD4, CD28, CD137, CD80, CD86, CD152, and PD1. In some ~~embodiment~~examples, the transmembrane domain is derived from CD8α.

[0018] In some ~~embodiment~~examples according to any one of the CARs described above, the intracellular signaling domain comprises a primary intracellular signaling domain of an immune effector cell. In some ~~embodiment~~examples, the primary intracellular signaling domain is derived from CD3ζ.

[0019] In other ~~embodiment~~examples according to any one of the CARs described above, the intracellular signaling domain further comprises a co-stimulatory signaling domain. In some ~~embodiment~~examples, the co-stimulatory signaling domain is derived from a co-stimulatory molecule selected from the group consisting of CD27, CD28, CD137, OX40, CD30, CD40, CD3, LFA-1, CD2, CD7, LIGHT, NKG2C, B7-H3, ligands of CD83 and combinations thereof. In some ~~embodiment~~examples, the co-stimulatory signaling domain is derived from CD137.

[0020] In some ~~embodiment~~examples, the CAR provided herein further comprises a hinge domain located between the C-terminus of the extracellular antigen binding domain and the N-terminus of the transmembrane domain. In some ~~embodiment~~examples, the hinge domain is derived from CD8α.

[0021] In some ~~embodiment~~examples, the CAR provided herein further comprises a signal peptide located at the N-terminus of the polypeptide. In some ~~embodiment~~examples, the signal peptide is derived from CD8α.

[0022] In certain aspect~~embodiments~~, provided herein is a chimeric antigen receptor (CAR), comprising (i) an amino acid sequence selected from the group consisting of SEQ ID NO: 81,

SEQ ID NO: 82, SEQ ID NO: 83, SEQ ID NO: 84, SEQ ID NO: 85, SEQ ID NO: 86, SEQ ID NO: 87, SEQ ID NO: 88, SEQ ID NO: 89, SEQ ID NO: 90, SEQ ID NO: 91, SEQ ID NO: 92, SEQ ID NO: 93, SEQ ID NO: 94, SEQ ID NO: 95, SEQ ID NO: 96, SEQ ID NO: 97, SEQ ID NO: 98, SEQ ID NO: 99, SEQ ID NO: 100, SEQ ID NO: 101, SEQ ID NO: 102, SEQ ID NO: 103, SEQ ID NO: 104, SEQ ID NO: 105, SEQ ID NO: 106, SEQ ID NO: 189, SEQ ID NO: 190, and SEQ ID NO: 191; or (ii) an amino acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or more sequence identity with the sequence of SEQ ID NO: 81, SEQ ID NO: 82, SEQ ID NO: 83, SEQ ID NO: 84, SEQ ID NO: 85, SEQ ID NO: 86, SEQ ID NO: 87, SEQ ID NO: 88, SEQ ID NO: 89, SEQ ID NO: 90, SEQ ID NO: 91, SEQ ID NO: 92, SEQ ID NO: 93, SEQ ID NO: 94, SEQ ID NO: 95, SEQ ID NO: 96, SEQ ID NO: 97, SEQ ID NO: 98, SEQ ID NO: 99, SEQ ID NO: 100, SEQ ID NO: 101, SEQ ID NO: 102, SEQ ID NO: 103, SEQ ID NO: 104, SEQ ID NO: 105, SEQ ID NO: 106, SEQ ID NO: 189, SEQ ID NO: 190, or SEQ ID NO: 191.

[0023] In another aspect, provided herein is an isolated nucleic acid comprising a nucleic acid sequence encoding the anti-CD20 sdAb provided herein. In another exampleaspect, provided herein is a vector comprising a nucleic acid encoding the anti-CD20 sdAb provided herein.

[0024] In yet another aspect, provided herein is an isolated nucleic acid comprising a nucleic acid sequence encoding the CAR provided herein. In another exampleaspect, provided herein is a vector comprising a nucleic acid encoding the CAR provided herein.

[0024a] In one aspect, provided herein is a vector comprising the isolated nucleic acid as disclosed herein.

[0025] In yet another exampleaspect, provided herein is an engineered immune effector cell, comprising the CAR, the isolated nucleic acid, or the vector provided herein. In one aspect, provided herein is an engineered immune effector cell, comprising the CAR as disclosed herein, the isolated nucleic acid as disclosed herein, or the vector as disclosed herein. In some embodimentexamples, the engineered immune effector cell is a T cell or B cell.

[0026] In another exampleaspect, provided herein is a pharmaceutical composition, comprising the anti-CD20 sdAb, the engineered immune effector cell, or the vector provided herein, and a pharmaceutically acceptable excipient. In one aspect, provided herein is a pharmaceutical composition, comprising the anti-CD20 sdAb as disclosed herein, the engineered immune effector cell as disclosed herein, or the vector as disclosed herein, and a pharmaceutically acceptable excipient.

[0027] In yet another exampleaspect, provided herein is a method of treating a disease or disorder in a subject, comprising administering to the subject an effective amount of the anti-CD20 sdAb, the engineered immune effector cell, or the pharmaceutical composition provided herein. In one aspect, provided herein is use of the anti-CD20 sdAb as disclosed herein, the engineered immune effector cell as disclosed herein, or the pharmaceutical composition as disclosed herein in the manufacture of a medicament for treating a disease or disorder in a subject. In some embodimentexamples, the disease or disorder is a B cell associated disease or disorder and/or CD20 associated disease or disorder. In some embodimentexamples, the disease or disorder is cancer. In other embodimentexamples, the disease or disorder is a B cell malignancy. In some embodimentexamples, the B cell malignancy is a B cell leukemia or B cell lymphoma. In some embodimentexamples, the disease or disorder is selected from a group consisting of marginal zone lymphoma (*e.g.*, splenic marginal zone lymphoma), diffuse large B cell lymphoma (DLBCL), mantle cell lymphoma (MCL), primary central nervous system (CNS) lymphoma, primary mediastinal B cell lymphoma (PMBL), small lymphocytic lymphoma (SLL), B cell prolymphocytic leukemia (B-PLL),

follicular lymphoma (FL), burkitt lymphoma, primary intraocular lymphoma, chronic lymphocytic leukemia (CLL), acute lymphoblastic leukemia (ALL), hairy cell leukemia (HCL), precursor B lymphoblastic leukemia, non-hodgkin lymphoma (NHL), high-grade B-cell lymphoma (HGBL), and multiple myelomia (MM). In other ~~embodiment~~example**s**, the disease or disorder is an autoimmune and/or inflammatory disease. In some ~~embodiment~~example**s**, the autoimmune and/or inflammatory disease is associated with inappropriate or enhanced B cell numbers and/or activation.

4. BRIEF DESCRIPTION OF THE FIGURES

[0028] FIGs.1A-1B show the exemplary transduction efficiency of VHH-based CAR-T cells (**FIG.1A**) and scFv-based CAR-T cells (**FIG.1B**). UnT refers to T cells un-transduced with CAR.

[0029] FIGs.2A-2F show *in vitro* cytotoxicity of VHH-based CAR-T cells compared to that of scFv-based CAR-T cells against CD20 positive cell lines (**FIGs.2A-2B and FIGs.2E-2F**) or CD20 negative cell lines (**FIGs.2C-2D**).

[0030] FIGs.3A-3D show *in vitro* cytotoxicity of mono-, bi- and tri-VHH CAR-T cells compared to that of CD20 scFv CAR-T cells against CD20 positive cell lines (**FIGs.3A-3B**) or CD20 negative cell lines (**FIGs.3C-3D**).

[0031] FIGs.4A-4B show *in vitro* cytotoxicity of bi-VHH CAR-T cells with different length of peptide linkers against Raji.Luc cells.

[0032] FIGs.5A-5B show IFN-γ release level of VHH-based CAR-T cells compared to that of scFv-based CAR-T cells after co-culture with Raji.Luc or K562-CD20.Luc cells at different E:T ratios for 24 hours.

[0033] FIG.6 shows IFN-γ release level of mono-, bi- and tri-VHH CAR-T cells compared to that of CD20 scFv CAR-T cells after co-culture with Raji.Luc or K562.Luc cells for 24 hours.

[0034] FIGs.7A-7D show the proliferation capacity and CAR positve ratio of the exemplary CD20 VHH CAR-T cells compared to that of CD20 scFv CAR-T cells with or without antigen specific stimulation.

[0035] FIG.8 shows the results of exhaustion markers assessment of exemplary CD20 VHH CAR-T cells and CD20 scFv CAR-T cells.

[0036] FIGs.9A-9D show the results of memory markers assessment of exemplary CD20 VHH CAR-T cells and CD20 scFv CAR-T cells.

[0037] FIGs.10A-10F show *in vivo* anti-tumor and re-challenging efficacy of VHH-273 CAR-T cells and VHH-496 CAR-T cells in a Raji xenograft NCG mouse model. Mice were assessed on a regular basis to monitor tumor growth by bioluminescence imaging (**FIGs.10A-10B**), and body weight (**FIG.10C**), as well as CAR copies in mouse peripheral blood (**FIG.10D**). Then, mice at least 5 weeks tumor-free with undetectable CAR copy number (copies/ng of genomic DNA) in