CHIMERIC ANTIGEN RECEPTORS TARGETING B-CELL MATURATION ANTIGEN

Field

The invention relates to chimeric antigen receptors (CAR). CARs are able to redirect immune cell specificity and reactivity toward a selected target exploiting the ligand-binding domain properties. In particular, the invention relates to CARs that specifically bind to B-Cell Maturation Antigen (BCMA specific CARs). The invention further relates to polynucleotides encoding BCMA specific CAR and isolated cells expressing BCMA specific CARs at their surface. The invention further relates to methods for engineering immune cells expressing BCMA specific CARs at their surface. The invention is particularly useful for the treatment of B-cell lymphomas and leukemia. The invention further relates to immune cells comprising the BCMA specific CARs (BCMA specific CAR-T cells), compositions comprising the BCMA specific CAR-T cells, and methods of using the BCMA specific CAR-T cells for treating conditions associated with malignant cells expressing BCMA (e.g., cancer).

Background

Multiple myeloma is a malignancy characterized by an accumulation of clonal plasma cells (see, e.g., Lonial et al., Clinical Cancer Res., 77(6): 1264-1277 (2011)). Current therapies for MM often cause remissions, but nearly all patients eventually relapse and die (see, e.g., Rajkumar, Nature Rev. Clinical Oncol, 5(8): 479-491 (2011)).

Adoptive transfer of T cells genetically modified to recognize malignancy-associated antigens is showing promise as a new approach to treating cancer (see, e.g., Brenner et al., Current Opinion in Immunology, 22(2): 251-257 (2010); Rosenberg et al., Nature Reviews Cancer, 8(4): 299-308 (2008)). T cells can be genetically modified to express chimeric antigen receptors (CARs), which are fusion proteins comprised of an antigen recognition moiety and T cell activation domains (see, e.g., Eshhar et al., Proc. Natl. Acad. Sci. USA, 90(2): 720-724 (1993), and Sadelain et al., Curr. Opin. Immunol, 21(2): 215-223 (2009)).

B-cell maturation antigen (BCMA, CD269, or TNFRSF17) is a member of the tumor necrosis factor receptor (TNFR) superfamily. BCMA was identified in a malignant human T cell lymphoma containing a t(4;16) translocation. The gene is selectively expressed in the

B-cell lineage with the highest expression in plasmablasts and plasma cells, antibody secreting cells. BCMA binds two ligands, B-cell activation factor (BAFF) (also called B-lymphoctye stimulator (BLyS) and APOL-related leukocyte expressed ligand (TALL-1)) and a proliferation-inducing ligand (APRIL) with affinity of 1uM and 16 nM, respectively. Binding of APRIL or BAFF to BCMA promotes a signaling cascade involving NF-kappa B, Elk-1, c-Jun N-terminal kinase and the p38 mitogen-activated protein kinase, which produce signals for cell survival and proliferation. BCMA is also expressed on malignant B cells and several cancers that involve B lymphocytes including multiple myeloma, plasmacytoma, Hodgkin's Lymphoma, and chronic lymphocytic leukemia. In autoimmune diseases where plasmablasts are involved such as systemic lupus erythematosus (SLE) and rheumatoid arthritis, BCMA expressing antibody-producing cells secrete autoantibodies that attack self.

In the case of multiple myeloma, about 24,000 new cases are newly diagnosed in the United States each year, and this number represents about 15% of the newly diagnosed hematological cancers in the United States. An average of 11,000 deaths result from multiple myeloma each year, and the average 5-year survival rate is about 44%, with median survival of 50-55 months. Current treatment for multiple myeloma is focused on plasma cells apoptosis and/or decreasing osteoclast activity (e.g., chemotherapy, thalidomide, lenalidomide, bisphosphonates, and/or proteasome inhibitors such as bortezomib (VELCADE®) or carfilzomib). However, multiple myeloma remains an incurable disease, and almost all patients have developed resistance to these agents and eventually relapse. Accordingly, an alternative treatment to multiple myeloma, such as using an anti-BCMA antagonist including BCMA specific CARs and BCMA specific CAR-T cells, would make a superior therapeutic agent.

Summary

Chimeric antigen receptors (CARs) that bind to BCMA are provided. It is demonstrated that certain BCMA specific CARs are effective when expressed in T cells to activate T cells upon contact with BCMA. Advantageously, the BCMA specific CARs provided herein bind human and cynomolgous monkey BCMA. Also advantageously, the BCMA specific CAR-T cells provided herein exhibit degranulation activity, increased

interferon gamma production, and/or cytotoxic activity upon contact with BCMA-expressing cells.

In one aspect, the invention provides a BCMA specific CAR comprising an extracellular ligand-binding domain, a first transmembrane domain, and an intracellular signaling domain, wherein the extracellular ligand-binding domain domain comprises (a) a heavy chain variable (VH) region comprising (i) a VH complementarity determining region one (CDR1) comprising the sequence SYX_1MX_2, wherein X_1 is A or P; and X_2 is T, N, or S (SEQ ID NO: 301), $GFTFX_1SY$, wherein X_1 is G or S (SEQ ID NO: 302), or $GFTFX_1SYX_2MX_3$, wherein X_1 is G or S, X_2 is A or P; and X_3 is T, N, or S (SEQ ID NO: 303); (ii) a VH CDR2 comprising the sequence $AX_1X_2X_3X_4GX_5X_6X_7X_8YADX_9X_{10}KG$, wherein X_1 is I, V, T, H, L, A, or C; X_2 is S, D, G, T, I, L, F, M, or V; X_3 is G, Y, L, H, D, A, S, or M; X_4 is S, Q, T, A, F, or W; X_5 is G or T;X_6 is N, S, P, Y, W, or F; X_7 is S, T, I, L, T, A, R, V, K, G, or C; X_8 is F, Y, P, W, H, or G; X_9 is V, R, or L; and X_{10} is G or T (SEQ ID NO: 305), or $X_1X_2X_3X_4X_5X_6$, wherein X_1 is S, V, I, D, G, T, L, F, or M; X_2 is G, Y, L, H, D, A, S, or M; X_3 is S, G, F, or W; X_4 is G or S; X_5 is G or T; and X_6 is N, S, P, Y, or W (SEQ ID NO: 306); and iii) a VH CDR3 comprising the sequence $VSPIX_1X_2X_3X_4$, wherein X_1 is A or Y; X_2 is A or S; and X_3 is G, Q, L, P, or E (SEQ ID NO: 307), or $YWPMX_1X_2$, wherein X_1 is D, S, T, or A; and X_2 is I, S, L, P, or D (SEQ ID NO: 308); and/or (b) a light chain variable (VL) region comprising (i) a VL CDR1 comprising the sequence $X_1X_2X_3X_4X_5X_6X_7X_8X_9X_{10}X_{11}X_{12}$, wherein X_1 is R, G, W, A, or C; X_2 is A, P, G, L, C, or S; X_3 is S, G, or R; X_4 is Q, C, E, V, or I; X_5 is S, L, P, G, A, R, or D; X_6 is V, G, or I; X_7 is S, E, D, or P; X_8 is S, P, F, A, M, E, V, N, D, or Y; X_9 is I, T, V, E, S, A, M, Q, Y, H, or R; X_{10} is Y or F; X_{11} is L, W, or P; and X_{12} is A, S, or G (SEQ ID NO: 309); (ii) a VL CDR2 comprising the sequence $X_1ASX_2RAX_3$, wherein X_1 is G or D; X_2 is S or I; and X_3 is T or P (SEQ ID NO: 310); and (iii) a VL CDR3 comprising the sequence $QQYX_1X_2X_3PX_4T$, wherein X_1 is G, Q, E, L, F, A, S, M, K, R, or Y; X_2 is S, R, T, G, V, F, Y, D, A, H, V, E, K, or C; X_3 is W, F, or S; and X_4 is L or I (SEQ ID NO: 311), or $QQYX_1X_2X_3PX_4$, wherein X_1 is G, Q, E, L, F, A, S, M, R, K, or Y; X_2 is S, R, T, G, R, V, D, A, H, E, K, C, F, or Y; X_3 is W, S, or F; and X_4 is L or I (SEQ ID NO: 312).

In another aspect, the invention provides a BCMA specific CAR comprising an extracellular ligand-binding domain, a first transmembrane domain, and an intracellular signaling domain, wherein the extracellular domain comprises a single chain Fv fragment

(scFv) comprising a heavy chain variable (VH) region comprising three CDRs from the VH region comprising the sequence shown in SEQ ID NO: 33, 72, 39, 76, 83, 92, 25, or 8; and a light chain variable (VL) region comprising three CDRs from the VL region shown in SEQ ID NO: 34, 73, 40, 77, 84, 93, 18, or 80. In some embodiments, the VH region can comprise the sequence shown in SEQ ID NO: 33, 72, 39, 76, 83, 92, 25, or 8, or a variant thereof with one or several conservative amino acid substitutions in residues that are not within a CDR and/or the VL region can comprise the amino acid sequence shown in SEQ ID NO: 34, 73, 40, 77, 84, 93, 18, or 80, or a variant thereof with one or several amino acid substitutions in amino acids that are not within a CDR.

In some embodiments, the extracellular ligand-binding domain domain of a BCMA specific CAR provided herein comprises (a) a heavy chain variable (VH) region comprising (i) a VH complementarity determining region one (CDR1) comprising the sequence shown in SEQ ID NO: 150, 151, 152, 156, 157, 129, 130, or 131; (ii) a VH CDR2 comprising the sequence shown in 153, 154, 187, 188, 165, 166, 162, 159, 190, 191, 169, 154, 139, 140, 132, or 133; and (iii) a VH CD3 comprising the sequence shown in 155, 161, 134, or 137; and/or (b) a light chain variable region (VL) comprising (i) a VL CDR1 comprising the sequence shown in SEQ ID NO: 209, 249, 226, 251, 262, 271, 217, or 377; (ii) a VL CDR2 comprising the sequence shown in SEQ ID NO: 221, 252, or 210; and (iii) a VL CDR3 comprising the sequence shown in SEQ ID NO: 222, 225, 227, 253, 263, 272, 216, or 214.

In some embodiments, the extracellular ligand-binding domain domain of a BCMA specific CAR provided herein comprises (a) a heavy chain variable (VH) region comprising (i) a VH complementarity determining region one (CDR1) comprising the sequence shown in SEQ ID NO: 150, 151, or 152; (ii) a VH CDR2 comprising the sequence shown in 153 or 154; and (iii) a VH CD3 comprising the sequence shown in 155; and/or (b) a light chain variable region (VL) comprising (i) a VL CDR1 comprising the sequence shown in SEQ ID NO: 209; (ii) a VL CDR2 comprising the sequence shown in SEQ ID NO: 221, and (iii) a VL CDR2 comprising the sequence shown in SEQ ID NO: 222.

In some embodiments, the extracellular ligand-binding domain domain of a BCMA specific CAR provided herein comprises (a) a heavy chain variable (VH) region comprising (i) a VH complementarity determining region one (CDR1) comprising the sequence shown in SEQ ID NO: 150, 151, or 152; (ii) a VH CDR2 comprising the sequence shown in 187 or

188; and (iii) a VH CD3 comprising the sequence shown in 155; and/or (b) a light chain variable region (VL) comprising (i) a VL CDR1 comprising the sequence shown in SEQ ID NO: 249; (ii) a VL CDR2 comprising the sequence shown in SEQ ID NO: 221, and (iii) a VL CDR3 comprising the sequence shown in SEQ ID NO: 225.

In some embodiments, the extracellular ligand-binding domain domain of a BCMA specific CAR provided herein comprises (a) a heavy chain variable (VH) region comprising (i) a VH complementarity determining region one (CDR1) comprising the sequence shown in SEQ ID NO: 150, 151, or 152; (ii) a VH CDR2 comprising the sequence shown in 165 or 166; and (iii) a VH CD3 comprising the sequence shown in 155; and/or (b) a light chain variable region (VL) comprising (i) a VL CDR1 comprising the sequence shown in SEQ ID NO: 226; (ii) a VL CDR2 comprising the sequence shown in SEQ ID NO: 221, and (iii) a VL CDR3 comprising the sequence shown in SEQ ID NO: 227.

In some embodiments, the extracellular ligand-binding domain domain of a BCMA specific CAR provided herein comprises (a) a heavy chain variable (VH) region comprising (i) a VH complementarity determining region one (CDR1) comprising the sequence shown in SEQ ID NO: 156, 151, or 157; (ii) a VH CDR2 comprising the sequence shown in 162 or 159; and (iii) a VH CD3 comprising the sequence shown in 161; and/or (b) a light chain variable region (VL) comprising (i) a VL CDR1 comprising the sequence shown in SEQ ID NO: 251; (ii) a VL CDR2 comprising the sequence shown in SEQ ID NO: 252, and (iii) a VL CDR3 comprising the sequence shown in SEQ ID NO: 253.

In some embodiments, the extracellular ligand-binding domain domain of a BCMA specific CAR provided herein comprises (a) a heavy chain variable (VH) region comprising (i) a VH complementarity determining region one (CDR1) comprising the sequence shown in SEQ ID NO: 156, 151, or 157; (ii) a VH CDR2 comprising the sequence shown in 190 or 191; and (iii) a VH CD3 comprising the sequence shown in 161; and/or (b) a light chain variable region (VL) comprising (i) a VL CDR1 comprising the sequence shown in SEQ ID NO: 262; (ii) a VL CDR2 comprising the sequence shown in SEQ ID NO: 252, and (iii) a VL CDR3 comprising the sequence shown in SEQ ID NO: 263.

In some embodiments, the extracellular ligand-binding domain domain of a BCMA specific CAR provided herein comprises (a) a heavy chain variable (VH) region comprising (i) a VH complementarity determining region one (CDR1) comprising the sequence shown

in SEQ ID NO: 150, 151, or 152; (ii) a VH CDR2 comprising the sequence shown in 169 or 154; and (iii) a VH CD3 comprising the sequence shown in 155; and/or (b) a light chain variable region (VL) comprising (i) a VL CDR1 comprising the sequence shown in SEQ ID NO: 271; (ii) a VL CDR2 comprising the sequence shown in SEQ ID NO: 221, and (iii) a VL CDR3 comprising the sequence shown in SEQ ID NO: 272.

In some embodiments, the extracellular ligand-binding domain domain of a BCMA specific CAR provided herein comprises (a) a heavy chain variable (VH) region comprising (i) a VH complementarity determining region one (CDR1) comprising the sequence shown in SEQ ID NO: 129, 130, or 131; (ii) a VH CDR2 comprising the sequence shown in 139 or 140; and (iii) a VH CD3 comprising the sequence shown in 134; and/or (b) a light chain variable region (VL) comprising (i) a VL CDR1 comprising the sequence shown in SEQ ID NO: 217; (ii) a VL CDR2 comprising the sequence shown in SEQ ID NO: 210, and (iii) a VL CDR3 comprising the sequence shown in SEQ ID NO: 216.

In some embodiments, the extracellular ligand-binding domain domain of a BCMA specific CAR provided herein comprises (a) a heavy chain variable (VH) region comprising (i) a VH complementarity determining region one (CDR1) comprising the sequence shown in SEQ ID NO: 129, 130, or 131; (ii) a VH CDR2 comprising the sequence shown in 132 or 133; and (iii) a VH CD3 comprising the sequence shown in 137; and/or (b) a light chain variable region (VL) comprising (i) a VL CDR1 comprising the sequence shown in SEQ ID NO: 377; (ii) a VL CDR2 comprising the sequence shown in SEQ ID NO: 210, and (iii) a VL CDR3 comprising the sequence shown in SEQ ID NO: 214.

In some embodiments, the intracellular signaling domain comprises a CD3ζ signalling domain. In some embodiments, the intracellular signaling domain comprises a 4-1BB domain. In some embodiments, the CAR can further comprise another intracellular signaling domain. In some embodiments, the additional intracellular signaling domain can comprise a 4-1BB domain.

In some embodiments, the CAR can comprise a stalk domain between the extracellular ligand-binding domain and the first transmembrane domain. In some embodiments, the stalk domain can be selected from the group consisting of: a human CD8α hinge, an IgG1 hinge, and an FcγRIIIα hinge.

In some embodiments, the first transmembrane domain can comprise a CD8α chain transmembrane domain.

In some embodiments, the CAR can comprise a CD20 epitope.

In some embodiments, the CAR can comprise another extracellular ligand-binding domain which is not specific for BCMA.

In some embodiments, the BCMA specific CAR can comprise the amino acid sequence shown in SEQ ID NO: 396.

In some embodiments of a CAR, the extracellular ligand-binding domain(s), the first transmembrane domain, and intracellular signaling domain(s) are on a single polypeptide.

In some embodiments, the CAR can comprise a second transmembrane domain, wherein the first transmembrane domain and the extracellular ligand-binding domain(s) are on a first polypeptide, and wherein the second transmembrane domain and the intracellular signaling domain(s) are on a second polypeptide, wherein the first transmembrane domain comprises a transmembrane domain from the α chain of the high-affinity IgE receptor (FcεRI) and the second transmembrane domain comprises a transmembrane domain from the γ or β chain of FcεRI. In some embodiments, the CAR can comprise a third polypeptide comprising a third transmembrane domain fused to an intracellular signaling domain from a co-stimulatory molecule, wherein the third transmembrane domain comprises a transmembrane domain from the γ or β chain of FcεRI.

In another aspect, the invention provides an isolated polynucleotide comprising a nucleic acid sequence encoding a BCMA specific CAR as described herein.

In another aspect, the invention provides an expression vector comprising a nucleic acid sequence encoding a BCMA specific CAR antibody as described herein.

In another aspect, the invention provides engineered immune cell expressing at its cell surface membrane a BCMA specific CAR as described herein. In some embodiments, the engineered immunce cell can comprise another CAR which is not specific for BCMA. In some embodiments, the engineered immunce cell can comprise a polynucleotide encoding a suicide polypeptide. In some embodiments, the suicide polypeptide is RQR8.

In some embodiments, the immune cell can be derived from an inflammatory T-lymphocyte, a cytotoxic T-lymphocyte, a regulatory T- lymphocyte, or a helper T-lymphocyte.

In some embodiments, the engineered immune cell can comprise a disruption one or more endogenous genes, wherein the endogenous gene encodes TCRα, TCRβ, CD52, glucocorticoid receptor (GR), deoxycytidine kinase (DCK), or an immune checkpoint protein such as for example programmed death-1 (PD-1).

In some embodiments, immune cell is obtained from a healthy donor. In some embodiments, the immune cell is obtained from a patient.

In another aspect, the invention provides an engineered immune cell expressing at its cell surface membrane a BCMA specific CAR as described herein for use as a medicament. In some embodiments, the medicament is for use in treatment of a B-cell related cancer selecting from the group consisting of multiple myeloma, malignant plasma cell neoplasm, Hodgkin's lymphoma, nodular lymphocyte predominant Hodgkin's lymphoma, Kahler's disease and Myelomatosis, plasma cell leukemia, plasmacytoma, B-cell prolymphocytic leukemia, hairy cell leukemia, B-cell non-Hodgkin's lymphoma (NHL), acute myeloid leukemia (AML), chronic lymphocytic leukemia (CLL), acute lymphocytic leukemia (ALL), chronic myeloid leukemia (CML), follicular lymphoma, Burkitt's lymphoma, marginal zone lymphoma, mantle cell lymphoma, large cell lymphoma, precursor B-lymphoblastic lymphoma, myeloid leukemia, Waldenstrom's macroglobulienemia, diffuse large B cell lymphoma, follicular lymphoma, marginal zone lymphoma, mucosa-associated lymphatic tissue lymphoma, small cell lymphocytic lymphoma, mantle cell lymphoma, Burkitt lymphoma, primary mediastinal (thymic) large B-cell lymphoma, lymphoplasmactyic lymphoma, Waldenström macroglobulinemia, nodal marginal zone B cell lymphoma, splenic marginal zone lymphoma, intravascular large B-cell lymphoma, primary effusion lymphoma, lymphomatoid granulomatosis, T cell/histiocyte-rich large B-cell lymphoma, primary central nervous system lymphoma, primary cutaneous diffuse large B-cell lymphoma (leg type), EBV positive diffuse large B-cell lymphoma of the elderly, diffuse large B-cell lymphoma associated with inflammation, intravascular large B-cell lymphoma, ALK-positive large B-cell lymphoma, plasmablastic lymphoma, large B-cell lymphoma arising in HHV8-associated multicentric Castleman disease, B-cell lymphoma unclassified with features intermediate between diffuse large B-cell lymphoma and Burkitt lymphoma, B-cell lymphoma unclassified with features intermediate between diffuse large B-cell lymphoma and classical Hodgkin lymphoma, and other B-cell related lymphoma.

In another aspect, the invention provides a method of engineering an immune cell comprising: providing an immune cell; and expressing at the surface of the cell at least one BCMA specific CAR as described herein.

In some embodiments, the method comprises: providing an immune cell; introducing into the cell at least one polynucleotide encoding said BCMA specific CAR; and expressing said polynucleotide into the cell.

In some embodiments, the method comprises providing an immune cell; introducing into the cell at least one polynucleotide encoding said BCMA specific CAR; and introducing at least one other CAR which is not specific for BCMA.

In another aspect, the invention provides a method of treating a subject suffering from a condition associated with malignant cells, the method comprising: providing a immune cell expressing at the surface a BCMA specific CAR as described herein; and administering said immune cells to said patient.

In another aspect, the invention provides a pharmaceutical composition comprising an engineered immune cell as described herein.

In another aspect, the invention provides a method of treating a condition associated with malignant cells expressing BCMA in a subject comprising administering to a subject in need thereof an effective amount of a pharmaceutical composition of claim comprising an engineered immune cell as described herein. In some embodiments, the condition is a cancer. In some embodiments, the cancer is a B-cell related cancer selecting from the group consisting of multiple myeloma, malignant plasma cell neoplasm, Hodgkin's lymphoma, nodular lymphocyte predominant Hodgkin's lymphoma, Kahler's disease and Myelomatosis, plasma cell leukemia, plasmacytoma, B-cell prolymphocytic leukemia, hairy cell leukemia, B-cell non-Hodgkin's lymphoma (NHL), acute myeloid leukemia (AML), chronic lymphocytic leukemia (CLL), acute lymphocytic leukemia (ALL), chronic myeloid leukemia (CML), follicular lymphoma, Burkitt's lymphoma, marginal zone lymphoma, mantle cell lymphoma, large cell lymphoma, precursor B-lymphoblastic lymphoma, myeloid leukemia, Waldenstrom's macroglobulienemia, diffuse large B cell lymphoma, follicular lymphoma, marginal zone lymphoma, mucosa-associated lymphatic tissue lymphoma, small cell lymphocytic lymphoma, mantle cell lymphoma, Burkitt lymphoma, primary mediastinal (thymic) large B-cell lymphoma, lymphoplasmactyic lymphoma, Waldenström

macroglobulinemia, nodal marginal zone B cell lymphoma, splenic marginal zone lymphoma, intravascular large B-cell lymphoma, primary effusion lymphoma, lymphomatoid granulomatosis, T cell/histiocyte-rich large B-cell lymphoma, primary central nervous system lymphoma, primary cutaneous diffuse large B-cell lymphoma (leg type), EBV positive diffuse large B-cell lymphoma of the elderly, diffuse large B-cell lymphoma associated with inflammation, intravascular large B-cell lymphoma, ALK-positive large B-cell lymphoma, plasmablastic lymphoma, large B-cell lymphoma arising in HHV8-associated multicentric Castleman disease, B-cell lymphoma unclassified with features intermediate between diffuse large B-cell lymphoma and Burkitt lymphoma, B-cell lymphoma unclassified with features intermediate between diffuse large B-cell lymphoma and classical Hodgkin lymphoma, and other B-cell related lymphoma.

In another aspect, the invention provides a method of inhibiting tumor growth or progression in a subject who has malignant cells expressing BCMA, comprising administering to the subject in need thereof an effective amount of a pharmaceutical composition comprising an engineered immune cell as described herein.

In another aspect, the invention provides a method inhibiting metastasis of malignant cells expressing BCMA in a subject, comprising administering to the subject in need thereof an effective amount of the pharmaceutical composition comprising an engineered immune cell as described herein.

In another aspect, the invention provides a method inducing tumor regression in a subject who has malignant cells expressing BCMA, comprising administering to the subject in need thereof an effective amount of the pharmaceutical composition of a pharmaceutical composition comprising an engineered immune cell as described herein.

In some embodiments, any of the above methods further comprises administering one or more additional therapies, such as for example, a monoclonal antibody and/or a chemotherapeutic. In some embodiments, the monoclonal antibody can be, for example, an antibody that binds to a checkpoint inhibitor such as, for example, an anti-PD-1 antibody or an anti-PD-L1 antibody. In some embodiments, any of the above methods further comprises administering a nucleoside analog therapy, such as for example fludarabine or clofarabine, to the subject.

Brief Description of the Drawings

FIG. 1 depicts a graph summarizing the results of treatment with BCMA specific CAR-T in the MM1.S tumor model.

FIG. 2 depicts a graph summarizing the results of treatment with BCMA specific CAR-T in the Molp8 tumor model.

Detailed Description

The invention disclosed herein provides chimeric antigen receptors (CARs) and immune cells comprising CARs (CAR-T cells) that specifically bind to BCMA (e.g., human BCMA). The invention also provides polynucleotides encoding these CARs, compositions comprising these CAR-T cells, and methods of making and using these CARs and CAR-T cells. The invention also provides methods for treating a condition associated with malignant BCMA expression in a subject, such as cancer.

General Techniques

The practice of the invention will employ, unless otherwise indicated, conventional techniques of molecular biology (including recombinant techniques), microbiology, cell biology, biochemistry and immunology, which are within the skill of the art. Such techniques are explained fully in the literature, such as, Molecular Cloning: A Laboratory Manual, second edition (Sambrook et al., 1989) Cold Spring Harbor Press; Oligonucleotide Synthesis (M.J. Gait, ed., 1984); Methods in Molecular Biology, Humana Press; Cell Biology: A Laboratory Notebook (J.E. Cellis, ed., 1998) Academic Press; Animal Cell Culture (R.I. Freshney, ed., 1987); Introduction to Cell and Tissue Culture (J.P. Mather and P.E. Roberts, 1998) Plenum Press; Cell and Tissue Culture: Laboratory Procedures (A. Doyle, J.B. Griffiths, and D.G. Newell, eds., 1993-1998) J. Wiley and Sons; Methods in Enzymology (Academic Press, Inc.); Handbook of Experimental Immunology (D.M. Weir and C.C. Blackwell, eds.); Gene Transfer Vectors for Mammalian Cells (J.M. Miller and M.P. Calos, eds., 1987); Current Protocols in Molecular Biology (F.M. Ausubel et al., eds., 1987); PCR: The Polymerase Chain Reaction, (Mullis et al., eds., 1994); Current Protocols in Immunology (J.E. Coligan et al., eds., 1991); Short Protocols in Molecular Biology (Wiley and Sons, 1999); Immunobiology (C.A. Janeway and P. Travers, 1997); Antibodies (P.

Finch, 1997); Antibodies: a practical approach (D. Catty., ed., IRL Press, 1988-1989); Monoclonal antibodies: a practical approach (P. Shepherd and C. Dean, eds., Oxford University Press, 2000); Using antibodies: a laboratory manual (E. Harlow and D. Lane (Cold Spring Harbor Laboratory Press, 1999); The Antibodies (M. Zanetti and J.D. Capra, eds., Harwood Academic Publishers, 1995).

Definitions

The term "extracellular ligand-binding domain" as used herein refers to an oligo- or polypeptide that is capable of binding a ligand. Preferably, the domain will be capable of interacting with a cell surface molecule. For example, the extracellular ligand-binding domain may be chosen to recognize a ligand that acts as a cell surface marker on target cells associated with a particular disease state.

The term "stalk domain" or "hinge domain" are used interchangeably herein to refer to any oligo- or polypeptide that functions to link the transmembrane domain to the extracellular ligand- binding domain. In particular, stalk domains are used to provide more flexibility and accessibility for the extracellular ligand-binding domain.

The term "intracellular signaling domain" refers to the portion of a protein which transduces the effector signal function signal and directs the cell to perform a specialized function.

A "co-stimulatory molecule" as used herein refers to the cognate binding partner on a T cell that specifically binds with a co-stimulatory ligand, thereby mediating a co-stimulatory response by the cell, such as, but not limited to proliferation. Co-stimulatory molecules include, but are not limited to an MHC class I molecule, BTLA and Toll ligand receptor. Examples of costimulatory molecules include CD27, CD28, CD8, 4-1BB (CD137), OX40, CD30, CD40, PD-1, ICOS, lymphocyte function-associated antigen-1 (LFA-1), CD2, CD7, LIGHT, NKG2C, B7-H3 and a ligand that specifically binds with CD83 and the like.

A "co-stimulatory ligand" refers to a molecule on an antigen presenting cell that specifically binds a cognate co-stimulatory signal molecule on a T cell, thereby providing a signal which, in addition to the primary signal provided by, for instance, binding of a TCR/CD3 complex with an MHC molecule loaded with peptide, mediates a T cell response, including, but not limited to, proliferation activation, differentiation and the like. A co-

stimulatory ligand can include but is not limited to CD7, B7-1 (CD80), B7-2 (CD86), PD-L1, PD-L2, 4-1BBL, OX40L, inducible costimulatory igand (ICOS-L), intercellular adhesion molecule (ICAM, CD30L, CD40, CD70, CD83, HLA-G, MICA, M1CB, HVEM, lymphotoxin β receptor, 3/TR6, ILT3, ILT4, an agonist or antibody that binds Toll ligand receptor and a ligand that specifically binds with B7-H3. A co-stimulatory ligand also encompasses, inter alia, an antibody that specifically binds with a co-stimulatory molecule present on a T cell, such as but not limited to, CD27, CD28, 4-1BB, OX40, CD30, CD40, PD-1, ICOS, lymphocyte function-associated antigen-1 (LFA-1), CD2, CD7, LTGHT, NKG2C, B7-H3, a ligand that specifically binds with CD83.

An "antibody" is an immunoglobulin molecule capable of specific binding to a target, such as a carbohydrate, polynucleotide, lipid, polypeptide, etc., through at least one antigen recognition site, located in the variable region of the immunoglobulin molecule. As used herein, the term encompasses not only intact polyclonal or monoclonal antibodies, but also fragments thereof (such as Fab, Fab', $F(ab')_2$, Fv), single chain (scFv) and domain antibodies (including, for example, shark and camelid antibodies), and fusion proteins comprising an antibody, and any other modified configuration of the immunoglobulin molecule that comprises an antigen recognition site. An antibody includes an antibody of any class, such as IgG, IgA, or IgM (or sub-class thereof), and the antibody need not be of any particular class. Depending on the antibody amino acid sequence of the constant region of its heavy chains, immunoglobulins can be assigned to different classes. There are five major classes of immunoglobulins: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into subclasses (isotypes), e.g., IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2. The heavy-chain constant regions that correspond to the different classes of immunoglobulins are called alpha, delta, epsilon, gamma, and mu, respectively. The subunit structures and three-dimensional configurations of different classes of immunoglobulins are well known.

The term "antigen binding fragment" or "antigen binding portion" of an antibody, as used herein, refers to one or more fragments of an intact antibody that retain the ability to specifically bind to a given antigen (e.g., BCMA). Antigen binding functions of an antibody can be performed by fragments of an intact antibody. Examples of binding fragments encompassed within the term "antigen binding fragment" of an antibody include Fab; Fab';

$F(ab')_2$; an Fd fragment consisting of the VH and CH1 domains; an Fv fragment consisting of the VL and VH domains of a single arm of an antibody; a single domain antibody (dAb) fragment (Ward et al., Nature 341:544-546, 1989), and an isolated complementarity determining region (CDR).

An antibody, an antibody conjugate, or a polypeptide that "preferentially binds" or "specifically binds" (used interchangeably herein) to a target (e.g., BCMA protein) is a term well understood in the art, and methods to determine such specific or preferential binding are also well known in the art. A molecule is said to exhibit "specific binding" or "preferential binding" if it reacts or associates more frequently, more rapidly, with greater duration and/or with greater affinity with a particular cell or substance than it does with alternative cells or substances. An antibody "specifically binds" or "preferentially binds" to a target if it binds with greater affinity, avidity, more readily, and/or with greater duration than it binds to other substances. For example, an antibody that specifically or preferentially binds to a BCMA epitope is an antibody that binds this epitope with greater affinity, avidity, more readily, and/or with greater duration than it binds to other BCMA epitopes or non-BCMA epitopes. It is also understood that by reading this definition, for example, an antibody (or moiety or epitope) that specifically or preferentially binds to a first target may or may not specifically or preferentially bind to a second target. As such, "specific binding" or "preferential binding" does not necessarily require (although it can include) exclusive binding. Generally, but not necessarily, reference to binding means preferential binding.

A "variable region" of an antibody refers to the variable region of the antibody light chain or the variable region of the antibody heavy chain, either alone or in combination. As known in the art, the variable regions of the heavy and light chain each consist of four framework regions (FR) connected by three complementarity determining regions (CDRs) also known as hypervariable regions. The CDRs in each chain are held together in close proximity by the FRs and, with the CDRs from the other chain, contribute to the formation of the antigen binding site of antibodies. There are at least two techniques for determining CDRs: (1) an approach based on cross-species sequence variability (i.e., Kabat et al. Sequences of Proteins of Immunological Interest, (5th ed., 1991, National Institutes of Health, Bethesda MD)); and (2) an approach based on crystallographic studies of antigen-antibody complexes (Al-lazikani et al., 1997, J. Molec. Biol. 273:927-948). As used herein,

a CDR may refer to CDRs defined by either approach or by a combination of both approaches.

A "CDR" of a variable domain are amino acid residues within the variable region that are identified in accordance with the definitions of the Kabat, Chothia, the accumulation of both Kabat and Chothia, AbM, contact, and/or conformational definitions or any method of CDR determination well known in the art. Antibody CDRs may be identified as the hypervariable regions originally defined by Kabat et al. See, e.g., Kabat et al., 1992, Sequences of Proteins of Immunological Interest, 5th ed., Public Health Service, NIH, Washington D.C. The positions of the CDRs may also be identified as the structural loop structures originally described by Chothia and others. See, e.g., Chothia et al., Nature 342:877-883, 1989. Other approaches to CDR identification include the "AbM definition," which is a compromise between Kabat and Chothia and is derived using Oxford Molecular's AbM antibody modeling software (now Accelrys®), or the "contact definition" of CDRs based on observed antigen contacts, set forth in MacCallum et al., J. Mol. Biol., 262:732-745, 1996. In another approach, referred to herein as the "conformational definition" of CDRs, the positions of the CDRs may be identified as the residues that make enthalpic contributions to antigen binding. See, e.g., Makabe et al., Journal of Biological Chemistry, 283:1156-1166, 2008. Still other CDR boundary definitions may not strictly follow one of the above approaches, but will nonetheless overlap with at least a portion of the Kabat CDRs, although they may be shortened or lengthened in light of prediction or experimental findings that particular residues or groups of residues or even entire CDRs do not significantly impact antigen binding. As used herein, a CDR may refer to CDRs defined by any approach known in the art, including combinations of approaches. The methods used herein may utilize CDRs defined according to any of these approaches. For any given embodiment containing more than one CDR, the CDRs may be defined in accordance with any of Kabat, Chothia, extended, AbM, contact, and/or conformational definitions.

As used herein, "monoclonal antibody" refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical except for possible naturally-occurring mutations that may be present in minor amounts. Monoclonal antibodies are highly specific, being directed against a single antigenic site. Furthermore, in contrast to polyclonal antibody

preparations, which typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody is directed against a single determinant on the antigen. The modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the invention may be made by the hybridoma method first described by Kohler and Milstein, Nature 256:495, 1975, or may be made by recombinant DNA methods such as described in U.S. Pat. No. 4,816,567. The monoclonal antibodies may also be isolated from phage libraries generated using the techniques described in McCafferty et al., Nature 348:552-554, 1990, for example.

As used herein, "humanized" antibody refers to forms of non-human (*e.g.* murine) antibodies that are chimeric immunoglobulins, immunoglobulin chains, or fragments thereof (such as Fv, Fab, Fab', $F(ab')_2$ or other antigen binding subsequences of antibodies) that contain minimal sequence derived from non-human immunoglobulin. Preferably, humanized antibodies are human immunoglobulins (recipient antibody) in which residues from a complementarity determining region (CDR) of the recipient are replaced by residues from a CDR of a non-human species (donor antibody) such as mouse, rat, or rabbit having the desired specificity, affinity, and capacity. In some instances, Fv framework region (FR) residues of the human immunoglobulin are replaced by corresponding non-human residues. Furthermore, the humanized antibody may comprise residues that are found neither in the recipient antibody nor in the imported CDR or framework sequences, but are included to further refine and optimize antibody performance. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin and all or substantially all of the FR regions are those of a human immunoglobulin consensus sequence. The humanized antibody optimally also will comprise at least a portion of an immunoglobulin constant region or domain (Fc), typically that of a human immunoglobulin. Preferred are antibodies having Fc regions modified as described in WO 99/58572. Other forms of humanized antibodies have one or more CDRs (CDR L1, CDR L2, CDR L3, CDR H1, CDR H2, or CDR H3) which are altered with respect

to the original antibody, which are also termed one or more CDRs "derived from" one or more CDRs from the original antibody.

As used herein, "human antibody" means an antibody having an amino acid sequence corresponding to that of an antibody produced by a human and/or which has been made using any of the techniques for making human antibodies known to those skilled in the art or disclosed herein. This definition of a human antibody includes antibodies comprising at least one human heavy chain polypeptide or at least one human light chain polypeptide. One such example is an antibody comprising murine light chain and human heavy chain polypeptides. Human antibodies can be produced using various techniques known in the art. In one embodiment, the human antibody is selected from a phage library, where that phage library expresses human antibodies (Vaughan et al., Nature Biotechnology, 14:309-314, 1996; Sheets et al., Proc. Natl. Acad. Sci. (USA) 95:6157-6162, 1998; Hoogenboom and Winter, J. Mol. Biol., 227:381, 1991; Marks et al., J. Mol. Biol., 222:581, 1991). Human antibodies can also be made by immunization of animals into which human immunoglobulin loci have been transgenically introduced in place of the endogenous loci, e.g., mice in which the endogenous immunoglobulin genes have been partially or completely inactivated. This approach is described in U.S. Pat. Nos. 5,545,807; 5,545,806; 5,569,825; 5,625,126; 5,633,425; and 5,661,016. Alternatively, the human antibody may be prepared by immortalizing human B lymphocytes that produce an antibody directed against a target antigen (such B lymphocytes may be recovered from an individual or from single cell cloning of the cDNA, or may have been immunized *in vitro).* See, e.g., Cole et al. Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, p. 77, 1985; Boerner et al., J. Immunol., 147 (1):86-95, 1991; and U.S. Pat. No. 5,750,373.

The term "chimeric antibody" is intended to refer to antibodies in which the variable region sequences are derived from one species and the constant region sequences are derived from another species, such as an antibody in which the variable region sequences are derived from a mouse antibody and the constant region sequences are derived from a human antibody.

The terms "polypeptide", "oligopeptide", "peptide" and "protein" are used interchangeably herein to refer to chains of amino acids of any length, preferably, relatively short (e.g., 10-100 amino acids). The chain may be linear or branched, it may comprise

modified amino acids, and/or may be interrupted by non-amino acids. The terms also encompass an amino acid chain that has been modified naturally or by intervention; for example, disulfide bond formation, glycosylation, lipidation, acetylation, phosphorylation, or any other manipulation or modification, such as conjugation with a labeling component. Also included within the definition are, for example, polypeptides containing one or more analogs of an amino acid (including, for example, unnatural amino acids, etc.), as well as other modifications known in the art. It is understood that the polypeptides can occur as single chains or associated chains.

A "monovalent antibody" comprises one antigen binding site per molecule (e.g., IgG or Fab). In some instances, a monovalent antibody can have more than one antigen binding sites, but the binding sites are from different antigens.

A "bivalent antibody" comprises two antigen binding sites per molecule (e.g., IgG). In some instances, the two binding sites have the same antigen specificities. However, bivalent antibodies may be bispecific.

A "bispecific," "dual-specific" or "bifunctional" antibody is a hybrid antibody having two different antigen binding sites. The two antigen binding sites of a bispecific antibody bind to two different epitopes, which may reside on the same or different protein targets.

Antibodies of the invention can be produced using techniques well known in the art, *e.g.*, recombinant technologies, phage display technologies, synthetic technologies or combinations of such technologies or other technologies readily known in the art (see, for example, Jayasena, S.D., Clin. Chem., 45: 1628-50, 1999 and Fellouse, F.A., et al, J. Mol. Biol., 373(4):924-40, 2007).

As known in the art, "polynucleotide," or "nucleic acid," as used interchangeably herein, refer to chains of nucleotides of any length, and include DNA and RNA. The nucleotides can be deoxyribonucleotides, ribonucleotides, modified nucleotides or bases, and/or their analogs, or any substrate that can be incorporated into a chain by DNA or RNA polymerase. A polynucleotide may comprise modified nucleotides, such as methylated nucleotides and their analogs. If present, modification to the nucleotide structure may be imparted before or after assembly of the chain. The sequence of nucleotides may be interrupted by non-nucleotide components. A polynucleotide may be further modified after polymerization, such as by conjugation with a labeling component. Other types of

modifications include, for example, "caps", substitution of one or more of the naturally occurring nucleotides with an analog, internucleotide modifications such as, for example, those with uncharged linkages (e.g., methyl phosphonates, phosphotriesters, phosphoamidates, carbamates, etc.) and with charged linkages (e.g., phosphorothioates, phosphorodithioates, etc.), those containing pendant moieties, such as, for example, proteins (e.g., nucleases, toxins, antibodies, signal peptides, poly-L-lysine, etc.), those with intercalators (e.g., acridine, psoralen, etc.), those containing chelators (e.g., metals, radioactive metals, boron, oxidative metals, etc.), those containing alkylators, those with modified linkages (e.g., alpha anomeric nucleic acids, etc.), as well as unmodified forms of the polynucleotide(s). Further, any of the hydroxyl groups ordinarily present in the sugars may be replaced, for example, by phosphonate groups, phosphate groups, protected by standard protecting groups, or activated to prepare additional linkages to additional nucleotides, or may be conjugated to solid supports. The 5' and 3' terminal OH can be phosphorylated or substituted with amines or organic capping group moieties of from 1 to 20 carbon atoms. Other hydroxyls may also be derivatized to standard protecting groups. Polynucleotides can also contain analogous forms of ribose or deoxyribose sugars that are generally known in the art, including, for example, 2'-O-methyl-, 2'-O-allyl, 2'-fluoro- or 2'-azido-ribose, carbocyclic sugar analogs, alpha- or beta-anomeric sugars, epimeric sugars such as arabinose, xyloses or lyxoses, pyranose sugars, furanose sugars, sedoheptuloses, acyclic analogs and abasic nucleoside analogs such as methyl riboside. One or more phosphodiester linkages may be replaced by alternative linking groups. These alternative linking groups include, but are not limited to, embodiments wherein phosphate is replaced by P(O)S("thioate"), P(S)S ("dithioate"), (O)NR_2 ("amidate"), P(O)R, P(O)OR', CO or CH_2 ("formacetal"), in which each R or R' is independently H or substituted or unsubstituted alkyl (1-20 C) optionally containing an ether (-O-) linkage, aryl, alkenyl, cycloalkyl, cycloalkenyl or araldyl. Not all linkages in a polynucleotide need be identical. The preceding description applies to all polynucleotides referred to herein, including RNA and DNA.

As known in the art a "constant region" of an antibody refers to the constant region of the antibody light chain or the constant region of the antibody heavy chain, either alone or in combination.

As used herein, "substantially pure" refers to material which is at least 50% pure (i.e., free from contaminants), more preferably, at least 90% pure, more preferably, at least 95% pure, yet more preferably, at least 98% pure, and most preferably, at least 99% pure.

A "host cell" includes an individual cell or cell culture that can be or has been a recipient for vector(s) for incorporation of polynucleotide inserts. Host cells include progeny of a single host cell, and the progeny may not necessarily be completely identical (in morphology or in genomic DNA complement) to the original parent cell due to natural, accidental, or deliberate mutation. A host cell includes cells transfected in vivo with a polynucleotide(s) of this invention.

As used herein, "immune cell" refers to a cell of hematopoietic origin functionally involved in the initiation and/or execution of innate and/or adaptative immune response.

As known in the art, the term "Fc region" is used to define a C-terminal region of an immunoglobulin heavy chain. The "Fc region" may be a native sequence Fc region or a variant Fc region. Although the boundaries of the Fc region of an immunoglobulin heavy chain might vary, the human IgG heavy chain Fc region is usually defined to stretch from an amino acid residue at position Cys226, or from Pro230, to the carboxyl-terminus thereof. The numbering of the residues in the Fc region is that of the EU index as in Kabat. Kabat et al., Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md., 1991. The Fc region of an immunoglobulin generally comprises two constant regions, CH2 and CH3.

As used in the art, "Fc receptor" and "FcR" describe a receptor that binds to the Fc region of an antibody. The preferred FcR is a native sequence human FcR. Moreover, a preferred FcR is one which binds an IgG antibody (a gamma receptor) and includes receptors of the FcγRI, FcγRII, and FcγRIII subclasses, including allelic variants and alternatively spliced forms of these receptors. FcγRII receptors include FcγRIIA (an "activating receptor") and FcγRIIB (an "inhibiting receptor"), which have similar amino acid sequences that differ primarily in the cytoplasmic domains thereof. FcRs are reviewed in Ravetch and Kinet, Ann. Rev. Immunol., 9:457-92, 1991; Capel et al., Immunomethods, 4:25-34, 1994; and de Haas et al., J. Lab. Clin. Med., 126:330-41, 1995. "FcR" also includes the neonatal receptor, FcRn, which is responsible for the transfer of maternal IgGs

to the fetus (Guyer et al., J. Immunol., 117:587, 1976; and Kim et al., J. Immunol., 24:249, 1994).

The term "compete", as used herein with regard to an antibody, means that a first antibody, or an antigen binding fragment (or portion) thereof, binds to an epitope in a manner sufficiently similar to the binding of a second antibody, or an antigen binding portion thereof, such that the result of binding of the first antibody with its cognate epitope is detectably decreased in the presence of the second antibody compared to the binding of the first antibody in the absence of the second antibody. The alternative, where the binding of the second antibody to its epitope is also detectably decreased in the presence of the first antibody, can, but need not be the case. That is, a first antibody can inhibit the binding of a second antibody to its epitope without that second antibody inhibiting the binding of the first antibody to its respective epitope. However, where each antibody detectably inhibits the binding of the other antibody with its cognate epitope or ligand, whether to the same, greater, or lesser extent, the antibodies are said to "cross-compete" with each other for binding of their respective epitope(s). Both competing and cross-competing antibodies are encompassed by the invention. Regardless of the mechanism by which such competition or cross-competition occurs (e.g., steric hindrance, conformational change, or binding to a common epitope, or portion thereof), the skilled artisan would appreciate, based upon the teachings provided herein, that such competing and/or cross-competing antibodies are encompassed and can be useful for the methods disclosed herein.

As used herein "autologous" means that cells, a cell line, or population of cells used for treating patients are originating from said patient or from a Human Leucocyte Antigen (HLA) compatible donor.

As used herein "allogeneic" means that cells or population of cells used for treating patients are not originating from said patient but from a donor.

As used herein, "treatment" is an approach for obtaining beneficial or desired clinical results. For purposes of this invention, beneficial or desired clinical results include, but are not limited to, one or more of the following: reducing the proliferation of (or destroying) neoplastic or cancerous cells, inhibiting metastasis of neoplastic cells, shrinking or decreasing the size of BCMA expressing tumor, remission of a BCMA associated disease (e.g., cancer), decreasing symptoms resulting from a BCMA associated disease (e.g.,

cancer), increasing the quality of life of those suffering from a BCMA associated disease (e.g., cancer), decreasing the dose of other medications required to treat a BCMA associated disease (e.g., cancer), delaying the progression of a BCMA associated disease (e.g., cancer), curing a BCMA associated disease (e..g, cancer), and/or prolong survival of patients having a BCMA associated disease (e.g., cancer).

"Ameliorating" means a lessening or improvement of one or more symptoms as compared to not administering a BCMA antibody or a BCMA antibody conjugate. "Ameliorating" also includes shortening or reduction in duration of a symptom.

As used herein, an "effective dosage" or "effective amount" of drug, compound, or pharmaceutical composition is an amount sufficient to effect any one or more beneficial or desired results. For prophylactic use, beneficial or desired results include eliminating or reducing the risk, lessening the severity, or delaying the outset of the disease, including biochemical, histological and/or behavioral symptoms of the disease, its complications and intermediate pathological phenotypes presenting during development of the disease. For therapeutic use, beneficial or desired results include clinical results such as reducing incidence or amelioration of one or more symptoms of various BCMA associated diseases or conditions (such as for example multiple myeloma), decreasing the dose of other medications required to treat the disease, enhancing the effect of another medication, and/or delaying the progression of the BCMA associated disease of patients. An effective dosage can be administered in one or more administrations. For purposes of this invention, an effective dosage of drug, compound, or pharmaceutical composition is an amount sufficient to accomplish prophylactic or therapeutic treatment either directly or indirectly. As is understood in the clinical context, an effective dosage of a drug, compound, or pharmaceutical composition may or may not be achieved in conjunction with another drug, compound, or pharmaceutical composition. Thus, an "effective dosage" may be considered in the context of administering one or more therapeutic agents, and a single agent may be considered to be given in an effective amount if, in conjunction with one or more other agents, a desirable result may be or is achieved.

An "individual" or a "subject" is a mammal, more preferably, a human. Mammals also include, but are not limited to, farm animals, sport animals, pets, primates, horses, dogs, cats, mice and rats.

As used herein, "vector" means a construct, which is capable of delivering, and, preferably, expressing, one or more gene(s) or sequence(s) of interest in a host cell. Examples of vectors include, but are not limited to, viral vectors, naked DNA or RNA expression vectors, plasmid, cosmid or phage vectors, DNA or RNA expression vectors associated with cationic condensing agents, DNA or RNA expression vectors encapsulated in liposomes, and certain eukaryotic cells, such as producer cells.

As used herein, "expression control sequence" means a nucleic acid sequence that directs transcription of a nucleic acid. An expression control sequence can be a promoter, such as a constitutive or an inducible promoter, or an enhancer. The expression control sequence is operably linked to the nucleic acid sequence to be transcribed.

As used herein, "pharmaceutically acceptable carrier" or "pharmaceutical acceptable excipient" includes any material which, when combined with an active ingredient, allows the ingredient to retain biological activity and is non-reactive with the subject's immune system. Examples include, but are not limited to, any of the standard pharmaceutical carriers such as a phosphate buffered saline solution, water, emulsions such as oil/water emulsion, and various types of wetting agents. Preferred diluents for aerosol or parenteral administration are phosphate buffered saline (PBS) or normal (0.9%) saline. Compositions comprising such carriers are formulated by well known conventional methods (see, for example, Remington's Pharmaceutical Sciences, 18th edition, A. Gennaro, ed., Mack Publishing Co., Easton, PA, 1990; and Remington, The Science and Practice of Pharmacy 21st Ed. Mack Publishing, 2005).

The term "k_{on}", as used herein, refers to the rate constant for association of an antibody to an antigen.

The term "k_{off} ", as used herein, refers to the rate constant for dissociation of an antibody from the antibody/antigen complex.

The term "K_D", as used herein, refers to the equilibrium dissociation constant of an antibody-antigen interaction.

Reference to "about" a value or parameter herein includes (and describes) embodiments that are directed to that value or parameter per se. For example, description referring to "about X" includes description of "X." Numeric ranges are inclusive of the numbers defining the range.

It is understood that wherever embodiments are described herein with the language "comprising," otherwise analogous embodiments described in terms of "consisting of" and/or "consisting essentially of" are also provided.

Where aspects or embodiments of the invention are described in terms of a Markush group or other grouping of alternatives, the invention encompasses not only the entire group listed as a whole, but each member of the group individually and all possible subgroups of the main group, but also the main group absent one or more of the group members. The invention also envisages the explicit exclusion of one or more of any of the group members in the claimed invention.

Unless otherwise defined, 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 invention belongs. In case of conflict, the present specification, including definitions, will control. Throughout this specification and claims, the word "comprise," or variations such as "comprises" or "comprising" will be understood to imply the inclusion of a stated integer or group of integers but not the exclusion of any other integer or group of integers. Unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular.

Exemplary methods and materials are described herein, although methods and materials similar or equivalent to those described herein can also be used in the practice or testing of the invention. The materials, methods, and examples are illustrative only and not intended to be limiting.

BCMA specific CARs and Methods of Making Thereof

The invention provides CARs that bind to BCMA (e.g., human BCMA (e.g., SEQ ID NO: 354 or accession number: Q02223-2). BCMA specific CARs provided herein include single chain CARS and multichain CARs. The CARs have the ability to redirect T cell specificity and reactivity toward BCMA in a non-MHC-restricted manner, exploiting the antigen-binding properties of monoclonal antibodies. The non-MHC-restricted antigen recognition gives T cells expressing CARs the ability to recognize an antigen independent of antigen processing, thus bypassing a major mechanism of tumor escape.

In some embodiments, CARs provided herein comprise an extracellular ligand-binding domain (e.g., a single chain variable fragment (scFv)), a transmembrane domain, and an intracellular signaling domain. In some embodiments, the extracellular ligand-binding domain, transmembrane domain, and intracellular signaling domain are in one polypeptide, i.e., in a single chain. Multichain CARs and polypeptides are also provided herein. In some embodiments, the mulitchain CARs comprise: a first polypeptide comprising a transmembrane domain and at least one extracellular ligand-binding domain, and a second polypeptide comprising a transmembrane domain and at least one intracellular signaling domain, wherein the polypeptides assemble together to form a multichain CAR.

In some embodiments, a BCMA specific multichain CAR is based on the high affinity receptor for IgE (FcεRI). The FcεRI expressed on mast cells and basophiles triggers allergic reactions. FcεRI is a tetrameric complex composed of a single α subunit, a single β subunit, and two disulfide-linked γ subunits. The α subunit contains the IgE-binding domain. The β and γ subunits contain ITAMs that mediate signal transduction. In some embodiments, the extracellular domain of the FcRα chain is deleted and replaced by a BCMA specific extracellular ligand-binding domain. In some embodiments, the multichain BCMA specific CAR comprises an scFv that binds specifically to BCMA, the CD8α hinge, and the ITAM of the FcRβ chain. In some embodiments, the CAR may or may not comprise the FcRγ chain.

In some embodiments, the extracellular ligand-binding domain comprises an scFv comprising the light chain variable (VL) region and the heavy chain variable (VH) region of a target antigen specific monoclonal antibody joined by a flexible linker. Single chain variable region fragments are made by linking light and/or heavy chain variable regions by using a short linking peptide (Bird et al., Science 242:423-426, 1988). An example of a linking peptide is the GS linker having the amino acid sequence $(GGGGS)_3$ (SEQ ID NO: 333), which bridges approximately 3.5 nm between the carboxy terminus of one variable region and the amino terminus of the other variable region. Linkers of other sequences have been designed and used (Bird et al., 1988, supra). In general, linkers can be short, flexible polypeptides and preferably comprised of about 20 or fewer amino acid residues. Linkers can in turn be modified for additional functions, such as attachment of drugs or

attachment to solid supports. The single chain variants can be produced either recombinantly or synthetically. For synthetic production of scFv, an automated synthesizer can be used. For recombinant production of scFv, a suitable plasmid containing polynucleotide that encodes the scFv can be introduced into a suitable host cell, either eukaryotic, such as yeast, plant, insect or mammalian cells, or prokaryotic, such as E. coli. Polynucleotides encoding the scFv of interest can be made by routine manipulations such as ligation of polynucleotides. The resultant scFv can be isolated using standard protein purification techniques known in the art.

In some embodiments, the extracellular ligand-binding domain comprises (a) a VH region comprising (i) a VH complementarity determining region one (CDR1) comprising the sequence SYX_1MX_2, wherein X_1 is A or P; and X_2 is T, N, or S (SEQ ID NO: 301), $GFTFX_1SY$, wherein X_1 is G or S (SEQ ID NO: 302), or $GFTFX_1SYX_2MX_3$, wherein X_1 is G or S, X_2 is A or P; and X_3 is T, N, or S (SEQ ID NO: 303); (ii) a VH CDR2 comprising the sequence $AX_1X_2X_3X_4GX_5X_6X_7X_8YADX_9X_{10}KG$, wherein X_1 is I, V, T, H, L, A, or C; X_2 is S, D, G, T, I, L, F, M, or V; X_3 is G, Y, L, H, D, A, S, or M; X_4 is S, Q, T, A, F, or W; X_5 is G or T;X_6 is N, S, P, Y, W, or F; X_7 is S, T, I, L, T, A, R, V, K, G, or C; X_8 is F, Y, P, W, H, or G; X_9 is V, R, or L; and X_{10} is G or T (SEQ ID NO: 305), or $X_1X_2X_3X_4X_5X_6$, wherein X_1 is S, V, I, D, G, T, L, F, or M; X_2 is G, Y, L, H, D, A, S, or M; X_3 is S, G, F, or W; X_4 is G or S; X_5 is G or T; and X_6 is N, S, P, Y, or W (SEQ ID NO: 306); and iii) a VH CDR3 comprising the sequence $VSPIX_1X_2X_3X_4$, wherein X_1 is A or Y; X_2 is A or S; and X_3 is G, Q, L, P, or E (SEQ ID NO: 307), or $YWPMX_1X_2$, wherein X_1 is D, S, T, or A; and X_2 is I, S, L, P, or D (SEQ ID NO: 308); and a VL region comprising (i) a VL CDR1 comprising the sequence $X_1X_2X_3X_4X_5X_6X_7X_8X_9X_{10}X_{11}X_{12}$, wherein X_1 is R, G, W, A, or C; X_2 is A, P, G, L, C, or S; X_3 is S, G, or R; X_4 is Q, C, E, V, or I; X_5 is S, L, P, G, A, R, or D; X_6 is V, G, or I; X_7 is S, E, D, or P; X_8 is S, P, F, A, M, E, V, N, D, or Y; X_9 is I, T, V, E, S, A, M, Q, Y, H, or R; X_{10} is Y or F; X_{11} is L, W, or P; and X_{12} is A, S, or G (SEQ ID NO: 309); (ii) a VL CDR2 comprising the sequence $X_1ASX_2RAX_3$, wherein X_1 is G or D; X_2 is S or I; and X_3 is T or P (SEQ ID NO: 310); and (iii) a VL CDR3 comprising the sequence $QQYX_1X_2X_3PX_4T$, wherein X_1 is G, Q, E, L, F, A, S, M, K, R, or Y; X_2 is S, R, T, G, V, F, Y, D, A, H, V, E, K, or C; X_3 is W, F, or S; and X_4 is L or I (SEQ ID NO: 311), or $QQYX_1X_2X_3PX_4$, wherein X_1 is G, Q, E, L, F, A, S, M, R, K, or Y; X_2 is S, R, T, G, R, V, D, A, H, E, K, C, F, or Y; X_3 is W, S, or F; and X_4 is L or I

(SEQ 10 NO: 312). In some embodiments, the VH and VL are linked together by a flexible linker. In some embodiments a flexible linker comprises the amino acid sequence shown in SEQ 10 NO: 333.

In another aspect, provided is CAR, which specifically binds to BCMA, wherein the CAR comprises an extracellular ligand-binding domain comprising: a VH region comprising a VH COR1, VH COR2, and VH COR3 of the VH sequence shown in SEQ 10 NO: 2, 3, 7, 8,24,25,26,27,28,29,30,31,32,33,35,37,39,42,44,46, 48, 50, 52, 54, 56,58,60, 62,64,66,68,70,72,74,76,78,83,87,92,78,95,97,99, 101, 104, 106, 110, 112, 114, 76,118,120,122,112,125,127,313, or314; and/or a VL region comprising VL COR1, VL COR2, and VL COR3 of the VL sequence shown in SEQ 10 NO: 1,4,5,6,9,10,11,12, 13,15,16,17,18,19,20,21,22,23,34,36,38,40,41,43, 45, 47, 49, 51, 53, 57, 59,61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 317, 81, 82, 84, 85, 86, 88, 89, 90, 91, 93, 94, 96, 98, 100,102,103,105,107,108,109,111,113,115,116,117, 119, 121, 123, 124, 126, 128, 315, or 316. In some embodiments, the VH and VL are linked together by a flexible linker. In some embodiments a flexible linker comprises the amino acid sequence shown in SEQ 10 NO: 333.

In some embodiments, a CAR of the invention comprises an extracellular ligand-binding domain having anyone of partial light chain sequence as listed in Table 1 and/or anyone of partial heavy chain sequence as listed in Table 1. In Table 1, the underlined sequences are CDR sequences according to Kabat and in bold according to Chothia, <u>except</u> for the following heavy chain CDR2 sequences, in which the Chothia CDR sequences are underlined and the Kabat CDR sequences are in bold: P5A2_VHVL, A02_Rd4_0.6nM_C06, A02_Rd4_0.6nM_C09

A02_Rd4_6nM_C16, A02_Rd4_6nM_C03, A02_Rd4_6nM_C01, A02_Rd4_6nM_C26

A02_Rd4_6nM_C25, A02_Rd4_6nM_C22, A02_Rd4_6nM_C19, A02_Rd4_0.6nM_C03

A02_Rd4_6nM_C07, A02_Rd4_6nM_C23, A02_Rd4_0.6nM_C18, A02_Rd4_6nM_C10,

A02_Rd4_6nM_C05, A02_Rd4_0.6nM_C10, A02_Rd4_6nM_C04, A02_Rd4_0.6nM_C26,

A02_Rd4_0.6nM_C13, A02_Rd4_0.6nM_C01, A02_Rd4_6nM_C08, P5C1_VHVL, C01_Rd4_6nM_C24, C01_Rd4_6nM_C26, C01_Rd4_6nM_C10, C01_Rd4_0.6nM_C27,

C01_Rd4_6nM_C20, C01_Rd4_6nM_C12, C01_Rd4_0.6nM_C16, C01_Rd4_0.6nM_C09, C01_Rd4_6nM_C09, C01_Rd4_0.6nM_C03, C01_Rd4_0.6nM_C06, C01_Rd4_6nM_C04, COMBO_Rd4_0.6nM_C22, COMBO_Rd4_6nM_C21, COMBO_Rd4_6nM_C10, COMBO_Rd4_0.6nM_C04, COMBO_Rd4_6nM_C25, COMBO_Rd4_0.6nM_C21, COMBO_Rd4_6nM_C11, COMBO_Rd4_0.6nM_C20, COMBO_Rd4_6nM_C09, COMBO_Rd4_6nM_C08, COMBO_Rd4_0.6nM_C19, COMBO_Rd4_0.6nM_C02, COMBO_Rd4_0.6nM_C23, COMBO_Rd4_0.6nM_C29, COMBO_Rd4_0.6nM_C09, COMBO_Rd4_6nM_C12, COMBO_Rd4_0.6nM_C30, COMBO_Rd4_0.6nM_C14, COMBO_Rd4_6nM_C07, COMBO_Rd4_6nM_C02, COMBO_Rd4_0.6nM_C05, COMBO_Rd4_0.6nM_C17, COMBO_Rd4_6nM_C22, and COMBO_Rd4_0.6nM_C11.

Table 1

mAb	Light Chain	Heavy Chain
P6E01/ P6E01	EIVLTQSPGTLSLSPGERATLSC**R ASQSVSSSYLA**WYQQKPGQAP RLLIY**GASSRAT**GIPDRFSGSGS GTDFTLTISRLEPEDFAVYYC**QH YGSPPSFT**FGQGTKVEIK (SEQ ID NO: 1)	EVQLLESGGGLVQPGGSLRLSCA ASGFTFG**SYAMT**WVRQAPGKGLE WVSA**ISGSGGNT**FYADSVKGRFTI SRDNSKNTLYLQMNSLRAEDTAV YYCAR**VSPIASGMDY**WGQGTLVT VSS (SEQ ID NO: 2)
P6E01/ H3.AQ	EIVLTQSPGTLSLSPGERATLSC**R ASQSVSSSYLA**WYQQKPGQAP RLLIY**GASSRAT**GIPDRFSGSGS GTDFTLTISRLEPEDFAVYYC**QH YGSPPSFT**FGQGTKVEIK (SEQ ID NO: 1)	EVQLLESGGGLVQPGGSLRLSCA ASGFTFG**SYAMT**WVRQAPGKGLE WVSA**ISGSGGNT**FYADSVKGRFTI SRDNSKNTLYLQMNSLRAEDTAV YYCAR**VSPIAAQMDY**WGQGTLVT VSS (SEQ ID NO: 3)
L1.LG F/L3.K W/P6E 01	EIVLTQSPGTLSLSPGERATLSC **RASQSLGSFYLA**WYQQKPGQA PRLLIY**GASSRAT**GIPDRFSGSG SGTDFTLTISRLEPEDFAVYYC**KH YGWPPSFT**FGQGTKVEIK (SEQ ID NO: 4)	EVQLLESGGGLVQPGGSLRLSCA ASGFTFG**SYAMT**WVRQAPGKGLE WVSA**ISGSGGNT**FYADSVKGRFTI SRDNSKNTLYLQMNSLRAEDTAV YYCAR**VSPIASGMDY**WGQGTLVT VSS (SEQ ID NO: 2)
L1.LG	EIVLTQSPGTLSLSPGERATLSC**R**	EVQLLESGGGLVQPGGSLRLSCA

mAb	Light Chain	Heavy Chain
L1.LG F/L3.P Y/H3.A P	EIVLTQSPGTLSLSPGERATLSCR ASQSLGSFYLAWYQQKPGQAP RLLIYGASSRATGIPDRFSGSGS GTDFTLTISRLEPEDFAVYYCQH YPYPPSFTFGQGTKVEIK (SEQ ID NO: 9)	EVQLLESGGGLVQPGGSLRLSCA ASGFTFGSYAMTWVRQAPGKGLE WVSAISGSGGNTFYADSVKGRFTI SRDNSKNTLYLQMNSLRAEDTAV YYCARVSPIAAPMDYWGQGTLVT VSS (SEQ ID NO: 8)
L1.LG F/L3.P Y/H3.A Q	EIVLTQSPGTLSLSPGERATLSCR ASQSLGSFYLAWYQQKPGQAP RLLIYGASSRATGIPDRFSGSGS GTDFTLTISRLEPEDFAVYYCQH YPYPPSFTFGQGTKVEIK (SEQ ID NO: 9)	EVQLLESGGGLVQPGGSLRLSCA ASGFTFGSYAMTWVRQAPGKGLE WVSAISGSGGNTFYADSVKGRFTI SRDNSKNTLYLQMNSLRAEDTAV YYCARVSPIAAQMDYWGQGTLVT VSS (SEQ ID NO: 3)
L1.LG F/L3.N Y/H3.A L	EIVLTQSPGTLSLSPGERATLSCR ASQSLGSFYLAWYQQKPGQAP RLLIYGASSRATGIPDRFSGSGS GTDFTLTISRLEPEDFAVYYCQH YNYPPSFTFGQGTKVEIK (SEQ ID NO: 10)	EVQLLESGGGLVQPGGSLRLSCA ASGFTFGSYAMTWVRQAPGKGLE WVSAISGSGGNTFYADSVKGRFTI SRDNSKNTLYLQMNSLRAEDTAV YYCARVSPIAALMDYWGQGTLVT VSS (SEQ ID NO: 7)
L1.LG F/L3.N Y/H3.A P	EIVLTQSPGTLSLSPGERATLSCR ASQSLGSFYLAWYQQKPGQAP RLLIYGASSRATGIPDRFSGSGS GTDFTLTISRLEPEDFAVYYCQH YNYPPSFTFGQGTKVEIK (SEQ ID NO: 10)	EVQLLESGGGLVQPGGSLRLSCA ASGFTFGSYAMTWVRQAPGKGLE WVSAISGSGGNTFYADSVKGRFTI SRDNSKNTLYLQMNSLRAEDTAV YYCARVSPIAAPMDYWGQGTLVT VSS (SEQ ID NO: 8)
L1.LG F/L3.N Y/H3.A Q	EIVLTQSPGTLSLSPGERATLSCR ASQSLGSFYLAWYQQKPGQAP RLLIYGASSRATGIPDRFSGSGS GTDFTLTISRLEPEDFAVYYCQH YNYPPSFTFGQGTKVEIK (SEQ ID NO: 10)	EVQLLESGGGLVQPGGSLRLSCA ASGFTFGSYAMTWVRQAPGKGLE WVSAISGSGGNTFYADSVKGRFTI SRDNSKNTLYLQMNSLRAEDTAV YYCARVSPIAAQMDYWGQGTLVT VSS (SEQ ID NO: 3)

mAb	Light Chain	Heavy Chain
L1.GDF/L3.KW/H3.AL	EIVLTQSPGTLSLSPGERATLSCR ASQSVGDFYLAWYQQKPGQAP RLLIYGASSRATGIPDRFSGSGS GTDFTLTISRLEPEDFAVYYCKHY GWPPSFTFGQGTKVEIK (SEQ ID NO: 11)	EVQLLESGGGLVQPGGSLRLSCA ASGFTFGSYAMTWVRQAPGKGLE WVSAISGSGGNTFYADSVKGRFTI SRDNSKNTLYLQMNSLRAEDTAV YYCARVSPIAALMDYWGQGTLVT VSS (SEQ ID NO: 7)
L1.GDF/L3.KW/H3.AP	EIVLTQSPGTLSLSPGERATLSCR ASQSVGDFYLAWYQQKPGQAP RLLIYGASSRATGIPDRFSGSGS GTDFTLTISRLEPEDFAVYYCKHY GWPPSFTFGQGTKVEIK (SEQ ID NO: 11)	EVQLLESGGGLVQPGGSLRLSCA ASGFTFGSYAMTWVRQAPGKGLE WVSAISGSGGNTFYADSVKGRFTI SRDNSKNTLYLQMNSLRAEDTAV YYCARVSPIAAPMDYWGQGTLVT VSS (SEQ ID NO: 8)
L1.GDF/L3.KW/H3.AQ	EIVLTQSPGTLSLSPGERATLSCR ASQSVGDFYLAWYQQKPGQAP RLLIYGASSRATGIPDRFSGSGS GTDFTLTISRLEPEDFAVYYCKHY GWPPSFTFGQGTKVEIK (SEQ ID NO: 11)	EVQLLESGGGLVQPGGSLRLSCA ASGFTFGSYAMTWVRQAPGKGLE WVSAISGSGGNTFYADSVKGRFTI SRDNSKNTLYLQMNSLRAEDTAV YYCARVSPIAAQMDYWGQGTLVT VSS (SEQ ID NO: 3)
L1.GDF/L3.PY/H3.AQ	EIVLTQSPGTLSLSPGERATLSCR ASQSVGDFYLAWYQQKPGQAP RLLIYGASSRATGIPDRFSGSGS GTDFTLTISRLEPEDFAVYYCQH YPYPPSFTFGQGTKVEIK (SEQ ID NO: 12)	EVQLLESGGGLVQPGGSLRLSCA ASGFTFGSYAMTWVRQAPGKGLE WVSAISGSGGNTFYADSVKGRFTI SRDNSKNTLYLQMNSLRAEDTAV YYCARVSPIAAQMDYWGQGTLVT VSS (SEQ ID NO: 3)
L1.GDF/L3.NY/H3.AL	EIVLTQSPGTLSLSPGERATLSCR ASQSVGDFYLAWYQQKPGQAP RLLIYGASSRATGIPDRFSGSGS GTDFTLTISRLEPEDFAVYYCQH YNYPPSFTFGQGTKVEIK (SEQ ID NO: 13)	EVQLLESGGGLVQPGGSLRLSCA ASGFTFGSYAMTWVRQAPGKGLE WVSAISGSGGNTFYADSVKGRFTI SRDNSKNTLYLQMNSLRAEDTAV YYCARVSPIAALMDYWGQGTLVT VSS (SEQ ID NO: 7)

mAb	Light Chain	Heavy Chain
L1.GDF/L3.NY/H3.AP	EIVLTQSPGTLSLSPGERATLSCRASQSVGDFYLAWYQQKPGQAPRLLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQHYNYPPSFTFGQGTKVEIK (SEQ ID NO: 13)	EVQLLESGGGLVQPGGSLRLSCAASGFTFGSYAMTWVRQAPGKGLEWVSAISGSGGNTFYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARVSPIAAPMDYWGQGTLVTVSS (SEQ ID NO: 8)
L1.GDF/L3.NY/H3.AQ	EIVLTQSPGTLSLSPGERATLSCRASQSVGDFYLAWYQQKPGQAPRLLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQHYNYPPSFTFGQGTKVEIK (SEQ ID NO: 14)	EVQLLESGGGLVQPGGSLRLSCAASGFTFGSYAMTWVRQAPGKGLEWVSAISGSGGNTFYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARVSPIAAQMDYWGQGTLVTVSS (SEQ ID NO: 3)
L3.KW/P6E01	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCKHYGWPPSFTFGQGTKVEIK (SEQ ID NO: 15)	EVQLLESGGGLVQPGGSLRLSCAASGFTFGSYAMTWVRQAPGKGLEWVSAISGSGGNTFYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARVSPIASGMDYWGQGTLVTVSS (SEQ ID NO: 2)
L3.PY/P6E01	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQHYPYPPSFTFGQGTKVEIK (SEQ ID NO: 16)	EVQLLESGGGLVQPGGSLRLSCAASGFTFGSYAMTWVRQAPGKGLEWVSAISGSGGNTFYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARVSPIASGMDYWGQGTLVTVSS (SEQ ID NO: 2)
L3.NY/P6E01	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQHYNYPPSFTFGQGTKVEIK (SEQ ID NO: 17)	EVQLLESGGGLVQPGGSLRLSCAASGFTFGSYAMTWVRQAPGKGLEWVSAISGSGGNTFYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARVSPIASGMDYWGQGTLVTVSS (SEQ ID NO: 2)

mAb	Light Chain	Heavy Chain
L3.PY/ L1.PS/ P6E01	EIVLTQSPGTLSLSPGERATLSCR **ASQSVSSSYPS**WYQQKPGQAP RLLIY**GASSRAT**GIPDRFSGSGS GTDFTLTISRLEPEDFAVYYC**QH YPYPPSFT**FGQGTKVEIK (SEQ ID NO: 18)	EVQLLESGGGLVQPGGSLRLSCA ASGFTFGSYAMTWVRQAPGKGLE WVSAISGSGGNTFYADSVKGRFTI SRDNSKNTLYLQMNSLRAEDTAV YYCAR**VSPIASGMDY**WGQGTLVT VSS (SEQ ID NO: 2)
L3.PY/ L1.AH/ P6E01	EIVLTQSPGTLSLSPGERATLSCR **ASQSVSAHYLA**WYQQKPGQAP RLLIY**GASSRAT**GIPDRFSGSGS GTDFTLTISRLEPEDFAVYYC**QH YPYPPSFT**FGQGTKVEIK (SEQ ID NO: 19)	EVQLLESGGGLVQPGGSLRLSCA ASGFTFGSYAMTWVRQAPGKGLE WVSAISGSGGNTFYADSVKGRFTI SRDNSKNTLYLQMNSLRAEDTAV YYCAR**VSPIASGMDY**WGQGTLVT VSS (SEQ ID NO: 2)
L3.PY/ L1.FF/ P6E01	EIVLTQSPGTLSLSPGERATLSCR **ASQSVSSFFLA**WYQQKPGQAPR LLIY**GASSRAT**GIPDRFSGSGSG TDFTLTISRLEPEDFAVYYC**QHYP YPPSFT**FGQGTKVEIK (SEQ ID NO: 20)	EVQLLESGGGLVQPGGSLRLSCA ASGFTFGSYAMTWVRQAPGKGLE WVSAISGSGGNTFYADSVKGRFTI SRDNSKNTLYLQMNSLRAEDTAV YYCAR**VSPIASGMDY**WGQGTLVT VSS (SEQ ID NO: 2)
L3.PY/ L1.PH/ P6E01	EIVLTQSPGTLSLSPGERATLSCR **ASQSVSPHYLA**WYQQKPGQAP RLLIY**GASSRAT**GIPDRFSGSGS GTDFTLTISRLEPEDFAVYYC**QH YPYPPSFT**FGQGTKVEIK (SEQ ID NO: 21)	EVQLLESGGGLVQPGGSLRLSCA ASGFTFGSYAMTWVRQAPGKGLE WVSAISGSGGNTFYADSVKGRFTI SRDNSKNTLYLQMNSLRAEDTAV YYCAR**VSPIASGMDY**WGQGTLVT VSS (SEQ ID NO: 2)
L3.PY/ L3.KY/ P6E01	EIVLTQSPGTLSLSPGERATLSCR **ASQSVSSSYLA**WYQQKPGQAP RLLIY**GASSRAT**GIPDRFSGSGS GTDFTLTISRLEPEDFAVYYC**KYY PYPPSFT**FGQGTKVEIK (SEQ ID NO: 22)	EVQLLESGGGLVQPGGSLRLSCA ASGFTFGSYAMTWVRQAPGKGLE WVSAISGSGGNTFYADSVKGRFTI SRDNSKNTLYLQMNSLRAEDTAV YYCAR**VSPIASGMDY**WGQGTLVT VSS (SEQ ID NO: 2)

mAb	Light Chain	Heavy Chain
L3.PY/ L3.KF/ P6E01	EIVLTQSPGTLSLSPGERATLSCR ASQSVSSSYLAWYQQKPGQAP RLLIYGASSRATGIPDRFSGSGS GTDFTLTISRLEPEDFAVYYCKFY PYPPSFTFGQGTKVEIK (SEQ ID NO: 23)	EVQLLESGGGLVQPGGSLRLSCA ASGFTFGSYAMTWVRQAPGKGLE WVSAISGSGGNTFYADSVKGRFTI SRDNSKNTLYLQMNSLRAEDTAV YYCARVSPIASGMDYWGQGTLVT VSS (SEQ ID NO: 2)
L3.PY/ H2.QR	EIVLTQSPGTLSLSPGERATLSCR ASQSVSSSYLAWYQQKPGQAP RLLIYGASSRATGIPDRFSGSGS GTDFTLTISRLEPEDFAVYYCQH YPYPPSFTFGQGTKVEIK (SEQ ID NO: 16)	EVQLLESGGGLVQPGGSLRLSCA ASGFTFGSYAMTWVRQAPGKGLE WVSAISGSGGNTFYADQRKGRFTI SRDNSKNTLYLQMNSLRAEDTAV YYCARVSPIASGMDYWGQGTLVT VSS (SEQ ID NO: 24)
L3.PY/ H2.DY	EIVLTQSPGTLSLSPGERATLSCR ASQSVSSSYLAWYQQKPGQAP RLLIYGASSRATGIPDRFSGSGS GTDFTLTISRLEPEDFAVYYCQH YPYPPSFTFGQGTKVEIK (SEQ ID NO: 16)	EVQLLESGGGLVQPGGSLRLSCA ASGFTFGSYAMTWVRQAPGKGLE WVSAIDYSGGNTFYADSVKGRFTI SRDNSKNTLYLQMNSLRAEDTAV YYCARVSPIASGMDYWGQGTLVT VSS (SEQ ID NO: 25)
L3.PY/ H2.YQ	EIVLTQSPGTLSLSPGERATLSCR ASQSVSSSYLAWYQQKPGQAP RLLIYGASSRATGIPDRFSGSGS GTDFTLTISRLEPEDFAVYYCQH YPYPPSFTFGQGTKVEIK (SEQ ID NO: 16)	EVQLLESGGGLVQPGGSLRLSCA ASGFTFGSYAMTWVRQAPGKGLE WVSAISYQGGNTFYADSVKGRFTI SRDNSKNTLYLQMNSLRAEDTAV YYCARVSPIASGMDYWGQGTLVT VSS (SEQ ID NO: 26)
L3.PY/ H2.LT	EIVLTQSPGTLSLSPGERATLSCR ASQSVSSSYLAWYQQKPGQAP RLLIYGASSRATGIPDRFSGSGS GTDFTLTISRLEPEDFAVYYCQH YPYPPSFTFGQGTKVEIK (SEQ ID NO: 16)	EVQLLESGGGLVQPGGSLRLSCA ASGFTFGSYAMTWVRQAPGKGLE WVSAISLTGGNTFYADSVKGRFTI SRDNSKNTLYLQMNSLRAEDTAV YYCARVSPIASGMDYWGQGTLVT VSS (SEQ ID NO: 27)

mAb	Light Chain	Heavy Chain
L3.PY/ H2.HA	EIVLTQSPGTLSLSPGERATLSCR ASQSVSSSYLAWYQQKPGQAP RLLIYGASSRATGIPDRFSGSGS GTDFTLTISRLEPEDFAVYYCQH YPYPPSFTFGQGTKVEIK (SEQ ID NO: 16)	EVQLLESGGGLVQPGGSLRLSCA ASGFTFGSYAMTWVRQAPGKGLE WVSAISHAGGNTFYADSVKGRFTI SRDNSKNTLYLQMNSLRAEDTAV YYCARVSPIASGMDYWGQGTLVT VSS (SEQ ID NO: 28)
L3.PY/ H2.QL	EIVLTQSPGTLSLSPGERATLSCR ASQSVSSSYLAWYQQKPGQAP RLLIYGASSRATGIPDRFSGSGS GTDFTLTISRLEPEDFAVYYCQH YPYPPSFTFGQGTKVEIK (SEQ ID NO: 16)	EVQLLESGGGLVQPGGSLRLSCA ASGFTFGSYAMTWVRQAPGKGLE WVSAISGSGGNTFYADQLKGRFTI SRDNSKNTLYLQMNSLRAEDTAV YYCARVSPIASGMDYWGQGTLVT VSS (SEQ ID NO: 29)
L3.PY/ H3.YA	EIVLTQSPGTLSLSPGERATLSCR ASQSVSSSYLAWYQQKPGQAP RLLIYGASSRATGIPDRFSGSGS GTDFTLTISRLEPEDFAVYYCQH YPYPPSFTFGQGTKVEIK (SEQ ID NO: 16)	EVQLLESGGGLVQPGGSLRLSCA ASGFTFGSYAMTWVRQAPGKGLE WVSAISGSGGNTFYADSVKGRFTI SRDNSKNTLYLQMNSLRAEDTAV YYCARVSPIYAGMDYWGQGTLVT VSS (SEQ ID NO: 30)
L3.PY/ H3.AE	EIVLTQSPGTLSLSPGERATLSCR ASQSVSSSYLAWYQQKPGQAP RLLIYGASSRATGIPDRFSGSGS GTDFTLTISRLEPEDFAVYYCQH YPYPPSFTFGQGTKVEIK (SEQ ID NO: 16)	EVQLLESGGGLVQPGGSLRLSCA ASGFTFGSYAMTWVRQAPGKGLE WVSAISGSGGNTFYADSVKGRFTI SRDNSKNTLYLQMNSLRAEDTAV YYCARVSPIAAEMDYWGQGTLVT VSS (SEQ ID NO: 31)
L3.PY/ H3.AQ	EIVLTQSPGTLSLSPGERATLSCR ASQSVSSSYLAWYQQKPGQAP RLLIYGASSRATGIPDRFSGSGS GTDFTLTISRLEPEDFAVYYCQH YPYPPSFTFGQGTKVEIK (SEQ ID NO: 16)	EVQLLESGGGLVQPGGSLRLSCA ASGFTFGSYAMTWVRQAPGKGLE WVSAISGSGGNTFYADSVKGRFTI SRDNSKNTLYLQMNSLRAEDTAV YYCARVSPIAAQMDYWGQGTLVT VSS (SEQ ID NO: 3)

mAb	Light Chain	Heavy Chain
L3.PY/ H3.TA Q	EIVLTQSPGTLSLSPGERATLSCR ASQSVSSSYLAWYQQKPGQAP RLLIYGASSRATGIPDRFSGSGS GTDFTLTISRLEPEDFAVYYCQH YPYPPSFTFGQGTKVEIK (SEQ ID NO: 16)	EVQLLESGGGLVQPGGSLRLSCA ASGFTFGSYAMTWVRQAPGKGLE WVSAISGSGGNTFYADSVKGRFTI SRDNSKNTLYLQMNSLRAEDTAV YYCTRVSPIAAQMDYWGQGTLVT VSS (SEQ ID NO: 32)
L3.PY/ P6E01	EIVLTQSPGTLSLSPGERATLSCR ASQSVSSSYLAWYQQKPGQAP RLLIYGASSRATGIPDRFSGSGS GTDFTLTISRLEPEDFAVYYCQH YPYPPSFTFGQGTKVEIK (SEQ ID NO: 16)	EVQLLESGGGLVQPGGSLRLSCA ASGFTFGSYAMTWVRQAPGKGLE WVSAISGSGGNTFYADSVKGRFTI SRDNSKNTLYLQMNSLRAEDTAV YYCARVSPIASGMDYWGQGTLVT VSS (SEQ ID NO: 2)
L3.PY/ L1.PS/ H2.QR	EIVLTQSPGTLSLSPGERATLSCR ASQSVSSSYPSWYQQKPGQAP RLLIYGASSRATGIPDRFSGSGS GTDFTLTISRLEPEDFAVYYCQH YPYPPSFTFGQGTKVEIK (SEQ ID NO: 18)	EVQLLESGGGLVQPGGSLRLSCA ASGFTFGSYAMTWVRQAPGKGLE WVSAISGSGGNTFYADQRKGRFTI SRDNSKNTLYLQMNSLRAEDTAV YYCARVSPIASGMDYWGQGTLVT VSS (SEQ ID NO: 24)
L3.PY/ L1.PS/ H2.DY	EIVLTQSPGTLSLSPGERATLSCR ASQSVSSSYPSWYQQKPGQAP RLLIYGASSRATGIPDRFSGSGS GTDFTLTISRLEPEDFAVYYCQH YPYPPSFTFGQGTKVEIK (SEQ ID NO: 18)	EVQLLESGGGLVQPGGSLRLSCA ASGFTFGSYAMTWVRQAPGKGLE WVSAIDYSGGNTFYADSVKGRFTI SRDNSKNTLYLQMNSLRAEDTAV YYCARVSPIASGMDYWGQGTLVT VSS (SEQ ID NO: 25)
L3.PY/ L1.PS/ H2.YQ	EIVLTQSPGTLSLSPGERATLSCR ASQSVSSSYPSWYQQKPGQAP RLLIYGASSRATGIPDRFSGSGS GTDFTLTISRLEPEDFAVYYCQH YPYPPSFTFGQGTKVEIK (SEQ ID NO: 18)	EVQLLESGGGLVQPGGSLRLSCA ASGFTFGSYAMTWVRQAPGKGLE WVSAISYQGGNTFYADSVKGRFTI SRDNSKNTLYLQMNSLRAEDTAV YYCARVSPIASGMDYWGQGTLVT VSS (SEQ ID NO: 26)

mAb	Light Chain	Heavy Chain
L3.PY/ L1.PS/ H2.LT	EIVLTQSPGTLSLSPGERATLSCR ASQSVSSSYPSWYQQKPGQAP RLLIYGASSRATGIPDRFSGSGS GTDFTLTISRLEPEDFAVYYCQH YPYPPSFTFGQGTKVEIK (SEQ ID NO: 18)	EVQLLESGGGLVQPGGSLRLSCA ASGFTFGSYAMTWVRQAPGKGLE WVSAISLTGGNTFYADSVKGRFTI SRDNSKNTLYLQMNSLRAEDTAV YYCARVSPIASGMDYWGQGTLVT VSS (SEQ ID NO: 27)
L3.PY/ L1.PS/ H2.HA	EIVLTQSPGTLSLSPGERATLSCR ASQSVSSSYPSWYQQKPGQAP RLLIYGASSRATGIPDRFSGSGS GTDFTLTISRLEPEDFAVYYCQH YPYPPSFTFGQGTKVEIK (SEQ ID NO: 18)	EVQLLESGGGLVQPGGSLRLSCA ASGFTFGSYAMTWVRQAPGKGLE WVSAISHAGGNTFYADSVKGRFTI SRDNSKNTLYLQMNSLRAEDTAV YYCARVSPIASGMDYWGQGTLVT VSS (SEQ ID NO: 28)
L3.PY/ L1.PS/ H2.QL	EIVLTQSPGTLSLSPGERATLSCR ASQSVSSSYPSWYQQKPGQAP RLLIYGASSRATGIPDRFSGSGS GTDFTLTISRLEPEDFAVYYCQH YPYPPSFTFGQGTKVEIK (SEQ ID NO: 18)	EVQLLESGGGLVQPGGSLRLSCA ASGFTFGSYAMTWVRQAPGKGLE WVSAISGSGGNTFYADQLKGRFTI SRDNSKNTLYLQMNSLRAEDTAV YYCARVSPIASGMDYWGQGTLVT VSS (SEQ ID NO: 29)
L3.PY/ L1.PS/ H3.YA	EIVLTQSPGTLSLSPGERATLSCR ASQSVSSSYPSWYQQKPGQAP RLLIYGASSRATGIPDRFSGSGS GTDFTLTISRLEPEDFAVYYCQH YPYPPSFTFGQGTKVEIK (SEQ ID NO: 18)	EVQLLESGGGLVQPGGSLRLSCA ASGFTFGSYAMTWVRQAPGKGLE WVSAISGSGGNTFYADSVKGRFTI SRDNSKNTLYLQMNSLRAEDTAV YYCARVSPIYAGMDYWGQGTLVT VSS (SEQ ID NO: 30)
L3.PY/ L1.PS/ H3.AE	EIVLTQSPGTLSLSPGERATLSCR ASQSVSSSYPSWYQQKPGQAP RLLIYGASSRATGIPDRFSGSGS GTDFTLTISRLEPEDFAVYYCQH YPYPPSFTFGQGTKVEIK (SEQ ID NO: 18)	EVQLLESGGGLVQPGGSLRLSCA ASGFTFGSYAMTWVRQAPGKGLE WVSAISGSGGNTFYADSVKGRFTI SRDNSKNTLYLQMNSLRAEDTAV YYCARVSPIAAEMDYWGQGTLVT VSS (SEQ ID NO: 31)

mAb	Light Chain	Heavy Chain
L3.PY/ L1.PS/ H3.AQ	EIVLTQSPGTLSLSPGERATLSCR ASQSVSSSYPSWYQQKPGQAP RLLIYGASSRATGIPDRFSGSGS GTDFTLTISRLEPEDFAVYYCQH YPYPPSFTFGQGTKVEIK (SEQ ID NO: 18)	EVQLLESGGGLVQPGGSLRLSCA ASGFTFGSYAMTWVRQAPGKGLE WVSAISGSGGNTFYADSVKGRFTI SRDNSKNTLYLQMNSLRAEDTAV YYCARVSPIAAQMDYWGQGTLVT VSS (SEQ ID NO: 3)
L3.PY/ L1.PS/ H3.TA Q	EIVLTQSPGTLSLSPGERATLSCR ASQSVSSSYPSWYQQKPGQAP RLLIYGASSRATGIPDRFSGSGS GTDFTLTISRLEPEDFAVYYCQH YPYPPSFTFGQGTKVEIK (SEQ ID NO: 18)	EVQLLESGGGLVQPGGSLRLSCA ASGFTFGSYAMTWVRQAPGKGLE WVSAISGSGGNTFYADSVKGRFTI SRDNSKNTLYLQMNSLRAEDTAV YYCTRVSPIAAQMDYWGQGTLVT VSS (SEQ ID NO: 32)
L3.PY/ L1.AH/ H2.QR	EIVLTQSPGTLSLSPGERATLSCR ASQSVSAHYLAWYQQKPGQAP RLLIYGASSRATGIPDRFSGSGS GTDFTLTISRLEPEDFAVYYCQH YPYPPSFTFGQGTKVEIK (SEQ ID NO: 19)	EVQLLESGGGLVQPGGSLRLSCA ASGFTFGSYAMTWVRQAPGKGLE WVSAISGSGGNTFYADQRKGRFTI SRDNSKNTLYLQMNSLRAEDTAV YYCARVSPIASGMDYWGQGTLVT VSS (SEQ ID NO: 24)
L3.PY/ L1.AH/ H2.DY	EIVLTQSPGTLSLSPGERATLSCR ASQSVSAHYLAWYQQKPGQAP RLLIYGASSRATGIPDRFSGSGS GTDFTLTISRLEPEDFAVYYCQH YPYPPSFTFGQGTKVEIK (SEQ ID NO: 19)	EVQLLESGGGLVQPGGSLRLSCA ASGFTFGSYAMTWVRQAPGKGLE WVSAIDYSGGNTFYADSVKGRFTI SRDNSKNTLYLQMNSLRAEDTAV YYCARVSPIASGMDYWGQGTLVT VSS (SEQ ID NO: 25)
L3.PY/ L1.AH/ H2.YQ	EIVLTQSPGTLSLSPGERATLSCR ASQSVSAHYLAWYQQKPGQAP RLLIYGASSRATGIPDRFSGSGS GTDFTLTISRLEPEDFAVYYCQH YPYPPSFTFGQGTKVEIK (SEQ ID NO: 19)	EVQLLESGGGLVQPGGSLRLSCA ASGFTFGSYAMTWVRQAPGKGLE WVSAISYQGGNTFYADSVKGRFTI SRDNSKNTLYLQMNSLRAEDTAV YYCARVSPIASGMDYWGQGTLVT VSS (SEQ ID NO: 26)

mAb	Light Chain	Heavy Chain
L3.PY/ L1.AH/ H2.LT	EIVLTQSPGTLSLSPGERATLSCR ASQSVSAHYLAWYQQKPGQAP RLLIYGASSRATGIPDRFSGSGS GTDFTLTISRLEPEDFAVYYCQH YPYPPSFTFGQGTKVEIK (SEQ ID NO: 19)	EVQLLESGGGLVQPGGSLRLSCA ASGFTFGSYAMTWVRQAPGKGLE WVSAISLTGGNTFYADSVKGRFTI SRDNSKNTLYLQMNSLRAEDTAV YYCARVSPIASGMDYWGQGTLVT VSS (SEQ ID NO: 27)
L3.PY/ L1.AH/ H2.HA	EIVLTQSPGTLSLSPGERATLSCR ASQSVSAHYLAWYQQKPGQAP RLLIYGASSRATGIPDRFSGSGS GTDFTLTISRLEPEDFAVYYCQH YPYPPSFTFGQGTKVEIK (SEQ ID NO: 19)	EVQLLESGGGLVQPGGSLRLSCA ASGFTFGSYAMTWVRQAPGKGLE WVSAISHAGGNTFYADSVKGRFTI SRDNSKNTLYLQMNSLRAEDTAV YYCARVSPIASGMDYWGQGTLVT VSS (SEQ ID NO: 28)
L3.PY/ L1.AH/ H2.QL	EIVLTQSPGTLSLSPGERATLSCR ASQSVSAHYLAWYQQKPGQAP RLLIYGASSRATGIPDRFSGSGS GTDFTLTISRLEPEDFAVYYCQH YPYPPSFTFGQGTKVEIK (SEQ ID NO: 19)	EVQLLESGGGLVQPGGSLRLSCA ASGFTFGSYAMTWVRQAPGKGLE WVSAISGSGGNTFYADQLKGRFTI SRDNSKNTLYLQMNSLRAEDTAV YYCARVSPIASGMDYWGQGTLVT VSS (SEQ ID NO: 29)
L3.PY/ L1.AH/ H3.YA	EIVLTQSPGTLSLSPGERATLSCR ASQSVSAHYLAWYQQKPGQAP RLLIYGASSRATGIPDRFSGSGS GTDFTLTISRLEPEDFAVYYCQH YPYPPSFTFGQGTKVEIK (SEQ ID NO: 19)	EVQLLESGGGLVQPGGSLRLSCA ASGFTFGSYAMTWVRQAPGKGLE WVSAISGSGGNTFYADSVKGRFTI SRDNSKNTLYLQMNSLRAEDTAV YYCARVSPIYAGMDYWGQGTLVT VSS (SEQ ID NO: 30)
L3.PY/ L1.AH/ H3.AE	EIVLTQSPGTLSLSPGERATLSCR ASQSVSAHYLAWYQQKPGQAP RLLIYGASSRATGIPDRFSGSGS GTDFTLTISRLEPEDFAVYYCQH YPYPPSFTFGQGTKVEIK (SEQ ID NO: 19)	EVQLLESGGGLVQPGGSLRLSCA ASGFTFGSYAMTWVRQAPGKGLE WVSAISGSGGNTFYADSVKGRFTI SRDNSKNTLYLQMNSLRAEDTAV YYCARVSPIAAEMDYWGQGTLVT VSS (SEQ ID NO: 31)

mAb	Light Chain	Heavy Chain
L3.PY/ L1.AH/ H3.AQ	EIVLTQSPGTLSLSPGERATLSCR ASQSVSAHYLAWYQQKPGQAP RLLIYGASSRATGIPDRFSGSGS GTDFTLTISRLEPEDFAVYYCQH YPYPPSFTFGQGTKVEIK (SEQ ID NO: 19)	EVQLLESGGGLVQPGGSLRLSCA ASGFTFGSYAMTWVRQAPGKGLE WVSAISGSGGNTFYADSVKGRFTI SRDNSKNTLYLQMNSLRAEDTAV YYCARVSPIAAQMDYWGQGTLVT VSS (SEQ ID NO: 3)
L3.PY/ L1.AH/ H3.TA Q	EIVLTQSPGTLSLSPGERATLSCR ASQSVSAHYLAWYQQKPGQAP RLLIYGASSRATGIPDRFSGSGS GTDFTLTISRLEPEDFAVYYCQH YPYPPSFTFGQGTKVEIK (SEQ ID NO: 19)	EVQLLESGGGLVQPGGSLRLSCA ASGFTFGSYAMTWVRQAPGKGLE WVSAISGSGGNTFYADSVKGRFTI SRDNSKNTLYLQMNSLRAEDTAV YYCTRVSPIAAQMDYWGQGTLVT VSS (SEQ ID NO: 32)
L3.PY/ L1.FF/ H2.QR	EIVLTQSPGTLSLSPGERATLSCR ASQSVSSFFLAWYQQKPGQAPR LLIYGASSRATGIPDRFSGSGSG TDFTLTISRLEPEDFAVYYCQHYP YPPSFTFGQGTKVEIK (SEQ ID NO: 20)	EVQLLESGGGLVQPGGSLRLSCA ASGFTFGSYAMTWVRQAPGKGLE WVSAISGSGGNTFYADQRKGRFTI SRDNSKNTLYLQMNSLRAEDTAV YYCARVSPIASGMDYWGQGTLVT VSS (SEQ ID NO: 24)
L3.PY/ L1.FF/ H2.DY	EIVLTQSPGTLSLSPGERATLSCR ASQSVSSFFLAWYQQKPGQAPR LLIYGASSRATGIPDRFSGSGSG TDFTLTISRLEPEDFAVYYCQHYP YPPSFTFGQGTKVEIK (SEQ ID NO: 20)	EVQLLESGGGLVQPGGSLRLSCA ASGFTFGSYAMTWVRQAPGKGLE WVSAIDYSGGNTFYADSVKGRFTI SRDNSKNTLYLQMNSLRAEDTAV YYCARVSPIASGMDYWGQGTLVT VSS (SEQ ID NO: 25)
L3.PY/ L1.FF/ H2.YQ	EIVLTQSPGTLSLSPGERATLSCR ASQSVSSFFLAWYQQKPGQAPR LLIYGASSRATGIPDRFSGSGSG TDFTLTISRLEPEDFAVYYCQHYP YPPSFTFGQGTKVEIK (SEQ ID	EVQLLESGGGLVQPGGSLRLSCA ASGFTFGSYAMTWVRQAPGKGLE WVSAISYQGGNTFYADSVKGRFTI SRDNSKNTLYLQMNSLRAEDTAV YYCARVSPIASGMDYWGQGTLVT

mAb	Light Chain	Heavy Chain
	NO: 20)	VSS (SEQ ID NO: 26)
L3.PY/ L1.FF/ H2.LT	EIVLTQSPGTLSLSPGERATLSCR ASQSVSSFFLAWYQQKPGQAPR LLIYGASSRATGIPDRFSGSGSG TDFTLTISRLEPEDFAVYYCQHYP YPPSFTFGQGTKVEIK (SEQ ID NO: 20)	EVQLLESGGGLVQPGGSLRLSCA ASGFTFGSYAMTWVRQAPGKGLE WVSAISLTGGNTFYADSVKGRFTI SRDNSKNTLYLQMNSLRAEDTAV YYCARVSPIASGMDYWGQGTLVT VSS (SEQ ID NO: 27)
L3.PY/ L1.FF/ H2.HA	EIVLTQSPGTLSLSPGERATLSCR ASQSVSSFFLAWYQQKPGQAPR LLIYGASSRATGIPDRFSGSGSG TDFTLTISRLEPEDFAVYYCQHYP YPPSFTFGQGTKVEIK (SEQ ID NO: 20)	EVQLLESGGGLVQPGGSLRLSCA ASGFTFGSYAMTWVRQAPGKGLE WVSAISHAGGNTFYADSVKGRFTI SRDNSKNTLYLQMNSLRAEDTAV YYCARVSPIASGMDYWGQGTLVT VSS (SEQ ID NO: 28)
L3.PY/ L1.FF/ H2.QL	EIVLTQSPGTLSLSPGERATLSCR ASQSVSSFFLAWYQQKPGQAPR LLIYGASSRATGIPDRFSGSGSG TDFTLTISRLEPEDFAVYYCQHYP YPPSFTFGQGTKVEIK (SEQ ID NO: 20)	EVQLLESGGGLVQPGGSLRLSCA ASGFTFGSYAMTWVRQAPGKGLE WVSAISGSGGNTFYADQLKGRFTI SRDNSKNTLYLQMNSLRAEDTAV YYCARVSPIASGMDYWGQGTLVT VSS (SEQ ID NO: 29)
L3.PY/ L1.FF/ H3.YA	EIVLTQSPGTLSLSPGERATLSCR ASQSVSSFFLAWYQQKPGQAPR LLIYGASSRATGIPDRFSGSGSG TDFTLTISRLEPEDFAVYYCQHYP YPPSFTFGQGTKVEIK (SEQ ID NO: 20)	EVQLLESGGGLVQPGGSLRLSCA ASGFTFGSYAMTWVRQAPGKGLE WVSAISGSGGNTFYADSVKGRFTI SRDNSKNTLYLQMNSLRAEDTAV YYCARVSPIYAGMDYWGQGTLVT VSS (SEQ ID NO: 30)
L3.PY/ L1.FF/ H3.AE	EIVLTQSPGTLSLSPGERATLSCR ASQSVSSFFLAWYQQKPGQAPR LLIYGASSRATGIPDRFSGSGSG TDFTLTISRLEPEDFAVYYCQHYP YPPSFTFGQGTKVEIK (SEQ ID	EVQLLESGGGLVQPGGSLRLSCA ASGFTFGSYAMTWVRQAPGKGLE WVSAISGSGGNTFYADSVKGRFTI SRDNSKNTLYLQMNSLRAEDTAV YYCARVSPIAAEMDYWGQGTLVT

mAb	Light Chain	Heavy Chain
	NO: 20)	VSS (SEQ ID NO: 31)
L3.PY/ L1.FF/ H3.AQ	EIVLTQSPGTLSLSPGERATLSCR ASQSVSSFFLAWYQQKPGQAPR LLIYGASSRATGIPDRFSGSGSG TDFTLTISRLEPEDFAVYYCQHYP YPPSFTFGQGTKVEIK (SEQ ID NO: 20)	EVQLLESGGGLVQPGGSLRLSCA ASGFTFGSYAMTWVRQAPGKGLE WVSAISGSGGNTFYADSVKGRFTI SRDNSKNTLYLQMNSLRAEDTAV YYCARVSPIAAQMDYWGQGTLVT VSS (SEQ ID NO: 3)
L3.PY/ L1.FF/ H3.TA Q	EIVLTQSPGTLSLSPGERATLSCR ASQSVSSFFLAWYQQKPGQAPR LLIYGASSRATGIPDRFSGSGSG TDFTLTISRLEPEDFAVYYCQHYP YPPSFTFGQGTKVEIK (SEQ ID NO: 20)	EVQLLESGGGLVQPGGSLRLSCA ASGFTFGSYAMTWVRQAPGKGLE WVSAISGSGGNTFYADSVKGRFTI SRDNSKNTLYLQMNSLRAEDTAV YYCTRVSPIAAQMDYWGQGTLVT VSS (SEQ ID NO: 32)
L3.PY/ L1.PH/ H2.QR	EIVLTQSPGTLSLSPGERATLSCR ASQSVSPHYLAWYQQKPGQAP RLLIYGASSRATGIPDRFSGSGS GTDFTLTISRLEPEDFAVYYCQH YPYPPSFTFGQGTKVEIK (SEQ ID NO: 21)	EVQLLESGGGLVQPGGSLRLSCA ASGFTFGSYAMTWVRQAPGKGLE WVSAISGSGGNTFYADQRKGRFTI SRDNSKNTLYLQMNSLRAEDTAV YYCARVSPIASGMDYWGQGTLVT VSS (SEQ ID NO: 24)
L3.PY/ L1.PH/ H2.HA	EIVLTQSPGTLSLSPGERATLSCR ASQSVSPHYLAWYQQKPGQAP RLLIYGASSRATGIPDRFSGSGS GTDFTLTISRLEPEDFAVYYCQH YPYPPSFTFGQGTKVEIK (SEQ ID NO: 21)	EVQLLESGGGLVQPGGSLRLSCA ASGFTFGSYAMTWVRQAPGKGLE WVSAISHAGGNTFYADSVKGRFTI SRDNSKNTLYLQMNSLRAEDTAV YYCARVSPIASGMDYWGQGTLVT VSS (SEQ ID NO: 28)
L3.PY/ L1.PH/ H3.AE	EIVLTQSPGTLSLSPGERATLSCR ASQSVSPHYLAWYQQKPGQAP RLLIYGASSRATGIPDRFSGSGS GTDFTLTISRLEPEDFAVYYCQH YPYPPSFTFGQGTKVEIK (SEQ	EVQLLESGGGLVQPGGSLRLSCA ASGFTFGSYAMTWVRQAPGKGLE WVSAISGSGGNTFYADSVKGRFTI SRDNSKNTLYLQMNSLRAEDTAV YYCARVSPIAAEMDYWGQGTLVT

mAb	Light Chain	Heavy Chain
	ID NO: 21)	VSS (SEQ ID NO: 31)
L3.PY/ L1.PH/ H3.AQ	EIVLTQSPGTLSLSPGERATLSCR ASQSVSPHYLAWYQQKPGQAP RLLIYGASSRATGIPDRFSGSGS GTDFTLTISRLEPEDFAVYYCQH YPYPPSFTFGQGTKVEIK (SEQ ID NO: 21)	EVQLLESGGGLVQPGGSLRLSCA ASGFTFGSYAMTWVRQAPGKGLE WVSAISGSGGNTFYADSVKGRFTI SRDNSKNTLYLQMNSLRAEDTAV YYCARVSPIAAQMDYWGQGTLVT VSS (SEQ ID NO: 3)
L3.PY/ L1.PH/ H3.TA Q	EIVLTQSPGTLSLSPGERATLSCR ASQSVSPHYLAWYQQKPGQAP RLLIYGASSRATGIPDRFSGSGS GTDFTLTISRLEPEDFAVYYCQH YPYPPSFTFGQGTKVEIK (SEQ ID NO: 21)	EVQLLESGGGLVQPGGSLRLSCA ASGFTFGSYAMTWVRQAPGKGLE WVSAISGSGGNTFYADSVKGRFTI SRDNSKNTLYLQMNSLRAEDTAV YYCTRVSPIAAQMDYWGQGTLVT VSS (SEQ ID NO: 32)
L3.PY/ L3.KY/ H2.QR	EIVLTQSPGTLSLSPGERATLSCR ASQSVSSSYLAWYQQKPGQAP RLLIYGASSRATGIPDRFSGSGS GTDFTLTISRLEPEDFAVYYCKYY PYPPSFTFGQGTKVEIK (SEQ ID NO: 22)	EVQLLESGGGLVQPGGSLRLSCA ASGFTFGSYAMTWVRQAPGKGLE WVSAISGSGGNTFYADQRKGRFTI SRDNSKNTLYLQMNSLRAEDTAV YYCARVSPIASGMDYWGQGTLVT VSS (SEQ ID NO: 24)
L3.PY/ L3.KY/ H2.DY	EIVLTQSPGTLSLSPGERATLSCR ASQSVSSSYLAWYQQKPGQAP RLLIYGASSRATGIPDRFSGSGS GTDFTLTISRLEPEDFAVYYCKYY PYPPSFTFGQGTKVEIK (SEQ ID NO: 22)	EVQLLESGGGLVQPGGSLRLSCA ASGFTFGSYAMTWVRQAPGKGLE WVSAIDYSGGNTFYADSVKGRFTI SRDNSKNTLYLQMNSLRAEDTAV YYCARVSPIASGMDYWGQGTLVT VSS (SEQ ID NO: 25)
L3.PY/ L3.KY/ H2.YQ	EIVLTQSPGTLSLSPGERATLSCR ASQSVSSSYLAWYQQKPGQAP RLLIYGASSRATGIPDRFSGSGS GTDFTLTISRLEPEDFAVYYCKYY PYPPSFTFGQGTKVEIK (SEQ ID	EVQLLESGGGLVQPGGSLRLSCA ASGFTFGSYAMTWVRQAPGKGLE WVSAISYQGGNTFYADSVKGRFTI SRDNSKNTLYLQMNSLRAEDTAV YYCARVSPIASGMDYWGQGTLVT

mAb	Light Chain	Heavy Chain
	NO: 22)	VSS (SEQ ID NO: 26)
L3.PY/ L3.KY/ H2.LT	EIVLTQSPGTLSLSPGERATLSCR ASQSVSSSYLAWYQQKPGQAP RLLIYGASSRATGIPDRFSGSGS GTDFTLTISRLEPEDFAVYYCKYY PYPPSFTFGQGTKVEIK (SEQ ID NO: 22)	EVQLLESGGGLVQPGGSLRLSCA ASGFTFGSYAMTWVRQAPGKGLE WVSAISLTGGNTFYADSVKGRFTI SRDNSKNTLYLQMNSLRAEDTAV YYCARVSPIASGMDYWGQGTLVT VSS (SEQ ID NO: 27)
L3.PY/ L3.KY/ H2.HA	EIVLTQSPGTLSLSPGERATLSCR ASQSVSSSYLAWYQQKPGQAP RLLIYGASSRATGIPDRFSGSGS GTDFTLTISRLEPEDFAVYYCKYY PYPPSFTFGQGTKVEIK (SEQ ID NO: 22)	EVQLLESGGGLVQPGGSLRLSCA ASGFTFGSYAMTWVRQAPGKGLE WVSAISHAGGNTFYADSVKGRFTI SRDNSKNTLYLQMNSLRAEDTAV YYCARVSPIASGMDYWGQGTLVT VSS (SEQ ID NO: 28)
L3.PY/ L3.KY/ H2.QL	EIVLTQSPGTLSLSPGERATLSCR ASQSVSSSYLAWYQQKPGQAP RLLIYGASSRATGIPDRFSGSGS GTDFTLTISRLEPEDFAVYYCKYY PYPPSFTFGQGTKVEIK (SEQ ID NO: 22)	EVQLLESGGGLVQPGGSLRLSCA ASGFTFGSYAMTWVRQAPGKGLE WVSAISGSGGNTFYADQLKGRFTI SRDNSKNTLYLQMNSLRAEDTAV YYCARVSPIASGMDYWGQGTLVT VSS (SEQ ID NO: 29)
L3.PY/ L3.KY/ H3.YA	EIVLTQSPGTLSLSPGERATLSCR ASQSVSSSYLAWYQQKPGQAP RLLIYGASSRATGIPDRFSGSGS GTDFTLTISRLEPEDFAVYYCKYY PYPPSFTFGQGTKVEIK (SEQ ID NO: 22)	EVQLLESGGGLVQPGGSLRLSCA ASGFTFGSYAMTWVRQAPGKGLE WVSAISGSGGNTFYADSVKGRFTI SRDNSKNTLYLQMNSLRAEDTAV YYCARVSPIYAGMDYWGQGTLVT VSS (SEQ ID NO: 30)
L3.PY/ L3.KY/ H3.TA Q	EIVLTQSPGTLSLSPGERATLSCR ASQSVSSSYLAWYQQKPGQAP RLLIYGASSRATGIPDRFSGSGS GTDFTLTISRLEPEDFAVYYCKYY PYPPSFTFGQGTKVEIK (SEQ ID	EVQLLESGGGLVQPGGSLRLSCA ASGFTFGSYAMTWVRQAPGKGLE WVSAISGSGGNTFYADSVKGRFTI SRDNSKNTLYLQMNSLRAEDTAV YYCTRVSPIAAQMDYWGQGTLVT

mAb	Light Chain	Heavy Chain
	NO: 22)	VSS (SEQ ID NO: 32)
L3.PY/ L3.KF/ H2.DY	EIVLTQSPGTLSLSPGERATLSCR **ASQSVSSSYLA**WYQQKPGQAP RLLIY**GASSRAT**GIPDRFSGSGS GTDFTLTISRLEPEDFAVYYC**KFY PYPPSFT**FGQGTKVEIK (SEQ ID NO: 23)	EVQLLESGGGLVQPGGSLRLSCA ASGFTFG**SY**AMTWVRQAPGKGLE WVSA**IDYSGGNT**FYADSVKGRFTI SRDNSKNTLYLQMNSLRAEDTAV YYCAR**VSPIASGMDY**WGQGTLVT VSS (SEQ ID NO: 25)
L3.PY/ L3.KF/ H2.YQ	EIVLTQSPGTLSLSPGERATLSCR **ASQSVSSSYLA**WYQQKPGQAP RLLIY**GASSRAT**GIPDRFSGSGS GTDFTLTISRLEPEDFAVYYC**KFY PYPPSFT**FGQGTKVEIK (SEQ ID NO: 23)	EVQLLESGGGLVQPGGSLRLSCA ASGFTFG**SY**AMTWVRQAPGKGLE WVSA**ISYQGGN**TFYADSVKGRFTI SRDNSKNTLYLQMNSLRAEDTAV YYCAR**VSPIASGMDY**WGQGTLVT VSS (SEQ ID NO: 26)
L3.PY/ L3.KF/ H2.LT	EIVLTQSPGTLSLSPGERATLSCR **ASQSVSSSYLA**WYQQKPGQAP RLLIY**GASSRAT**GIPDRFSGSGS GTDFTLTISRLEPEDFAVYYC**KFY PYPPSFT**FGQGTKVEIK (SEQ ID NO: 23)	EVQLLESGGGLVQPGGSLRLSCA ASGFTFG**SY**AMTWVRQAPGKGLE WVSA**ISLTGGN**TFYADSVKGRFTI SRDNSKNTLYLQMNSLRAEDTAV YYCAR**VSPIASGMDY**WGQGTLVT VSS (SEQ ID NO: 27)
L3.PY/ L3.KF/ H2.QL	EIVLTQSPGTLSLSPGERATLSCR **ASQSVSSSYLA**WYQQKPGQAP RLLIY**GASSRAT**GIPDRFSGSGS GTDFTLTISRLEPEDFAVYYC**KFY PYPPSFT**FGQGTKVEIK (SEQ ID NO: 23)	EVQLLESGGGLVQPGGSLRLSCA ASGFTFG**SY**AMTWVRQAPGKGLE WVSA**ISGSGGN**TFYADQLKGRFTI SRDNSKNTLYLQMNSLRAEDTAV YYCAR**VSPIASGMDY**WGQGTLVT VSS (SEQ ID NO: 29)
L3.PY/ L3.KF/ H3.YA	EIVLTQSPGTLSLSPGERATLSCR **ASQSVSSSYLA**WYQQKPGQAP RLLIY**GASSRAT**GIPDRFSGSGS GTDFTLTISRLEPEDFAVYYC**KFY**	EVQLLESGGGLVQPGGSLRLSCA ASGFTFG**SY**AMTWVRQAPGKGLE WVSA**ISGSGGN**TFYADSVKGRFTI SRDNSKNTLYLQMNSLRAEDTAV

mAb	Light Chain	Heavy Chain
	PYPPSFTFGQGTKVEIK (SEQ ID NO: 23)	YYCARVSPIYAGMDYWGQGTLVT VSS (SEQ ID NO: 30)
L3.PY/ L3.KF/ H3.AE	EIVLTQSPGTLSLSPGERATLSCR ASQSVSSSYLAWYQQKPGQAP RLLIYGASSRATGIPDRFSGSGS GTDFTLTISRLEPEDFAVYYCKFY PYPPSFTFGQGTKVEIK (SEQ ID NO: 23)	EVQLLESGGGLVQPGGSLRLSCA ASGFTFGSYAMTWVRQAPGKGLE WVSAISGSGGNTFYADSVKGRFTI SRDNSKNTLYLQMNSLRAEDTAV YYCARVSPIAAEMDYWGQGTLVT VSS (SEQ ID NO: 31)
L3.PY/ L3.KF/ H3.AQ	EIVLTQSPGTLSLSPGERATLSCR ASQSVSSSYLAWYQQKPGQAP RLLIYGASSRATGIPDRFSGSGS GTDFTLTISRLEPEDFAVYYCKFY PYPPSFTFGQGTKVEIK (SEQ ID NO: 23)	EVQLLESGGGLVQPGGSLRLSCA ASGFTFGSYAMTWVRQAPGKGLE WVSAISGSGGNTFYADSVKGRFTI SRDNSKNTLYLQMNSLRAEDTAV YYCARVSPIAAQMDYWGQGTLVT VSS (SEQ ID NO: 3)
L3.PY/ L3.KF/ H3.TA Q	EIVLTQSPGTLSLSPGERATLSCR ASQSVSSSYLAWYQQKPGQAP RLLIYGASSRATGIPDRFSGSGS GTDFTLTISRLEPEDFAVYYCKFY PYPPSFTFGQGTKVEIK (SEQ ID NO: 23)	EVQLLESGGGLVQPGGSLRLSCA ASGFTFGSYAMTWVRQAPGKGLE WVSAISGSGGNTFYADSVKGRFTI SRDNSKNTLYLQMNSLRAEDTAV YYCTRVSPIAAQMDYWGQGTLVT VSS (SEQ ID NO: 32)
P5A2_ VHVL	EIVLTQSPGTLSLSPGERATLSCR ASQSVSSSYLAWYQQKPGQAP RLLMYDASIRATGIPDRFSGSGS GTDFTLTISRLEPEDFAVYYCQQ YGSWPLTFGQGTKVEIK (SEQ ID NO: 34)	EVQLLESGGGLVQPGGSLRLSCA ASGFTFSSYAMNWVRQAPGKGLE WVSAISDSGGSTYYADSVKGRFTI SRDNSKNTLYLQMNSLRAEDTAV YYCARYWPMDIWGQGTLVTVSS (SEQ ID NO: 33)
A02_R d4_0.6 nM_C0 6	EIVLTQSPGTLSLSPGERATLSCR ASQSVSVIYLAWYQQKPGQAPR LLMYDASIRATGIPDRFSGSGSG TDFTLTISRLEPEDFAVYYCQQY	EVQLLESGGGLVQPGGSLRLSCA ASGFTFSSYAMNWVRQAPGKGLE WVSAISDSGGSAWYADSVKGRFT ISRDNSKNTLYLQMNSLRAEDTAV

mAb	Light Chain	Heavy Chain
	QRWPLTFGQGTKVEIK (SEQ ID NO: 36)	YYCARYWPMSLWGQGTLVTVSS (SEQ ID NO: 35)
A02_Rd4_0.6nM_C09	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLMYDASIRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYQSWPLTFGQGTKVEIK (SEQ ID NO: 38)	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMNWVRQAPGKGLEWVSAISDSGGSMWYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARYWPMSLWGQGTLVTVSS (SEQ ID NO: 37)
A02_Rd4_6nM_C16 (P5AC16)	EIVLTQSPGTLSLSPGERATLSCRASQSVSDIYLAWYQQKPGQAPRLLMYDASIRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYQTWPLTFGQGTKVEIK (SEQ ID NO: 40)	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMNWVRQAPGKGLEWVSAISdFGGSTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARYWPMDIWGQGTLVTVSS (SEQ ID NO: 39)
A02_Rd4_6nM_C03	EIVLTQSPGTLSLSPGERATLSCRASQSVSNLYLAWYQQKPGQAPRLLMYDASIRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYQGWPLTFGQGTKVEIK (SEQ ID NO: 41)	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMNWVRQAPGKGLEWVSAISDSGGSTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARYWPMDIWGQGTLVTVSS (SEQ ID NO: 33)
A02_Rd4_6nM_C01	EIVLTQSPGTLSLSPGERATLSCRASQSVSAYYLAWYQQKPGQAPRLLMYDASIRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYERWPLTFGQGTKVEIK (SEQ ID NO: 43)	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMNWVRQAPGKGLEWVSAITASGGSTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARYWPMSLWGQGTLVTVSS (SEQ ID NO: 42)
A02_Rd4_6nM_C26	EIVLTQSPGTLSLSPGERATLSCRASQSVSSLYLAWYQQKPGQAPRLLMYDASIRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQY	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMNWVRQAPGKGLEWVSAISDSGGSTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAV

mAb	Light Chain	Heavy Chain
	QVWPLTFGQGTKVEIK (SEQ ID NO: 45)	YYCARYWPMSLWGQGTLVTVSS (SEQ ID NO: 44)
A02_Rd4_6nM_C25	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLMYDASIRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYLDWPLTFGQGTKVEIK (SEQ ID NO: 47)	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMNWVRQAPGKGLEWVSAISdSGGSRWYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARYWPMTPWGQGTLVTVSS (SEQ ID NO: 46)
A02_Rd4_6nM_C22	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLMYDASIRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYQVWPLTFGQGTKVEIK (SEQ ID NO: 49)	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMNWVRQAPGKGLEWVSAVLdSGGSTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARYWPMTPWGQGTLVTVSS (SEQ ID NO: 48)
A02_Rd4_6nM_C19	EIVLTQSPGTLSLSPGERATLSCRASQSVSVIYLAWYQQKPGQAPRLLMYDASIRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYLAWPLTFGQGTKVEIK (SEQ ID NO: 51)	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMNWVRQAPGKGLEWVSAISdSGGSRWYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARYWPMSDWGQGTLVTVSS (SEQ ID NO: 50)
A02_Rd4_0.6nM_C03	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLMYDASIRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYFTWPLTFGQGTKVEIK (SEQ ID NO: 53)	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMNWVRQAPGKGLEWVSAISdSGGSKWYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARYWPMSLWGQGTLVTVSS (SEQ ID NO: 52)
A02_Rd4_6nM_C07	EIVLTQSPGTLSLSPGERATLSCRASQSVSPyYLAWYQQKPGQAPRLLMYDASIRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYE	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMNWVRQAPGKGLEWVSAIGGSGGSLPYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAV

mAb	Light Chain	Heavy Chain
	RWPLTFGQGTKVEIK (SEQ ID NO: 55)	YYCARYWPMDSWGQGTLVTVSS (SEQ ID NO: 54)
A02_Rd4_6nM_C23	EIVLTQSPGTLSLSPGERATLSCRASQSVSVEYLAWYQQKPGQAPRLLMYDASIRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYARWPLTFGQGTKVEIK (SEQ ID NO: 57)	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMNWVRQAPGKGLEWVSAISdSGGSGWYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARYWPMSLWGQGTLVTVSS (SEQ ID NO: 56)
A02_Rd4_0.6nM_C18	EIVLTQSPGTLSLSPGERATLSCRASQSVSEIYLAWYQQKPGQAPRLLMYDASIRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYFGWPLTFGQGTKVEIK (SEQ ID NO: 59)	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMNWVRQAPGKGLEWVSAVLdSGGSTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARYWPMSLWGQGTLVTVSS (SEQ ID NO: 58)
A02_Rd4_6nM_C10	EIVLTQSPGTLSLSPGERATLSCRASQSVEMSYLAWYQQKPGQAPRLLMYDASIRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYAHWPLTFGQGTKVEIK (SEQ ID NO: 61)	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMNWVRQAPGKGLEWVSAISdSGGSCWYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARYWPMTPWGQGTLVTVSS (SEQ ID NO: 60)
A02_Rd4_6nM_C05	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLMYDASIRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYQRWPLTFGQGTKVEIK (SEQ ID NO: 63)	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMNWVRQAPGKGLEWVSAIFaSGGSTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARYWPMTPWGQGTLVTVSS (SEQ ID NO: 62)
A02_Rd4_0.6nM_C10	EIVLTQSPGTLSLSPGERATLSCRASQSVSAQYLAWYQQKPGQAPRLLMYDASIRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQ	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMNWVRQAPGKGLEWVSAISgWGGSLPYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAV

mAb	Light Chain	Heavy Chain
	YQRWPLTFGQGTKVEIK (SEQ ID NO: 65)	YYCARYWPMDSWGQGTLVTVSS (SEQ ID NO: 64)
A02_Rd4_6nM_C04	EIVLTQSPGTLSLSPGERATLSCRASQSVSAIYLAWYQQKPGQAPRLLMYDASIRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYQVWPLTFGQGTKVEIK (SEQ ID NO: 67)	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMNWVRQAPGKGLEWVSAIMsSGGPLYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARYWPMALWGQGTLVTVSS (SEQ ID NO: 66)
A02_Rd4_0.6nM_C26	EIVLTQSPGTLSLSPGERATLSCGPSQSVSSSYLAWYQQKPGQAPRLLMYDASIRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYQSWPLTFGQGTKVEIK (SEQ ID NO: 69)	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMNWVRQAPGKGLEWVSAILmSGGSTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARYWPMSLWGQGTLVTVSS (SEQ ID NO: 68)
A02_Rd4_0.6nM_C13	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYWAWYQQKPGQAPRLLMYDASIRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYESWPLTFGQGTKVEIK (SEQ ID NO: 71)	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMNWVRQAPGKGLEWVSAISdSGGYRYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARYWPMSLWGQGTLVTVSS (SEQ ID NO: 70)
A02_Rd4_0.6nM_C01 (P5AC1)	EIVLTQSPGTLSLSPGERATLSCRGGQSVSSSYLAWYQQKPGQAPRLLMYDASIRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYQSWPLTFGQGTKVEIK (SEQ ID NO: 73)	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMNWVRQAPGKGLEWVSAILsSGGSTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARYWPMDIWGQGTLVTVSS (SEQ ID NO: 72)
A02_Rd4_6nM_C08	EIVLTQSPGTLSLSPGERATLSCRASQSVSFIYLAWYQQKPGQAPRLLMYDASIRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQY	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMNWVRQAPGKGLEWVSAILdSGGSTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAV

mAb	Light Chain	Heavy Chain
	GSWPLTFGQGTKVEIK (SEQ ID NO: 75)	YYCARYWPMSPWGQGTLVTVSS (SEQ ID NO: 74)
P5C1_VHVL (PC1)	EIVLTQSPGTLSLSPGERATLSCRASQSVSSTYLAWYQQKPGQAPRLLIYDASSRAPGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYSTSPLTFGQGTKVEIK (SEQ ID NO: 77)	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYPMSWVRQAPGKGLEWVSAIGGSGGSTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARYWPMDSWGQGTLVTVSS (SEQ ID NO: 76)
C01_Rd4_6nM_C24	EIVLTQSPGTLSLSPGERATLSCRASQSVSPEYLAWYQQKPGQAPRLLIYDASSRAPGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYSVWPLTFGQGTKVEIK (SEQ ID NO: 79)	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYPMSWVRQAPGKGLEWVSAIGGSGGSLPYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARYWPMDSWGQGTLVTVSS (SEQ ID NO: 78)
C01_Rd4_6nM_C26	EIVLTQSPGTLSLSPGERATLSCRASQSVSAIYLAWYQQKPGQAPRLLIYDASSRAPGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYSAWPLTFGQGTKVEIK (SEQ ID NO: 317)	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYPMSWVRQAPGKGLEWVSAIGGSGGSLPYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARYWPMDSWGQGTLVTVSS (SEQ ID NO: 78)
C01_Rd4_6nM_C10	EIVLTQSPGTLSLSPGERATLSCRASQSVSSvYLAWYQQKPGQAPRLLIYDASSRAPGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYSTWPLTFGQGTKVEIK (SEQ ID NO: 79)	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYPMSWVRQAPGKGLEWVSAIGgSGGSLPYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARYWPMDSWGQGTLVTVSS (SEQ ID NO: 78)
C01_Rd4_0.6nM_C27	EIVLTQSPGTLSLSPGERATLSCRASQSVSSTYLAWYQQKPGQAPRLLIYDASSRAPGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYS	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYPMSWVRQAPGKGLEWVSAIGgSGGSLPYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAV

mAb	Light Chain	Heavy Chain
	RWPLTFGQGTKVEIK (SEQ ID NO: 81)	YYCARYWPMDSWGQGTLVTVSS (SEQ ID NO: 78)
C01_Rd4_6nM_C20	EIVLTQSPGTLSLSPGERATLSCRASQSVSPIYLAWYQQKPGQAPRLLIYDASSRAPGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYSAFPLTFGQGTKVEIK (SEQ ID NO: 82)	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYPMSWVRQAPGKGLEWVSAIGgSGGSLPYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARYWPMDSWGQGTLVTVSS (SEQ ID NO: 78)
C01_Rd4_6nM_C12 (PC1C12)	EIVLTQSPGTLSLSPGERATLSCWLSQSVSSTYLAWYQQKPGQAPRLLIYDASSRAPGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYSEWPLTFGQGTKVEIK (SEQ ID NO: 84)	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYPMSWVRQAPGKGLEWVSAIGgSGGWSYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARYWPMDSWGQGTLVTVSS (SEQ ID NO: 83)
C01_Rd4_0.6nM_C16	EIVLTQSPGTLSLSPGERATLSCRASQSVSSTYLAWYQQKPGQAPRLLIYDASSRAPGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYSSWPLTFGQGTKVEIK (SEQ ID NO: 85)	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYPMSWVRQAPGKGLEWVSAIGgSGGSLPYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARYWPMDSWGQGTLVTVSS (SEQ ID NO: 78)
C01_Rd4_0.6nM_C09	EIVLTQSPGTLSLSPGERATLSCRASQSVSSIFLAWYQQKPGQAPRLLIYDASSRAPGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYSAWPLTFGQGTKVEIK (SEQ ID NO: 86)	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYPMSWVRQAPGKGLEWVSAIGgSGGSLPYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARYWPMDSWGQGTLVTVSS (SEQ ID NO: 78)
C01_Rd4_6nM_C09	EIVLTQSPGTLSLSPGERATLSCACSQSVSSTYLAWYQQKPGQAPRLLIYDASSRAPGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYS	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYPMSWVRQAPGKGLEWVSATVgSGGSIGYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAV

mAb	Light Chain	Heavy Chain
	AWPLTFGQGTKVEIK (SEQ ID NO: 88)	YYCARYWPMDSWGQGTLVTVSS (SEQ ID NO: 87)
C01_Rd4_0.6nM_C03	EIVLTQSPGTLSLSPGERATLSCRASCDVSSTYLAWYQQKPGQAPRLLIYDASSRAPGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYMRSPLTFGQGTKVEIK (SEQ ID NO: 89)	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYPMSWVRQAPGKGLEWVSAIGgSGGSLPYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARYWPMDSWGQGTLVTVSS (SEQ ID NO: 78)
C01_Rd4_0.6nM_C06	EIVLTQSPGTLSLSPGERATLSCRASEAVPSTYLAWYQQKPGQAPRLLIYDASSRAPGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYSAFPLTFGQGTKVEIK (SEQ ID NO: 90)	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYPMSWVRQAPGKGLEWVSAIGgSGGSLPYADSVKGTISRDNSKNTLYLQMNSLRAEDTAVYYCARYWPMDSWGQGTLVTVSS (SEQ ID NO: 78)
C01_Rd4_6nM_C04	EIVLTQSPGTLSLSPGERATLSCCSSQSVSSTYLAWYQQKPGQAPRLLIYDASSRAPGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYSAFPLTFGQGTKVEIK (SEQ ID NO: 91)	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYPMSWVRQAPGKGLEWVSAIGgSGGSLPYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARYWPMDSWGQGTLVTVSS (SEQ ID NO: 78)
COMBO_Rd4_0.6nM_C22 (COM22)	EIVLTQSPGTLSLSPGERATLSCRASVRVSSTYLAWYQQKPGQAPRLLMYDASIRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYMKWPLTFGQGTKVEIK (SEQ ID NO: 93)	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMNWVRQAPGKGLEWVSAISdSGGSRWYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCTRYWPMDIWGQGTLVTVSS (SEQ ID NO: 92)
COMBO_Rd4_6nM_C21	EIVLTQSPGTLSLSPGERATLSCRASQSVSAAYLAWYQQKPGQAPRLLMYDASIRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQ	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYPMSWVRQAPGKGLEWVSAIGgSGGSLPYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAV

mAb	Light Chain	Heavy Chain
	YMCWPLTFGQGTKVEIK (SEQ ID NO: 94)	YYCARYWPMDSWGQGTLVTVSS (SEQ ID NO: 78)
COMBO_Rd4_6nM_C10	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYWGWYQQKPGQAPRLLMYDASIRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYQCWPLTFGQGTKVEIK (SEQ ID NO: 96)	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYPMSWVRQAPGKGLEWVSAIGgSGGSIHYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARYWPMDSWGQGTLVTVSS (SEQ ID NO: 95)
COMBO_Rd4_0.6nM_C04	EIVLTQSPGTLSLSPGERATLSCRASQSVSSTYLAWYQQKPGQAPRLLMYDASIRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYQSWPLTFGQGTKVEIK (SEQ ID NO: 98)	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYPMSWVRQAPGKGLEWVSAHIgSGGSTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARYWPMDSWGQGTLVTVSS (SEQ ID NO: 97)
COMBO_Rd4_6nM_C25	EIVLTQSPGTLSLSPGERATLSCRASQSVSSpYLAWYQQKPGQAPRLLMYDASIRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYQSWPLTFGQGTKVEIK (SEQ ID NO: 100)	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYPMSWVRQAPGKGLEWVSAIGgSGGSTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARYWPMDPWGQGTLVTVSS (SEQ ID NO: 99)
COMBO_Rd4_0.6nM_C21	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLMYDASIRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYQSWPLTFGQGTKVEIK (SEQ ID NO: 38)	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYPMSWVRQAPGKGLEWVSAIGgSGGSLPYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARYWPMDSWGQGTLVTVSS (SEQ ID NO: 78)
COMBO_Rd4_6nM_C11	EIVLTQSPGTLSLSPGERATLSCRASQSVSPIYLAWYQQKPGQAPRLLMYDASIRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQY	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYPMSWVRQAPGKGLEWVSAIGGSGGSLGYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAV

mAb	Light Chain	Heavy Chain
	KAWPLTFGQGTKVEIK (SEQ ID NO: 102)	YYCARYWPMDSWGQGTLVTVSS (SEQ ID NO: 101)
COMBO_Rd4_0.6nM_C20	EIVLTQSPGTLSLSPGERATLSCRASQSVSYLYLAWYQQKPGQAPRLLMYDASIRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYMEWPLTFGQGTKVEIK (SEQ ID NO: 103)	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYPMSWVRQAPGKGLEWVSAIGGSGGSLPYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARYWPMDSWGQGTLVTVSS (SEQ ID NO: 78)
COMBO_Rd4_6nM_C09	EIVLTQSPGTLSLSPGERATLSCRASQSVSAQYLAWYQQKPGQAPRLLMYDASIRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYQAWPLTFGQGTKVEIK (SEQ ID NO: 105)	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYPMSWVRQAPGKGLEWVSAIFASGGSTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARYWPMDSWGQGTLVTVSS (SEQ ID NO: 104)
COMBO_Rd4_6nM_C08	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLMYDASIRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYQKWPLTFGQGTKVEIK (SEQ ID NO: 107)	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYPMSWVRQAPGKGLEWVSAIGGSGGTWTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARYWPMDSWGQGTLVTVSS (SEQ ID NO: 106)
COMBO_Rd4_0.6nM_C19	EIVLTQSPGTLSLSPGERATLSCRASQSVSAVYLAWYQQKPGQAPRLLMYDASIRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYRAWPLTFGQGTKVEIK (SEQ ID NO: 108)	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYPMSWVRQAPGKGLEWVSAIGGSGGSLPYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARYWPMDSWGQGTLVTVSS (SEQ ID NO: 78)
COMBO_Rd4_0.6nM_C02	EIVLTQSPGTLSLSPGERATLSCRASIAVSSTYLAWYQQKPGQAPRLLMYDASIRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQY	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYPMSWVRQAPGKGLEWVSAIGGSGGSLPYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAV

mAb	Light Chain	Heavy Chain
	MVWPLTFGQGTKVEIK (SEQ ID NO: 109)	YYCARYWPMDSWGQGTLVTVSS (SEQ ID NO: 78)
COMBO_Rd4_0.6nM_C23	EIVLTQSPGTLSLSPGERATLSCRPRQSVSSSYLAWYQQKPGQAPRLLMYDASIRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYQDWPLTFGQGTKVEIK (SEQ ID NO: 111)	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYPMSWVRQAPGKGLEWVSALFGSGGSTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARYWPMDSWGQGTLVTVSS (SEQ ID NO: 110)
COMBO_Rd4_0.6nM_C29	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLMYDASIRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYQSWPLTFGQGTKVEIK (SEQ ID NO: 38)	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYPMSWVRQAPGKGLEWVSAIGGSGGSLPYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARYWPMDIWGQGTLVTVSS (SEQ ID NO: 112)
COMBO_Rd4_0.6nM_C09	EIVLTQSPGTLSLSPGERATLSCRASQSVSSTYLAWYQQKPGQAPRLLMYDASIRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYQEWPLTFGQGTKVEIK (SEQ ID NO: 113)	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYPMSWVRQAPGKGLEWVSAIGGSGGSLPYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARYWPMDIWGQGTLVTVSS (SEQ ID NO: 112)
COMBO_Rd4_6nM_C12	EIVLTQSPGTLSLSPGERATLSCRASQSVSASYLAWYQQKPGQAPRLLMYDASIRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYMSWPLTFGQGTKVEIK (SEQ ID NO: 115)	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYPMSWVRQAPGKGLEWVSAALGSGGSTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARYWPMDSWGQGTLVTVSS (SEQ ID NO: 114)
COMBO_Rd4_0.6nM_C30	EIVLTQSPGTLSLSPGERATLSCRASQSVSYMYLAWYQQKPGQAPRLLIYDASIRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQY	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYPMSWVRQAPGKGLEWVSAIGGSGGSTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAV

mAb	Light Chain	Heavy Chain
	KSWPLTFGQGTKVEIK (SEQ ID NO: 116)	YYCARYWPMDSWGQGTLVTVSS (SEQ ID NO: 76)
COMBO_Rd4_0.6nM_C14	EIVLTQSPGTLSLSPGERATLSCRASQSVSALYLAWYQQKPGQAPRLLMYDASIRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYYGWPLTFGQGTKVEIK (SEQ ID NO: 117)	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYPMSWVRQAPGKGLEWVSAIGGSGGSLPYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARYWPMDIWGQGTLVTVSS (SEQ ID NO: 112)
COMBO_Rd4_6nM_C07	EIVLTQSPGTLSLSPGERATLSCRASQPISSSYLAWYQQKPGQAPRLLMYDASIRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYQGWPLTFGQGTKVEIK (SEQ ID NO: 119)	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYPMSWVRQAPGKGLEWVSAIGGSGGSLPYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARYWPMADWGQGTLVTVSS (SEQ ID NO: 118)
COMBO_Rd4_6nM_C02	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLMYDASIRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYEFWPLTFGQGTKVEIK (SEQ ID NO: 121)	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMNWVRQAPGKGLEWVSAISDSGGFVYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARYWPMDSWGQGTLVTVSS (SEQ ID NO: 120)
COMBO_Rd4_0.6nM_C05	EIVLTQSPGTLSLSPGERATLSCRASQSVSSTYLAWYQQKPGQAPRLLMYDASIRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYMSWPLTFGQGTKVEIK (SEQ ID NO: 123)	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMNWVRQAPGKGLEWVSAIGGSGGSTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARYWPMSLWGQGTLVTVSS (SEQ ID NO: 122)
COMBO_Rd4_0.6nM_C17	EIVLTQSPGTLSLSPGERATLSCRASQGISSTYLAWYQQKPGQAPRLLMYDASIRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQY	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYPMSWVRQAPGKGLEWVSAIGGSGGSLPYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAV

mAb	Light Chain	Heavy Chain
	AYWPLTFGQGTKVEIK (SEQ ID NO: 124)	YYCARYWPMDIWGQGTLVTVSS (SEQ ID NO: 112)
COMBO_Rd4_6nM_C22	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLMYDASIRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYQGWPLTFGQGTKVEIK (SEQ ID NO: 126)	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMNWVRQAPGKGLEWVSACLDSGGSTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARYWPMDSWGQGTLVTVSS (SEQ ID NO: 125)
COMBO_Rd4_0.6nM_C11	EIVLTQSPGTLSLSPGERATLSCRASQSVSVRYLAWYQQKPGQAPRLLMYDASIRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYGSWPITFGQGTKVEIK (SEQ ID NO: 128)	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYPMSWVRQAPGKGLEWVSAALGSGGSTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARYWPMSLWGQGTLVTVSS (SEQ ID NO: 127)
P6DY	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYPSWYQQKPGQAPRLLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQHYPYPPSFTFGQGTKVEIK (SEQ ID NO: 18)	EVQLLESGGGLVQPGGSLRLSCAASGFTFGSYAMTWVRQAPGKGLEWVSAIDYSGGNTFYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARVSPIASGMDYWGQGTLVTVSS (SEQ ID NO: 25)
P6AP	EIVLTQSPGTLSLSPGERATLSCRASQLGSFYLAWYQQKPGQAPRLLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQHYNYPPSFTFGQGTKVEIK (SEQ ID NO: 80)	EVQLLESGGGLVQPGGSLRLSCAASGFTFGSYAMTWVRQAPGKGLEWVSAISGSGGNTFYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARVSPIAAPMDYWGQGTLVTVSS (SEQ ID NO: 8)
Consensus	EIVLTQSPGTLSLSPGERATLSC $X_1X_2X_3X_4X_5X_6X_7X_8X_9X_{10}X_{11}X_{12}$WYQQKPGQAPRLLMY$X_{13}ASX_{14}RAX_{15}$GIPDRFSGSGSGTDFTLTISRLE	EVQLLESGGGLVQPGGSLRLSCAASGFTFX_1SYX_2MX_3WVRQAPGKGLEWVSA$X_4X_5X_6X_7$G$X_8X_9X_{10}X_{11}$YAD$X_{12}X_{13}$KGRFTISRDNSKNTLYLQMN

mAb	Light Chain	Heavy Chain
	PEDFAVYYCX$_{16}$X$_{17}$YX$_{18}$X$_{19}$PPSF TFGQGTKVEIK, wherein X$_1$ is R, G, W, A, or C; X$_2$ is A, P, G, L, C, or S; X$_3$ is S, G, or R; X$_4$ is Q, C, E, V, or I; X$_5$ is S, P, G, A, R, or D; X$_6$ is V, G, I, or L; X$_7$ is S, E, D, P, or G; X$_8$ is S, P, F, A, M, E, V, N, D, or Y; X$_9$ is I, T, V, E, S, A, M, Q, Y, H, R, or F; X$_{10}$ is Y or F; X$_{11}$ is L, W, or P; X$_{12}$ is A, S, or G, X$_{13}$ is G or D; X$_{14}$ is S or I; X$_{15}$ is T or P; X$_{16}$ is Q or K; X$_{17}$ is H or Y; X$_{18}$ is G, N, or P; and X$_{19}$ is S, W, or Y (SEQ ID NO: 315); or EIVLTQSPGTLSLSPGERATLSC X$_1$X$_2$X$_3$X$_4$X$_5$X$_6$X$_7$X$_8$X$_9$X$_{10}$X$_{11}$X$_{12}$WY QQKPGQAPRLLMYX$_{13}$ASX$_{14}$RAX $_{15}$GIPDRFSGSGSGTDFTLTISRLE PEDFAVYYCQQYX$_{16}$X$_{17}$X$_{18}$PX$_{19}$F GQGTKVEIK, wherein X$_1$ is R, G, W, A, or C; X$_2$ is A, P, G, L, C, or S; X$_3$ is S, G, or R; X$_4$ is Q, C, E, V, or I; X$_5$ is S, L, P, G, A, R, or D; X$_6$ is V, G, or I; X$_7$ is S, E, D, or P; X$_8$ is S, P, F, A, M, E, V, N, D, or Y; X$_9$ is I, T, V, E, S, A, M, Q, Y, H, or R; X$_{10}$ is Y or F; X$_{11}$ is L, W, or P; X$_{12}$ is A, S, or G, X$_{13}$ is G or D; X$_{14}$ is S or I; X$_{15}$ is T or P; X$_{16}$ is G, Q, E, L, F, A, S, M, R, K, or Y; X$_{17}$	SLRAEDTAVYYCARVSPIX$_{14}$X$_{15}$X$_{16}$ MDYWGQGTLVTVSS, wherein X$_1$ is G or S, X$_2$ is A or P; X$_3$ is T, N, or S; X$_4$ is I, V, T, H, L, A, or C; X$_5$ is S, D, G, T, I, L, F, M, or V; X$_6$ is G, Y, L, H, D, A, S, or M; X$_7$ is S, Q, T, A, F, or W; X$_8$ is G or T; X$_9$ is N, S, P, Y, W, or F; X$_{10}$ is S, T, I, L, T, A, R, V, K, G, or C; X$_{11}$ is F, Y, P, W, H, or G; X$_{12}$ is V, R, or L; X$_{13}$ is G or T; X$_{14}$ is A or Y; X$_{15}$ is A or S; and X$_{16}$ is G, Q, L, P, or E (SEQ ID NO: 313); or EVQLLESGGGLVQPGGSLRLSCA ASGFTFX$_1$SYX$_2$MX$_3$WVRQAPGKG LEWVSAX$_4$X$_5$X$_6$X$_7$GX$_8$X$_9$X$_{10}$X$_{11}$YAD X$_{12}$X$_{13}$KGRFTISRDNSKNTLYLQMN SLRAEDTAVYYCARYWPMX$_{14}$X$_{15}$ WGQGTLVTVSS, wherein X$_1$ is G or S, X$_2$ is A or P; X$_3$ is T, N, or S; X$_4$ is I, V, T, H, L, A, or C; X$_5$ is S, D, G, T, I, L, F, M, or V; X$_6$ is G, Y, L, H, D, A, S, or M; X$_7$ is S, Q, T, A, F, or W; X$_8$ is G or T; X$_9$ is N, S, P, Y, W, or F; X$_{10}$ is S, T, I, L, T, A, R, V, K, G, or C; X$_{11}$ is F, Y, P, W, H, or G; X$_{12}$ is V, R, or L; X$_{13}$ is G or T; X$_{14}$ is D, S, T, or A; and X$_{15}$ is I, S, L, P, or D (SEQ ID NO: 314)

mAb	Light Chain	Heavy Chain
	is S, R, T, G, R, V, D, A, H, E, K, C, F, or Y; X_{18} is W, S, or F; and X_{19} is L or I (SEQ ID NO: 316)	
P4G4	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLIYGASSRAYGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQHYGSPPLFTFGQGTKVEIK (SEQ ID NO: 401)	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAISASGGSTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARLSWSGAFDNWGQGTLVTVSS (SEQ ID NO: 378)
P1A11	EIVLTQSPGTLSLSPGERATLSCRASQNVSSSYLAWYQQKPGQAPRLLIYGASYRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQHYGSPPSFTFGQGTKVEIK (SEQ ID NO: 379)	EVQLLESGGGLVQPGGSLRLSCAASGFTFRSYAMSWVRQAPGKGLEWVSAISGSGGSTFYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCATVGTSGAFGIWGQGTLVTVSS (SEQ ID NO: 380)

Also provided herein are CDR portions of extracellular ligand-binding domains of CARs to BCMA (including Chothia, Kabat CDRs, and CDR contact regions). Determination of CDR regions is well within the skill of the art. It is understood that in some embodiments, CDRs can be a combination of the Kabat and Chothia CDR (also termed "combined CRs" 5 or "extended CDRs"). In some embodiments, the CDRs are the Kabat CDRs. In other embodiments, the CDRs are the Chothia CDRs. In other words, in embodiments with more than one CDR, the CDRs may be any of Kabat, Chothia, combination CDRs, or combinations thereof. Table 2 provides examples of CDR sequences provided herein.

Table 2

Heavy Chain			
mAb	**CDRH1**	**CDRH2**	**CDRH3**
P6E01 For the following mAbs: P6E01/P6E01;L1.LGF/L3.KW/P6E01; L1.LGF/L3.NY/P6E01; L1.GDF/L3.NY/P6E01; L3.KW/P6E01; L3.PY/P6E01; L3.NY/P6E01;	SYAMT (SEQ ID NO: 129) (Kabat); GFTFGSY (SEQ ID NO: 130) (Chothia); GFTFGSYAMT (SEQ ID NO: 131) (extended)	AISGSGGNTFYADSVKG (SEQ ID NO: 132) (Kabat) SGSGGN (SEQ ID NO:133) (Chothia)	VSPIASGMDY (SEQ ID NO: 134)

L3.PY/L1.PS/P6E01; L3.PY/L1.AH/P6E01; L3.PY/L1.FF/P6E01; L3.PY/L1.PH/P6E01; L3.PY/L3.KY/P6E01; L3.PY/L3.KF/P6E01; and L3.PY/P6E01.			
H3.AQ For the following mAbs: P6E01/H3.AQ; L1.LGF/L3.KW/H3.AQ; L1.LGF/L3.PY/H3.AQ	SYAMT (SEQ ID NO: 129) (Kabat); GFTFGSY (SEQ ID NO: 130) (Chothia); GFTFGSYAMT (SEQ ID NO: 131) (extended)	AISGSGGNTFYA DSVKG (SEQ ID NO: 132) (Kabat) SGSGGN (SEQ ID NO:133) (Chothia)	VSPIAAQMD Y (SEQ ID NO: 135)
H3.AL For the following mAbs: L1.LGF/L3.KW/H3.AL; L1.LGF/L3.NY/H3.AL; and L1.GDF/L3.NY/H3.AL.	SYAMT (SEQ ID NO: 129) (Kabat); GFTFGSY (SEQ ID NO: 130) (Chothia); GFTFGSYAMT (SEQ ID NO: 131) (extended)	AISGSGGNTFYA DSVKG (SEQ ID NO: 132) (Kabat) SGSGGN (SEQ ID NO:133) (Chothia)	VSPIAALMD Y (SEQ ID NO: 136)
H3.AP For the following mAbs:	SYAMT (SEQ ID NO: 129) (Kabat);	AISGSGGNTFYA DSVKG (SEQ ID	VSPIAAPMD Y

L1.LGF/L3.KW/H3.AP; L1.LGF/L3.PY/H3.AP; L1.LGF/L3NY/H3.AP; L1.GDF/L3.KW/H3.AP; L1.GDF/L3NY/H3.AP; P6AP.	GFTFGSY (SEQ ID NO: 130) (Chothia); GFTFGSYAMT (SEQ ID NO: 131) (extended)	NO: 132) (Kabat) SGSGGN (SEQ ID NO:133) (Chothia)	(SEQ ID NO: 137)
H2.QR For the following mAbs: L3.PY/H2.QR; L3.PY/L1.PS/H2.QR; L3.PY/L1.AH/H2.QR; L3.PY/L1.FF/H2.QR; L3.PY/L1.PH/H2.QR; and L3.PY/L3.KY/H2.QR.	SYAMT (SEQ ID NO: 129) (Kabat); GFTFGSY (SEQ ID NO: 130) (Chothia); GFTFGSYAMT (SEQ ID NO: 131) (extended)	AISGSGGNTFYA DQRKG (SEQ ID NO: 138) (Kabat) SGSGGN (SEQ ID NO:133) (Chothia)	VSPIASGMD Y (SEQ ID NO: 134)
H2.DY For the following mAbs: L3.PY/H2.DY; P6DY; L3.PY/L1.PS/H2.DY; L3.PY/L1.AH/H2.DY; L3.PY/L1.FF/H2.DY; L3.PY/L3.KY/H2.DY; and L3.PY/L3.KF/H2.DY.	SYAMT (SEQ ID NO: 129) (Kabat); GFTFGSY (SEQ ID NO: 130) (Chothia); GFTFGSYAMT (SEQ ID NO: 131) (extended)	AIDYSGGNTFYA DSVKG (SEQ ID NO: 139) (Kabat) DYSSGN (SEQ ID NO:140) (Chothia)	VSPIASGMD Y (SEQ ID NO: 134)

H2.YQ For the following mAbs: L3.PY/H2.YQ; L3.PY/L1.PS/H2.YQ; L3.PY/L1.AH/H2.YQ; L3.PY/L1.FF/H2.YQ; L3.PY/L3.KY/H2.YQ; and L3.PY/L3.KF/H2.YQ.	SYAMT (SEQ ID NO: 129) (Kabat); GFTFGSY (SEQ ID NO: 130) (Chothia); GFTFGSYAMT (SEQ ID NO: 131) (extended)	AISYQGGNTFYA DSVKG (SEQ ID NO: 141) (Kabat) SYQGGN (SEQ ID NO:142) (Chothia)	VSPIASGMD Y (SEQ ID NO: 134)
H2.LT For the following mAbs: L3.PY/H2.LT; L3.PY/L1.PS/H2.LT; L3.PY/L1.AH/H2.LT; L3.PY/L1.FF/H2.LT; L3.PY/L3.KY/H2.LT; and L3.PY/L3.KF/H2.LT.	SYAMT (SEQ ID NO: 129) (Kabat); GFTFGSY (SEQ ID NO: 130) (Chothia); GFTFGSYAMT (SEQ ID NO: 131) (extended)	AISLTGGNTFYA DSVKG (SEQ ID NO: 143) (Kabat) SLTGGN (SEQ ID NO:144) (Chothia)	VSPIASGMD Y (SEQ ID NO: 134)
H2.HA For the following mAbs: L3.PY/H2.HA; L3.PY/L1.AH/H2.HA; L3.PY/L1.FF/H2.HA; L3.PY/L1.PH/H2.HA; and L3.PY/L3.KY/H2.HA.	SYAMT (SEQ ID NO: 129) (Kabat); GFTFGSY (SEQ ID NO: 130) (Chothia); GFTFGSYAMT (SEQ ID NO: 131) (extended)	AISHAGGNTFYA DSVKG (SEQ ID NO: 145) (Kabat) SHAGGN (SEQ ID NO:146) (Chothia)	VSPIASGMD Y (SEQ ID NO: 134)
H2.QL For the following mAbs:	SYAMT (SEQ ID NO: 129) (Kabat);	AISGSGGNTFYA DQLKG (SEQ ID	VSPIASGMD Y (SEQ ID

L3.PY/H2.QL; L3.PY/L1.PS/H2.QL; L3.PY/L1.AH/H2.QL; L3.PY/L1.FF/H2.QL; L3.PY/L3.KY/H2.QL; and L3.PY/L3.KF/H2.QL.	GFTFGSY (SEQ ID NO: 130) (Chothia); GFTFGSYAMT (SEQ ID NO: 131) (extended)	NO: 147) **(Kabat)** SGSGGN (SEQ ID NO:133) **(Chothia)**	NO: 134)
H3.YA For the following mAbs: L3.PY/H3.YA; L3.PY/L1.PS/H3.YA; L3.PY/L1.AH/H3.YA; L3.PY/L1.FF/H3.YA; L3.PY/L3.KY/H3.YA; and L3.PY/L3.KF/H3.YA.	SYAMT (SEQ ID NO: 129) (Kabat); GFTFGSY (SEQ ID NO: 130) (Chothia); GFTFGSYAMT (SEQ ID NO: 131) (extended)	AISGSGGNTFYA DSVKG (SEQ ID NO: 132) **(Kabat)** SGSGGN (SEQ ID NO:133) **(Chothia)**	VSPIYAGMD Y (SEQ ID NO: 148)
H3.AE For the following mAbs: L3.PY/H3.AE; L3.PY/L1.AH/H3.AE; L3.PY/L1.FF/H3.AE; L3.PY/L1.PH/H3.AE; and L3.PY/L3.KF/H3.AE.	SYAMT (SEQ ID NO: 129) (Kabat); GFTFGSY (SEQ ID NO: 130) (Chothia); GFTFGSYAMT (SEQ ID NO: 131) (extended)	AISGSGGNTFYA DSVKG (SEQ ID NO: 132) **(Kabat)** SGSGGN (SEQ ID NO:133) **(Chothia)**	VSPIAAEMD Y (SEQ ID NO: 149)

H3.TAQ For the following mAbs: L3.PY/H3.TAQ; L3.PY/L1.PS/H3.TAQ; L3.PY/L1.AH/H3.TAQ; L3.PY/L1.FF/H3.TAQ; L3.PY/L1.PH/H3.TAQ; and L3.PY/L3.KF/H3.TAQ.	SYAMT (SEQ ID NO: 129) (Kabat); GFTFGSY (SEQ ID NO: 130) (Chothia); GFTFGSYAMT (SEQ ID NO: 131) (extended)	AISGSGGNTFYA DSVKG (SEQ ID NO: 132) (Kabat) SGSGGN (SEQ ID NO:133) (Chothia)	VSPIAAQMD Y (SEQ ID NO: 135)
P5A2_VHVL and A02_Rd4_6nM_C03	SYAMN (SEQ ID NO: 150) (Kabat); GFTFSSY (SEQ ID NO: 151) (Chothia); GFTFSSYAMN (SEQ ID NO: 152) (extended)	AISDSGGSTYYA DSVKG (SEQ ID NO: 153) (Kabat) SDSGGS (SEQ ID NO: 154) (Chothia)	YWPMDI (SEQ ID NO: 155)
COMBO_Rd4_0.6nM_C 17; COMBO_Rd4_0.6nM_C 14; COMBO_Rd4_0.6nM_C 29; and COMBO_Rd4_0.6nM_C 09	SYPMS (SEQ ID NO: 156) (Kabat); GFTFSSY (SEQ ID NO: 151) (Chothia); GFTFSSYPMS (SEQ ID NO: 157) (extended)	AIGGSGGSLPYA DSVKG (SEQ ID NO: 158) (Kabat) GGSGGS (SEQ ID NO: 159) (Chothia)	YWPMDI (SEQ ID NO: 155)
C01_Rd4_6nM_C04; C01_Rd4_0.6nM_C03; C01_Rd4_0.6nM_C06; COMBO_Rd4_0.6nM_C	SYPMS (SEQ ID NO: 156) (Kabat); GFTFSSY (SEQ ID NO: 151) (Chothia);	AIGGSGGSLPYA DSVKG (SEQ ID NO: 158) (Kabat)	YWPMDS (SEQ ID NO: 161)

02; COMBO_Rd4_6nM_C21; C01_Rd4_6nM_C26; COMBO_Rd4_0.6nM_C19; C01_Rd4_6nM_C24; C01_Rd4_6nM_C20; C01_Rd4_0.6nM_C09; COMBO_Rd4_0.6nM_C21; C01_Rd4_0.6nM_C04_C27; C01_Rd4_0.6nM_C16; C01_Rd4_6nM_C10; COMBO_Rd4_0.6nM_C20	GFTFSSYPMS (SEQ ID NO: 157) (extended)	GGSGGS (SEQ ID NO: 159) (Chothia)	
P5C1_VHVL (PC1) and COMBO_Rd4_0.6nM_C30	SYPMS (SEQ ID NO: 156) (Kabat); GFTFSSY (SEQ ID NO: 151) (Chothia); GFTFSSYPMS (SEQ ID NO: 157) (extended)	AIGGSGGSTYYADSVKG (SEQ ID NO: 162) (Kabat) GGSGGS (SEQ ID NO: 159) (Chothia)	YWPMDS (SEQ ID NO: 161)
A02_Rd4_0.6nM_C06	SYAMN (SEQ ID NO: 150) (Kabat); GFTFSSY (SEQ ID NO: 151) (Chothia); GFTFSSYAMN (SEQ ID NO: 152) (extended)	AISDSGGSAWYADSVKG (SEQ ID NO: 163) (Kabat) SDSGGS (SEQ ID NO: 154) (Chothia)	YWPMSL (SEQ ID NO: 164)

A02_Rd4_0.6nM_C09	SYAMN (SEQ ID NO: 150) (Kabat); GFTFSSY (SEQ ID NO: 151) (Chothia); GFTFSSYAMN (SEQ ID NO: 152) (extended)	AISDSGGSAWY ADSVKG (SEQ ID NO: 163) (Kabat) SDSGGS (SEQ ID NO: 154) (Chothia)	YWPMSL (SEQ ID NO: 164)
A02_Rd4_0.6nM_C16; A02_Rd4_6nM_C16 (P5A16)	SYAMN (SEQ ID NO: 150) (Kabat); GFTFSSY (SEQ ID NO: 151) (Chothia); GFTFSSYAMN (SEQ ID NO: 152) (extended)	AISDFGGSTYYA DSVKG (SEQ ID NO: 165) (Kabat) SDFGGS (SEQ ID NO: 166) (Chothia)	YWPMDI (SEQ ID NO: 155)
A02_Rd4_6nM_C01	SYAMN (SEQ ID NO: 150) (Kabat); GFTFSSY (SEQ ID NO: 151) (Chothia); GFTFSSYAMN (SEQ ID NO: 152) (extended)	AITASGGSTYYA DSVKG (SEQ ID NO: 167) (Kabat) TASGGS (SEQ ID NO: 168) (Chothia)	YWPMSL (SEQ ID NO: 164)
A02_Rd4_6nM_C26	SYAMN (SEQ ID NO: 150) (Kabat); GFTFSSY (SEQ ID NO: 151) (Chothia); GFTFSSYAMN (SEQ ID NO: 152) (extended)	AISDSGGSTYYA DSVKG (SEQ ID NO: 153) (Kabat) SDSGGS (SEQ ID NO: 154) (Chothia)	YWPMSL (SEQ ID NO: 164)
A02_Rd4_6nM_C25	SYAMN (SEQ ID NO: 150) (Kabat); GFTFSSY (SEQ ID	AISDSGGSRWY ADSVKG (SEQ ID NO: 169)	YWPMTP (SEQ ID NO: 170)

	NO: 151) (Chothia); GFTFSSYAMN (SEQ ID NO: 152) (extended)	(Kabat) SDSGGS (SEQ ID NO: 154) (Chothia)	
A02_Rd4_6nM_C22	SYAMN (SEQ ID NO: 150) (Kabat); GFTFSSY (SEQ ID NO: 151) (Chothia); GFTFSSYAMN (SEQ ID NO: 152) (extended)	AVLDSGGSTYY ADSVKG (SEQ ID NO: 171) (Kabat) LDSGGS (SEQ ID NO: 172) (Chothia)	YWPMTP (SEQ ID NO: 170)
A02_Rd4_6nM_C19	SYAMN (SEQ ID NO: 150) (Kabat); GFTFSSY (SEQ ID NO: 151) (Chothia); GFTFSSYAMN (SEQ ID NO: 152) (extended)	AISDSGGSRWY ADSVKG (SEQ ID NO: 169) (Kabat) SDSGGS (SEQ ID NO: 154) (Chothia)	YWPMSD (SEQ ID NO: 173)
A02_Rd4_0.6nM_C03	SYAMN (SEQ ID NO: 150) (Kabat); GFTFSSY (SEQ ID NO: 151) (Chothia); GFTFSSYAMN (SEQ ID NO: 152) (extended)	AISDSGGSKWY ADSVKG (SEQ ID NO: 174) (Kabat) SDSGGS (SEQ ID NO: 154) (Chothia)	YWPMSL (SEQ ID NO: 164)
A02_Rd4_6nM_C07	SYAMN (SEQ ID NO: 150) (Kabat); GFTFSSY (SEQ ID NO: 151) (Chothia); GFTFSSYAMN (SEQ ID NO: 152)	AIGGSGGSLPYA DSVKG(SEQ ID NO: 158) (Kabat) GGSGGS (SEQ ID NO: 159)	YWPMDS (SEQ ID NO: 161)

	(extended)	(Chothia)	
A02_Rd4_6nM_C23	SYAMN (SEQ ID NO: 150) (Kabat); GFTFSSY (SEQ ID NO: 151) (Chothia); GFTFSSYAMN (SEQ ID NO: 152) (extended)	AISDSGGSGWY ADSVKG (SEQ ID NO: 175)(Kabat) SDSGGS (SEQ ID NO: 154) (Chothia)	YWPMSL (SEQ ID NO: 164)
A02_Rd4_0.6nM_C18	SYAMN (SEQ ID NO: 150) (Kabat); GFTFSSY (SEQ ID NO: 151) (Chothia); GFTFSSYAMN (SEQ ID NO: 152) (extended)	AVLDSGGSTYY ADSVKG (SEQ ID NO: 171) (Kabat) LDSGGS (SEQ ID NO: 172) (Chothia)	YWPMSL (SEQ ID NO: 164)
A02_Rd4_6nM_C10	SYAMN (SEQ ID NO: 150) (Kabat); GFTFSSY (SEQ ID NO: 151) (Chothia); GFTFSSYAMN (SEQ ID NO: 152) (extended)	AISDSGGSCWY ADSVKG (SEQ ID NO: 176) (Kabat) SDSGGS (SEQ ID NO: 154) (Chothia)	YWPMTP (SEQ ID NO: 170)
A02_Rd4_6nM_C05	SYAMN (SEQ ID NO: 150) (Kabat); GFTFSSY (SEQ ID NO: 151) (Chothia); GFTFSSYAMN (SEQ ID NO: 152) (extended)	AIFASGGSTYYA DSVKG (SEQ ID NO: 177) (Kabat) FASGGS (SEQ ID NO: 178) (Chothia)	YWPMTP (SEQ ID NO: 170)
A02_Rd4_0.6nM_C10	SYAMN (SEQ ID NO:	AISGWGGSLPY	YWPMDS

	150) (Kabat); GFTFSSY (SEQ ID NO: 151) (Chothia); GFTFSSYAMN (SEQ ID NO: 152) (extended)	ADSVKG (SEQ ID NO: 304) **(Kabat)** SGWGGS (SEQ ID NO: 179) **(Chothia)**	(SEQ ID NO: 161)
A02_Rd4_6nM_C04	SYAMN (SEQ ID NO: 150) (Kabat); GFTFSSY (SEQ ID NO: 151) (Chothia); GFTFSSYAMN (SEQ ID NO: 152) (extended)	AIMSSGGPLYYA DSVKG (SEQ ID NO: 180) **(Kabat)** MSSGGP (SEQ ID NO: 181) **(Chothia)**	YWPMAL (SEQ ID NO: 182)
A02_Rd4_0.6nM_C26	SYAMN (SEQ ID NO: 150) (Kabat); GFTFSSY (SEQ ID NO: 151) (Chothia); GFTFSSYAMN (SEQ ID NO: 152) (extended)	AILMSGGSTYYA DSVKG (SEQ ID NO: 183) **(Kabat)** LMSGGS (SEQ ID NO: 184) **(Chothia)**	YWPMSL (SEQ ID NO: 164)
A02_Rd4_0.6nM_C13	SYAMN (SEQ ID NO: 150) (Kabat); GFTFSSY (SEQ ID NO: 151) (Chothia); GFTFSSYAMN (SEQ ID NO: 152) (extended)	AISDSGGYRYYA DSVKG (SEQ ID NO: 185) **(Kabat)** SDSGGY (SEQ ID NO: 186) **(Chothia)**	YWPMSL (SEQ ID NO: 164)
A02_Rd4_0.6nM_C01 (P5AC1)	SYAMN (SEQ ID NO: 150) (Kabat); GFTFSSY (SEQ ID NO: 151) (Chothia);	AILSSGGSTYYA DSVKG (SEQ ID NO: 187) **(Kabat)**	YWPMDI (SEQ ID NO: 155)

	GFTFSSYAMN (SEQ ID NO: 152) (extended)	LSSGGS (SEQ ID NO: 188) (Chothia)	
A02_Rd4_6nM_C08	SYAMN (SEQ ID NO: 150) (Kabat); GFTFSSY (SEQ ID NO: 151) (Chothia); GFTFSSYAMN (SEQ ID NO: 152) (extended)	AILDSGGSTYYA DSVKG (SEQ ID NO: 160)((Kabat) LDSGGS (SEQ ID NO: 172) (Chothia)	YWPMSP (SEQ ID NO: 189)
C01_Rd4_6nM_C12 (PC1C12)	SYPMS (SEQ ID NO: 156) (Kabat); GFTFSSY (SEQ ID NO: 151) (Chothia); GFTFSSYPMS (SEQ ID NO: 157) (extended)	AIGGSGGWSYY ADSVKG (SEQ ID NO: 190) (Kabat) GGSGGW (SEQ ID NO: 191) (Chothia)	YWPMDS (SEQ ID NO: 161)
C01_Rd4_6nM_C09	SYPMS (SEQ ID NO: 156) (Kabat); GFTFSSY (SEQ ID NO: 151) (Chothia); GFTFSSYPMS (SEQ ID NO: 157) (extended)	ATVGSGGSIGYA DSVKG (SEQ ID NO: 192) (Kabat) VGSGGS (SEQ ID NO: 193) (Chothia)	YWPMDS (SEQ ID NO: 161)
COMBO_Rd4_0.6nM_C 22 (COM22)	SYAMN (SEQ ID NO: 150) (Kabat); GFTFSSY (SEQ ID NO: 151) (Chothia); GFTFSSYAMN (SEQ	AISDSGGSRWY ADSVKG (SEQ ID NO: 169) (Kabat) SDSGGS (SEQ	YWPMDI (SEQ ID NO: 155)

	ID NO: 152) (extended)	ID NO: 154) (Chothia)	
COMBO_Rd4_0.6nM_C10	SYPMS (SEQ ID NO: 156) (Kabat); GFTFSSY (SEQ ID NO: 151) (Chothia); GFTFSSYPMS (SEQ ID NO: 157) (extended)	AIGGSGGSIHYA DSVKG (SEQ ID NO: 194) (Kabat) GGSGGS (SEQ ID NO: 159) (Chothia)	YWPMDS (SEQ ID NO: 161)
COMBO_Rd4_0.6nM_C04	SYPMS (SEQ ID NO: 156) (Kabat); GFTFSSY (SEQ ID NO: 151) (Chothia); GFTFSSYPMS (SEQ ID NO: 157) (extended)	AHIGSGGSTYYA DSVKG (SEQ ID NO: 195)I (Kabat) IGSGGS (SEQ ID NO: 196) . (Chothia)	YWPMDS (SEQ ID NO: 161)
COMBO_Rd4_0.6nM_C25	SYPMS (SEQ ID NO: 156) (Kabat); GFTFSSY (SEQ ID NO: 151) (Chothia); GFTFSSYPMS (SEQ ID NO: 157) (extended)	AIGGSGGSTYYA DSVKG (SEQ ID NO: 162) (Kabat) GGSGGS (SEQ ID NO: 159) (Chothia)	YWPMDP (SEQ ID NO: 197)
COMBO_Rd4_6nM_C21	SYPMS (SEQ ID NO: 156) (Kabat); GFTFSSY (SEQ ID NO: 151) (Chothia); GFTFSSYPMS (SEQ ID NO: 157)	AIGGSGGSLPYA DSVKG (SEQ ID NO: 158) (Kabat) GGSGGS (SEQ ID NO: 159)	YWPMDS (SEQ ID NO: 161)

	(extended)	(Chothia)	
COMBO_Rd4_6nM_C11	SYPMS (SEQ ID NO: 156) (Kabat); GFTFSSY (SEQ ID NO: 151) (Chothia); GFTFSSYPMS (SEQ ID NO: 157) (extended)	AIGGSGGSLGYADSVKG (SEQ ID NO: 198) (Kabat) GGSGGS (SEQ ID NO: 159) (Chothia)	YWPMDS (SEQ ID NO: 161)
COMBO_Rd4_6nM_C09	SYPMS (SEQ ID NO: 156) (Kabat); GFTFSSY (SEQ ID NO: 151) (Chothia); GFTFSSYPMS (SEQ ID NO: 157) (extended)	AIFASGGSTYYADSVKG (SEQ ID NO: 177) (Kabat) FASGGS (SEQ ID NO: 178) (Chothia)	YWPMDS (SEQ ID NO: 161)
COMBO_Rd4_6nM_C08	SYPMS (SEQ ID NO: 156) (Kabat); GFTFSSY (SEQ ID NO: 151) (Chothia); GFTFSSYPMS (SEQ ID NO: 157) (extended)	AIGGSGTWTYYADSVKG (SEQ ID NO: 199) (Kabat) GGSGTW (SEQ ID NO: 200) (Chothia)	YWPMDS (SEQ ID NO: 161)
COMBO_Rd4_0.6nM_C23	SYPMS (SEQ ID NO: 156) (Kabat); GFTFSSY (SEQ ID NO: 151) (Chothia); GFTFSSYPMS (SEQ ID NO: 157) (extended)	ALFGSGGSTYYADSVKG (SEQ ID NO: 201) (Kabat) FGSGGS (SEQ ID NO: 202) (Chothia)	YWPMDS (SEQ ID NO: 161)
COMBO_Rd4_0.6nM_C12	SYPMS (SEQ ID NO: 156) (Kabat);	AALGSGGSTYYADSVKG (SEQ	YWPMDS (SEQ ID NO:

	GFTFSSY (SEQ ID NO: 151) (Chothia); GFTFSSYPMS (SEQ ID NO: 157) (extended)	ID NO: 203) (Kabat) LGSGGS (SEQ ID NO: 204) (Chothia)	161)
COMBO_Rd4_6nM_C07	SYPMS (SEQ ID NO: 156) (Kabat); GFTFSSY (SEQ ID NO: 151) (Chothia); GFTFSSYPMS (SEQ ID NO: 157) (extended)	AIGGSGGSLPYA DSVKG (SEQ ID NO: 158) (Kabat) GGSGGS (SEQ ID NO: 159) (Chothia)	YWPMAD (SEQ ID NO: 205)
COMBO_Rd4_6nM_C02	SYAMN (SEQ ID NO: 150) (Kabat); GFTFSSY (SEQ ID NO: 151) (Chothia); GFTFSSYAMN (SEQ ID NO: 152) (extended)	AISDSGGFVYYA DSVKG (SEQ ID NO: 206) (Kabat) SDSGGF (SEQ ID NO: 207) (Chothia)	YWPMDS (SEQ ID NO: 161)
COMBO_Rd4_6nM_C05	SYAMN (SEQ ID NO: 150) (Kabat); GFTFSSY (SEQ ID NO: 151) (Chothia); GFTFSSYAMN (SEQ ID NO: 152) (extended)	AIGGSGGSTYYA DSVKG (SEQ ID NO: 162) (Kabat) GGSGGS (SEQ ID NO: 159) (Chothia)	YWPMSL (SEQ ID NO: 164)
COMBO_Rd4_6nM_C22	SYAMN (SEQ ID NO: 150) (Kabat); GFTFSSY (SEQ ID NO: 151) (Chothia); GFTFSSYAMN (SEQ	ACLDSGGSTYY ADSVKG (SEQ ID NO: 208) (Kabat) LDSGGS (SEQ	YWPMDS (SEQ ID NO: 161)

	ID NO: 152) (extended)	ID NO: 172) (Chothia)	
COMBO_Rd4_6nM_C11	SYPMS (SEQ ID NO: 156) (Kabat); GFTFSSY (SEQ ID NO: 151) (Chothia); GFTFSSYPMS (SEQ ID NO: 157) (extended)	AALGSGGSTYY ADSVKG (SEQ ID NO: 203) (Chothia) LGSGGS (SEQ ID NO: 204) (Chothia)	YWPMSL (SEQ ID NO: 164)
Heavy chain consensus	SYX_1MX_2, wherein X_1 is A or P; and X_2 is T, N, or S (Kabat) (SEQ ID NO: 301) $GFTFX_1SY$, wherein X_1 is G or S (Chothia) (SEQ ID NO: 302) $GFTFX_1SYX_2MX_3$, wherein X_1 is G or S, X_2 is A or P; and X_3 is T, N, or S (SEQ ID NO: 303) (extended)	$AX_1X_2X_3X_4GX_5X_6X_7X_8YADX_9X_{10}KG$, wherein X_1 is I, V, T, H, L, A, or C; X_2 is S, D, G, T, I, L, F, M, or V; X_3 is G, Y, L, H, D, A, S, or M; X_4 is S, Q, T, A, F, or W; X_5 is G or T;X_6 is N, S, P, Y, W, or F; X_7 is S, T, I, L, T, A, R, V, K, G, or C; X_8 is F, Y, P, W, H, or G; X_9 is V, R, or L; and X_{10} is G or T (Kabat) (SEQ ID NO: 305) $X_1X_2X_3X_4X_5X_6$,	$VSPIX_1X_2X_3$ MDY, wherein X_1 is A or Y; X_2 is A or S; and X_3 is G, Q, L, P, or E (SEQ ID NO: 307) $YWPMX_1X_2$, wherein X_1 is D, S, T, or A; and X_2 is I, S, L, P, or D (SEQ ID NO: 308)

		wherein X_1 is S, V, I, D, G, T, L, F, or M; X_2 is G, Y, L, H, D, A, S, or M; X_3 is S, G, F, or W; X_4 is G or S; X_5 is G or T; and X_6 is N, S, P, Y, or W (Chothia) (SEQ ID NO: 306)	
P4G4	SYAMS (SEQ ID NO: 381) (Kabat); GFTFSSY (SEQ ID NO: 151) (Chothia); GFTFSSYAMS (SEQ ID NO: 382) (extended)	SASGGS (SEQ ID NO: 383) (Chothia) AISASGGSTYYA DSVKG (SEQ ID NO: 384) (Kabat)	LSWSGAFD N (SEQ ID NO: 385)
P1A11	SYAMS (SEQ ID NO: 386) (Kabat); GFTFRSY (SEQ ID NO: 387) GFTFRSYAMS (SEQ ID NO: 388)	SGSGGS (SEQ ID NO: 389) (Chothia) AISGSGGSTFYA DSVKG (SEQ ID NO: 390) (Kabat)	VGTSGAFGI (SEQ ID NO: 391)
Light Chain			
mAb	**CDRL1**	**CDRL2**	**CDRL3**
P6E01 For the following mAbs: P6E01/P6E01; and	RASQSVSSSYLA (SEQ ID NO: 209)	GASSRAT (SEQ ID NO: 210)	QHYGSPPSF T (SEQ ID NO: 211)

P6E01/H3.AQ.			
L1.LGF/L3.KW For the following mAbs: L1.LGF/L3.KW/P6E01; L1.LGF/L3.KW/H3.AL; L1.LGF/L3.KW/H3.AP; and L1.LGF/L3.KW/H3.AQ	RASQSLGSFYLA (SEQ ID NO: 212)	GASSRAT (SEQ ID NO: 210)	KHYGWPPSFT (SEQ ID NO: 213)
L1.LGF/L3.NY For the following mAbs: L1.LGF/L3.NY/P6E01; L1.LGF/L3.NY/H3.AL; L1.LGF/L3.NY/H3.AP; and L1.LGF/L3.NY/H3AQ	RASQSLGSFYLA (SEQ ID NO: 212)	GASSRAT (SEQ ID NO: 210)	QHYNYPPSFT (SEQ ID NO: 214)
L1.GDF/L3.NY For the following mAbs: L1.GDF/L3.NY/P6E01; L1.GDF/L3.NY/H3.AL; L1.GDF/L3.NY/H3.AP; and L1.GDF/L3.NY/H3.AQ	RASQSVGDFYLA (SEQ ID NO: 215)	GASSRAT (SEQ ID NO: 210)	QHYNYPPSFT (SEQ ID NO: 214)
L1.LGF/L3.PY For the following mAbs: L1.LGF/L3.PY/H3.AP; and L1.LGF/L3.PY/H3.AQ	RASQSLGSFYLA (SEQ ID NO: 212)	GASSRAT (SEQ ID NO: 210)	QHYPYPPSFT (SEQ ID NO: 216)
L1.GDF /L3.KW For the following mAbs: L1.GDF /L3.KW/H3.AL; L1.GDF /L3.KW/H3.AP;	RASQSVGDFYLA (SEQ ID NO: 215)	GASSRAT (SEQ ID NO: 210)	KHYGWPPSFT (SEQ ID NO: 213)

and L1.GDF /L3.KW/H3.AQ			
L1.GDF /L3.PY/H3.AQ	RASQSVGDFYLA (SEQ ID NO: 215)	GASSRAT (SEQ ID NO: 210)	QHYPYPPSFT (SEQ ID NO: 216)
L3.KW/P6E01	RASQSVSSSYLA (SEQ ID NO: 209)	GASSRAT (SEQ ID NO: 210)	KHYGWPPSFT (SEQ ID NO: 213)
L3.PY For the following mAbs: L3.PY/P6E01; L3.PY/H2.QR; L3.PY/H2.DY; L3.PY/H2.YQ; L3.PY/H2.LT; L3.PY/H2.HA; L3.PY/H2.QL; L3.PY/H3.YA; L3.PY/H3.AE; L3.PY/H3.AQ; L3.PY/H3.TAQ	RASQSVSSSYLA (SEQ ID NO: 209)	GASSRAT (SEQ ID NO: 210)	QHYPYPPSFT (SEQ ID NO: 216)
L3.NY/P6E01	RASQSVSSSYLA (SEQ ID NO: 209)	GASSRAT (SEQ ID NO: 210)	QHYNYPPSFT (SEQ ID NO: 214)
L3.PY/L1.PS For the following mAbs: L3.PY/L1.PS/P6E01; P6DY;	RASQSVSSSYPS (SEQ ID NO: 217)	GASSRAT (SEQ ID NO: 210)	QHYPYPPSFT (SEQ ID NO: 216)

L3.PY/L1.PS/H2.QR; L3.PY/L1.PS/H2.DY; L3.PY/L1.PS/H2.YQ; L3.PY/L1.PS/H2.LT; L3.PY/L1.PS/H2.HA; L3.PY/L1.PS/H2.QL; L3.PY/L1.PS/H3.YA; L3.PY/L1.PS/H3.AE; L3.PY/L1.PS/H3.AQ; L3.PY/L1.PS/H3.TAQ;			
L3.PY/L1.AH For the following mAbs: L3.PY/L1.AH/P6E01; L3.PY/L1.AH/H2.QR; L3.PY/L1.AH/H2.DY; L3.PY/L1.AH/H2.YQ; L3.PY/L1.AH/H2.LT; L3.PY/L1.AH/H2.HA; L3.PY/L1.AH/H2.QL; L3.PY/L1.AH/H3.YA; L3.PY/L1.AH/H3.AE; L3.PY/L1.AH/H3.AQ; L3.PY/L1.AH/H3.TAQ	RASQSVSAHYLA (SEQ ID NO: 218)	GASSRAT (SEQ ID NO: 210)	QHYPYPPSFT (SEQ ID NO: 216)
L3.PY/L1.FF For the following mAbs: L3.PY/L1.FF/P6E01; L3.PY/L1.FF/H2.QR; L3.PY/L1.FF/H2.DY;	RASQSVSSFFLA (SEQ ID NO: 219)	GASSRAT (SEQ ID NO: 210)	QHYPYPPSFT (SEQ ID NO: 216)

L3.PY/L1.FF/H2.YQ; L3.PY/L1.FF/H2.LT; L3.PY/L1.FF/H2.HA; L3.PY/L1.FF/H2.QL; L3.PY/L1.FF/H3.YA; L3.PY/L1.FF/H3.AE; L3.PY/L1.FF/H3.AQ; and L3.PY/L1.FF/H3.TAQ			
L3.PY/L1.PH For the following mAbs: L3.PY/L1.PH/P6E01; L3.PY/L1.PH/H2.QR; L3.PY/L1.PH/H2.HA; L3.PY/L1.PH/H3.AE; L3.PY/L1.PH/H3.AQ; and L3.PY/L1.PH/H3.TAQ	RASQSVSPHYLA (SEQ ID NO: 219)	GASSRAT (SEQ ID NO: 210)	QHYPYPPSFT (SEQ ID NO: 216)
L3.PY/L3.KY For the following mAbs: L3.PY/L3.KY/P6E01; L3.PY/L3.KY/H2.QR; L3.PY/L3.KY/H2.DY; L3.PY/L3.KY/H2.YQ; L3.PY/L3.KY/H2.LT; L3.PY/L3.KY/H2.HA; L3.PY/L3.KY/H2.QL; L3.PY/L3.KY/H3.YA; and	RASQSVSSSYLA (SEQ ID NO: 209)	GASSRAT (SEQ ID NO: 210)	KYYPYPPSFT (SEQ ID NO: 220)

L3.PY/L3.KY/H3.TAQ			
L3.PY/L3.KF For the following mAbs: L3.PY/L3.KF/H2.DY; L3.PY/L3.KF/H2.YQ; L3.PY/L3.KF/H2.LT; L3.PY/L3.KF/H2.QL; L3.PY/L3.KF/H3.YA; L3.PY/L3.KF/H3.AE; L3.PY/L3.KF/H3.AQ; and L3.PY/L3.KF/H3.TAQ	RASQSVSSSYLA (SEQ ID NO: 209)	GASSRAT (SEQ ID NO: 210)	KFYPYPPSFT (SEQ ID NO: 220)
P5A2_VHVL (P5A)	RASQSVSSSYLA (SEQ ID NO: 209)	DASIRAT (SEQ ID NO: 221)	QQYGSWPLT (SEQ ID NO: 222)
A02_Rd4_0.6nM_C06	RASQSVSVIYLA (SEQ ID NO: 223)	DASIRAT (SEQ ID NO: 221)	QQYQRWPLT (SEQ ID NO: 224)
A02_Rd4_0.6nM_C09; COMBO_Rd_0.6nM_C29; and COMBO_Rd4_0.6nM_C21	RASQSVSSSYLA (SEQ ID NO: 209)	DASIRAT (SEQ ID NO: 221)	QQYQSWPLT (SEQ ID NO: 225)
A02_Rd4_6nM_C16 (P5AC16)	RASQSVSDIYLA (SEQ ID NO: 226)	DASIRAT (SEQ ID NO: 221)	QQYQTWPLT (SEQ ID NO: 227)
A02_Rd4_6nM_C03	RASQSVSNIYLA	DASIRAT	QQYQGWPL

	(SEQ ID NO: 228)	(SEQ ID NO: 221)	T (SEQ ID NO: 229)
A02_Rd4_6nM_C01	RASQSVSAYYLA (SEQ ID NO: 230)	DASIRAT (SEQ ID NO: 221)	QQYERWPL T (SEQ ID NO: 231)
A02_Rd4_6nM_C26	RASQSVSSIYLA (SEQ ID NO: 232)	DASIRAT (SEQ ID NO: 221)	QQYQVWPL T (SEQ ID NO: 233)
A02_Rd4_6nM_C25	RASQSVSSSYLA (SEQ ID NO: 209)	DASIRAT (SEQ ID NO: 221)	QQYLDWPL T (SEQ ID NO: 234)
A02_Rd4_6nM_C22	RASQSVSSSYLA (SEQ ID NO: 209)	DASIRAT (SEQ ID NO: 221)	QQYQVWPL T (SEQ ID NO: 233)
A02_Rd4_6nM_C19	RASQSVSVIYLA (SEQ ID NO: 223)	DASIRAT (SEQ ID NO: 221)	QQYLAWPLT (SEQ ID NO: 236)
A02_Rd4_0.6nM_C03	RASQSVSSSYLA (SEQ ID NO: 209)	DASIRAT (SEQ ID NO: 221)	QQYFTWPLT (SEQ ID NO: 237)
A02_Rd4_6nM_C07	RASQSVSPYYLA (SEQ ID NO: 238)	DASIRAT (SEQ ID NO: 221)	QQYERWPL T (SEQ ID NO: 231)
A02_Rd4_6nM_C23	RASQSVSVEYLA (SEQ ID NO: 239)	DASIRAT (SEQ ID NO: 221)	QQYARWPL T (SEQ ID NO:

			240)
A02_Rd4_0.6nM_C18	RASQSVSEIYLA (SEQ ID NO: 241)	DASIRAT (SEQ ID NO: 221)	QQYFGWPL T (SEQ ID NO: 242)
A02_Rd4_6nM_C10	RASQSVEMSYLA (SEQ ID NO: 243)	DASIRAT (SEQ ID NO: 221)	QQYAHWPL T (SEQ ID NO: 244)
A02_Rd4_6nM_C05	RASQSVSSSYLA (SEQ ID NO: 209)	DASIRAT (SEQ ID NO: 221)	QQYQRWPL T (SEQ ID NO: 224)
A02_Rd4_0.6nM_C10	RASQSVSAQYLA (SEQ ID NO: 245)	DASIRAT (SEQ ID NO: 221)	QQYQRWPL T (SEQ ID NO: 224)
A02_Rd4_6nM_C04	RASQSVSAIYLA (SEQ ID NO: 235)	DASIRAT (SEQ ID NO: 221)	QQYQVWPL T (SEQ ID NO: 233)
A02_Rd4_0.6nM_C26	GPSQSVSSSYLA (SEQ ID NO: 246)	DASIRAT (SEQ ID NO: 221)	QQYQSWPL T (SEQ ID NO: 225)
A02_Rd4_0.6nM_C13	RASQSVSSSYWA (SEQ ID NO: 247)	DASIRAT (SEQ ID NO: 221)	QQYESWPL T (SEQ ID NO: 248)
A02_Rd4_0.6nM_C01 (P5AC1)	RGGQSVSSSYLA (SEQ ID NO: 249)	DASIRAT (SEQ ID NO: 221)	QQYQSWPL T

			(SEQ ID NO: 225)
A02_Rd4_6nM_C08	RASQSVSFIYLA (SEQ ID NO: 250)	DASIRAT (SEQ ID NO: 221)	QQYGSWPLT (SEQ ID NO: 222)
P5C1_VHVL (PC1)	RASQSVSSTYLA (SEQ ID NO: 251)	DASSRAP (SEQ ID NO: 252)	QQYSTSPLT (SEQ ID NO: 253)
C01_Rd4_6nM_C24	RASQSVSPEYLA (SEQ ID NO: 254)	DASSRAP (SEQ ID NO: 252)	QQYSVWPLT (SEQ ID NO: 255)
C01_Rd4_6nM_C26	RASQSVSAIYLA (SEQ ID NO: 235)	DASSRAP (SEQ ID NO: 252)	QQYSAWPLT (SEQ ID NO: 256)
C01_Rd4_6nM_C10	RASQSVSSVYLA (SEQ ID NO: 257)	DASSRAP (SEQ ID NO: 252)	QQYSTWPLT (SEQ ID NO: 258)
C01_Rd4_0.6nM_C27	RASQSVSSTYLA (SEQ ID NO: 251)	DASSRAP (SEQ ID NO: 252)	QQYSRWPLT (SEQ ID NO: 259)
C01_Rd4_6nM_C20	RASQSVSPIYLA (SEQ ID NO: 260)	DASSRAP (SEQ ID NO: 252)	QQYSAFPLT (SEQ ID NO: 261)
C01_Rd4_6nM_C12 (PC1C12)	WLSQSVSSTYLA (SEQ ID NO: 262)	DASSRAP (SEQ ID NO: 252)	QQYSEWPLT (SEQ ID NO: 263)

C01_Rd4_0.6nM_C16	RASQSVSSTYLA (SEQ ID NO: 251)	DASSRAP (SEQ ID NO: 252)	QQYSSWPLT (SEQ ID NO: 264)
C01_Rd4_0.6nM_C09	RASQSVSSIFLA (SEQ ID NO: 265)	DASSRAP (SEQ ID NO: 252)	QQYSAWPLT (SEQ ID NO: 256)
C01_Rd4_6nM_C09	ACSQSVSSTYLA (SEQ ID NO: 266)	DASSRAP (SEQ ID NO: 252)	QQYSAWPLT (SEQ ID NO: 256)
C01_Rd4_0.6nM_C03	RASCDVSSTYLA (SEQ ID NO: 267)	DASSRAP (SEQ ID NO: 252)	QQYMRSPLT (SEQ ID NO: 268)
C01_Rd4_0.6nM_C06	RASEAVPSTYLA (SEQ ID NO: 269)	DASSRAP (SEQ ID NO: 252)	QQYSAFPLT (SEQ ID NO: 261)
C01_Rd4_0.6nM_C04	CSSQSVSSTYLA (SEQ ID NO: 270)	DASSRAP (SEQ ID NO: 252)	QQYSAFPLT (SEQ ID NO: 261)
COMBO_Rd4_0.6nM_C22 (COM22)	RASVRVSSTYLA (SEQ ID NO: 271)	DASIRAT (SEQ ID NO: 221)	QQYMKWPLT (SEQ ID NO: 272)
COMBO_Rd4_6nM_C21	RASQSVSAAYLA (SEQ ID NO: 273)	DASIRAT (SEQ ID NO: 221)	QQYMCWPLT (SEQ ID NO: 274)
COMBO_Rd4_6nM_C1	RASQSVSSSYWG	DASIRAT	QQYQCWPL

0	(SEQ ID NO: 275)	(SEQ ID NO: 221)	T (SEQ ID NO: 276)
COMBO_Rd4_0.6nM_C04	RASQSVSSTYLA (SEQ ID NO: 251)	DASIRAT (SEQ ID NO: 221)	QQYQSWPLT (SEQ ID NO: 225)
COMBO_Rd4_6nM_C25	RASQSVSSPYLA (SEQ ID NO: 277)	DASIRAT (SEQ ID NO: 221)	QQYQSWPLT (SEQ ID NO: 225)
COMBO_Rd4_6nM_C11	RASQSVSPIYLA (SEQ ID NO: 260)	DASIRAT (SEQ ID NO: 221)	QQYKAWPLT (SEQ ID NO: 278)
COMBO_Rd4_0.6nM_C20	RASQSVSYLYLA (SEQ ID NO: 279)	DASIRAT (SEQ ID NO: 221)	QQYMEWPLT (SEQ ID NO: 280)
COMBO_Rd4_6nM_C09	RASQSVSAQYLA (SEQ ID NO: 245)	DASIRAT (SEQ ID NO: 221)	QQYQAWPLT (SEQ ID NO: 281)
COMBO_Rd4_6nM_C08	RASQSVSSSYLA (SEQ ID NO: 209)	DASIRAT (SEQ ID NO: 221)	QQYQKWPLT (SEQ ID NO: 282)
COMBO_Rd4_0.6nM_C19	RASQSVSAVYLA (SEQ ID NO: 283)	DASIRAT (SEQ ID NO: 221)	QQYRAWPLT (SEQ ID NO: 284)

COMBO_Rd4_0.6nM_C02	RASIAVSSTYLA (SEQ ID NO: 285)	DASIRAT (SEQ ID NO: 221)	QQYMVWPLT (SEQ ID NO: 286)
COMBO_Rd4_0.6nM_C23	RPRQSVSSSYLA (SEQ ID NO: 287)	DASIRAT (SEQ ID NO: 221)	QQYQDWPLT (SEQ ID NO: 288)
COMBO_Rd4_0.6nM_C09	RASQSVSSTYLA (SEQ ID NO: 251)	DASIRAT (SEQ ID NO: 221)	QQYQEWPLT (SEQ ID NO: 289)
COMBO_Rd4_6nM_C12	RASQSVSASYLA (SEQ ID NO: 290)	DASIRAT (SEQ ID NO: 221)	QQYMSWPLT (SEQ ID NO: 291)
COMBO_Rd4_0.6nM_C30	RASQSVSYMYLA (SEQ ID NO: 292)	DASIRAT (SEQ ID NO: 221)	QQYKSWPLT (SEQ ID NO: 293)
COMBO_Rd4_0.6nM_C14	RASQSVSAIYLA (SEQ ID NO: 235)	DASIRAT (SEQ ID NO: 221)	QQYYGWPLT (SEQ ID NO: 294)
COMBO_Rd4_6nM_C07	RASQPISSSYLA (SEQ ID NO: 295)	DASIRAT (SEQ ID NO: 221)	QQYQGWPLT (SEQ ID NO: 229)
COMBO_Rd4_6nM_C02	RASQSVSSSYLA (SEQ ID NO: 209)	DASIRAT (SEQ ID NO: 221)	QQYEFWPLT (SEQ ID NO:

			296)
COMBO_Rd4_0.6nM_C05	RASQSVSSTYLA (SEQ ID NO: 251)	DASIRAT (SEQ ID NO: 221)	QQYMSWPLT (SEQ ID NO: 291)
COMBO_Rd4_0.6nM_C17	RASQGISSTYLA (SEQ ID NO: 297)	DASIRAT (SEQ ID NO: 221)	QQYAYWPLT (SEQ ID NO: 298)
COMBO_Rd4_6nM_C22	RASQSVSSSYLA (SEQ ID NO: 209)	DASIRAT (SEQ ID NO: 221)	QQYQGWPLT (SEQ ID NO: 229)
COMBO_Rd4_0.6nM_C11	RASQSVSVRYLA (SEQ ID NO: 299)	DASIRAT (SEQ ID NO: 221)	QQYGSWPIT (SEQ ID NO: 300)
Light chain consensus	$X_1X_2X_3X_4X_5X_6X_7X_8X_9X_{10}X_{11}X_{12}$, wherein X_1 is R, G, W, A, or C; X_2 is A, P, G, L, C, or S; X_3 is S, G, or R; X_4 is Q, C, E, V, or I; X_5 is S, P, G, A, R, or D; X_6 is V, G, I, or L; X_7 is S, E, D, P, or G; X_8 is S, P, F, A, M, E, V, N, D, or Y; X_9 is I, T, V, E, F S, A, M, Q, Y, H, or R; X_{10} is Y or F; X_{11} is L, W, or P; and X_{12} is A, S, or G (SEQ ID NO:	$X_1ASX_2RAX_3$, wherein X_1 is G or D; X_2 is S or I; and X_3 is T or P (SEQ ID NO: 310)	$X_1X_2YX_3X_4PPSFT$, wherein X_1 is Q or K; X_2 is H or Y; X_3 is G, N, or P; and X_4 is S, W, or Y (SEQ ID NO: 311) $QQYX_1X_2X_3PX_4T$, wherein X_1 is G, Q, E, L, F, A, S, M, K, R, or Y; X_2 is S, R, T, G,

	309)		V, F, Y, D, A, H, V, E, K, or C; X_3 is W, F, or S; and X_4 is L or I (SEQ ID NO: 312)
P4G4	RASQSVSSSYLA (SEQ ID NO: 209)	GASSRAY (SEQ ID NO: 392)	QHYGSPPLFT (SEQ ID NO: 393)
P1A11	RASQNVSSSYLA (SEQ ID NO: 379)	GASYRAT (SEQ ID NO: 395)	QHYGSPPSFT (SEQ ID NO: 211)
P6AP	RASQLGSFYLA (SEQ ID NO: 377)	GASSRAT (SEQ ID NO: 210)	QHYNYPPSFT (SEQ ID NO: 214)

The invention encompasses modifications to the CARs and polypeptides of the invention variants shown in Table 1, including functionally equivalent CARs having modifications which do not significantly affect their properties and variants which have enhanced or decreased activity and/or affinity. For example, the amino acid sequence may be mutated to obtain an antibody with the desired binding affinity to BCMA. Modification of polypeptides is routine practice in the art and need not be described in detail herein. Examples of modified polypeptides include polypeptides with conservative substitutions of amino acid residues, one or more deletions or additions of amino acids which do not significantly deleteriously change the functional activity, or which mature (enhance) the affinity of the polypeptide for its ligand, or use of chemical analogs.

Amino acid sequence insertions include amino- and/or carboxyl-terminal fusions ranging in length from one residue to polypeptides containing a hundred or more residues, as well as intrasequence insertions of single or multiple amino acid residues. Examples of terminal insertions include an antibody with an N-terminal methionyl residue or the antibody fused to an epitope tag. Other insertional variants of the antibody molecule include the fusion to the N- or C-terminus of the antibody of an enzyme or a polypeptide which increases the half-life of the antibody in the blood circulation.

Substitution variants have at least one amino acid residue in the antibody molecule removed and a different residue inserted in its place. The sites of greatest interest for substitutional mutagenesis include the hypervariable regions, but FR alterations are also contemplated. Conservative substitutions are shown in Table 2.1 under the heading of "conservative substitutions." If such substitutions result in a change in biological activity,

then more substantial changes, denominated "exemplary substitutions" in Table 2.1, or as further described below in reference to amino acid classes, may be introduced and the products screened.

Table 2.1: Amino Acid Substitutions

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

In some embodiments, the invention provides a CAR comprising an extracellular ligand-binding domain that binds to BCMA and competes for binding to BCMA with a CAR

described herein, including P6E01/P6E01, P6E01/H3.AQ, L1.LGF/L3.KW/P6E01; L1.LGF/L3.NY/P6E01, L1.GDF/L3.NY/P6E01, L1.LGF/L3.KW/H3.AL, L1.LGF/L3.KW/H3.AP, L1.LGF/L3.KW/H3.AQ, L1.LGF/L3.PY/H3.AP, L1.LGF/L3.PY/H3.AQ, L1.LGF/L3.NY/H3.AL, L1.LGF/L3.NY/H3.AP, L1.LGF/L3.NY/H3.AQ, L1.GDF/L3.KW/H3.AL, L1.GDF/L3.KW/H3.AP, L1.GDF/L3.KW/H3.AQ, L1.GDF/L3.PY/H3.AQ, L1.GDF/L3.NY/H3.AL, L1.GDF/L3.NY/H3.AP, L1.GDF/L3.NY/H3.AQ, L3.KW/P6E01, L3.PY/P6E01, L3.NY/P6E01, L3.PY/L1.PS/P6E01, L3.PY/L1.AH/P6E01, L3.PY/L1.FF/P6E01, L3.PY/L1.PH/P6E01, L3.PY/L3.KY/P6E01, L3.PY/L3.KF/P6E01, L3.PY/H2.QR, L3.PY/H2.DY, L3.PY/H2.YQ, L3.PY/H2.LT, L3.PY/H2.HA, L3.PY/H2.QL, L3.PY/H3.YA, L3.PY/H3.AE, L3.PY/H3.AQ, L3.PY/H3.TAQ, L3.PY/P6E01, L3.PY/L1.PS/H2.QR, L3.PY/L1.PS/H2.DY, L3.PY/L1.PS/H2.YQ, L3.PY/L1.PS/H2.LT, L3.PY/L1.PS/H2.HA, L3.PY/L1.PS/H2.QL, L3.PY/L1.PS/H3.YA, L3.PY/L1.PS/H3.AE, L3.PY/L1.PS/H3.AQ, L3.PY/L1.PS/H3.TAQ, L3.PY/L1.AH/H2.QR, L3.PY/L1.AH/H2.DY, L3.PY/L1.AH/H2.YQ, L3.PY/L1.AH/H2.LT, L3.PY/L1.AH/H2.HA, L3.PY/L1.AH/H2.QL, L3.PY/L1.AH/H3.YA, L3.PY/L1.AH/H3.AE, L3.PY/L1.AH/H3.AQ, L3.PY/L1.AH/H3.TAQ, L3.PY/L1.FF/H2.QR, L3.PY/L1.FF/H2.DY, L3.PY/L1.FF/H2.YQ, L3.PY/L1.FF/H2.LT, L3.PY/L1.FF/H2.HA, L3.PY/L1.FF/H2.QL, L3.PY/L1.FF/H3.YA, L3.PY/L1.FF/H3.AE, L3.PY/L1.FF/H3.AQ, L3.PY/L1.FF/H3.TAQ, L3.PY/L1.PH/H2.QR, L3.PY/L1.PH/H2.HA, L3.PY/L1.PH/H3.AE, L3.PY/L1.PH/H3.AQ, L3.PY/L1.PH/H3.TAQ, L3.PY/L3.KY/H2.QR, L3.PY/L3.KY/H2.DY, L3.PY/L3.KY/H2.YQ L3.PY/L3.KY/H2.LT, L3.PY/L3.KY/H2.HA, L3.PY/L3.KY/H2.QL, L3.PY/L3.KY/H3.YA L3.PY/L3.KY/H3.TAQ, L3.PY/L3.KF/H2.DY, L3.PY/L3.KF/H2.YQ, L3.PY/L3.KF/H2.LT L3.PY/L3.KF/H2.QL, L3.PY/L3.KF/H3.YA, L3.PY/L3.KF/H3.AE, L3.PY/L3.KF/H3.AQ L3.PY/L3.KF/H3.TAQ, P5A2_VHVL, A02_Rd4_0.6nM_C06, A02_Rd4_0.6nM_C09 A02_Rd4_6nM_C16, A02_Rd4_6nM_C03, A02_Rd4_6nM_C01, A02_Rd4_6nM_C26 A02_Rd4_6nM_C25, A02_Rd4_6nM_C22, A02_Rd4_6nM_C19, A02_Rd4_0.6nM_C03 A02_Rd4_6nM_C07, A02_Rd4_6nM_C23, A02_Rd4_0.6nM_C18, A02_Rd4_6nM_C10 A02_Rd4_6nM_C05, A02_Rd4_0.6nM_C10, A02_Rd4_6nM_C04, A02_Rd4_0.6nM_C26 A02_Rd4_0.6nM_C13, A02_Rd4_0.6nM_C01, A02_Rd4_6nM_C08, P5C1_VHVL, C01_Rd4_6nM_C24, C01_Rd4_6nM_C26, C01_Rd4_6nM_C10, C01_Rd4_0.6nM_C27 C01_Rd4_6nM_C20, C01_Rd4_6nM_C12, C01_Rd4_0.6nM_C16, C01_Rd4_0.6nM_C09

C01_Rd4_6nM_C09, C01_Rd4_0.6nM_C03, C01_Rd4_0.6nM_C06, C01_Rd4_6nM_C04 COMBO_Rd4_0.6nM_C22, COMBO_Rd4_6nM_C21, COMBO_Rd4_6nM_C10, COMBO_Rd4_0.6nM_C04, COMBO_Rd4_6nM_C25, COMBO_Rd4_0.6nM_C21, COMBO_Rd4_6nM_C11, COMBO_Rd4_0.6nM_C20, COMBO_Rd4_6nM_C09, COMBO_Rd4_6nM_C08, COMBO_Rd4_0.6nM_C19, COMBO_Rd4_0.6nM_C02, COMBO_Rd4_0.6nM_C23, COMBO_Rd4_0.6nM_C29, COMBO_Rd4_0.6nM_C09, COMBO_Rd4_6nM_C12, COMBO_Rd4_0.6nM_C30, COMBO_Rd4_0.6nM_C14, COMBO_Rd4_6nM_C07, COMBO_Rd4_6nM_C02, COMBO_Rd4_0.6nM_C05, COMBO_Rd4_0.6nM_C17, COMBO_Rd4_6nM_C22, COMBO_Rd4_0.6nM_C11, or COMBO_Rd4_0.6nM_C29.

In some embodiments, the invention provides a CAR, which specifically binds to BCMA, wherein the CAR comprises a VH region comprising a sequence shown in SEQ ID NO: 33; and/or a VL region comprising a sequence shown in SEQ ID NO: 34. In some embodiments, the invention provides a CAR, which specifically binds to BCMA, wherein the CAR comprises a VH region comprising a sequence shown in SEQ ID NO: 33, 72, 39, 76, 83, 92, 25, or 8; and/or a VL region comprising a sequence shown in SEQ ID NO: 34, 73, 40, 77, 84, 93, 18, or 80. In some embodiments, the invention also provides CARs comprising CDR portions of antibodies to BCMA antibodies based on CDR contact regions. CDR contact regions are regions of an antibody that imbue specificity to the antibody for an antigen. In general, CDR contact regions include the residue positions in the CDRs and Vernier zones which are constrained in order to maintain proper loop structure for the antibody to bind a specific antigen. See, e.g., Makabe et al., J. Biol. Chem., 283:1156-1166, 2007. Determination of CDR contact regions is well within the skill of the art.

The binding affinity (K_D) of the BCMA specific CAR as described herein to BCMA (such as human BCMA (e.g., (SEQ ID NO: 354) can be about 0.002 to about 6500 nM. In some embodiments, the binding affinity is about any of 6500 nm, 6000 nm, 5986 nm, 5567 nm, 5500 nm, 4500 nm, 4000 nm, 3500 nm, 3000 nm, 2500 nm, 2134 nm, 2000 nm, 1500 nm, 1000 nm, 750 nm, 500 nm, 400 nm, 300 nm, 250 nm, 200 nM, 193 nM, 100 nM, 90 nM, 50 nM, 45 nM, 40 nM, 35 nM, 30 nM, 25 nM, 20 nM, 19 nm, 18 nm, 17 nm, 16 nm, 15 nM, 10 nM, 8 nM, 7.5 nM, 7 nM, 6.5 nM, 6 nM, 5.5 nM, 5 nM, 4 nM, 3 nM, 2 nM, 1 nM, 0.5 nM, 0.3 nM, 0.1 nM, 0.01 nM, or 0.002 nM. In some embodiments, the binding affinity is

less than about any of 6500 nm, 6000 nm, 5500 nm, 5000 nm, 4000 nm, 3000 nm, 2000 nm, 1000 nm, 900 nm, 800 nm, 250 nM, 200 nM, 100 nM, 50 nM, 30 nM, 20 nM, 10 nM, 7.5 nM, 7 nM, 6.5 nM, 6 nM, 5 nM, 4.5 nM, 4 nM, 3.5 nM, 3 nM, 2.5 nM, 2 nM, 1.5 nM, 1 nM, or 0.5 nM.

The intracellular signaling domain of a CAR according to the invention is responsible for intracellular signaling following the binding of extracellular ligand-binding domain to the target resulting in the activation of the immune cell and immune response. The intracellular signaling domain has the ability to activate of at least one of the normal effector functions of the immune cell in which the CAR is expressed. For example, the effector function of a T cell can be a cytolytic activity or helper activity including the secretion of cytokines.

In some embodiments, an intracellular signaling domain for use in a CAR can be the cytoplasmic sequences of, for example without limitation, the T cell receptor and co-receptors that act in concert to initiate signal transduction following antigen receptor engagement, as well as any derivative or variant of these sequences and any synthetic sequence that has the same functional capability. Intracellular signaling domains comprise two distinct classes of cytoplasmic signaling sequences: those that initiate antigen-dependent primary activation, and those that act in an antigen- independent manner to provide a secondary or co-stimulatory signal. Primary cytoplasmic signaling sequences can comprise signaling motifs which are known as immunoreceptor tyrosine-based activation motifs of ITAMs. ITAMs are well defined signaling motifs found in the intracytoplasmic tail of a variety of receptors that serve as binding sites for syk/zap70 class tyrosine kinases. Examples of ITAM used in the invention can include as non limiting examples those derived from TCRζ, FcRγ, FcRβ, FcRε, CD3γ, CD3δ, CD3ε, CD5, CD22, CD79a, CD79b and CD66d. In some embodiments, the intracellular signaling domain of the CAR can comprise the CD3ζ signaling domain which has amino acid sequence with at least about 70%, preferably at least 80%, more preferably at least 90%, 95%, 97%, or 99% sequence identity with an amino acid sequence shown in SEQ. ID NO: 324. In some embodiments the intracellular signaling domain of the CAR of the invention comprises a domain of a co-stimulatory molecule.

In some embodiments, the intracellular signaling domain of a CAR of the invention comprises a part of co-stimulatory molecule selected from the group consisting of fragment

of 41BB (GenBank: AAA53133.) and CD28 (NP_006130.1). In some embodiments, the intracellular signaling domain of the CAR of the invention comprises amino acid sequence which comprises at least 70%, preferably at least 80%, more preferably at least 90%, 95%, 97%, or 99% sequence identity with an amino acid sequence shown in SEQ. ID NO: 323 and SEQ. ID NO: 327.

CARs are expressed on the surface membrane of the cell. Thus, the CAR can comprise a transmembrane domain. Suitable transmembrane domains for a CAR disclosed herein have the ability to (a) be expressed at the surface of a cell, preferably an immune cell such as, for example without limitation, lymphocyte cells or Natural killer (NK) cells, and (b) interact with the ligand-binding domain and intracellular signaling domain for directing cellular response of immune cell against a predefined target cell. The transmembrane domain can be derived either from a natural or from a synthetic source. The transmembrane domain can be derived from any membrane-bound or transmembrane protein. As non-limiting examples, the transmembrane polypeptide can be a subunit of the T cell receptor such as α, β, γ or δ, polypeptide constituting CD3 complex, IL-2 receptor p55 (a chain), p75 (β chain) or γ chain, subunit chain of Fc receptors, in particular Fcγ receptor III or CD proteins. Alternatively, the transmembrane domain can be synthetic and can comprise predominantly hydrophobic residues such as leucine and valine. In some embodiments said transmembrane domain is derived from the human CD8α chain (e.g., NP_001139345.1). The transmembrane domain can further comprise a stalk domain between the extracellular ligand-binding domain and said transmembrane domain. A stalk domain may comprise up to 300 amino acids, preferably 10 to 100 amino acids and most preferably 25 to 50 amino acids. Stalk region may be derived from all or part of naturally occurring molecules, such as from all or part of the extracellular region of CD8, CD4, or CD28, or from all or part of an antibody constant region. Alternatively the stalk domain may be a synthetic sequence that corresponds to a naturally occurring stalk sequence, or may be an entirely synthetic stalk sequence. In some embodiments said stalk domain is a part of human CD8α chain (e.g., NP_001139345.1). In another particular embodiment, said transmembrane and hinge domains comprise a part of human CD8α chain, preferably which comprises at least 70%, preferably at least 80%, more preferably at least 90%, 95% 97%, or 99% sequence identity with amino acid sequence selected from the group

consisting of SEQ ID NO: 318. In some embodiments, CARs disclosed herein can comprise an extracellular ligand-binding domain that specifically binds BCMA, CD8α human hinge and transmembrane domains, the CD3ζ signaling domain, and 4-1BB signaling domain.

Table 3 provides exemplary sequences of domains which can be used in the CARs disclosed herein.

Table 3: Exemplary sequences of CAR Components

Domain	Amino Acid Sequence	SEQ ID NO:
CD8α signal peptide	MALPVTALLLPLALLLHAARP	318
FcγRIIIα hinge	GLAVSTISSFFPPGYQ	319
CD8α hinge	TTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACD	320
IgG1 hinge	EPKSPDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMIARTPEVTCVVVDV SHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWL NGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSL TCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKS RWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK	321
CD8α transmembrane (TM) domain	IYIWAPLAGTCGVLLLSLVITLYC	322
41BB intracellular signaling domain (ISD)	KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCEL	323
CD3ζ intracellular signaling domain (ISD)	RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGG KPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLST ATKDTYDALHMQALPPR	324

Domain	Amino Acid Sequence	SEQ ID NO:
FcεRI α-TM-IC (FcεRI α chain transmembrane and intracellular domain)	FFIPLLVVILFAVDTGLFISTQQQVTFLLKIKRTRKGFRLLNPHPKPNPKNN	325
FcεRIβ-ΔITAM (FcεRI β chain without ITAM)	MDTESNRRANLALPQEPSSVPAFEVLEISPQEVSSGRLLKSASSPPLHTWL TVLKKEQEFLGVTQILTAMICLCFGTVVCSVLDISHIEGDIFSSFKAGYPFW GAIFFSISGMLSIISERRNATYLVRGSLGANTASSIAGGTGITILIINLKKSLAY IHIHSCQKFFETKCFMASFSTEIVVMMLFLTILGLGSAVSLTICGAGEELKG NKVPE	326
41BB-IC (41BB co-stimulatory domain)	KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCEL	327
CD28-IC (CD28 co-stimulatory domain)	RSKRSRGGHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRS	328
FcεRIγ-SP (signal peptide)	MIPAVVLLLLLVEQAAA	329
FcεRI γ-ΔITAM (FcεRI γ chain without ITAM)	LGEPQLCYILDAILFLYGIVLTLLYCRLKIQVRKAAITSYEKS	330
GSG-P2A (GSG-P2A ribosomal skip polypeptide)	GSGATNFSLLKQAGDVEENPGP	331
GSG-T2A (GSG-T2A ribosomal skip polypeptide)	GSGEGRGSLLTCGDVEENPGP	332

Downregulation or mutation of target antigens is commonly observed in cancer cells, creating antigen-loss escape variants. Thus, to offset tumor escape and render immune cell

more specific to target, the BCMA specific CAR can comprise one or more additional extracellular ligand-binding domains, to simultaneously bind different elements in target thereby augmenting immune cell activation and function. In one embodiment, the extracellular ligand-binding domains can be placed in tandem on the same transmembrane polypeptide, and optionally can be separated by a linker. In some embodiments, said different extracellular ligand- binding domains can be placed on different transmembrane polypeptides composing the CAR. In some embodiments, the invention relates to a population of CARs, each CAR comprising a different extracellular ligand-binding domain. In a particular, the invention relates to a method of engineering immune cells comprising providing an immune cell and expressing at the surface of the cell a population of CARs, each CAR comprising different extracellular ligand-binding domains. In another particular embodiment, the invention relates to a method of engineering an immune cell comprising providing an immune cell and introducing into the cell polynucleotides encoding polypeptides composing a population of CAR each one comprising different extracellular ligand-binding domains. By population of CARs, it is meant at least two, three, four, five, six or more CARs each one comprising different extracellular ligand-binding domains. The different extracellular ligand-binding domains according to the invention can preferably simultaneously bind different elements in target thereby augmenting immune cell activation and function. The invention also relates to an isolated immune cell which comprises a population of CARs each one comprising different extracellular ligand-binding domains.

In another aspect, the invention provides polynucleotides encoding any of the CARs and polypeptides described herein. Polynucleotides can be made and expressed by procedures known in the art.

In another aspect, the invention provides compositions (such as a pharmaceutical compositions) comprising any of the cells of the invention. In some embodiments, the composition comprises a cell comprising a polynucleotide encoding any of the CARs described herein. In still other embodiments, the composition comprises either or both of the polynucleotides shown in SEQ ID NO: 367 and SEQ ID NO:368 below:

P5A heavy chain variable region

GAGGTGCAGCTGCTGGAATCTGGCGGAGGACTGGTGCAGCCTGGCGGCTCTCTGAG
ACTGTCTTGTGCCGCCAGCGGCTTCACCTTCAGCAGCTACGCCATGAACTGGGTGCG
CCAGGCCCCTGGCAAAGGCCTGGAATGGGTGTCCGCCATCAGCGATAGCGGCGGCA
GCACCTACTACGCCGATAGCGTGAAGGGCCGGTTCACCATCAGCCGGGACAACAGCA
AGAACACCCTGTACCTGCAGATGAACAGCCTGCGGGCCGAGGACACCGCCGTGTACT
ACTGTGCCANATACTGGCCCATGGACATCTGGGGCCAGGGAACCTTGGTCACCGTCT
CCTCA (SEQ ID NO: 367)

P5A light chain variable region

GAGATCGTGCTGACACAGAGCCCTGGCACCCTGAGCCTGTCTCCAGGCGAAAGAGC
CACCCTGTCCTGCAAAGCCAGCCAGAGCGTGTCCAGCAGCTACCTGGCCTGGTATCA
GCAAAAGCCCGGCCAGGCTCCCCGGCTGCTGATGTACGATGCCAGCATCAGAGCCA
CCGGCATCCCCGACAGATTTTCCGGCTCTGGCAGCGGCACCGACTTCACCCTGACCA
TCAGCAGACTGGAACCCGAGGACTTCGCCGTGTACTACTGCCAGCAGTACGGCAGCT
GGCCCCTGACATTTGGCCAGGGCACAAAGGTGGAGATCAAA (SEQ ID NO: 368)

In other embodiments, the composition comprises either or both of the polynucleotides shown in SEQ ID NO: 369 and SEQ ID NO: 370 below:

P5AC1 heavy chain variable region

GAGGTGCAGCTGCTGGAATCTGGCGGAGGACTGGTGCAGCCTGGCGGCTCTCTGAG
ACTGTCTTGTGCCGCCAGCGGCTTCACCTTCAGCAGCTACGCCATGAACTGGGTGCG
CCAGGCCCCTGGTAAAGGTTTGGAATGGGTTTCTGCTATTCTGTCGTCTGGTGGTTCT
ACTTACTATGCCGATTCTGTTAAGGGTAGATTCACCATTTCTAGAGACAACTCTAAGAA
CACCTTGTACTTGCAAATGAACTCCTTGAGAGCTGAAGATACTGCTGTTTATTACTGTG
CTAGATACTGGCCAATGGATATTTGGGGTCAAGGTACTCTGGTCACCGTCTCCTCA
(SEQ ID NO: 369)

P5AC1 light chain variable region

GAGATCGTGCTGACACAGAGCCCTGGCACCCTGAGCCTGTCTCCTGGTGAAAGAGCT
ACTTTGTCTTGTAGAGGGGGTCAATCCGTTTCCTCTTCTTATTTGGCTTGGTATCAACA
AAAACCAGGTCAAGCTCCAAGATTATTGATGTACGATGCTTCTATTAGAGCCACCGGT
ATTCCAGATAGATTTTCTGGTTCTGGTTCCGGTACTGATTTCACTTTGACTATCTCTAGA
TTGGAACCAGAAGATTTCGCTGTTTACTACTGTCAACAATATCAGTCTTGGCCATTGAC
TTTTGGTCAAGGTACAAAGGTTGAAATCAAA (SEQ ID NO: 370)

In other embodiments, the composition comprises either or both of the polynucleotides shown in SEQ ID NO: 371 and SEQ ID NO: 372 below:

PC1 *heavy chain variable region*

GAGGTGCAGCTGCTGGAATCTGGCGGAGGACTGGTGCAGCCTGGCGGCTCTCTGAG
ACTGTCTTGTGCCGCCAGCGGCTTCACCTTCAGCAGCTACCCTATGAGCTGGGTGCG
CCAGGCCCCTGGCAAAGGACTGGAATGGGTGTCCGCCATCGGAGGCTCTGGCGGCA
GCACCTACTACGCCGATAGCGTGAAGGGCCGGTTCACCATCAGCCGGGACAACAGCA
AGAACACCCTGTACCTGCAAATGAACAGCCTGCGGGCCGAGGACACCGCCGTGTACT
ACTGTGCCAGATACTGGCCCATGGACAGCTGGGGCCAGGGAACTTTGGTCACCGTCT
CCTCA (SEQ ID NO: 371)

PC1 *light chain variable region*

GAGATCGTGCTGACACAGAGCCCTGGCACCCTGAGCCTGTCTCCAGGCGAAAGAGC
CACCCTGTCCTGCAAAGCCAGCCAGAGCGTGTCCAGCACATACCTGGCCTGGTATCA
GCAAAAGCCCGGCCAGGCTCCCCGGCTGCTGATCTACGATGCCTCTTCTAGAGCCCC
TGGCATCCCCGACAGATTCAGCGGCTCTGGCAGCGGCACCGACTTCACCCTGACCAT
CAGCAGACTGGAACCCGAGGACTTCGCCGTGTACTACTGCCAGCAGTACAGCACCAG
CCCCCTGACCTTTGGCCAGGGCACAAAGGTGGAGATCAAA (SEQ ID NO: 372).

In other embodiments, the composition comprises either or both of the polynucleotides shown in SEQ ID NO: 373 and SEQ ID NO: 374 below:

PC1C12 *heavy chain variable region*

GAGGTGCAGCTGCTGGAATCTGGCGGAGGACTGGTGCAGCCTGGCGGCTCTCTGAG
ACTGTCTTGTGCCGCCAGCGGCTTCACCTTCAGCAGCTACCCTATGAGCTGGGTGCG
CCAGGCCCCTGGTAAAGGTTTGGAATGGGTTTCTGCTATTGGTGGTTCAGGTGGTTG
GAGTTATTATGCCGATTCTGTTAAGGGTAGATTCACCATTTCTAGAGACAACTCTAAGA
ACACCTTGTACTTGCAAATGAACTCCTTGAGAGCTGAAGATACTGCTGTTTATTACTGT
GCTAGATACTGGCCAATGGATTCTTGGGGTCAAGGTACTCTGGTCACCGTCTCCTCA
(SEQ ID NO: 373)

PC1C12 *light chain variable region*

GAGATCGTGCTGACACAGAGCCCTGGCACCCTGAGCCTGTCTCCTGGTGAAAGAGCT
ACTTTGTCTTGTTGGTTGTCTCAATCTGTTTCCTCTACTTACTTGGCTTGGTATCAACAA
AAACCAGGTCAAGCTCCAAGATTATTGATCTACGATGCTTCTTCTAGAGCACCAGGTAT

TCCAGATAGATTTTCTGGTTCTGGTTCCGGTACTGATTTCACTTTGACTATCTCTAGATT
GGAACCAGAAGATTTCGCTGTTTACTACTGCCAACAATACTCTGAGTGGCCATTGACT
TTTGGTCAAGGTACAAAGGTTGAAATCAAA (SEQ ID NO: 374).

In other embodiments, the composition comprises either or both of the polynucleotides shown in SEQ ID NO: 375 and SEQ ID NO: 376 below:

COM22 *heavy chain variable region*

GAGGTGCAGCTGCTGGAATCTGGCGGAGGACTGGTGCAGCCTGGCGGCTCTCTGAG
ACTGTCTTGTGCCGCCAGCGGCTTCACCTTCAGCAGCTACGCCATGAACTGGGTGCG
CCAGGCCCCTGGTAAAGGTTTGGAATGGGTTTCTGCTATTTCTGATTCTGGTGGTTCT
AGGTGGTATGCCGATTCTGTTAAGGGTAGATTCACCATTTCTAGAGACAACTCTAAGA
ACACCTTGTACTTGCAAATGAACTCCTTGAGAGCTGAAGATACTGCTGTTTATTACTGT
ACGCGGTACTGGCCAATGGATATTTGGGGTCAAGGTACTCTGGTCACCGTCTCCTCA
(SEQ ID NO: 375)

COM22 *light chain variable region*

GAGATCGTGCTGACACAGAGCCCTGGCACCCTGAGCCTGTCTCCTGGTGAAAGAGCT
ACTTTGTCTTGTTGGTTGTCTCAATCTGTTTCCTCTACTTACTTGGCTTGGTATCAACAA
AAACCAGGTCAAGCTCCAAGATTATTGATCTACGATGCTTCTTCTAGAGCACCAGGTAT
TCCAGATAGATTTTCTGGTTCTGGTTCCGGTACTGATTTCACTTTGACTATCTCTAGATT
GGAACCAGAAGATTTCGCTGTTTACTACTGCCAACAATACTCTGAGTGGCCATTGACT
TTTGGTCAAGGTACAAAGGTTGAAATCAAA (SEQ ID NO: 376).

Expression vectors, and administration of polynucleotide compositions are further described herein.

In another aspect, the invention provides a method of making any of the polynucleotides described herein.

Polynucleotides complementary to any such sequences are also encompassed by the invention. Polynucleotides may be single-stranded (coding or antisense) or double-stranded, and may be DNA (genomic, cDNA or synthetic) or RNA molecules. RNA molecules include HnRNA molecules, which contain introns and correspond to a DNA molecule in a one-to-one manner, and mRNA molecules, which do not contain introns. Additional coding or non-coding sequences may, but need not, be present within a

polynucleotide of the invention, and a polynucleotide may, but need not, be linked to other molecules and/or support materials.

Polynucleotides may comprise a native sequence (i.e., an endogenous sequence that encodes an antibody or a portion thereof) or may comprise a variant of such a sequence. Polynucleotide variants contain one or more substitutions, additions, deletions and/or insertions such that the immunoreactivity of the encoded polypeptide is not diminished, relative to a native immunoreactive molecule. The effect on the immunoreactivity of the encoded polypeptide may generally be assessed as described herein. Variants preferably exhibit at least about 70% identity, more preferably, at least about 80% identity, yet more preferably, at least about 90% identity, and most preferably, at least about 95% identity to a polynucleotide sequence that encodes a native antibody or a portion thereof.

Two polynucleotide or polypeptide sequences are said to be "identical" if the sequence of nucleotides or amino acids in the two sequences is the same when aligned for maximum correspondence as described below. Comparisons between two sequences are typically performed by comparing the sequences over a comparison window to identify and compare local regions of sequence similarity. A "comparison window" as used herein, refers to a segment of at least about 20 contiguous positions, usually 30 to about 75, or 40 to about 50, in which a sequence may be compared to a reference sequence of the same number of contiguous positions after the two sequences are optimally aligned.

Optimal alignment of sequences for comparison may be conducted using the Megalign program in the Lasergene suite of bioinformatics software (DNASTAR, Inc., Madison, WI), using default parameters. This program embodies several alignment schemes described in the following references: Dayhoff, M.O., 1978, A model of evolutionary change in proteins - Matrices for detecting distant relationships. In Dayhoff, M.O. (ed.) Atlas of Protein Sequence and Structure, National Biomedical Research Foundation, Washington DC Vol. 5, Suppl. 3, pp. 345-358; Hein J., 1990, Unified Approach to Alignment and Phylogenes pp. 626-645 Methods in Enzymology vol. 183, Academic Press, Inc., San Diego, CA; Higgins, D.G. and Sharp, P.M., 1989, CABIOS 5:151-153; Myers, E.W. and Muller W., 1988, CABIOS 4:11-17; Robinson, E.D., 1971, Comb. Theor. 11:105; Santou, N., Nes, M., 1987, Mol. Biol. Evol. 4:406-425; Sneath, P.H.A. and Sokal,

R.R., 1973, Numerical Taxonomy the Principles and Practice of Numerical Taxonomy, Freeman Press, San Francisco, CA; Wilbur, W.J. and Lipman, D.J., 1983, Proc. Natl. Acad. Sci. USA 80:726-730.

Preferably, the "percentage of sequence identity" is determined by comparing two optimally aligned sequences over a window of comparison of at least 20 positions, wherein the portion of the polynucleotide or polypeptide sequence in the comparison window may comprise additions or deletions (i.e., gaps) of 20 percent or less, usually 5 to 15 percent, or 10 to 12 percent, as compared to the reference sequences (which does not comprise additions or deletions) for optimal alignment of the two sequences. The percentage is calculated by determining the number of positions at which the identical nucleic acid bases or amino acid residue occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the reference sequence (i.e. the window size) and multiplying the results by 100 to yield the percentage of sequence identity.

Variants may also, or alternatively, be substantially homologous to a native gene, or a portion or complement thereof. Such polynucleotide variants are capable of hybridizing under moderately stringent conditions to a naturally occurring DNA sequence encoding a native antibody (or a complementary sequence).

Suitable "moderately stringent conditions" include prewashing in a solution of 5 X SSC, 0.5% SDS, 1.0 mM EDTA (pH 8.0); hybridizing at 50°C-65°C, 5 X SSC, overnight; followed by washing twice at 65°C for 20 minutes with each of 2X, 0.5X and 0.2X SSC containing 0.1 % SDS.

As used herein, "highly stringent conditions" or "high stringency conditions" are those that: (1) employ low ionic strength and high temperature for washing, for example 0.015 M sodium chloride/0.0015 M sodium citrate/0.1% sodium dodecyl sulfate at 50°C; (2) employ during hybridization a denaturing agent, such as formamide, for example, 50% (v/v) formamide with 0.1% bovine serum albumin/0.1% Ficoll/0.1% polyvinylpyrrolidone/50 mM sodium phosphate buffer at pH 6.5 with 750 mM sodium chloride, 75 mM sodium citrate at 42°C; or (3) employ 50% formamide, 5 x SSC (0.75 M NaCl, 0.075 M sodium citrate), 50 mM sodium phosphate (pH 6.8), 0.1% sodium pyrophosphate, 5 x Denhardt's solution, sonicated salmon sperm DNA (50 µg/ml), 0.1% SDS, and 10% dextran sulfate at 42°C, with

washes at 42°C in 0.2 x SSC (sodium chloride/sodium citrate) and 50% formamide at 55°C, followed by a high-stringency wash consisting of 0.1 x SSC containing EDTA at 55°C. The skilled artisan will recognize how to adjust the temperature, ionic strength, etc. as necessary to accommodate factors such as probe length and the like.

It will be appreciated by those of ordinary skill in the art that, as a result of the degeneracy of the genetic code, there are many nucleotide sequences that encode a polypeptide as described herein. Some of these polynucleotides bear minimal homology to the nucleotide sequence of any native gene. Nonetheless, polynucleotides that vary due to differences in codon usage are specifically contemplated by the invention. Further, alleles of the genes comprising the polynucleotide sequences provided herein are within the scope of the invention. Alleles are endogenous genes that are altered as a result of one or more mutations, such as deletions, additions and/or substitutions of nucleotides. The resulting mRNA and protein may, but need not, have an altered structure or function. Alleles may be identified using standard techniques (such as hybridization, amplification and/or database sequence comparison).

The polynucleotides of this invention can be obtained using chemical synthesis, recombinant methods, or PCR. Methods of chemical polynucleotide synthesis are well known in the art and need not be described in detail herein. One of skill in the art can use the sequences provided herein and a commercial DNA synthesizer to produce a desired DNA sequence.

For preparing polynucleotides using recombinant methods, a polynucleotide comprising a desired sequence can be inserted into a suitable vector, and the vector in turn can be introduced into a suitable host cell for replication and amplification, as further discussed herein. Polynucleotides may be inserted into host cells by any means known in the art. Cells are transformed by introducing an exogenous polynucleotide by direct uptake, endocytosis, transfection, F-mating or electroporation. Once introduced, the exogenous polynucleotide can be maintained within the cell as a non-integrated vector (such as a plasmid) or integrated into the host cell genome. The polynucleotide so amplified can be isolated from the host cell by methods well known within the art. See, e.g., Sambrook et al., 1989.

Alternatively, PCR allows reproduction of DNA sequences. PCR technology is well known in the art and is described in U.S. Patent Nos. 4,683,195, 4,800,159, 4,754,065 and 4,683,202, as well as PCR: The Polymerase Chain Reaction, Mullis et al. eds., Birkauswer Press, Boston, 1994.

RNA can be obtained by using the isolated DNA in an appropriate vector and inserting it into a suitable host cell. When the cell replicates and the DNA is transcribed into RNA, the RNA can then be isolated using methods well known to those of skill in the art, as set forth in Sambrook et al., 1989, supra, for example.

Suitable cloning vectors may be constructed according to standard techniques, or may be selected from a large number of cloning vectors available in the art. While the cloning vector selected may vary according to the host cell intended to be used, useful cloning vectors will generally have the ability to self-replicate, may possess a single target for a particular restriction endonuclease, and/or may carry genes for a marker that can be used in selecting clones containing the vector. Suitable examples include plasmids and bacterial viruses, e.g., pUC18, pUC19, Bluescript (e.g., pBS SK+) and its derivatives, mp18, mp19, pBR322, pMB9, ColE1, pCR1, RP4, phage DNAs, and shuttle vectors such as pSA3 and pAT28. These and many other cloning vectors are available from commercial vendors such as BioRad, Strategene, and Invitrogen.

Expression vectors generally are replicable polynucleotide constructs that contain a polynucleotide according to the invention. It is implied that an expression vector must be replicable in the host cells either as episomes or as an integral part of the chromosomal DNA. Suitable expression vectors include but are not limited to plasmids, viral vectors, including adenoviruses, adeno-associated viruses, retroviruses, cosmids, and expression vector(s) disclosed in PCT Publication No. WO 87/04462. Vector components may generally include, but are not limited to, one or more of the following: a signal sequence; an origin of replication; one or more marker genes; suitable transcriptional controlling elements (such as promoters, enhancers and terminator). For expression (i.e., translation), one or more translational controlling elements are also usually required, such as ribosome binding sites, translation initiation sites, and stop codons.

The vectors containing the polynucleotides of interest can be introduced into the host cell by any of a number of appropriate means, including electroporation, transfection

employing calcium chloride, rubidium chloride, calcium phosphate, DEAE-dextran, or other substances; microprojectile bombardment; lipofection; and infection (e.g., where the vector is an infectious agent such as vaccinia virus). The choice of introducing vectors or polynucleotides will often depend on features of the host cell.

A polynucleotide encoding a BCMA specific CAR disclosed herein may exist in an expression cassette or expression vector (e.g., a plasmid for introduction into a bacterial host cell, or a viral vector such as a baculovirus vector for transfection of an insect host cell, or a plasmid or viral vector such as a lentivirus for transfection of a mammalian host cell). In some embodiments, a polynucleotide or vector can include a nucleic acid sequence encoding ribosomal skip sequences such as, for example without limitation, a sequence encoding a 2A peptide. 2A peptides, which were identified in the Aphthovirus subgroup of picornaviruses, causes a ribosomal "skip" from one codon to the next without the formation of a peptide bond between the two amino acids encoded by the codons (see (Donnelly and Elliott 2001; Atkins, Wills et al. 2007; Doronina, Wu et al. 2008)). By "codon" is meant three nucleotides on an mRNA (or on the sense strand of a DNA molecule) that are translated by a ribosome into one amino acid residue. Thus, two polypeptides can be synthesized from a single, contiguous open reading frame within an mRNA when the polypeptides are separated by a 2A oligopeptide sequence that is in frame. Such ribosomal skip mechanisms are well known in the art and are known to be used by several vectors for the expression of several proteins encoded by a single messenger RNA.

To direct transmembrane polypeptides into the secretory pathway of a host cell, in some embodiments, a secretory signal sequence (also known as a leader sequence, prepro sequence or pre sequence) is provided in a polynucleotide sequence or vector sequence. The secretory signal sequence is operably linked to the transmembrane nucleic acid sequence, i.e., the two sequences are joined in the correct reading frame and positioned to direct the newly synthesized polypeptide into the secretory pathway of the host cell. Secretory signal sequences are commonly positioned 5' to the nucleic acid sequence encoding the polypeptide of interest, although certain secretory signal sequences may be positioned elsewhere in the nucleic acid sequence of interest (see, e.g., Welch et al., U.S. Patent No. 5,037,743; Holland et al., U.S. Patent No. 5,143,830). In some embodiments the signal peptide comprises the amino acid sequence shown in SEQ ID NO:

318 or 329. Those skilled in the art will recognize that, in view of the degeneracy of the genetic code, considerable sequence variation is possible among these polynucleotide molecules. In some embodiments, nucleic acid sequences of the invention are codon-optimized for expression in mammalian cells, preferably for expression in human cells. Codon-optimization refers to the exchange in a sequence of interest of codons that are generally rare in highly expressed genes of a given species by codons that are generally frequent in highly expressed genes of such species, such codons encoding the amino acids as the codons that are being exchanged.

In some embodiments, a polynucleotide according to the invention comprises the nucleic acid sequence selected from the group consisting of: SEQ. ID NO: 1397. The invention relates to polynucleotides comprising a nucleic acid sequence that has at least 70%, preferably at least 80%, more preferably at least 90%, 95%, 97%, or 99 % sequence identity with nucleic acid sequence selected from the group consisting of SEQ ID NO: 1397.

Methods of engineering an immune cell

Methods of preparing immune cells for use in immunotherapy are provided herein. In some embodiments, the methods comprise introducing a CAR according to the invention into immune cells, and expanding the cells. In some embodiments, the invention relates to a method of engineering an immune cell comprising: providing a cell and expressing at the surface of the cell at least one CAR as described above. Methods for engineering immune cells are described in, for example, PCT Patent Application Publication Nos. WO/2014/039523, WO/2014/184741, WO/2014/191128, WO/2014/184744, and WO/2014/184143, each of which is incorporated herein by reference in its entirety. In some embodiments, the method comprises: transfecting the cell with at least one polynucleotide encoding CAR as described above, and expressing the polynucleotides in the cell.

In some embodiments, the polynucleotides are present in lentiviral vectors for stable expression in the cells.

In some embodiments, the method can further comprise a step of genetically modifying a cell by inactivating at least one gene expressing, for example without limitation, a component of the TCR, a target for an immunosuppressive agent, an HLA gene, and/or

an immune checkpoint protein such as, for example, PDCD1 or CTLA-4. By inactivating a gene it is intended that the gene of interest is not expressed in a functional protein form. In some embodiments, the gene to be inactivated is selected from the group consisting of, for example without limitation, TCRα, TCRβ, CD52, GR, PD-1, and CTLA-4. In some embodiments the method comprises inactivating one or more genes by introducing into the cells a rare-cutting endonuclease able to selectively inactivate a gene by selective DNA cleavage. In some embodiments the rare-cutting endonuclease can be, for example, a transcription activator-like effector nuclease (TALE-nuclease) or Cas9 endonuclease.

In some embodiments, an additional catalytic domain is used with a rare-cutting endonuclease to enhance its capacity to inactivate targeted genes. For example, an additional catalytic domain can be a DNA end-processing enzyme. Non-limiting examples of DNA end-processing enzymes include 5-3' exonucleases, 3-5' exonucleases, 5-3' alkaline exonucleases, 5' flap endonucleases, helicases, hosphatase, hydrolases and template-independent DNA polymerases. Non-limiting examples of such catalytic domain comprise of a protein domain or catalytically active derivate of the protein domain selected from the group consisting of hExol (EXO1_HUMAN), Yeast Exol (EXO1_YEAST), *E. coli* Exol, Human TREX2, Mouse TREX1, Human TREX1, Bovine TREX1, Rat TREX1, TdT (terminal deoxynucleotidyl transferase) Human DNA2, Yeast DNA2 (DNA2_YEAST). In some embodiments, an additional catalytic domain can have a 3'-5'-exonuclease activity, and In some embodiments, said additional catalytic domain is TREX, more preferably TREX2 catalytic domain (WO2012/058458). In some embodiments, said catalytic domain is encoded by a single chain TREX polypeptide. The additional catalytic domain may be fused to a nuclease fusion protein or chimeric protein. In some embodiments, the additional catalytic domain is fused using, for example, a peptide linker.

In some embodiments, the method further comprises a step of introducing into cells an exogeneous nucleic acid comprising at least a sequence homologous to a portion of the target nucleic acid sequence, such that homologous recombination occurs between the target nucleic acid sequence and the exogeneous nucleic acid. In some embodimentss, said exogenous nucleic acid comprises first and second portions which are homologous to region 5' and 3' of the target nucleic acid sequence, respectively. The exogenous nucleic acid may also comprise a third portion positioned between the first and the second portion

which comprises no homology with the regions 5' and 3' of the target nucleic acid sequence. Following cleavage of the target nucleic acid sequence, a homologous recombination event is stimulated between the target nucleic acid sequence and the exogenous nucleic acid. In some embodiments, homologous sequences of at least about 50 bp, greater than about 100 bp, or greater than about 200 bp can be used within the donor matrix. The exogenous nucleic acid can be, for example without limitation, from about 200 bp to about 6000 bp, more preferably from about 1000 bp to about 2000 bp. Shared nucleic acid homologies are located in regions flanking upstream and downstream the site of the break, and the nucleic acid sequence to be introduced is located between the two arms.

In some embodiments, a nucleic acid successively comprises a first region of homology to sequences upstream of said cleavage; a sequence to inactivate a targeted gene selected from the group consisting of TCRα, TCRβ, CD52, glucocorticoid receptor (GR), deoxycytidine kinase (DCK), and an immune checkpoint protein such as for example programmed death-1 (PD-1); and a second region of homology to sequences downstream of the cleavage. The polynucleotide introduction step can be simultaneous, before or after the introduction or expression of the rare-cutting endonuclease. Depending on the location of the target nucleic acid sequence wherein break event has occurred, such exogenous nucleic acid can be used to knock-out a gene, e.g. when exogenous nucleic acid is located within the open reading frame of the gene, or to introduce new sequences or genes of interest. Sequence insertions by using such exogenous nucleic acid can be used to modify a targeted existing gene, by correction or replacement of the gene (allele swap as a non-limiting example), or to up- or down-regulate the expression of the targeted gene (promoter swap as non-limiting example), the targeted gene correction or replacement. In some embodiments, inactivation of a genes selected from the group consisting of TCRα, TCRβ, CD52, GR, DCK, and immune checkpoint proteins, can be done at a precise genomic location targeted by a specific TALE-nuclease, wherein said specific TALE-nuclease catalyzes a cleavage and wherein the exogenous nucleic acid successively comprising at least a region of homology and a sequence to inactivate one targeted gene selected from the group consisting of TCRα, TCRβ, CD52, GR, DCK, immune checkpoint proteins which is integrated by homologous recombination. In some embodiments, several genes can be,

successively or at the same time, inactivated by using several TALE-nucleases respectively and specifically targeting one defined gene and several specific polynucleotides for specific gene inactivation.

In some embodiments, the method comprises inactivation of one or more additional genes selected from the group consisting of TCRα, TCRβ, CD52, GR, DCK, and immune checkpoint proteins. In some embodiments, inactivation of a gene can be accomplished by introducing into the cells at least one rare-cutting endonuclease such that the rare-cutting endonuclease specifically catalyzes cleavage in a targeted sequence of the cell genome; and optionally, introducing into the cells an exogenous nucleic acid successively comprising a first region of homology to sequences upstream of the cleavage, a sequence to be inserted in the genome of the cell, and a second region of homology to sequences downstream of the cleavage; wherein the introduced exogenous nucleic acid inactivates a gene and integrates at least one exogenous polynucleotide sequence encoding at least one recombinant protein of interest. In some embodiments, theexogenous polynucleotide sequence is integrated within a gene encoding a protein selected from the group consisting of TCRα, TCRβ, CD52, GR, DCK, and immune checkpoint protein.

In another aspect, a step of genetically modifying cells can comprise : modifying T cells by inactivating at least one gene expressing a target for an immunosuppressive agent, and; expanding the cells, optionally in presence of the immunosuppressive agent. An immunosuppressive agent is an agent that suppresses immune function by one of several mechanisms of action. An immunosuppressive agent can diminish the extent and/or voracity of an immune response. Non-limiting examples of immunosuppressive agents iinclude calcineurin inhibitors, targets of rapamycin, interleukin-2 α-chain blockers, inhibitors of inosine monophosphate dehydrogenase, inhibitors of dihydrofolic acid reductase, corticosteroids, and immunosuppressive antimetabolites. Some cytotoxic immunosuppressants act by inhibiting DNA synthesis. Others may act through activation of T cells or by inhibiting the activation of helper cells. The methods according to the invention allow conferring immunosuppressive resistance to T cells for immunotherapy by inactivating the target of the immunosuppressive agent in T cells. As non-limiting examples, targets for immunosuppressive agent can be a receptor for an immunosuppressive agent

such as for example without limtiation CD52, glucocorticoid receptor (GR), FKBP family gene members, and cyclophilin family gene members.

In some embodiments, the genetic modification of the method invovles expression, in provided cells to engineer, of one rare-cutting endonuclease such that the rare-cutting endonuclease specifically catalyzes cleavage in one targeted gene, thereby inactivating the targeted gene. In some embodiments, a method of engineering cells comprises at least one of the following steps: providing a T cell, such as from a cell culture or from a blood sample; selecting a gene in the T cell expressing a target for an immunosuppressive agent; introducing into the T cell a rare-cutting endonuclease able to selectively inactivate by DNA cleavage, preferably by double-strand break the gene encoding a target for the immunosuppressive agent, and expanding the cells, optionally in presence of the immunosuppressive agent.

In some embodiments, the method comprises: providing a T cell, such as from a cell culture or from a blood sample; selecting a gene in the T cell wherein the gene expresses a target for an immunosuppressive agent; transfecting the T cell with nucleic acid encoding a rare-cutting endonuclease able to selectively inactivate by DNA cleavage, preferably by double-strand break the gene encoding a target for the immunosuppressive agent, and expressing the rare-cutting endonucleases into the T cells; and expanding the cells, optionally in presence of the immunosuppressive agent.

In some embodiments, the rare-cutting endonuclease specifically targets CD52 or GR. In some embodiments, the gene selected for inactivation encodes CD52, and the immunosuppressive treatment comprises a humanized antibody targeting CD52 antigen. In some embodiments, the gene selected for inactivation encodes GR, and the immunosuppressive treatment comprises a corticosteroid such as dexamethasone. In some embodiments, the gene selected for inactivation is a FKBP family gene member or a variant thereof and the immunosuppressive treatment comprises FK506, also known as Tacrolimus or fujimycin. In some embodiments, the FKBP family gene member is FKBP12 or a variant thereof. In some embodiments, gene selected for inactivation is a cyclophilin family gene member or a variant thereof and the immunosuppressive treatment comprises cyclosporine.

In some embodiments, the rare-cutting endonuclease can be, for example, a meganuclease, a zinc finger nuclease, or a TALE-nuclease (TALEN). In some embodiments, the rare-cutting endonuclease is a TALE-nuclease.

Also provided herein are methods of engineering T cells, suitable for immunotherapy, wherein the methods comprise: genetically modifying T cells by inactivating at least immune checkpoint protein. In some embodiments the immune checkpoint protein is, for example, PD-1 and/or CTLA-4.In some embodiments, methods of genetically modifying a cell comprises: modifying T cells by inactivating at least one immune checkpoint protein; and expanding the cells. Immune checkpoint proteins include, but are not limited to Programmed Death 1 (PD-1, also known as PDCD1 or CD279, accession number: NM_005018), Cytotoxic T-Lymphocyte Antigen 4 (CTLA-4, also known as CD152, GenBank accession number AF414120.1), LAG3 (also known as CD223, accession number: NM_002286.5), Tim3 (also known as HAVCR2, GenBank accession number: JX049979.1), BTLA (also known as CD272, accession number: NM_181780.3), BY55 (also known as CD160, GenBank accession number: CR541888.1), TIGIT (also known as VSTM3, accession number: NM_173799), B7H5 (also known as C10orf54, homolog of mouse vista gene, accession number: NM_022153.1), LAIR1 (also known as CD305, GenBank accession number: CR542051.1), SIGLEC10 (GeneBank accession number: AY358337.1), 2B4 (also known as CD244, accession number: NM_001166664.1), which directly inhibit immune cells. For example, CTLA-4 is a cell-surface protein expressed on certain CD4 and CD8 T cells; when engaged by its ligands (B7-1 and B7-2) on antigen presenting cells, T cell activation and effector function are inhibited.

In some embodiments, said method to engineer cells comprises at least one of the following steps: providing a T cell, such as from a cell culture or from a blood sample; introducing into the T cell a rare-cutting endonuclease able to selectively inactivate by DNA cleavage, preferably by double-strand break one gene encoding a immune checkpoint protein; and expanding the cells. In some embodiments, the method comprises: providing a T cell, such as from a cell culture or from a blood sample; transfecting said T cell with nucleic acid encoding a rare-cutting endonuclease able to selectively inactivate by DNA cleavage, preferably by double-strand break a gene encoding a immune checkpoint protein; expressing the rare-cutting endonucleases into the T cells; expanding the cells. In

some embodiments, the rare-cutting endonuclease specifically targets a gene selected from the group consisting of: PD-1, CTLA-4, LAG3, Tim3, BTLA, BY55, TIGIT, B7H5, LAIR1, SIGLEC10, 2B4, TCRα, and TCRβ. In some embodiments, the rare-cutting endonuclease can be a meganuclease, a zinc finger nuclease or a TALE-nuclease. In some embodiments, the rare-cutting endonuclease is a TALE-nuclease.

In some embodiments, the present invention can be particularly suitable for allogeneic immunotherapy. In such embodiments, cells may be modified by a method comprising: inactivating at least one gene encoding a component of the T cell receptor (TCR) in T cells; and expanding the T cells. In some embodiments, the genetic modification of the method relies on the expression, in provided cells to engineer, of one rare-cutting endonuclease such that the rare-cutting endonuclease specifically catalyzes cleavage in one targeted gene thereby inactivating the targeted gene. In some embodiments, said method to engineer cells comprises at least one of the following steps: providing a T cell, such as from a cell culture or from a blood sample; introducing into the T cell a rare-cutting endonuclease able to selectively inactivate by DNA cleavage, preferably by double-strand break at least one gene encoding a component of the T cell receptor (TCR), and expanding the cells.

In some embodiments, the method comprises: providing a T cell, such as from a cell culture or from a blood sample; transfecting said T cell with nucleic acid encoding a rare-cutting endonuclease able to selectively inactivate by DNA cleavage, preferably by double-strand break at least one gene encoding a component of the T cell receptor (TCR); expressing the rare-cutting endonucleases into the T cells; sorting the transformed T cells, which do not express TCR on their cell surface;and expanding the cells.

In some embodiments, the rare-cutting endonuclease can be a meganuclease, a zinc finger nucleasec or a TALE-nuclease. In some embodiments, the rare-cutting endonuclease is a TALE-nuclease. In some embodiments the TALE-nucleases recognize and cleave a sequence encoding TCRα or TCRβ. In some embodiments a TALE-nuclease comprises a polypeptide sequence selected from the amino acid sequence shown in SEQ ID NO: 334, 335, 336, 337, 338, 339, 340, or 341

TALE-nuclease polypeptide sequences:

Repeat TRAC_T01-L

LTPQQVVAIASNGGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNNGGKQALETVQRLL
PVLCQAHGLTPQQVVAIASNGGGKQALETVQRLLPVLCQAHGLTPEQVVAIASHDGGKQA
LETVQRLLPVLCQAHGLTPEQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPEQVVAIA
SHDGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNIGGKQALETVQALLPVLCQAHGLT
PEQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNIGGKQALETVQALLPV
LCQAHGLTPQQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNIGGKQALE
TVQALLPVLCQAHGLTPQQVVAIASNGGGKQALETVQRLLPVLCQAHGLTPEQVVAIASN
IGGKQALETVQALLPVLCQAHGLTPQQVVAIASNGGGKQALETVQRLLPVLCQAHGLTPE
QVVAIASHDGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNGGGRPALE (SEQ ID NO:
334)

Repeat TRAC_T01-R

LTPEQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNGGGKQALETVQRLL
PVLCQAHGLTPEQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNIGGKQA
LETVQALLPVLCQAHGLTPQQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIA
SHDGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNGGGKQALETVQRLLPVLCQAHGLT
PQQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNNGGKQALETVQRLLPV
LCQAHGLTPQQVVAIASNGGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNIGGKQALE
TVQALLPVLCQAHGLTPEQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPEQVVAIASN
IGGKQALETVQALLPVLCQAHGLTPEQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPQ
QVVAIASNNGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNGGGRPALE (SEQ ID NO:
335)

Repeat TRBC_T01-L

LTPQQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNGGGKQALETVQRLL
PVLCQAHGLTPQQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNGGGKQ
ALETVQRLLPVLCQAHGLTPQQVVAIASNGGGKQALETVQRLLPVLCQAHGLTPQQVVAI
ASNGGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNNGGKQALETVQRLLPVLCQAHG
LTPEQVVAIASNIGGKQALETVQALLPVLCQAHGLTPQQVVAIASNNGGKQALETVQRLLP
VLCQAHGLTPEQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPEQVVAIASHDGGKQAL
ETVQRLLPVLCQAHGLTPEQVVAIASNIGGKQALETVQALLPVLCQAHGLTPQQVVAIASN

GGGKQALETVQRLLPVLCQAHGLTPEQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPE
QVVAIASNIGGKQALETVQALLPVLCQAHGLTPQQVVAIASNGGGRPALE (SEQ ID NO:
336)

Repeat TRBC T01-R
NPQRSTVWYLTPQQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNGGGK
QALETVQRLLPVLCQAHGLTPQQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPQQVVA
IASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASHDGGKQALETVQRLLPVLCQAHG
LTPEQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNGGGKQALETVQRLL
PVLCQAHGLTPQQVVAIASNGGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNGGGKQ
ALETVQRLLPVLCQAHGLTPQQVVAIASNGGGKQALETVQRLLPVLCQAHGLTPQQVVAI
ASNNGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNNGGKQALETVQRLLPVLCQAHGL
TPQQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNGGGKQALETVQRLLP
VLCQAHGLTPQQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNGGGRPAL
E (SEQ ID NO: 337)

Repeat TRBC T02-L
LTPEQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPEQVVAIASHDGGKQALETVQRLL
PVLCQAHGLTPEQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNIGGKQA
LETVQALLPVLCQAHGLTPEQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPEQVVAIA
SHDGGKQALETVQRLLPVLCQAHGLTPEQVVAIASHDGGKQALETVQRLLPVLCQAHGLT
PQQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNIGGKQALETVQALLPV
LCQAHGLTPQQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNNGGKQALE
TVQRLLPVLCQAHGLTPQQVVAIASNGGGKQALETVQRLLPVLCQAHGLTPEQVVAIASH
DGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPE
QVVAIASHDGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNGGGRPALE (SEQ ID NO:
338)

Repeat TRBC T02-R
LTPQQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNNGGKQALETVQRLL
PVLCQAHGLTPQQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNIGGKQA

LETVQALLPVLCQAHGLTPQQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIA
SNIGGKQALETVQALLPVLCQAHGLTPQQVVAIASNGGGKQALETVQRLLPVLCQAHGLT
PEQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNGGGKQALETVQRLLPV
LCQAHGLTPEQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNGGGKQALE
TVQRLLPVLCQAHGLTPQQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASH
DGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNGGGKQALETVQRLLPVLCQAHGLTP
QQVVAIASNGGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNGGGRPALE
(SEQ ID NO: 339)

Repeat CD52_T02-L
LTPQQVVAIASNGGGKQALETVQRLLPVLCQAHGLTPEQVVAIASHDGGKQALETVQRLL
PVLCQAHGLTPEQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNGGGKQA
LETVQRLLPVLCQAHGLTPEQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPEQVVAIA
SHDGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNGGGKQALETVQRLLPVLCQAHGLT
PEQVVAIASNIGGKQALETVQALLPVLCQAHGLTPEQVVAIASHDGGKQALETVQRLLPV
LCQAHGLTPQQVVAIASNGGGKQALETVQRLLPVLCQAHGLTPEQVVAIASHDGGKQALE
TVQRLLPVLCQAHGLTPEQVVAIASNIGGKQALETVQALLPVLCQAHGLTPEQVVAIASH
DGGKQALETVQRLLPVLCQAHGLTPEQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPE
QVVAIASNIGGKQALETVQALLPVLCQAHGLTPQQVVAIASNGGGRPALE (SEQ ID NO:
340)

Repeat CD52_T02-R
LTPQQVVAIASNGGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNNGGKQALETVQRLL
PVLCQAHGLTPEQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNGGGKQA
LETVQRLLPVLCQAHGLTPEQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPQQVVAIA
SNGGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNGGGKQALETVQRLLPVLCQAHGL
TPEQVVAIASNIGGKQALETVQALLPVLCQAHGLTPEQVVAIASHDGGKQALETVQRLLPV
LCQAHGLTPEQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNGGGKQALE
TVQRLLPVLCQAHGLTPQQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPQQVVAIASN
GGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNIGGKQALETVQALLPVLCQAHGLTPE
QVVAIASHDGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNGGGRPALE

(SEQ ID NO: 341)

In another aspect, one another step of genetically modifying cell can be a method of expanding TCRα deficient T cells comprising introducing into the T cell pTα (also known as preTCRα) or a functional variant thereof and expanding the cells, optionally through stimulation of the CD3 complex. In some embodiments, the method comprises: a) transfecting the cells with nucleic acid encoding at least a fragment of pTα to support CD3 surface expression; b) expressing said pTα into the cells; and c) expanding the cells, optionally through stimulation of the CD3 complex.

Also provided are methods of preparing T cells for immunotherapy comprising steps of the method for expansion for T cell. In some embodiments, the pTα polynucleotide sequence can be introduced randomly or by homologous recombination. In some embodiments, the insertion can be associated with the inactivation of the TCRα gene.
Different functional variants of pTα can be used. A "functional variant" of the peptide refers to a molecule substantially similar to either the entire peptide or a fragment thereof. A "fragment" of the pTα or functional variant thereof refers to any subset of the molecule, that is, a shorter peptide than the full-length pTα. In some embodiments, pTα or functional variants can be, for example, full-length pTα or a C-terminal truncated pTα version. C-terminal truncated pTα lacks in C-terminal end one or more residues. As non limiting examples, C-terminal truncated pTα version lacks 18, 48, 62, 78, 92, 110 or 114 residues from the C-terminus of the protein. Amino acid sequence variants of the peptide can be prepared by mutations in the DNA which encodes the peptide. Such functional variants include, for example, deletions from, or insertions or substitutions of, residues within the amino acid sequence. Any combination of deletion, insertion, and substitution may also be made to arrive at the final construct, provided that the final construct possesses the desired activity, in particular the restoration of a functional CD3 complex. In preferred embodiment, at least one mutation is introduced in the different pTα versions as described above to affect dimerization. As non limiting example, mutated residue can be at least W46R, D22A, K24A, R102A or R117A of the human pTα protein or aligned positions using CLUSTALW method on pTα family or homologue member. Preferably pTα or variant thereof as described above comprise the mutated residue W46R or the mutated residues D22A, K24A, R102A and R117A. In some embodiments, said pTα or variants are also fused to a

signal-transducing domain such as CD28, OX40, ICOS, CD27, CD137 (4-1BB) and CD8 as non limiting examples. The extracellular domain of pTα or variants as described above can be fused to a fragment of the TCRα protein, particularly the transmembrane and intracellular domain of TCRα. pTα variants can also be fused to the intracellular domain of TCRα.

In some embodiments, pTα versions can be fused to an extracellular ligand-binding domain. In some embodiments, pTα or functional variant thereof is fused to a single chain antibody fragment (scFv) comprising the light and the heavy variable fragment of a target antigen specific monoclonal antibody joined by a flexible linker.

The term "TCRα deficient T cell" refers to an isolated T cell that lacks expression of a functional TCRα chain. This may be accomplished by different means, as non limiting examples, by engineering a T cell such that it does not express any functional TCRα on its cell surface or by engineering a T cell such that it produces very little functional TCRα chain on its surface or by engineering a T cell to express mutated or truncated form of TCRα chain. TCRα deficient cells can no longer be expanded through CD3 complex. Thus, to overcome this problem and to allow proliferation of TCRα deficient cells, pTα or functional variant thereof is introduced into the cells, thus restoring a functional CD3 complex. In some embodiments, the method further comprises introducing into said T cells rare-cutting endonucleases able to selectively inactivate by DNA cleavage one gene encoding one component of the T cell receptor (TCR). In some embodiments, the rare-cutting endonuclease is a TALE-nuclease.

In another aspect, engineered T cells obtained by the methods described herein can be contacted with bispecific antibodies. For example, the T cells can be contacted with bispecific antibodies ex vivo prior to administration to a patient, or in vivo following administration to a patient. Bispecific antibodies comprise two variable regions with distinct antigen properties that facilitate bringing the engineered cells into proximity to a target antigen. As a non-limiting example, a bispecific antibody can be directed against a tumor marker and lymphocyte antigen, such as for example without limitation CD3, and has the potential to redirect and activate any circulating T cells against tumors.

In some embodiments, polynucleotides encoding polypeptides according to the present invention can be mRNA which is introduced directly into the cells, for example by

electroporation. In some embodiments, cytoPulse technology can be used to transiently permeabilize living cells for delivery of material into the cells. Parameters can be modified in order to determine conditions for high transfection efficiency with minimal mortality.

Also provided herein are methods of transfecting T cell. In some embodiments, the method comprises: contacting a T cell with RNA and applying to T cell an agile pulse sequence consisting of: (a) an electrical pulse with a voltage range from about 2250 to 3000 V per centimeter; (b) a pulse width of 0.1 ms; (c) a pulse interval of about 0.2 to 10 ms between the electrical pulses of step (a) and (b); (d) an electrical pulse with a voltage range from about 2250 to 3000 V with a pulse width of about 100 ms and a pulse interval of about 100 ms between the electrical pulse of step (b) and the first electrical pulse of step (c); and (e) four electrical pulses with a voltage of about 325 V with a pulse width of about 0.2 ms and a pulse interval of 2 ms between each of 4 electrical pulses. In some embodiments, a method of transfecting T cell comprising contacting said T cell with RNA and applying to T cell an agile pulse sequence comprising: (a) an electrical pulse with a voltage of about 2250, 2300, 2350, 2400, 2450, 2500, 2550, 2400, 2450, 2500, 2600, 2700, 2800, 2900 or 3000V per centimeter; (b) a pulse width of 0.1 ms; (c) and a pulse interval of about 0.2, 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 ms between the electrical pulses of step (a) and (b); (d) one electrical pulse with a voltage range from about 2250, of 2250, 2300, 2350, 2400, 2450, 2500, 2550, 2400, 2450, 2500, 2600, 2700, 2800, 2900 or 3000V with a pulse width of 100 ms and a pulse interval of 100 ms between the electrical pulse of step (b) and the first electrical pulse of step (c); and (e) 4 electrical pulses with a voltage of about 325 V with a pulse width of about 0.2 ms and a pulse interval of about 2 ms between each of 4 electrical pulses. Any values included in the value range described above are disclosed in the present application. Electroporation medium can be any suitable medium known in the art. In some embodiments, the electroporation medium has conductivity in a range spanning about 0.01 to about 1.0 milliSiemens.

In some embodiments, as non limiting examples, an RNA encodes a rare-cutting endonuclase, one monomer of the rare-cutting endonuclease such as half-TALE-nuclease, a CAR, at least one component of the multi-chain chimeric antigen receptor, a pTα or functional variant thereof, an exogenous nucleic acid, and/or one additional catalytic domain.

Engineered immune cells

The invention also provides engineered immune cells comprising any of the CAR polynucleotides described herein. In some embodiments, a CAR can be introduced into an immune cell as a transgene via a plasmid vector. In some embodiments, the plasmid vector can also contain, for example, a selection marker which provides for identification and/or selection of cells which received the vector.

CAR polypeptides may be synthesized in situ in the cell after introduction of polynucleotides encoding the CAR polypeptides into the cell. Alternatively, CAR polypeptides may be be produced outside of cells, and then introduced into cells. Methods for introducing a polynucleotide construct into cells are known in the art. In some embodiments, stable transformation methods can be used to integrate the polynucleotide construct into the genome of the cell. In other embodiments, transient transformation methods can be used to transiently express the polynucleotide construct, and the polynucleotide construct not integrated into the genome of the cell. In other embodiments, virus-mediated methods can be used. The polynucleotides may be introduced into a cell by any suitable means such as for example, recombinant viral vectors (e.g. retroviruses, adenoviruses), liposomes, and the like. Transient transformation methods include, for example without limitation, microinjection, electroporation or pa rticle bombardment. Polynucleotides may be included in vectors, such as for example plasmid vectors or viral vectors.

Also provided herein are isolated cells and cell lines obtained by the above-described methods of engineering cells provided herein. In some embodiments, an isolated cell comprises at least one CAR as described above. In some embodiments, an isolated cell comprises a population of CARs, each CAR comprising different extracellular ligand-binding domains.

Also provided herein are isolated immune cells obtained according to any one of the methods described above. Any immune cell capable of expressing heterologous DNAs can be used for the purpose of expressing the CAR of interest. In some embodiments, the immune cell is a T cell. In some embodiments, an immune cell can be derived from, for example without limitation, a stem cell. The stem cells can be adult stem cells, non-human

embryonic stem cells, more particularly non-human stem cells, cord blood stem cells, progenitor cells, bone marrow stem cells, induced pluripotent stem cells, totipotent stem cells or hematopoietic stem cells. Representative human cells are CD34+ cells. The isolated cell can also be a dendritic cell, killer dendritic cell, a mast cell, a NK-cell, a B-cell or a T cell selected from the group consisting of inflammatory T-lymphocytes, cytotoxic T-lymphocytes, regulatory T-lymphocytes or helper T-lymphocytes. I n some embodiments, the cell can be derived from the group consisting of CD4+ T-lymphocytes and CD8+ T-lymphocytes.

Prior to expansion and genetic modification, a source of cells can be obtained from a subject through a variety of non-limiting methods. Cells can be obtained from a number of non-limiting sources, including peripheral blood mononuclear cells, bone marrow, lymph node tissue, cord blood, thymus tissue, tissue from a site of infection, ascites, pleural effusion, spleen tissue, and tumors. In some embodiments, any number of T cell lines available and known to those skilled in the art, may be used. In some embodiments, cells can be derived from a healthy donor, from a patient diagnosed with cancer or from a patient diagnosed with an infection. In some embodiments, cells can be part of a mixed population of cells which present different phenotypic characteristics.

Also provided herein are cell lines obtained from a transformed T cell according to any of the above-described methods. Also provided herein are modified cells resistant to an immunosuppressive treatment. In some embodiments, an isolated cell according to the invention comprises a polynucleotide encoding a CAR.

The immune cells of the invention can be activated and expanded, either prior to or after genetic modification of the T cells, using methods as generally described, for example without limitation, in U.S. Patents 6,352,694; 6,534,055; 6,905,680; 6,692,964; 5,858,358; 6,887,466; 6,905,681; 7,144,575; 7,067,318; 7,172,869; 7,232,566; 7,175,843; 5,883,223; 6,905,874; 6,797,514; 6,867,041; and U.S. Patent Application Publication No. 20060121005. T cells can be expanded in vitro or in vivo. Generally, the T cells of the invention can be expanded, for example, by contact with an agent that stimulates a CD3 TCR complex and a co-stimulatory molecule on the surface of the T cells to create an activation signal for the T cell. For example, chemicals such as calcium ionophore A23187,

phorbol 12-myristate 13-acetate (PMA), or mitogenic lectins like phytohemagglutinin (PHA) can be used to create an activation signal for the T cell.

In some embodiments, T cell populations may be stimulated in vitro by contact with, for example, an anti-CD3 antibody, or antigen-binding fragment thereof, or an anti-CD2 antibody immobilized on a surface, or by contact with a protein kinase C activator (e.g., bryostatin) in conjunction with a calcium ionophore. For co-stimulation of an accessory molecule on the surface of the T cells, a ligand that binds the accessory molecule is used. For example, a population of T cells can be contacted with an anti-CD3 antibody and an anti-CD28 antibody, under conditions appropriate for stimulating proliferation of the T cells. Conditions appropriate for T cell culture include an appropriate media (e.g., Minimal Essential Media or RPMI Media 1640 or, X-vivo 5, (Lonza)) that may contain factors necessary for proliferation and viability, including serum (e.g., fetal bovine or human serum), interleukin-2 (IL-2), insulin, IFN-γ, IL-4, IL-7, GM-CSF, IL-10, IL-2, IL-15, TGFp, and TNF, or any other additives for the growth of cells known to the skilled artisan. Other additives for the growth of cells include, but are not limited to, surfactant, plasmanate, and reducing agents such as N-acetyl- cysteine and 2-mercaptoethanol. Media can include RPMI 1640, A1M-V, DMEM, MEM, a- MEM, F-12, X-Vivo 1, and X-Vivo 20, Optimizer, with added amino acids, sodium pyruvate, and vitamins, either serum-free or supplemented with an appropriate amount of serum (or plasma) or a defined set of hormones, and/or an amount of cytokine(s) sufficient for the growth and expansion of T cells. Antibiotics, e.g., penicillin and streptomycin, are included only in experimental cultures, not in cultures of cells that are to be infused into a subject. The target cells are maintained under conditions necessary to support growth, for example, an appropriate temperature (e.g., 37° C) and atmosphere (e.g., air plus 5% CO_2). T cells that have been exposed to varied stimulation times may exhibit different characteristics

In some embodiments, the cells of the invention can be expanded by co-culturing with tissue or cells. The cells can also be expanded in vivo, for example in the subject's blood after administrating the cell into the subject.

In some embodiments, an isolated cell according to the present invention comprises one inactivated gene selected from the group consisting of CD52, GR, PD-1, CTLA-4, LAG3, Tim3, BTLA, BY55, TIGIT, B7H5, LAIR1, SIGLEC10, 2B4, HLA, TCRα and TCRβ

and/or expresses a CAR, a multi-chain CAR and/or a pTα transgene. In some embodiments, an isolated cell comprises polynucleotides encoding polypeptides comprising a multi-chain CAR. In some embodiments, the isolated cell according to the present invention comprises two inactivated genes selected from the group consisting of: CD52 and GR, CD52 and TCRα, CDR52 and TCRβ, GR and TCRα, GR and TCRβ, TCRα and TCRβ, PD-1 and TCRα, PD-1 and TCRβ, CTLA-4 and TCRα, CTLA-4 and TCRβ, LAG3 and TCRα, LAG3 and TCRβ, Tim3 and TCRα, Tim3 and TCRβ, BTLA and TCRα, BTLA and TCRβ, BY55 and TCRα, BY55 and TCRβ, TIGIT and TCRα, TIGIT and TCRβ, B7H5 and TCRα, B7H5 and TCRβ, LAIR1 and TCRα, LAIR1 and TCRβ, SIGLEC10 and TCRα, SIGLEC10 and TCRβ, 2B4 and TCRα, 2B4 and TCRβ and/or expresses a CAR, a multi-chain CAR and a pTα transgene.

In some embodiments, TCR is rendered not functional in the cells according to the invention by inactivating TCRα gene and/or TCRβ gene(s). In some embodiments, a method to obtain modified cells derived from an individual is provided, wherein the cells can proliferate independently of the major histocompatibility complex (MHC) signaling pathway. Modified cells, which can proliferate independently of the MHC signaling pathway, susceptible to be obtained by this method are encompassed in the scope of the present invention. Modified cells disclosed herein can be used in for treating patients in need thereof against Host versus Graft (HvG) rejection and Graft versus Host Disease (GvHD); therefore in the scope of the present invention is a method of treating patients in need thereof against Host versus Graft (HvG) rejection and Graft versus Host Disease (GvHD) comprising treating said patient by administering to said patient an effective amount of modified cells comprising inactivated TCRα and/or TCRβ genes.

In some embodiments, the immune cells are engineered to be resistant to one or more chemotherapy drugs. The chemotherapy drug can be, for example, a purine nucleotide analogue (PNA), thus making the immune cell suitable for cancer treatment combining adoptive immunotherapy and chemotherapy. Exemplary PNAs include, for example, clofarabine, fludarabine, and cytarabine, alone or in combination. PNAs are metabolized by deoxycytidine kinase (dCK) into mono-, di-, and tri-phosphate PNA. Their tri-phosphate forms compete with ATP for DNA synthesis, act as pro-apoptotic agents, and are potent inhibitors of ribonucleotide reductase (RNR), which is involved in trinucleotide

production. Provided herein are BCMA specific CAR-T cells comprising an inactivated dCK gene. In some embodiments, the dCK knockout cells are made by transfection of T cells using polynucleotides encoding specific TAL-nulcease directed against dCK genes by, for example, electroporation of mRNA. The dCK knockout BCMA specific CAR-T cells are resistant to PNAs, including for example clorofarabine and/or fludarabine, and maintain T cell cytotoxic activity toward BCMA-expressing cells.

In some embodiments, isolated cells or cell lines of the invention can comprise a pTα or a functional variant thereof. In some embodiments, an isolated cell or cell line can be further genetically modified by inactivating the TCRα gene.

In some embodiments, the CAR-T cell comprises a polynucleotide encoding a suicide polypeptide, such as for example RQR8. See, e.g., WO2013153391A, which is hereby incorporated by reference in its entirety. In CAR-T cells comprising the polynucleotide, the suicide polypeptide is expressed at the surface of a CAR-T cell. In some embodiments, the suicide polypeptide comprises the amino acid sequence shown in SEQ ID NO: 342.

CPYSNPSLCSGGGGSELPTQGTFSNVSTNVSPAKPTTTACPYSNPSLCSGGGGSP
APRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLS
LVITLYCNHRNRRRVCKCPRPVV (SEQ ID NO: 342)

The suicide polypeptide may also comprise a signal peptide at the amino terminus. In some embodiments, the suicide polypeptide comprises the amino acid sequence shown in SEQ ID NO: 400.

MGTSLLCWMALCLLGADHADACPYSNPSLCSGGGGSELPTQGTFSNVSTNVSPAKPTTT
ACPYSNPSLCSGGGGSPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYI
WAPLAGTCGVLLLSLVITLYCNHRNRRRVCKCPRPVV (SEQ ID NO: 400)

When the suicide polypeptide is expressed at the surface of a CAR-T cell, binding of rituximab to the R epitopes of the polypeptide causes lysis of the cell. More than one molecule of rituximab may bind per polypeptide expressed at the cell surface. Each R epitope of the polypeptide may bind a separate molecule of rituximab. Deletion of BCMA specific CAR-T cells may occur in vivo, for example by administering rituximab to a patient. The decision to delete the transferred cells may arise from undesirable effects being

detected in the patient which are attributable to the transferred cells, such as for example, when unacceptable levels of toxicity are detected.

In some embodiments, the CAR-T cell comprises a selected epitope within the scFv having a specificity to be recognized by a specific antibody. See, e.g., PCT application "mAb-DRIVEN CHIMERIC ANTIGEN RECEPTOR SYSTEMS FOR SORTING/DEPLETING ENGINEERED IMMUNE CELLS," filed on January 25, 2016, which is hereby incorporated by reference in its entirety. Such an epitope facilitates sorting and/or depleting the CAR-T cells. The epitope can be selected from any number of epitopes known in the art. In some embodiments, the epitope can be a target of a monoclonal antibody approved for medical use, such as, for example without limitation, the CD20 epitope recognized by rituximab. In some embodiments, the epitope comprises the amino acid sequence shown in SEQ ID NO: 397.

CPYSNPSLC (SEQ ID NO: 397)

In some embodiments, the epitope is located within the CAR. For example without limitation, the epitope can be located between the scFv and the hinge of a CAR. In some embodiments, two instances of the same epitope, separate by linkers, may be used in the CAR. For example, the polypeptide comprising the amino acid sequence shown in SEQ ID NO: 398 can be used within a CAR, located between the light chain variable region and the hinge.

GSGGGGSCPYSNPSLCSGGGGSCPYSNPSLCSGGGGS (SEQ ID NO: 398)

In some embodiments, the epitope-specific antibody may be conjugated with a cytotoxic drug. It is also possible to promote CDC cytotoxicity by using engineered antibodies on which are grafted component(s) of the complement system. In some embodiments, activation of the CAR-T cells can be modulated by depleting the cells using an antibody which recognizes the epitope.

Therapeutic applications

Isolated cells obtained by the methods described above, or cell lines derived from such isolated cells, can be used as a medicament. In some embodiments, such a medicament can be used for treating cancer. In some embodiments, the cancer is multiple myeloma malignant plasma cell neoplasm, Hodgkin's lymphoma, nodular lymphocyte

predominant Hodgkin's lymphoma, Kahler's disease and Myelomatosis, plasma cell leukemia, plasmacytoma, B-cell prolymphocytic leukemia, hairy cell leukemia, B-cell non-Hodgkin's lymphoma (NHL), acute myeloid leukemia (AML), chronic lymphocytic leukemia (CLL), acute lymphocytic leukemia (ALL), chronic myeloid leukemia (CML), follicular lymphoma, Burkitt's lymphoma, marginal zone lymphoma, mantle cell lymphoma, large cell lymphoma, precursor B-lymphoblastic lymphoma, myeloid leukemia, Waldenstrom's macroglobulienemia, diffuse large B cell lymphoma, follicular lymphoma, marginal zone lymphoma, mucosa-associated lymphatic tissue lymphoma, small cell lymphocytic lymphoma, mantle cell lymphoma, Burkitt lymphoma, primary mediastinal (thymic) large B-cell lymphoma, lymphoplasmactyic lymphoma, Waldenström macroglobulinemia, nodal marginal zone B cell lymphoma, splenic marginal zone lymphoma, intravascular large B-cell lymphoma, primary effusion lymphoma, lymphomatoid granulomatosis, T cell/histiocyte-rich large B-cell lymphoma, primary central nervous system lymphoma, primary cutaneous diffuse large B-cell lymphoma (leg type), EBV positive diffuse large B-cell lymphoma of the elderly, diffuse large B-cell lymphoma associated with inflammation, intravascular large B-cell lymphoma, ALK-positive large B-cell lymphoma, plasmablastic lymphoma, large B-cell lymphoma arising in HHV8-associated multicentric Castleman disease, B-cell lymphoma unclassified with features intermediate between diffuse large B-cell lymphoma and Burkitt lymphoma, B-cell lymphoma unclassified with features intermediate between diffuse large B-cell lymphoma and classical Hodgkin lymphoma, or another B-cell related lymphomas.

In some embodiments, an isolated cell according to the invention, or cell line derived from the isolated cells, can be used in the manufacture of a medicament for treatment of a cancer in a patient in need thereof.

Also provided herein are methods for treating patients. In some embodiments the method comprises.providing an immune cell of the invention to a patient in need thereof. In some embodiments, the method comprises a step of administrating transformed immune cells of the invention to a patient in need thereof.

In some embodiments, T cells of the invention can undergo robust in vivo T cell expansion and can persist for an extended amount of time.

Methods of treatment of the invention can be ameliorating, curative or prophylactic. The method of the invention may be either part of an autologous immunotherapy or part of an allogenic immunotherapy treatment. The invention is particularly suitable for allogeneic immunotherapy. T cells from donors can be transformed into non-alloreactive cells using standard protocols and reproduced as needed, thereby producing CAR-T cells which may be administered to one or several patients. Such CAR-T cell therapy can be made available as an "off the shelf" therapeutic product.

Cells that can be used with the disclosed methods are described in the previous section. Treatment can be used to treat patients diagnosed with, for example, cancer. Cancers that may be treated include, for example without limitation, cancers that involve B lymphocytes, including any of the above-listed cancers. Types of cancers to be treated with the CARs and CAR-T cells of the invention include, but are not limited to certain leukemia or lymphoid malignancies. Adult tumors/cancers and pediatric tumors/cancers are also included. In some embodiments, the treatment can be in combination with one or more therapies against cancer selected from the group of antibodies therapy, chemotherapy, cytokines therapy, dendritic cell therapy, gene therapy, hormone therapy, laser light therapy and radiation therapy.

In some embodiments, treatment can be administrated into patients undergoing an immunosuppressive treatment. Indeed, the invention preferably relies on cells or population of cells, which have been made resistant to at least one immunosuppressive agent due to the inactivation of a gene encoding a receptor for such immunosuppressive agent. In this aspect, the immunosuppressive treatment should help the selection and expansion of the T cells according to the invention within the patient. The administration of the cells or population of cells according to the invention may be carried out in any convenient manner, including by aerosol inhalation, injection, ingestion, transfusion, implantation or transplantation. The compositions described herein may be administered to a patient subcutaneously, intradermally, intratumorally, intranodally, intramedullary, intramuscularly, by intravenous or intralymphatic injection, or intraperitoneally. In one embodiment, the cell compositions of the invention are preferably administered by intravenous injection.

In some embodiments the administration of the cells or population of cells can comprise administration of, for example, about 10^4 to about 10^9 cells per kg body weight

including all integer values of cell numbers within those ranges. In some embodiments the administration of the cells or population of cells can comprise administration of about 10^5 to 10^6 cells per kg body weight including all integer values of cell numbers within those ranges. The cells or population of cells can be administrated in one or more doses. In some embodiments, said effective amount of cells can be administrated as a single dose. In some embodiments, said effective amount of cells can be administrated as more than one dose over a period time. Timing of administration is within the judgment of managing physician and depends on the clinical condition of the patient. The cells or population of cells may be obtained from any source, such as a blood bank or a donor. While individual needs vary, determination of optimal ranges of effective amounts of a given cell type for a particular disease or conditions within the skill of the art. An effective amount means an amount which provides a therapeutic or prophylactic benefit. The dosage administrated will be dependent upon the age, health and weight of the recipient, kind of concurrent treatment, if any, frequency of treatment and the nature of the effect desired. In some embodimetns, an effective amount of cells or composition comprising those cells are administrated parenterally. In some embodiments, administration can be an intravenous administration. In some embodimetns, administration can be directly done by injection within a tumor.

In some embodiments of the invention, cells are administered to a patient in conjunction with (e.g., before, simultaneously or following) any number of relevant treatment modalities, including but not limited to treatment with agents such as monoclonal antibody therapy, CCR2 antagonist (e.g., INC-8761), antiviral therapy, cidofovir and interleukin-2, Cytarabine (also known as ARA-C) or natalizilmab treatment for MS patients or efaliztimab treatment for psoriasis patients or other treatments for PML patients. In some embodiments, BCMA specific CAR-T cells are administered to a patient in conjunction with one or more of the following: an anti-PD-1 antibody (e.g., nivolumab, pembrolizumab, or PF-06801591), an anti-PD-L1 antibody (e.g., avelumab, atezolizumab, or durvalumab), an anti-OX40 antibody (e.g., PF-04518600), an anti-4-1BB antibody (e.g., PF-05082566), an anti-MCSF antibody (e.g., PD-0360324), an anti-GITR antibody, and/or an anti-TIGIT antibody. In some embodiments, a BCMA specific CAR comprising the amino acid sequence shown in SEQ ID NO: 396 is administered to a patient in conjunction with anti-

PD-L1 antibody avelumab. In further embodiments, the T cells of the invention may be used in combination with chemotherapy, radiation, immunosuppressive agents, such as cyclosporin, azathioprine, methotrexate, mycophenolate, and FK506, antibodies, or other immunoablative agents such as CAMPATH, anti-CD3 antibodies or other antibody therapies, cytoxin, fludaribine, cyclosporin, FK506, rapamycin, mycoplienolic acid, steroids, FR901228, cytokines, and/or irradiation. These drugs inhibit either the calcium dependent phosphatase calcineurin (cyclosporine and FK506) or inhibit the p70S6 kinase that is important for growth factor induced signaling (rapamycin) (Henderson, Naya et al. 1991; Liu, Albers et al. 1992; Bierer, Hollander et al. 1993). In a further embodiment, the cell compositions of the invention are administered to a patient in conjunction with (e.g., before, simultaneously or following) bone marrow transplantation, T cell ablative therapy using either chemotherapy agents such as, fludarabine, external-beam radiation therapy (XRT), cyclophosphamide, or antibodies such as OKT3 or CAMPATH, In some embodiments, the cell compositions of the invention are administered following B-cell ablative therapy such as agents that react with CD20, e.g., Rituxan. For example, in one embodiment, subjects may undergo standard treatment with high dose chemotherapy followed by peripheral blood stem cell transplantation. In certain embodiments, following the transplant, subjects receive an infusion of the expanded immune cells of the invention. In some embodiments, expanded cells are administered before or following surgery.

Kits

The invention also provides kits for use in the instant methods. Kits of the invention include one or more containers comprising a polynucleotide encoding a BCMA specific CAR, or an engineered immune cell comprising a polynucleotide encoding a BCMA specific CAR as described herein, and instructions for use in accordance with any of the methods of the invention described herein. Generally, these instructions comprise a description of administration of the engineered immune cell for the above described therapeutic treatments.

The instructions relating to the use of the engineered immune cells as described herein generally include information as to dosage, dosing schedule, and route of administration for the intended treatment. The containers may be unit doses, bulk

packages (e.g., multi-dose packages) or sub-unit doses. Instructions supplied in the kits of the invention are typically written instructions on a label or package insert (e.g., a paper sheet included in the kit), but machine-readable instructions (e.g., instructions carried on a magnetic or optical storage disk) are also acceptable.

The kits of this invention are in suitable packaging. Suitable packaging includes, but is not limited to, vials, bottles, jars, flexible packaging (e.g., sealed Mylar or plastic bags), and the like. Also contemplated are packages for use in combination with a specific device, such as an inhaler, nasal administration device (e.g., an atomizer) or an infusion device such as a minipump. A kit may have a sterile access port (for example the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). The container may also have a sterile access port (for example the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). At least one active agent in the composition is a BCMA antibody. The container may further comprise a second pharmaceutically active agent.

Kits may optionally provide additional components such as buffers and interpretive information. Normally, the kit comprises a container and a label or package insert(s) on or associated with the container.

The following examples are offered for illustrative purposes only, and are not intended to limit the scope of the invention in any way. Indeed, various modifications of the invention in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description and fall within the scope of the appended claims.

Representative materials of the present invention were deposited in the American Type Culture Collection (ATCC) on February 9, 2016. The biological deposit having ATCC Accession No. PTA-122834 is a vector comprising a polynucleotide encoding a BCMA specific CAR. The deposit was made under the provisions of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purpose of Patent Procedure and Regulations thereunder (Budapest Treaty). This assures maintenance of a viable culture of the deposit for 30 years from the date of deposit. The deposit will be made available by ATCC under the terms of the Budapest Treaty, and subject to an agreement between Pfizer, Inc. and ATCC, which assures permanent and unrestricted availability of

the progeny of the culture of the deposit to the public upon issuance of the pertinent U.S. patent or upon laying open to the public of any U.S. or foreign patent application, whichever comes first, and assures availability of the progeny to one determined by the U.S. Commissioner of Patents and Trademarks to be entitled thereto according to 35 U.S.C. Section 122 and the Commissioner's rules pursuant thereto (including 37 C.F.R. Section 1.14 with particular reference to 886 OG 638).

The assignee of the present application has agreed that if a culture of the materials on deposit should die or be lost or destroyed when cultivated under suitable conditions, the materials will be promptly replaced on notification with another of the same. Availability of the deposited material is not to be construed as a license to practice the invention in contravention of the rights granted under the authority of any government in accordance with its patent laws.

Examples

Example 1: Determination of kinetics and affinity of BCMA/human IgG interactions at 25°C and/or 37°C

This example determines the kinetics and affinity of various anti-BCMA antibodies at 25°C and 37°C.

All experiments were performed on a Bio-Rad Proteon XPR36 surface Plasmon resonance biosensor (Bio-Rad, Hercules, CA). An array of anti-BCMA antibodies was prepared using an amine-coupling method on a Bio-Rad GLC Sensor Chip similar to that described in Abdiche, et al., Anal. Biochem. 411, 139-151 (2011). The analysis temperature for the immobilization was 25°C and the running buffer was HBS-T+ (10 mM HEPES, 150 mM NaCl, 0.05% Tween-20, pH 7.4). Channels were activated in the analyte (horizontal) direction by injecting a mixture of 1 mM ECD and 0.25 mM NHS for 3 minutes at a flow rate of 30 µL/min. IgGs were immobilized on the activated spots by injecting them in the ligand (vertical) direction at 20 µg/mL in 10 mM Acetate pH 4.5 buffer for 1.5 minutes at 30 µg/mL. The activated surfaces were blocked by injecting 1M ethanolamine, pH 8.5 in the analyte direction for 3 minutes at 30 µL/min.

The analysis temperature for the BCMA binding analysis was 37°C or 25°C in a running buffer of HBS-T+, supplemented with 1 mg/mL BSA. A kinetic titration method was employed for the interaction analysis as described in Abdiche, et al. Human BCMA (huBCMA) or cynomolgus monkey BCMA (cyBCMA) analyte was injected in the analyte direction using a series of injections from low to high concentration. The concentrations used were 0.08 nM, 0.4 nM, 2 nM, 10 nM and 50 nM (a 5-membered series, with a 5-fold dilution factor and top concentration of 50 nM). The association time for a given analyte dilution was two minutes. Immediately after the 50 nM BCMA injection, dissociation was monitored for 2 hours. Prior to the BCMA analyte injections, buffer was injected 5 times using the same association and dissociation times at the BCMA analyte cycles to prepare a buffer blank sensorgram for double-referencing purposes (double referencing as described in Myszka, J. Mol. Recognit. 12, 279-284 (1999).

The sensorgrams were double-referenced and fit to a *1:1 Langmuir with mass transport* kinetic titration model in BIAevaluation Software version 4.1.1 (GE Lifesciences, Piscataway, NJ). The kinetics and affinity parameters for various anti-BCMA antibodies of the invention are shown in Tables 4A-4C. The antibodies shown in Tables 4A-4C share the same VH and VL regions as the CARs shown in Table 1 having the same name.

Table 4A

Sample	k_a (1/Ms)	k_d (1/s)	$t_{1/2}$ (min)	K_D (pM)
A02_Rd4_6nM_C01	1.2E+06	2.8E-05	411	24
A02_Rd4_6nM_C16	1.1E+06	6.2E-05	187	59
Combo_Rd4_0.6nM_C29	6.6E+06	1.4E-04	83	21
L3PY/H3TAQ	2.6E+06	1.4E-04	84	53

Table 4B

Antibody	ka (1/Ms) huBCMA @ 25°C	kd (1/s) huBCMA @25°C	T½ (min) to huBCMA @25°C	KD (nM) to huBCMA @ 25°C
P6E01/P6E01	1.04E+06	4.15E-03	2.8	4.0
P6E01/H3.AQ	8.35E+05	3.45E-04	33.53	0.41
L1.LGF/L3.KW/P6E01	8.31E+05	7.55E-03	1.53	9.08
L1.LGF/L3.NY/P6E01	1.33E+06	4.40E-03	2.63	3.32
L1.GDF/L3.NY/P6E01	1.60E+06	5.92E-03	1.95	3.70
L1.LGF/L3.KW/H3.AL	4.28E+05	1.23E-03	9.40	2.87
L1.LGF/L3.KW/H3.AP	9.28E+05	2.27E-03	5.10	2.44
L1.LGF/L3.KW/H3.AQ	5.24E+05	9.56E-04	12.09	1.82

L1.LGF/L3.PY/H3.AP	4.57E+05	9.69E-04	11.92	2.12
L1.LGF/L3.PY/H3.AQ	9.31E+05	8.86E-04	13.04	0.95
L1.LGF/L3.NY/H3.AL	7.63E+05	9.70E-04	11.91	1.27
L1.LGF/L3.NY/H3.AP	9.36E+05	5.33E-04	21.67	0.57
L1.LGF/L3.NY/H3.AQ	6.66E+05	2.99E-04	38.61	0.45
L1.GDF/L3.KW/H3.AL	4.45E+05	3.90E-03	2.96	8.76
L1.GDF/L3.KW/H3.AP	1.17E+06	4.61E-03	2.51	3.93
L1.GDF/L3.KW/H3.AQ	7.97E+05	3.48E-03	3.32	4.37
L1.GDF/L3.PY/H3.AQ	1.42E+06	1.35E-02	0.86	9.49
L1.GDF/L3.NY/H3.AL	9.07E+05	4.03E-03	2.87	4.44
L1.GDF/L3.NY/H3.AP	1.41E+06	1.41E-03	8.21	1.00
L1.GDF/L3.NY/H3.AQ	9.84E+05	7.22E-04	16.00	0.73
L3.KW/P6E01	7.40E+05	3.15E-04	36.66	0.43
L3.PY/P6E01	7.12E+05	2.28E-04	50.74	0.32
L3.NY/P6E01	8.76E+05	3.84E-04	30.08	0.44
Antibody	ka (1/Ms) huBCMA @ 37°C	kd (1/s) huBCMA @37°C	T½ (min) to huBCMA @37°C	KD (nM) to huBCMA @ 37°C
L3.PY/L1.PS/P6E01	2.49E+06	1.13E-03	10.21	0.45
L3.PY/L1.AH/P6E01	2.55E+06	1.26E-03	9.19	0.49
L3.PY/L1.FF/P6E01	2.39E+06	1.41E-03	8.18	0.59
L3.PY/L1.PH/P6E01	2.81E+06	9.13E-04	12.65	0.32
L3.PY/L3.KY/P6E01	3.18E+06	1.09E-03	10.65	0.34
L3.PY/L3.KF/P6E01	2.88E+06	2.08E-03	5.56	0.72
L3.PY/H2.QR	2.56E+06	1.19E-03	9.75	0.46
L3.PY/H2.DY	2.60E+06	1.38E-03	8.37	0.53
L3.PY/H2.YQ	2.58E+06	1.56E-03	7.41	0.60
L3.PY/H2.LT	2.40E+06	1.29E-03	8.95	0.54
L3.PY/H2.HA	2.43E+06	1.47E-03	7.89	0.60
L3.PY/H2.QL	2.64E+06	2.18E-03	5.31	0.82
L3.PY/H3.YA	3.15E+06	1.18E-03	9.82	0.37
L3.PY/H3.AE	3.29E+06	1.39E-03	8.32	0.42
L3.PY/H3.AQ	3.08E+06	1.73E-03	6.69	0.56
L3.PY/H3.TAQ	3.08E+06	1.14E-03	10.13	0.37
L3.PY/P6E01	2.65E+06	1.96E-03	5.91	0.74
L3.PY/L1.PS/H2.QR	3.97E+06	1.03E-01	0.11	25.85
L3.PY/L1.PS/H2.DY	3.22E+06	3.61E-03	3.20	1.12
L3.PY/L1.PS/H2.YQ	3.35E+06	4.30E-03	2.69	1.28
L3.PY/L1.PS/H2.LT	3.40E+06	4.65E-03	2.49	1.37
L3.PY/L1.PS/H2.HA	3.30E+06	1.06E-02	1.09	3.21
L3.PY/L1.PS/H2.QL	1.52E+07	3.14E-01	0.04	20.64
L3.PY/L1.PS/H3.YA	3.07E+06	9.05E-03	1.28	2.95
L3.PY/L1.PS/H3.AE	3.14E+06	1.46E-03	7.93	0.46
L3.PY/L1.PS/H3.AQ	3.26E+06	1.79E-03	6.46	0.55
L3.PY/L1.PS/H3.TAQ	3.25E+06	2.46E-03	4.70	0.76
L3.PY/L1.AH/H2.QR	3.13E+06	1.81E-03	6.39	0.58
L3.PY/L1.AH/H2.DY	3.05E+06	1.52E-03	7.62	0.50
L3.PY/L1.AH/H2.YQ	2.42E+06	1.93E-03	6.00	0.80
L3.PY/L1.AH/H2.LT	3.16E+06	1.23E-03	9.38	0.39
L3.PY/L1.AH/H2.HA	3.33E+06	1.81E-03	6.37	0.54

L3.PY/L1.AH/H2.QL	3.04E+06	1.60E-03	7.22	0.53
L3.PY/L1.AH/H3.YA	3.00E+06	1.50E-03	7.73	0.50
L3.PY/L1.AH/H3.AE	3.32E+06	1.73E-03	6.70	0.52
L3.PY/L1.AH/H3.AQ	3.03E+06	1.97E-03	5.85	0.65
L3.PY/L1.AH/H3.TAQ	3.27E+06	1.19E-03	9.68	0.37
L3.PY/L1.FF/H2.QR	3.47E+06	1.77E-03	6.54	0.51
L3.PY/L1.FF/H2.DY	4.14E+06	2.71E-03	4.27	0.65
L3.PY/L1.FF/H2.YQ	3.32E+06	1.52E-03	7.61	0.46
L3.PY/L1.FF/H2.LT	3.30E+06	1.67E-03	6.92	0.51
L3.PY/L1.FF/H2.HA	3.49E+06	2.19E-03	5.29	0.63
L3.PY/L1.FF/H2.QL	3.48E+06	1.40E-03	8.28	0.40
L3.PY/L1.FF/H3.YA	3.50E+06	1.80E-03	6.41	0.51
L3.PY/L1.FF/H3.AE	3.82E+06	2.63E-03	4.39	0.69
L3.PY/L1.FF/H3.AQ	3.32E+06	1.54E-03	7.51	0.46
L3.PY/L1.FF/H3.TAQ	3.52E+06	1.89E-03	6.12	0.54
L3.PY/L1.PH/H2.QR	3.69E+06	2.36E-03	4.89	0.64
L3.PY/L1.PH/H2.HA	2.37E+06	1.16E-03	9.99	0.49
L3.PY/L1.PH/H3.AE	3.68E+06	1.34E-03	8.61	0.36
L3.PY/L1.PH/H3.AQ	3.08E+06	1.59E-03	7.27	0.52
L3.PY/L1.PH/H3.TAQ	3.58E+06	2.13E-03	5.43	0.59
L3.PY/L3.KY/H2.QR	2.95E+06	9.90E-04	11.67	0.34
L3.PY/L3.KY/H2.DY	3.19E+06	6.42E-04	18.00	0.20
L3.PY/L3.KY/H2.YQ	2.14E+06	1.65E-03	7.02	0.77
L3.PY/L3.KY/H2.LT	2.92E+06	9.06E-04	12.75	0.31
L3.PY/L3.KY/H2.HA	3.29E+06	1.63E-03	7.10	0.49
L3.PY/L3.KY/H2.QL	3.65E+06	2.08E-03	5.56	0.57
L3.PY/L3.KY/H3.YA	3.30E+06	9.12E-04	12.67	0.28
L3.PY/L3.KY/H3.TAQ	2.79E+06	6.49E-04	17.79	0.23
L3.PY/L3.KF/H2.DY	2.74E+06	1.82E-03	6.35	0.67
L3.PY/L3.KF/H2.YQ	1.96E+06	2.23E-03	5.18	1.14
L3.PY/L3.KF/H2.LT	2.75E+06	1.91E-03	6.05	0.69
L3.PY/L3.KF/H2.QL	2.07E+06	1.25E-03	9.26	0.60
L3.PY/L3.KF/H3.YA	3.12E+06	1.47E-03	7.85	0.47
L3.PY/L3.KF/H3.AE	3.07E+06	1.55E-03	7.44	0.51
L3.PY/L3.KF/H3.AQ	3.48E+06	2.27E-03	5.09	0.65
L3.PY/L3.KF/H3.TAQ	2.82E+06	1.62E-03	7.12	0.58
Antibody	**ka (1/Ms) cyBCMA @ 25°C**	**kd (1/s) cyBCMA @25°C**	**T ½ (min) to cyBCMA @25°C**	**KD (nM) to cyBCMA @ 25°C**
P6E01/P6E01		*7.02E-02*	*0.16*	*115.4*
P6E01/H3.AQ	1.08E+06	7.40E-03	1.6	6.9
L1.LGF/L3.KW/P6E01	4.55E+05	1.95E-02	0.6	42.8
L1.LGF/L3.NY/P6E01	9.20E+05	1.05E-02	1.1	11.4
L1.GDF/L3.NY/P6E01	1.20E+06	7.67E-03	1.5	6.4
L1.LGF/L3.KW/H3.AL	2.90E+05	1.21E-02	1.0	41.8
L1.LGF/L3.KW/H3.AP	5.54E+05	1.54E-02	0.7	27.8
L1.LGF/L3.KW/H3.AQ	5.27E+05	3.55E-03	3.3	6.7
L1.LGF/L3.PY/H3.AP	3.64E+05	1.30E-02	0.9	35.8
L1.LGF/L3.PY/H3.AQ	1.00E+06	4.77E-03	2.4	4.8
L1.LGF/L3.NY/H3.AL	6.35E+05	1.48E-02	0.8	23.2

L1.LGF/L3.NY/H3.AP	8.30E+05	5.57E-03	2.1	6.7
L1.LGF/L3.NY/H3.AQ	7.51E+05	1.48E-03	7.8	2.0
L1.GDF/L3.KW/H3.AL	3.18E+05	1.80E-02	0.6	56.7
L1.GDF/L3.KW/H3.AP	8.14E+05	2.03E-02	0.6	24.9
L1.GDF/L3.KW/H3.AQ	8.02E+05	5.65E-03	2.0	7.0
L1.GDF/L3.PY/H3.AQ	1.55E+06	1.66E-02	0.7	10.7
L1.GDF/L3.NY/H3.AL	9.00E+05	2.19E-02	0.5	24.3
L1.GDF/L3.NY/H3.AP	1.36E+06	7.02E-03	1.6	5.2
L1.GDF/L3.NY/H3.AQ	1.18E+06	1.36E-03	8.5	1.2
L3.KW/P6E01	7.63E+05	2.57E-03	4.5	3.4
L3.PY/P6E01	8.55E+05	2.93E-03	3.9	3.4
L3.NY/P6E01	1.01E+06	2.87E-03	4.0	2.8

Antibody	ka (1/Ms) cyBCMA @ 37°C	kd (1/s) cyBCMA @37°C	T ½ (min) to cyBCMA @37°C	KD (nM) to cyBCMA @ 37°C
L3.PY/L1.PS/P6E01	2.17E+06	6.06E-03	1.91	2.79
L3.PY/L1.AH/P6E01	2.16E+06	5.72E-03	2.02	2.65
L3.PY/L1.FF/P6E01	2.45E+06	5.91E-03	1.96	2.41
L3.PY/L1.PH/P6E01	2.17E+06	7.89E-03	1.46	3.63
L3.PY/L3.KY/P6E01	2.27E+06	5.02E-03	2.30	2.21
L3.PY/L3.KF/P6E01	2.39E+06	8.30E-03	1.39	3.48
L3.PY/H2.QR	2.18E+06	6.58E-03	1.76	3.02
L3.PY/H2.DY	2.24E+06	6.18E-03	1.87	2.76
L3.PY/H2.YQ	2.46E+06	6.21E-03	1.86	2.53
L3.PY/H2.LT	2.09E+06	7.57E-03	1.53	3.63
L3.PY/H2.HA	1.99E+06	7.55E-03	1.53	3.79
L3.PY/H2.QL	2.05E+06	1.26E-02	0.91	6.16
L3.PY/H3.YA	2.87E+06	5.40E-03	2.14	1.88
L3.PY/H3.AE	2.82E+06	5.04E-03	2.29	1.79
L3.PY/H3.AQ	2.77E+06	5.39E-03	2.14	1.94
L3.PY/H3.TAQ	2.57E+06	4.37E-03	2.64	1.70
L3.PY/P6E01	2.20E+06	1.31E-02	0.88	5.96
L3.PY/L1.PS/H2.QR	5.25E+05	6.70E-04	17.23	1.28
L3.PY/L1.PS/H2.DY	1.90E+06	3.78E-03	3.06	1.99
L3.PY/L1.PS/H2.YQ	2.00E+06	3.74E-03	3.09	1.87
L3.PY/L1.PS/H2.LT	2.17E+06	4.11E-03	2.81	1.89
L3.PY/L1.PS/H2.HA	1.45E+06	2.69E-03	4.30	1.86
L3.PY/L1.PS/H2.QL	6.57E+05	6.36E-04	18.17	0.97
L3.PY/L1.PS/H3.YA	1.77E+06	9.98E-03	1.16	5.65
L3.PY/L1.PS/H3.AE	2.46E+06	4.13E-03	2.80	1.68
L3.PY/L1.PS/H3.AQ	2.52E+06	4.33E-03	2.67	1.72
L3.PY/L1.PS/H3.TAQ	2.58E+06	5.52E-03	2.09	2.14
L3.PY/L1.AH/H2.QR	2.20E+06	4.91E-03	2.35	2.23
L3.PY/L1.AH/H2.DY	2.32E+06	4.51E-03	2.56	1.95
L3.PY/L1.AH/H2.YQ	1.58E+06	4.31E-03	2.68	2.74
L3.PY/L1.AH/H2.LT	2.19E+06	2.96E-03	3.91	1.35
L3.PY/L1.AH/H2.HA	2.58E+06	4.39E-03	2.63	1.70
L3.PY/L1.AH/H2.QL	2.62E+06	9.55E-03	1.21	3.65
L3.PY/L1.AH/H3.YA	2.37E+06	5.26E-03	2.20	2.22

L3.PY/L1.AH/H3.AE	2.25E+06	3.56E-03	3.25	1.58
L3.PY/L1.AH/H3.AQ	2.24E+06	3.99E-03	2.90	1.78
L3.PY/L1.AH/H3.TAQ	2.28E+06	3.02E-03	3.83	1.32
L3.PY/L1.FF/H2.QR	2.55E+06	4.21E-03	2.75	1.65
L3.PY/L1.FF/H2.DY	2.66E+06	5.00E-03	2.31	1.88
L3.PY/L1.FF/H2.YQ	2.19E+06	3.26E-03	3.55	1.49
L3.PY/L1.FF/H2.LT	2.19E+06	3.41E-03	3.38	1.56
L3.PY/L1.FF/H2.HA	2.33E+06	4.17E-03	2.77	1.79
L3.PY/L1.FF/H2.QL	2.36E+06	4.49E-03	2.57	1.91
L3.PY/L1.FF/H3.YA	2.46E+06	4.16E-03	2.77	1.69
L3.PY/L1.FF/H3.AE	2.85E+06	5.01E-03	2.31	1.76
L3.PY/L1.FF/H3.AQ	2.18E+06	3.29E-03	3.51	1.51
L3.PY/L1.FF/H3.TAQ	2.32E+06	3.76E-03	3.07	1.62
L3.PY/L1.PH/H2.QR	2.42E+06	4.36E-03	2.65	1.80
L3.PY/L1.PH/H2.HA	1.61E+06	5.53E-03	2.09	3.44
L3.PY/L1.PH/H3.AE	2.61E+06	2.02E-03	5.72	0.77
L3.PY/L1.PH/H3.AQ	2.28E+06	3.41E-03	3.39	1.50
L3.PY/L1.PH/H3.TAQ	2.51E+06	3.20E-03	3.61	1.28
L3.PY/L3.KY/H2.QR	2.05E+06	7.74E-03	1.49	3.78
L3.PY/L3.KY/H2.DY	1.96E+06	2.43E-03	4.75	1.24
L3.PY/L3.KY/H2.YQ	1.27E+06	2.58E-03	4.47	2.04
L3.PY/L3.KY/H2.LT	1.82E+06	2.32E-03	4.98	1.27
L3.PY/L3.KY/H2.HA	2.28E+06	3.18E-03	3.63	1.40
L3.PY/L3.KY/H2.QL	2.75E+06	4.09E-03	2.83	1.49
L3.PY/L3.KY/H3.YA	1.84E+06	4.28E-03	2.70	2.33
L3.PY/L3.KY/H3.TAQ	1.81E+06	1.92E-03	6.03	1.06
L3.PY/L3.KF/H2.DY	2.08E+06	3.68E-03	3.14	1.77
L3.PY/L3.KF/H2.YQ	1.41E+06	5.01E-03	2.30	3.55
L3.PY/L3.KF/H2.LT	1.91E+06	4.13E-03	2.80	2.16
L3.PY/L3.KF/H2.QL	1.42E+06	3.10E-03	3.73	2.18
L3.PY/L3.KF/H3.YA	2.10E+06	7.96E-03	1.45	3.78
L3.PY/L3.KF/H3.AE	1.85E+06	5.64E-03	2.05	3.05
L3.PY/L3.KF/H3.AQ	2.55E+06	2.38E-03	4.85	0.93
L3.PY/L3.KF/H3.TAQ	2.01E+06	1.91E-03	6.05	0.95

Table 4C

	Human BCMA			Cyno BCMA		
Antibody	ka (1/Ms)	kd (1/s)	KD (pM)	ka (1/Ms)	kd (1/s)	KD (pM)
P5A2_VHVL (**P5A**)	6.96E+06	3.87E-02	5567	1.61E+06	1.64E-02	10230
A02_Rd4_0.6nM_C06	3.49E+06	7.37E-05	21	1.81E+06	1.05E-04	58
A02_Rd4_0.6nM_C09	5.50E+06	9.75E-05	18	2.13E+06	1.74E-04	82
A02_Rd4_6nM_C16 (**P5AC16**)	1.56E+06	1.41E-04	90	1.34E+06	1.58E-04	118

Antibody	Human BCMA			Cyno BCMA		
	ka (1/Ms)	kd (1/s)	KD (pM)	ka (1/Ms)	kd (1/s)	KD (pM)
A02_Rd4_6nM_C03	1.69E+06	1.26E-04	75	1.17E+06	1.85E-04	158
A02_Rd4_6nM_C01	3.11E+06	9.20E-05	30	1.45E+06	5.83E-04	401
A02_Rd4_6nM_C26	4.26E+06	1.39E-04	33	2.21E+06	4.48E-04	203
A02_Rd4_6nM_C25	2.75E+06	1.80E-04	65	1.50E+06	3.30E-04	220
A02_Rd4_6nM_C22	3.38E+06	1.82E-04	54	1.84E+06	3.24E-04	176
A02_Rd4_6nM_C19	3.00E+06	1.48E-04	49	2.54E+06	6.61E-04	260
A02_Rd4_0.6nM_C03	4.27E+06	1.82E-04	43	2.12E+06	4.26E-04	201
A02_Rd4_6nM_C07	1.48E+06	1.89E-04	128	6.91E+05	7.86E-04	1138
A02_Rd4_6nM_C23	1.22E+07	2.55E-04	21	2.63E+06	4.14E-04	157
A02_Rd4_0.6nM_C18	4.73E+06	2.29E-04	48	3.24E+06	6.39E-04	197
A02_Rd4_6nM_C10	4.51E+06	3.15E-04	70	1.90E+06	8.98E-04	472
A02_Rd4_6nM_C05	3.10E+06	3.08E-04	99	1.36E+06	1.29E-03	950
A02_Rd4_0.6nM_C10	2.30E+06	2.96E-04	129	8.83E+05	1.63E-03	1842
A02_Rd4_6nM_C04	4.47E+06	6.03E-04	135	2.18E+06	8.31E-04	381
A02_Rd4_0.6nM_C26	7.26E+06	4.43E-04	61	2.71E+06	2.56E-03	941
A02_Rd4_0.6nM_C13	8.53E+06	5.66E-04	66	2.29E+06	1.28E-03	560
A02_Rd4_0.6nM_C01 (P5AC1)	4.74E+06	9.15E-04	193	2.39E+06	1.57E-03	655
A02_Rd4_6nM_C08	3.92E+06	7.38E-04	188	2.23E+06	1.13E-02	5072
P5C1_VHVL (PC1)	1.16E+07	6.92E-02	5986	3.53E+06	5.38E-02	15231
C01_Rd4_6nM_C24	7.47E+06	3.48E-03	467	3.17E+06	8.91E-04	281
C01_Rd4_6nM_C26	1.50E+07	1.36E-03	90	4.75E+06	1.99E-03	419
C01_Rd4_6nM_C02	1.61E+07	1.44E-03	89	5.12E+06	2.18E-03	426
C01_Rd4_6nM_C10	1.31E+07	2.12E-03	162	4.44E+06	2.19E-03	493
C01_Rd4_0.6nM_C27	1.23E+07	3.74E-03	303	3.34E+06	2.85E-03	852
C01_Rd4_6nM_C20	6.02E+06	2.76E-03	459	3.60E+06	6.25E-03	1737
C01_Rd4_6nM_C12	1.21E+07	6.49E-03	535	4.51E+06	3.70E-03	820
C01_Rd4_0.6nM_C16	1.55E+07	6.30E-03	407	4.95E+06	4.64E-03	939
C01_Rd4_0.6nM_C09	1.51E+07	8.25E-03	545	5.28E+06	9.36E-03	1773

Antibody	Human BCMA			Cyno BCMA		
	ka (1/Ms)	kd (1/s)	KD (pM)	ka (1/Ms)	kd (1/s)	KD (pM)
C01_Rd4_6nM_C09	1.58E+07	1.28E-02	811	3.73E+06	8.68E-03	2328
C01_Rd4_0.6nM_C03	1.55E+07	1.50E-02	964	4.72E+06	1.19E-02	2528
C01_Rd4_0.6nM_C06	1.82E+07	1.54E-02	847	6.22E+06	1.21E-02	1948
C01_Rd4_6nM_C04	2.33E+07	4.97E-02	2134	6.34E+06	3.27E-02	5156
COMBO_Rd4_0.6nM_C22	1.97E+06	7.15E-05	36	1.34E+06	6.66E-05	50
COMBO_Rd4_6nM_C21	1.17E+07	7.34E-05	6	3.17E+06	2.48E-04	78
COMBO_Rd4_6nM_C10	5.47E+06	9.72E-05	18	1.52E+06	1.60E-04	105
COMBO_Rd4_0.6nM_C04	1.07E+07	1.58E-04	15	3.52E+06	1.37E-04	39
COMBO_Rd4_6nM_C25	7.98E+06	1.13E-04	14	2.85E+06	2.26E-04	79
COMBO_Rd4_0.6nM_C21	1.34E+07	1.15E-04	9	3.63E+06	3.04E-04	84
COMBO_Rd4_6nM_C11	6.74E+06	1.24E-04	18	2.64E+06	4.12E-04	156
COMBO_Rd4_0.6nM_C20	7.65E+06	1.46E-04	19	3.09E+06	2.84E-04	92
COMBO_Rd4_6nM_C09	8.85E+06	1.43E-04	16	2.37E+06	3.18E-04	134
COMBO_Rd4_6nM_C08	8.99E+06	1.69E-04	19	3.06E+06	4.28E-04	140
COMBO_Rd4_0.6nM_C19	7.86E+06	1.55E-04	20	2.92E+06	9.79E-04	336
COMBO_Rd4_0.6nM_C02	8.57E+06	1.85E-04	22	3.01E+06	4.94E-04	164
COMBO_Rd4_0.6nM_C23	7.39E+06	2.10E-04	28	2.81E+06	5.31E-04	189
COMBO_Rd4_0.6nM_C29	1.47E+07	2.77E-04	19	4.00E+06	3.36E-04	84
COMBO_Rd4_0.6nM_C09	1.04E+07	3.19E-04	31	3.77E+06	3.46E-04	92
COMBO_Rd4_6nM_C12 (**PC1C12**)	1.38E+07	2.70E-04	20	3.29E+06	4.86E-04	148
COMBO_Rd4_0.6nM_C30	4.35E+06	2.82E-04	65	1.68E+06	8.08E-04	481
COMBO_Rd4_0.6nM_C14	8.66E+06	3.28E-04	38	3.48E+06	6.45E-04	185
COMBO_Rd4_6nM_C07	1.05E+07	3.71E-04	35	3.94E+06	9.34E-04	237
COMBO_Rd4_6nM_C02	1.05E+06	4.43E-04	422	7.95E+05	1.36E-03	1714
COMBO_Rd4_0.6nM_C05	4.32E+06	4.97E-04	115	1.94E+06	1.72E-03	886
COMBO_Rd4_0.6nM_C17	8.68E+06	8.01E-04	92	3.06E+06	1.01E-03	330
COMBO_Rd4_6nM_C22 (**COM22**)	3.03E+06	7.75E-04	256	1.70E+06	1.65E-03	972

Antibody	Human BCMA			Cyno BCMA		
	ka (1/Ms)	kd (1/s)	KD (pM)	ka (1/Ms)	kd (1/s)	KD (pM)
COMBO_Rd4_0.6nM_C11	5.11E+06	1.06E-03	207	2.20E+06	4.23E-03	1924

Example 2: BCMA Specific CAR-T Cells

This example demonstrates functional activity of BCMA specific CAR-T cells against BCMA positive (BCMA+) tumor cells.

Among all the BCMA specific CAR molecules generated, eight were selected for further activity tests based on affinity to BCMA, cross-reactivity to human BCMA and cyno BCMA, and epitope. The CAR molecules tested included: P5A, P5AC1, P5AC16, PC1, PC1C12, COM22, P6DY, and P6AP. Three different architectures were designed: version 1 (v1) comprises an FcγRIIIα hinge, version 2 (v2) comprises a CD8α hinge, and version 3 (v3) comprises and IgG1 hinge. The chimeric antigen receptors (CARs) shown in Table 5 were prepared and used and assessed for their degranulation activity towards BCMA+ cells. Degranulation activity was determined upon transient expression of each CAR in human T cells.

Table 5: Exemplary BCMA specific CARs

CAR	CAR Amino Acid Sequence	Components
P5A-V1	MALPVTALLLPLALLLHAARPEVQLLESGGGLVQPGGSLRLSCA ASGFTFSSYAMNWVRQAPGKGLEWVSAISDSGGSTYYADSVK GRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARYWPMDIWGQ GTLVTVSSGGGGSGGGGSGGGGSEIVLTQSPGTLSLSPGERATL SCRASQSVSSSYLAWYQQKPGQAPRLLMYDASIRATGIPDRFS GSGSGTDFTLTISRLEPEDFAVYYCQQYGSWPLTFGQGTKVEIK GLAVSTISSFFPPGYQIYIWAPLAGTCGVLLLSLVITLYCKRGRKKL LYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSAD APAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPR RKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQ	CD8α signal peptide; P5A2_VHVL VH (Table 1 SEQ ID NO: 33); GS linker; P5A2_VHVL VL (SEQ ID NO: 34); FcγRIIIα hinge; CD8α TM domain; 41BB ISD;

CAR	CAR Amino Acid Sequence	Components
	GLSTATKDTYDALHMQALPPR (SEQ ID NO: 343)	CD3ζ ISD
P5A-V2	MALPVTALLLPLALLLHAARPEVQLLESGGGLVQPGGSLRLSCA ASGFTFSSYAMNWVRQAPGKGLEWVSAISDSGGSTYYADSVK GRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARYWPMDIWGQ GTLVTVSSGGGGSGGGGSGGGGSEIVLTQSPGTLSLSPGERATL SCRASQSVSSSYLAWYQQKPGQAPRLLMYDASIRATGIPDRFS GSGSGTDFTLTISRLEPEDFAVYYCQQYGSWPLTFGQGTKVEIK TTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFAC DIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQT TQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLY NELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQ KDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALH MQALPPR(SEQ ID NO: 344)	CD8α signal peptide; P5A2_VHVL VH; GS linker; P5A2_VHVL VL; CD8α hinge; CD8α TM domain; 41BB ISD; CD3ζ ISD
P5A-V3	MALPVTALLLPLALLLHAARPEVQLLESGGGLVQPGGSLRLSCA ASGFTFSSYAMNWVRQAPGKGLEWVSAISDSGGSTYYADSVK GRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARYWPMDIWGQ GTLVTVSSGGGGSGGGGSGGGGSEIVLTQSPGTLSLSPGERATL SCRASQSVSSSYLAWYQQKPGQAPRLLMYDASIRATGIPDRFS GSGSGTDFTLTISRLEPEDFAVYYCQQYGSWPLTFGQGTKVEIK EPKSPDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMIARTPEVT CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV VSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREP QVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN NYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEAL HNHYTQKSLSLSPGKIYIWAPLAGTCGVLLLSLVITLYCKRGRKKL LYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSAD APAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPR	CD8α signal peptide; P5A2_VHVL VH ; GS linker; P5A2_VHVL VL; IgG1 hinge; CD8α TM domain; 41BB ISD; CD3ζ ISD

CAR	CAR Amino Acid Sequence	Components
	RKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQ GLSTATKDTYDALHMQALPPR(SEQ ID NO: 345)	
P5AC1-V1	MALPVTALLLPLALLLHAARPEVQLLESGGGLVQPGGSLRLSCA ASGFTFSSYAMNWVRQAPGKGLEWVSAILsSGGSTYYADSVK GRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARYWPMDIWGQ GTLVTVSSGGGGSGGGGSGGGGSEIVLTQSPGTLSLSPGERATL SCRGGQSVSSSYLAWYQQKPGQAPRLLMYDASIRATGIPDRFS GSGSGTDFTLTISRLEPEDFAVYYCQQYQSWPLTFGQGTKVEIK GLAVSTISSFFPPGYQIYIWAPLAGTCGVLLLSLVITLYCKRGRKKL LYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSAD APAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPR RKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQ GLSTATKDTYDALHMQALPPR (SEQ ID NO: 346)	CD8α signal peptide; A02_Rd4_0.6nM_C01 VH (SEQ ID NO: 72); GS linker; A02_Rd4_0.6nM_C01 VL (SEQ ID NO: 73); FcγRIIIα hinge; CD8α TM domain; 41BB ISD; CD3ζ ISD
P5AC1-V2	MALPVTALLLPLALLLHAARPEVQLLESGGGLVQPGGSLRLSCA ASGFTFSSYAMNWVRQAPGKGLEWVSAILsSGGSTYYADSVK GRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARYWPMDIWGQ GTLVTVSSGGGGSGGGGSGGGGSEIVLTQSPGTLSLSPGERATL SCRGGQSVSSSYLAWYQQKPGQAPRLLMYDASIRATGIPDRFS GSGSGTDFTLTISRLEPEDFAVYYCQQYQSWPLTFGQGTKVEIK TTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFAC DIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQT TQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLY NELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQ KDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALH MQALPPR (SEQ ID NO: 347)	CD8α signal peptide; A02_Rd4_0.6nM_C01 VH; GS linker; A02_Rd4_0.6nM_C01 VL; CD8α hinge; CD8α TM domain; 41BB ISD; CD3ζ ISD

CAR	CAR Amino Acid Sequence	Components
P5AC1-V2.1	MALPVTALLLPLALLLHAARPEVQLLESGGGLVQPGGSLRLSCA ASGFTFSSYAMNWVRQAPGKGLEWVSAILSSGGSTYYADSVK GRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARYWPMDIWGQ GTLVTVSSGGGGSGGGGSGGGGSEIVLTQSPGTLSLSPGERATL SCRGGQSVSSSYLAWYQQKPGQAPRLLMYDASIRATGIPDRFS GSGSGTDFTLTISRLEPEDFAVYYCQQYQSWPLTFGQGTKVEIK GSGGGGSCPYSNPSLCSGGGGSCPYSNPSLCSGGGGSTTTPAP RPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWA PLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEED GCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLG RREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMA EAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALP PR (SEQ ID NO: 396)	CD8α signal peptide; A02_Rd4_0.6nM_C01 VH; GS linker; A02_Rd4_0.6nM_C01 VL; rituximab epitope; CD8α hinge; CD8α TM domain; 41BB ISD; CD3ζ ISD
P5AC1-V3	MALPVTALLLPLALLLHAARPEVQLLESGGGLVQPGGSLRLSCA ASGFTFSSYAMNWVRQAPGKGLEWVSAILSSGGSTYYADSVK GRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARYWPMDIWGQ GTLVTVSSGGGGSGGGGSGGGGSEIVLTQSPGTLSLSPGERATL SCRGGQSVSSSYLAWYQQKPGQAPRLLMYDASIRATGIPDRFS GSGSGTDFTLTISRLEPEDFAVYYCQQYQSWPLTFGQGTKVEIK EPKSPDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMIARTPEVT CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV VSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREP QVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN NYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEAL HNHYTQKSLSLSPGKIYIWAPLAGTCGVLLLSLVITLYCKRGRKKL LYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSAD APAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPR	CD8α signal peptide; A02_Rd4_0.6nM_C01 VH; GS linker; A02_Rd4_0.6nM_C01 VL; IgG1 hinge; CD8α TM domain; 41BB ISD; CD3ζ ISD

CAR	CAR Amino Acid Sequence	Components
	RKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQ GLSTATKDTYDALHMQALPPR (SEQ ID NO: 348)	
P5AC16-V1	MALPVTALLLPLALLLHAARPEVQLLESGGGLVQPGGSLRLSCA ASGFTFSSYAMNWVRQAPGKGLEWVSAISdFGGSTYYADSVK GRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARYWPMDIWGQ GTLVTVSSGGGGSGGGGSGGGGSEIVLTQSPGTLSLSPGERATL SCRASQSVSDIYLAWYQQKPGQAPRLLMYDASIRATGIPDRFS GSGSGTDFTLTISRLEPEDFAVYYCQQYQTWPLTFGQGTKVEIK GLAVSTISSFFPPGYQIYIWAPLAGTCGVLLLSLVITLYCKRGRKKL LYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSAD APAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPR RKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQ GLSTATKDTYDALHMQALPPR (SEQ ID NO: 349)	CD8α signal peptide; A02_Rd4_6nM_C16 VH (SEQ ID NO: 39); GS linker; A02_Rd4_6nM_C16 VL (SEQ ID NO: 40); FcγRIIIα hinge; CD8α TM domain; 41BB ISD; CD3ζ ISD;
P5AC16-V2	MALPVTALLLPLALLLHAARPEVQLLESGGGLVQPGGSLRLSCA ASGFTFSSYAMNWVRQAPGKGLEWVSAISdFGGSTYYADSVK GRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARYWPMDIWGQ GTLVTVSSGGGGSGGGGSGGGGSEIVLTQSPGTLSLSPGERATL SCRASQSVSDIYLAWYQQKPGQAPRLLMYDASIRATGIPDRFS GSGSGTDFTLTISRLEPEDFAVYYCQQYQTWPLTFGQGTKVEIK TTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFAC DIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQT TQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLY NELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQ KDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALH MQALPPR (SEQ ID NO: 350)	CD8α signal peptide; A02_Rd4_6nM_C16 VH; GS linker; A02_Rd4_6nM_C16 VL; CD8α hinge; CD8α TM domain; 41BB ISD; CD3ζ ISD

CAR	CAR Amino Acid Sequence	Components
P5AC16-V3	MALPVTALLLPLALLLHAARPEVQLLESGGGLVQPGGSLRLSCA ASGFTFSSYAMNWVRQAPGKGLEWVSAISdFGGSTYYADSVK GRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARYWPMDIWGQ GTLVTVSSGGGGSGGGGSGGGGSEIVLTQSPGTLSLSPGERATL SCRASQSVSDIYLAWYQQKPGQAPRLLMYDASIRATGIPDRFS GSGSGTDFTLTISRLEPEDFAVYYCQQYQTWPLTFGQGTKVEIK EPKSPDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMIARTPEVT CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV VSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREP QVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN NYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEAL HNHYTQKSLSLSPGKIYIWAPLAGTCGVLLLSLVITLYCKRGRKKL LYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSAD APAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPR RKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQ GLSTATKDTYDALHMQALPPR (SEQ ID NO: 351)	CD8α signal peptide; A02_Rd4_6nM_C16 VH; GS linker; A02_Rd4_6nM_C16 VL; IgG1 hinge; CD8α TM domain; 41BB ISD; CD3ζ ISD
PC1-V1	MALPVTALLLPLALLLHAARPEVQLLESGGGLVQPGGSLRLSCA ASGFTFSSYPMSWVRQAPGKGLEWVSAIGGSGGSTYYADSVK GRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARYWPMDSWG QGTLVTVSSGGGGSGGGGSGGGGSEIVLTQSPGTLSLSPGERA TLSCRASQSVSSTYLAWYQQKPGQAPRLLIYDASSRAPGIPDRF SGSGSGTDFTLTISRLEPEDFAVYYCQQYSTSPLTFGQGTKVEIK GLAVSTISSFFPPGYQIYIWAPLAGTCGVLLLSLVITLYCKRGRKKL LYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSAD APAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPR RKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQ GLSTATKDTYDALHMQALPPR (SEQ ID NO: 352)	CD8α signal peptide; P5C1_VHVL VH (SEQ ID NO: 76); GS linker; P5C1_VHVL VL (SEQ ID NO: 77); FcγRIIIα hinge; CD8α TM domain; 41BB ISD;

CAR	CAR Amino Acid Sequence	Components
		CD3ζ ISD
PC1-V2	MALPVTALLLPLALLLHAARPEVQLLESGGGLVQPGGSLRLSCA ASGFTFSSYPMSWVRQAPGKGLEWVSAIGGSGGSTYYADSVK GRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARYWPMDSWG QGTLVTVSSGGGGSGGGGSGGGGSEIVLTQSPGTLSLSPGERA TLSCRASQSVSSTYLAWYQQKPGQAPRLLIYDASSRAPGIPDRF SGSGSGTDFTLTISRLEPEDFAVYYCQQYSTSPLTFGQGTKVEIK TTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFAC DIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQT TQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLY NELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQ KDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALH MQALPPR (SEQ ID NO: 353)	CD8α signal peptide; P5C1_VHVL VH; GS linker; P5C1_VHVL VL; CD8α hinge; CD8α TM domain; 41BB ISD; CD3ζ ISD
PC1-V3	MALPVTALLLPLALLLHAARPEVQLLESGGGLVQPGGSLRLSCA ASGFTFSSYPMSWVRQAPGKGLEWVSAIGGSGGSTYYADSVK GRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARYWPMDSWG QGTLVTVSSGGGGSGGGGSGGGGSEIVLTQSPGTLSLSPGERA TLSCRASQSVSSTYLAWYQQKPGQAPRLLIYDASSRAPGIPDRF SGSGSGTDFTLTISRLEPEDFAVYYCQQYSTSPLTFGQGTKVEIK EPKSPDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMIARTPEVT CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV VSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREP QVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN NYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEAL HNHYTQKSLSLSPGKIYIWAPLAGTCGVLLLSLVITLYCKRGRKKL LYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSAD APAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPR	CD8α signal peptide; P5C1_VHVL VH; GS linker; P5C1_VHVL VL; IgG1 hinge; CD8α TM domain; 41BB ISD; CD3ζ ISD

CAR	CAR Amino Acid Sequence	Components
	RKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQ GLSTATKDTYDALHMQALPPR (SEQ ID NO: 354)	
PC1C12-V1	MALPVTALLLPLALLLHAARPEVQLLESGGGLVQPGGSLRLSCA ASGFTFSSYPMSWVRQAPGKGLEWVSAIGgSGGWSYYADSVK GRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARYWPMDSWG QGTLVTVSSGGGGSGGGGSGGGGSEIVLTQSPGTLSLSPGERA TLSCWLSQSVSSTYLAWYQQKPGQAPRLLIYDASSRAPGIPDRF SGSGSGTDFTLTISRLEPEDFAVYYCQQYSEWPLTFGQGTKVEIK GLAVSTISSFFPPGYQIYIWAPLAGTCGVLLLSLVITLYCKRGRKKL LYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSAD APAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPR RKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQ GLSTATKDTYDALHMQALPPR (SEQ ID NO: 355)	CD8α signal peptide; C01_Rd4_6nM_C12 VH (SEQ ID NO: 83); GS linker; C01_Rd4_6nM_C12 VL (SEQ ID NO: 84); FcγRIIIα hinge; CD8α TM domain; 41BB ISD; CD3ζ ISD
PC1C12-V2	MALPVTALLLPLALLLHAARPEVQLLESGGGLVQPGGSLRLSCA ASGFTFSSYPMSWVRQAPGKGLEWVSAIGgSGGWSYYADSVK GRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARYWPMDSWG QGTLVTVSSGGGGSGGGGSGGGGSEIVLTQSPGTLSLSPGERA TLSCWLSQSVSSTYLAWYQQKPGQAPRLLIYDASSRAPGIPDRF SGSGSGTDFTLTISRLEPEDFAVYYCQQYSEWPLTFGQGTKVEIK TTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFAC DIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQT TQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLY NELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQ KDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALH MQALPPR (SEQ ID NO: 356)	CD8α signal peptide; C01_Rd4_6nM_C12 VH; GS linker; C01_Rd4_6nM_C12 VL; CD8α hinge; CD8α TM domain; 41BB ISD; CD3ζ ISD

CAR	CAR Amino Acid Sequence	Components
PC1C12-V3	MALPVTALLLPLALLLHAARPEVQLLESGGGLVQPGGSLRLSCA ASGFTFSSYPMSWVRQAPGKGLEWVSAIGgSGGWSYYADSVK GRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARYWPMDSWG QGTLVTVSSGGGGSGGGGSGGGGSEIVLTQSPGTLSLSPGERA TLSCWLSQSVSSTYLAWYQQKPGQAPRLLIYDASSRAPGIPDRF SGSGSGTDFTLTISRLEPEDFAVYYCQQYSEWPLTFGQGTKVEIK EPKSPDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMIARTPEVT CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV VSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREP QVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN NYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEAL HNHYTQKSLSLSPGKIYIWAPLAGTCGVLLLSLVITLYCKRGRKKL LYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSAD APAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPR RKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQ GLSTATKDTYDALHMQALPPR (SEQ ID NO: 357)	CD8α signal peptide; C01_Rd4_6nM_C12 VH; GS linker; C01_Rd4_6nM_C12 VL; IgG1 hinge; CD8α TM domain; 41BB ISD; CD3ζ ISD
COM22-V1	MALPVTALLLPLALLLHAARPEVQLLESGGGLVQPGGSLRLSCA ASGFTFSSYAMNWVRQAPGKGLEWVSAISdSGGSRWYADSV KGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCTRYWPMDIWG QGTLVTVSSGGGGSGGGGSGGGGSEIVLTQSPGTLSLSPGERA TLSCRASVRVSSTYLAWYQQKPGQAPRLLMYDASIRATGIPDRF SGSGSGTDFTLTISRLEPEDFAVYYCQQYMKWPLTFGQGTKVEI KGLAVSTISSFFPPGYQIYIWAPLAGTCGVLLLSLVITLYCKRGRK KLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRS ADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGK PRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLY QGLSTATKDTYDALHMQALPPR (SEQ ID NO: 358)	CD8α signal peptide; COMBO_Rd4_0.6nM_C 22 VH (SEQ ID NO: 92); GS linker; COMBO_Rd4_0.6nM_C 22 VL (SEQ ID NO: 93); FcγRIIIα hinge; CD8α TM domain; 41BB ISD;

CAR	CAR Amino Acid Sequence	Components
		CD3ζ ISD
COM22-V2	MALPVTALLLPLALLLHAARPEVQLLESGGGLVQPGGSLRLSCA ASGFTFSSYAMNWVRQAPGKGLEWVSAISdSGGSRWYADSV KGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCTRYWPMDIWG QGTLVTVSSGGGGSGGGGSGGGGSEIVLTQSPGTLSLSPGERA TLSCRASVRVSSTYLAWYQQKPGQAPRLLMYDASIRATGIPDRF SGSGSGTDFTLTISRLEPEDFAVYYCQQYMKWPLTFGQGTKVEI KTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFA CDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPV QTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQ LYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNE LQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDA LHMQALPPR (SEQ ID NO: 359)	CD8α signal peptide; COMBO_Rd4_0.6nM_C 22 VH; GS linker; COMBO_Rd4_0.6nM_C 22 VL; CD8α hinge; CD8α TM domain; 41BB ISD; CD3ζ ISD
COM22-V3	MALPVTALLLPLALLLHAARPEVQLLESGGGLVQPGGSLRLSCA ASGFTFSSYAMNWVRQAPGKGLEWVSAISdSGGSRWYADSV KGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCTRYWPMDIWG QGTLVTVSSGGGGSGGGGSGGGGSEIVLTQSPGTLSLSPGERA TLSCRASVRVSSTYLAWYQQKPGQAPRLLMYDASIRATGIPDRF SGSGSGTDFTLTISRLEPEDFAVYYCQQYMKWPLTFGQGTKVEI KEPKSPDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMIARTPEV TCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYR VVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPRE PQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPE NNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHE ALHNHYTQKSLSLSPGKIYIWAPLAGTCGVLLLSLVITLYCKRGRK KLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRS ADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGK	CD8α signal peptide; COMBO_Rd4_0.6nM_C 22 VH; GS linker; COMBO_Rd4_0.6nM_C 22 VL; IgG1 hinge; CD8α TM domain; 41BB ISD; CD3ζ ISD

CAR	CAR Amino Acid Sequence	Components
	PRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLY QGLSTATKDTYDALHMQALPPR (SEQ ID NO: 360)	
P6DY-V1	MALPVTALLLPLALLLHAARPEVQLLESGGGLVQPGGSLRLSCA ASGFTFGSYAMTWVRQAPGKGLEWVSAIDYSGGNTFYADSVK GRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARVSPIASGMDY WGQGTLVTVSSGGGGSGGGGSGGGGSEIVLTQSPGTLSLSPG ERATLSCRASQSVSSSYPSWYQQKPGQAPRLLIYGASSRATGIP DRFSGSGSGTDFTLTISRLEPEDFAVYYCQHYPYPPSFTFGQGTK VEIKGLAVSTISSFFPPGYQIYIWAPLAGTCGVLLLSLVITLYCKRG RKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFS RSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMG GKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHD GLYQGLSTATKDTYDALHMQ (SEQ ID NO: 361)	CD8α signal peptide; L3.PY/H2.DY VH (SEQ ID NO: 25); GS linker; L3.PY/L1.PS/P6E01 VL (SEQ ID NO: 18); FcγRIIIα hinge; CD8α TM domain; 41BB ISD; CD3ζ ISD
P6DY-V2	MALPVTALLLPLALLLHAARPEVQLLESGGGLVQPGGSLRLSCA ASGFTFGSYAMTWVRQAPGKGLEWVSAIDYSGGNTFYADSVK GRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARVSPIASGMDY WGQGTLVTVSSGGGGSGGGGSGGGGSEIVLTQSPGTLSLSPG ERATLSCRASQSVSSSYPSWYQQKPGQAPRLLIYGASSRATGIP DRFSGSGSGTDFTLTISRLEPEDFAVYYCQHYPYPPSFTFGQGTK VEIKTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLD FACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRP VQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQN QLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYN ELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYD ALHMQALPPR (SEQ ID NO: 362)	CD8α signal peptide; L3.PY/H2.DY VH; GS linker; L3.PY/L1.PS/P6E01 VL; CD8α hinge; CD8α TM domain; 41BB ISD; CD3ζ ISD

CAR	CAR Amino Acid Sequence	Components
P6DY-V3	MALPVTALLLPLALLLHAARPEVQLLESGGGLVQPGGSLRLSCA ASGFTFGSYAMTWVRQAPGKGLEWVSAIDYSGGNTFYADSVK GRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARVSPIASGMDY WGQGTLVTVSSGGGGSGGGGSGGGGSEIVLTQSPGTLSLSPG ERATLSCRASQSVSSSYPSWYQQKPGQAPRLLIYGASSRATGIP DRFSGSGSGTDFTLTISRLEPEDFAVYYCQHYPYPPSFTFGQGTK VEIKEPKSPDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMIARTP EVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST YRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQP REPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQP ENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMH EALHNHYTQKSLSLSPGKIYIWAPLAGTCGVLLLSLVITLYCKRGR KKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSR SADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGG KPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGL YQGLSTATKDTYDALHMQALPPR (SEQ ID NO: 363)	CD8α signal peptide; L3.PY/H2.DY VH; GS linker; L3.PY/L1.PS/P6E01 VL; IgG1 hinge; CD8α TM domain; 41BB ISD; CD3ζ ISD
P6AP-V1	MALPVTALLLPLALLLHAARPEVQLLESGGGLVQPGGSLRLSCA ASGFTFGSYAMTWVRQAPGKGLEWVSAISGSGGNTFYADSVK GRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARVSPIAAPMDY WGQGTLVTVSSGGGGSGGGGSGGGGSEIVLTQSPGTLSLSPG ERATLSCRASQLGSFYLAWYQQKPGQAPRLLIYGASSRATGIPD RFSGSGSGTDFTLTISRLEPEDFAVYYCQHYNYPPSFTFGQGTKV EIKGLAVSTISSFFPPGYQIYIWAPLAGTCGVLLLSLVITLYCKRGR KKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSR SADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGG KPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGL YQGLSTATKDTYDALHMQA (SEQ ID NO: 364)	CD8α signal peptide; P6AP-V1 VH (SEQ ID NO: 8); GS linker; P6AP-V1 VL (SEQ ID NO: 80) FcγRIIIα hinge; CD8α TM domain; 41BB ISD;

CAR	CAR Amino Acid Sequence	Components
		CD3ζ ISD
P6AP-V2	MALPVTALLLPLALLLHAARPEVQLLESGGGLVQPGGSLRLSCA ASGFTFGSYAMTWVRQAPGKGLEWVSAISGSGGNTFYADSVK GRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARVSPIAAPMDY WGQGTLVTVSSGGGGSGGGGSGGGGSEIVLTQSPGTLSLSPG ERATLSCRASQLGSFYLAWYQQKPGQAPRLLIYGASSRATGIPD RFSGSGSGTDFTLTISRLEPEDFAVYYCQHYNYPPSFTFGQGTKV EIKTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDF ACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPV QTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQ LYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNE LQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDA LHMQALPPR (SEQ ID NO: 365)	CD8α signal peptide; L1.LGF/L3.KW/H3.AP VH; GS linker; P6AP-V1 VL; CD8α hinge; CD8α TM domain; 41BB ISD; CD3ζ ISD
P6AP-V3	MALPVTALLLPLALLLHAARPEVQLLESGGGLVQPGGSLRLSCA ASGFTFGSYAMTWVRQAPGKGLEWVSAISGSGGNTFYADSVK GRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARVSPIAAPMDY WGQGTLVTVSSGGGGSGGGGSGGGGSEIVLTQSPGTLSLSPG ERATLSCRASQLGSFYLAWYQQKPGQAPRLLIYGASSRATGIPD RFSGSGSGTDFTLTISRLEPEDFAVYYCQHYNYPPSFTFGQGTKV EIKEPKSPDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMIARTPE VTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTY RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPR EPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPE NNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHE ALHNHYTQKSLSLSPGKIYIWAPLAGTCGVLLLSLVITLYCKRGRK KLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRS ADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGK	CD8α signal peptide; L1.LGF/L3.KW/H3.AP VH; GS linker; P6AP-V1 VL; IgG1 hinge; CD8α TM domain; 41BB ISD; CD3ζ ISD

CAR	CAR Amino Acid Sequence	Components
	PRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLY QGLSTATKDTYDALHMQALPPR (SEQ ID NO: 366)	

For the activity assays, T cells from thirteen healthy donors (Donors 1-13) were obtained. Briefly, the T cells were purified from buffy-coat samples and activated using CD3/CD28 beads. Cells were transiently transfected with mRNAs encoding the different CAR molecules at D11/12 after activation. CAR activity was assessed by measuring their degranulation capacity, the inteferon-γ (IFNγ) release, and the cytotoxic activity when co-cultured with (a) cells expressing BCMA (MM1S, KMS12BM, and L363), or (b) cells that do not express the BCMA protein (K562). Also included for each assay were mock transfected T cells (T cells in in buffer) to determine baseline activity of T cells that do not express a CAR.

CAR detection was done using a fusion protein in which the extracellular domain of the human BCMA protein was fused to a mouse IgG1 derived Fc fragment. Binding of the CAR at the cell surface with the BCMA portion of the fusion protein was detected with anti-Fc PE-conjugated antibody and analyzed by flow cytometry.

Materials and Methods

Primary T cell cultures

T cells were purified from Buffy coat samples provided by EFS (Etablissement Français du Sang, Paris, France) using Ficoll gradient density medium (Ficoll Paque PLUS / GE Healthcare Life Sciences). The PBMC layer was recovered and T cells were purified using a commercially available T cell enrichment kit (Stem Cell Technologies). Purified T cells were activated in X-Vivo™-15 medium (Lonza) supplemented with 20ng/mL Human IL-2 (Miltenyi Biotech), 5% Human Serum (Sera Laboratories), and Dynabeads Human T activator CD3/CD28 at a bead:cell ratio 1:1 (Life Technologies). After activation cells were grown and maintained in X-Vivo™-15 medium (Lonza) supplemented with 20ng/mL Human IL-2 (Miltenyi Biotec) and 5% Human Serum (Sera Laboratories)

CAR mRNA transfection

Transfections were done at Day 4/5 or Day 11/12 after T cell purification and activation. 5 millions of cells were transfected with 15µg of mRNA encoding the different CAR constructs. CAR mRNAs were produced using the mMESSAGE mMACHINE T7 Kit (Life Technologies) and purified using RNeasy Mini Spin Columns (Qiagen). Transfections were done using PulseAgile™ Cytopulse technology, by applying two 0.1 mS pulses at 3000V/cm followed by four 0.2 mS pulses at 325V/cm in 0.4cm gap cuvettes in a final volume of 200µl of "Cytoporation buffer T" (BTX Harvard Apparatus). Cells were immediately diluted in X-Vivo™-15 media (Lonza) and incubated at 37°C with 5% CO_2. IL-2 (from Miltenyi Biotec was added 2h after electroporation at 20ng/mL.

Degranulation assay (CD107a mobilization)

T cells were incubated in 96-well plates (50,000 cells/well), together with an equal amount of cells expressing or not the BCMA protein. Co-cultures were maintained in a final volume of 100µl of X-Vivo™-15 medium (Lonza) for 6 hours at 37°C with 5% CO_2. CD107a staining was done during cell stimulation, by the addition of a fluorescent anti-CD107a antibody (APC conjugated, from Miltenyi Biotec) at the beginning of the co-culture, together with 1µg/ml of anti-CD49d (BD Pharmingen), 1µg/ml of anti-CD28 (Miltenyi Biotec), and 1x Monensin solution (eBioscience). After the 6h incubation period, cells were stained with a fixable viability dye (eFluor 780, from eBioscience) and fluorochrome-conjugated anti-CD8 (PE conjugated Miltenyi Biotec) and analyzed by flow cytometry. The degranulation activity was determined as the % of CD8+/CD107a+ cells, and by determining the mean fluorescence intensity signal (MFI) for CD107a staining among CD8+ cells. Degranulation assays were carried out 24h after mRNA transfection. Results are summarized in the Tables 6A-9H and 9A-9C below. In the tables, the second column (labeled "CAR-T cell") indicates the BCMA specific CAR being expressed in the transfected T cells.

CD107a expression on cells is a marker of antigen specific activation. The percent and MFI of CD107a on CD8 T cells expressing BCMA specific CARs increase when incubated with BCMA high (H929), medium (MM1S) and low (KMS12BM, L363) expressing cells but not BCMA negative cells (K562 and Daudi) (Tables 6A-9H and 9A-9C). CD107a expression levels did not increase on mock transfected T cells contacted with BCMA. Thus,

the BCMA specific CAR-T cells are activated in the presence of BCMA-expressing cells but not in the presence of cells that do not express BCMA.

These results demonstrate that T cells expressing BCMA specific CARs are activated when incubated with BCMA expressing cells, and that the activation is antigen-specific.

IFN γ release assay

T cells were incubated in 96-well plates (50,000 cells/well), together with (a) cells expressing BCMA (MM1S, KMS12BM, and L363) or (b) cells that do not express the BCMA protein (K562). Co-cultures were maintained in a final volume of 100µl of X-Vivo™-15 medium (Lonza) for 24 hours at 37°C with 5% CO_2. After this incubation period the plates were centrifuged at 1500 rpm for 5 minutes and the supernatants were recovered in a new plate. IFNγ detection in the cell culture supernatants was done by ELISA assay (Human IFNγ Quantikine ELISA Kit, from R&D Systems). The IFNγ release assays were carried by starting the cell co-cultures 24h after mRNA transfection. Results are summarized in the Tables 8A-8D and 10 below.

As shown in Tables 8A-8D and 10, CD8 T cells expressing BCMA specific CARs produce IFNγ when incubated with either medium BCMA-expressing cells (MM1S) or low BCMA-expressing cells (KMS12BM, L363). In contrast, CD8 T cells expressing BCMA specific CARs produce negligible IFNγ when incubated with BCMA negative cells (K562).

These results demonstrate that T cells expressing BCMA specific CARs are activated when incubated with BCMA expressing cells, and that the activation is antigen-specific.

- *Cytotoxicity assay*

T cells were incubated in 96-well plates (100,000 cells/well), together with 10,000 target cells (expressing BCMA) and 10,000 control (BCMAneg) cells in the same well. Target and control cells were labelled with fluorescent intracellular dyes (CFSE or Cell Trace Violet, from Life Technologies) before co-culturing them with CAR+ T cells. The co-cultures were incubated for 4 hours at 37°C with 5% CO_2. After this incubation period, cells were labelled with a fixable viability dye (eFluor 780, from eBioscience) and analyzed by flow cytometry.

Viability of each cellular population (target cells or BCMAneg control cells) was determined and the % of specific cell lysis was calculated. Cytotoxicity assays were carried out 48h after mRNA transfection. Results are summarized in the Tables 7A-7H below. In the tables, the cytotoxicity data are shown as percent viable cells, then calculated as a ratio of live BCMA positive cells/live BCMA negative cells. Cell lysis is calculated as 100 – mock transfected T cells.

As shown in Tables 7A-7H, T cells expressing BCMA specific CARs exhibit killing activity when incubated with either medium BCMA-expressing cells (MM1S) or low BCMA-expressing cells (L363). In contrast, CD8 T cells expressing BCMA specific CARs do not exhibit killing activity when incubated with BCMA negative cells (K562).

In summary, T cells expressing the selected BCMA specific CARs shown in Table 5 are selectively activated upon contact with BCMA-expressing cells. While all versions of the BCMA specific CARs exhibited BCMA-specific activiation, BCMA specific CARs comprising a CD8α hinge (v2) exhibited increased activation levels compared to BCMA specific CARs comprising a FcγRIIIα (v1) hinge or IgG1 (v3) hinge.

Table 6A: Degranulation Assay Results, Donor 1

				MFI CD107a+	% CD107a+ (in CD8+)
Donor 1	mock transfected T cells		T cells	410	2.45
			PMA/Iono	4038	76.1
			MM1S	547	6.78
			K562	610	7.55
	P6DY	v1	T cells	588	5.19
			PMA/Iono	3758	75.1
			MM1S	850	14.9
			K562	829	9.76
		v2	T cells	756	6.86
			PMA/Iono	4103	75.5
			MM1S	3872	75.4
			K562	1130	20.7
		v3	T cells	707	7.71
			PMA/Iono	4336	78.7
			MM1S	3665	72.6

			K562	612	7.7
	P6AP	v1	T cells	604	4.61
			PMA/Iono	3526	72.8
			MM1S	1847	46.4
			K562	503	4.28
		v2	T cells	1380	27.8
			PMA/Iono	2504	58
			MM1S	5299	83.9
			K562	949	14.6
		v3	T cells	856	12.6
			PMA/Iono	2500	58.9
			MM1S	3638	73
			K562	718	9.15

Table 6B: Degranulation Assay Results, Donor 2

				MFI CD107a+	% CD107a+ (in CD8+)
Donor 2	mock transfected T cells		T cells	270	1.66
			PMA/Iono	3872	88.3
			MM1S	499	11
			K562	492	8.78
	P5A	v1	T cells	423	7.2
			PMA/Iono	6034	96.3
			MM1S	2670	77.6
			K562	648	16.6
		v2	T cells	428	7.14
			PMA/Iono	4420	90.7
			MM1S	5019	91.8
			K562	620	13.8
		v3	T cells	451	8.87
			PMA/Iono	4835	93.2
			MM1S	4191	88.5
			K562	607	14.1
	P5A_C1	v1	T cells	315	4.12
			PMA/Iono	3567	85.8
			MM1S	2193	68.6
			K562	537	10.1

		v2	T cells	413	7.46
			PMA/Iono	4423	91.1
			MM1S	4575	90.6
			K562	660	17.2
		v3	T cells	429	7.82
			PMA/Iono	4442	93.5
			MM1S	3710	84.4
			K562	597	13.9
	P5A_C16	v1	T cells	424	7.95
			PMA/Iono	4325	91.1
			MM1S	1858	61.6
			K562	636	14.9
		v2	T cells	401	5.69
			PMA/Iono	3007	80
			MM1S	4228	87.9
			K562	696	17.6
		v3	T cells	372	5.25
			PMA/Iono	3611	86.6
			MM1S	3372	83.6
			K562	476	7.72

Table 6C: Degranulation Assay Results, Donor 3

				MFI CD107a+	% CD107a+ (in CD8+)
Donor 3	mock transfected T cells		T cells	338	3.61
			PMA/Iono	7111	98.1
			MM1S	464	9.44
			K562	533	9.73
	PC1	v1	T cells	454	6.67
			PMA/Iono	5226	96.5
			MM1S	2178	75.6
			K562	753	22.3
		v2	T cells	507	13
			PMA/Iono	4743	95.2
			MM1S	759	25.5
			K562	649	15.5

		v3	T cells	463	6.84
			PMA/Iono	7092	98.1
			MM1S	2857	87.2
			K562	665	15
	PC1C12	v1	T cells	373	3.35
			PMA/Iono	6214	97.2
			MM1S	1960	68.2
			K562	513	7.61
		v2	T cells	579	11.5
			PMA/Iono	6341	97.5
			MM1S	4478	95.1
			K562	680	15
		v3	T cells	533	10.1
			PMA/Iono	5785	97.4
			MM1S	3739	91
			K562	648	13.2
	COM22	v1	T cells	354	2.74
			PMA/Iono	5894	96.7
			MM1S	2219	76.1
			K562	445	5.62
		v2	T cells	401	6.52
			PMA/Iono	5802	94.6
			MM1S	2372	79.2
			K562	534	8.9
		v3	T cells	501	10.4
			PMA/Iono	6387	97.6
			MM1S	2780	85.9
			K562	648	13.8

Table 6D: Degranulation Assay Results, Donor 4

			MFI CD107a+	% CD107a+ (in CD8+)
Donor 4 (v3 only)	mock transfected T cells	T cells	248	2.64
		PMA/Iono	5750	94.9
		MM1S	363	8.89
		K562	368	6.86

	P5A	T cells	335	3.82
		PMA/Iono	6025	93
		MM1S	3150	86.7
		K562	418	9.91
	P5AC1	T cells	505	22.1
		PMA/Iono	6950	98.3
		MM1S	2975	84.7
		K562	575	23.3
	P5AC16	T cells	368	6.2
		PMA/Iono	5775	97.7
		MM1S	3675	86.8
		K562	420	9.73
	PC1	T cells	403	9.05
		PMA/Iono	6975	97.8
		MM1S	4625	93
		K562	543	15.8
	PC1C12	T cells	485	12.9
		PMA/Iono	6400	96.5
		MM1S	3575	90.4
		K562	585	18.9
	COM22	T cells	535	20.5
		PMA/Iono	7250	98.3
		MM1S	3725	91.4
		K562	533	16.9
	P6DY	T cells	313	3.08
		PMA/Iono	5125	94.3
		MM1S	2435	79.9
		K562	438	10.4
	P6AP	T cells	430	10.4
		PMA/Iono	6100	94.2
		MM1S	3800	91.7
		K562	478	14.6

Table 6E: Degranulation Assay Results, Donor 5

			% CD107a+ (in CD8+)	MFI CD107a+
Donor	CAR-	L363	47	917

5 (v3 only)	BCMA-P5A	MM1S	65.3	1713
		K562	3.65	247
		T cells	1.71	199
		PMA/iono	98.6	4797
	CAR-BCMA-P5AC1	L363	50.6	1117
		MM1S	65.5	1753
		K562	5.29	265
		T cells	1.93	213
		PMA/iono	99.1	5755
	CAR-BCMA-P5AC16	L363	57.2	1392
		MM1S	73.9	2520
		K562	4.13	273
		T cells	2.57	232
		PMA/iono	98.1	5120
	CAR-BCMA-PC1	L363	71.9	2167
		MM1S	82.9	2987
		K562	4.5	316
		T cells	2.47	273
		PMA/iono	98.5	5556
	CAR-BCMA-PC1C12	L363	57.8	1492
		MM1S	71.5	2094
		K562	3.72	313
		T cells	2.53	272
		PMA/iono	98.2	4480
	CAR-BCMA-COM22	L363	61.3	1574
		MM1S	78.1	2602
		K562	5.84	296
		T cells	5.26	284
		PMA/iono	98.3	4434
	CAR-BCMA-P6DY	L363	43.4	859
		MM1S	63.6	1624
		K562	3.99	256
		T cells	1.95	228
		PMA/iono	98.1	4075
	CAR-BCMA-P6AP	L363	63.4	1745
		MM1S	77.8	2461
		K562	4.81	310
		T cells	4.74	300
		PMA/iono	98.9	32

	mock transfected T cells	L363	2.54	200
		MM1S	5.19	233
		K562	4.02	201
		T cells	1.95	192
		PMA/iono	97.7	3216

Table 6F: Degranulation Assay Results, Donor 6

			MFI CD107a+	% CD107a+ (in CD8+)
Donor 6	BCMA_BC30_v3 (18)	Tcells alone	121	1.04
		T cells PMA IONO	5253	87.4
		T cells K562	230	3.21
		T cells MM1S	1321	50.4
		T cells L363	986	41.8
	CAR_BCMA_P5AC1_v2	Tcells alone	150	1.07
		T cells PMA IONO	4701	83.2
		T cells K562	256	5.5
		T cells MM1S	2193	63.8
		T cells L363	1400	50.9
	CAR_BCMA_P5AC1_v3	Tcells alone	166	0.96
		T cells PMA IONO	4518	80.2
		T cells K562	301	6.87
		T cells MM1S	1101	40.7
		T cells L363	728	29.8
	CAR_BCMA_PC1_v3	Tcells alone	217	1.63
		T cells PMA IONO	4711	82.4
		T cells K562	329	6.36
		T cells MM1S	2083	60.3
		T cells L363	1500	52.1
	CAR_BCMA_PC1C12_v2	Tcells alone	209	2.01
		T cells PMA IONO	5401	87.8
		T cells K562	332	7.7
		T cells MM1S	2588	68.4
		T cells L363	1976	59.5

	CAR_BCMA_PC1C12_v3	Tcells alone	162	1.72
		T cells PMA IONO	5299	85.3
		T cells K562	266	6.25
		T cells MM1S	669	28.8
		T cells L363	414	18.6
	CAR_BCMA_COM22_v3	Tcells alone	193	3.23
		T cells PMA IONO	4750	82.7
		T cells K562	288	5.13
		T cells MM1S	814	35.7
		T cells L363	606	26.8
	CAR_BCMA_P6AP_v2	Tcells alone	359	9.69
		T cells PMA IONO	5521	87.4
		T cells K562	327	7.69
		T cells MM1S	2289	63.8
		T cells L363	1876	56.9
	CAR_BCMA_P6AP_v3	Tcells alone	284	4.87
		T cells PMA IONO	4480	82.7
		T cells K562	331	5.9
		T cells MM1S	1409	46.9
		T cells L363	926	35.3
	mock transfected T cells	Tcells alone	184	0.92
		T cells PMA IONO	3955	78.6
		T cells K562	278	3.58
		T cells MM1S	393	4.7
		T cells L363	190	1.12

Table 6G: Degranulation Assay Results, Donor 7

			MFI CD107a+	% CD107a+ (In CD8+)
Donor 7	mock transfected T cells	Tcells alone	68.3	1.55
		T cells PMA IONO	3097	94.6
		T cells MM1S	118	7.15
		T cells L363	90.3	2.63

		T cells K562	144	3.4
		T cells Daudi	117	1.93
	BCMA_BC30_v3 (18)	Tcells alone	69.7	2.69
		T cells PMA IONO	2864	94.9
		T cells MM1S	1630	68.9
		T cells L363	529	43.8
		T cells K562	125	3.85
		T cells Daudi	426	38.5
	P5AC1_v2	Tcells alone	111	3.67
		T cells PMA IONO	2859	95.6
		T cells MM1S	2305	71.5
		T cells L363	877	53.1
		T cells K562	166	8.54
		T cells Daudi	770	51.5
	P5AC1_v3	Tcells alone	70.8	1.04
		T cells PMA IONO	2740	94.6
		T cells MM1S	526	43.3
		T cells L363	209	20.4
		T cells K562	118	8.32
		T cells Daudi	450	35.9
	PC1_v3	Tcells alone	61	1.37
		T cells PMA IONO	2786	94.6
		T cells MM1S	1027	56.3
		T cells L363	314	29.9
		T cells K562	140	12.1
		T cells Daudi	536	39.6
	PC1C12_v2	Tcells alone	98	5.95
		T cells PMA IONO	3493	95.3
		T cells MM1S	1917	73.7
		T cells L363	939	56.2
		T cells K562	192	11.5
		T cells Daudi	1485	64.6
	PC1C12_v3	Tcells alone	84.2	2.28
		T cells PMA IONO	3017	95.2

		T cells MM1S	342	28.2
		T cells L363	145	8.72
		T cells K562	186	7.53
		T cells Daudi	223	11.8
	COM22_v3	Tcells alone	93.6	5.32
		T cells PMA IONO	2989	96.3
		T cells MM1S	540	40
		T cells L363	154	12.5
		T cells K562	138	8.29
		T cells Daudi	93.5	3.99
	P6AP_v2	Tcells alone	164	13.7
		T cells PMA IONO	3303	95.9
		T cells MM1S	2755	76
		T cells L363	859	50.3
		T cells K562	287	15.8
		T cells Daudi	1263	58.2
	P6AP_v3	Tcells alone	114	10.5
		T cells PMA IONO	3084	94.5
		T cells MM1S	849	51.6
		T cells L363	380	30.9
		T cells K562	211	8.46
		T cells Daudi	678	42.7

Table 6H: Degranulation Assay Results, Donor 8

			MFI · CD107a+	% CD107a+ (In CD8+)
Donor 8	mock transfected T cells	Tcells alone	154	0.67
		T cells PMA IONO	3777	66.2
		T cells MM1S	229	2.16
		T cells L363	166	1.51
		T cells K562	220	2.08
	BCMA_BC30_v3 (18)	Tcells alone	210	1.05
		T cells PMA IONO	4302	70.6
		T cells MM1S	1661	42

		T cells L363	1049	26.5
		T cells K562	262	3.46
	P5AC1_v2	Tcells alone	207	0.86
		T cells PMA IONO	4298	71.5
		T cells MM1S	1648	40.8
		T cells L363	1099	26.5
		T cells K562	232	1.72
	P5AC1_v3	Tcells alone	187	0.84
		T cells PMA IONO	3989	68.8
		T cells MM1S	766	21.2
		T cells L363	521	14.2
		T cells K562	258	2.05
	PC1_v3	Tcells alone	242	1.23
		T cells PMA IONO	4256	70.6
		T cells MM1S	1046	23.1
		T cells L363	1183	27.4
		T cells K562	283	2.97
	PC1C12_v2	Tcells alone	257	1.87
		T cells PMA IONO	3487	60.2
		T cells MM1S	2463	51.2
		T cells L363	1657	35.4
		T cells K562	314	4.05
	PC1C12_v3	Tcells alone	166	0.86
		T cells PMA IONO	4238	69.1
		T cells MM1S	641	17.3
		T cells L363	507	14.2
		T cells K562	296	3.52
	COM22_v3	Tcells alone	283	2.55
		T cells PMA IONO	4800	75.9
		T cells MM1S	1035	27.9
		T cells L363	704	22.7
		T cells K562	334	4.82
	P6AP_v2	Tcells alone	545	8.33
		T cells PMA IONO	4362	68.6

		T cells MM1S	2273	46.7
		T cells L363	1671	34.7
		T cells K562	629	9.71
	P6AP_v3	Tcells alone	360	3.87
		T cells PMA IONO	3584	61.5
		T cells MM1S	1553	34.5
		T cells L363	1045	23
		T cells K562	595	7.4

Table 7A: Cytotoxicity Data, Donor 6

		Viability (mean)			
	CAR	L363	K562	MM1S	K562
Donor 6	BC30_v3	22.93	89.90	16.30	88.43
	P5AC1_v2	27.27	90.07	21.47	90.17
	P5AC1_v3	36.03	89.30	19.80	88.50
	PC1_v3	19.03	88.23	13.57	87.50
	PC1C12_v2	19.60	86.13	14.67	84.67
	PC1C12_v3	55.50	89.33	41.33	88.67
	COM22_v3	42.00	90.33	25.67	88.30
	P6AP_v2	29.40	80.27	21.07	82.10
	P6AP_v3	48.53	85.20	25.57	81.30
	mock transfected T cells	90.90	88.20	91.77	86.30

Table 7B: Cytotoxicity Data, Donor 6

		BCMA+/BCMA-		Ratio to Mock transfected T cells		Cell lysis	
	CAR	L363	MM1S	L363	MM1S	L363	MM1S
Donor 6	BC30_v3	25.51	18.43	0.24752108	0.17333946	75.2	82.7
	P5AC1_v2	30.27	23.81	0.29374647	0.22389502	70.6	77.6
	P5AC1_v3	40.35	22.37	0.39152336	0.21040098	60.8	79.0
	PC1_v3	21.57	15.50	0.2093085	0.14581122	79.1	85.4
	PC1C12_v2	22.76	17.32	0.22079514	0.1629089	77.9	83.7
	PC1C12_v3	62.13	46.62	0.60281513	0.4383953	39.7	56.2
	COM22_v3	46.49	29.07	0.45113441	0.27335977	54.9	72.7

	P6AP_v2	36.63	25.66	0.35539949	0.24131177	64.5	75.9
	P6AP_v3	56.96	31.45	0.55272006	0.29573955	44.7	70.4
	mock transfected T cells	103.06	106.33	1	1	0.0	0.0

Table 7C: Cytotoxicity Data, Donor 7

		Viability (mean)			
	CAR	L363	K562	MM1S	K562
	mock transfected T cells	92.53	92.80	90.70	92.33
	BC30_v3	46.00	90.40	34.00	89.83
	P5AC1_v2	50.50	90.73	35.17	89.40
	P5AC1_v3	60.20	89.97	43.03	89.53
	PC1_v3	49.43	89.67	37.33	88.97
	PC1C12_v2	40.23	88.50	22.53	87.53
	PC1C12_v3	81.03	91.30	71.70	89.83
	COM22_v3	67.87	90.00	52.97	89.20
Donor 7	P6AP_v2	57.33	89.93	32.87	87.10
	P6AP_v3	66.37	91.60	46.35	94.00

Table 7D: Cytotoxicity Data, Donor 7

		BCMA+/BCMA-		Ratio to Mock transfected T cells		Cell lysis	
	CAR	L363	MM1S	L363	MM1S	L363	MM1S
	mock transfected T cells	99.71	98.23	1	1	0.0	0.0
	BC30_v3	50.88	37.85	0.51031598	0.38529434	49.0	61.5
	P5AC1_v2	55.66	39.34	0.55818001	0.40044688	44.2	60.0
	P5AC1_v3	66.91	48.06	0.67106507	0.48929577	32.9	51.1
	PC1_v3	55.13	41.96	0.55288988	0.4271896	44.7	57.3
	PC1C12_v2	45.46	25.74	0.45592406	0.26206149	54.4	73.8
	PC1C12_v3	88.76	79.81	0.89010798	0.81251777	11.0	18.7
	COM22_v3	75.41	59.38	0.7562472	0.60448985	24.4	39.6
Donor 7	P6AP_v2	63.75	37.73	0.63934647	0.38413929	36.1	61.6
	P6AP_v3	72.45	49.31	0.7266149	0.50196463	27.3	49.8

Table 7E: Cytotoxicity Data, Donor 8

		Viability (mean)			
	CAR	L363	K562	MM1S	K562
Donor 8	mock transfected T cells	93.97	91.13	95.97	88.07
	BC30_v3	67.97	86.80	46.40	78.87
	P5AC1_v2	69.80	85.37	47.13	79.17
	P5AC1_v3	77.90	88.77	62.70	84.40
	PC1_v3	61.67	86.60	41.67	78.97
	PC1C12_v2	62.43	85.27	35.27	78.20
	PC1C12_v3	85.17	85.27	78.87	77.77
	COM22_v3	76.70	87.87	56.40	84.50
	P6AP_v2	77.23	84.90	61.47	83.47
	P6AP_v3	83.23	85.67	72.57	84.63
	cell lines	95.20	94.97	96.97	94.20

Table 7F: Cytotoxicity Data, Donor 8

		BCMA+/BCMA-		Ratio to Mock transfected T cells		Cell lysis	
	CAR	L363	MM1S	L363	MM1S	L363	MM1S
Donor 8	mock transfected T cells	1.03	0.95	1	1	0.0	0.0
	BC30_v3	0.78	0.59	0.75941589	0.61953757	24.1	38.0
	P5AC1_v2	0.82	0.60	0.79299515	0.62694429	20.7	37.3
	P5AC1_v3	0.88	0.74	0.85112036	0.78229085	14.9	21.8
	PC1_v3	0.71	0.53	0.69061501	0.5556331	30.9	44.4
	PC1C12_v2	0.73	0.45	0.7101346	0.47489852	29.0	52.5
	PC1C12_v3	1.00	1.01	0.96871004	1.06793091	3.1	-6.8
	COM22_v3	0.87	0.67	0.84659295	0.70285469	15.3	29.7
	P6AP_v2	0.91	0.74	0.88226799	0.77547847	11.8	22.5
	P6AP_v3	0.97	0.86	0.94229927	0.9028984	5.8	9.7
	cell lines	1.00	1.03	0.97223038	1.08396365	2.8	-8.4

Table 7G: Cytotoxicity Data, Donor 9

		Viability (mean)			
Donor	CAR	L363	K562	MM1S	K562

9	mock transfected T cells	86.3	87.8	69.6	86.5
	BC30_v3	27.1	86.6	16.0	86.6
	P5AC1_v2	31.9	87.9	21.0	87.2
	P5AC1_v3	46.9	85.1	36.3	84.0
	PC1_v3	27.8	85.3	25.4	85.0
	PC1C12_v2	29.3	88.7	15.0	86.0
	COM22_v3	49.0	88.8	35.7	87.5
	P6AP_v2	41.4	85.7	22.8	84.0
	P6AP_v3	56.4	84.3	44.9	84.4
	Cell lines	92.3	91.7	83.5	91.8

Table 7H: Cytotoxicity Data, Donor 9

		BCMA+/BCMA-		Ratio to Mock transfected T cells		Cell lysis	
	CAR	L363	MM1S	L363	MM1S	L363	MM1S
	mock transfected T cells	0.98216319	0.80469954	1	1	0	0
	BC30_v3	0.31331794	0.18444359	0.31900802	0.22920802	68.10	77.08
	P5AC1_v2	0.3631539	0.24111578	0.36974905	0.29963455	63.03	70.04
	P5AC1_v3	0.55133229	0.43231441	0.56134489	0.53723706	43.87	46.28
	PC1_v3	0.32551778	0.29831439	0.33142942	0.37071525	66.86	62.93
	PC1C12_v2	0.3298272	0.17473847	0.3358171	0.21714748	66.42	78.29
	COM22_v3	0.55159475	0.40746382	0.56161212	0.50635523	43.84	49.36
	P6AP_v2	0.48289269	0.27092424	0.49166238	0.33667751	50.83	66.33
Donor 9	P6AP_v3	0.66903915	0.53199052	0.68118939	0.66110454	31.88	33.89
	Cell lines	1.00690909	0.90889292	1.02519531	1.1294811	-2.52	-12.95

Table 8A: IFNγ Production (pg/mL), Donor 6

Donor 6		
CAR		pg/ml
mock transfected T cells	Tcells alone	155.1
BCMA_BC30_v3 (18)		654.71
P5AC1_v2		174.035
P5AC1_v3		61.215

PC1_v3		255.045
PC1C12_v2		481.595
PC1C12_v3		463.08
COM22_v3		2996.305
P6AP_v2		1294.055
P6AP_v3		500.435
mock transfected T cells	T cells PMA IONO	81654.2
BCMA_BC30_v3 (18)		49368.7
P5AC1_v2		49102.7
P5AC1_v3		66837.7
PC1_v3		70798.2
PC1C12_v2		56402.2
PC1C12_v3		121954.7
COM22_v3		125878.7
P6AP_v2		73577.2
P6AP_v3		51242.7
mock transfected T cells	T cells K562	-83.215
BCMA_BC30_v3 (18)		265.565
P5AC1_v2		-10.05
P5AC1_v3		36.475
PC1_v3		-74.04
PC1C12_v2		344.72
PC1C12_v3		583.99
COM22_v3		610.97
P6AP_v2		40.66
P6AP_v3		36.775
mock transfected T cells	T cells MM1S	660.33
BCMA_BC30_v3 (18)		8004.42
P5AC1_v2		5667.72
P5AC1_v3		2619.735
PC1_v3		6152.67
PC1C12_v2		8526.27
PC1C12_v3		1405.945
COM22_v3		3330.27

P6AP_v2		5436.27
P6AP_v3		3881.115
mock transfected T cells	T cells 1363	1287.38
BCMA_BC30_v3 (18)		6363.72
P5AC1_v2		3116.725
P5AC1_v3		2720.52
PC1_v3		6661.97
PC1C12_v2		9478.72
PC1C12_v3		1707.885
COM22_v3		2397.83
P6AP_v2		5911.97
P6AP_v3		3470.38

Table 8B: IFNγ Production (pg/mL), Donor 7

Donor 7		
CAR		pg/ml
mock transfected T cells	Tcells alone	-3.1
BCMA_BC30_v3 (18)		64.1
P5AC1_v2		-18.0
P5AC1_v3		-73.0
PC1_v3		6.1
PC1C12_v2		156.5
PC1C12_v3		100.1
COM22_v3		182.9
P6AP_v2		564.7
P6AP_v3		107.0
mock transfected T cells	T cells PMA IONO	44970.8
BCMA_BC30_v3 (18)		32725.3
P5AC1_v2		27476.6
P5AC1_v3		13100.5
PC1_v3		40824.4
PC1C12_v2		39884.0
PC1C12_v3		30245.2
COM22_v3		62690.4

P6AP_v2		69923.2
P6AP_v3		88578.4
mock transfected T cells	T cells MM1S	29.9
BCMA_BC30_v3 (18)		4662.6
P5AC1_v2		3420.3
P5AC1_v3		1173.7
PC1_v3		2478.5
PC1C12_v2		5314.6
PC1C12_v3		809.9
COM22_v3		1344.6
P6AP_v2		3020.3
P6AP_v3		2166.7
mock transfected T cells	T cells L363	15.6
BCMA_BC30_v3 (18)		2360.2
P5AC1_v2		2576.3
P5AC1_v3		582.7
PC1_v3		1723.3
PC1C12_v2		2962.9
PC1C12_v3		136.6
COM22_v3		467.4
P6AP_v2		2081.4
P6AP_v3		1119.0
mock transfected T cells	T cells K562	-80.5
BCMA_BC30_v3 (18)		-127.2
P5AC1_v2		-124.4
P5AC1_v3		-47.9
PC1_v3		-93.6
PC1C12_v2		21.8
PC1C12_v3		-55.4
COM22_v3		-36.1
P6AP_v2		83.8
P6AP_v3		83.8
mock transfected T cells	T cells Daudi	335.1
BCMA_BC30_v3		7794.8

(18)		
P5AC1_v2		8093.7
P5AC1_v3		3870.6
PC1_v3		6068.9
PC1C12_v2		10190.2
PC1C12_v3		1638.8
COM22_v3		4287.6
P6AP_v2		6971.6
P6AP_v3		5280.0

Table 8C: IFN-γ Production (pg/mL), Donor 8

Donor 8		
CAR		pg/ml
mock transfected T cells	Tcells alone	-697.44
BCMA_BC30_v3 (18)		-660.92
P5AC1_v2		-603.38
P5AC1_v3		-543.44
PC1_v3		-552.22
PC1C12_v2		-399.26
PC1C12_v3		-652.73
COM22_v3		-530.09
P6AP_v2		17.24
P6AP_v3		-289.82
mock transfected T cells	T cells PMA IONO	37206.73
BCMA_BC30_v3 (18)		53311.73
P5AC1_v2		57732.14
P5AC1_v3		52577.56
PC1_v3		48925.48
PC1C12_v2		38310.06
PC1C12_v3		71881.73
COM22_v3		61941.73
P6AP_v2		82339.64
P6AP_v3		63337.14
mock transfected T cells	T cells MM1S	-684.65

BCMA_BC30_v3 (18)		2976.34
P5AC1_v2		2727.71
P5AC1_v3		769.05
PC1_v3		2682.98
PC1C12_v2		5019.05
PC1C12_v3		-198.04
COM22_v3		1155.19
P6AP_v2		2945.65
P6AP_v3		671.21
mock transfected T cells	T cells L363	-664.74
BCMA_BC30_v3 (18)		2934.77
P5AC1_v2		2342.50
P5AC1_v3		579.85
PC1_v3		2232.65
PC1C12_v2		3676.59
PC1C12_v3		-303.86
COM22_v3		695.72
P6AP_v2		1612.74
P6AP_v3		311.07
mock transfected T cells	T cells K562	-672.42
BCMA_BC30_v3 (18)		-583.71
P5AC1_v2		-631.02
P5AC1_v3		-650.83
PC1_v3		-615.50
PC1C12_v2		-501.18
PC1C12_v3		-615.17
COM22_v3		-596.02
P6AP_v2		-393.94
P6AP_v3		-476.71

Table 8D: IFN-γ Production (pg/mL), Donor 9

Donor 9	
CAR	pg/ml
mock transfected T cells (tcells alone)	93.2

BCMA_BC30_v3 (18)		1225.2
P5AC1_v2		1344.5
P5AC1_v3		632.3
PC1_v3		2745.7
PC1C12_v2		48.1
COM22_v3		2656.5
P6AP_v2		566.5
P6AP_v3		-335.8
mock transfected T cells	T cells PMA IONO	12505.8
BCMA_BC30_v3 (18)		12312.2
P5AC1_v2		10607.5
P5AC1_v3		12014.7
PC1_v3		12829.9
PC1C12_v2		13829.5
COM22_v3		13489.5
P6AP_v2		13182.1
P6AP_v3		13506.3
mock transfected T cells	T cells MM1S	1006.4
BCMA_BC30_v3 (18)		2376.8
P5AC1_v2		-359.5
P5AC1_v3		97.8
PC1_v3		290.1
PC1C12_v2		752.7
COM22_v3		-601.0
P6AP_v2		-304.1
P6AP_v3		-394.9
mock transfected T cells	T cells L363	-228.2
BCMA_BC30_v3 (18)		3000.2
P5AC1_v2		2314.0
P5AC1_v3		1646.4
PC1_v3		-15.4
PC1C12_v2		2796.5
COM22_v3		320.6
P6AP_v2		-163.0
P6AP_v3		-233.9
mock transfected T cells	T cells K562	-227.9
BCMA_BC30_v3 (18)		2027.5
P5AC1_v2		3928.4

P5AC1_v3		300.2
PC1_v3		74.9
PC1C12_v2		1835.7
COM22_v3		45.0
P6AP_v2		51.4
P6AP_v3		158.3

Table 9A: Degranulation Assay Results, Donor 10

Donor 10		MFI CD107a+	% CD107a+ (in CD8+)
LT alone	mock transfected T cells	82.2	1.95
	26859 P5AC1-V2	83.8	1.47
	26868 PC1C12-V2	94.2	3.21
	26871 COM22-V2	107	5.96
PMA Iono	mock transfected T cells	5933	99
	26859 P5AC1-V2	5863	99
	26868 PC1C12-V2	6366	99.4
	26871 COM22-V2	6149	99
MM1S	mock transfected T cells	211	16.5
	26859 P5AC1-V2	1377	74.4
	26868 PC1C12-V2	1760	79.1
	26871 COM22-V2	1470	76.5
H929	mock transfected T cells	141	6.09
	26859 P5AC1-V2	1026	65.4
	26868 PC1C12-V2	1262	71.1
	26871 COM22-V2	784	59.2

L363	mock transfected T cells	153	6.48
	26859 P5AC1-V2	793	60.1
	26868 PC1C12-V2	1054	67.3
	26871 COM22-V2	827	61.7
MM1S GFP LUC	mock transfected T cells	187	9.88
	26859 P5AC1-V2	1228	70.5
	26868 PC1C12-V2	1476	74.9
	26871 COM22-V2	1095	68.5
H929 GFP LUC	mock transfected T cells	153	9.48
	26859 P5AC1-V2	1648	77.8
	26868 PC1C12-V2	1960	84
	26871 COM22-V2	1029	69.4
L363 GFP LUC	mock transfected T cells	104	3.06
	26859 P5AC1-V2	753	60.7
	26868 PC1C12-V2	873	64.6
	26871 COM22-V2	766	61.1
KMS12BM GFP LUC	mock transfected T cells	91.3	2.67
	26859 P5AC1-V2	945	67.2
	26868 PC1C12-V2	1192	71.2
	26871 COM22-V2	961	67.2
K562	mock transfected T cells	127	6.06
	26859 P5AC1-V2	136	9.1

	26868 PC1C12-V2	119	9.49
	26871 COM22-V2	135	9.55

Table 9B: Degranulation Assay Results, Donor 11

Donor 11		MFI CD107a+	% CD107a+ (in CD8+)
LT alone	mock transfected T cells	69.9	0.57
	26859 P5AC1-V2	68.3	0.62
	26868 PC1C12-V2	67.2	0.88
	26871 COM22-V2	80.9	3.95
PMA Iono	mock transfected T cells	5511	91.7
	26859 P5AC1-V2	5360	97.4
	26868 PC1C12-V2	4741	96.1
	26871 COM22-V2	5066	95.7
KMS12BM GFP LUC	mock transfected T cells	77.8	1.81
	26859 P5AC1-V2	1304	68.3
	26868 PC1C12-V2	650	45.5
	26871 COM22-V2	986	62.5
H929 GFP LUC	mock transfected T cells	73	1.04
	26859 P5AC1-V2	738	49.6
	26868 PC1C12-V2	428	30.9
	26871 COM22-V2	468	35.5
MM1S	mock transfected T cells	121	2.67

	26859 P5AC1-V2	854	52
	26868 PC1C12-V2	399	26.4
	26871 COM22-V2	486	33.4
K562	mock transfected T cells	125	3.08
	26859 P5AC1-V2	140	3.35
	26868 PC1C12-V2	123	1.84
	26871 COM22-V2	161	4.11

Table 9C: Degranulation Assay Results, Donor 12

Donor 11		MFI CD107a+	% CD107a+ (in CD8+)
LT alone	mock transfected T cells	69.9	0.57
	26859 P5AC1-V2	68.3	0.62
	26868 PC1C12-V2	67.2	0.88
	26871 COM22-V2	80.9	3.95
PMA Iono	mock transfected T cells	5511	91.7
	26859 P5AC1-V2	5360	97.4
	26868 PC1C12-V2	4741	96.1
	26871 COM22-V2	5066	95.7
KMS12BM GFP LUC	mock transfected T cells	77.8	1.81
	26859 P5AC1-V2	1304	68.3
	26868 PC1C12-V2	650	45.5
	26871 COM22-V2	986	62.5
H929 GFP LUC	mock transfected T	73	1.04

	cells		
	26859 P5AC1-V2	738	49.6
	26868 PC1C12-V2	428	30.9
	26871 COM22-V2	468	35.5
MM1S	mock transfected T cells	121	2.67
	26859 P5AC1-V2	854	52
	26868 PC1C12-V2	399	26.4
	26871 COM22-V2	486	33.4
K562	mock transfected T cells	125	3.08
	26859 P5AC1-V2	140	3.35
	26868 PC1C12-V2	123	1.84
	26871 COM22-V2	161	4.11

Table 10: IFN gamma release assay results, Donor 10

Donor 10		
mock transfected T cells	T cells alone	871.8 pg/mL
pCLS26859 CAR_BCMA_P5AC1_v2		1466.2 pg/mL
pCLS26868 CAR_BCMA_PC1C12_v2		1172.2 pg/mL
pCLS26871 CAR_BCMA_COM22_v2		1873.1 pg/mL
mock transfected T cells	MM1S LucGFP	1436.5 pg/mL
pCLS26859 CAR_BCMA_P5AC1_v2		12208.4 pg/mL
pCLS26868 CAR_BCMA_PC1C12_v2		13695.3 pg/mL
pCLS26871 CAR_BCMA_COM22_v2		10784.1 pg/mL
mock transfected T cells	MM1S	5329.0 pg/mL
pCLS26859 CAR_BCMA_P5AC1_v2		6060.3 pg/mL
pCLS26868 CAR_BCMA_PC1C12_v2		6776.1 pg/mL
pCLS26871 CAR_BCMA_COM22_v2		7827.0 pg/mL
mock transfected T cells	H929 LucGFP	754.2 pg/mL
pCLS26859 CAR_BCMA_P5AC1_v2		16589.9 pg/mL

pCLS26868 CAR_BCMA_PC1C12_v2		15989.7 pg/mL
pCLS26871 CAR_BCMA_COM22_v2		14410.4 pg/mL
mock transfected T cells	H929	809.8 pg/mL
pCLS26859 CAR_BCMA_P5AC1_v2		18072.7 pg/mL
pCLS26868 CAR_BCMA_PC1C12_v2		17948.1 pg/mL
pCLS26871 CAR_BCMA_COM22_v2		14437.3 pg/mL
mock transfected T cells	L363 LucGFP	1184.5 pg/mL
pCLS26859 CAR_BCMA_P5AC1_v2		11556.9 pg/mL
pCLS26868 CAR_BCMA_PC1C12_v2		13254.5 pg/mL
pCLS26871 CAR_BCMA_COM22_v2		11384.1 pg/mL
mock transfected T cells	L363	1777.3 pg/mL
pCLS26859 CAR_BCMA_P5AC1_v2		15685.1 pg/mL
pCLS26868 CAR_BCMA_PC1C12_v2		14929.1 pg/mL
pCLS26871 CAR_BCMA_COM22_v2		14995.7 pg/mL
mock transfected T cells	L363 LucGFP	1184.5 pg/mL
pCLS26859 CAR_BCMA_P5AC1_v2		11556.9 pg/mL
pCLS26868 CAR_BCMA_PC1C12_v2		13254.5 pg/mL
pCLS26871 CAR_BCMA_COM22_v2		11384.1 pg/mL
mock transfected T cells	KMS12BM LucGFP	1283.2 pg/mL
pCLS26859 CAR_BCMA_P5AC1_v2		9073.3 pg/mL
pCLS26868 CAR_BCMA_PC1C12_v2		10060.6 pg/mL
pCLS26871 CAR_BCMA_COM22_v2		10687.2 pg/mL
mock transfected T cells	K562	691.6 pg/mL
pCLS26859 CAR_BCMA_P5AC1_v2		684.1 pg/mL
pCLS26868 CAR_BCMA_PC1C12_v2		904.2 pg/mL
pCLS26871 CAR_BCMA_COM22_v2		969.0 pg/mL

Example 3: BCMA specific CAR-T cells induce tumor regression in MM1.S tumor model

This example illustrates treatment of tumors with BCMA specific CAR-T cells using the MM1.S tumor model.

In vivo efficacy study of BCMA specific CAR-T cells was performed with MM1.S, expressing luciferase and GFP, orthotopic model. Five million MM1.S Luc2AGFP cells were injected intravenously through the tail vein into 6-8 weeks old female Nod/Scid/IL2Rg-/-(NSG) animals. Intraperitoneal injection of D-luciferin (Regis Technologies, Morton Grove, IL) (200uL per animal at 15mg/mL), followed by anesthesia with isofluorane and subsequent whole body bioluminescence imaging (BLI) enable

monitoring of tumor burden. Bioluminescent signals emitted by the interaction between luciferase expressed by the tumor cells and luciferin were captured by imaging using an IVIS Spectrum CT (Perkin Elmer, MA) and quantified as total flux (photons/sec) using Living Image 4.4 (Caliper Life Sciences, Alameda, CA).

Three different BCMA specific CAR-T cells were used in this study: T cells expressing the BCMA specific CAR contructs P5AC1-V2, PC1C12-V2, or COM22-V2 (see, Table 5 above). Non-transduced control T cells were used as the negative control. All T cells were engineered to be TCRα deficient.

When the total flux reached an average of 45E6 for all animals (day 20 post tumor implant), the animals were randomized into four groups. A single dose of human either BCMA specific CAR-T cells or non-transduced control T cells was administered through bolus tail vein injection. Animals were terminated when they exhibit hindlimb paralysis or a 20% loss of body weight, an endpoint for MM1.S orthotopic models.

Results of this study are summarized in Figure 1. In Figure 1, total flux [p/s] represents tumor progression. Treatment with BCMA specific CAR-T cells (triangles, diamonds, squares) resulted in lower total flux as compared to the negative control (circles). Thus, treatment with BCMA specific CAR-T cells inhibited tumor progression as compared to the negative control.

These results demonstrate BCMA specific CAR-T cells are effective to induce tumor regression.

Example 4: Treatment of Multiple Myeloma with BCMA specific CAR-T Cells

This example illustrates treatment of multiple myeloma with BCMA specific CAR-T cells using the Molp8 orthotopic model.

In vivo efficacy study of BCMA specific CAR-T cells was performed with Molp8, expressing luciferase and GFP, orthotopic model. Two million Molp8 Luc2AGFP cells were injected intravenously through the tail vein into 6-8 weeks old female NSG animals. Intraperitoneal injection of D-luciferin (Regis Technologies, Morton Grove, IL) (200uL per animal at 15mg/mL), followed by anesthesia with isofluorane and subsequent whole body bioluminescence imaging (BLI) enable monitoring of tumor burden. Bioluminescent signals emitted by the interaction between luciferase expressed by the tumor cells and luciferin

were captured by imaging using an IVIS Spectrum CT (Perkin Elmer, MA) and quantified as total flux (photons/sec) using Living Image 4.4 (Caliper Life Sciences, Alameda, CA).

When the total flux reached an average of 30E6 for all animals (day 8 post tumor implant), the animals were randomized into three groups. Each group was administered one of the following cells: 1) non-transduced T cells TCR KO ("TCR KO") used as a control, 2) BCMA specific CAR-T cells expressing P5AC1-V2.1 ("P5AC1 V2 R2 TCR KO"), or 3) BCMA specific CAR-T cells expressing P5AC1-V2 and the RQR8 suicide polypeptide ("P5AC1 V2 RQR8 TCR KO"). All of cells 1-3 are TCRα deficient. The BCMA specific CAR-T cells were prepared as described in example above. BCMA specific CAR constructs P5AC1-V2.1 and P5AC1-V2 are shown in Table 5 above. A single dose of 3 million control (TCR KO) or BCMA specific CAR-T (P5AC1 V2 R2 TCR KO or P5AC1 V2 RQR8 TCR KO) cells were administered through bolus tail vein injection. Animals were terminated when they lose more than 15% of total body weight, an endpoint for Molp8 orthotopic models.

Results from the study are summarized in Figure 2. A single dose of 3 million P5AC1 R2 TCRKO BCMA specific CAR-T cells (squares) or P5AC1 RQR8 TCRKO CAR-T cells (triangles) BCMA specific CAR-T cells resulted in lower total flux from days 10-35 post tumor implant as compared to the negative control (circles) (Figure 2). Thus, treatment with BCMA specific CAR-T cells inhibited tumor progression as compared to the negative control.

These results demonstrate BCMA specific CAR-T cells are effective to inhibit tumor progression.

Example 5: Treatment of multiple myeloma with BCMA specific CAR-T cells

This example illustrates the therapeutic activity of BCMA specific CAR-T cells in orthotopic mouse models of multiple myeloma.

Two humanized mouse models were used to evaluate the efficacy of BCMA specific CAR-T cells against human myeloma cell lines expressing BCMA. Six (6) to eight (8) week old female Nod/Scid IL2rg-/- (NSG) mice were purchased from the Jackson Laboratories. All animals were housed in a pathogen free vivarium facility at Rinat and experiments were conducted according to the protocols in accordance with the Institutional Animal Care and Use Committee (IACUC) guidelines.

The MM1.S and Molp-8 cell lines were purchased from the American Type Culture Collection (ATCC.org) and the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ.de). Cell lines were engineered to express a Luc-GFP fusion protein using lentiviral particles (amsbio). Cells were cultured in RPMI 1640 medium with L-glutamine supplemented with either 10% fetal calf serum for MM1.S or with 20% FCS for Molp-8 cells at 37°C in 5% carbon dioxide (CO_2). Cells growing in an exponential growth phase were harvested and used for tumor inoculation.

Therapeutic BCMA specific CAR-T cells were produced as described. Healthy human donor cells, peripheral blood mononuclear cells (PBMC) or purified pan-T cells, are activated and transduced with lentiviral particles encoding a BCMA specific CAR and RQR8 driven by EF-1a promoter. Three different BCMA specific CARs were used in this study: P5AC1-V2, PC1C12-V2 and COM22-V2 (see, Table 5 above). T cells were gene edited for deletion of the TCRα gene. Cells were cultured for 14 to 17 days and then cryopreserved in 90% FCS/10% DMSO. For T cell injection, T cells were rapidly thawed in a 37°C waterbath and washed twice with RPMI 1640 medium containing 25mM Hepes. Cells were injected in 0.2 ml RPMI 1640 with 25 mM Hepes into the tail vein of tumor-bearing animals.

NSG mice were irradiated with 1 Gy total body irradiation (RAD Source Technologies) one day prior to tumor cell inoculation. $5x10^6$ MM1.S/Luc2-EGFP cells or $2x10^6$ Molp-8/Luc2-EGFP cells were injected into the tail vein in 0.1 ml of phosphate-buffered saline (PBS). Tumor burden was measured twice weekly using bioluminescence imaging. Mice were injected with 3 ug D-Luciferin dissolved in 0.2 ml PBS and anesthetized using isofluorane. 7 minutes after injection animals were imaged using a Perkin Elmer IVIS Spectrum camera system. The total body luminescence with the exception of the mouse tail was measured and tumor burden is reported as total flux (photons per second). Tumors were allowed to establish until exponential growth occurred. Animals were randomized into treatment groups based on total flux and treated with BCMA specific CAR-T cells or untransduced control T cells from the same donor. The effect of CAR-T treatment was assessed twice weekly using bioluminescence imaging and body weight measurements. The study endpoint was reached when the first animal exhibited end-stage disease as indicated by body weight loss (>20% of initial body weight), hindleg paralysis, or other signs

of animal distress. Statistical analysis was performed using GraphPad Prism 6. Repeated measures one-way ANOVA with Tukey's correction was used to compare anti-tumor efficacy between all groups. $P<0.05$ was considered significant.

Results are summarized in Table 11 (MM1.S) and Table 12 (Molp-8) below (log_{10} values of total flux in photons per second +/- SEM). A suboptimal CAR-T cell dose was used to compare BCMA specific CAR-T cells having different scFvs. The BCMA specific CAR-T cell groups are P5AC1-V2, PC1C12-V2 and COM22-V2 (see, Table 5 above). In the MM1.S model, $3.5x10^6$ CAR-expressing T cells were injected on day 17 after tumor implantation. In the Molp8 model, $4x10^6$ CAR-expressing T cells were injected on day 7 after tumor implantation. Transduction efficiencies ranged from 19% to 29% for BCMA specific CAR-T cells dosed in MM1.S mouse model and 31% to 36% for BCMA specific CAR-T cells dosed in Molp8 mouse model. An equivalent total dose of untransduced T cells was used for the control group. The control T cell-treated group exhibited progressive tumor growth until the study endpoint was reached at day 35 for MM1.S and day 23 for Molp8. Statistical analysis of the tumor burden using the RM-ANOVA test with Dunnets correction showed that in all three BCMA specific CAR-T treated groups, tumor burden was significantly lower compared to the tumor burden in the control group ($p<0.01$) (Tables 11 and 12). For example, in the MM1.S tumor model, mean total flux in animals treated with P5AC1-V2 BCMA specific CAR-T cells was 6.44 log10 photons/s at day 25, compared to 9.22 log10 photons/s in animals given control T cells (Table 11). At day 35 post tumor implantation, mean total flux in animals treated with P5AC1-V2 BCMA specific CAR-T cells was 6.82 log10 photons/s, compared to 10.18 log10 photons/s in animals given control T cells (Table 11). In the Molp8 tumor model, mean total flux in animals treated with P5AC1-V2 BCMA specific CAR-T cells was 7.88 log10 photons/s at day 14, compared to 9.39 log10 photons/s in animals given control T cells (Table 12). At day 23 post tumor implantation, mean total flux in animals treated with P5AC1-V2 BCMA specific CAR-T cells was 9.29 log10 photons/s, compared 10.37 log10 photons/s in animals given control T cells (Table 12).

These results demonstrate that treatments with BCMA specific CAR-T cells are effective to induce tumor regression.

Table 11: Tumor bioluminescence measurements of orthotopic MM1.S tumor model

Group 1: Control T cells			
Days after tumor implantation	Mean total flux (log10 photons/s)	SEM	N
17	7.84	0.04	10
21	8.16	0.19	10
25	9.22	0.02	10
28	9.53	0.02	10
32	9.96	0.05	10
35	10.18	0.07	10
Group 2: P5AC1-V2 BCMA specific CAR-T cells			
Days after tumor implantation	Mean total flux (log10)	SEM	N
17	7.84	0.03	10
21	8.14	0.11	10
25	6.44	0.16	10
28	6.51	0.09	10
32	6.72	0.10	10
35	6.82	0.09	10
Group 3: PC1C12-V2 BCMA specific CAR-T cells			
Days after tumor implantation	Mean total flux (log10)	SEM	N
17	7.86	0.04	10
21	8.56	0.15	10
25	6.85	0.26	10
28	6.41	0.30	10
32	6.64	0.29	10
35	6.62	0.30	10
Group 4: COM22-V2 BCMA specific CAR-T cells			
Days after tumor implantation	Mean total flux (log10)	SEM	N
17	7.84	0.04	10
21	8.49	0.10	10
25	6.55	0.08	10
28	6.40	0.09	10
32	6.98	0.14	10
35	6.87	0.22	10

Table 12: Tumor bioluminescence measurements of orthotopic Molp-8 tumor model

Group 1: T cell only control			
Days after tumor implantation	Mean total flux (log10)	SEM	N
0	5.80	0.02	10
7	7.48	0.04	10
10	8.24	0.06	10
14	9.39	0.04	10
17	9.88	0.03	10
21	10.12	0.04	10
23	10.37	0.03	10

Group 2: P5AC1-V2 BCMA specific CAR-T cells			
Days after tumor implantation	Mean total flux (log10)	SEM	N
0	5.80	0.02	10
7	7.48	0.04	10
10	8.41	0.05	10
14	7.88	0.18	10
17	7.39	0.21	10
21	7.98	0.12	10
23	8.29	0.11	10

Group 3: PC1C12-V2 BCMA specific CAR-T cells			
Days after tumor implantation	Mean total flux (log10)	SEM	N
0	5.80	0.02	10
7	7.51	0.04	10
10	8.31	0.07	10
14	7.07	0.21	10
17	6.51	0.15	10
21	7.37	0.13	10
23	7.75	0.13	10

Group 4: COM22-V2 BCMA specific CAR-T cells			
Days after tumor implantation	Mean total flux (log10	SEM	N
0	5.80	0.02	10

7	7.49	0.04	10
10	8.39	0.07	10
14	7.78	0.16	10
17	7.51	0.21	10
21	7.89	0.17	10
23	8.32	0.14	10

Example 6: Treatment of multiple myeloma with TCRα/dCK knockout BCMA specific CAR-T cells

This example illustrates the therapeutic activity of BCMA specific CAR-T cells in orthotopic mouse models of multiple myeloma.

A humanized mouse model was used to evaluate the efficacy of BCMA CAR-T cells against human myeloma cell lines expressing BCMA. 6 to 8 week old female Nod/Scid IL2rg-/- (NSG) mice were purchased from the Jackson Laboratories. All animals were housed in a pathogen free vivarium facility at Rinat and experiments were conducted according to the protocols in accordance with the Institutional Animal Care and Use Committee (IACUC) guidelines.

The MM1.S cell lines was purchased from the American Type Culture Collection (ATCC.org). The Cell line was engineered to express a Luc-GFP fusion protein using lentiviral particles (amsbio)and gene edited using TALEN nucleases to disable the deoxycytidine (dCK) gene. Cells were cultured in RPMI 1640 medium with L-glutamine supplemented with 10% fetal calf serum at 37°C in 5% carbon dioxide (CO_2). Cells growing in an exponential growth phase were harvested and used for tumor inoculation.

Therapeutic CAR-T cells were produced as described. Healthy human donor cells, peripheral blood mononuclear cells (PBMC) or purified pan-T cells, are activated and transduced with lentiviral particles encoding for BCMA scFV, CD8 hinge, CD8 transmembrane, 41BB and CD3ζ with RQR8 genes under the control of the EF-1a promoter. The BCMA specific CAR-T cells were gene edited to delete the TCRα and/or the dCK gene using a combination of TCRα and dCK TALEN, or TCRα TALEN alone. Transduction efficiency for all T cells was 70%. TCRα knockout T cells were purified using magnetic selection kits for CD3-positive cells (Miltenyi); dCK knockout T cells were purified by expansion in the presence of 0.5 μM clofarabine. Cells were cultured for 14 to 17 days

and then cryopreserved in 90% FCS/10% DMSO. For T cell injection, T cells were rapidly thawed in a 37°C water bath and washed twice with RPMI 1640 medium containing 25mM Hepes. For treatment, T cells were injected in 0.2 ml RPMI 1640 with 25 mM Hepes into the tail vein of tumor-bearing animals.

For the mouse tumor model, animals were injected with MM1.S/dCK KO tumor cells. Mice were then treated with $2.5x10^6$ BCMA specific CAR-T cells on day 18 post tumor cell implantation. An equivalent dose of untransduced T cells that received TCRα and dCK TALEN was used as control. Animals were treated with clofarabine or vehicle for five days after T cell injection.

Results: the control T cell-treated group exhibited progressive tumor growth until the study endpoint was reached at day 35 (Table 13, Group 1). Compared against control, groups treated with TCRα knockout BCMA specific CAR-T cells and vehicle exhibited a significant decrease in tumor burden ($p<0.05$) that was diminished upon coadministration of clofarabine ($p<0.05$) (Table 13, Groups 2 and 3). Tumor burden was significantly reduced in animals treated with TCRα/dCK double knockout CAR-T cells, irrespective of whether the animals received vehicle or clofarabine ($p<0.05$) (Table 13, Groups 4 and 5). Reduction of tumor burden in the groups receiving TCRα/dCK double knockout T cells did not differ from the group receiving TCRα single knockout T cells and vehicle ($p>0.1$) (Table 13, Groups 2, 4, and 5).

These results demonstrate that treatments with TCRα/dCK double knockout BCMA CAR-T cells are effective to induce tumor regression in the presence of nucleoside analog therapies such as fludarabine and clofarabine.

Table 13: Tumor bioluminescence measurements of nucleoside analog therapy-resistant orthotopic MM1.S tumor model.

Group 1: TCRα/dCK KO control T cells + clofarabine			
Days after T cell administration	Mean total flux (log10 photons/s)	SEM	N
0	7.87	0.04	10
4	8.94	0.08	10
8	9.22	0.05	10
11	9.52	0.04	10
15	10.00	0.04	10
18	10.38	0.04	10

Group 2: TCRα KO BCMA specific CAR-T cells + vehicle			
Days after T cell administration	Mean total flux (log10)	SEM	N
0	7.86	0.04	10
4	9.28	0.07	10
8	8.58	0.12	10
11	8.04	0.14	10
15	8.14	0.15	10
18	8.24	0.15	10
Group 3: TCRα KO BCMA specific CAR-T cells + clofarabine			
Days after T cell administration	Mean total flux (log10)	SEM	N
0	7.87	0.04	10
4	9.33	0.07	10
8	9.17	0.07	10
11	8.95	0.14	10
15	9.36	0.08	10
18	9.50	0.07	10
Group 4: TCRα/dCK KO BCMA specific CAR-T cells + vehicle			
Days after T cell administration	Mean total flux (log10)	SEM	N
0	7.86	0.04	10
4	9.19	0.08	10
8	9.08	0.12	10
11	8.59	0.18	10
15	8.60	0.21	10
18	8.69	0.18	10
Group 5: TCRα/dCK KO BCMA specific CAR-T cells + clofarabine			
Days after T cell administration	Mean total flux (log10)	SEM	N
0	7.87	0.04	10
4	9.26	0.09	10
8	9.07	0.10	10
11	8.51	0.14	10
15	8.42	0.21	10
18	8.49	0.18	10

Although the disclosed teachings have been described with reference to various applications, methods, kits, and compositions, it will be appreciated that various changes and modifications can be made without departing from the teachings herein and the claimed invention below. The foregoing examples are provided to better illustrate the

disclosed teachings and are not intended to limit the scope of the teachings presented herein. While the present teachings have been described in terms of these exemplary embodiments, the skilled artisan will readily understand that numerous variations and modifications of these exemplary embodiments are possible without undue experimentation. All such variations and modifications are within the scope of the current teachings.

All references cited herein, including patents, patent applications, papers, text books, and the like, and the references cited therein, to the extent that they are not already, are hereby incorporated by reference in their entirety. In the event that one or more of the incorporated literature and similar materials differs from or contradicts this application, including but not limited to defined terms, term usage, described techniques, or the like, this application controls.

The foregoing description and Examples detail certain specific embodiments of the invention and describes the best mode contemplated by the inventors. It will be appreciated, however, that no matter how detailed the foregoing may appear in text, the invention may be practiced in many ways and the invention should be construed in accordance with the appended claims and any equivalents thereof.

Claims

It is claimed:

1. A B-cell maturation antigen (BCMA) specific chimeric antigen receptor (CAR) comprising an extracellular ligand-binding domain, a first transmembrane domain, and an intracellular signaling domain, wherein the extracellular domain comprises a single chain Fv fragment (scFv) comprising a heavy chain variable (VH) region comprising three CDRs from the VH region comprising the sequence shown in SEQ ID NO: 33, 72, 39, 76, 83, 92, 25, or 8; and a light chain variable (VL) region comprising three CDRs from the VL region shown in SEQ ID NO: 34, 73, 40, 77, 84, 93, 18, or 80.

2. The BCMA specific CAR of claim 1, wherein the VH region comprises the sequence shown in SEQ ID NO: 33, 72, 39, 76, 83, 92, 25, or 8, or a variant thereof with one or several conservative amino acid substitutions in residues that are not within a CDR and/or the VL region comprises the amino acid sequence shown in SEQ ID NO: 34, 73, 40, 77, 84, 93, 18, or 80, or a variant thereof with one or several amino acid substitutions in amino acids that are not within a CDR.

3. The BCMA specific CAR of claim 1 or 2, wherein the VH region comprises a VH CDR1 comprising the amino acid sequence shown in SEQ ID NO: 150, 151, or 152; a VH CDR2 comprising the amino acid sequence shown in SEQ ID NO: 187 or 188; and a VH CDR3 comprising the amino acid sequence shown in SEQ ID NO: 155; and a light chain variable region (VL) comprising the following CDRs: a VL CDR1 comprising the amino acid sequence shown in SEQ ID NO: 249; a VL CDR2 comprising the amino acid sequence shown in SEQ ID NO: 221; and a VL CDR3 comprising the amino acid sequence shown in SEQ ID NO: 225.

4. The BCMA specific CAR of claim 3, wherein the VH region comprises the amino acid sequence shown in SEQ ID NO: 72 and the VL region comprises the amino acid sequence shown in SEQ ID NO: 73.

5. The BCMA specific CAR of claim claim 1 or 2, wherein each CDR is defined in accordance with the Kabat definition, the Chothia definition, the combination of the Kabat definition and the Chothia definition, the AbM definition, or the contact definition of CDR.

6. The BCMA specific CAR of any one of claims 1 to 5, wherein the intracellular signaling domain comprises a CD3ζ signalling domain.

7. The BCMA specific CAR of any one of claims 1 to 6, wherein the intracellular signaling domain comprises a 4-1BB domain.

8. The BCMA specific CAR of claim of any one of claims 1 to 7, further comprising a second intracellular signaling domain.

9. The BCMA specific CAR of claim 8, wherein the second intracellular signaling domain comprises a 4-1BB domain.

10. The BCMA specific CAR of any one of claims 1 to 9, further comprising a stalk domain between the extracellular ligand-binding domain and the first transmembrane domain.

11. The BCMA specific CAR of claim 10, wherein the stalk domain is selected from the group consisting of: a human CD8α hinge, an IgG1 hinge, and an FcγRIIIα hinge.

12. The BCMA specific CAR of any one of claims 1 to 11, further comprising a CD20 epitope.

13. The BCMA specific CAR of claim 12, wherein the CD20 epitope comprises the amino acid sequence shown in SEQ ID NO: 397 or SEQ ID NO: 398.

14. The BCMA specific CAR of claim 13, wherein the BCMA specific CAR comprises the amino acid sequence shown in SEQ ID NO: 396.

15. The BCMA specific CAR of any one of claims 1 to 14, wherein the first transmembrane domain comprises a CD8α chain transmembrane domain.

16. The BCMA specific CAR of any one of claims 1 to 15, further comprising another extracellular ligand-binding domain which is not specific for BCMA.

17. The BCMA specific CAR of any one of claims 1 to 16, wherein the extracellular ligand-binding domain(s), the first transmembrane domain, and intracellular signaling domain(s) are on a single polypeptide.

18. The BCMA specific CAR of any one of claims 1 to 9, further comprising a second transmembrane domain, wherein the first transmembrane domain and the extracellular ligand-binding domain(s) are on a first polypeptide, and wherein the second transmembrane domain and the intracellular signaling domain(s) are on a second polypeptide, wherein the first transmembrane domain comprises a transmembrane domain from the α chain of the high-affinity IgE receptor (FcεRI) and the second transmembrane domain comprises a transmembrane domain from the γ or β chain of FcεRI.

19. The BCMA specific CAR of claim 18, further comprising a third polypeptide comprising a third transmembrane domain fused to an intracellular signaling domain from a co-stimulatory molecule, wherein the third transmembrane domain comprises a transmembrane domain from the γ or β chain of FcεRI.

20. A polynucleotide comprising a nucleic acid sequence encoding the BCMA specific CAR of any one of claims 1 to 19.

21. The polynucleotide of claim 20, wherein the polynucleotide encodes the BCMA specific CAR encoded by the nucleic acid sequence of the vector having ATCC Accession No. PTA-122834.

22. The polynucleotide of claim 20, wherein the polynucleotide comprises the nucleic acid sequence shown in SEQ ID NO: 399.

23. An expression vector comprising the polynucleotide of any one of claims 20 to 22.

24. An engineered immune cell expressing at its cell surface membrane a BCMA specific CAR of any one of claims 1 to 19.

25. The engineered immune cell of claim 24, further comprising another CAR which is not specific for BCMA.

26. The engineered immune cell of claim 24 or 25, further comprising a polynucleotide encoding a suicide polypeptide.

27. The engineered immune cell of claim 26, wherein the suicide polypeptide is RQR8.

28. The engineered immune cell of any one of claims 24 to 27, wherein the immune cell is derived from an inflammatory T-lymphocyte, a cytotoxic T-lymphocyte, a regulatory T-lymphocyte, or a helper T-lymphocyte.

29. The engineered immune cell of claim any one of claims 24 to 28, further comprising a disruption one or more endogenous genes, wherein the endogenous gene encodes TCRα, TCRβ, CD52, glucocorticoid receptor (GR), deoxycytidine kinase (dCK), or an immune checkpoint protein such as for example programmed death-1 (PD-1).

30. The engineered immune cell of any one of claims 24 to 29, wherein the immune cell is obtained from a healthy donor.

31. The engineered immune cell of any one of claims 24 to 30, wherein the immune cell is obtained from a patient.

32. The engineered immune cell of any one of claims 24 to 31 for use as a medicament.

33. The engineered immune cell of claim 32, wherein the medicament is for use in treatment of a B-cell related cancer selecting from the group consisting of multiple myeloma, malignant plasma cell neoplasm, Hodgkin's lymphoma, nodular lymphocyte predominant Hodgkin's lymphoma, Kahler's disease and Myelomatosis, plasma cell leukemia, plasmacytoma, B-cell prolymphocytic leukemia, hairy cell leukemia, B-cell non-Hodgkin's lymphoma (NHL), acute myeloid leukemia (AML), chronic lymphocytic leukemia (CLL), acute lymphocytic leukemia (ALL), chronic myeloid leukemia (CML), follicular lymphoma, Burkitt's lymphoma, marginal zone lymphoma, mantle cell lymphoma, large cell lymphoma, precursor B-lymphoblastic lymphoma, myeloid leukemia, Waldenstrom's

macroglobulienemia, diffuse large B cell lymphoma, follicular lymphoma, marginal zone lymphoma, mucosa-associated lymphatic tissue lymphoma, small cell lymphocytic lymphoma, mantle cell lymphoma, Burkitt lymphoma, primary mediastinal (thymic) large B-cell lymphoma, lymphoplasmactyic lymphoma, Waldenström macroglobulinemia, nodal marginal zone B cell lymphoma, splenic marginal zone lymphoma, intravascular large B-cell lymphoma, primary effusion lymphoma, lymphomatoid granulomatosis, T cell/histiocyte-rich large B-cell lymphoma, primary central nervous system lymphoma, primary cutaneous diffuse large B-cell lymphoma (leg type), EBV positive diffuse large B-cell lymphoma of the elderly, diffuse large B-cell lymphoma associated with inflammation, intravascular large B-cell lymphoma, ALK-positive large B-cell lymphoma, plasmablastic lymphoma, large B-cell lymphoma arising in HHV8-associated multicentric Castleman disease, B-cell lymphoma unclassified with features intermediate between diffuse large B-cell lymphoma and Burkitt lymphoma, B-cell lymphoma unclassified with features intermediate between diffuse large B-cell lymphoma and classical Hodgkin lymphoma, and other B-cell related lymphoma.

34. A method of engineering an immune cell comprising:

a. providing an immune cell; and

b. expressing at the surface of the cell at least one BCMA specific CAR according to any one of claims 1 to 19.

35. The method of engineering an immune cell of claim 34 comprising:

a. providing an immune cell;

b. introducing into the cell at least one polynucleotide encoding said BCMA specific CAR; and

c. expressing said polynucleotide into the cell.

36. The method of engineering an immune cell of claim 34 comprising:

a. providing an immune cell;

b. introducing into the cell at least one polynucleotide encoding said BCMA specific CAR; and

c. introducing at least one other CAR which is not specific for BCMA.

37. A method of treating a subject in need thereof comprising:

a. providing a immune cell expressing at the surface a BCMA specific CAR according to any one of claims 1 to 19; and

b. administering said immune cells to said patient.

38. A pharmaceutical composition comprising the engineered immune cell of any one of claims 24 to 31.

39. A method of treating a condition associated with malignant cells expressing BCMA in a subject comprising administering to a subject in need thereof an effective amount of the pharmaceutical composition of claim 38.

40. The method of claim 39, wherein the condition is a cancer.

41. The method of claim 40, wherein the cancer is a B-cell related cancer selecting from the group consisting of multiple myeloma, malignant plasma cell neoplasm, Hodgkin's lymphoma, nodular lymphocyte predominant Hodgkin's lymphoma, Kahler's disease and Myelomatosis, plasma cell leukemia, plasmacytoma, B-cell prolymphocytic leukemia, hairy cell leukemia, B-cell non-Hodgkin's lymphoma (NHL), acute myeloid leukemia (AML), chronic lymphocytic leukemia (CLL), acute lymphocytic leukemia (ALL), chronic myeloid leukemia (CML), follicular lymphoma, Burkitt's lymphoma, marginal zone lymphoma, mantle cell lymphoma, large cell lymphoma, precursor B-lymphoblastic lymphoma, myeloid leukemia, Waldenstrom's macroglobulienemia, diffuse large B cell lymphoma, follicular lymphoma, marginal zone lymphoma, mucosa-associated lymphatic tissue lymphoma, small cell lymphocytic lymphoma, mantle cell lymphoma, Burkitt lymphoma, primary

mediastinal (thymic) large B-cell lymphoma, lymphoplasmactyic lymphoma, Waldenström macroglobulinemia, nodal marginal zone B cell lymphoma, splenic marginal zone lymphoma, intravascular large B-cell lymphoma, primary effusion lymphoma, lymphomatoid granulomatosis, T cell/histiocyte-rich large B-cell lymphoma, primary central nervous system lymphoma, primary cutaneous diffuse large B-cell lymphoma (leg type), EBV positive diffuse large B-cell lymphoma of the elderly, diffuse large B-cell lymphoma associated with inflammation, intravascular large B-cell lymphoma, ALK-positive large B-cell lymphoma, plasmablastic lymphoma, large B-cell lymphoma arising in HHV8-associated multicentric Castleman disease, B-cell lymphoma unclassified with features intermediate between diffuse large B-cell lymphoma and Burkitt lymphoma, B-cell lymphoma unclassified with features intermediate between diffuse large B-cell lymphoma and classical Hodgkin lymphoma, and other B-cell related lymphoma.

42. A method of inhibiting tumor growth or progression in a subject who has malignant cells expressing BCMA, comprising administering to the subject in need thereof an effective amount of the pharmaceutical composition of claim 38 to the subject.

43. A method of inhibiting metastasis of malignant cells expressing BCMA in a subject, comprising administering to the subject in need thereof an effective amount of the pharmaceutical composition of claim 38 to the subject.

44. A method of inducing tumor regression in a subject who has malignant cells expressing BCMA, comprising administering to the subject in need thereof an effective amount of the pharmaceutical composition of claim 38 to the subject.

45. The method of any one of claims 39 to 44, wherein the method further comprises administering a nucleoside analog therapy to the subject.

46. The method of claim 45, wherein the nucleoside analog therapy is fludarabine or clofarabine.