

CHIMERIC ANTIGEN AND T CELL RECEPTORS AND METHODS OF USE

CROSS-REFERENCE TO RELATED APPLICATIONS

[1] The present application claims the priority benefit of U.S. Provisional Application No. 62/778,893, filed December 12, 2018, which is hereby incorporated by reference in its entirety.

BACKGROUND OF THE DISCLOSURE

[2] Human cancers are by their nature comprised of normal cells that have undergone a genetic or epigenetic conversion to become abnormal cancer cells. In doing so, cancer cells begin to express proteins and other antigens that are distinct from those expressed by normal cells. These aberrant tumor antigens can be used by the body's innate immune system to specifically target and kill cancer cells. However, cancer cells employ various mechanisms to prevent immune cells, such as T and B lymphocytes, from successfully targeting cancer cells.

[3] Current therapies T cell therapies rely on enriched or modified human T cells to target and kill cancer cells in a patient. To increase the ability of T cells to target and kill a particular cancer cell, methods have been developed to engineer T cells to express constructs which direct T cells to a particular target cancer cell. Chimeric antigen receptors (CARs) and engineered T cell receptors (TCRs), which comprise binding domains capable of interacting with a particular tumor antigen, allow T cells to target and kill cancer cells that express the particular tumor antigen. A need exists for CARs and TCRs for targeting and killing cancer cells.

SUMMARY

[4] In at least a first aspect, the present disclosure comprises an antigen binding system, antibody, or antigen binding fragment thereof comprising an anti-CD20 binding motif, wherein the anti-CD20 binding motif comprises sequences of three heavy chain complementarity determining regions (HCDRs) of any one heavy chain variable region (HCVR) selected from the group consisting of SEQ ID NOs: 1, 23, 45, 67, 89, 111, 133, 155, 177, and 199, and sequences of three light chain CDRs (LCDRs) of any one light chain variable region (LCVR) selected from the group consisting of SEQ ID NOs: 12, 34, 56, 78, 100, 122, 144, 166, 188, and 210. In some embodiments, the anti-CD20 binding motif comprises a first domain comprising three heavy chain complementarity determining regions (HCDR1, HCDR2 and HCDR3) and a second domain comprising three light chain complementarity determining regions (LCDR1, LCDR2 and LCDR3), wherein (i) the HCDR1 has a sequence according to any one of SEQ ID NOs: 3-5, 25-27, 47-49, 69-71, 91-93, 113-115, 135-137, 157-159, 179-181; and 201-203; (ii) the HCDR2 has a sequence according to any one of SEQ ID NOs: 6-8,

28-30, 50-52, 72-74, 94-96, 116-118, 138-140, 160-162, 182-184, and 204-206; (iii) the HCDR3 has a sequence according to any one of SEQ ID NOs: 9-11, 31-33, 53-55, 75-77, 97-99, 119-121, 141-143, 163-165, 185-187, and 207-209; (iv) the LCDR1 has a sequence according to any one of SEQ ID NOs: 14-16, 36-38, 58-60, 80-82, 102-104, 124-126, 146-148, 168-170, 190-192, and 212-214; (v) the LCDR2 has a sequence according to any one of SEQ ID NOs: 17-19, 39-41, 61-63, 83-85, 105-107, 127-129, 149-151, 171-173, 193-195, and 215-217; and (vi) the LCDR3 has a sequence according to any one of SEQ ID NOs: 20-22, 42-44, 64-66, 86-88, 108-110, 130-132, 152-154, 174-176, 196-198, and 218-220. In some embodiments, the HCDRs comprise: (i) an HCDR1 according to any of SEQ ID NOs: 3-5; an HCDR2 according to any of SEQ ID NOs: 6-8; an HCDR3 according to any one of SEQ ID NOs: 9-11; (ii) an HCDR1 according to any of SEQ ID NOs: 25-27; an HCDR2 according to any of SEQ ID NOs: 28-30; an HCDR3 according to any one of SEQ ID NOs: 31-33; (iii) an HCDR1 according to any of SEQ ID NOs: 47-49; an HCDR2 according to any of SEQ ID NOs: 50-52; an HCDR3 according to any one of SEQ ID NOs: 53-55; (iv) an HCDR1 according to any of SEQ ID NOs: 69-71; an HCDR2 according to any of SEQ ID NOs: 72-74; an HCDR3 according to any one of SEQ ID NOs: 75-77; (v) an HCDR1 according to any of SEQ ID NOs: 91-93; an HCDR2 according to any of SEQ ID NOs: 94-96; an HCDR3 according to any one of SEQ ID NOs: 97-99; (vi) an HCDR1 according to any of SEQ ID NOs: 113-115; an HCDR2 according to any of SEQ ID NOs: 116-118; an HCDR3 according to any one of SEQ ID NOs: 119-121; (vii) an HCDR1 according to any of SEQ ID NOs: 135-137; an HCDR2 according to any of SEQ ID NOs: 138-140; an HCDR3 according to any one of SEQ ID NOs: 141-143; (viii) an HCDR1 according to any of SEQ ID NOs: 157-159; an HCDR2 according to any of SEQ ID NOs: 160-162; an HCDR3 according to any one of SEQ ID NOs: 163-165; (ix) an HCDR1 according to any of SEQ ID NOs: 179-181; an HCDR2 according to any of SEQ ID NOs: 182-184; an HCDR3 according to any one of SEQ ID NOs: 185-187; or (x) an HCDR1 according to any of SEQ ID NOs: 201-203; an HCDR2 according to any of SEQ ID NOs: 204-206; an HCDR3 according to any one of SEQ ID NOs: 207-209; and the LCDRs comprise: (i) an LCDR1 according to any of SEQ ID NOs: 14-16; an LCDR2 according to any of SEQ ID NOs: 17-19; an LCDR3 according to any one of SEQ ID NOs: 20-22; (ii) an LCDR1 according to any of SEQ ID NOs: 36-38; an LCDR2 according to any of SEQ ID NOs: 39-41; an LCDR3 according to any one of SEQ ID NOs: 42-44; (iii) an LCDR1 according to any of SEQ ID NOs: 58-60; an LCDR2 according to any of SEQ ID NOs: 61-63; an LCDR3 according to any one of SEQ ID NOs: 64-66; (iv) an LCDR1 according to any of SEQ ID NOs:

80-82; an LCDR2 according to any of SEQ ID NOs: 83-85; an LCDR3 according to any one of SEQ ID NOs: 86-88; (v) an LCDR1 according to any of SEQ ID NOs: 102-104; an LCDR2 according to any of SEQ ID NOs: 105-107; an LCDR3 according to any one of SEQ ID NOs: 108-110; (vi) an LCDR1 according to any of SEQ ID NOs: 124-126; an LCDR2 according to any of SEQ ID NOs: 127-129; an LCDR3 according to any one of SEQ ID NOs: 130-132; (vii) an LCDR1 according to any of SEQ ID NOs: 146-148; an LCDR2 according to any of SEQ ID NOs: 149-151; an LCDR3 according to any one of SEQ ID NOs: 152-154; (viii) an LCDR1 according to any of SEQ ID NOs: 168-170; an LCDR2 according to any of SEQ ID NOs: 171-173; an LCDR3 according to any one of SEQ ID NOs: 174-176; (ix) an LCDR1 according to any of SEQ ID NOs: 190-192; an LCDR2 according to any of SEQ ID NOs: 193-195; an LCDR3 according to any one of SEQ ID NOs: 196-198; or (x) an LCDR1 according to any of SEQ ID NOs: 212-214; an LCDR2 according to any of SEQ ID NOs: 215-217; an LCDR3 according to any one of SEQ ID NOs: 218-220.

[5] In various embodiments, an antigen binding system, antibody, or antigen binding fragment thereof of the present disclosure comprises a first domain comprising three heavy chain complementarity determining regions (HCDRs) and a second domain comprising three light chain complementarity determining regions (LCDRs), wherein: the HCDRs and LCDRs comprise: (i) an HCDR1 according to any of SEQ ID NOs: 3-5; an HCDR2 according to any of SEQ ID NOs: 6-8; an HCDR3 according to any one of SEQ ID NOs: 9-11; an LCDR1 according to any of SEQ ID NOs: 14-16; an LCDR2 according to any of SEQ ID NOs: 17-19; an LCDR3 according to any one of SEQ ID NOs: 20-22; (ii) an HCDR1 according to any of SEQ ID NOs: 25-27; an HCDR2 according to any of SEQ ID NOs: 28-30; an HCDR3 according to any one of SEQ ID NOs: 31-33; an LCDR1 according to any of SEQ ID NOs: 36-38; an LCDR2 according to any of SEQ ID NOs: 39-41; an LCDR3 according to any one of SEQ ID NOs: 42-44; (iii) an HCDR1 according to any of SEQ ID NOs: 47-49; an HCDR2 according to any of SEQ ID NOs: 50-52; an HCDR3 according to any one of SEQ ID NOs: 53-55; an LCDR1 according to any of SEQ ID NOs: 58-60; an LCDR2 according to any of SEQ ID NOs: 61-63; an LCDR3 according to any one of SEQ ID NOs: 64-66; (iv) an HCDR1 according to any of SEQ ID NOs: 69-71; an HCDR2 according to any of SEQ ID NOs: 72-74; an HCDR3 according to any one of SEQ ID NOs: 75-77; an LCDR1 according to any of SEQ ID NOs: 80-82; an LCDR2 according to any of SEQ ID NOs: 83-85; an LCDR3 according to any one of SEQ ID NOs: 86-88; (v) an HCDR1 according to any of SEQ ID NOs: 91-93; an HCDR2 according to any of SEQ ID NOs: 94-96; an HCDR3 according to any one of SEQ ID

NOs: 97-99; an LCDR1 according to any of SEQ ID NOs: 102-104; an LCDR2 according to any of SEQ ID NOs: 105-107; an LCDR3 according to any one of SEQ ID NOs: 108-110; (vi) an HCDR1 according to any of SEQ ID NOs: 113-115; an HCDR2 according to any of SEQ ID NOs: 116-118; an HCDR3 according to any one of SEQ ID NOs: 119-121; an LCDR1 according to any of SEQ ID NOs: 124-126; an LCDR2 according to any of SEQ ID NOs: 127-129; an LCDR3 according to any one of SEQ ID NOs: 130-132; (vii) an HCDR1 according to any of SEQ ID NOs: 135-137; an HCDR2 according to any of SEQ ID NOs: 138-140; an HCDR3 according to any one of SEQ ID NOs: 141-143; an LCDR1 according to any of SEQ ID NOs: 146-148; an LCDR2 according to any of SEQ ID NOs: 149-151; an LCDR3 according to any one of SEQ ID NOs: 152-154; (viii) an HCDR1 according to any of SEQ ID NOs: 157-159; an HCDR2 according to any of SEQ ID NOs: 160-162; an HCDR3 according to any one of SEQ ID NOs: 163-165; an LCDR1 according to any of SEQ ID NOs: 168-170; an LCDR2 according to any of SEQ ID NOs: 171-173; an LCDR3 according to any one of SEQ ID NOs: 174-176; (ix) an HCDR1 according to any of SEQ ID NOs: 179-181; an HCDR2 according to any of SEQ ID NOs: 182-184; an HCDR3 according to any one of SEQ ID NOs: 185-187; an LCDR1 according to any of SEQ ID NOs: 190-192; an LCDR2 according to any of SEQ ID NOs: 193-195; an LCDR3 according to any one of SEQ ID NOs: 196-198; or (x) an HCDR1 according to any of SEQ ID NOs: 201-203; an HCDR2 according to any of SEQ ID NOs: 204-206; an HCDR3 according to any one of SEQ ID NOs: 207-209; an LCDR1 according to any of SEQ ID NOs: 212-214; an LCDR2 according to any of SEQ ID NOs: 215-217; an LCDR3 according to any one of SEQ ID NOs: 218-220. In some embodiments, the antigen binding system, antibody, or antigen binding fragment thereof comprises a first heavy chain variable domain comprising the three HCDRs and a light chain variable domain comprising the three LCDRs, wherein: (i) the heavy chain variable domain is at least 80% identical to SEQ ID NO: 1, SEQ ID NO: 23, SEQ ID NO: 45, SEQ ID NO: 67, SEQ ID NO: 89, SEQ ID NO: 111, SEQ ID NO: 133, SEQ ID NO: 155, SEQ ID NO: 177, or SEQ ID NO: 199; and (ii) the light chain variable domain is at least 80% identical to SEQ ID NO: 12, SEQ ID NO: 34, SEQ ID NO: 56, SEQ ID NO: 78, SEQ ID NO: 100, SEQ ID NO: 122, SEQ ID NO: 144, SEQ ID NO: 166, SEQ ID NO: 188, or SEQ ID NO: 210. In some embodiments, the antigen binding system, antibody, or antigen binding fragment thereof comprises a first heavy chain variable domain comprising the three HCDRs and a light chain variable domain comprising the three LCDRs, wherein: (i) the heavy chain variable domain is at least 80% identical to SEQ ID NO: 1 and the light chain variable domain is at least 80% identical to SEQ

ID NO: 12; (ii) the heavy chain variable domain is at least 80% identical to SEQ ID NO: 23 and the light chain variable domain is at least 80% identical to SEQ ID NO: 34; (iii) the heavy chain variable domain is at least 80% identical to SEQ ID NO: 45 and the light chain variable domain is at least 80% identical to SEQ ID NO: 56; (iv) the heavy chain variable domain is at least 80% identical to SEQ ID NO: 67 and the light chain variable domain is at least 80% identical to SEQ ID NO: 78; (v) the heavy chain variable domain is at least 80% identical to SEQ ID NO: 89 and the light chain variable domain is at least 80% identical to SEQ ID NO: 100; (vi) the heavy chain variable domain is at least 80% identical to SEQ ID NO: 111 and the light chain variable domain is at least 80% identical to SEQ ID NO: 122; (vii) the heavy chain variable domain is at least 80% identical to SEQ ID NO: 133 and the light chain variable domain is at least 80% identical to SEQ ID NO: 144; (viii) the heavy chain variable domain is at least 80% identical to SEQ ID NO: 155 and the light chain variable domain is at least 80% identical to SEQ ID NO: 166; (ix) the heavy chain variable domain is at least 80% identical to SEQ ID NO: 177 and the light chain variable domain is at least 80% identical to SEQ ID NO: 188; or (x) the heavy chain variable domain is at least 80% identical to SEQ ID NO: 199 and the light chain variable domain is at least 80% identical to SEQ ID NO: 210.

[6] In some embodiments of the present disclosure comprising the three HCDRs and the three LCDRs, the three HCDRs and the three LCDRs are comprised by a single polypeptide. In some embodiments of the present disclosure comprising the three HCDRs and the three LCDRs, the three HCDRs are comprised by a first polypeptide and the three LCDRs are comprised by a second polypeptide. In some embodiments, the first polypeptide is an antibody heavy chain and the second polypeptide is an antibody light chain.

[7] In some embodiments, the antigen binding system, antibody, or antigen binding fragment thereof further comprises: (i) a binding motif that binds an antigen selected from the group consisting of 5T4, alphafetoprotein, B cell maturation antigen (BCMA), B cell receptor, CA-125, carcinoembryonic antigen, CD19, CD20, CD22, CD23, CD30, CD33, CD40, CD56, CD79, CD78, CD123, CD138, c-Met, CSPG4, IgM, C-type lectin-like molecule 1 (CLL-1), EGFRvIII, epithelial tumor antigen, ERBB2, FLT3, folate binding protein, GD2, GD3, HER1-HER2 in combination, HER2-HER3 in combination, HER2/Neu, HERV-K, HIV-1 envelope glycoprotein gp41, HIV-1 envelope glycoprotein gp120, IL-11Ralpha, kappa chain, lambda chain, melanoma-associated antigen, mesothelin, MUC-1, mutated p53, mutated ras, prostate-specific antigen, ROR1, VEGFR2, EphA3 (EPH receptor A3), BAFFR (B-cell activating factor receptor), and combinations thereof; and/or (ii) a binding motif that binds an antigen that is

characteristic of B-cells, optionally wherein the antigen that is characteristic of B-cells is not CD19 or CD20. In some embodiments, the antigen binding system, antibody, or antigen binding fragment thereof further comprises an anti-CD19 binding motif. In some embodiments, the anti-CD19 binding motif comprises a first domain comprising three HCDRs and a second domain comprising three LCDRs, wherein: the three HCDRs of the anti-CD19 binding motif comprise an HCDR1, an HCDR2, and an HCDR3; the three LCDRs of the anti-CD19 binding motif comprise an LCDR1, an LCDR2, and an LCDR3; and the HCDRs and LCDRs of the anti-CD19 binding motif comprise an HCDR1 according to any of SEQ ID NOs: 223-225; an HCDR2 according to any of SEQ ID NOs: 226-228; an HCDR3 according to any one of SEQ ID NOs: 229-231; an LCDR1 according to any of SEQ ID NOs: 234-236; an LCDR2 according to any of SEQ ID NOs: 237-239; an LCDR3 according to any one of SEQ ID NOs: 240-242. In some embodiments, the anti-CD19 binding motif comprises a first heavy chain variable domain comprising the three HCDRs of the anti-CD19 binding motif and a light chain variable domain comprising the three LCDRs of the anti-CD19 binding motif, wherein the heavy chain variable domain of the anti-CD19 binding motif is at least 80% identical to SEQ ID NO: 221 and the light chain variable domain of the anti-CD19 binding motif is at least 80% identical to SEQ ID NO: 232. In some embodiments, the three HCDRs of the anti-CD19 binding motif and the three LCDRs of the anti-CD19 binding motif are comprised by a single polypeptide. In some embodiments, the three HCDRs of the anti-CD20 binding motif, the three LCDRs of the anti-CD20 binding motif, the three HCDRs of the anti-CD19 binding motif, and the three LCDRs of the anti-CD19 binding motif are together comprised by a single polypeptide.

[8] In various embodiments, the antigen binding system, antibody, or antigen binding fragment thereof is, or is comprised by, a chimeric antigen receptor. In some embodiments, the antigen binding system, antibody, or antigen binding fragment thereof is a single polypeptide that is, or is comprised by, a chimeric antigen receptor, which chimeric antigen receptor is a bispecific chimeric antigen receptor. In some embodiments, the chimeric antigen receptor comprises a transmembrane domain that is a transmembrane domain of 4-1BB/CD137, an alpha chain of a T cell receptor, a beta chain of a T cell receptor, CD3 epsilon, CD4, CD5, CD8 alpha, CD9, CD16, CD19, CD22, CD28, CD33, CD37, CD45, CD64, CD80, CD86, CD134, CD137, CD154, or a zeta chain of a T cell receptor, or any combination thereof. In some embodiments, (i) the three HCDRs of the anti-CD20 binding motif and the three LCDRs of the anti-CD20 binding motif are present in a first polypeptide and (ii) the three HCDRs of the anti-

CD19 binding motif and the three LCDRs of the anti-CD19 binding motif are together comprised by a second different polypeptide. In some embodiments, the first polypeptide is, or is comprised by, a first chimeric antigen receptor. In some embodiments, the second polypeptide is, or is comprised by, a second chimeric antigen receptor.

[9] In various embodiments, the present disclosure comprises a nucleic acid encoding at least one polypeptide of the present disclosure and/or a vector comprising such a nucleic acid. The present disclosure further comprises a method of generating an engineered cell, the method comprising transfecting or transducing a cell with a nucleic acid encoding at least one polypeptide of the present disclosure. Further provided herein is a cell encoding or expressing an antigen binding system, antibody, or antigen binding fragment provided herein, optionally wherein the cell is an immune cell, optionally wherein the cell is a T cell.

[10] The present disclosure further comprises a method of treating cancer in a subject in need thereof, the method comprising administering to the subject a cell therapy composition comprising one or more cells that encode or comprise an antigen binding system, antibody, or antigen binding fragment thereof of the present disclosure. Also provided herein is a method of inducing an immune response in a subject or immunizing a subject against a cancer, the method comprising administering to the subject a cell therapy composition comprising one or more cells that encode or comprise an antigen binding system, antibody, or antigen binding fragment thereof of the present disclosure. In some embodiments, the cells are CAR-T cells. In various embodiments, the cancer is acute lymphoblastic leukemia (ALL) (including non T cell ALL), acute myeloid leukemia, B cell prolymphocytic leukemia, B cell acute lymphoid leukemia ("BALL"), blastic plasmacytoid dendritic cell neoplasm, Burkitt's lymphoma, chronic lymphocytic leukemia (CLL), chronic myelogenous leukemia (CML), chronic myeloid leukemia, chronic or acute leukemia, diffuse large B cell lymphoma (DLBCL), follicular lymphoma (FL), hairy cell leukemia, Hodgkin's Disease, malignant lymphoproliferative conditions, MALT lymphoma, mantle cell lymphoma, Marginal zone lymphoma, monoclonal gammopathy of undetermined significance (MGUS), multiple myeloma, myelodysplasia and myelodysplastic syndrome, non-Hodgkin's lymphoma (NHL), plasma cell proliferative disorder (including asymptomatic myeloma (smoldering multiple myeloma or indolent myeloma), plasmablastic lymphoma, plasmacytoid dendritic cell neoplasm, plasmacytomas (including plasma cell dyscrasia; solitary myeloma; solitary plasmacytoma; extramedullary plasmacytoma; and multiple plasmacytoma), POEMS syndrome (also known as Crow-Fukase syndrome; Takatsuki disease; and PEP syndrome), primary mediastinal large B cell lymphoma

(PMBC), small cell- or a large cell-follicular lymphoma, splenic marginal zone lymphoma (SMZL), systemic amyloid light chain amyloidosis, T cell acute lymphoid leukemia ("TALL"), T cell lymphoma, transformed follicular lymphoma, or Waldenstrom macroglobulinemia, Mantle cell lymphoma (MCL), Transformed follicular lymphoma (TFL), Primary mediastinal B cell lymphoma (PMBCL), Multiple myeloma, Hairy cell lymphoma/leukemia, or a combination thereof. In some embodiments, cell therapy is an allogeneic cell therapy or an autologous cell therapy.

[11] At least one aspect of the present disclosure includes a chimeric antigen receptor comprising an anti-CD20 binding motif, the anti-CD20 binding motif comprising three heavy chain complementarity determining regions (HCDRs) contained within any one of the heavy chain variable region (HCVR) sequences selected from the group consisting of SEQ ID NOs: 1, 23, 45, 67, 89, 111, 133, 155, 177, and 199, and three light chain CDRs (LCDRs) contained within any one of the light chain variable region (LCVR) sequences selected from the group consisting of SEQ ID NOs: 12, 34, 56, 78, 100, 122, 144, 166, 188, and 210. In some embodiments, a first domain comprising the three heavy chain complementarity determining regions (HCDRs) and a second domain comprising the three light chain complementarity determining regions (LCDRs), wherein (i) the HCDR1 has a sequence according to any one of SEQ ID NOs: 3-5, 25-27, 47-49, 69-71, 91-93, 113-115, 135-137, 157-159, 179-181; and 201-203; (ii) the HCDR2 has a sequence according to any one of SEQ ID NOs: 6-8, 28-30, 50-52, 72-74, 94-96, 116-118, 138-140, 160-162, 182-184; and 204-206; (iii) the HCDR3 has a sequence according to any one of SEQ ID NOs: 9-11, 31-33, 53-55, 75-77, 97-99, 119-121, 141-143, 163-165, 185-187; and 207-209; (iv) the LCDR1 has a sequence according to any one of SEQ ID NOs: 14-16, 36-38, 58-60, 80-82, 102-104, 124-126, 146-148, 168-170, 190-192; and 212-214; (v) the LCDR2 has a sequence according to any one of SEQ ID NOs: 17-19, 39-41, 61-63, 83-85, 105-107, 127-129, 149-151, 171-173, 193-195; and 215-217; and (vi) the LCDR3 has a sequence according to any one of SEQ ID NOs: 20-22, 42-44, 64-66, 86-88, 108-110, 130-132, 152-154, 174-176, 196-198; and 218-220. In some embodiments, the HCDRs comprise: (i) an HCDR1 according to any of SEQ ID NOs: 3-5; an HCDR2 according to any of SEQ ID NOs: 6-8; an HCDR3 according to any one of SEQ ID NOs: 9-11; (ii) an HCDR1 according to any of SEQ ID NOs: 25-27; an HCDR2 according to any of SEQ ID NOs: 28-30; an HCDR3 according to any one of SEQ ID NOs: 31-33; (iii) an HCDR1 according to any of SEQ ID NOs: 47-49; an HCDR2 according to any of SEQ ID NOs: 50-52; an HCDR3 according to any one of SEQ ID NOs: 53-55; (iv) an HCDR1

according to any of SEQ ID NOs: 69-71; an HCDR2 according to any of SEQ ID NOs: 72-74; an HCDR3 according to any one of SEQ ID NOs: 75-77; (v) an HCDR1 according to any of SEQ ID NOs: 91-93; an HCDR2 according to any of SEQ ID NOs: 94-96; an HCDR3 according to any one of SEQ ID NOs: 97-99; (vi) an HCDR1 according to any of SEQ ID NOs: 113-115; an HCDR2 according to any of SEQ ID NOs: 116-118; an HCDR3 according to any one of SEQ ID NOs: 119-121; (vii) an HCDR1 according to any of SEQ ID NOs: 135-137; an HCDR2 according to any of SEQ ID NOs: 138-140; an HCDR3 according to any one of SEQ ID NOs: 141-143; (viii) an HCDR1 according to any of SEQ ID NOs: 157-159; an HCDR2 according to any of SEQ ID NOs: 160-162; an HCDR3 according to any one of SEQ ID NOs: 163-165; (ix) an HCDR1 according to any of SEQ ID NOs: 179-181; an HCDR2 according to any of SEQ ID NOs: 182-184; an HCDR3 according to any one of SEQ ID NOs: 185-187; or (x) an HCDR1 according to any of SEQ ID NOs: 201-203; an HCDR2 according to any of SEQ ID NOs: 204-206; an HCDR3 according to any one of SEQ ID NOs: 207-209; and the LCDRs comprise: (i) an LCDR1 according to any of SEQ ID NOs: 14-16; an LCDR2 according to any of SEQ ID NOs: 17-19; an LCDR3 according to any one of SEQ ID NOs: 20-22; (ii) an LCDR1 according to any of SEQ ID NOs: 36-38; an LCDR2 according to any of SEQ ID NOs: 39-41; an LCDR3 according to any one of SEQ ID NOs: 42-44; (iii) an LCDR1 according to any of SEQ ID NOs: 58-60; an LCDR2 according to any of SEQ ID NOs: 61-63; an LCDR3 according to any one of SEQ ID NOs: 64-66; (iv) an LCDR1 according to any of SEQ ID NOs: 80-82; an LCDR2 according to any of SEQ ID NOs: 83-85; an LCDR3 according to any one of SEQ ID NOs: 86-88; (v) an LCDR1 according to any of SEQ ID NOs: 102-104; an LCDR2 according to any of SEQ ID NOs: 105-107; an LCDR3 according to any one of SEQ ID NOs: 108-110; (vi) an LCDR1 according to any of SEQ ID NOs: 124-126; an LCDR2 according to any of SEQ ID NOs: 127-129; an LCDR3 according to any one of SEQ ID NOs: 130-132; (vii) an LCDR1 according to any of SEQ ID NOs: 146-148; an LCDR2 according to any of SEQ ID NOs: 149-151; an LCDR3 according to any one of SEQ ID NOs: 152-154; (viii) an LCDR1 according to any of SEQ ID NOs: 168-170; an LCDR2 according to any of SEQ ID NOs: 171-173; an LCDR3 according to any one of SEQ ID NOs: 174-176; (ix) an LCDR1 according to any of SEQ ID NOs: 190-192; an LCDR2 according to any of SEQ ID NOs: 193-195; an LCDR3 according to any one of SEQ ID NOs: 196-198; or (x) an LCDR1 according to any of SEQ ID NOs: 212-214; an LCDR2 according to any of SEQ ID NOs: 215-217; an LCDR3 according to any one of SEQ ID NOs: 218-220.

[12] In some embodiments, the chimeric antigen receptor comprises a first domain comprising three heavy chain complementarity determining regions (HCDRs) and a second domain comprising three light chain complementarity determining regions (LCDRs), wherein: the three HCDRs comprise an HCDR1, an HCDR2, and an HCDR3; the three LCDRs comprise an LCDR1, an LCDR2, and an LCDR3; and the HCDRs and LCDRs comprise: (i) an HCDR1 according to any of SEQ ID NOs: 3-5; an HCDR2 according to any of SEQ ID NOs: 6-8; an HCDR3 according to any one of SEQ ID NOs: 9-11; an LCDR1 according to any of SEQ ID NOs: 14-16; an LCDR2 according to any of SEQ ID NOs: 17-19; an LCDR3 according to any one of SEQ ID NOs: 20-22; (ii) an HCDR1 according to any of SEQ ID NOs: 25-27; an HCDR2 according to any of SEQ ID NOs: 28-30; an HCDR3 according to any one of SEQ ID NOs: 31-33; an LCDR1 according to any of SEQ ID NOs: 36-38; an LCDR2 according to any of SEQ ID NOs: 39-41; an LCDR3 according to any one of SEQ ID NOs: 42-44; (iii) an HCDR1 according to any of SEQ ID NOs: 47-49; an HCDR2 according to any of SEQ ID NOs: 50-52; an HCDR3 according to any one of SEQ ID NOs: 53-55; an LCDR1 according to any of SEQ ID NOs: 58-60; an LCDR2 according to any of SEQ ID NOs: 61-63; an LCDR3 according to any one of SEQ ID NOs: 64-66; (iv) an HCDR1 according to any of SEQ ID NOs: 69-71; an HCDR2 according to any of SEQ ID NOs: 72-74; an HCDR3 according to any one of SEQ ID NOs: 75-77; an LCDR1 according to any of SEQ ID NOs: 80-82; an LCDR2 according to any of SEQ ID NOs: 83-85; an LCDR3 according to any one of SEQ ID NOs: 86-88; (v) an HCDR1 according to any of SEQ ID NOs: 91-93; an HCDR2 according to any of SEQ ID NOs: 94-96; an HCDR3 according to any one of SEQ ID NOs: 97-99; an LCDR1 according to any of SEQ ID NOs: 102-104; an LCDR2 according to any of SEQ ID NOs: 105-107; an LCDR3 according to any one of SEQ ID NOs: 108-110; (vi) an HCDR1 according to any of SEQ ID NOs: 113-115; an HCDR2 according to any of SEQ ID NOs: 116-118; an HCDR3 according to any one of SEQ ID NOs: 119-121; an LCDR1 according to any of SEQ ID NOs: 124-126; an LCDR2 according to any of SEQ ID NOs: 127-129; an LCDR3 according to any one of SEQ ID NOs: 130-132; (vii) an HCDR1 according to any of SEQ ID NOs: 135-137; an HCDR2 according to any of SEQ ID NOs: 138-140; an HCDR3 according to any one of SEQ ID NOs: 141-143; an LCDR1 according to any of SEQ ID NOs: 146-148; an LCDR2 according to any of SEQ ID NOs: 149-151; an LCDR3 according to any one of SEQ ID NOs: 152-154; (viii) an HCDR1 according to any of SEQ ID NOs: 157-159; an HCDR2 according to any of SEQ ID NOs: 160-162; an HCDR3 according to any one of SEQ ID NOs: 163-165; an LCDR1 according to any of SEQ ID NOs: 168-170; an LCDR2 according to any of SEQ

ID NOs: 171-173; an LCDR3 according to any one of SEQ ID NOs: 174-176; (ix) an HCDR1 according to any of SEQ ID NOs: 179-181; an HCDR2 according to any of SEQ ID NOs: 182-184; an HCDR3 according to any one of SEQ ID NOs: 185-187; an LCDR1 according to any of SEQ ID NOs: 190-192; an LCDR2 according to any of SEQ ID NOs: 193-195; an LCDR3 according to any one of SEQ ID NOs: 196-198; or (x) an HCDR1 according to any of SEQ ID NOs: 201-203; an HCDR2 according to any of SEQ ID NOs: 204-206; an HCDR3 according to any one of SEQ ID NOs: 207-209; an LCDR1 according to any of SEQ ID NOs: 212-214; an LCDR2 according to any of SEQ ID NOs: 215-217; an LCDR3 according to any one of SEQ ID NOs: 218-220. In various embodiments, the chimeric antigen receptor comprises a first heavy chain variable domain comprising the three HCDRs and a light chain variable domain comprising the three LCDRs, wherein: (i) the heavy chain variable domain is at least 80% identical to SEQ ID NO: 1, SEQ ID NO: 23, SEQ ID NO: 45, SEQ ID NO: 67, SEQ ID NO: 89, SEQ ID NO: 111, SEQ ID NO: 133, SEQ ID NO: 155, SEQ ID NO: 177, or SEQ ID NO: 199; and (ii) the light chain variable domain is at least 80% identical to SEQ ID NO: 12, SEQ ID NO: 34, SEQ ID NO: 56, SEQ ID NO: 78, SEQ ID NO: 100, SEQ ID NO: 122, SEQ ID NO: 144, SEQ ID NO: 166, SEQ ID NO: 188, or SEQ ID NO: 210. In some embodiments, the chimeric antigen receptor comprises a first heavy chain variable domain comprising the three HCDRs and a light chain variable domain comprising the three LCDRs, wherein: (i) the heavy chain variable domain is at least 80% identical to SEQ ID NO: 1 and the light chain variable domain is at least 80% identical to SEQ ID NO: 12; (ii) the heavy chain variable domain is at least 80% identical to SEQ ID NO: 23 and the light chain variable domain is at least 80% identical to SEQ ID NO: 34; (iii) the heavy chain variable domain is at least 80% identical to SEQ ID NO: 45 and the light chain variable domain is at least 80% identical to SEQ ID NO: 56; (iv) the heavy chain variable domain is at least 80% identical to SEQ ID NO: 67 and the light chain variable domain is at least 80% identical to SEQ ID NO: 78; (v) the heavy chain variable domain is at least 80% identical to SEQ ID NO: 89 and the light chain variable domain is at least 80% identical to SEQ ID NO: 100; (vi) the heavy chain variable domain is at least 80% identical to SEQ ID NO: 111 and the light chain variable domain is at least 80% identical to SEQ ID NO: 122; (vii) the heavy chain variable domain is at least 80% identical to SEQ ID NO: 133 and the light chain variable domain is at least 80% identical to SEQ ID NO: 144; (viii) the heavy chain variable domain is at least 80% identical to SEQ ID NO: 155 and the light chain variable domain is at least 80% identical to SEQ ID NO: 166; (ix) the heavy chain variable domain is at least 80% identical to SEQ ID NO: 177 and the light

chain variable domain is at least 80% identical to SEQ ID NO: 188; or (x) the heavy chain variable domain is at least 80% identical to SEQ ID NO: 199 and the light chain variable domain is at least 80% identical to SEQ ID NO: 210.

[13] In some embodiments comprising three HCDRs and the three LCDRs, the three HCDRs and the three LCDRs are comprised by a single polypeptide. In some embodiments comprising three HCDRs and the three LCDRs, the three HCDRs are comprised by a first polypeptide and the three LCDRs are comprised by a second polypeptide. In some embodiments, the first polypeptide is an antibody heavy chain and the second polypeptide is an antibody light chain. In some embodiments, the chimeric antigen receptor further comprises: (i) a binding motif that specifically binds an antigen selected from the group consisting of 5T4, alphafetoprotein, B cell maturation antigen (BCMA), B cell receptor, CA-125, carcinoembryonic antigen, CD19, CD20, CD22, CD23, CD30, CD33, CD40, CD56, CD79, CD78, CD123, CD138, c-Met, CSPG4, IgM, C-type lectin-like molecule 1 (CLL-1), EGFRvIII, epithelial tumor antigen, ERBB2, FLT3, folate binding protein, GD2, GD3, HER1-HER2 in combination, HER2-HER3 in combination, HER2/Neu, HERV-K, HIV-1 envelope glycoprotein gp41, HIV-1 envelope glycoprotein gpl20, IL-IIIRalpha, kappa chain, lambda chain, melanoma-associated antigen, mesothelin, MUC-1, mutated p53, mutated ras, prostate-specific antigen, ROR1, VEGFR2, EphA3 (EPH receptor A3), BAFFR (B-cell activating factor receptor), and combinations thereof; and/or (ii) a binding motif that specifically binds an antigen that is characteristic of B-cells, optionally wherein the antigen that is characteristic of B-cells is not CD19 or CD20. In some embodiments, the chimeric antigen receptor further comprises an anti-CD19 binding motif.

[14] In some embodiments, the chimeric antigen receptor of claim 40, wherein the anti-CD19 binding motif comprises a first domain comprising three HCDRs and a second domain comprising three LCDRs, wherein: the three HCDRs of the anti-CD19 binding motif comprise an HCDR1, an HCDR2, and an HCDR3; the three LCDRs of the anti-CD19 binding motif comprise an LCDR1, an LCDR2, and an LCDR3; and the HCDRs and LCDRs of the anti-CD19 binding motif comprise an HCDR1 according to any of SEQ ID NOs: 223-225; an HCDR2 according to any of SEQ ID NOs: 226-228; an HCDR3 according to any one of SEQ ID NOs: 229-231; an LCDR1 according to any of SEQ ID NOs: 234-236; an LCDR2 according to any of SEQ ID NOs: 237-239; an LCDR3 according to any one of SEQ ID NOs: 240-242. In some embodiments, the anti-CD19 binding motif comprises a first heavy chain variable domain comprising the three HCDRs of the anti-CD19 binding motif and a light chain variable

domain comprising the three LCDRs of the anti-CD19 binding motif, wherein the heavy chain variable domain of the anti-CD19 binding motif is at least 80% identical to SEQ ID NO: 221 and the light chain variable domain of the anti-CD19 binding motif is at least 80% identical to SEQ ID NO: 232. In some embodiments, the three HCDRs of the anti-CD19 binding motif and the three LCDRs of the anti-CD19 binding motif are comprised by a single polypeptide.

[15] In some embodiments, the three HCDRs of the anti-CD20 binding motif, the three LCDRs of the anti-CD20 binding motif, the three HCDRs of the anti-CD19 binding motif, and the three LCDRs of the anti-CD19 binding motif are together comprised by a single polypeptide. In some embodiments, the chimeric antigen receptor comprises a transmembrane domain that is a transmembrane domain of 4-1BB/CD137, an alpha chain of a T cell receptor, a beta chain of a T cell receptor, CD3 epsilon, CD4, CD5, CD8 alpha, CD9, CD16, CD19, CD22, CD28, CD33, CD37, CD45, CD64, CD80, CD86, CD134, CD137, CD154, or a zeta chain of a T cell receptor, or any combination thereof.

[16] In various embodiments, the present disclosure comprises a bicistronic chimeric antigen receptor comprising a first chimeric antigen receptor of the present disclosure and a second chimeric antigen receptor that comprises a binding motif that specifically binds an antigen selected from the group consisting of 5T4, alphafetoprotein, B cell maturation antigen (BCMA), B cell receptor, CA-125, carcinoembryonic antigen, CD19, CD20, CD22, CD23, CD30, CD33, CD40, CD56, CD79, CD78, CD123, CD138, c-Met, CSPG4, IgM, C-type lectin-like molecule 1 (CLL-1), EGFRvIII, epithelial tumor antigen, ERBB2, FLT3, folate binding protein, GD2, GD3, HER1-HER2 in combination, HER2-HER3 in combination, HER2/Neu, HERV-K, HIV-1 envelope glycoprotein gp41, HIV-1 envelope glycoprotein gp120, IL-11Ralpha, kappa chain, lambda chain, melanoma-associated antigen, mesothelin, MUC-1, mutated p53, mutated ras, prostate-specific antigen, ROR1, VEGFR2, EphA3 (EPH receptor A3), BAFFR (B-cell activating factor receptor), and combinations thereof; and/or a binding motif that specifically binds an antigen that is characteristic of B-cells, optionally wherein the antigen that is characteristic of B-cells is not CD19 or CD20. In some embodiments, the second chimeric antigen receptor comprises an anti-CD19 binding motif.

[17] The present disclosure further provides a nucleic acid encoding at least one polypeptide of the present disclosure and/or a vector comprising such nucleic acid. The present disclosure also comprises a method of generating an engineered cell, the method comprising transfecting or transducing a cell with a nucleic acid of the present disclosure. In various embodiments, the present disclosure comprises a cell encoding or expressing the chimeric antigen receptor

provided herein, optionally wherein the cell is an immune cell, optionally wherein the cell is a T cell.

[18] The present disclosure further provides a method of treating cancer in a subject in need thereof, the method comprising administering to the subject a cell therapy composition comprising one or more cells that encode or comprise an chimeric antigen receptor of the present disclosure. The present disclosure further provides a method of inducing an immune response in a subject or immunizing a subject against a cancer, the method comprising administering to the subject a cell therapy composition comprising one or more cells that encode or comprise an chimeric antigen receptor of the present disclosure. In various embodiments, the cells are CAR-T cells. In various embodiments, the cancer is acute lymphoblastic leukemia (ALL) (including non T cell ALL), acute myeloid leukemia, B cell prolymphocytic leukemia, B cell acute lymphoid leukemia ("BALL"), blastic plasmacytoid dendritic cell neoplasm, Burkitt's lymphoma, chronic lymphocytic leukemia (CLL), chronic myelogenous leukemia (CML), chronic myeloid leukemia, chronic or acute leukemia, diffuse large B cell lymphoma (DLBCL), follicular lymphoma (FL), hairy cell leukemia, Hodgkin's Disease, malignant lymphoproliferative conditions, MALT lymphoma, mantle cell lymphoma, Marginal zone lymphoma, monoclonal gammopathy of undetermined significance (MGUS), multiple myeloma, myelodysplasia and myelodysplastic syndrome, non-Hodgkin's lymphoma (NHL), plasma cell proliferative disorder (including asymptomatic myeloma (smoldering multiple myeloma or indolent myeloma), plasmablastic lymphoma, plasmacytoid dendritic cell neoplasm, plasmacytomas (including plasma cell dyscrasia; solitary myeloma; solitary plasmacytoma; extramedullary plasmacytoma; and multiple plasmacytoma), POEMS syndrome (also known as Crow-Fukase syndrome; Takatsuki disease; and PEP syndrome), primary mediastinal large B cell lymphoma (PMBC), small cell- or a large cell-follicular lymphoma, splenic marginal zone lymphoma (SMZL), systemic amyloid light chain amyloidosis, T cell acute lymphoid leukemia ("TALL"), T cell lymphoma, transformed follicular lymphoma, or Waldenstrom macroglobulinemia, Mantle cell lymphoma (MCL), Transformed follicular lymphoma (TFL), Primary mediastinal B cell lymphoma (PMBCL), Multiple myeloma, Hairy cell lymphoma/leukemia, or a combination thereof. In some embodiments, the cell therapy is an allogeneic cell therapy or an autologous cell therapy.

[19] In at least one aspect, the present disclosure comprises a chimeric antigen receptor comprising an anti-CD20 binding motif and a CD19 binding motif, wherein the anti-CD20 binding motif comprising amino acid sequences selected from the group consisting of SEQ ID

NOs: 1, 23, 45, 67, 89, 111, 133, 155, 177, 199, 12, 34, 56, 78, 100, 122, 144, 166, 188, and 210. In some embodiments, the CD19 binding motif comprising amino acid sequences selected from the group consisting of SEQ ID NOs: 221 and 232. In some embodiments, the anti-CD20 binding motif comprising amino acid sequences selected from the group consisting of SEQ ID NOs: 1, 23, 45, 67, 89, 111, 133, 155, 177, 199, 12, 34, 56, 78, 100, 122, 144, 166, 188, and 210; wherein the CD19 binding motif comprising amino acid sequences selected from the group consisting of SEQ ID NOs: 221 and 232. In some embodiments, the anti-CD20 binding motif and the CD19 binding motif are comprised by one polypeptide. In some embodiments, the anti-CD20 binding motif and the CD19 binding motif are comprised by different polypeptides.

[20] In at least one aspect, the present disclosure comprises a polynucleotide encoding amino acid sequences selected from the group consisting of SEQ ID NOs: 1, 23, 45, 67, 89, 111, 133, 155, 177, 199, 12, 34, 56, 78, 100, 122, 144, 166, 188, and 210. In at least one aspect, the present disclosure comprises a pharmaceutical composition comprising a chimeric antigen receptor comprising an anti-CD20 binding motif having amino acid sequences selected from the group consisting of SEQ ID NOs: 1, 23, 45, 67, 89, 111, 133, 155, 177, 199, 12, 34, 56, 78, 100, 122, 144, 166, 188, and 210. In some embodiments, the composition further comprises a CD19 binding motif.

DETAILED DESCRIPTION OF THE DISCLOSURE

[21] The present disclosure relates to novel polypeptides comprising novel antigen binding molecules and polynucleotides encoding the same. Some aspects of the disclosure relate to a polynucleotide encoding a chimeric antigen receptor (CAR) comprising at least one of the heavy chain and light chains disclosed herein (or CDRs thereof). The present disclosure also provides vectors (*e.g.*, viral vectors) comprising such polynucleotides and compositions comprising such vectors. The present disclosure further provides polynucleotides encoding such CARs or TCRs and compositions comprising such polynucleotides. The present disclosure additionally provides engineered cells (*e.g.*, T cells) comprising such polynucleotides and/or transduced with such viral vectors and compositions comprising such engineered cells. The present disclosure provides compositions (*e.g.*, pharmaceutical compositions) including a plurality of engineered T cells. The present disclosure provides methods for manufacturing such engineered T cells and compositions and uses (*e.g.*, in treating a melanoma) of such engineered T cells and compositions. And, the present disclosure provides

a method of inducing an immunity against a tumor comprising administering to a subject an effective amount of a cell comprising a polynucleotide, a vector, or a polypeptide of the present disclosure. Other aspects of the disclosure relate to cells comprising the CAR and their use in a T cell therapy for the treatment of a patient suffering from a cancer.

[22] Any aspect or embodiment described herein may be combined with any other aspect or embodiment as disclosed herein. While the present disclosure has been described in conjunction with the detailed description thereof, the foregoing description is intended to illustrate and not limit the scope of the present disclosure, which is defined by the scope of the appended claims. Other aspects, advantages, and modifications are within the scope of the following claims. The patent and scientific literature referred to herein establishes the knowledge that is available to those with skill in the art. All United States patents and published or unpublished United States patent applications cited herein are incorporated by reference. All published foreign patents and patent applications cited herein are hereby incorporated by reference. All other published references, dictionaries, documents, manuscripts and scientific literature cited herein are hereby incorporated by reference. Other features and advantages of the disclosure will be apparent from the following Detailed Description, comprising the Examples, and the claims.

DEFINITIONS

[23] In order for the present disclosure to be more readily understood, certain terms are first defined below. Additional definitions for the following terms and other terms are set forth throughout the Specification.

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

[25] Unless specifically stated or obvious from context, as used herein, the term “or” is understood to be inclusive and covers both “or” and “and”.

[26] The term “and/or” where used herein is to be taken as specific disclosure of each of the two specified features or components with or without the other. Thus, the term “and/or” as used in a phrase such as “A and/or B” herein is intended to include A and B; A or B; A (alone); and B (alone). Likewise, the term “and/or” as used in a phrase such as “A, B, and/or C” is intended to encompass each of the following aspects: A, B, and C; A, B, or C; A or C; A or B; B or C; A and C; A and B; B and C; A (alone); B (alone); and C (alone).

[27] The terms “*e.g.*,” and “*i.e.*” as used herein, are used merely by way of example, without limitation intended, and should not be construed as referring only those items explicitly enumerated in the specification.

[28] The terms “or more”, “at least”, “more than”, and the like, *e.g.*, “at least one” are understood to include but not be limited to at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149 or 150, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 2000, 3000, 4000, 5000 or more than the stated value. Also included is any greater number or fraction in between.

[29] Conversely, the term “no more than” includes each value less than the stated value. For example, “no more than 100 nucleotides” includes 100, 99, 98, 97, 96, 95, 94, 93, 92, 91, 90, 89, 88, 87, 86, 85, 84, 83, 82, 81, 80, 79, 78, 77, 76, 75, 74, 73, 72, 71, 70, 69, 68, 67, 66, 65, 64, 63, 62, 61, 60, 59, 58, 57, 56, 55, 54, 53, 52, 51, 50, 49, 48, 47, 46, 45, 44, 43, 42, 41, 40, 39, 38, 37, 36, 35, 34, 33, 32, 31, 30, 29, 28, 27, 26, 25, 24, 23, 22, 21, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, 1, and 0 nucleotides. Also included is any lesser number or fraction in between.

[30] The terms “plurality”, “at least two”, “two or more”, “at least second”, and the like, are understood to include but not limited to at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149 or 150, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 2000, 3000, 4000, 5000 or more. Also included is any greater number or fraction in between.

[31] Throughout the specification the word “comprising,” or variations such as “comprises” or “comprising,” will be understood to imply the inclusion of a stated element, integer or step, or group of elements, integers or steps, but not the exclusion of any other element, integer or

step, or group of elements, integers or steps. It is understood that wherever aspects are described herein with the language “comprising,” otherwise analogous aspects described in terms of “consisting of” and/or “consisting essentially of” are also provided.

[32] Unless specifically stated or evident from context, as used herein, the term “about” refers to a value or composition that is within an acceptable error range for the particular value or composition as determined by one of ordinary skill in the art, which will depend in part on how the value or composition is measured or determined, *i.e.*, the limitations of the measurement system. For example, “about” or “comprising essentially of” can mean within one or more than one standard deviation per the practice in the art. “About” or “comprising essentially of” can mean a range of up to 10% (*i.e.*, $\pm 10\%$). Thus, “about” can be understood to be within 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, 0.5%, 0.1%, 0.05%, 0.01%, or 0.001% greater or less than the stated value. For example, about 5 mg can include any amount between 4.5 mg and 5.5 mg. Furthermore, particularly with respect to biological systems or processes, the terms can mean up to an order of magnitude or up to 5-fold of a value. When particular values or compositions are provided in the instant disclosure, unless otherwise stated, the meaning of “about” or “comprising essentially of” should be assumed to be within an acceptable error range for that particular value or composition.

[33] As described herein, any concentration range, percentage range, ratio range or integer range is to be understood to be inclusive of the value of any integer within the recited range and, when appropriate, fractions thereof (such as one-tenth and one-hundredth of an integer), unless otherwise indicated.

[34] Units, prefixes, and symbols used herein are provided using their Système International de Unites (SI) accepted form. Numeric ranges are inclusive of the numbers defining the range.

[35] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure is related. For example, Juo, “The Concise Dictionary of Biomedicine and Molecular Biology”, 2nd ed., (2001), CRC Press; “The Dictionary of Cell & Molecular Biology”, 5th ed., (2013), Academic Press; and “The Oxford Dictionary Of Biochemistry And Molecular Biology”, Cammack *et al.* eds., 2nd ed, (2006), Oxford University Press, provide those of skill in the art with a general dictionary for many of the terms used in this disclosure.

[36] “Administering” refers to the physical introduction of an agent to a subject, using any of the various methods and delivery systems known to those skilled in the art. Exemplary routes of administration for the formulations disclosed herein include intravenous, intramuscular,

subcutaneous, intraperitoneal, spinal or other parenteral routes of administration, for example by injection or infusion. The phrase “parenteral administration” means modes of administration other than enteral and topical administration, usually by injection, and includes, without limitation, intravenous, intramuscular, intraarterial, intrathecal, intralymphatic, intralesional, intracapsular, intraorbital, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal, epidural and intrasternal injection and infusion, as well as *in vivo* electroporation. In some embodiments, the formulation is administered via a non-parenteral route, *e.g.*, orally. Other non-parenteral routes include a topical, epidermal or mucosal route of administration, for example, intranasally, vaginally, rectally, sublingually or topically. Administering can also be performed, for example, once, a plurality of times, and/or over one or more extended periods.

[37] The term “antibody” (Ab) includes, without limitation, a glycoprotein immunoglobulin which binds specifically to an antigen. In general, an antibody can comprise at least two heavy (H) chains and two light (L) chains interconnected by disulfide bonds, or an antigen-binding molecule thereof. Each H chain comprises a heavy chain variable region (abbreviated herein as VH) and a heavy chain constant region. The heavy chain constant region comprises three constant domains, CH1, CH2 and CH3. Each light chain comprises a light chain variable region (abbreviated herein as VL) and a light chain constant region. The light chain constant region comprises one constant domain, CL. The VH and VL regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDRs), interspersed with regions that are more conserved, termed framework regions (FR). Each VH and VL comprises three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, and FR4. The variable regions of the heavy and light chains contain a binding domain that interacts with an antigen. The constant regions of the Abs may mediate the binding of the immunoglobulin to host tissues or factors, including various cells of the immune system (*e.g.*, effector cells) and the first component (C1q) of the classical complement system. In general, human antibodies are approximately 150 kD tetrameric agents composed of two identical heavy (H) chain polypeptides (about 50 kD each) and two identical light (L) chain polypeptides (about 25 kD each) that associate with each other into what is commonly referred to as a “Y-shaped” structure. The heavy and light chains are linked or connected to one another by a single disulfide bond; two other disulfide bonds connect the heavy chain hinge regions to one another, so that the dimers are connected to one

another and the tetramer is formed. Naturally-produced antibodies are also glycosylated, *e.g.*, on the CH₂ domain.

[38] The term “human antibody” is intended to comprise antibodies having variable and constant domain sequences generated, assembled, or derived from human immunoglobulin sequences, or sequences indistinguishable therefrom. In some embodiments, antibodies (or antibody components) may be considered to be “human” even though their amino acid sequences comprise residues or elements not encoded by human germline immunoglobulin sequences (*e.g.*, variations introduced by *in vitro* random or site-specific mutagenesis or introduced by *in vivo* somatic mutation). The term “humanized” is intended to comprise antibodies having a variable domain with a sequence derived from a variable domain of a non-human species (*e.g.*, a mouse), modified to be more similar to a human germline encoded sequence. In some embodiments, a “humanized” antibody comprises one or more framework domains having substantially the amino acid sequence of a human framework domain, and one or more complementary determining regions having substantially the amino acid sequence as that of a non-human antibody. In some embodiments, a humanized antibody comprises at least a portion of an immunoglobulin constant region (Fc), generally that of a human immunoglobulin constant domain. In some embodiments, a humanized antibodies may comprise a C_H1, hinge, C_H2, C_H3, and, optionally, a C_H4 region of a human heavy chain constant domain.

[39] Antibodies can include, for example, monoclonal antibodies, recombinantly produced antibodies, monospecific antibodies, multispecific antibodies (including bispecific antibodies), human antibodies, engineered antibodies, humanized antibodies, chimeric antibodies, immunoglobulins, synthetic antibodies, tetrameric antibodies comprising two heavy chain and two light chain molecules, an antibody light chain monomer, an antibody heavy chain monomer, an antibody light chain dimer, an antibody heavy chain dimer, an antibody light chain- antibody heavy chain pair, intrabodies, antibody fusions (sometimes referred to herein as “antibody conjugates”), heteroconjugate antibodies, single domain antibodies, monovalent antibodies, single chain antibodies or single-chain Fvs (scFv), camelized antibodies, affybodies, Fab fragments, F(ab')₂ fragments, disulfide-linked Fvs (sdFv), anti-idiotypic (anti-Id) antibodies (including, *e.g.*, anti-anti-Id antibodies), minibodies, domain antibodies, synthetic antibodies (sometimes referred to herein as “antibody mimetics”), and antigen binding fragments of any of the above. In certain embodiments, antibodies described herein refer to polyclonal antibody populations. Antibodies may also comprise, for example, Fab'

fragments, Fd' fragments, Fd fragments, isolated CDRs, single chain Fvs, polypeptide-Fc fusions, single domain antibodies (e.g., shark single domain antibodies such as IgNAR or fragments thereof), camelid antibodies, single chain or Tandem diabodies (TandAb®), Anticalins®, Nanobodies® minibodies, BiTE®, ankyrin repeat proteins or DARPINs®, Avimers®, DARTs, TCR-like antibodies, Adnectins®, Affilins®, Trans-bodies®, Affibodies®, TrimerX®, MicroProteins, Fynomers®, Centyrins®, and KALBITOR®.

[40] An immunoglobulin may derive from any of the commonly known isotypes, including but not limited to IgA, secretory IgA, IgG, IgE and IgM. IgG subclasses are also well known to those in the art and include but are not limited to human IgG1, IgG2, IgG3 and IgG4. "Isotype" refers to the Ab class or subclass (e.g., IgM or IgG1) that is encoded by the heavy chain constant region genes. The term "antibody" includes, by way of example, both naturally occurring and non-naturally occurring Abs; monoclonal and polyclonal Abs; chimeric and humanized Abs; human or nonhuman Abs; wholly synthetic Abs; and single chain Abs. A nonhuman Ab may be humanized by recombinant methods to reduce its immunogenicity in man. Where not expressly stated, and unless the context indicates otherwise, the term "antibody" also includes an antigen binding fragment or an antigen-binding portion of any of the aforementioned immunoglobulins, and includes a monovalent and a divalent fragment or portion, and a single chain Ab.

[41] An "antigen binding molecule," "antigen binding portion," or "antibody fragment" refers to any molecule that comprises the antigen binding parts (e.g., CDRs) of the antibody from which the molecule is derived. An antigen binding molecule can include the antigenic complementarity determining regions (CDRs). Examples of antibody fragments include, but are not limited to, Fab, Fab', F(ab')₂, and Fv fragments, dAb, linear antibodies, scFv antibodies, and multispecific antibodies formed from antigen binding molecules. Peptibodies (i.e., Fc fusion molecules comprising peptide binding domains) are another example of suitable antigen binding molecules. In some embodiments, the antigen binding molecule binds to an antigen on a tumor cell. In some embodiments, the antigen binding molecule binds to an antigen on a cell involved in a hyperproliferative disease or to a viral or bacterial antigen. In certain embodiments, the antigen binding molecule binds to BCMA, CLL-1, or FLT3. In certain embodiments, the antigen binding molecule binds to CD19, CD20, or both. In further embodiments, the antigen binding molecule is an antibody fragment that specifically binds to the antigen, including one or more of the complementarity determining regions (CDRs) thereof.

In further embodiments, the antigen binding molecule is a single chain variable fragment (scFv). In some embodiments, the antigen binding molecule comprises or consists of avimers.

[42] In some instances, a CDR is substantially identical to one found in a reference antibody (e.g., an antibody of the present disclosure) and/or the sequence of a CDR provided in the present disclosure. In some embodiments, a CDR is substantially identical to a reference CDR (e.g., a CDR provided in the present disclosure) in that it is either identical in sequence or contains between 1, 2, 3, 4, or 5 (e.g. 1-5) amino acid substitutions as compared with the reference CDR. In some embodiments a CDR is substantially identical to a reference CDR in that it shows at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity with the reference CDR (e.g., 85-90%, 85-95%, 85-100%, 90-95%, 90-100%, or 95-100%). In some embodiments a CDR is substantially identical to a reference CDR in that it shows at least 96%, 96%, 97%, 98%, 99%, or 100% sequence identity with the reference CDR. In some embodiments a CDR is substantially identical to a reference CDR in that one amino acid within the CDR is deleted, added, or substituted as compared with the reference CDR while the CDR has an amino acid sequence that is otherwise identical with that of the reference CDR. In some embodiments a CDR is substantially identical to a reference CDR in that 2, 3, 4, or 5 (e.g. 2-5) amino acids within the CDR are deleted, added, or substituted as compared with the reference CDR while the CDR has an amino acid sequence that is otherwise identical to the reference CDR. In various embodiments, an antigen binding fragment binds a same antigen as a reference antibody.

[43] An antigen binding fragment may be produced by any means. For example, in some embodiments, an antigen binding fragment may be enzymatically or chemically produced by fragmentation of an intact antibody. In some embodiments, an antigen binding fragment may be recombinantly produced (i.e., by expression of an engineered nucleic acid sequence. In some embodiments, an antigen binding fragment may be wholly or partially synthetically produced. In some embodiments, an antigen binding fragment may have a length of at least about 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190 amino acids or more; in some embodiments at least about 200 amino acids (e.g., 50-100, 50-150, 50-200, or 100-200 amino acids).

[44] The term “variable region” or “variable domain” is used interchangeably and are common in the art. The variable region typically refers to a portion of an antibody, generally, a portion of a light or heavy chain, typically about the amino-terminal 110 to 120 amino acids in the mature heavy chain and about 90 to 115 amino acids in the mature light chain, which

differ extensively in sequence among antibodies and are used in the binding and specificity of a particular antibody for its particular antigen. The variability in sequence is concentrated in those regions called complementarity determining regions (CDRs) while the more highly conserved regions in the variable domain are called framework regions (FR). Without wishing to be bound by any particular mechanism or theory, it is believed that the CDRs of the light and heavy chains are primarily responsible for the interaction and specificity of the antibody with antigen. In certain embodiments, the variable region is a human variable region. In certain embodiments, the variable region comprises rodent or murine CDRs and human framework regions (FRs). In particular embodiments, the variable region is a primate (*e.g.*, non-human primate) variable region. In certain embodiments, the variable region comprises rodent or murine CDRs and primate (*e.g.*, non-human primate) framework regions (FRs).

[45] The terms “VL” and “VL domain” are used interchangeably to refer to the light chain variable region of an antibody or an antigen-binding molecule thereof.

[46] The terms “VH” and “VH domain” are used interchangeably to refer to the heavy chain variable region of an antibody or an antigen-binding molecule thereof.

[47] A number of definitions of the CDRs are commonly in use: Kabat numbering, Chothia numbering, AbM numbering, or contact numbering. The AbM definition is a compromise between the two used by Oxford Molecular’s AbM antibody modelling software. The contact definition is based on an analysis of the available complex crystal structures.

[48] Table 1. CDR Numbering

Loop	Kabat	AbM	Chothia	Contact
L1	L24--L34	L24--L34	L24--L34	L30--L36
L2	L50--L56	L50--L56	L50--L56	L46--L55
L3	L89--L97	L89--L97	L89--L97	L89--L96
H1	H31--H35B (Kabat Numbering)	H26--H35B	H26--H32..34	H30--H35B
H1	H31--H35 (Chothia Numbering)	H26--H35	H26--H32	H30--H35
H2	H50--H65	H50--H58	H52--H56	H47--H58
H3	H95--H102	H95--H102	H95--H102	H93--H101

[49] The term “Kabat numbering” and like terms are recognized in the art and refer to a system of numbering amino acid residues in the heavy and light chain variable regions of an antibody, or an antigen-binding molecule thereof. In certain aspects, the CDRs of an antibody can be determined according to the Kabat numbering system (*see, e.g.*, Kabat EA & Wu TT

(1971) *Ann NY Acad Sci* 190: 382-391 and Kabat EA *et al.*, (1991) *Sequences of Proteins of Immunological Interest*, Fifth Edition, U.S. Department of Health and Human Services, NIH Publication No. 91-3242). Using the Kabat numbering system, CDRs within an antibody heavy chain molecule are typically present at amino acid positions 31 to 35, which optionally can include one or two additional amino acids, following 35 (referred to in the Kabat numbering scheme as 35A and 35B) (CDR1), amino acid positions 50 to 65 (CDR2), and amino acid positions 95 to 102 (CDR3). Using the Kabat numbering system, CDRs within an antibody light chain molecule are typically present at amino acid positions 24 to 34 (CDR1), amino acid positions 50 to 56 (CDR2), and amino acid positions 89 to 97 (CDR3). In a specific embodiment, the CDRs of the antibodies described herein have been determined according to the Kabat numbering scheme.

[50] In certain aspects, the CDRs of an antibody can be determined according to the Chothia numbering scheme, which refers to the location of immunoglobulin structural loops (*see, e.g.*, Chothia C & Lesk AM, (1987), *J Mol Biol* 196: 901-917; Al-Lazikani B *et al.*, (1997) *J Mol Biol* 273: 927-948; Chothia C *et al.*, (1992) *J Mol Biol* 227: 799-817; Tramontano A *et al.*, (1990) *J Mol Biol* 215(1): 175-82; and U.S. Patent No. 7,709,226). Typically, when using the Kabat numbering convention, the Chothia CDR-H1 loop is present at heavy chain amino acids 26 to 32, 33, or 34, the Chothia CDR-H2 loop is present at heavy chain amino acids 52 to 56, and the Chothia CDR-H3 loop is present at heavy chain amino acids 95 to 102, while the Chothia CDR-L1 loop is present at light chain amino acids 24 to 34, the Chothia CDR-L2 loop is present at light chain amino acids 50 to 56, and the Chothia CDR-L3 loop is present at light chain amino acids 89 to 97. The end of the Chothia CDR-HI loop when numbered using the Kabat numbering convention varies between H32 and H34 depending on the length of the loop (this is because the Kabat numbering scheme places the insertions at H35A and H35B; if neither 35A nor 35B is present, the loop ends at 32; if only 35A is present, the loop ends at 33; if both 35A and 35B are present, the loop ends at 34). In a specific embodiment, the CDRs of the antibodies described herein have been determined according to the Chothia numbering scheme.

[51] The terms “constant region” and “constant domain” are interchangeable and have a meaning common in the art. The constant region is an antibody portion, *e.g.*, a carboxyl terminal portion of a light and/or heavy chain which is not directly involved in binding of an antibody to antigen but which can exhibit various effector functions, such as interaction with

the Fc receptor. The constant region of an immunoglobulin molecule generally has a more conserved amino acid sequence relative to an immunoglobulin variable domain.

[52] The term “heavy chain” when used in reference to an antibody can refer to any distinct type, *e.g.*, alpha (α), delta (δ), epsilon (ϵ), gamma (γ) and mu (μ), based on the amino acid sequence of the constant domain, which give rise to IgA, IgD, IgE, IgG and IgM classes of antibodies, respectively, including subclasses of IgG, *e.g.*, IgG₁, IgG₂, IgG₃ and IgG₄.

[53] The term “light chain” when used in reference to an antibody can refer to any distinct type, *e.g.*, kappa (κ) or lambda (λ) based on the amino acid sequence of the constant domains. Light chain amino acid sequences are well known in the art. In specific embodiments, the light chain is a human light chain.

[54] “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) and its binding partner (*e.g.*, an antigen). Unless indicated otherwise, as used herein, “binding affinity” refers to intrinsic binding affinity which reflects a 1:1 interaction between members of a binding pair (*e.g.*, antibody and antigen). The affinity of a molecule X for its partner Y can generally be represented by the dissociation constant (K_D). Affinity can be measured and/or expressed in a number of ways known in the art, including, but not limited to, equilibrium dissociation constant (K_D), and equilibrium association constant (K_A). The K_D is calculated from the quotient of k_{off}/k_{on} , whereas K_A is calculated from the quotient of k_{on}/k_{off} . k_{on} refers to the association rate constant of, *e.g.*, an antibody to an antigen, and k_{off} refers to the dissociation of, *e.g.*, an antibody to an antigen. The k_{on} and k_{off} can be determined by techniques known to one of ordinary skill in the art, such as BIACORE® or KinExA.

[55] A “conservative amino acid substitution” is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having side chains have been defined in the art. These families include amino acids with basic side chains (*e.g.*, lysine, arginine, histidine), acidic side chains (*e.g.*, aspartic acid, glutamic acid), uncharged polar side chains (*e.g.*, glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine, tryptophan), nonpolar side chains (*e.g.*, alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine), beta-branched side chains (*e.g.*, threonine, valine, isoleucine) and aromatic side chains (*e.g.*, tyrosine, phenylalanine, tryptophan, histidine). In certain embodiments, one or more amino acid residues within a CDR(s) or within a framework region(s) of an antibody or antigen-binding molecule thereof can be replaced with an amino acid residue with a similar side chain. In general, two sequences are generally

considered to be “substantially similar” if they contain a conservative amino acid substitution in corresponding positions. For example, certain amino acids are generally classified as “hydrophobic” or “hydrophilic” amino acids, and/or as having “polar” or “non-polar” side chains. Substitution of one amino acid for another of the same type may be considered a conservative substitution. Exemplary amino acid categorizations are summarized in Tables 2 and 3 below:

Table 2

Amino Acid	3-Letter	1-Letter	Property	Property	Hydropathy Index
Alanine	Ala	A	nonpolar	neutral	1.8
Arginine	Arg	R	polar	positive	-4.5
Asparagine	Asn	N	polar	neutral	-3.5
Aspartic acid	Asp	D	polar	negative	-3.5
Cysteine	Cys	C	nonpolar	neutral	2.5
Glutamic acid	Glu	E	polar	negative	-3.5
Glutamine	Gln	Q	polar	neutral	-3.5
Glycine	Gly	G	nonpolar	neutral	-0.4
Histidine	His	H	polar	positive	-3.2
Isoleucine	Ile	I	nonpolar	neutral	4.5
Leucine	Leu	L	nonpolar	neutral	3.8
Lysine	Lys	K	polar	positive	-3.9
Methionine	Met	M	nonpolar	neutral	1.9
Phenylalanine	Phe	F	nonpolar	neutral	2.8
Proline	Pro	P	nonpolar	neutral	-1.6
Serine	Ser	S	polar	neutral	-0.8
Threonine	Thr	T	polar	neutral	-0.7
Tryptophan	Trp	W	nonpolar	neutral	-0.9
Tyrosine	Tyr	Y	polar	neutral	-1.3
Valine	Val	V	nonpolar	neutral	4.2

Table 3

Ambiguous Amino Acids	3-Letter	1-Letter
Asparagine or aspartic acid	Asx	B
Glutamine or glutamic acid	Glx	Z
Leucine or Isoleucine	Xle	J
Unspecified or unknown amino acid	Xaa	X

[56] The term “heterologous” means from any source other than naturally occurring sequences. For example, a heterologous sequence included as a part of a costimulatory protein having the amino acid sequence of SEQ ID NO: 232, e.g., the corresponding human costimulatory protein, is amino acids that do not naturally occur as, i.e., do not align with, the wild type human costimulatory protein. For example, a heterologous nucleotide sequence refers to a nucleotide sequence other than that of the wild type human costimulatory protein-encoding sequence.

[57] An “epitope” is a term in the art and refers to a localized region of an antigen to which an antibody can specifically bind. An epitope can be, for example, contiguous amino acids of a polypeptide (linear or contiguous epitope) or an epitope can, for example, come together from two or more non-contiguous regions of a polypeptide or polypeptides (conformational, non-linear, discontinuous, or non-contiguous epitope). In certain embodiments, the epitope to which an antibody binds can be determined by, e.g., NMR spectroscopy, X-ray diffraction crystallography studies, ELISA assays, hydrogen/deuterium exchange coupled with mass spectrometry (e.g., liquid chromatography electrospray mass spectrometry), array-based oligopeptide scanning assays, and/or mutagenesis mapping (e.g., site-directed mutagenesis mapping). For X-ray crystallography, crystallization may be accomplished using any of the known methods in the art (e.g., Giegé R *et al.*, (1994) *Acta Crystallogr D Biol Crystallogr* 50(Pt 4): 339-350; McPherson A (1990) *Eur J Biochem* 189: 1-23; Chayen NE (1997) *Structure* 5: 1269-1274; McPherson A (1976) *J Biol Chem* 251: 6300-6303). Antibody:antigen crystals may be studied using well known X-ray diffraction techniques and may be refined using computer software such as X-PLOR (Yale University, 1992, distributed by Molecular Simulations, Inc.; see e.g. *Meth Enzymol* (1985) volumes 114 & 115, eds Wyckoff HW *et al.*; U.S. 2004/0014194), and BUSTER (Bricogne G (1993) *Acta Crystallogr D Biol Crystallogr* 49(Pt

1): 37-60; Bricogne G (1997) *Meth Enzymol* 276A: 361-423, ed Carter CW; Roversi P *et al.*, (2000) *Acta Crystallogr D Biol Crystallogr* 56(Pt 10): 1316-1323). Mutagenesis mapping studies may be accomplished using any method known to one of skill in the art. *See, e.g.*, Champe M *et al.*, (1995) *J Biol Chem* 270: 1388-1394 and Cunningham BC & Wells JA (1989) *Science* 244: 1081-1085 for a description of mutagenesis techniques, including alanine scanning mutagenesis techniques.

[58] An antigen binding molecule, an antibody, or an antigen binding molecule thereof “cross-competes” with a reference antibody or an antigen binding molecule thereof if the interaction between an antigen and the first binding molecule, an antibody, or an antigen binding molecule thereof blocks, limits, inhibits, or otherwise reduces the ability of the reference binding molecule, reference antibody, or an antigen binding molecule thereof to interact with the antigen. Cross competition can be complete, *e.g.*, binding of the binding molecule to the antigen completely blocks the ability of the reference binding molecule to bind the antigen, or it can be partial, *e.g.*, binding of the binding molecule to the antigen reduces the ability of the reference binding molecule to bind the antigen. In certain embodiments, an antigen binding molecule that cross-competes with a reference antigen binding molecule binds the same or an overlapping epitope as the reference antigen binding molecule. In other embodiments, the antigen binding molecule that cross-competes with a reference antigen binding molecule binds a different epitope as the reference antigen binding molecule. Numerous types of competitive binding assays can be used to determine if one antigen binding molecule competes with another, for example: solid phase direct or indirect radioimmunoassay (RIA); solid phase direct or indirect enzyme immunoassay (EIA); sandwich competition assay (Stahli *et al.*, 1983, *Methods in Enzymology* 9:242-253); solid phase direct biotin-avidin EIA (Kirkland *et al.*, 1986, *J. Immunol.* 137:3614-3619); solid phase direct labeled assay, solid phase direct labeled sandwich assay (Harlow and Lane, 1988, *Antibodies, A Laboratory Manual*, Cold Spring Harbor Press); solid phase direct label RIA using 1-125 label (Morel *et al.*, 1988, *Molec. Immunol.* 25:7-15); solid phase direct biotin-avidin EIA (Cheung, *et al.*, 1990, *Virology* 176:546-552); and direct labeled RIA (Moldenhauer *et al.*, 1990, *Scand. J. Immunol.* 32:77-82).

[59] The term “binding” generally refers to a non-covalent association between or among two or more entities. Direct binding involves physical contact between entities or moieties. “Indirect” binding involves physical interaction by way of physical contact with one or more intermediate entities. Binding between two or more entities may be assessed in any of a variety

of contexts, *e.g.*, where interacting entities or moieties are studied in isolation or in the context of more complex systems (*e.g.*, while covalently or otherwise associated with a carrier entity and/or in a biological system such as a cell).

[60] The terms “immunospecifically binds,” “immunospecifically recognizes,” “specifically binds,” and “specifically recognizes” are analogous terms in the context of antibodies and refer to molecules that bind to an antigen (*e.g.*, epitope or immune complex) as such binding is understood by one skilled in the art. For example, a molecule that specifically binds to an antigen may bind to other peptides or polypeptides, generally with lower affinity as determined by, *e.g.*, immunoassays, BIACORE[®], KinExA 3000 instrument (Sapidyne Instruments, Boise, ID), or other assays known in the art. In a specific embodiment, molecules that specifically bind to an antigen bind to the antigen with a K_A that is at least 2 logs, 2.5 logs, 3 logs, 4 logs or greater than the K_A when the molecules bind to another antigen. Binding may comprise preferential association of a binding motif, antibody, or antigen binding system with a target of the binding motif, antibody, or antigen binding system as compared to association of the binding motif, antibody, or antigen binding system with an entity that is not the target (*i.e.* non-target). In some embodiments, a binding motif, antibody, or antigen binding system selectively binds a target if binding between the binding motif, antibody, or antigen binding system and the target is greater than 2-fold, greater than 5-fold, greater than 10-fold, 20-fold, 30-fold, 40-fold, 50-fold, 60-fold, 70-fold, 80-fold, 90-fold, or greater than 100-fold as compared with binding of the binding motif, antibody, or antigen binding system and a non-target. In some embodiments, a binding motif, antibody, or antigen binding system selectively binds a target if the binding affinity is less than about 10^{-5} M, less than about 10^{-6} M, less than about 10^{-7} M, less than about 10^{-8} M, or less than about 10^{-9} M.

[61] In another embodiment, molecules that specifically bind to an antigen bind with a dissociation constant (K_d) of about 1×10^{-7} M. In some embodiments, the antigen binding molecule specifically binds an antigen with “high affinity” when the K_d is about 1×10^{-9} M to about 5×10^{-9} M. In some embodiments, the antigen binding molecule specifically binds an antigen with “very high affinity” when the K_d is 1×10^{-10} M to about 5×10^{-10} M. In one embodiment, the antigen binding molecule has a K_d of 10^{-9} M. In one embodiment, the off-rate is less than about 1×10^{-5} . In other embodiments, the antigen binding molecule binds human BCMA with a K_d of between about 1×10^{-7} M and about 1×10^{-13} M. In yet another embodiment, the antigen binding molecule binds human BCMA with a K_d of about 1×10^{-10} M to about 5×10^{-10} M. In some embodiments, the antigen binding molecule binds human CD19,

CD20, or both with a K_d of between about 1×10^{-7} M and about 1×10^{-13} M. In yet another embodiment, the antigen binding molecule binds human CD19, CD20, or both with a K_d of about 1×10^{-10} M to about 5×10^{-10} M.

[62] In a specific embodiment, provided herein is an antibody or an antigen binding molecule thereof that binds to a target human antigen, *e.g.*, human BCMA or human CLL-1, with higher affinity than to another species of the target antigen, *e.g.*, a non-human BCMA or a non-human CLL-1. In some embodiments, provided herein is an antibody or an antigen binding molecule thereof that binds to human CD19, human CD20, or both, with higher affinity than to another species of one or both target antigens, *e.g.*, a non-human CD19, non-human CD20, or both. In certain embodiments, provided herein is an antibody or an antigen binding molecule thereof that binds to the target human antigen, *e.g.*, human BCMA or human CLL-1, with a 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70% or higher affinity than to another species of the target antigen as measured by, *e.g.*, a radioimmunoassay, surface plasmon resonance, or kinetic exclusion assay. In certain embodiments, provided herein is an antibody or an antigen binding molecule thereof that binds to human CD19, human CD20, or both, with a 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70% or higher affinity than to another species of one or both target antigens as measured by, *e.g.*, a radioimmunoassay, surface plasmon resonance, or kinetic exclusion assay. In a specific embodiment, an antibody or an antigen binding molecule thereof described herein, which binds to a target human antigen, will bind to another species of the target antigen with less than 10%, 15%, or 20% of the binding of the antibody or an antigen binding molecule thereof to the human antigen as measured by, *e.g.*, a radioimmunoassay, surface plasmon resonance, or kinetic exclusion assay.

[63] “Chimeric antigen receptor” or “CAR” refers to a molecule engineered to comprise a binding motif and a means of activating immune cells (for example T cells such as naive T cells, central memory T cells, effector memory T cells or combination thereof) upon antigen binding. CARs are also known as artificial T cell receptors, chimeric T cell receptors or chimeric immunoreceptors. In some embodiments, a CAR comprises a binding motif, an extracellular domain, a transmembrane domain, one or more co-stimulatory domains, and an intracellular signaling domain. A T cell that has been genetically engineered to express a chimeric antigen receptor may be referred to as a CAR T cell. “Extracellular domain” (or “ECD”) refers to a portion of a polypeptide that, when the polypeptide is present in a cell membrane, is understood to reside outside of the cell membrane, in the extracellular space.

[64] An “antigen” refers to any molecule that provokes an immune response or is capable of being bound by an antibody or an antigen binding molecule. The immune response may involve either antibody production, or the activation of specific immunologically-competent cells, or both. A person of skill in the art would readily understand that any macromolecule, including virtually all proteins or peptides, can serve as an antigen. An antigen can be endogenously expressed, *i.e.* expressed by genomic DNA, or can be recombinantly expressed. An antigen can be specific to a certain tissue, such as a cancer cell, or it can be broadly expressed. In addition, fragments of larger molecules can act as antigens. In one embodiment, antigens are tumor antigens. In one particular embodiment, the antigen is all or a fragment of CD19 or CD20. A “target” is any molecule bound by a binding motif, antigen binding system, or binding agent, *e.g.*, an antibody. In some embodiments, a target is an antigen or epitope of the present disclosure.

[65] The term “neutralizing” refers to an antigen binding molecule, scFv, antibody, or a fragment thereof, that binds to a ligand and prevents or reduces the biological effect of that ligand. In some embodiments, the antigen binding molecule, scFv, antibody, or a fragment thereof, directly blocking a binding site on the ligand or otherwise alters the ligand's ability to bind through indirect means (such as structural or energetic alterations in the ligand). In some embodiments, the antigen binding molecule, scFv, antibody, or a fragment thereof prevents the protein to which it is bound from performing a biological function.

[66] The term “autologous” refers to any material derived from the same individual to which it is later to be re-introduced. For example, the engineered autologous cell therapy (eACT™) method described herein involves collection of lymphocytes from a patient, which are then engineered to express, *e.g.*, a CAR construct, and then administered back to the same patient.

[67] The term “allogeneic” refers to any material derived from one individual which is then introduced to another individual of the same species, *e.g.*, allogeneic T cell transplantation.

[68] The terms “transduction” and “transduced” refer to the process whereby foreign DNA is introduced into a cell via viral vector (*see* Jones et al., “Genetics: principles and analysis,” Boston: Jones & Bartlett Publ. (1998)). In some embodiments, the vector is a retroviral vector, a DNA vector, a RNA vector, an adenoviral vector, a baculoviral vector, an Epstein Barr viral vector, a papovaviral vector, a vaccinia viral vector, a herpes simplex viral vector, an adenovirus associated vector, a lentiviral vector, or any combination thereof.

[69] “Transformation” refers to any process by which exogenous DNA is introduced into a host cell. Transformation may occur under natural or artificial conditions using various

methods. Transformation may be achieved using any known method for the insertion of foreign nucleic acid sequences into a prokaryotic or eukaryotic host cell. In some embodiments, some transformation methodology is selected based on the host cell being transformed and/or the nucleic acid to be inserted. Methods of transformation may comprise, yet are not limited to, viral infection, electroporation, and lipofection. In some embodiments, a “transformed” cell is stably transformed in that the inserted DNA is capable of replication either as an autonomously replicating plasmid or as part of the host chromosome. In some embodiments, a transformed cell may express introduced nucleic acid.

[70] Term “vector” refers to a recipient nucleic acid molecule modified to comprise or incorporate a provided nucleic acid sequence. One type of vector is a “plasmid,” which refers to a circular double stranded DNA molecule into which additional DNA may be ligated. Another type of vector is a viral vector, wherein additional DNA segments may be ligated into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (e.g., bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (e.g., non-episomal mammalian vectors) may be integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. Moreover, certain vectors comprise sequences that direct expression of inserted genes to which they are operatively linked. Such vectors may be referred to herein as “expression vectors.” Standard techniques may be used for engineering of vectors, e.g., as found in Sambrook et al., *Molecular Cloning: A Laboratory Manual* (2d ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989)), which is incorporated herein by reference for any purpose.

[71] A “cancer” refers to a broad group of various diseases characterized by the uncontrolled growth of abnormal cells in the body. Unregulated cell division and growth results in the formation of malignant tumors that invade neighboring tissues and may also metastasize to distant parts of the body through the lymphatic system or bloodstream. A “cancer” or “cancer tissue” can include a tumor. Examples of cancers that can be treated by the methods of the present disclosure include, but are not limited to, cancers of the immune system including lymphoma, leukemia, myeloma, and other leukocyte malignancies. In some embodiments, the methods of the present disclosure can be used to reduce the tumor size of a tumor derived from, for example, bone cancer, pancreatic cancer, skin cancer, cancer of the head or neck, cutaneous or intraocular malignant melanoma, uterine cancer, ovarian cancer, rectal cancer, cancer of the anal region, stomach cancer, testicular cancer, uterine cancer, carcinoma of the fallopian tubes,

carcinoma of the endometrium, carcinoma of the cervix, carcinoma of the vagina, carcinoma of the vulva, multiple myeloma, Hodgkin's Disease, non-Hodgkin's lymphoma (NHL), primary mediastinal large B cell lymphoma (PMBC), diffuse large B cell lymphoma (DLBCL), follicular lymphoma (FL), transformed follicular lymphoma, splenic marginal zone lymphoma (SMZL), cancer of the esophagus, cancer of the small intestine, cancer of the endocrine system, cancer of the thyroid gland, cancer of the parathyroid gland, cancer of the adrenal gland, sarcoma of soft tissue, cancer of the urethra, cancer of the penis, chronic or acute leukemia, acute myeloid leukemia, chronic myeloid leukemia, acute lymphoblastic leukemia (ALL) (including non T cell ALL), chronic lymphocytic leukemia (CLL), solid tumors of childhood, lymphocytic lymphoma, cancer of the bladder, cancer of the kidney or ureter, carcinoma of the renal pelvis, neoplasm of the central nervous system (CNS), primary CNS lymphoma, tumor angiogenesis, spinal axis tumor, brain stem glioma, pituitary adenoma, Kaposi's sarcoma, epidermoid cancer, squamous cell cancer, T-cell lymphoma, environmentally induced cancers including those induced by asbestos, other B cell malignancies, and combinations of said cancers. In one particular embodiment, the cancer is multiple myeloma. The particular cancer can be responsive to chemo- or radiation therapy or the cancer can be Arefractory. A refractory cancer refers to a cancer that is not amendable to surgical intervention and the cancer is either initially unresponsive to chemo- or radiation therapy or the cancer becomes unresponsive over time. Cancer further includes relapsed or refractory large B-cell lymphoma after two or more lines of systemic therapy, including diffuse large B-cell lymphoma (DLBCL) not otherwise specified, primary mediastinal large B-cell lymphoma after two or more lines of systemic therapy, high grade B-cell lymphoma, and DLBCL arising from follicular lymphoma.

[72] An “anti-tumor effect” as used herein, refers to a biological effect that can present as a decrease in tumor volume, a decrease in the number of tumor cells, a decrease in tumor cell proliferation, a decrease in the number of metastases, an increase in overall or progression-free survival, an increase in life expectancy, or amelioration of various physiological symptoms associated with the tumor. An anti-tumor effect can also refer to the prevention of the occurrence of a tumor, *e.g.*, a vaccine.

[73] A “cytokine,” as used herein, refers to a non-antibody protein that is released by one cell in response to contact with a specific antigen, wherein the cytokine interacts with a second cell to mediate a response in the second cell. A cytokine can be endogenously expressed by a cell or administered to a subject. Cytokines may be released by immune cells, including macrophages, B cells, T cells, and mast cells to propagate an immune response. Cytokines can

induce various responses in the recipient cell. Cytokines can include homeostatic cytokines, chemokines, pro-inflammatory cytokines, effectors, and acute-phase proteins. For example, homeostatic cytokines, including interleukin (IL) 7 and IL-15, promote immune cell survival and proliferation, and pro-inflammatory cytokines can promote an inflammatory response. Examples of homeostatic cytokines include, but are not limited to, IL-2, IL-4, IL-5, IL-7, IL-10, IL-12p40, IL-12p70, IL-15, and interferon (IFN) gamma. Examples of pro-inflammatory cytokines include, but are not limited to, IL-1a, IL-1b, IL-6, IL-13, IL-17a, tumor necrosis factor (TNF)-alpha, TNF-beta, fibroblast growth factor (FGF) 2, granulocyte macrophage colony-stimulating factor (GM-CSF), soluble intercellular adhesion molecule 1 (sICAM-1), soluble vascular adhesion molecule 1 (sVCAM-1), vascular endothelial growth factor (VEGF), VEGF-C, VEGF-D, and placental growth factor (PLGF). Examples of effectors include, but are not limited to, granzyme A, granzyme B, soluble Fas ligand (sFasL), and perforin. Examples of acute phase-proteins include, but are not limited to, C-reactive protein (CRP) and serum amyloid A (SAA).

[74] “Chemokines” are a type of cytokine that mediates cell chemotaxis, or directional movement. Examples of chemokines include, but are not limited to, IL-8, IL-16, eotaxin, eotaxin-3, macrophage-derived chemokine (MDC or CCL22), monocyte chemotactic protein 1 (MCP-1 or CCL2), MCP-4, macrophage inflammatory protein 1α (MIP-1α, MIP-1a), MIP-1β (MIP-1b), gamma-induced protein 10 (IP-10), and thymus and activation regulated chemokine (TARC or CCL17).

[75] A “therapeutically effective amount,” “effective dose,” “effective amount,” or “therapeutically effective dosage” of a therapeutic agent, *e.g.*, engineered CAR T cells, is any amount that, when used alone or in combination with another therapeutic agent, protects a subject against the onset of a disease or promotes disease regression evidenced by a decrease in severity of disease symptoms, an increase in frequency and duration of disease symptom-free periods, or a prevention of impairment or disability due to the disease affliction. The ability of a therapeutic agent to promote disease regression can be evaluated using a variety of methods known to the skilled practitioner, such as in human subjects during clinical trials, in animal model systems predictive of efficacy in humans, or by assaying the activity of the agent in *in vitro* assays.

[76] The term “lymphocyte” includes natural killer (NK) cells, T cells, or B cells. NK cells are a type of cytotoxic (cell toxic) lymphocyte that represent a major component of the inherent immune system. NK cells reject tumors and cells infected by viruses. It works through the

process of apoptosis or programmed cell death. They were termed “natural killers” because they do not require activation in order to kill cells. T-cells play a major role in cell-mediated-immunity (no antibody involvement). Its T-cell receptors (TCR) differentiate themselves from other lymphocyte types. The thymus, a specialized organ of the immune system, is primarily responsible for the T cell’s maturation. There are six types of T-cells, namely: Helper T-cells (*e.g.*, CD4⁺ cells), Cytotoxic T-cells (also known as TC, cytotoxic T lymphocyte, CTL, T-killer cell, cytolytic T cell, CD8⁺ T-cells or killer T cell), Memory T-cells ((i) stem memory T_{SCM} cells, like naive cells, are CD45RO[−], CCR7⁺, CD45RA⁺, CD62L⁺ (L-selectin), CD27⁺, CD28⁺ and IL-7Rα⁺, but they also express large amounts of CD95, IL-2Rβ, CXCR3, and LFA-1, and show numerous functional attributes distinctive of memory cells); (ii) central memory T_{CM} cells express L-selectin and the CCR7, they secrete IL-2, but not IFNγ or IL-4, and (iii) effector memory T_{EM} cells, however, do not express L-selectin or CCR7 but produce effector cytokines like IFNγ and IL-4), Regulatory T-cells (Tregs, suppressor T cells, or CD4⁺CD25⁺ regulatory T cells), Natural Killer T-cells (NKT) and Gamma Delta T-cells. B-cells, on the other hand, play a principal role in humoral immunity (with antibody involvement). It makes antibodies and antigens and performs the role of antigen-presenting cells (APCs) and turns into memory B-cells after activation by antigen interaction. In mammals, immature B-cells are formed in the bone marrow, where its name is derived from.

[77] “Linker” (L) or “linker domain” or “linker region” refers to an oligo- or polypeptide region from about 1 to 100 amino acids in length, which links together any of the domains/regions of the CAR of the invention. Linkers may be composed of flexible residues like glycine and serine so that the adjacent protein domains are free to move relative to one another. Longer linkers may be used when it is desirable to ensure that two adjacent domains do not sterically interfere with one another. Linkers may be cleavable or non-cleavable. Examples of cleavable linkers include 2A linkers (for example T2A), 2A-like linkers or functional equivalents thereof and combinations thereof. In some embodiments, the linkers include the picornaviral 2A-like linker, CHYSEL sequences of porcine teschovirus (P2A), virus (T2A) or combinations, variants and functional equivalents thereof. In other embodiments, the linker sequences may comprise Asp-Val/Ile-Glu-X-Asn-Pro-Gly.^(2A)-Pro.^(2B) motif (SEQ ID NO: 314), which results in cleavage between the 2A glycine and the 2B proline. Other linkers will be apparent to those of skill in the art and may be used in connection with alternate embodiments of the invention. By way of example, in some examples, a linker may be used to connect or link different antigen binding systems such as two CARs of a

bicistronic CAR. A linker may be a portion of a multi-element agent that connects different elements to one another. For example, a polypeptide comprises two or more functional or structural domains may comprise a stretch of amino acids between such domains that links them to one another. In some embodiments, a polypeptide comprising a linker element has an overall structure of the general form S1-L-S2, wherein S1 and S2 may be the same or different and represent two domains associated with one another by the linker. A linker may connect or link together any of the domains/regions of a CAR of the present disclosure. In some embodiments, a polypeptide linker is at least 2, 3, 4, 5, 6, 7, 8, 9, 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, 75, 80, 85, 90, 95, 100 or more amino acids in length (*e.g.*, 1 to 10, 1 to 20, 1 to 30, 1 to 40, 1 to 50, 1 to 60, 1 to 70, 1 to 80, 1 to 90, 1 to 100, 10 to 20, 10 to 30, 10 to 40, 10 to 50, 10 to 60, 10 to 70, 10 to 80, 10 to 90, or 10 to 100 amino acids in length). In some embodiments, a linker is characterized in that it tends not to adopt a rigid three-dimensional structure, and instead provides flexibility to the polypeptide.

[78] "Single chain variable fragment", "single-chain antibody variable fragments" or "scFv" antibodies refer to forms of antibodies comprising the variable regions of only the heavy and light chains, connected by a linker peptide.

[79] The term "genetically engineered" or "engineered" refers to a method of modifying the genome of a cell, including, but not limited to, deleting a coding or non-coding region or a portion thereof or inserting a coding region or a portion thereof. In some embodiments, the cell that is modified is a lymphocyte, *e.g.*, a T cell, which can either be obtained from a patient or a donor. The cell can be modified to express an exogenous construct, such as, *e.g.*, a chimeric antigen receptor (CAR) or a T cell receptor (TCR), which is incorporated into the cell's genome. Engineering generally comprises manipulation by the hand of man. For example, a polynucleotide is considered to be "engineered" when two or more sequences, that are not linked or connected together in that order in nature, are manipulated by the hand of man to be directly linked or connected to one another in the engineered polynucleotide. In the context of manipulation of cells by techniques of molecular biology, a cell or organism is considered to be "engineered" if it has been manipulated so that its genetic information is altered (*e.g.*, new genetic material not previously present has been introduced, for example by transformation, somatic hybridization, transfection, transduction, or other mechanism, or previously present genetic material is altered or removed, for example by substitution or deletion mutation, or by other protocols). In some embodiments, a binding agent is a modified lymphocyte, *e.g.*, a T

cell, may be obtained from a patient or a donor. An engineered cell may be modified to express an exogenous construct, such as, *e.g.*, a chimeric antigen receptor (CAR) or a T cell receptor (TCR), which is incorporated into the cell's genome. Progeny of an engineered polynucleotide or binding agent are generally referred to as “engineered” even though the actual manipulation was performed on a prior entity. In some embodiments, “engineered” refers to an entity that has been designed and produced. The term “designed” refers to an agent (i) whose structure is or was selected by the hand of man; (ii) that is produced by a process requiring the hand of man; and/or (iii) that is distinct from natural substances and other known agents. A “T cell receptor” or “TCR” refers to antigen-recognition molecules present on the surface of T-cells. During normal T-cell development, each of the four TCR genes, α , β , γ , and δ , may rearrange leading to highly diverse TCR proteins.

[80] An “immune response” refers to the action of a cell of the immune system (for example, T lymphocytes, B lymphocytes, natural killer (NK) cells, macrophages, eosinophils, mast cells, dendritic cells and neutrophils) and soluble macromolecules produced by any of these cells or the liver (including Abs, cytokines, and complement) that results in selective targeting, binding to, damage to, destruction of, and/or elimination from a vertebrate's body of invading pathogens, cells or tissues infected with pathogens, cancerous or other abnormal cells, or, in cases of autoimmunity or pathological inflammation, normal human cells or tissues.

[81] The term “immunotherapy” refers to the treatment of a subject afflicted with, or at risk of contracting or suffering a recurrence of, a disease by a method comprising inducing, enhancing, suppressing or otherwise modifying an immune response. Examples of immunotherapy include, but are not limited to, T cell therapies. T cell therapy can include adoptive T cell therapy, tumor-infiltrating lymphocyte (TIL) immunotherapy, autologous cell therapy, engineered autologous cell therapy (eACT™), and allogeneic T cell transplantation. However, one of skill in the art would recognize that the conditioning methods disclosed herein would enhance the effectiveness of any transplanted T cell therapy. Examples of T cell therapies are described in U.S. Patent Publication Nos. 2014/0154228 and 2002/0006409, U.S. Patent No. 5,728,388, and International Publication No. WO 2008/081035.

[82] The T cells of the immunotherapy can come from any source known in the art. For example, T cells can be differentiated *in vitro* from a hematopoietic stem cell population, or T cells can be obtained from a subject. T cells can be obtained from, *e.g.*, peripheral blood mononuclear cells (PBMCs), bone marrow, lymph node tissue, cord blood, thymus tissue, tissue from a site of infection, ascites, pleural effusion, spleen tissue, and tumors. In addition,

the T cells can be derived from one or more T cell lines available in the art. T cells can also 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 and/or apheresis. Additional methods of isolating T cells for a T cell therapy are disclosed in U.S. Patent Publication No. 2013/0287748, which is herein incorporated by references in its entirety.

[83] The term “engineered Autologous Cell Therapy,” which can be abbreviated as “eACT™,” also known as adoptive cell transfer, is a process by which a patient's own T cells are collected and subsequently genetically altered to recognize and target one or more antigens expressed on the cell surface of one or more specific tumor cells or malignancies. T cells can be engineered to express, for example, chimeric antigen receptors (CAR) or T cell receptor (TCR). CAR positive (+) T cells are engineered to express an extracellular single chain variable fragment (scFv) with specificity for a particular tumor antigen linked to an intracellular signaling part comprising at least one costimulatory domain and at least one activating domain. The costimulatory domain can be derived from a naturally-occurring costimulatory domain, *e.g.*, having the amino acid sequence of SEQ ID NO: 232, or a variant thereof, *e.g.*, a variant having a truncated hinge domain (“THD”), and the activating domain can be derived from, *e.g.*, CD3-zeta. In certain embodiments, the CAR is designed to have two, three, four, or more costimulatory domains. The CAR scFv can be designed to target, for example, CD19, which is a transmembrane protein expressed by cells in the B cell lineage, including all normal B cells and B cell malignancies, including but not limited to NHL, CLL, and non-T cell ALL. In some embodiments, the CAR is engineered such that the costimulatory domain is expressed as a separate polypeptide chain. Example CAR T cell therapies and constructs are described in U.S. Patent Publication Nos. 2013/0287748, 2014/0227237, 2014/0099309, and 2014/0050708, and these references are incorporated by reference in their entirety. “Adoptive cell therapy” or “ACT” involves transfer of immune cells with anti-tumor activity into a subject, *e.g.*, a cancer patient. In some embodiments, ACT is a treatment approach that involves the use of lymphocytes (*e.g.*, engineered lymphocytes) with anti-tumor activity.

[84] A “patient” includes any human who is afflicted with a cancer (*e.g.*, a lymphoma or a leukemia). The terms “subject” and “patient” are used interchangeably herein.

[85] The term “*in vitro*” refers to events occurring in an artificial environment, *e.g.*, in a test tube, reaction vessel, cell culture, etc., rather than within a multi-cellular organism. The term “*in vitro* cell” refers to any cell which is cultured *ex vivo*. In particular, an *in vitro* cell can

include a T cell. The term “*in vivo*” refers to events that occur within a multi-cellular organism, such as a human or a non-human animal.

[86] “Antigen-specific targeting region” (ASTR) refers to the region of the CAR which targets specific antigens. The CARs of the invention comprise at least two targeting regions which target at least two different antigens. In an embodiment, CARs comprise three or more targeting regions which target at least three or more different antigens. The targeting regions on the CAR are extracellular. In some embodiments, the antigen-specific targeting regions comprise an antibody or a functional equivalent thereof or a fragment thereof or a derivative thereof and each of the targeting regions target a different antigen. The targeting regions may comprise full length heavy chain, Fab fragments, single chain Fv (scFv) fragments, divalent single chain antibodies or diabodies, each of which are specific to the target antigen. There are, however, numerous alternatives, such as linked cytokines (which leads to recognition of cells bearing the cytokine receptor), affibodies, ligand binding domains from naturally occurring receptors, soluble protein/peptide ligand for a receptor (for example on a tumor cell), peptides, and vaccines to prompt an immune response, which may each be used in various embodiments of the invention. In fact, almost any molecule that binds a given antigen with high affinity can be used as an antigen-specific targeting region, as will be appreciated by those of skill in the art.

[87] “Antigen presenting cell” or “APC” refers to cells that process and present antigens to T-cells. Exemplary APCs comprise dendritic cells, macrophages, B cells, certain activated epithelial cells, and other cell types capable of TCR stimulation and appropriate T cell costimulation.

[88] The terms “peptide,” “polypeptide,” and “protein” are used interchangeably, and refer to a compound comprised of amino acid residues covalently linked by peptide bonds. A protein or peptide contains at least two amino acids, and no limitation is placed on the maximum number of amino acids that can comprise a protein's or peptide's sequence. Polypeptides include any peptide or protein comprising two or more amino acids joined to each other by peptide bonds. As used herein, the term refers to both short chains, which also commonly are referred to in the art as peptides, oligopeptides and oligomers, for example, and to longer chains, which generally are referred to in the art as proteins, of which there are many types. “Polypeptides” include, for example, biologically active fragments, substantially homologous polypeptides, oligopeptides, homodimers, heterodimers, variants of polypeptides, modified

polypeptides, derivatives, analogs, fusion proteins, among others. The polypeptides include natural peptides, recombinant peptides, synthetic peptides, or a combination thereof.

[89] “Stimulation,” as used herein, refers to a primary response induced by binding of a stimulatory molecule with its cognate ligand, wherein the binding mediates a signal transduction event. A “stimulatory molecule” is a molecule on a T cell, *e.g.*, the T cell receptor (TCR)/CD3 complex, that specifically binds with a cognate stimulatory ligand present on an antigen presenting cell. A “stimulatory ligand” is a ligand that when present on an antigen presenting cell (*e.g.*, an APC, a dendritic cell, a B-cell, and the like) can specifically bind with a stimulatory molecule on a T cell, thereby mediating a primary response by the T cell, including, but not limited to, activation, initiation of an immune response, proliferation, and the like. Stimulatory ligands include, but are not limited to, an anti-CD3 antibody (such as OKT3), an MHC Class I molecule loaded with a peptide, a superagonist anti-CD2 antibody, and a superagonist anti-CD28 antibody.

[90] A “costimulatory signal,” as used herein, refers to a signal, which in combination with a primary signal, such as TCR/CD3 ligation, leads to a T cell response, such as, but not limited to, proliferation and/or upregulation or down regulation of key molecules.

[91] A “costimulatory ligand” as used herein, includes a molecule on an antigen presenting cell that specifically binds a cognate co-stimulatory molecule on a T cell. Binding of the costimulatory ligand provides a signal that mediates a T cell response, including, but not limited to, proliferation, activation, differentiation, and the like. A costimulatory ligand induces a signal that is in addition to the primary signal provided by a stimulatory molecule, for instance, by binding of a T cell receptor (TCR)/CD3 complex with a major histocompatibility complex (MHC) molecule loaded with peptide. A co-stimulatory ligand can include, but is not limited to, 3/TR6, 4-1BB ligand, agonist or antibody that binds Toll ligand receptor, B7-1 (CD80), B7-2 (CD86), CD30 ligand, CD40, CD7, CD70, CD83, herpes virus entry mediator (HVEM), human leukocyte antigen G (HLA-G), ILT4, immunoglobulin-like transcript (ILT) 3, inducible costimulatory ligand (ICOS-L), intercellular adhesion molecule (ICAM), ligand that specifically binds with B7-H3, lymphotoxin beta receptor, MHC class I chain-related protein A (MICA), MHC class I chain-related protein B (MICB), OX40 ligand, PD-L2, or programmed death (PD) L1. A co-stimulatory ligand includes, without limitation, an antibody that specifically binds with a co-stimulatory molecule present on a T cell, such as, but not limited to, 4-1BB, B7-H3, CD2, CD27, CD28, CD30, CD40, CD7, ICOS, ligand that specifically binds with CD83, lymphocyte function-associated antigen-1 (LFA-1), natural killer cell receptor C

(NKG2C), OX40, PD-1, or tumor necrosis factor superfamily member 14 (TNFSF14 or LIGHT).

[92] A “costimulatory molecule” is a cognate binding partner on a T cell that specifically binds with a costimulatory ligand, thereby mediating a costimulatory response by the T cell, such as, but not limited to, proliferation. Costimulatory molecules include, but are not limited to, A “costimulatory molecule” is a cognate binding partner on a T cell that specifically binds with a costimulatory ligand, thereby mediating a costimulatory response by the T cell, such as, but not limited to, proliferation. Costimulatory molecules include, but are not limited to, 4-1BB/CD137, B7-H3, BAFFR, BLAME (SLAMF8), BTLA, CD 33, CD 45, CD100 (SEMA4D), CD103, CD134, CD137, CD154, CD16, CD160 (BY55), CD18, CD19, CD19a, CD2, CD22, CD247, CD27, CD276 (B7-H3), CD28, CD29, CD3 (alpha; beta; delta; epsilon; gamma; zeta), CD30, CD37, CD4, CD4, CD40, CD49a, CD49D, CD49f, CD5, CD64, CD69, CD7, CD80, CD83 ligand, CD84, CD86, CD8alpha, CD8beta, CD9, CD96 (Tactile), CDI-la, CDI-lb, CDI-lc, CDI-lc, CDS, CEACAM1, CRT AM, DAP-10, DNAM1 (CD226), Fc gamma receptor, GADS, GITR, HVEM (LIGHTR), IA4, ICAM-1, ICAM-1, ICOS, Ig alpha (CD79a), IL2R beta, IL2R gamma, IL7R alpha, integrin, ITGA4, ITGA4, ITGA6, ITGAD, ITGAE, ITGAL, ITGAM, ITGAX, ITGB2, ITGB7, ITGB1, KIRDS2, LAT, LFA-1, LFA-1, LIGHT, LIGHT (tumor necrosis factor superfamily member 14; TNFSF14), LTBR, Ly9 (CD229), lymphocyte function-associated antigen-1 (LFA-1 (CD1 la/CD18), MHC class I molecule, NKG2C, NKG2D, NKp30, NKp44, NKp46, NKp80 (KLRF1), OX40, PAG/Cbp, PD-1, PSGL1, SELPLG (CD162), signaling lymphocytic activation molecule, SLAM (SLAMF1; CD150; IPO-3), SLAMF4 (CD244; 2B4), SLAMF6 (NTB-A; Ly108), SLAMF7, SLP-76, TNF, TNFr, TNFR2, Toll ligand receptor, TRANCE/RANKL, VLA1, or VLA-6, or fragments, truncations, or combinations thereof.

[93] The terms “reducing” and “decreasing” are used interchangeably herein and indicate any change that is less than the original. “Reducing” and “decreasing” are relative terms, requiring a comparison between pre- and post- measurements. “Reducing” and “decreasing” include complete depletions.

[94] The terms “improve,” “increase,” “inhibit,” and “reduce” indicate values that are relative to a baseline or other reference measurement. In some embodiments, an appropriate reference measurement may comprise a measurement in certain system (*e.g.*, in a single individual) under otherwise comparable conditions absent presence of (*e.g.*, prior to and/or after) an agent or treatment, or in presence of an appropriate comparable reference agent. In

some embodiments, an appropriate reference measurement may comprise a measurement in comparable system known or expected to respond in a comparable way, in presence of the relevant agent or treatment.

[95] “Treatment” or “treating” of a subject refers to any type of intervention or process performed on, or the administration of an active agent to, the subject with the objective of reversing, alleviating, ameliorating, inhibiting, slowing down or preventing the onset, progression, development, severity or recurrence of a symptom, complication or condition, or biochemical indicia associated with a disease. In one embodiment, “treatment” or “treating” includes a partial remission. In another embodiment, “treatment” or “treating” includes a complete remission. In some embodiments, treatment may be of a subject who does not exhibit signs of the relevant disease, disorder and/or condition and/or of a subject who exhibits only early signs of the disease, disorder, and/or condition. In some embodiments, such treatment may be of a subject who exhibits one or more established signs of the relevant disease, disorder and/or condition. In some embodiments, treatment may be of a subject who has been diagnosed as suffering from the relevant disease, disorder, and/or condition. In some embodiments, treatment may be of a subject known to have one or more susceptibility factors that are statistically correlated with increased risk of development of the relevant disease, disorder, and/or condition.

[96] The term “agent” may refer to a molecule or entity of any class comprising, or a plurality of molecules or entities, any of which may be, for example, a polypeptide, nucleic acid, saccharide, lipid, small molecule, metal, cell, or organism (for example, a fraction or extract thereof) or component thereof. In some embodiments, an agent may be utilized in isolated or pure form. In some embodiments, an agent may be utilized in a crude or impure form. In some embodiments, an agent may be provided as a population, collection, or library, for example that may be screened to identify or characterize members present therein.

[97] Two events or entities are “associated” with one another if the presence, level, and/or form of one is correlated with that of the other. For example, an entity (*e.g.*, polypeptide, genetic signature, metabolite, microbe, etc.) is considered to be associated with a disease, disorder, or condition, if its presence, level, and/or form correlates with incidence of and/or susceptibility to the disease, disorder, or condition (*e.g.*, across a relevant population). For example, two or more entities are physically “associated” with one another if they interact, directly or indirectly, so that they are and/or remain in physical proximity with one another (*e.g.*, bind). In additional examples, two or more entities that are physically associated with one

another are covalently linked or connected to one another, or non-covalently associated, for example by means of hydrogen bonds, van der Waals interaction, hydrophobic interactions, magnetism, and combinations thereof.

[98] Term “identity” refers to the overall relatedness between polymeric molecules, *e.g.*, between nucleic acid molecules (*e.g.*, DNA molecules and/or RNA molecules) and/or between polypeptide molecules. Methods for the calculation of a percent identity as between two provided polypeptide sequences are known. Calculation of the percent identity of two nucleic acid or polypeptide sequences, for example, may be performed by aligning the two sequences for optimal comparison purposes (*e.g.*, gaps may be introduced in one or both of a first and a second sequences for optimal alignment and non-identical sequences may be disregarded for comparison purposes). The nucleotides or amino acids at corresponding positions are then compared. When a position in the first sequence is occupied by the same residue (*e.g.*, nucleotide or amino acid) as the corresponding position in the second sequence, then the molecules are identical at that position. The percent identity between the two sequences is a function of the number of identical positions shared by the sequences, optionally taking into account the number of gaps, and the length of each gap, which may need to be introduced for optimal alignment of the two sequences. Comparison or alignment of sequences and determination of percent identity between two sequences may be accomplished using a mathematical algorithm, such as BLAST (basic local alignment search tool). In some embodiments, polymeric molecules are considered to be “homologous” to one another if their sequences are at least 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 99% identical (*e.g.*, 85-90%, 85-95%, 85-100%, 90-95%, 90-100%, or 95-100%).

[99] To calculate percent identity, the sequences being compared are typically aligned in a way that gives the largest match between the sequences. One example of a computer program that can be used to determine percent identity is the GCG program package, which includes GAP (Devereux et al., 1984, Nucl. Acid Res. 12:387; Genetics Computer Group, University of Wisconsin, Madison, Wis.). The computer algorithm GAP is used to align the two polypeptides or polynucleotides for which the percent sequence identity is to be determined. The sequences are aligned for optimal matching of their respective amino acid or nucleotide (the “matched span,” as determined by the algorithm). In certain embodiments, a standard comparison matrix (*see*, Dayhoff et al., 1978, Atlas of Protein Sequence and Structure 5:345-352 for the PAM 250 comparison matrix; Henikoff et al., 1992, Proc. Natl. Acad. Sci. U.S.A. 89:10915-10919 for

the BLOSUM 62 comparison matrix) is also used by the algorithm. Other algorithms are also available for comparison of amino acid or nucleic acid sequences, comprising those available in commercial computer programs such as BLASTN for nucleotide sequences and BLASTP, gapped BLAST, and PSI-BLAST for amino acid sequences. Exemplary such programs are described in Altschul, et al., Basic local alignment search tool, J. Mol. Biol., 215(3): 403-410, 1990; Altschul, et al., Methods in Enzymology; Altschul, et al., "Gapped BLAST and PSI-BLAST: a new generation of protein database search programs," Nucleic Acids Res. 25:3389-3402, 1997; Baxevanis, et al., Bioinformatics : A Practical Guide to the Analysis of Genes and Proteins, Wiley, 1998; and Misener, et al., (eds.), Bioinformatics Methods and Protocols (Methods in Molecular Biology, Vol. 132), Humana Press, 1999. In addition to identifying similar sequences, the programs mentioned above generally provide an indication of the degree of similarity. In some embodiments, two sequences are considered to be substantially similar if at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or more of their corresponding residues are similar and/or identical over a relevant stretch of residues (e.g., 85-90%, 85-95%, 85-100%, 90-95%, 90-100%, or 95-100%). In some embodiments, the relevant stretch is a complete sequence. In some embodiments, the relevant stretch is at least 10, at least 15, at least 20, at least 25, at least 30, at least 35, at least 40, at least 45, at least 50, at least 55, at least 60, at least 65, at least 70, at least 75, at least 80, at least 85, at least 90, at least 95, at least 100, at least 125, at least 150, at least 175, at least 200, at least 225, at least 250, at least 275, at least 300, at least 325, at least 350, at least 375, at least 400, at least 425, at least 450, at least 475, at least 500 or more residues. Sequences with substantial sequence similarity may be homologs of one another.

[100] "Combination therapy" refers to those situations in which a subject is simultaneously exposed to two or more therapeutic regimens (*e.g.*, two or more therapeutic moieties). In some embodiments, the two or more regimens may be administered simultaneously; in some embodiments, such regimens may be administered sequentially (*e.g.*, all "doses" of a first regimen are administered prior to administration of any doses of a second regimen); in some embodiments, such agents are administered in overlapping dosing regimens. In some embodiments, "administration" of combination therapy may involve administration of one or more agent(s) or modality(ies) to a subject receiving the other agent(s) or modality(ies) in the combination. For clarity, combination therapy does not require that individual agents be

administered together in a single composition (or even necessarily at the same time), although in some embodiments, two or more agents, or active moieties thereof, may be administered together in a combination composition, or even in a combination compound (*e.g.*, as part of a single chemical complex or covalent entity).

[101] “Corresponding to” may be used to designate the position/identity of a structural element in a molecule or composition through comparison with an appropriate reference molecule or composition. For example, in some embodiments, a monomeric residue in a polymer (*e.g.*, an amino acid residue in a polypeptide or a nucleic acid residue in a polynucleotide) may be identified as “corresponding to” a residue in an appropriate reference polymer. For example, for purposes of simplicity, residues in a polypeptide may be designated using a canonical numbering system based on a reference related polypeptide, so that an amino acid “corresponding to” a residue at position 100, for example, need not actually be the 100th amino acid in an amino acid chain provided it corresponds to the residue found at position 100 in the reference polypeptide. Various sequence alignment strategies are available, comprising software programs such as, for example, BLAST, CS-BLAST, CUDASW++, DIAMOND, FASTA, GGSEARCH/GLSEARCH, Genoogle, HMMER, HHpred/HHsearch, IDF, Infernal, KLAST, USEARCH, parasail, PSI-BLAST, PSI-Search, ScalaBLAST, Sequilab, SAM, SSEARCH, SWAPHI, SWAPHI-LS, SWIMM, or SWIPE that may be utilized, for example, to identify “corresponding” residues in polypeptides and/or nucleic acids in accordance with the present disclosure.

[102] The term “domain” refers to a portion of an entity. In some embodiments, a “domain” is associated with a structural and/or functional feature of the entity, *e.g.*, so that, when the domain is physically separated from the rest of its parent entity, it substantially or entirely retains the structural and/or functional feature. In some embodiments, a domain may comprise a portion of an entity that, when separated from that (parent) entity and linked or connected with a different (recipient) entity, substantially retains and/or imparts on the recipient entity one or more structural and/or functional features, *e.g.*, that characterized it in the parent entity. In some embodiments, a domain is a portion of a molecule (*e.g.*, a small molecule, carbohydrate, lipid, nucleic acid, or polypeptide). In some embodiments, a domain is a section of a polypeptide; in some such embodiments, a domain is characterized by a structural element (*e.g.*, an amino acid sequence or sequence motif, α -helix character, β -sheet character, coiled-coil character, random coil character, etc.), and/or by a functional feature (*e.g.*, binding activity, enzymatic activity, folding activity, signaling activity, etc.).

[103] The term “dosage form” may be used to refer to a physically discrete unit of an active agent (*e.g.*, an antigen binding system or antibody) for administration to a subject. Generally, each such unit contains a predetermined quantity of active agent. In some embodiments, such quantity is a unit dosage amount (or a whole fraction thereof) appropriate for administration in accordance with a dosing regimen that has been determined to correlate with a desired or beneficial outcome when administered to a relevant population. The total amount of a therapeutic composition or agent administered to a subject is determined by one or more medical practitioners and may involve administration of more than one dosage forms.

[104] The term “dosing regimen” may be used to refer to a set of one or more unit doses that are administered individually to a subject. In some embodiments, a given therapeutic agent has a recommended dosing regimen, which may involve one or more doses. In some embodiments, a dosing regimen comprises a plurality of doses each of which is separated in time from other doses. In some embodiments, a dosing regimen comprises a plurality of doses and consecutive doses are separated from one another by time periods of equal length; in some embodiments, a dosing regimen comprises a plurality of doses and consecutive doses are separated from one another by time periods of at least two different lengths. In some embodiments, all doses within a dosing regimen are of the same unit dose amount. In some embodiments, different doses within a dosing regimen are of different amounts. In some embodiments, a dosing regimen comprises a first dose in a first dose amount, followed by one or more additional doses in a second dose amount different from the first dose amount. In some embodiments, a dosing regimen is periodically adjusted to achieve a desired or beneficial outcome.

[105] “Effector function” refers to a biological result of interaction of an antibody Fc region with an Fc receptor or ligand. Effector functions comprise, without limitation, antibody-dependent cell-mediated cytotoxicity (ADCC), antibody-dependent cell-mediated phagocytosis (ADCP), and complement-mediated cytotoxicity (CMC). An effector function may be antigen binding dependent, antigen binding independent, or both. ADCC refers to lysis of antibody-bound target cells by immune effector cells. Without wishing to be bound by any theory, ADCC is generally understood to involve Fc receptor (FcR)-bearing effector cells recognizing and subsequently killing antibody-coated target cells (*e.g.*, cells that express on their surface antigens to which an antibody is bound). Effector cells that mediate ADCC may comprise immune cells, comprising yet not limited to, one or more of natural killer (NK) cells, macrophages, neutrophils, eosinophils.

[106] “Effector cell” refers to a cell of the immune system that expresses one or more Fc receptors and mediates one or more effector functions. In some embodiments, effector cells may comprise, without limitation, one or more of monocytes, macrophages, neutrophils, dendritic cells, eosinophils, mast cells, platelets, large granular lymphocytes, Langerhans' cells, natural killer (NK) cells, T-lymphocytes, and B-lymphocytes. Effector cells may be of any organism comprising, without limitation, humans, mice, rats, rabbits, and monkeys.

[107] The term “excipient” refers to an agent that may be comprised in a composition, for example to provide or contribute to a desired consistency or stabilizing effect. In some embodiments, a suitable excipient may comprise, for example, starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol, or the like.

[108] A “fragment” or “portion” of a material or entity as described herein has a structure that comprises a discrete portion of the whole, *e.g.*, of a physical entity or abstract entity. In some embodiments, a fragment lacks one or more moieties found in the whole. In some embodiments, a fragment consists of or comprises a characteristic structural element, domain or moiety found in the whole. In some embodiments, a polymer fragment comprises or consists of at least 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 210, 220, 230, 240, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 500 or more monomeric units (*e.g.*, residues) as found in the whole polymer. In some embodiments, a polymer fragment comprises or consists of at least about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or more of the monomeric units (*e.g.*, residues) found in the whole polymer (*e.g.*, 85-90%, 85-95%, 85-100%, 90-95%, 90-100%, or 95-100%). The whole material or entity may in some embodiments be referred to as the “parent” of the fragment.

[109] The term “fusion polypeptide” or “fusion protein” generally refers to a polypeptide comprising at least two segments. Generally, a polypeptide containing at least two such segments is considered to be a fusion polypeptide if the two segments are moieties that (1) are not comprised in nature in the same peptide, and/or (2) have not previously been linked or connected to one another in a single polypeptide, and/or (3) have been linked or connected to one another through action of the hand of man.

[110] The term “gene product” or “expression product” generally refers to an RNA transcribed from the gene (pre-and/or post-processing) or a polypeptide (pre- and/or post-modification) encoded by an RNA transcribed from the gene.

[111] The term “isolated” refers to a substance that (1) has been separated from at least some components with which it was associated at an earlier time or with which the substance would otherwise be associated, and/or (2) is present in a composition that comprises a limited or defined amount or concentration of one or more known or unknown contaminants. An isolated substance, in some embodiments, may be separated from about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or more than about 99% (e.g., 85-90%, 85-95%, 85-100%, 90-95%, 90-100%, or 95-100%) of other non-substance components with which the substance was associated at an earlier time, *e.g.*, other components or contaminants with which the substance was previously or otherwise would be associated. In certain instances, a substance is isolated if it is present in a composition that comprises a limited or reduced amount or concentration of molecules of a same or similar type. For instance, in certain instances, a nucleic acid, DNA, or RNA substance is isolated if it is present in a composition that comprises a limited or reduced amount or concentration of non-substance nucleic acid, DNA, or RNA molecules. For instance, in certain instances, a polypeptide substance is isolated if it is present in a composition that comprises a limited or reduced amount or concentration of non-substance polypeptide molecules. In certain embodiments, an amount may be, *e.g.*, an amount measured relative to the amount of a desired substance present in a composition. In certain embodiments, a limited amount may be an amount that is no more than 100% of the amount of substance in a composition, *e.g.*, no more than 1%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 95% of the amount of substance in a composition (e.g., 85-90%, 85-95%, 85-100%, 90-95%, 90-100%, or 95-100%). In certain instances, a composition is pure or substantially pure with respect to a selected substance. In some embodiments, an isolated substance is about 80%, about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or more than about 99% pure (e.g., 85-90%, 85-95%, 85-100%, 90-95%, 90-100%, or 95-100%). A substance is “pure” if it is substantially free of other components or of contaminants. In some embodiments, a substance may still be considered “isolated” or even “pure,” after having been combined with certain other components such as, for example, one

or more carriers or excipients (*e.g.*, buffer, solvent, water, etc.); in such embodiments, percent isolation or purity of the substance is calculated without comprising such carriers or excipients.

[112] “Nucleic acid” refers to any polymeric chain of nucleotides. A nucleic acid may be DNA, RNA, or a combination thereof. In some embodiments, a nucleic acid comprises one or more natural nucleic acid residues. In some embodiments, a nucleic acid comprises of one or more nucleic acid analogs. In some embodiments, nucleic acids are prepared by one or more of isolation from a natural source, enzymatic synthesis by polymerization based on a complementary template (*in vivo* or *in vitro*), reproduction in a recombinant cell or system, and chemical synthesis. In some embodiments, a nucleic acid is at least 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 500, 600, 700, 800, 900, 1000, 1500, 2000, 2500, 3000, 3500, 4000, 4500, 5000 or more residues long (*e.g.*, 20 to 100, 20 to 500, 20 to 1000, 20 to 2000, or 20 to 5000 or more residues). In some embodiments, a nucleic acid is partly or wholly single stranded; in some embodiments, a nucleic acid is partly or wholly double stranded. In some embodiments a nucleic acid has a nucleotide sequence comprising at least one element that encodes, or is the complement of a sequence that encodes, a polypeptide.

[113] “Operably linked” refers to a juxtaposition where the components described are in a relationship permitting them to function in their intended manner. For example, a control element “*operably linked*” to a functional element is associated in such a way that expression and/or activity of the functional element is achieved under conditions compatible with the control element.

[114] The term “pharmaceutically acceptable” refers to a molecule or composition that, when administered to a recipient, is not deleterious to the recipient thereof, or that any deleterious effect is outweighed by a benefit to the recipient thereof. With respect to a carrier, diluent, or excipient used to formulate a composition as disclosed herein, a pharmaceutically acceptable carrier, diluent, or excipient must be compatible with the other ingredients of the composition and not deleterious to the recipient thereof, or any deleterious effect must be outweighed by a benefit to the recipient. The term “pharmaceutically acceptable carrier” means a pharmaceutically-acceptable material, composition or vehicle, such as a liquid or solid filler, diluent, excipient, or solvent encapsulating material, involved in carrying or transporting an agent from one portion of the body to another (*e.g.*, from one organ to another). Each carrier present in a pharmaceutical composition must be “acceptable” in the sense of being compatible

with the other ingredients of the formulation and not deleterious to the patient, or any deleterious effect must be outweighed by a benefit to the recipient. Some examples of materials which may serve as pharmaceutically acceptable carriers comprise: sugars, such as lactose, glucose and sucrose; starches, such as corn starch and potato starch; cellulose, and its derivatives, such as sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate; powdered tragacanth; malt; gelatin; talc; excipients, such as cocoa butter and suppository waxes; oils, such as peanut oil, cottonseed oil, safflower oil, sesame oil, olive oil, corn oil and soybean oil; glycols, such as propylene glycol; polyols, such as glycerin, sorbitol, mannitol and polyethylene glycol; esters, such as ethyl oleate and ethyl laurate; agar; buffering agents, such as magnesium hydroxide and aluminum hydroxide; alginic acid; pyrogen-free water; isotonic saline; Ringer's solution; ethyl alcohol; pH buffered solutions; polyesters, polycarbonates and/or polyanhydrides; and other non-toxic compatible substances employed in pharmaceutical formulations.

[115] The term “pharmaceutical composition” refers to a composition in which an active agent is formulated together with one or more pharmaceutically acceptable carriers. In some embodiments, the active agent is present in a unit dose amount appropriate for administration in a therapeutic regimen that shows a statistically significant probability of achieving a predetermined therapeutic effect when administered to a relevant subject or population. In some embodiments, a pharmaceutical composition may be formulated for administration in solid or liquid form, comprising, without limitation, a form adapted for the following: oral administration, for example, drenches (aqueous or non-aqueous solutions or suspensions), tablets, *e.g.*, those targeted for buccal, sublingual, and systemic absorption, boluses, powders, granules, pastes for application to the tongue; parenteral administration, for example, by subcutaneous, intramuscular, intravenous or epidural injection as, for example, a sterile solution or suspension, or sustained-release formulation; topical application, for example, as a cream, ointment, or a controlled-release patch or spray applied to the skin, lungs, or oral cavity; intravaginally or intrarectally, for example, as a pessary, cream, or foam; sublingually; ocularly; transdermally; or nasally, pulmonary, and to other mucosal surfaces.

[116] The term “reference” describes a standard or control relative to which a comparison is performed. For example, in some embodiments, an agent, animal, individual, population, sample, sequence, or value of interest is compared with a reference or control that is an agent, animal, individual, population, sample, sequence, or value. In some embodiments, a reference or control is tested, measured, and/or determined substantially simultaneously with the testing,

measuring, or determination of interest. In some embodiments, a reference or control is a historical reference or control, optionally embodied in a tangible medium. Generally, a reference or control is determined or characterized under comparable conditions or circumstances to those under assessment. When sufficient similarities are present to justify reliance on and/or comparison to a selected reference or control.

[117] “Regulatory T cells” (“Treg”, “Treg cells”, or “Tregs”) refer to a lineage of CD4⁺ T lymphocytes that participate in controlling certain immune activities, *e.g.*, autoimmunity, allergy, and response to infection. Regulatory T cells may regulate the activities of T cell populations, and may also influence certain innate immune system cell types. Tregs may be identified by the expression of the biomarkers CD4, CD25 and Foxp3, and low expression of CD127. Naturally occurring Treg cells normally constitute about 5-10% of the peripheral CD4⁺ T lymphocytes. However, Treg cells within a tumor microenvironment (*i.e.* tumor-infiltrating Treg cells), Treg cells may make up as much as 20-30% of the total CD4⁺ T lymphocyte population.

[118] The term “sample” generally refers to an aliquot of material obtained or derived from a source of interest. In some embodiments, a source of interest is a biological or environmental source. In some embodiments, a source of interest may comprise a cell or an organism, such as a cell population, tissue, or animal (*e.g.*, a human). In some embodiments, a source of interest comprises biological tissue or fluid. In some embodiments, a biological tissue or fluid may comprise amniotic fluid, aqueous humor, ascites, bile, bone marrow, blood, breast milk, cerebrospinal fluid, cerumen, chyle, chime, ejaculate, endolymph, exudate, feces, gastric acid, gastric juice, lymph, mucus, pericardial fluid, perilymph, peritoneal fluid, pleural fluid, pus, rheum, saliva, sebum, semen, serum, smegma, sputum, synovial fluid, sweat, tears, urine, vaginal secretions, vitreous humour, vomit, and/or combinations or component(s) thereof. In some embodiments, a biological fluid may comprise an intracellular fluid, an extracellular fluid, an intravascular fluid (blood plasma), an interstitial fluid, a lymphatic fluid, and/or a transcellular fluid. In some embodiments, a biological fluid may comprise a plant exudate. In some embodiments, a biological tissue or sample may be obtained, for example, by aspirate, biopsy (*e.g.*, fine needle or tissue biopsy), swab (*e.g.*, oral, nasal, skin, or vaginal swab), scraping, surgery, washing or lavage (*e.g.*, bronchoalveolar, ductal, nasal, ocular, oral, uterine, vaginal, or other washing or lavage). In some embodiments, a biological sample comprises cells obtained from an individual. In some embodiments, a sample is a “primary sample” obtained directly from a source of interest by any appropriate means. In some embodiments, as

will be clear from context, the term “sample” refers to a preparation that is obtained by processing (*e.g.*, by removing one or more components of and/or by adding one or more agents to) a primary sample. Such a “processed sample” may comprise, for example nucleic acids or proteins extracted from a sample or obtained by subjecting a primary sample to one or more techniques such as amplification or reverse transcription of nucleic acid, isolation and/or purification of certain components, *etc.*

[119] The term “stage of cancer” refers to a qualitative or quantitative assessment of the level of advancement of a cancer. In some embodiments, criteria used to determine the stage of a cancer may comprise, without limitation, one or more of where the cancer is located in a body, tumor size, whether the cancer has spread to lymph nodes, whether the cancer has spread to one or more different parts of the body, *etc.* In some embodiments, cancer may be staged using the so-called TNM System, according to which T refers to the size and extent of the main tumor, usually called the primary tumor; N refers to the number of nearby lymph nodes that have cancer; and M refers to whether the cancer has metastasized. In some embodiments, a cancer may be referred to as Stage 0 (abnormal cells are present without having spread to nearby tissue, also called carcinoma *in situ*, or CIS; CIS is not cancer, though could become cancer), Stage I-III (cancer is present; the higher the number, the larger the tumor and the more it has spread into nearby tissues), or Stage IV (the cancer has spread to distant parts of the body). In some embodiments, a cancer may be assigned to a stage selected from the group consisting of: *in situ*; localized (cancer is limited to the place where it started, with no sign that it has spread); regional (cancer has spread to nearby lymph nodes, tissues, or organs); distant (cancer has spread to distant parts of the body); and unknown (there is not enough information to determine the stage).

[120] The phrase “therapeutic agent” may refer to any agent that elicits a desired pharmacological effect when administered to an organism. In some embodiments, an agent is considered to be a therapeutic agent if it demonstrates a statistically significant effect across an appropriate population. In some embodiments, the appropriate population may be a population of model organisms or human subjects. In some embodiments, an appropriate population may be defined by various criteria, such as a certain age group, gender, genetic background, preexisting clinical conditions, in accordance with presence or absence of a biomarker, *etc.* In some embodiments, a therapeutic agent is a substance that may be used to alleviate, ameliorate, relieve, inhibit, prevent, delay onset of, reduce severity of, and/or reduce incidence of one or more symptoms or features of a disease, disorder, and/or condition. In some embodiments, a

therapeutic agent is an agent that has been or is required to be approved by a government agency before it may be marketed for administration to humans. In some embodiments, a therapeutic agent is an agent for which a medical prescription is required for administration to humans.

[121] Various aspects of the disclosure are described in further detail in the following subsections. The present disclosure provides antigen binding systems and binding agents comprising at least an anti-CD20 binding motif. Among other things, the present disclosure provides methods and compositions useful for treatment of cancer and/or for initiating or modulating immune responses. In certain embodiments, the present disclosure comprises antigen binding systems and binding agents that are dual-targeted in that they comprise an anti-CD20 binding motif and a second binding motif against a second antigen or epitope. In some instances, the second binding motif selectively binds CD19. In various embodiments, one or more binding motifs is an scFv. Exemplary binding motif amino acid sequences, and nucleic acid sequences encoding the same, are provided herein. In some embodiments, an antigen binding system of the present disclosure is a chimeric antigen receptor. In some embodiments, an antigen binding system of the present disclosure is a bispecific or bicistronic chimeric antigen receptor. In some embodiments, a binding agent of the present disclosure is an engineered T cell receptor.

[122] Various embodiments of the present disclosure provide a vector encoding a binding motif or antigen binding system provided herein, *e.g.*, a vector encoding an anti-CD20/anti-CD19 antigen binding system, such as a bispecific or bicistronic anti-CD20/anti-CD19 chimeric antigen receptor. Various embodiments of the present disclosure provide binding agent that is a cell encoding or expressing an antigen binding system or binding motif provided herein, *e.g.*, a T cell engineered to encode or express an anti-CD20/anti-CD19 chimeric antigen receptor, such as a bispecific or bicistronic anti-CD20/anti-CD19 chimeric antigen receptor. The present disclosure provides binding agents, *e.g.*, comprising binding agents that are immune cells genetically modified with an integrated gene, *e.g.*, a nucleotide sequence of interest (*e.g.*, a constitutive expression construct and/or an inducible expression construct that comprises such nucleotide sequence). In some embodiments, the present disclosure provides methods of treating a subject having a tumor, comprising administering to the subject a binding agent therapy described herein and/or a protein therapeutic described herein. In some embodiments, methods further comprise administration of one or more additional therapies (*e.g.*, a second binding agent (*e.g.*, CAR-T cell, CAR-NK cell, TCR-T cell, TIL cell, allogeneic NK cell, and autologous NK cell), an antibody-drug conjugate, an antibody, a bispecific

antibody, a T cell-engaging bispecific antibody, an engineered antibody, and/or a polypeptide described herein).

[123] Other features, objects, and advantages of the present disclosure are apparent in the detailed description that follows. It should be understood, however, that the detailed description, while indicating embodiments of the present disclosure, is given by way of illustration only, not limitation.

[124] An anti-CD20 binding motif of the present disclosure may comprise antigen-binding sequences as found in an antibody described herein. In some instances, an anti-CD20 binding motif of the present disclosure comprises an antigen binding fragment described herein. Unless otherwise indicated, it is to be appreciated the references to CD20 in the present disclosure relate to human CD20. In various embodiments, an anti-CD20 binding motif of the present disclosure comprises at least one heavy chain CDR (HCDR) provided herein, *e.g.*, at least one HCDR disclosed in any one of Tables 4-13. In various embodiments, an anti-CD20 binding motif of the present disclosure comprises two HCDRs provided herein, *e.g.*, at least two HCDRs disclosed in any one of Tables 4-13. In various embodiments, an anti-CD20 binding motif of the present disclosure comprises three HCDRs provided herein, *e.g.*, three HCDRs disclosed in any one of Tables 4-13. In various embodiments, an anti-CD20 binding motif of the present disclosure comprises at least one light chain CDR (LCDR) provided herein, *e.g.*, at least one LCDR disclosed in any one of Tables 4-13. In various embodiments, an anti-CD20 binding motif of the present disclosure comprises two LCDRs provided herein, *e.g.*, at least two LCDRs disclosed in any one of Tables 4-13. In various embodiments, an anti-CD20 binding motif of the present disclosure comprises three LCDRs provided herein, *e.g.*, three LCDRs disclosed in any one of Tables 4-13.

[125] In various embodiments, an anti-CD20 binding motif of the present disclosure comprises at least one HCDR provided herein, *e.g.*, at least one HCDR disclosed in any one of Tables 4-13, and at least one LCDR provided herein, *e.g.*, at least one LCDR disclosed in any one of Tables 4-13. In various embodiments, an anti-CD20 binding motif of the present disclosure comprises one HCDR provided herein, *e.g.*, at least one HCDR disclosed in any one of Tables 4-13, and one LCDR provided herein, *e.g.*, derived from the same Table of Tables 4-13 as the HCDR(s). In various embodiments, an anti-CD20 binding motif of the present disclosure comprises two HCDRs provided herein, *e.g.*, at least two HCDRs disclosed in any one of Tables 4-13, and two LCDRs provided herein, *e.g.*, at least two LCDRs disclosed in any one of Tables 4-13. In various embodiments, an anti-CD20 binding motif of the present

disclosure comprises two HCDRs provided herein, *e.g.*, at least two HCDRs disclosed in any one of Tables 4-13, and two LCDRs provided herein, *e.g.*, derived from the same Table of Tables 4-13 as the HCDR(s). In various embodiments, an anti-CD20 binding motif of the present disclosure comprises three HCDRs provided herein, *e.g.*, three HCDRs disclosed in any one of Tables 4-13, and three LCDRs provided herein, *e.g.*, three LCDRs disclosed in any one of Tables 4-13. In various embodiments, an anti-CD20 binding motif of the present disclosure comprises three HCDRs provided herein, *e.g.*, three HCDRs disclosed in any one of Tables 4-13, and three LCDRs derived from the same Table of Tables 4-13 as the HCDR(s).

[126] In various embodiments, an anti-CD20 binding motif of the present disclosure comprises at least one heavy chain framework region (heavy chain FR) of a heavy chain variable domain disclosed herein, *e.g.*, at least one heavy chain FR of a heavy chain variable domain disclosed in any one of Tables 4-13. In various embodiments, an anti-CD20 binding motif of the present disclosure comprises two heavy chain FRs of a heavy chain variable domain disclosed herein, *e.g.*, at least two heavy chain FRs of a heavy chain variable domain disclosed in any one of Tables 4-13. In various embodiments, an anti-CD20 binding motif of the present disclosure comprises three heavy chain FRs of a heavy chain variable domain disclosed herein, *e.g.*, three heavy chain FRs of a heavy chain variable domain disclosed in any one of Tables 4-13.

[127] In various embodiments, an anti-CD20 binding motif of the present disclosure comprises at least one light chain FR of a light chain variable domain disclosed herein, *e.g.*, at least one light chain FR of a light chain variable domain disclosed in any one of Tables 4-13. In various embodiments, an anti-CD20 binding motif of the present disclosure comprises two light chain FRs of a light chain variable domain disclosed herein, *e.g.*, at least two light chain FRs of a light chain variable domain disclosed in any one of Tables 4-13. In various embodiments, an anti-CD20 binding motif of the present disclosure comprises three light chain FRs of a light chain variable domain disclosed herein, *e.g.*, three light chain FRs of a light chain variable domain disclosed in any one of Tables 4-13.

[128] In various embodiments, an anti-CD20 binding motif of the present disclosure comprises at least one heavy chain FR of a heavy chain variable domain disclosed herein, *e.g.*, at least one heavy chain FR of a heavy chain variable domain disclosed in any one of Tables 4-13, and at least one light chain FR of a light chain variable domain disclosed herein, *e.g.*, at least one light chain FR of a light chain variable domain disclosed in any one of Tables 4-13. In various embodiments, an anti-CD20 binding motif of the present disclosure comprises one

heavy chain FR of a heavy chain variable domain disclosed herein, *e.g.*, at least one heavy chain FR of a heavy chain variable domain disclosed in any one of Tables 4-13, and one light chain FR of a light chain variable domain disclosed herein, *e.g.*, derived from the same Table of Tables 4-13 as the heavy chain FR(s). In various embodiments, an anti-CD20 binding motif of the present disclosure comprises two heavy chain FRs of a heavy chain variable domain disclosed herein, *e.g.*, at least two heavy chain FRs of a heavy chain variable domain disclosed in any one of Tables 4-13, and two light chain FRs of a light chain variable domain disclosed herein, *e.g.*, at least two light chain FRs of a light chain variable domain disclosed in any one of Tables 4-13. In various embodiments, an anti-CD20 binding motif of the present disclosure comprises two heavy chain FRs of a heavy chain variable domain disclosed herein, *e.g.*, at least two heavy chain FRs of a heavy chain variable domain disclosed in any one of Tables 4-13, and two light chain FRs of a light chain variable domain disclosed herein, *e.g.*, derived from the same Table of Tables 4-13 as the heavy chain FR(s). In various embodiments, an anti-CD20 binding motif of the present disclosure comprises three heavy chain FRs of a heavy chain variable domain disclosed herein, *e.g.*, three heavy chain FRs of a heavy chain variable domain disclosed in any one of Tables 4-13, and three light chain FRs of a light chain variable domain disclosed herein, *e.g.*, three light chain FRs of a light chain variable domain disclosed in any one of Tables 4-13. In various embodiments, an anti-CD20 binding motif of the present disclosure comprises three heavy chain FRs of a heavy chain variable domain disclosed herein, *e.g.*, three light chain FRs of a light chain variable domain disclosed in any one of Tables 4-13, and three light chain FRs derived from the same Table of Tables 4-13 as the heavy chain FR(s).

[129] Exemplary antibody sequences provided in Tables 4-13 are suitable for use in any antibody format, comprising, *e.g.*, a tetrameric antibody, a monospecific antibody, a bispecific antibody, an antigen binding fragment, or a binding motif. Heavy chain variable domains and light chain variable domains and portions thereof provided in Tables 4-13 may be comprised in a binding motif.

[130] In various embodiments, an anti-CD20 binding motif of the present disclosure comprises one, two, or three FRs that together or each individually have at least 75% identity (*e.g.*, at least 75%, at least 80%, at least 90%, at least 95%, or 100%, *e.g.*, 85-90%, 85-95%, 85-100%, 90-95%, 90-100%, or 95-100%) to corresponding FR(s) of a heavy chain variable domain of a heavy chain variable domain disclosed in in any one of Tables 4-13. In various embodiments, an anti-CD20 binding motif of the present disclosure comprises one, two, or three FRs that together or each individually have at least 75% identity (*e.g.*, at least 75%, at

least 80%, at least 90%, at least 95%, or 100%) to corresponding FR(s) of a light chain variable domain of a light chain variable domain disclosed in any one of Tables 4-13.

[131] In various embodiments, an anti-CD20 binding motif of the present disclosure comprises at least one heavy chain variable domain having at least 75% sequence identity to a heavy chain variable domain disclosed in any one of Tables 4-13 (*e.g.*, at least 75%, at least 80%, at least 90%, at least 95%, or 100% identity; *e.g.*, 85-90%, 85-95%, 85-100%, 90-95%, 90-100%, or 95-100%). In various embodiments, an anti-CD20 binding motif of the present disclosure comprises two heavy chain variable domains each having at least 75% sequence identity to a heavy chain variable domain disclosed in Tables 4-13 (*e.g.*, at least 75%, at least 80%, at least 90%, at least 95%, or 100% identity; *e.g.*, 85-90%, 85-95%, 85-100%, 90-95%, 90-100%, or 95-100%), which heavy chain variable domains may be same or different.

[132] In various embodiments, an anti-CD20 binding motif of the present disclosure comprises at least one light chain variable domain having at least 75% sequence identity to a light chain variable domain disclosed in any one of Tables 4-13 (*e.g.*, at least 75%, at least 80%, at least 90%, at least 95%, or 100% identity; *e.g.*, 85-90%, 85-95%, 85-100%, 90-95%, 90-100%, or 95-100%). In various embodiments, an anti-CD20 binding motif of the present disclosure comprises two light chain variable domains each having at least 75% sequence identity to a light chain variable domain disclosed in any one of Tables 4-13 (*e.g.*, at least 75%, at least 80%, at least 90%, at least 95%, or 100% identity; *e.g.*, 85-90%, 85-95%, 85-100%, 90-95%, 90-100%, or 95-100%), which light chain variable domains may be same or different.

[133] In various embodiments, an anti-CD20 binding motif of the present disclosure comprises at least one heavy chain variable domain having at least 75% sequence identity to a heavy chain variable domain disclosed in any one of Tables 4-13 (*e.g.*, at least 75%, at least 80%, at least 90%, at least 95%, or 100% identity; *e.g.*, 85-90%, 85-95%, 85-100%, 90-95%, 90-100%, or 95-100%) and at least one light chain variable domain having at least 75% sequence identity to a light chain variable domain disclosed in any one of Tables 4-13 (*e.g.*, at least 75%, at least 80%, at least 90%, at least 95%, or 100% identity; *e.g.*, 85-90%, 85-95%, 85-100%, 90-95%, 90-100%, or 95-100%). In certain embodiments, an anti-CD20 binding motif of the present disclosure comprises one heavy chain variable domain having at least 75% sequence identity to a heavy chain variable domain disclosed in any one of Tables 4-13 (*e.g.*, at least 75%, at least 80%, at least 90%, at least 95%, or 100% identity; *e.g.*, 85-90%, 85-95%, 85-100%, 90-95%, 90-100%, or 95-100%) and one light chain variable domain having at least 75% sequence identity to a light chain variable domain disclosed in any one of Tables 4-13

(*e.g.*, at least 75%, at least 80%, at least 90%, at least 95%, or 100% identity; *e.g.*, 85-90%, 85-95%, 85-100%, 90-95%, 90-100%, or 95-100%), where the heavy chain variable domain and light chain variable domain are optionally derived from the same Table of Tables 4-13.

[134] In various embodiments, an anti-CD20 binding motif of the present disclosure comprises two heavy chain variable domains each having at least 75% sequence identity to a heavy chain variable domain disclosed in Tables 4-13 (*e.g.*, at least 75%, at least 80%, at least 90%, at least 95%, or 100% identity; *e.g.*, 85-90%, 85-95%, 85-100%, 90-95%, 90-100%, or 95-100%) and two light chain variable domains each having at least 75% sequence identity to a light chain variable domain disclosed in Tables 4-13 (*e.g.*, at least 75%, at least 80%, at least 90%, at least 95%, or 100% identity; *e.g.*, 85-90%, 85-95%, 85-100%, 90-95%, 90-100%, or 95-100%), where, in various embodiments, (i) each of the heavy chain variable domains may be same or different; (ii) each of the light chain variable domains may be same or different; (iii) at least one heavy chain variable domain and at least one light chain variable domain may be derived from the same Table of Tables 4-13; or (iv) the two heavy chain variable domains and the two light chain variable domains are all derived from the same Table of Tables 4-13. Each of Tables 4-13 represents the heavy chain variable domain and light chain variable domain sequences of an exemplary antibody, comprising (i) the heavy chain variable domain of the exemplary antibody; (ii) a DNA sequence encoding the heavy chain variable domain (iii) three heavy chain variable domain CDRs of the heavy chain variable domain, according to IMGT, Kabat, and Chothia numbering; (iv) the light chain variable domain of the exemplary antibody; (v) a DNA sequence encoding the light chain variable domain; and (vi) three light chain variable domain CDRs of the light chain variable domain, according to IMGT, Kabat, and Chothia numbering. Information provided in each table provides framework amino acid sequences, as well as nucleotide sequences encoding each CDR amino acid sequence and nucleotide sequences encoding corresponding FR amino acid sequence.

[135] In various embodiments a binding motif may comprise a heavy chain variable domain of the present disclosure (*e.g.*, having at least 75% sequence identity to a heavy chain variable domain of any one of Tables 4-13, *e.g.*, at least 80%, 85%, 90%, 95%, or 100% identity; *e.g.*, 85-90%, 85-95%, 85-100%, 90-95%, 90-100%, or 95-100%), a light chain variable domain of the present disclosure (*e.g.*, having at least 75% sequence identity to a light chain variable domain of any one of Tables 4-13, *e.g.*, at least 80%, 85%, 90%, 95%, or 100% identity; *e.g.*, 85-90%, 85-95%, 85-100%, 90-95%, 90-100%, or 95-100%), and a linker (*e.g.*, a linker according to SEQ ID NO: 247 and/or a linker according to any one of SEQ ID NOs: 307-313;

see, e.g., Whitlow et al. Protein Eng. 1993 Nov;6(8):989-95.). In various embodiments a binding motif may comprise a leader sequence, a heavy chain variable domain of the present disclosure (e.g., having at least 75% sequence identity to a heavy chain variable domain of any one of Tables 4-13, e.g., at least 80%, 85%, 90%, 95%, or 100% identity; e.g., 85-90%, 85-95%, 85-100%, 90-95%, 90-100%, or 95-100%), a light chain variable domain of the present disclosure (e.g., having at least 75% sequence identity to a light chain variable domain of any one of Tables 4-13, e.g., at least 80%, 85%, 90%, 95%, or 100% identity; e.g., 85-90%, 85-95%, 85-100%, 90-95%, 90-100%, or 95-100%), and a linker. If provided with an amino acid or nucleotide sequence of a binding motif comprising a heavy chain variable domain of the present disclosure and a light chain variable domain of the present disclosure, the linker joining the two variable domains will be apparent from the sequence in view of the present disclosure. If provided with an amino acid or nucleotide sequence of a binding motif comprising a heavy chain variable domain of the present disclosure and a light chain variable domain of the present disclosure, the leader sequence will be apparent in view of the present disclosure. For the avoidance of doubt, a heavy chain variable domain and a light chain variable domain of the present disclosure may be present in any orientation, e.g., an orientation in which the heavy chain variable domain is C terminal of the light chain variable domain or in which the heavy chain variable domain is N terminal of the light chain variable domain. In various embodiments a binding motif may comprise a linker according to SEQ ID NO: 247 and/or a linker according to any one of SEQ ID NOs: 307-313 adjacent to one or more additional linkers.

[136] In certain embodiments, an anti-CD20 binding motif of the present disclosure comprises a binding motif that comprises a heavy chain variable domain of the present disclosure, a light chain variable domain of the present disclosure, and a linker having at least 75% sequence identity to SEQ ID NO: 247 (e.g., at least 75%, at least 80%, at least 90%, at least 95%, or 100% identity; e.g., 85-90%, 85-95%, 85-100%, 90-95%, 90-100%, or 95-100%). In certain embodiments, an anti-CD20 binding motif of the present disclosure comprises a binding motif that comprises a linker according to SEQ ID NO: 247 and/or a linker according to any one of SEQ ID NOs: 307-313. In certain embodiments, an anti-CD20 binding motif of the present disclosure comprises a binding motif that comprises a heavy chain variable domain of the present disclosure, a light chain variable domain of the present disclosure, and a leader sequence having at least 75% sequence identity to SEQ ID NO: 245 (e.g., at least 75%, at least 80%, at least 90%, at least 95%, or 100% identity; e.g., 85-90%, 85-95%, 85-100%, 90-95%, 90-100%, or 95-100%). In certain embodiments, an anti-CD20 binding motif of the present

disclosure comprises a binding motif that comprises a CSF2RA leader sequence according to SEQ ID NO: 245. In certain embodiments, an anti-CD20 binding motif of the present disclosure comprises a binding motif that comprises a heavy chain variable domain of the present disclosure, a light chain variable domain of the present disclosure, a linker of the present disclosure, and a leader sequence of the present disclosure. Exemplary nucleotide sequences encoding an anti-CD19 binding motif and components thereof are found in SEQ ID NOs: 246, and 248. In various embodiments, a binding motif of the present disclosure has a sequence according to any one of the sequences of Table 53 (SEQ ID NOs: 251-260).

[137] A binding agent of the present disclosure that is based on an exemplary antibody provided herein, such as for example Ab1, may be provided in any fragment or format, comprising a heavy chain variable domain according to the indicated exemplary antibody and a light chain variable domain according to the indicated exemplary antibody.

[138] Table 4: Exemplary Antibody Sequences 1 (Ab1)

SEQ ID NO:	Description	Sequence
1	Heavy Chain Variable Domain	QVQLQQWGAGLLKPSETLSLTCAVYGGSFSGYYWSWI RQPPGKGLEWIGEIDHSGSTNYNPSLKSRVTISVDTSKN QFSLKLSSVTAADTAVYYCARGGGSWYSNWFDPWGQ GTMVTVSS
2	VH (DNA)	CAGGTGCAGCTACAGCAGTGGGGCGCAGGACTGTTG AAGCCTTCGGAGACCCCTGTCCCTCACCTGCGCTGTCT ATGGTGGGTCCTTCAGTGGTTACTACTGGAGCTGGAT CCGCCAGCCCCAGGGAAGGGGCTGGAGTGGATTGG GGAAATCGACCATAGTGGAAGCACCAACTACAACCC GTCCCTCAAGAGTCGAGTCACCATATCAGTAGACACG TCCAAGAACCAGTTCTCCCTGAAGCTGAGCTCTGTGA CCGCCGCGGACACGGCGGTGTACTACTGCGCCAGAG GTGGAGGAAGTTGGTACAGCAACTGGTTCGACCCATG GGGACAGGGTACAATGGTCACCGTCTCCTCA
3	CDRH1 IMGT (Prot)	GGSFSGYY
4	CDRH1 Kabat (Prot)	GYYS

5	CDRH1 Chothia (Prot)	GGSFSG
6	CDRH2 IMGT (Prot)	IDHSGST
7	CDRH2 Kabat (Prot)	EIDHSGSTNYNPSLKS
8	CDRH2 Chothia (Prot)	DHSGS
9	CDRH3 IMGT (Prot)	ARGGGSWYSNWFD
10	CDRH3 Kabat (Prot)	GGGSWYSNWFD
11	CDRH3 Chothia (Prot)	GGGSWYSNWFD
12	Light Chain Variable Domain	DIQMTQSPSTLSASVGDRVTITCRASQSISSWLAWYQQK PGKAPKLLIYDASSLESGVPSRFSGSGSGTEFTLTISLQP DDFATYYCQQDRSLPPTFGGGTKVEIK
13	VL (DNA)	GACATCCAGATGACCCAGTCTCCTTCCACCCTGTCTG CATCTGTAGGAGACAGAGTCACCATCACTTGCCGGGC CAGTCAGAGTATTAGTAGCTGGTTGGCCTGGTATCAG CAGAAACCAGGGAAAGCCCCTAAGCTCCTGATCTATG ATGCCTCCAGTTTGGAAAGTGGGGTCCCATCAAGTT CAGCGGCAGTGGATCTGGGACAGAATTCCTCTCACC ATCAGCAGCCTGCAGCCTGATGATTTTGCAACTTATT ACTGCCAGCAGGACCGAAGTCTCCCTCCTACTTTTGG CGGAGGGACCAAGGTTGAGATCAAA
14	CDRL1 IMGT (Prot)	RASQSISSWLA
15	CDRL1 Kabat (Prot)	RASQSISSWLA
16	CDRL1 Chothia (Prot)	RASQSISSWLA
17	CDRL2 IMGT (Prot)	DASSLES
18	CDRL2 Kabat (Prot)	DASSLES
19	CDRL2 Chothia (Prot)	DASSLES
20	CDRL3 IMGT (Prot)	QQDRSLPPT
21	CDRL3 Kabat (Prot)	QQDRSLPPT
22	CDRL3 Chothia (Prot)	QQDRSLPPT

[140] Table 5: Exemplary Antibody Sequences 2 (Ab2)

SEQ ID NO:	Description	Sequence
23	Heavy Chain Variable Domain	QVQLQQWGAGLLKPSETLSLTCAVYGGSFSGIHWNWIR QPPGKGLEWIGDIDTSGSTNYNPSLKS RVTISVDTSKNQF SLKLSSVTAADTAVYYCARLGQESATYLGMDVWGQGT TVTVSS
24	VH (DNA)	CAGGTGCAGCTACAGCAGTGGGGCGCAGGACTGTTG AAGCCTTCGGAGACCCTGTCCCTCACCTGCGCTGTCT ATGGTGGGTCTTCAGTGGTATCCACTGGAAGTGGAT CCGCCAGCCCCAGGGAAGGGGCTGGAGTGGATTGG GGACATCGACACAAGTGAAGCACCAACTACAACCC GTCCCTCAAGAGTCGAGTCACCATATCCGTAGACACG TCCAAGAACCAGTTCTCCCTGAAGCTGAGTTCTGTGA CCGCCGCAGACACGGCGGTGTACTACTGCGCCAGATT GGGACAGGAGTCAGCCACCTATCTCGGAATGGACGT ATGGGGCCAGGGAACAAGTGCACCGTCTCCTCA
25	CDRH1 IMGT (Prot)	GGSFSGIH
26	CDRH1 Kabat (Prot)	GIHWN
27	CDRH1 Chothia (Prot)	GGSFSG
28	CDRH2 IMGT (Prot)	IDTSGST
29	CDRH2 Kabat (Prot)	DIDTSGSTNYNPSLKS
30	CDRH2 Chothia (Prot)	DTSGS
31	CDRH3 IMGT (Prot)	ARLGQESATYLGMDV
32	CDRH3 Kabat (Prot)	LGQESATYLGMDV
33	CDRH3 Chothia (Prot)	LGQESATYLGMDV
34	Light Chain Variable Domain	DIVMTQSPDSLAVSLGERATINCKSSQSVLYSSNNKNYL AWYQQKPGQPPKLLIYWASTRESGVPDRFSGSGSGTDF TLTISSLQAEDVAVYYCQQLYTPFTFGGGTKVEIK
35	VL (DNA)	GACATCGTGATGACCCAGTCTCCAGACTCCCTGGCTG TGTCTCTGGGCGAGAGGGCCACCATCAACTGCAAGTC CAGCCAGAGTGTTTTATACAGCTCCAACAATAAGAAC

		TACTTAGCTTGGTACCAGCAGAAACCAGGACAGCCTC CTAAGCTGCTCATTTACTGGGCATCTACCCGGGAATC CGGGGTCCCTGACCGATTCACTGGCAGCGGGTCTGGG ACAGATTTCACTCTCACCATCAGCAGCCTGCAGGCTG AAGATGTGGCAGTTTATTACTGTCAGCAGCTCTACAC CTACCCTTTCACTTTTGGCGGAGGGACCAAGGTTGAG ATCAAA
36	CDRL1 IMGT (Prot)	KSSQSVLYSSNNKNYLA
37	CDRL1 Kabat (Prot)	KSSQSVLYSSNNKNYLA
38	CDRL1 Chothia (Prot)	KSSQSVLYSSNNKNYLA
39	CDRL2 IMGT (Prot)	WASTRES
40	CDRL2 Kabat (Prot)	WASTRES
41	CDRL2 Chothia (Prot)	WASTRES
42	CDRL3 IMGT (Prot)	QQLYTPFT
43	CDRL3 Kabat (Prot)	QQLYTPFT
44	CDRL3 Chothia (Prot)	QQLYTPFT

[142] Table 6: Exemplary Antibody Sequences 3 (Ab3)

SEQ ID NO:	Description	Sequence
45	Heavy Chain Variable Domain	QLQLQESGPGLVKPSETLSLTCTVSGGSISSSSYWGWIRQPPGKGLEWIGSIYYSGSTYYNPSLKSRTISVDTSKNQFSLKLSSVTAADTAVYYCARETDYSSGMGYGMDVWGQGTTVTVSS
46	VH (DNA)	CAGCTGCAGCTGCAGGAGTCGGGCCAGGACTGGTG AAGCCTTCGGAGACCCTGTCCCTCACCTGCACTGTCT CTGGTGGCTCCATCAGCAGTAGTAGTTACTACTGGGG CTGGATCCGCCAGCCCCAGGGAAGGGGCTGGAGTG GATTGGGAGTATCTATTATAGTGGGAGCACCTACTAC AACCCGTCCCTCAAGAGTCGAGTCACCATATCCGTAG ACACGTCCAAGAACCAGTTCTCCCTGAAGCTGAGTTC TGTGACCGCCGCAGACACGGCGGTGTACTACTGCGCC AGAGAGACTGACTACAGCAGCGGAATGGGATACGGA ATGGACGTATGGGGCCAGGGAACAACCTGTCACCGTCT CCTCA
47	CDRH1 IMGT (Prot)	GGSISSSSYY
48	CDRH1 Kabat (Prot)	SSSYWG
49	CDRH1 Chothia (Prot)	GGSISSSS
50	CDRH2 IMGT (Prot)	IYYSGST
51	CDRH2 Kabat (Prot)	SIYYSGSTYYNPSLKS
52	CDRH2 Chothia (Prot)	YYSGS
53	CDRH3 IMGT (Prot)	ARETDYSSGMGYGMDV
54	CDRH3 Kabat (Prot)	ETDYSSGMGYGMDV
55	CDRH3 Chothia (Prot)	ETDYSSGMGYGMDV
56	Light Chain Variable Domain	DIQMTQSPSSLSASVGDRVTITCRASQSINSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSTDFLTISLQPEDFATYYCQQLADPFTFGGGTKVEIK
57	VL (DNA)	GACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGAGTCACCATCACTTGCCGGGC

		AAGTCAGAGCATTAACAGCTATTTAAATTGGTATCAG CAGAAACCAGGGAAAGCCCCTAAGCTCCTGATCTATG CTGCATCCAGTTTGCAAAGTGGGGTCCCATCAAGGTT CAGTGGCAGTGGATCTGGGACAGATTTCACTCTCACC ATCAGCAGTCTGCAACCTGAAGATTTTGCAACTTACT ACTGCCAGCAAAGCCTCGCCGACCCTTTCACTTTTGG CGGAGGGACCAAGGTTGAGATCAAA
58	CDRL1 IMGT (Prot)	RASQSINSYLN
59	CDRL1 Kabat (Prot)	RASQSINSYLN
60	CDRL1 Chothia (Prot)	RASQSINSYLN
61	CDRL2 IMGT (Prot)	AASSLQS
62	CDRL2 Kabat (Prot)	AASSLQS
63	CDRL2 Chothia (Prot)	AASSLQS
64	CDRL3 IMGT (Prot)	QQLADPFT
65	CDRL3 Kabat (Prot)	QQLADPFT
66	CDRL3 Chothia (Prot)	QQLADPFT

[144] Table 7: Exemplary Antibody Sequences 4 (Ab4)

SEQ ID NO:	Description	Sequence
67	Heavy Chain Variable Domain	QVQLVQSGAEVKKPGASVKVSCKASGYTFKEYGISWV RQAPGQGLEWMGWISAYSGHTYYAQKLQGRVTMTTD TSTSTAYMELRSLRSDDTAVYYCARGPHYDDWSGFIIW FDPWGQGTLLVTVSS
68	VH (DNA)	CAGGTTTCAGCTGGTGCAGTCTGGAGCTGAGGTGAAGA AGCCTGGGGCCTCAGTGAAGGTCTCCTGCAAGGCTTC TGGTTACACCTTTAAAGAATATGGTATCAGCTGGGTG CGACAGGCCCTGGACAAGGGCTTGAGTGGATGGGA TGGATCAGCGCTTACAGTGGTCACACATACTATGCAC AGAAGCTCCAGGGCAGAGTCACCATGACCACAGACA CATCCACGAGCACAGCCTACATGGAGCTGAGGAGCC TGAGATCTGACGACACGGCGGTGTACTACTGCGCCAG AGGGCCTCACTACGACGACTGGAGCGGATTTATCATA TGGTTCGACCCATGGGGACAGGGTACATTGGTCACCG TCTCCTCA
69	CDRH1 IMGT (Prot)	GYTFKEYG
70	CDRH1 Kabat (Prot)	EYGIS
71	CDRH1 Chothia (Prot)	GYTFKE
72	CDRH2 IMGT (Prot)	ISAYSGHT
73	CDRH2 Kabat (Prot)	WISAYSGHTYYAQKLQ
74	CDRH2 Chothia (Prot)	SAYSG
75	CDRH3 IMGT (Prot)	ARGPHYDDWSGFIIWFDP
76	CDRH3 Kabat (Prot)	GPHYDDWSGFIIWFDP
77	CDRH3 Chothia (Prot)	GPHYDDWSGFIIWFDP
78	Light Chain Variable Domain	DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQK PGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFLTITSLQP EDFATYYCQSYRFPPTFGQGTKVEIK

79	VL (DNA)	GACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTG CATCTGTAGGAGACAGAGTCACCATCACTTGCCGGGC AAGTCAGAGCATTAGCAGCTATTTAAATTGGTATCAG CAGAAACCAGGGAAAGCCCCTAAGCTCCTGATCTATG CTGCATCCAGTTTGCAAAGTGGGGTCCCTTCAAGGTT CAGTGGCAGTGGATCTGGGACAGATTTCACTCTCACC ATCAGCAGTCTGCAACCTGAAGATTTTGCAACTTACT ACTGTCAACAGAGTTACAGGTTTCCTCCTACCTTTGGC CAAGGGACCAAGGTTGAGATCAAA
80	CDRL1 IMGT (Prot)	RASQSISSYLN
81	CDRL1 Kabat (Prot)	RASQSISSYLN
82	CDRL1 Chothia (Prot)	RASQSISSYLN
83	CDRL2 IMGT (Prot)	AASSLQS
84	CDRL2 Kabat (Prot)	AASSLQS
85	CDRL2 Chothia (Prot)	AASSLQS
86	CDRL3 IMGT (Prot)	QQSYRFPPT
87	CDRL3 Kabat (Prot)	QQSYRFPPT
88	CDRL3 Chothia (Prot)	QQSYRFPPT

[146] Table 8: Exemplary Antibody Sequences 5 (Ab5)

SEQ ID NO:	Description	Sequence
89	Heavy Chain Variable Domain	QVQLQESGPGLVKPSSETLSLTCTVSGGSISPDHYWGWI RQPPGKGLEWIGSIYASGSTFYNPSLKSRTISVDTSKNQ FSLKLSSVTAADTAVYYCARETDYSSGMGYGMDVWG QGTITVTVSS
90	VH (DNA)	CAGGTGCAGCTGCAGGAGTCGGGCCAGGACTGGTG AAGCCTTCGGAGACCCTGTCCCTCACCTGCACTGTCT CTGGTGGCTCCATCAGCAGTCCCGACCACTACTGGGG CTGGATCCGCCAGCCCCAGGGAAGGGGCTGGAGTG GATTGGGTCCATCTACGCCAGTGGGAGCACCTTCTAC AACCCGTCCCTCAAGAGTCGAGTCACCATATCCGTAG ACACGTCCAAGAACCAGTTCTCCCTGAAGCTGAGCTC TGTGACCGCCGCGGACACGGCGGTGTACTACTGCGCC AGAGAGACTGACTACAGCAGCGGAATGGGATACGGA ATGGACGTATGGGGCCAGGGAACAACCTGTCACCGTCT CCTCA
91	CDRH1 IMGT (Prot)	GGSISPDHY
92	CDRH1 Kabat (Prot)	SPDHYWG
93	CDRH1 Chothia (Prot)	GGSISPD
94	CDRH2 IMGT (Prot)	IYASGST
95	CDRH2 Kabat (Prot)	SIYASGSTFYNPSLKS
96	CDRH2 Chothia (Prot)	YASGS
97	CDRH3 IMGT (Prot)	ARETDYSSGMGYGMDV
98	CDRH3 Kabat (Prot)	ETDYSSGMGYGMDV
99	CDRH3 Chothia (Prot)	ETDYSSGMGYGMDV
100	Light Chain Variable Domain	DIQMTQSPSSLSASVGDRVTITCRASQSINSYLNWYQQK PGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISLQP EDFATYYCQQLADPFTFGGGTKVEIK
101	VL (DNA)	GACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTG CATCTGTAGGAGACAGAGTCACCATCACTTGCCGGGC

		AAGTCAGAGCATTAACAGCTATTTAAATTGGTATCAG CAGAAACCAGGGAAAGCCCCTAAGCTCCTGATCTATG CTGCATCCAGTTTGCAAAGTGGGGTCCCATCAAGGTT CAGTGGCAGTGGATCTGGGACAGATTTCACTCTCACC ATCAGCAGTCTGCAACCTGAAGATTTTGCAACTTACT ACTGCCAGCAAAGCCTCGCCGACCCTTTCACTTTTGG CGGAGGGACCAAGGTTGAGATCAAA
102	CDRL1 IMGT (Prot)	RASQSINSYLN
103	CDRL1 Kabat (Prot)	RASQSINSYLN
104	CDRL1 Chothia (Prot)	RASQSINSYLN
105	CDRL2 IMGT (Prot)	AASSLQS
106	CDRL2 Kabat (Prot)	AASSLQS
107	CDRL2 Chothia (Prot)	AASSLQS
108	CDRL3 IMGT (Prot)	QQLADPFT
109	CDRL3 Kabat (Prot)	QQLADPFT
110	CDRL3 Chothia (Prot)	QQLADPFT

[148] Table 9: Exemplary Antibody Sequences 6 (Ab6)

SEQ ID NO:	Description	Sequence
111	Heavy Chain Variable Domain	QITLKESGPTLVKPTQTLTLCTFSGFSLDTEGVGVGWIR QPPGKALEWLALIYFNDQKRYSPSLKSRLTTTKDTSKNQ VVLMTNMDPVDTA VYYCARDTGYSRWYYGMDVWG QGTTVTVSS
112	VH (DNA)	CAGATCACCTTGAAGGAGTCTGGTCCTACGCTGGTGA AACCACACAGACCCCTACGCTGACCTGCACCTTCTC TGGGTTCTCACTCGACACTGAAGGAGTGGGTGTGGGC TGGATCCGTCAGCCCCAGGAAAGGCCCTGGAGTGG CTTGCACTCATTTATTTCAATGATCAAAAGCGCTACA GCCCATCTCTGAAGAGCAGGCTCACCATCACCAAGGA CACCTCCAAAAACCAGGTGGTCCTTACAATGACCAAC ATGGACCCTGTGGACACGGCGGTGTACTACTGCGCCA GAGACACGGGATACAGCCGATGGTACTACGGCATGG ATGTATGGGGCCAGGGAACAACGTGCACCGTCTCCTC A
113	CDRH1 IMGT (Prot)	GFSLDTEGVG
114	CDRH1 Kabat (Prot)	TEGVGVG
115	CDRH1 Chothia (Prot)	GFSLDTEG
116	CDRH2 IMGT (Prot)	IYFNDQK
117	CDRH2 Kabat (Prot)	LIYFNDQKRYSPSLKS
118	CDRH2 Chothia (Prot)	YFNDQ
119	CDRH3 IMGT (Prot)	ARDTGYSRWYYGMDV
120	CDRH3 Kabat (Prot)	DTGYSRWYYGMDV
121	CDRH3 Chothia (Prot)	DTGYSRWYYGMDV
122	Light Chain Variable Domain	DIQMTQSPSSVSASVGDRVTITCRASQGISSWLAWYQQ KPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFLTISL QPEDFATYYCQAYAYPITFGGGTKVEIK
123	VL (DNA)	GACATCCAGATGACCCAGTCTCCATCTTCCGTGTCTG CATCTGTAGGAGACAGAGTCACCATCACTTGTCTGGGC

		GAGTCAGGGTATTAGCAGCTGGTTAGCCTGGTATCAG CAGAAACCAGGGAAAGCCCCTAAGCTCCTGATCTATG CTGCATCCAGTTTGCAAAGTGGGGTCCCATCAAGGTT CAGCGGCAGTGGATCTGGGACAGATTTCACTCTCACC ATCAGCAGCCTGCAGCCTGAAGATTTTGCAACTTATT ACTGTCAGCAGGCATACGCCTACCCTATCACTTTTGG CGGAGGGACCAAGGTTGAGATCAAA
124	CDRL1 IMGT (Prot)	RASQGISSWLA
125	CDRL1 Kabat (Prot)	RASQGISSWLA
126	CDRL1 Chothia (Prot)	RASQGISSWLA
127	CDRL2 IMGT (Prot)	AASSLQS
128	CDRL2 Kabat (Prot)	AASSLQS
129	CDRL2 Chothia (Prot)	AASSLQS
130	CDRL3 IMGT (Prot)	QQAYAYPIT
131	CDRL3 Kabat (Prot)	QQAYAYPIT
132	CDRL3 Chothia (Prot)	QQAYAYPIT

[150] Table 10: Exemplary Antibody Sequences 7 (Ab7)

SEQ ID NO:	Description	Sequence
133	Heavy Chain Variable Domain	QVQLQQWGAGLLKPSETLSLTCAVYGGSFEEKYYWSWI RQPPGKGLEWIGEIIYHSGLTNYPNPSLKSRTISVDTSKN QFSLKLSSVTAADTAVYYCARVRYDSSDSYYSYDYG MDVWGQGTTTVTVSS
134	VH (DNA)	CAGGTGCAGCTACAGCAGTGGGGCGCAGGACTGTTG AAGCCTTCGGAGACCCTGTCCCTCACCTGCGCTGTCT ATGGTGGGTCTTCGAAAAATACTACTGGAGCTGGAT CCGCCAGCCCCAGGGAAGGGGCTGGAGTGGATTGG GGAAATCTACCATAGTGGACTCACCACTACAACCCG TCCCTCAAGAGTCGAGTCACCATATCAGTAGACACGT CCAAGAACCAGTTCTCCCTGAAGCTGAGCTCTGTGAC CGCCGCGGACACGGCGGTGTACTACTGCGCCAGGGTC AGATACGACAGCAGCGACTCCTACTACTATAGCTACG ATTATGGAATGGACGTATGGGGCCAGGGAACAACCTG TCACCGTCTCCTCA
135	CDRH1 IMGT (Prot)	GGSFEEKYY
136	CDRH1 Kabat (Prot)	KYYWS
137	CDRH1 Chothia (Prot)	GGSFEEK
138	CDRH2 IMGT (Prot)	IYHSGLT
139	CDRH2 Kabat (Prot)	EIYHSGLTNYPNPSLKS
140	CDRH2 Chothia (Prot)	YHSGLT
141	CDRH3 IMGT (Prot)	ARVRYDSSDSYYSYDYGMDV
142	CDRH3 Kabat (Prot)	VRYDSSDSYYSYDYGMDV
143	CDRH3 Chothia (Prot)	VRYDSSDSYYSYDYGMDV
144	Light Chain Variable Domain	DIVLTQSPDLSAVSLGERATINCKSSQSVLYSSNNKNYL AWYQQKPGQPPKLLIYWASSRESGVPDFSGSGSGTDF TLTISSLQAEDVAVYYCQQSYSPFWTFGGGTKVEIK
145	VL (DNA)	GACATCGTGCTGACCCAGTCTCCAGACTCCCTGGCTG TGTCTCTGGGCGAGAGGGCCACCATCAACTGCAAGTC

		CAGCCAGAGTGTTTTATACAGCTCCAACAATAAGAAC TACTTAGCTTGGTACCAGCAGAAACCAGGACAGCCTC CTAAGCTGCTCATTTACTGGGCATCTAGCCGGGAATC CGGGGTCCCTGACCGATTTCAGTGGCAGCGGGTCTGGG ACAGATTTCACTCTCACCATCAGCAGCCTGCAGGCTG AAGATGTGGCAGTTTATTACTGTCAGCAGTCCTACTC CTTCCCTTGGACTTTTGGCGGAGGGACCAAGGTTGAG ATCAAA
146	CDRL1 IMGT (Prot)	KSSQSVLYSSNNKNYLA
147	CDRL1 Kabat (Prot)	KSSQSVLYSSNNKNYLA
148	CDRL1 Chothia (Prot)	KSSQSVLYSSNNKNYLA
149	CDRL2 IMGT (Prot)	WASSRES
150	CDRL2 Kabat (Prot)	WASSRES
151	CDRL2 Chothia (Prot)	WASSRES
152	CDRL3 IMGT (Prot)	QQSYSPWT
153	CDRL3 Kabat (Prot)	QQSYSPWT
154	CDRL3 Chothia (Prot)	QQSYSPWT

[152] Table 11: Exemplary Antibody Sequences 8 (Ab8)

SEQ ID NO:	Description	Sequence
155	Heavy Chain Variable Domain	QVQLQQWGAGLLKPSETLSLTCAVYGGSFsRYVWSWIR QPPGKGLEWIGEIDSSGKTNYNPSLKSrVTISVDTSKNQF SLKLSSVTAADTAVYYCARVRYDSSDSYYYsYDYGMD VWGQGTTVTVSS
156	VH (DNA)	CAGGTGCAGCTACAGCAGTGGGGCGCAGGACTGTTG AAGCCTTCGGAGACCCTGTCCCTCACCTGCGCTGTCT ATGGTGGGTCTTCAGTCGATACGTATGGAGCTGGAT CCGCCAGCCCCAGGGAAGGGGCTGGAGTGGATTGG GGAAATCGACTCCAGTGGA AAAACCAACTACAACCC GTCCCTCAAGAGTCGAGTCACCATATCAGTAGACACG TCCAAGAACCAGTTCTCCCTGAAGCTGAGCTCTGTGA CCGCCGCGGACACGGCGGTGTACTACTGCGCCAGGGT CAGATACGACAGCAGCGACTCCTACTACTATAGCTAC GATTATGGAATGGACGTATGGGGCCAGGGAACAAC GTCACCGTCTCCTCA
157	CDRH1 IMGT (Prot)	GGSFsRYV
158	CDRH1 Kabat (Prot)	RYVWS
159	CDRH1 Chothia (Prot)	GGSFsR
160	CDRH2 IMGT (Prot)	IDSSGKT
161	CDRH2 Kabat (Prot)	EIDSSGKTNYNPSLKS
162	CDRH2 Chothia (Prot)	DSSGK
163	CDRH3 IMGT (Prot)	ARVRYDSSDSYYYsYDYGMDV
164	CDRH3 Kabat (Prot)	VRYDSSDSYYYsYDYGMDV
165	CDRH3 Chothia (Prot)	VRYDSSDSYYYsYDYGMDV
166	Light Chain Variable Domain	DIVLTQSPDLSAVSLGERATINCKSSQSVLYSSNNKNYL AWYQQKPGQPPKLLIYWASSRESGVPDFSGSGSGTDF TLTISSLQAEDVAVYYCQQSYSFPWTFGGGTKVEIK
167	VL (DNA)	GACATCGTGCTGACCCAGTCTCCAGACTCCCTGGCTG TGTCTCTGGGCGAGAGGGCCACCATCAACTGCAAGTC

		CAGCCAGAGTGTTTTATACAGCTCCAACAATAAGAAC TACTTAGCTTGGTACCAGCAGAAACCAGGACAGCCTC CTAAGCTGCTCATTTACTGGGCATCTAGCCGGAATC CGGGGTCCCTGACCGATTTCAGTGGCAGCGGGTCTGGG ACAGATTTCACTCTCACCATCAGCAGCCTGCAGGCTG AAGATGTGGCAGTTTATTACTGTCAGCAGTCCTACTC CTTCCCTTGGACTTTTGGCGGAGGGACCAAGGTTGAG ATCAAA
168	CDRL1 IMGT (Prot)	KSSQSVLYSSNNKNYLA
169	CDRL1 Kabat (Prot)	KSSQSVLYSSNNKNYLA
170	CDRL1 Chothia (Prot)	KSSQSVLYSSNNKNYLA
171	CDRL2 IMGT (Prot)	WASSRES
172	CDRL2 Kabat (Prot)	WASSRES
173	CDRL2 Chothia (Prot)	WASSRES
174	CDRL3 IMGT (Prot)	QQSYSPWT
175	CDRL3 Kabat (Prot)	QQSYSPWT
176	CDRL3 Chothia (Prot)	QQSYSPWT

[154] Table 12: Exemplary Antibody Sequences 9 (Ab9)

SEQ ID NO:	Description	Sequence
177	Heavy Chain Variable Domain	QVQLQQWGAGLLKPSETLSLTCAVYGGSFSGYAWSWI RQPPGKGLEWIGEIDHRGFTNYPNPSLKSRTVISVDTSKN QFSLKLSSVTAADTAVYYCARVRYDSSDSYYSYDYG MDVWGQGTTVTVSS
178	VH (DNA)	CAGGTGCAGCTACAGCAGTGGGGCGCAGGACTGTTG AAGCCTTCGGAGACCCTGTCCCTCACCTGCGCTGTCT ATGGTGGGTCTTCTCCGGTTACGCATGGAGCTGGAT CCGCCAGCCCCAGGGAAGGGGCTGGAGTGGATTGG GGAAATCGACCATCGAGGATTCACCAACTACAACCC GTCCCTCAAGAGTCGAGTCACCATATCAGTAGACACG TCCAAGAACCAGTTCTCCCTGAAGCTGAGCTCTGTGA CCGCCGCGGACACGGCGGTGTACTACTGCGCCAGGGT CAGATACGACAGCAGCGACTCCTACTACTATAGCTAC GATTATGGAATGGACGTATGGGGCCAGGGAACAACCT GTCACCGTCTCCTCA
179	CDRH1 IMGT (Prot)	GGSFSGYA
180	CDRH1 Kabat (Prot)	GYAWS
181	CDRH1 Chothia (Prot)	GGSFSG
182	CDRH2 IMGT (Prot)	IDHRGFT
183	CDRH2 Kabat (Prot)	EIDHRGFTNYPNPSLKS
184	CDRH2 Chothia (Prot)	DHRGF
185	CDRH3 IMGT (Prot)	ARVRYDSSDSYYSYDYGMDV
186	CDRH3 Kabat (Prot)	VRYDSSDSYYSYDYGMDV
187	CDRH3 Chothia (Prot)	VRYDSSDSYYSYDYGMDV
188	Light Chain Variable Domain	DIVLTQSPDLSAVSLGERATINCKSSQSVLYSSNNKNYL AWYQQKPGQPPKLLIYWASSRESGVPDFSGSGSGTDF TLTISSLQAEDVAVYYCQQSYSPFWTFGGGTKVEIK
189	VL (DNA)	GACATCGTGCTGACCCAGTCTCCAGACTCCCTGGCTG TGTCTCTGGGCGAGAGGGCCACCATCAACTGCAAGTC

		CAGCCAGAGTGTTTTATACAGCTCCAACAATAAGAAC TACTTAGCTTGGTACCAGCAGAAACCAGGACAGCCTC CTAAGCTGCTCATTTACTGGGCATCTAGCCGGGAATC CGGGGTCCCTGACCGATTTCAGTGGCAGCGGGTCTGGG ACAGATTTCACTCTCACCATCAGCAGCCTGCAGGCTG AAGATGTGGCAGTTTATTACTGTCAGCAGTCCTACTC CTTCCCTTGGACTTTTGGCGGAGGGACCAAGGTTGAG ATCAAA
190	CDRL1 IMGT (Prot)	KSSQSVLYSSNNKNYLA
191	CDRL1 Kabat (Prot)	KSSQSVLYSSNNKNYLA
192	CDRL1 Chothia (Prot)	KSSQSVLYSSNNKNYLA
193	CDRL2 IMGT (Prot)	WASSRES
194	CDRL2 Kabat (Prot)	WASSRES
195	CDRL2 Chothia (Prot)	WASSRES
196	CDRL3 IMGT (Prot)	QQSYSPWT
197	CDRL3 Kabat (Prot)	QQSYSPWT
198	CDRL3 Chothia (Prot)	QQSYSPWT

[156] Table 13: Exemplary Antibody Sequences 10 (Ab10)

SEQ ID NO:	Description	Sequence
199	Heavy Chain Variable Domain	QVQLQQWGAGLLKPSETLSLTCAVYGGSFQKYYWSWI RQPPGKGLEWIGEIDTSGFTNYNPSLKSRTISVDTSKNQ FSLKLSSVTAADTAVYYCARVGRYSYGYITAFDIWGQ GTTVTVSS
200	VH (DNA)	CAGGTGCAGCTACAGCAGTGGGGCGCAGGACTGTTG AAGCCTTCGGAGACCCTGTCCCTCACCTGCGCTGTCT ATGGTGGGTCTTCCAAAAATACTACTGGAGCTGGAT CCGCCAGCCCCAGGGAAGGGGCTGGAGTGGATTGG GGAAATCGACACCAGTGGATTACCAACTACAACCC GTCCCTCAAGAGTCGAGTCACCATATCAGTAGACACG TCCAAGAACCAGTTCTCCCTGAAGCTGAGCTCTGTGA CCGCCGCGGACACGGCGGTGTACTACTGCGCCAGAGT GGGAAGGTACAGCTACGGATACTATATCACCGCATTC GACATATGGGGTCAGGGTACAACCTGTCACCGTCTCCT CA
201	CDRH1 IMGT (Prot)	GGSFQKYY
202	CDRH1 Kabat (Prot)	KYYWS
203	CDRH1 Chothia (Prot)	GGSFQK
204	CDRH2 IMGT (Prot)	IDTSGFT
205	CDRH2 Kabat (Prot)	EIDTSGFTNYNPSLKS
206	CDRH2 Chothia (Prot)	DTSGF
207	CDRH3 IMGT (Prot)	ARVGRYSYGYITAFDI
208	CDRH3 Kabat (Prot)	VGRYSYGYITAFDI
209	CDRH3 Chothia (Prot)	VGRYSYGYITAFDI
210	Light Chain Variable Domain	DIVMTQSPDSLAVSLGERATINCKSSQSVLYSSNNKNYL AWYQQKPGQPPKLLIYWASTRESGVPDRFSGSGSTDF TLTISSLQAEDVAVYYCQQHYSPFTFGGGTKVEIK
211	VL (DNA)	GACATCGTGATGACCCAGTCTCCAGACTCCCTGGCTG TGTCTCTGGGCGAGAGGGCCACCATCAACTGCAAGTC

		CAGCCAGAGTGTTTTATACAGCTCCAACAATAAGAAC TACTTAGCTTGGTACCAGCAGAAACCAGGACAGCCTC CTAAGCTGCTCATTTACTGGGCATCTACCCGGGAATC CGGGGTCCCTGACCGATTCACTGGCAGCGGGTCTGGG ACAGATTTCACTCTCACCATCAGCAGCCTGCAGGCTG AAGATGTGGCAGTTTATTACTGTCAGCAGCACTACTC CTTCCCTTTCACTTTTGGCGGAGGGACCAAGGTTGAG ATCAAA
212	CDRL1 IMGT (Prot)	KSSQSVLYSSNNKNYLA
213	CDRL1 Kabat (Prot)	KSSQSVLYSSNNKNYLA
214	CDRL1 Chothia (Prot)	KSSQSVLYSSNNKNYLA
215	CDRL2 IMGT (Prot)	WASTRES
216	CDRL2 Kabat (Prot)	WASTRES
217	CDRL2 Chothia (Prot)	WASTRES
218	CDRL3 IMGT (Prot)	QQHYSFPFT
219	CDRL3 Kabat (Prot)	QQHYSFPFT
220	CDRL3 Chothia (Prot)	QQHYSFPFT

[157] The present disclosure comprises antibodies and antigen binding systems that comprise an anti-CD20 binding motif and a second binding motif that binds a second target antigen or epitope, *e.g.*, an antigen that is not CD20 (*e.g.*, CD19). Dual-targeted antigen binding systems comprise bispecific CARs and bicistronic CARs. Many antigen binding motifs are known. In various embodiments, the second target antigen is CD19. The present specification comprises a variety of second target antigens, comprising, without limitation, a second antigen that is 5T4, alphafetoprotein, B cell maturation antigen (BCMA), CA-125, carcinoembryonic antigen, CD19, CD20, CD22, CD23, CD30, CD33, CD56, CD123, CD138, c-Met, CSPG4, C-type lectin-like molecule 1 (CLL-1), EGFRvIII, epithelial tumor antigen, ERBB2, FLT3, folate binding protein, GD2, GD3, HER1-HER2 in combination, HER2-HER3 in combination, HER2/Neu, HERV-K, HIV-1 envelope glycoprotein gp41, HIV-1 envelope glycoprotein gp120, IL-11Ralpha, kappa chain, lambda chain, melanoma-associated antigen, mesothelin, MUC-1, mutated p53, mutated ras, prostate-specific antigen, ROR1, VEGFR2, or a combination thereof. Accordingly in various embodiments, an antigen binding system or antibody of the present disclosure may comprise a first binding motif that is an anti-CD20

binding motif and a second binding motif that binds a second antigen that is 5T4, alphafetoprotein, B cell maturation antigen (BCMA), CA-125, carcinoembryonic antigen, CD19, CD20, CD22, CD23, CD30, CD33, CD56, CD123, CD138, c-Met, CSPG4, C-type lectin-like molecule 1 (CLL-1), EGFRvIII, epithelial tumor antigen, ERBB2, FLT3, folate binding protein, GD2, GD3, HER1-HER2 in combination, HER2-HER3 in combination, HER2/Neu, HERV-K, HIV-1 envelope glycoprotein gp41, HIV-1 envelope glycoprotein gp120, IL-11Ralpha, kappa chain, lambda chain, melanoma-associated antigen, mesothelin, MUC-1, mutated p53, mutated ras, prostate-specific antigen, ROR1, VEGFR2, EphA3 (EPH receptor A3), BAFFR (B-cell activating factor receptor), or a combination thereof. In some embodiments, a second antigen is an antigen that is characteristic of B-cells or of a subset thereof, optionally wherein the second antigen is not CD19 or CD20. Examples of binding motifs that target these second antigens are known and/or provided herein.

[158] In some instances, in an antigen binding system such as a bispecific CAR that comprises an anti-CD20 binding motif (*e.g.*, comprising a heavy chain variable domain and/or a light chain variable domain of the present disclosure) and an anti-CD19 binding motif (*e.g.*, comprising a heavy chain variable domain and/or a light chain variable domain of the present disclosure), the anti-CD20 binding motif (or a heavy chain variable domain and/or a light chain variable domain thereof) is closer to the C-terminus of the chimeric antigen receptor than the anti-CD19 binding motif (or than a heavy chain variable domain and/or a light chain variable domain thereof). In some instances, in an antigen binding system such as a bispecific CAR comprising an anti-CD20 binding motif (*e.g.*, comprising a heavy chain variable domain and/or a light chain variable domain of the present disclosure) and an anti-CD19 binding motif (*e.g.*, comprising a heavy chain variable domain and/or a light chain variable domain of the present disclosure), the anti-CD20 binding motif (or a heavy chain variable domain and/or a light chain variable domain thereof) is closer to the N-terminus of the agent than the anti-CD19 binding motif (or than a heavy chain variable domain and/or a light chain variable domain thereof).

[159] CD19 (also known as Cluster of Differentiation 19, B-lymphocyte antigen CD19, B-lymphocyte surface antigen B4, B4, CVID3, Differentiation antigen CD19) is a protein that is encoded by the CD19 gene in humans. Unless otherwise indicated, it is to be appreciated the references to CD19 in the present disclosure relate to human CD19. It is found on the surface of B cells. Since CD19 expression is a hallmark of B cells, it may be useful as an antigen, *e.g.*, in recognizing B cells and cancer cells that arise from B cells, *e.g.*, B-cell lymphomas. Anti-CD19 antibodies may bind CD19 expressed on, *e.g.*, B lymphocytes in peripheral blood and

spleen, B cell chronic lymphocytic leukemia (B-CLL) cells, pro lymphocytic leukemia (PLL) cells, hairy cell leukemia (HCL) cells, common acute lymphoblastic leukemia (CALL) cells, pre-B acute lymphoblastic leukemia (pre-B-ALL) cells, and NULL-acute lymphoblastic leukemia (NULL-ALL) cells, to provide a few non limiting examples. An exemplary pharmaceutical product that comprises an antigen binding system that comprises an anti-CD19 binding motif is the pharmaceutical product YESCARTA®. YESCARTA® is a CD19-directed genetically modified autologous T cell immunotherapy indicated for the treatment of adult patients with relapsed or refractory large B-cell lymphoma after two or more lines of systemic therapy, comprising diffuse large B-cell lymphoma (DLBCL) not otherwise specified, primary mediastinal large B-cell lymphoma, high grade B-cell lymphoma, and DLBCL arising from follicular lymphoma (See YESCARTA® FDA-approved package insert, the entirety of which is incorporated herein by reference with respect to methods and compositions relating to immunotherapy). Another exemplary pharmaceutical product that comprises an antigen binding system that comprises an anti-CD19 binding motif is the pharmaceutical product KYMRIA®. KYMRIA® is a CD19-directed genetically modified autologous T-cell immunotherapy indicated for the treatment of: (1) Patients up to 25 years of age with B-cell precursor acute lymphoblastic leukemia (ALL) that is refractory or in second or later relapse; and (2) Adult patients with relapsed or refractory (r/r) large B-cell lymphoma after two or more lines of systemic therapy comprising diffuse large B-cell lymphoma (DLBCL) not otherwise specified, high grade B-cell lymphoma and DLBCL arising from follicular lymphoma (See KYMRIA® FDA-approved package insert, the entirety of which is incorporated herein by reference with respect to methods and compositions relating to immunotherapy).

[160] Both YESCARTA® and KYMRIA® comprise antibody binding domains derived from an anti-human CD19 antibody. Many anti-CD19 antibodies are thought to bind an epitope of CD19 encoded in exon 4 of the CD19 gene. Other anti-CD19 binding motifs may recognize different epitopes of CD19, or the same epitope with differential affinities. Antigen binding systems may comprise antigen binding domains derived, for example, from SJ25C1. The CD19 antibody, clone SJ25C1 was derived from hybridization of Sp2/0 mouse myeloma cells with spleen cells isolated from BALB/c mice immunized with NALM1 and NALM16 cells. SJ25C1 antigen binding domains were used in other investigational CD19-targeting chimeric antigen receptor (CAR) T-cell therapy.

[161] An anti-CD19 binding motif of the present disclosure may comprise antigen-binding sequences as found in an antibody described herein. In some embodiments, an anti-CD19 binding motif of the present disclosure comprises an antigen binding fragment provided herein.

[162] In various embodiments, an anti-CD19 binding motif of the present disclosure comprises at least one HCDR provided herein, *e.g.*, at least one HCDR disclosed in Table 14. In various embodiments, an anti-CD19 binding motif of the present disclosure comprises two HCDRs provided herein, *e.g.*, at least two HCDRs disclosed in Table 14. In various embodiments, an anti-CD19 binding motif of the present disclosure comprises three HCDRs provided herein, *e.g.*, three HCDRs disclosed in Table 14.

[163] In various embodiments, an anti-CD19 binding motif of the present disclosure comprises at least one LCDR provided herein, *e.g.*, at least one LCDR disclosed in Table 14. In various embodiments, an anti-CD19 binding motif of the present disclosure comprises two LCDRs provided herein, *e.g.*, at least two LCDRs disclosed in Table 14. In various embodiments, an anti-CD19 binding motif of the present disclosure comprises three LCDRs provided herein, *e.g.*, three LCDRs disclosed in Table 14.

[164] In various embodiments, an anti-CD19 binding motif of the present disclosure comprises at least one HCDR provided herein, *e.g.*, at least one HCDR disclosed in Table 14, and at least one LCDR provided herein, *e.g.*, at least one LCDR disclosed in Table 14. In various embodiments, an anti-CD19 binding motif of the present disclosure comprises two HCDRs provided herein, *e.g.*, at least two HCDRs disclosed in Table 14, and two LCDRs provided herein, *e.g.*, at least two LCDRs disclosed in Table 14. In various embodiments, an anti-CD19 binding motif of the present disclosure comprises three HCDRs provided herein, *e.g.*, three HCDRs disclosed in Table 14, and three LCDRs provided herein, *e.g.*, three LCDRs disclosed in Table 14.

[165] In various embodiments, an anti-CD19 binding motif of the present disclosure comprises at least one heavy chain framework region (heavy chain FR) of a heavy chain variable domain disclosed herein, *e.g.*, at least one heavy chain FR of a heavy chain variable domain disclosed in Table 14. In various embodiments, an anti-CD19 binding motif of the present disclosure comprises two heavy chain FRs of a heavy chain variable domain disclosed herein, *e.g.*, at least two heavy chain FRs of a heavy chain variable domain disclosed in Table 14. In various embodiments, an anti-CD19 binding motif of the present disclosure comprises three heavy chain FRs of a heavy chain variable domain disclosed herein, *e.g.*, three heavy chain FRs of a heavy chain variable domain disclosed in Table 14.

[166] In various embodiments, an anti-CD19 binding motif of the present disclosure comprises at least one light chain FR of a light chain variable domain disclosed herein, *e.g.*, at least one light chain FR of a light chain variable domain disclosed in Table 14. In various embodiments, an anti-CD19 binding motif of the present disclosure comprises two light chain FRs of a light chain variable domain disclosed herein, *e.g.*, at least two light chain FRs of a light chain variable domain disclosed in Table 14. In various embodiments, an anti-CD19 binding motif of the present disclosure comprises three light chain FRs of a light chain variable domain disclosed herein, *e.g.*, three light chain FRs of a light chain variable domain disclosed in Table 14.

[167] In various embodiments, an anti-CD19 binding motif of the present disclosure comprises at least one heavy chain FR of a heavy chain variable domain disclosed herein, *e.g.*, at least one heavy chain FR of a heavy chain variable domain disclosed in Table 14, and at least one light chain FR of a light chain variable domain disclosed herein, *e.g.*, at least one light chain FR of a light chain variable domain disclosed in Table 14. In various embodiments, an anti-CD19 binding motif of the present disclosure comprises two heavy chain FRs of a heavy chain variable domain disclosed herein, *e.g.*, at least two heavy chain FRs of a heavy chain variable domain disclosed in Table 14, and two light chain FRs of a light chain variable domain disclosed herein, *e.g.*, at least two light chain FRs of a light chain variable domain disclosed in Table 14. In various embodiments, an anti-CD19 binding motif of the present disclosure comprises three heavy chain FRs of a heavy chain variable domain disclosed herein, *e.g.*, three heavy chain FRs of a heavy chain variable domain disclosed in Table 14, and three light chain FRs of a light chain variable domain disclosed herein, *e.g.*, three light chain FRs of a light chain variable domain disclosed in Table 14.

[168] In various embodiments, an anti-CD19 binding motif of the present disclosure comprises one, two, or three FRs that together or each individually have at least 75% identity (*e.g.*, at least 75%, at least 80%, at least 90%, at least 95%, or 100% identity; *e.g.*, 85-90%, 85-95%, 85-100%, 90-95%, 90-100%, or 95-100%) to corresponding FR(s) of a heavy chain variable domain of a heavy chain variable domain disclosed in in Table 14. In various embodiments, an anti-CD19 binding motif of the present disclosure comprises one, two, or three FRs that together or each individually have at least 75% identity (*e.g.*, at least 75%, at least 80%, at least 90%, at least 95%, or 100% identity; *e.g.*, 85-90%, 85-95%, 85-100%, 90-95%, 90-100%, or 95-100%) to corresponding FR(s) of a light chain variable domain of a light chain variable domain disclosed in Table 14.

[169] In various embodiments, an anti-CD19 binding motif of the present disclosure comprises at least one heavy chain variable domain having at least 75% sequence identity to a heavy chain variable domain disclosed in Table 14 (*e.g.*, at least 75%, at least 80%, at least 90%, at least 95%, or 100% identity; *e.g.*, 85-90%, 85-95%, 85-100%, 90-95%, 90-100%, or 95-100%). In various embodiments, an anti-CD19 binding motif of the present disclosure comprises two heavy chain variable domains each having at least 75% sequence identity to a heavy chain variable domain disclosed in Table 14 (*e.g.*, at least 75%, at least 80%, at least 90%, at least 95%, or 100% identity; *e.g.*, 85-90%, 85-95%, 85-100%, 90-95%, 90-100%, or 95-100%), which heavy chain variable domains may be same or different.

[170] In various embodiments, an anti-CD19 binding motif of the present disclosure comprises at least one light chain variable domain having at least 75% sequence identity to a light chain variable domain disclosed in Table 14 (*e.g.*, at least 75%, at least 80%, at least 90%, at least 95%, or 100% identity; *e.g.*, 85-90%, 85-95%, 85-100%, 90-95%, 90-100%, or 95-100%). In various embodiments, an anti-CD19 binding motif of the present disclosure comprises two light chain variable domains each having at least 75% sequence identity to a light chain variable domain disclosed in Table 14 (*e.g.*, at least 75%, at least 80%, at least 90%, at least 95%, or 100% identity; *e.g.*, 85-90%, 85-95%, 85-100%, 90-95%, 90-100%, or 95-100%), which light chain variable domains may be same or different.

[171] In various embodiments, an anti-CD19 binding motif of the present disclosure comprises at least one heavy chain variable domain having at least 75% sequence identity to a heavy chain variable domain disclosed in Table 14 (*e.g.*, at least 75%, at least 80%, at least 90%, at least 95%, or 100% identity; *e.g.*, 85-90%, 85-95%, 85-100%, 90-95%, 90-100%, or 95-100%) and at least one light chain variable domain having at least 75% sequence identity to a light chain variable domain disclosed in Table 14 (*e.g.*, at least 75%, at least 80%, at least 90%, at least 95%, or 100% identity; *e.g.*, 85-90%, 85-95%, 85-100%, 90-95%, 90-100%, or 95-100%).

In various embodiments, an anti-CD19 binding motif of the present disclosure comprises two heavy chain variable domains each having at least 75% sequence identity to a heavy chain variable domain disclosed in Table 14 (*e.g.*, at least 75%, at least 80%, at least 90%, at least 95%, or 100% identity; *e.g.*, 85-90%, 85-95%, 85-100%, 90-95%, 90-100%, or 95-100%) and two light chain variable domains each having at least 75% sequence identity to a light chain variable domain disclosed in Table 14 (*e.g.*, at least 75%, at least 80%, at least 90%, at least 95%, or 100% identity; *e.g.*, 85-90%, 85-95%, 85-100%, 90-95%, 90-100%, or 95-

100%), where, in various embodiments, (i) each of the heavy chain variable domains may be same or different; or (ii) each of the light chain variable domains may be same or different.

[172] In certain embodiments, an anti-CD19 binding motif of the present disclosure comprises a binding motif that comprises a heavy chain variable domain of the present disclosure, a light chain variable domain of the present disclosure, and a linker having at least 75% sequence identity to SEQ ID NO: 247 (*e.g.*, at least 75%, at least 80%, at least 90%, at least 95%, or 100% identity; *e.g.*, 85-90%, 85-95%, 85-100%, 90-95%, 90-100%, or 95-100%). In certain embodiments, an anti-CD19 binding motif of the present disclosure comprises a binding motif that comprises a linker according to SEQ ID NO: 247 and/or a linker according to any one of SEQ ID NOs: 307-313. In certain embodiments, an anti-CD19 binding motif of the present disclosure comprises a binding motif that comprises a heavy chain variable domain of the present disclosure, a light chain variable domain of the present disclosure, and a leader sequence having at least 75% sequence identity to SEQ ID NO: 245 (*e.g.*, at least 75%, at least 80%, at least 90%, at least 95%, or 100% identity; *e.g.*, 85-90%, 85-95%, 85-100%, 90-95%, 90-100%, or 95-100%). In certain embodiments, an anti-CD19 binding motif of the present disclosure comprises a binding motif that comprises a CSF2RA leader sequence according to SEQ ID NO: 245. In certain embodiments, an anti-CD19 binding motif of the present disclosure comprises a binding motif that comprises a heavy chain variable domain of the present disclosure, a light chain variable domain of the present disclosure, a linker of the present disclosure, and a leader sequence of the present disclosure. In certain embodiments a binding motif has the sequence set forth in SEQ ID NO: 243. Exemplary nucleotide sequences encoding an anti-CD19 binding motif and components thereof are found in SEQ ID NOs: 244, 246, and 248. In various embodiments a binding motif may comprise a linker according to SEQ ID NO: 247 and/or a linker according to any one of SEQ ID NOs: 307-313 adjacent to one or more additional linkers.

Table 14: Exemplary anti-CD19 Antibody Sequences (Ab11)

SEQ ID NO:	Description	Sequence
221	Heavy Chain Variable Domain	EVKLQESGPGVLVAPSQSLSVTCTVSGVSLPDYGVSWIRQ PPRKGLEWLGVWGSSETTYNSALKSRLTHKDNSKSQV FLKMNSLQTDDTAIYYCAKHYYYGGSYAMDYWGQGT SVTVSS

222	VH (DNA)	GAGGTGAAACTGCAGGAGTCAGGACCTGGCCTGGTG GCGCCCTCACAGAGCCTGTCCGTCACATGCACTGTCT CAGGGGTCTCATTACCCGACTATGGTGTAAAGCTGGAT TCGCCAGCCTCCACGAAAGGGTCTGGAGTGGCTGGG AGTAATATGGGGTAGTGAAACCACATACTATAATTCA GCTCTCAAATCCAGACTGACCATCATCAAGGACAACT CCAAGAGCCAAGTTTTCTTAAAAATGAACAGTCTGCA AACTGATGACACAGCCATTTACTACTGTGCCAAACAT TATTACTACGGTGGTAGCTATGCTATGGACTACTGGG GTCAAGGAACCTCAGTCACCGTCTCCTCA
223	CDRH1 IMGT (Prot)	GVSLPDYG
224	CDRH1 Kabat (Prot)	DYGVS
225	CDRH1 Chothia (Prot)	GVSLPDY
226	CDRH2 IMGT (Prot)	IWGSETT
227	CDRH2 Kabat (Prot)	VIWGSETTYNSALKS
228	CDRH2 Chothia (Prot)	WGSET
229	CDRH3 IMGT (Prot)	AKHYYYGGSYAMDY
230	CDRH3 Kabat (Prot)	HYYYGGSYAMDY
231	CDRH3 Chothia (Prot)	HYYYGGSYAMDY
232	Light Chain Variable Domain	DIQMTQTTSSLSASLGDRVITSCRASQDISKYLNWYQQK PDGTVKLLIYHTSRLHSGVPSRFSGSGSGTDYSLTISNLE QEDIATYFCQQGNTLPYTFGGGTKLEIT
233	VL (DNA)	GACATCCAGATGACACAGACTACATCCTCCCTGTCTG CCTCTCTGGGAGACAGAGTCACCATCAGTTGCAGGGC AAGTCAGGACATTAGTAAATATTTAAATTGGTATCAG CAGAAACCAGATGGAAGTGTAAACTCCTGATCTACC ATACATCAAGATTACACTCAGGAGTCCCATCAAGGTT

		CAGTGGCAGTGGGTCTGGAACAGATTATTCTCTCACC ATTAGCAACCTGGAGCAAGAAGATATTGCCACTTACT TTTGCCAACAGGGTAATACGCTTCCGTACACGTTCCG AGGGGGGACTAAGTTGGAATAACA
234	CDRL1 IMGT (Prot)	RASQDISKYN
235	CDRL1 Kabat (Prot)	RASQDISKYN
236	CDRL1 Chothia (Prot)	RASQDISKYN
237	CDRL2 IMGT (Prot)	HTSRLHS
238	CDRL2 Kabat (Prot)	HTSRLHS
239	CDRL2 Chothia (Prot)	HTSRLHS
240	CDRL3 IMGT (Prot)	QQGNTLPYT
241	CDRL3 Kabat (Prot)	QQGNTLPYT
242	CDRL3 Chothia (Prot)	QQGNTLPYT
243	binding motif (Prot)	DIQMTQTSSLSASLGDRV TISCRASQDISKYNWYQQK PDGTVKLLIYHTSRLHSGVPSRFSGSGSDYSLTISNLE QEDIATYFCQQGNTLPYTFGGGKLEITGSTSGSGKPGS GEGSTKGEVKLQESGPGLVAPSQSLSVTCTVSGVSLPDY GVSWIRQPPRKGLEWLGVIWGSETTYNSALKSRLTIK DNSKSQVFLKMNSLQTD DTAIYYCAKHYYYGGSYAMD YWGQGTSVTVSS
244	binding motif (DNA)	gacatccagatgacacagactacatcctcctgtctgcctctctgggagacagagtcacc atcagttgcagggaagtcaggacattagtaaattttaattggtatcagcagaaaccag atggaactgttaactcctgatctaccatacatcaagattacactcaggagtcctcatcaag

		gttcagtggcagtgggtctggaacagattattctctaccattagcaacctggagcaaga agatattgccacttacttttgccaacagggtataacgcttccttacacgttcggagggggg actaagttggaataacaggctccacctctggatccggcaagcccggatctggcgagg gatccaccaagggcgaggtgaaactgcaggagtgcaggacctggcctggcgccct cacagagcctgtccgtcacatgcactgtctcaggggtctcattaccgactatgggttaa gctggattcgccagcctccacgaaagggtctggagtggctgggagtaatatgggtagt gaaaccacatactataattcagctctcaaatccagactgaccatcatcaaggacaactcca agagccaagtttcttaaaaatgaacagtctgcaaaactgatgacacagccatttactactgt gccaaacattattactacggtggtagctatgctatggactactgggggtcaaggaacctca gtcaccgtctcctca
245	Leader (CSF2RA) (Prot)	MLLLVTSLLLCELPHPAFLIP
246	Leader (CSF2RA) (DNA)	atgcttctcctgggtgacaagccttctgctctgtgagttaccacaccagcattctctctgat cca
247	Linker (Prot)	GSTSGSGKPGSGEGSTKG
248	Linker (DNA)	ggctccacctctggatccggcaagcccggatctggcgagggatccaccaagggc

[173] Antigen binding systems comprise, as examples, bispecific and bicistronic chimeric antigen receptors (CARs). The present disclosure provides, among other things, antigen binding systems that target both CD20 and a second target antigen, *e.g.*, CD19. In some embodiments, an antigen binding system of the present disclosure comprises a bispecific antigen binding system. In some embodiments, an antigen binding system of the present disclosure comprises a bicistronic antigen binding system (*e.g.*, a system comprising a first CAR and a second CAR, which first and second CARs are expressed in the same cell). Bicistronic CARs can comprise two CARs that bind different targets and are encoded by a single vector. Bicistronic CARs may comprise a first CAR comprising an anti-CD19 binding motif and a second CAR comprising an anti-CD20 binding motif. The binding motif associated with various CAR frameworks is interchangeable, and combinations of features provided in the present Example are exemplary, not limiting. In some embodiments, a first CAR and a second CAR of a bicistronic CAR (*e.g.*, an anti-CD20 CAR and an anti-CD19 CAR) are

encoded by separate genes and/or expressed as separate mRNA molecules. In some embodiments, a first CAR and a second CAR of a bicistronic CAR (*e.g.*, an anti-CD20 CAR and an anti-CD19 CAR) are encoded by a single gene and/or expressed together in a single mRNA molecule, where a protein expressed comprises the first CAR, a cleavable linker domain, and a second CAR. First and second CARs of a bicistronic CAR are generally to be expressed together in immune cells, *e.g.*, in CAR-T cells, so that individual CAR-T cells expressed CARs targeting each of the target antigens (*e.g.*, each of CD20 and CD19).

[174] In various embodiments, a bicistronic CAR vector utilizes a ribosomal skip sequence or internal ribosomal entry sites. A single vector encoding two independent CAR molecules separated by a ribosomal skip sequence may express a bicistronic CAR. In various embodiments, a bicistronic CAR comprises a first CAR and a second CAR where the sequence of the first CAR and the second CAR differ only with respect to the binding motif. In various embodiments, a bicistronic CAR comprises a first CAR and a second CAR where the sequence of the first CAR and the second CAR differ only with respect to a heavy chain variable domain sequence and/or a light chain variable domain sequence. Thus, in some embodiments, a first CAR and a second CAR of a bicistronic CAR may have same or different sequences for any or all of one or more components thereof, *e.g.*, same or different costimulatory domains. For example, one or both of a first CAR and a second CAR of a bicistronic CAR may comprise a costimulatory domain provided herein, such as a CD28, 41BB, OX40, or ICOS costimulatory domain.

[175] A CAR of a bicistronic CAR may comprise a binding motif, a hinge, a transmembrane domain, and an intracellular domain comprising a costimulatory domain and an activation domain. The binding motif may be an anti-CD19 or an anti-CD20 binding motif of the present disclosure. A hinge and transmembrane domain may be a 28T (CD28) domain or a CD8K domain that comprises a hinge domain and a transmembrane domain. A costimulatory domain may be a CD28 or 41BB costimulatory domain. An activation domain may be a CD3z activation domain.

[176] In some embodiments, a first binding motif and a second binding motif (*e.g.*, distinct anti-CD20 and anti-CD19 binding motifs) are both comprised in single bispecific CAR. In such bispecific CARs, a CAR molecule itself may be engineered to recognize more than one antigen. In tandem bispecific CARs, the first and second binding motifs are extracellular and may be characterized as a membrane-proximal binding motif and a membrane-distal binding motif. In some embodiments, an anti-CD20 binding motif is membrane-proximal and an anti-CD19

binding motif is membrane-distal. In other embodiments, an anti-CD19 binding motif is membrane-distal and an anti-CD20 binding motif is membrane proximal.

[177] Chimeric antigen receptors (CARs) are engineered receptors that may direct or redirect T cells (*e.g.*, patient or donor T cells) to target a selected antigen. A CAR may be engineered to recognize an antigen and, when bound to that antigen, activate the immune cell to attack and destroy the cell bearing that antigen. When these antigens exist on tumor cells, an immune cell that expresses the CAR may target and kill the tumor cell. CARs generally comprise an extracellular binding motif that mediates antigen binding (*e.g.*, an anti-CD20 and/or an anti-CD19 binding motif), a transmembrane domain that spans, or is understood to span, the cell membrane when the antigen binding system is present at a cell surface or cell membrane, and an intracellular (or cytoplasmic) signaling domain.

[178] According to at least one non-limiting view, there have been at least three “generations” of CAR compositions. In a first generation of CARs, a binding motif (*e.g.*, a single chain fragment variable, binding motif) is linked or connected to a signaling domain (*e.g.*, CD3 ζ) via a transmembrane domain, optionally comprising a hinge domain and one or more spacers. In a second generation of CARs, a costimulatory domain (CM1, such as CD28, 4-1BB, or OX-40) is introduced with the signaling domain (*e.g.*, CD3 ζ). In a third generation of CARs, a second costimulatory domain (CM2) is comprised.

[179] TCRs are heterodimers composed of an α -chain and a β -chain. TCR signaling requires recruitment of signaling proteins that generate an immune synapse. In addition, TCR localization at the plasma membrane depends on CD3 complex, which is expressed in T cells. Engineered single chain TCRs may be generated, *e.g.*, using transmembrane and signaling domains of CAR constructs, methods and constructs for which are known (*e.g.*, sTCR and TCR-CAR molecules, *e.g.*, fusion of a TCR β chain with CD28 TM and CD28 and CD3 ζ signaling modules). An anti-CD20 and/or anti-CD19 Antigen binding system of the present disclosure may comprise one or more antigen binding motifs that bind CD20 and/or CD19. In some embodiments, an antigen binding system further comprises a costimulatory domain, and/or an extracellular domain (*e.g.*, a “hinge” or “spacer” region), and/or a transmembrane domain, and/or an intracellular (signaling) domain, and/or a CD3-zeta or CD3-epsilon activation domain. In some embodiments, an anti-CD20 and/or anti-CD19 Antigen binding system of the present disclosure comprises at least a binding motif that binds human CD20, a costimulatory domain, an extracellular domain, a transmembrane domain, and a CD3-zeta or CD3-epsilon activating domain.

[180] In some embodiments, an antigen binding system of the present disclosure may comprise an antigen binding system that comprises one or more, or all, of a leader peptide (P), a binding motif (B), a costimulatory protein's extracellular domain (E), a transmembrane domain (T), a costimulatory domain (C), a second costimulatory domain (C'), and an activation domain (A). In some instances, an antigen binding system is configured according to the following: B E T A. In some instances, an antigen binding system is configured according to the following: P B E T A. In some instances, an antigen binding system is configured according to the following: B E T C A. In some instances, an antigen binding system is configured according to the following: P B E T C A. In some instances, an antigen binding system is configured according to the following: B E T C C' A. In some instances, an antigen binding system is configured according to the following: P B E T C C' A. In some embodiments, the antigen binding system comprises a VH and a VL, optionally wherein the CAR is configured according to the following: P-VH-VL-E-T-C-A or P-VL-VH-E-T-C-A. In some embodiments, the VH and the VL are connected by a linker (L), optionally wherein the CAR is configured according to the following, from N-terminus to C-terminus: P-VH-L-VL-E-T-C-A or P-VH-L-VL-E-T-C-A.

[181] One or more antigen binding motifs determine the target(s) of an antigen binding system. A binding motif of an antigen binding system may comprise any binding motif, *e.g.*, an antibody provided by the present disclosure, *e.g.*, a binding motif of the present disclosure. In some embodiments, a binding motif may comprise an anti-CD20 binding motif and/or anti-CD19 binding motif. In some embodiments, a binding motif may comprise an anti-CD20 binding motif and/or anti-CD19 binding motif.

[182] Binding motifs are used in chimeric antigen receptors at least in part because they may be engineered to be expressed as part of a single chain along with the other CAR components. See, for example, U.S. Pat. Nos. 7,741,465, and 6,319,494 as well as Eshhar et al., *Cancer Immunol Immunotherapy* (1997) 45: 131-136, Krause et al., *J. Exp. Med.*, Volume 188, No. 4, 1998 (619-626); Finney et al., *Journal of Immunology*, 1998, 161: 2791-2797, each of which is incorporated herein by reference with respect to binding motif domains in CARs. A binding motif, or scFv, is a single chain antigen binding fragment comprising a heavy chain variable domain and a light chain variable domain, which heavy chain variable domain and light chain variable domain are linked or connected together. See, for example, U.S. Pat. Nos. 7,741,465, and 6,319,494 as well as Eshhar et al., *Cancer Immunol Immunotherapy* (1997) 45: 131-136, each of which is incorporated herein by reference with respect to binding motif domains. When

derived from a parent antibody, a binding motif may retain some of, retain all of, or essentially retain the parent antibody's binding of a target antigen.

[183] A hinge may be an extracellular domain of an antigen binding system positioned between the binding motif and the transmembrane domain. A hinge may also be referred to as an extracellular domain or as a “spacer.” A hinge may contribute to receptor expression, activity, and/or stability. In some embodiments, a hinge domain is positioned between a binding motif and a transmembrane domain. A hinge may also provide flexibility to access the targeted antigen. Hinges comprise immunoglobulin-like hinge domains.

[184] In some embodiments, an Antigen binding system of the present disclosure may comprise a hinge that is, is from, or is derived from (*e.g.*, comprises all or a fragment of) an immunoglobulin-like hinge domain. In some embodiments, a hinge domain is from or derived from an immunoglobulin. In some embodiments, a hinge domain is selected from the hinge of IgG1, IgG2, IgG3, IgG4, IgA, IgD, IgE, or IgM, or a fragment thereof.

[185] A hinge may be derived from a natural source or from a synthetic source. In some embodiments, an Antigen binding system of the present disclosure may comprise a hinge that is, is from, or is derived from (*e.g.*, comprises all or a fragment of) CD2, CD3 delta, CD3 epsilon, CD3 gamma, CD4, CD7, CD8.alpha., CD8.beta., CD11a (ITGAL), CD11b (ITGAM), CD11c (ITGAX), CD11d (ITGAD), CD18 (ITGB2), CD19 (B4), CD27 (TNFRSF7), CD28, CD28T, CD29 (ITGB1), CD30 (TNFRSF8), CD40 (TNFRSF5), CD48 (SLAMF2), CD49a (ITGA1), CD49d (ITGA4), CD49f (ITGA6), CD66a (CEACAM1), CD66b (CEACAM8), CD66c (CEACAM6), CD66d (CEACAM3), CD66e (CEACAM5), CD69 (CLEC2), CD79A (B-cell antigen receptor complex-associated alpha chain), CD79B (B-cell antigen receptor complex-associated beta chain), CD84 (SLAMF5), CD96 (Tactile), CD100 (SEMA4D), CD103 (ITGAE), CD134 (OX40), CD137 (4-1BB), CD150 (SLAMF1), CD158A (KIR2DL1), CD158B1 (KIR2DL2), CD158B2 (KIR2DL3), CD158C (KIR3DP1), CD158D (KIRDL4), CD158F1 (KIR2DL5A), CD158F2 (KIR2DL5B), CD158K (KIR3DL2), CD160 (BY55), CD162 (SELPLG), CD226 (DNAM1), CD229 (SLAMF3), CD244 (SLAMF4), CD247 (CD3-zeta), CD258 (LIGHT), CD268 (BAFFR), CD270 (TNFSF14), CD272 (BTLA), CD276 (B7-H3), CD279 (PD-1), CD314 (NKG2D), CD319 (SLAMF7), CD335 (NK-p46), CD336 (NK-p44), CD337 (NK-p30), CD352 (SLAMF6), CD353 (SLAMF8), CD355 (CRTAM), CD357 (TNFRSF18), inducible T cell co-stimulator (ICOS), LFA-1 (CD11a/CD18), NKG2C, DAP-10, ICAM-1, NKp80 (KLRP1), IL-2R beta, IL-2R gamma, IL-7R alpha, LFA-1, SLAMF9, LAT, GADS (GrpL), SLP-76 (LCP2), PAG1/CBP, a CD83 ligand, Fc gamma receptor, MHC

class 1 molecule, MHC class 2 molecule, a TNF receptor protein, an immunoglobulin protein, a cytokine receptor, an integrin, activating NK cell receptors, or Toll ligand receptor, or which is a fragment or combination thereof. In certain embodiments, a CAR does not comprise a CD28 hinge.

[186] In some embodiments, an antigen binding system of the present disclosure may comprise a hinge that is, is from, or is derived from (*e.g.*, comprises all or a fragment of) a hinge of CD8 alpha. In some embodiments a hinge is, is from, or is derived from a hinge of CD28. In some embodiments, a hinge is, is from, or is derived from a fragment of a hinge of CD8 alpha or a fragment of a hinge of CD28, wherein the fragment is anything less than the whole. In some embodiments, a fragment of a CD8 alpha hinge or a fragment of a CD28 hinge comprises an amino acid sequence that excludes at least 1, at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, or at least 20 amino acids at the N-terminus or C-Terminus, or both, of a CD8 alpha hinge, or of a CD28 hinge. Exemplary hinge sequences comprise those provided in Table 54 (SEQ ID NOs: 261-269).

[187] Polynucleotide and polypeptide sequences of these hinge domains are known. In some embodiments, the polynucleotide encoding a hinge domain comprises a nucleotide sequence at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or about 100% (*e.g.*, 85-90%, 85-95%, 85-100%, 90-95%, 90-100%, or 95-100%) identical to a nucleotide sequence known. In some embodiments, the polypeptide sequence of a hinge domain comprises a polypeptide sequence at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or about 100% (*e.g.*, 85-90%, 85-95%, 85-100%, 90-95%, 90-100%, or 95-100%) identical to a known polypeptide sequence.

[188] In general, a “transmembrane domain” (*e.g.*, of an antigen binding system) refers to a domain having an attribute of being present in the membrane when present in a molecule at a cell surface or cell membrane (*e.g.*, spanning a portion or all of a cellular membrane). A costimulatory domain for an antigen binding system of the present disclosure may further comprise a transmembrane domain and/or an intracellular signaling domain. It is not required that every amino acid in a transmembrane domain be present in the membrane. For example, in some embodiments, a transmembrane domain is characterized in that a designated stretch or

portion of a protein is substantially located in the membrane. Amino acid or nucleic acid sequences may be analyzed using a variety of algorithms to predict protein subcellular localization (*e.g.*, transmembrane localization). The programs psort (PSORT.org) and Prosite (prosite.expasy.org) are exemplary of such programs.

[189] The type of transmembrane domain comprised in an antigen binding system described herein is not limited to any type. In some embodiments, a transmembrane domain is selected that is naturally associated with a binding motif and/or intracellular domain. In some instances, a transmembrane domain comprises a modification of one or more amino acids (*e.g.*, deletion, insertion, and/or substitution), *e.g.*, to avoid binding of such domains to a transmembrane domain of the same or different surface membrane proteins to minimize interactions with other members of the receptor complex.

[190] A transmembrane domain may be derived either from a natural or from a synthetic source. Where the source is natural, a domain may be derived from any membrane-bound or transmembrane protein. Exemplary transmembrane domains may be derived from (*e.g.*, may comprise at least a transmembrane domain of) an alpha, beta or zeta chain of a T-cell receptor, CD28, CD3 epsilon, CD3 delta, CD3 gamma, CD45, CD4, CD5, CD7, CD8, CD8 alpha, CD8beta, CD9, CD11a, CD11b, CD11c, CD11d, CD16, CD22, CD27, CD33, CD37, CD64, CD80, CD86, CD134, CD137, TNFSFR25, CD154, 4-1BB/CD137, activating NK cell receptors, an Immunoglobulin protein, B7-H3, BAFFR, BLAME (SLAMF8), BTLA, CD100 (SEMA4D), CD103, CD160 (BY55), CD18, CD19, CD19a, CD2, CD247, CD276 (B7-H3), CD29, CD30, CD40, CD49a, CD49D, CD49f, CD69, CD84, CD96 (Tactile), CDS, CEACAM1, CRT AM, cytokine receptor, DAP-10, DNAM1 (CD226), Fc gamma receptor, GADS, GITR, HVEM (LIGHTR), IA4, ICAM-1, ICAM-1, Ig alpha (CD79a), IL-2R beta, IL-2R gamma, IL-7R alpha, inducible T cell costimulator (ICOS), integrins, ITGA4, ITGA4, ITGA6, ITGAD, ITGAE, ITGAL, ITGAM, ITGAX, ITGB2, ITGB7, ITGB1, KIRDS2, LAT, LFA-1, LFA-1, a ligand that binds with CD83, LIGHT, LIGHT, LTBR, Ly9 (CD229), lymphocyte function-associated antigen-1 (LFA-1; CD1-1a/CD18), MHC class 1 molecule, NKG2C, NKG2D, NKp30, NKp44, NKp46, NKp80 (KLRF1), OX-40, PAG/Cbp, programmed death-1 (PD-1), PSGL1, SELPLG (CD162), Signaling Lymphocytic Activation Molecules (SLAM proteins), SLAM (SLAMF1; CD150; IPO-3), SLAMF4 (CD244; 2B4), SLAMF6 (NTB-A; Ly108), SLAMF7, SLP-76, TNF receptor proteins, TNFR2, TNFSF14, a Toll ligand receptor, TRANCE/RANKL, VLA1, or VLA-6, or a fragment, truncation, or a combination thereof. In some embodiments, a transmembrane domain may be synthetic (and

can, *e.g.*, comprise predominantly hydrophobic residues such as leucine and valine). In some embodiments, a triplet of phenylalanine, tryptophan and valine are comprised at each end of a synthetic transmembrane domain. In some embodiments, a transmembrane domain is directly linked or connected to a cytoplasmic domain. In some embodiments, a short oligo- or polypeptide linker (*e.g.*, between 2 and 10 amino acids in length) may form a linkage between a transmembrane domain and an intracellular domain. In some embodiments, a linker is a glycine-serine doublet.

[191] Polynucleotide and polypeptide sequences of transmembrane domains provided herein are known. In some embodiments, the polynucleotide encoding a transmembrane domain comprises a nucleotide sequence at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or about 100% (*e.g.*, 85-90%, 85-95%, 85-100%, 90-95%, 90-100%, or 95-100%) identical to a nucleotide sequence known. In some embodiments, the polypeptide sequence of a transmembrane domain comprises a polypeptide sequence at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or about 100% (*e.g.*, 85-90%, 85-95%, 85-100%, 90-95%, 90-100%, or 95-100%) identical to a polypeptide sequence known. Optionally, short spacers may form linkages between any or some of the extracellular, transmembrane, and intracellular domains of the CAR.

[192] The intracellular domain (or cytoplasmic domain) comprises one or more signaling domains that, upon binding of target antigen to the binding motif, cause and/or mediate an intracellular signal, *e.g.*, that activates one or more immune cell effector functions (*e.g.*, native immune cell effector functions). In some embodiments, signaling domains of an intracellular domain mediate activation at least one of the normal effector functions of the immune cell. Effector function of a T cell, for example, may be cytolytic activity or helper activity comprising the secretion of cytokines. In some embodiments, signaling domains of an intracellular domain mediate T cell activation, proliferation, survival, and/or other T cell function. An intracellular domain may comprise a signaling domain that is an activating domain. An intracellular domain may comprise a signaling domain that is a costimulatory signaling domain.

[193] Intracellular signaling domains that may transduce a signal upon binding of an antigen to an immune cell are known, any of which may be comprised in an antigen binding system of

the present disclosure. For example, cytoplasmic sequences of a T cell receptor (TCR) are known to initiate signal transduction following TCR binding to an antigen (see, *e.g.*, Brownlie et al., *Nature Rev. Immunol.* 13:257-269 (2013)).

[194] In some embodiments, a signaling domain and/or activation domain comprises an immunoreceptor tyrosine-based activation motif (ITAM). Examples of ITAM containing cytoplasmic signaling sequences comprise those derived from TCR zeta, FcR gamma, FcR beta, CD3 zeta, CD3 gamma, CD3 delta, CD3 epsilon, CD5, CD22, CD79a, CD79b, and CD66d (see, *e.g.*, Love et al., *Cold Spring Harb. Perspect. Biol.* 2:a002485 (2010); Smith-Garvin et al., *Annu. Rev. Immunol.* 27:591-619 (2009)).

[195] In certain embodiments, suitable signaling domains comprise, without limitation, 4-1BB/CD137, activating NK cell receptors, an Immunoglobulin protein, B7-H3, BAFFR, BLAME (SLAMF8), BTLA, CD100 (SEMA4D), CD103, CD160 (BY55), CD18, CD19, CD19a, CD2, CD247, CD27, CD276 (B7-H3), CD28, CD29, CD3 delta, CD3 epsilon, CD3 gamma, CD30, CD4, CD40, CD49a, CD49D, CD49f, CD69, CD7, CD84, CD8alpha, CD8beta, CD96 (Tactile), CD11a, CD11b, CD11c, CD11d, CDS, CEACAM1, CRT AM, cytokine receptor, DAP-10, DNAM1 (CD226), Fc gamma receptor, GADS, GITR, HVEM (LIGHTR), IA4, ICAM-1, ICAM-1, Ig alpha (CD79a), IL-2R beta, IL-2R gamma, IL-7R alpha, inducible T cell costimulator (ICOS), integrins, ITGA4, ITGA4, ITGA6, ITGAD, ITGAE, ITGAL, ITGAM, ITGAX, ITGB2, ITGB7, ITGB1, KIRDS2, LAT, LFA-1, LFA-1, ligand that binds with CD83, LIGHT, LIGHT, LTBR, Ly9 (CD229), Ly108), lymphocyte function-associated antigen-1 (LFA-1; CD1-1a/CD18), MHC class 1 molecule, NKG2C, NKG2D, NKp30, NKp44, NKp46, NKp80 (KLRF1), OX-40, PAG/Cbp, programmed death-1 (PD-1), PSGL1, SELPLG (CD162), Signaling Lymphocytic Activation Molecules (SLAM proteins), SLAM (SLAMF1; CD150; IPO-3), SLAMF4 (CD244; 2B4), SLAMF6 (NTB-A, SLAMF7, SLP-76, TNF receptor proteins, TNFR2, TNFSF14, a Toll ligand receptor, TRANCE/RANKL, VLA1, or VLA-6, or a fragment, truncation, or a combination thereof.

[196] A CAR may comprise a costimulatory signaling domain, *e.g.*, to increase signaling potency. See U.S. Pat. Nos. 7,741,465, and 6,319,494, as well as Krause et al. and Finney et al. (*supra*), Song et al., *Blood* 119:696-706 (2012); Kalos et al., *Sci Transl. Med.* 3:95 (2011); Porter et al., *N. Engl. J. Med.* 365:725-33 (2011), and Gross et al., *Annu. Rev. Pharmacol. Toxicol.* 56:59-83 (2016). Signals generated through a TCR alone may be insufficient for full activation of a T cell and a secondary or co-stimulatory signal may increase activation. Thus, in some embodiments, a signaling domain further comprises one or more additional signaling

domains (*e.g.*, costimulatory signaling domains) that activate one or more immune cell effector functions (*e.g.*, a native immune cell effector function described herein). In some embodiments, a portion of such costimulatory signaling domains may be used, as long as the portion transduces the effector function signal. In some embodiments, a cytoplasmic domain described herein comprises one or more cytoplasmic sequences of a T cell co-receptor (or fragment thereof). Non-limiting examples of such T cell co-receptors comprise CD27, CD28, 4-1BB (CD137), OX40, CD30, CD40, PD-1, ICOS, lymphocyte function-associated antigen-1 (LFA-1), MYD88, CD2, CD7, LIGHT, NKG2C, B7-H3, and a ligand that binds with CD83. An exemplary costimulatory protein has the amino acid sequence of a costimulatory protein found naturally on T cells, the complete native amino acid sequence of which costimulatory protein is described in NCBI Reference Sequence: NP_006130.1. In certain instances, a CAR comprises a 41BB costimulatory domain encoded by the sequence according to SEQ ID NO: 270, as shown below:

[197] SEQ ID NO: 270

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AGATTCAGCGTTGTGAAGAGAGGCCGGAAGAAGCTGCTGTACATCTTCAAGCAGCCCTT
CATGAGACCTGTGCAGACCACACAGGAGGAAGACGGCTGCAGCTGTAGATTCCCCGAGG
AAGAGGAGGGCGGCTGTGAGCTGAGAGTTAAGTTCAGCAGGAGCGCCGACGCCCTGCC
TACCAGCAAGGACAGAATCAACTGTACAACGAGCTGAACCTGGGCAGACGGGAGGAAT
ACGATGTGCTGGACAAGAGGAGAGGCAGAGACCCCGAGATGGGCGGCAAACCTAGAAG
AAAGAACCCCCAGGAGGGCCTGTATAACGAGCTCCAGAAGGACAAGATGGCCGAGGCC
TACAGCGAGATCGGCATGAAGGGCGAAAGAAGAAGAGGCAAGGGCCACGACGGCCTCT
ACCAGGGCTTAAGCACAGCTACAAAGGACACCTACGACGCCCTGCACATGCAGGCCCTG
CCCCCTAGATGATTAATTAAatcgat
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[198] The polynucleotide and polypeptide sequences of signaling domains provided herein are known. In some embodiments, the polynucleotide encoding a signaling domain comprises a nucleotide sequence at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or about 100% (*e.g.*, 85-90%, 85-95%, 85-100%, 90-95%, 90-100%, or 95-100%) identical to a known nucleotide sequence. In some embodiments, the polypeptide sequence of a signaling domain comprises a polypeptide sequence at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or about 100% (*e.g.*, 85-

90%, 85-95%, 85-100%, 90-95%, 90-100%, or 95-100%) identical to a known polypeptide sequence.

[199] In various embodiments, a mechanism of modulating (*e.g.*, decreasing) antigen binding system activity is desired, *e.g.*, to minimize or curtail adverse events resulting from antigen binding system activity. It may also be desired to comprise an inducible "on" or "accelerator" switch in immune cells. Suitable techniques comprise use of inducible caspase-9 (U.S. Appl. 2011/0286980) or a thymidine kinase, before, after, or at the same time, as the cells are transduced with the CAR construct of the present disclosure. Additional methods for introducing suicide genes and/or "on" switches comprise TALENS, zinc fingers, RNAi, siRNA, shRNA, antisense technology, and other techniques.

[200] In accordance with the present disclosure, on-off or other types of control switch techniques may be incorporated herein. These techniques may comprise use of dimerization domains and optional activators of such domain dimerization, *e.g.*, as disclosed by Wu et al., Science 2014 350 (6258) utilizing FKBP/Rapalog dimerization systems in certain cells, the contents of which are incorporated by reference herein in their entirety. Additional dimerization technology is described in, *e.g.*, Fegan et al. Chem. Rev. 2010, 110, 3315-3336 as well as U.S. Pat. Nos. 5,830,462; 5,834,266; 5,869,337; and 6,165,787, the contents of each of which is also incorporated by reference herein with respect to dimerization technology. Additional dimerization pairs may comprise cyclosporine-A/cyclophilin, receptor, estrogen/estrogen receptor (optionally using tamoxifen, 4-hydroxytamoxifen, or endoxifen), glucocorticoids/glucocorticoid receptor, tetracycline/tetracycline receptor, and/or vitamin D/vitamin D receptor. Further examples of dimerization technology may be found in *e.g.*, WO 2014/127261, WO 2015/090229, US 2014/0286987, US 2015/0266973, US 2016/0046700, U.S. Pat. No. 8,486,693, US 2014/0171649, and US 2012/0130076, the contents of which are further incorporated by reference herein in their entirety.

[201] In some embodiments, an antigen binding system of the present disclosure comprises a leader peptide (also referred to herein as a "signal peptide" or "leader sequence"). In certain embodiments, a leader peptide comprises an amino acid sequence that is at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% (*e.g.*, 85-90%, 85-95%, 85-100%, 90-95%, 90-100%, or 95-100%) identical to the amino acid sequence MEWTWVFLFLLSVTAGVHS (SEQ ID NO: 249), MALPVTALLLPLALLLHAARP (SEQ ID NO: 250), or MLLLVTSLLLCELPHPAFLIP (SEQ ID NO: 295)..

[202] Components of a CAR may be exchanged or “swapped” using routine techniques of biotechnology for equivalent components. To provide just a few non-limiting and partial examples, a CAR of the present disclosure may comprise a binding motif as provided herein in combination with a hinge provided herein and a costimulatory domain provided herein. In certain examples, a CAR of the present disclosure may comprise a leader sequence as provided herein together with a binding motif as provided herein in combination with a hinge provided herein and a costimulatory domain provided herein. In various embodiments, the present disclosure provides a binding motif according to any one of SEQ ID NOs: 251-260 in combination with (*e.g.*, adjacently fused to) a hinge according to any one of SEQ ID NOs: 261-269, optionally in further combination with (*e.g.*, adjacently fused to) a 41BB costimulatory domain according to SEQ ID NO: 270. A few, non-limiting examples thereof are provided in SEQ ID NOs: 271-290.

[203] A bicistronic CAR may comprise a first CAR sequence and a second CAR sequence expressed as a single polypeptide comprising a cleavable linker between the first and second CARs. An exemplary cleavable linker is Furin-GSG-T2A (see, *e.g.*, Chng et al. *MAbs*. 2015 Mar-Apr; 7(2): 403–412, which is herein incorporated by reference with respect to cleavable linkers; see also Guedan et al. *Mol Ther Methods Clin Dev*. 2019 Mar 15; 12: 145–156, which is incorporated herein by reference with respect to bicistronic CAR design). To provide just one non-limiting example of a bicistronic CAR structure, a bicistronic CAR may comprise (a) a first CAR comprising (i) a signal peptide (*e.g.*, a CSF2RA signal peptide); (ii) an anti-CD19 light chain variable domain; (iii) a linker (*e.g.*, a G4S linker or plurality thereof); (iv) an anti-CD19 heavy chain variable domain; (v) a spacer or hinge (*e.g.*, a CD28T spacer); (vi) a transmembrane domain (*e.g.*, a CD28 transmembrane domain); (vii) a costimulatory domain (*e.g.*, a CD28 costimulatory domain); (viii) a stimulatory domain (*e.g.*, a CD3z stimulatory domain); (b) a cleavable linker (*e.g.*, a Furin GSG-T2A linker); and (c) a second CAR comprising (i) a signal peptide (*e.g.*, a CD8a signal peptide); (ii) an anti-CD20 heavy chain variable domain of the present disclosure; (iii) a linker (*e.g.*, a linker according to SEQ ID NO: 247 and/or a linker according to any one of SEQ ID NOs: 307-313); (iv) an anti-CD20 light chain variable domain of the present disclosure; (v) a spacer or hinge (*e.g.*, a CD8a spacer); (vi) a transmembrane domain (*e.g.*, a CD8 transmembrane domain); (vii) a costimulatory domain (*e.g.*, a 41bb costimulatory domain); and (viii) a stimulatory domain (*e.g.*, a CD3z stimulatory domain). Thus, without limitation, an exemplary anti-CD20/anti-CD19 bicistronic

CAR may have or comprise the nucleotide and amino acid sequences set forth in SEQ ID NOs: 291 and 292.

[204] A bispecific CAR may be a single polypeptide that comprises a first binding motif of the present disclosure and a second binding motif that is an anti-CD19 binding motif. In a non-limiting exemplary embodiment, a bispecific CAR may comprise (i) a leader (*e.g.*, a CSF2RA signal peptide), (ii) an anti-CD20 light chain variable domain of the present disclosure; (iii) a linker (iv) an anti-CD20 heavy chain variable domain; (v) a linker (*e.g.*, a truncated linker); (vi) an anti-CD19 light chain variable domain; (vii) a linker; (viii) an anti-CD19 heavy chain variable domain; (ix) an extracellular domain (*e.g.*, a CD28T hinge or IgG4 hinge); (x) a transmembrane domain (*e.g.*, a CD28 transmembrane domain); (xi) an intracellular region (*e.g.*, a CD28 intracellular costimulatory domain and/or 41bb costimulatory domain); and a stimulatory domain (*e.g.*, a CD3z stimulatory domain). Thus, without limitation, an exemplary anti-CD20/anti-CD19 bispecific CAR may have or comprise the nucleotide and amino acid sequences set forth in SEQ ID NOs: 293-306.

[205] Various CAR sequences, components, and/or frameworks are known, comprising without limitation sequences of hinges, spacers, transmembrane domains, costimulatory domains, stimulatory domains, binding motifs, and variants of each, and a CAR with desired binding and components or architecture can be readily constructed if, *e.g.*, a heavy chain variable domain sequence or CDR sequences and a light chain variable domain sequence or CDR sequences are provided.

[206] The present disclosure provides, among other things, bispecific antibodies that bind CD20 and a second target antigen, *e.g.*, CD19. Bispecific antibodies comprise antibodies having a first binding motif that binds a first target antigen and a second binding motif that binds a second target antigen. In some embodiments, a bispecific antibody comprises an anti-CD20 binding motif of the present and an anti-CD19 binding motif of the present disclosure. In some embodiments, a bispecific antibody comprises an anti-CD20 binding motif that comprises an anti-CD20 heavy chain variable domain of the present disclosure and an anti-CD20 light chain variable domain of the present disclosure, as well as an anti-CD19 binding motif that comprises an anti-CD19 heavy chain variable domain and an anti-CD19 light chain variable domain.

[207] The present disclosure comprises conjugates in which an antibody of the present disclosure is associated with a therapeutic agent or a detectable moiety. In various embodiments, the therapeutic agent is an anti-cancer agent as provided herein. In certain

embodiments, provided conjugate comprises one or more detectable moieties, i.e., is “labeled” with one or more such moieties. In some such embodiments, a conjugate of the present disclosure is useful in diagnostic or imaging applications, *e.g.*, diagnosing or imaging cancer. Any of a wide variety of detectable moieties may be used in labeled antibody conjugates described herein. Suitable detectable moieties comprise, without limitation: various ligands, radionuclides; fluorescent dyes; chemiluminescent agents (such as, for example, acridinum esters, stabilized dioxetanes, and the like); bioluminescent agents; spectrally resolvable inorganic fluorescent semiconductor nanocrystals (i.e., quantum dots); microparticles; metal nanoparticles (*e.g.*, gold, silver, copper, platinum, etc.); nanoclusters; paramagnetic metal ions; enzymes; colorimetric labels (such as, for example, dyes, colloidal gold, and the like); biotin; dioxigenin; haptens; and proteins for which antisera or monoclonal antibodies are available.

[208] The present disclosure comprises nucleic acids encoding anti-CD20 binding motifs and/or anti-CD19 binding motifs provided herein. The present disclosure comprises nucleic acids encoding antibodies of the provided herein, comprising, without limitation, nucleic acids encoding binding motifs (*e.g.*, anti-CD20 binding motifs and anti-CD19 binding motifs). The present disclosure comprises nucleic acids encoding antigen binding systems provided herein, comprising without limitation nucleic acids encoding biscistronic and bispecific chimeric antigen receptors (*e.g.*, bicistronic and bispecific chimeric antigen receptors that bind CD20 and CD19). The nucleic acid sequence of SEQ ID NO: 2 comprises and provides exemplary nucleic acid sequences corresponding to and encoding each of SEQ ID NOs: 1 and 3-11. The nucleic acid sequence of SEQ ID NO: 13 comprises and provides exemplary nucleic acid sequences corresponding to and encoding each of SEQ ID NOs: 12 and 14-22. The nucleic acid sequence of SEQ ID NO: 24 comprises and provides exemplary nucleic acid sequences corresponding to and encoding each of SEQ ID NOs: 23 and 25-33. The nucleic acid sequence of SEQ ID NO: 35 comprises and provides exemplary nucleic acid sequences corresponding to and encoding each of SEQ ID NOs: 34 and 36-44. The nucleic acid sequence of SEQ ID NO: 46 comprises and provides exemplary nucleic acid sequences corresponding to and encoding each of SEQ ID NOs: 45 and 47-55. The nucleic acid sequence of SEQ ID NO: 57 comprises and provides exemplary nucleic acid sequences corresponding to and encoding each of SEQ ID NOs: 56 and 58-66. The nucleic acid sequence of SEQ ID NO: 68 comprises and provides exemplary nucleic acid sequences corresponding to and encoding each of SEQ ID NOs: 67 and 69-77. The nucleic acid sequence of SEQ ID NO: 79 comprises and provides exemplary nucleic acid sequences corresponding to and encoding each of SEQ ID NOs: 78 and

80-88. The nucleic acid sequence of SEQ ID NO: 90 comprises and provides exemplary nucleic acid sequences corresponding to and encoding each of SEQ ID NOs: 89 and 91-99. The nucleic acid sequence of SEQ ID NO: 101 comprises and provides exemplary nucleic acid sequences corresponding to and encoding each of SEQ ID NOs: 100 and 102-110. The nucleic acid sequence of SEQ ID NO: 112 comprises and provides exemplary nucleic acid sequences corresponding to and encoding each of SEQ ID NOs: 111 and 113-121. The nucleic acid sequence of SEQ ID NO: 123 comprises and provides exemplary nucleic acid sequences corresponding to and encoding each of SEQ ID NOs: 122 and 124-132. The nucleic acid sequence of SEQ ID NO: 134 comprises and provides exemplary nucleic acid sequences corresponding to and encoding each of SEQ ID NOs: 133 and 135-143. The nucleic acid sequence of SEQ ID NO: 145 comprises and provides exemplary nucleic acid sequences corresponding to and encoding each of SEQ ID NOs: 144 and 146-154. The nucleic acid sequence of SEQ ID NO: 156 comprises and provides exemplary nucleic acid sequences corresponding to and encoding each of SEQ ID NOs: 155 and 157-165. The nucleic acid sequence of SEQ ID NO: 167 comprises and provides exemplary nucleic acid sequences corresponding to and encoding each of SEQ ID NOs: 166 and 168-176. The nucleic acid sequence of SEQ ID NO: 178 comprises and provides exemplary nucleic acid sequences corresponding to and encoding each of SEQ ID NOs: 177 and 179-187. The nucleic acid sequence of SEQ ID NO: 189 comprises and provides exemplary nucleic acid sequences corresponding to and encoding each of SEQ ID NOs: 188 and 190-198. The nucleic acid sequence of SEQ ID NO: 200 comprises and provides exemplary nucleic acid sequences corresponding to and encoding each of SEQ ID NOs: 199 and 201-209. The nucleic acid sequence of SEQ ID NO: 211 comprises and provides exemplary nucleic acid sequences corresponding to and encoding each of SEQ ID NOs: 210 and 212-220. The present disclosure comprises nucleic acids encoding anti-CD19 binding motifs provided herein. The nucleic acid sequence of SEQ ID NO: 222 comprises and provides exemplary nucleic acid sequences corresponding to and encoding each of SEQ ID NOs: 221 and 223-231. The nucleic acid sequence of SEQ ID NO: 233 comprises and provides exemplary nucleic acid sequences corresponding to and encoding each of SEQ ID NOs: 232 and 234-242.

[209] The present disclosure comprises vectors that comprise nucleic acids of the present disclosure and/or that encode polypeptides of the present disclosure. In various embodiments, the present disclosure comprises a vector that comprises a nucleic acid encoding an anti-CD20 binding motif and/or an anti-CD19 binding motif provided herein. In various embodiments, the

present disclosure comprises a vector that comprises a nucleic acid encoding an antibody provided herein, comprising, without limitation, a nucleic acid encoding a binding motif molecule (*e.g.*, an anti-CD20 binding motif or an anti-CD19 binding motif). In various embodiments, the present disclosure comprises a vector that comprises a nucleic acid encoding one or more antigen binding systems provided herein, comprising without limitation nucleic acids encoding a bicistronic or bispecific chimeric antigen receptor (*e.g.*, a bicistronic and bispecific chimeric antigen receptor that bind CD20 and CD19).

[210] Any vector may be suitable for the present disclosure. In some embodiments, the vector is a viral vector. In some embodiments, the vector is a retroviral vector, a DNA vector, a murine leukemia virus vector, an SFG vector, a plasmid, a RNA vector, an adenoviral vector, a baculoviral vector, an Epstein Barr viral vector, a papovaviral vector, a vaccinia viral vector, a herpes simplex viral vector, an adenovirus associated vector (AAV), a lentiviral vector, or any combination thereof. Suitable exemplary vectors include *e.g.*, pGAR, pBABE-puro, pBABE-neo largeTcDNA, pBABE-hygro-hTERT, pMKO.1 GFP, MSCV-IRES-GFP, pMSCV PIG (Puro IRES GFP empty plasmid), pMSCV-loxp-dsRed-loxp-eGFP-Puro-WPRE, MSCV IRES Luciferase, pMIG, MDH1-PGK-GFP_2.0, TtRMPVIR, pMSCV-IRES-mCherry FP, pRetroX GFP T2A Cre, pRXTN, pLncEXP, and pLXIN-Luc.

[211] A recombinant expression vector may be any suitable recombinant expression vector. Suitable vectors comprise those designed for propagation and expansion or for expression or both, such as plasmids and viruses. For example, a vector may be selected from the pUC series (Fermentas Life Sciences, Glen Burnie, Md.), the pBluescript series (Stratagene, LaJolla, Calif.), the pET series (Novagen, Madison, Wis.), the pGEX series (Pharmacia Biotech, Uppsala, Sweden), and the pEX series (Clontech, Palo Alto, Calif.). Bacteriophage vectors, such as λ GT10, λ GT11, λ ZapII (Stratagene), λ EMBL4, and λ NM1149, also may be used. Examples of plant expression vectors useful in the context of the disclosure comprise pBI01, pBI101.2, pBI101.3, pBI121 and pBIN19 (Clontech). Examples of animal expression vectors useful in the context of the disclosure comprise pcDNA, pEUK-Cl, pMAM, and pMAMneo (Clontech). In some embodiments, a bicistronic IRES vector (*e.g.*, from Clontech) is used to comprise both a nucleic acid encoding an antigen binding system and an inducible expression construct described herein.

[212] In some embodiments, a recombinant expression vector is a viral vector. Suitable viral vectors comprise, without limitation, retroviral vectors, alphaviral, vaccinal, adenoviral,

adeno-associated viral, herpes viral, and fowl pox viral vectors, and preferably have a native or engineered capacity to transform an immune cell (*e.g.*, T cell).

[213] Recombinant expression vectors may be prepared using standard recombinant DNA techniques described in, for example, Sambrook et al., *Molecular Cloning: A Laboratory Manual*, 3rd ed., Cold Spring Harbor Press, Cold Spring Harbor, N.Y. 2001; and Ausubel et al., *Current Protocols in Molecular Biology*, Greene Publishing Associates and John Wiley & Sons, NY, 1994. Constructs of expression vectors, which are circular or linear, may be prepared to contain a replication system functional in a prokaryotic or eukaryotic host cell. Replication systems may be derived, *e.g.*, from ColEI, 2 μ plasmid, λ , SV40, bovine papilloma virus, and the like.

[214] A recombinant expression vector may comprise one or more marker genes, which allow for selection of transformed or transfected hosts. Marker genes comprise biocide resistance, *e.g.*, resistance to antibiotics, heavy metals, etc., complementation in an auxotrophic host to provide prototrophy, and the like. Suitable marker genes for the recombinant expression vectors comprise, for instance, neomycin/G418 resistance genes, puromycin resistance genes, hygromycin resistance genes, histidinol resistance genes, tetracycline resistance genes, and ampicillin resistance genes.

[215] Vectors useful in the context of the disclosure may be “naked” nucleic acid vectors (*i.e.*, vectors having little or no proteins, sugars, and/or lipids encapsulating them), or vectors complexed with other molecules. Other molecules that may be suitably combined with the vectors comprise without limitation viral coats, cationic lipids, liposomes, polyamines, gold particles, and targeting moieties such as ligands, receptors, or antibodies that target cellular molecules.

[216] Vector DNA may be introduced into a cell, *e.g.*, an immune cell, via conventional transformation, transfection, or transduction techniques. The terms “transformation” and “transfection” encompass a variety of art-recognized techniques for introducing foreign nucleic acid (*e.g.*, DNA) into a cell, such as calcium phosphate or calcium chloride co-precipitation, DEAE-dextran-mediated transfection, lipofection, gene gun, nanoparticle-mediated delivery, or electroporation. Transduction comprises viral delivery of a vector to a cell, *e.g.*, by a vector disclosed herein, comprising without limitation retrovirus, lentivirus, and AAV.

[217] The present disclosure comprises cells that comprise, express, or are engineered (*e.g.*, transformed or transduced) to comprise or express, at least one vector or nucleic acid of the present disclosure. In some embodiments, a method comprises transducing a cell with a vector

that comprises a polynucleotide encoding at least one antigen binding system. The present disclosure comprises cells that comprise, or are transformed to comprise, at least one vector that encodes one or more polypeptides of the present disclosure. The present disclosure comprises cells that comprise, or are transformed to comprise, at least one vector that encodes an anti-CD20 binding motif and/or an anti-CD19 binding motif provided herein. The present disclosure comprises cells that comprise, or are transformed to comprise, at least one vector that encodes an antibody provided herein, comprising, without limitation, a binding motif molecule (*e.g.*, an anti-CD20 binding motif or an anti-CD19 binding motif). The present disclosure comprises cells that comprise, or are transformed to comprise, at least one vector that encodes one or more antigen binding systems provided herein, comprising without limitation a biscistronic or bispecific chimeric antigen receptor (*e.g.*, a bicistronic or bispecific chimeric antigen receptor that bind CD20 and CD19). In some embodiments, cells are co-transfected or co-transduced with two vectors, each vector encoding a different CAR, which two different CARs together are a bicistronic CAR. Transfection or transduction of cells with two different vectors encoding two different CARs that together are a bicistronic CAR may be performed simultaneously on a single population of cells, simultaneously on two different populations of cells with each population transduced with only one of the two vectors, or independently on two different populations of cells each transduced with only one of the two vectors.

[218] The present disclosure comprises cells that comprise one or more polypeptides of the present disclosure. The present disclosure comprises cells that comprise (*e.g.*, express) an anti-CD20 binding motif and/or an anti-CD19 binding motif provided herein. The present disclosure comprises cells that comprise (*e.g.*, express) an antibody provided herein, comprising, without limitation, a binding motif (*e.g.*, an anti-CD20 binding motif or an anti-CD19 binding motif). The present disclosure comprises cells that comprise (*e.g.*, express) one or more antigen binding systems provided herein, comprising without limitation a biscistronic or bispecific chimeric antigen receptor (*e.g.*, a bicistronic and bispecific chimeric antigen receptor that bind CD20 and CD19).

[219] In other aspects, provided herein are cells comprising a polynucleotide or a vector of the present disclosure. In some embodiments, the present disclosure is directed to cells, *e.g.*, in vitro cells, comprising a polynucleotide encoding a CAR or a TCR comprising one or two of the scfv disclosed herein. In other embodiments, the present disclosure is directed to cells, *e.g.*, in vitro cells, comprising a polypeptide encoded by a CAR or a TCR comprising one or two of

the scfv disclosed herein. In some embodiments, the polypeptide comprise the amino acid sequence set forth below, or any combination thereof.

SEQ ID NO: 232 (anti-CD19 scFv light chain):

DIQMTQTTSSLSASLGDRVTISCRASQDISKYLNWYQQKPDGTVKLLIYHTSRLHS
GVPSRFGSGSGTDYSLTISNLEQEDIATYFCQQGNTLPYTFGGGTKLEIT

SEQ ID NO: 221 (anti-CD19 scFv heavy chain):

EVKLQESGPGLVAPSQSLSVTCTVSGVSLPDYGVSWIRQPPRKGLEWLGVIWGSE
TTYNSALKSRLTIHKDNSKSQVFLKMNSLQTDDTAIYYCAKHYYYGGSYAMDY
WGQGTSVTVSS

SEQ ID NO: 56 (anti-CD20 light chain):

DIQMTQSPSSLSASVGDRVTITCRASQSINSYLNWYQQKPGKAPKLLIYAASSLQS
GVPSRFGSGSGTDFTLTISLQPEDFATYYCQQSLADPFTFGGGTKVEIK

SEQ ID NO: 45 (anti-CD20 heavy chain):

QLQLQESGPGLVKPSETLSLTCTVSGSISSSSYWGWIRQPPGKGLEWIGSIYYSG
STYYNPSLKSRVTISVDTSKNQFSLKLSSVTAADTAVYYCARETDYSSGMGYGMD
VWGQGTITVTVSS

SEQ ID NO: 56 (anti-CD20 scFv 2 light chain):

DIQMTQSPSSLSASVGDRVTITCRASQSINSYLNWYQQKPGKAPKLLIYAASSLQS
GVPSRFGSGSGTDFTLTISLQPEDFATYYCQQSLADPFTFGGGTKVEIK

SEQ ID NO: 155 (anti-CD20 scFv 2 heavy chain):

QVQLQQWGAGLLKPSETLSLTCAVYGGSFsRYVWSWIRQPPGKGLEWIGEIDSSG
KTNYNPSLKSRVTISVDTSKNQFSLKLSSVTAADTAVYYCARVRYDSSDSYYSY
DYGMDVWGQGTITVTVSS

SEQ ID NO: 144 (anti-CD20 scFv 3 light chain):

DIVLTQSPDSLAVSLGERATINCKSSQSVLYSSNNKNYLAWYQQKPGQPPKLLIY
WASSRESGVPDRFSGSGSGTDFTLTISLQAEDVAVYYCQQSYSPWTFGGGTKV
EIK

SEQ ID NO: 177 (anti-CD20 scFv 3 heavy chain):

QVQLQQWGAGLLKPSETLSLTCAVYGGSFsGYAWSWIRQPPGKGLEWIGEIDHR
GFTNYNPSLKSRVTISVDTSKNQFSLKLSSVTAADTAVYYCARVRYDSSDSYYSY
YDYGMDVWGQGTITVTVSS

SEQ ID NO: 78 (anti-CD20 scFv 4 light chain):

DIQMTQSPSSLSASVGDRVITTCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSG
VPSRFGSGSGTDFTLTISLQPEDFATYYCQQSYRFPPTFGQGTKVEIK

SEQ ID NO: 67 (anti-CD20 scFv 4 heavy chain):

QVQLVQSGAEVKKPGASVKVSCKASGYTFKEYGISWVRQAPGGGLEWMGWISA
YSGHTYYAQKLQGRVTMTTDTSTSTAYMELRSLRSDDTAVYYCARGPHYDDWS
GFIWFDPPWGQGLTVTVSS

[220] Any cell may be used as a host cell for the polynucleotides, the vectors, or the polypeptides of the present disclosure. In some embodiments, the cell can be a prokaryotic cell, fungal cell, yeast cell, or higher eukaryotic cells such as a mammalian cell. Suitable prokaryotic cells include, without limitation, eubacteria, such as Gram-negative or Gram-positive organisms, for example, *Enterobacteriaceae* such as *Escherichia*, e.g., *E. coli*; *Enterobacter*; *Erwinia*; *Klebsiella*; *Proteus*; *Salmonella*, e.g., *Salmonella typhimurium*; *Serratia*, e.g., *Serratia marcescans*, and *Shigella*; *Bacilli* such as *B. subtilis* and *B. licheniformis*; *Pseudomonas* such as *P. aeruginosa*; and *Streptomyces*. In some embodiments, the cell is a human cell. In some embodiments, the cell is an immune cell. In some embodiments, the immune cell is selected from the group consisting of a T cell, a B cell, a tumor infiltrating lymphocyte (TIL), a TCR expressing cell, a natural killer (NK) cell, a dendritic cell, a granulocyte, an innate lymphoid cell, a megakaryocyte, a monocyte, a macrophage, a platelet, a thymocyte, and a myeloid cell. In one embodiment, the immune cell is a T cell. In another embodiment, the immune cell is an NK cell. In certain embodiments, the T cell is a tumor-infiltrating lymphocyte (TIL), autologous T cell, engineered autologous T cell (eACT™), an allogeneic T cell, a heterologous T cell, or any combination thereof.

[221] Chimeric antigen receptors (CARs or CAR-Ts) and engineered T cell receptors (TCRs) may be readily inserted into and expressed by immune cells, e.g., T cells, producing binding agents. In certain embodiments, cells (e.g., immune cells such as T cells) are obtained from a donor subject. In some embodiments, the donor subject is human patient afflicted with a cancer or a tumor. In other embodiments, the donor subject is a human patient not afflicted with a cancer or a tumor. In some embodiments, an engineered cell is autologous to a subject. In some embodiments, an engineered cell is allogeneic to a subject.

[222] The cell of the present disclosure may be obtained through any source known in the art. For example, T cells can be differentiated in vitro from a hematopoietic stem cell population, or T cells can be obtained from a subject. T cells can be obtained from, e.g., 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 addition, the T cells can be derived from one or more T cell lines available in the art. T cells can also 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 and/or apheresis. In certain embodiments, the cells collected by apheresis are washed to remove the plasma fraction, and placed in an appropriate buffer or media for subsequent processing. In some embodiments, the cells are washed with PBS. As will be appreciated, a washing step can be used, such as by using a semiautomated flowthrough centrifuge, *e.g.*, the Cobe™ 2991 cell processor, the Baxter CytoMate™, or the like. In some embodiments, the washed cells are resuspended in one or more biocompatible buffers, or other saline solution with or without buffer. In certain embodiments, the undesired components of the apheresis sample are removed. Additional methods of isolating T cells for a T cell therapy are disclosed in U.S. Patent Publication No. 2013/0287748, which is herein incorporated by references in its entirety.

[223] In certain embodiments, T cells are isolated from PBMCs by lysing the red blood cells and depleting the monocytes, *e.g.*, by using centrifugation through a PERCOLL™ gradient. In some embodiments, a specific subpopulation of T cells, such as CD4⁺, CD8⁺, CD28⁺, CD45RA⁺, and CD45RO⁺ T cells is further isolated by positive or negative selection techniques known in the art. For example, enrichment of a T cell population by negative selection can be accomplished with a combination of antibodies directed to surface markers unique to the negatively selected cells. In some embodiments, 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 can be used. For example, to enrich for CD4⁺ cells by negative selection, a monoclonal antibody cocktail typically includes antibodies to CD8, CD11b, CD14, CD16, CD20, and HLA-DR. In certain embodiments, flow cytometry and cell sorting are used to isolate cell populations of interest for use in the present disclosure.

[224] In some embodiments, PBMCs are used directly for genetic modification with the immune cells (such as CARs or TCRs) using methods as described herein. In certain embodiments, after isolating the PBMCs, T lymphocytes are further isolated, and both cytotoxic and helper T lymphocytes are sorted into naive, memory, and effector T cell subpopulations either before or after genetic modification and/or expansion.

[225] In some embodiments, CD8⁺ cells are further sorted into naive, central memory, and effector cells by identifying cell surface antigens that are associated with each of these types of CD8⁺ cells. In some embodiments, the expression of phenotypic markers of central memory T cells includes CCR7, CD3, CD28, CD45RO, CD62L, and CD127 and are negative for granzyme B. In some embodiments, central memory T cells are CD8⁺, CD45RO⁺, and CD62L⁺ T cells. In some embodiments, effector T cells are negative for CCR7, CD28, CD62L, and CD127 and positive for granzyme B and perforin. In certain embodiments, CD4⁺ T cells are further sorted into subpopulations. For example, CD4⁺ T helper cells can be sorted into naive, central memory, and effector cells by identifying cell populations that have cell surface antigens.

[226] In some embodiments, the immune cells, *e.g.*, T cells, are genetically modified following isolation using known methods, or the immune cells are activated and expanded (or differentiated in the case of progenitors) *in vitro* prior to being genetically modified. In another embodiment, the immune cells, *e.g.*, T cells, are genetically modified with the chimeric antigen receptors described herein (*e.g.*, transduced with a viral vector comprising one or more nucleotide sequences encoding a CAR) and then are activated and/or expanded *in vitro*. Methods for activating and expanding T cells are known in the art and are described, *e.g.*, in U.S. Patent Nos. 6,905,874; 6,867,041; and 6,797,514; and PCT Publication No. WO 2012/079000, the contents of which are hereby incorporated by reference in their entirety. Generally, such methods include contacting PBMC or isolated T cells with a stimulatory agent and costimulatory agent, such as anti-CD3 and anti-CD28 antibodies, generally attached to a bead or other surface, in a culture medium with appropriate cytokines, such as IL-2. Anti-CD3 and anti-CD28 antibodies attached to the same bead serve as a “surrogate” antigen presenting cell (APC). One example is The Dynabeads® system, a CD3/CD28 activator/stimulator system for physiological activation of human T cells. In other embodiments, the T cells are activated and stimulated to proliferate with feeder cells and appropriate antibodies and cytokines using methods such as those described in U.S. Patent Nos. 6,040,177 and 5,827,642 and PCT Publication No. WO 2012/129514, the contents of which are hereby incorporated by reference in their entirety.

[227] In various aspects of the present disclosure, a cell that comprises, expresses, encodes, or is transformed to encode a vector or polypeptide of the present disclosure (*e.g.*, an anti-CD20 binding motif of the present disclosure and/or an anti-CD20/anti-CD19 antigen binding system) is a binding agent. A binding agent, or population of binding agents, can be used as a (*e.g.*, as

the active agent of) a binding agent (a composition that comprises cells useful as a treatment, *e.g.*, for a cancer).

[228] The present disclosure further comprises methods and process for producing antibody agents as disclosed herein, *e.g.*, by transformation (*e.g.*, transduction) of a cell with a vector or nucleic acid of the present disclosure. In some embodiments, a method or process for producing antibody agents as disclosed herein comprises transforming (*e.g.*, transducing) a cell with a nucleic acid (*e.g.*, a nucleic acid present in a vector) encoding at least one antigen binding system provided herein. In general, antibody agents described herein may be produced from an immune cell, *e.g.*, a cell useful in or capable of use in adoptive cell therapy. In some embodiments, a binding agent is produced from a cell type selected from a group consisting of TILs, T-cells, CD8⁺ cells, CD4⁺ cells, NK-cells, gamma-delta T-cells, regulatory T-cells or peripheral blood mononuclear cells. “Tumor-infiltrating lymphocytes” or TILs refer to white blood cells that have left the bloodstream and migrated into a tumor. Lymphocytes may be divided into three groups comprising B cells, T cells and natural killer cells. “T-cells” refers to CD3⁺ cells, comprising CD4⁺ helper cells, CD8⁺ cytotoxic T-cells and gamma-delta T cells.

[229] In certain embodiments a binding agent is produced by genetically modifying (*e.g.*, transforming) a cell, *e.g.*, an immune cell, with a nucleic acid encoding an antigen binding system and/or an expression construct described herein (*e.g.*, (i) a first recombinant expression vector that comprises a nucleic acid encoding an antigen binding system and a second recombinant expression vector that comprises an inducible expression construct, (ii) a single recombinant expression vector that comprises both a nucleic acid encoding an antigen binding system and an inducible expression construct; or (iii) a recombinant expression vector that comprises a constitutive expression construct). The recombinant expression vector may comprise any type of nucleotides, comprising, without limitation, DNA and RNA, which may be single-stranded or double-stranded, synthesized or obtained in part from natural sources, and which may contain natural, non-natural or altered nucleotides. A recombinant expression vector may comprise naturally-occurring or non-naturally-occurring internucleotide linkages, or both types of linkages.

[230] In some embodiments, a method comprises transducing a cell with a polynucleotide encoding an antigen binding system, as disclosed herein. In some embodiments, a method comprises transducing a cell with a vector comprising the polynucleotide encoding an antigen binding system.

[231] In some embodiments, the donor T cells for use in the T cell therapy are obtained from the patient (*e.g.*, for an autologous T cell therapy). In other embodiments, the donor T cells for use in the T cell therapy are obtained from a subject that is not the patient. In an exemplary aspect, subjects may undergo leukapheresis, wherein leukocytes are collected, enriched, or depleted *ex vivo* to select and/or isolate the cells of interest, *e.g.*, T cells. These T cell isolates may be expanded by methods and treated such that one or more CAR constructs of the disclosure may be introduced, thereby creating a CAR T cell of the disclosure.

[232] In some embodiments, an immune cell is obtained from a subject and is transformed, *e.g.*, transduced, with inducible expression construct or a constitutive expression construct described herein, *e.g.*, an expression vector comprising an inducible expression construct or a constitutive expression construct described herein, to obtain a binding agent. Thus, in some embodiments, a binding agent comprises an autologous cell that is administered into the same subject from which an immune cell was obtained. In some embodiments, an immune cell is obtained from a subject and is transformed, *e.g.*, transduced, with an inducible expression construct or a constitutive expression construct described herein, *e.g.*, an expression vector comprising an inducible expression construct or a constitutive expression construct described herein, to obtain a binding agent that is allogeneically transferred into another subject.

[233] In certain embodiments, the T cells are obtained from a donor subject. In some embodiments, the donor subject is human patient afflicted with a cancer or a tumor. In other embodiments, the donor subject is a human patient not afflicted with a cancer or a tumor.

[234] Various compositions of the present disclosure comprise populations of engineered cells, which may be produced by any means. In some embodiments, the present disclosure provides populations of human cells engineered to express an antigen binding system as described herein. In some embodiments, such a population comprises binding agents. In some embodiments, such a population comprises a cultured population. In some embodiments, such a population is a cultured population of cells from a single human source who may, in some embodiments, receive administration of the cultured population. As disclosed herein, a binding agent may comprise any single cell or population of cells, *e.g.* population of engineered cells, as provided herein.

[235] Other aspects of the present disclosure are directed to compositions comprising a polynucleotide described herein, a vector described herein, a polypeptide described herein, or an *in vitro* cell described herein. In some embodiments, the composition comprises a pharmaceutically acceptable carrier, diluent, solubilizer, emulsifier, preservative and/or

adjuvant. In some embodiments, the composition comprises an excipient. In one embodiment, the composition comprises a polynucleotide encoding a CAR or a TCR comprising antigen binding molecules described herein. In another embodiment, the composition comprises a CAR or a TCR comprising a TCD encoded by a polynucleotide of the present disclosure. In another embodiment, the composition comprises a T cell comprising a CAR or a TCR comprising one or two of the scfv disclosed herein.

[236] In other embodiments, the composition is selected for parenteral delivery, for inhalation, or for delivery through the digestive tract, such as orally. The preparation of such pharmaceutically acceptable compositions is within the ability of one skilled in the art. In certain embodiments, buffers are used to maintain the composition at physiological pH or at a slightly lower pH, typically within a pH range of from about 5 to about 8. In certain embodiments, when parenteral administration is contemplated, the composition is in the form of a pyrogen-free, parenterally acceptable aqueous solution comprising a composition described herein, with or without additional therapeutic agents, in a pharmaceutically acceptable vehicle. In certain embodiments, the vehicle for parenteral injection is sterile distilled water in which composition described herein, with or without at least one additional therapeutic agent, is formulated as a sterile, isotonic solution, properly preserved. In certain embodiments, the preparation involves the formulation of the desired molecule with polymeric compounds (such as polylactic acid or polyglycolic acid), beads or liposomes, that provide for the controlled or sustained release of the product, which are then be delivered via a depot injection. In certain embodiments, implantable drug delivery devices are used to introduce the desired molecule.

[237] In some embodiments, the present disclosure provides pharmaceutical compositions that comprise and/or deliver one or more of the present disclosure, *e.g.*, an antigen binding systems of the present disclosure, nucleic acids that encode them, and/or cell(s) or populations thereof that comprise and/or express them.

[238] In some embodiments, the present disclosure provides pharmaceutical compositions that comprise and or deliver one or more cells as provided herein, *e.g.*, a binding agent that encodes or expresses a polypeptide provided herein, *e.g.*, an anti-CD20/anti-CD19 CAR (*i.e.*, a “binding agent pharmaceutical compositions”). A binding agent pharmaceutical composition may comprise one or a plurality of cells, as described herein, in combination with one or more pharmaceutically or physiologically acceptable carriers, diluents or excipients. Such compositions may comprise buffers such as neutral buffered saline, phosphate buffered saline

and the like; carbohydrates such as glucose, mannose, sucrose or dextrans, mannitol; proteins; polypeptides or amino acids such as glycine; antioxidants; chelating agents such as EDTA or glutathione; adjuvants (*e.g.*, aluminum hydroxide); and preservatives.

[239] Binding agent pharmaceutical composition of the present disclosure may be formulated for administration according to any embodiment set forth herein, at least one non-limiting example of which is intravenous administration. A composition may be formulated for intravenous, intratumoral, intraarterial, intramuscular, intraperitoneal, intrathecal, epidural, and/or subcutaneous administration routes. Preferably, the composition is formulated for a parenteral route of administration. A composition suitable for parenteral administration may be an aqueous or nonaqueous, isotonic sterile injection solution, which may contain antioxidants, buffers, bacteriostats, and solutes, for example, that render the composition isotonic with the blood of the intended recipient. An aqueous or nonaqueous sterile suspension may contain one or more suspending agents, solubilizers, thickening agents, stabilizers, and preservatives. Binding agent pharmaceutical compositions of the present disclosure may be administered in a manner appropriate to the disease to be treated (or prevented).

[240] The sterile composition for injection may be formulated in accordance with conventional pharmaceutical practices using distilled water for injection as a vehicle. For example, physiological saline or an isotonic solution containing glucose and other supplements such as D-sorbitol, D-mannose, D-mannitol, and sodium chloride may be used as an aqueous solution for injection, optionally in combination with a suitable solubilizing agent, for example, alcohol such as ethanol and polyalcohol such as propylene glycol or polyethylene glycol, and a nonionic surfactant such as polysorbate 80™, HCO-50 and the like.

[241] Non-limiting examples of oily liquid comprise sesame oil and soybean oil, and it may be combined with benzyl benzoate or benzyl alcohol as a solubilizing agent. Other items that may be comprised are a buffer such as a phosphate buffer, or sodium acetate buffer, a soothing agent such as procaine hydrochloride, a stabilizer such as benzyl alcohol or phenol, and an antioxidant. The formulated injection may be packaged in a suitable ampule.

[242] In one embodiment, a binding agent pharmaceutical composition is substantially free of detectable levels of a contaminant, *e.g.*, of endotoxin, mycoplasma, replication competent lentivirus (RCL), p24, VSV-G nucleic acid, HIV gag, residual anti-CD3/anti-CD28 coated beads, mouse antibodies, pooled human serum, bovine serum albumin, bovine serum, culture media components, vector packaging cell or plasmid components, a bacterium and a fungus. In one embodiment, the bacterium is at least one selected from the group consisting of

Alcaligenes faecalis, *Candida albicans*, *Escherichia coli*, *Haemophilus influenzae*, *Neisseria meningitidis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Streptococcus pneumoniae*, and/or *Streptococcus pyogenes* group A.

[243] In various embodiments, cells provided herein (*e.g.*, binding agents, *e.g.*, engineered T cells or engineered NK cells) may be activated and/or expanded from, and/or to produce, a binding agent pharmaceutical composition. In some embodiments, additional steps may be performed prior to administration to a subject. For instance, a binding agent may be expanded in vitro after contacting (*e.g.*, transducing or transfecting) an immune cell with an inducible expression construct or a constitutive expression construct described herein (*e.g.*, an expression vector comprising an inducible expression construct or a constitutive expression construct), prior to the administration to a subject. In vitro expansion may proceed for 1 day or more, *e.g.*, 2 days or more, 3 days or more, 4 days or more, 6 days or more, or 8 days or more, prior to the administration to a subject. In some embodiments, in vitro expansion may proceed for 21 days or less, *e.g.*, 18 days or less, 16 days or less, 14 days or less, 10 days or less, 7 days or less, or 5 days or less, prior to administration to a subject. For example, in vitro expansion may proceed for 1-7 days, 2-10 days, 3-5 days, or 8-14 days prior to the administration to a subject. A binding agent pharmaceutical composition comprising, *e.g.*, binding agents (*e.g.*, engineered T cells or engineered NK cells), may be formulated for administration at a desired dosage, *e.g.*, a dosage of 10^4 to 10^9 cells/kg body weight (*e.g.*, 10^5 to 10^6 cells/kg body weight). Certain embodiments of the disclosure comprise methods of administering to a subject a pharmaceutical composition as described herein, such as, for example, a binding agent described (*e.g.*, a population of engineered cells of the present disclosure) herein, a protein therapeutic described herein, a composition comprising a binding agent, and/or a composition comprising a protein therapeutic, *e.g.*, in an amount effective to treat a subject, when administered in an appropriate dosing regimen.

[244] In some embodiments, a binding agent is autologous to a subject, and the subject may be immunologically naive, immunized, diseased, or in another condition prior to isolation of an immune cell from the subject. In some embodiments, during in vitro expansion, a binding agent may be stimulated with an antigen (*e.g.*, a TCR antigen). Antigen-stimulated expansion optionally may be supplemented with expansion under conditions that non-specifically stimulate lymphocyte proliferation such as, for example, anti-CD3 antibody, anti-Tac antibody, anti-CD28 antibody, or phytohemagglutinin (PHA). The expanded binding agent may be

directly administered into a subject or may be frozen for future use, i.e., for subsequent administrations to a subject.

[245] In some embodiments, a binding agent is treated *ex vivo* with interleukin-2 (IL-2) prior to infusion into a cancer patient, and the cancer patient is treated with IL-2 after infusion. Furthermore, in some embodiments, a cancer patient may undergo preparative lymphodepletion--the temporary ablation of the immune system--prior to administration of a binding agent. A combination of IL-2 treatment and preparative lymphodepletion may enhance persistence of a binding agent. In some embodiments, a binding agent is transduced or transfected with a nucleic acid encoding a cytokine, which nucleic acid may be engineered to provide for constitutive, regulatable, or temporally-controlled expression of the cytokine. Suitable cytokines comprise, for example, cytokines which act to enhance the survival of T lymphocytes during the contraction phase, which may facilitate the formation and survival of memory T lymphocytes.

[246] In certain embodiments, a binding agent is administered prior to, substantially simultaneously with, or after the administration of another therapeutic agent, such as a cancer therapeutic agent. The cancer therapeutic agent may be, *e.g.*, a chemotherapeutic agent, a biological agent, or radiation treatment. In some embodiments, a subject receiving a binding agent is not administered a treatment which is sufficient to cause a depletion of immune cells, such as lymphodepleting chemotherapy or radiation therapy.

[247] Dosage administered to a subject in some embodiments, may vary with the embodiment, the composition employed, the method of administration, and the site and subject being treated. However, a dose should be sufficient to provide a therapeutic response. A clinician may determine the therapeutically effective amount of a composition to be administered to a human or other subject in order to treat or prevent a medical condition. The precise amount of the composition required to be therapeutically effective may depend upon numerous factors, *e.g.*, such as the activity of the binding agent, and the route of administration.

[248] A suitable number binding agent cells may be administered to a subject. While a single binding agent cell described herein is capable of expanding and providing a therapeutic benefit, in some embodiments, 10^2 or more, *e.g.*, 10^3 or more, 10^4 or more, 10^5 or more, or 10^8 or more, binding agent cells are administered. In some embodiments, 10^{12} or less, *e.g.*, 10^{11} or less, 10^9 or less, 10^7 or less, or 10^5 or less, binding agent cells described herein are administered to a subject. In some embodiments, 10^2 - 10^5 , 10^4 - 10^7 , 10^3 - 10^9 , or 10^5 - 10^{10} binding agent cells described herein are administered. A binding agent pharmaceutical composition may be

administered, *e.g.*, a dosage of 10^4 to 10^9 cells/kg body weight (*e.g.*, 10^5 to 10^6 cells/kg body weight). A binding agent pharmaceutical composition may be administered at a dosage of, *e.g.*, about 2×10^6 cells/kg, about 3×10^6 cells/kg, about 4×10^6 cells/kg, about 5×10^6 cells/kg, about 6×10^6 cells/kg, about 7×10^6 cells/kg, about 8×10^6 cells/kg, about 9×10^6 cells/kg, about 1×10^7 cells/kg, about 2×10^7 cells/kg, about 3×10^7 cells/kg, about 4×10^7 cells/kg, about 5×10^7 cells/kg, about 6×10^7 cells/kg, about 7×10^7 cells/kg, about 8×10^7 cells/kg, or about 9×10^7 cells/kg.

[249] A dose of a binding agent described herein may be administered to a mammal at one time or in a series of subdoses administered over a suitable period of time, *e.g.*, on a daily, semi-weekly, weekly, bi-weekly, semi-monthly, bi-monthly, semi-annual, or annual basis, as needed. A dosage unit comprising an effective amount of a binding agent may be administered in a single daily dose, or the total daily dosage may be administered in two, three, four, or more divided doses administered daily, as needed.

[250] A suitable means of administration may be selected by a medical practitioner. Route of administration may be parenteral, for example, administration by injection, transnasal administration, transpulmonary administration, or transcutaneous administration. Administration may be systemic or local by intravenous injection, intramuscular injection, intraperitoneal injection, subcutaneous injection. In some embodiments, a composition is selected for parenteral delivery, for inhalation, or for delivery through the digestive tract, such as orally. Dose and method of administration may vary depending on the weight, age, condition, and the like of the subject, and may be suitably selected.

[251] In various embodiments, a binding agent described herein may be incorporated into a pharmaceutical composition. Pharmaceutical compositions comprising a binding agent of the present disclosure may be formulated by known methods (such as described in Remington's Pharmaceutical Sciences, 17th edition, ed. Alfonso R. Gennaro, Mack Publishing Company, Easton, Pa. (1985)). In various instances, a pharmaceutical composition comprising a binding agent of the present disclosure may be formulated to comprise a pharmaceutically acceptable carrier or excipient. Examples of pharmaceutically acceptable carriers comprise, without limitation, any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like that are physiologically compatible. Compositions comprising a binding agent of the present disclosure may comprise a pharmaceutically acceptable salt, *e.g.*, an acid addition salt or a base addition salt.

[252] In various embodiments, a composition comprising a binding agent as described herein, *e.g.*, a sterile formulation for injection, may be formulated in accordance with conventional pharmaceutical practices using distilled water for injection as a vehicle. For example, physiological saline or an isotonic solution containing glucose and other supplements such as D-sorbitol, D-mannose, D-mannitol, and sodium chloride may be used as an aqueous solution for injection, optionally in combination with a suitable solubilizing agent, for example, alcohol such as ethanol and polyalcohol such as propylene glycol or polyethylene glycol, and a nonionic surfactant such as polysorbate 80™, HCO-50 and the like. As disclosed herein, a pharmaceutical composition comprising a binding agent may be in any form. Such forms comprise, *e.g.*, liquid, semi-solid and solid dosage forms, such as liquid solutions (*e.g.*, injectable and infusible solutions), dispersions or suspensions, tablets, pills, powders, liposomes and suppositories.

[253] Selection or use of any form may depend, in part, on the intended mode of administration and therapeutic application. For example, a composition comprising a binding agent of the present disclosure intended for systemic or local delivery may be in the form of injectable or infusible solutions. Accordingly, the compositions comprising a binding agent of the present disclosure may be formulated for administration by a parenteral mode (*e.g.*, intravenous, subcutaneous, intraperitoneal, or intramuscular injection). Parenteral administration refers to modes of administration other than enteral and topical administration, usually by injection, and comprise, without limitation, intravenous, intranasal, intraocular, pulmonary, intramuscular, intraarterial, intrathecal, intracapsular, intraorbital, intracardiac, intradermal, intrapulmonary, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal, epidural, intracerebral, intracranial, intracarotid and intrasternal injection and infusion.

[254] Route of administration may be parenteral, for example, administration by injection, transnasal administration, transpulmonary administration, or transcutaneous administration. Administration may be systemic or local by intravenous injection, intramuscular injection, intraperitoneal injection, subcutaneous injection.

[255] In various embodiments, a pharmaceutical composition comprising a binding agent of the present disclosure may be formulated as a solution, microemulsion, dispersion, liposome, or other ordered structure suitable for stable storage at high concentration. Sterile injectable solutions may be prepared by incorporating a composition comprising a binding agent of the present disclosure in the required amount in an appropriate solvent with one or a combination

of ingredients enumerated above, as required, followed by filter sterilization. Generally, dispersions are prepared by incorporating a composition comprising a binding agent of the present disclosure into a sterile vehicle that contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, methods for preparation comprise vacuum drying and freeze-drying that yield a powder of a composition comprising a binding agent of the present disclosure plus any additional desired ingredient (see below) from a previously sterile-filtered solution thereof. The proper fluidity of a solution may be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prolonged absorption of injectable compositions comprising a binding agent of the present disclosure may be brought about by comprising in the composition comprising a binding agent of the present disclosure a reagent that delays absorption, for example, monostearate salts, and gelatin.

[256] A pharmaceutical composition comprising a binding agent of the present disclosure may be administered parenterally in the form of an injectable formulation comprising a sterile solution or suspension in water or another pharmaceutically acceptable liquid. For example, the pharmaceutical composition comprising a binding agent of the present disclosure may be formulated by suitably combining the therapeutic molecule with pharmaceutically acceptable vehicles or media, such as sterile water and physiological saline, vegetable oil, emulsifier, suspension agent, surfactant, stabilizer, flavoring excipient, diluent, vehicle, preservative, binder, followed by mixing in a unit dose form required for generally accepted pharmaceutical practices. The amount of active ingredient comprised in the pharmaceutical preparations is such that a suitable dose within the designated range is provided. Nonlimiting examples of oily liquid comprise sesame oil and soybean oil, and it may be combined with benzyl benzoate or benzyl alcohol as a solubilizing agent. Other items that may be comprised are a buffer such as a phosphate buffer, or sodium acetate buffer, a soothing agent such as procaine hydrochloride, a stabilizer such as benzyl alcohol or phenol, and an antioxidant. The formulated injection may be packaged in a suitable ampule.

[257] In some embodiments, a composition comprising a binding agent of the present disclosure may be formulated for storage at a temperature below 0°C (*e.g.*, -20°C or -80°C). In some embodiments, the composition comprising a binding agent of the present disclosure may be formulated for storage for up to 2 years (*e.g.*, one month, two months, three months, four months, five months, six months, seven months, eight months, nine months, 10 months, 11

months, 1 year, 1 1/2 years, or 2 years) at 2-8°C (*e.g.*, 4°C). Thus, in some embodiments, the compositions comprising a binding agent of the present disclosure are stable in storage for at least 1 year at 2-8°C (*e.g.*, 4°C).

[258] In some instances, a pharmaceutical composition comprising a binding agent of the present disclosure may be formulated as a solution. In some embodiments, a composition comprising a binding agent of the present disclosure may be formulated, for example, as a buffered solution at a suitable concentration and suitable for storage at 2-8°C (*e.g.*, 4°C). Pharmaceutical compositions comprising a binding agent as described herein may be formulated in immunoliposome compositions. Liposomes with enhanced circulation time are disclosed in, *e.g.*, U.S. Pat. No. 5,013,556.

[259] In certain embodiments, compositions comprising a binding agent of the present disclosure may be formulated with a carrier that will protect the composition against rapid release, such as a controlled release formulation, comprising implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers may be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Many methods for the preparation of such formulations are known. See, *e.g.*, J. R. Robinson (1978) "Sustained and Controlled Release Drug Delivery Systems," Marcel Dekker, Inc., New York.

[260] In some embodiments, compositions comprising a binding agent of the present disclosure may be formulated in a composition suitable for intrapulmonary administration (*e.g.*, for administration via an inhaler or nebulizer) to a mammal such as a human. Methods for formulating such compositions are known. Dry powder inhaler formulations and suitable systems for administration of the formulations are also known. Pulmonary administration may be oral and/or nasal. Examples of pharmaceutical devices for pulmonary delivery comprise metered dose inhalers, dry powder inhalers (DPIs), and nebulizers. For example, a composition comprising a binding agent of the present disclosure may be administered to the lungs of a subject by way of a dry powder inhaler. These inhalers are propellant-free devices that deliver dispersible and stable dry powder formulations to the lungs. Dry powder inhalers are known and comprise, without limitation: the TURBOHALER® (AstraZeneca; London, England) the AIR® inhaler (ALKERMES®; Cambridge, Mass.); ROTAHALER® (GlaxoSmithKline; London, England); and ECLIPSE™ (Sanofi-Aventis; Paris, France). See also, *e.g.*, PCT Publication Nos. WO 04/026380, WO 04/024156, and WO 01/78693. DPI devices have been used for pulmonary administration of polypeptides such as insulin and growth hormone. In

some embodiments, a composition comprising a binding agent of the present disclosure may be intrapulmonarily administered by way of a metered dose inhaler. These inhalers rely on a propellant to deliver a discrete dose of a molecule to the lungs. Additional devices and intrapulmonary administration methods are set forth in, *e.g.*, U.S. Patent Application Publication Nos. 20050271660 and 20090110679, the disclosures of each of which are incorporated herein by reference in their entirety.

[261] In some embodiments, compositions comprising a binding agent of the present disclosure may be formulated for delivery to the eye, *e.g.*, in the form of a pharmaceutically acceptable solution, suspension or ointment. A preparation for use in treating an eye may be in the form of a sterile aqueous solution containing, *e.g.*, additional ingredients such as, without limitation, preservatives, buffers, tonicity agents, antioxidants and stabilizers, nonionic wetting or clarifying agents, and viscosity-increasing agents. A preparation as described herein may be administered topically to the eye of the subject in need of treatment (*e.g.*, a subject afflicted with AMD) by conventional methods, *e.g.*, in the form of drops, or by bathing the eye in a therapeutic solution, containing one or more compositions.

[262] A variety of devices for introducing drugs into the vitreal cavity of the eye may be appropriate, in certain embodiments, for administration of a composition comprising a binding agent of the present disclosure. For example, U.S. Publication No. 2002/0026176 describes a pharmaceutical-containing plug that may be inserted through the sclera such that it projects into the vitreous cavity to deliver the pharmaceutical agent into the vitreous cavity. In another example, U.S. Patent No. 5,443,505 describes an implantable device for introduction into a suprachoroidal space or an avascular region for sustained release of drug into the interior of the eye. U.S. Patent Nos. 5,773,019 and 6,001,386 each disclose an implantable drug delivery device attachable to the scleral surface of an eye. Additional methods and devices (*e.g.*, a transscleral patch and delivery via contact lenses) for delivery of a therapeutic agent to the eye are described in, *e.g.*, Ambati and Adamis (2002) *Prog Retin Eye Res* 21(2):145-151; Ranta and Urtti (2006) *Adv Drug Delivery Rev* 58(11):1164-1181; Barocas and Balachandran (2008) *Expert Opin Drug Delivery* 5(1):1-10(10); Gulsen and Chauhan (2004) *Invest Ophthalmol Vis Sci* 45:2342-2347; Kim et al. (2007) *Ophthalmic Res* 39:244-254; and PCT publication no. WO 04/073551, the disclosures of which are incorporated herein by reference in their entirety.

[263] In various embodiments, subcutaneous administration may be accomplished by means of a device, such as a syringe, a prefilled syringe, an auto-injector (*e.g.*, disposable or reusable), a pen injector, a patch injector, a wearable injector, an ambulatory syringe infusion pump with

subcutaneous infusion sets, or other device for combining with binding agent drug for subcutaneous injection.

[264] An injection system of the present disclosure may employ a delivery pen as described in U.S. Pat. No. 5,308,341. Pen devices are commonly used for self-delivery of insulin to patients with diabetes. Such devices may comprise at least one injection needle (*e.g.*, a 31 gauge needle of about 5 to 8 mm in length), are generally pre-filled with one or more therapeutic unit doses of a therapeutic solution, and are useful for rapidly delivering solution to a subject with as little pain as possible. One medication delivery pen comprises a vial holder into which a vial of a therapeutic or other medication may be received. The pen may be an entirely mechanical device or it may be combined with electronic circuitry to accurately set and/or indicate the dosage of medication that is injected into the user. See, *e.g.*, U.S. Pat. No. 6,192,891. In some embodiments, the needle of the pen device is disposable and the kits comprise one or more disposable replacement needles. Pen devices suitable for delivery of any one of the presently featured compositions comprising a binding agent of the present disclosure are also described in, *e.g.*, U.S. Pat. Nos. 6,277,099; 6,200,296; and 6,146,361, the disclosures of each of which are incorporated herein by reference in their entirety. A microneedle-based pen device is described in, *e.g.*, U.S. Pat. No. 7,556,615, the disclosure of which is incorporated herein by reference in its entirety. See also the Precision Pen Injector (PPI) device, MOLLY™, manufactured by Scandinavian Health Ltd.

[265] In some embodiments, a composition comprising a binding agent of the present disclosure may be delivered to a subject by way of local administration that does not rely upon transport of the binding agent to its intended target tissue or site via the vascular system. For example, the composition comprising a binding agent of the present disclosure may be delivered by injection or implantation of the composition comprising a binding agent of the present disclosure or by injection or implantation of a device containing the composition comprising a binding agent of the present disclosure. In certain embodiments, following local administration in the vicinity of a target tissue or site, the composition comprising a binding agent of the present disclosure, or one or more components thereof, may diffuse to an intended target tissue or site that is not the site of administration.

[266] In some embodiments, a composition comprising a binding agent of the present disclosure may be locally administered to a joint, *e.g.*, directly to a joint (*e.g.*, into a joint space) or in the vicinity of a joint. Examples of intraarticular joints to which a composition comprising a binding agent of the present disclosure may be locally administered comprise, *e.g.*, the hip,

knee, elbow, wrist, sternoclavicular, temporomandibular, carpal, tarsal, ankle, and any other joint subject to arthritic conditions. A composition comprising a binding agent of the present disclosure may also be administered to bursa such as, *e.g.*, acromial, bicipitoradial, cubitoradial, deltoid, infrapatellar, ischial, and any other bursa.

[267] In some embodiments, the compositions comprising a binding agent of the present disclosure provided herein are present in unit dosage form, which unit dosage form may be suitable for self-administration. Such a unit dosage form may be provided within a container, generally, for example, a vial, cartridge, prefilled syringe or disposable pen. A doser such as the doser device described in U.S. Pat. No. 6,302,855, may also be used, for example, with an injection system as described herein.

[268] A pharmaceutical solution may comprise a therapeutically effective amount of a composition comprising a binding agent of the present disclosure. Such effective amounts may be readily determined based, in part, on the effect of the administered composition comprising a binding agent of the present disclosure, or the combinatorial effect of the composition comprising a binding agent of the present disclosure and one or more additional active agents, if more than one agent is used. A therapeutically effective amount of a composition comprising a binding agent of the present disclosure may also vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the composition (and one or more additional active agents) to elicit a desired response in the individual, *e.g.*, amelioration of at least one condition parameter, *e.g.*, amelioration of at least one symptom of the complement-mediated disorder. For example, a therapeutically effective amount of a composition comprising a binding agent of the present disclosure may inhibit (lessen the severity of or eliminate the occurrence of) and/or prevent a disorder, and/or any one of the symptoms of the disorder. A therapeutically effective amount is also one in which any toxic or detrimental effects of the composition comprising a binding agent of the present disclosure are outweighed by the therapeutically beneficial effects.

[269] A composition comprising a binding agent of the present disclosure may be administered as a fixed dose, or in a milligram per kilogram (mg/kg) dose. In some embodiments, the dose may also be chosen to reduce or avoid production of antibodies or other host immune responses against one or more of the antigen-binding molecules in the composition comprising a binding agent of the present disclosure. While in no way intended to be limiting, exemplary dosages of a binding agent, such as a composition comprising a binding agent of the present disclosure comprise, *e.g.*, 1-1000 mg/kg, 1-100 mg/kg, 0.5-50 mg/kg, 0.1-

100 mg/kg, 0.5-25 mg/kg, 1-20 mg/kg, and 1-10 mg/kg. Exemplary dosages of a composition comprising a binding agent of the present disclosure comprise, without limitation, 0.1 mg/kg, 0.5 mg/kg, 1.0 mg/kg, 2.0 mg/kg, 4 mg/kg, 8 mg/kg, or 20 mg/kg.

[270] Suitable human doses of any of the compositions comprising a binding agent of the present disclosure may further be evaluated in, *e.g.*, Phase I dose escalation studies. See, *e.g.*, van Gurp et al. (2008) *Am J Transplantation* 8(8):1711-1718; Hanouska et al. (2007) *Clin Cancer Res* 13(2, part 1):523-531; and Hetherington et al. (2006) *Antimicrobial Agents and Chemotherapy* 50(10): 3499-3500.

[271] In various embodiments, a pharmaceutical composition may comprise a nucleic acid of the present disclosure, *e.g.*, a vector. Methods of formulating pharmaceutical compositions comprising a nucleic acid of the present disclosure are known, *e.g.*, in the books in the series *Drugs and the Pharmaceutical Sciences: a Series of Textbooks and Monographs* (Dekker, N.Y.). For example, solutions or suspensions used for parenteral, intradermal, or subcutaneous application may comprise the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose. pH may be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. Parenteral preparations may be enclosed, *e.g.*, in ampoules, disposable syringes, or more than one dose vials made of glass or plastic.

[272] Pharmaceutical compositions comprising a nucleic acid of the present disclosure suitable for injection may comprise sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. For intravenous administration, suitable carriers may comprise physiological saline, bacteriostatic water, Cremophor EL.TM. (BASF, Parsippany, N.J.) or phosphate buffered saline (PBS). The carrier may be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof.

[273] Sterility, stability, viscosity and other factors relating to effective therapeutic use may be considered. One method of maintaining fluidity, for example, comprises the use of a coating such as lecithin, the maintenance of required particle size, or the use of surfactants. In some instances, it will be preferable to comprise isotonic agents, for example, sugars, polyalcohols

such as mannitol, sorbitol, sodium chloride in the composition. In some instances, prolonged absorption of injectable compositions comprising a nucleic acid of the present disclosure may be brought about by comprising in the composition an agent that delays absorption, for example, aluminum monostearate and gelatin.

[274] Sterile injectable solutions may be prepared by incorporating one or a combination of ingredients such as antibacterial and antifungal agents (for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like), and/or by filtered sterilization. In some instances, dispersions are prepared by incorporating the active molecule into a sterile vehicle, which contains a basic dispersion medium and antibacterial or antifungal agents. In the case of sterile powders for the preparation of sterile injectable solutions, methods of preparation comprise vacuum drying and freeze-drying from a previously sterile-filtered solution thereof.

[275] Oral compositions comprising a nucleic acid of the present disclosure may comprise an inert diluent or an edible carrier. For the purpose of oral therapeutic administration, a nucleic acid of the present disclosure may be incorporated with excipients and used, *e.g.*, in the form of tablets, troches, or capsules, *e.g.*, gelatin capsules. Oral compositions comprising a nucleic acid of the present disclosure may also be prepared using a fluid carrier for use as a mouthwash. Pharmaceutically compatible binding agents, and/or adjuvant materials may be comprised as part of the composition. The tablets, pills, capsules, troches and the like may contain any of the following ingredients, or molecules of a similar nature: a binder such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose, a disintegrating agent such as alginic acid, Primogel®, or corn starch; a lubricant such as magnesium stearate or Sterotes®; a glidant such as colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; or a flavoring agent such as peppermint, methyl salicylate, or orange flavoring.

[276] In some embodiments, nucleic acids may be administered by any method suitable for administration of nucleic acid agents, such as a DNA vaccine. These methods comprise, *e.g.*, gene guns, bio injectors, and skin patches as well as needle-free methods such as the micro-particle DNA vaccine technology disclosed in U.S. Pat. No. 6,194,389, and the mammalian transdermal needle-free vaccination with powder-form vaccine as disclosed in U.S. Pat. No. 6,168,587. Additionally, intranasal delivery is possible, as described in, *e.g.*, Hamajima et al., (1998) Clin. Immunol. Immunopathol. 88(2), 205-10. Liposomes (*e.g.*, as described in U.S. Pat. No. 6,472,375). In certain instances, microencapsulation may be used. In addition, biodegradable targetable microparticle delivery systems may be used (*e.g.*, as described in U.S. Pat. No. 6,471,996).

[277] Compositions comprising a nucleic acid of the present disclosure may comprise components such as adjuvants, diluents, binders, stabilizers, buffers, salts, lipophilic solvents, preservatives, mixtures thereof, or any component for inclusion in therapeutic compositions comprising a nucleic acid. A nucleic acid composition may comprise, *e.g.*, saline, solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, a phosphate buffered saline solution, water, and emulsions, such as an oil/water or water/oil emulsion, and various types of wetting agents, compatible with pharmaceutical administration. Supplementary active molecules may also be incorporated into compositions comprising a nucleic acid of the present disclosure of the present disclosure. Compositions comprising a nucleic acid of the present disclosure of the present disclosure may comprise stabilizers and preservatives and any of the carriers described herein with the optional additional proviso that they be acceptable for use *in vivo*. For examples of additional carriers, stabilizers, and adjuvants, see Martin REMINGTON'S PHARM. SCI., 15th Ed. (Mack Publ. Co., Easton (1975) and Williams & Williams, (1995), and in the "PHYSICIAN'S DESK REFERENCE," 52nd ed., Medical Economics, Montvale, N.J. (1998).

[278] Methods described herein comprise the manufacture and use of pharmaceutical compositions comprising a nucleic acid of the present disclosure. Pharmaceutical compositions comprising a nucleic acid of the present disclosure are generally formulated to be compatible with their intended route of administration. Examples of routes of administration comprise parenteral, *e.g.*, intravenous, intracranial, intradermal, subcutaneous, oral (*e.g.*, inhalation), transdermal (topical), transmucosal, and rectal administration.

[279] A nucleic acid composition may comprise a buffer or a pH adjusting agent. A buffer may be a salt prepared from an organic acid or base. Buffers of the present disclosure comprise organic acid salts such as salts of citric acid, ascorbic acid, gluconic acid, carbonic acid, tartaric acid, succinic acid, acetic acid, or phthalic acid, Tris, tromethamine hydrochloride, and phosphate buffers. Additional carriers comprise polymeric excipients or additives such as polyvinylpyrrolidones, ficolls (a polymeric sugar), dextrates (*e.g.*, cyclodextrins, such as 2-hydroxypropyl- β -cyclodextrin), polyethylene glycols, flavoring agents, antimicrobial agents, sweeteners, antioxidants, antistatic agents, surfactants (*e.g.*, polysorbates such as TWEEN 20® and TWEEN 80®), lipids (*e.g.*, phospholipids, fatty acids), steroids (*e.g.*, cholesterol), and chelating agents (*e.g.*, EDTA).

[280] In certain embodiments in which a nucleic acid of the present disclosure is a vector, the present disclosure comprises a composition for gene transduction and/or gene therapy, *e.g.*, a

composition comprising viral particles, *e.g.*, AAV particles and/or retroviral particles such as lentiviral particles. The terms "genome particles (gp)," or "genome equivalents," as used in reference to a viral titer, refer to the number of virions containing the recombinant viral genome, *e.g.*, recombinant AAV DNA genome, regardless of infectivity or functionality. The number of genome particles in a vector preparation may be measured. The terms "infection unit (IU)," "infectious particle," or "replication unit," as used in reference to a viral titer, refer to the number of infectious and replication-competent recombinant virus, *e.g.*, of recombinant AAV vector particles, as may be measured by the infectious center assay, also known as replication center assay. The term "transducing unit (TU)" as used in reference to a viral titer, refers to the number of infectious recombinant vector particles, *e.g.*, recombinant AAV vector particles, that result in the production of a functional transgene product.

[281] In some embodiments, a composition comprises, *e.g.*, 2×10^6 to 2×10^{12} , 2×10^7 to 2×10^{11} , or 2×10^8 to 2×10^{11} DNA-containing viral particles per dose. In certain embodiments, the concentration or titer of vector in a unit dosage form is, *e.g.*, at least: (a) 1×10^{12} particles per mL, 2×10^{12} particles per mL, 3×10^{12} particles per mL, 4×10^{12} particles per mL, 5×10^{12} particles per mL, 6×10^{12} particles per mL, 7×10^{12} particles per mL, 8×10^{12} particles per mL, 9×10^{12} particles per mL, 10×10^{12} particles per mL, 15×10^{12} particles per mL, 20×10^{12} particles per mL, 25×10^{12} particles per mL, or 50×10^{12} particles per mL; (b) 1×10^9 TU/mL, 2×10^9 TU/mL, 3×10^9 TU/mL, 4×10^9 TU/mL, 5×10^9 TU/mL, 6×10^9 TU/mL, 7×10^9 TU/mL, 8×10^9 TU/mL, 9×10^9 TU/mL, 10×10^9 TU/mL, 15×10^9 TU/mL, 20×10^9 TU/mL, 25, or 50×10^9 TU/mL; or (c) 1×10^{10} IU/mL, 2×10^{10} IU/mL, 3×10^{10} IU/mL, 4×10^{10} IU/mL, 5×10^{10} IU/mL, 6×10^{10} IU/mL, 7×10^{10} IU/mL, 8×10^{10} IU/mL, 9×10^{10} IU/mL, 10×10^{10} IU/mL, 15×10^{10} IU/mL, 20×10^{10} IU/mL, 25×10^{10} IU/mL, or 50×10^{10} IU/mL. Such embodiments do not limit the unit dosages encompassed by the present disclosure and do not limit the various measures of dosage that may be used in conjunction with various compositions comprising a nucleic acid of the present disclosure of the present disclosure. For instance, a particle dosage, concentration, or amount may be measured and/or expressed in terms of vector genomes per kilogram subject (Vg/Kg) or Vg/dose. The preferred means of measuring and/or expressing particle dosage, concentration, or amount may vary depending upon various factors, *e.g.*, route of administration.

[282] The present disclosure provides technologies for simultaneously targeting CD20 and another antigen, *e.g.*, CD19. In some embodiments, the present disclosure provides technologies for initiating and/or modulating immune responses. In some embodiments, the

present disclosure provides technologies for treating cancer (*e.g.*, cancer characterized by cell(s) with surface-expressed CD20).

[283] The present specification comprises use of a binding agent pharmaceutical composition provided herein to treat or prevent cancer. Another aspect of the present disclosure is directed to a method for treating or preventing a malignancy, said method comprising administering to a subject in need thereof an effective amount of a binding agent pharmaceutical composition, *e.g.*, where the cells comprises at least one Antigen binding system provided herein. Methods of the present disclosure comprising administration of an pharmaceutically effective amount of a binding agent pharmaceutical composition of the present disclosure may be used to treat a cancer in a subject, reduce the size of a tumor, kill tumor cells, prevent tumor cell proliferation, prevent growth of a tumor, eliminate a tumor from a patient, prevent relapse of a tumor, prevent tumor metastasis, induce remission in a patient, or any combination thereof. In certain embodiments, a method provided herein induces a complete response. In some embodiments, a method provided herein induces a partial response. In certain embodiments the binding agent pharmaceutical composition is, comprises, comprises as the active agent, or comprises as the sole active agent, cells provided herein, *e.g.*, cells that comprise or express at least one CAR of the present disclosure. In some embodiments, the binding agent pharmaceutical composition comprises a bicistronic CAR system comprising an anti-CD20 car and an anti-CD19 CAR, or the binding agent pharmaceutical composition comprises a bispecific anti-CD20/anti-CD19 CAR of the present disclosure.

[284] In various embodiments, the present disclosure comprises use of a binding agent pharmaceutical composition provided herein to induce in a subject, or provide a subject with, immunity against a cancer. The present disclosure further comprises a method of preventing cancer in a subject by administering to the subject a binding agent pharmaceutical composition provided herein. The present disclosure further comprises a method of inducing an immune response in a subject by administering to the subject a binding agent pharmaceutical composition provided herein. In certain embodiments the binding agent pharmaceutical composition is, comprises, comprises as the active agent, or comprises as the sole active agent, cells provided herein, *e.g.*, cells that comprise or express at least one CAR of the present disclosure. In some embodiments, the binding agent pharmaceutical composition comprises a bicistronic CAR system comprising an anti-CD20 car and an anti-CD19 CAR, or the binding agent pharmaceutical composition comprises a bispecific anti-CD20/anti-CD19 CAR of the present disclosure.

[285] In certain embodiments, a method of treating a cancer in a subject in need thereof comprises administering to the subject a polynucleotide, vector, antibody, or antigen binding system disclosed herein. In one embodiment, the method comprises administering a polynucleotide encoding an antigen binding system or antibody (*e.g.*, an antigen binding system). In another embodiment, the method comprises administering a vector comprising a polynucleotide encoding an antigen binding system or antibody. In another embodiment, the method comprises administering an antigen binding system or antibody to the subject.

[286] Another aspect of the disclosure is directed to a method of making a cell expressing a CAR or a TCR comprising transducing a cell with a polynucleotide disclosed herein under suitable conditions. In some embodiments, the method comprises transducing a cell with a polynucleotide encoding a CAR or a TCR, as disclosed herein. In some embodiments, the method comprises transducing a cell with a vector comprising the polynucleotide encoding a CAR or a TCR. In certain embodiments, the present disclosure provides a T cell therapy in which a binding agent pharmaceutical composition comprises T cells transfected or transduced with a vector comprising a polynucleotide sequence encoding an antigen-binding agent of the present disclosure (*e.g.*, an antigen binding system). In some embodiments, the donor T cells for use in the T cell therapy are obtained from the patient (*e.g.*, for an autologous T cell therapy). In other embodiments, the donor T cells for use in the T cell therapy are obtained from a subject that is not the patient. In one embodiment, the T cell therapy of the present disclosure is an Autologous Cell Therapy (eACT™). According to this embodiment, the method may comprise collecting blood cells from the patient. The isolated blood cells (*e.g.*, T cells) may then be engineered to express an antigen binding system of the present disclosure. In certain embodiments, the binding agents are administered to the patient. In some embodiments, the binding agents treat or are intended to treat or a cancer in the patient. For instance, in one embodiment the binding agents reduce the size of a tumor. In various embodiments, cells of the present disclosure may be cells freshly isolated from a human subject, cells freshly isolated from a cell culture, or cells having been stored, *e.g.*, frozen.

[287] Another aspect of the present disclosure is directed to a method of inducing an immunity against a tumor comprising administering to a subject an effective amount of a cell comprising a polynucleotide described herein, a vector described herein, or a CAR or a TCR described herein. In one embodiment, the method comprises administering to a subject an effective amount of a cell comprising a polynucleotide encoding a CAR or a TCR disclosed herein. In another embodiment, the method comprises administering to a subject an effective

amount of a cell comprising a vector comprising a polynucleotide encoding a CAR or a TCR disclosed herein. In another embodiment, the method comprises administering to a subject an effective amount of a cell comprising a CAR or a TCR encoded by a polynucleotide disclosed herein.

[288] Another aspect of the present disclosure is directed to a method of inducing an immune response in a subject comprising administering an effective amount of the engineered immune cells of the present application. In some embodiments, the immune response is a T cell-mediated immune response. In some embodiments, the T cell-mediated immune response is directed against one or more target cells. In some embodiments, the engineered immune cell comprises a CAR or a TCR, wherein the CAR or the TCR comprises a THD described in the present disclosure. In some embodiments, the target cell is a tumor cell.

[289] Another aspect of the present disclosure is directed to a method for treating or preventing a malignancy, said method comprising administering to a subject in need thereof an effective amount of at least one immune cell, wherein the immune cell comprises at least one CAR or TCR, and wherein the CAR or the TCR comprises one or two of the scfv disclosed herein.

[290] Another aspect of the present disclosure is directed to a method of treating a cancer in a subject in need thereof comprising administering to the subject a polynucleotide, a vector, a CAR or a TCR, a cell, or a composition disclosed herein. In one embodiment, the method comprises administering a polynucleotide encoding a CAR or a TCR. In another embodiment, the method comprises administering a vector comprising a polynucleotide encoding a CAR or a TCR. In another embodiment, the method comprises administering a CAR or a TCR encoded by a polynucleotide disclosed herein. In another embodiment, the method comprises administering a cell comprising the polynucleotide, or a vector comprising the polynucleotide, encoding a CAR or a TCR.

[291] In some embodiments, the methods of treating a cancer in a subject in need thereof comprise a T cell therapy. In one embodiment, the T cell therapy of the present disclosure is engineered Autologous Cell Therapy (eACT™). According to this embodiment, the method can include collecting blood cells from the patient. The isolated blood cells (*e.g.*, T cells) can then be engineered to express a CAR or a TCR of the present disclosure. In a particular embodiment, the CAR T cells or the TCR T cells are administered to the patient. In some embodiments, the CAR T cells or the TCR T cells treat a tumor or a cancer in the patient. In one embodiment the CAR T cells or the TCR T cells reduce the size of a tumor or a cancer.

[292] In some embodiments, the donor T cells for use in the T cell therapy are obtained from the patient (*e.g.*, for an autologous T cell therapy). In other embodiments, the donor T cells for use in the T cell therapy are obtained from a subject that is not the patient.

[293] The T cells can be administered at a therapeutically effective amount. For example, a therapeutically effective amount of the T cells can be at least about 10^4 cells, at least about 10^5 cells, at least about 10^6 cells, at least about 10^7 cells, at least about 10^8 cells, at least about 10^9 , or at least about 10^{10} . In another embodiment, the therapeutically effective amount of the T cells is about 10^4 cells, about 10^5 cells, about 10^6 cells, about 10^7 cells, or about 10^8 cells. In one particular embodiment, the therapeutically effective amount of the CAR T cells or the TCR T cells is about 2×10^6 cells/kg, about 3×10^6 cells/kg, about 4×10^6 cells/kg, about 5×10^6 cells/kg, about 6×10^6 cells/kg, about 7×10^6 cells/kg, about 8×10^6 cells/kg, about 9×10^6 cells/kg, about 1×10^7 cells/kg, about 2×10^7 cells/kg, about 3×10^7 cells/kg, about 4×10^7 cells/kg, about 5×10^7 cells/kg, about 6×10^7 cells/kg, about 7×10^7 cells/kg, about 8×10^7 cells/kg, or about 9×10^7 cells/kg.

[294] The methods of the disclosure can be used to treat a cancer in a subject, reduce the size of a tumor, kill tumor cells, prevent tumor cell proliferation, prevent growth of a tumor, eliminate a tumor from a patient, prevent relapse of a tumor, prevent tumor metastasis, induce remission in a patient, or any combination thereof. In certain embodiments, the methods induce a complete response. In other embodiments, the methods induce a partial response.

[295] In certain embodiments, the cancer comprises cells that express CD19, *e.g.*, on the surface of the cell. In certain embodiments the cancer comprises cells that express CD20, *e.g.*, on the surface of the cell. In certain embodiments the cancer comprises cells that each individually express both CD19 and CD20, *e.g.*, on the surface of the cell.

[296] Cancers that may be treated include tumors that are not vascularized, not yet substantially vascularized, or vascularized. The cancer may also include solid or non-solid tumors. In some embodiments, the cancer is a hematologic cancer. In some embodiments, the cancer is of the white blood cells. In other embodiments, the cancer is of the plasma cells. In some embodiments, the cancer is leukemia, lymphoma, or myeloma. In certain embodiments, the cancer is acute lymphoblastic leukemia (ALL) (including non T cell ALL), acute lymphoid leukemia (ALL), and hemophagocytic lymphohistocytosis (HLH), B cell prolymphocytic leukemia, B-cell acute lymphoid leukemia ("BALL"), blastic plasmacytoid dendritic cell neoplasm, Burkitt's lymphoma, chronic lymphocytic leukemia (CLL), chronic myelogenous leukemia (CML), chronic myeloid leukemia (CML), chronic or acute granulomatous disease,

chronic or acute leukemia, diffuse large B cell lymphoma, diffuse large B cell lymphoma (DLBCL), follicular lymphoma, follicular lymphoma (FL), hairy cell leukemia, hemophagocytic syndrome (Macrophage Activating Syndrome (MAS), Hodgkin's Disease, large cell granuloma, leukocyte adhesion deficiency, malignant lymphoproliferative conditions, MALT lymphoma, mantle cell lymphoma, Marginal zone lymphoma, monoclonal gammopathy of undetermined significance (MGUS), multiple myeloma, myelodysplasia and myelodysplastic syndrome (MDS), myeloid diseases including but not limited to acute myeloid leukemia (AML), non-Hodgkin's lymphoma (NHL), plasma cell proliferative disorders (e.g., asymptomatic myeloma (smoldering multiple myeloma or indolent myeloma), plasmablastic lymphoma, plasmacytoid dendritic cell neoplasm, plasmacytomas (e.g., plasma cell dyscrasia; solitary myeloma; solitary plasmacytoma; extramedullary plasmacytoma; and multiple plasmacytoma), POEMS syndrome (Crow-Fukase syndrome; Takatsuki disease; PEP syndrome), primary mediastinal large B cell lymphoma (PMBC), small cell- or a large cell-follicular lymphoma, splenic marginal zone lymphoma (SMZL), systemic amyloid light chain amyloidosis, T-cell acute lymphoid leukemia ("TALL"), T-cell lymphoma, transformed follicular lymphoma, Waldenstrom macroglobulinemia, or a combination thereof. In one embodiment, the cancer is a myeloma. In one particular embodiment, the cancer is multiple myeloma. In another embodiment, the cancer is a leukemia. In one embodiment, the cancer is acute myeloid leukemia.

[297] In various instances, a method of using a binding agent pharmaceutical composition provided herein to treat cancer is an autologous cell therapy. In various instances, a method of using a binding agent pharmaceutical composition provided herein to treat cancer is an allogeneic cell therapy.

[298] Certain method using a binding agent pharmaceutical composition provided herein comprise collecting blood cells from the subject. Isolated subject blood cells (*e.g.*, T cells) may then be engineered to express, *e.g.*, an antigen binding system of the present disclosure. In some embodiments, the binding agents are administered to the subject. In some embodiments, the binding agents treat cancer in the subject. In one embodiment the binding agents reduce the size of a tumor.

[299] In various embodiments, a cell therapy provided herein for use in the present disclosure may be administered to a subject in a course of treatment that further comprises administration of one or more additional therapeutic agents or therapies that are not a cell therapy provided herein. In certain embodiments, the present disclosure provides combination therapy for the

treatment of cancer, the treatment comprising administering an anti-cancer agent to a subject receiving and/or in need of a binding agent provided herein.

[300] In certain embodiments, administration of a binding agent provided herein may be to a subject having previously received, scheduled to receive, or in the course of a treatment regimen comprising an additional anti-cancer therapy. In various embodiments, an additional agent or therapy administered in combination with a binding agent provided herein as described herein may be administered at the same time as binding agent provided herein, on the same day as binding agent provided herein, or in the same week as binding agent provided herein. In various embodiments, an additional agent or therapy administered in combination with a binding agent provided herein as described herein may be administered such that administration of the binding agent provided herein and the additional agent or therapy are separated by one or more hours before or after, one or more days before or after, one or more weeks before or after, or one or more months before or after administration of binding agent provided herein. In various embodiments, the administration frequency of one or more additional agents may be the same as, similar to, or different from the administration frequency of a binding agent provided herein.

[301] An agent or therapy used in combination with binding agent provided herein may be administered in a single therapeutic composition or dose together with binding agent provided herein, at the same time as binding agent provided herein in the form of a separate composition, or in a manner temporally distinct from the administration of binding agent provided herein. When a binding agent provided herein is to be used in combination with an additional agent, the binding agent provided herein may be co-formulated with the additional agent or the binding agent provided herein may be formulated separately from the additional agent formulation.

[302] In some embodiments, the methods further comprise administering a chemotherapeutic. In certain embodiments, the chemotherapeutic selected is a lymphodepleting (preconditioning) chemotherapeutic. Beneficial preconditioning treatment regimens, along with correlative beneficial biomarkers are described in U.S. Provisional Patent Applications 62/262,143 and 62/167,750 which are hereby incorporated by reference in their entirety herein. These describe, *e.g.*, methods of conditioning a patient in need of a T cell therapy comprising administering to the patient specified beneficial doses of cyclophosphamide (between 200 mg/m²/day and 2000 mg/m²/day) and specified doses of fludarabine (between 20 mg/m²/day and 900 mg/m²/day). One such dose regimen involves treating a patient comprising administering daily to the patient

about 500 mg/m²/day of cyclophosphamide and about 60 mg/m²/day of fludarabine for three days prior to administration of a therapeutically effective amount of engineered T cells to the patient. In other embodiments, the antigen binding molecule, transduced (or otherwise engineered) cells (such as CARs or TCRs), and the chemotherapeutic agent are administered each in an amount effective to treat the disease or condition in the subject.

[303] In certain embodiments, compositions comprising CAR- and/or TCR-expressing immune effector cells disclosed herein may be administered in conjunction with any number of chemotherapeutic agents. Examples of chemotherapeutic agents include alkylating agents such as thiopeta and cyclophosphamide (CYTOXANTM); alkyl sulfonates such as busulfan, improsulfan and piposulfan; aziridines such as benzodopa, carboquone, meturedopa, and uredopa; ethylenimines and methylamelamines including altretamine, triethylenemelamine, triethylenephosphoramide, triethylenethiophosphoramide and trimethylolomelamine resins; nitrogen mustards such as chlorambucil, chlornaphazine, chlorthophosphamide, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, melphalan, novembichin, phenesterine, prednimustine, trofosfamide, uracil mustard; nitrosureas such as carmustine, chlorozotocin, fotemustine, lomustine, nimustine, ranimustine; antibiotics such as aclacinomysins, actinomycin, anthramycin, azaserine, bleomycins, cactinomycin, calicheamicin, carabycin, carminomycin, carzinophilin, chromomycins, dactinomycin, daunorubicin, detorubicin, 6-diazo-5-oxo-L-norleucine, doxorubicin, epirubicin, esorubicin, idarubicin, marcellomycin, mitomycins, mycophenolic acid, nogalamycin, olivomycins, peplomycin, potfiromycin, puromycin, quelamycin, rodorubicin, streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin, zorubicin; anti-metabolites such as methotrexate and 5-fluorouracil (5-FU); folic acid analogues such as denopterin, methotrexate, pteropterin, trimetrexate; purine analogs such as fludarabine, 6-mercaptopurine, thiamiprine, thioguanine; pyrimidine analogs such as ancitabine, azacitidine, 6-azauridine, carmofur, cytarabine, dideoxyuridine, doxifluridine, enocitabine, floxuridine, 5-FU; androgens such as calusterone, dromostanolone propionate, epitostanol, mepitiostane, testolactone; anti-adrenals such as aminoglutethimide, mitotane, trilostane; folic acid replenisher such as frolinic acid; aceglutone; aldophosphamide glycoside; aminolevulinic acid; amsacrine; bestabucil; bisantrene; edatraxate; defofamine; demecolcine; diaziquone; elformithine; elliptinium acetate; etoglucid; gallium nitrate; hydroxyurea; lentinan; lonidamine; mitoguazone; mitoxantrone; mopidamol; nitracrine; pentostatin; phenamet; pirarubicin; podophyllinic acid; 2-ethylhydrazide; procarbazine; PSK[®]; razoxane; sizofiran; spirogermanium; tenuazonic acid; triaziquone; 2,

2',2''-trichlorotriethylamine; urethan; vindesine; dacarbazine; mannomustine; mitobronitol; mitolactol; pipobroman; gacytosine; arabinoside ("Ara-C"); cyclophosphamide; thiotepa; taxoids, e.g. paclitaxel (TAXOL™, Bristol-Myers Squibb) and doxorubicin (TAXOTERE®, Rhone-Poulenc Rorer); chlorambucil; gemcitabine; 6-thioguanine; mercaptopurine; methotrexate; platinum analogs such as cisplatin and carboplatin; vinblastine; platinum; etoposide (VP-16); ifosfamide; mitomycin C; mitoxantrone; vincristine; vinorelbine; navelbine; novantrone; teniposide; daunomycin; aminopterin; xeloda; ibandronate; CPT-11; topoisomerase inhibitor RFS2000; difluoromethylomithine (DMFO); retinoic acid derivatives such as Targretin™ (bexarotene), Panretin™, (alitretinoin); ONTAK™ (denileukin diftitox); esperamicins; capecitabine; and pharmaceutically acceptable salts, acids or derivatives of any of the above. In some embodiments, compositions comprising CAR- and/or TCR-expressing immune effector cells disclosed herein may be administered in conjunction with an anti-hormonal agent that acts to regulate or inhibit hormone action on tumors such as anti-estrogens including for example tamoxifen, raloxifene, aromatase inhibiting 4(5)-imidazoles, 4-hydroxytamoxifen, trioxifene, keoxifene, LY117018, onapristone, and toremifene (Fareston); and anti-androgens such as flutamide, nilutamide, bicalutamide, leuprolide, and goserelin; and pharmaceutically acceptable salts, acids or derivatives of any of the above. Combinations of chemotherapeutic agents are also administered where appropriate, including, but not limited to CHOP, i.e., Cyclophosphamide (Cytosan®), Doxorubicin (hydroxydoxorubicin), Vincristine (Oncovin®), and Prednisone.

[304] In some embodiments, the chemotherapeutic agent is administered at the same time or within one week after the administration of the engineered cell or nucleic acid. In other embodiments, the chemotherapeutic agent is administered from 1 to 4 weeks or from 1 week to 1 month, 1 week to 2 months, 1 week to 3 months, 1 week to 6 months, 1 week to 9 months, or 1 week to 12 months after the administration of the engineered cell or nucleic acid. In some embodiments, the chemotherapeutic agent is administered at least 1 month before administering the cell or nucleic acid. In some embodiments, the methods further comprise administering two or more chemotherapeutic agents.

[305] A variety of additional therapeutic agents may be used in conjunction with the compositions described herein. For example, potentially useful additional therapeutic agents include PD-1 inhibitors such as nivolumab (OPDIVO®), pembrolizumab (KEYTRUDA®), pembrolizumab, pidilizumab (CureTech), and atezolizumab (Roche). Additional therapeutic agents suitable for use in combination with the disclosure include, but are not limited to,

ibrutinib (IMBRUVICA[®]), ofatumumab (ARZERRA[®]), rituximab (RITUXAN[®]), bevacizumab (AVASTIN[®]), trastuzumab (HERCEPTIN[®]), trastuzumab emtansine (KADCYLA[®]), imatinib (GLEEVEC[®]), cetuximab (ERBITUX[®]), panitumumab (VECTIBIX[®]), catumaxomab, ibritumomab, ofatumumab, tositumomab, brentuximab, alemtuzumab, gemtuzumab, erlotinib, gefitinib, vandetanib, afatinib, lapatinib, neratinib, axitinib, masitinib, pazopanib, sunitinib, sorafenib, toceranib, lestaurtinib, axitinib, cediranib, lenvatinib, nintedanib, pazopanib, regorafenib, semaxanib, sorafenib, sunitinib, tivozanib, toceranib, vandetanib, entrectinib, cabozantinib, imatinib, dasatinib, nilotinib, ponatinib, radotinib, bosutinib, lestaurtinib, ruxolitinib, pacritinib, cobimetinib, selumetinib, trametinib, binimetinib, alectinib, ceritinib, crizotinib, aflibercept, adipotide, denileukin diftitox, mTOR inhibitors such as Everolimus and Temsirolimus, hedgehog inhibitors such as sonidegib and vismodegib, CDK inhibitors such as CDK inhibitor (palbociclib).

[306] In additional embodiments, the composition comprising CAR- and/or TCR-containing immune are administered with an anti-inflammatory agent. Anti-inflammatory agents or drugs can include, but are not limited to, steroids and glucocorticoids (including betamethasone, budesonide, dexamethasone, hydrocortisone acetate, hydrocortisone, hydrocortisone, methylprednisolone, prednisolone, prednisone, triamcinolone), nonsteroidal anti-inflammatory drugs (NSAIDs) including aspirin, ibuprofen, naproxen, methotrexate, sulfasalazine, leflunomide, anti-TNF medications, cyclophosphamide and mycophenolate. Exemplary NSAIDs include ibuprofen, naproxen, naproxen sodium, Cox-2 inhibitors, and sialylates. Exemplary analgesics include acetaminophen, oxycodone, tramadol or propoxyphene hydrochloride. Exemplary glucocorticoids include cortisone, dexamethasone, hydrocortisone, methylprednisolone, prednisolone, or prednisone. Exemplary biological response modifiers include molecules directed against cell surface markers (*e.g.*, CD4, CD5, etc.), cytokine inhibitors, such as the TNF antagonists, (*e.g.*, etanercept (ENBREL[®]), adalimumab (HUMIRA[®]) and infliximab (REMICADE[®]), chemokine inhibitors and adhesion molecule inhibitors. The biological response modifiers include monoclonal antibodies as well as recombinant forms of molecules. Exemplary DMARDs include azathioprine, cyclophosphamide, cyclosporine, methotrexate, penicillamine, leflunomide, sulfasalazine, hydroxychloroquine, Gold (oral (auranofin) and intramuscular), and minocycline.

[307] In certain embodiments, the compositions described herein are administered in conjunction with a cytokine. "Cytokine" is meant to refer to proteins released by one cell population that act on another cell as intercellular mediators. Examples of cytokines are

lymphokines, monokines, and traditional polypeptide hormones. Included among the cytokines are growth hormones such as human growth hormone, N-methionyl human growth hormone, and bovine growth hormone; parathyroid hormone; thyroxine; insulin; proinsulin; relaxin; prorelaxin; glycoprotein hormones such as follicle stimulating hormone (FSH), thyroid stimulating hormone (TSH), and luteinizing hormone (LH); hepatic growth factor (HGF); fibroblast growth factor (FGF); prolactin; placental lactogen; mullerian-inhibiting substance; mouse gonadotropin-associated peptide; inhibin; activin; vascular endothelial growth factor; integrin; thrombopoietin (TPO); nerve growth factors (NGFs) such as NGF-beta; platelet-growth factor; transforming growth factors (TGFs) such as TGF-alpha and TGF-beta; insulin-like growth factor-I and -II; erythropoietin (EPO); osteoinductive factors; interferons such as interferon-alpha, beta, and -gamma; colony stimulating factors (CSFs) such as macrophage-CSF (M-CSF); granulocyte-macrophage-CSF (GM-CSF); and granulocyte-CSF (G-CSF); interleukins (ILs) such as IL-1, IL-1alpha, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12; IL-15, a tumor necrosis factor such as TNF-alpha or TNF-beta; and other polypeptide factors including LIF and kit ligand (KL). As used herein, the term cytokine includes proteins from natural sources or from recombinant cell culture, and biologically active equivalents of the native sequence cytokines. In various embodiments, a binding agent provided herein for use in the present disclosure may be administered to a subject in a course of treatment that further comprises administration of an anti-inflammatory agent. Anti-inflammatory agents may comprise, without limitation, steroids and glucocorticoids (comprising betamethasone, budesonide, dexamethasone, hydrocortisone acetate, hydrocortisone, hydrocortisone, methylprednisolone, prednisolone, prednisone, triamcinolone), nonsteroidal anti-inflammatory drugs (NSAIDs) comprising aspirin, ibuprofen, naproxen, methotrexate, sulfasalazine, leflunomide, anti-TNF medications, cyclophosphamide and mycophenolate. Exemplary NSAIDs comprise ibuprofen, naproxen, naproxen sodium, Cox-2 inhibitors, and sialylates. Exemplary analgesics comprise acetaminophen, oxycodone, tramadol or propoxyphene hydrochloride. Exemplary glucocorticoids comprise cortisone, dexamethasone, hydrocortisone, methylprednisolone, prednisolone, or prednisone. Exemplary biological response modifiers comprise molecules directed against cell surface markers (*e.g.*, CD4, CD5, etc.), cytokine inhibitors, such as the TNF antagonists, (*e.g.*, etanercept (ENBREL[®]), adalimumab (HUMIRA[®]) and infliximab (REMICADE[®]), chemokine inhibitors and adhesion molecule inhibitors. The biological response modifiers comprise monoclonal antibodies as well as recombinant forms of

molecules. Exemplary DMARDs comprise azathioprine, cyclophosphamide, cyclosporine, methotrexate, penicillamine, leflunomide, sulfasalazine, hydroxychloroquine, Gold (oral (auranofin) and intramuscular), and minocycline.

[308] In various embodiments, a binding agent provided herein for use in the present disclosure may be administered to a subject in a course of treatment that further comprises administration of a CHOP. CHOP consists of (C)yclophosphamide, an alkylating agent which damages DNA by binding to it and causing the formation of cross-links; (H)ydroxydaunorubicin (also called doxorubicin or adriamycin), an intercalating agent which damages DNA by inserting itself between DNA bases; (O)ncovin (vincristine), which prevents cells from duplicating by binding to the protein tubulin; and (P)rednisone or (P)rednisolone, which are corticosteroids.

Additional Exemplary Embodiments. The present disclosure comprises, without limitation, the following exemplary embodiments:

Embodiment 1. An isolated polynucleotide encoding a chimeric antigen receptor (CAR) or a T cell receptor (TCR), which comprises (i) an antigen binding molecule, (ii) a costimulatory domain, and (iii) an activating domain, wherein the costimulatory domain comprises an extracellular domain, a transmembrane domain, and an intracellular domain, wherein the extracellular domain comprises an antigen binding molecule consisting essentially of or consisting of (i) an amino acid sequence at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or about 100% identical to the amino acid sequence of any of the constructs described in SEQ ID NO. 232, 221, 56, 45, 155, 144, 177, 78, and 67.

Embodiment 2. The polynucleotide of embodiment 1, wherein the transmembrane domain is a transmembrane domain of 4-1BB/CD137, an alpha chain of a T cell receptor, a beta chain of a T cell receptor, CD3 epsilon, CD4, CD5, CD8 alpha, CD9, CD16, CD19, CD22, CD33, CD37, CD45, CD64, CD80, CD86, CD134, CD137, CD154, or a zeta chain of a T cell receptor, or any combination thereof.

Embodiment 3. The polynucleotide of embodiment 1 or 2, wherein the intracellular domain comprises a signaling region of 4-1BB/CD137, activating NK cell receptors, B7-H3, BAFFR, BLAME (SLAMF8), BTLA, CD100 (SEMA4D), CD103, CD160 (BY55), CD18, CD19, CD19a, CD2, CD247, CD27, CD276 (B7-H3), CD29, CD3 delta, CD3 epsilon, CD3 gamma, CD30, CD4, CD40, CD49a, CD49D, CD49f, CD69, CD7, CD84,

CD8alpha, CD8beta, CD96 (Tactile), CD11a, CD11b, CD11c, CD11d, CDS, CEACAM1, CRTAM, cytokine receptors, DAP-10, DNAM1 (CD226), Fc gamma receptor, GADS, GATR, HVEM (LIGHTR), IA4, ICAM-1, ICAM-1, Ig alpha (CD79a), IL2R beta, IL2R gamma, IL7R alpha, Immunoglobulin-like proteins, inducible T cell costimulator (ICOS), integrins, ITGA4, ITGA4, ITGA6, ITGAD, ITGAE, ITGAL, ITGAM, ITGAX, ITGB2, ITGB7, ITGB1, KIRDS2, LAT, LFA-1, LFA-1, a ligand that specifically binds with CD83, LIGHT, LIGHT (tumor necrosis factor superfamily member 14; TNFSF14), LTBR, Ly9 (CD229), lymphocyte function-associated antigen-1 (LFA-1 (CD11a/CD18), MHC class I molecule, NKG2C, NKG2D, NKp30, NKp44, NKp46, NKp80 (KLRF1), OX-40, PAG/Cbp, programmed death-1 (PD-1), PSGL1, SELPLG (CD162), signaling lymphocytic activation molecules (SLAM proteins), SLAM (SLAMF1; CD150; IPO-3), SLAMF4 (CD244; 2B4), SLAMF6 (NTB-A; Lyl08), SLAMF7, SLP-76, TNF receptor proteins, TNFR2, a Toll ligand receptor, TRANCE/RANKL, VLA1, or VLA-6, or a combination thereof.

Embodiment 4. The polynucleotide of embodiments 1 to 3, wherein at least one of the antigen binding molecule specifically binds an antigen selected from the group consisting of 5T4, alphafetoprotein, B cell maturation antigen (BCMA), CA-125, carcinoembryonic antigen, CD19, CD20, CD22, CD23, CD30, CD33, CD56, CD123, CD138, c-Met, CSPG4, C-type lectin-like molecule 1 (CLL-1), EGFRvIII, epithelial tumor antigen, ERBB2, FLT3, folate binding protein, GD2, GD3, HER1-HER2 in combination, HER2-HER3 in combination, HER2/Neu, HERV-K, HIV-1 envelope glycoprotein gp41, HIV-1 envelope glycoprotein gp120, IL-11Ralpha, kappa chain, lambda chain, melanoma-associated antigen, mesothelin, MUC-1, mutated p53, mutated ras, prostate-specific antigen, ROR1, or VEGFR2, or a combination thereof.

Embodiment 5. The polynucleotide of any of embodiments 1 to 4, wherein the polynucleotide encodes a polypeptide comprising the amino acid sequence set forth in SEQ ID NOs 232, 221, 56, 45, 155, 144, 177, 78, and 67.

Embodiment 6. A polypeptide encoded by the polynucleotide of any one of embodiments 1 to 5.

Embodiment 7. A polypeptide comprising the amino acid sequence as set forth in SEQ ID NOs. 232, 221, 56, 45, 155, 144, 177, 78, and 67.

Embodiment 8. A vector comprising the polynucleotide of any one of embodiments 1 to 5.

Embodiment 9. The vector of embodiment 7, wherein the vector is an adenoviral vector, an adenovirus-associated vector, a DNA vector, a lentiviral vector, a plasmid, a retroviral vector, or an RNA vector, or any combination thereof.

Embodiment 10. A cell comprising the polynucleotide of any one of embodiments 1 to 5, the polypeptide of claim 6 or 7, the vector of claim 8 or 9, or any combination thereof.

Embodiment 11. A composition comprising the polynucleotide of any one of embodiments 1 to 5, the polypeptide of claim 6 or 7, the vector of claim 8 or 9, the cell of claim 10, or any combination thereof.

Embodiment 12. A method of making a cell comprising the polynucleotide of any one of claims 1 to 5, the polypeptide of embodiment 6 or 7, the vector of claim 8 or 9, or any combination thereof.

Embodiment 13. A method of inducing an immunity against a tumor comprising administering to a subject an effective amount the polynucleotide of any one of claims 1 to 5, the polypeptide of embodiment 6 or 7, the vector of embodiment 8 or 9, the cell of embodiment 10, the composition of embodiment 11 or any combination thereof.

Embodiment 14. Use of the polynucleotide of any one of embodiments 1 to 5, the polypeptide of embodiment 6 or 7, the vector of embodiment 8 or 9, the cell of embodiment 11, or the composition of embodiment 12 for the manufacture of a medicament for treating a cancer in a subject in need thereof.

Embodiment 15. The use of embodiment 15, wherein the cancer is acute lymphoblastic leukemia (ALL) (including non T cell ALL), acute myeloid leukemia, B cell prolymphocytic leukemia, B-cell acute lymphoid leukemia ("BALL"), blastic plasmacytoid dendritic cell neoplasm, Burkitt's lymphoma, chronic lymphocytic leukemia (CLL), chronic myelogenous leukemia (CML), chronic myeloid leukemia, chronic or acute leukemia, diffuse large B cell lymphoma (DLBCL), follicular lymphoma (FL), hairy cell leukemia, Hodgkin's Disease, malignant lymphoproliferative conditions, MALT lymphoma, mantle cell lymphoma, Marginal zone lymphoma, monoclonal gammopathy of undetermined significance (MGUS), multiple myeloma, myelodysplasia and myelodysplastic syndrome, non-Hodgkin's lymphoma (NHL), plasma cell proliferative disorder (including asymptomatic myeloma (smoldering multiple myeloma or indolent myeloma), plasmablastic lymphoma, plasmacytoid dendritic cell neoplasm, plasmacytomas (including plasma cell dyscrasia; solitary myeloma; solitary plasmacytoma; extramedullary plasmacytoma; and multiple plasmacytoma), POEMS syndrome (also known as Crow-Fukase syndrome; Takatsuki disease; and PEP syndrome),

primary mediastinal large B cell lymphoma (PMBC), small cell- or a large cell-follicular lymphoma, splenic marginal zone lymphoma (SMZL), systemic amyloid light chain amyloidosis, T-cell acute lymphoid leukemia (“TALL”), T-cell lymphoma, transformed follicular lymphoma, or Waldenstrom macroglobulinemia, or a combination thereof.

[309] All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference. However, the citation of a reference herein should not be construed as an acknowledgement that such reference is prior art to the present disclosure. To the extent that any of the definitions or terms provided in the references incorporated by reference differ from the terms and discussion provided herein, the present terms and definitions control. The contents of all references cited throughout this application are expressly incorporated herein by reference.

EXAMPLES

Example 1

[310] The present Example provides exemplary produced anti-CD20 heavy chain variable domains and light chain variable domains, and combinations thereof. CDR sequences of exemplary anti-CD20 heavy chain variable domains and anti-CD20 light chain variable domains, and thereby exemplary combinations of HCDRs and LCDRs, are also provided in below Tables 4-13. Tables 4-13 comprise exemplary nucleic acid sequences encoding exemplary variable domains (and thus also provides exemplary nucleic acid sequences encoding the identified CDRs of the exemplary variable domains).

To determine cell binding by exemplary heavy chain variable domains and light chain variable domains of the present disclosure, purified IgGs were characterized for cell binding at a concentration of 10 nM by flow cytometry. Antibodies were incubated with CHO-S cells overexpressing CD20, Raji, and Namalwa CD20+ cell lines; and with EoL-1 and CHO-S CD20– cell lines. FITC-LC was used to detect IgGs. The ratio of binding over negative controls was calculated for each antibody. Exemplary cell binding by selected anti-CD20 antibodies is provided in Table 15.

Table 15

Ab	CHO-CD20 Cell Binding FOB (Fold Over Background)	Raji Cell Binding FON (Fold Over Negative)	Namalwa Cell Binding FON (Fold Over Negative)	EOL-1 Cell Binding FON (Fold Over Negative)
Ab1	813	266	12	1
Ab2	2790	101	3	1
Ab3	2002	316	16	1
Ab4	3617	479	19	1
Ab5	2480	185	17	1
Ab6	2525	13	2	1
Ab7	4083	549	11	1
Ab8	3831	562	10	1
Ab9	3618	562	11	1
Ab10	3159	186	4	1

Example 2

[311] The present Example provides bicistronic and bispecific CARs. Bicistronic and bispecific CARs comprise two binding motifs (in two CAR molecules or on a single CAR molecule, respectively). A first binding motif binds CD20 and a second binding motif binds CD19. Antibody sequences that bind CD19, and/or are useful in constructing binding motifs, antibodies, and antigen binding systems that bind CD19 are known. The present Example uses antibody sequences of an anti-CD19 binding agent referred to here as antibody Ab11, which are set forth in Table 14.

[312] Bispecific CARs of the present Example were generated to comprise the following domains: a first binding motif, a second binding motif, a hinge, a transmembrane domain, and an intracellular domain comprising a costimulatory domain and an activation domain. In the present Example, each bispecific CAR comprised a first binding motif comprising a heavy chain variable domain and a light chain variable domain, which heavy chain and light chain variable domains are the heavy chain variable domain and the light chain variable domain from a single set of exemplary antibody sequences of Example 1 (i.e., both are from the same table

of Tables 4-13, corresponding to Ab1-Ab10). Thus, binding motifs can be identified by reference to a source antibody or source table, and refer to a binding motif having the heavy chain variable domain and light chain variable domain of the source antibody as set out in the corresponding table. In the present Example, each bispecific CAR comprised a second binding motif comprising a heavy chain variable domain and a light chain variable domain, which heavy chain and light chain variable domains are the heavy chain variable domain and the light chain variable domain of antibody Ab11 (i.e., SEQ ID NOs: 221 and 232). Bispecific CARs of the present Example comprised a 28T (CD28) domain that comprises a hinge domain and a transmembrane domain. Bispecific CARs of the present Example comprised a CD28 costimulatory domain. Bispecific CARs of the present Example comprised a CD3z activation domain.

[313] The present Example comprises four bicistronic CARs, identified as Bic-2, Bic-8, Bic-9, and Bic-14. Each of the four bicistronic CARs comprised a first CAR construct comprising an anti-CD20 binding motif that comprises a heavy chain variable domain and light chain variable domain pair of Example 1, as set forth in Table 16 below, and a second CAR construct comprising an anti-CD19 binding motif.

Table 16: anti-CD20 binding motif sequences of bicistronic CARs

CAR	Binding motif	VH SEQ ID	VL SEQ ID
Bic-2	Ab3	45	56
Bic-8	Ab8	155	166
Bic-9	Ab9	177	188
Bic-14	Ab4	67	78

Example 3

[314] CD4⁺ and CD8⁺ T cells were isolated by positive selection from apheresis material from healthy donors and used to generate anti-CD20 monovalent or anti-CD20/anti-CD19 bicistronic CAR T-cell products. T cells were activated with bound-anti-CD3 and soluble CD28 antibodies and transduced with a lentiviral vector encoding for a CAR construct. As a control, non-transduced (NTD) T cells were generated from the same donor T cells in parallel. On the harvest day (Days 8-10 of manufacture), CAR T-cell products were stained and analyzed by flow cytometry to assess transduction efficiency and used in co-culture assays.

Transduction efficiency of T cells with vector encoding monovalent CAR and vector encoding bicistronic CAR were monitored.

[315] To determine the T cell transduction efficiency of vector encoding a monovalent CAR, CAR-T products were stained with a panel of antibodies (anti-CD3, anti-CD4, anti-CD8, and anti-linker antibodies) in the presence of a fixable viability dye and analyzed by flow cytometry to assess the percentage of viable CAR-positive cells (see WO/US2017/041534, which is incorporated herein by reference with respect to the anti-linker antibody). The anti-linker antibody is an antibody that binds the linker between the heavy and light chains of the binding motif of the anti-CD20 CAR and is used to measure transduction efficiency. Controls comprised non-transduced cells (NTD), cells transduced with a retrovirus comprising a control anti-CD19 binding agent, and cells transduced with a control anti-CD20 binding agent (Ab12 binding motif).

[316] To determine the T cell transduction efficiency of vector encoding a bicistronic CAR, CAR-T products were stained with a panel of antibodies (comprising anti-CD3, anti-CD4, anti-CD8, anti-idiotypic, and anti-linker antibodies) in the presence of a fixable viability dye and analyzed by flow cytometry to assess the percentage of viable CAR-positive cells. The anti-idiotypic antibody binds the binding motif of the Ab11 anti-CD19 binding motif. Thus, the anti-idiotypic antibody binds the anti-CD19 CAR. It is used to measure the transduction efficiency of the anti-CD19 CAR. The anti-linker antibody is used to measure transduction efficiency of the anti-CD20 CAR. Controls comprised non-transduced cells (NTD), cells transduced with a retrovirus comprising a control anti-CD19 binding agent, and cells transduced with control anti-CD20/anti-CD19 bispecific CARs (Ab13/Ab14 bispecific; Ab11/Ab12 bispecific).

Table 17A: Transduction efficiency of anti-CD20 monovalent CARs

binding motif #	Transduction Efficiency (% CD20 CAR+)
Ab3	65.6
Ab5	60.9
Ab6	70.7
Ab10	51.1
Ab7	52.8
Ab8	40.6

Ab9	33
Ab1	44.4
Ab4	62.6
Ab2	35.8
NTD	0.25
Ab11	47.8
Ab12	60.7

Table 17B: Transduction Efficiency of anti-CD20/anti-CD19 Bicistronic CARs

Bic-binding motif #	Anti-CD20 binding motif	Transduction Efficiency (%)	
		% CD19 CAR+	% CD20 CAR+
Bic-2	Ab3	52.72	46.42
Bic-8	Ab8	50.2	45.57
Bic-9	Ab9	44.72	41.36
Bic-14	Ab4	40.28	37.04
NTD	N/A	0.56	0.09
Ab11	N/A	59.52	69.3
Ab13/Ab14 bispecific	N/A	56.77	60.99
Ab11/Ab12 bispecific	N/A	47.54	49.33

Example 4

[317] The present Example provides, among other things, an exemplary method of co-culturing CAR-T cells with cells expressing CAR-T target antigens. To facilitate tracking of T cells in culture, CAR-T cells were labeled with CellTrace™ Violet (CTV) reagent according to the manufacturer's instructions and subsequently washed with R-10% media. To facilitate tracking of cells expressing CAR-T target antigens ("target cells"), target cells were engineered to express luciferase. Luciferase-expressing target cells comprised Nalm6 and Raji, both of which expresses both the CD19 and CD20 antigens. In addition, Nalm6 and Raji cells not expressing CD19 or CD20 (knockout cells, or KO) were prepared. These CD19KO and CD20KO cells were clonally selected from Nalm6 and Raji parental cells and express CD20 but not CD19, or CD19 but not CD20, respectively. The CD19KO and CD20KO strains were

generated and used as controls to functionally assess antigen binding of each CAR of cells expressing bicistronic anti-CD20/anti-CD19 CAR.

[318] Luciferase-expressing target cells were plated together with CTV labeled CAR-T cells at various ratios in R-10% media (Day 0 of co-culture). The ratio may be referred to as the ratio of effector (CAR-T) cells to target cells (effector:target or E:T). To plate cells at desired ratios, CAR-T cells were serially diluted 2 to 3-fold while the number of target cells per well was held constant at 25,000 cells. Co-cultures were incubated at 37°C for either 16 hours (h) or 4 days and functional assessments were performed as described below.

Example 5

[319] In this example, T cells were co-cultured with target cells as described in Example 4. T-cell mediated cytotoxicity was measured as a function of the reduction in target luciferase signal in co-culture wells compared to the signal emitted by target cells plated alone. On Day 4 after co-culture initiation, D-luciferin substrate was added to the co-culture wells at a final concentration of 0.14 mg/mL and plates were incubated at 37°C in the dark for 10 minutes. Luminescent signal was read immediately after in a VarioSkan™ LUX or VarioSkan® Flash multimode microplate reader. T cell-mediated cytotoxicity was calculated as follows: % Cytotoxicity = $[1 - \text{luciferase signal of (sample of interest/target alone control)}] * 100$.

[320] Controls comprised non-transduced (NTD) T cells (i.e., T cells not expressing a CAR) as a negative control, cells transduced with a retrovirus comprising a control anti-CD19 binding agent, cells transduced with a control anti-CD20 binding agent, cells transduced with control anti-CD20/anti-CD19 bispecific CARs (Ab13/Ab14 bispecific; Ab15/Ab16 bispecific).

Table 18: Percent cytotoxicity of anti-CD20 CAR-T in co-culture after 4 days (Nalm6 wild type cells)

binding motif #	Ab #	Nalm6 WT Cells at Various E:T Ratios								
		3 to 1			1 to 1			1 to 3		
2	Ab3	92	91	92	72	73	77	64	62	53
3	Ab5	97	96	97	65	74	77	68	67	60
5	Ab6	12	21	23	-11	-10	-13	-12	-20	-22
6	Ab10	37	46	55	11	4	6	-4	-8	-8
7	Ab7	98	98	98	89	91	90	79	76	75

8	Ab8	99	99	99	86	91	93	89	86	90
9	Ab9	70	68	66	71	72	72	91	90	92
10	Ab1	23	23	27	7	4	5	-8	-6	-17
14	Ab4	100	100	100	52	70	68	65	70	70
16	Ab2	22	20	27	-13	-7	-13	-20	-23	-30
NTD	N/A	17	20	24	-7	0	4	-4	-2	-18
Ab11	N/A	100	100	100	93	95	96	96	94	96
Ab12	N/A	100	100	100	82	90	88	83	86	83
binding motif #	Ab #	1 to 9			1 to 27					
2	Ab3	19	21	20	13	4	2			
3	Ab5	21	18	22	21	12	7			
5	Ab6	-8	-12	-3	-9	-21	-21			
6	Ab10	-7	-8	-1	-2	-15	-18			
7	Ab7	32	34	40	28	24	21			
8	Ab8	66	63	59	63	58	59			
9	Ab9	88	84	78	99	100	99			
10	Ab1	-16	-9	3	-8	3	-9			
14	Ab4	36	37	40	65	64	59			
16	Ab2	-9	-8	0	-18	-4	-9			
NTD	N/A	0	-2	1	-4	1	-3			
Ab11	N/A	73	65	68	84	79	76			
Ab12	N/A	45	43	41	58	62	56			

Table 19: Percent cytotoxicity of anti-CD20 CAR-T in co-culture after 4 days (Raji wild type cells)

		Raji WT Cells at Various E:T Ratios								
binding motif #	Ab #	3 to 1			1 to 1			1 to 3		
2	Ab3	100	100	100	61	75	82	58	53	50
3	Ab5	100	100	100	61	55	59	55	60	57
5	Ab6	33	40	46	5	2	5	-7	-9	-8
6	Ab10	99	99	99	57	62	71	36	34	30
7	Ab7	100	100	100	65	70	78	55	44	43

8	Ab8	100	100	100	70	68	57	67	62	65
9	Ab9	100	100	100	63	60	63	73	72	61
10	Ab1	18	17	23	-15	-28	-25	-24	-12	-10
14	Ab4	100	100	100	17	-1	5	31	36	31
16	Ab2	60	63	69	2	-6	-6	-6	-2	-8
NTD	N/A	29	24	27	-8	4	5	11	18	3
Ab11	N/A	100	100	100	47	42	43	61	64	58
Ab12	N/A	100	100	100	40	30	27	47	52	40
binding motif #	Ab #	1 to 9			1 to 27					
2	Ab3	15	16	29	10	6	0			
3	Ab5	12	14	28	10	4	-6			
5	Ab6	2	2	8	-16	-15	-29			
6	Ab10	9	7	21	2	-3	-16			
7	Ab7	9	21	32	6	7	0			
8	Ab8	40	30	40	39	30	24			
9	Ab9	33	37	55	37	33	21			
10	Ab1	0	-1	7	-13	-10	1			
14	Ab4	6	19	28	24	35	26			
16	Ab2	15	13	12	11	7	2			
NTD	N/A	9	16	13	0	12	7			
Ab11	N/A	10	18	29	40	40	31			
Ab12	N/A	12	22	29	25	26	15			

Table 20: Percent cytotoxicity of anti-CD20/anti-CD19 bicistronic CAR-T in co-culture after 4 days (Nalm6 wild type cells)

		Nalm6 WT Cells at Various E:T Ratios					
Bic-binding motif #	Ab #	3 to 1		1 to 1		1 to 3	
Bic-2	Ab3	100.11	100.05	100.08	100.02	99.54	98.84
Bic-8	Ab8	100.2	100.18	100.19	100.13	100.06	99.54
Bic-9	Ab9	100.21	100.19	100.19	100.17	99.45	96.37
Bic-14	Ab4	100.21	100.18	100.17	100.13	99.86	99.69
NTD	N/A	8.92	-12.24	-17.23	-29.28	-17.13	-30.68

Ab11	N/A	100.21	100.17	100.19	100.16	99.79	99.01
Ab13/Ab14 bispecific	N/A	100.17	100.06	99.91	99.48	93.02	83.24
Ab15/Ab16 bispecific	N/A	100.05	100.06	99.89	99.89	98.89	96.03
Bic-binding motif #	Ab #	1 to 9		1 to 27		1 to 81	
Bic-2	Ab3	77.98	72.64	46.65	33.58	20.07	9.87
Bic-8	Ab8	97.6	87.64	67.03	51.79	28.36	12.92
Bic-9	Ab9	91.03	77.15	53.63	32.1	21.94	2.87
Bic-14	Ab4	99.5	99.49	84.68	76.42	48.83	38.37
NTD	N/A	-10.7	-19.68	-3.76	-11.49	0.02	-8.9
Ab11	N/A	89.83	87.75	55.83	43.85	22.23	0.48
Ab13/Ab14 bispecific	N/A	77.28	50.19	22.73	11.85	11.58	-2.9
Ab15/Ab16 bispecific	N/A	66.63	40.98	25.75	10.27	5.66	-9.98

Table 21: Percent cytotoxicity of anti-CD20/anti-CD19 bicistronic CAR-T in co-culture after 4 days (Raji wild type cells)

		Raji WT Cells at Various E:T Ratios					
Bic-binding motif #	Ab #	3 to 1		1 to 1		1 to 3	
Bic-2	Ab3	99.96	100.01	88.29	85.71	53.5	42.14
Bic-8	Ab8	100.02	100.05	70.5	74.72	53.41	56.52
Bic-9	Ab9	100.06	100.06	77.3	64.45	27.42	43.62
Bic-14	Ab4	100.09	100.11	99.58	99.47	78.71	90.26
NTD	N/A		14.29		7.61		-20.48
Ab11	N/A	100.1	100.11	96.49	98.25	71.1	70.95
Ab13/Ab14 bispecific	N/A	99.96	100.07	96.49	97.86	33.42	34.31
Ab15/Ab16 bispecific	N/A	99.6	99.08	85.33	89.26	29.88	32.49
Bic-binding motif #	Ab #	1 to 9		1 to 27		1 to 81	
Bic-2	Ab3	24.84	34.73	-14.98	-2.59	-25.65	3.89
Bic-8	Ab8	20.34	8.78	-1.02	4.33	-0.83	7.66
Bic-9	Ab9	18.64	18.79	-6.64	1.03	-11.66	-5.01
Bic-14	Ab4	40.22	30.49	14.88	15.82	-0.97	4.59
NTD	N/A		-6.05		-4.56		-8.23

Ab11	N/A	23.88	14.55	11.65	5.89	-8.43	-0.33
Ab13/Ab14 bispecific	N/A	10.08	30.72	12.69	10.41	-7.6	-7.03
Ab15/Ab16 bispecific	N/A	5.83	9.17	-8.37	8.85	-18.35	-7.06

Table 22: Percent cytotoxicity of anti-CD20/anti-CD19 bicistronic CAR-T in co-culture after 4 days (Nalm6 CD19KO cells)

		Nalm6 CD19KO Cells at Various E:T Ratios					
Bic-binding motif #	Ab #	3 to 1		1 to 1		1 to 3	
Bic-2	Ab3	41.94	47.16	22.68	21.26	-16.73	-4.21
Bic-8	Ab8	43.75	40.8	16.79	1.89	-22.77	-11.37
Bic-9	Ab9	43.71	39.92	0.98	-4.54	-17.33	-20.52
Bic-14	Ab4	75.1	74.88	39.07	26.69	10.6	31.47
NTD	N/A	-16.73	-5.35	-31.94	-30.48	-23.75	-36.72
Ab11	N/A	31.93	36.32	-8.57	-7.77	-26.58	-23.61
Ab13/Ab14 bispecific	N/A	81.71	85.04	72.06	51.9	39.52	38.16
Ab15/Ab16 bispecific	N/A	67.95	73.67	47.26	54.21	20	32.61
Bic-binding motif #	Ab #	1 to 9		1 to 27		1 to 81	
Bic-2	Ab3	-22.58	-8.65	-26.2	-22.17	-11.82	-18.02
Bic-8	Ab8	-28.81	-21.81	-34.06	-28.68	-29.45	-20.23
Bic-9	Ab9	-26.39	-22.98	-41.15	-32.2	-34.02	-29.96
Bic-14	Ab4	2.6	4.87	-12.92	-12.22	-26.69	-10.49
NTD	N/A	-27.18	-14.79	-15.71	-22.76	-15.37	-17.73
Ab11	N/A	-30.02	-24.23	-37.34	-25.77	-28.09	-15.96
Ab13/Ab14 bispecific	N/A	7.05	7.22	-16.35	-23.2	-25.86	-25.63
Ab15/Ab16 bispecific	N/A	9.13	-6.85	-22.84	-15.85	-33.56	-18.83

Table 23: Percent cytotoxicity of anti-CD20/anti-CD19 bicistronic CAR-T in co-culture after 4 days (Nalm6 CD20KO cells)

		Nalm6 CD20KO Cells at Various E:T Ratios					
Bic-binding motif #	Ab #	3 to 1		1 to 1		1 to 3	
Bic-2	Ab3	100.01	100.06	100.02	100.05	97.34	97.11

Bic-8	Ab8	100.14	100.17	100	100.12	95.96	94.9
Bic-9	Ab9	100.15	100.19	100.13	100.13	84.99	81.09
Bic-14	Ab4	100.15	100.2	100.11	100.13	99.31	99.01
NTD	N/A	13.47	20.49	-5.54	4.54	-11.02	-3.62
Ab11	N/A	100.14	100.15	100.11	100.16	89.94	90.48
Ab13/Ab14 bispecific	N/A	100.13	100.12	99.25	99.19	72.92	79.13
Ab15/Ab16 bispecific	N/A	100.01	100.06	99.54	99.63	90.53	87.4
Bic-binding motif #	Ab #	1 to 9		1 to 27		1 to 81	
Bic-2	Ab3	75.37	74.98	30.97	14.79	3.49	19.71
Bic-8	Ab8	59.25	71.18	16.46	24.12	3.67	9.64
Bic-9	Ab9	43.28	48.23	11.87	13.08	-0.46	5.06
Bic-14	Ab4	96.4	96.13	58.68	58.6	17.97	20.79
NTD	N/A	-6.55	2.96	-3.3	2.3	8.61	11
Ab11	N/A	82.02	74.55	12.37	7.52	-8.48	-1.93
Ab13/Ab14 bispecific	N/A	38.53	30.15	-5.74	1.34	-18.63	-18.22
Ab15/Ab16 bispecific	N/A	34.92	32.63	0.2	9.2	-5.64	1.78

Table 24: Percent cytotoxicity of anti-CD20/anti-CD19 bicistronic CAR-T in co-culture after 4 days (Raji CD19KO cells)

		Raji CD19KO Cells at Various E:T Ratios					
Bic-binding motif #	Ab #	3 to 1		1 to 1		1 to 3	
Bic-2	Ab3	100.02	100.1	91.85	89.69	55.92	52.39
Bic-8	Ab8	100.14	100.16	68.63	67.49	57.76	55.82
Bic-9	Ab9	100.09	100.16	91.11	83.72	33.32	45.71
Bic-14	Ab4	100.03	100.05	97.97	97.47	64.86	69.55
NTD	N/A		40.71		15.06		3.97
Ab11	N/A	33.35	27.24	12.88	16.32	-8.36	3.73
Ab13/Ab14 bispecific	N/A	100.03	100.01	76.44	82.17	56.77	55.08
Ab15/Ab16 bispecific	N/A	99.84	99.92	92.45	90.15	42.9	52.01
Bic-binding motif #	Ab #	1 to 9		1 to 27		1 to 81	
Bic-2	Ab3	24.24	44.1	-3.13	21.88	-7.78	15.73
Bic-8	Ab8	30.49	32.83	23.56	33.43	6.7	19.54

Bic-9	Ab9	20.8	32.03	12.07	17.72	-4.15	14.19
Bic-14	Ab4	47.46	54.56	33.42	36.33	9.45	22.34
NTD	N/A		6.28		-1.56		-2.01
Ab11	N/A	-15.98	0.93	-6.53	-6.94	-3.47	-2.64
Ab13/Ab14 bispecific	N/A	26.72	35.95	11.49	25.38	20.53	19.5
Ab15/Ab16 bispecific	N/A	33.11	35.35	12.34	22.23	-0.75	5.69

Table 25: Percent cytotoxicity of anti-CD20/anti-CD19 bicistronic CAR-T in co-culture after 4 days (Raji CD20KO cells)

		Raji CD20KO Cells at Various E:T Ratios					
Bic-binding motif #	Ab #	3 to 1		1 to 1		1 to 3	
Bic-2	Ab3	100.04	100.1	95	90.48	67.63	74.09
Bic-8	Ab8	100.15	100.18	84.19	84.25	81.09	80.16
Bic-9	Ab9	100.19	100.18	97.38	93.04	63.87	58.26
Bic-14	Ab4	100.18	100.19	98.61	97.05	85.53	82.29
NTD	N/A		56.92		28.9		16.55
Ab11	N/A	100.19	100.16	99.93	99.39	81.15	76.78
Ab13/Ab14 bispecific	N/A	99.24	99.36	86.31	86.76	77.15	75.46
Ab15/Ab16 bispecific	N/A	99.16	99.74	87.16	79.93	67.07	67.48
Bic-binding motif #	Ab #	1 to 9		1 to 27		1 to 81	
Bic-2	Ab3	60.58	60.16	31.83	46.37	0.15	39.97
Bic-8	Ab8	65.44	64.02	48.52	51.56	23.53	35.21
Bic-9	Ab9	49.76	54.83	25.93	30.53	20.54	23.32
Bic-14	Ab4	73.45	74	55.92	57.01	31.8	36.38
NTD	N/A		8.06		-2.8		-5.43
Ab11	N/A	60.19	62.53	47.63	39.67	10.91	15.16
Ab13/Ab14 bispecific	N/A	58.77	52.6	32.16	39.92	15.56	20.28

Ab15/Ab16 bispecific	N/A	49.49	54.48	18.17	19.02	-3.47	-3.58
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Example 6

[321] In this example, T cells were co-cultured with target cells as described in Example 4. After 16h in co-culture, supernatants were collected and analyzed for cytokine levels using the Meso Scale Discovery V-PLEX Proinflammatory Panel 1 human kit according to the manufacturer's instructions. Supernatants from the co-cultures of T-cell products plated at the 1:1 E:T ratio with antigen-expressing target cells were analyzed for levels of interferon gamma (IFN- γ), IL-2, tumor necrosis factor alpha (TNF- α), and IL-10 secretion mediated by antigen engagement. All samples were diluted to be within the range of detection. The level of each cytokine is reported as pg/mL and the lower limit of quantitation and upper limit of quantitation of each assay is reported.

[322] Controls comprised non-transduced (NTD) T cells (i.e., T cells not expressing a CAR) as a negative control, cells transduced with a retrovirus comprising a control anti-CD19 binding agent, cells transduced with a control anti-CD20 binding agent, cells transduced with a control bispecific antigen binding system (Ab13/Ab14 bispecific), and cells transduced with a bispecific antigen binding system (Ab15/Ab16 bispecific).

Table 26: Cytokine production (pg/mL) in 16h co-culture of anti-CD20 CAR-T with Nalm6 wild type cells

binding motif #	Ab #	Cytokines (pg/mL) Upon Co-culture with Nalm6 WT					
		IL-10			IL-2		
2	Ab3	2.886	2.251	3.438	110.474	86.758	70.6158
3	Ab5	4.305	4.194	3.125	163.198	174.192	167.524
5	Ab6	0.277	0.147	0.327	1.39082	1.08314	2.446
6	Ab10	0.769	0.772	0.832	2.70375	6.86764	4.13888
7	Ab7	3.170	4.423	6.179	209.604	272.439	233.01
8	Ab8	5.736	4.562	5.283	314.496	273.505	259.131
9	Ab9	7.970	8.754	6.446	455.254	445.985	408.829
10	Ab1	0.321	0.573	0.296	1.25038	2.8819	0.3113
14	Ab4	5.948	5.325	3.942	383.166	351.627	364.473

16	Ab2	0.276	0.312	0.191	1.52526	1.84837	0.5235
NTD	N/A	0.157	0.348	0.451	4.83766	2.43654	5.10316
Ab11	N/A	19.959	13.241	16.798	860.093	843.101	690.879
Ab12	N/A	7.613	6.145	5.710	750.441	802.013	704.947
	LLOQ	1.360			1.78		
	ULOQ	466.000			1876		
binding motif #	Ab #	TNF-a			IFN-g		
2	Ab3	87.1829	70.8648	71.7046	5669.7	4265.89	4974.73
3	Ab5	154.755	143.572	142.014	9479.79	8824.55	9192.87
5	Ab6	1.1906	1.2789	1.53004	67.7508	61.6543	75.6417
6	Ab10	5.08074	6.14829	5.80572	172.603	188.02	229.674
7	Ab7	112.546	143.963	137.234	5556.7	7514.95	6939.41
8	Ab8	249.849	201.326	194.1	12452.8	12117.7	10956.6
9	Ab9	263.572	286.889	267.453	12689.3	12716.4	10847.6
10	Ab1	2.51653	2.46732	1.80637	134.553	103.288	90.2425
14	Ab4	314.402	240.486	251.363	17113.1	14123.9	13754.6
16	Ab2	2.05431	2.63529	2.19805	107.334	136.239	100.073
NTD	N/A	1.72614	1.68826	1.3141	83.4983	106.559	77.1905
Ab11	N/A	392.426	395.229	347.665	38674.9	37969.3	32962.3
Ab12	N/A	381.969	387.54	307.418	20023.6	18118.9	15659.7
	LLOQ	1.38			149.4		
	ULOQ	496			18760		

Table 27: Cytokine production (pg/mL) in 16h co-culture of anti-CD20 CAR-T with Raji wild type cells

binding motif #	Ab #	Cytokines (pg/mL) Upon Co-culture with Raji WT					
		IL-10			IL-2		
2	Ab3	4.22791	4.06553	4.09989	1470.48	1168.14	1572.25
3	Ab5	4.71127	4.54175	5.42819	1098.24	959.202	1242.67
5	Ab6	2.94802	3.02925	3.27569	48.5778	46.0853	36.4492
6	Ab10	4.12995	6.20356	4.34137	532.235	638.548	477.869
7	Ab7	6.22517	4.41875	5.68705	1632.41	1256.81	1629.67

8	Ab8	6.82197	5.50928	5.61023	1406.26	1167.16	1552.86
9	Ab9	8.0439	6.16812	8.28169	1493.89	1541.38	1341.7
10	Ab1	2.57139	2.58163	2.76773	1.61246	1.07776	2.95043
14	Ab4	3.83274	4.91531	4.91929	559.513	846.005	1004.86
16	Ab2	4.33862	4.97592	5.1657	44.1423	63.8978	75.554
NTD	N/A	3.54859	3.26674	3.67311	4.17588	5.40813	2.67597
Ab11	N/A	8.76434	7.89571	10.5552	562.35	531.706	574.047
Ab12	N/A	5.66881	4.89856	4.90972	1309.6	1156.2	1335.23
	LLOQ	0.600			17.8		
	ULOQ	466.000			18760		
binding motif #	Ab #	TNF-a			IFN-g		
2	Ab3	355.825	330.247	415.31	34934.1	27937.3	40433.3
3	Ab5	299.426	287.003	372.212	35868.4	29774.6	42922.3
5	Ab6	49.2653	47.2393	44.2108	1325.8	2259.4	1672.66
6	Ab10	230.666	295.155	213.26	17114.2	24139.8	18852.1
7	Ab7	385.919	272.502	371.498	30569.3	21404.7	30464.6
8	Ab8	400.397	322.357	409.831	30576.4	23842.2	38718.6
9	Ab9	379.925	424.475	338.892	34630.1	39572.9	35143.5
10	Ab1	10.2538	11.2538	10.461	64.3593	84.505	87.5275
14	Ab4	189.652	276.922	313.618	15464.9	22870.4	26823.3
16	Ab2	76.1237	81.5253	92.838	1755.37	2782.59	3461.08
NTD	N/A	8.11105	6.99595	7.40474	52.2049	42.1524	51.0898
Ab11	N/A	216.851	209.783	211.514	35496.6	33980.6	32858.6
Ab12	N/A	354.528	350.431	328.904	30852.3	26033	28850.7
	LLOQ	13.8			352		
	ULOQ	4960			187600		

Table 28: Cytokine production (pg/mL) in 16h co-culture of anti-CD20/anti-CD19 bicistronic CAR-T with Nalm6 wild type cells

		Nalm6 WT			
Bic-binding motif #	Ab #	IL-10		IL-2	
Bic-2	Ab3	319.96	148.07	3841.67	997.02

Bic-8	Ab8	340.60	198.04	4787.34	2351.16
Bic-9	Ab9	339.47	228.56	4472.49	3070.04
Bic-14	Ab4	310.71	306.43	3456.34	2075.77
NTD	N/A	22.11	26.07	47.13	57.14
Ab11	N/A	156.72	129.75	5898.60	5164.57
Ab13/Ab14 bispecific	N/A	175.34	137.17	466.18	462.89
Ab15/Ab16 bispecific	N/A	220.27	184.11	914.97	609.44
	LLOQ	13.60		17.80	
	ULOQ	4660.00		18760.00	
Bic-binding motif #	Ab #	TNF-a		IFN-g	
Bic-2	Ab3	1162.13	547.11	31344.55	17704.04
Bic-8	Ab8	1326.76	943.35	41201.40	26645.47
Bic-9	Ab9	1297.88	1123.15	44205.89	30919.65
Bic-14	Ab4	1314.55	1092.37	41473.90	32712.72
NTD	N/A	15.38	17.73	127.03	310.15
Ab11	N/A	1765.65	1481.87	50877.54	46996.98
Ab13/Ab14 bispecific	N/A	716.26	700.12	33318.42	26294.09
Ab15/Ab16 bispecific	N/A	845.64	801.45	44585.96	36507.97
	LLOQ	13.80		1494.00	
	ULOQ	4960.00		187600.00	

Table 29: Cytokine production (pg/mL) in 16h co-culture of anti-CD20/anti-CD19 bicistronic CAR-T with Raji wild type cells

		Raji WT			
Bic-binding motif #	Ab #	IL-10		IL-2	
Bic-2	Ab3	249.61	302.79	5716.65	6324.53
Bic-8	Ab8	286.93	375.44	5657.78	6398.44
Bic-9	Ab9	199.97	364.30	5311.75	6221.35
Bic-14	Ab4	289.02	441.49	4524.40	5178.06
NTD	N/A	20.08	31.64	52.78	68.18
Ab11	N/A	158.93	168.48	6913.98	6266.77
Ab13/Ab14 bispecific	N/A	104.07	120.62	7388.26	7908.03

Ab15/Ab16 bispecific	N/A	152.99	196.19	6620.35	7598.18
	LLOQ	13.60		17.80	
	ULOQ	4660.00		18760.00	
Bic-binding motif #	Ab #	TNF-a		IFN-g	
Bic-2	Ab3	1276.98	1348.49	46275.13	39485.80
Bic-8	Ab8	1248.22	1538.31	44611.85	52535.86
Bic-9	Ab9	1329.21	1649.67	37559.20	49128.37
Bic-14	Ab4	1500.30	1815.74	50827.49	65974.91
NTD	N/A	24.85	31.77	132.97	290.37
Ab11	N/A	1833.09	1748.65	43391.37	47478.31
Ab13/Ab14 bispecific	N/A	2599.76	2726.70	84185.42	118333.60
Ab15/Ab16 bispecific	N/A	2270.58	2434.51	62974.89	85048.84
	LLOQ	13.80		1494.00	
	ULOQ	4960.00		187600.00	

Table 30: Cytokine production (pg/mL) in 16h co-culture of anti-CD20/anti-CD19 bicistronic CAR-T with Nalm6 CD19KO cells

		Nalm6 CD19KO			
Bic-binding motif #	Ab #	IL-10		IL-2	
Bic-2	Ab3	20.85	21.32	415.03	397.66
Bic-8	Ab8	25.07	23.96	371.09	419.08
Bic-9	Ab9	17.46	16.68	315.63	308.79
Bic-14	Ab4	27.34	37.57	239.42	263.16
NTD	N/A	12.07	12.33	13.90	5.93
Ab11	N/A	11.94	10.61	7.24	8.06
Ab13/Ab14 bispecific	N/A	22.57	25.85	1373.50	1344.97
Ab11/Ab12 bispecific	N/A	19.22	27.92	758.03	769.46
	LLOQ	3.4		4.45	
	ULOQ	1165		4690	
Bic-binding motif #	Ab #	TNF-a		IFN-g	
Bic-2	Ab3	317.89	371.73	33687.05	41534.70
Bic-8	Ab8	414.53	293.49	33669.96	46572.37

Bic-9	Ab9	386.27	293.61	41476.85	32400.90
Bic-14	Ab4	418.64	307.18	44847.72	41189.26
NTD	N/A	21.43	30.02	2183.86	1360.11
Ab11	N/A	33.88	28.23	1707.39	2083.68
Ab13/Ab14 bispecific	N/A	836.78	842.73	113383.20	130673.40
Ab11/Ab12 bispecific	N/A	720.37	614.86	98809.63	79846.05
	LLOQ	17.25		933.75	
	ULOQ	13700		117250	

Table 31: Cytokine production (pg/mL) in 16h co-culture of anti-CD20/anti-CD19 bicistronic CAR-T with Nalm6 CD20KO cells

		Nalm6 CD20KO			
Bic-binding motif #	Ab #	IL-10		IL-2	
Bic-2	Ab3	12.82183	8.967694	300.3656	255.746
Bic-8	Ab8	3.530049	4.734808	158.655	112.5326
Bic-9	Ab9	13.69715	12.26638	464.8549	435.2715
Bic-14	Ab4	27.21842	23.67642	238.8009	202.8983
NTD	N/A	3.795072	3.924667	5.336147	10.97881
Ab11	N/A	13.97866	10.62249	170.2153	136.2659
Ab13/Ab14 bispecific	N/A	5.964617	6.354307	192.2036	214.7087
Ab15/Ab16 bispecific	N/A		25.07804		389.0046
	LLOQ	1.36		1.78	
	ULOQ	466		1876	
Bic-binding motif #	Ab #	TNF-a		IFN-g	
Bic-2	Ab3	208.4781	184.026	25169.65	20518.19
Bic-8	Ab8	173.3662	143.7201	17698.8	14222.19
Bic-9	Ab9	302.2407	268.2183	174.1232	143.1469
Bic-14	Ab4	295.3972	261.3406	46810.13	41911.35
NTD	N/A	7.415782	7.415782	108.3741	3143.67
Ab11	N/A	448.6204	337.0853	81984.54	65671.96
Ab13/Ab14 bispecific	N/A	182.4278	144.6086	23278.19	20362.21

Ab15/Ab16 bispecific	N/A		419.7012		81159.51
	LLOQ	13.8		1494	
	ULOQ	4960		187600	

Example 7

[323] T cells were co-cultured with target cells as described in Example 4. After 16h co-culture, T-cell products plated at the specified E:T ratio with antigen-positive target cells were harvested, stained with a panel of antibody-fluorophores to identify T cells (CD3, CD4, CD8) and 4-1BB, an activation marker, and analyzed by flow cytometry. A fixable viability dye allowed analysis of viable cells. Events were systematically gated on live cells (viability dye-negative), lymphocytes (using forward scatter [FSC]-area by side scatter [SSC]-area plot), single cells (using FSC-area by FSC-height plot), and then T cells (CD3⁺). T cells were then analyzed for level of activation (ie, percentage of 4-1BB⁺ cells); the 4-1BB gating threshold was set based on the level expressed by the NTD control T cells.

[324] Controls comprised non-transduced (NTD) T cells (i.e., T cells not expressing a CAR) as a negative control, cells transduced with a retrovirus comprising a control anti-CD19 binding agent, cells transduced with a control anti-CD20 binding agent, cells transduced with a control bispecific antigen binding system (Ab13/Ab14 bispecific), and cells transduced with a bispecific antigen binding system.

Table 32: Activation of anti-CD20 CAR-T cells in 16h co-culture with Nalm6 wild type and Raji wild type cells

binding motif #	Ab #	%4-1BB Upon 16h Co-culture with Target Cells	
		Nalm6	Raji
2	Ab3	32.54	36.85
3	Ab5	35.72	38.89
5	Ab6	8.99	16.81
6	Ab10	14.62	33.19
7	Ab7	36.28	35.65
8	Ab8	46.66	58.46
9	Ab9	56.88	61.44
10	Ab1	6.48	8.66

14	Ab4	39.49	36.66
16	Ab2	8.29	32.31
NTD	N/A	5.6	6.08
Ab11	N/A	57.68	45.09
Ab12	N/A	50.26	40.1

Table 33: Activation of anti-CD20/anti-CD19 bicistronic CAR-T cells in 16h co-culture with Nalm6 wild type, Nalm6 CD20KO, and Nalm6CD19KO cells

Bic-binding motif #	Ab #	%4-1BB Upon 16h Co-culture with Target Cells					
		Nalm6 WT		Nalm6 CD19KO		Nalm6 CD20KO	
Bic-2	Ab3	31.93	33.75	42.84	31.46	28.57	22.82
Bic-8	Ab8	25.23	27.71	39.2	40.04	25.57	27.1
Bic-9	Ab9	24.35	32.54	25.95	26.99	18.87	21.66
Bic-14	Ab4	28.33	27.05	27.81	28.21	18.65	22.4
NTD	N/A	1.31	1.51	5.01	6.1	2.66	2.23
Ab11	N/A	23.63	20.6	5.39	5.58	24.33	19.05
Ab13/Ab14 bispecific	N/A	17.56	19.25	18.85	19.06	15.22	13.31
Ab11/Ab12 bispecific	N/A	25.89	22.71	22.24	22.93	19.82	15.46

Example 8

[325] T cells were co-cultured with target cells as described in Example 4. The proliferative capacity of the T-cell products was determined by flow cytometric analysis of the cell division-driven dilution of CTV dye compared with NTD control T cells in response to antigen-expressing target cells. On Day 4 after co-culture initiation, T-cell products plated at the 3:1 E:T ratio with antigen-expressing target cells were harvested, stained with a panel of antibody-fluorophores (CD3, CD4, CD8) in the presence of a fixable viability dye to identify viable T cells and analyzed by flow cytometry. The percentage of proliferating cells as well as the mean fluorescence intensity (MFI) of CTV signal is reported. The decrease in MFI of CTV is proportional to the number of rounds of cell division the product has undergone (i.e., the lower the CTV MFI, the more proliferation the cell has undergone).

[326] Controls comprised non-transduced (NTD) T cells (i.e., T cells not expressing a CAR) as a negative control, cells transduced with a retrovirus comprising a control anti-CD19 binding

agent, cells transduced with a control anti-CD20 binding agent, cells transduced with a control bispecific antigen binding system (Ab13/Ab14 bispecific), and cells transduced with a Ab11/Ab12 bispecific antigen binding system.

Table 34: Fluorescence of anti-CD20 CAR-T cells in four day co-culture with Nalm6 wild type and Raji wild type cells

		Nalm6 WT	Raji WT
binding motif #	Ab #	CTV MFI	CTV MFI
2	Ab3	4902.56	3100.8
3	Ab5	3426.83	2640.14
5	Ab6	6396.87	3041.46
6	Ab10	5645.62	2177.05
7	Ab7	4698.94	2483.31
8	Ab8	4905.7	2068.97
9	Ab9	5039.64	2033.59
10	Ab1	10316.89	5653.56
14	Ab4	4640.49	2200.1
16	Ab2	9129.9	3097.49
NTD	N/A	7739.37	4816.32
Ab11	N/A	3548.72	2113.72
Ab12	N/A	2909.96	2498.54

Table 34: Percent proliferation of anti-CD20 CAR-T cells in four day co-culture with Nalm6 wild type and Raji wild type cells

		Nalm6 WT	Raji WT
binding motif #	Ab #	% Proliferation	% Proliferation
2	Ab3	68.77	74.95
3	Ab5	81.21	78.35
5	Ab6	40.43	78.68
6	Ab10	52.31	87.53
7	Ab7	76.23	78.48

8	Ab8	76.6	84.79
9	Ab9	75.91	87.42
10	Ab1	33.94	53.61
14	Ab4	76.1	80.6
16	Ab2	38.16	85.05
NTD	N/A	37.38	48.15
Ab11	N/A	74.96	86.98
Ab12	N/A	86.32	72.9

Table 35: Fluorescence of anti-CD20/anti-CD19 bicistronic CAR-T cells in four day co-culture with Nalm6 wild type, Nalm6 CD19KO, Raji wild type, and Raji CD19KO cells

		CellTrace Violet (MFI)			
Bic-binding motif #	Ab #	Nalm6 WT		Nalm6 CD19KO	
Bic-2	Ab3	1196.99	1191.69	1433.19	1433.24
Bic-8	Ab8	1266.27	1297.78	1503.07	1486.28
Bic-9	Ab9	1533.09	1503.48	1747.27	1815.87
Bic-14	Ab4	1242.68	1241.04	1421.57	1405.64
NTD	N/A	2823.57	2775.76	2269.11	2500.3
Ab11	N/A	1307.52	1273.51	2392.63	2350.37
Ab13/Ab14 bispecific	N/A	1434.19	1406.81	1409.42	1614.71
Ab11/Ab12 bispecific	N/A	1207.67	1358.15	1319.45	1448.75
Bic-binding motif #	Ab #	Raji WT		Raji CD19KO	
Bic-2	Ab3	1166.68	1277.03	1227.25	1249.14
Bic-8	Ab8	1286.29	1426.14	1285.56	1271.65
Bic-9	Ab9	1613.51	1474.92	1453.13	1456.33
Bic-14	Ab4	1767.75	1820.79	1115.55	1113.95
NTD	N/A	2150.62	2065.69	2050.38	2016.25
Ab11	N/A	1575.17	1438.12	2011.85	1793.87
Ab13/Ab14 bispecific	N/A	1330.39	1708.95	1407.7	1628.62
Ab11/Ab12 bispecific	N/A	1636.56	1773.56	1347.75	1426.61

Table 36: Percent proliferation of anti-CD20/anti-CD19 bicistronic CAR-T cells in four day co-culture with Nalm6 wild type, Nalm6 CD19KO, Raji wild type, and Raji CD19KO cells

Bic-binding motif #	Ab #	% Proliferation			
		Nalm6 WT		Nalm6 CD19KO	
Bic-2	Ab3	91.59	92.65	87.03	87.51
Bic-8	Ab8	90.61	90.43	81.44	81.91
Bic-9	Ab9	86.97	86.6	78.09	79.86
Bic-14	Ab4	90.56	88.55	84.14	83.74
NTD	N/A	32.78	35.81	55.57	50.62
Ab11	N/A	90.4	90.37	43.6	46.56
Ab13/Ab14 bispecific	N/A	87.37	86.42	89.06	80.19
Ab11/Ab12 bispecific	N/A	91.92	89.53	89.29	86.6
Bic-binding motif #	Ab #	Raji WT		Raji CD19KO	
Bic-2	Ab3	92.78	89.58	92.52	92.77
Bic-8	Ab8	84.65	87.63	88.89	90.11
Bic-9	Ab9	85.06	77.69	87.2	87.81
Bic-14	Ab4	83.43	88.9	91.96	91.38
NTD	N/A	64.5	65.09	61.81	60.57
Ab11	N/A	92.01	88.46	57.51	56.55
Ab13/Ab14 bispecific	N/A	92.56	77.42	90.38	81.8
Ab11/Ab12 bispecific	N/A	89.16	87.23	91.48	88.6

Example 9

[327] The present Example provides data relating to hinges for use in anti-CD20 antigen binding systems. CAR T cells of the present Example comprise a monovalent anti-CD20 CAR. The present Example tests CAR-T constructs against a disseminated luciferase-expressing Raji human B-Cell lymphoma model in NSG mice. Weekly blood samples were monitored for CAR-T persistence. Response was evaluated based on bioluminescence imaging (BLI) and survival endpoints. Whole blood was drawn for analysis at days 7, 13, 20, 27, 34, and 41. BLI was performed on days 5, 12, 19, 26, 33, and 47. Study was terminated on day 55.

[328] CAR-T cells of this study are shown in Table 37 below, each of which CAR-T cell types of Table 37 was administered to six mice. Table 37 provides the experimental conditions or groups referred to in subsequent tables of this Example. All CAR-T cells were administered intravenously (QDx1) at a dose of 2×10^7 cells/mL. All mice also received a human IL-2 dosage of $36 \mu\text{g}$ ((Q12Hx2)QDx3). Human IL-2 promotes CAR-T persistence and survival. Constructs of CAR T cells identified as groups 4, 5, and 7-10 of Table 37 comprised a 41BB costimulatory domain. CAR T cells identified as group 6 of Table 37 comprised a CD28 costimulatory domain.

Table 37:

1	PBS control
2	Mock CAR-T cells control (non-transduced T cells)
3	CAR-T cells comprising anti-CD19 control CAR
4	CAR-T cells comprising anti-CD20 CAR with CD8 hinge (8k) and an anti-CD19 CAR (bicistronic CAR)
5	CAR-T cells comprising anti-CD20 CAR with IgG4 hinge and an anti-CD19 CAR (bicistronic CAR)
6	CAR-T cells comprising anti-CD20 CAR with 28T (CD28) hinge and an anti-CD19 CAR (bicistronic CAR)
7	CAR-T cells comprising anti-CD20 CAR with 28T (CD28) hinge and an anti-CD19 CAR (bicistronic CAR)
8	CAR-T cells comprising anti-CD20 CAR with G4sx1 linker and an anti-CD19 CAR (bicistronic CAR)
9	CAR-T cells comprising anti-CD20 CAR with G4sx2 linker and an anti-CD19 CAR (bicistronic CAR)
10	CAR-T cells comprising anti-CD20 CAR with G4sx3 linker and an anti-CD19 CAR (bicistronic CAR)

[329] All CAR-T cell treatments were well tolerated by study mice. Within each group, a number of metrics were monitored, comprising treatment related weight change, treatment related deaths, median tumor growth delay, median tumor burden as compared to control (%T/C), percent of animals showing partial tumor regression (% PR), percent of animals showing complete tumor regression (% CR), percent of tumor free survivors (% TFS), and percent increased lifespan. Results are shown in Table 38 below, noting that data reflect monitoring through termination of the experiment at day 55.

Table 38

Group #	Treatment Related Weight Change (%)	Treatment Related Deaths (%)	Median Tumor Growth Delay (Days)	Median % T/C (Day 12)	%PR	%CR	%TFS	Increased Life Span %
1	-6.6	0	0	100	0	0	0	0.0
2	-15.9	0	0.4	73.8	0	0	0	9.1
3	5.6	0	>37.3	2.6	0	83	83	>345.5
4	-6	0	>37.3	2.1	0	100	100	>345.5
5	5.5	0	>37.3	2.4	0	100	83	>345.5
6	-2.7	0	15.5	4.6	0	0	0	131.6
7	4	0	>37.3	2.4	0	100	93	>345.5
8	1.3	0	35.6	3.8	0	33	0	331.6
9	3	0	23.3	2.3	0	100	0	166.4
10	-3.1	0	15.3	2.6	0	67	17	200.0

Example 10

[330] The present Example provides data relating to the anti-tumor efficacy of CAR-T cells comprising bicistronic CARs comprising an anti-CD19 CAR and an anti-CD20 CAR, the anti-CD20 CARs comprising various anti-CD20 binding motifs and/or hinges.

[331] Bicistronic anti-CD20/anti-CD19 CAR-T cells were tested against disseminated luciferase-expressing Raji CD19KO B cell lymphoma tumors in NSG mice. Weekly blood samples were monitored for CAR-T persistence. Response was evaluated based on bioluminescence imaging (BLI) and survival endpoints. Whole blood was drawn for analysis

at days 7, 13, 20, 27, 34, and 41. BLI was performed on days 5, 12, 19, 26, 33, 40, and 48. Study was terminated on day 55. CAR-T cells of this study as shown in rows 5-14 of Table 39 below are bicistronic CAR-T cells comprising an anti-CD20 CAR as indicated in Table 39 and an anti-CD19 CAR (Ab11 CAR comprising a 28T (CD28) hinge and a CD28 costimulatory domain). Table 39 provides the experimental conditions or groups referred to in subsequent tables of this Example. CAR-T cells were administered to six mice (except that four mice received non-transduced T cells (row 2 of Table 39)). All CAR-T cells were administered intravenously (QDx1) at a dose of $1\text{E}+07$ cells/mL (rows 2-7, 9, 11, and 13) or $4\text{E}+06$ (rows 8, 10, 12, and 14). All mice also received a human IL-2 dosage of $36\mu\text{g}$ ((Q12Hx2)QDx3).

Table 39

1	PBS control
2	Mock CAR-T cells control (non-transduced T cells)
3	CAR-T cells comprising anti-CD20 control CAR (CD8 hinge (8k), 41BB costimulatory domain)
4	CAR-T cells comprising anti-CD20 control CAR
5	CAR-T cells comprising anti-CD20 control CAR (CD8 hinge (8k), 41BB costimulatory domain) and an anti-CD19 CAR
6	CAR-T cells comprising anti-CD20 CAR comprising binding motif #9 (CD28 hinge, 41BB costimulatory domain) and an anti-CD19 CAR (bicistronic CAR)
7	CAR-T cells comprising anti-CD20 CAR comprising binding motif #2 (CD8 hinge (8k), 41BB costimulatory domain) and an anti-CD19 CAR (bicistronic CAR)
8	CAR-T cells comprising anti-CD20 CAR comprising binding motif #2 (CD8 hinge (8k), 41BB costimulatory domain) and an anti-CD19 CAR (bicistronic CAR)
9	CAR-T cells comprising anti-CD20 CAR comprising binding motif #8 (CD8 hinge (8k), 41BB costimulatory domain) and an anti-CD19 CAR (bicistronic CAR)
10	CAR-T cells comprising anti-CD20 CAR comprising binding motif #8 (CD8 hinge (8k), 41BB costimulatory domain) and an anti-CD19 CAR (bicistronic CAR)
11	CAR-T cells comprising anti-CD20 CAR comprising binding motif #9 (CD8 hinge (8k), 41BB costimulatory domain) and an anti-CD19 CAR (bicistronic CAR)
12	CAR-T cells comprising anti-CD20 CAR comprising binding motif #9 (CD8 hinge (8k), 41BB costimulatory domain) and an anti-CD19 CAR (bicistronic CAR)

13	CAR-T cells comprising anti-CD20 CAR comprising binding motif #14 (CD8 hinge (8k), 41BB costimulatory domain) and an anti-CD19 CAR (bicistronic CAR)
14	CAR-T cells comprising anti-CD20 CAR comprising binding motif #14 (CD8 hinge (8k), 41BB costimulatory domain) and an anti-CD19 CAR (bicistronic CAR)

[332] A number of metrics were monitored, comprising treatment related weight change, treatment related deaths, median tumor growth delay, median tumor burden as compared to control (%T/C), percent of animals showing partial tumor regression (% PR), percent of animals showing complete tumor regression (% CR), percent of tumor free survivors (% TFS), and percent increased lifespan (ILS %). Results are shown in Table 40 below, noting that data reflect monitoring through termination of the experiment at day 55.

[333] All CAR-T cell treatments were well tolerated by study mice. All CAR-T cell treatments tested elicited an anti-tumor response. All treatment regimens resulted in median tumor growth delays of >30.2 days and increase in life spans (ILS) of >226.7%. All treatment regimens resulted in median %T/C on Day 19 of 0%, except treatment with non-transduced (NTD) cells (24%).

Table 40

Group #	Treatment Related Weight Change (%)	Treatment Related Deaths (%)	Median Tumor Growth Delay (Days)	Median %T/C (Day 19)	%PR	%CR	%TFS	Increased Life Span (ILS) %
1	-18.0	0.0	0.0	100.0	0.0	0.0	0.0	0.0
2	-2.1	0.0	>30.2	23.8	0.0	0.0	0.0	>226.7
3	-5.2	0.0	>30.2	0.0	0.0	100.0	100.0	>226.7
4	1.1	16.7	>30.2	0.0	0.0	100.0	100.0	>226.7
5	-1.0	0.0	>30.2	0.0	16.7	66.7	66.7	>226.7
6	2.0	0.0	>30.2	0.0	50.0	50.0	16.7	>226.7
7	-4.4	0.0	>30.2	0.0	0.0	66.7	66.7	>226.7
8	-2.4	0.0	>30.2	0.0	0.0	100.0	33.3	>226.7
9	-3.0	0.0	>30.2	0.0	33.3	66.7	50.0	>226.7
10	2.5	0.0	>30.2	0.0	0.0	83.3	33.3	>226.7
11	-2.7	0.0	>30.2	0.0	0.0	100.0	50.0	>226.7
12	4.6	0.0	>30.2	0.0	0.0	66.7	33.3	>226.7
13	4.0	0.0	>30.2	0.0	16.7	83.3	66.7	>226.7
14	5.4	0.0	>30.2	0.0	0.0	100.0	66.7	>226.7

Example 11

[334] The present Example provides data relating to the anti-tumor efficacy of CAR-T cells comprising bispecific CARs comprising an anti-CD19 binding motif and an anti-CD20 binding motif. Bispecific anti-CD20/anti-CD19 CAR-T cells were tested against disseminated luciferase-expressing Raji B cell lymphoma tumors in NSG mice. Weekly blood samples were monitored for CAR-T persistence. Response was evaluated based on bioluminescence imaging (BLI) and survival endpoints. Whole blood was drawn for analysis at days 7, 13, 20, 27, 32, and 41. BLI was performed on days 5, 12, 19, 26, 33, 40, and 47. The study was terminated on day 54.

[335] CAR-T cells of this study as shown in rows 4-8 of Table 41 below are bispecific CAR-T cells comprising an anti-CD19 binding motif and an anti-CD20 binding motif, where the anti-CD19 binding motif is Ab11. Table 41 provides the experimental conditions or groups referred to in subsequent tables of this Example. CAR-T cells were administered at a dose, and

to a number of mice, shown in Table 41. All CAR-T cells were administered intravenously (QDx1). All mice also received a human IL-2 dosage of 36 μ g ((Q12Hx2)QDx3).

Table 41

1	PBS control (6 mice)
2	Mock CAR-T cells control (non-transduced T cells) (1.5E+07 cells; 6 mice)
3	CAR-T cells comprising anti-CD19 control CAR (1.2E+07 cells; 6 mice)
4	Monospecific ant-CD19 CAR (1.5E+07 cells; 6 mice)
5	CAR-T cells comprising anti-CD20/anti-CD19 bispecific CAR comprising an anti-CD20 binding motif #2 (1.5E+07 cells; 5 mice)
6	CAR-T cells comprising anti-CD20/anti-CD19 bispecific CAR comprising an anti-CD20 binding motif #2 (1.0E+07 cells; 4 mice)
7	CAR-T cells comprising anti-CD20/anti-CD19 bispecific CAR comprising an anti-CD20 binding motif #8 (7.0E+06 cells; 6 mice)
8	CAR-T cells comprising anti-CD20/anti-CD19 bispecific CAR comprising an anti-CD20 binding motif #8 (1.5E+07 cells; 6 mice)

[336] A number of metrics were monitored, comprising treatment related weight change, treatment related deaths, median tumor growth delay, median tumor burden as compared to control (%T/C), percent of animals showing partial tumor regression (% PR), percent of animals showing complete tumor regression (% CR), percent of tumor free survivors (% TFS), and percent increased lifespan (ILS %). Results are shown in Table 42 below. Anti-tumor efficacy was observed.

Table 42

Group #	Treatment Related Weight Change (%)	Treatment Related Deaths (%)	Median Tumor Growth Delay (Days)	Median %T/C (Day 12)	%PR	%CR	%TFS	Increased Life Span (ILS) %
1	-10.2	0.0	0.0	100.0	0.0	0.0	0.0	0.0
2	-15.7	0.0	1.3	58.7	0.0	0.0	0.0	21.7
3	-4.1	0.0	25.5	6.0	0.0	83.3	0.0	295.7
4	-8.8	0.0	7.2	13.2	0.0	0.0	0.0	73.9
5	-3.5	0.0	11.7	12.2	0.0	0.0	0.0	113.0
6	0.2	0.0	10.4	9.6	0.0	0.0	0.0	160.9
7	1.9	50.0	>25.5	5.4	0.0	100.0	0.0	195.7
8	1.3	17.0	15.0	7.1	0.0	0.0	0.0	126.1

Example 12

[337] The present Example provides data relating to anti-tumor efficacy of (i) CAR-T cells comprising bicistronic CARs comprising an anti-CD19 CAR and an anti-CD20 CAR; and (ii) bispecific CARs comprising an anti-CD19 binding motif and an anti-CD20 binding motif. Bicistronic and bispecific anti-CD20/anti-CD19 CAR-T cells were tested against disseminated luciferase-expressing Raji B cell lymphoma tumors in NSG mice. Weekly blood samples were monitored for CAR-T persistence. Response was evaluated based on bioluminescence imaging (BLI) and survival endpoints. Whole blood was drawn for analysis at days 7, 13, 20, 27, 34, and 40. BLI was performed on days 5, 12, 19, 26, 33, and 40. Study was terminated on day 48.

[338] CAR-T cells of this study as shown in Table 43 below are bicistronic or bispecific CAR-T cells comprising an anti-CD20 binding motif an anti-CD19 binding motif (Ab11). Table 43 provides the experimental conditions or groups referred to in subsequent tables of this Example. CAR-T cells were administered at a dose of 6.0E+06, each of which CAR-T cell

types was administered to six mice. All CAR-T cells were administered intravenously (QDx1). All mice also received a human IL-2 dosage of 36 μ g ((Q12Hx2)QDx3) intraperitoneally.

Table 43

1	PBS control
2	Mock CAR-T cells control (non-transduced T cells) (60.0E+06 cells)
3	CAR-T cells comprising anti-CD19 control CAR (2.6E+06 cells)
4	CAR-T cells comprising an anti-CD19 CAR with a linker according to SEQ ID NO: 247
5	CAR-T cells comprising an anti-CD19 CAR with a G4S linker
6	CAR-T cells comprising a bicistronic CAR comprising binding motif #2
7	CAR-T cells comprising a bicistronic CAR comprising binding motif #9
8	CAR-T cells comprising a bicistronic CAR comprising binding motif #14
9	CAR-T cells comprising a bispecific CAR comprising binding motif #2
10	CAR-T cells comprising a bispecific CAR comprising binding motif #9
11	CAR-T cells comprising a bispecific CAR comprising binding motif #14
12	CAR-T cells comprising a control bispecific anti-CD20/anti-CD19 CAR
13	CAR-T cells comprising a control bispecific anti-CD20/anti-CD19 CAR

[339] A number of metrics were monitored, comprising treatment related weight change, treatment related deaths, median tumor growth delay, median tumor burden as compared to control (%T/C), percent of animals showing partial tumor regression (% PR), percent of animals showing complete tumor regression (% CR), percent of tumor free survivors (% TFS), and percent increased lifespan (ILS %). Results are shown in Table 44 below, noting that data reflect monitoring through termination of the experiment at day 48.

[340] All treatments were tolerated. All treatment regimens, excluding non-transduced cells and LG cells, resulted in median tumor growth delays of at least 21 days, increase in life span (ILS) of >223%, median %T/C on Day 12 of approximately 0%, and many resulted partial and/or complete tumor regressions.

Table 44

Group #	Treatment Related Weight Change (%)	Treatment Related Deaths (%)	Median Tumor Growth Delay (Days)	Median %T/C (Day 12)	%PR	%CR	%TFS	Increased Life Span (LS) %
1	-21.8	0.0	0.0	100.0	0.0	0.0	0.0	0.0
2	-22.2	0.0	-0.1	108.0	0.0	0.0	0.0	0.0
3	-7	0.0	>28.5	0.1	50.0	33.3	0.0	>223.1
4	-6.8	0.0	>28.5	0.2	33.3	66.6	50.0	>223.1
5	-4.8	0.0	>28.5	0.3	67.0	33.3	0.0	>223.1
6	-5.8	0.0	>28.5	0.4	16.6	66.6	0.0	>223.1
7	-5.4	0.0	>28.5	0.6	50.0	16.6	0.0	>223.1
8	-3.3	0.0	>28.5	0.1	33.3	66.6	0.0	>223.1
9	-5.1	0.0	>28.5	0.7	16.6	0.0	0.0	>223.1
10	-4.6	0.0	25.2	0.5	0.0	0.0	0.0	>223.1
11	-4.4	0.0	>28.5	0.3	16.6	33.3	0.0	>223.1
12	-8.8	0.0	3.8	28.7	0.0	0.0	0.0	61.5
13	-3.3	0.0	20.8	3.2	16.6	0.0	0.0	>223.1

Example 13

[341] The present Example provides data relating to the anti-tumor efficacy of CAR-T cells comprising monovalent anti-CD20 CARs. Anti-CD20 CAR-T cells were tested against disseminated luciferase-expressing Raji B cell lymphoma tumors (CD19 WT) in NSG mice. Weekly blood samples were monitored for CAR-T persistence. Response was evaluated based on bioluminescence imaging (BLI) and survival endpoints. BLI was performed on days 5, 12, 21, 28, 33, 40, and 47. Study was terminated on day 55. CAR-T cells of this study as shown in Table 45 below, administered at the dosage and number of mice shown in Table 45. Table 45 provides the experimental conditions or groups referred to in subsequent tables of this

Example. All CAR-T cells were administered intravenously (QDx1). All mice received a human IL-2 dosage of 36µg ((Q12Hx2)QDx3).

Table 45

1	PBS control (6 mice)
2	Mock CAR-T cells control (non-transduced T cells) (1.5E+07 cells) (6 mice)
3	CAR-T cells comprising anti-CD19 CAR (6.58E+06 cells) + non-transduced T cells (8.39E+06 cells) (5 mice)
4	CAR-T cells comprising anti-CD19 CAR (1.32E+06 cells) + non-transduced T cells (1.37E+07 cells) (6 mice)
5	CAR-T cells comprising anti-CD20 CAR (1.1E+07 cells) + non-transduced T cells (4.1E+06 cells) (5 mice)
6	CAR-T cells comprising anti-CD20 CAR (2.2E+06 cells) + non-transduced T cells (1.3E+07 cells) (5 mice)
7	CAR-T cells comprising anti-CD20 CAR comprising binding motif #8 (1.5 E+07 cells) (5 mice)
8	CAR-T cells comprising anti-CD20 CAR comprising binding motif #8 (3.0E+06 cells) (5 mice)
9	CAR-T cells comprising anti-CD20 CAR comprising binding motif #9 (1.1E+07 cells) (5 mice)
10	CAR-T cells comprising anti-CD20 CAR comprising binding motif #9 (1.3E+06 cells) (4 mice)
11	CAR-T cells comprising anti-CD20 CAR comprising binding motif #14 (1.1E+07 cells) + non-transduced T cells (4.0E+06 cells) (6 mice)
12	CAR-T cells comprising anti-CD20 CAR comprising binding motif #14 (2.2E+06 cells) + non-transduced T cells (1.3E+07 cells) (5 mice)

[342] A number of metrics were monitored, comprising treatment related weight change, treatment related deaths, median tumor growth delay, median tumor burden as compared to control (%T/C), percent of animals showing partial tumor regression (% PR), percent of animals showing complete tumor regression (% CR), percent of tumor free survivors (% TFS), and percent increased lifespan (ILS %). Results are shown in Table 46 below.

Table 46

Group #	Treatment Related Weight Change (%)	Increased Life Span (%)	Median %T/C Day 12	Tumor Growth Delay (days)	% CR	% PR	% TFS
1	-17.1	0.0	100.0	0.0	0.0	0.0	0.0
2	-23.6	27.3	61.7	0.9	0.0	0.0	0.0
3	-4.9	215.2	3.7	20.5	0.0	0.0	0.0
4	-9.4	113.2	26.6	4.1	0.0	0.0	0.0
5	-6.4	>290.9	36.3	>35.9	60.0	0.0	0.0
6	-10.3	>290.9	59.2	29.5	0.0	0.0	0.0
7	-6.7	>290.9	4.3	>35.9	0.0	0.0	0.0
8	-5.1	>290.9	42.7	5.7	0.0	0.0	0.0
9	-10.2	>290.9	3.3	>35.9	40.0	0.0	40.0
10	-11.2	263.6	56.0	4.4	0.0	0.0	0.0
11	-6.9	227.3	24.0	18.7	0.0	0.0	0.0
12	-10.0	254.6	50.7	2.9	0.0	0.0	0.0

Example 14

[343] The present Example provides data relating to the anti-tumor efficacy of CAR-T cells comprising bicistronic CARs comprising an anti-CD19 CAR and an anti-CD20 CAR, and to the anti-tumor efficacy of CAR-T cells comprising monovalent anti-CD20 CARs. CAR-T cells were tested against disseminated luciferase-expressing Raji B cell lymphoma tumors in NSG mice. Weekly blood samples were monitored for CAR-T persistence. Response was evaluated based on bioluminescence imaging (BLI) and survival endpoints. BLI was performed on days 5, 12, 19, 26, 33, and 40.

[344] CAR-T cells of this study are shown in Table 47 below. Table 47 provides the experimental conditions or groups referred to in subsequent tables of this Example. Groups 1-10 comprise only controls and monospecific CAR-T cells, while groups 11-16 comprise bicistronic CAR-T cells. Bicistronic CAR-T cells comprise an anti-CD20 CAR as indicated in Table 39 and an anti-CD19 CAR (Ab11 CAR). Each condition shown in Table 47 comprised 6 mice. All CAR-T cells were administered intravenously (QDx1) at the indicated dose. All mice also received intraperitoneal human IL-2 at a dosage of 36 μ g ((Q12Hx2)QDx3).

Table 47

1	PBS control
2	Mock CAR-T cells control (non-transduced T cells) (9.0E+06 cells)
3	Mock CAR-T cells control (non-transduced T cells) (1.4E+07 cells)

4	CAR-T cells comprising anti-CD19 control CAR comprising an Ab11 binding motif (1.40E+07 cells)
5	CAR-T cells comprising anti-CD19 control CAR comprising an Ab11 binding motif (7.0E+06 cells) + Non-transduced T cells (2.00E+06 cells)
6	CAR-T cells comprising anti-CD19 control CAR comprising an Ab11 binding motif (1.40E+06 cells) + Non-transduced T cells (7.60E+06 cells)
7	CAR-T cells comprising anti-CD20 CAR comprising binding motif #14 (6.80E+06 cells) + Non-transduced T cells (2.20E+06 cells)
8	CAR-T cells comprising anti-CD20 CAR comprising binding motif #14 (1.40E+06 cells) + Non-transduced T cells (7.60E+06 cells)
9	CAR-T cells comprising control anti-CD20 CAR (7.0E+06 cells) + Non-transduced T cells (2.00E+06 cells)
10	CAR-T cells comprising control anti-CD20 CAR (1.40E+06 cells) + Non-transduced T cells (7.60E+06 cells)
11	CAR-T cells comprising anti-CD20 CAR comprising binding motif #14 and an anti-CD19 CAR (bicistronic CAR) (7.50E+06 cells) + Non-transduced T cells (1.40E+06 cells)
12	CAR-T cells comprising anti-CD20 CAR comprising binding motif #14 and an anti-CD19 CAR (bicistronic CAR) (1.50E+06 cells) + Non-transduced T cells (7.50E+06 cells)
13	CAR-T cells comprising anti-CD20 CAR control and an anti-CD19 CAR (bicistronic CAR) (8.0E+06 cells) + Non-transduced T cells (9.50E+06 cells)
14	CAR-T cells comprising anti-CD20 CAR control and an anti-CD19 CAR (bicistronic CAR) (1.60E+06 cells) + Non-transduced T cells (7.40E+06 cells)
15	CAR-T cells comprising anti-CD20 CAR comprising binding motif #14 and an anti-CD19 CAR (bicistronic CAR) (9.0E+06 cells)
16	CAR-T cells comprising anti-CD20 CAR comprising binding motif #14 and an anti-CD19 CAR (bicistronic CAR) (1.80E+06 cells) + Non-transduced T cells (7.20E+06 cells)

[345] A number of metrics were monitored, comprising treatment related weight change, treatment related deaths, median tumor growth delay, median tumor burden as compared to control (%T/C), percent of animals showing partial tumor regression (% PR), percent of animals showing complete tumor regression (% CR), percent of tumor free survivors (% TFS), and percent increased lifespan (ILS %). Results are shown in Table 48 below. All CAR-T cell treatments were well tolerated by study mice.

Table 48

Group #	Treatment Related Weight Change (%)	Treatment Related Death (%)	Increased Life Span (%)	Median $\Delta T/\Delta C$ Day 12 (%)	Tumor Growth Delay (days)	% CR	% PR	% TFS
1	-16.5	0.0	0.0	100.0	0.0	0.0	0.0	0.0
2	-23.9	0.0	28.0	67.3	0.9	0.0	0.0	0.0
3	-23.0	0.0	28.0	84.8	0.3	0.0	0.0	0.0
4	-10.8	0.0	>188.0	4.6	>28.3	33.3	0.0	0.0
5	-2.6	0.0	>188.0	5.6	>28.3	0.0	0.0	0.0
6	-4.2	0.0	164.0	6.9	20.4	0.0	0.0	0.0
7	-9.0	0.0	>188.0	4.9	>28.3	16.6	0.0	16.6
8	-4.4	0.0	>188.0	9.0	>28.3	0.0	0.0	0.0
9	-9.1	0.0	>188.0	4.5	>28.3	0.0	0.0	0.0
10	-0.5	0.0	183.0	9.0	24.5	0.0	0.0	0.0
11	-7.3	0.0	>188.0	4.8	>28.3	0.0	0.0	0.0
12	-2.9	0.0	>188.0	7.6	24.5	0.0	0.0	0.0
13	-4.6	0.0	>188.0	4.4	>28.3	0.0	0.0	0.0
14	-0.8	0.0	>188.0	7.2	>28.3	0.0	0.0	0.0
15	-6.2	0.0	>188.0	5.0	>28.3	0.0	0.0	0.0
16	-4.9	0.0	>188.0	5.0	>28.3	0.0	0.0	0.0

Example 15

[346] The present Example provides data relating to the anti-tumor efficacy of CAR-T cells comprising bicistronic CARs comprising an anti-CD19 CAR and an anti-CD20 CAR, and to the anti-tumor efficacy of CAR-T cells comprising monovalent anti-CD20 CARs. CAR-T cells were tested against a luciferase-expressing NALM6 human acute lymphoblastic leukemia model in NSG mice. Blood samples were monitored for CAR-T persistence. Response was evaluated based on bioluminescence imaging (BLI) and survival endpoints. Whole blood was drawn for analysis at days 4, 11, 20, 25, 32, and 39. BLI was performed on days 3, 10, 18, 24, 31, 38, and 45. The study was terminated on day 48.

[347] CAR-T cells of this study are shown in Table 49 below. Table 49 provides the experimental conditions or groups referred to in subsequent tables of this Example. Groups 1-8 comprise only controls and monospecific CAR-T cells, while groups 9-12 comprise bicistronic CAR-T cells. Bicistronic CAR-T cells comprise an anti-CD20 CAR as indicated in

Table 49 and an anti-CD19 CAR (Ab11 CAR). Each condition shown in Table 49 comprised 6 mice. All CAR-T cells were administered intravenously (QDx1) at the indicated dose.

Table 49

1	PBS control
2	Mock CAR-T cells control (non-transduced T cells) (1.1E+07 cells)
3	CAR-T cells comprising anti-CD19 control CAR comprising an Ab11 binding motif (9.70E+06 cells) + Non-transduced T cells (8.90E+05 cells)
4	CAR-T cells comprising anti-CD19 control CAR comprising an Ab11 binding motif (1.90E+06 cells) + Non-transduced T cells (8.70E+06 cells)
5	CAR-T cells comprising anti-CD20 CAR comprising binding motif #14 (6.50E+06 cells) + Non-transduced T cells (4.10E+06 cells)
6	CAR-T cells comprising anti-CD20 CAR comprising binding motif #14 (1.30E+06 cells) + Non-transduced T cells (9.30E+06 cells)
7	CAR-T cells comprising control anti-CD20 CAR (7.00E+06 cells) + Non-transduced T cells (3.60E+06 cells)
8	CAR-T cells comprising control anti-CD20 CAR (1.40E+06 cells) + Non-transduced T cells (9.20E+06 cells)
9	CAR-T cells comprising anti-CD20 CAR comprising binding motif #14 and an anti-CD19 CAR (bicistronic CAR) (1.10E+07 cells)
10	CAR-T cells comprising anti-CD20 CAR comprising binding motif #14 and an anti-CD19 CAR (bicistronic CAR) (2.10E+06 cells) + Non-transduced T cells (8.50E+06 cells)
11	CAR-T cells comprising anti-CD20 CAR control and an anti-CD19 CAR (bicistronic CAR) (8.4E+06 cells) + Non-transduced T cells (2.30E+06 cells)
12	CAR-T cells comprising anti-CD20 CAR control and an anti-CD19 CAR (bicistronic CAR) (1.70E+06 cells) + Non-transduced T cells (9.00E+06 cells)

[348] A number of metrics were monitored, comprising treatment related weight change, treatment related deaths, median tumor growth delay, median tumor burden as compared to control (%T/C), percent of animals showing partial tumor regression (% PR), percent of animals showing complete tumor regression (% CR), percent of tumor free survivors (% TFS), and percent increased lifespan (ILS %). Results are shown in Table 50 below. All CAR-T cell treatments were tolerated by study mice.

Table 50

Group #	Treatment Related Weight Change (%)	Treatment Related Deaths (%)	Increased Life Span (ILS) %	Median Tumor Growth Delay (Days)	Median %T/C (Day 18)	%PR	%CR	%TFS
1	2.9	0.0	0.0	0.0	100.0	0.0	0.0	0.0
2	3.8	0.0	0.0	0.0	121.6	0.0	0.0	0.0
3	-2.2	0.0	>100.0	27.0	0.0	50.0	50.0	16.6
4	3.9	0.0	72.2	13.0	0.2	33.3	0.0	0.0
5	-8.7	0.0	>100.0	27.0	0.0	66.6	0.0	0.0
6	-2.2	0.0	86.1	16.5	13.5	0.0	0.0	0.0
7	-5.4	0.0	105.6	19.0	1.5	0	0.0	0.0
8	-2.4	0.0	97.2	17.5	39.4	0.0	0.0	0.0
9	-2.2	0.0	>100.0	27.0	0.0	83.3	16.6	16.6
10	-2.2	0.0	>100.0	27.0	0.1	100.0	0.0	0.0
11	6.5	0.0	>100.0	27.0	0.0	100.0	0.0	0.0
12	10.3	0.0	111.1	20.0	0.2	15.6	0.0	0.0

Example 16

[349] The present Example provides data relating to sequences for use as components of CARs. Four anti-CD20 binding motifs are tested in combination with 4 hinges (CD8 hinge (8k), truncated CD28 hinge (28T), truncated CD28 hinge with a G4S linker; and IgG4 hinge (I4)). All bispecific and bicistronic CARs comprise an Ab11 anti-CD19 binding motif. CD4⁺ and CD8⁺ T cells were isolated by positive selection from apheresis material from healthy donors and used to generate anti-CD20 monovalent or anti-CD20/anti-CD19 bicistronic CAR T-cell products. T cells were activated with bound-anti-CD3 and soluble CD28 antibodies and transduced with a lentiviral vector encoding for a CAR construct. As a control, non-transduced

(NTD) T cells were generated from the same donor T cells in parallel. On the harvest day (Days 8-10 of manufacture), CAR T-cell products were stained and analyzed by flow cytometry to assess transduction efficiency and used in co-culture assays. Transduction efficiency of T cells with vector encoding monovalent CAR and vector encoding bicistronic CAR were monitored. [350] To determine the T cell transduction efficiency of vector encoding a monovalent CAR, CAR-T products were stained with a panel of antibodies (anti-CD3, anti-CD4, anti-CD8, and anti-linker) in the presence of a fixable viability dye and analyzed by flow cytometry to assess the percentage of viable CAR-positive cells. The anti-linker antibody is an antibody that binds the linker between the heavy and light chains of the binding motif of the anti-CD20 CAR and is used to measure transduction efficiency. Controls comprised non-transduced cells (NTD), cells transduced with a retrovirus comprising a control anti-CD19 binding agent, and cells transduced with a control anti-CD20 binding agent (Ab12 binding motif).

[351] To determine the T cell transduction efficiency of vector encoding a bicistronic CAR, CAR-T products were stained with a panel of antibodies (comprising anti-CD3, anti-CD4, anti-CD8, anti-idiotypic, and anti-linker) in the presence of a fixable viability dye and analyzed by flow cytometry to assess the percentage of viable CAR-positive cells. The anti-idiotypic antibody binds the binding motif of the Ab11 anti-CD19 binding motif. Thus, the anti-idiotypic antibody binds the anti-CD19 CAR. It is used to measure the transduction efficiency of the anti-CD19 CAR. The anti-linker antibody is used to measure transduction efficiency of the anti-CD20 CAR. Controls comprised non-transduced cells (NTD), cells transduced with a retrovirus comprising a control anti-CD19 binding agent, and cells transduced with control anti-CD20/anti-CD19 bispecific CARs (Ab13/Ab14 bispecific; Ab11/Ab12 bispecific).

[352] To facilitate tracking of T cells in culture, CAR-T cells were labeled with CellTrace™ Violet (CTV) reagent according to the manufacturer's instructions and subsequently washed with R-10% media. To facilitate tracking of cells expressing CAR-T target antigens ("target cells"), target cells were engineered to express luciferase. Luciferase-expressing target cells comprised Nalm6 and Raji, both of which expresses both the CD19 and CD20 antigens. In addition, Nalm6 and Raji cells not expressing CD19 or CD20 (knockout cells, or KO) were prepared. These CD19KO and CD20KO cells were clonally selected from Nalm6 and Raji parental cells and express CD20 but not CD19, or CD19 but not CD20, respectively. The CD19KO and CD20KO strains were generated and used as controls to functionally assess antigen binding of each CAR of cells expressing bicistronic anti-CD20/anti-CD19 CAR.

[353] Luciferase-expressing target cells were plated together with CTV labeled CAR-T cells at various ratios in R-10% media (Day 0 of co-culture). The ratio may be referred to as the ratio of effector (CAR-T) cells to target cells (effector:target or E:T). To plate cells at desired ratios, CAR-T cells were serially diluted 2 to 3-fold while the number of target cells per well was held constant at 25,000 cells. Co-cultures were incubated at 37°C for either 16 hours (h) or 4 days and functional assessments were performed as described below.

[354] T-cell mediated cytotoxicity was measured as a function of the reduction in target luciferase signal in co-culture wells compared to the signal emitted by target cells plated alone. On Day 4 after co-culture initiation, D-luciferin substrate was added to the co-culture wells at a final concentration of 0.14 mg/mL and plates were incubated at 37°C in the dark for 10 minutes. Luminescent signal was read immediately after in a VarioSkan™ LUX or VarioSkan® Flash multimode microplate reader. T cell-mediated cytotoxicity was calculated as follows: % Cytotoxicity = $[1 - \text{luciferase signal of (sample of interest/target alone control)}] * 100$.

[355] Cytokine production was measured after 16h in co-culture, at which time supernatants were collected and analyzed for cytokine levels using the Meso Scale Discovery V-PLEX Proinflammatory Panel 1 human kit according to the manufacturer's instructions. Supernatants from the co-cultures of T-cell products plated at the 1:1 E:T ratio with antigen-expressing target cells were analyzed for levels of interferon gamma (IFN- γ), IL-2, tumor necrosis factor alpha (TNF- α), and IL-10 secretion mediated by antigen engagement. All samples were diluted to be within the range of detection. The level of each cytokine is reported as pg/mL and the lower limit of quantitation and upper limit of quantitation of each assay is reported.

[356] To determine T cell activation after 16h co-culture, T-cell products were plated at the specified E:T ratio with antigen-positive target cells were harvested, stained with a panel of antibody-fluorophores to identify T cells (CD3, CD4, CD8) and 4-1BB, an activation marker, and analyzed by flow cytometry. A fixable viability dye allowed analysis of viable cells. Events were systematically gated on live cells (viability dye-negative), lymphocytes (using forward scatter [FSC]-area by side scatter [SSC]-area plot), single cells (using FSC-area by FSC-height plot), and then T cells (CD3⁺). T cells were then analyzed for level of activation (ie, percentage of 4-1BB⁺ cells); the 4-1BB gating threshold was set based on the level expressed by the NTD control T cells.

[357] The proliferative capacity of the T-cell products was determined by flow cytometric analysis of the cell division-driven dilution of CTV dye compared with NTD control T cells in response to antigen-expressing target cells. On Day 4 after co-culture initiation, T-cell products plated at the 3:1 E:T ratio with antigen-expressing target cells were harvested, stained with a panel of antibody-fluorophores (CD3, CD4, CD8) in the presence of a fixable viability dye to identify viable T cells and analyzed by flow cytometry. The percentage of proliferating cells as well as the mean fluorescence intensity (MFI) of CTV signal is reported. The decrease in MFI of CTV is proportional to the number of rounds of cell division the product has undergone.

[358] CAR-T cells used in this Example comprised the CARs identified in Table 51 below. Table 51 provides the experimental conditions or groups referred to in subsequent tables of this Example. Transduction efficiency is shown in Table 52. Day 4 cytotoxicity in Nalm6 (CD19+; CD20low), Nalm6CD19KO (CD19-; CD20+), Raji (CD19+, CD20+) and Raji CD19KO (CD19-, CD20+) is shown in Tables 53A-53D. Cytokine production at 14h post-co-culture at a 1:3 E:T ratio in Nalm6 CD19KO target cells is shown in Tables 54A-54D, as measured in pg/mL (2 replicates per condition). T cell activation as measured by upregulation of 41BB (percent 41BB+ live T cells) at 16h post co-culture is shown in Tables 55A-D (2 replicates per condition). Proliferation of T cells at 4d post co-culture is shown in Table 56 (percent proliferating CD3+, two replicates per condition).

Table 51

	Type (<i>e.g.</i> , Bicistronic v. Bispecific)
1	Non-transduced control
2	Monospecific anti-CD19 CAR comprising G4S linker
3	Monospecific anti-CD19 CAR comprising G4S linker
4	Monospecific anti-CD19 CAR comprising G4S linker
5	Monospecific anti-CD20 CAR comprising CD8 hinge (8k)
6	Bicistronic anti-CD20/anti-CD19 CAR, the anti-CD20 CAR comprising CD8 hinge (8k)
7	Bicistronic anti-CD20/anti-CD19 CAR, the anti-CD20 CAR comprising IgG4 hinge
8	Bicistronic anti-CD20/anti-CD19 CAR

9	Bicistronic anti-CD20/anti-CD19 CAR, the anti-CD20 CAR comprising binding motif #2 and a CD8 hinge (8k)
10	Bicistronic anti-CD20/anti-CD19 CAR, the anti-CD20 CAR comprising binding motif #8 and a CD8 hinge (8k)
11	Bicistronic anti-CD20/anti-CD19 CAR, the anti-CD20 CAR comprising binding motif #9 and a CD8 hinge (8k)
12	Bicistronic anti-CD20/anti-CD19 CAR, the anti-CD20 CAR comprising binding motif #14 and a CD8 hinge (8k)
13	Bicistronic anti-CD20/anti-CD19 CAR, the anti-CD20 CAR comprising binding motif #2 and an CD28 hinge
14	Bicistronic anti-CD20/anti-CD19 CAR, the anti-CD20 CAR comprising binding motif #8 and an CD28 hinge
15	Bicistronic anti-CD20/anti-CD19 CAR, the anti-CD20 CAR comprising binding motif #9 and an CD28 hinge
16	Bicistronic anti-CD20/anti-CD19 CAR, the anti-CD20 CAR comprising binding motif #14 and an CD28 hinge
17	Bicistronic anti-CD20/anti-CD19 CAR, the anti-CD20 CAR comprising binding motif #2 and an CD28 hinge with a G4S linker
18	Bicistronic anti-CD20/anti-CD19 CAR, the anti-CD20 CAR comprising binding motif #8 and an CD28 hinge
19	Bicistronic anti-CD20/anti-CD19 CAR, the anti-CD20 CAR comprising binding motif #9 and an CD28 hinge with a G4S linker
20	Bicistronic anti-CD20/anti-CD19 CAR, the anti-CD20 CAR comprising binding motif #14 and an CD28 hinge with a G4S linker
21	Bicistronic anti-CD20/anti-CD19 CAR, the anti-CD20 CAR comprising binding motif #2 and an IgG4 hinge
22	Bicistronic anti-CD20/anti-CD19 CAR, the anti-CD20 CAR comprising binding motif #8 and an IgG4 hinge
23	Bicistronic anti-CD20/anti-CD19 CAR, the anti-CD20 CAR comprising binding motif #9 and an IgG4 hinge

24	Bicistronic anti-CD20/anti-CD19 CAR, the anti-CD20 CAR comprising binding motif #14 and an IgG4 hinge
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Table 52

Group	1	2	5	6	7	8
% CD19 CAR+ Live T Cells	0.56	59.52	2.35	47.54	29.05	56.77
% CD20 CAR+ Live T Cells	0.09	69.3	75.76	49.33	40.13	60.99
Group	9	10	11	12	13	14
% CD19 CAR+ Live T Cells	52.72	50.2	44.72	40.28	38.99	34.12
% CD20 CAR+ Live T Cells	46.42	45.57	41.36	37.04	37.15	34.7
Group	15	16	17	18	19	20
% CD19 CAR+ Live T Cells	34.87	51.32	28.89	41.69	41.34	36.22
% CD20 CAR+ Live T Cells	33.78	47.97	28.87	41.82	41.95	35.14
Group	21	22	23	24		
% CD19 CAR+ Live T Cells	37.07	30.62	32.18	39.93		
% CD20 CAR+ Live T Cells	38.69	41	41.23	46.43		

Table 53A

Nalm6 WT	Group							
ET Ratio	1		2		3		4	
1 to 243	30.2	21.9	-22.9	-11.5	-0.5	-12.1	-13.8	-11.7
1 to 81	-0.8	-7.5	-20.9	-16.4	-8.1	-10.2	-27.6	-11.9
1 to 27	-19.5	-37.2	-5.7	-17.2	-13.3	-15.9	-2.1	-13.2
1 to 9	-13.6	-20.2	14.9	-1.5	8.7	-5.9	13.4	1.2
1 to 3	-7.1	-28.9	100	100	100.1	100.1	100.1	100.1
3 to 1	16	4.6	100.4	100.1	100.5	100.1	100.5	100.1
ET Ratio	5		6		7		8	
1 to 243	-14.6	-12.6	-4	-10.2	-7.3	-11.6	-9.8	-9.9
1 to 81	5.2	-20.1	-6.2	-20.5	-5.7	-15	-2.2	-7
1 to 27	-8	-21.2	3.2	-10.9	6	-5.5	-8.1	-8.9
1 to 9	-1.8	0.6	19.4	16.1	19	1.7	18.7	11
1 to 3	100.1	99.9	100.1	100.1	100.1	100.1	100	99.9
3 to 1	100.5	100.1	100.5	100.1	100.5	100.1	100.4	100
ET Ratio	9		10		11		12	
1 to 243	43.6	48.1	-16	0.7	-8.3	-14.3	-3.1	-19.9
1 to 81	9.5	20.1	-7.5	-2.4	-10.7	-22.4	-10.2	-14.2
1 to 27	-8.2	0.9	-8.9	-1.6	-12.8	-9.7	3	-13
1 to 9	6.5	10.3	0.3	5	-2.3	-10.9	-0.4	-16.9
1 to 3	100	100.1	100.1	100.1	100.1	100.1	100.1	100.1
3 to 1	100.1	100.1	100.1	100.1	100.1	100.1	100.1	100.1
ET Ratio	13		14		15		16	
1 to 243	-11.6	-21.9	-13.8	-21.2	-9	-16.6	-2.9	-12.6
1 to 81	-11	-31.6	-20.8	-25	-15.7	-20.6	-6.3	-8
1 to 27	-13.6	-10.1	-22.3	-4.6	-15.4	-11.1	0.9	3.2
1 to 9	-6.5	-20.4	-7.1	-7.5	-13.3	-17.6	14.1	17.3
1 to 3	100.1	100.1	100.1	100.1	100.1	100.1	100	99.9
3 to 1	100.1	100.1	100.1	100.1	100.1	100.1	100	100
ET Ratio	17		18		19		20	
1 to 243	30.4	32.6	-1.9	-6.4	-2.2	-16	-18.5	-13
1 to 81	8.7	9.1	-8.1	-5.6	-20.8	-19.4	-31.6	-26.6
1 to 27	1.5	-3.7	-0.9	-6	-4.1	-7	-15.6	-10.9
1 to 9	6.5	0	-4.7	-1.4	-0.6	11.7	-22	-4.2
1 to 3	100.1	100	100.1	100.1	100.1	100.1	100.1	100
3 to 1	100.1	100.5	100.1	100.5	100.1	100.5	100.1	100.5
ET Ratio	21		22		23		24	
1 to 243	-11.2	-12.1	-9.4	-32.5	-10.9	-29.1	-17.9	-19.8
1 to 81	-22.6	-17.9	-20.9	-26.9	-17.6	-24.6	-17.4	-19.2
1 to 27	-5.1	-8.3	6.1	-7.6	-2.2	-9.4	3.6	-2.6
1 to 9	-8.6	-2.4	0.4	14.4	22.5	-3.5	19.5	5.3
1 to 3	100.1	100.1	100.1	100.1	100.1	100.1	100	99.9
3 to 1	100.1	100.5	100.1	100.5	100.1	100.5	100	100.4

Table 53B

Nalm6 CD19KO	Group							
ET Ratio	1		2		3		4	
1 to 243	-10.2	-10.7	-14.4	-14.8	-7.3	-11.3	-15.8	-14.1
1 to 81	-8.7	-21.5	-5	-14.6	-5.8	-16.6	-7.8	-12.2
1 to 27	3.8	-18.2	-2.3	-13.6	-4.7	-21.8	-1	-18.8
1 to 9	-0.2	-11.2	-3.5	-10.3	-3.8	-13.7	-4.8	-3.6
1 to 3	39.2	30.5	33.6	19.8	33.2	24.5	33.9	26.7
3 to 1	56.2	41.9	39.4	32.6	43.8	40.7	38	32.9
ET Ratio	5		6		7		8	
1 to 243	-9	-15.4	-8.2	-13.9	-12.9	-11.9	-11.1	-9.2
1 to 81	6.3	-4.5	-1.9	-9.9	-3.6	-13.4	5.3	5.3
1 to 27	13.6	16.5	18.8	5.5	10	-0.6	23.1	19.3
1 to 9	40.5	33.2	45.3	44.7	35.7	20.2	59.2	46.4
1 to 3	100.1	99.9	100.3	100.1	99.8	99.7	100.1	99.8
3 to 1	100.9	101.1	101.1	101.2	101.1	101.2	101	101
ET Ratio	9		10		11		12	
1 to 243	-6	-8	-6	-11.4	-8.2	-7.6	-7.8	-4.3
1 to 81	4.4	10.8	-1.5	-0.1	2.3	0.3	8.5	10.4
1 to 27	-2.7	6.1	12	-5.9	4.5	21.5	38.2	21.7
1 to 9	32.2	34.6	17.6	14.1	17.3	11.4	30.8	22.7
1 to 3	100.2	100.8	100.1	100.9	100.2	100.8	100.1	100.8
3 to 1	101	101.4	101	101.4	101	101.5	101	101.4
ET Ratio	13		14		15		16	
1 to 243	-5.3	-7.2	-8.6	5.8	-5.3	18.7	-7.4	8.1
1 to 81	5	0.5	-2.5	9.6	-1.7	5.8	7.1	13
1 to 27	13.6	18.6	10.4	40.6	11.9	27.8	27.8	28.2
1 to 9	8.4	3.1	31.3	20.3	2.2	7.9	32.3	42.4
1 to 3	98.2	99.8	100.2	100.8	100.1	100.8	100	100.6
3 to 1	99.6	100.1	101.1	101.4	101.1	101.3	101	101.3
ET Ratio	17		18		19		20	
1 to 243	-1.9	-5	-8.5	-12.4	-9	-7.7	-12.7	-14.3
1 to 81	-5.9	-10.3	-10.6	-10.5	-4.8	-11	-11.2	-15.9
1 to 27	-5.6	-2.8	1.4	-0.4	16.1	14.8	-0.7	2.4
1 to 9	21.5	4.9	8.9	3.7	20.6	35.4	1.3	7
1 to 3	97	94.4	100.2	100	100.3	100.1	100.2	99.3
3 to 1	99.6	101.3	100.2	101.9	100.2	101.9	100.2	101.9
ET Ratio	21		22		23		24	
1 to 243	-5.8	0.2	-4.2	-9.4	7	-12.5	-2	-10.2
1 to 81	-0.5	-5.4	8.3	-4.3	4.1	-10.1	6.2	2.1
1 to 27	32.9	13.9	41.7	27.8	34.2	14.4	35.6	26.8
1 to 9	22.9	32.9	38.3	46	44.5	19.7	43.5	25.5
1 to 3	100.3	100.1	100.3	100.1	100.2	100	100.2	100
3 to 1	100.2	101.9	100.2	101.8	100.1	101.7	100	101.7

Table 53C

Raji WT	Group							
ET Ratio	1		2		3		4	
1 to 243	2.2	-33.8	-12.6	-23	-19.4	-30.3	-8.1	-22.8
1 to 81	16.3	-29.4	-2.5	-15	-2.8	-16.3	-6.4	-17.7
1 to 27	-9.2	-48.8	1.6	-33.1	-2	-29.4	2.7	-25.3
1 to 9	-6.5	-35.9	13.5	-9.7	14	-10.5	11.6	-5.5
1 to 3	8.9	-17.9	99.8	99.9	99.9	99.9	100.1	100
3 to 1	19.5	5.9	103.8	100.1	103.9	100.2	103.9	100.2
ET Ratio	5		6		7		8	
1 to 243	-11	-21.7	-7.9	-25.1	-6.5	-18.8	-5.1	-14.7
1 to 81	-4.6	-14.9	-0.5	-19.2	0.4	-2	-0.1	-0.1
1 to 27	-0.1	-14.1	8.6	-20.6	7.9	-6.6	10.4	-8.8
1 to 9	13.6	-2.6	16.5	5.1	22.2	-3.3	23.8	-0.5
1 to 3	100.2	99.9	100.1	100.1	100.1	100	99.9	99.8
3 to 1	104	100.2	103.9	100.2	103.9	100.1	103.8	100
ET Ratio	9		10		11		12	
1 to 243	-30.8	-24.1	-30.9	-12.5	-17.6	-14.4	-23.9	-6.8
1 to 81	-26.7	-15.7	-9.8	-14.6	-6.9	-6	-6.5	-13.2
1 to 27	-20.6	-22.8	-6.5	-3.3	-4.1	-4	0.3	2.3
1 to 9	-7.4	-15.1	-1.5	-14.6	-2.5	-12.8	9.3	-3.7
1 to 3	104.4	104.6	104.4	104.6	104.4	104.5	104.5	104.5
3 to 1	108	105.9	108.1	105.9	108.1	105.8	108.1	105.9
ET Ratio	13		14		15		16	
1 to 243	-28.5	-8.4	-17.4	-11.5	-11.1	-11	-9.9	-5.6
1 to 81	-11.4	-7.2	-16.4	-13.8	-9.9	-10.4	4.7	1.7
1 to 27	1	0.7	-1.6	5.2	6.9	-3.3	10.2	13.5
1 to 9	3.3	-12	7.7	-11	4	-2.3	14.4	9.9
1 to 3	104.4	104.6	104.4	104.5	104.4	104.5	104.3	104.4
3 to 1	108.1	105.9	108	105.9	108	105.8	107.8	105.7
ET Ratio	17		18		19		20	
1 to 243	-28.3	-21	-22.1	-16.4	-11.4	-11.8	-10.6	-11.1
1 to 81	-18.5	-22.7	-14.6	-26.2	-11.4	-34.7	-12.6	-28.9
1 to 27	-23	-21.7	-14.5	-26.5	8.2	-5.9	-5.3	-14.9
1 to 9	-9.5	-34.2	-11.2	-39.6	-6.9	-15.5	-17.8	-27.7
1 to 3	100	100.2	100	100.2	100.1	100.2	100.1	100.2
3 to 1	101.2	120.1	101.2	120.1	101.2	120.1	101.2	120.1
ET Ratio	21		22		23		24	
1 to 243	-7	-4.1	-9.6	-6.3	-8.1	-11.1	-7.5	-1.1
1 to 81	-5.8	-14.4	-2.6	-19.2	9	-19.9	19.2	-4.6
1 to 27	5.3	-6.3	-5.1	2.4	4.9	0.7	13.3	5.4
1 to 9	-6.7	-14.2	-7.4	-6.1	9.6	-16.6	15.3	-9
1 to 3	100.1	100.2	100.1	100.2	100.1	100.2	99.9	100.1
3 to 1	101.2	120.1	101.2	120.1	101.2	120.1	101	119.9

Table 53D

Raji CD19KO	Group							
ET Ratio	1		2		3		4	
1 to 243	51	55	36.7	26.2	-18.2	12.5	-44.9	0
1 to 81	-0.4	33.7	-85.1	3.4	-143.7	3	-190.3	9.3
1 to 27	15.3	-0.9	4.5	6.3	-0.5	2.7	4	1.1
1 to 9	-1.9	3.3	2.9	2	8.4	6.5	1.5	15.2
1 to 3	34.5	20.9	37.3	20.8	16.3	16	5.5	11.7
3 to 1	41.3	14.2	14.6	2.1	11.2	19.7	4.6	3.8
ET Ratio	5		6		7		8	
1 to 243	-15.1	-3.2	22.8	-1.4	-5.5	0.2	-85.7	-2.4
1 to 81	-217.9	3.3	-201.5	-2.2	-227.2	-0.9	-210.3	2.5
1 to 27	-0.6	7	11.8	3.4	1.9	-1.6	1.4	9.5
1 to 9	8.5	17	22.1	21.5	13.5	12.6	30.9	21
1 to 3	98.8	99.8	99.4	100.6	91.9	93.5	98.8	99.6
3 to 1	99.6	99.7	99.8	100.1	99.1	99.6	99.7	99.9
ET Ratio	9		10		11		12	
1 to 243	9	11.5	-2.9	10.2	-0.6	2.2	-2.4	17.6
1 to 81	10.3	17.5	6.6	9.6	8.1	8.5	-2.6	3.2
1 to 27	15.7	21.5	16	21.2	20.2	32	16.2	22
1 to 9	25.6	35	38.1	32.2	32.9	27.9	23.7	28.8
1 to 3	99.7	100.2	99.7	100	99.9	100	99.8	100.1
3 to 1	99.9	100.9	99.8	100.9	100	101	99.9	101
ET Ratio	13		14		15		16	
1 to 243	-1.8	9.8	-3	2.6	5.6	1.9	-7.7	14.5
1 to 81	-2.8	0.5	-0.4	-2.7	-6.3	-11.5	-3.3	-2.5
1 to 27	10.5	19.6	-0.8	11.7	-4.4	8.6	8.7	16.7
1 to 9	14.7	15.7	24.3	22.5	7.3	10.8	15	12.3
1 to 3	96.1	98.5	97.2	99.7	95.3	97	92.3	97.7
3 to 1	96.2	96.2	98.4	98.7	97.5	97.5	96.1	95.7
ET Ratio	17		18		19		20	
1 to 243	7.6	20.4	5.1	6.1	-0.1	8.8	4.6	11.8
1 to 81	9.7	13.2	11.1	11.8	8.9	13.2	3	6.8
1 to 27	18.1	10.1	29.9	18.7	24.4	21.3	13.8	15.8
1 to 9	44.9	19.3	37	26.8	33.6	32.3	24.1	16
1 to 3	87.9	84.7	99.7	99.1	98.7	97.5	93.8	92.5
3 to 1	95	96.5	99.7	100	99.7	99.9	96.1	95
ET Ratio	21		22		23		24	
1 to 243	-5.9	6.6	2.9	-9.9	2.7	-2.6	-4.3	-3.4
1 to 81	11.5	-5.2	10.7	-0.3	8	-3.2	7.3	0.4
1 to 27	26	20.7	21.9	16.7	22.1	14.1	31.7	20.6
1 to 9	18.6	28.9	21.2	25.8	32.1	9.2	28.7	19.7
1 to 3	100.4	100.2	100.4	100.1	100.4	100.2	100.2	100
3 to 1	100	100.1	100	100.1	100	100.1	99.8	100

Table 54A

TNF α							
Group							
1		2		3		4	
21.43362	30.0185	33.8795	28.22578	23.07486	21.60766	28.62412	32.4843
5		6		7		8	
769.5068	750.8404	720.3671	614.8629	229.2041	233.5543	836.7802	842.7271
9		10		11		12	
317.8869	371.7266	414.5337	293.488	386.2704	293.6139	418.6442	307.1845
13		14		15		16	
252.4179	152.0566	185.1981	253.8267	180.7021	249.8017	208.5401	327.4072
17		18		19		20	
203.6143	217.2118	405.7839	345.8976	467.5809	425.1003	269.5771	258.1286
21		22		23		24	
472.2237	421.9478	446.7417	402.7078	430.8759	441.7219	432.4396	494.9866

Table 54B

IL10							
Group							
1		2		3		4	
12.06837	12.32633	11.93813	10.60854	11.04015	12.18301	9.930545	14.15293
5		6		7		8	
17.26261	17.49864	19.22346	27.91504	28.00769	25.40533	22.57109	25.85191
9		10		11		12	
20.85123	21.31715	25.06719	23.96362	17.45668	16.68065	27.34339	37.56894
13		14		15		16	
15.01034	17.85805	21.4962	15.99171	17.25999	16.67803	29.79311	30.33925
17		18		19		20	
20.35921	24.0903	22.76352	23.08782	15.64091	19.16565	25.07248	18.56677
21		22		23		24	
16.28242	12.91556	15.43415	18.99226	16.57846	14.00925	22.48675	23.85015

Table 54C

IL-2							
Group							
1		2		3		4	
13.90412	5.925647	7.239758	8.057854	3.416301	10.05507	5.481665	4.24322
5		6		7		8	
1979.747	1880.755	758.0331	769.4632	136.3706	143.4531	1373.496	1344.97
9		10		11		12	
415.0335	397.6617	371.0906	419.0813	315.6276	308.785	239.4187	263.1596
13		14		15		16	
81.99199	101.7831	191.2544	191.099	258.0696	200.4818	149.9144	150.8354
17		18		19		20	
83.13918	96.59951	234.2623	273.2762	400.7794	439.0895	132.4347	146.6177
21		22		23		24	
324.6525	284.3169	312.9986	304.5192	284.8684	245.4556	171.9664	220.1387

Table 54D

IFNg							
Group							
1		2		3		4	
2183.862	1360.111	1707.394	2083.675	1077.22	1338.666	1977.427	1416.691
5		6		7		8	
82883.42	90801.34	98809.63	79846.05	25763.3	22756.31	113383.2	130673.4
9		10		11		12	
33687.05	41534.7	33669.96	46572.37	41476.85	32400.9	44847.72	41189.26
13		14		15		16	
18695.39	13259.19	22300.69	18887.22	19159.02	26725.05	27437.53	38194.28
17		18		19		20	
19441.39	19547.47	36846.64	34373.3	30178.64	49566.75	27345.52	24419.34
21		22		23		24	
42811.57	31839.23	44792.28	37959.75	40016.19	41814.98	56819.12	56847.75

Table 55A

Raji CD19KO							
Group							
1		2		3		4	
5.09	4.2	3.34	4.04	3.54	3.98	4.31	3.94
5		6		7		8	
21.8	17.92	18.12	18.53	15.05	12.81	18.42	15.43
9		10		11		12	
17.44	21.84	18.46	14.89	17.75	16.01	20.06	16.96
13		14		15		16	
9.52	9.53	10.54	11.11	12	11.99	16.69	16.19
17		18		19		20	
8.38	7.48	16.6	14.92	15.64	11.99	13.78	14.05
21		22		23		24	
18.7	18.72	13.26	14.9	16.55	18.4	17.12	17.54

Table 55B

Raji parental							
Group							
1		2		3		4	
24.53	4.51	22.54	23.61	17.08	18.23	23.65	24.2
5		6		7		8	
21.84	23.46	25.65	19.57	20.96	20.04	17.69	17.27
9		10		11		12	
25.04	24.38	22.53	25.75	25.69	21.61	23.93	27.4
13		14		15		16	
21.25	23.01	19.43	21.15	18.99	21.92	27.95	30.26
17		18		19		20	
15.13	15.72	26.86	28	27.46	25.72	24.49	23.03
21		22		23		24	
25.59	25.04	20.98	19.29	23.01	20.78	22.14	21.49

Table 55C

Nalm6 CD19KO							
Group							
1		2		3		4	
5.12	5.19	3.6	3.55	5.1	4.64	5.12	5.07
5		6		7		8	
14.47	20.84	18.69	19.81	13.33	17.17	18.12	17.87
9		10		11		12	
22.37	23.08	19.86	19.82	19.28	18.98	19.01	23.35
13		14		15		16	
8.87	10.25	10.83	12.05	13.18	14.8	17.6	17.86
17		18		19		20	
9.04	8.8	19.73	18.54	18.22	16.83	16.11	14.89
21		22		23		24	
19.29	18.03	18.25	15.5	18.01	15.89	15.89	16.97

Table 55D

Nalm6 parental							
Group							
1		2		3		4	
1.95	1.53	23.14	25.28	19.79	19.69	23.06	23.75
5		6		7		8	
19.06	23.01	21.27	20.07	18.62	20.4	15.82	14.64
9		10		11		12	
25.8	28.75	26.91	26.98	32.3	27.59	23.53	25.26
13		14		15		16	
24.38	28.81	21.38	21.64	21.2	23.73	23.59	28.61
17		18		19		20	
20.24	19.63	28.58	30.28	28.35	26.96	26.32	23.5
21		22		23		24	
26.96	24.02	24.71	20.78	23.5	24.06	23.58	20.73

Table 56

	Raji parental		Raji CD19KO		Nalm6 CD19KO		Nalm6 parental	
Group	replicate 1	replicate 2	replicate 1	replicate 2	replicate 1	replicate 2	replicate 1	replicate 2
1	64.5	65.1	61.8	60.6	55.6	50.6	32.8	35.8
2	92	88.5	57.5	56.6	43.6	46.6	90.4	72.1
3	84.1	84.6	58.3	59.8	43.4	50.3	87.1	83.9
4	91.8	90.4	63.5	59.9	44.3	47.6	91	89.7
5	77	69.1	78.6	78.4	64.7	70.7	90	88.7
6	89.2	87.2	91.5	88.6	89.3	86.6	91.9	89.5
7	87.2	83	88.8	86.4	84	82	91.7	90.2
8	92.6	77.4	90.4	81.8	89.1	80.2	87.4	86.4
9	92.8	89.6	92.5	92.8	87	87.5	91.6	92.6
10	84.6	87.6	88.9	90.1	81.4	81.9	90.6	90.4
11	85.1	77.7	87.2	87.8	78.1	79.9	87	86.6
12	83.4	88.9	92	91.4	84.1	83.7	90.6	88.5
13	88	81.8	80.4	78.2	65.2	66.6	89.1	88.5
14	81	74.4	81.4	80.6	77.3	76.8	88.5	89.2
15	74.8	72.5	81	81	75.9	77.5	85.5	84.2
16	91.8	91.6	87.9	88.1	81.7	81.4	92.4	92.1
17	83.3	84.3	77.6	80.7	62.2	62.9	88.1	86.8
18	70.2	76.4	82	83.4	74.5	78.1	85.6	108
19	81.8	85.6	88.9	89.6	82.4	82.7	88	90.4
20	77.5	81.2	83.6	84.4	78.1	79.6	88.1	88.5
21	80.1	86.8	90.5	90.2	85.3	84.6	90.9	91.9
22	82.6	77.9	88.5	88.4	83.7	83.7	90.3	89.7
23	75.8	71.2	86.8	85.7	79.2	80.1	84.6	89.4
24	84.6	84.9	89.7	89.7	82.6	84.2	89.7	89.5

Example 17

[359] Comparison of hinges was undertaken in anti-CD20 monovalent CARs. Data presented in this Example was obtained using the methods set forth in the Example above. CAR-T cells used in this Example comprised the CARs identified in Table 57 below. Table 57 provides the experimental conditions or groups referred to in subsequent tables of this Example. The Example utilized four anti-CD20 binding motifs, identified in this Example as binding motif A, binding motif B, binding motif C, and binding motif D. Hinges tested comprise a truncated CD28 hinge (28T), a CD8 hinge (8k), and an IgG4 hinge (I4).

[360] Transduction efficiency is shown in Table 58. Day 4 cytotoxicity in Raji (CD19+, CD20high), Raji CD20KO (CD19+, CD20-), Namalwa (CD19+, CD20low) and Nalm6 (CD19+, CD20low) is shown in Tables 59 and 60. Cytokine production at 14h post-co-culture at a 1:1 E:T ratio is shown in Tables 61A-D. T cell activation as measured by upregulation of 41BB at 16h post co-culture is shown at E:T 1:1 or 1:4 in Tables 62 and 63 respectively. Proliferation of T cells at 4d post co-culture is shown at E:T 1:1 or 1:4 in Tables 64 and 65, respectively.

Table 57

	Type (e.g., Bicistronic v. Bispecific)
1	Non-transduced control
2	Monovalent anti-CD20 CAR comprising scFv#A and a CD28 hinge
3	Monovalent anti-CD20 CAR comprising binding motif #A and a CD8 hinge
4	Monovalent anti-CD20 CAR comprising binding motif #A and a IgG4 hinge
5	Monovalent anti-CD20 CAR comprising binding motif #B and a CD28 hinge
6	Monovalent anti-CD20 CAR comprising binding motif #B and a CD8 hinge
7	Monovalent anti-CD20 CAR comprising binding motif #B and a IgG4 hinge
8	Monovalent anti-CD20 CAR comprising binding motif #C and a CD28 hinge
9	Monovalent anti-CD20 CAR comprising binding motif #C and a CD8 hinge
10	Monovalent anti-CD20 CAR comprising binding motif #C and a IgG4 hinge
11	Monovalent anti-CD20 CAR comprising binding motif #D and a CD28 hinge
12	Monovalent anti-CD20 CAR comprising binding motif #D and a CD8 hinge
13	Monovalent anti-CD20 CAR comprising binding motif #D and a IgG4 hinge

Table 58

Group	Transduction efficiency (% CAR+)
1	0.15
2	62.3
3	56.3
4	89.2
5	51.6
6	66.5
7	71.6
8	73.9
9	77.0
10	85.4
11	66.8
12	80.8
13	84.8

Table 59

	Group											
Cell Type	Targets Alone			1			2			3		
Raji	79202.8	70452	71294.3	59370.9	60525.3	62135.5	20933.5	16844.6	18439.3	11943.1	11014.1	12652.8
Raji CD20KO	94864.6	93729.3	107518	80627	79824.4	59886.5	56081.3	52181.7	57347.3	4569.03	5957.66	3498.72
Namalwa	38530.1	44751.2	48600.5	33016.5	37313.6	30919.3	4394.22	2694.15	3891.21	519.957	3115.59	3057.82
Nalm6	90735.3	91482.2	98863.8	59924.4	59786.6	64853.3	2658.44	3592.39	5366.27	305.796	2897.74	3346.3
T Cells Alone				31.3053	78.4287	81.2964	25.6924	18.7526	11.3603	26.524	20.1956	-1.40636
	4			5			6			7		
Raji	22070.7	28253	17675.7	14077.7	14314.2	10548.3	18323.8	20951.1	13738	12898.1	13121.6	11526.1
Raji CD20KO	10671	12115.3	7939.32	5497.05	4740.32	3396.25	16686.9	15579.5	10419.2	17695.6	15896.4	12334.8
Namalwa	2134.36	1335.68	1012.9	209.453	3599.39	112.932	294.986	262.702	290.627	156.625	84.3594	212.663
Nalm6	379.48	231.061	1459.7	386.438	393.678	259.554	274.516	183.039	319.083	185.179	188.334	200.887
T Cells Alone	76.2154	50.5227	19.5781	61.926	24.5794	4.43293	48.7191	15.8604	32.1614	35.2493	59.7065	25.6558
	8			9			10			11		
Raji	20733.1	21458.2	17483	23005.8	20136	21075.7	15456.7	17261	13376.7	9450.65	6450.55	3648.15
Raji CD20KO	52536.7	49496	44758.5	9961.2	10555.9	5704.38	1032.2	1686.96	867.172	23411.8	46859.6	39609.5
Namalwa	847.869	815.512	533.335	532.712	306.157	265.491	173.011	170.523	157.236	180.832	376.613	147.429
Nalm6	427.827	272.767	816.448	248.126	248.976	188.004	309.551	236.001	148.273	1231.56	1128.79	1102.86
T Cells Alone	31.0058	17.5848	1.5163	34.0876	48.9268	4.17612	48.6274	28.5599	17.4688	55.8118	16.6736	7.52665
	12			13								
Raji	14053.5	17263.2	11094.7	15470.9	13763.4	10694						
Raji CD20KO	15535.6	17771.9	13844.9	14099.5	14913.9	8969.65						
Namalwa	326.695	492.742	221.914	487.203	504.029	297.42						
Nalm6	718.3	704.494	680.758	925.057	960.085	641.792						
T Cells Alone	25.8577	13.3904	24.335	47.1967	22.3783	-3.57676						

Table 60

	Group											
Cell Type	Targets Alone			1			2			3		
Raji	79202.8	70452	71294.3	70496.5	65321.5	58423.9	42813	44348.5	37624.9	39257.9	38721.7	30611
Namalwa	38530.1	44751.2	48600.5	33819.1	32454.5	41126	22632.1	19889.3	18162	22050.6	12958.9	7359.29
Nalm6	90735.3	91482.2	98863.8	73391.3	65922.4	72170.8	46819.9	38689.7	34293.5	21699.7	33426.3	9867.04
	4			5			6			7		
Raji	44017.5	43902.4	33164	38984.7	36843.5	36870.2	40930.5	39351.8	36485.6	39262.2	41832.7	34967.1
Namalwa	22821.8	19476.6	18126.5	13029.1	11967.4	9916	11781.4	9822.72	9658.94	7294.71	7892.54	12538.3
Nalm6	44203.2	41959.1	35773	21237.1	33388	20677.9	28267.6	23091.1	34779.9	20386.4	18653.5	27414.2
	8			9			10			11		
Raji	41713	45451.7	32434	39503.7	46552.6	32453.8	39272	45873.5	27479.8	41628.7	48931.1	30158.6
Namalwa	17375.7	15523.3	16927.4	15573.5	13148.5	10366.2	13962.5	14478.1	7466.35	17478.4	11443.5	2857.6
Nalm6	40265.3	34385.2	44712.1	29146.2	33328.6	45078.6	27153.9	24678.3	23030.7	35739.8	25712.2	19693.8
	12			13								
Raji	35491	40527.2	26702.8	40345.8	43589.9	32952.6						
Namalwa	12422.4	9309.45	5395.27	20039.8	18096.3	8713.27						
Nalm6	32197.1	22364.4	6859.16	21170.7	23463	18225.7						

Table 61A

TNFA								
Cell type	Group	Replicates			Group	Replicates		
Raji	1	742.7049	719.9322	860.8748	2	13789.44	1000.926	13787.09
					3	40482.31	10509.95	22032.99
					4	29963.57	10729.04	30770.2
Raji CD20KO	1	141.045	123.2006	193.527	2	2335.533	136.1735	1378.167
					3	12412.38	1591.951	6519.058
					4	19806.9	5900.966	28857.1
Namalwa	1	51.37436	54.13314	67.40804	2	5755.176	760.4728	4287.225
					3	13230.78	5171.471	4564.486
					4	16464.95	1756.426	29002.14
Nalm6	1	85.77411	41.52716	65.44027	2	2071.754	243.4781	1436.396
					3	5188.394	354.1513	3276.759
					4	8136.712	3462.309	9516.77
T cells alone	1	32.09018	27.34532	41.75542	2	60.93963		73.35283
					3	53.7447	132.6905	40.34432
					4	202.1004	49.30805	404.2379
Cell type	Group	Replicates			Group	Replicates		
Raji	5	5234.629	29643.24	25173.66	8	11804.04	5948.771	17987.23
	6	5881.73	11238.04	12382.32	9	23295.94	32399.1	41903.58
	7	14913.76	7566.033	20158.56	10	25189.57	6263.5	30133.63
Raji CD20KO	5	5938.115	6402.741	5130.22	8	215.2762	905.5388	2441.275
	6	13127.4	3087.198	2251.109	9	1131.756	11010.04	17227.01
	7	5314.634	3215.46	968.1883	10	1335.315	4004.466	19501.31
Namalwa	5	10163.08	41246.69	4330.718	8	769.9154	1797.08	12106.37
	6	16230.87	15604.86	3141.588	9	1521.87	8562.425	28414.64
	7	12714.45	16044.57	5474.505	10	2997.275	2239.813	23060.28
Nalm6	5	2356.156	601.0444	4148.512	8	2480.164	547.2608	1625.393
	6	4695.265	555.9875	735.1625	9	5150.879	998.5473	7296.929
	7	3560.739	2614.049	485.996	10	5296.607	666.9747	3631.616
T cells alone	5	35.14156	55.05909	86.07184	8	60.31223	26.13958	107.2801
	6	79.07053	56.76139	11.75329	9	48.80656	33.28641	122.8503
	7	94.67347	121.4341	87.89045	10	54.93238	77.29811	41.86264
Cell type	Group	Replicates						
Raji	11	2228.672	3952.296	5817.774				
	12	3639.337	7400.496	9585.174				
	13	4699.947	13583.39	10646.23				
Raji CD20KO	11	343.0228	138.8956	455.7918				
	12	666.3561	1570.956	2033.204				
	13	904.006	2134.385	1311.669				
Namalwa	11	712.1625	1751.303	1156.45				
	12	1605.915	5712.698	1989.63				
	13	1850.41	7176.859	1563.88				
Nalm6	11	1952.569	90.90757	194.5827				
	12	1909.742	237.1462	229.7622				
	13	623.5514	305.8666	204.3989				
T cells alone	11	53.96276	48.59063	37.73721				
	12	73.1479	38.83681	45.07434				
	13	59.63715	65.16515	32.03268				

Table 61B

IL-2								
Cell type	Group	Replicates			Group	Replicates		
Raji	1	322.5795	320.5048	350.4306	2	2073.658	100.1265	1416.118
					3	20921.12	8886.709	26954.08
					4	10616.69	2267.245	8153.757
Raji CD20KO	1	144.0881	141.6475	157.9271	2	114.382	35.60637	55.48347
					3	6247.868	1515.275	6207.359
					4	3882.511	839.9909	3086.204
Namalwa	1	143.7966	154.0605	106.577	2	85.0148	24.75363	71.28991
					3	2232.844	952.9922	4293.162
					4	668.272	175.3299	638.2828
Nalm6	1	83.28817	95.77933	86.58723	2	39.02308	28.937	30.45196
					3	345.5094	81.29567	483.7254
					4	55.70095	10.65965	45.28578
T cells alone	1	2.475707	2.907949	3.047796	2	7.311224	14.58527	15.62037
					3	2.694135	186.4649	6.853129
					4	2.898734	7.362667	5.307659
Cell type	Group	Replicates			Group	Replicates		
Raji	5	3510.081	9491.165	16208.74	8	9435.531	3440.359	7830.216
	6	3804.56	15811.23	31095.21	9	36918.38	16658.88	38763.1
	7	1753.378	7711.317	11902.51	10	14906.08	5990.056	18862.47
Raji CD20KO	5	2277.17	1268.5	1623.264	8	70.68176	108.5994	239.3027
	6	5142.875	3411.332	4541.088	9	1584.179	2889.009	8581.598
	7	2575.962	1500.105	1613.56	10	666.248	3000.571	9240.457
Namalwa	5	2547.378	3062.228	1450.611	8	303.5536	593.7966	1560.495
	6	6034.566	10971.02	6869.738	9	2305.998	3875.078	11899.19
	7	2975.146	4558.808	1835.84	10	595.2925	1550.197	4864.39
Nalm6	5	132.1701	71.77628	102.7936	8	72.12333	50.96007	80.62564
	6	183.3285	176.8246	267.3849	9	823.2917	299.8667	901.03
	7	51.11399	50.24134	57.06661	10	221.3179	102.692	234.6751
T cells alone	5	12.70908	18.65894	16.35375	8	17.00011	13.59572	15.75731
	6	8.719928	7.591335	11.91575	9	8.642956	4.919328	11.89729
	7	5.717691	4.289614	6.980622	10	5.387739	3.932472	9.043607
Cell type	Group	Replicates						
Raji	11	823.133	3402.324	896.7429				
	12	2799.535	6101.386	1873.887				
	13	2180.811	6525.733	2506.268				
Raji CD20KO	11	43.58357	76.1947	47.89087				
	12	84.85969	225.7324	65.41539				
	13	101.4673	179.8601	106.4787				
Namalwa	11	96.9067	180.8989	87.23379				
	12	228.7018	503.392	198.3201				
	13	201.064	208.7059	125.427				
Nalm6	11	36.04238	35.06366	29.59289				
	12	6.91882	11.08133	4.670726				
	13	12.17589	7.01667	5.499756				
T cells alone	11	14.98063	15.91492	13.15346				
	12	5.901473	6.110295	5.688816				
	13	4.758223	3.894212	3.511602				

Table 61C

IL-10								
Cell type	Group	Replicates			Group	Replicates		
Raji	1	178.0432	142.8539	120.4695	2	895.8653	69.6716	892.6929
					3	1097.383	275.2268	630.683
					4	1609.907	633.7046	1807.731
Raji CD20KO	1	97.01133	85.2923	53.7706	2	309.4966	28.25922	239.7828
					3	606.8257	91.35382	309.2822
					4	1253.292	391.0293	1863.251
Namalwa	1	33.10979	35.6513	34.88744	2	573.4806	68.91302	432.3923
					3	646.463	239.0274	221.7614
					4	1527.354	192.0951	2717.487
Nalm6	1	50.10573	56.39945	79.07014	2	185.7021	24.16594	125.913
					3	443.9515	46.61747	273.2845
					4	1161.58	507.9296	1320.574
T cells alone	1	30.33024	36.6716	31.08655	2	30.82579	5.067228	35.02344
					3	32.25264	2.559772	29.35922
					4	64.27	10.88138	106.1506
Cell type	Group	Replicates			Group	Replicates		
Raji	5	251.3409	1515.368	1375.689	8	510.7076	293.819	798.0907
	6	161.179	290.4449	338.9459	9	273.8515	394.2801	513.9037
	7	773.2684	422.0451	1022.366	10	463.0497	110.0162	621.5802
Raji CD20KO	5	872.8176	934.0822	703.4425	8	47.28079	112.9426	333.4111
	6	1060.602	244.7751	222.7141	9	48.86976	422.8751	674.2297
	7	807.5648	530.7835	124.8675	10	79.00288	134.7437	641.66
Namalwa	5	1079.3	3961.233	446.361	8	92.83561	137.2657	913.6063
	6	953.3019	746.7817	191.549	9	67.28244	274.0883	832.4924
	7	1364.544	1637.356	531.6147	10	150.1814	100.8329	968.0775
Nalm6	5	412.9091	114.515	600.812	8	317.099	52.03268	148.2565
	6	908.1756	76.49075	123.8227	9	394.3369	82.53729	417.2169
	7	853.5691	573.8378	106.7992	10	553.2771	50.80476	227.2295
T cells alone	5	22.4375	26.96257	45.5054	8	22.21889	10.15072	41.45767
	6	28.556	26.16897	37.44123	9	20.68183	22.8389	39.23846
	7	33.4457	30.93852	32.09581	10	17.24112	20.44047	27.8371
Cell type	Group	Replicates						
Raji	11	95.38296	176.1182	207.3491				
	12	73.72115	147.9041	157.5053				
	13	183.7121	443.0117	331.2405				
Raji CD20KO	11	66.01768	35.65559	60.18955				
	12	83.15986	186.4922	223.6873				
	13	168.1965	401.8826	210.6137				
Namalwa	11	69.36119	159.804	92.54174				
	12	108.0373	334.7655	119.5407				
	13	192.2065	1887.085	163.9822				
Nalm6	11	249.6969	34.90282	21.92475				
	12	219.2402	53.1071	41.7788				
	13	124.2308	84.31646	49.04896				
T cells alone	11	27.12001	36.7858	18.54694				
	12	20.80955	28.15541	15.18784				
	13	26.48491	31.52268	22.26345				

Table 61D

IFNg								
Cell type	Group	Replicates			Group	Replicates		
Raji	1	41464.96	30963.75	25447.23	2	1432613	84135.23	1386317
					3	2397286	821594.6	1846574
					4	2274190	942782.2	1937710
Raji CD20K	1	11561.27	8586.159	5026.661	2	213743.2	12565.5	131488.3
					3	664001.4	69248.71	373145.3
					4	1163951	304488.7	1465425
Namalwa	1	3530.81	1842.586	2716.459	2	510701.3	52054.34	360549.4
					3	904990.4	300162.3	336275.7
					4	1253120	116275.2	1855904
Nalm 6	1	3899.43	3281.217	6749.83	2	163300.5	13171.62	96551.14
					3	294888.9	14905.42	202445.1
					4	503047.8	189820.4	597772.9
T cells alone	1	1753.5	1876.623	1557.067	2	5403.774	1746.713	7399.695
					3	5454.525	6663.711	5861.042
					4	23112.12	5606.156	49759.7
Cell type	Group	Replicates			Group	Replicates		
Raji	5	575698	1687352	1965687	8	1078730	402925.4	1348360
	6	519005.7	614491.4	1018404	9	1803657	1518159	2003596
	7	1578042	517803	1788915	10	2490064	419647.4	1932058
Raji CD20K	5	513259.5	347633.9	357939.6	8	24994.79	67790.24	231769.2
	6	784775.3	111826.9	115041.6	9	59348.85	419743.6	852087.2
	7	487082.8	204193.3	69028.4	10	108512.4	237310.2	1327924
Namalwa	5	1095254	1828112	422922.4	8	70351.05	121957.9	1029824
	6	1460396	852559.7	222410.3	9	100939.4	489723.7	1834780
	7	1421146	1201823	475557.9	10	309207.5	160166.7	1874998
Nalm 6	5	219984.2	32995.87	314350.1	8	220363.4	32273.67	135918
	6	418594.9	25470.36	50276.03	9	361549.7	49278.98	433392.1
	7	364977.2	182266.8	39687.06	10	554730	52797.1	337819.6
T cells alone	5	1805.136	1126.776	6229.361	8	5965.458	948.2716	8793.781
	6	10846.88	2035.132	8678.811	9	10854	1365.383	13795.46
	7	10220.63	7642.1	8708.371	10	7452.874	5353.539	6430.798
Cell type	Group	Replicates						
Raji	11	205423.6	305476.1	512645.3				
	12	392821.5	750606.6	903119.7				
	13	554100.1	1314131	1028525				
Raji CD20K	11	34116.67	12335.53	45684.74				
	12	69699.52	134538	208660.6				
	13	100355.9	206666.2	149680.4				
Namalwa	11	80511.69	155485.4	106748				
	12	175944.5	544014	210348.9				
	13	264515.8	565138.4	187873.3				
Nalm 6	11	176019.5	5267.248	15715.26				
	12	229324.8	24222.55	26877.11				
	13	76258.82	31998.6	26876.26				
T cells alone	11	2279.092	180.2089	392.9496				
	12	9621.94	1391.83	1901.967				
	13	1775.067	2816.077	947.6876				

Table 62

	Group											
Cell Type	1			2			3			4		
Raji	11.9	11.8	12.9	45	31	48.7	54.1	39.9	59.7	44.2	34.3	51.2
Raji CD20KO	12.1	12	13.2	24.7	22.4	30.2	54.7	36.9	56.4	45	37.7	52.2
Namalwa	6.78	6.78	6.72	17.9	18	32.3	37.5	26.7	43.1	36.9	28.7	41.8
Nalm6	6.75	7.04	6.92	19.3	13.3	20.5	26.5	19.7	30.1	25.7	20.7	31.3
T Cells Alone	3.23	2.78	3.12	7.4	6.33	9.02	6.79	6.13	8.11	13.3	11.6	16.4
Cell Type	5			6			7			8		
Raji	56.6	43.3	59.9	55.1	40.8	61.6	53.7	42.2	59.1	44.2	32.2	53.4
Raji CD20KO	45	33.7	47.8	44.7	34.5	48.2	46.5	35.1	48.5	26.2	24.8	29.5
Namalwa	41.4	26	42.7	45.1	31	50.8	44.1	28.8	48.5	33.4	20.7	35.3
Nalm6	23.3	17.7	25.5	23.9	18.7	28.2	24.6	18.6	28.7	19.1	15.4	20.8
T Cells Alone	3.78	3.97	5.09	5.62	5.91	7.36	8.13	8.18	9.92	4.53	4.68	4.6
Cell Type	9			10			11			12		
Raji	53.1	40.8	57.6	55.2	42.2	60.1	49.2	35.8	55.7	51.9	37.3	58
Raji CD20KO	48.2	38.8	53	56.4	41.8	56.3	28.2	25.3	32.6	42.3	33.8	48.6
Namalwa	44.2	30.9	41.2	42.2	30.6		31.9	24.8		38.9	33.2	46.5
Nalm6	28.6	22.3	33	28.1	23.5	32.5	17.2	17.4	22.1	21.2	19.1	28.2
T Cells Alone	5.01	5.5	7.29	5.37	6.24	7.4	2.9	4.18	3.67	6.67	7.27	9.39
Cell Type	13											
Raji	57	42	61.1									
Raji CD20KO	41.8	32.7	49.4									
Namalwa	38	28.1	43.6									
Nalm6	19.9	17.9	25.1									
T Cells Alone	3.8	4.76	5.87									

Table 63

	Group											
Cell Type	1			2			3			4		
Raji 1:4	14.8	13.4	14.7	31.6	18.5	31.1	38.8	23.7	35.8	39.2	24.5	33.8
Namalwa 1:4	6.6	6.61	6.55	25.5	15.4	23	38.1	20.9	34.5	34.1	20.8	29.8
Nalm6 1:4	9.92	9.96	10.7	21.7	16.2	19.5	31.2	22	27.8	27.5	20.7	28.5
T Cells Alone	3.23	2.78	3.12	7.4	6.33	9.02	6.79	6.13	8.11	13.3	11.6	16.4
Cell Type	5			6			7			8		
Raji 1:4	35.9	26.4	32.5	39.9	28	36.3	37.4	27.3	37.6	26.3	21.6	30.3
Namalwa 1:4	32	20	32.7	37.7	23.9	42.4	36.3	20.8	36.6	25.2	17.8	25.7
Nalm6 1:4	26	18.7	26.8	30.3	20.9	32.8	27.5	21.1	28.9	20.3	16.1	21.3
T Cells Alone	3.78	3.97	5.09	5.62	5.91	7.36	8.13	8.18	9.92	4.53	4.68	4.6
Cell Type	9			10			11			12		
Raji 1:4	35.3	28.6	39.5	33.1	28.8	40	28.2	27.9	35.6	39.3	33.7	42
Namalwa 1:4	33.8	22	33.8	29.9	20.4	31.2	25	19.3	27	32.2	24.6	36.4
Nalm6 1:4	27.5	19.9	29	26.5	20.8	26	20.4	18.6	21.8	27.4	19.5	27.9
T Cells Alone	5.01	5.5	7.29	5.37	6.24	7.4	2.9	4.18	3.67	6.67	7.27	9.39
Cell Type	13											
Raji 1:4	40	36.3	44.4									
Namalwa 1:4	31	22.9	36.9									
Nalm6 1:4	27.8	22.4	29									
T Cells Alone	3.8	4.76	5.87									

Table 64

	Group											
Cell Type	1			2			3			4		
Raji	1.49	1.1	1.28	9.95	8.23	8.26	8.36	6.88	4.62	29.5	28.5	27.8
Raji CD20KO	6.95	6.65	6.57	15.9	14.1	31.3	19.8	12	25.4	39.4	38	48.7
Namalwa	1.11	1.26	1.6	28.7	28.5	29.1	23.2	19.4	19	41.7	38.6	39.8
Nalm6	5.44	5.04	4.13	52.2	50.4	55.8	58.5	63.8	63	69.3	70.7	72.6
T Cells Alone	4.65	2.28	3.88	15.9	14.9	16.8	22.2	23.1	22.7	67.2	68.1	70.3
Cell Type	5			6			7			8		
Raji	23	17.5	11.2	14.3	10.9	10.4	15.1	11.9	10.7	15	12.8	9.6
Raji CD20KO	34.9	34.3	51	21.7	16.3	30.5	13.8	11.5	23	16.3	19.6	29.8
Namalwa	39.8	31.7	38.3	30.3	22.5	28.1	30	25.3	26.2	28.4	23.7	24.8
Nalm6	68.5	72.6	74.6	60	62.2	64.6	60.5	65.3	69.4	58.6	63.5	67.1
T Cells Alone	11.7	14	14.7	26.2	27	28.7	23.9	28.9	30.2	11.2	13.4	15.1
Cell Type	9			10			11			12		
Raji	9.59	9.11	7.33	18.4	17.3	15.6	17.4	17.4	12.6	13.1	13.4	10.1
Raji CD20KO	21	14.7	21.4	33.3	31.1	37.5	18.6	19.2	32.1	28.7	23.7	35.7
Namalwa	19.5	14.7	14.6	32.4	30	30.8	38.7	44.1	40.8	34.2	33.1	31.7
Nalm6	43.5	44.1	49.2	61.7	69.8	68.8	48.6	47.8	56.2	65.8	68.3	67.8
T Cells Alone	23.3	28.1	30	22.6	28.1	29	5.18	5.6	7.22	36.9	43.5	45.2
Cell Type	13											
Raji	9.02	8.5	5.41									
Raji CD20KO	14.4	10.4	21.3									
Namalwa	16	17.2	18.3									
Nalm6	53.1	58	58.2									
T Cells Alone	8.53	10.8	13.7									

Table 65

	Group											
Cell Type	1			2			3			4		
Raji	1.66	1.42	1.4	11.3	9.83	18.6	12.8	11.2	18.3	36	31.4	44.3
Namalwa	1.2	1.41	1.05	18.8	17.4	25.2	20.8	19.2	32.8	33.3	37.8	44.2
Nalm6	2.34	2.23	2.22	31	28.9	38.3	28.7	30.4	39.1	40.2	46.1	51.9
T Cells Alone	4.65	2.28	3.88	15.9	14.9	16.8	22.2	23.1	22.7	67.2	68.1	70.3
Cell Type	5			6			7			8		
Raji	18.6	20.5	25.8	14.8	13	21.3	15.6	13.2	21.2	11.3	12.7	20.4
Namalwa	22.5	25.8	31.3	23.6	25.6	34.4	22.2	22.9	31	17.1	16.9	24.8
Nalm6	45.9	43.3	48.4	39.9	43.2	44.6	37.6	45.6	49.7	27.3	31.2	39.4
T Cells Alone	11.7	14	14.7	26.2	27	28.7	23.9	28.9	30.2	11.2	13.4	15.1
Cell Type	9			10			11			12		
Raji	9.42	9.78	15.3	17.6	15.3	22.6	12.2	16.5	18.3	10.2	11.4	18.1
Namalwa	13.3	14.5	22.6	23.6	23.5	29.5	20.3	17.1	26.9	20.7	20.2	31
Nalm6	30.3	27.4	34.9	35.6	36.8	50.7	32.9	48.3	65.4	41	50	60.7
T Cells Alone	23.3	28.1	30	22.6	28.1	29	5.18	5.6	7.22	36.9	43.5	45.2
Cell Type	13											
Raji	6.57	8.41	8.1									
Namalwa	9.64	7.92	14.8									
Nalm6	22.1	22.9	38.9									
T Cells Alone	8.53	10.8	13.7									

Example 18

Table 66: Exemplary Binding motif sequences

SEQ ID NO	VH/VL Of Table:	Sequence
251	Table 4	ggtaccCCCGGgCCCATGGCTCTTCCTGTGACAGCTCTTCTGCTGC CCCTGGCCCTGCTTCTGCATGCTGCTAGACCTCAGGTTCAAGTTG CAGCAATGGGGAGCTGGCCTGTAAAGCCCAGCGAAACCTGT CCCTCACCTGCGCTGTGTATGGCGGAAGCTTCAGCGGCTATTA CTGGAGCTGGATCCGGCAGCCTCCTGGAAAAGGATTAGAATGG ATCGGCGAGATAGACCACAGCGGGAGCACAACTACAACCCC AGCCTGAAATCGCGGGTTACAATCTCTGTGGACACAAGCAAGA ATCAGTTCTCCCTGAAGCTGAGCAGCGTTACTGCCGCCGACAC AGCTGTGTACTATTGCGCCAGAGGCGGAGGCTCCTGGTACAGC AACTGGTTCGATCCTTGGGGCCAAGGCACCATGGTGACCGTTT CCAGCGGCTCTACAAGCGGCAGCGGGAACCTGGTTCTGGAGA GGGCAGCACAAAGGGCGACATCCAGATGACACAGAGCCCCAG CACCCTTAGCGCCTCTGTGGGAGATAGGGTTACCATTACCTGC AGGGCTTCCCAGAGCATCAGCAGCTGGCTGGCATGGTATCAAC AGAAGCCTGGCAAGGCTCCCAAGCTGCTCATCTATGACGCCTC CAGCCTGGAAAGCGGGGTTCCCTCCAGATTAGCGGCTCAGGC TCCGGAACAGAGTTACCCCTTACCATCTCTAGCCTGCAACCCG ACGACTTCGCTACTTATTACTGTCAACAAGACAGAAGCTTGCC CCCCACATTGCGCGGAGGGACCAAGGTTGAGATCAAG
252	Table 5	ggtaccCCCGGgCCCATGGCTCTTCCTGTGACAGCTCTTCTGCTGC CCCTGGCCCTGCTTCTGCATGCTGCTAGACCTCAGGTTCAAGTTG CAGCAATGGGGAGCTGGCCTGTAAAGCCCAGCGAAACCTGT CCCTCACCTGCGCTGTGTATGGCGGAAGCTTCAGCGGCATCCA CTGGAACTGGATCCGGCAGCCTCCTGGCAAAGGCCTTGAATGG ATCGGCGATATCGACACCAGCGGCTCCACCAACTACAACCCCA GCCTGAAATCGAGGGTTACAATCTCTGTGGACACAAGCAAGAA TCAGTTCTCCCTGAAGCTGAGCAGCGTTACTGCCGCCGACACA GCTGTGTACTATTGCGCCAGACTGGGCCAGGAAAGCGCTACCT

		ACCTTGGCATGGATGTGTGGGGCAGGGCACCACCGTTACTGT TAGCTCTGGCTCAACAAGCGGCAGCGGCAAGCCTGGCTCAGGA GAAGGAAGCACAAAGGGCGACATTGTAATGACTCAGAGCCCC GACAGCCTGGCCGTTAGCTTAGGCGAAAGGGCTACAATCAATT GCAAGAGCAGCCAGAGCGTTCTGTACAGCAGCAACAACAAGA ACTACCTCGCATGGTATCAACAGAAGCCAGGCCAGCCTCCCAA GCTGCTCATCTACTGGGCTTCCACCAGAGAGAGCGGGGTTCCC GATAGATTCTCCGGCTCCGGTTCTGGAACAGATTTACGCTCAC AATCAGCAGCTTACAGGCCGAGGATGTGGCTGTCTACTATTGT CAGCAGTTGTACACCTACCCCTTACATTTCGGCGGAGGCACCA AGGTTGAGATCAAG
253	Table 6	ggtaccCCCGGgCCCATGGCTCTTCCTGTGACAGCTCTTCTGCTGC CCCTGGCCCTGCTTCTGCATGCTGCTAGACCTCAGCTTCAGCTC CAAGAGAGCGGACCTGGCTTAGTGAAGCCCAGCGAAACCCTGT CCCTCACCTGCACCGTTTCTGGCGGAAGCATCAGCAGCTCCAG CTATTACTGGGGATGGATCAGGCAGCCCCCTGGCAAGGGTTTA GAATGGATCGGCTCGATATATTACTCCGGCAGCACCTACTATA ACCCAGCTTGAAGAGCCGGGTACCATTCTGTGGACACATC AAAGAACCAGTTCAGCCTGAAGCTGAGCTCTGTGACTGCCGCC GACACAGCTGTGTACTACTGTGCCAGAGAGACAGACTACTCCA GCGGCATGGGCTACGGCATGGATGTGTGGGGACAAGGAACCA CCGTTACTGTGAGCAGCGTTCCACCAGCGGCTCAGGCAAGCC TGGCTCAGGAGAAGGAAGCACCAAGGGGGATATACAGATGAC ACAGAGCCCCCTCAGCCTGTCCGCCAGCGTTGGCGATCGTGTA ACGATCACCTGCCGGGCCTCTCAGAGCATCAACTCCTACCTCA ATTGGTATCAACAGAAGCCAGGCAAGGCCCCCAAATTACTCAT CTACGCCGCCAGCAGCTTACAGAGCGGGGTTCCCTCTAGATT TCCGGCTCCGGTTCTGGAACAGATTTACCCTCACTATCTCCAG CTTGCAGCCCGAGGATTTGCCACTTATTACTGTCAGCAGAGC CTGGCCGACCCCTTACATTTCGGCGGAGGCACAAAGGTTGAGA TCAAG

254	Table 7	ggtaccCCCGGgCCCATGGCTCTTCCTGTGACAGCTCTTCTGCTGC CCCTGGCCCTGCTTCTGCATGCTGCTAGACCTCAGGTTCACTT GTGCAGAGCGGAGCTGAAGTTAAGAAGCCTGGCGCCTCTGTGA AGGTTAGCTGCAAGGCCAGCGGCTACACATTCAAGGAATATGG CATCTCCTGGGTAGGCAGGCTCCCGGCCAAGGCTTAGAATGG ATGGGCTGGATCTCCGCCTACTCCGGCCACACCTACTACGCCC AGAAGCTTCAGGGCAGGGTTACCATGACCACCGACACCAGCAC CTCTACCGCCTATATGGAGCTGAGGAGCCTGAGATCGGACGAC ACAGCTGTGTATTACTGCGCCAGAGGCCCCACTACGACGACT GGTCTGGATTTATCATCTGGTTCGACCCCTGGGGGCAGGGCAC CCTGGTCACAGTTTCTTCTGGCTCCACCAGCGGAAGCGGCAAG CCAGGCTCAGGCGAAGGATCTACAAAAGGCGACATCCAAATG ACACAGAGCCCCAGCAGCTTGAGCGCCTCCGTTGGCGACAGAG TTACAATCACCTGCAGGGCCTCTCAGAGCATCAGCAGCTATTT GAATTGGTATCAACAGAAGCCAGGAAAGGCCCTAAGCTGCTC ATCTACGCTGCCAGCTCGCTCCAATCTGGCGTTCCTAGCAGATT TAGCGGCTCCGGCAGCGGCACAGACTTTACTCTTACCATTAGC TCCCTGCAGCCCGAGGACTTCGCTACCTACTATTGCCAGCAAA GCTACAGATTCCCTCCCACCTTTGGCCAGGGCACAAAGGTTGA GATCAAG
255	Table 8	ggtaccCCCGGgCCCATGGCTCTTCCTGTGACAGCTCTTCTGCTGC CCCTGGCCCTGCTTCTGCATGCTGCTAGACCTCAGGTTCACTTA CAAGAGAGCGGACCTGGCTTAGTGAAGCCCAGCGAAACCCTGT CCCTCACCTGCACCGTTTCTGGCGGAAGCATCAGCTCTCCCGAC CATTACTGGGGATGGATCAGGCAGCCCCCTGGCAAGGGTTTGG AATGGATCGGCAGCATCTACGCCAGCGGCAGCACATTCTACAA CCCCTCGCTCAAAAGCAGGGTTACTATTTCTGTGGACACAAGC AAAAATCAGTTCAGCCTGAAGCTGAGCTCTGTGACTGCCGCCG ACACAGCTGTGTACTACTGTGCCAGAGAGACAGACTACTCCAG CGGGATGGGCTACGGCATGGATGTGTGGGGACAAGGAACCAC CGTTACTGTGAGCAGCGGCTCCACAAGCGGCTCAGGCAAGCCT GGCTCAGGAGAAGGAAGCACCAAGGGGGACATTCAATGACC

		CAAAGCCCCTCCAGCCTGTCCGCCAGCGTTGGCGATAGGGTTA CCATTACCTGCAGGGCCAGCCAAAGCATCAACTCCTACCTAAA TTGGTATCAACAGAAGCCAGGCAAGGCCCCCAAACACTACTCATT TACGCCGCCAGCAGCTTACAGAGCGGGGTTCCCTCTAGATTCT CCGGCAGCGGTTCTGGAACAGATTTCACTCTCACAAATATCTTCG CTGCAGCCCGAGGATTTCTGCTACCTACTATTGCCAGCAATCCCT GGCCGACCCCTTCACATTCGGCGGAGGCACAAAGGTTGAGATC AAG
256	Table 9	ggtaccCCCGGgCCCATGGCTCTTCCTGTGACAGCTCTTCTGCTGC CCCTGGCCCTGCTTCTGCATGCTGCTAGACCTCAGATCACATTA AAAGAGAGCGGACCTACACTGGTGAAGCCCACCCAAACGCTT ACCCTCACCTGCACCTTTAGCGGGTTCAGCCTGGACACAGAGG GCGTTGGCGTTGGATGGATCAGGCAGCCTCCTGGCAAAGCCCT CGAATGGCTTGCCCTCATCTACTTCAACGACCAGAAGAGATAC AGCCCCCTCCTTAAATCTCGGCTCACAAATCACCAAAGACACAA GCAAAAATCAGGTTGTGCTCACCATGACCAACATGGACCCTGT GGACACCGCTGTGTACTACTGTGCCAGAGACACCGGCTACAGC AGATGGTACTACGGGATGGACGTTTGGGGCCAAGGCACCACTG TGACCGTTTCCAGCGGCTCTACAAGCGGCAGCGGGAACCTGG TTCTGGAGAGGGCAGCACAAAGGGCGACATCCAGATGACGCA ATCCCCCAGCTCTGTGAGCGCCTCTGTGGGAGACAGAGTTACA ATCACATGCCGGGCCTCCCAGGGCATCAGCTCTTGGCTGGCAT GGTATCAACAGAAGCCTGGCAAGGCTCCCAAGCTGCTCATCTA TGCCGCCTCCTCCTTACAATCTGGAGTTCCTCCAGGTTACAGCG GGAGCGGCTCAGGAACAGACTTCACCTTACCATCTCTAGCCT GCAACCCGAGGACTTCGCTACTTATTACTGTCAGCAGGCCTAC GCCTACCCCATCACATTCGGCGGAGGAACAAAGGTTGAGATCA AG
257	Table 10	ggtaccCCCGGgCCCATGGCTCTTCCTGTGACAGCTCTTCTGCTGC CCCTGGCCCTGCTTCTGCATGCTGCTAGACCTCAGGTTCAAGTTG CAGCAATGGGGAGCTGGCCTGTAAAGCCCAGCGAAACCTGT CCCTCACCTGCGCTGTGTATGGCGGAAGCTTCGAGAAATACTA

		CTGGAGCTGGATCCGGCAGCCTCCCGGCAAAGGCTTAGAATGG ATCGGCGAGATTTATCACAGCGGGCTCACCAACTACAACCCCA GCCTGAAATCTCGAGTTACAATCTCTGTGGACACAAGCAAGAA TCAGTTCTCCCTGAAGCTGAGCAGCGTTACTGCCGCCGACACA GCTGTGTACTATTGCGCCAGAGTTAGATACGACAGCAGCGACA GCTATTACTACAGCTATGACTACGGCATGGATGTGTGGGGGCA GGGCACCACCGTTACTGTCTCCTCTGGATCTACCAGCGGCAGC GGCAAGCCTGGATCTGGCGAAGGAAGCACAAAGGGCGACATT GTGCTCACCCAGAGCCCCGACAGCCTGGCTGTGTCTTTAGGCG AAAGGGCTACCATCAACTGCAAGAGCAGCCAGAGCGTTCTGTA CAGCAGCAACAACAAGAACTACCTTGCTTGGTATCAACAGAAG CCTGGCCAGCCCCCTAAGCTGCTCATCTACTGGGCCTCTAGCA GAGAGAGCGGGGTTCCCGATCGGTTTAGCGGCTCCGGCTCAGG AACCGATTTCACCCTCACTATCTCCAGCCTCCAGGCCGAGGAT GTGGCTGTCTACTATTGTCAGCAGAGCTATAGCTTCCCCTGGAC ATTCGGCGGAGGCACCAAGGTTGAGATCAAG
258	Table 11	ggtaccCCCGGgCCCATGGCTCTTCCTGTGACAGCTCTTCTGCTGC CCCTGGCCCTGCTTCTGCATGCTGCTAGACCTCAGGTTCAAGTTA CAACAATGGGGAGCTGGCCTGTAAAGCCCAGCGAAACCTGT CCCTCACCTGCGCTGTGTATGGCGGAAGCTTCAGCCGCTATGT GTGGAGCTGGATCCGGCAGCCTCCTGGCAAAGGCCTTGAATGG ATCGGAGAGATAGACAGCAGCGGCAAGACCAACTACAACCCC AGCCTGAAATCACGCGTTACAATCTCTGTGGACACAAGCAAGA ATCAGTTCTCCCTGAAGCTGAGCAGCGTTACTGCCGCCGACAC AGCTGTGTACTATTGCGCCAGAGTTAGATACGACAGCTCCGAC AGCTATTACTACAGCTATGACTACGGCATGGATGTGTGGGGGC AGGGCACCACCGTTACAGTTAGCTCTGGAAGCACCAGCGGCTC CGGCAAGCCTGGATCTGGTGAAGGAAGCACAAAGGGCGACAT TGTGCTCACCCAGAGCCCCGACAGCCTGGCTGTGTCTTTAGGC GAAAGGGCTACCATCAACTGCAAGAGCAGCCAGAGCGTTCTGT ACAGCAGCAACAACAAGAACTACCTTGCTATGGTATCAACAGA AGCCTGGCCAGCCTCCCAAGCTGCTCATCTACTGGGCCTCTAG

		CAGAGAGAGCGGGGTTCCCGATCGCTTTAGCGGCAGCGGTTCT GGCACCGATTTCCTTACAATCAGCAGCTTACAGGCCGAGG ATGTGGCTGTCTACTATTGTCAGCAGAGCTATAGCTTCCCCTGG ACATTCGGCGGAGGCACCAAGGTTGAGATCAAG
259	Table 12	ggtaccCCCGGgCCCATGGCTCTTCCTGTGACAGCTCTTCTGCTGC CCCTGGCCCTGCTTCTGCATGCTGCTAGACCTCAGGTTCAAGT CAACAATGGGGAGCTGGCCTGTAAAGCCCAGCGAAACCCTGT CCCTCACCTGCGCTGTGTATGGCGGAAGCTTCAGCGGCTACGC TTGGAGCTGGATTAGACAGCCTCCTGGCAAAGGACTAGAATGG ATCGGAGAGATCGACCACAGAGGCTTCACCAACTACAACCCCA GCCTGAAATCCAGAGTTACAATCTCTGTGGACACAAGCAAGAA TCAGTTCTCCCTGAAGCTGAGCAGCGTTACTGCCGCCGACACA GCTGTGTACTATTGCGCCAGGGTTAGATACGACAGCAGCGACA GCTATTACTACAGCTATGACTACGGCATGGATGTGTGGGGGCA GGGCACCACCGTTACGGTTAGCTCTGGATCTACCAGCGGCAGC GGCAAGCCTGGCTCAGGAGAAGGAAGCACAAAGGGCGACATT GTGCTCACCCAGAGCCCCGACAGCCTGGCCGTTTCTTTAGGCG AAAGGGCTACCATCAACTGCAAGAGCAGCCAGAGCGTTCTGTGA CAGCAGCAACAACAAGAACTACCTTGCATGGTATCAACAGAA GCCAGGCCAGCCTCCCAAGCTGCTCATCTACTGGGCCTCTAGC AGAGAGAGCGGGGTTCCCGATAGATTTTCGGGATCAGGCTCCG GCACCGATTTCCTTACGATCAGCAGCTTACAGGCCGAGGA TGTGGCTGTCTACTATTGTCAGCAGAGCTATAGCTTCCCCTGGA CATTCGGCGGAGGCACCAAGGTTGAGATCAAG
260	Table 13	ggtaccCCCGGgCCCATGGCTCTTCCTGTGACAGCTCTTCTGCTGC CCCTGGCCCTGCTTCTGCATGCTGCTAGACCTCAGGTTCAAGT CAACAATGGGGAGCTGGCCTGTAAAGCCCAGCGAAACCCTGT CCCTCACCTGCGCTGTGTATGGCGGAAGCTTCAGAAATACTA CTGGAGCTGGATCCGGCAGCCTCCCGGCAAAGGCTTAGAATGG ATCGGAGAGATAGACACCAGCGGCTTCACCAACTACAACCCCA GCCTGAAATCTAGGGTTACAATCTCTGTGGACACAAGCAAGAA TCAGTTCTCCCTGAAGCTGAGCAGCGTTACTGCCGCCGACACA

		GCTGTGTACTATTGCGCCAGAGTTGGCAGATACAGCTACGGCT ACTACATCACCGCCTTCGACATTTGGGGCCAAGGCACCACTGT GACCGTTTCCAGCGGAAGCACTAGCGGCAGCGGAAACCTGGT TCTGGAGAGGGGCTCAACCAAGGGCGACATCGTGATGACACAG AGCCCCGACTCTCTGGCTGTGTCCCTGGGAGAGAGAGCCACCA TCAACTGCAAGAGCAGCCAGAGCGTTCTGTACAGCAGCAACAA CAAGAACTACCTGGCATGGTATCAACAGAAGCCTGGCCAGCCC CCTAAGCTGCTCATCTACTGGGCTTCCACCAGAGAATCAGGCG TTCCAGACAGGTTCTCCGGCTCGGGTTCAGGCACAGACTTCAC CCTTACCATCTCTTCCCTGCAGGCCGAAGATGTGGCCGTTTACT ACTGTCAGCAGCACTACAGCTTCCCTTTCACATTCGGCGGAGG CACCAAGGTTGAGATCAAG
--	--	--

Example 19

Table 67: Exemplary hinge sequences

SEQ ID NO	Hinge	Sequence
261	C8K	GCAGCTGCTTTCGTGCCTGTGTTCTGCCTGCTAAGCCCACCAC CACTCCTGCTCCAAGACCTCCTACCCCCGCTCCTACAATCGCCA GCCAACCTCTGAGCCTGAGACCGGAGGCATGCAGACCTGCGGC AGGGGGAGCAGTTCACACAAGAGGCTTGGACTTCGCTTGCGAC ATCTACATCTGGGCCCCCTCTGGCCGGCACATGCGGAGTTCTTCT TCTTAGCCTGGTGATCACCTGTACTGCAACCACAGAAAC
262	C28T	GCTGCTGCATTGGATAATGAAAAATCGAACGGCACAATCATTC ATGTGAAGGGCAAACACCTGTGTCCCAGCCCCCTTGTTCCCAGG ACCTAGCAAGCCTTTTTGGGTTCTCGTGGTGGTGGGCGGCGTTC TGGCTTGCTACTCTCTACTTGTAAGTGTGCGATTTATTATATTCT GGGTT
263	C28T1 x	GGAGGAGGAGGATCTCTGGATAACGAGAAAAGCAACGGGACC ATCATTCATGTGAAGGGAAAACATCTGTGTCCCAGCCCCCTTGTT CCCCGGACCTAGCAAGCCGTTTTGGGTTCTCGTGGTGGTGGGC

		GGCGTTCTGGCTTGCTACTCTCTGCTTGTGACCGTTGCCTTCAT TATCTTCTGGGT
264	C28T2 x	GGAGGAGGAGGATCTGGTGGAGGAGGTTCTCTGGACAATGAG AAATCAAATGGAACGATCATCCATGTGAAGGGGAAGCACCTCT GCCCCCTCTCCCCTGTTTCCTGGTCCTAGCAAGCCCTTCTGGGT TTGGTGGTCGTGGGCGGCCTTCTGGCTTGCTACAGCCTGTAGT GACCGTTGCATTTATCATATTTTGGGT
265	C28T2 x_NC	GGAGGAGGAGGATCTGGTGGAGGAGGTTCTCTGGACAATGAG AAATCGAATGGGACAATCATCCATGTGAAGGGGAAGCACCTG AGCCCCCTCTCCCCTGTTTCCTGGTCCTAGCAAGCCCTTCTGGGT TTTGGTGGTCGTGGGCGGCCTTCTGGCCGTTTACAGCCTGTAG TGACCGTTGCTTTTATCATATTTTGGGT
266	C28T2 x_NG	GGAGGAGGAGGATCTGGTGGAGGAGGTTCTCTGGACAATGAA AAGAGCAATGGCACAATCATCCATGTGAAGGGGAAGCACCTG AACGGCTCCGCCCTGTTTCCTGGTCCTAGCAAGCCATTTTGGGT TCTCGTGGTGGTGGGCGGCCTTCTGGCCGTTTACAGCCTGTAG TGACCGTTGCGTTCATAATCTTCTGGGT
267	C28T3 x	GGAGGAGGAGGATCTGGTGGAGGAGGTTCTGGAGGAGGCGGC TCTCTCGACAACGAAAAGAGCAATGGCACCATTATTCACGTTA AAGGCAAGCATCTGTGCCCCCTCCCCCTGTTCCCCGGACCTTCA AAACCTTTTTGGGTCTCGTGGTGGTGGGCGGCCTTCTGGCCTG CTATTCTTTGCTGGTAACTGTAGCCTTCATTATCTTCTGGGT
268	I4	GAGAGCAAGTACGGACCTCCTTGTCTCCATGTCCTGCTCCCG AGTTCGAGGGCGGACCTTCAGTGTTCTGTTCCCCCTAAACCC AAGGATACTCTTATGATCAGCCGACCCCCGAGGTCACCTGTG TGGTGGTAGATGTTAGCCAGGAGGATCCCGAGGTGCAGTTCAA CTGGTACGTCGACGGCGTCGAGGTACACAACGCCAAGACCAA GCCTAGGGAGGAGCAGTTCCAGTCCACCTATAGGGTCGTGAGC GTGCTTACCGTGCTGCACCAGGACTGGTTGAACGGCAAGGAGT ACAAGTGCAAGGTGTCCAACAAGGGCCTCCCCAGCAGCATCGA GAAGACCATTAGCAAGGCAAAGGGACAGCCCAGGGAGCCCCA GGTGTACACATTACCTCCTTCCCAGGAAGAGATGACCAAGAAC

		CAGGTGTCGCTTACCTGCCTGGTCAAGGGCTTCTACCCCTCCGA CATTGCAGTTGAATGGGAGTCAAACGGCCAGCCGGAGAACAA TTACAAGACCACCCCCCAGTCTTGGACAGCGACGGCTCTTTCT TCCTCTACTCGCGGCTTACTGTAGATAAAAGTCGTTGGCAGGA GGGAAACGTGTTTCAGCTGCTCTGTGATGCACGAGGCCCTCCAT AACCCTACACCCAGAAGAGCCTCTCCCTGTCTCTGGGCAAGA TGTCTGGGTGCTGGTTCGTGGTGGGCGGAGTTCTTGCTTGCTAC TCCCTGCTCGTGACCGTCGCTTTCATTATATTCTGGGTC
269	II-2	GAGAGAAAGTGTTGTGTTGAGTGTCTCCTTGTCTCCTCCCTGCCC TGCTCCCGAGTTACTTGGCGGACCTTCAGTGTTCTGTTCCTCC CCAAGCCCAAGGATACTCTCATGATCAGCCGGACCCCGAGGT CACCTGTGTGGTGGTAGATGTTAGCCACGAGGACCTGAGGTC AAGTTCAACTGGTACGTCGACGGCGTCGAGGTGCACAACGCCA AGACCAAGCCTCGTGAAGAACAGTACCAGTCCACCTACAGAGT TGTGAGCGTGCTTACCGTGCTGCACCAGGACTGGCTGAACGGC AAGGAGTACAAGTGCAAGGTGTCCAACAAGGCCCTCCCCGCTC CCATCGAGAAGACAATCAGCAAGGCCAAGCCCTGTCCAGCCCC TGAGCTCTTAGGAGGACCTAGCGTTTTCTTTTCCCTCCCAAGC CTAAGGACACTCTTATGATCTCCAGAACACCAGAGGTTACCTG CGTCGTGGTGGACGTGTCCCATGAGGACCCAGAAGTCAAATTC AATTGGTATGTAGATGGGGTCGAGGTCCACAACGCTAAGACAA AGCCCCGCGAGGAGCAGTACAACCTCTACCTACAGGGTCGTGTC CGTGCTCACAGTGCTGCATCAGGATTGGCTCAACGGGAAGGAG TATAAGTGCAAAGTGTCCAATAAGGCCCTTCCCGCCCCCTATCG AGAAAACCATCTCTAAGGCCAAATTCTGGGTGCTGGTGGTTGT GGGCGGCGTGCTTGCTTGTTACTCCCTGCTGGTCACTGTAGCTT TCATCATATTTTGGGTG

Example 20

Table 68: Exemplary nucleotide sequences encoding a binding motif, hinge, and 41BB costimulatory domain

SEQ ID NO	Sequence
271	ggtaccCCCGGgCCCATGGCTCTTCCTGTGACAGCTCTTCTGCTGCCCCCTGGCCC TGCTTCTGCATGCTGCTAGACCTCAGCTTCAGCTCCAAGAGAGCGGACCTGG CTTAGTGAAGCCCAGCGAAACCCTGTCCCTCACCTGCACCGTTTCTGGCGGA AGCATCAGCAGCTCCAGCTATTACTGGGGATGGATCAGGCAGCCCCCTGGCA AGGGTTTGAATGGATCGGCTCGATATATTACTCCGGCAGCACCTACTATAA CCCCAGCTTGAAGAGCCGGGTACCATTCTGTGGACACATCAAAGAACCAG TTCAGCCTGAAGCTGAGCTCTGTGACTGCCGCCGACACAGCTGTGTACTACT GTGCCAGAGAGACAGACTACTCCAGCGGCATGGGCTACGGCATGGATGTGT GGGGACAAGGAACCAACCGTTACTGTGAGCAGCGTTCCACCAGCGGCTCAG GCAAGCCTGGCTCAGGAGAAGGAAGCACCAGGGGGATATACAGATGACAC AGAGCCCCCTCAGCCTGTCCGCCAGCGTTGGCGATCGTGTAACGATCACCTG CCGGGCCTCTCAGAGCATCAACTCCTACCTCAATTGGTATCAACAGAAGCCA GGCAAGGCCCCCAAATTACTCATCTACGCCGCCAGCAGCTTACAGAGCGGG GTTCCCTCTAGATTCTCCGGCTCCGGTTCTGGAACAGATTTCACCCTCACTAT CTCCAGCTTGACAGCCGAGGATTTGCCACTTATTACTGTCAGCAGAGCCTG GCCGACCCCTTACATTCGGCGGAGGCACAAAGGTTGAGATCAAGGCAGCT GCTTTCGTGCCTGTGTTCCCTGCCTGCTAAGCCCACCACCACTCCTGCTCCAAG ACCTCCTACCCCCGCTCCTACAATCGCCAGCCAACCTCTGAGCCTGAGACCG GAGGCATGCAGACCTGCGGCAGGGGGAGCAGTTCACACAAGAGGCTTGGAC TTCGCTTGCGACATCTACATCTGGGCCCTCTGGCCGGCACATGCGGAGTTCT TCTTCTTAGCCTGGTGATCACCTGTACTGCAACCACAGAAACAGATTACAGC GTTGTGAAGAGAGGCCGGAAGAAGCTGCTGTACATCTTCAAGCAGCCCTTCA TGAGACCTGTGCAGACCACACAGGAGGAAGACGGCTGCAGCTGTAGATTCC CCGAGGAAGAGGAGGGCGGCTGTGAGCTGAGAGTTAAGTTCAGCAGGAGCG CCGACGCCCCCTGCCTACCAGCAAGGACAGAATCAACTGTACAACGAGCTGA ACCTGGGCAGACGGGAGGAATACGATGTGCTGGACAAGAGGAGAGGCAGA GACCCCGAGATGGGCGGCAAACCTAGAAGAAAGAACCCCAAGGAGGGCCTG TATAACGAGCTCCAGAAGGACAAGATGGCCGAGGCCTACAGCGAGATCGGC ATGAAGGGCGAAAGAAGAAGAGGCAAGGGCCACGACGGCCTTACCAGGG CTTAAGCACAGCTACAAAGGACACCTACGACGCCCTGCACATGCAGGCCCT GCCCCCTAGATGATTAATTAAatcgat
272	ggtaccCCCGGgCCCATGGCTCTTCCTGTGACAGCTCTTCTGCTGCCCCCTGGCCC TGCTTCTGCATGCTGCTAGACCTCAGGTTTCAGTTACAAGAGAGCGGACCTGG CTTAGTGAAGCCCAGCGAAACCCTGTCCCTCACCTGCACCGTTTCTGGCGGA AGCATCAGCTCTCCCGACCACTACTGGGGATGGATCAGGCAGCCCCCTGGCA AGGGTTTGAATGGATCGGCAGCATCTACGCCAGCGGCAGCACATTCTACAA CCCCTCGCTCAAAAGCAGGGTTACTATTTCTGTGGACACAAGCAAAAATCAG

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273	ggtaccCCCGGgCCCATGGCTCTTCTGTGACAGCTCTTCTGCTGCCCCCTGGCCC TGCTTCTGCATGCTGCTAGACCTCAGATCACATTAAGAGAGCGGACCTAC ACTGGTGAAGCCCACCCAAACGCTTACCCTCACCTGCACCTTTAGCGGGTTC AGCCTGGACACAGAGGGCGTTGGCGTTGGATGGATCAGGCAGCCTCCTGGC AAAGCCCTCGAATGGCTTGCCCTCATCTACTTCAACGACCAGAAGAGATACA GCCCCCTCCTAAAAATCTCGGCTCACAATCACCAAAGACACAAGCAAAAATCA GGTTGTGCTCACCATGACCAACATGGACCCTGTGGACACCGCTGTGTACTAC TGTGCCAGAGACACCGGCTACAGCAGATGGTACTACGGGATGGACGTTTGG GGCCAAGGCACCACTGTGACCGTTTCCAGCGGCTCTACAAGCGGCAGCGGG AAACCTGGTTCTGGAGAGGGCAGCACAAAGGGCGACATCCAGATGACGCAA TCCCCCAGCTCTGTGAGCGCCTCTGTGGGAGACAGAGTTACAATCACATGCC GGGCCTCCAGGGCATCAGCTCTTGGCTGGCATGGTATCAACAGAAGCCTGG CAAGGCTCCCAAGCTGCTCATCTATGCCGCCTCCTCCTTACAATCTGGAGTTC CCTCCAGGTTACGCGGGAGCGGCTCAGGAACAGACTTCACCTTACCATCTC

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274	ggtaccCCCGGgCCCATGGCTCTTCTGTGACAGCTCTTCTGCTGCCCCCTGGCCC TGCTTCTGCATGCTGCTAGACCTCAGGTTCAAGTTACAACAATGGGGAGCTGG CCTGTAAAGCCCAGCGAAACCCTGTCCCTCACCTGCGCTGTGTATGGCGGA AGCTTCCAGAAATACTACTGGAGCTGGATCCGGCAGCCTCCCGGCAAAGGCT TAGAATGGATCGGAGAGATAGACACCAGCGGCTTCACCAACTACAACCCCA GCCTGAAATCTAGGGTTACAATCTCTGTGGACACAAGCAAGAATCAGTTCTC CCTGAAGCTGAGCAGCGTTACTGCCGCCGACACAGCTGTGTACTATTGCGCC AGAGTTGGCAGATACAGCTACGGCTACTACATCACCGCCTTCGACATTTGGG GCCAAGGCACCACTGTGACCGTTTCCAGCGGAAGCACTAGCGGCAGCGGGA AACCTGGTTCTGGAGAGGGCTCAACCAAGGGCGACATCGTGATGACACAGA GCCCCGACTCTCTGGCTGTGTCCCTGGGAGAGAGAGCCACCATCAACTGCAA GAGCAGCCAGAGCGTTCTGTACAGCAGCAACAACAAGAACTACCTGGCATG GTATCAACAGAAGCCTGGCCAGCCCCCTAAGCTGCTCATCTACTGGGCTTCC ACCAGAGAATCAGGCGTTCCAGACAGGTTCTCCGGCTCGGGTTCAGGCACAG ACTTCACCCTTACCATCTCTCCCTGCAGGCCGAAGATGTGGCCGTTTACTAC TGTCAGCAGCACTACAGCTTCCCTTTCACATTCGGCGGAGGCACCAAGGTTG AGATCAAGGCAGCTGCTTTCGTGCCTGTGTTCTGCTGCTAAGCCCACCAC CACTCCTGCTCCAAGACCTCCTACCCCGCTCCTACAATCGCCAGCCAACCT CTGAGCCTGAGACCGGAGGCATGCAGACCTGCGGCAGGGGGAGCAGTTCAC ACAAGAGGCTTGGACTTCGCTTGCACATCTACATCTGGGCCCCCTCTGGCCG GCACATGCGGAGTTCTTCTTCTTAGCCTGGTGATCACCTGTACTGCAACCAC AGAAACAGATTACAGCGTTGTGAAGAGAGGCCGGAAGAAGCTGCTGTACATC

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275	ggtaccCCCGGgCCCATGGCTCTTCTGTGACAGCTCTTCTGCTGCCCTGGCCC TGCTTCTGCATGCTGCTAGACCTCAGGTTCAAGTTGCAGCAATGGGGAGCTGG CCTGTTAAAGCCAGCGAAACCCTGTCCCTCACCTGCGCTGTGTATGGCGGA AGCTTCGAGAAATACTACTGGAGCTGGATCCGGCAGCCTCCCGGCAAAGGCT TAGAATGGATCGGCGAGATTTATCACAGCGGGCTCACCAACTACAACCCAG CCTGAAATCTCGAGTTACAATCTCTGTGGACACAAGCAAGAATCAGTTCTCC CTGAAGCTGAGCAGCGTTACTGCCGCCGACACAGCTGTGTACTATTGCGCCA GAGTTAGATACGACAGCAGCGACAGCTATTACTACAGCTATGACTACGGCAT GGATGTGTGGGGCAGGGCACCACCGTTACTGTCTCCTCTGGATCTACCAGC GGCAGCGGCAAGCCTGGATCTGGCGAAGGAAGCACAAAGGGCGACATTGTG CTCACCCAGAGCCCCGACAGCCTGGCTGTGTCTTTAGGCGAAAGGGCTACCA TCAACTGCAAGAGCAGCCAGAGCGTTCTGTACAGCAGCAACAACAAGAACT ACCTTGCTTGGTATCAACAGAAGCCTGGCCAGCCCCCTAAGCTGCTCATCTA CTGGGCCTCTAGCAGAGAGAGCGGGGTTCGGATCGGTTTAGCGGCTCCGGC TCAGGAACCGATTTACCCCTCACTATCTCCAGCCTCCAGGCCGAGGATGTGG CTGTCTACTATTGTGACAGAGCTATAGCTTCCCCTGGACATTCGGCGGAGG CACCAAGGTTGAGATCAAGGCAGCTGCTTTCGTGCCTGTGTTCTGCCTGCT AAGCCCACCACCACTCCTGCTCCAAGACCTCCTACCCCGCTCCTACAATCG CCAGCCAACCTCTGAGCCTGAGACCGGAGGCATGCAGACCTGCGGCAGGGG GAGCAGTTCACACAAGAGGCTTGGACTTCGCTTGCGACATCTACATCTGGGC CCCTCTGGCCGGCACATGCGGAGTTCTTCTTCTTAGCCTGGTGATCACCTGT ACTGCAACCACAGAAACAGATTCAGCGTTGTGAAGAGAGGCCGGAAGAAGC TGCTGTACATCTTCAAGCAGCCCTTCATGAGACCTGTGCAGACCACACAGGA GGAAGACGGCTGCAGCTGTAGATTCCCCGAGGAAGAGGAGGGCGGCTGTGA GCTGAGAGTTAAGTTCAGCAGGAGCGCCGACGCCCTGCCTACCAGCAAGG ACAGAATCAACTGTACAACGAGCTGAACCTGGGCAGACGGGAGGAATACGA TGTGCTGGACAAGAGGAGAGGCAGAGACCCCGAGATGGGCGGCAAACCTAG AAGAAAGAACCCCAAGAGGGCCTGTATAACGAGCTCCAGAAGGACAAGAT GGCCGAGGCCTACAGCGAGATCGGCATGAAGGGCGAAAGAAGAAGAGGCA

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277	ggtaccCCCGGgCCCATGGCTCTTCCTGTGACAGCTCTTCTGCTGCCCCCTGGCCC TGCTTCTGCATGCTGCTAGACCTCAGGTTCAAGTTACAACAATGGGGAGCTGG CCTGTTAAAGCCCAGCGAAACCCTGTCCCTCACCTGCGCTGTGTATGGCGGA AGCTTCAGCGGCTACGCTTGGAGCTGGATTAGACAGCCTCCTGGCAAAGGAC TAGAATGGATCGGAGAGATCGACCACAGAGGCTTCACCAACTACAACCCCA GCCTGAAATCCAGAGTTACAATCTCTGTGGACACAAGCAAGAATCAGTTCTC

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278	ggtaccCCCGGgCCCATGGCTCTTCTGTGACAGCTCTTCTGCTGCCCCCTGGCCC TGCTTCTGCATGCTGCTAGACCTCAGGTTCAAGTTGCAGCAATGGGGAGCTGG CCTGTTAAAGCCAGCGAAACCTGTCCCTCACCTGCGCTGTGTATGGCGGA AGCTTCAGCGGCTATTACTGGAGCTGGATCCGGCAGCCTCCTGGAAGAGGAT TAGAATGGATCGGCGAGATAGACCACAGCGGGAGCACAACTACAACCCCA GCCTGAAATCGCGGGTTACAATCTCTGTGGACACAAGCAAGAATCAGTTCTC CCTGAAGCTGAGCAGCGTTACTGCCGCCGACACAGCTGTGTACTATTGCGCC AGAGGCGGAGGCTCCTGGTACAGCAACTGGTTCGATCCTTGGGGCCAAGGC ACCATGGTGACCGTTTCCAGCGGCTCTACAAGCGGCAGCGGGAACCTGGTT CTGGAGAGGGCAGCACAAAGGGCGACATCCAGATGACACAGAGCCCCAGCA CCCTTAGCGCCTCTGTGGGAGATAGGGTTACCATTACCTGCAGGGCTTCCCA GAGCATCAGCAGCTGGCTGGCATGGTATCAACAGAAGCCTGGCAAGGCTCC CAAGCTGCTCATCTATGACGCCTCCAGCCTGGAAAGCGGGGTTCCCTCCAGA TTTAGCGGCTCAGGCTCCGGAACAGAGTTCACCCTTACCATCTCTAGCCTGC

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279	ggtaccCCCGGgCCCATGGCTCTTCTGTGACAGCTCTTCTGCTGCCCCCTGGCCC TGCTTCTGCATGCTGCTAGACCTCAGGTTACAGCTTGTGCAGAGCGGAGCTGA AGTTAAGAAGCCTGGCGCCTCTGTGAAGGTTAGCTGCAAGGCCAGCGGCTAC ACATTCAAGGAATATGGCATCTCCTGGGTTAGGCAGGCTCCCGGCCAAGGCT TAGAATGGATGGGCTGGATCTCCGCCTACTCCGCCACACCTACTACGCCCA GAAGCTTCAGGGCAGGGTTACCATGACCACCGACACCAGCACCTCTACCGCC TATATGGAGCTGAGGAGCCTGAGATCGGACGACACAGCTGTGTATTACTGCG CCAGAGGCCCCCACTACGACGACTGGTCTGGATTTATCATCTGGTTCGACCC CTGGGGGCAGGGCACCTGGTCACAGTTTCTTCTGGCTCCACCAGCGGAAGC GGCAAGCCAGGCTCAGGCGAAGGATCTACAAAAGGCGACATCCAAATGACA CAGAGCCCCAGCAGCTTGAGCGCCTCCGTTGGCGACAGAGTTACAATCACCT GCAGGGCCTCTCAGAGCATCAGCAGCTATTTGAATTGGTATCAACAGAAGCC AGGAAAGGCCCTAAGCTGCTCATCTACGCTGCCAGCTCGCTCCAATCTGGC GTTCTTAGCAGATTTAGCGGCTCCGGCAGCGGCACAGACTTTACTCTTACCA TTAGCTCCCTGCAGCCCCGAGGACTTCGCTACCTACTATTGCCAGCAAAGCTA CAGATTCCCTCCCACCTTTGGCCAGGGCACAAAGGTTGAGATCAAGGCAGCT GCTTTCGTGCCTGTGTTCTGCCTGCTAAGCCCACCACCTCCTGCTCCAAG ACCTCCTACCCCCGCTCCTACAATCGCCAGCCAACCTCTGAGCCTGAGACCG GAGGCATGCAGACCTGCGGCAGGGGGAGCAGTTCACACAAGAGGCTTGGAC TTCGCTTGGGACATCTACATCTGGGCCCCCTCTGGCCGGCACATGCGGAGTTCT TCTTCTTAGCCTGGTGATCACCTGTACTGCAACCACAGAAACAGATTACAGC GTTGTGAAGAGAGGCCGGAAGAAGCTGCTGTACATCTTCAAGCAGCCCTTCA

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281	ggtaccCCCGGgCCCATGGCTCTTCCTGTGACAGCTCTTCTGCTGCCCCCTGGCCC TGCTTCTGCATGCTGCTAGACCTCAGCTTCAGCTCCAAGAGAGCGGACCTGG CTTAGTGAAGCCCAGCGAAACCCTGTCCCTCACCTGCACCGTTTCTGGCGGA AGCATCAGCAGCTCCAGCTATTACTGGGGATGGATCAGGCAGCCCCCTGGCA AGGGTTTAGAATGGATCGGCTCGATATATTACTCCGGCAGCACCTACTATAA CCCCAGCTTGAAGAGCCGGTTACCATTCTGTGGACACATCAAAGAACCAG TTCAGCCTGAAGCTGAGCTCTGTGACTGCCGCCGACACAGCTGTGTACTACT GTGCCAGAGAGACAGACTACTCCAGCGGCATGGGCTACGGCATGGATGTGT GGGGACAAGGAACCAACCGTTACTGTGAGCAGCGGTTCCACCAGCGGCTCAG GCAAGCCTGGCTCAGGAGAAGGAAGCACCAGGGGGATATACAGATGACAC AGAGCCCCCTCAGCCTGTCCGCCAGCGTTGGCGATCGTGTAACGATCACCTG CCGGGCCTCTCAGAGCATCAACTCCTACCTCAATTGGTATCAACAGAAGCCA GGCAAGGCCCCCAAATTACTCATCTACGCCGCCAGCAGCTTACAGAGCGGG GTTCCCTCTAGATTCTCCGGCTCCGGTTCTGGAACAGATTTCACCCTCACTAT CTCCAGCTTGACAGCCCGAGGATTTCCGCACTTATTACTGTCAGCAGAGCCTG GCCGACCCCTTCACATTCGGCGGAGGCACAAAGGTTGAGATCAAGGCTGCTG CATTGGATAATGAAAAATCGAACGGCACAATCATTGATGTGAAGGGCAAAAC ACCTGTGTCCAGCCCCCTTGTTCAGGACCTAGCAAGCCTTTTGGGTTCTC GTGGTGGTGGGCGGCGTTCTGGCTTGCTACTCTCTACTTGTAAGTGTGCGATT TATTATATTCTGGGTTAGATTAGCGTTGTGAAGAGAGGCCGGAAGAAGCTG CTGTACATCTTCAAGCAGCCCTTCATGAGACCTGTGCAGACCACACAGGAGG AAGACGGCTGCAGCTGTAGATTCCCCGAGGAAGAGGAGGGCGGCTGTGAGC TGAGAGTTAAGTTCAGCAGGAGCGCCGACGCCCTGCCTACCAGCAAGGAC AGAATCAACTGTACAACGAGCTGAACCTGGGCAGACGGGAGGAATACGATG TGCTGGACAAGAGGAGAGGCAGAGACCCCGAGATGGGCGGCAAAACCTAGA AGAAAGAACCCCCAGGAGGGCCTGTATAACGAGCTCCAGAAGGACAAGATG GCCGAGGCCTACAGCGAGATCGGCATGAAGGGCGAAAGAAGAAGAGGCAA GGGCCACGACGGCCTCTACCAGGGCTTAAGCACAGCTACAAAGGACACCTA CGACGCCCTGCACATGCAGGCCCTGCCCCCTAGATGATTAATTAAatcgat
282	ggtaccCCCGGgCCCATGGCTCTTCCTGTGACAGCTCTTCTGCTGCCCCCTGGCCC TGCTTCTGCATGCTGCTAGACCTCAGGTTTCAGTTACAAGAGAGCGGACCTGG CTTAGTGAAGCCCAGCGAAACCCTGTCCCTCACCTGCACCGTTTCTGGCGGA AGCATCAGCTCTCCCGACCACTACTGGGGATGGATCAGGCAGCCCCCTGGCA AGGGTTTGGAATGGATCGGCAGCATCTACGCCAGCGGCAGCACATTCTACAA CCCCCTCGCTCAAAGCAGGGTTACTATTTCTGTGGACACAAGCAAAAATCAG TTCAGCCTGAAGCTGAGCTCTGTGACTGCCGCCGACACAGCTGTGTACTACT GTGCCAGAGAGACAGACTACTCCAGCGGGATGGGCTACGGCATGGATGTGT

	GGGGACAAGGAACCAACCGTTACTGTGAGCAGCGGCTCCACAAGCGGCTCAG GCAAGCCTGGCTCAGGAGAAGGAAGCACCAAGGGGGACATTCAAATGACCC AAAGCCCCCTCCAGCCTGTCCGCCAGCGTTGGCGATAGGGTTACCATTACCTG CAGGGCCAGCCAAAGCATCAACTCCTACCTAAATTGGTATCAACAGAAGCC AGGCAAGGCCCCCAAATACTACTCATTTACGCCGCCAGCAGCTTACAGAGCGG GGTTCCCTCTAGATTCTCCGGCAGCGGTTCTGGAACAGATTTCCTCTCACAA TATCTTCGCTGCAGCCCGAGGATTTTCGCTACCTACTATTGCCAGCAATCCCTG GCCGACCCCTTCACATTCGGCGGAGGCACAAAGGTTGAGATCAAGGCTGCTG CATTGGATAATGAAAAATCGAACGGCACAATCATTATGTGAAGGGCAAAC ACCTGTGTCCAGCCCCCTTGTTCAGGACCTAGCAAGCCTTTTGGGTTCTC GTGGTGGTGGGCGGCGTTCTGGCTTGCTACTCTCTACTTGTAACTGTGCGATT TATTATATTCTGGGTTAGATTGAGCGTTGTGAAGAGAGGCCGGAAGAAGCTG CTGTACATCTTCAAGCAGCCCTTCATGAGACCTGTGCAGACCACACAGGAGG AAGACGGCTGCAGCTGTAGATTCCCCGAGGAAGAGGAGGGCGGCTGTGAGC TGAGAGTTAAGTTCAGCAGGAGCGCCGACGCCCTGCCTACCAGCAAGGAC AGAATCAACTGTACAACGAGCTGAACCTGGGCAGACGGGAGGAATACGATG TGCTGGACAAGAGGAGAGGCAGAGACCCCGAGATGGGCGGCAAACCTAGA AGAAAGAACCCCCAGGAGGGCCTGTATAACGAGCTCCAGAAGGACAAGATG GCCGAGGCCTACAGCGAGATCGGCATGAAGGGCGAAAGAAGAGAGGCAA GGGCCACGACGGCCTCTACCAGGGCTTAAGCACAGCTACAAAGGACACCTA CGACGCCCTGCACATGCAGGCCCTGCCCCCTAGATGATTAATTAAatcgat
283	ggtaccCCCGGgCCCATGGCTCTTCTGTGACAGCTCTTCTGCTGCCCTGGCCC TGCTTCTGCATGCTGCTAGACCTCAGATCACATTAAGAGAGCGGACCTAC ACTGGTGAAGCCACCCAAACGCTTACCCTCACCTTTCAGCGGGTTC AGCCTGGACACAGAGGGCGTTGGCGTTGGATGGATCAGGCAGCCTCCTGGC AAAGCCCTCGAATGGCTTGCCCTCATCTACTTCAACGACCAGAAGAGATACA GCCCCCTCCTTAAATCTCGGCTCACAATCACCAAAGACACAAGCAAAAATCA GGTTGTGCTCACCATGACCAACATGGACCCTGTGGACACCGCTGTGTACTAC TGTGCCAGAGACACCGGCTACAGCAGATGGTACTACGGGATGGACGTTTGG GGCCAAGGCACCACTGTGACCGTTTCCAGCGGCTCTACAAGCGGCAGCGGG AAACCTGGTTCTGGAGAGGGCAGCACAAAGGGCGACATCCAGATGACGCAA TCCCCCAGCTCTGTGAGCGCCTCTGTGGGAGACAGAGTTACAATCACATGCC GGGCCTCCCAGGGCATCAGCTCTTGGCTGGCATGGTATCAACAGAAGCCTGG CAAGGCTCCCAAGCTGCTCATCTATGCCGCCTCCTCCTTACAATCTGGAGTTC CCTCCAGGTTTCAGCGGGAGCGGCTCAGGAACAGACTTCACCTTACCATCTC TAGCCTGCAACCCGAGGACTTCGCTACTTATTACTGTCAGCAGGCCTACGCC TACCCCATCACATTTCGGCGGAGGAACAAAGGTTGAGATCAAGGCTGCTGCAT TGGATAATGAAAAATCGAACGGCACAATCATTATGTGAAGGGCAAACACC TGTGTCCAGCCCCTTGTTCAGGACCTAGCAAGCCTTTTGGGTTCTCGTG

	GTGGTGGGCGGCGTTCTGGCTTGCTACTCTCTACTTGTAAGTGTGCGATTTAT TATATTCTGGGTTAGATTACGCGTTGTGAAGAGAGGCCGGAAGAAGCTGCTG TACATCTTCAAGCAGCCCTTCATGAGACCTGTGCAGACCACACAGGAGGAAG ACGGCTGCAGCTGTAGATTCCCCGAGGAAGAGGAGGGCGGCTGTGAGCTGA GAGTTAAGTTCAGCAGGAGCGCCGACGCCCTGCCTACCAGCAAGGACAGA ATCAACTGTACAACGAGCTGAACCTGGGCAGACGGGAGGAATACGATGTGC TGGACAAGAGGAGAGGCAGAGACCCCGAGATGGGCGGCAAACCTAGAAGA AAGAACCCCAAGGAGGGCCTGTATAACGAGCTCCAGAAGGACAAGATGGCC GAGGCCTACAGCGAGATCGGCATGAAGGGCGAAAGAAGAAGAGGCAAGGG CCACGACGGCCTCTACCAGGGCTTAAGCACAGCTACAAAGGACACCTACGA CGCCCTGCACATGCAGGCCCTGCCCCCTAGATGATTAATTAAatcat
284	ggtaccCCCCGgCCCATGGCTCTTCTGTGACAGCTCTTCTGCTGCCCCCTGGCCC TGCTTCTGCATGCTGCTAGACCTCAGGTTCAAGTTACAACAATGGGGAGCTGG CCTGTAAAGCCCAGCGAAACCTGTCCCTCACCTGCGCTGTGTATGGCGGA AGCTTCCAGAAATACTACTGGAGCTGGATCCGGCAGCCTCCCGCAAAGGCT TAGAATGGATCGGAGAGATAGACACCAGCGGCTTCACCAACTACAACCCCA GCCTGAAATCTAGGGTTACAATCTCTGTGGACACAAGCAAGAATCAGTTCTC CCTGAAGCTGAGCAGCGTTACTGCCGCCGACACAGCTGTGTACTATTGCGCC AGAGTTGGCAGATACAGCTACGGCTACTACATCACCGCCTTCGACATTTGGG GCCAAGGCACCACTGTGACCGTTTCCAGCGGAAGCACTAGCGGCAGCGGGA AACCTGGTTCTGGAGAGGGCTCAACCAAGGGCGACATCGTGATGACACAGA GCCCCGACTCTCTGGCTGTGTCCCTGGGAGAGAGAGCCACCATCAACTGCAA GAGCAGCCAGAGCGTTCTGTACAGCAGCAACAACAAGAACTACCTGGCATG GTATCAACAGAAGCCTGGCCAGCCCCCTAAGCTGCTCATCTACTGGGCTTCC ACCAGAGAATCAGGCGTTCAGACAGGTTCTCCGGCTCGGGTTCAGGCACAG ACTTCACCCCTTACCATCTCTCCCTGCAGGCCGAAGATGTGGCCGTTTACTAC TGTCAGCAGCACTACAGCTTCCCTTTCACATTCGGCGGAGGCACCAAGGTTG AGATCAAGGCTGCTGCATTGGATAATGAAAAATCGAACGGCACAATCATTC ATGTGAAGGGCAAACACCTGTGTCCAGCCCCCTGTTCACAGGACCTAGCAA GCCTTTTGGGTTCTCGTGGTGGTGGGCGGCGTTCTGGCTTGCTACTCTCTAC TTGTAAGTGTGCGATTTATTATATTCTGGGTTAGATTACGCGTTGTGAAGAGA GGCCGGAAGAAGCTGCTGTACATCTTCAAGCAGCCCTTCATGAGACCTGTGC AGACCACACAGGAGGAAGACGGCTGCAGCTGTAGATTCCCCGAGGAAGAGG AGGGCGGCTGTGAGCTGAGAGTTAAGTTCAGCAGGAGCGCCGACGCCCTG CCTACCAGCAAGGACAGAATCAACTGTACAACGAGCTGAACCTGGGCAGAC GGGAGGAATACGATGTGCTGGACAAGAGGAGAGGCAGAGACCCCGAGATG GGCGGCAAACCTAGAAGAAAGAACCCCAAGGAGGGCCTGTATAACGAGCTC CAGAAGGACAAGATGGCCGAGGCCTACAGCGAGATCGGCATGAAGGGCGA AAGAAGAAGAGGCAAGGGCCACGACGGCCTCTACCAGGGCTTAAGCACAGC

	TACAAAGGACACCTACGACGCCCTGCACATGCAGGCCCTGCCCCCTAGATGATTAATTAAatcgat
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287	ggtaccCCCGGgCCCATGGCTCTTCTGTGACAGCTCTTCTGCTGCCCCCTGGCCC TGCTTCTGCATGCTGCTAGACCTCAGGTTACAGTTACAACAATGGGGAGCTGG CCTGTTAAAGCCCAGCGAAACCCTGTCCCTCACCTGCGCTGTGTATGGCGGA AGCTTCAGCGGCTACGCTTGGAGCTGGATTAGACAGCCTCCTGGCAAAGGAC TAGAATGGATCGGAGAGATCGACCACAGAGGCTTCACCAACTACAACCCCA GCCTGAAATCCAGAGTTACAATCTCTGTGGACACAAGCAAGAATCAGTTCTC CCTGAAGCTGAGCAGCGTTACTGCCGCCGACACAGCTGTGTACTATTGCGCC AGGGTTAGATACGACAGCAGCGACAGCTATTACTACAGCTATGACTACGGC ATGGATGTGTGGGGGCAGGGCACCACCGTTACGGTTAGCTCTGGATCTACCA GCGGCAGCGGCAAGCCTGGCTCAGGAGAAGGAAGCACAAAGGGCGACATTG TGCTACCCAGAGCCCCGACAGCCTGGCCGTTTCTTTAGGCGAAAGGGCTAC CATCAACTGCAAGAGCAGCCAGAGCGTTCTGTACAGCAGCAACAACAAGAA CTACCTTGCATGGTATCAACAGAAGCCAGGCCAGCCTCCCAAGCTGCTCATC TACTGGGCCTCTAGCAGAGAGAGCGGGGTTCGGATAGATTTTCGGGATCAG GCTCCGGCACCGATTCTACTCTTACGATCAGCAGCTTACAGGCCGAGGATGT GGCTGTCTACTATTGTCAGCAGAGCTATAGCTTCCCCTGGACATTCGGCGGA

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288	ggtaccCCCGGgCCCATGGCTCTTCTGTGACAGCTCTTCTGCTGCCCCCTGGCCC TGCTTCTGCATGCTGCTAGACCTCAGGTTCAAGTTGCAGCAATGGGGAGCTGG CCTGTAAAGCCCAGCGAAACCTGTCCCTCACCTGCGCTGTGTATGGCGGA AGCTTCAGCGGCTATTACTGGAGCTGGATCCGGCAGCCTCCTGGAAGAGGAT TAGAATGGATCGGCGAGATAGACCACAGCGGGAGCACAACTACAACCCCA GCCTGAAATCGCGGGTTACAATCTCTGTGGACACAAGCAAGAATCAGTTCTC CCTGAAGCTGAGCAGCGTTACTGCCGCCGACACAGCTGTGTACTATTGCGCC AGAGGCGGAGGCTCCTGGTACAGCAACTGGTTCGATCCTTGGGGCCAAGGC ACCATGGTGACCGTTTCCAGCGGCTCTACAAGCGGCAGCGGGAACCTGGTT CTGGAGAGGGCAGCACAAAGGGCGACATCCAGATGACACAGAGCCCCAGCA CCCTTAGCGCCTCTGTGGGAGATAGGGTTACCATTACCTGCAGGGCTTCCCA GAGCATCAGCAGCTGGCTGGCATGGTATCAACAGAAGCCTGGCAAGGCTCC CAAGCTGCTCATCTATGACGCCTCCAGCCTGGAAAGCGGGGTTCCCTCCAGA TTTAGCGGCTCAGGCTCCGGAACAGAGTTCACCTTACCATCTCTAGCCTGC AACCCGACGACTTCGCTACTTATTACTGTCAACAAGACAGAAGCTTGCCCCC CACATTCGGCGGAGGGACCAAGGTTGAGATCAAGGCTGCTGCATTGGATAA TGAAAAATCGAACGGCACAAATCATTATGTGAAGGGCAAACACCTGTGTCCC AGCCCCCTGTTCAGGACCTAGCAAGCCTTTTGGGTTCTCGTGGTGGTGGG CGGCGTTCTGGCTTGCTACTCTCTACTTGTAACTGTCGCATTTATTATATTCTG GGTTAGATTACAGCTTGTGAAGAGAGGGCCGGAAGAAGCTGCTGTACATCTTC AAGCAGCCCTTCATGAGACCTGTGCAGACCACACAGGAGGAAGACGGCTGC AGCTGTAGATTCCCCGAGGAAGAGGAGGGCGGCTGTGAGCTGAGAGTTAAG TTCAGCAGGAGCGCCGACGCCCTGCCTACCAGCAAGGACAGAATCAACTG TACAACGAGCTGAACCTGGGCAGACGGGAGGAATACGATGTGCTGGACAAG AGGAGAGGCAGAGACCCCCGAGATGGGCGGCAAACCTAGAAGAAAGAACCC

	CCAGGAGGGCCTGTATAACGAGCTCCAGAAGGACAAGATGGCCGAGGCCTA CAGCGAGATCGGCATGAAGGGCGAAAGAAGAAGAGGCAAGGGCCACGACG GCCTCTACCAGGGCTTAAGCACAGCTACAAAGGACACCTACGACGCCCTGC ACATGCAGGCCCTGCCCCCTAGATGATTAATTAAatcgat
289	ggtaccCCCGGgCCCATGGCTCTTCCTGTGACAGCTCTTCTGCTGCCCCCTGGCCC TGCTTCTGCATGCTGCTAGACCTCAGGTTTCAGTTGTGCAGAGCGGAGCTGA AGTTAAGAAGCCTGGCGCCTCTGTGAAGGTTAGCTGCAAGGCCAGCGGCTAC ACATTCAAGGAATATGGCATCTCCTGGGTTAGGCAGGCTCCCCGGCCAAGGCT TAGAATGGATGGGCTGGATCTCCGCCTACTCCGGCCACACCTACTACGCCCA GAAGCTTCAGGGCAGGGTTACCATGACCACCGACACCAGCACCTCTACCGCC TATATGGAGCTGAGGAGCCTGAGATCGGACGACACAGCTGTGTATTACTGCG CCAGAGGCCCCCACTACGACGACTGGTCTGGATTTATCATCTGGTTCGACCC CTGGGGGCAGGGCACCTGGTCACAGTTTCTTCTGGCTCCACCAGCGGAAGC GGCAAGCCAGGCTCAGGCGAAGGATCTACAAAAGGCGACATCCAAATGACA CAGAGCCCCAGCAGCTTGAGCGCCTCCGTTGGCGACAGAGTTACAATCACCT GCAGGGCCTCTCAGAGCATCAGCAGCTATTTGAATTGGTATCAACAGAAGCC AGGAAAGGCCCCTAAGCTGCTCATCTACGCTGCCAGCTCGCTCCAATCTGGC GTTCTTAGCAGATTTAGCGGCTCCGGCAGCGGCACAGACTTTACTCTTACCA TTAGCTCCCTGCAGCCCCGAGGACTTCGCTACCTACTATTGCCAGCAAAGCTA CAGATTCCCTCCACCTTTGGCCAGGGCACAAAGGTTGAGATCAAGGCTGCT GCATTGGATAATGAAAAATCGAACGGCACAATCATTTCATGTGAAGGGCAAA CACCTGTGTCCCAGCCCCCTTGTTCAGGACCTAGCAAGCCTTTTGGGTTCT CGTGGTGGTGGGCGCGTTCTGGCTTGCTACTCTCTACTTGTAACTGTGCGAT TTATTATATTCTGGGTTAGATTCAGCGTTGTGAAGAGAGGCCGGAAGAAGCT GCTGTACATCTTCAAGCAGCCCTTCATGAGACCTGTGCAGACCACACAGGAG GAAGACGGCTGCAGCTGTAGATTCCCCGAGGAAGAGGAGGGCGGCTGTGAG CTGAGAGTTAAGTTCAGCAGGAGCGCCGACGCCCCCTGCCTACCAGCAAGGA CAGAATCAACTGTACAACGAGCTGAACCTGGGCAGACGGGAGGAATACGAT GTGCTGGACAAGAGGAGAGGCAGAGACCCCCGAGATGGGCGGCAAAACCTAG AAGAAAGAACCCCCAGGAGGGCCTGTATAACGAGCTCCAGAAGGACAAGAT GGCCGAGGCCTACAGCGAGATCGGCATGAAGGGCGAAAGAAGAAGAGGCA AGGGCCACGACGGCCTCTACCAGGGCTTAAGCACAGCTACAAAGGACACCT ACGACGCCCTGCACATGCAGGCCCTGCCCCCTAGATGATTAATTAAatcgat
290	ggtaccCCCGGgCCCATGGCTCTTCCTGTGACAGCTCTTCTGCTGCCCCCTGGCCC TGCTTCTGCATGCTGCTAGACCTCAGGTTTCAGTTGCAGCAATGGGGAGCTGG CCTGTTAAAGCCCAGCGAAACCCTGTCCCTCACCTGCGCTGTGTATGGCGGA AGCTTCAGCGGCATCCACTGGAACCTGGATCCGGCAGCCTCCTGGCAAAGGCC TTGAATGGATCGGCATATCGACACCAGCGGCTCCACCAACTACAACCCCAG CCTGAAATCGAGGGTTACAATCTCTGTGGACACAAGCAAGAATCAGTTCTCC

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271	ggtaccCCCGGgCCCATGGCTCTTCTGTGACAGCTCTTCTGCTGCCCCCTGGCCC TGCTTCTGCATGCTGCTAGACCTCAGCTTCAGCTCCAAGAGAGCGGACCTGG CTTAGTGAAGCCCAGCGAAACCCTGTCCCTCACCTGCACCGTTTCTGGCGGA AGCATCAGCAGCTCCAGCTATTACTGGGGATGGATCAGGCAGCCCCCTGGCA AGGGTTTAGAATGGATCGGCTCGATATATTACTCCGGCAGCACCTACTATAA CCCCAGCTTGAAGAGCCGGGTTACCATTCTGTGGACACATCAAAGAACCAG TTCAGCCTGAAGCTGAGCTCTGTGACTGCCGCCGACACAGCTGTGTACTACT GTGCCAGAGAGACAGACTACTCCAGCGGCATGGGCTACGGCATGGATGTGT GGGGACAAGGAACCACCGTTACTGTGAGCAGCGGTTCCACCAGCGGCTCAG GCAAGCCTGGCTCAGGAGAAGGAAGCACCAAGGGGGATATACAGATGACAC AGAGCCCCCTCCAGCCTGTCCGCCAGCGTTGGCGATCGTGTAACGATCACCTG CCGGGCCTCTCAGAGCATCAACTCCTACCTCAATTGGTATCAACAGAAGCCA GGCAAGGGCCCCAAATTACTCATCTACGCCGCCAGCAGCTTACAGAGCGGG GTTCCCTCTAGATTCTCCGGCTCCGGTTCTGGAACAGATTTACCCTCACTAT CTCCAGCTTGAGCCCGAGGATTTCCGCACTTATTACTGTCAGCAGAGCCTG

	GCCGACCCCTTCACATTCGGCGGAGGCACAAAGGTTGAGATCAAGGCAGCT GCTTTCGTGCCTGTGTTCTGCCTGCTAAGCCCACCACCACTCCTGCTCCAAG ACCTCCTACCCCGCTCCTACAATCGCCAGCCAACCTCTGAGCCTGAGACCG GAGGCATGCAGACCTGCGGCAGGGGGAGCAGTTCACACAAGAGGCTTGGAC TTCGCTTGGCAGATCTACATCTGGGCCCCTCTGGCCGGCACATGCGGAGTTCT TCTTCTTAGCCTGGTGATCACCTGTACTGCAACCACAGAAACAGATTACAGC GTTGTGAAGAGAGGCCGGAAGAAGCTGCTGTACATCTTCAAGCAGCCCTTCA TGAGACCTGTGCAGACCACACAGGAGGAAGACGGCTGCAGCTGTAGATTCC CCCGAGGAAGAGGAGGGCGGCTGTGAGCTGAGAGTTAAGTTCAGCAGGAGCG CCCGACGCCCCTGCCTACCAGCAAGGACAGAATCAACTGTACAACGAGCTGA ACCTGGGCAGACGGGAGGAATACGATGTGCTGGACAAGAGGAGAGGCAGA GACCCCGAGATGGGCGGCAAACCTAGAAGAAAGAACCCCGAGGAGGGCCTG TATAACGAGCTCCAGAAGGACAAGATGGCCGAGGCCTACAGCGAGATCGGC ATGAAGGGCGAAAGAAGAAGAGGCAAGGGCCACGACGGCCTCTACCAGGG CTTAAGCACAGCTACAAAGGACACCTACGACGCCCTGCACATGCAGGCCCT GCCCCCTAGATGATTAATTAAtcga
272	ggtaccCCCGGgCCCATGGCTCTTCTGTGACAGCTCTTCTGCTGCCCCTGCCCC TGCTTCTGCATGCTGCTAGACCTCAGGTTCAAGAGAGCGGACCTGG CTTAGTGAAGCCCAGCGAAACCTGTCCCTCACCTGCACCGTTTCTGGCGGA AGCATCAGCTCTCCGACCACTACTGGGGATGGATCAGGCAGCCCCCTGGCA AGGGTTTGGAATGGATCGGCAGCATCTACGCCAGCGGCAGCACATTCTACAA CCCCTCGCTCAAAAGCAGGGTTACTATTTCTGTGGACACAAGCAAAAATCAG TTCAGCCTGAAGCTGAGCTCTGTGACTGCCGCCGACACAGCTGTGTACTACT GTGCCAGAGAGACAGACTACTCCAGCGGGATGGGCTACGGCATGGATGTGT GGGGACAAGGAACCAACCGTTACTGTGAGCAGCGGCTCCACAAGCGGCTCAG GCAAGCCTGGCTCAGGAGAAGGAAGCACCAAGGGGGACATTCAAATGACCC AAAGCCCCCTCCAGCCTGTCCGCCAGCGTTGGCGATAGGGTTACCATTACCTG CAGGGCCAGCCAAAGCATCAACTCCTACCTAAATTGGTATCAACAGAAGCC AGGCAAGGCCCCCAAATACTCATTTACGCCGCCAGCAGCTTACAGAGCGG GGTTCCTCTAGATTCTCCGGCAGCGTTCTGGAACAGATTTCACTCTCACAA TATCTTCGCTGCAGCCCGAGGATTTGCTACCTACTATTGCCAGCAATCCCTG GCCGACCCCTTCACATTCGGCGGAGGCACAAAGGTTGAGATCAAGGCAGCT GCTTTCGTGCCTGTGTTCTGCCTGCTAAGCCCACCACCACTCCTGCTCCAAG ACCTCCTACCCCGCTCCTACAATCGCCAGCCAACCTCTGAGCCTGAGACCG GAGGCATGCAGACCTGCGGCAGGGGGAGCAGTTCACACAAGAGGCTTGGAC TTCGCTTGGCAGATCTACATCTGGGCCCCTCTGGCCGGCACATGCGGAGTTCT TCTTCTTAGCCTGGTGATCACCTGTACTGCAACCACAGAAACAGATTACAGC GTTGTGAAGAGAGGCCGGAAGAAGCTGCTGTACATCTTCAAGCAGCCCTTCA TGAGACCTGTGCAGACCACACAGGAGGAAGACGGCTGCAGCTGTAGATTCC

	CCGAGGAAGAGGAGGGCGGCTGTGAGCTGAGAGTTAAGTTCAGCAGGAGCG CCGACGCCCCCTGCCTACCAGCAAGGACAGAATCAACTGTACAACGAGCTGA ACCTGGGCAGACGGGAGGAATACGATGTGCTGGACAAGAGGAGAGGCAGA GACCCCGAGATGGGCGGCAAACCTAGAAGAAAGAACCCCAAGGAGGGCCTG TATAACGAGCTCCAGAAGGACAAGATGGCCGAGGCCTACAGCGAGATCGGC ATGAAGGGCGAAAGAAGAAGAGGCAAGGGCCACGACGGCCTCTACCAGGG CTTAAGCACAGCTACAAAGGACACCTACGACGCCCTGCACATGCAGGCCCT GCCCCCTAGATGATTAATTAAtcgat
273	ggtaccCCCGGgCCCATGGCTCTTCTGTGACAGCTCTTCTGCTGCCCCCTGGCCC TGCTTCTGCATGCTGCTAGACCTCAGATCACATTAAGAGAGCGGACCTAC ACTGGTGAAGCCACCCAAACGCTTACCCTCACCTGCACCTTTAGCGGGTTC AGCCTGGACACAGAGGGCGTTGGCGTTGGATGGATCAGGCAGCCTCCTGGC AAAGCCCTCGAATGGCTTGCCCTCATCTACTTCAACGACCAGAAGAGATACA GCCCCCTCCTAAAATCTCGGCTCACAATCACCAAAGACACAAGCAAAAATCA GGTGTGTCTACCATGACCAACATGGACCCTGTGGACACCGCTGTGTACTAC TGTGCCAGAGACACCGGCTACAGCAGATGGTACTACGGGATGGACGTTTGG GGCCAAGGCACCACTGTGACCGTTTCCAGCGGCTCTACAAGCGGCAGCGGG AAACCTGGTTCTGGAGAGGGCAGCACAAAGGGCGACATCCAGATGACGCAA TCCCCCAGCTCTGTGAGCGCCTCTGTGGGAGACAGAGTTACAATCACATGCC GGGCCTCCCAGGGCATCAGCTCTTGGCTGGCATGGTATCAACAGAAGCCTGG CAAGGCTCCCAAGCTGCTCATCTATGCCGCCTCCTCCTTACAATCTGGAGTTC CCTCCAGGTTTACGCGGAGCGGCTCAGGAACAGACTTCACCTTACCATCTC TAGCCTGCAACCCGAGGACTTCGCTACTTATTACTGTCAGCAGGCCTACGCC TACCCCATCACATTCGGCGGAGGAACAAAGGTTGAGATCAAGGCAGCTGCTT TCGTGCCTGTGTTCTGCTGCTAAGCCCAACCACTCCTGCTCCAAGACCT CCTACCCCGCTCCTACAATCGCCAGCCAACCTCTGAGCCTGAGACCGGAGG CATGCAGACCTGCGGCAGGGGAGCAGTTCACACAAGAGGCTTGGACTTCG CTTGCGACATCTACATCTGGGCCCCCTCTGGCCGGCACATGCGGAGTTCTTCTT CTTAGCCTGGTGATCACCTGTACTGCAACCACAGAAAACAGATTCAGCGTTG TGAAGAGAGGCCGGAAGAAGCTGCTGTACATCTTCAAGCAGCCCTTCATGA GACCTGTGCAGACCACACAGGAGGAAGACGGCTGCAGCTGTAGATTCCCCG AGGAAGAGGAGGGCGGCTGTGAGCTGAGAGTTAAGTTCAGCAGGAGCGCCG ACGCCCCTGCCTACCAGCAAGGACAGAATCAACTGTACAACGAGCTGAACC TGGGCAGACGGGAGGAATACGATGTGCTGGACAAGAGGAGAGGCAGAGAC CCCGAGATGGGCGGCAAACCTAGAAGAAAGAACCCCAAGGAGGGCCTGTAT AACGAGCTCCAGAAGGACAAGATGGCCGAGGCCTACAGCGAGATCGGCATG AAGGGCGAAAGAAGAAGAGGCAAGGGCCACGACGGCCTCTACCAGGGCTTA AGCACAGCTACAAAGGACACCTACGACGCCCTGCACATGCAGGCCCTGCCC CCTAGATGATTAATTAAtcgat

274	ggtaccCCCGGgCCCATGGCTCTTCCTGTGACAGCTCTTCTGCTGCCCCTGCCCC TGCTTCTGCATGCTGCTAGACCTCAGGTTCAAGTTACAACAATGGGGAGCTGG CCTGTAAAGCCCAGCGAAACCCTGTCCCTCACCTGCGCTGTGTATGGCGGA AGCTTCCAGAAATACTACTGGAGCTGGATCCGGCAGCCTCCCGGCAAAGGCT TAGAATGGATCGGAGAGATAGACACCAGCGGCTTCACCAACTACAACCCCA GCCTGAAATCTAGGGTTACAATCTCTGTGGACACAAGCAAGAATCAGTTCTC CCTGAAGCTGAGCAGCGTTACTGCCGCCGACACAGCTGTGTACTATTGCGCC AGAGTTGGCAGATACAGCTACGGCTACTACATCACCGCCTTCGACATTTGGG GCCAAGGCACCACTGTGACCGTTTCCAGCGGAAGCACTAGCGGCAGCGGGA AACCTGGTTCTGGAGAGGGCTCAACCAAGGGCGACATCGTGATGACACAGA GCCCCGACTCTCTGGCTGTGTCCCTGGGAGAGAGAGCCACCATCAACTGCAA GAGCAGCCAGAGCGTTCTGTACAGCAGCAACAACAAGAACTACCTGGCATG GTATCAACAGAAGCCTGGCCAGCCCCCTAAGCTGCTCATCTACTGGGCTTCC ACCAGAGAATCAGGCGTTCCAGACAGGTTCTCCGGCTCGGGTTCAGGCACAG ACTTCACCCCTTACCATCTCTCCCTGCAGGCCGAAGATGTGGCCGTTTACTAC TGTCAGCAGCACTACAGCTTCCCTTTCACATTGCGCGGAGGCACCAAGGTTG AGATCAAGGCAGCTGCTTTCGTGCCTGTGTTCTGCCTGCTAAGCCCACCAC CACTCTGTCTCAAGACCTCCTACCCCCGCTCCTACAATCGCCAGCCAACCT CTGAGCCTGAGACCGGAGGCATGCAGACCTGCGGCAGGGGGAGCAGTTAC ACAAGAGGCTTGGACTTCGCTTGCGACATCTACATCTGGGCCCCCTCTGGCCG GCACATGCGGAGTTCTTCTTCTTAGCCTGGTGATCACCTGTACTGCAACCAC AGAAACAGATTCAAGCGTTGTGAAGAGAGGCCGGAAGAAGCTGCTGTACATC TTCAAGCAGCCCTTCATGAGACCTGTGCAGACCACACAGGAGGAAGACGGC TGCAGCTGTAGATTCCCCGAGGAAGAGGAGGGCGGCTGTGAGCTGAGAGTT AAGTTCAGCAGGAGCGCCGACGCCCTGCCTACCAGCAAGGACAGAATCAA CTGTACAACGAGCTGAACCTGGGCAGACGGGAGGAATACGATGTGCTGGAC AAGAGGAGAGGCAGAGACCCCGAGATGGGCGGCAAACCTAGAAGAAAGAA CCCCAGGAGGGCCTGTATAACGAGCTCCAGAAGGACAAGATGGCCGAGGC CTACAGCGAGATCGGCATGAAGGGCGAAAGAAGAAGAGGCAAGGGCCACG ACGGCCTCTACCAGGGCTTAAGCACAGCTACAAAGGACACCTACGACGCCC TGCACATGCAGGCCCTGCCCCCTAGATGATTAATTAAtcgat
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Example 21

Table 69: Exemplary anti-CD20/anti-CD19 bicistronic CAR nucleotide and amino acid sequences set forth in SEQ ID NOs: 291 and 292.

SEQ ID NO: 291	ATGCTGCTGCTGGTGACATCTCTGCTGCTTTGCGAGCTGCCCCACCCT GCCTTCCTGCTTATCCCCGACATTCAGATGACCCAGACCACCAGCAGC CTGAGCGCCAGCTTAGGAGATAGAGTTACCATCAGCTGCAGAGCCAG CCAGGACATCAGCAAATACCTGAACTGGTATCAGCAGAAGCCCCGACG GCACTGTGAAACTGCTTATTTACCACACCTCCAGACTGCACAGCGGCG TTCCAGCAGATTCTCTGGCAGCGGATCTGGAACCGACTACAGCCTCA CCATCTCCAACCTGGAGCAGGAGGACATCGCCACCTACTTCTGCCAGC AGGGCAACACACTGCCCTACACCTTCGGAGGAGGAACCAAGCTGGAG ATCACCGGGGGAGGAGGCTCTGGAGGCGGCGGATCAGGAGGAGGGG GATCTGAGGTAAAGCTGCAGGAGAGCGGCCCTGGCCTGGTGGCTCCT AGCCAATCTTTATCTGTGACCTGCACTGTGTCCGGCGTTAGCCTGCCC GATTATGGCGTTTCCTGGATCAGACAGCCCCCAGAAAGGGCCTGGA ATGGCTGGGCGTTATCTGGGGCAGCGAGACCACATACTACAACAGCG CCCTGAAGAGCAGACTTACGATTATCAAGGACAACAGCAAGAGCCAG GTTTTCCTGAAGATGAACAGCCTGCAGACCGACGACACCGCCATCTAC TACTGCGCTAAGCACTACTACTACGGCGGCAGCTACGCCATGGACTAC TGGGGCCAGGGAACAAGCGTTACCGTTAGCAGCGCTGCTGCACTGGA CAACGAGAAGAGCAACGGCACCATCATCCACGTTAAGGGCAAGCACC TGTGCCCCAGCCCTCTGTTCCCTGGACCTTCTAAGCCTTTCTGGGTTCT GGTGGTGGTCGGCGGCGTTTTAGCCTGTTACAGCCTTCTGGTGACTGT GGCTTCATCATCTTTTGGGTTAGAAGCAAGAGAAGCAGACTGCTCCA CAGCGACTACATGAACATGACCCCCAGACGGCCTGGCCCCACCAGAA AGCATTACCAGCCCTACGCTCCTCCAGAGACTTCGCCGCTACAGGA GCAGAGTTAAATTCAGCAGATCCGCCGATGCCCCCGCTTACCAACAG GGACAAAACCAGCTGTACAATGAGCTCAACCTGGGGAGAAGAGAAG AATACGACGTTCTGGATAAGAGAAGGGGCAGAGATCCCGAAATGGGG GGCAAGCCCAGACGCAAGAACCCTCAGGAGGGGCTTTACAACGAAT GCAGAAGGATAAGATGGCTGAGGCTTACTCGGAGATTGGGATGAAGG GGGAGAGAAGGCGGGGCAAGGGACACGATGGCTTATACCAGGGCT GAGCACCGCCACCAAGGACACATACGACGCTCTTCATATGCAGGCTC TGCCCCCAAGAAGGGCTAAGAGATCTGGCTCTGGCGAGGGCAGAGGC AGCTTGCTTACATGTGGCGATGTGGAGGAGAACCCCGGGCCCATGGC TCTTCCTGTGACAGCTCTTCTGCTGCCCCTGGCCCTGCTTCTGCATGCT GCTAGACCTCAGCTTCAGCTCCAAGAGAGCGGACCTGGCTTAGTGAA GCCAGCGAAACCCTGTCCCTCACCTGCACCGTTTCTGGCGGAAGCAT CAGCAGCTCCAGCTATTACTGGGGATGGATCAGGCAGCCCCCTGGCA AGGGTTTAGAATGGATCGGCTCGATATATTACTCCGGCAGCACCTACT ATAACCCAGCTTGAAGAGCCGGGTTACCATTTCTGTGGACACATCAA AGAACCAGTTCAGCCTGAAGCTGAGCTCTGTGACTGCCGCCGACACA GCTGTGTACTACTGTGCCAGAGAGACAGACTACTCCAGCGGCATGGG CTACGGCATGGATGTGTGGGGACAAGGAACCAACCGTTACTGTGAGCA GCGGTTCCACCAGCGGCTCAGGCAAGCCTGGCTCAGGAGAAGGAAGC ACCAAGGGGGATATACAGATGACACAGAGCCCCCTCAGCCTGTCCGC CAGCGTTGGCGATCGTGTAACGATCACCTGCCGGGCCTCTCAGAGCAT
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	CAACTCCTACCTCAATTGGTATCAACAGAAGCCAGGCAAGGCCCCCA AATTACTCATCTACGCCGCCAGCAGCTTACAGAGCGGGGTTCCCTCTA GATTCTCCGGCTCCGGTTCTGGAACAGATTTCACCCTCACTATCTCCA GCTTGCAGCCCGAGGATTTCCGCCACTTATTACTGTCAGCAGAGCCTGG CCGACCCCTTCACATTCGGCGGAGGCACAAAGGTTGAGATCAAGGCA GCTGCTTTTCGTGCCTGTGTTCTGCTGCTAAGCCCACCACCCTCCTG CTCCAAGACCTCCTACCCCGCTCCTACAATCGCCAGCCAACCTCTGA GCCTGAGACCGGAGGCATGCAGACCTGCGGCAGGGGGAGCAGTTCAC ACAAGAGGCTTGGACTTCGCTTGCACATCTACATCTGGGCCCCCTCTG GCCGGCACATGCGGAGTTCTTCTTCTTAGCCTGGTGATCACCTGTAC TGCAACCACAGAAACAGATTTCAGCGTTGTGAAGAGAGGCCGGAAGAA GCTGCTGTACATCTTCAAGCAGCCCTTCATGAGACCTGTGCAGACCAC ACAGGAGGAAGACGGCTGCAGCTGTAGATTCCCCGAGGAAGAGGAG GGCGGCTGTGAGCTGAGAGTTAAGTTCAGCAGGAGCGCCGACGCCCC TGCCTACCAGCAAGGACAGAATCAACTGTACAACGAGCTGAACCTGG GCAGACGGGAGGAATACGATGTGCTGGACAAGAGGAGAGGCAGAGA CCCCGAGATGGGCGGCAAACCTAGAAAGAAAGAACCCCCAGGAGGGC CTGTATAACGAGCTCCAGAAGGACAAGATGGCCGAGGCCTACAGCGA GATCGGCATGAAGGGCGAAAGAAAGAGGCAAGGGCCACGACGGC CTCTACCAGGGCTTAAGCACAGCTACAAAGGACACCTACGACGCCCT GCACATGCAGGCCCTGCCCCCTAGATGA
SEQ ID NO: 292	MLLLVTSLLLCELPHPAFLIPDIQMTQTSSLSASLGDRVTISCRASQDIS KYLNWYQQKPDGTVKLLIYHTSRLHSGVPSRFSGSGSGTDYSLTISNLEQ EDIATYFCQQGNTLPYTFGGGKLEITGGGGSGGGGSGGGGSEVKLQESG PGLVAPSQSLSVTCTVSGVSLPDYGVSWIRQPPRKGLEWLGVWGETTY YNSALKSRLTHKDNSKSQVFLKMNSLQTDDBTAIYYCAKHYYYGGSYAM DYWGQTSVTVSSAAALDNEKSNGTIIHVKGKHLCPSPFLPGPSKPFWVL VVVGGVLACYSLLVTVAFIIFWVRSKRSLHSDYMNMTPRRPGPTRKH YQPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGRREEYD VLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERR RGKGHDLGYQGLSTATKDTYDALHMQALPPRAKRSGSGEGRGSLLTC GDVEENPGPMALPVTALLLPLALLHAARPQLQLQESGPGLVKPSETLSL TCTVSGGSISSSSYWGWIRQPPGKGLEWIGSIYYSGSTYYNPSLKSRVTI SVDTSKNQFSLKLSSVTAADTAVYYCARETDYSSGMGYGMDVWGQGT VTVSSGSTSGSGKPGSGEGSTKGDQMTQSPSSLSASVGDRVTITCRASQS INSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISLQ PEDFATYYCQQLADPFTFGGGTKVEIKAAAFVPVFLPAKPTTTPAPRPPT PAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLL

	LSLVITLYCNHRNRFVVKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRF PEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRR GRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGH DGLYQGLSTATKDTYDALHMQALPPR
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Example 22

Table 70: Exemplary anti-CD20/anti-CD19 bispecific CAR and components thereof

	SEQ ID NO:	Sequence
Bispecific CAR (nt sequence)	293	ATGCTTCTCCTGGTGACAAGCCTTCTGCTCTGTGAGTTAC CACACCCAGCATTCTCCTGATCCCAGACATCCAGATGA CCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAG AGTCACCATCACTTGCCGGGCAAGTCAGAGCATTAACAG CTATTTAAATTGGTATCAGCAGAAACCAGGGAAAGCCCC TAAGCTCCTGATCTATGCTGCATCCAGTTTGCAAAGTGGG GTCCCATCAAGGTTCAAGTGGCAGTGGATCTGGGACAGAT TTCACTCTCACCATCAGCAGTCTGCAACCTGAAGATTTTG CAACTTACTACTGCCAGCAAAGCCTCGCCGACCCTTTTCAC TTTTGGCGGAGGGACCAAGGTTGAGATCAAAGGGGGGG GTGGAAGTGGGAAGCCTGGCAGCGGCAGGGCGGCAGT CAGCTGCAGCTGCAGGAGTCGGGCCAGGACTGGTGAAG CCTTCGGAGACCCTGTCCCTCACCTGCACTGTCTCTGGTG GCTCCATCAGCAGTAGTAGTTACTACTGGGGCTGGATCC GCCAGCCCCAGGGAAGGGGCTGGAGTGGATTGGGAGT ATCTATTATAGTGGGAGCACCTACTACAACCCGTCCCTCA AGAGTCGAGTCACCATATCCGTAGACACGTCCAAGAACC AGTTCTCCCTGAAGCTGAGTTCTGTGACCGCCGCAGACA CGGCGGTGTACTACTGCGCCAGAGAGACTGACTACAGCA GCGGAATGGGATACGGAATGGACGTATGGGGCCAGGGA ACAACTGTCACCGTCTCCTCAGGCGGTGGCGGCAGTGGG AAGCCTGGCAGCGATATTCAAATGACCCAGTCCCCGTCC TCCCTGAGTGCCTCCGTGCGGTGACCGTGTTACGATTACCT GCCGTGCGAGCCAAGACATCTCTAAATACCTGAAGTGGT ATCAGCAAAAACCGGATCAGGCACCGAACTGCTGATCA AACATACCTCACGTCTGCACTCGGGTGTGCCGAGCCGCTT TAGTGGTTCCGGCTCAGGTACCGATTACACCCTGACGATC AGCTCTCTGCAGCCGGAAGACTTTGCCACGTATTACTGCC AGCAAGGTAATACCCTGCCGTATACGTTTCGGCCAAGGTA CCAAACCTGGAAATCAAAGGGGGGGGTGGAAGTGGGGGC GGTGGCAGCGGCGGTGGCGGCAGTGAAGTGCAGCTGGTT GAAAGCGGTGGTGGTCTGGTTCAACCGGGTCGTTCCCTG CGTCTGTCATGTACGGCGAGTGGTGTCTCCCTGCCGACT ATGGCGTGTCTGGATTCTGTCAGCCGCCGGGTAAAGGCC TGGAATGGATTGGTGTCTGTCATCTGGGGCAGTGAACACGT ATTACAACCTCGGCCCTGAAAAGCCGTTTACCATCTCTCG CGATAACAGTAAAAATACGCTGTACCTGCAGATGAATAG CCTGCGCGCGGAAGACACCGCCGTTTACTACTGCGCAA ACATTACTACTACGGTGGCAGCTATGCTATGGATTACTGG GGTCAAGGCACGCTGGTCACCGTTTCGTCAGCCGCTGCC CTAGACAATGAGAAGAGCAATGGAACCATATCCATGTG AAAGGGAAACACCTTTGTCCAAGTCCCCTATTTCCCGGA CCTTCTAAGCCCTTTTGGGTGCTGGTGGTGGTTGGGGGAG

		TCCTGGCTTGCTATAGCTTGCTAGTAACAGTGGCCTTTAT TATTTTCTGGGTGAGGAGTAAGAGGAGCAGGCTCCTGCA CAGTGACTACATGAACATGACTCCCCGCCGCCCGGGCC CACCCGCAAGCATTACCAGCCCTATGCCCCACCACGCGA CTTCGCAGCCTATCGCTCCAGAGTGAAGTTCAGCAGGAG CGCAGACGCCCCGCGTACCAGCAGGGCCAGAACCAGCT CTATAACGAGCTCAATCTAGGACGAAGAGAGGAGTACGA TGTTTTGGACAAGAGACGTGGCCGGGACCCTGAGATGGG GGGAAAGCCGAGAAGGAAGAACCCTCAGGAAGGCCTGT ACAATGAACTGCAGAAAGATAAGATGGCGGAGGCCTAC AGTGAGATTGGGATGAAAGGCGAGCGCCGGAGGGGCAA GGGGCACGATGGCCTTTACCAGGGTCTCAGTACAGCCAC CAAGGACACCTACGACGCCCTTCACATGCAGGCCCTGCC CCCTCGATGA
Bispecific CAR (AA sequence)	294	MLLLVTSLLLCELPHPAFLIPDIQMTQSPSSLSASVGDRTI TCRASQSINSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFS GSGSGTDFTLTISSLQPEDFATYYCQQSLADPFTFGGGTKVE IKGGGSGKPGSGEGGSQLQLQESGPGLVKPSSETLSLTCTVS GGSISSSSYWGWIRQPPGKGLEWIGSIYYSGSTYYNPSLKS RVTISVDTSKNQFSLKLSSVTAADTAVYYCARETDYSSGMG YGM DVWGQGT TVTVSSGGGGSGKPGSDIQMTQSPSSLSAS VGDRVTITCRASQDISKYLWYQQKPDQAPKLLIKHTSR LH SGVPSRFSGSGSGTDYTLTISSLQPEDFATYYCQQGNTLPYT FGQGTKLEIKGGGGSGGGSGGGGSEVQLVESGGGLVQPG RSLRLSCTASGVSLPDYGVSWIRQPPGKGLEWIGVIWGSETT YYNSALKSRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAK HYYYGGSYAMDYWGQGLTVTVSSAAALDNEKSNGTIIHV KGKHLCPSPFLPGPSKPFWVLVVGGLVACYSLLVTVAFIIF WVRSKRSRLHSDYMNMTPRRPGPTRKHYPYAPPRDFAA YRSRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVL DK RRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGM KGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR
CSF2RA Signal Peptide	295	MLLLVTSLLLCELPHPAFLIP
Ab3 VL	296	DIQMTQSPSSLSASVGDRTITCRASQSINSYLNWYQQKPG KAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFA TYYCQQSLADPFTFGGGTKVEIK

KL2 linker	297	GGGGSGKPGSGEGGS
Ab3 VH	298	QLQLQESGPGLVKPSSETLSLTCTVSGGSISSSSYYWGWIRQP PGKGLEWIGSIYYSGSTYYNPSLKSRVTISVDTSKNQFSLKL SSVTAADTAVYYCARETDYSSGMGYGMDVWGQGTTVTVS S
truncated linker	299	GGGGSGKPGS
Anti-CD19 VL	300	DIQMTQSPSSLSASVGDRVTITCRASQDISKYLNWYQQKPDQAPK LLIKHTSRLHSGVPSRFSGSGSGTDYTLTISSLQPEDFATYYCQQG NTLPYTFGQGTKLEIK
G4S linker	301	GGGGSGGGGSGGGGS
Anti-CD19 VH	302	EVQLVESGGGLVQPGRSLRLSCTASGVSLPDYGVSWIRQPPGKGL EWIGVIWGSETTYNSALKSRFTISRDNSKNTLYLQMNSLRAEDT AVYYCAKHYYGGSYAMDYWGQGLVTVSS
CD28T extracellular region	303	LDNEKSNGTIIHVKGKHLCPSPFPGPSKP
CD28 transmembrane region	304	FWVLVVVGGLACYSLLVTVAFIIFWV
CD28 intracellular region	305	RSKRSRLLHSDYMNMTPRRPGPTRKHYPYAPPRDFAAYR S
CD3z	306	RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRG RDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGE RRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

Example 23

Table 71: Exemplary linkers

	SEQ ID NO:	Sequence
G4S (G4Sx1)	307	GGGGS
G4Sx2	308	GGGGS GGGGS
G4Sx3	309	GGGGS GGGGS GGGGS
G4Sx4	310	GGGGS GGGGS GGGGS GGGGS
IgA	311	PSTPPTPSPSTPPTSPS
PAPAP	312	PAPAP (optionally comprising 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 adjacent copies of PAPAP)
EAAAK	313	EAAAK (optionally comprising 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 adjacent copies of EAAAK)

[361] While a number of embodiments have been described, it is apparent that the disclosure and examples may provide other embodiments that utilize or are encompassed by the compositions and methods described herein. Therefore, it will be appreciated that the scope of is to be defined by that which may be understood from the disclosure and the appended claims rather than by the embodiments that have been represented by way of example.

CLAIMS

What is claimed is:

1. An antigen binding system, antibody, or antigen binding fragment thereof comprising an anti-CD20 binding motif, wherein the anti-CD20 binding motif comprising sequences of three heavy chain complementarity determining regions (HCDRs) of any one of the heavy chain variable region (HCVR) selected from the group consisting of SEQ ID NOs: 1, 23, 45, 67, 89, 111, 133, 155, 177, and 199, and sequences of three light chain CDRs (LCDRs) of the light chain variable region (LCVR) selected from the group consisting of SEQ ID NOs: 12, 34, 56, 78, 100, 122, 144, 166, 188, and 210.

2. The antigen binding system, antibody, or antigen binding fragment thereof of claim 1, wherein the anti-CD20 binding motif comprising a first domain comprising three heavy chain complementarity determining regions (HCDR1, HCDR2 and HCDR3) and a second domain comprising three light chain complementarity determining regions (LCDR1, LCDR2 and LCDR3), wherein

- (i) the HCDR1 has a sequence according to any one of SEQ ID NOs: 3-5, 25-27, 47-49, 69-71, 91-93, 113-115, 135-137, 157-159, 179-181; and 201-203;
 - (ii) the HCDR2 has a sequence according to any one of SEQ ID NOs: 6-8, 28-30, 50-52, 72-74, 94-96, 116-118, 138-140, 160-162, 182-184; and 204-206;
 - (iii) the HCDR3 has a sequence according to any one of SEQ ID NOs: 9-11, 31-33, 53-55, 75-77, 97-99, 119-121, 141-143, 163-165, 185-187; and 207-209;
 - (iv) the LCDR1 has a sequence according to any one of SEQ ID NOs: 14-16, 36-38, 58-60, 80-82, 102-104, 124-126, 146-148, 168-170, 190-192; and 212-214;
 - (v) the LCDR2 has a sequence according to any one of SEQ ID NOs: 17-19, 39-41, 61-63, 83-85, 105-107, 127-129, 149-151, 171-173, 193-195; and 215-217;
- and
- (vi) the LCDR3 has a sequence according to any one of SEQ ID NOs: 20-22, 42-44, 64-66, 86-88, 108-110, 130-132, 152-154, 174-176, 196-198; and 218-220.

3. The antigen binding system, antibody, or antigen binding fragment thereof of claim 1 or 2 wherein the HCDRs comprise:

- (i) an HCDR1 according to any of SEQ ID NOs: 3-5; an HCDR2 according to any of SEQ ID NOs: 6-8; an HCDR3 according to any one of SEQ ID NOs: 9-11;
 - (ii) an HCDR1 according to any of SEQ ID NOs: 25-27; an HCDR2 according to any of SEQ ID NOs: 28-30; an HCDR3 according to any one of SEQ ID NOs: 31-33;
 - (iii) an HCDR1 according to any of SEQ ID NOs: 47-49; an HCDR2 according to any of SEQ ID NOs: 50-52; an HCDR3 according to any one of SEQ ID NOs: 53-55;
 - (iv) an HCDR1 according to any of SEQ ID NOs: 69-71; an HCDR2 according to any of SEQ ID NOs: 72-74; an HCDR3 according to any one of SEQ ID NOs: 75-77;
 - (v) an HCDR1 according to any of SEQ ID NOs: 91-93; an HCDR2 according to any of SEQ ID NOs: 94-96; an HCDR3 according to any one of SEQ ID NOs: 97-99;
 - (vi) an HCDR1 according to any of SEQ ID NOs: 113-115; an HCDR2 according to any of SEQ ID NOs: 116-118; an HCDR3 according to any one of SEQ ID NOs: 119-121;
 - (vii) an HCDR1 according to any of SEQ ID NOs: 135-137; an HCDR2 according to any of SEQ ID NOs: 138-140; an HCDR3 according to any one of SEQ ID NOs: 141-143;
 - (viii) an HCDR1 according to any of SEQ ID NOs: 157-159; an HCDR2 according to any of SEQ ID NOs: 160-162; an HCDR3 according to any one of SEQ ID NOs: 163-165;
 - (ix) an HCDR1 according to any of SEQ ID NOs: 179-181; an HCDR2 according to any of SEQ ID NOs: 182-184; an HCDR3 according to any one of SEQ ID NOs: 185-187; or
 - (x) an HCDR1 according to any of SEQ ID NOs: 201-203; an HCDR2 according to any of SEQ ID NOs: 204-206; an HCDR3 according to any one of SEQ ID NOs: 207-209;
- and

the LCDRs comprise:

- (i) an LCDR1 according to any of SEQ ID NOs: 14-16; an LCDR2 according to any of SEQ ID NOs: 17-19; an LCDR3 according to any one of SEQ ID NOs: 20-22;
- (ii) an LCDR1 according to any of SEQ ID NOs: 36-38; an LCDR2 according to any of SEQ ID NOs: 39-41; an LCDR3 according to any one of SEQ ID NOs: 42-44;
- (iii) an LCDR1 according to any of SEQ ID NOs: 58-60; an LCDR2 according to any of SEQ ID NOs: 61-63; an LCDR3 according to any one of SEQ ID NOs: 64-66;
- (iv) an LCDR1 according to any of SEQ ID NOs: 80-82; an LCDR2 according to any of SEQ ID NOs: 83-85; an LCDR3 according to any one of SEQ ID NOs: 86-88;
- (v) an LCDR1 according to any of SEQ ID NOs: 102-104; an LCDR2 according to any of SEQ ID NOs: 105-107; an LCDR3 according to any one of SEQ ID NOs: 108-110;

- (vi) an LCDR1 according to any of SEQ ID NOs: 124-126; an LCDR2 according to any of SEQ ID NOs: 127-129; an LCDR3 according to any one of SEQ ID NOs: 130-132;
- (vii) an LCDR1 according to any of SEQ ID NOs: 146-148; an LCDR2 according to any of SEQ ID NOs: 149-151; an LCDR3 according to any one of SEQ ID NOs: 152-154;
- (viii) an LCDR1 according to any of SEQ ID NOs: 168-170; an LCDR2 according to any of SEQ ID NOs: 171-173; an LCDR3 according to any one of SEQ ID NOs: 174-176;
- (ix) an LCDR1 according to any of SEQ ID NOs: 190-192; an LCDR2 according to any of SEQ ID NOs: 193-195; an LCDR3 according to any one of SEQ ID NOs: 196-198; or
- (x) an LCDR1 according to any of SEQ ID NOs: 212-214; an LCDR2 according to any of SEQ ID NOs: 215-217; an LCDR3 according to any one of SEQ ID NOs: 218-220.

4. The antigen binding system, antibody, or antigen binding fragment thereof of any one of claims 1-3, wherein the antigen binding system, antibody, or antigen binding fragment thereof comprises a first domain comprising three heavy chain complementarity determining regions (HCDRs) and a second domain comprising three light chain complementarity determining regions (LCDRs), wherein:

the HCDRs and LCDRs comprise:

- (i) an HCDR1 according to any of SEQ ID NOs: 3-5; an HCDR2 according to any of SEQ ID NOs: 6-8; an HCDR3 according to any one of SEQ ID NOs: 9-11; an LCDR1 according to any of SEQ ID NOs: 14-16; an LCDR2 according to any of SEQ ID NOs: 17-19; an LCDR3 according to any one of SEQ ID NOs: 20-22;
- (ii) an HCDR1 according to any of SEQ ID NOs: 25-27; an HCDR2 according to any of SEQ ID NOs: 28-30; an HCDR3 according to any one of SEQ ID NOs: 31-33; an LCDR1 according to any of SEQ ID NOs: 36-38; an LCDR2 according to any of SEQ ID NOs: 39-41; an LCDR3 according to any one of SEQ ID NOs: 42-44;
- (iii) an HCDR1 according to any of SEQ ID NOs: 47-49; an HCDR2 according to any of SEQ ID NOs: 50-52; an HCDR3 according to any one of SEQ ID NOs: 53-55; an LCDR1 according to any of SEQ ID NOs: 58-60; an LCDR2 according to any of SEQ ID NOs: 61-63; an LCDR3 according to any one of SEQ ID NOs: 64-66;
- (iv) an HCDR1 according to any of SEQ ID NOs: 69-71; an HCDR2 according to any of SEQ ID NOs: 72-74; an HCDR3 according to any one of SEQ ID NOs: 75-77; an LCDR1 according to any of SEQ ID NOs: 80-82; an LCDR2 according to any of SEQ ID NOs: 83-85; an LCDR3 according to any one of SEQ ID NOs: 86-88;

(v) an HCDR1 according to any of SEQ ID NOs: 91-93; an HCDR2 according to any of SEQ ID NOs: 94-96; an HCDR3 according to any one of SEQ ID NOs: 97-99; an LCDR1 according to any of SEQ ID NOs: 102-104; an LCDR2 according to any of SEQ ID NOs: 105-107; an LCDR3 according to any one of SEQ ID NOs: 108-110;

(vi) an HCDR1 according to any of SEQ ID NOs: 113-115; an HCDR2 according to any of SEQ ID NOs: 116-118; an HCDR3 according to any one of SEQ ID NOs: 119-121; an LCDR1 according to any of SEQ ID NOs: 124-126; an LCDR2 according to any of SEQ ID NOs: 127-129; an LCDR3 according to any one of SEQ ID NOs: 130-132;

(vii) an HCDR1 according to any of SEQ ID NOs: 135-137; an HCDR2 according to any of SEQ ID NOs: 138-140; an HCDR3 according to any one of SEQ ID NOs: 141-143; an LCDR1 according to any of SEQ ID NOs: 146-148; an LCDR2 according to any of SEQ ID NOs: 149-151; an LCDR3 according to any one of SEQ ID NOs: 152-154;

(viii) an HCDR1 according to any of SEQ ID NOs: 157-159; an HCDR2 according to any of SEQ ID NOs: 160-162; an HCDR3 according to any one of SEQ ID NOs: 163-165; an LCDR1 according to any of SEQ ID NOs: 168-170; an LCDR2 according to any of SEQ ID NOs: 171-173; an LCDR3 according to any one of SEQ ID NOs: 174-176;

(ix) an HCDR1 according to any of SEQ ID NOs: 179-181; an HCDR2 according to any of SEQ ID NOs: 182-184; an HCDR3 according to any one of SEQ ID NOs: 185-187; an LCDR1 according to any of SEQ ID NOs: 190-192; an LCDR2 according to any of SEQ ID NOs: 193-195; an LCDR3 according to any one of SEQ ID NOs: 196-198; or

(x) an HCDR1 according to any of SEQ ID NOs: 201-203; an HCDR2 according to any of SEQ ID NOs: 204-206; an HCDR3 according to any one of SEQ ID NOs: 207-209; an LCDR1 according to any of SEQ ID NOs: 212-214; an LCDR2 according to any of SEQ ID NOs: 215-217; an LCDR3 according to any one of SEQ ID NOs: 218-220.

5. The antigen binding system, antibody, or antigen binding fragment thereof of any one of claims 1-4, wherein the antigen binding system, antibody, or antigen binding fragment thereof comprises a first heavy chain variable domain comprising the three HCDRs and a light chain variable domain comprising the three LCDRs, wherein:

(i) the heavy chain variable domain is at least 80% identical to SEQ ID NO: 1, SEQ ID NO: 23, SEQ ID NO: 45, SEQ ID NO: 67, SEQ ID NO: 89, SEQ ID NO: 111, SEQ ID NO: 133, SEQ ID NO: 155, SEQ ID NO: 177, or SEQ ID NO: 199; and

(ii) the light chain variable domain is at least 80% identical to SEQ ID NO: 12,

SEQ ID NO: 34, SEQ ID NO: 56, SEQ ID NO: 78, SEQ ID NO: 100, SEQ ID NO: 122, SEQ ID NO: 144, SEQ ID NO: 166, SEQ ID NO: 188, or SEQ ID NO: 210.

6. The antigen binding system, antibody, or antigen binding fragment thereof of claim 5, wherein the antigen binding system, antibody, or antigen binding fragment thereof comprises a first heavy chain variable domain comprising the three HCDRs and a light chain variable domain comprising the three LCDRs, wherein:

(i) the heavy chain variable domain is at least 80% identical to SEQ ID NO: 1 and the light chain variable domain is at least 80% identical to SEQ ID NO: 12;

(ii) the heavy chain variable domain is at least 80% identical to SEQ ID NO: 23 and the light chain variable domain is at least 80% identical to SEQ ID NO: 34;

(iii) the heavy chain variable domain is at least 80% identical to SEQ ID NO: 45 and the light chain variable domain is at least 80% identical to SEQ ID NO: 56;

(iv) the heavy chain variable domain is at least 80% identical to SEQ ID NO: 67 and the light chain variable domain is at least 80% identical to SEQ ID NO: 78;

(v) the heavy chain variable domain is at least 80% identical to SEQ ID NO: 89 and the light chain variable domain is at least 80% identical to SEQ ID NO: 100;

(vi) the heavy chain variable domain is at least 80% identical to SEQ ID NO: 111 and the light chain variable domain is at least 80% identical to SEQ ID NO: 122;

(vii) the heavy chain variable domain is at least 80% identical to SEQ ID NO: 133 and the light chain variable domain is at least 80% identical to SEQ ID NO: 144;

(viii) the heavy chain variable domain is at least 80% identical to SEQ ID NO: 155 and the light chain variable domain is at least 80% identical to SEQ ID NO: 166;

(ix) the heavy chain variable domain is at least 80% identical to SEQ ID NO: 177 and the light chain variable domain is at least 80% identical to SEQ ID NO: 188; or

(x) the heavy chain variable domain is at least 80% identical to SEQ ID NO: 199 and the light chain variable domain is at least 80% identical to SEQ ID NO: 210.

7. The antigen binding system, antibody, or antigen binding fragment thereof of any one of claims 1-6, wherein the three HCDRs and the three LCDRs are comprised by a single polypeptide.

8. The antigen binding system, antibody, or antigen binding fragment thereof of any one of claims 1-6, wherein the three HCDRs are comprised by a first polypeptide and the three LCDRs are comprised by a second polypeptide.

9. The antigen binding system, antibody, or antigen binding fragment thereof of claim 8, wherein the first polypeptide is an antibody heavy chain and the second polypeptide is an antibody light chain.

10. The antigen binding system, antibody, or antigen binding fragment thereof of any one of claims 1-9, wherein the antigen binding system, antibody, or antigen binding fragment thereof further comprises:

- (i) a binding motif that specifically binds an antigen selected from the group consisting of 5T4, alphafetoprotein, B cell maturation antigen (BCMA), B cell receptor, CA-125, carcinoembryonic antigen, CD19, CD20, CD22, CD23, CD30, CD33, CD40, CD56, CD79, CD78, CD123, CD138, c-Met, CSPG4, IgM, C-type lectin-like molecule 1 (CLL-1), EGFRvIII, epithelial tumor antigen, ERBB2, FLT3, folate binding protein, GD2, GD3, HER1-HER2 in combination, HER2-HER3 in combination, HER2/Neu, HERV-K, HIV-1 envelope glycoprotein gp41, HIV-1 envelope glycoprotein gpl20, IL-11Ralpha, kappa chain, lambda chain, melanoma-associated antigen, mesothelin, MUC-1, mutated p53, mutated ras, prostate-specific antigen, ROR1, VEGFR2, EphA3 (EPH receptor A3), BAFFR (B-cell activating factor receptor), and combinations thereof; and/or
- (ii) a binding motif that specifically binds an antigen that is characteristic of B-cells, optionally wherein the antigen that is characteristic of B-cells is not CD19 or CD20.

11. The antigen binding system, antibody, or antigen binding fragment thereof of any one of claims 1-9, wherein the antigen binding system, antibody, or antigen binding fragment thereof further comprises an anti-CD19 binding motif.

12. The antigen binding system, antibody, or antigen binding fragment thereof of claim 11, wherein the anti-CD19 binding motif comprises a first domain comprising three HCDRs and a second domain comprising three LCDRs, wherein:

the three HCDRs of the anti-CD19 binding motif comprise an HCDR1, an HCDR2, and an HCDR3;

the three LCDRs of the anti-CD19 binding motif comprise an LCDR1, an LCDR2, and an LCDR3; and

the HCDRs and LCDRs of the anti-CD19 binding motif comprise an HCDR1 according to any of SEQ ID NOs: 223-225; an HCDR2 according to any of SEQ ID NOs: 226-228; an HCDR3 according to any one of SEQ ID NOs: 229-231; an LCDR1 according to any of SEQ ID NOs: 234-236; an LCDR2 according to any of SEQ ID NOs: 237-239; an LCDR3 according to any one of SEQ ID NOs: 240-242.

13. The antigen binding system, antibody, or antigen binding fragment thereof of claim 12, wherein the anti-CD19 binding motif comprises a first heavy chain variable domain comprising the three HCDRs of the anti-CD19 binding motif and a light chain variable domain comprising the three LCDRs of the anti-CD19 binding motif, wherein the heavy chain variable domain of the anti-CD19 binding motif is at least 80% identical to SEQ ID NO: 221 and the light chain variable domain of the anti-CD19 binding motif is at least 80% identical to SEQ ID NO: 232.

14. The antigen binding system, antibody, or antigen binding fragment thereof of claim 12 or claim 13, wherein the three HCDRs of the anti-CD19 binding motif and the three LCDRs of the anti-CD19 binding motif are comprised by a single polypeptide.

15. The antigen binding system, antibody, or antigen binding fragment thereof of any one of claims 12-14, wherein the three HCDRs of the anti-CD20 binding motif, the three LCDRs of the anti-CD20 binding motif, the three HCDRs of the anti-CD19 binding motif, and the three LCDRs of the anti-CD19 binding motif are together comprised by a single polypeptide.

16. The antigen binding system, antibody, or antigen binding fragment thereof of any one of claims 1-15, wherein the antigen binding system, antibody, or antigen binding fragment thereof is, or is comprised by, a chimeric antigen receptor.

17. The antigen binding system, antibody, or antigen binding fragment thereof of claim 16, wherein the chimeric antigen receptor comprises a transmembrane domain that is a transmembrane domain of 4-1BB/CD137, an alpha chain of a T cell receptor, a beta chain of a T cell receptor, CD3 epsilon, CD4, CD5, CD8 alpha, CD9, CD16, CD19, CD22, CD28, CD33, CD37, CD45, CD64, CD80, CD86, CD134, CD137, CD154, or a zeta chain of a T cell receptor, or any combination thereof.

18. The antigen binding system, antibody, or antigen binding fragment thereof of any one of claims 11-14, wherein (i) the three HCDRs of the anti-CD20 binding motif and the three LCDRs of the anti-CD20 binding motif are present in a first polypeptide and (ii) the three HCDRs of the anti-CD19 binding motif and the three LCDRs of the anti-CD19 binding motif are together comprised by a second different polypeptide.

19. The antigen binding system, antibody, or antigen binding fragment thereof of claim 18, wherein the first polypeptide is, or is comprised by, a first chimeric antigen receptor.

20. The antigen binding system, antibody, or antigen binding fragment thereof of claims 18 or claim 19, wherein the second polypeptide is, or is comprised by, a second chimeric antigen receptor.

21. A nucleic acid encoding at least one polypeptide of any one of claims 1-20.

22. A vector comprising the nucleic acid of claim 21.

23. A method of generating an engineered cell, the method comprising transfecting or transducing a cell with a nucleic acid according to claim 21 or a vector according to claim 22.

24. A cell encoding or expressing the antigen binding system, antibody, or antigen binding fragment thereof of any one of claims 1-20, optionally wherein the cell is an immune cell, optionally wherein the cell is a T cell.

25. A method of treating cancer in a subject in need thereof, the method comprising administering to the subject a cell therapy composition comprising one or more cells that encode or comprise an antigen binding system, antibody, or antigen binding fragment thereof of any one of claims 1-20.

26. A method of inducing an immune response in a subject or immunizing a subject against a cancer, the method comprising administering to the subject a cell therapy composition comprising one or more cells that encode or comprise an antigen binding system, antibody, or antigen binding fragment thereof of any one of claims 1-20.

27. The method of claim 25 or 26, wherein the cells are CAR-T cells.

28. The method of any one of claims 25-27, wherein the cancer is acute lymphoblastic leukemia (ALL) (including non T cell ALL), acute myeloid leukemia, B cell prolymphocytic leukemia, B cell acute lymphoid leukemia ("BALL"), blastic plasmacytoid dendritic cell neoplasm, Burkitt's lymphoma, chronic lymphocytic leukemia (CLL), chronic myelogenous leukemia (CML), chronic myeloid leukemia, chronic or acute leukemia, diffuse large B cell lymphoma (DLBCL), follicular lymphoma (FL), hairy cell leukemia, Hodgkin's Disease, malignant lymphoproliferative conditions, MALT lymphoma, mantle cell lymphoma, Marginal zone lymphoma, monoclonal gammopathy of undetermined significance (MGUS), multiple myeloma, myelodysplasia and myelodysplastic syndrome, non-Hodgkin's lymphoma (NHL), plasma cell proliferative disorder (including asymptomatic myeloma (smoldering multiple myeloma or indolent myeloma), plasmablastic lymphoma, plasmacytoid dendritic cell neoplasm, plasmacytomas (including plasma cell dyscrasia; solitary myeloma; solitary plasmacytoma; extramedullary plasmacytoma; and multiple plasmacytoma), POEMS syndrome (also known as Crow-Fukase syndrome; Takatsuki disease; and PEP syndrome), primary mediastinal large B cell lymphoma (PMBC), small cell- or a large cell-follicular lymphoma, splenic marginal zone lymphoma (SMZL), systemic amyloid light chain amyloidosis, T cell acute lymphoid leukemia ("TALL"), T cell lymphoma, transformed follicular lymphoma, or Waldenstrom macroglobulinemia, Mantle

cell lymphoma (MCL), Transformed follicular lymphoma (TFL), Primary mediastinal B cell lymphoma (PMBCL), Multiple myeloma, Hairy cell lymphoma/leukemia, or a combination thereof.

29. The method of any one of claims 25-28, wherein the cell therapy is an allogeneic cell therapy or an autologous cell therapy.