

MODIFIED B CELLS AND METHODS OF USE THEREOF

BACKGROUND OF THE INVENTION

[0001] Most cellular immunotherapies to date have focused in T cells. For example, cancer immunotherapies are primarily focused on modification and administration of T cells – enhancing the killer T cell response to a tumor. Modifying B cells for the treatment of various disease, however, is a technique that has not been extensively studied, despite the critical role of B cells in immune responses.

[0002] B cells, also known as B lymphocytes, are a type of white blood cell responsible for, among other things, helping the body resist infection and diseases. They are part of our adaptive immune system, and are capable of various immune responses, for example, secreting antibodies in response to a recognized antigen. Additionally, B cells are capable of presenting antigens, and can also secrete cytokines.

[0003] Many B cells mature into plasma cells that produce antibodies (proteins) capable of fighting off infections. Other B cells mature into memory B cells. All plasma cells descended from a single B cell produce the same antibody that is directed against the antigen that stimulated it to mature. The same principle holds with memory B cells. Thus, all plasma cells and memory cells "remember" the stimulus that led to their formation. The B cell, or B lymphocyte, is not thymus-dependent, has a short lifespan, and is responsible for the production of immunoglobulins. *See e.g.*, <https://www.medicinenet.com/script/main/art.asp?articlekey=2413>. The B cell is thus an immunologically important cell.

[0004] B cells appear to be associated with patient outcomes in the treatment of cancer. For example, the presence of tertiary lymphoid structures (TLSs) is associated with better patient outcomes. *See, e.g.*, Helmink, B.A., *et al.*, *Nature*, 2020, 577(7791), 549-555; Petitprez F *et al.*, *Nature*, 2020, 577(7791), 556-560. TLSs are aggregates of immune cells (mostly T and B cells) that arise in response to immunological stimuli. While TLSs that surround tumor cells include B cells, the role of B cells in antitumor responses have been unclear. B cells found in tumors can produce inhibitory factors that hinder the function of immune cells. *See, e.g.*, Kessel, A., *et al.*, *Autoimmun Rev.*, 2012, 11(9), 670-677; Khan, A.R., *et al.*, *Nature Commun.*, 2015, 6, 5997. Further, current evidence indicates that B cells impede antitumor responses in most mouse models of cancer. Affara, N.I., *et al.* *Cancer Cell*, 2014, 25(6), 809-821; Shalapour, S., *et al.*, *Nature*, 2017, 551, 340-345; Ammirante, M. *et al.*, *Nature*, 2010, 464, 302-305. Yet, the presence of B cells in TLS structures is correlated with positive clinical outcomes to cancer immunotherapy. Petitprez 2020. Intratumoral injection of LPS-activated spleen cells, which include B cells, in combination

with checkpoint inhibitors has been shown to produce anti-tumor responses. Soldevilla *et al.*, *Oncoimmunology*, 2018, 7:8, e1450711.

[0005] CD79 (also termed “Cluster of Differentiation 79”) is a transmembrane protein that forms a complex with the B-cell receptor and is capable of generating a signal following recognition of an antigen by the B-cell receptor.¹ CD79 is comprised of two different chains known as CD79A and CD79B (also termed Ig α and Ig β). CD79a and CD79b are both members of the immunoglobulin superfamily. These form a heterodimer on the surface of B cells stabilized by disulfide bonding. Both CD79 chains contain an immunoreceptor tyrosine-based activation motif (“ITAM”) in their intracellular tail regions that propagate a signal in a B cell.²

[0006] Given the natural ability of B cells to present antigens and secrete proteins, there is great potential as a cellular therapy for targeting certain diseased cell types and secreting therapeutic payloads. There thus exists a need for alternative treatments beyond T cell therapies, such as engineered B cells, for the treatment of a variety of diseases and disorders, including cancer, heart disease, inflammatory disease, muscle wasting disease, neurological disease, and the like.

SUMMARY OF THE INVENTION

[0007] It has now been found that engineered B cells can be efficacious in the treatment of various diseases and disorders as recited herein. The invention therefore relates to modified B cells.

[0008] It has also been found that CD79a (Immunoglobulin α) when incorporated into the intracellular signaling domain of the CAR-B constructs of the invention exhibits superior qualities over CD79b (Immunoglobulin β). Further, it has further been found that when used in the CAR-B constructs described herein, intracellular CD79b (Immunoglobulin β) displays no (or even a negative effect) on efficacy. The invention thus relates to, *inter alia*, CAR-B constructs comprising the CD79a intracellular signaling domain.

[0009] In certain embodiments, the invention relates to an isolated modified B cell (“CAR-B cell”), capable of expressing a chimeric receptor (“CAR-B receptor”), wherein said chimeric receptor comprises (a) an extracellular domain; (b) a transmembrane domain; and (c) a cytoplasmic domain that comprises at least one signaling domain. The cytoplasmic domain preferably comprises CD79a. In various embodiments, the extracellular domain comprises an extracellular binding domain and a hinge domain. In various embodiments, the extracellular binding domain(s) recognizes at least one antigen or protein expressed on the surface of a target cell. In various

¹ See Chu PG, Arber DA (June 2001); CD79: a review; *Applied Immunohistochemistry & Molecular Morphology*. 9 (2): 97–106. doi:10.1097/00022744-200106000-00001. PMID 11396639. See also <https://en.wikipedia.org/wiki/CD79>.

² See Müller B, Cooper L, Terhorst C (Jan. 1995), Interplay between the human TCR/CD3 epsilon and the B-cell antigen receptor associated Ig-beta (B29); *Immunology Letters*. 44 (2–3): 97–103. doi:10.1016/0165-2478(94)00199-2. PMID 7541024.

embodiments, the target cell is selected from the group consisting of a tumor cell, cardiac muscle cell, a skeletal muscle cell, a bone cell, a blood cell, a nerve cell, a fat cell, a skin cell, and an endothelial cell. In various embodiments, the B cell expresses more than one CAR-B receptor construct. In various embodiments, the CAR-B receptor comprises more than one extracellular binding domain. In various embodiments, the extracellular binding domain is a single chain variable fragment (scFv), or a full-length antibody, or the extracellular domain of a receptor or ligand. In various embodiments, the extracellular binding domain is capable of binding to an antigen or protein selected from the group consisting of: PSMA, GPC3, ASGR1, ASGR2, Sarcoglycan, Corin, FAP (fibroblast activation protein) and Her2. In various embodiments, the hinge domain is derived from the group consisting of IgG, CD28 and CD8. In various embodiments, the hinge domain is comprised of a nucleic acid sequence selected from the group consisting of SEQ ID Nos. 27, 29, 31. In various embodiments, the cytoplasmic domain comprises at least one signaling domain native to B cell receptors. In various embodiments, the cytoplasmic domain comprises a domain that is selected from the group consisting of: CD79a (Immunoglobulin α), CD79b (Immunoglobulin β), CD40, CD19, CD137, Fc γ 2a, MyD88, CD21, Syk, FYN, LYN, PI3K, BTK, PLC γ 2, CD3 ζ and BLNK. In various embodiments, the cytoplasmic domain further comprises a costimulatory domain.

[0010] In various embodiments, the invention comprises an isolated modified B cell, wherein said B cell is capable of expressing and secreting a payload, wherein the payload is not naturally expressed in a B cell or is expressed at higher levels than is naturally expressed in a B cell. In various embodiments, the payload is an antibody or fragment thereof. In various embodiments, the antibody is a secreted antibody and can include blocking antibodies (eg anti-PD-1) or agonist antibodies (anti-CD137, GITR, OX40) engineered to contain native or engineered Fc regions and can be soluble or membrane-bound. In various embodiments, the payload(s) can be immune modifiers such as chemokines or cytokines. In various embodiments, the payload is selected from the group consisting of: IL-1, IL-7, IL-8, IL-10, IL-12, IL-13, IL-17, IL18, IL-21, interferon α , interferon β , interferon γ , TSLP, CCL21, FLT3L, XCL1, LIGHT(TNFSF14), OX40L, CD137L, CD40L, ICOSL, anti-CD3 antibody, CD47, TIM4-FC, CXCL13, CCL21, CD80, CD40L, IFN α A2, LIGHT, 4-1BBL, MDGF (C19orf10), FGF10, PDGF, agrin, TNF- α , GM-CSF, an anti-FAP antibody, an anti-TGF- β antibody; a TGF- β trap, decoy or other inhibitory molecule; an anti-BMP antibody; a BMP trap, decoy or other inhibitory molecule. In various embodiments, the B cell is capable of expressing more than one payload. In various embodiments, the B cell is capable of expressing more than 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 payloads.

[0011] In various embodiments, the invention relates to a method of treating a patient comprising administering the modified B cell of the present invention. In various embodiments, the modified

B cell is administered intra-tumorally, intravenously, subcutaneously, or intradermally. In various embodiments, the method further comprises administering a checkpoint inhibitor. In various embodiments, the checkpoint inhibitor to a checkpoint molecule that is selected from the group consisting of PD-1, PD-L1, CTLA-4, LAG3, TIM-3 and NKG2A proteins. In various embodiments, the checkpoint inhibitor is a monoclonal antibody.

[0012] In various embodiments, the invention relates to an isolated modified B cell, capable of expressing a chimeric receptor, wherein said chimeric receptor comprises (a) an extracellular domain, wherein the extracellular domain comprises an extracellular binding domain and a hinge domain; (b) a transmembrane domain; and (c) a cytoplasmic domain that comprises at least one signaling domain, wherein said modified B cell is further capable of expressing a payload, wherein the payload is not naturally expressed on the surface of a cell. In various embodiments, the extracellular binding domain recognizes an antigen or protein expressed on the surface of a target cell. In various embodiments, the target cell is selected from the group consisting of a tumor cell, a cardiac muscle cell, a skeletal muscle cell, a bone cell, a blood cell, a nerve cell, a fat cell, a skin cell and an endothelial cell. In various embodiments, the B cell expresses more than one CAR-B receptor construct. In various embodiments, the CAR-B receptor comprises more than one extracellular binding domain. In various embodiments, the extracellular binding domain is a single chain variable fragment (scFv), an antibody, or the extracellular domain of a receptor or ligand. In various embodiments, the extracellular binding domain is capable of binding to an antigen or protein selected from the group consisting of PSMA, GP3, ASGR1, ASGR2, Sarcoglycan, Corin, FAP and Her2. In various embodiments, the hinge domain is derived from the group consisting of IgG, CD28 and CD8. In various embodiments, the hinge domain is comprised of a nucleic acid sequence selected from the group consisting of SEQ ID Nos. 27, 29, and 31. In various embodiments, the cytoplasmic domain comprises at least one signaling domain native to B cells. In various embodiments, the cytoplasmic domain comprises a domain selected from the group consisting of: CD79a (Immunoglobulin α), CD79b (Immunoglobulin β), CD40, CD19, CD137, Fc γ 2a, MyD88, CD21, Syk, FYN, LYN, PI3K, BTK, PLC γ 2, CD3 ζ and BLNK. In various embodiments, the cytoplasmic domain further comprises a costimulatory domain. In various embodiments, the payload is a secreted or membrane bound antibody or fragment thereof. In various embodiments, the payload is selected from the group consisting of: IL-1, IL-7, IL-8, IL-10, IL-12, IL-13, IL-17, IL-18, IL-21, interferon α , interferon β , interferon γ , TSLP, CCL21, FLT3L, XCL1, LIGHT(TNFSF14), OX40L, CD137L, CD40L, ICOSL, anti-CD3 antibody, CD47, TIM4-FC, CXCL13, CCL21, CD80, CD40L, IFN α A2, LIGHT, 4-1BBL, MDGF (C19orf10), FGF10, PDGF, agrin, TNF- α , GM-CSF, an anti-FAP antibody, an anti-TGF- β antibody; a TGF- β trap, decoy or other inhibitory molecule; an anti-BMP antibody; a BMP trap, decoy or other inhibitory

molecule. In various embodiments, the B cell is capable of expressing more than one payload. In various embodiments, the B cell is capable of expressing more than 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 payloads. In various embodiments, the modified B cell further encodes at least one protein selected from the group consisting of: the cytoplasmic domains of CD79a, CD79b, CD40, CD19, CD137, Fcγ2a, CD38 and MyD88. In various embodiments, the invention relates to a method of treating a patient comprising administering the modified B cell. In various embodiments, the method further comprises administering a checkpoint inhibitor. In various embodiments, the checkpoint inhibitor is selected from inhibitors to one or more checkpoint molecules from the group consisting of: PD-1, PD-L1, CTLA-4, LAG3, TIM-3 and NKG2A. In various embodiments, the checkpoint inhibitor is a monoclonal antibody. In various embodiments, the present invention relates to an isolated modified B cell, capable of expressing a chimeric receptor, wherein said chimeric receptor comprises an extracellular domain, wherein said extracellular domain comprises a hinge domain and an extracellular binding domain, wherein said extracellular binding domain is not naturally expressed on a B cell; and wherein said extracellular binding domain is capable of recognizing a target of interest. In various embodiments, the binding domain is a single chain variable fragment (scFv), antibody, ligand or receptor. In various embodiments, the binding domain is an scFv. In various embodiments, the binding domain is a receptor, a ligand, or a fragment thereof. In various embodiments, the B cell is further capable of expressing a payload. In various embodiments, the invention comprises a method of treating a patient comprising administering the modified B cell to a patient.

[0013] In various embodiments, the present invention comprises a nucleic acid capable of expressing a chimeric B cell receptor, wherein said chimeric receptor comprises: (a) an extracellular domain, wherein said extracellular domain comprises an extracellular binding domain and a hinge domain; (b) a transmembrane domain; and (c) a cytoplasmic domain that comprises at least one signaling domain. In various embodiments, the extracellular binding domain, recognizes an antigen or protein expressed on the surface of a target cell. In various embodiments, the extracellular binding domain is a single chain variable fragment (scFv), antibody, receptor or ligand. In various embodiments, the target cell is selected from the group consisting of a tumor cell, a cardiac muscle cell, a skeletal muscle cell, a bone cell, a blood cell, a nerve cell, a fat cell, a skin cell and an endothelial cell. In various embodiments, the vector expresses more than one CAR-B receptor. In various embodiments, the CAR-B receptor expresses more than one extracellular binding domain. In various embodiments, the extracellular binding domain is capable of binding to an antigen or protein selected from the group consisting of: PSMA, GP3, ASGR1, ASGR2, Sarcoglycan, Corin, Her2, FAP, MUC1, CEA153, JAM-1, and LFA-1. In various embodiments, the hinge domain is derived from the group consisting of IgG, CD28 and CD8. In various embodiments, the hinge

domain is comprised of a nucleic acid sequence selected from the group consisting of SEQ ID Nos. 27, 29, and 31. In various embodiments, the cytoplasmic domain comprises at least one signaling domain native to B cell receptors. In various embodiments, the cytoplasmic domain comprises a domain selected from the group consisting of: CD79a (Immunoglobulin α), CD79b (Immunoglobulin β), CD40, CD19, CD137, Fc γ 2a, MyD88, CD21, Syk, FYN, LYN, PI3K, BTK, PLC γ 2, CD3 ζ and BLNK. In various embodiments, the cytoplasmic domain further comprises a costimulatory domain.

[0014] In various embodiments, the invention relates to a vector comprising a nucleic acid capable of expressing a chimeric B cell receptor, wherein said chimeric receptor comprises: (a) an extracellular domain, wherein said extracellular domain comprises an extracellular binding domain and a hinge domain; (b) a transmembrane domain; and (c) a cytoplasmic domain that comprises at least one signaling domain. In various embodiments, the extracellular binding domain recognizes an antigen or protein. In various embodiments, the target cell is selected from the group consisting of a tumor cell, a cardiac muscle cell, a skeletal muscle cell, a bone cell, a blood cell, a nerve cell, a fat cell, a skin cell and an endothelial cell. In various embodiments, the vector expresses more than one CAR-B receptor. In various embodiments, the CAR-B expresses more than one extracellular binding domain. In various embodiments, the extracellular binding domain is a single chain variable fragment (scFv), antibody, receptor or ligand. In various embodiments, the extracellular binding domain is capable of binding to an antigen or protein selected from the group consisting of: PSMA, GPC3, ASGR1, AGSR2, Sarcoglycan, Corin, Her2, FAP, MUC1, CEA153, JAM-1, and LFA-1. In various embodiments, the hinge domain is derived from the group consisting of IgG, CD28 and CD8. In various embodiments, the hinge domain is comprised of a nucleic acid sequence selected from the group consisting of SEQ ID Nos. 27, 29, and 31. In various embodiments, the cytoplasmic domain comprises at least one signaling domain native to B cells. In various embodiments, the cytoplasmic domain comprises a signaling domain selected from the group consisting of: CD79a (Immunoglobulin α), CD79b (Immunoglobulin β), CD40, CD19, CD137, Fc γ 2a, MyD88, CD21, Syk, FYN, LYN, PI3K, BTK, PLC γ 2, CD3 ζ and BLNK. In various embodiments, the cytoplasmic domain further comprises a costimulatory domain. The various embodiments, the cytoplasmic region is comprised of multiple, 2 or more, domains, being either identical or unique.

[0015] In various embodiments, the invention relates to an isolated modified B cell, capable of expressing an integrin, a homing antibody, protein, a receptor, or combinations thereof, wherein said integrin, homing antibody, protein, or receptor is not naturally expressed in a B cell or is expressed at higher levels than is naturally expressed in a B cell; and wherein said integrin, homing antibody, protein, receptor, or combinations thereof is attracted to a site or target of interest. In

various embodiments, the integrin, homing antibody, protein, and receptor is selected from CLA (PSGL-1 glycoform), CLA (PSGL-1 glycoform), CCR10, CCR3, CCR4, CCR5, CCR6, CCR9, CD43E, CD44, c-Met, CXCR3, CXCR4, LFA-1, LFA-1 (α L β 2), selectin ligands, VLA-4, VLA-4 (α 4 β 1), and α 4 β 7, or combinations thereof. In various embodiments, the site of interest is a homing or target tissue, an inflammatory site in a specific location or tissue, or a tumor or tumor microenvironment, where delivery of payloads is desirable. In various embodiments, the homing or target tissue is selected from skin, gut (intestine, colon, mesenteric lymph nodes (mLN), Peyer's Patch (PP), small intestine), liver, lung, bone marrow, heart, peripheral lymph node (LN), CNS, thymus, and bone marrow. In various embodiments, the target of interest is selected from CXCL16, CCL17, CCL17(22), CCL20 (MIP-3 α), CCL21, CCL25, CCL27, CCL28, CCL4, CCL5, CD62E, CD62P, CXCL10, CXCL12, CXCL13, CXCL16, CXCL9/CXCL10, CXCR3, E/P-selectin, E-selectin, GPR15L, HGF, Hyaluronate, ICAM-1, ligands for CCR1, 2, 5, MAdCAM, MAdCAM-1, PNA^d, VAP-1, VCAM, and VCAM-1, or combinations thereof. In various embodiments, the method comprises treating a patient by administering the isolated modified B cell. In various embodiments, the method involves further administering a compound or a derivative thereof, wherein the compound or derivative thereof is capable of increasing the expression of the integrin, homing antibody, protein, and receptor, or combinations thereof. In various embodiments, the compound or a derivative thereof is capable of altering trafficking of B cells to a site or target of interest in the patient. In various embodiments, the compound is all-trans-retinoic acid (ATRA) or a derivative thereof.

[0016] In various embodiments, the invention relates to an isolated modified B cell, capable of expressing an immune inhibitory molecule, wherein said immune inhibitory molecule is not naturally expressed in a B cell or is expressed at higher levels than is naturally expressed in a B cell. In various embodiments, said immune inhibitory molecule is selected from IL-10, TGF- β , PD-L1, PD-L2, LAG-3, and TIM-3, or combinations thereof. In various embodiments, said immune inhibitory molecule is capable of decreasing inflammation and autoimmune activity of B cells at a site or target of interest in a patient. In various embodiments, the invention relates to a method of treating a patient comprising administering said isolated modified B cell. In various embodiments, said immune inhibitory molecule is selected from IL-10, TGF- β , PD-L1, PD-L2, LAG-3, and TIM-3, or combinations thereof. In various embodiments, said immune inhibitory molecule is capable of decreasing inflammation and autoimmune activity of B cells at a site or target of interest in the patient. In various embodiments, the invention relates to further administering a compound or a derivative thereof capable of increasing the expression of an integrin, a homing antibody, a protein, a receptor, or combinations thereof in the B cell. In various embodiments, said compound or derivative thereof is capable of altering trafficking of B cells to a

site or target of interest in the patient. In various embodiments, said compound is all-trans-retinoic acid (ATRA) or a derivative thereof. In various embodiments, the invention relates to an isolated modified B cell, wherein the isolated modified B cell is treated with a compound or a derivative thereof, wherein said compound or derivative thereof is capable of increasing the expression of an integrin, a homing antibody, a protein, a receptor, or combinations thereof in B cells. In various embodiments, said compound or derivative thereof is capable of altering trafficking of B cells to a site or target of interest in the patient. In various embodiments, said compound is all-trans-retinoic acid (ATRA) or a derivative thereof. In various embodiments, said compound or derivative thereof is capable of (i) increasing the expression of an integrin, a homing antibody, a protein, a receptor, or combinations thereof in B cells, and (ii) altering trafficking of B cells to a site or target of interest in the patient. In various embodiments, said compound is all-trans-retinoic acid (ATRA) or a derivative thereof.

[0017] In various embodiments, the invention relates to an isolated modified B cell, capable of expressing at least one or more of a constitutively active Toll-like receptor (TLR), wherein said TLR is not naturally expressed in a B cell or is expressed at higher levels than is naturally expressed in a B cell. In various embodiments, said TLR is selected from TLR1, TLR2, TLR3, TLR4, TLR5, TLR6, TLR7, TLR8, TLR9, TLR10, TLR11, TLR12, and TLR13, or combinations thereof. In various embodiments, said TLR is capable of potentiating B cells for increasing immune responses in a patient. In various embodiments, said TLR is capable of producing potent effector B cells for increasing immune responses in a patient. In various embodiments, said immune inhibitory molecule is capable of decreasing inflammation and autoimmune activity of B cells at a site or target of interest in a patient. In various embodiments, said TLR is selected from TLR1, TLR2, TLR3, TLR4, TLR5, TLR6, TLR7, TLR8, TLR9, TLR10, TLR11, TLR12, and TLR13, or combinations thereof. In various embodiments, said TLR is capable of (i) potentiating B cells, and (ii) producing potent effector B cells, for increasing immune responses in a patient. In various embodiments, at least one or more of a TLR agonist is administered to the patient. In various embodiments, the isolated modified B cell is treated with at least one or more of a TLR agonist. In various embodiments, said TLR agonist is capable of (i) potentiating B cells, and (ii) producing potent effector B cells, for increasing immune responses in a patient. In various embodiments, said TLR agonist binds to one or more TLRs selected from TLR1, TLR2, TLR3, TLR4, TLR5, TLR6, TLR7, TLR8, TLR9, TLR10, TLR11, TLR12, and TLR13, or combinations thereof. In various embodiments, said TLR agonist is selected from CpG-rich oligonucleotides, double-stranded RNA mimic, polyinosinic acid:polycytidylic acid (poly-I:C). In various embodiments, said TLR agonist comprises CpG oligonucleotides. In various embodiments, said TLR agonist is capable of (i) potentiating B cells, and (ii) producing potent effector B cells, for increasing immune

responses in the patient. In various embodiments, said TLR agonist binds to one or more TLRs selected from TLR1, TLR2, TLR3, TLR4, TLR5, TLR6, TLR7, TLR8, TLR9, TLR10, TLR11, TLR12, and TLR13, or combinations thereof. In various embodiments, said TLR agonist is selected from CpG-rich oligonucleotides, double-stranded RNA mimic, polyinosinic acid:polycytidylic acid (poly-I:C). In various embodiments, said TLR agonist comprises CpG oligonucleotides.

[0018] In various embodiments, the invention relates to an isolated modified B cell, wherein said B cell is electroporated with an mRNA encoding at least one or more of an antigen fused to a targeting signal. In various embodiments, said antigen is (i) not naturally presented by a B cell, (ii) not presented by a B cell simultaneously in both HLA class I and class II molecules naturally, or (iii) not presented by a B cell with high efficiencies in both HLA class I and class II molecules naturally. In various embodiments, said targeting signal is targeting signal of a lysosomal protein. In various embodiments, said targeting signal is a targeting signal of lysosome-associated membrane protein-1 (LAMP1). In various embodiments, said antigen is capable of being targeted to the lysosomes and presented simultaneously and efficiently in both HLA class I and class II molecules. In various embodiments, said B cells is capable of increasing antigen-specific immune responses in a patient. In various embodiments, said antigen is (i) not naturally presented by a B cell, (ii) not presented by a B cell simultaneously in both HLA class I and class II molecules naturally, or (iii) not presented by a B cell with high efficiencies in both HLA class I and class II molecules naturally. In various embodiments, said targeting signal is targeting signal of a lysosomal protein. In various embodiments, said targeting signal is a targeting signal of lysosome-associated membrane protein-1 (LAMP1). In various embodiments, said antigen is capable of being targeted to the lysosomes and presented simultaneously and efficiently in both HLA class I and class II molecules. In various embodiments, said B cells is capable of increasing antigen-specific immune responses in the patient.

BRIEF DESCRIPTION OF THE FIGURES

[0019] FIG. 1 sets forth an example of a chimeric B Cell Receptor (CAR-B) of the present invention. In certain embodiments, the CAR-B construct will comprise an extracellular domain, a transmembrane domain, and a cytoplasmic domain. As depicted in FIG. 1, the extracellular domain may in certain embodiments comprise a binding domain and a hinge region. In certain embodiments, the binding region may be an scFv. CAR-B constructs are cloned into a vector for expression.

[0020] FIGS. 2A-2C show examples of engineered B cells with homing domains. In various embodiments, the engineered B cells may comprise (a) an scFv binding domain and optional hinge

region; (b) an scFv binding domain directly linked to the cell through a transmembrane domain, or (c) a ligand/receptor binding domain directly linked to a cell through a transmembrane domain.

[0021] FIG. 3 shows examples of certain CAR-B constructs of the present invention. (A) CAR-B that binds GPC3. (B) CAR-B that binds PSMA.

[0022] FIG. 4 shows examples of CAR-B receptors of the present invention capable of binding (A) GPC3 and (B) PSMA. The "C" domain corresponds to the native BCR C-terminus.

[0023] FIG. 5 sets forth expression of various anti-PSMA CARs on the surface of HEK-293 cells.

[0024] FIGS. 6A-6C set forth a FACS Plot illustrating interrogation of binding of anti-PSMA CAR and of anti-sarcoglycan CAR to PSMA. B cells expressing anti-PSMA CAR-B constructs pWF396 and pWF397 bound PSMA whereas the B cells expressing pWF398 (anti-sarcoglycan CAR-B) did not bind PSMA.

[0025] FIG. 7 illustrates the ability of adenovirus F35 encoding GFP to transduce human B cells. Human B cells were isolated from peripheral blood. The B cells were infected with adenovirus encoding GFP. 0, 1, 3, 10 ul, representing the microliter volume of the adenovirus preparation used to infect human B cells. The titer of the adenovirus preparations were approximately 1×10^{12} particles/ml.

[0026] FIG. 8 describes an experiment where BALB/c mice were injected with CT26 bilateral tumors at day zero. At day 12 and day 16, tumor-bearing mice were injected intra-tumorally with payload-expressing cells at a volume of 10^6 in 50 μ L.

[0027] FIG. 9 illustrates the effect of 12 different combinations of payloads injected intra-tumorally on tumor volume over 30-35 days as compared to saline and 3T3 cells (without a payload).

[0028] FIG. 10 illustrates the effect of 12 different combinations of payloads injected intra-tumorally on tumor volume over 30-35 days as compared to saline and 3T3 cells (without a payload).

[0029] FIGS. 11A-11C illustrate the effect of the top three combinations of payloads injected intra-tumorally on tumor volume over 30 days as compared to saline and 3T3 cells (without a payload).

[0030] FIG. 12 illustrates the abscopal effect of intratumorally injected B cells. B cells were then injected either (i) fresh or (ii) first stimulated for 16-24 hours in growth media (RPMI, 10% FBS, 1% Pen/Strep, 5ng/ml recombinant mouse IL-4, 100uM beta-mercaptoethanol) with 5 μ g/ml Lipopolysaccharide. 5×10^6 B cells were then intratumorally injected into the CT26 mouse model, and anti-tumor responses in the distal (abscopal) tumor were measured. Tumors were implanted at day 0, and at day 6 palpable tumor mass was observed. Treatment was initiated on day 6 intratumorally.

[0031] FIGS. 13A-13C illustrates expression of three CAR-B receptors (also referred to as CAR-B receptors) in mouse B cells 24 hours post transfection.

[0032] FIG. 14 illustrates the efficacy of PSMA-specific CAR engineered murine B cells on tumor volume and survival in BALB/c mice with CT26-PSMA tumors.

[0033] FIG. 15 illustrates the efficacy of PSMA-specific CAR engineered allogenic B cells on tumor volume and survival in BALB/c mice with CT26-PSMA tumors.

[0034] FIGS. 16 illustrates the efficacy of PSMA-specific CAR engineered murine B cells on immunocompromised BALB/c mice with CT26-PSMA tumors.

[0035] FIG. 17 illustrates the efficacy of murine B cells on tumor volume and survival in C57B1/6 mice with HEPA 1-6 GPC3 tumors.

[0036] FIG. 18 illustrates NFKb signaling in luciferase reporter cells in B cells engineered with four different CAR constructs that recognized GPC3, using GFP as a control.

[0037] FIG. 19 illustrates basal or tonic NFKb activity in the absence of cognate target antigen in CAR constructs expressed in human B cell reporter line.

[0038] FIG. 20 illustrates the efficacy of murine B cells electroporated with anti-GPC3CAR-CD79a and a CD80 payload mRNAs in syngeneic C57B1/6 mice with HEPA1-6GPC3 tumors.

[0039] FIGS. 21A-21C illustrate the responses of the saline control, anti-GPC3CAR-CD79a, and anti-GPC3CAR-CD79a plus CD80 combo B cell groups.

[0040] FIGS. 22A-22C illustrate the expression of the GPC3 CAR post-electroporation, using FACS plots.

DETAILED DESCRIPTION

[0041] The invention disclosed herein relates to several embodiments of engineered or modified B cells:

1. B cells that have been modified to home to a site/target of interest, using, e.g., a binding domain such as an scFv, antibody, ligand, receptor, or fragments thereof;
2. B cells that have been modified with a homing domain, further comprising an activation, and optionally a costimulatory domain, such that the B cells can home and activate upon interaction with a desired target;
3. B cells engineered to be capable of making a desired protein payload, such as an antibody, therapeutic protein, polypeptide, nucleic acid sequence (such as RNAi) or the like;
4. Engineered B cells comprising a homing/binding domain, an activating domain, an optional costimulatory domain, and further engineered to express a desired protein payload, such as an antibody, therapeutic protein, polypeptide, nucleic acid sequence (such as RNAi) or the like;

5. B cells that have been modified to express an integrin, a homing antibody, protein, or a receptor, such that the B cells are attracted to specific ligands, chemokines, or attractants at a specific site/target of interest (e.g., a homing tissue) and can thereby home to the site/target of interest, for example, to deliver a desired payload;
6. B cells that have been modified to express an immune inhibitory molecule, such that the inflammation and autoimmune activity of B cells localized to a site/target of interest is decreased, thereby leading to a positive therapeutic response;
7. B cells that have been treated with a compound or derivatives thereof, such that trafficking of the B cells is altered by expression of specific B cell integrins and/or homing receptors;
8. B cells that have been (i) treated with a Toll-like receptor (TLR) agonist, and/or (ii) engineered to express a constitutively active TLR, for potentiating B cells and/or producing potent effector B cells for increasing immune responses in a subject;
9. B cells that have been electroporated with an mRNA encoding specific antigens of interest fused to a targeting signal of a lysosomal protein, such that the B cells can simultaneously and efficiently present the specific antigens and/or antigen-derived epitopes of interest in both HLA class I and class II molecules.
10. B cells that have been electroporated with a self-amplifying RNA that encodes any items noted heretofore in 1-9.

[0042] It is understood that the various embodiments of engineered or modified B cells of the present application are not mutually exclusive and can be combined with each other in any way and without any restriction unless explicitly indicated, for achieving or facilitating any of the results and/or therapeutic responses contemplated herein.

[0043] **Tumor Antigen.** In certain embodiments, the site/target of interest is a tumor antigen. The selection of the antigen-binding domain (moiety) of the invention will depend on the particular type of cancer to be treated. Some tumor antigens may be membrane bound, whereas other may be secreted. For example, a tumor antigen may be secreted and accumulate in the extracellular matrix, or the tumor antigen may be expressed as part of the MHC complex. Tumor antigens are well known in the art and may include, for example, CD19, KRAS, HGF, CLL, a glioma-associated antigen, carcinoembryonic antigen (CEA); β -human chorionic gonadotropin, alphafetoprotein (AFP), lectin-reactive AFP, thyroglobulin, RAGE-1, MN-CA IX, human telomerase reverse transcriptase, RU1, RU2 (AS), intestinal carboxyl esterase, mut hsp70-2, M-CSF, prostase, prostate-specific antigen (PSA), PAP, NY-ESO-1, LAGE-1a, p53, protein, PSMA, Her2/neu, survivin and telomerase, prostate-carcinoma tumor antigen-1 (PCTA-1), MAGE, ELF2M, neutrophil elastase, ephrinB2, CD22, insulin growth factor (IGF)-I, IGF-II, IGF-I receptor, mesothelin, EGFR, BCMA, KIT and IL-13.

[0044] Infectious Disease Antigen. In certain embodiments, the site/target of interest is an infectious disease antigen against which an immune response may be desired. Infectious disease antigens are well known in the art and may include, but are not limited to, viruses, bacteria, protists, and parasitic antigens, such as parasites, fungi, yeasts, mycoplasma, viral proteins, bacterial proteins and carbohydrates, and fungal proteins and carbohydrates. In addition, the type of infectious disease of the infectious disease antigen is not particularly limited, and may include, but are not limited to, intractable diseases among viral infectious diseases such as AIDS, hepatitis B, Epstein Barr Virus (EBV) infection, HPV infection, HCV infection, SARS, SARS-CoV2, etc. Parasitic antigens may include, but are not limited to, the malaria parasite sporozoite protein.

[0045] In certain embodiments the modified B cells express an engineered B cell receptor (CAR-B) comprising an extracellular domain, a transmembrane domain and an intracellular domain. In certain embodiments, the extracellular domain comprises a binding domain and a hinge domain. In certain embodiments, the extracellular domain comprises a binding domain, such as an scFv, ligand, antibody, receptor, or fragment thereof which allows the modified B cell to target specific target cells by binding to proteins expressed on the surface of those cells. In certain embodiments, the modified tumor cells target and bind to proteins/antigens expressed on the surface of tumor cells. In certain embodiments, the modified B cell further expresses a payload. In certain embodiments, the payload is capable of increasing the number of cross-presenting antigen or antigenic fragments to dendritic cells (DC) in tumors or in lymph nodes. In certain embodiments, the payload is capable of activating and attracting T cells into tumors. In certain embodiments, the payload is capable of fomenting the formation of tertiary lymphoid structures (TLS) in tumors. In certain embodiments of the invention, the modified B cell expresses both a CAR-B and a payload. In certain embodiments, the CAR-B comprises stimulatory domains that activate expression of the payload when bound to an antigen or protein expressed on the surface of a tumor cell.

1. Design and domain orientation of Chimeric Antigen Receptors in B Cells (CAR-Bs)

[0046] In various embodiments, the invention provides a chimeric B Cell Receptor (CAR-B). It will be appreciated that chimeric B cell receptors (CAR-Bs) are genetically engineered receptors. These engineered receptors can be readily inserted into and expressed by B cells in accordance with techniques known in the art. With a CAR-B, a single receptor can be programmed to both recognize a specific protein or antigen expressed on a tumor cell, and when bound to said protein or antigen elicit an anti-tumor response. In various embodiments, the CAR-Bs serve in part as a homing mechanism to deliver B cells to target tissue.

[0047] It will be appreciated that relative to the cell bearing the receptor, the chimeric B cell receptor of the invention will comprise an extracellular domain (which will comprise an antigen-binding domain and may comprise an extracellular signaling domain and/or a hinge domain), a

transmembrane domain, and an intracellular domain. The intracellular domain comprises at least an activating domain, preferably comprised of CD79a (Immunoglobulin α), CD79b (Immunoglobulin β), CD40, CD19, CD137, CD137 ζ , Fc γ r2a and/or MyD88. It will further be appreciated that the antigen-binding domain is engineered such that it is located in the extracellular portion of the molecule/construct, such that it is capable of recognizing and binding to its target or targets.

[0048] Structurally it will be appreciated that these domains correspond to locations relative to the immune cell. Exemplary CAR-B constructs in accordance with the invention are set forth in Table 1:

TABLE 1

Construct Name	Extracellular Domain	Hinge	TM	Signal 1	Signal 2
pWF-82	anti-PSMA	CD8	CD28	hCD19	
pWF-83	anti-PSMA	CD8	CD28	hCD40	
pWF-84	anti-PSMA	CD8	CD28	hCD40	CD79b
pWF-85	anti-PSMA	CD8	CD28	hCD40	CD137
pWF-86	anti-PSMA	CD8	CD28	hCD40	Fc γ r2a
pWF-87	anti-PSMA	CD8	CD28	hMyd88	hCD40
pWF-88	anti-PSMA	CD8	CD28	CD79a	
pWF-89	anti-PSMA	CD8	CD28	CD79b	
pWF-391	anti-PSMA	3x strep II tag	CD28	CD79b	
pWF-394	anti-Sarcoglycan	3x strep II tag	CD28	CD79b	
pWF-396	anti-GPC-3	CD8	CD28	CD79a	
pWF-397	anti-GPC-3	CD8	CD28	CD79b	
pWF-460	anti-GPC-3	Human IgG1 Fc	CD28	CD79a	
pWF428	anti-GPC-3	Human Lambda Constant region	Human Lambda Constant region		
pWF429	anti-GPC-3	Human IgG1 Fc	Human IgG1 Fc		
pWF-521	Anti-GPC3 ν L-hclambda constant region-linker- ν H-hcH1-cH2-cH3	Human IgG1 Fc	Human IgG1	Endogenous BCR complex	
pWF-533	Anti-GPC3- ν L-hcH1		Human IgG1 (complex with pWF534)	Endogenous BCR complex	
pWF-534	Anti-GPC3- ν H-hcKappa-hcH2-cH3	Human IgG1 Fc	Human IgG1	Endogenous BCR complex	

[0049] In various embodiments, chimeric B cell receptors are comprised of an extracellular domain, a transmembrane domain and a cytoplasmic domain. In various embodiments, the cytoplasmic domain comprises an activating domain. In various embodiments, the cytoplasmic domain may also comprise a co-stimulatory domain. In various embodiments, the extracellular domain comprises an antigen-binding domain. In various embodiments, the extracellular domain further comprises a hinge region between the antigen-binding domain and the transmembrane domain. FIG. 1 provides a schematic representation of a chimeric B cell receptor of various embodiments of the present invention.

[0050] **Extracellular Domain.** A number of extracellular domains may be used with the present invention. In various embodiments, the extracellular domain comprises an antigen-binding domain. In various embodiments, the extracellular domain may also comprise a hinge region and/or a signaling domain. In various embodiments, the extracellular domains containing IgG1 constant domain may also comprise either IgG1 (hole) or IgG1 (knob) to facilitate directed CAR-B formation.

[0051] **Antigen-Binding Domain and Binding Domain.** As used herein, an “antigen binding domain,” “antigen-binding domain” or “binding domain” refers to a portion of the CAR-B capable of binding an antigen or protein expressed on the surface of a cell. In some embodiments, the antigen-binding domain binds to an antigen or protein on a cell involved in a hyperproliferative disease. In preferred embodiments, the antigen-binding domain binds to an antigen or protein expressed on the surface of a tumor cell. The antigen-binding molecules will be further understood in view of the definitions and descriptions below.

[0052] An antigen-binding domain is said to “specifically bind” its target antigen or protein when the dissociation constant (K_d) is 1×10^{-7} M. The antigen-binding domain specifically binds antigen with “high affinity” when the K_d is $1-5 \times 10^{-9}$ M, and with “very high affinity” when the K_d is $1-5 \times 10^{-10}$ M. In one embodiment, the antigen-binding domain has a K_d of 10^{-9} M. In one embodiment, the off-rate is $< 1 \times 10^{-5}$. In other embodiments, the antigen-binding domain will bind to antigen or protein with a K_d of between about 10^{-7} M and 10^{-13} M, and in yet another embodiment the antigen-binding domain will bind with a K_d $1.0-5.0 \times 10^{-10}$.

[0053] An antigen-binding domain is said to be “selective” when it binds to one target more tightly than it binds to a second target.

[0054] The term “neutralizing” refers to an antigen-binding domain that binds to a ligand and prevents or reduces the biological effect of that ligand. This can be done, for example, by directly blocking a binding site on the ligand or by binding to the ligand and altering the ligand’s ability to bind through indirect means (such as structural or energetic alterations in the ligand). In some

embodiments, the term can also denote an antigen-binding domain that prevents the protein to which it is bound from performing a biological function.

[0055] The term “target” or “antigen” refers to a molecule or a portion of a molecule capable of being bound by an antigen-binding molecule. In certain embodiments, a target can have one or more epitopes.

[0056] The term “antibody” refers to what are known as immunoglobulins, Y-shaped proteins that are produced by the immune system to recognize a particular antigen. The term “antibody fragment” refers to antigen-binding fragments and Fc fragments of antibodies. Types of antigen-binding fragments include: F(ab')₂, Fab, Fab' and scFv molecules. Fc fragments are generated entirely from the heavy chain constant region of an immunoglobulin.

[0057] Extracellular Signaling Domains. The extracellular domain is beneficial for signaling and for an efficient response of lymphocytes to an antigen. Extracellular domains of particular use in this invention may be derived from (i.e., comprise) CD28, CD28T (*See e.g.*, U.S. Patent Application US2017/0283500A1), OX40, 4-1BB/CD137, CD2, CD7, CD27, CD30, CD40, programmed death-1 (PD-1), inducible T cell costimulator (ICOS), lymphocyte function-associated antigen-1 (LFA-1, CD1-1a/CD18), CD3 gamma, CD3 delta, CD3 epsilon, CD247, CD276 (B7-H3), LIGHT, (TNFSF14), NKG2C, CD79a (Immunoglobulin α), CD79b (Immunoglobulin β), DAP-10, Fc gamma receptor, MHC class I molecule, TNF receptor proteins, an Immunoglobulin protein, cytokine receptor, integrins, Signaling Lymphocytic Activation Molecules (SLAM proteins), activating NK cell receptors, BTLA, a Toll ligand receptor, ICAM-1, B7-H3, CDS, ICAM-1, GITR, BAFFR, LIGHT, HVEM (LIGHTR), KIRDS2, SLAMF7, NKp80 (KLRF1), NKp44, NKp30, NKp46, CD19, CD4, CD8alpha, CD8beta, IL-2R beta, IL-2R gamma, IL-7R alpha, ITGA4, VLA1, CD49a, ITGA4, IA4, CD49D, ITGA6, VLA-6, CD49f, ITGAD, CD11d, ITGAE, CD103, ITGAL, CD11a, LFA-1, ITGAM, CD11b, ITGAX, CD11c, ITGB1, CD29, ITGB2, CD18, LFA-1, ITGB7, NKG2D, TNFR2, TRANCE/RANKL, DNAM1 (CD226), SLAMF4 (CD244, 2B4), CD84, CD96 (Tactile), CEACAM1, CRT AM, Ly9 (CD229), CD160 (BY55), PSGL1, CD100 (SEMA4D), CD69, SLAMF6 (NTB-A, Ly108), SLAM (SLAMF1, CD150, IPO-3), BLAME (SLAMF8), SELPLG (CD162), LTBR, LAT, GADS, SLP-76, PAG/Cbp, CD19a, a ligand that specifically binds with CD83, or any combination thereof. The extracellular domain may be derived either from a natural or from a synthetic source.

[0058] Hinge Domains. As described herein, extracellular domains often comprise a hinge portion. This is a portion of the extracellular domain proximal to the cell membrane. The extracellular domain may further comprise a spacer region. A variety of hinges can be employed in accordance with the invention, including costimulatory molecules as discussed above, as well as immunoglobulin (Ig) sequences a 3X strep II spacer or other suitable molecules to achieve the

desired special distance from the target cell. In some embodiments, the hinge region comprises the extracellular domain of CD28, or CD8 or a portion thereof as described herein.

[0059] Transmembrane Domains. The CAR-B can be designed to comprise a transmembrane domain that is fused or otherwise linked to the extracellular domain of the CAR-B-B. It can similarly be fused to the intracellular domain of the CAR-B. In one embodiment, the transmembrane domain that naturally is associated with one of the domains in a CAR-B is used. In some instances, the transmembrane domain can be selected or modified by amino acid substitution to avoid binding of such domains to the transmembrane domains of the same or different surface membrane proteins to minimize interactions with other members of the receptor complex. The transmembrane domain may be derived either from a natural or from a synthetic source. Where the source is natural, the domain may be derived from any membrane-bound or transmembrane protein. Transmembrane regions of particular use in this invention may be derived from (*i.e.* comprise) CD28, CD28T, OX-40, 4-1BB/CD137, CD2, CD7, CD27, CD30, CD40, programmed death-1 (PD-1), inducible T cell costimulator (ICOS), lymphocyte function-associated antigen-1 (LFA-1, CD1-1a/CD18), CD3 gamma, CD3 delta, CD3 epsilon, CD247, CD276 (B7-H3), LIGHT, (TNFSF14), NKG2C, CD79a (Immunoglobulin α), CD79b (Immunoglobulin β), DAP-10, Fc gamma receptor, MHC class I molecule, TNF receptor proteins, an Immunoglobulin protein, cytokine receptor, integrins, Signaling Lymphocytic Activation Molecules (SLAM proteins), activating NK cell receptors, BTLA, a Toll ligand receptor, ICAM-1, B7-H3, CDS, ICAM-1, GITR, BAFFR, LIGHT, HVEM (LIGHTR), KIRDS2, SLAMF7, NKp80 (KLRF1), NKp44, NKp30, NKp46, CD19, CD4, CD8alpha, CD8beta, IL-2R beta, IL-2R gamma, IL-7R alpha, ITGA4, VLA1, CD49a, ITGA4, IA4, CD49D, ITGA6, VLA-6, CD49f, ITGAD, CD11d, ITGAE, CD103, ITGAL, CD1 1a, LFA-1, ITGAM, CD1 1b, ITGAX, CD1 1c, ITGB1, CD29, ITGB2, CD18, LFA-1, ITGB7, NKG2D, TNFR2, TRANCE/RANKL, DNAM1 (CD226), SLAMF4 (CD244, 2B4), CD84, CD96 (Tactile), CEACAM1, CRT AM, Ly9 (CD229), CD160 (BY55), PSGL1, CD100 (SEMA4D), CD69, SLAMF6 (NTB-A, Ly108), SLAM (SLAMF1, CD150, IPO-3), BLAME (SLAMF8), SELPLG (CD162), LTBR, LAT, GADS, SLP-76, PAG/Cbp, CD19a, a ligand that specifically binds with CD83, or any combination thereof.

[0060] Optionally, short linkers may form linkages between any or some of the extracellular, transmembrane, and intracellular domains of the CAR-B.

[0061] In certain embodiments, the transmembrane domain in the CAR-B of the invention is the CD28 transmembrane domain. In one embodiment, the CD28 transmembrane domain comprises the nucleic acid sequence of SEQ ID NO: 1. In one embodiment, the CD28 transmembrane domain comprises the nucleic acid sequence that encodes the amino acid sequence of SEQ ID NO: 2. In one embodiment, the CD28 transmembrane domain comprises the nucleic acid sequence of SEQ

ID NO: 3. In another embodiment, the CD28 transmembrane domain comprises the amino acid sequence of SEQ ID NO: 4.

[0062] In one embodiment, the transmembrane domain in the CAR-B of the invention is a CD8 transmembrane domain.

[0063] **Intracellular (Cytoplasmic) Domains.** The intracellular (IC, or cytoplasmic) domain of the CAR-B receptors of the invention can provide activation of at least one of the normal effector functions of the immune cell.

[0064] It will be appreciated that suitable intracellular molecules, include, but are not limited to CD79a (Immunoglobulin α), CD79b (Immunoglobulin β), CD40, CD19, CD137, Fc γ 2a CD3 ζ and MyD88. Intracellular molecules may further include CD28, CD28T, OX-40, 4-1BB/CD137, CD2, CD7, CD27, CD30, CD40, programmed death-1 (PD-1), inducible T cell costimulator (ICOS), lymphocyte function-associated antigen-1 (LFA-1, CD1-1a/CD18), CD3 gamma, CD3 delta, CD3 epsilon, CD247, CD276 (B7-H3), LIGHT, (TNFSF14), NKG2C, Ig alpha (CD79a), DAP-10, Fc gamma receptor, MHC class 1 molecule, TNF receptor proteins, an Immunoglobulin protein, cytokine receptor, integrins, Signaling Lymphocytic Activation Molecules (SLAM proteins), activating NK cell receptors, BTLA, a Toll ligand receptor, ICAM-1, B7-H3, CDS, ICAM-1, GITR, BAFR, LIGHT, HVEM (LIGHTR), KIRDS2, SLAMF7, NKp80 (KLRF1), NKp44, NKp30, NKp46, CD19, CD4, CD8alpha, CD8beta, IL-2R beta, IL-2R gamma, IL-7R alpha, ITGA4, VLA1, CD49a, ITGA4, IA4, CD49D, ITGA6, VLA-6, CD49f, ITGAD, CD11d, ITGAE, CD103, ITGAL, CD11a, LFA-1, ITGAM, CD11b, ITGAX, CD11c, ITGB1, CD29, ITGB2, CD18, LFA-1, ITGB7, NKG2D, TNFR2, TRANCE/ RANKL, DNAM1 (CD226), SLAMF4 (CD244, 2B4), CD84, CD96 (Tactile), CEACAM1, CRT AM, Ly9 (CD229), CD160 (BY55), PSGL1, CD100 (SEMA4D), CD69, SLAMF6 (NTB-A, Ly108), SLAM (SLAMF1, CD150, IPO-3), BLAME (SLAMF8), SELPLG (CD162), LTBR, LAT, GADS, SLP-76, PAG/Cbp, CD19a, a ligand that specifically binds with CD83, or any combination thereof. The cytoplasmic signaling sequences within the cytoplasmic signaling portion of the CAR-B of the invention may be linked to each other in a random or specified order.

[0065] The term “co-stimulatory” domain or molecule as used herein refers to a heterogeneous group of cell surface molecules that act to amplify or counteract initial activating signals of the cell.

[0066] In one preferred embodiment, the cytoplasmic domain is designed to comprise the signaling domain of hCD19, wherein the hCD19 domain comprises the nucleic acid sequence set forth in SEQ ID NO. 5. In another embodiment, the cytoplasmic domain is designed to comprise the signaling domain of hCD40, wherein the hCD40 domain comprises the nucleic acid sequence set forth in SEQ ID NO. 7. In another embodiment, the cytoplasmic domain is designed to comprise the signaling domain of hCD40 and hCD79b, wherein the hCD40 domain comprises the nucleic

acid sequence set forth in SEQ ID NO. 7 and the hCD79b domain comprises the nucleic acid sequence set forth in SEQ ID NO. 25. In another embodiment, the cytoplasmic domain is designed to comprise the signaling domain of hCD40 and hCD137, wherein the hCD40 domain comprises the nucleic acid sequence set forth in SEQ ID NO. 7 and the hCD137 domain comprises the nucleic acid sequence set forth in SEQ ID NO. 13. In another embodiment, the cytoplasmic domain is designed to comprise the signaling domain of hCD40 and hFcγ2a, wherein the hCD40 domain comprises the nucleic acid sequence set forth in SEQ ID NO. 7 and the hFcγ2a domain comprises the nucleic acid sequence set forth in SEQ ID NO. 17. In another embodiment, the cytoplasmic domain is designed to comprise the signaling domain of hCD40 and hMyd88, wherein the hCD40 domain comprises the nucleic acid sequence set forth in SEQ ID NO. 7 and the hMyd88 domain comprises the nucleic acid sequence set forth in SEQ ID NO. 21. In another embodiment, the cytoplasmic domain is designed to comprise the signaling domain of hCD79a, wherein the hCD79a domain comprises the nucleic acid sequence set forth in SEQ ID NO. 23. In another embodiment, the cytoplasmic domain is designed to comprise the signaling domain of hCD79b, wherein the hCD79b domain comprises the nucleic acid sequence set forth in SEQ ID NO. 25. These embodiments are preferably of human origin but may be derived from other species. In various embodiments the signaling domain comprises both hCD79a in tandem with hCD79b or another hCD79a domain. In various embodiments the signaling domain comprises both hCD79b in tandem with hCD79a or another hCD79b domain.

2. Modified B Cells

[0067] Modified B Cells that Express Payloads. In various embodiments of the present invention a modified B cell is provided that is capable of expressing a payload. As used herein the term “payload” refers to an amino acid sequence, a nucleic acid sequence encoding a peptide or protein, or an RNA molecule, for use as a therapeutic agent. In certain embodiments the payload is for delivery to the tumor or tumor microenvironment or the draining lymph node. In certain embodiments, it is desirable that the B cell deliver to the tumor or tumor microenvironment or draining lymph node a payload capable of, for example, increasing the number of cross-presenting dendritic cells (DCs) in tumors. Cross-presenting DCs will allow for improved presentation of tumor antigens. In various embodiments, the payload may be capable of activating and attracting T cells into tumors. Activating more T cells in tumors will complement the cross-presenting DCs to remold the tumor environment to have more potent antitumor immune capabilities. Payloads may also foment the formation of tertiary lymphoid structures (TLS) in tumors. Clinical studies have demonstrated that there is a relationship between B cells, TLS and responses to immune checkpoint blockade.

[0068] Nonexclusive examples of payloads of the present invention include: IL-1, IL-7, IL-8, IL-10, IL-12, IL-13, IL-17, IL-18, IL-21, interferon α , interferon β , interferon γ , TSLP, CCL21, FLT3L, XCL1, LIGHT(TNFSF14), OX40L, CD137L, CD40L, ICOSL, anti-CD3 antibody, CD47, TIM4-FC, CXCL13, CCL21, CD80, CD40L, IFN α A2, LIGHT, 4-1BBL, MDGF (C19orf10), FGF10, PDGF, agrin, TNF- α , GM-CSF, an anti-FAP antibody, an anti-TGF- β antibody; a TGF- β trap, decoy or other inhibitory molecule; an anti-BMP antibody; a BMP trap, decoy or other inhibitory molecule.

[0069] Signaling for Payload Expression. In various embodiments of the present invention, the payload is expressed in the modified B cell as a DNA construct under the control of an activated transcriptional pathway. In certain embodiments, the expression of the payload is controlled of the Nuclear Factor of Activated T cell ("NFAT") pathway. The NFAT pathway is a transcription factor pathway activated during an immune response and is activated by the NF κ B. In various embodiments, the modified B cell expresses both a payload and a CAR-B. In various embodiments, where the modified B cell expresses both a payload and a CAR-B, the CAR-B may further encode signaling molecules that induce activation of the NF κ B pathway. Such molecules include but are not limited to: CD79a (Immunoglobulin α), CD79b (Immunoglobulin β), CD40, CD19, CD137, Fc γ 2a, CD3 ζ and MyD88.

[0070] In various embodiments, the invention relates to isolated B cells that express at least one payload. In various embodiments, the invention relates to isolated B cells that express more than one payload. In various embodiments, the invention relates to isolated B cells that express 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 or 12 different payloads.

[0071] Modification of B Cells for homing. In various embodiments of the present invention, the engineered B cells can be modified with homing domains (e.g., as illustrated in FIG. 2) such that the B cells can home to a site/target of interest and activate upon interaction with the target. Additionally, B cell homing receptors expressed on B cell membranes that recognize addressins and ligands on target tissues, compound or derivatives thereof that alter the trafficking of B cells to a particular site, and inhibitory molecules inflammation and autoimmune activity of the B cells, can play a role in B cell homing and development of specialized immune responses.

[0072] Modified B cells that Express Integrin of Interest. The major homing receptors expressed by lymphocytes are the integrins, which are a large class of molecules characterized by a heterodimeric structure of α and β chains. In general, the pairing of specific α and β chains of the integrin determines the type of the homing receptor. For example, pairing of the α 4 chain with β 7 chain characterizes the major integrin molecule (α 4 β 7) responsible for lymphocyte binding to Mucosal addressin cell adhesion molecule 1 (MAdCAM-1) expressed on high endothelial venules (HEVs) in Peyer's patches (PP) and gastrointestinal (GI) tract lamina propria endothelial venules

(LPVs). Similarly, pairing of the $\alpha 4$ chain with $\beta 1$ chain characterizes the homing receptor ($\alpha 4\beta 1$) for the skin.

[0073] In various embodiments of the present inventions, a B cell to be modified can be selected for in advance, with specific traits that mediate preferred localizations. For example, memory B cells expressing CXCR3 may be enriched for and then subjected to engineering. CXCR3 cells may be attracted to ligands expressed at sites of inflammation. As such, modified B cells can preferentially localize to such sites.

[0074] In various embodiments of the present invention, a modified B cell is provided that expresses the $\alpha 4$ and $\beta 7$ chains of an integrin. It is desirable that expression of the $\alpha 4\beta 7$ integrin will promote homing of the modified B cell to the colon. In various embodiments, a modified B cell is provided that expresses the $\alpha 4$ and $\beta 1$ chains of an integrin. It is desirable that expression of the $\alpha 4\beta 1$ integrin will promote homing of the modified B cell to the skin. In various embodiments, a modified B cell is provided that expresses a desired pairing of an α and a β chain of an integrin, such that the expressed integrin promotes homing of the modified B cell to a desired site/target of interest. Accordingly, in various embodiments, any desired combination of the α and β chains of an integrin is contemplated for expression in the B cells, such that the modified B cells expressing the specific integrin is targeted to a desired site/target of interest.

[0075] **Modified B cells that Express Homing Receptors of Interest.** B cells have an ability to home to inflammatory tissues and altering their homing receptor expression can complement their native homing tendencies. B cell localization is also driven by expression of attractant molecules (e.g., targets such as ligands and chemokines) at inflammatory sites in specific locations or tissues. Such molecules can also include antibodies, such as the MECA79 antibody that targets cells to peripheral node addressin (PNAd). Bahmani et al., J Clin Invest. 2018;128(11):4770-4786; Azzi et al., Cell Rep. 2016;15(6):1202-13. Accordingly, B cells can be engineered to express certain antibodies, proteins, and receptors that facilitate B cell homing to a site/target of interest and interactions of such B cells with the desired target. In certain instances, expression of such receptors redirects the B cells to the tissue of interest.

[0076] In various embodiments of the present invention, a modified B cell is provided that is capable of expressing a homing antibody, protein, or a receptor, expression of which is capable of directing the B cell to a specific site/target of interest. Exemplary homing of T cells to specific homing tissues (target tissues) using specific homing receptor/ligand pairs are set forth in Table 2. The same specific homing receptor/ligand pairs are also capable of facilitating homing of B cells to a specific homing tissue (target tissue). Accordingly, in various embodiments of the present invention, homing of the modified B cells to an exemplary homing tissue (target tissue) is facilitated using the corresponding homing receptor/ligand pairs as set forth in Table 2.

TABLE 2

T_{eff} cell homing receptors and their cognate ligands mediating organotropic targeting		
Homing Tissue Type	T_{eff} Cell Homing Receptor	Cognate Ligand
Skin	CLA (PSGL-1 glycoform)	E/P-selectin
	CD43E	E-selectin
	VLA-4 ($\alpha_4\beta_1$)	VCAM-1
	LFA-1 ($\alpha_L\beta_2$)	ICAM-1
	CCR4	CCL17
	CCR10	CCL27
	$\alpha_4\beta_7$	MAdCAM-1
Gut (intestine, colon, mLN, PP)	CCR9 ^a	CCL25 ^a
	CXCR4	CXCL12
	Selectin ligands ^b	E/P-selectin ^b
	VLA-4 ^b	VCAM-1 ^b
	LFA-1 ^b	ICAM-1 ^b
	CCR6 ^b	CCL20 (MIP-3 α) ^b
Liver	CD44	Hyaluronate
	VLA-4	VCAM-1
	CCR5	CCL5
		VAP-1
	Selectin ligands ^b	E/P-selectin
	$\alpha_4\beta_7$ ^b	MAdCAM-1 ^b
Lung	LFA-1	ICAM-1
	CCR3	CCL28
	CCR4	CCL17
	CXCR4	CXCL12
	Selectin ligands ^b	E/P-selectin ^b
	VLA-4 ^b	VCAM-1 ^b
	LFA-1 ^b	ICAM-1 ^b
Bone Marrow	CLA (PSGL-1 glycoform)	E/P-selectin
	CD43E	E-selectin
	VLA-4	VCAM-1
	LFA-1	ICAM-1

	CXCR4	CXCL12
	$\alpha_4\beta_7^b$	MAdCAM-1 ^b
Heart	CCR5	CCL4, CCL5
	CCR4	?
	CXCR3	CXCL10
	c-Met	HGF
Brain	VLA-4 ^b	VCAM-1 ^b
	LFA-1 ^b	ICAM-1 ^b
	CXCR3 ^b	CXCL9/CXCL10 ^b
Peripheral LN^c	Selectin ligands ^b	E/P-selectin ^b
	LFA-1 ^b	ICAM-1 ^b
	CXCR3 ^b	CXCL9/CXCL10 ^b
^aInvolved in T_{eff} cell homing to the intestine but not colon. ^bInflammatory reactions, tissue injury. ^cUnder non-inflamed, steady-state conditions, T_{eff} cells typically lose L-selectin and CCR7 expression and are largely restricted from LN access though may enter during inflammatory reactions (b) as shown. In contrast, both naïve T cells and T_{cm} cells express L-selectin, CCR7, and CXCR4 and engage PNA^d, CCL19/CCL21, and CXCL12, respectively, to undergo T-cell rolling and LFA-1/ICAM-1/2- mediated adhesion and transmigration into LNs.		

[0077] Exemplary homing tissue (target tissue) type and ligand or chemokine that enables tissue-restricted B cell homing in accordance with the invention are set forth in Table 3.

TABLE 3

Homing Tissue Type	Ligand/Chemokines
CNS	VCAM-1, CD62P, ligands for CCR1,2, 5, CXCR3
Liver	CD62P, VAP-1, CXCL16
Small Intestine	MAdCAM, CD62P, CCL25
Colon	MAdCAM, CD62P, CCL20, GPR15L
Skin	CD62E, CD62P, CCL17(22), ICAM-1
Thymus	VCAM, CD62P, CCL25
Peripheral Lymph Node	PNA ^d , CCL21, ICAM-1
Peyer's Patch	MAdCAM, CCL21, CXCL13
Bone Marrow	VCAM, CD62P, CXCL12, ICAM-1

[0078] In various embodiments of the present invention, a modified B cell is provided that expresses one or more of an antibody, a protein, or a receptor that facilitate homing of the modified B cell to the exemplary target/homing tissues using the specific homing receptor/ligand pairs as set forth in Table 2. In various embodiments of the present invention, a modified B cell is provided that expresses one or more of a homing receptor that facilitate homing of the modified B cell to the exemplary target/homing tissue using the ligand or chemokines are set forth in Tables 2 and/or 3. As used herein, the term “B cell homing” refers to localizing, targeting, trafficking, directing, or redirecting of the B cell of the present application to a site/target of interest, for example, a homing or target tissue, an inflammatory site in a specific location or tissue, or a tumor or tumor microenvironment, where delivery of therapeutic payloads is desirable. As used in the context of B cell homing, the term “antibody”, “protein” or a “receptor” refers to an amino acid sequence, a nucleic acid sequence encoding a peptide or protein, or an RNA molecule, for use as a therapeutic agent, which when expressed in a modified B cell of the present invention will direct the B cell to a site/target of interest.

[0079] In certain embodiments, the homing antibody, protein, or receptor molecule is for homing/targeting the modified B cell expressing such a molecule to a site/target of interest. In certain embodiments, the homing antibody, protein, or receptor molecule is for homing/targeting the modified B cell expressing such a molecule to inflammatory sites in specific locations or tissues. In certain embodiments, the homing antibody, protein or receptor is for targeting the B cell to a tumor or tumor microenvironment and to the tumor draining lymph node. In certain embodiments, targeting B cells to particular locations is desirable so that the engineered or modified B cells of the present invention can deliver therapeutic payloads to desired locations of interest, for example, a homing or target tissue, an inflammatory site in a specific location or tissue, or a tumor or tumor microenvironment. Accordingly, in certain embodiments, it is desirable that the B cells home to a site/target of interest, for example, a tumor or tumor microenvironment and tumor-draining lymph node and deliver to the site/target of interest a payload capable of, for example, increasing the number of cross-presenting dendritic cells (DCs) at the site/target of interest (e.g., in tumors).

[0080] In various embodiments, the homing antibody, protein, or receptor is expressed in the modified or engineered B cell as a DNA construct. In various embodiments, the homing antibody, protein, or receptor is expressed in the modified B cell as a DNA construct under the control of a constitutively activated transcriptional pathway. In various embodiments, the homing antibody, protein, or receptor involved in the B cell homing/targeting is either not naturally expressed in a B cell or is expressed at higher levels than is naturally expressed in a B cell. Exemplary homing of the modified B cells to specific homing/target tissues using specific homing receptor/ligand pairs

in accordance with the present invention is set forth in Table 4. It should be understood that, notwithstanding the exemplary homing tissues, homing receptor, and ligand pairs set forth in Table 4, a modified B cell of the present invention may be engineered to express any homing antibody, protein, or a receptor (e.g., any homing receptor set forth in Table 2), such that the modified B cell can be directed to a specific site/target of interest.

TABLE 4

Homing Tissue Type	Homing Receptor	Ligand/Chemokine
Liver	CXCR6	CXCL16
Small Intestine	CCR9	CCL25
Large Intestine (Colon)	CCR6	CCL20
Lymph Node	CCR7	CCL21
Bone Marrow	CXCR4	CXCL12
Peyer's Patch	CCR7 and CXCR5	CCL21 and CXCL13, respectively
Skin	CCR4	CCL17(22)

[0081] Nonexclusive examples of homing (target) tissue types for the specific homing receptor/ligand pairs of the present invention include: skin, gut (intestine, colon, mesenteric lymph nodes (mLN), Peyer's Patch (PP), small intestine), liver, lung, bone marrow, heart, peripheral lymph node (LN), CNS, thymus, and bone marrow.

[0082] Nonexclusive examples of homing receptors that can be paired with specific or corresponding attractants/ligands/chemokines of the present invention include: CLA (PSGL-1 glycoform), CLA (PSGL-1 glycoform), CCR10, CCR3, CCR4, CCR5, CCR6, CCR9, CD43E, CD44, c-Met, CXCR3, CXCR4, LFA-1, LFA-1 (α L β 2), Selectin ligands, VLA-4, VLA-4 (α 4 β 1), and α 4 β 7.

[0083] Nonexclusive examples of ligands/chemokines that can be paired with specific or corresponding homing receptors of the present invention include: CXCL16, CCL17, CCL17(22), CCL20 (MIP-3 α), CCL21, CCL25, CCL27, CCL28, CCL4, CCL5, CD62E, CD62P, CXCL10, CXCL12, CXCL13, CXCL16, CXCL9/CXCL10, CXCR3, E/P-selectin, E-selectin, GPR15L, HGF, Hyaluronate, ICAM-1, ligands for CCR1,2, 5, MAdCAM, MAdCAM-1, PNA α , VAP-1, VCAM, and VCAM-1.

[0084] In certain embodiments of the present invention, a modified B cell is provided that express or have increased expression of the exemplary B cell homing receptors (e.g., as set forth in Table 2), such that the modified B cell is targeted to the corresponding homing tissue of interest that

expresses the corresponding ligand/chemokines (*e.g.*, as set forth in Tables 2 and/or 3). In certain embodiments of the present invention, a modified B cell is provided that co-expresses an integrin with a specific α and β chain pairing and a specific B cell homing receptor (*e.g.*, as set forth in Tables 2 and/or 3), expression of which integrin and/or homing receptor promote or facilitate homing/targeting of the modified B cell to a site/target of interest. In some embodiments, a modified B cell is provided that co-expresses an $\alpha 4\beta 7$ integrin and CCR9. It is desirable that co-expression of $\alpha 4\beta 7$ and CCR9 will promote small intestine homing of the modified B cells of the present invention. In some embodiments, a modified B cell is provided that co-expresses an $\alpha 4\beta 1$ integrin and CCR4. It is desirable that co-expression of $\alpha 4\beta 1$ and CCR4 will promote small intestine homing of the modified B cells of the present invention.

[0085] Modified B cells that Express Immune Inhibitory Molecules. B cells are key contributors to many autoimmune diseases. However, B cells can be used therapeutically to antagonize autoimmunity. Specifically, B cells can be engineered to express at least one or more immune inhibitory molecules, which may decrease the autoimmune activity of the B cells, leading to decrease in an autoimmune disease. Immune inhibitory molecules are well known in the art. Such inhibitory molecules may include, but are not limited to, IL-10, TGF- β , PD-L1, PD-L2, LAG-3, and TIM-3. In certain embodiments of the present invention, a modified B cell is provided that is engineered to express at least one or more of an inhibitory molecule selected from IL-10, TGF- β , PD-L1, PD-L2, LAG-3, and TIM-3, or any combinations thereof, such that the inflammation at the site and autoimmune activity of the B cells localized to the site are decreased, thereby leading to a positive therapeutic response.

[0086] Compounds that alter B cell Trafficking. In certain embodiments of the present invention, a modified B cell is provided that is treated with at least one or more compound or derivatives thereof that alter the trafficking of B cells by inducing expression of a specific B cell integrin and/or a homing receptor. Compounds or derivatives thereof that alter the trafficking of B cells are well known in the art. In certain embodiments, a modified B cell is provided that is treated with all-trans-retinoic acid (ATRA) or derivatives thereof that promote homing of the B cells to gut (small intestine) due to the increased expression of $\alpha 4\beta 7$ integrin and CCR9 homing receptor. As used herein, the term “compound” refers to a chemical, drug, a therapeutic agent, or derivatives thereof, that alter the trafficking of B cells in a desired manner.

[0087] In various embodiments of the present invention, a modified B cell engineered to co-express a specific integrin (*e.g.*, with a specific α and β chain pairing) and a specific B cell homing receptor of interest is treated with at least one or more compounds or derivatives thereof that alter the trafficking of the modified B cells and promote homing of the cells to a specific site/target of interest due to the increased expression of the specific integrin and/or the homing receptor. In

various embodiments, a B cell modified to co-express an integrin with a specific α and β chain pairings and a specific B cell homing receptor further expresses at least one or more immune inhibitory molecules, such that the autoimmune activity of the modified B cells targeted to a specific site of inflammation is decreased, leading to a decrease in the autoimmune disease. In some embodiments, a modified B cell engineered to express one or more immune inhibitory molecules, for example IL-10, TGF- β , PD-L1, PD-L2, LAG-3, and TIM-3, or combinations thereof, is treated with ATRA or derivatives thereof for a specified period of time, such that expression of the $\alpha 4\beta 7$ integrin and CCR9 homing receptor is induced to promote B cell homing to a specific site/target of interest (e.g., the gut), but the inflammation at the site and autoimmune activity of B cells localized to the site are decreased, leading to a positive therapeutic response. In one embodiment, a modified B cell engineered to express one or more immune inhibitory molecules, for example IL-10, TGF- β , or combinations thereof, is treated with ATRA or derivatives thereof for a specified period of time, such that expression of the $\alpha 4\beta 7$ integrin and CCR9 homing receptor is induced to promote B cell homing to a specific site/target of interest (e.g., the gut), but the inflammation at the site and autoimmune activity of B cells localized to the site are decreased, leading to a positive therapeutic response.

[0088] It is understood that, any B cell of the present invention modified to co-express a specific B cell integrin and homing receptor that targets the B cell to a particular homing/target tissue of interest, may be further engineered to express one or more immune inhibitory molecules for reducing inflammation and autoimmune activity of the B cells localized to the site, and/or treated with a compound that alter the homing/targeting of the modified B cells by inducing expression of the specific B cell integrin and/or the homing receptor.

[0089] Activation of B cells with TLR agonists and TLRs. B cells have a natural ability to uptake and present antigens recognized by their specific B cell receptors (BCRs). B cells activated by Toll-like receptors (TLRs) result in potent effector B cells in defending the body in an immune response. Expression of or increasing the expression of TLRs in B cells can provide a mechanism for potentiating B cells for innate signals regulating adaptive immune responses.

[0090] Activation of B cells with TLR agonists. In various embodiments of the present invention, a B cell is provided, where the B cell is treated *in vitro* with at least one TLR agonist. In various embodiments, the TLR can be a TLR1, TLR2, TLR3, TLR4, TLR5, TLR6, TLR7, TLR8, TLR9, TLR10, TLR11, TLR12, and/or a TLR13. In various embodiments, the TLR agonist preferentially binds to one or more TLR selected from the group consisting of TLR1, TLR2, TLR3, TLR4, TLR5, TLR6, TLR7, TLR8, TLR9, TLR10, TLR11, TLR12, and TLR13. TLR agonists are well known in the art and may include, but are not limited to, CpG-rich oligonucleotides and the

double-stranded RNA mimic, polyinosinic acid:polycytidylic acid (poly-I:C). In various embodiments, the TLR agonist can be CpG oligonucleotides.

[0091] In various embodiments, each B cell may be treated with one TLR agonist. In various embodiments, each B cell may be treated with more than one TLR agonist. For example, each B cell may be treated 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 different TLR agonists. Alternatively, the patient may be administered a heterogeneous population of B cells, each B cell treated with a unique TLR agonist or a combination of TLR agonists. In some embodiments, the B cells for use a therapeutic agent is treated with one or more TLR agonists at the same time or in advance of the administration of the B cells to a subject or patient in need thereof. In certain embodiments, treatment with one or more TLR agonist is capable of producing more potent effector B cells for defending the body in an immune response. In certain embodiments, treatment with one or more TLR agonist is capable of potentiating B cells for immune responses. In some embodiments, treating a B cell of the present invention with at least one or more TLR agonists induces expression or activation of one or more TLRs.

[0092] Activation of B cells with TLR Expression. In various embodiments of the present invention, a modified B cell is provided that is capable of expressing a constitutively active TLR. In various embodiments, the TLR is expressed in the modified or engineered B cell as a DNA construct under the control of a constitutively activated transcriptional pathway. In various embodiments, the TLR is either not naturally expressed in a B cell or is expressed at higher levels than is naturally expressed in a B cell. In various embodiments, the TLR can be a TLR1, TLR2, TLR3, TLR4, TLR5, TLR6, TLR7, TLR8, TLR9, TLR10, TLR11, TLR12, and/or a TLR13.

[0093] In various embodiments, each B cell may express more than one constitutively active TLR. For example, each B cell may express 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 or 13 different constitutively active TLRs. Alternatively, the patient may be administered a heterogeneous population of B cells, each B cell capable of expressing and/or secreting a unique TLR or combination of TLRs, which are constitutively active. In various embodiments, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 or 13 different constitutively active TLRs may be administered to the subject or patient through a heterogeneous population of B cells.

[0094] In certain embodiments of the present invention, the B cell is a modified B cell that expresses at least one constitutively active TLR. In certain embodiments, the modified B cell that expresses at least one constitutively active TLR is treated with one or more TLR agonist. In certain embodiments, the expression of the constitutively active TLR is capable of producing more potent effector B cells for defending the body in an immune response. In certain embodiments, the expression of the constitutively active TLR is capable of potentiating B cells for immune responses. In certain embodiments, the modified B cell expresses both a TLR that is constitutively active and

any CAR-B of the present application. In various embodiments, the modified B cell expressing a TLR that is constitutively active and/or a CAR-B is further treated with one or more TLR agonist at the same time or in advance of the administration of the modified B cells to a subject or patient in need thereof. In certain embodiments, B cells may be engineered to express payloads and modifiers, such as TLRs, in the absence of CAR-B, for intratumoral administration.

[0095] Modified B Cells that Present Antigens Simultaneously in HLA Class I and Class II Molecules. B cells, in addition to their function in antibody production, also express high level of Human Leukocyte Antigen (HLA) class II molecules and can present antigens to CD4+ T cells (Hong et al., 2018, Immunity 49, 695–708). In various embodiments of the present invention, a modified B cell is provided that is capable of presenting specific antigens and/or antigen-derived epitopes of interest, such as tumor antigens or infectious disease antigens, simultaneously in both HLA class I and class II molecules. Tumor antigens and infectious disease antigens are well known in the art and are described in the foregoing sections. In certain embodiments, a specific antigen of interest, e.g., a tumor antigen or an infectious disease antigen, is fused to a targeting signal of a lysosomal protein that targets the antigen to the lysosomes and presents the antigen simultaneously and efficiently in both HLA class I and class II molecules. In some embodiments, the targeting signal is the targeting signal of lysosome-associated membrane protein-1 (LAMP1). In some embodiments, the targeting signal is capable of entering endosomal recycling compartments. The c-terminal sequence of Clec9A is such a targeting moiety. As used herein, a specific tumor antigen or an infectious disease antigen fused to a targeting signal refers to an amino acid sequence, a nucleic acid sequence encoding a peptide or protein, or an RNA molecule (e.g., an mRNA molecule), for use as a therapeutic agent. In one embodiment, a specific tumor antigen or an infectious disease antigen fused to a targeting signal refers to an mRNA molecule for use as a therapeutic agent. In certain embodiments, it is desirable that the specific tumor antigens and/or infectious disease antigens fused to a targeting signal, such as the targeting signal of LAMP1 or Clec9A, be targeted to the lysosomes or endosomes and presented simultaneously and efficiently in both HLA class I and class II molecules. In certain embodiments, it is desirable that electroporation of B cells (e.g., human B cells), before or after maturation, with an mRNA encoding specific tumor antigens and/or infectious disease antigens of interest fused to a targeting signal, such as the targeting signal of LAMP1 or Clec9A, be capable of simultaneously and efficiently presenting the specific antigens and/or antigen-derived epitopes in both HLA class I and class II molecules. In various embodiments, the specific tumor antigens and/or infectious disease antigens of interest is either not naturally presented by a B cell, is not presented by a B cell simultaneously in both HLA class I and class II molecules naturally, or is not presented by a B cell with high efficiencies in both HLA class I and class II molecules naturally. It is contemplated that,

introduction of such electroporated B cells into a subject, e.g., a human host, will promote development of or potentiate antigen-specific immune responses by presenting specific antigens and/or antigen-derived epitopes of interest simultaneously and efficiently in both HLA class I and class II molecules.

[0096] In various embodiments, the invention relates to a nucleic acid sequence, e.g., an mRNA sequence, encoding at least one specific antigen of interest, e.g., a tumor antigen or an infectious disease antigen, fused to a targeting signal, such as the targeting signal of LAMP1, for use as a therapeutic agent in electroporation of B cells for simultaneously and efficiently presenting the specific antigen and/or antigen-derived epitopes in both HLA class I and class II molecules. In various embodiments, the invention relates to nucleic acid sequence, e.g., an mRNA sequence, encoding more than one (e.g., 1, 2, 3, 4, 5, or more) specific tumor antigen and/or an infectious disease antigen of interest fused to a targeting signal. In various embodiments, the invention relates to pools of different nucleic acid sequences, e.g., pools of different mRNA sequences, for use as a therapeutic agent in electroporation of B cells as described above, where each pool encodes at least one specific antigen of interest, e.g., a tumor antigen or an infectious disease antigen, fused to a targeting signal that is different from the other pools of the mRNA sequences. Accordingly, in some embodiments, the subject may be administered a homogeneous population of B cells, where each B cell is electroporated with an mRNA encoding at least one specific antigen of interest fused to a targeting signal. In some embodiments, the subject may be administered a homogeneous a population of B cells, where each B cell is electroporated with an mRNA encoding more than one specific antigen of interest fused to targeting signal. In some embodiments, the subject may be administered a heterogeneous population of B cells, where each B cell is electroporated with a combination of mRNAs each encoding at least one specific antigen of interest fused to a different targeting signal.

[0097] In some embodiments, the B cells for use in electroporation as described above may be any of the modified B cells of the present application. In some embodiments, the modified B cell comprises a chimeric antigen receptor for B cells (CAR-B). In various embodiments, the modified B cell can express a CAR-B and simultaneously and efficiently present specific antigen and/or antigen-derived epitopes of interest in both HLA class I and class II molecules.

[0098] In various embodiments, the invention relates to a method of administering an isolated B cell to a patient in need thereof. In various embodiments, a population of B cells may be administered to the patient. In various embodiments, each B cell may express more than one payload peptide or protein. For example, each B cell may express 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 or 12 different payloads. Alternatively, the patient may be administered a heterogeneous population of B cells, each B cell capable of expressing and/or secreting a unique payload or combination of

payloads. In various embodiments, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 or 12 different payloads may be administered to the patient through a heterogeneous population of B cells.

3. Methods of Treatment

[0099] In some aspects, the invention therefore comprises a method for treating or preventing a tumor or cancerous tissue, comprising administering to a patient in need thereof an effective amount of at least one CAR-B disclosed herein.

[0100] Methods are provided for treating diseases or disorders, including cancer. In some embodiments, the invention relates to creating a B cell-mediated immune response in a subject, comprising administering an effective amount of the engineered immune cells of the present application to the subject. In some embodiments, the B cell-mediated immune response is directed against a target cell or cells. In some embodiments, the engineered immune cell comprises a chimeric antigen receptor for B cells (CAR-B). In some embodiments, the target cell is a tumor cell. In some aspects, the invention comprises a method for treating or preventing a malignancy, said method comprising administering to a subject in need thereof an effective amount of at least one isolated antigen-binding molecule described herein. In some aspects, the invention comprises a method for treating or preventing a malignancy, said method comprising administering to a subject in need thereof an effective amount of at least one immune cell, wherein the immune cell comprises at least one chimeric antigen receptor.

[0101] In some aspects, the invention comprises a pharmaceutical composition comprising at least one antigen-binding molecule as described herein and a pharmaceutically acceptable excipient. In some embodiments, the pharmaceutical composition further comprises an additional active agent.

[0102] In some embodiments, the subject is diagnosed with a metastatic disease localized to the liver. In other embodiments, the metastatic disease is a cancer. In still other embodiments, the cancer metastasized from a primary tumor in the breast, colon, rectum, esophagus, lung, pancreas and/or stomach. In still other embodiments, the subject is diagnosed with unresectable metastatic liver tumors. In yet other embodiments, the subject is diagnosed with unresectable metastatic liver tumors from primary colorectal cancer. In some embodiments, the subject is diagnosed with hepatocellular carcinoma.

[0103] It will be appreciated that target doses for modified B cells can range from 1×10^6 - 2×10^{10} cells/kg, preferably 2×10^6 cells/kg, more preferably. It will be appreciated that doses above and below this range may be appropriate for certain subjects, and appropriate dose levels can be determined by the healthcare provider as needed. Additionally, multiple doses of cells can be provided in accordance with the invention.

[0104] Also provided are methods for reducing the size of a tumor in a subject, comprising administering to the subject a modified B cell of the present invention, wherein the cell comprises a CAR-B receptor comprising an antigen-binding domain that binds to an antigen on a tumor, a payload or both a CAR-B and a payload. In some embodiments, the subject has a solid tumor, or a blood malignancy such as lymphoma or leukemia. In some embodiments, the modified B cell is delivered to a tumor bed. In some embodiments, the cancer is present in the bone marrow of the subject.

[0105] Also provided are methods for homing B cells to a site/target of interest in a subject, comprising administering to the subject a modified B cell of the present invention, wherein the cell comprises an integrin, a homing antibody, protein, or a receptor that is attracted to a ligand, chemokine, or an attractant at the site/target of interest. In some embodiments, the site/target of interest is, for example, a homing or target tissue, an inflammatory site in a specific location or tissue, or a tumor or tumor microenvironment, where delivery of therapeutic payloads is desirable.

[0106] Also provided are methods for decreasing inflammation and autoimmune activity of B cells at a site/target of interest in a subject, comprising administering to the subject a modified B cell of the present invention, wherein the cell comprises an immune inhibitory molecule. In some embodiments, the site/target of interest is, for example, a homing or target tissue, an inflammatory site in a specific location or tissue, or a tumor or tumor microenvironment, where delivery of therapeutic payloads is desirable.

[0107] Also provided are methods for altering trafficking of B cells to a site/target of interest in a subject, comprising treating a B cell of the present invention with a compound or derivatives thereof suitable for altering B cell trafficking, and administering the treated B cell to the subject in need thereof. In some instances, the compound or derivatives thereof alters B cell trafficking by increasing the expression of an integrin, homing antibody, protein, receptor, or combinations thereof, expressed by the B cells.

[0108] Also provided are methods for potentiating B cells and/or producing potent effector B cells for increasing immune responses in a subject, comprising treating a B cell of the present invention with at least one or more TLR agonists, and administering the treated B cell to the subject in need thereof. In some embodiments, treating a B cell of the present invention with at least one or more TLR agonists induces expression or activation of one or more TLRs. In some embodiments, the method for potentiating B cells and/or producing potent effector B cells for increasing immune responses in a subject, further comprises administering to the subject a modified B cell of the present invention that expresses at least one or more constitutively active TLRs. Also provided are methods for potentiating B cells and/or producing potent effector B cells for increasing immune responses in a subject, comprising administering to the subject a modified B cell of the

present invention, wherein the cell expresses a CAR-B receptor comprising an antigen-binding domain that binds to an antigen on a tumor, a constitutively active TLR or both a CAR-B and a constitutively active TLR, where the cell is treated with at least one or more TLR agonists at the same time or in advance of the administration of the cells to the subject.

[0109] Also provided are methods for increasing antigen-specific immune responses in a subject, comprising administering to the subject a modified B cell of the present invention, wherein the cell is electroporated with a nucleic acid sequence, e.g., an mRNA, encoding specific tumor antigens and/or infectious disease antigens fused to a targeting signal, such as the targeting signal of LAMP1 or Clec9A, for simultaneously and efficiently presenting the specific antigens and/or antigen-derived epitopes in both HLA class I and class II molecules. In some embodiments, the subject has a solid tumor, or a blood malignancy such as lymphoma or leukemia.

[0110] It is understood that the various embodiments of the methods of treatment using the engineered or modified B cells of the present application are not mutually exclusive and can be combined with each other in any way and without any restriction unless explicitly indicated, for achieving or facilitating any of the results and/or therapeutic responses contemplated herein.

[0111] In some embodiments, the modified B cells are autologous B cells. In some embodiments, the modified B cells are allogeneic B cells. In some embodiments, the modified B cells are heterologous B cells. In some embodiments, the modified B cells of the present application are transfected or transduced in vivo. In other embodiments, the engineered cells are transfected or transduced ex vivo.

[0112] As used herein, the term "subject" or "patient" means an individual. In some aspect, a subject is a mammal such as a human. In some aspect, a subject can be a non-human primate. Non-human primates include marmosets, monkeys, chimpanzees, gorillas, orangutans, and gibbons, to name a few. The term "subject" also includes domesticated animals, such as cats, dogs, *etc.*, livestock (e.g., llama, horses, cows), wild animals (e.g., deer, elk, moose, *etc.*), laboratory animals (e.g., mouse, rabbit, rat, gerbil, guinea pig, *etc.*) and avian species (e.g., chickens, turkeys, ducks, *etc.*). Preferably, the subject is a human subject. More preferably, the subject is a human patient.

[0113] The methods can further comprise administering one or more chemotherapeutic agents. In certain embodiments, the chemotherapeutic agent is a lymphodepleting (preconditioning) chemotherapeutic. Beneficial preconditioning treatment regimens, along with correlative beneficial biomarkers are described in U.S. Provisional Patent Applications 62/262,143 and 62/167,750, which are hereby incorporated by reference in their entirety herein. These describe, e.g., methods of conditioning a patient in need of a T cell therapy comprising administering to the patient specified beneficial doses of cyclophosphamide (between 200 mg/m²/day and 2000 mg/m²/day) and specified doses of fludarabine (between 20 mg/m²/day and 900 mg/m²/day). A

preferred dose regimen involves treating a patient comprising administering daily to the patient about 500 mg/m²/day of cyclophosphamide and about 60 mg/m²/day of fludarabine for three days prior to administration of a therapeutically effective amount of engineered B cells to the patient.

[0114] In other embodiments, the antigen-binding molecule, transduced (or otherwise engineered) cells (such as CARs), and the chemotherapeutic agent are administered each in an amount effective to treat the disease or condition in the subject.

[0115] In certain embodiments, compositions comprising CAR-expressing immune effector cells disclosed herein may be administered in conjunction with any number of chemotherapeutic agents. Examples of chemotherapeutic agents include alkylating agents such as thiotepea and cyclophosphamide (CYTOXAN™); alkyl sulfonates such as busulfan, improsulfan and piposulfan; aziridines such as benzodopa, carboquone, meturedopa, and uredopa; ethylenimines and methylamelamines including altretamine, triethylenemelamine, trietylenephosphoramide, triethylenethiophosphoramide and trimethylolomelamine resins; nitrogen mustards such as chlorambucil, chlornaphazine, cholophosphamide, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, melphalan, novembichin, phenesterine, prednimustine, trofosfamide, uracil mustard; nitrosureas such as carmustine, chlorozotocin, fotemustine, lomustine, nimustine, ranimustine; antibiotics such as aclacinomysins, actinomycin, anthramycin, azaserine, bleomycins, cactinomycin, calicheamicin, carabycin, carminomycin, carzinophilin, chromomycins, dactinomycin, daunorubicin, detorubicin, 6-diazo-5-oxo-L-norleucine, doxorubicin, epirubicin, esorubicin, idarubicin, marcellomycin, mitomycins, mycophenolic acid, nogalamycin, olivomycins, peplomycin, potfiromycin, puromycin, que-lamycin, rodorubicin, streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin, zorubicin; anti-metabolites such as methotrexate and 5-fluorouracil (5-FU); folic acid analogues such as denopterin, methotrexate, pteropterin, trimetrexate; purine analogs such as fludarabine, 6-mercaptopurine, thiamiprine, thioguanine; pyrimidine analogs such as ancitabine, azacitidine, 6-azauridine, carmofur, cytarabine, dideoxyuridine, doxifluridine, enocitabine, floxuridine, 5-FU; androgens such as calusterone, dromostanolone propionate, epitostanol, mepitostane, testolactone; anti-adrenals such as aminoglutethimide, mitotane, trilostane; folic acid replenisher such as frolinic acid; aceglatone; aldophosphamide glycoside; aminolevulinic acid; amsacrine; bestrabucil; bisantrene; edatraxate; defofamine; demecolcine; diaziquone; elformithine; elliptinium acetate; etoglucid; gallium nitrate; hydroxyurea; lentinan; lonidamine; mitoguazone; mitoxantrone; mopidamol; nitracrine; pentostatin; phenamet; pirarubicin; podophyllinic acid; 2-ethylhydrazide; procarbazine; PSK®; razoxane; sizofiran; spirogermanium; tenuazonic acid; triaziquone; 2, 2', 2''-trichlorotriethylamine; urethan; vindesine; dacarbazine; mannomustine; mitobronitol; mitolactol; pipobroman; gacytosine; arabinoside ("Ara-C"); cyclophosphamide; thiotepea; taxoids, e.g. paclitaxel (Taxol®, Bristol-

Myers Squibb) and doxorubicin (Taxotere®, Rhone-Poulenc Rorer); chlorambucil; gemcitabine; 6-thioguanine; mercaptopurine; methotrexate; platinum analogs such as cisplatin and carboplatin; vinblastine; platinum; etoposide (VP-16); ifosfamide; mitomycin C; mitoxantrone; vincristine; vinorelbine; navelbine; novantrone; teniposide; daunomycin; aminopterin; xeloda; ibandronate; CPT-11; topoisomerase inhibitor RFS2000; difluoromethylomithine (DMFO); retinoic acid derivatives such as Targretin™ (bexarotene), Panretin™, (alitretinoin); Ontak™ (denileukin diftitox); esperamicins; capecitabine; and pharmaceutically acceptable salts, acids or derivatives of any of the above. Also included in this definition are anti-hormonal agents that act to regulate or inhibit hormone action on tumors such as anti-estrogens including for example tamoxifen, raloxifene, aromatase inhibiting 4(5)-imidazoles, 4-hydroxytamoxifen, trioxifene, keoxifene, LY117018, onapristone, and toremifene (Fareston); and anti-androgens such as flutamide, nilutamide, bicalutamide, leuprolide, and goserelin; and pharmaceutically acceptable salts, acids or derivatives of any of the above. Combinations of chemotherapeutic agents are also administered where appropriate, including, but not limited to CHOP, i.e., Cyclophosphamide (Cytosan®), Doxorubicin (hydroxydoxorubicin), Fludarabine, Vincristine (Oncovin®), and Prednisone.

[0116] In some embodiments, the chemotherapeutic agent is administered at the same time or within one week after the administration of the engineered cell or nucleic acid. In other embodiments, the chemotherapeutic agent is administered from 1 to 4 weeks or from 1 week to 1 month, 1 week to 2 months, 1 week to 3 months, 1 week to 6 months, 1 week to 9 months, or 1 week to 12 months after the administration of the engineered cell or nucleic acid. In other embodiments, the chemotherapeutic agent is administered at least 1 month before administering the cell or nucleic acid. In some embodiments, the methods further comprise administering two or more chemotherapeutic agents.

[0117] A variety of additional therapeutic agents may be used in conjunction with the compositions described herein. For example, potentially useful additional therapeutic agents include PD-1 (or PD-L1) inhibitors such as nivolumab (Opdivo®), pembrolizumab (Keytruda®), pembrolizumab, pidilizumab, and atezolizumab (Tecentriq®). Other additional therapeutics include anti-CTLA-4 antibodies (e.g., Ipilimumab®), anti-LAG-3 antibodies (e.g., Relatlimab, BMS), alone or in combination with PD-1 and/or PD-L1 inhibitors.

[0118] Additional therapeutic agents suitable for use in combination with the invention include, but are not limited to, ibrutinib (Imbruvica®), ofatumumab (Arzerra®), rituximab (Rituxan®), bevacizumab (Avastin®), trastuzumab (Herceptin®), trastuzumab emtansine (KADCYLA®), imatinib (Gleevec®), cetuximab (Erbix®), panitumumab (Vectibix®), catumaxomab, ibritumomab, ofatumumab, tositumomab, brentuximab, alemtuzumab, gemtuzumab, erlotinib, gefitinib, vandetanib, afatinib, lapatinib, neratinib, axitinib, masitinib, pazopanib, sunitinib,

sorafenib, toceranib, lestaurtinib, axitinib, cediranib, lenvatinib, nintedanib, pazopanib, regorafenib, semaxanib, sorafenib, sunitinib, tivozanib, toceranib, vandetanib, entrectinib, cabozantinib, imatinib, dasatinib, nilotinib, ponatinib, radotinib, bosutinib, lestaurtinib, ruxolitinib, pacritinib, cobimetinib, selumetinib, trametinib, binimetinib, alectinib, ceritinib, crizotinib, aflibercept, adipotide, denileukin diftitox, mTOR inhibitors such as Everolimus and Temsirolimus, hedgehog inhibitors such as sonidegib and vismodegib, CDK inhibitors such as CDK inhibitor (palbociclib).

[0119] In additional embodiments, the composition comprising CAR-containing B cells can be administered with an anti-inflammatory agent. Anti-inflammatory agents or drugs include, but are not limited to, steroids and glucocorticoids (including betamethasone, budesonide, dexamethasone, hydrocortisone acetate, hydrocortisone, hydrocortisone, methylprednisolone, prednisolone, prednisone, triamcinolone), nonsteroidal anti-inflammatory drugs (NSAIDs) including aspirin, ibuprofen, naproxen, methotrexate, sulfasalazine, leflunomide, anti-TNF medications, cyclophosphamide and mycophenolate. Exemplary NSAIDs include ibuprofen, naproxen, naproxen sodium, Cox-2 inhibitors, and sialylates. Exemplary analgesics include acetaminophen, oxycodone, tramadol or propoxyphene hydrochloride. Exemplary glucocorticoids include cortisone, dexamethasone, hydrocortisone, methylprednisolone, prednisolone, or prednisone. Exemplary biological response modifiers include molecules directed against cell surface markers (e.g., CD4, CD5, etc.), cytokine inhibitors, such as the TNF antagonists, (e.g., etanercept (ENBREL®), adalimumab (HUMIRA®) and infliximab (REMICADE®)), chemokine inhibitors and adhesion molecule inhibitors. The biological response modifiers include monoclonal antibodies as well as recombinant forms of molecules. Exemplary DMARDs include azathioprine, cyclophosphamide, cyclosporine, methotrexate, penicillamine, leflunomide, sulfasalazine, hydroxychloroquine, Gold (oral (auranofin) and intramuscular) and minocycline.

[0120] In certain embodiments, the compositions described herein are administered in conjunction with a cytokine. "Cytokine" as used herein is meant to refer to proteins released by one cell population that act on another cell as intercellular mediators. Examples of cytokines are lymphokines, monokines, and traditional polypeptide hormones. Included among the cytokines are growth hormones such as human growth hormone, N-methionyl human growth hormone, and bovine growth hormone; parathyroid hormone; thyroxine; insulin; proinsulin; relaxin; prolaxin; glycoprotein hormones such as follicle stimulating hormone (FSH), thyroid stimulating hormone (TSH), and luteinizing hormone (LH); hepatic growth factor (HGF); fibroblast growth factor (FGF); prolactin; placental lactogen; mullerian-inhibiting substance; mouse gonadotropin-associated peptide; inhibin; activin; vascular endothelial growth factor; integrin; thrombopoietin (TPO); nerve growth factors (NGFs) such as NGF-beta; platelet-growth factor; transforming growth factors

(TGFs) such as TGF-alpha and TGF-beta; insulin-like growth factor-I and -II; erythropoietin (EPO); osteoinductive factors; interferons such as interferon-alpha, beta, and -gamma; colony stimulating factors (CSFs) such as macrophage-CSF (M-CSF); granulocyte-macrophage-CSF (GM-CSF); and granulocyte-CSF (G-CSF); interleukins (ILs) such as IL-1, IL-1 alpha, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12; IL-15, a tumor necrosis factor such as TNF-alpha or TNF-beta; and other polypeptide factors including LIF and kit ligand (KL). As used herein, the term cytokine includes proteins from natural sources or from recombinant cell culture, and biologically active equivalents of the native sequence cytokines.

4. Methods of Making

[0121] A variety of known techniques can be utilized in making the polynucleotides, polypeptides, vectors, antigen-binding molecules, immune cells, compositions, and the like according to the invention.

[0122] Prior to the in vitro manipulation or genetic modification of the immune cells described herein, the cells may be obtained from a subject. In some embodiments, the immune cells comprise B cells. B cells can be obtained from a number of sources, including peripheral blood mononuclear cells (PBMCs), bone marrow, lymph nodes tissue, cord blood, thymus tissue, tissue from a site of infection, ascites, pleural effusion, spleen tissue, and tumors. In certain embodiments, B cells can be obtained from a unit of blood collected from the subject using any number of techniques known to the skilled person, such as FICOLL™ separation. Cells may preferably be obtained from the circulating blood of an individual by apheresis. The apheresis product typically contains lymphocytes, including T cells, monocytes, granulocytes, B cells, other nucleated white blood cells, red blood cells, and platelets. In certain embodiments, the cells collected by apheresis may be washed to remove the plasma fraction, and placed in an appropriate buffer or media for subsequent processing. The cells may be washed with PBS. As will be appreciated, a washing step may be used, such as by using a semiautomated flowthrough centrifuge for example, the Cobe™ 2991 cell processor, the Baxter Cyto-Mate™, or the like. After washing, the cells may be resuspended in a variety of biocompatible buffers, or other saline solution with or without buffer. In certain embodiments, the undesired components of the apheresis sample may be removed.

[0123] The immune cells, such as B cells, can be genetically modified following isolation using known methods, or the immune cells can be activated and expanded (or differentiated in the case of progenitors) in vitro prior to being genetically modified. In another embodiment, the immune cells, such as B cells, are genetically modified with the chimeric B cell receptors described herein (e.g., transduced with a viral vector comprising one or more nucleotide sequences encoding a CAR-B) and then are activated and/or expanded in vitro. Methods for activating and expanding B cells

are known in the art and are described, for example, in U.S. Pat. Nos. 6,905,874; 6,867,041; 6,797,514; and PCT WO 2012/079000, the contents of which are hereby incorporated by reference in their entirety. Generally, such methods include contacting PBMC or isolated B cells with a stimulatory agent and costimulatory agent generally attached to a bead or other surface, in a culture medium with appropriate cytokines, such as IL-2.

[0124] In other embodiments, the B cells may be activated and stimulated to proliferate with feeder cells and appropriate antibodies and cytokines using methods such as those described in U.S. Pat. Nos. 6,040,177; 5,827,642; and WO/2012129514, the contents of which are hereby incorporated by reference in their entirety.

[0125] Certain methods for making the constructs and engineered immune cells of the invention are described in PCT application PCT/US2015/14520, the contents of which are hereby incorporated by reference in their entirety. Additional methods of making the constructs and cells can be found in U.S. provisional patent application No. 62/244,036 the contents of which are hereby incorporated by reference in their entirety.

[0126] For cloning of polynucleotides, the vector may be introduced into a host cell (an isolated host cell) to allow replication of the vector itself and thereby amplify the copies of the polynucleotide contained therein. The cloning vectors may contain sequence components generally include, without limitation, an origin of replication, promoter sequences, transcription initiation sequences, enhancer sequences, and selectable markers. These elements may be selected as appropriate by a person of ordinary skill in the art. For example, the origin of replication may be selected to promote autonomous replication of the vector in the host cell.

[0127] In certain embodiments, the present disclosure provides isolated host cells containing the vector provided herein. The host cells containing the vector may be useful in expression or cloning of the polynucleotide contained in the vector. Suitable host cells can include, without limitation, prokaryotic cells, fungal cells, yeast cells, or higher eukaryotic cells such as mammalian cells. Suitable prokaryotic cells for this purpose include, without limitation, eubacteria, such as Gram-negative or Gram-positive organisms, for example, Enterobacteriaceae such as *Escherichia*, e.g., *E. coli*, *Enterobacter*, *Erwinia*, *Klebsiella*, *Proteus*, *Salmonella*, e.g., *Salmonella typhimurium*, *Serratia*, e.g., *Serratia marc-escans*, and *Shigella*, as well as Bacilli such as *B. subtilis* and *B. licheniformis*, *Pseudomonas* such as *P. aeruginosa*, and *Streptomyces*.

[0128] The vector can be introduced to the host cell using any suitable methods known in the art, including, without limitation, DEAE-dextran mediated delivery, calcium phosphate precipitate method, cationic lipids mediated delivery, liposome mediated transfection, electroporation, microprojectile bombardment, receptor-mediated gene delivery, delivery mediated by polylysine, histone, chitosan, and peptides. Standard methods for transfection and transformation of cells for

expression of a vector of interest are well known in the art. In a further embodiment, a mixture of different expression vectors can be used in genetically modifying a donor population of immune effector cells wherein each vector encodes a different CAR-Bs as disclosed herein. The resulting transduced immune effector cells form a mixed population of engineered cells, with a proportion of the engineered cells expressing more than one different CAR-Bs.

[0129] In one embodiment, the invention provides a method of storing genetically engineered cells expressing CAR-Bs that target a protein. This involves cryopreserving the immune cells such that the cells remain viable upon thawing. A fraction of the immune cells expressing the CAR-Bs can be cryopreserved by methods known in the art to provide a permanent source of such cells for the future treatment of patients afflicted with a malignancy. When needed, the cryopreserved transformed immune cells can be thawed, grown and expanded for more such cells.

[0130] As used herein, “cryopreserve” refers to the preservation of cells by cooling to sub-zero temperatures, such as (typically) 77 Kelvin or 196° C. (the boiling point of liquid nitrogen). Cryoprotective agents are often used at sub-zero temperatures to prevent the cells being preserved from damage due to freezing at low temperatures or warming to room temperature. Cryopreservative agents and optimal cooling rates can protect against cell injury. Cryoprotective agents which can be used in accordance with the invention include but are not limited to: dimethyl sulfoxide (DMSO) (Lovelock & Bishop, *Nature*, 1959, 183, 1394-1395; Ashwood-Smith, *Nature*, 1961, 190, 1204-1205), glycerol, polyvinylpyrrolidone (Rinfret, *Ann. N.Y. Acad. Sci.*, 1960, 85, 576), and polyethylene glycol (Sloviter & Ravdin, *Nature*, 1962, 196, 48). The preferred cooling rate is 1°-3° C/minute.

[0131] The term, “substantially pure,” is used to indicate that a given component is present at a high level. The component is desirably the predominant component present in a composition. Preferably it is present at a level of more than 30%, of more than 50%, of more than 75%, of more than 90%, or even of more than 95%, said level being determined on a dry weight/dry weight basis with respect to the total composition under consideration. At very high levels (*e.g.* at levels of more than 90%, of more than 95% or of more than 99%) the component can be regarded as being in “pure form.” Biologically active substances of the present invention (including polypeptides, nucleic acid molecules, antigen-binding molecules, moieties) can be provided in a form that is substantially free of one or more contaminants with which the substance might otherwise be associated. When a composition is substantially free of a given contaminant, the contaminant will be at a low level (*e.g.*, at a level of less than 10%, less than 5%, or less than 1% on the dry weight/dry weight basis set out above).

[0132] In some embodiments, the cells are formulated by first harvesting them from their culture medium, and then washing and concentrating the cells in a medium and container system suitable

for administration (a “pharmaceutically acceptable” carrier) in a treatment-effective amount. Suitable infusion media can be any isotonic medium formulation, typically normal saline, Normosol™ R (Abbott) or Plasma-Lyte™ A (Baxter), but also 5% dextrose in water or Ringer’s lactate can be utilized. The infusion medium can be supplemented with human serum albumin.

[0133] Desired treatment amounts of cells in the composition is generally at least 2 cells or is more typically greater than 10^2 cells, and up to 10^6 , up to and including 10^8 or 10^9 cells and can be more than 10^{10} cells. The number of cells will depend upon the desired use for which the composition is intended, and the type of cells included therein. The density of the desired cells is typically greater than 10^6 cells/ml and generally is greater than 10^7 cells/ml, generally 10^8 cells/ml or greater. The clinically relevant number of immune cells can be apportioned into multiple infusions that cumulatively equal or exceed 10^5 , 10^6 , 10^7 , 10^8 , 10^9 , 10^{10} , 10^{11} , or 10^{12} cells. In some aspects of the present invention, particularly since all the infused cells will be redirected to a particular target antigen, lower numbers of cells, in the range of 10^6 /kilogram (10^6 - 10^{11} per patient) may be administered. CAR-B treatments may be administered multiple times at dosages within these ranges. The cells may be autologous, allogeneic, or heterologous to the patient undergoing therapy. In some aspects, different CAR-B cells are found in a single product. The composition can be as few as 2, 3, 4, 5, 6, 7, 8, 9 or up to 10 different CAR-B cells. These can consist of cells expressing a chimeric CAR protein and B cells expressing other CARs and/or payloads.

[0134] The B cells of the present invention may be administered either alone, or as a pharmaceutical composition in combination with diluents and/or with other components such as IL-2 or other cytokines or cell populations. Pharmaceutical compositions of the present invention may comprise a CAR-B expressing cell population, such as B cells, as described herein, in combination with one or more pharmaceutically or physiologically acceptable carriers, diluents or excipients. Such compositions may comprise buffers such as neutral buffered saline, phosphate buffered saline and the like; carbohydrates such as glucose, mannose, sucrose or dextrans, mannitol; proteins; polypeptides or amino acids such as glycine; antioxidants; chelating agents such as EDTA or glutathione; adjuvants (e.g., aluminum hydroxide); and preservatives. Compositions of the present invention are preferably formulated for intravenous administration. Treatment may also include one or more corticosteroid treatment, such as dexamethasone and/or methylprednisolone.

[0135] The compositions of the present application can comprise, consist essentially of, or consist of, the components disclosed.

[0136] The pharmaceutical compositions of the invention (solutions, suspensions or the like), may include one or more of the following: sterile diluents such as water for injection, saline solution, preferably physiological saline, Ringer’s solution, isotonic sodium chloride, fixed oils such as synthetic mono or diglycerides which may serve as the solvent or suspending medium,

polyethylene glycols, glycerin, propylene glycol or other solvents; antibacterial agents such as benzyl alcohol or methyl paraben; antioxidants such as ascorbic acid or sodium bisulfate; chelating agents such as ethylene-diaminetetraacetic acid; buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose. The parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic. An injectable pharmaceutical composition is preferably sterile.

[0137] It will be appreciated that adverse events may be minimized by transducing the immune cells (containing one or more CAR-B) with a suicide gene. It may also be desired to incorporate an inducible “on” or “accelerator” switch into the immune cells. These techniques may employ the use of dimerization domains and optional activators of such domain dimerization. These techniques include, e.g., those described by Wu et al., Science 2014, 350(6258) utilizing FKBP/Rapalog dimerization systems in certain cells, the contents of which are incorporated by reference herein in their entirety. Additional dimerization technology is described in, e.g., Fegan *et al.* Chem. Rev. 2010, 110, 3315-3336 as well as U.S. Patent Nos. 5,830,462; 5,834,266; 5,869,337; and 6,165,787, the contents of which are also incorporated by reference herein in their entirety. Additional dimerization pairs may include cyclosporine-A/cyclophilin, receptor, estrogen/estrogen receptor (optionally using tamoxifen), glucocorticoids/glucocorticoid receptor, tetracycline/tetracycline receptor, vitamin D/vitamin D receptor. Further examples of dimerization technology can be found in e.g., WO 2014/127261, WO 2015/090229, US 2014/0286987, US 2015/0266973, US 2016/0046700, U.S. Patent No. 8,486,693, US 2014/0171649, and US 2012/0130076, the contents of which are further incorporated by reference herein in their entirety.

[0138] Suitable techniques include use of inducible caspase-9 (U.S. Appl. Pub. No. 2011/0286980) or a thymidine kinase, before, after or at the same time, as the cells are transduced with the CAR-B construct of the present invention. Additional methods for introducing suicide genes and/or “on” switches include CRISPR, TALENS, MEGATALEN, zinc fingers, RNAi, siRNA, shRNA, antisense technology, and other techniques known in the art.

[0139] Anti-CD20 or anti-CD19 represent additional means to reduce or eliminate engineered B cells if such cells are responsible for adverse events or pathologies.

[0140] It will be understood that descriptions herein are exemplary and explanatory only and are not restrictive of the invention as claimed. In this application, the use of the singular includes the plural unless specifically stated otherwise.

[0141] The section headings used herein are for organizational purposes only and are not to be construed as limiting the subject matter described. All documents, or portions of documents, cited in this application, including but not limited to patents, patent applications, articles, books, and treatises, are hereby expressly incorporated by reference in their entirety for any purpose. As

utilized in accordance with the present disclosure, the following terms, unless otherwise indicated, shall be understood to have the following meanings:

[0142] In this application, the use of “or” means “and/or” unless stated otherwise. Furthermore, the use of the term “including”, as well as other forms, such as “includes” and “included”, is not limiting. Also, terms such as “element” or “component” encompass both elements and components comprising one unit and elements and components that comprise more than one subunit unless specifically stated otherwise.

[0143] The term “polynucleotide”, “nucleotide”, or “nucleic acid” includes both single-stranded and double-stranded nucleotide polymers. The nucleotides comprising the polynucleotide can be ribonucleotides or deoxyribonucleotides or a modified form of either type of nucleotide. Said modifications include base modifications such as bromouridine and inosine derivatives, ribose modifications such as 2', 3'-dideoxyribose, and internucleotide linkage modifications such as phosphorothioate, phosphorodithioate, phosphoroselenoate, phosphoro-diselenoate, phosphoro-anilothioate, phosphoraniladate and phosphoroamidate.

[0144] The term “oligonucleotide” refers to a polynucleotide comprising 200 or fewer nucleotides. Oligonucleotides can be single stranded or double stranded, e.g., for use in the construction of a mutant gene. Oligonucleotides can be sense or antisense oligonucleotides. An oligonucleotide can include a label, including a radiolabel, a fluorescent label, a hapten or an antigenic label, for detection assays. Oligonucleotides can be used, for example, as PCR primers, cloning primers or hybridization probes.

[0145] The term “control sequence” refers to a polynucleotide sequence that can affect the expression and processing of coding sequences to which it is ligated. The nature of such control sequences can depend upon the host organism. In particular embodiments, control sequences for prokaryotes can include a promoter, a ribosomal binding site, and a transcription termination sequence. For example, control sequences for eukaryotes can include promoters comprising one or a plurality of recognition sites for transcription factors, transcription enhancer sequences, and transcription termination sequence. “Control sequences” can include leader sequences (signal peptides) and/or fusion partner sequences.

[0146] As used herein, “operably linked” means that the components to which the term is applied are in a relationship that allows them to carry out their inherent functions under suitable conditions.

[0147] The term “vector” means any molecule or entity (*e.g.*, nucleic acid, plasmid, bacteriophage or virus) used to transfer protein coding information into a host cell. The term “expression vector” or “expression construct” refers to a vector that is suitable for transformation of a host cell and contains nucleic acid sequences that direct and/or control (in conjunction with the host cell) expression of one or more heterologous coding regions operatively linked thereto. An

expression construct can include, but is not limited to, sequences that affect or control transcription, translation, and, if introns are present, affect RNA splicing of a coding region operably linked thereto.

[0148] The term “host cell” refers to a cell that has been transformed, or is capable of being transformed, with a nucleic acid sequence and thereby expresses a gene of interest. The term includes the progeny of the parent cell, whether or not the progeny is identical in morphology or in genetic make-up to the original parent cell, so long as the gene of interest is present.

[0149] The term “transformation” refers to a change in a cell’s genetic characteristics, and a cell has been transformed when it has been modified to contain new DNA or RNA. For example, a cell is transformed where it is genetically modified from its native state by introducing new genetic material via transfection, transduction, or other techniques. Following transfection or transduction, the transforming DNA can recombine with that of the cell by physically integrating into a chromosome of the cell, or can be maintained transiently as an episomal element without being replicated, or can replicate independently as a plasmid. A cell is considered to have been “stably transformed” when the transforming DNA is replicated with the division of the cell.

[0150] The term “transfection” refers to the uptake of foreign or exogenous DNA by a cell. A number of transfection techniques are well known in the art and are disclosed herein. *See, e.g.,* Graham *et al.*, *VIROLOGY*, 1973, 52:456; Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual*, 2001, *supra*; Davis *et al.*, *Basic Methods in Molecular Biology*, 1986, Elsevier; Chu *et al.*, *Gene*, 1981, 13:197.

[0151] The term “transduction” refers to the process whereby foreign DNA is introduced into a cell via viral vector. *See, e.g.,* Jones *et al.*, *Genetics: principles and analysis*, 1998, Boston: Jones & Bartlett Publ.

[0152] The terms “polypeptide” or “protein” refer to a macromolecule having the amino acid sequence of a protein, including deletions from, additions to, and/or substitutions of one or more amino acids of the native sequence. The terms “polypeptide” and “protein” specifically encompass antigen-binding molecules, antibodies, or sequences that have deletions from, additions to, and/or substitutions of one or more amino acid of antigen-binding protein. The term “polypeptide fragment” refers to a polypeptide that has an amino-terminal deletion, a carboxyl-terminal deletion, and/or an internal deletion as compared with the full-length native protein. Such fragments can also contain modified amino acids as compared with the native protein. Useful polypeptide fragments include immunologically functional fragments of antigen-binding molecules.

[0153] The term “isolated” means (i) free of at least some other proteins with which it would normally be found, (ii) is essentially free of other proteins from the same source, *e.g.,* from the same species, (iii) separated from at least about 50 percent of polynucleotides, lipids, carbohydrates, or

other materials with which it is associated in nature, (iv) operably associated (by covalent or noncovalent interaction) with a polypeptide with which it is not associated in nature, or (v) does not occur in nature.

[0154] A “variant” of a polypeptide (*e.g.*, an antigen-binding molecule) comprises an amino acid sequence wherein one or more amino acid residues are inserted into, deleted from and/or substituted into the amino acid sequence relative to another polypeptide sequence. Variants include fusion proteins.

[0155] The term “identity” refers to a relationship between the sequences of two or more polypeptide molecules or two or more nucleic acid molecules, as determined by aligning and comparing the sequences. “Percent identity” means the percent of identical residues between the amino acids or nucleotides in the compared molecules and is calculated based on the size of the smallest of the molecules being compared. For these calculations, gaps in alignments (if any) are preferably addressed by a particular mathematical model or computer program (*i.e.*, an “algorithm”).

[0156] To calculate percent identity, the sequences being compared are typically aligned in a way that gives the largest match between the sequences. One example of a computer program that can be used to determine percent identity is the GCG program package, which includes GAP (Devereux *et al.*, Nucl. Acid Res., 1984, 12, 387; Genetics Computer Group, University of Wisconsin, Madison, Wis.). The computer algorithm GAP is used to align the two polypeptides or polynucleotides for which the percent sequence identity is to be determined. The sequences are aligned for optimal matching of their respective amino acid or nucleotide (the “matched span”, as determined by the algorithm). In certain embodiments, a standard comparison matrix (*see, e.g.*, Dayhoff *et al.*, 1978, Atlas of Protein Sequence and Structure, 1978, 5:345-352 for the PAM 250 comparison matrix; Henikoff *et al.*, Proc. Natl. Acad. Sci. U.S.A. 1992, 89, 10915-10919 for the BLO-SUM 62 comparison matrix) is also used by the algorithm.

[0157] As used herein, the twenty conventional (*e.g.*, naturally occurring) amino acids and their abbreviations follow conventional usage. *See, e.g.*, Immunology A Synthesis (2nd Edition, Golub and Green, Eds., Sinauer Assoc., Sunderland, Mass. (1991)), which is incorporated herein by reference for any purpose. Stereoisomers (*e.g.*, D-amino acids) of the twenty conventional amino acids, unnatural amino acids such as alpha-, alpha-disubstituted amino acids, N-alkyl amino acids, lactic acid, and other unconventional amino acids can also be suitable components for polypeptides of the present invention. Examples of unconventional amino acids include: 4-hydroxyproline, gamma.-carboxy-glutamate, epsilon-N,N,N-trimethyllysine, epsilon-N-acetyllysine, 0-phosphoserine, N-acetyls erine, N-formylmethionine, 3-methylhistidine, 5-hydroxylysine, sigma.-N-methylarginine, and other similar amino acids and imino acids (*e.g.*, 4-hydroxyproline). In the

polypeptide notation used herein, the left-hand direction is the amino terminal direction and the right-hand direction is the carboxy-terminal direction, in accordance with standard usage and convention.

[0158] Conservative amino acid substitutions can encompass non-naturally occurring amino acid residues, which are typically incorporated by chemical peptide synthesis rather than by synthesis in biological systems. These include peptidomimetics and other reversed or inverted forms of amino acid moieties. Naturally occurring residues can be divided into classes based on common side chain properties:

- a) hydrophobic: norleucine, Met, Ala, Val, Leu, Ile;
- b) neutral hydrophilic: Cys, Ser, Thr, Asn, Gln;
- c) acidic: Asp, Glu;
- d) basic: His, Lys, Arg;
- e) residues that influence chain orientation: Gly, Pro; and
- f) aromatic: Trp, Tyr, Phe.

[0159] For example, non-conservative substitutions can involve the exchange of a member of one of these classes for a member from another class.

[0160] In making changes to the antigen-binding molecule, the costimulatory or activating domains of the engineered T cell, according to certain embodiments, the hydropathic index of amino acids can be considered. Each amino acid has been assigned a hydropathic index on the basis of its hydrophobicity and charge characteristics. They are: isoleucine (+4.5); valine (+4.2); leucine (+3.8); phenylalanine (+2.8); cysteine/cystine (+2.5); methionine (+1.9); alanine (+1.8); glycine (-0.4); threonine (-0.7); serine (-0.8); tryptophan (-0.9); tyrosine (-1.3); proline (-1.6); histidine (-3.2); glutamate (-3.5); glutamine (-3.5); aspartate (-3.5); asparagine (-3.5); lysine (-3.9); and arginine (-4.5). *See, e.g., Kyte et al., J. Mol. Biol., 1982, 157, 105-131.* It is known that certain amino acids can be substituted for other amino acids having a similar hydropathic index or score and still retain a similar biological activity. It is also understood in the art that the substitution of like amino acids can be made effectively on the basis of hydrophilicity, particularly where the biologically functional protein or peptide thereby created is intended for use in immunological embodiments, as in the present case. Exemplary amino acid substitutions are set forth in Table 5.

TABLE 5

Original Residues	Exemplary Substitutions	Preferred Substitutions
Ala	Val, Leu, Ile	Val
Arg	Lys, Gln, Asn	Lys
Asn	Gln	Gln
Asp	Glu	Glu

Cys	Ser, Ala	Ser
Gln	Asn	Asn
Glu	Asp	Asp
Gly	Pro, Ala	Ala
His	Asn, Gln, Lys, Arg	Arg
Ile	Leu, Val, Met, Ala, Phe, Norleucine	Leu
Leu	Norleucine, Ile, Va, Met, Ala, Phe	Ile
Lys	Arg, 1, 4 Diamino-butyric Acid, Gin, Asn	Arg
Met	Leu, Phe, Ile	Leu
Phe	Leu, Val, Ile, Ala, Tyr	Leu
Pro	Ala	Gly
Ser	Thr, Ala, Cys	Thr
Thr	Ser	Ser
Trp	Tyr, Phe	Tyr
Tyr	Trp, Phe, Thr, Ser	Phe
Val	Ile, Met, Leu, Phe, Ala, Norleucine	Leu

[0161] The term “derivative” refers to a molecule that includes a chemical modification other than an insertion, deletion, or substitution of amino acids (or nucleic acids). In certain embodiments, derivatives comprise covalent modifications, including, but not limited to, chemical bonding with polymers, lipids, or other organic or inorganic moieties. In certain embodiments, a chemically modified antigen-binding molecule can have a greater circulating half-life than an antigen-binding molecule that is not chemically modified. In some embodiments, a derivative antigen-binding molecule is covalently modified to include one or more water soluble polymer attachments, including, but not limited to, polyethylene glycol, polyoxyethylene glycol, or polypropylene glycol.

[0162] Peptide analogs are commonly used in the pharmaceutical industry as non-peptide drugs with properties analogous to those of the template peptide. These types of non-peptide compound are termed “peptide mimetics” or “peptidomimetics.” Fauchere, J. L., *Adv. Drug Res.*, 1986, 15, 29; Veber, D. F. & Freidinger, R. M., *Trends in Neuroscience*, 1985, 8, 392-396; and Evans, B. E., *et al.*, *J. Med. Chem.*, 1987, 30, 1229-1239, which are incorporated herein by reference for any purpose.

[0163] The term “therapeutically effective amount” refers to the amount of CAR-B cells determined to produce a therapeutic response in a mammal. Such therapeutically effective amounts are readily ascertained by one of ordinary skill in the art.

[0164] The terms “patient” and “subject” are used interchangeably and include human and non-human animal subjects as well as those with formally diagnosed disorders, those without formally

recognized disorders, those receiving medical attention, those at risk of developing the disorders, etc.

[0165] The term “treat” and “treatment” includes therapeutic treatments, prophylactic treatments, and applications in which one reduces the risk that a subject will develop a disorder or other risk factor. Treatment does not require the complete curing of a disorder and encompasses embodiments in which one reduces symptoms or underlying risk factors. The term “prevent” does not require the 100% elimination of the possibility of an event. Rather, it denotes that the likelihood of the occurrence of the event has been reduced in the presence of the compound or method.

[0166] Standard techniques can be used for recombinant DNA, oligonucleotide synthesis, and tissue culture and transformation (*e.g.*, electroporation, lipofection). Enzymatic reactions and purification techniques can be performed according to manufacturer’s specifications or as commonly accomplished in the art or as described herein. The foregoing techniques and procedures can be generally performed according to conventional methods well known in the art and as described in various general and more specific references that are cited and discussed throughout the present specification. *See, e.g.*, Sambrook *et al.*, Molecular Cloning: A Laboratory Manual (2d ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989)), which is incorporated herein by reference for any purpose.

5. Sequences

[0167] The following sequences will further exemplify the invention:

CD28 transmembrane domain - mouse

(SEQ ID NO: 1)

TTCTGGGCCCTTGTGGTGGTTGCCGAGTGCTGTTTTGCTATGGGCTCCTGGTTACCGT
TGCCCTTTGTGTGATTTGGACC

CD28 transmembrane domain - mouse

(SEQ ID NO: 2)

FWALVVVAGVLCYGLLVTVALCVIWT

CD28 transmembrane domain - human

(SEQ ID NO: 3)

TTTTGGGTATTGGTAGTGGTGGGCGGAGTTTTAGCCTGCTACAGCCTCCTGGTAACAGT
GGCTTTTATCATCTTTTGGGTG

CD28 transmembrane domain - human

(SEQ ID NO: 4)

FWVLVVVGGVLACYSLLVTVAFIIFWV

CD19 cytoplasmic domain – human

(SEQ ID NO: 5)

CAGCGGGCTTTAGTCTTGCGGCGTAAACGTAAAAGAATGACAGATCCAACCTCGCAGGT
TCTTCAAAGTGACCCCCCACCTGGGTCCGGACCGCAGAACCAATATGGGAATGTCCT
GTCTCTGCCTACGCCTACAAGTGGACTGGGTAGGGCTCAGAGGTGGGCTGCCGGTCTC

GGCGGAACTGCGCCATCTTACGGAAATCCCTCCTCCGACGTTTCAGGCAGACGGGGCCC
TGGGGTCTCGATCCCCGCCTGGTGTGGACCAGAAGAGGAAGAGGGCGAGGGCTACG
AAGAGCCCCGACTCCGAAGAGGACAGTGAGTTTTACGAGAACGACAGCAACCTGGGGC
AGGATCAGCTGTACAGGATGGCTCAGGATATGAAAACCTGAGGACGAGCCTTTGGG
GCCTGAAGATGAGGACTCCTTTTCTAATGCAGAGTCATATGAGAATGAGGACGAAGAA
TTGACTCAACCCGTGGCAAGAACAATGGATTTCTCAGTCCACACGGGAGTGCATGGG
ACCCCTCCAGAGAGGCTACTAGCCTCGGTCTCAAAGCTATGAGGACATGAGGGGTAT
TCTGTACGCAGCGCCTCAGTTGAGGTCCATCCGCGGCCAGCCAGGCCCCAAACCATGAG
GAAGATGCCGATTCTTACGAAAACATGGACAACCCCGATGGTCCTGACCCCGCATGGG
GGGGCGGCGGGAGGATGGGCACCTGGTCTACTCGC

CD19 cytoplasmic domain – human

(SEQ ID NO: 6)

QRALVLRKRKRMTDPTRRFFKVTTPPGSGPQNQYGNVLSLPTPTSLGRAQRWAAGLGG
TAPSYGNPSSDVQADGALGSRPPGVGPEEEEGEGYEEDSEEDSEFYENDSNLQDQLSQ
DGSGYENPEDEPLGPEDEDSFSNAESYENEDEELTPVARTMDFLSPHGSADWDPSREATSL
GSQSYEDMRGILYAAPQLRSIRGQPGPNHEEDADSYENMDNPDGPDPAWGGGGRMGTS
TR

CD40 cytoplasmic domain – human

(SEQ ID NO: 7)

AAGAAGGTTGCAAAAAACCTACTAATAAGGCTCCCCATCCTAAGCAAGAGCCCCAA
GAAATTAACCTTTCCCGATGATCTTCCGGGTTCTAACACGGCAGCCCCGGTGCAGGAGA
CCCTGCATGGTTGTCAACCCGTCACTCAGGAGGACGGGAAAGAGTCTCGTATCTCCGT
CCAGGAGAGACAG

CD40 cytoplasmic domain – human

(SEQ ID NO: 8)

KKVAKKPTNKAPHPKQEPQEINFPDDLPGSNTAAPVQETLHGCQPVTQEDGKESRISVQER
Q

CD40 + CD79b cytoplasmic domain – human

(SEQ ID NO: 9)

AAGAAGGTTGCAAAAAACCTACTAATAAGGCTCCCCATCCTAAGCAAGAGCCCCAA
GAAATTAACCTTTCCCGATGATCTTCCGGGTTCTAACACGGCAGCCCCGGTGCAGGAGA
CCCTGCATGGTTGTCAACCCGTCACTCAGGAGGACGGGAAAGAGTCTCGTATCTCCGT
CCAGGAGAGACAGGACAAGGACGATAGTAAAGCAGGGATGGAGGAGGACCATACAT
ACGAGGGACTGGATATCGATCAGACAGCCACGTACGAAGACATTGTGACACTGAGAA
CTGGCGAGGTGAAGTGGTCAGTGGGAGAACATCCGGGGCAGGAA

CD40 + CD79b cytoplasmic domain – human

(SEQ ID NO: 10)

KKVAKKPTNK AHPKQEPQE INFPDDLPGS NTAAPVQETL HGCQPVTQED
GKESRISVQE RQDKDDSKAG MEEDHTYEG L DIDQTATYED IVTLRTGEVK
WSVGEHPGQE

CD40 + CD137 cytoplasmic domain – human

(SEQ ID NO: 11)

AAGAAGGTTGCAAAAAACCTACTAATAAGGCTCCCCATCCTAAGCAAGAGCCCCAA
GAAATTAACCTTTCCCGATGATCTTCCGGGTTCTAACACGGCAGCCCCGGTGCAGGAGA
CCCTGCATGGTTGTCAACCCGTCACTCAGGAGGACGGGAAAGAGTCTCGTATCTCCGT
CCAGGAGAGACAGAAAAGAGGCCGAAAAAAGCTGCTGTACATCTTCAAACAACCCTT

CATGCGACCTGTTACAGACGACACAGGAGGAGGACGGCTGCAGCTGTAGGTTTCCCGAA
GAAGAGGAGGGAGGATGCGAACTT

CD40 + CD137 cytoplasmic domain – human

(SEQ ID NO: 12)

KKVAKKPTNKAPHPKQEPQEINFPDDLPGSNTAAPVQETLHGCQPVTQEDGKESRISVQER
QKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEEGGCEL

CD137 cytoplasmic domain – human

(SEQ ID NO: 13)

AAAAGAGGCCGAAAAAAGCTGCTGTACATCTTCAAACAACCCTTCATGCGACCTGTTT
AGACGACACAGGAGGAGGACGGCTGCAGCTGTAGGTTTCCCGAAGAAGAGGAGGGAG
GATGCGAACTT

CD137 cytoplasmic domain – human

(SEQ ID NO: 14)

KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEEGGCEL

CD40 and Fc gamma receptor 2a cytoplasmic domain – human

(SEQ ID NO: 15)

AAGAAGGTTGCAAAAAACCTACTAATAAGGCTCCCCATCCTAAGCAAGAGCCCCAA
GAAATTAACCTTTCCCGATGATCTTCCGGGTTCTAACACGGCAGCCCCGGTGCAGGAGA
CCCTGCATGGTTGTCAACCCGTCACCTCAGGAGGACGGGAAAGAGTCTCGTATCTCCGT
CCAGGAGAGACAGCGCAAAAAACGTATAAGCGCAAACTCTACAGATCCAGTAAAAGC
CGCGCAATTCGAGCCTCCCGGCCGCCAGATGATTGCAATACGGAAACGTCAACTGGAG
GAACTAATAATGACTATGAGACGGCCGACGGTGGATACATGACCCTTAATCCCCGCG
CGCCAACCGACGATGATAAGAACATATATCTGACGCTCCCCCTAACGATCACGTAA
CAGTAATAAT

CD40 and Fc gamma receptor 2a cytoplasmic domain – human

(SEQ ID NO: 16)

KKVAKKPTNKAPHPKQEPQEINFPDDLPGSNTAAPVQETLHGCQPVTQEDGKESRISVQER
QRKKRISANSTDPVKAAQFEPGRQMIAIRKRQLEETNNDYETADGGYMTLNPRAPTDDD
KNIYLTLPNDHVNSNN

Fc gamma receptor 2a cytoplasmic domain – human

(SEQ ID NO: 17)

CGAAAAAACGTATAAGCGCAAACTCTACAGATCCAGTAAAAGCCGCGCAATTCGAG
CCTCCCGGCCGCCAGATGATTGCAATACGGAAACGTCAACTGGAGGAACTAATAATG
ACTATGAGACGGCCGACGGTGGATACATGACCCTTAATCCCCGCGCGCCAACCGACGA
TGATAAGAACATATATCTGACGCTCCCCCTAACGATCACGTAAACAGTAATAAT

Fc gamma receptor 2a cytoplasmic domain – human

(SEQ ID NO: 18)

RKKRISANSTDPVKAAQFEPGRQMIAIRKRQLEETNNDYETADGGYMTLNPRAPTDDDK
NIYLTLPNDHVNSNN

Myd88 + CD40 cytoplasmic domain – human

(SEQ ID NO: 19)

ATGGCGGCGGGCGGGCCCGCGCCGGAAGCGCCGCGCCAGTCTCATCTACGTCCAGTC
TGCCACTGGCTGCCCTGAACATGAGAGTGAGACGCCGTTTATCCCTCTTCTGAATGTG
CGGACCCAGGTCGCCGCTGATTGGACCGCCCTGGCCGAAGAGATGGACTTTGAATACT
TGGAATCAGACAGCTGGAAACACAGGCAGACCCAACGGGAGACTGCTTGACGCTT
GGCAGGGACGCCAGGGGCAAGTGTGGTTCGGTTACTGGAGCTTTTAACTAAGTTGGG

CCGCGATGACGTGCTGTTGGAGTTAGGACCCAGTATCGAGGAGGATTGTCAGAAATAC
ATCTTGAACAGCAGCAGGAGGAGGCGGAAAAGCCCCCTGCAGGTGGCGGCCGTTGAC
AGCAGTGTACCCAGAACAGCTGAGCTGGCCGGCATCACAACCCTGGATGATCCCCCTGG
GCCACATGCCTGAGAGGTTTCGACGCTTTCATAAAGAAGGTTGCAAAAAACCTACTAA
TAAGGCTCCCCATCCTAAGCAAGAGCCCCAAGAAATTAACCTTCCCGATGATCTTCCG
GGTTCTAACACGGCAGCCCCGGTGCAGGAGACCCTGCATGGTTGTCAACCCGTCACCTC
AGGAGGACGGGAAAGAGTCTCGTATCTCCGTCCAGGAGAGACAG

Myd88 + CD40 cytoplasmic domain – human

(SEQ ID NO: 20)

MAAGGPGAGSAAPVSSTSSLPLAALNMRVRRRLSLFLNVRTQVAADWTALAEEMDFEYL
EIRQLETQADPTGRLLDAWQGRPGASVGRLLLELLTKLGRDDVLLELGPSIEEDCQKYILKQ
QQEEAEKPLQVAAVDSSVPRTAELAGITTLDDPLGHMPERFD AFIKKVAKKPTNKAPHPK
QEPQEINFDDPLPGSNTAAPVQETLHGCQPVTQEDGKESRISVQERQ

Myd88 cytoplasmic domain – human

(SEQ ID NO: 21)

ATGGCGGCGGGCGGGCCCCGGCGCCGGAAGCGCCGCGCCAGTCTCATCTACGTCCAGTC
TGCCACTGGCTGCCCTGAACATGAGAGTGAGACGCCGTTTATCCCTCTTCCTGAATGTG
CGGACCCAGGTCGCCGCTGATTGGACCGCCCTGGCCGAAGAGATGGACTTTGAATACT
TGGAATCAGACAGCTGGAAACACAGGCAGACCCAACCGGGAGACTGCTTGACGCCT
GGCAGGGACGCCCAGGGGCAAGTGTTGGTTCGGTTACTGGAGCTTTTAACTAAGTTGGG
CCGCGATGACGTGCTGTTGGAGTTAGGACCCAGTATCGAGGAGGATTGTCAGAAATAC
ATCTTGAACAGCAGCAGGAGGAGGCGGAAAAGCCCCCTGCAGGTGGCGGCCGTTGAC
AGCAGTGTACCCAGAACAGCTGAGCTGGCCGGCATCACAACCCTGGATGATCCCCCTGG
GCCACATGCCTGAGAGGTTTCGACGCTTTCATA

Myd88 cytoplasmic domain – human

(SEQ ID NO: 22)

MAAGGPGAGSAAPVSSTSSLPLAALNMRVRRRLSLFLNVRTQVAADWTALAEEMDFEYL
EIRQLETQADPTGRLLDAWQGRPGASVGRLLLELLTKLGRDDVLLELGPSIEEDCQKYILKQ
QQEEAEKPLQVAAVDSSVPRTAELAGITTLDDPLGHMPERFD AFI

CD79a cytoplasmic domain – human

(SEQ ID NO: 23)

AGGAAACGATGGCAGAACGAGAAGCTCGGGTTGGATGCCGGGGATGAATATGAAGAT
GAAAACCTTTATGAAGGCCTGAACCTGGACGACTGCTCCATGTATGAGGACATCTCCC
GGGGCCTCCAGGGCACCTACCAGGATGTGGGCAGCCTAACATAGGAGATGTCCAGCT
GGAGAAGCCG

CD79a cytoplasmic domain – human

(SEQ ID NO: 24)

RKRWQNEKLGLDAGDEYEDENLYEGLNLDDCSMYEDISRGLQGTQYQDVGSLNIGDVQLE
KP

CD79b cytoplasmic domain – human

(SEQ ID NO: 25)

CTGGACAAGGATGACAGCAAGGCTGGCATGGAGGAAGATCACACCTACGAGGGCCTG
GACATTGACCAGACAGCCACCTATGAGGACATAGTGACGCTGCGGACAGGGGAAGTG
AAGTGGTCTGTAGGTGAGCACCCAGGCCAGGAG

CD79b cytoplasmic domain – human

(SEQ ID NO: 26)

LDKDDSKAGMEEDHTYEGLDIDQTATYEDIVTLRTGEVKWSVGEHPGQE

CD8 hinge domain - human

(SEQ ID NO: 27)

TTCGTGCCTGTGTTCTCCAGCTAAGCCCACTACCACCCCGCTCCAAGGCCGCCAC
GCCCCGCTCCTACTATTGCTAGTCAGCCTTTAAGTTTACGACCCGAAGCTTGCAGGCCG
CCGCCGGCGGCGCTGTGCACACCAGGGGGCTTGATTTTGCCTGCGAC

CD8 hinge domain - human

(SEQ ID NO: 28)

FVPVFLPAKPTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACD

Spacer with 3X strep II tag

(SEQ ID NO: 29)

GGCGCTGGTAGTGGCGGTAAGTGGAGCCACCCTCAATTTGAGAAGGGCGGGTCAGGC
GGATCAGGTGGTAGTGGTGGGTCCAAGTGGAGCCATCCGCAATTTGAAAAGGGCGGA
AGCGGCGGTTCCGGCGGTTTCAAGCGGTAGCAACTGGTCACATCCGCAATTTGAGAAAG
GCGGGTCAGGCGGCGGG

Spacer with 3X strep II tag

(SEQ ID NO: 30)

GAGSGGNWSHPQFEKGGSGGSGGSGGSNWSHPQFEKGGSGGSGGSGGSNWSHPQFEKGG
SGGG

human IgG1 Fc (transmembrane form)

(SEQ ID NO: 31)

CCCAAGAGCTGCGACAAGACCCACACCTGCCCCCCTGCCAGCCCCAGAGCTGCTGG
GCGGACCCTCCGTGTTCTGTTCCCCCAAGCCCAAGGACACCCTGATGATCAGCAG
GACCCCGAGGTGACCTGCGTGGTGGTGGACGTGAGCCACGAGGACCCAGAGGTGAA
GTTCAACTGGTACGTGGACGCGGTGGAGGTGCACAACGCCAAGACCAAGCCCAGAGA
GGAGCAGTACAACAGCACCTACAGGGTGGTGTCCGTGCTGACCGTGCTGCACCAGGAC
TGGCTGAACGGCAAGGAATACAAGTGCAAGGTCTCCAACAAGGCCCTGCCAGCCCC
ATCGAAAAGACCATCAGCAAGGCCAAGGGCCAGCCACGGGAGCCCCAGGTGTACACC
CTGCCCCCCTCCCGGGAGGAGATGACCAAGAACCAGGTGTCCCTGACCTGTCTGGTGA
AGGGCTTCTACCCAGCGACATCGCCGTGGAGTGGGAGAGCAACGGCCAGCCCGAGA
ACAATAACAAGACCACCCCCCAGTGCTGGACAGCGACGGCAGCTTCTTCTGTACAG
CAAGCTGACCGTGGACAAGTCCAGGTGGCAGCAGGGCAACGTGTTTCAAGCTGCAGCGT
GATGCACGAGGCCCTGCACAACCACTACACCCAGAAGAGCCTGAGCCTGTCCCCGAG
CTGCAACTGGAGGAGAGCTGTGCGGAGGCGCAGGACGGGGAGCTGGACGGGCTGTGG
ACGACCATCACCATCTTCATCACACTCTTCTGTGTAAGCGTGTGCTACAGTGCCACCGT
CACCTTCTTCAAGGTGAAGTGGATCTTCTCTCGGTGGTGGACCTGAAGCAGACCATC
ATCCCCGACTACAGGAACATGATCGGACAGGGGGCCTGA

human IgG1 Fc (transmembrane form)

(SEQ ID NO: 32)

PKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNW
YVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTIS
KAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPV
LDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSPQLQLEESCAEAQ
DGELDGLWTTITIFITLFLLSVCYSATVTFFKVKWIFSSVVDLKQTIIIPDYRNMIGQGA

anti-huPSMA scFv

(SEQ ID NO: 33)

GAGGTTCAACTTGTTCAATCTGGGGCAGAAGTGAAGAAGCCCGGGGCATCTGTGAAAG
TATCATGCAAAACATCCGGCTATACGTTTACCGAATACACCATTCACTGGGTCAGACA
GGCTCCCGGTCAAAGCCTCGAATGGATGGGAAATATTAACCCTAACAAATGGCGGAACC
ACATATAATCAGAAATTCCAAGGCCGAGTGACGATAACTGTCGATAAGAGTACGTCCA
CAGCTTACATGGAACCTCAGCTCTTTGAGATCCGAAGACACTGCAGTTTATTATTGTGCA
GCTGGATGGAACCTTCGACTATTGGGGACAAGGGACTCTTGTTACGGTGTCCAGTGGCA
AACCAGGTAGTGGTAAACCCGGAAGCGGCAAGCCCGGGAGCGGTAAACCTGGTAGCG
ACATCGTCATGACTCAAAGCCCTGACTCACTCGCCGTGAGCCTGGGAGAGCGTGCAAC
GCTATCTTGTGCGGGCCTCTCAGGATGTCGGAACCTGCTGTAGACTGGTATCAACAGAAA
CCTGACCAATCACCAAACTCCTGATTTATTGGGCCTCAACACGTCACACAGGAGTGC
CAGATAGGTTACAGGTAGTGGCAGTGGAACCTGATTTTACTTTGACAATTAGCAGCCT
GCAAGCCGAAGATGTAGCCGTTTACTTCTGTCAACAATATAACTCATACCCACTAACG
TTCGGTGCCGGGACGAAGGTAGAGATTAAA

anti-huPSMA scFv

(SEQ ID NO: 34)

EVQLVQSGAE VKKPGASVKV SCKTSGYTFT EYTIHWVRQA PGQSLEWMGN
INPNNGGTTY NQKFQGRVTI TVDKSTSTAY MELSSLRSED TAVYYCAAGW
NFDYWGQGTI VTVSSGKPGS GKPGSGKPGS GKPGSDIVMT QSPDSLAVSL
GERATLSCRA SQDVGTAVDW YQKPKDQSPK LLIYWASTRH TGVDPDRFTGS
GSGTDFTLTI SSLQAEDVAV YFCQQYNSYP LTFGAGTKVE IK

anti-Sarcoglycan scFv

(SEQ ID NO: 35)

GAAGTCCAATTGGTTGAAAGCGGTGGTGGACTCGTCAAACCTGGCGGTAGCCTTAAAC
TTTCATGTGCCGCAAGCGGCTTCACGTTTAGTAACTATGCTATGAGTTGGGTCCGCCAA
AGTCCAGAAAAGCGCCTCGAATGGGTGGCGGAGATCTCTGGAGGAGGAACATATACA
TATTATCCAGACACCATGACCGGTAGGTTTACAATCTCAAGAGACAACGCTAAGAACA
CCCTGTACCTGGAATGTCAAGCCTGAGATCAGAAGATACGGCCATGTATTATTGTAC
GCGCCTACTCGACTATTGGGGTCAAGGAACTTCCGTGACGGTGTCAAGCGGAGGAGGT
GGGAGCGGAGGAGGCGGAAGTGGCGGTGGTGGCTCTGGTGGCGGTGGAAGTGATATA
GTGATGACGCAAGCTGCCTTTTCAAACCCTGTTACTTTGGGGACTAGCGCATCAATCTC
CTGTAGGTCCAGCAAATCTTTGCTGCACAGTAATGGAATCACCTATCTTTTCTGGTATT
TGCAAAAGCCTGGGCAGAGCCCGCAACTGCTGATCTATCAAATGTCAAATCTTGCTTC
CGGAGTTCAGACCGCTTCTCAAGTTCCGGGTCCGGCACTGATTTTACCTTGAGAAATT
CTAGGGTCAAGCTGAAGACGTCGGTGTCTATTATTGCGCGCAAAACCTTGAGCTTCC
ATACACCTTCGGGGGGGGGCACAAAACCTTGAGATCAAG

anti-Sarcoglycan scFv

(SEQ ID NO: 36)

EVQLVESGGGLVKPGGSLKLSAASGFTFSNYAMSWVRQSPEKRLEWVAEISGGGTYTY
PDTMTGRFTISRDNKNTLYLEMSSLRSEDAMYCTRLLDYWGQGTSTVTVSSGGGSG
GGSGGGGSGGGGSDIVMTQAAFSNPVTLGTSASISCRSSKLLHSNGITYLFWYLQKPGQ
SPQLLIYQMSNLASGVPDRFSSSGSGTDFTLRISRVEAEDVGVYYCAQNLELPYTFGGG
TKLEIK

anti-hu GPC3 scFv

(SEQ ID NO: 37)

CAGTCTGTGTTGACGCAGCCGCCCTCAGTGTCTGCGGCCCCAGGACAGAGGGTCACCA
TCTCCTGTCTGGAACCAGGTCCAACATTGGGAGTGATTATGTTTCCTGGTACCAACAC
CTCCCAGGAACAGCCCCCAAACCTCCTCGTTTATGGCGATAATCTGCGACCCTCAGGGA
TTCCTGACCGATTCTCTGCCTCCAAGTCTGGCACGTCAGCCACCCTGGGCATCACCGGA

CTCCAGACTGGGGACGAGGCCGATTATTACTGCGGCACATGGGATTACACCCTGAATG
GTGTGGTGTTCGGCGGAGGGACCAAGCTGACCGTCCTAGGTTCTAGAGGTGGTGGTGG
TAGCGGCGGGCGGCGGCTCTGGTGGTGGTGGATCCCTCGAGATGGCCCAGGTGCAGCTG
GTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGGGGGTCCCTGAGACTCTCCTGTGCAG
CCTCTGGATTACCTTTAGCAGCTATGCCATGAGCTGGGTCCGCCAGGCTCCAGGGAA
GGGGCTGGAGTGGGTCTCAGTTATTTATAGCGGTGGTAGTAGCACATACTATGCAGAC
TCCGTGAAGGGCCGGTTCACCATCTCCAGAGATAATTCCAAGAACACGCTGTATCTGC
AAATGAACAGCCTGAGAGCCGAGGACACGGCCGTATATTACTGTGCGCGCACTTCTTA
CCTGAACCATGGTGATTACTGGGGTCAAGGTACTCTGGTGACCGTGTCTAGCGCCGCT
GCA

anti-hu GPC3 scFv

(SEQ ID NO: 38)

QSVLTQPPSVSAAPGQRTVITSCSGTRSNIGSDYVSWYQHLPGTAPKLLVYGDNLRPSGIPDR
FSASKSGTSATLGITGLQTGDEADYYCGTWDYTLNGVVFGGGTKLTVLGSRGGGGSGGG
GSGGGGSLEMAQVQLVESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWV
SVIYSGGSSTYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCARTSYLNHGDIY
QGQTLVTVSSAAA

pWF-82

(SEQ ID NO: 39)

GAGGTTCAACTTGTTCAATCTGGGGCAGAAGTGAAGAAGCCCGGGGCATCTGTGAAAG
TATCATGCAAAACATCCGGCTATACGTTTACCGAATACACCATTCACTGGGTCAGACA
GGCTCCCGGTCAAAGCCTCGAATGGATGGGAAATATTAACCCTAACAATGGCGGAACC
ACATATAATCAGAAATTCCAAGGCCGAGTGACGATAACTGTGCGATAAGAGTACGTCCA
CAGCTTACATGGAACCTCAGCTCTTTGAGATCCGAAGACACTGCAGTTTATTATTGTGCA
GCTGGATGGAACCTTCGACTATTGGGGACAAGGGACTCTTGTTACGGTGTCCAGTGGCA
AACCAGGTAGTGGTAAACCCGGAAGCGGCAAGCCCGGGAGCGGTAAACCTGGTAGCG
ACATCGTCGACTCAAAGCCCTGACTCACTCGCCGTGAGCCTGGGAGAGCGGTGCAAC
GCATCTTTGTTCGGGCTCTCAGGATGTTCGGAAGTCTGTAGACTGGTATCAACAGAAA
CCTGACCAATCACCAAACTCCTGATTTATTGGGCCTCAACACGTCACACAGGAGTGC
CAGATAGGTTACAGGTAGTGGCAGTGGAAGTGAATTTACTTTGACAATTAGCAGCCT
GCAAGCCGAAGATGTAGCCGTTTACTTCTGTCAACAATATAACTCATACCCACTAACG
TTCGGTGCCGGGACGAAGGTAGAGATTAAATTCGTGCCTGTGTTCTCCAGCTAAGC
CCACTACCACCCCGCTCCAAGGCCGCCACGCCCGCTCCTACTATTGCTAGTCAGCCT
TTAAGTTTACGACCCGAAGCTTGCAGGCCCGCCGCCGGCGGCGCTGTGCACACCAGGG
GGCTTGATTTTGCCTGCGACTTTTGGGTATTGGTAGTGGTGGGCGGAGTTTATGCTGC
TACAGCCTCCTGGTAACAGTGGCTTTTATCATCTTTTGGGTGCAGCGGGCTTTAGTCTT
GCGGCGTAAACGTAAAAGAATGACAGATCCAACCTCGCAGGTTCTTCAAAGTGACCCCC
CCACCTGGGTCCGGACCGCAGAACCAATATGGGAATGTCCTGTCTCTGCCTACGCCTA
CAAGTGGACTGGGTAGGGCTCAGAGGTGGGCTGCCGGTCTCGGCGGAACTGCGCCATC
TTACGGAAATCCCTCCTCCGACGTTTACGGCAGACGGGGCCCTGGGGTCTCGATCCCCG
CCTGGTGTGGACCAGAAGAGGAAGAGGGCGAGGGCTACGAAGAGCCCGACTCCGAA
GAGGACAGTGAGTTTACGAGAACGACAGCAACCTGGGGCAGGATCAGCTGTCACAG
GATGGCTCAGGATATGAAAACCTGAGGACGAGCCTTTGGGGCCTGAAGATGAGGAC
TCCTTTTCTAATGCAGAGTCATATGAGAATGAGGACGAAGAATTGACTCAACCCGTGG
CAAGAACAATGGATTTCTCAGTCCACACGGGAGTGCATGGGACCCCTCCAGAGAGGC
TACTAGCCTCGGTTCTCAAAGCTATGAGGACATGAGGGGTATTCTGTACGCAGCGCCT
CAGTTGAGGTCCATCCGCGGCCAGCCAGGCCCAAACCATGAGGAAGATGCCGATTCTT
ACGAAAACATGGACAACCCCGATGGTCCTGACCCCGCATGGGGGGGCGGCGGGAGGA
TGGGCACCTGGTCTACTCGCTAG

pWF-82

(SEQ ID NO: 40)

EVQLVQSGAE VKKPGASVKV SCKTSGYTFT EYTIHWVRQA PGQSLEWMGN
INPNNGGTTY NQKFQGRVTI TVDKSTSTAY MELSSLRSED TAVYYCAAGW
NFDYWGQGTI VTVSSGKPGS GKPGSGKPGS GKPGSDIVMT QSPDSLAVSL
GERATLSCRA SQDVGTAVDW YQQKPDQSPK LLIYWASTRH TGVDPDRFTGS
GSGTDFTLTI SSLQAEDVAV YFCQQYNSYP LTFGAGTKVE IKFVPVFLPA KPTTTPAPRP
PTPAPTIASQ PLSLRPEACR PAAGGAVHTR GLDFACDFWV LVVVGGVLAC
YSLLVTVAFI IFWVQRALVL RRRKRKRMTDP TRRFFKVTPP PGSGPQNQYG NVLSLPTPTS
GLGRAQRWAA GLGGTAPSYG NPSSDVQADG ALGSRSPPGV GPEEEEEEGGY
EEDPSEEDSE FYENDSNLQ DQLSQDGSY ENPEDEPLGP EDEDSFSNAE SYENEDEELT
QPVARMTDFL SPHGSAWDPS REATSLGSQS YEDMRGILYA APQLRSIRGQ
PGPNHEEDAD SYENMDNPDG PDPAWGGGGR MGTWSTR-

pWF-83

(SEQ ID NO: 41)

GAGGTTCAACTTGTTCAATCTGGGGCAGAAGTGAAGAAGCCCGGGGCATCTGTGAAAG
TATCATGCAAAACATCCGGCTATACGTTTACCGAATACACCATTCACTGGGTCAGACA
GGCTCCCGGTCAAAGCCTCGAATGGATGGGAAATATTAACCCTAACAATGGCGGAACC
ACATATAATCAGAAATTCCAAGGCCGAGTGACGATAACTGTGCGATAAGAGTACGTCCA
CAGCTTACATGGAAGTCAGCTCTTTGAGATCCGAAGACACTGCAGTTTATTATTGTGCA
GCTGGATGGAAGTTGCGACTATTGGGGACAAGGGACTCTTGTTACGGTGTCCAGTGGCA
AACCAGGTAGTGGTAAACCCGGAAGCGGCAAGCCCGGGAGCGGTAAACCTGGTAGCG
ACATCGTCATGACTCAAAGCCCTGACTCACTCGCCGTGAGCCTGGGAGAGCGTGCAAC
GCTATCTTGTCTGGGCCTCTCAGGATGTCGGAAGTGTGTAGACTGGTATCAACAGAAA
CCTGACCAATCACAAAACCTCTGATTTATTGGGCCTCAACACGTCACACAGGAGTGC
CAGATAGGTTACAGGTAGTGGCAGTGGAAGTGTATTTACTTTGACAATTAGCAGCCT
GCAAGCCGAAGATGTAGCCGTTTACTTCTGTCAACAATATAACTCATACCCACTAAGC
TTCGGTGCCGGGACGAAGGTAGAGATTAAATTCGTGCCTGTGTTCTCCAGCTAAGC
CCACTACCACCCCGCTCCAAGGCCGCCACGCCCGCTCTACTATTGTAGTCAGCCT
TTAAGTTTACGACCCGAAGCTTGCAGGCCCGCCCGCGCGCGCTGTGCACACCAGGG
GGCTTGATTTTGCCTGCGACTTTTGGGTATTGGTAGTGGTGGGCGGAGTTTATGCTGC
TACAGCCTCCTGGTAACAGTGGCTTTTATCATCTTTTGGGTGCTGGACAAGGATGACAG
CAAGGCTGGCATGGAGGAAGATCACACCTACGAGGGCCTGGACATTGACCAGACAGC
CACCTATGAGGACATAGTGACGCTGCGGACAGGGGAAGTGAAGTGGTCTGTAGGTGA
GCACCCAGGCCAGGAGTGA

pWF-83

(SEQ ID NO: 42)

EVQLVQSGAE VKKPGASVKV SCKTSGYTFT EYTIHWVRQA PGQSLEWMGN
INPNNGGTTY NQKFQGRVTI TVDKSTSTAY MELSSLRSED TAVYYCAAGW
NFDYWGQGTI VTVSSGKPGS GKPGSGKPGS GKPGSDIVMT QSPDSLAVSL
GERATLSCRA SQDVGTAVDW YQQKPDQSPK LLIYWASTRH TGVDPDRFTGS
GSGTDFTLTI SSLQAEDVAV YFCQQYNSYP LTFGAGTKVE IKFVPVFLPA KPTTTPAPRP
PTPAPTIASQ PLSLRPEACR PAAGGAVHTR GLDFACDFWV LVVVGGVLAC
YSLLVTVAFI IFWVLDKDDS KAGMEEDHTY EGLDIDQTAT YEDIVTLRTG
EVKWSVGEHP GQE-

pWF-84:

(SEQ ID NO: 43)

GAGGTTCAACTTGTTCAATCTGGGGCAGAAGTGAAGAAGCCCGGGGCATCTGTGAAAG
TATCATGCAAAACATCCGGCTATACGTTTACCGAATACACCATTCACTGGGTCAGACA
GGCTCCCGGTCAAAGCCTCGAATGGATGGGAAATATTAACCCTAACAATGGCGGAACC
ACATATAATCAGAAATTCCAAGGCCGAGTGACGATAACTGTGCGATAAGAGTACGTCCA

CAGCTTACATGGAAGCTCAGCTCTTTGAGATCCGAAGACACTGCAGTTTATTATTGTGCA
GCTGGATGGAAGTTTCGACTATTGGGGACAAGGGACTCTTGTTACGGTGTCCAGTGGCA
AACCAGGTAGTGGTAAACCCGGAAGCGGCAAGCCCCGGGAGCGGTAAACCTGGTAGCG
ACATCGTCATGACTCAAAGCCCTGACTCACTCGCCGTGAGCCTGGGAGAGCGTGCAAC
GCTATCTTGTGGGCCTCTCAGGATGTCGGAAGTGTGTAGACTGGTATCAACAGAAA
CCTGACCAATCACCAAACTCCTGATTTATTGGGCCTCAACACGTCACACAGGAGTGC
CAGATAGGTTTACAGGTAGTGGCAGTGGAAGTGTATTTACTTTGACAATTAGCAGCCT
GCAAGCCGAAGATGTAGCCGTTTACTTCTGTCAACAATATAACTCATACCCACTAAGC
TTCGGTGCCGGGACGAAGGTAGAGATTAAATTCGTGCCTGTGTTCTCCAGCTAAGC
CCACTACCACCCCGCTCCAAGGCCGCCACGCCCGCTCCTACTATTGCTAGTCAGCCT
TTAAGTTTACGACCCGAAGCTTGCAGGCCCGCCGCCGGCGCGCTGTGCACACCAGGG
GGCTTGATTTTGCCTGCGACTTTTGGGTATTGGTAGTGGTGGGCGGAGTTTATAGCCTGC
TACAGCCTCCTGGTAACAGTGGCTTTTATCATCTTTTGGGTGAAGAAGGTTGCAAAAA
AACCTACTAATAAGGCTCCCCATCCTAAGCAAGAGCCCCAAGAAATTAACCTTTCCCGA
TGATCTTCCGGGTTCTAACACGGCAGCCCCGGTGCAGGAGACCCTGCATGGTTGTCAA
CCCGTCACTCAGGAGGACGGGAAAGAGTCTCGTATCTCCGTCCAGGAGAGACAGGAC
AAGGACGATAGTAAAGCAGGGATGGAGGAGGACCATACATACGAGGGACTGGATATC
GATCAGACAGCCACGTACGAAGACATTGTGACACTGAGAACTGGCGAGGTGAAGTGG
TCAGTGGGAGAACATCCGGGGCAGGAATAA

pWF-84:

(SEQ ID NO: 44)

EVQLVQSGAE VKKPGASVKV SCKTSGYTFT EYTIHWVRQA PGQSLEWMGN
INPNNGGTTY NQKFQGRVTI TVDKSTSTAY MELSSLRSED TAVYYCAAGW
NFDYWQGTL VTVSSGKPGS GKPGSGKPGS GKPGSDIVMT QSPDSLAVSL
GERATLSCRA SQDVGTAVDW YQQKPDQSPK LLIYWASTRH TGVDPDRFTGS
GSGTDFTLTI SSLQAEDVAV YFCQQYNSYP LTFGAGTKVE IKFVPVFLPA KPTTTPAPRP
PTPAPTIASQ PLSLRPEACR PAAGGAVHTR GLDFACDFWV LVVVGGVLAC
YSLLVTVAFI IFWVKVAKK PTNKAPHPKQ EPQEINFDD LPGSNTAAPV
QETLHGCQPV TQEDGKESRI SVQERQDKDD SKAGMEEDHT YEGLDIDQTA
TYEDIVTLRT GEVKWSVGEH PGQE-

pWF-85:

(SEQ ID NO: 45)

GAGGTTCAACTTGTTCAATCTGGGGCAGAAGTGAAGAAGCCCCGGGGCATCTGTGAAAG
TATCATGCAAAACATCCGGCTATACGTTTACCGAATACACCATTCACTGGGTCAGACA
GGCTCCCGGTCAAAGCCTCGAATGGATGGGAAATATTAACCCTAACAATGGCGGAACC
ACATATAATCAGAAATTCCAAGGCCGAGTGACGATAACTGTCGATAAGAGTACGTCCA
CAGCTTACATGGAAGCTCAGCTCTTTGAGATCCGAAGACACTGCAGTTTATTATTGTGCA
GCTGGATGGAAGTTTCGACTATTGGGGACAAGGGACTCTTGTTACGGTGTCCAGTGGCA
AACCAGGTAGTGGTAAACCCGGAAGCGGCAAGCCCCGGGAGCGGTAAACCTGGTAGCG
ACATCGTCATGACTCAAAGCCCTGACTCACTCGCCGTGAGCCTGGGAGAGCGTGCAAC
GCTATCTTGTGGGCCTCTCAGGATGTCGGAAGTGTGTAGACTGGTATCAACAGAAA
CCTGACCAATCACCAAACTCCTGATTTATTGGGCCTCAACACGTCACACAGGAGTGC
CAGATAGGTTTACAGGTAGTGGCAGTGGAAGTGTATTTACTTTGACAATTAGCAGCCT
GCAAGCCGAAGATGTAGCCGTTTACTTCTGTCAACAATATAACTCATACCCACTAAGC
TTCGGTGCCGGGACGAAGGTAGAGATTAAATTCGTGCCTGTGTTCTCCAGCTAAGC
CCACTACCACCCCGCTCCAAGGCCGCCACGCCCGCTCCTACTATTGCTAGTCAGCCT
TTAAGTTTACGACCCGAAGCTTGCAGGCCCGCCGCCGGCGCGCTGTGCACACACAGGG
GGCTTGATTTTGCCTGCGACTTTTGGGTATTGGTAGTGGTGGGCGGAGTTTATAGCCTGC
TACAGCCTCCTGGTAACAGTGGCTTTTATCATCTTTTGGGTGAAGAAGGTTGCAAAAA
AACCTACTAATAAGGCTCCCCATCCTAAGCAAGAGCCCCAAGAAATTAACCTTTCCCGA
TGATCTTCCGGGTTCTAACACGGCAGCCCCGGTGCAGGAGACCCTGCATGGTTGTCAA
CCCGTCACTCAGGAGGACGGGAAAGAGTCTCGTATCTCCGTCCAGGAGAGACAGAAA

AGAGGCCGAAAAAAGCTGCTGTACATCTTCAAACAACCCTTCATGCGACCTGTTTCAGA
CGACACAGGAGGAGGACGGCTGCAGCTGTAGGTTTCCCGAAGAAGAGGAGGGAGGAT
GCCAACTTTAA

pWF-85:

(SEQ ID NO: 46)

EVQLVQSGAE VKKPGASVKV SCKTSGYTFT EYTIHWVRQA PGQSLEWMGN
INPNNGGTTY NQKFQGRVTI TVDKSTSTAY MELSSLRSED TAVYYCAAGW
NFDYWGGTL VTVSSGKPGS GKPGSGKPGS GKPGSDIVMT QSPDSLAVSL
GERATLSCRA SQDVGTAVDW YQQKPDQSPK LLIYWASTRH TGVDPDRFTGS
GSGTDFTLTI SSLQAEDVAV YFCQQYNSYP LTFGAGTKVE IKFVPVFLPA KPTTTTPAPRP
PTPAPTIASQ PLSLRPEACR PAAGGAVHTR GLDFACDFWV
LVVVGGVLAC YSLLVTVAFI IFWVKVAKK PTNKAPHPKQ EPQEINFPDD
LPGSNTAAPV QETLHGCQPV TQEDGKESRI SVQERQKRGR KKLLYIFKQP
FMRPVQTTQE EDGCSCRFPE EEEGGCEL-

pWF-86

(SEQ ID NO: 150)

GAGGTTCAACTTGTTCAATCTGGGGCAGAAGTGAAGAAGCCCGGGGCATCTGTGAAA
GTATCATGCAAAACATCCGGCTATACGTTTACCGAATACACCATTCACTGGGTCAGA
CAGGCTCCCGGTCAAAGCCTCGAATGGATGGGAAATATTAACCCTAACAAATGGCGGA
ACCACATATAATCAGAAATTCCAAGGCCGAGTGACGATAACTGTCGATAAGAGTACG
TCCACAGCTTACATGGAACCTCAGCTCTTTGAGATCCGAAGACACTGCAGTTTATTATT
GTGCAGCTGGATGGAACCTTCGACTATTGGGGACAAGGGACTCTTGTTACGGTGTCCA
GTGGCAAACCAGGTAGTGGTAAACCCGGAAGCGGCAAGCCCGGGAGCGGTAAACCT
GGTAGCGACATCGTCATGACTCAAAGCCCTGACTCACTCGCCGTGAGCCTGGGAGAG
CGTGCAACGCTATCTTGTCTGGGCCTCTCAGGATGTCGGAAGTGTGTAGACTGGTATC
AACAGAAACCTGACCAATCACCAAAACCTCTGATTTATTGGGCCTCAACACGTCACA
CAGGAGTGCCAGATAGGTTTACAGGTAGTGGCAGTGGAACTGATTTTACTTTGACAA
TTAGCAGCTGCAAGCCGAAGATGTAGCCGTTTACTTCTGTCAACAATATAACTATA
CCCACTAACGTTTCGGTGCCGGGACGAAGGTAGAGATTAAATTCGTGCCTGTGTTCT
CCCAGCTAAGCCCACTACCACCCCGCTCCAAGGCCGCCACGCCCGCTCCTACTATT
GCTAGTCAGCCTTTAAGTTTACGACCCGAAGCTTGCAGGCCCGCCGCCGGCGCGCT
GTGCACACCAGGGGGCTTGATTTTGCCTGCGACTTTTGGGTATTGGTAGTGGTGGGCG
GAGTTTTAGCCTGCTACAGCCTCCTGGTAACAGTGGCTTTTATCATCTTTTGGGTGAA
GAAGGTTGCAAAAAAACCTACTAATAAGGCTCCCCATCCTAAGCAAGAGCCCCAAG
AAATTAACTTTCCCGATGATCTTCCGGGTTCTAACACGGCAGCCCCGGTGCAGGAGA
CCCTGCATGGTTGTCAACCCGTCCTCAGGAGGACGGGAAAGAGTCTCGTATCTCCG
TCCAGGAGAGACAGCGCAAAAAACGTATAAGCGCAAACTCTACAGATCCAGTAAAA
GCCGCGCAATTCGAGCCTCCCGGCCGCCAGATGATTGCAATACGGAACGTCAACTG
GAGGAACTAATAATGACTATGAGACGGCCGACGGTGGATACATGACCCTTAATCCC
CGCGCGCCAACCGACGATGATAAGAACATATATCTGACGCTCCCCCCTAACGATCAC
GTTAACAGTAATAATTA

pWF-86:

(SEQ ID NO: 47)

EVQLVQSGAE VKKPGASVKV SCKTSGYTFT EYTIHWVRQA PGQSLEWMGN
INPNNGGTTY NQKFQGRVTI TVDKSTSTAY MELSSLRSED TAVYYCAAGW
NFDYWGGTL VTVSSGKPGS GKPGSGKPGS GKPGSDIVMT QSPDSLAVSL
GERATLSCRA SQDVGTAVDW YQQKPDQSPK LLIYWASTRH TGVDPDRFTGS
GSGTDFTLTI SSLQAEDVAV YFCQQYNSYP LTFGAGTKVE IKFVPVFLPA KPTTTTPAPRP
PTPAPTIASQ PLSLRPEACR PAAGGAVHTR GLDFACDFWV LVVVGGVLAC
YSLLVTVAFI IFWVKVAKK PTNKAPHPKQ EPQEINFPDD LPGSNTAAPV

QETLHGCQPV TQEDGKESRI SVQERQRKKR ISANSTDPVK AAQFEPPGRQ
MIAIRKRQLE ETNNDYETAD GGYMTLNRA PTDDDKNIYL TLPPNDHVNS NN-

pWF-87:

(SEQ ID NO: 48)

GAGGTTCAACTTGTTCAATCTGGGGCAGAAGTGAAGAAGCCCGGGGCATCTGTGAAAG
TATCATGCAAAACATCCGGCTATACGTTTACCGAATACACCATTCACTGGGTCAGACA
GGCTCCCGGTCAAAGCCTCGAATGGATGGGAAATATTAACCCTAACAAATGGCGGAACC
ACATATAATCAGAAATTCCAAGGCCGAGTGACGATAAAGTGTGATAAGAGTACGTCCA
CAGCTTACATGGAACCTCAGCTCTTTGAGATCCGAAGACACTGCAGTTTATTATTGTGCA
GCTGGATGGAACCTTCGACTATTGGGGACAAGGGACTCTTGTTACGGTGTCCAGTGGCA
AACCAGGTAGTGGTAAACCCGGAAGCGGCAAGCCCGGGAGCGGTAAACCTGGTAGCG
ACATCGTCATGACTCAAAGCCCTGACTCACTCGCCGTGAGCCTGGGAGAGCGTGCAAC
GCTATCTTGTGGGCCTCTCAGGATGTGCGAACTGCTGTAGACTGGTATCAACAGAAA
CCTGACCAATCACCAAAACCTCCTGATTTATTGGGCCTCAACACGTCACACAGGAGTGC
CAGATAGGTTACAGGTAGTGGCAGTGGAACTGATTTTACTTTGACAATTAGCAGCCT
GCAAGCCGAAGATGTAGCCGTTTACTTCTGTCAACAATATAACTCATACCCACTAACG
TTCGGTGCCGGGACGAAGGTAGAGATTAAATTCGTGCCTGTGTTCTCCAGCTAAGC
CCACTACCACCCCGCTCCAAGGCCGCCACGCCCGCTCCTACTATTGCTAGTCAGCCT
TTAAGTTTACGACCCGAAGCTTGCAGGCCCGCCGCCGGCGCGCTGTGCACACCAGGG
GGCTTGATTTTGCCTGCGACTTTTGGGTATTGGTAGTGGTGGGCGGAGTTTATAGCCTGC
TACAGCCTCCTGGTAACAGTGGCTTTTATCATCTTTTGGGTGATGGCGGCGGGCGGGCC
CGGCGCCGGAAGCGCCGCGCCAGTCTCATCTACGTCCAGTCTGCCACTGGCTGCCCTG
AACATGAGAGTGAGACGCCGTTTATCCCTCTTCTGAATGTGCGGACCCAGGTCGCCG
CTGATTGGACCGCCCTGGCCGAAGAGATGGACTTTGAATACTTGGAATCAGACAGCT
GGAAACACAGGCAGACCCAACCGGGAGACTGCTTGACGCCTGGCAGGGACGCCAGG
GGCAAGTGTTGGTCGGTTACTGGAGCTTTTAACTAAGTTGGGCGCGATGACGTGCTG
TTGGAGTTAGGACCCAGTATCGAGGAGGATTGTCAGAAATACATCTTGAAACAGCAGC
AGGAGGAGGCGGAAAAGCCCTGCAGGTGGCGGCCGTTGACAGCAGTATACCCAGAA
CAGCTGAGCTGGCCGCGCATCACAAACCCTGGATGATCCCCTGGGCCACATGCCTGAGAG
GTTTCGACGCTTTCATAAAGAAGGTTGCAAAAAAACCTACTAATAAGGCTCCCCATCCT
AAGCAAGAGCCCCAAGAAATTAACCTTTCCCGATGATCTTCCGGGTTCTAACACGGCAG
CCCCGGTGCAGGAGACCCTGCATGGTTGTCAACCCGTCACCTCAGGAGGACGGGAAAG
AGTCTCGTATCTCCGTCCAGGAGAGACAGTGA

pWF-87:

(SEQ ID NO: 49)

EVQLVQSGAEVKKPGASVKVSCKTSGYTFTEYTIHWVRQAPGQSLEWMGNINPNNGGTT
YNQKFQGRVTITVDKSTSTAYMELSSLRSEDTAVYYCAAGWNFDYWGQGTLLTVSSGKP
GSGKPGSGKPGSGKPGSDIVMTQSPDSLAVSLGERATLSCRASQDVGTAVDWYQQKPDQS
PKLLIYWASTRHTGVPDRFTGSGSGTDFTLTISSLQAEDVAVYFCQQYNSYPLTFGAGTKV
EIKFVPVFLPAKPTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDFWVL
VVVGGVLACYSLLVTVAFIIFWVMAAGGPGAGSAAPVSSTSSLPLAALNMRVRRRLSLFL
NVRTQVAADWTALAEEMDFEYLEIRQLETQADPTGRLLDAWQGRPGASVGRLLLELLTKL
GRDDVLLELGPSIEEDCQKYILKQQEEAEKPLQVAADVSSVPRTAELAGITTLDDPLGHM
PERFDAFIKKVAKKPTNKAPHPKQEPQEINFDDLPGSNTAAPVQETLHGCQPV TQEDGKE
SRISVQERQ-

pWF-88:

(SEQ ID NO: 50)

GAGGTTCAACTTGTTCAATCTGGGGCAGAAGTGAAGAAGCCCGGGGCATCTGTGAAAG
TATCATGCAAAACATCCGGCTATACGTTTACCGAATACACCATTCACTGGGTCAGACA
GGCTCCCGGTCAAAGCCTCGAATGGATGGGAAATATTAACCCTAACAAATGGCGGAACC

ACATATAATCAGAAATTCCAAGGCCGAGTGACGATAAACTGTCGATAAGAGTACGTCCA
CAGCTTACATGGAAGCTCAGCTCTTTGAGATCCGAAGACACTGCAGTTTATTATTGTGCA
GCTGGATGGAAGTTTCGACTATTGGGGACAAGGGACTCTTGTTACGGTGTCCAGTGGCA
AACCAGGTAGTGGTAAACCCGGAAGCGGCAAGCCCGGGAGCGGTAAACCTGGTAGCG
ACATCGTCATGACTCAAAGCCCTGACTCACTCGCCGTGAGCCTGGGAGAGCGTGCAAC
GCTATCTTGTCTGGGCCTCTCAGGATGTCGGAAGTCTGTAGACTGGTATCAACAGAAA
CCTGACCAATCACCAAACTCCTGATTTATTGGGCCTCAACACGTCACACAGGAGTGC
CAGATAGGTTACAGGTAGTGGCAGTGGAAGTGAATTTACTTTGACAATTAGCAGCCT
GCAAGCCGAAGATGTAGCCGTTTACTTCTGTCAACAATATAAACTCATACCCACTAAGC
TTCGGTGCCGGGACGAAGGTAGAGATTAAATTCGTGCCTGTGTTCCCTCCCAGCTAAGC
CCACTACCACCCCGCTCCAAGGCCGCCACGCCCGCTCCTACTATTGCTAGTCAGCCT
TTAAGTTTACGACCCGAAGCTTGCAGGCCCGCCGCCGGCGCGCTGTGCACACCAGGG
GGCTTGATTTTGCCTGCGACTTTTGGGTATTGGTAGTGGTGGGCGGAGTTTATAGCCTGC
TACAGCCTCCTGGTAACAGTGGCTTTTATCATCTTTTGGGTGAGGAAACGATGGCAGA
ACGAGAAGCTCGGGTTGGATGCCGGGGATGAATATGAAGATGAAAACCTTTATGAAG
GCCTGAACCTGGACGACTGCTCCATGTATGAGGACATCTCCCGGGGCCTCCAGGGCAC
CTACCAGGATGTGGGCAGCCTCAACATAGGAGATGTCCAGCTGGAGAAGCCGTGA

pWF-88:

(SEQ ID NO: 51)

EVQLVQSGAEVKKPGASVKVSCKTSGYTFTEYTIHWVRQAPGQSLEWMGNINPNNGGTT
YNQKFQGRVITITVDKSTSTAYMELSSLRSEDTAVYYCAAGWNFDYWGQGLTVTVSSGKP
GSGKPGSGKPGSGKPGSDIVMTQSPDSLAVSLGERATLSCRASQDVGTAVDWYQQKPDQS
PKLLIYWASTRHTGVPDRFTGSGSGTDFTLTISSLQAEDVAVYFCQQYNSYPLTFGAGTKV
EIKFVPVFLPAKPTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDFWVL
VVVGGVLACYSLLVTVAFIIFWVRKRWQNEKLGLDAGDEYEDENLYEGLNLDDCSMYED
ISRGLQGTQYQDVGSLNIGDVQLEKP-

pWF-89:

(SEQ ID NO: 52)

GAGGTTCAACTTGTTCAATCTGGGGCAGAAGTGAAGAAGCCCGGGGCATCTGTGAAAG
TATCATGCAAAACATCCGGCTATACGTTTACCGAATACACCATTCACTGGGTCAGACA
GGCTCCCGGTCAAAGCCTCGAATGGATGGGAAATATTAACCCTAACAATGGCGGAACC
ACATATAATCAGAAATTCCAAGGCCGAGTGACGATAAACTGTCGATAAGAGTACGTCCA
CAGCTTACATGGAAGCTCAGCTCTTTGAGATCCGAAGACACTGCAGTTTATTATTGTGCA
GCTGGATGGAAGTTTCGACTATTGGGGACAAGGGACTCTTGTTACGGTGTCCAGTGGCA
AACCAGGTAGTGGTAAACCCGGAAGCGGCAAGCCCGGGAGCGGTAAACCTGGTAGCG
ACATCGTCATGACTCAAAGCCCTGACTCACTCGCCGTGAGCCTGGGAGAGCGTGCAAC
GCTATCTTGTCTGGGCCTCTCAGGATGTCGGAAGTCTGTAGACTGGTATCAACAGAAA
CCTGACCAATCACCAAACTCCTGATTTATTGGGCCTCAACACGTCACACAGGAGTGC
CAGATAGGTTACAGGTAGTGGCAGTGGAAGTGAATTTACTTTGACAATTAGCAGCCT
GCAAGCCGAAGATGTAGCCGTTTACTTCTGTCAACAATATAAACTCATACCCACTAAGC
TTCGGTGCCGGGACGAAGGTAGAGATTAAATTCGTGCCTGTGTTCCCTCCCAGCTAAGC
CCACTACCACCCCGCTCCAAGGCCGCCACGCCCGCTCCTACTATTGCTAGTCAGCCT
TTAAGTTTACGACCCGAAGCTTGCAGGCCCGCCGCCGGCGCGCTGTGCACACCAGGG
GGCTTGATTTTGCCTGCGACTTTTGGGTATTGGTAGTGGTGGGCGGAGTTTATAGCCTGC
TACAGCCTCCTGGTAACAGTGGCTTTTATCATCTTTTGGGTGCTGGACAAGGATGACAG
CAAGGCTGGCATGGAGGAAGATCACACCTACGAGGGCCTGGACATTGACCAGACAGC
CACCTATGAGGACATAGTGACGCTGCGGACAGGGGAAGTGAAGTGGTCTGTAGGTGA
GCACCCAGGCCAGGAGTGA

pWF-89:

(SEQ ID NO: 53)

EVQLVQSGAEVKKPGASVKVSCKTSGYTFTEYTIHWVRQAPGQSLEWMGNINPNNGGTT
YNQKFQGRVTITVDKSTSTAYMELSSLRSEDTAVYYCAAGWNFDYWGQGLTVTVSSGKP
GSGKPGSGKPGSGKPGSDIVMTQSPDSLAVSLGERATLSCRASQDVGTAVDWYQQKPDQS
PKLLIYWASTRHTGVPDRFTGSGSGTDFTLTISSLQAEDVAVYFCQQYNSYPLTFGAGTKV
EIKFVPVFLPAKPTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDFWVL
VVVGGVLACYSLLVTVAFIIFWVLDKDDSKAGMEEDHTYEGLDIDQTATYEDIVTLRTGE
VKWSVGEHPGQE

pWF-391:

(SEQ ID NO: 54)

GAGGTTCAACTTGTTCAATCTGGGGCAGAAGTGAAGAAGCCCGGGGCATCTGTGAAAG
TATCATGCAAAACATCCGGCTATACGTTTACCGAATACACCATTCACTGGGTCAGACA
GGCTCCCGGTCAAAGCCTCGAATGGATGGGAAATATTAACCCTAACAATGGCGGAACC
ACATATAATCAGAAATTCCAAGGCCGAGTGACGATAACTGTCGATAAGAGTACGTCCA
CAGCTTACATGGAACCTCAGCTCTTTGAGATCCGAAGACACTGCAGTTTATTATTGTGCA
GCTGGATGGAACCTTCGACTATTGGGGACAAGGGACTCTTGTTACGGTGTCCAGTGGCA
AACCAGGTAGTGGTAAACCCGGAAGCGGCAAGCCCGGGAGCGGTAAACCTGGTAGCG
ACATCGTCATGACTCAAAGCCCTGACTCACTCGCCGTGAGCCTGGGAGAGCGTGCAAC
GCTATCTTGTGGGCCTCTCAGGATGTCGGAACCTGCTGTAGACTGGTATCAACAGAAA
CCTGACCAATCACCAAACTCCTGATTTATTGGGCCTCAACACGTCACACAGGAGTGC
CAGATAGGTTACAGGTAGTGGCAGTGGAACCTGATTTTACTTTGACAATTAGCAGCCT
GCAAGCCGAAGATGTAGCCGTTTACTTCTGTCAACAATATAACTCATACCCACTAACG
TTCGGTGCCGGGACGAAGGTAGAGATTAAAGGCGCTGGTAGTGGCGGTAACCTGGAGC
CACCTCAATTTGAGAAGGGCGGGTCAGGCGGATCAGGTGGTAGTGGTGGGTCCAAC
GGAGCCATCCGCAATTTGAAAAGGGCGGAAGCGGCGGTTCCGGCGGTTACAGGCGGTA
GCAACTGGTCACATCCGCAATTTGAGAAAGGCGGGTCAGGCGGCGGGTTTTGGGCTCT
CGTGGTGGTGGCTGGAGTGCTTTTCTGCTATGGCCTGCTGGTAACCGTGGCCCTTTGTG
TAATCTGGACCGATAAAGACGATGGAAAAGCCGGGATGGAAGAAGACCATACTACG
AGGGGCTCAATATTGATCAAACCGCCACGTATGAAGACATTGTAACACTGCGCACAGG
TGAGGTCAAGTGGTCCGTCGGTGAACACCCAGGACAAGAATAA

pWF-391:

(SEQ ID NO: 55)

EVQLVQSGAEVKKPGASVKVSCKTSGYTFTEYTIHWVRQAPGQSLEWMGNINPNNGGTT
YNQKFQGRVTITVDKSTSTAYMELSSLRSEDTAVYYCAAGWNFDYWGQGLTVTVSSGKP
GSGKPGSGKPGSGKPGSDIVMTQSPDSLAVSLGERATLSCRASQDVGTAVDWYQQKPDQS
PKLLIYWASTRHTGVPDRFTGSGSGTDFTLTISSLQAEDVAVYFCQQYNSYPLTFGAGTKV
EIKGAGSGGNWSHPQFEKGGSGSGSGSGSNWSHPQFEKGGSGSGSGSGSGSNWSHPQFE
KGGSGGGFWALVVVAGVLFYGLLVTVALCVIWTDKDDGKAGMEEDHTYEGLNIDQTA
TYEDIVTLRTGEVKWSVGEHPGQE

pWF-394:

(SEQ ID NO: 56)

GAAGTCCAATTGGTTGAAAGCGGTGGTGGACTCGTCAAACCTGGCGGTAGCCTTAAAC
TTTCATGTGCCGCAAGCGGCTTCACGTTTAGTAACTATGCTATGAGTTGGGTCCGCCAA
AGTCCAGAAAAGCGCCTCGAATGGGTGGCGGAGATCTCTGGAGGAGGAACATATACA
TATTATCCAGACACCATGACCGGTAGGTTTACAATCTCAAGAGACAACGCTAAGAACA
CCCTGTACCTGGAATGTCAAGCCTGAGATCAGAAGATACGGCCATGTATTATTGTAC
CGCCTACTCGACTATTGGGGTCAAGGAACTTCCGTGACGGTGTCAAGCGGAGGAGGT
GGGAGCGTAGGAGGCGGAAGTGGCGGTGGTGGCTCTGGTGGCGGTGGAAGTGATATA
GTGATGACGCAAGCTGCCTTTTCAAACCCTGTTACTTTGGGGACTAGCGCATCAATCTC
CTGTAGGTCCAGCAAATCTTTGCTGCACAGTAATGGAATCACCTATCTTTTCTGGTATT
TGCAAAAGCCTGGGCAGAGCCCGCAACTGCTGATCTATCAAATGTCAAATCTTGCTTC

CGGAGTTCCAGACCGCTTCTCAAGTTCCGGGTCCGGCACTGATTTTACCTTGAGAATTT
CTAGGGTCGAAGCTGAAGACGTCCGTGTCTATTATTGCGCGCAAAACCTTGAGCTTCC
ATACACCTTCGGGGGGGGGCACAAAACCTTGAGATCAAGGGCGCTGGGAGCGGCGGGAA
TTGGAGTCATCCACAATTCGAAAAGGGTGGGTCCGGCGGCAGTGGTGGAAAGCGGCGG
GAGTAACTGGTCACATCCCCAGTTTGAGAAAGGCGGTAGTGGTGGCAGCGGCGGTAGT
GGTGGCAGTAATTGGAGCCATCCCCAATTCGAAAAGGGCGGTTCCGGCGGCGGATTTT
GGGCTCTTGTGTGGTGGCCGGAGTATTGTTTTGCTATGGCCTGCTCGTTACAGTGGCA
TTGTGCGTAATTTGGACTGATAAAGACGACGGCAAAGCCGGGATGGAAGAAGATCAC
ACCTATGAGGGGGCTTAATATAGATCAAACAGCCACATATGAAGATATTGTGACTCTAA
GGACTGGAGAGGTTAAATGGAGTGTGGGTGAGCATCCAGGACAAGAATAA

pWF-394:

(SEQ ID NO: 57)

EVQLVESGGGLVKPGGSLKLSCAASGFTFSNYAMSWVRQSPPEKRLWVAEISGGGTYTY
PDTMTGRFTISRDNKNTLYLEMSSLRSEDTAMYYCTRLLDYWGQTSVTVSSGGGSG
GGGSGGGSGGGSDIVMTQAAFSNPVTLGTSASISCRSSKSLHNSGITYLFWYLQKPGQ
SPQLLIYQMSNLASGVPDRFSSSGSGTDFTLRISRVEAEDVGVYYCAQNLELPYTFGGG
TKLEIKGAGSGGNWSHPQFEKGGSGSGSGSGSNWSHPQFEKGGSGSGSGSGSNWSHPQ
FEKGGSGGGFWALVAVVAGVLCYGLLVTVVLCVWTDKDDGKAGMEEDHTYEGLNIDQT
ATYEDIVTLRTGEVKWSVGEHPGQE

pWF-396:

(SEQ ID NO: 58)

CAGTCTGTGTTGACGCAGCCGCCCTCAGTGTCTGCGGCCCCAGGACAGAGGGTCACCA
TCTCCTGCTCTGGAACCAGGTCCAACATTGGGAGTGATTATGTTTCCTGGTACCAACAC
CTCCCAGGAACAGCCCCAACTCCTCGTTTATGGCGATAATCTGCGACCCCTCAGGGA
TTCCTGACCGATTCTCTGCCTCCAAGTCTGGCACGTCAGCCACCCTGGGCATCACCGGA
CTCCAGACTGGGGACGAGGCCGATTATTACTGCGGCACATGGGATTACACCCTGAATG
GTGTGGTGTTCGGCGGAGGGACCAAGCTGACCGTCCTAGGTTCTAGAGGTGGTGGTGG
TAGCGGCGGGCGGCGGCTCTGGTGGTGGTGGATCCCTCGAGATGGCCAGGTGCAGCTG
GTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGGGGGTCCCTGAGACTCTCCTGTGCAG
CCTCTGGATTACCTTTAGCAGCTATGCCATGAGCTGGGTCCGCCAGGCTCCAGGGAA
GGGGCTGGAGTGGGTCTCAGTTATTTATAGCGGTGGTAGTAGCACATACTATGCAGAC
TCCGTGAAGGGCCGGTTCACCATCTCCAGAGATAATTCCAAGAACACGCTGTATCTGC
AAATGAACAGCCTGAGAGCCGAGGACACGGCCGTATATTACTGTGCGCGCACTTCTTA
CCTGAACCATGGTGATTACTGGGGTCAAGGTACTCTGGTGACCGTGTCTAGCGCCGCT
GCATTTCGTGCCTGTGTTCCCTCCAGCTAAGCCCACTACCACCCCGCTCCAAGGCCGCC
CACGCCCGCTCCTACTATTGCTAGTCAGCCTTTAAGTTTACGACCCGAAGCTTGCAGGC
CCGCCCGCGGCGGCGCTGTGCACACCAGGGGGCTTGATTTTGCCTGCGACTTTTGGGT
ATTGGTAGTGGTGGGCGGAGTTTATGCCTGCTACAGCCTCCTGGTAACAGTGGCTTTTA
TCATCTTTTGGGTGAGGAAACGATGGCAGAACGAGAAGCTCGGGTGGATGCCGGGG
ATGAATATGAAGATGAAAACCTTTATGAAGGCCTGAACCTGGACGACTGCTCCATGTA
TGAGGACATCTCCCGGGGCTCCAGGGCACCTACCAGGATGTGGGCAGCCTCAACATA
GGAGATGTCCAGCTGGAGAAGCCGTGA

pWF-396:

(SEQ ID NO: 59)

QSVLTQPPSVSAAPGQRVTISCSGTRSNIGSDYVSWYQHLPGTAPKLLVYGDNLRPSGIPDR
FSASKSGTSATLGITGLQTGDEADYYCGTWDYTLNGVVFGGGTKLTVLGSRRGGGSGGG
GSGGGGSLEMAQVQLVESGGGLVQPGGSLRLSCAASGFTFSYAMSWVRQAPGKGLEWV
SVIYSGGSSTYYADSVKGRFTISRDNKNTLYLQMNSLRAEDTAVYYCARTSYLNHGDIW
GQGTLVTVSSAAAFVPLPAKPTTTPAPRPPTPAPTASQPLSLRPEACRPAAGGAVHTRG

LDFACDFWVLVVVGGVLACYSLLVTVAFIIFWVRKRWQNEKLGLDAGDEYEDENLYEGL
NLDDCSMYEDISRGLQGTYQDVGSLNIGDVQLEKP

pWF-397:

(SEQ ID NO: 60)

CAGTCTGTGTTGACGCAGCCGCCCTCAGTGTCTGCGGCCCCAGGACAGAGGGTCACCA
TCTCCTGCTCTGGAACCAGGTCCAACATTGGGAGTGATTATGTTTCCTGGTACCAACAC
CTCCCAGGAACAGCCCCCAAACCTCCTCGTTTATGGCGATAATCTGCGACCCTCAGGGA
TTCCTGACCGATTCTCTGCCTCCAAGTCTGGCACGTCAGCCACCCTGGGCATCACCGGA
CTCCAGACTGGGGACGAGGCCGATTATTACTGCGGCACATGGGATTACACCCTGAATG
GTGTGGTGTTCGGCGGAGGGACCAAGCTGACCGTCCTAGGTTCTAGAGGTGGTGGTGG
TAGCGGCGGGCGGCGGCTCTGGTGGTGGTGGATCCCTCGAGATGGCCCAGGTGCAGCTG
GTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGGGGGTCCCTGAGACTCTCCTGTGCAG
CCTCTGGATTCACCTTTAGCAGCTATGCCATGAGCTGGGTCCGCCAGGCTCCAGGGAA
GGGGCTGGAGTGGGTCTCAGTTATTTATAGCGGTGGTAGTAGCACATACTATGCAGAC
TCCGTGAAGGGCCGGTTCACCATCTCCAGAGATAATTCCAAGAACACGCTGTATCTGC
AAATGAACAGCCTGAGAGCCGAGGACACGGCCGTATATTACTGTGCGCGCACTTCTTA
CCTGAACCATGGTGATTACTGGGGTCAAGGTACTCTGGTGACCGTGTCTAGCGCCGCT
GCATTCTGTCCTGTGTTCTCCAGCTAAGCCCACTACCACCCCGCTCCAAGGCCGCC
CACGCCCGCTCCTACTATTGCTAGTCAGCCTTTAAGTTTACGACCCGAAGCTTGCAGGC
CCGCCCGCGGCGGCGCTGTGCACACCAGGGGGCTTGATTTTGCCTGCGACTTTTGGGT
ATTGGTAGTGGTGGGCGGAGTTTLAGCCTGCTACAGCCTCCTGGTAACAGTGGCTTTTA
TCATCTTTTGGGTGCTGGACAAGGATGACAGCAAGGCTGGCATGGAGGAAGATCACAC
CTACGAGGGCCTGGACATTGACCAGACAGCCACCTATGAGGACATAGTGACGCTGCGG
ACAGGGGAAGTGAAGTGGTCTGTAGGTGAGCACCCAGGCCAGGAGTGA

pWF-397:

(SEQ ID NO: 61)

QSVLTQPPSVSAAPGQRTVITSCGSTRSNIGSDYVSWYQHLPGTAPKLLVYGDNLRPSGIPDR
FSASKSGTSATLGITGLQTGDEADYYCGTWDYTLNGVVFGGGTKLTVLGSRRGGGSGGG
GSGGGGSLEMAQVQLVESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWV
SVIYSGGSSTYYADSVKGRFTISRDNKNTLYLQMNSLRAEDTAVYYCARTSYLNHGDIW
GQGTLLTVSSAAAFVPLPAKPTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRG
LDFACDFWVLVVVGGVLACYSLLVTVAFIIFWVLDDKDDSKAGMEEDHTYEGLDIDQTAT
YEDIVTLRTGEVKWSVGEHPGQE

pWF-460:

(SEQ ID NO: 62)

CAGTCTGTGTTGACGCAGCCGCCCTCAGTGTCTGCGGCCCCAGGACAGAGGGTCACCA
TCTCCTGCTCTGGAACCAGGTCCAACATTGGGAGTGATTATGTTTCCTGGTACCAACAC
CTCCCAGGAACAGCCCCCAAACCTCCTCGTTTATGGCGATAATCTGCGACCCTCAGGGA
TTCCTGACCGATTCTCTGCCTCCAAGTCTGGCACGTCAGCCACCCTGGGCATCACCGGA
CTCCAGACTGGGGACGAGGCCGATTATTACTGCGGCACATGGGATTACACCCTGAATG
GTGTGGTGTTCGGCGGAGGGACCAAGCTGACCGTCCTAGGTTCTAGAGGTGGTGGTGG
TAGCGGCGGGCGGCGGCTCTGGTGGTGGTGGATCCCTCGAGATGGCCCAGGTGCAGCTG
GTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGGGGGTCCCTGAGACTCTCCTGTGCAG
CCTCTGGATTCACCTTTAGCAGCTATGCCATGAGCTGGGTCCGCCAGGCTCCAGGGAA
GGGGCTGGAGTGGGTCTCAGTTATTTATAGCGGTGGTAGTAGCACATACTATGCAGAC
TCCGTGAAGGGCCGGTTCACCATCTCCAGAGATAATTCCAAGAACACGCTGTATCTGC
AAATGAACAGCCTGAGAGCCGAGGACACGGCCGTATATTACTGTGCGCGCACTTCTTA
CCTGAACCATGGTGATTACTGGGGTCAAGGTACTCTGGTGACCGTGTCTAGCCCCAAG
AGCTGCGACAAGACCCACACCTGCCCCCCTGCCAGCCCCAGAGCTGCTGGGCGGAC
CCTCCGTGTTCTGTTCCCCCCCCAAGCCCAAGGACACCCTGATGATCAGCAGGACCCCC

GAGGTGACCTGCGTGGTGGTGGACGTGAGCCACGAGGACCCAGAGGTGAAGTTCAAC
TGGTACGTGGACGGCGTGGAGGTGCACAACGCCAAGACCAAGCCCAGAGAGGAGCAG
TACAACAGCACCTACAGGGTGGTGTCCGTGCTGACCGTGCTGCACCAGGACTGGCTGA
ACGGCAAGGAATACAAGTGCAAGGTCTCCAACAAGGCCCTGCCAGCCCCATCGAAA
AGACCATCAGCAAGGCCAAGGGCCAGCCACGGGAGCCCCAGGTGTACACCCTGCCCC
CCTCCCGGGAGGAGATGACCAAGAACCAGGTGTCCCTGACCTGTCTGGTGAAGGGCTT
CTACCCAGCGACATCGCCGTGGAGTGGGAGAGCAACGGCCAGCCCCGAGAACAATA
CAAGACCACCCCCCAGTGCTGGACAGCGACGGCAGCTTCTTCCTGTACAGCAAGCTG
ACCGTGGACAAGTCCAGGTGGCAGCAGGGCAACGTGTTACGTGCAGCGTGATGCAC
GAGGCCCTGCACAACCACTACACCCAGAAAGAGCCTGAGCCTGTCCCCGAGCTGCAAC
TGGAGGAGAGCTGTGCGGAGGCGCAGGACGGGGAGCTGGACGGGCTGTGGACGACCA
TCACCATCTTCATCACACTCTTCCTGTAAAGCGTGTGCTACAGTGCCACCGTCACCTTCT
TCAAGGTGAAGTGGATCTTCTCCTCGGTGGTGGACCTGAAGCAGACCATCATCCCCGA
CTACAGGAACATGATCGGACAGGGGGCCTGA

pWF-460:

(SEQ ID NO: 63)

QSVLTQPPSVSAAPGQRTISCSGTRSNIGSDYVSWYQHLPGTAPKLLVYGDNLPSGIPDR
FSASKSGTSATLGITGLQTGDEADYYCGTWDYTLNGVVFGGGTKLTVLGSRRGGGSGGG
GSGGGGSLEMAQVQLVESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWV
SVIYSGGSSTYYADSVKGRFTISRDNKNTLYLQMNSLRAEDTAVYYCARTSYLNHGDYW
GQGLTVTVSSPKCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSH
EDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK
ALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPE
NNYKTTTPVLDSGDSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPQL
LEESCAEAQDGLDGLWTTITIFITLFLLSVCYSATVTFFKVKWIFSSVVDLKQTIIPDYRNM
IGGA

pWF-428:

(SEQ ID NO: 64)

CAGTCTGTGTTGACGCAGCCGCCCTCAGTGTCTGCGGCCCCAGGACAGAGGGTCACCA
TCTCCTGCTCTGGAACCAGGTCCAACATTGGGAGTGATTATGTTTCCTGGTACCAACAC
CTCCCAGGAACAGCCCCCAAACCTCTCGTTTATGGCGATAATCTGCGACCCTCAGGGA
TTCCTGACCGATTCTCTGCCTCCAAGTCTGGCACGTCAGCCACCCTGGGCATCACCGGA
CTCCAGACTGGGGACGAGGCCGATTATTACTGCGGCACATGGGATTACACCCTGAATG
GTGTGGTGTTCGGCGGAGGGACCAAGCTGACCGTCCTAGGTCAGCCCAAGGCCAACCC
CACTGTCACTCTGTTCCCGCCCTCCTCTGAGGAGCTCCAAGCCAACAAGGCCACACTA
GTGTGTCTGATCAGTGAATTCTACCCGGGAGCTGTGACAGTGGCCTGGAAGGCAGATG
GCAGCCCCGTCAAGGCGGGAGTGGAGACCACCAAACCCTCCAAACAGAGCAACAACA
AGTACGCGGCCAGCAGCTACCTGAGCCTGACGCCCAGCAGTGGAAGTCCCACAGAA
GCTACAGCTGCCAGGTCACGCATGAAGGGAGCACCGTGGAGAAGACAGTGGCCCCCTA
CAGAATGTTCA

pWF-428:

(SEQ ID NO: 65)

QSVLTQPPSVSAAPGQRTISCSGTRSNIGSDYVSWYQHLPGTAPKLLVYGDNLPSGIPDR
FSASKSGTSATLGITGLQTGDEADYYCGTWDYTLNGVVFGGGTKLTVLGQPKANPTVTLF
PPSSEELQANKATLVCLISDFYPGAVTVAWKADGSPVKAGVETTKPSKQSNNKYAASSYL
SLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS

pWF-429:

(SEQ ID NO: 66)

CAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGGGGGTCCCTGAGAC
TCTCCTGTGCAGCCTCTGGATTACCTTTAGCAGCTATGCCATGAGCTGGGTCCGCCAG
GCTCCAGGGAAGGGGCTGGAGTGGGTCTCAGTTATTTATAGCGGTGGTAGTAGCACAT
ACTATGCAGACTCCGTGAAGGGCCGGTTCACCATCTCCAGAGATAATTCCAAGAACAC
GCTGTATCTGCAAATGAACAGCCTGAGAGCCGAGGACACGGCCGTATATTACTGTGCG
CGCACTTCTTACCTGAACCATGGTGATTACTGGGGTCAAGGTACTCTGGTGACCGTGTG
TAGCGCCTCCACCAAGGGCCCATCGGTCTTCCCCCTGGCACCCCTCCTCCAAGAGCACCT
CTGGGGGACACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGAC
GGTGTCTGTGGAAGTCAAGGCGCCCTGACCAGCGGCGTGCACACCTTCCCGGTGTCTTA
CAGTCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGG
GCACCCAGACCTACATCTGCAACGTGAACCACAAGCCCAGCAACACCAAGGTGGACA
AGAGAGTGGAGCCCAAGAGCTGCGACAAGACCCACACCTGCCCCCCTGCCAGCCCC
AGAGCTGCTGGGCGGACCCTCCGTGTTCTGTTCCCCCCCCAAGCCCAAGGACACCCTG
ATGATCAGCAGGACCCCCGAGGTGACCTGCGTGGTGGTGGACGTGAGCCACGAGGAC
CCAGAGGTGAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCACAACGCCAAGACC
AAGCCCAGAGAGGAGCAGTACAACAGCACCTACAGGGTGGTGTCCGTGCTGACCGTG
CTGCACCAGGACTGGCTGAACGGCAAGGAATACAAGTGCAAGGTCTCCAACAAGGCC
CTGCCAGCCCCCATCGAAAAGACCATCAGCAAGGCCAAGGGCCAGCCACGGGAGCCC
CAGGTGTACACCCTGCCCCCCTCCCGGGAGGAGATGACCAAGAACCAGGTGTCCCTGA
CCTGTCTGGTGAAGGGCTTCTACCCCAGCGACATCGCCGTGGAGTGGGAGAGCAACGG
CCAGCCCGAGAACAACACTACAAGACCACCCCCCAGTGCTGGACAGCGACGGCAGCTTC
TTCCTGTACAGCAAGCTGACCGTGGACAAGTCCAGGTGGCAGCAGGGCAACGTGTTCA
GCTGCAGCGTGATGCACGAGGCCCTGCACAACCACTACACCCAGAAGAGCCTGAGCCT
GTCCCCCGAGCTGCAACTGGAGGAGAGCTGTGCGGAGGCGCAGGACGGGGAGCTGGA
CGGGCTGTGGACGACCATCACCATCTTCATCACACTCTTCCTGTTAAGCGTGTGCTACA
GTGCCACCGTCACCTTCTTCAAGGTGAAGTGGATCTTCTCCTCGGTGGTGACCTGAAG
CAGACCATCATCCCCGACTACAGGAACATGATCGGACAGGGGGCCTGA

pWF-429:

(SEQ ID NO: 67)

QVQLVESGGGLVQPGLSLRLSCAASGFTFSYAMSWVRQAPGKGLEWVSVIYSGGSSTYY
ADSVKGRFTISRDNKNTLYLQMNSLRAEDTAVYYCARTSYLNHGDYWGQGLTVTVSSA
STKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPTVSWNSGALTSGVHTFPAVLQSSGL
YSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVF
LFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYR
VVSVELTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKN
QVSLTCLVKGFYPDSIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGN
VFSCVMHEALHNHYTQKSLSLSPQLLESCAEAQDGELDGLWTTITIFITLFLLSVCYSA
TVTFFKVKWIFSSVVDLKQTIIPDYRNMIGQGA-

mu CXCL13

(SEQ ID NO: 68)

ATGAGACTTTCAACAGCAACACTCCTCCTGTTGCTGGCTTCATGTCTGAGCCCTGGTCA
TGGTATTTTGGAGGCCCACTATACAAATCTCAAATGTCGGTGTTCAGGCGTAATATCCA
CCGTAGTCGGCCTGAACATTATCGATAGGATTACAGGTACACCCCCCGGAACGGATG
TCCTAAGACCGAGGTGGTGATTTGGACCAAGATGAAGAAGGTCATTTGTGTGAACCCA
CGGGCTAAATGGCTGCAGCGTCTTTTGCAGACACGTGCAGTCCAAGAGCTTGTCAGCA
CACCTCAGGCCCCAGTTAGCAAGCGACGTGCAGCC

mu CXCL13

(SEQ ID NO: 69)

MRLSTATLLLLLASCLSPGHGILEAHYTNLKCRCSGVISTVVGLNIIDRIQVTPPGNGCPKTE
VVIWTKMKKVICVNPRAKWLQRLLRHVQSKLSSTPQ APVSKRRRA

mu FLT3LG

(SEQ ID NO: 70)

ATGACAGTGCTGGCCCCCGCGTGGTCTCCCAATAGCTCACTCCTCCTCTTGCTGCTACT
GCTCAGCCCATGCCTCAGGGGCACCCCGATTGTTACTTCAGCCACAGCCCAATCTCCT
CCAACTTCAAAGTGAAATTTAGGGAACTGACCGACCACCTGCTGAAAGATTATCCTGT
GACTGTGGCAGTGAACCTGCAAGACGAAAAGCATTGTAAGGCGCTATGGAGCCTCTTT
CTTGCCCAACGATGGATTGAGCAACTCAAACTGTAGCCGGAAGCAAAATGCAGACG
CTACTGGAGGACGTGAATACTGAGATTCACTTCGTTACCAGTTGTACTTTCCAGCCACT
GCCAGAGTGTCTCAGGTTTGTGCAGACTAATATCAGCCACCTGCTGAAGGATACTTGC
ACCCAGCTCCTGGCTCTCAAGCCTTGTATAGGCAAGGCTTGTCAAAATTTAGCAGGTG
TCTCGAAGTCCAGTGCCAGCCAGATTCATCCACACTGCTGCCGCCCGAAGCCCTATC
GCACTCGAAGCGACAGAGTTGCCAGAGCCTCGTCCCAGACAGCTTCTGCTGCTGCTAC
TTCTGCTGCTGCCGCTAACTCTGGTGCTACTTGCTGCCGCCTGGGGCCTCAGATGGCAA
CGCGCCAGACGCCGAGGCGAACTCCACCCTGGGGTGCCACTGCCATCCCACCA

mu FLT3LG

(SEQ ID NO: 71)

MTVLAPAWSPNSSLLLLLLLLLSPCLRGTPDCYFSSHPISSNFKVKFRELTDHLLKDYPVTV
VNLQDEKHCKALWSLFLAQRWIEQLKTVAGSKMQTLLEDVNTEIHFTVTSCTFQPLPECLRF
VQTNISHLKDTCTQLLALKPCIGKACQNFRCLEVQCQPDSSLLPPRSPIALEATELPEPR
PRQLLLLLLLLLLPLTLVLLAAAWGLRWQRARRRGELHPGVPLPS HP

mu XCL1

(SEQ ID NO: 72)

ATGCGACTCTTGTTGTTGACTTTTTCTCGGAGTGTGCTGCCTGACACCCTGGGTCGTAGA
GGGAGTTGGCACTGAAGTACTAGAAGAGTCTCCTGCGTTAACCTGCAGACACAGCGG
CTCCCAGTCCAGAAAATTAAGACCTACATTATATGGGAAGGAGCAATGCGAGCGGTGA
TTTTGTGACCAAGAGGGGTCTCAAGATTTGCGCGGACCCTGAGGCCAAGTGGGTCAA
AGCAGCTATTAAGACAGTAGACGGAAGAGCCTCCACCAGGAAGAATATGGCAGAAAC
TGTACCGACCGGTGCGCAGCGGTCAACATCTACCGCAATCACACTACCGGC

mu XCL1

(SEQ ID NO: 73)

MRLLLLTLFLGVCCLTPWVVEGVGTEVLEESSCVNLQTQRLPVQKIKTYIHWEGAMRAVIFV
TKRGLKICADPEAKWVKAAIKTVDGRASTRKNMAETVPTGAQRSTSTAI TLTG

mu Tim4(ECD)-mulgG2a Fc

(SEQ ID NO: 74)

ATGAGCAAGGGCCTTCTCCTGCTGTGGCTAGTAACTGAATTGTGGTGGTTGTACCTGAC
ACCTGCCGCTAGTGAGGACACCATCATTGGTTTTCCTTGGGCAGCCCGTCACCCTCCCTT
GCCATTACCTAAGCTGGAGCCAGTCACGGAAGTCTATGTGCTGGGGAAAGGGGTCATG
CCCTAATTCCAAGTGCAACGCCGAGCTGTTGCGCACGGACGGCACCAGAATAATCTCA
AGAAAGTCCACCAAGTATACGCTGCTCGGCAAGGTGCAATTCGGTGAAGTGAGCTTGA
CCATAAGTAACACCAACCGCGGTGACTCCGGAGTTTATTGTTGCAGGATCGAAGTGCC
AGGCTGGTTTAAACGACGTGAAGAAAAACGTGCGGCTGGAAGTGAAGAGGGCAACTAC
GACCAAGAAACCAACAACCACGACGAGACCTACCACCACTCCTTACGTGACAACCAC
GACACCGGAGCTGTTGCCAACTACCGTCATGACAACATCTGTGTTGCCAACTACCACC
CCCCCAAACGCTCGCGACAAGTGCCTTTTCCACAGCCGTTACCACATGTCCTTCCAC
CACCCAGGCTCTTTTTCTCAAGAACTACCAAGGGATCAGCTTTTACCACCGAGTCTG
AAACTCTCCAGCAAGTAATCACTCACAGCGGTCAATGATGACCATCAGCACAGACAT
CGCTGTCTTGAGACCTACTGGCAGCAATCCAGGCATTCTGCCCTCCACTTCACAGCTGA
CTACCCAAAAGACTACACTAACCACCAGCGAAAGTCTGCAGAAAAGTACAAAGAGCC

ATCAAATAAACTCCCGGCAGACTCCCAGAGGGGCCACAAATCAAGCCCTGTCTCCATG
CAAATGCCCAGCACCTAACCTCTTGGGTGGACCATCCGTCTTCATCTTCCCTCCAAAGA
TCAAGGATGTACTCATGATCTCCCTGAGCCCCATAGTCACATGTGTGGTGGTGGATGTG
AGCGAGGATGACCCAGATGTCCAGATCAGCTGGTTTGTGAACAACGTGGAAGTACACA
CAGCTCAGACACAAACCCATAGAGAGGATTACAACAGTACTCTCCGGGTGGTCAGTGC
CCTCCCCATCCAGCACCAGGACTGGATGAGTGGCAAGGAGTTCAAATGCAAGGTCAAC
AACAAAGACCTCCAGCGCCCATCGAGAGAACCATCTCAAAACCCAAAGGTGAGTA
AGAGCTCCACAGGTATATGTCTTGCCTCCACCAGAAGAAGAGATGACTAAGAAACAG
GTCACCTCTGAGTGCATGGTCCAGACTTCATGCCTGAAGACATTTACGTGGAGTGGGA
CCAACAACGGGAAAACAGAGCTAAACTACAAGAACACTGAACCAGTCTGGACTCTG
ATGGTTCTTACTTCATGTACAGCAAGCTGAGAGTGGAAAAGAAGAACTGGGTGGAAA
GAAATAGCTACTCCTGTTCAGTGGTCCACGAGGGTCTGCACAATCACCACACGACTAA
GAGCTTCTCCCGGACTCCGGGTAAA

mu Tim4(ECD)-mulgG2a Fc

(SEQ ID NO: 75)

MSKGLLLLWLVTELWWLYLTPAASEDTIIGFLGQPVTLPCHYLSWSQSRNSMCWKGSCP
NSKCNAELLRTDGTRIISRKSTKYTLGKVQFGEVSLTISNTNRGDSGVYCCRIEVPWFND
VKKNVRELELRATTTTKPTTTTRPTTTPYVTTTTPELLPTTVMTTSVLPTTTPPQTLATTA
STAVTTCPTSTTPGSFSQETTKGSAFTTESETLPASNHSQRSMMTISTDIAVLRPTGSNPILPS
TSQLTTQKTTLTSESLLQKTKSHQINSRQTPRGPTIKPCPPCKCPAPNLLGGPSVFIFPPKIK
DVLMSLSPIVTCVVVDVSEDDPDVQISWVFNVEVHTAQTQTHREDYNSTLRVVSALPIQ
HQDWMSGKEFKCKVNNKDLPAPIERTISKPKGSVRAPQVYVLPPEEEMTKKQVTLTCMV
TDFMPEDIYVEWTNNGKTELNYKNTEPVLDSDGSYFMYSKLRVEKKNWVERNSYSCSVV
HEGLHNHHTTKSFSRTPGK

mu 4-1BB-L

(SEQ ID NO: 76)

ATGGATCAGCATACACTGGACGTGGAAGATACAGCCGATGCCAGACACCTGCTGGA
ACGTCTGTCCCAGCGACGCTGCCCTGCTCAGAGACACCGGGCTGCTCGCAGATGCTG
CTCTGCTGAGTGATACCGTTCGGCCAACTAACCGGGCCCTACCCACAGATGCCGCATA
TCCCGCGGTAAATGTCAGGGACCGGGAAGCTGCCTGGCCACCGGGCCCTCAATTTCTGC
TCTAGACATCCGAAACTGTACGGTCTGGTTCGCACTGGTACTGCTGCTACTTATAGCAGC
TTGTGTTCCCATATTTACCCGCACTGAACCCAGACCCGCTCTCACTATTACAACCTTCAC
CAAACCTGGGCACACGTGAAAACAATGCAGATCAGGTTACCCCTGTAAGTCATATTGG
ATGCCCCAACACCACACAACAGGGAAGTCCGGTGTGTTGCAAACTCCTTGCTAAGAAT
CAGGCTTCACTGTGTAACTACTCTTAATTGGCACTCACAAGACGGGGCCGGGAGTA
GCTATCTCAGCCAAGGTCTCCGCTATGAAGAAGATAAGAAAGAGTTGGTGGTGGACAG
CCCAGGACTCTACTACGTCTTCTGAGCTAAAATAAGCCCCACTTTTACTAACACTG
GACATAAGGTCCAAGGTGGGTGTCCCTCGTACTTCAAGCTAAACCCAGGTGGACGA
CTTCGATAACCTGGCGTTGACAGTTGAGCTCTTTCCTTGCTCTATGGAAAATAAGCTCG
TGGATCGGAGCTGGTCTCAACTGTTGCTGCTTAAAGCCGGTCATCGTCTGTCTGTTGGA
CTACGCGCATACTTGCATGGAGCCCAGGACGCATATCGTGATTGGGAAGTGGAGCTACC
CGAATACCACTAGCTTTGGACTATTTCTTGTTAAACCAGATAATCCTTGGGAG

mu 4-1BB-L

(SEQ ID NO: 77)

MDQHTLDVEDTADARHPAGTSCPSDAALLRDTGLLADAALLSDTVRPTNAALPTDAAYP
AVNVRDREAAWPPALNFCSRHPKLYGLVALVLLLLIAACVPIFTRTEPRPALTITSPNLGT
RENNADQVTPVSHIGCPNTTQQGSVPFAKLLAKNQASLCNTLNWHSQDAGSSYLSQGL
RYEEDKKELVVDSPGLYYVFLELKLSPFTNTGHKVQGWVSLVLQAKPQVDDFDNLALT
VELFPCSMENKLVDRWSQLLLLKAGHRLSVGLRAYLHGAQDAYRDWELSYPNNTTSFGL
FLVKPDNPWE

mu LIGHT (cleavage-deficient mutant)

(SEQ ID NO: 78)

ATGGAGAGCGTAGTGCAACCCAGCGTATTTGTGGTGGATGGACAGACCGACATCCCAT
TCAGACGCTTGGAACAGAACCACCGAAGAAGGCGGTGCGGCACCGTCCAGGTGTCCCT
CGCTCTCGTGCTGCTGCTTGGTGTGCTGGCCTCGCAACACAAGGGTGGTTTCTTTTGAGAC
TCCATCAACGCTTGGGAGACATAGTGGCCACCTGCCTGATGGTGGGAAGGGCTCTTG
GCAGGACCAGCGATCACACCAGGCTAACCCCGCCGCTCACCTGACAGGGGCGAATGC
CAGCTTGATCGGAATAGGTGGGCCGCTGCTGTGGGAACTAGGCTTGGACTTGCCTTT
CTGAGAGGGCTTACATACCATGACGGAGCCCTCGTAACAATGGAGCCTGGTTATTACT
ACGTGTACAGTAAGGTGCAGCTTTCTGGAGTCGGGTGTCCCCAGGGGCTGGCTAACGG
ACTGCCCATCACTCATGGACTATACAAACGCACATCCAGATATCCTAAAGAGCTGGAA
CTGTTGGTGTCCCGTAGGAGCCCGTGTGGCAGGGCCAACTCTTCCCGTGTGTGGTGGG
ACTCCTCTTTTCTGGGCGGCGTGGTCCATCTGGAAGCTGGTGAGGAAGTCGTCGTAAG
AGTACCTGGAAACCGTCTGGTTCGCCCCCGCGATGGCACCAGGTCCTACTTCGGAGCT
TTCATGGTA

mu LIGHT (cleavage-deficient mutant)

(SEQ ID NO: 79)

MESVVQPSVFVVDGQTDIPFRRLEQNHRRRRCGTVQVSLALVLLLGAGLATQGWFLRLH
QRLGDIVAHLPGGKGSWQDQRSHQANPAHLTGANASLIGIGGPLLWETRLGLAFLRGL
TYHDGALVTMEPGYVVYSKVQLSGVGCPOQLANGLPITHGLYKRTSRYPKLELLVSR
SPCGRANSSRVWWDSSFLGGVVHLEAGEEVVVRVPGNRLVRPRDGRSYFGAFMV

mu IL12 (transmembrane form)

(SEQ ID NO: 80)

ATGTGCCACAGAACTCACAATTTCTTGGTTCGCAATCGTCCTGCTGGTGTCAACCCCT
GATGGCAATGTGGGAGTTGGAAAAGGATGTATACGTCGTCGAGGTGACATGGACACCT
GACGCTCCGGGTGAACTGTCAACCTCACTTGCGATACTCCTGAAGAGGACGACATCA
CGTGGACGAGCGACCAGCGACATGGAGTGATAGGGTCTGGCAAGACGCTTACTATCAC
GGTTAAGGAATTTCTCGACGCAGGGCAGTACACATGTACAAGGGCGCGAGACTCTG
AGCCACTCCCATTTGCTGCTGCACAAGAAGGAGAATGGTATCTGGTCTACCGAAATCC
TGAAGAATTTTAAAGAACAAGACTTTTCTGAAATGCGAGGCCCCAAATTATTCCGGACG
TTTCACTTGCAGTTGGCTCGTTCAAAGAAATATGGACTTGAAATTTAACATTAAATCCA
GCTCTTCATCTCCTGACAGCAGGGCCGTAACCTTGTTGAATGGCTTCATTGTCAGCTGAG
AAAGTTACGCTTGACCAAAGGGATTATGAGAAATACAGCGTGAGTTGCCAGGAAGAT
GTGACATGTCCAACGGCAGAGGAAACGTTGCCAATTGAGCTCGCTTTGGAAGCTCGTC
AACAAAACAAGTATGAAAACATAAGTACTAGCTTCTTCATACGGGACATCATCAAACC
AGATCCACCTAAGAATTTGCAGATGAAGCCTCTGAAGAATTCACAAGTCGAGGTATCC
TGGAATACCCAGATTTCATGGTCCACTCCTCATAGTTACTTTAGCCTGAAATCTTTGT
ACGCATACAGCGGAAGAAGGAGAAAATGAAGGAGACGGAAGAAGGCTGCAATCAGA
AAGGCGCTTTTCTTGTGAAAAGACGAGCACTGAGGTTCAATGCAAAGGCGGGAATGT
ATGTGTTCAAGCCCAAGATAGGTATTATAATAGCTCCTGCTCTAAGTGGGCTTGCGTAC
CATGCAGAGTTAGAAGTGGCTCAACCTCAGGCTCCGGAACCTGGTTCCGGTGAAGG
TTCCACAAAAGGGCGTGTGATTCCTGTGTCCGGCCCAGCTAGGTGTCTCTCCAGTCAC
GGAATCTCCTGAAAACACGGATGACATGGTAAAGACAGCTAGGGAGAACTCAAGC
ACTACTCCTGCACAGCTGAGGATATCGATCATGAGGACATCACAGGGACCAGACATC
CACTCTGAAAACCTTGCCTGCCTTTGGAACCTCCACAAGAACGAATCTTGTCTGGCAACG
CGTGAAACGAGTTCTACTACAAGAGGGTCTGTCTTCCCCCTCAAAAGACAAAGCCTTA
TGATGACCTGTGTCTCGGTAGCATTTATGAGGACCTAAAGATGTATCAAACCGAGTTT
CAGGCTATCAATGCAGCGCTCCAGAATCATAACCATCAGCAGATCATTCTTGACAAAG
GAATGCTCGTGGCCATTGATGAACTAATGCAGAGCCTAAACCACAATGGCGAGACTCT
TCGACAGAAACCGCCTGTGGGCGAGGCCGATCCATATAGAGTCAAAATGAACTGTGT
ATTCTCCTGCATGCATTTAGTACTCGTGTAGTGACTATTAACAGAGTGATGGGTTACCT

TTCCTCAGCTAATACACTTGTCTCTTTGGCGCTGGGTTCCGGCGCCGTCATAACGGTTG
TTGTCATCGTGGTAATAATCAAGTGCTTTTGCAAGCACAGGTCTTGTTTTTCGCAGGAAT
GAAGCCTCTAGAGAAACAAATAATTCACCTGACCTTTGGCCCCGAAGAAGCTCTTGCGAG
AGCAAACGGTGTTTCTC

mu IL12 (transmembrane form)

(SEQ ID NO: 81)

MCPQKLTIWFAIVLLVSPLMAMWELEKDVYVVEVDWTPDAPGETVNLTCDTPEEDDIT
WTSQQRHGVIGSGKLTITVKEFLDAGQYTCHKGGETLSHSHLLLHKKENGWSTEILKNF
KNKTFLKCEAPNYSGRFTCSWLVRNMDLKFNIKSSSSSPDSRAVTCGMASLSAEKVTLD
QRDYEKYSVSCQEDVTCPTAEETLPIELALEARQQNKYENYSTSFIRDIIKPDPPKNLQMK
PLKNSQVEVSWEYPDSWSTPHSYFSLKFFVRIQRKKEKMKETEEGCNQKGAFLVEKTSTE
VQCKGGNVVCVQAQDRYNNSSCSKWACVPCRVRSGSTSGSGKPGSGEGSTKGRVIPVSGPA
RCLSQSRNLLKTTDDMVKTAREKLKHYSCTAEDIDHEDITRDQTSTLKTCLPLELHKNESE
LATRETSSTTRGSCLPPQKTSMMTLCLGSIYEDLKMYQTEFQAINAALQNHNHQQIILDK
GMLVAIDELMQSLNHNGETLRQKPPVGEADPYRVKMKLCILLHAFSTRVVTINRVMGYLS
SANTLVLFAGFGAVITVVVIVVVIKCFCKHRSCFRRNEASRETNNSLTFGPPEALAEQTVF
L

mu IL12 (secreted form)

(SEQ ID NO: 82)

ATGTGTCAGTCACGCTATCTTCTCTTCTTGCTACTCTGGCCTTGCTCAATCACTTGTC
CTTGCTCGTGTGATTCTGTGTCGGGCCAGCTAGGTGTCTCTCCAGTCACGGAATCT
CCTGAAAACACGGATGACATGGTAAAGACAGCTAGGGAGAACTCAAGCACTACTC
CTGCACAGCTGAGGATATCGATCATGAGGACATCACCAGGGACCAGACATCCACTCTG
AAAACCTTGCCTTGTGAACTCCACAAGAACGAATCTTGTCTGGCAACGCGTGAAA
CGAGTTCTACTACAAGAGGGTCTGTCTTCCCCCTCAAAGACAAGCCTTATGATGAC
CTTGTGTCTCGGTAGCATTTATGAGGACCTAAAGATGTATCAAACCGAGTTTCAGGCTA
TCAATGCAGCGCTCCAGAATCATAACCATCAGCAGATCATTCTTGACAAAGGAATGCT
CGTGGCCATTGATGAACATAATGCAGAGCCTAAACCACAATGGCGAGACTCTTCGACAG
AAACCGCCTGTGGGCGAGGCCGATCCATATAGAGTCAAATGAACTGTGTATTCTCC
TGCATGCATTTAGTACTCGTGTAGTGACTATTAACAGAGTGATGGGTTACCTTTCCTCA
GCTGGAAGCGGCGCCACCAACTTCTCCCTGCTGAAGCAGGCCGGCGACGTGGAGGAG
AACCCCGGCCCATGTGCCCACAGAACTCACAATTTCTTGGTTTCGCAATCGTCCTGCT
GGTGTACCCCTGATGGCAATGTGGGAGTTGGAAAAGGATGTATACGTCGTCGAGGTC
GACTGGACACCTGACGCTCCGGGTGAACTGTCAACCTCACTTGCATACTCCTGAAG
AGGACGACATCACGTGGACGAGCGACCAGCGACATGGAGTGATAGGGTCTGGCAAGA
CGCTTACTATCACGGTTAAGGAATTTCTCGACGCGAGGGCAGTACACATGTCACAAGGG
CGGCGAGACTCTGAGCCACTCCATTTGCTGCTGCACAAGAAGGAGAATGGTATCTGG
TCTACCGAAATCCTGAAGAATTTTAAGAACAAGACTTTTCTGAAATGCGAGGCCCCAA
ATTATTCGGGACGTTTCACTTGCAGTTGGCTCGTTCAAAGAAATATGGACTTGAAATTT
AACATTAAATCCAGCTCTTCATCTCCTGACAGCAGGGCCGTAACCTTGTTGGAATGGCTTC
ATTGTACAGCTGAGAAAGTTACGCTTGACCAAAGGGATTATGAGAAATACAGCGTGAGT
TGCCAGGAAGATGTGACATGTCCAACGGCAGAGGAAACGTTGCCAATTGAGCTCGCTT
TGGAAGCTCGTCAACAAAACAAGTATGAAAACCTATAGTACTAGCTTCTTCATACGGGA
CATCATCAAACCAGATCCACCTAAGAATTTGCAGATGAAGCCTCTGAAGAATTCACAA
GTCGAGGTATCCTGGGAATACCCAGATTTCATGGTCCACTCCTCATAGTTACTTTAGCCT
GAAATTTCTTGTACGCATACAGCGGAAGAAGGAGAAAATGAAGGAGACGGAAGAAGG
CTGCAATCAGAAAGGCGCTTTTCTTGTGAAAAGACGAGCACTGAGGTTCAATGCAAA
GGCGGAATGTATGTGTTCAAGCCCAAGATAGGTATTATAATAGCTCCTGCTCTAAGT
GGGCTTGCGTACCATGCAGAGTTAGAAGT

mu IL12 (secreted form)

(SEQ ID NO: 83)

MCQSRYLFLATLALLNHLSTARVIPVSGPARCLSQSRNLLKTDDMVKTAREKLKHYSC
TAEDIDHEDITRDQTSTLKTCLPLELHKNESCLATRETSSTTRGSCLPPQKTSLMMTLCLGSI
YEDLKMYQTEFQAINAALQNHNHQQIILDKGMLVAIDELMQSLNHNGETLRQKPPVGEAD
PYRVKMKLCILLHAFSTRVVTINRVMGYLSSAGSGATNFSLLKQAGDVEENPGPMCPQKL
TISWFAIVLLVSPLMAMWELEKD VYVVEVDWTPDAPGETVNLTCDTPEEDDITWTS DQRH
GVIGSGKTLTITVKEFLDAGQYTCHKGGETLSHSHLLLHKKENGIWSTEILKNFNKNTFLK
CEAPNYSGRFTCSWL VQRNMDLKFNIKSSSSSPDSRAVTCGMASLSAEKVTL DQRDY EKY
SVSCQEDVTCPTAEETLPIELALEARQQNKYENYSTSFFIRDIIKPDPPKNLQMKPLKNSQVE
VSWEYPDSWSTPHSYFSLKFFVRIQRKKEKMKETEEGCNQKGAFLVEKTS TEVQCKGGNV
CVQ AQDRYYNSSCSKWACVPCRVS RS

mu IFN alpha A2

(SEQ ID NO: 84)

ATGGCCAGGCTTTGCGCTTTTCTCGTCATGCTGATCGTCATGAGTTACTGGTCCATTTG
CAGCCTCGGATGTGATCTGCCCCACACCTACAACCTGCGCAACAAACGAGCTCTCAA
GTGTTGGCCCAAATGAGGCGGTTGCCCTTCTTCTGTCTCAAAGACAGGCAAGATTT
TGGATTTCCACTAGAGAAAGTAGACAATCAACAGATACAGAAAGCTCAAGCTATCCCC
GTGTTGAGGGACTTGACTCAACAGACGTTGAATCTATTTACTAGCAAGGCCAGCTCTG
CTGCTTGGAATGCCACCCTTCTTGACTCATTTTGAATGACCTACATCAACA ACTGAAT
GATCTCCAAACATGTTTGATGCAGCAGGTAGGTGTCCAAGAACCCCGCTTACTCAGG
AAGACGCCCTTCTGGCTGTCCGCAAGTACTTTACAGAATCACAGTGTACCTGCGCGA
AAAGAAACACTCCCCCTGCGCTTGGGAAGTGGTCAGGGCCGAGGTTTGGCGAGCCCTG
AGTAGCTCCGTCAATCTCCTTCCTCGGTTGTCCGAGGAGAAAGAG

mu IFN alpha A2

(SEQ ID NO: 85)

MARLCAFLVMLIVMSYWSICSLGCDLPHTYNLRNKRALKVLAQMRRLPFLSCLKDRQDF
GFPLEKVDNQIQKAQAIPVLRDLTQQTLNLFTSKASSAAWNATLLDSFCNDLHQQLNDL
QTCLMQQVG VQEPPLTQEDALLAVRKYFHRITVYLREKKHSPCAWEVVRAEVWRALSS
VNLLPRLSEEKE

mu CD80

(SEQ ID NO: 86)

ATGGCTTGCAACTGTCAGCTCATGCAAGATACTCCCCTGCTTAAGTTTCCCTGCCCTAG
ACTCATTCTCCTCTTCGTCCTTCTCATTCGCCTAAGCCAGGTGAGTCCGATGTGGATG
AACA ACTGAGTAAATCTGTCAAGGATAAAGTTCTGCTCCCATGCCGCTACAATAGCCC
CCATGAGGACGAGTCCGAAGATAGGATTTACTGGCAGAAACATGATAAGGTGGTGCT
ATCCGTCATTGCCGGTAAATTGAAGGTGTGGCCCGAATATAAGAATAGAACCCCTGTAT
GACAACACA ACTTATAGCCTAATCATCCTCGGTCTCGTACTGAGCGACCGAGGTACTT
ACTCATGCGTTGTGCAGAAGAAGGAGCGCGGAACATACGAAGTCAAGCACCTTGCATT
GGTGAAATTGTCAATAAAAAGCTGACTTTTCAACTCCTAATATTACTGAATCAGGTAACC
CTTCCGCAGACACTAAAAGAATTACATGCTTCGCCTCTGGCGGGTTTCCCAAACCACG
GTTCTCTTGGCTAGAGAATGGGAGAGAACTTCCAGGTATCAATACAACCATCTCTCAA
GACCCAGAATCAGAACTGTACACCATCTCCAGCCAACTCGATTTCAATACCACAAGAA
ATCATACAATAAAAATGTCTGATAAAGTACGGAGATGCACATGTCTCTGAAGATTTTAC
ATGGGAGAAACCACAGAGGACCCGCCAGACAGCAAGAATACACTTGTCTCTTTGGC
GCTGGGTTTCGGCGCCGTCATAACGGTTGTTGTATCGTGGTAATAATCAAGTGCTTTTG
CAAGCACAGGTCTTGTTCGCAGGAATGAAGCCTCTAGAGAAACAATAATTCAC TG
ACCTTTGGCCCCGAAGAAGCTCTTGACAGAGCAAACGGTGTTTCTC

mu CD80

(SEQ ID NO: 87)

MACNCQLMQDTPLLKFPCPRLILLFVLLIRLSQVSSDVDEQLSKSVKDKVLLPCRYNSPHE
DESEDRIYWQKHDKVVL SVIAGKLKVWPEYKNRTLYDNTTYSLIILGLVLSDRGTYSCVV
QKKERGTYEVKHLALVKLSIKADFSTPNITESGNPSADTKRITCFASGGFPKPRFSWLENGR
ELPGINTTISQDPESELYTISSQLDFNTTRNHTIKCLIKYGDAHVSEDFTWEKPPEDPPDSKN
TLVLFGAGFGAVITVVVIVVIKCFCKHRSCFRR NEASRETNNS LTFGP EEALAEQTVFL

mu CD40-L

(SEQ ID NO: 88)

ATGATCGAAACTTATTCCCAACCCCTACCGCGCTCAGTAGCAACTGGCCTACCAGCCA
GCATGAAGATATTCATGTACCTCTTGACTGTATTCTTGATCACGCAAATGATTGGTAGT
GTTTTGTTCCGCGTTTATCTCCACAGGCGCCTGGATAAAGTTGAAGAAGAGGTTAATCT
CCATGAAGACTTTCGTGTTCAATTAAGAACTCAAAAGATGTAACAAAGGTGAGGGATCT
CTGTCTCTTCTGAACTGTGAGGAGATGCGACGGCAATTCGAGGACCTCGTAAAAGACA
TAACTCTCAACAAAGAAGAGAAGAAAGAAAAGAACTCTTTCGAGATGCAACGGGGCGACG
AGGACCCTCAAATTGCCGCACATGTCGTTTCTGAAGCGAATTCCAATGCCGCGTCCGT
GCTCCAGTGGGCGAAGAAGGGATACTACACGATGAAGAGCAACCTTGTGATGCTTGA
AAATGGCAAGCAGCTCACAGTTAAACGCGAGGGACTCTACTATGTATACACCCAAGTG
ACCTTTTGTTCACCGGGAGCCAAGTAGCCAACGCCCGTTTCATCGTTGGGCTGTGGCT
CAAGCCTTCTTCAGGGAGTGAACGAATCCTTCTCAAGGCAGCCAACACGCATTCCAGC
AGCCAACCTGTGTGAGCAACAATCCGTGCATCTTGGCGGGGTCTTTGAGCTGCAAGCGG
GCGCCTCTGTGTTCTGTAATGTTACCGAAGCCAGCCAGGTTATCCACCGCGTGGGTTTC
AGTAGTTTTGGCCTGCTCAAGCTG

mu CD40-L

(SEQ ID NO: 89)

MIETYSQPSPRSVATGLPASMKIFMYLLTVFLITQMIGSVLFAVYLHRRLDKVEEEVNLHED
FVFIKKLKR CNKGEGLSLLNCEEMRRQFEDLVKDITLNKEEK KENSFEMQRGDED PQIAA
HVVSEANSNAASVLQWAKKGYTMTKSNLVMLENGKQLTVKREGLYYVYTQVTFCSNRE
PSSQRPFIVGLWLKPSSG SERILLKAANTHSSSQLCEQQSVHLGGVFELQAGASVFNVT EAA
SQVIHRVGFSSFGLLKL

mu IL21

(SEQ ID NO: 90)

ATGGAGCGTACTCTGGTCTGCCTTGTTGTGATATTCTTGGGGACAGTTGCACACAAATC
ATCACCCCAAGGACCGGATAGACTCCTCATACGCTGCGCCATCTGATTGACATTGTC
GAGCAGTTGAAGATTTATGAGAACGACCTGGACCCTGAACTATTGAGCGCGCCTCAAG
ACGTCAAAGGGCATTGCGAGCATGCTGCATTTGCATGTTTTTCAGAAAGCTAAGCTCAA
ACCAAGTAATCCCGGTAACAATAAAACATTTCATCATCGACCTGGTGGCCCAACTAAGA
CGCCGGTTGCCGGCGCGCCGGGTGTAAGAAACAGAAACATATTGCTAAATGCCCT
CTTGCGACTCTTACGAGAAAAGGACACCTAAGGAATTCCTCGAACGATTGAAATGGTT
GTTGCAGAAGATGATCCATCAACATCTGAGC

mu IL21

(SEQ ID NO: 91)

MERTLVCLVVIFLGTVAHKSSPQGPDRLLIRLRHLIDIVEQLKIYENDLDPELLSAPQDVKG
HCEHA AFACFQKAKLKPSNPGNNKTFIIDLVAQLRRRLPARRGGKKQK
HIAKCPSCDSYEK RTPKEFLERLKWLLQKM IHQHLS

mu CCL21

(SEQ ID NO: 92)

ATGGCACAAATGATGACACTGTCCCTACTTAGTCTAGTTCTAGCTTTGTGATTCCCTG
GACTCAAGGCAGTGACGGAGGAGGACAAGACTGCTGCCTCAAATATTCTCAAAAGAA
AATCCCTTATTCTATAGTCCGAGGTTACCGTAAGCAAGAACCGAGTCTAGGTTGTCCTA
TCCCCGCAATCCTCTTTCTACCACGGAAACATAGCAAACCAGAATTGTGCGCCAACCC

AGAAGAGGGTTGGGTCCAAAATTTGATGAGGCGCCTTGACCAACCACCGGCCCCGGGT
AAACAATCACCGGGGTGTCTGGAAGAATAGGGGTACATCCAAATCCGGGAAGAAAGGG
AAGGGGAGTAAGGGCTGTAAGAGAACGGAACAACTCAACCTAGCAGAGGT

mu CCL21

(SEQ ID NO: 93)

MAQMMTSLLSLVLALCIPWTQGSDDGGQDCCLKYSQKKIPYSIVRGYRKQEPSLGCPIPA
ILFLPRKHSKPELCANPEEGWVQNLMMRLDQPPAPGKQSPGCRKNRGTSKSGKKGKSGSKG
CKRTEQTQPSRG

anti-mu CD3 scFv-transmembrane

(SEQ ID NO: 94)

ATGGAACCCGACACATTGCTCCTCTGGGTTCTCCTTCTATGGGTCCCCGGTTCCACCGG
AGATATCCAAATGACACAATACCCAGCAGCCTGCCTGCCTCTCTGGGCGACCGCGTT
ACCATCAATTGTCAAGCTTCCCAAGATATAAGTAATTATCTCAACTGGTACCAGCAAA
AGCCCCGTAAAGCGCCTAAATTGCTGATTTATTATACTAATAAACTCGCAGATGGAGT
TCCTAGTAGATTTTCTGGTTCAGGGAGTGGACGGGACTCCAGTTTTACCATATCAAGTC
TGGAATCCGAGGATATCGGCAGCTACTATTGCCAGCAATATTATAATTACCCTTGGACT
TTTGGACCCGGGACTAAACTTGAGATCAAAAGAGGCGGAGGAGGCAGTGGTGGTGGT
GGATCAGGCGGGCGGTGGTAGTGAGGTACAACCTCGTGGAATCAGGCGGCGGACTGGTC
CAACCCGGCAAGAGCCTTAAACTCTCTTGTGAGGCCAGTGGATTACATTCAGCGGTT
ATGGAATGCACTGGGTGAGACAAGCTCCCGGCAGGGGCCCTAGAATCAGTGGCGTACA
TCACCAGCTCATCAATAAACATTAAATACGCTGATGCAGTCAAGGGCCGGTTTACTGT
ATCCCGCGACAACGCTAAGAATCTTCTCTTTCTGCAAATGAACATACTTAAGAGCGAG
GATACTGCCATGTATTATTGTGCCCCTTCGATTGGGATAAGAATTATTGGGGACAAG
GCACCATGGTTACCGTTAGTAGTCCAAACATCACATCAAATAATAGCAACCCCGTGGA
AGGGGACGACTCTGTTTCACTCACCTGTGATTCTTATACCGATCCTGATAATATCAACT
ATCTATGGTCTCGTAACGGTGAAAGTCTCAGCGAAGGCGACCGGTTGAAACTCTCCGA
AGGTAACAGAACCCTTACGCTTCTGAACGTCACCCGGAACGATACCGGGCCCTATGTT
TGCGAAACTAGGAACCCTGTTAGCGTGAATCGTAGCGACCCTTTCTCCCTAAATAATA
CTCTAGTGCTATTTCGGAGCGGGATTCTGGTGCCGTATCACAGTAGTCGTTATTGTAGTC
ATTATTAATGCTTTTGTAAACATAGGTCTTGCTTCAGAAGAAATGAGGCCAGCCGTG
AAACTAATAATTCCCTGACCTTTGGGCCCCAAGAAGCTTTGGCTGAACAGACTGTGTTT
CTC

anti-mu CD3 scFv-transmembrane

(SEQ ID NO: 95)

METDTLLLWVLLLWVPGSTGDIQMTQSPSSLPASLGDRVTINCQASQDISNYLNWYQQKP
GKAPKLLIYYTNKLADGVPSRFSGSGSGRDSSTISSLESEDIGSYQCQQYYNYPWTFGP
KLEIKRGGGSGGGSGGGGSEVQLVESGGGLVQPGKSLKLSCEASGFTFSGYGMHWVR
QAPGRGLESVAYITSSSINIKYADAVKGRFTVSRDNAKNLLFLQMNILKSEDTAMYYCARF
DWDKNYWGQGTMTVTVSSPNITSNNSNPVEGDDSVSLTCDSTDPDNINYLWSRNGESLSE
GDRLKLSEGNRTLTLNVTRNDTGPHYVCETRPVSVNRSDFSLNNTLVLFAGAGFGAVITV
VVIVVIHKCFCKHRSCFRRNEASRETNNSLTFGPPEALAEQTVFL

mu TSLP

(SEQ ID NO: 96)

ATGGTTCTTCTCAGGAGCCTCTTCATCCTGCAAGTACTAGTACGGATGGGGCTAACTTA
CAACTTTTCTAACTGCAACTTCACGTCAATTACGAAAATATATTGTAACATAATTTTTC
ATGACCTGACTGGAGATTTGAAAGGGGCTAAGTTCGAGCAAATCGAGGACTGTGAGA
GCAAGCCAGCTTGTCTCCTGAAAATCGAGTACTATACTCTCAATCCTATCCCTGGCTGC
CCTTCACTCCCCGACAAAACATTTGCCCGGAGAACAAAGAGAAGCCCTCAATGACCACT
GCCCAGGCTACCCTGAAACTGAGAGAAATGACGGTACTCAGGAAATGGCACAAGAAG

TCCAAAACATCTGCCTGAATCAAACCTCACAAATTCTAAGATTGTGGTATTCCTTCATG
CAATCTCCAGAA

mu TSLP

(SEQ ID NO: 97)

MVLLRSLFILQVLVRMGLTYNFSNCNFTSITKIYCNIFHDLTGDLKGAKFEQIEDCESKPAC
LLKIEYYTLNPIPGCPSLPDKTFARRTREALNDHCPGYPPETERNDGTQEMAQEVQNICLNQ
TSQILRLWYSFMQSPE

mu GM-CSF

(SEQ ID NO: 98)

ATGTGGCTGCAGAATTTACTTTTCTGGGCATTGTGGTCTACAGCCTCTCAGCACCCAC
CCGCTCACCCATCACTGTCACCCGGCCTTGAAGCATGTAGAGGCCATCAAAGAAGCC
CTGAACCTCCTGGATGACATGCCTGTCACGTTGAATGAAGAGGTAGAAGTCGTCTCTA
ACGAGTTCTCCTTCAAGAAGCTAACATGTGTGCAGACCCGCCTGAAGATATTCGAGCA
GGGTCTACGGGGCAATTTACCAAACCTCAAGGGCGCCTTGAACATGACAGCCAGCTAC
TACCAGACATACTGCCCCCACTCCGGAAACGGACTGTGAAACACAAGTTACCACCT
ATGCGGATTTTCATAGACAGCCTTAAACCTTTCTGACTGATATCCCTTTGAATGCAAA
AAACCAGGCCAAAAA

mu GM-CSF

(SEQ ID NO: 99)

MWLQNLLFLGIVVYSLAPTRSPITVTRPWKHVEAIKEALNLLDDMPVTLNEEVEVVSNEF
SFKKLTCVQTRLKIFEQGLRGNFTKLKGALNMTASYYYQTYCPPTPETDCETQVTTYADFIG
SLKTFLTDIPFECKKPGQK

mu IFN gamma

(SEQ ID NO: 100)

ATGAACGCTACACACTGCATCTTGGCTTTGCAGCTCTTCCTCATGGCTGTTTCTGGCTG
TACTGCCACGGCACAGTCATTGAAAGCCTAGAAAGTCTGAATAACTATTTAACTCA
AGTGGCATAGATGTGGAAGAAAAGAGTCTCTTCTTGGATATCTGGAGGAACTGGCAAA
AGGATGGTGACATGAAAATCCTGCAGAGCCAGATTATCTCTTTCTACCTCAGACTCTTT
GAAGTCTTGAAAGACAATCAGGCCATCAGCAACAACATAAGCGTCATTGAATCACACC
TGATTACTACCTTCTTCAGCAACAGCAAGGCGAAAAAGGATGCATTCATGAGTATTGC
CAAGTTTGAGGTCAACAACCCACAGGTCCAGCGCCAAGCATTCAATGAGCTCATCCGA
GTGGTCCACCAGCTGTTGCCGGAATCCAGCCTCAGGAAGCGGAAAAGGAGTCGCTGC

mu IFN gamma

(SEQ ID NO: 101)

MNATHCILALQLFLMAVSGCYCHGTVIESLESNNYFNSSGIDVEEKSLFLDIWRNWQKDG
DMKILQSQIISFYLRLEVLKDNQAISNNISVIESHLITTFNSNSKAKKDAFMSIAKFEVNNPQ
VQRQAFNELIRVVHQLLPESSLRKRKRSRC

mu IL7

(SEQ ID NO: 102)

ATGTTCCATGTTTCTTTAGATATATCTTTGGAATTCCTCCACTGATCCTTGTCTGCTG
CCTGTCACATCATCTGAGTGCCACATTAAAGACAAAGAAGGTAAAGCATATGAGAGTG
TACTGATGATCAGCATCGATGAATTGGACAAAATGACAGGAACTGATAGTAATTGCC
GAATAATGAACCAAACCTTTTTAGAAAACATGTATGTGATGATACAAAGGAAGCTGCT
TTTCTAAATCGTGCTGCTCGCAAGTTGAAGCAATTTCTTAAAATGAATATCAGTGAAGA
ATTCAATGTCCACTTACTAACAGTATCACAAGGCACACAAACACTGGTGAACCTGCACA
AGTAAGGAAGAAAAAACGTAAAGGAACAGAAAAAGAATGATGCATGTTTCTTAAAG
AGACTACTGAGAGAAATAAAAACCTTGTGGAATAAAATTTTGAAGGGCAGTATA

mu IL7

(SEQ ID NO: 103)

MFHVSFRYIFGIPPLILVLLPVTSSSECHIKDKEGKAYESVLMISIDELDKMTGTDSNCPNNEP
NFFRKHVCDDTKEAAFLNRAARKLKQFLKMNISEEFNVHLLTVSQGTQTLVNCTSKEEKN
VKEQKKNDACFLKRLLREIKTCWNKILKGS

mu ICOS-L

(SEQ ID NO: 104)

ATGCAGCTAAAGTGTCCCTGTTTTGTGTCCTTGGGAACCAGGCAGCCTGTTTGGAAGA
AGCTCCATGTTTCTAGCGGGTCTTTTCTGGTCTTGGTCTGTTCTTGCTGCTGTTGAGCA
GCCTCTGTGCTGCCTCTGCAGAGACTGAAGTCGGTGCAATGGTGGGCAGCAATGTGGT
GCTCAGCTGCATTGACCCCCACAGACGCCATTTCAACTTGAGTGGTCTGTATGTCTATT
GGCAAATCGAAAACCCAGAAGTTTCGGTGACTTACTACCTGCCTTACAAGTCTCCAGG
GATCAATGTGGACAGTTCCTACAAGAACAGGGGCCATCTGTCCCTGGACTCCATGAAG
CAGGGTAACTTCTCTGTACCTGAAGAATGTCACCCCTCAGGATACCCAGGAGTTCA
CATGCCGGGTATTTATGAATACAGCCACAGAGTTAGTCAAGATCTTGGAAGAGGTGGT
CAGGCTGCGTGTGGCAGCAAACCTTCAGTACACCTGTCATCAGCACCTCTGATAGCTCC
AACCCGGGCCAGGAACGTACCTACACCTGCATGTCCAAGAATGGCTACCCAGAGCCCA
ACCTGTATTGGATCAACACAACGGACAATAGCCTAATAGACACGGCTCTGCAGAATAA
CACTGTCTACTTGAACAAGTTGGGCCTGTATGATGTAATCAGCACATTAAGGCTCCCTT
GGACATCTCGTGGGGATGTTCTGTGCTGCGTAGAGAATGTGGCTCTCCACCAGAACAT
CACTAGCATTAGCCAGGCAGAAAGTTTCACTGGAAATAACACAAAGAACCCACAGGA
AACCACAATAATGAGTTAAAAGTCCTTGTCCTCGTCTGCTGTACTGGCGGCAGCG
GCATTGTTTTCTTCATCATATACAGACGCACGCGTCCCCACCGAAGCTATACAGGACC
CAAGACTGTACAGCTTGAACCTACAGACCACGCC

mu ICOS-L

(SEQ ID NO: 105)

MQLKCPCFVSLGTRQPVWKKLHVSSGFFSGLGLFLLLLSSLCAASAETEVGAMVGSNVVL
SCIDPHRRHFNLISGLYVYWQIENPEVSVTYLPHYKSPGINVDSSYKNRGHLSLDSMKQGNF
SLYLKNVTPQDTQEFTCRVFMNTATELVKILEEVVRLRVAANFSTPVISTSDSSNPGQERTY
TCMSKNGYPEPNLYWINTTDNSLIDTALQNNTVYLNKLGLYDVISTLRLPWTSRGDVLCC
VENVALHQNITSISQAESFTGNNTKNPQETHNNELKVLVPVLAVLAAAFVSFIYRRTRPH
RSYTGPKTQLELTD HA

mu CD47

(SEQ ID NO: 106)

ATGTGGCCCTTGGCGGCGGCGCTGTTGCTGGGCTCCTGCTGCTGCGGTTACAGTCAACT
ACTGTTTAGTAACGTCAACTCCATAGAGTTCACTTCATGCAATGAACTGTGGTCATCC
CTTGCAATCGTCCGTAATGTGGAGGCGCAAAGCACCGAAGAAATGTTTGTAAGTGGAA
GTTGAACAAATCGTATATTTTCATCTATGATGGAAATAAAAATAGCACTACTACAGAT
CAAACTTTACCAGTGCAAAAATCTCAGTCTCAGACTTAATCAATGGCATTGCCCTTTT
GAAAATGGATAAGCGCGATGCCATGGTGGGAACTACACTTGCGAAGTGACAGAGTT
ATCCAGAGAAGGCAAAACAGTTATAGAGCTGAAAAACCGCACGGTTTCGTGGTTTTCT
CCAAATGAAAAGATCCTCATTGTTATTTTCCCAATTTTGGCTATACTCCTGTTCTGGGG
AAAGTTTGGTATTTTAACTCAAATATAAATCCAGCCATACGAATAAGAGAATCATT
CTGCTGCTCGTTGCCGGGCTGGTGCTCACAGTCATCGTGGTTGTTGGAGCCATCCTTCT
CATCCAGGAGAAAAGCCCCGTGAAGAATGCTTCTGGACTTGGCCTCATTGTAATCTCT
ACGGGGATATTAATACTACTTCAGTACAATGTGTTTATGACAGCTTTTGGAAATGACCTC
TTTACCATTTGCCATATTGATCACTCAAGTGCTGGGCTACGTCCTTGCTTTGGTCGGGC
TGTGTCTCTGCATCATGGCATGTGAGCCAGTGCACGGCCCCCTTTTGATTTACAGGTTTG

GGGATCATAGCTCTAGCAGAACTACTTGGATTAGTTTATATGAAGTTTGTCTGCTTCCAA
CCAGAGGACTATCCAACCTCCTAGGAATAGG

mu CD47

(SEQ ID NO: 107)

MWPLAAALLLGSCCCGSAQLLFSNVNSIEFTSCNETVVIPCIVRNVEAQSTEEMFVKWKLN
KSYIFIYDGNKNSTTTDQNFSAKISVSDLINGIASLKMDKRDAMVGNYTCEVTELSREGK
TVIELKNRTVSWFSPNEKILIVIFPILAILLFWGKFGILTLKYKSSHTNKRILLVAGLVLTVI
VVVGAILLIPGEKPVKNASGLGLIVISTGILILLQYNVFMATFGMTSFTIAILITQVLGYVLAL
VGLCLCIMACEPVHGPLLISGLGHIALAELLGLVYMKFVASNQRTIQPPRNR

Mu Sarcoglycan alpha:

(SEQ ID NO: 108)

ATGGCAGCAGCAGTAACTTGGATACCTCTCCTGGCAGGTCTCCTGGCAGGACTGAGGG
ACACCAAGGCCAGCAGACAACTTTACACCTACTTGTGGGTCGTGTGTTTGTGCATCCT
TTGGAACATGCCACCTTCCTGCGCCTTCCAGAACACGTTGCGGTGCCACCCACTGTCCG
ACTCACCTACCACGCTCACCTCCAGGGACATCCAGACCTGCCAGGTGGCTGCACTAC
ACACAGCGCAGTCCCTATAACCCTGGCTTCTCTACGGCTCCCCACTCCAGAAGATCG
TGGGTACCAAGTCATCGAGGTCACAGCCTACAATCGAGACAGTTTTGACACCACTAGA
CAGAGGCTGCTGCTGCTGATTGGGGACCCCGAAGGTCCCCGGTTGCCATACCAAGCTG
AGTTCCTGGTGCGCAGCCATGATGTGGAGGAGGTGCTGCCACCCACACCTGCCAACCG
CTTCCTCACCGCCTTGGGGGGACTGTGGGAGCCAGGAGAGCTTCAGCTGCTCAACATC
ACTTCCGCCTTGGACCGGGGAGGCCGAGTCCCTCTTCTATTGAGGGACGGAAGGAAG
GGGTATACATTAAGGTAGGCTCTGCCACACCCTTCTCCACCTGCCTGAAGATGGTGGC
GTCGCCCCGACAGCTATGCCCGTTGTGCCAGGGACAGCCTCCACTACTGTCTGCTACG
ACACTTTGGCACCCCACTTCCGCGTTGACTGGTGCAATGTGTCTCTGGTAGACAAGTCA
GTACCCGAGCCCCCTGGATGAGGTACCTACTCCAGGCGATGGGATCTTGGAGCACGACC
CGTCTCTTGTGCCACCCACTGAAGCCACAGACCGAGACTTCCTGACAGATGCCTTGGTG
ACCCTCTTGGTGCCTTTTGTGGTGGCTCTGCTGCTTACTCTGTTGCTGGCTTACATCATG
TGCTTTCCGCGTGAAGGACGGCTGAAGAGAGACATGGCCACCTCTGACATCCAGATGT
TTCACCACTGTTCCATCCATGGGAATACAGAAGAGCTTCGGCAGATGGCAGCCAGCCG
AGAGGTGCCCCGGCCTCTTCCACCTTGCCCATGTTTAATGTTCTGACAGGAGAGCGGT
TACCTCCCCGAGTAGACAGCGCACAGATGCCTCTTATCCTGGACCAGCAC

Mu Sarcoglycan alpha:

(SEQ ID NO: 109)

MAAAVTWIPLLAGLLAGLRDTKAQQTTLHLLVGRVVFHLEHATFLRLPEHVAVPPTVRL
TYHAHLQGHPLDPRWLHYTQRSPYNPGFLYGSPTPEDRGYQVIEVTAYNRDSFDTTRQRL
LLLIGDPEGPRLPYQAEFLVRSHDVEEVLPTTPANRFLTALGGLWEPGELQLLNITSALDRG
GRVPLPIEGRKEGVYIKVGSATPFSTCLKMVASPDYARCAQGPPLSCYDTLAPHFRVD
WCNVSLVDKSVPEPLDEVPTPGDGILEHDPFFCPPTTEATDRDFLTALVTLVPLLVALLLT
LLLAYIMCFRREGRLKRD MATSDIQMFHHC SIHGNT EELRQMAASREVPRPLSTLPMFNV
R TGERLPPRVDSAQM PLILDQH

Mu FGF10

(SEQ ID NO: 110)

ATGTGGAAATGGATACTGACACATTGTGCCTCAGCCTTTCCCCACCTGCCGGGCTGCTG
TTGCTGCTTCTTGTGCTCTTTTTGGTGTCTTCGTTCCCTGTACCTGCCAAGCTCTTGGT
CAGGACATGGGTGCACAGGAGGCCACCAACTGCTCTTCTCCTCCTCGTCTTCTCCTC
TCCTTCCAGTGCGGGAAGGCATGTGCGGAGCTACAATCACCTCCAAGGAGATGTCCGC
TGGAGAAGGCTGTTCTCCTTACCAAGTACTTTCTCACGATTGAGAAGAACGGCAAGG
TCAGCGGGACCAAGAATGAAGACTGTCCGTACAGTGTCTGAGATAACATCAGTGGA
AATCGGAGTTGTTGCCGTCAAAGCCATCAACAGCAACTATTACTTAGCCATGAACAAG

AAGGGGAAACTCTATGGCTCAAAAGAGTTTAAACAACGACTGTAAGCTGAAAGAGAGA
ATAGAGGAAAATGGATACAACACCTATGCATCTTTTAACTGGCAGCACAAATGGCAGGC
AAATGTATGTGGCATTGAATGGAAAAGGAGCTCCCAGGAGAGGACAAAAACAAGAA
GGAAAAACACCTCTGCTCACTTCTCCCATGACGATCCAAACA

Mu FGF10

(SEQ ID NO: 111)

MWKWILTHCASAFPHLPGCCCCFLLLFLVSSFPVTCQALGQDMVSQEATNCSSSSSFSSPS
SAGRHVRSYNHLQGDVRWRRLFSFTKYFLTIEKNGKVSGTKNEDCPYSVLEITSVEIGVVA
VKAINSYYLAMNKKGKLYGSKEFNNDCKLKERIEENGYNTYASFNWQHNGRQMYVAL
NGKGAPRRGQKTRRKNTSAHFLPMTIQT

Mu Agrin

(SEQ ID NO: 112)

ATGCCTCCTCTGCCACTGGAACACAGACCCAGGCAGCAGCCTGGTGCCTCCGTGCTGG
TTCGGTACTTCATGATCCCCTGCAACATCTGCTTGATCCTCTTGGCTACTTCTACGTTGG
GCTTTGCGGTGCTGCTTTTCTCAGCAACTACAAACCTGGGATCCACTTCACAGCAGCG
CCTTCTATGCCTCCTGATGTGTGCAGGGGAATGTTATGTGGCTTTGGTGCTGTGTGTGA
ACCTAGTGTTGAGGATCCAGGCCGGGCCTCCTGTGTGTGCAAGAAGAATGTCTGCCCT
GCTATGGTAGCTCCTGTGTGTGGCTCAGATGCTTCCACCTATAGCAACGAGTGTGAGCT
ACAGCGTGCACAGTGCAACCAGCAACGGCGCATCCGCCTACTCCGCCAAGGGCCATGT
GGGTCCCGGGACCCCTGTGCCAATGTGACCTGCAGTTTCGGTAGTACCTGTGTACCTTC
AGCCGATGGACAGACCGCCTCGTGTCTGTGTCTACAACCTGCTTTGGGGCCCTGATG
GCACAGTGTGTGGCAGTGATGGTGTTGACTACCCTAGTGAGTGCCAGCTGCTCCGTCA
TGCCTGTGCCAACCAGGAGCACATCTTTAAGAAGTTCGATGGTCCTTGTGACCCCTGCC
AGGGCAGCATGTCAGACCTGAATCATATTTGCCGGGTGAACCCACGTACACGGCACCC
AGAAATGCTTCTGCGGCCTGAGAACTGCCCCGCCAACACACACCTATCTGTGGAGAT
GATGGGTCACCTATGAAAACGACTGTGTCAATGCCAGACTCGAGACCAGTGCCCCGAGAC
TGCTTCTCCAGAAAGTGCGCTCTGGTCAATGCCAGACTCGAGACCAGTGCCCCGAGAC
CTGCCAGTTTAACTCTGTATGCCTGTCCCGCCGCGCGCTCCCCACTGTTCTCGGATC
GCGTCACCTGTGATGGGGCTTACAGGCCAGTGTGTGCCCAGGATGGGCACACGTATGA
CAATGACTGTTGGCGCCAACAGGCCGAGTGTGACAACAGCAGACCATTCCCCCAAG
CACCAGGGCCCGTGTGACCAGACCCCATCCCCGTGCCGTGGAGCGCAGTGTGCATTTG
GGGCAACATGCACAGTGAAGAATGGGAAAGCTGTGTGCGAGTGCCAGCGGGTGTGCT
CGGGCGGCTACGATCCTGTGTGCGGCAGTGATGGTGTCACCTACGGCAGTGTGTGCGA
GCTGGAATCCATGGCCTGTACCCTTGGGCGGGAAATCCGAGTGGCCCGCAGAGGACCG
TGTGACCGATGTGGGCAGTGCCGTTTGGATCCTTGTGCGAGGTGGAGACTGGACGCT
GTGTGTGCCCTCTGAGTGTGTGGAGTCAGCCCAGCCCGTATGTGGCTCTGACGGACA
CACATATGCTAGTGAATGTGAGCTGCATGTCCACGCCTGTACACACCAGATCAGCCTA
TACGTGGCCTCAGCCGACACTGCCAGACCTGTGGAGAAACAGTTTGTACCTTTGGGG
CTGTGTGCTCAGCTGGACAGTGTGTATGTCCCCGTGTGTGAGCACCCCCACCTGGCCCT
GTGTGCGGCAGTGATGGCGTCACCTACCTCAGTGCCTGTGAGCTCCGAGAGGCTGCCT
GTCAGCAGCAGGTACAAATTGAGGAGGCCCGTGCAGGGCCGTGTGAGCCGGCTGAGT
GTGGCTCAGGGGGCTCTGGGTCTGGGGAAGACAATGCGTGTGAGCAGGAGCTGTGTG
GCAGCATGGTGGTGTCTGGGATGAGGACTCAGAAGACGGGCCGTGTGTCTGTGACTTT
AGTTGCCAGAGTGTCTTAAAAGCCCAGTGTGTGGCTCAGATGGAGTCACCTATAGCA
CGGAGTGCCATCTGAAGAAGGCCAGATGTGAAGCGCGGCAAGAGCTGTACGTCGCTG
CTCAGGGAGCCTGCCGGGGCCCTACCTTGGCTCCACTGCTACCTATGGCCTCCCCAC
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GCTTGGCTGGCTGTCCAGCACCTGCCATTGCAACCCACACGGCTCCTATAGCGGCACT
TGTGACCCAGTCACAGGGCAGTGCTCCTGCCGACCAGGTGTAGGAGGCCTCAGGTGTG
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TGCACTCCCTGCAGCTGTGACCCTCGGGGTGCTGTAAGAGATGACTGTGAGCAGATGA

CTGGATTGTGTTCTGTAGACCTGGTGTGGCTGGTCCCAAGTGTGGGCAGTGTCCAGAT
GGTCAAGCCCTGGGCCATCTTGGCTGTGAAGCAGATCCCACAACACCAGTGAAGTGTG
TGGAATGCACTGTGAGTTTGGCGCCTCTGCGTAGAGGAGGCTGGTTTTGCCAGTG
TGTCTGCCAACTCTCACATGTCCAGAGGCTAACTCTACCAAGGTCTGTGGATCAGATG
GTGTCACATACGGCAATGAATGCCAGCTGAAGACCATTGCCTGCCGCCAGCGTCTGGA
CATCTCCATTACAGAGTCTTGGTCCATGCCGGGAGAGTGTGCTCCTGGGGTTTCCCTA
CATCTGCATCTATGACCACCCCAAGGCATATCCTGAGCAGGACACTGGCGTCTCCCA
CAGCAGCCTTCTCTGTCTCCAGCACTACTGCCATGATTGGCCACCCCAATTACCA
CATCACCTCAGACCGTAGTCGGCACCCCAAGGAGCACTGCAGCCACACCTCTGATGT
GGCCAGTCTTGCTACAGCGATCTTCAGGGAATCTGGCAGCACCAACGGCAGTGGCGAT
GAGGAGCTCAGTGGCGATGAGGAGGCCAGTGGGGGCGGGTCTGGGGGACTTGAGCCC
CCGGTGGGCAGCGTTGTGGTGACCCACGGGCCACCCATCGAGAGGGCTTCTGTTACA
ACTCACCTTTGGGCTGCTGCTCAGATGGCAAGACACCTCACTGGACTCAGAAGGCTC
CAACTGTCCAGCTACCAAGGCATTCCAGGGCGTGCTGGAGCTTGAGGGGGTCGAGGGA
CAGGAAGTGTCTACACACCAGAGATGGCTGACCCCAAGTCAGAGTTGTTTGGGGAGA
CTGCAAGGAGCATTGAGAGCACGCTGGACGACCTGTTCCGGAATTTCGGATGTTAAGAA
GGACTTCTGGAGCATCCGCCTACGGGAAGTGGGGCCTGGCAAATTAGTCCGTGCCATT
GTGGATGTTCACTTTGACCCACACAGCCTTCCAGGCACCAGATGTGGGTGAGGCCTT
GCTCCAACAGATCCAGGTATCCAGGCCGTGGGCCCTGGCAGTGAGGAGGCCTCTGCGG
GAGCATGTGCGATTCTTGACTTTGACTGGTTTCCCACTTTTTTTACGGGAGCTGCAAC
AGGAACCACAGCTGCTGTGGCCACAGCCAGAGCCACCACTGTGAGCCGACTGTCTGCC
TCTTCTGTCAACCCACGAGTCTACCCAGTTACACCAGCCGGCCTGTTGGCAGAACTAC
GGCACCGCTAACCACTCGCCGGCCACCAACCACTACCGCCAGTATTGACCGACCTCGG
ACTCCAGGCCCGCAACGGCCCCAAAGTCCTGTGATTCCCAGCCTTGCTCCACGGAG
GTACCTGCCAGGACCTGGATTCTGGCAAGGGTTTCAGCTGCAGCTGTACTGCAGGCAG
GGCTGGCACTGTCTGTGAGAAAGTGCAGCTCCCTCTGTGCCAGCTTTTAAGGGCCACT
CCTTCTTGCCCTTCCCAACCTCCGAGCCTACCACACGCTGCGTCTGGCACTAGAATTC
CGGGCGCTGGAGACAGAGGGACTGCTGCTCTACAATGGCAATGCACGTGGCAAAGAT
TTCTTGGCTCTGGCTCTGTTGGATGGTCATGTACAGTTACAGTTTCAGGTTGACAGGGCTCAGG
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GGTGAAAGTCCGAGTGGCACTGATGGCCTCAACTTGGACACGAAGCTCTATGTGGGTG
GTCTCCAGAGAAGAACAAGTTGCCACGGTGCTTGATCGGACCTCTGTGGGCATCGGCCT
GAAAGGATGCATTCGTATGTTGGACATCAACAACAGCAGCTGGAGCTGAGCGATTGG
CAGAGGGCTGTGGTTCAAAGCTCTGGTGTGGGGGAATGCGGAGACCATCCCTGCTCAC
CTAACCCCTGCCATGGCGGGGCCCTCTGCCAGGCCCTGGAGGCTGGCGTGTCTCTGT
CAGTGCCACCTGGCCGCTTTGGCCCAACTTGTGCAGATGAAAAGAACCCTGCCAAC
CGAACCCCTGCCACGGGTCAGCCCCCTGCCATGTGCTTTCCAGGGGTGGGGCCAAGTG
TGCGTGCCCCCTGGGACGCAGTGGTTCTTCTGTGAGACAGTCCTGGAGAATGCTGGC
TCCCGGCCCTTCTGGCTGACTTTAATGGCTTCTCCTACCTGGAAGTGAAGGCTTGCA
CACCTTCGAGAGAGACCTAGGGGAGAAGATGGCGCTGGAGATGGTGTTCTTGCTCGT
GGGCCCAGTGGCTTACTCCTCTACAATGGGCAGAAGACGGATGGCAAGGGGGACTTTG
TATCCCTGGCCCTGCATAACCGGCACCTAGAGTTCCGCTATGACCTTGGCAAGGGGGC
TGCAATCATCAGGAGCAAAGAGCCCATAGCCCTGGGCACCTGGGTAGGGTATTCCTG
GAACGAAATGGCCGCAAGGGTGCCCTTCAAGTGGGTGATGGGCCCGTGTGCTAGGG
GAATCTCCGAAATCCCGCAAGGTCCCGCACACCATGCTCAACCTCAAGGAGCCCTCT
ATGTGGGGGAGCTCCTGACTTCAGCAAGCTGGCTCGGGGCGCTGCAGTGGCCTCCGG
CTTTGATGGTGCCATCCAGCTGGTGTCTCTAAGAGGCCATCAGCTGTGACTCAGGAG
CATGTGTTGCGGGCAGTAGATGTAGCGCTTTTGCAGGCCACCCTGTACCAAGGCCGT
GGACAACCCCTGCCTTAATGGGGGCTCCTGTATCCCGAGGGAAGCCACTTATGAGTGC
CTGTGTCTGGGGGCTTCTCTGGGCTGCACTGCGAGAAGGGGATAGTTGAGAAGTCAG
TGGGGGACCTAGAAACACTGGCCTTTGATGGGCGGACCTACATCGAGTACCTCAATGC
TGTGACTGAGAGCGAGCTGACCAATGAGATCCAGCCCCCGAACTCTGGATTCCCGG

GCCCTTTTCAGTGAGAAAGCGCTGCAGAGCAACCACTTTGAGCTGAGCTTACGCACTG
AGGCCACGCAGGGGCTGGTGCTGTGGATTGGAAAAGGTTGGAGAACGTGCAGACTACA
TGGCTCTGGCCATTGTGGATGGGCACCTACAACCTGAGCTATGACCTAGGCTCCCAGCC
AGTTGTGCTGCGCTCCACTGTGAAGGTCAACACCAACCGCTGGCTTCGAGTCAGGGCT
CACAGGGAGCACAGGGAAGGTTCCCTTCAGGTGGGCAATGAAGCCCCTGTGACTGGCT
CTCCCCGCTGGGTGCCACACAATTGGACACAGATGGAGCCCTGTGGCTTGGAGGCCT
ACAGAAAGCTTCTGTGGGGCAGGCTCTCCCCAAGGCCTATGGCACGGGTTTTGTGGGC
TGTCTGCGGGACGTGGTAGTGGGCCATCGCCAGCTGCATCTGCTGGAGGACGCTGTCA
CCAAACCAGAGCTAAGACCCTGCCCCACTCTCTGA

Mu Agrin

(SEQ ID NO: 113)

MPPLPLEHRPRQQPGASVLVRYFMIPCNICLILLATSTLGFVLLFLSNYKPGIHFTAAPSM
PDVCRGMLCGFVAVCEPSVEDPGRASCVCKKNVCPAMVAPVCGSDASTYSNECELQRAQ
CNQQRIRLLRQGPCSRDPCANVTCSFGSTCVPSADGQTASCLCPTTCFGAPDGTVCSD
GVDYPSECQLLRHACANQEHIFKKFDGPCDPCQGSMSDLNHICRVNPRTRHPEMLLRPEN
CPAQHTPICGDDGVTYENDCVMSRIGAARGLLLQKVRSGQCQTRDQCPETCQFNSVCLSR
RGRPHCSCDRVTCDGAYRPVCAQDGHTYDNDWCWRQQAECRQQQTIPPKHQGPCDQTPSP
CRGAQCAFGATCTVKNGKAVCECQRVCSGGYDPVCGSDGVTYGSVCELESMAC TLGREI
RVARRGPCDRGQCRFGSLCEVETGRCVCPSECVESAQPVCGSDGHTYASECELHVHACT
HQISLYVASAGHCQTCGETVCTFGAVCSAGQCVCPRCEHPPPGPVCGSDGVTYLSACELRE
AACQQQVQIEEARAGPCEPAECGSGSGSGEDNACEQELCRQHGGVWDEDSGDGPCVCD
FSCQSVLKSPVCGSDGVTYSTEHLKKARCEARQELYVAAQGACRGPTLAPLLPMASPHC
AQTPYGCCQDNVTAAQGVGLAGCPSTCHCNPHGSYSGTCDPVTGQCSCRPGVGGLRCDR
CEPGFWNFRGIVTDGHSCTPCSCDPRGAVRDDCEQMTGLCSCRPGVAGPKCGQCPDGQ
ALGHLGCEADPTTPVTCVEMHCEFGASCVEEAGFAQCVCPTLTCPEANSTKVCSDGVTY
GNECQLKTIACRQRDLISIQSLGPCRESVAPGVSPTSASMTTPRHILSR TLASPHSSLPLSPST
TAHDWPTPLPTSPQTVVGTTPRSTAATPSDVASLATAIFRESGSTNGSGDEELSGDEEASGGG
SGGLEPPVGSVVVTHGPPIERASCYNSPLGCCSDGKTPSLDSEGSNCPATKAFQGVLELEGV
EGQELFYTPEMADPKSELFGETARSIESTLDDLFRNSDVKKDFWSIRLRELGPGLVRAIVD
VHFDPTTAFQAPDVGQALLOQQIQRVSRPWALAVRRPLREHVRFLDFDWFPTFTTGAATGTT
AAVATARATTVSRLSASSVTPRVYPSYTSRPVGRRTTAPLTTRRPPTTTASIDRPRTPGPQRPP
KSCDSQPCLHGGTCQDLDSGKGFSCSCTAGRAGTVCEKVQLPSVPAFKGHSFLAFPTLRAY
HTLRLALEFRALETEGLLLYNGNARGKDFLALALLDGHVQFRFDTGSGPAVLTS LVPVEPG
RWHRLERSRHWQGTLSVDGEAPVVGESPSGTDGLNLDTKLYVGGLPEEQVATVLDRTS
VGIGLKGCI RMLDINNQQLELSDWQRAVVQSSGVGECGDHPCSPNPCHGGALCQALEAGV
FLCQCPPGRFGPTCADEKNPCQPNPCHGSA PCHVLSRGGAKCACPLGRSGSFCE TVLENAG
SRPFADFNGFSYLELKG LHTFERDLGEKMALEMVFLARGPSGLLLYNGQKTDGKGDFVSL
ALHNRHLEFRYDLGKGAAIIRSKEPIALGTWVRVFLERNRKGALQVGDGPRVLGESPKSR
KVPHTMLNLKEPLYVGGAPDFSKLARGAAVASGFDGAIQLVSLRGHQLLTQEHVLR AIVD
VAPFAGHPCTQAVDNPCLNGGSCIPREATYECLCPGGFSGLHCEKGIVEKSVGDLETLAFD
GRTYIEYLN AVTESEL TNEIPAPETLDSRALFSEKALQSNHFELSLRTEATQGLVLWIGKVG
ERADYMALAIVDGHLLQSLYDLGSQPVVLRSTVKVNTNRWLRVRAHREHREGSLQVGNEA
PVTGSSPLGATQLD TDGALWLGGLQKL PVGQALPKAYGTGFVGC LRDVVVGHRQLHLL E
DAVTKEPRLPCPTL

Mu IL10

(SEQ ID NO: 114)

ATGCCTGGCTCAGCACTGCTATGCTGCCTGCTCTTACTGACTGGCATGAGGATCAGCAG
GGGCCAGTACAGCCGGGAAGACAATAACTGCACCCACTTCCCAGTCGCCAGAGCCA
CATGCTCCTAGAGCTGCGGACTGCCTTCAGCCAGGTGAAGACTTTCTTTCAAACAAAG
GACCAGCTGGACAACATACTGCTAACCGACTCCTTAATGCAGGACTTTAAGGGTTACT
TGGGTTGCCAAGCCTTATCGGAAATGATCCAGTTTTACCTGGTAGAAGTGATGCCCCA

GGCAGAGAAGCATGGCCCAGAAATCAAGGAGCATTTGAATTCCCTGGGTGAGAAGCT
GAAGACCCCTCAGGATGCGGCTGAGGCGCTGTCATCGATTTCTCCCCTGTGAAAATAAG
AGCAAGGCAGTGGAGCAGGTGAAGAGTGATTTTAATAAGCTCCAAGACCAAGGTGTC
TACAAGGCCATGAATGAATTTGACATCTTCATCAACTGCATAGAAGCATACATGATGA
TCAAATGAAAAGCTAA

Mu IL10

(SEQ ID NO: 115)

MPGSALLCCLLLLTGMRISRGQYSREDNNCTHFPVGQSHMLLELRTAFSQVKTFQTKDQ
LDNILLTDSLMDQDFKGYLGQCQALSEMIQFYLVVMPQAEKHGPEIKEHLNSLGEKLTLR
MRLRRCHRFLPCENKSKAVEQVKSDFNKLQDQGVYKAMNEFDIFINCIEAYMMIKMKS

Mu MYDGF (C19orf10)

(SEQ ID NO: 116)

ATGGCAGCCCCAGCGGAGGCTTCTGGACTGCGGTGGTCCTGGCGGCCGAGCGCTGA
AATTGGCCGCCGCTGTGTCCGAGCCCACCACCGTGCCATTTGACGTGAGGCCCGGAGG
GGTCGTGCATTCTGTTCTCCAGGACGTAGGACCCGGGAACAAGTTTACATGTACATTC
ACCTACGCTTCCCAAGGAGGGACCAACGAGCAATGGCAGATGAGCCTGGGGACAAGT
GAAGACAGCCAGCACTTTACCTGTACCATCTGGAGGCCCCAGGGGAAATCCTACCTCT
ACTTCACACAGTTCAAGGCTGAGTTGCGAGGTGCTGAGATCGAGTATGCCATGGCCTA
CTCCAAAGCCGCATTTGAGAGAGAGAGTGATGTCCCCCTGAAAAGTGAGGAGTTTGAA
GTGACCAAGACAGCAGTGCTCACAGGCCTGGGGCCTTCAAAGCTGAGCTCTCCAAGC
TGGTGATCGTAGCCAAGGCGGCACGCTCGGAGCTGTGA

Mu MYDGF (C19orf10)

(SEQ ID NO: 117)

MAAPSGGFWTAVVLAALAAKLAAVSEPTTVPFVDPGPGVVHVSFSDVGPNGKFTCTFT
YASQGGTNEQWQMSLGTSEDSQHFTCTIWRPQGKSYLYFTQFKAELRGAIEIYAMAYSK
AAFERESDVPLKSEEFVTKTAVSHRPGAFKAELSKLVIVAKAARSEL

pWF-521

(SEQ ID NO: 118)

CAGTCTGTGTTGACGCAGCCGCCCTCAGTGTCTGCGGCCCCAGGACAGAGGGTCAACA
TCTCCTGCTCTGGAACCAGGTCCAACATTGGGAGTGATTATGTTTCCTGGTACCAACAC
CTCCCAGGAACAGCCCCCAAACCTCTCGTTTATGGCGATAATCTGCGACCCTCAGGGA
TTCCTGACCGATTCTCTGCCTCCAAGTCTGGCACGTCAGCCACCCTGGGCATCACCGGA
CTCCAGACTGGGGACGAGGCCGATTATTACTGCGGCACATGGGATTACACCCTGAATG
GTGTGGTGTTTCGGCGGAGGGACCAAGCTGACCGTCCTAGGTCAGCCCAAGGCCAACCC
CACTGTCACTCTGTTCCCGCCCTCCTCTGAGGAGCTCCAAGCCAACAAGGCCACACTA
GTGTGTCTGATCAGTGACTTCTACCCGGGAGCTGTGACAGTGGCCTGGAAGGCAGATG
GCAGCCCCGTCAAGGCGGGAGTGAGAGACCACCAAACCTCCAAACAGAGCAACAACA
AGTACGCGGCCAGCAGCTACCTGAGCCTGACGCCCAGCAGTGGAAGTCCCACAGAA
GCTACAGCTGCCAGGTCACGCATGAAGGGAGCACCGTGGAAGACAGTGGCCCCCTA
CAGAATGTTCAAGGCGCCGGATCTGGTGGAAGTGGAGTCAATCCCAATTGAGAAGGG
CGGAAGCGGTGGGAGTGGCGGGTCCGGTGGAAGCAACTGGTCAACCCACAATTCGA
GAAAGGCGGTTCTGGCGGATCTGGTGATCTGGCGGAAGTAACTGGTCTCATCCTCAA
TTCGAAAAGGGCGGAAGCGGTGGCGGCAGGCTAGGTGGAGGCTCAGTGCAGGTGCAG
CTGGTGGAGTCTGGGGAGGCTTGGTACAGCCTGGGGGGTCCCTGAGACTCTCCTGTG
CAGCCTCTGATTACCTTTAGCAGCTATGCCATGAGCTGGGTCCGCGAGGCTCCAGG
GAAGGGGCTGGAGTGGGTCTCAGTTATTTATAGCGGTGGTAGTAGCACATACTATGCA
GACTCCGTGAAGGGCCGGTTCACCATCTCCAGAGATAATTCCAAGAACACGCTGTATC
TGCAAATGAACAGCCTGAGAGCCGAGGACACGGCCGTATATTACTGTGCGCGCACTTC
TTACCTGAACCATGGTGATTACTGGGGTCAAGGTACTCTGGTGACCGTGTCTAGCGCCT

CCACCAAGGGCCCATCGGTCTTCCCCCTGGCACCCCTCCTCCAAGAGCACCTCTGGGGG
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TGGAATCAGGCGCCCTGACCAGCGGCGTGCACACCTTCCCGGCTGTCTACAGTCTT
CAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCA
GACCTACATCTGCAACGTGAACCAAGCCCCAGCAACACCAAGGTGGACAAGAGAGT
GGAGCCCAAGAGCTGCGACAAGACCCACACCTGCCCCCCTGCCAGCCCCAGAGCTG
CTGGGCGGACCCCTCCGTGTTCTGTTCCTCCCCCAAGCCCAAGGACACCCTGATGATCA
GCAGGACCCCCGAGGTGACCTGCGTGGTGGTGGACGTGAGCCACGAGGACCCAGAGG
TGAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCAACAACGCCAAGACCAAGCCCC
GAGAGGAGCAGTACAACAGCACCTACAGGGTGGTGTCCGTGCTGACCGTGTGCACCA
GGACTGGCTGAACGGCAAGGAATACAAGTGCAAGGTCTCCAACAAGGCCCTGCCAGC
CCCCATCGAAAAGACCATCAGCAAGGCCAAGGGCCAGCCACGGGAGCCCCAGGTGTA
CACCCTGCCCCCTCCCGGGAGGAGATGACCAAGAACCAGGTGTCCCTGACCTGTCTG
GTGAAGGGCTTCTACCCCAGCGACATCGCCGTGGAGTGGGAGAGCAACGGCCAGCCC
GAGAACAACCTACAAGACCACCCCCCAGTGCTGGACAGCGACGGCAGCTTCTTCTGT
ACAGCAAGCTGACCGTGGACAAGTCCAGGTGGCAGCAGGGCAACGTGTTCAGCTGCA
GCGTGATGCACGAGGCCCTGCACAACCACTACACCCAGAAGAGCCTGAGCCTGTCCCC
CGAGCTGCAACTGGAGGAGAGCTGTGCGGAGGCGCAGGACGGGGAGCTGGACGGGCT
GTGGACGACCATCACCATCTTCATCACACTCTTCTGTAAAGCGTGTGCTACAGTGCCA
CCGTCACCTTCTTCAAGGTGAAGTGGATCTTCTCCTCGGTGGTGGACCTGAAGCAGACC
ATCATCCCCGACTACAGGAACATGATCGGACAGGGGGCCTGA

pWF-521

(SEQ ID NO: 119)

QSVLTQPPSVSAAPGQRTVITSCGTRSNIGSDYVSWYQHLPGTAPKLLVYGDNLRPSPGIPDR
FSASKSGTSATLGITGLQTGDEADYYCGTWDTLNGVVFGGGKLTVLGQPKANPTVTLF
PPSSEELQANKATLVCLISDFYPGAVTVAWKADGSPVKAGVETTKPSKQSNKNKYAASSYL
SLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECSGAGSGGNWSHPQFEKGGSGSGSGG
SNWSHPQFEKGGSGSGSGSGSNWSHPQFEKGGSGGGRLGGGSVQVQLVESGGGLVQPG
GSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSVIYSGGSSTYYADSVKGRFTISRDNK
NTLYLQMNSLRAREDYAVYYCARTSYLNHGDYWGQGTLLVTVSSASTKGPSVFPLAPSSKST
SGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQ
TYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPE
VTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG
KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIA
VEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYT
QKSLSLSPQLLESCAEAQDGELDGLWTTITITITLFLLSVCYSATVTFKVKWIFSSVVDL
KQTIIPDYRNMIGQGA

pWF-533

(SEQ ID NO: 120)

CAGTCTGTGTTGACGCAGCCGCCCTCAGTGTCTGCGGCCCCAGGACAGAGGGTCACCA
TCTCCTGCTCTGGAACCAGGTCCAACATTGGGAGTGATTATGTTTCCTGGTACCAACAC
CTCCCAGGAACAGCCCCCAAACCTCCTCGTTTATGGCGATAATCTGCGACCCCTCAGGGA
TTCCTGACCGATTCTCTGCCTCCAAGTCTGGCACGTCAGCCACCCTGGGCATCACCGGA
CTCCAGACTGGGGACGAGGCCGATTATTACTGCGGCACATGGGATTACACCCTGAATG
GTGTGGTGTTCGGCGGAGGGACCAAGCTGACCGTCCTAtcttcaGCCTCCACCAAGGGCCC
ATCGGTCTTCCCCCTGGCACCCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTG
GGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCTGTGGAACCTCAGGCG
CCCTGACCAGCGGCGTGCACACCTTCCCGGCTGTCTACAGTCCTCAGGACTCTACTCC
CTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCA
ACGTGAACCACAAGCCCAGCAACACCAAGGTGGACAAGAGAGTGGAGCCCAAGAGCT
GC

pWF-533

(SEQ ID NO: 121)

QSVLTQPPSVSAAPGQRTISCSGTRSNIGSDYVSWYQHLPGTAPKLLVYGDNLRPSGIPDR
FSASKSGTSATLGITGLQTGDEADYYCGTWDYTLNGVVFGGGTKLTVLSSASTKGPSVFPL
APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVP
SSSLGTQTYICNVNHKPSNTKVDKRVEPKSC

pWF-534

(SEQ ID NO: 122)

CAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGGGGGTCCCTGAGAC
TCTCCTGTGCAGCCTCTGGATTACCTTTAGCAGCTATGCCATGAGCTGGGTCCGCCAG
GCTCCAGGGAAGGGGCTGGAGTGGGTCTCAGTTATTTATAGCGGTGGTAGTAGCACAT
ACTATGCAGACTCCGTGAAGGGCCGGTTCACCATCTCCAGAGATAATTCCAAGAACAC
GCTGTATCTGCAAATGAACAGCCTGAGAGCCGAGGACACGGCCGTATATTACTGTGCG
CGCACTTCTTACCTGAACCATGGTGATTACTGGGGTCAAGGTACTCTGGTGACCGTGTCT
TAGCGCCTCCGTGGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGA
AATCTGGAAGTGCCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAA
GTACAGTGGAAGGTGGATAACGCCCTCCAATCGGGTAAGTCCCAGGAGAGTGTACACAG
AGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCTGACGCTGAGCAAAG
CAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCAACCATCAGGGCCTGAGCTC
GCCCCGTCACAAAGAGCTTCAACAGGGGAGAGTGTGACAAGACCCACACCTGCCCCCCC
TGCCAGCCCCAGAGCTGCTGGGCGGACCCTCCGTGTTCTTCCCCCCCCAAGCCCAA
GGACACCTGATGATCAGCAGGACCCCCGAGGTGACCTGCGTGGTGGTGGACGTGAGC
CACGAGGACCCAGAGGTGAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCACAAC
GCCAAGACCAAGCCCAGAGAGGAGCAGTACAACAGCACCTACAGGGTGGTGTCCGTG
CTGACCGTGTCTGCACAGGACTGGCTGAACGGCAAGGAATACAAGTGCAAGGTCTCCA
ACAAGGCCCTGCCAGCCCCCATCGAAAAGACCATCAGCAAGGCCAAGGGCCAGCCAC
GGGAGCCCCAGGTGTACACCTGCCCCCTCCCGGGAGGAGATGACCAAGAACCAGC
TGTCCCTGACCTGTCTGGTGAAGGGCTTCTACCCAGCGACATCGCCGTGGAGTGGGA
GAGCAACGGCCAGCCCGAGAACAATAAGACCACCCCCCAGTGCTGGACAGCGA
CGGCAGCTTCTTCTGTACAGCAAGCTGACCGTGGACAAGTCCAGGTGGCAGCAGGGC
AACGTGTTACAGCTGCAGCGTGATGCACGAGGCCCTGCACAACCACTACACCCAGAAGA
GCCTGAGCCTGTCCCCGAGCTGCAACTGGAGGAGAGCTGTGCGGAGGCGCAGGACG
GGGAGCTGGACGGGCTGTGGACGACCATCACCATCTTCATCACACTTCTCTGTAAAG
CGTGTGCTACAGTGCCACCGTCACCTTCTTCAAGGTGAAGTGGATCTTCTCTCGGTG
TGGACCTGAAGCAGACCATCATCCCCGACTACAGGAACATGATCGGACAGGGGGCCT
GA

pWF-534

(SEQ ID NO: 123)

QVQLVESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSVIYSGGSSTYY
ADSVKGRFTISRDNKNTLYLQMNSLRAEDTAVYYCARTSYLNHGDYWGQGLTVTVSSA
SVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSK
DSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECDKTHTCPPCPAPELLG
GPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQY
NSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREE
MTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPVLDSGDSFFLYSKLTVDKSRW
QQGNVFSCSVMHEALHNHYTQKSLSLSPQLLEESCAEAQDGELDGLWTTITIFITLFLLSV
CYSATVTFKVKWIFSSVVDLKQTIPDYRNMIQQA

mu IL15

(SEQ ID NO: 124)

ATGAAAATTTTGAAACCATATATGAGGAATACATCCATCTCGTGCTACTTGTGTTTCCT
TCTAAACAGTCACTTTTTAACTGAGGCTGGCATTTCATGTCTTCATTTTGGGCTGTGTCA
GTGTAGGTCTCCCTAAAACAGAGGCCAACTGGATAGATGTAAGATATGACCTGGAGAA
AATTGAAAGCCTTATTCAATCTATTCATATTGACACCACTTTATACACTGACAGTGACT
TTCATCCCAGTTGCAAAGTTACTGCAATGAACTGCTTTCTCCTGGAATTGCAGGTTATT
TTACATGAGTACAGTAACATGACTCTTAATGAAACAGTAAGAAACGTGCTCTACCTTG
CAAACAGCACTCTGTCTTCTAACAAGAATGTAGCAGAATCTGGCTGCAAGGAATGTGA
GGAGCTGGAGGAGAAAACCTTCACAGAGTTTTTGCAAAGCTTTATACGCATTGTCCAA
ATGTTTCATCAACACGTCC

mu IL15

(SEQ ID NO: 125)

MKILKPYMRNTSISCYLCFLLNSHFLTEAGIHVFI LGCVSVGLPKTEANWIDVRYDLEKIESL
IQSIHIDTTLTYDSDFHPSCKVTAMNCFLLLEQVILHEYSNMTLNETVRNVLYLANSTLSSN
KNVAESGCKECEELEKTFTEFLQS FIRIVQMFINTS
anti-human GPC3 CAR (79a)

(SEQ ID NO: 126)

CAGTCTGTGTTGACGCAGCCGCCCTCAGTGTCTGCGGCCCCAGGACAGAGGGTCACCA
TCTCCTGCTCTGGAACCAGGTCCAACATTGGGAGTGATTATGTTTCCTGGTACCAACAC
CTCCCAGGAACAGCCCCCAAACCTCCTCGTTTATGGCGATAATCTGCGACCCTCAGGGA
TTCCTGACCGATTCTCTGCCTCCAAGTCTGGCAGTCAGCCACCCTGGGCATCACCGGA
CTCCAGACTGGGGACGAGGCCGATTATTACTGCGGCACATGGGATTACACCCTGAATG
GTGTGGTGTTTCGGCGGAGGGACCAAGCTGACCGTCCTAGGTTCTAGAGGTGGTGGTGG
TAGCGGCGGCGGCGGCTCTGGTGGTGGTGGATCCCTCGAGATGGCCCAGGTGCAGCTG
GTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGGGGGTCCCTGAGACTCTCCTGTGCAG
CCTCTGGATTACCTTTAGCAGCTATGCCATGAGCTGGGTCCGCCAGGCTCCAGGGAA
GGGGCTGGAGTGGGTCTCAGTTATTTATAGCGGTGGTAGTAGCACATACTATGCAGAC
TCCGTGAAGGGCCGGTTCACCATCTCCAGAGATAATTCCAAGAACACGCTGTATCTGC
AAATGAACAGCCTGAGAGCCGAGGACACGGCCGTATATTACTGTGCGCGCACTTCTTA
CCTGAACCATGGTGATTACTGGGGTCAAGGTACTCTGGTGACCGTGTCTAGCGCCGCT
GCAGTGGTCCCCGTGCTGCAGAAAGTTAATAGCACCACTAAACCTGTCTGAGGACTCC
TAGTCCAGTGACCCCAACAGGGACCAGTCAGCCACAGAGACCGGAAGACTGCAGACCAAGA
GGTTCAGTGAAGGGAACCGGCCTGGATTTTCGCTGCGATTTTTGGGCCCTGGTCTGTCGTC
GCAGGAGTTTTGTTTTGCTATGGACTGCTCGTCACAGTTGCTTTGTGTGTTATCTGGAC
AAGGAAACGGTGGCAAATGAGAAGTTTGGGGTGGACATGCCAGATGACTATGAAGATGAA
AATCTCTATGAGGGCCTGAACCTTGATGACTGTTCTATGTATGAGGACATCTCCAGGGGACT
CCAGGGCACCTACCAGGATGTGGGCAACCTCCACATTGGAGATGCCAGCTGGAAAAGCCA
TGA

anti-human GPC3 CAR (79a)

(SEQ ID NO: 127)

QSVLTQPPSVSAAPGQRVITISCSGTRSNIGSDYVSWYQHLPGTAPKLLVYGDNL RPSGIPDR
FSASKSGTSATLGITGLQTGDEADYYCGTWDYTLNGVVFGGGTKLTVLGSRRGGGSGGG
GSGGGGSLEMAQVQLVESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWV
SVIYSGGSSTYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCARTSYLNHG DYW
GQGT LTVTVSSAAAVVPVLQKVNSTTKPVL RTPSPVHPTGTSQPQRPEDCRPRGSVKGTGLDF
ACDFWALVVVAGVLCYGLLVTV ALCVIWTRKRWQNEKFGVDMPDDYEDENLYEGLNLD
DCSMYEDISRGLQGTYQDVGNLHIGDAQLEKP

anti-human PSMA(XENP14484) CAR 79a

(SEQ ID NO: 128)

GAGGTTCAACTTGTTCAATCTGGGGCAGAAGTGAAGAAGCCCGGGGCATCTGTGAAAG
TATCATGCAAAACATCCGGCTATACGTTTACCGAATACACCATTCACTGGGTCAGACA
GGCTCCCGGTCAAAGCCTCGAATGGATGGGAAATATTAACCCTAACAATGGCGGAACC
ACATATAATCAGAAATTCCAAGGCCGAGTGACGATAACTGTCGATAAGAGTACGTCCA

CAGCTTACATGGAACCTCAGCTCTTTGAGATCCGAAGACACTGCAGTTTATTATTGTGCA
GCTGGATGGAACCTTCGACTATTGGGGACAAGGGACTCTTGTTACGGTGTCCAGTGGCA
AACCAGGTAGTGGTAAACCCGGAAGCGGCAAGCCCCGGGAGCGGTAAACCTGGTAGCG
ACATCGTCATGACTCAAAGCCCTGACTCACTCGCCGTGAGCCTGGGAGAGCGTGCAAC
GCTATCTTGTCTGGGCCTCTCAGGATGTCTGGAACCTGCTGTAGACTGGTATCAACAGAAA
CCTGACCAATCACCAAACTCCTGATTTATTGGGCCTCAACACGTCACACAGGAGTGC
CAGATAGGTTACAGGTAGTGGCAGTGGAACTGATTTTACTTTGACAATTAGCAGCCT
GCAAGCCGAAGATGTAGCCGTTTACTTCTGTCAACAATATAACTCATACCCACTAACG
TTCGGTGCCCGGACGAAGGTAGAGATTAAAGTGGTCCCCGTGCTGCAGAAAGTTAATAG
CACCACCAGTAAACCTGTCTGAGGACTCCTAGTCCAGTGCACCCAACAGGGACCTAGTGC
CCACAGAGACCGGAAGACTGCAGACCAAGAGGTTTCAGTGAAGGGAACCGGCCTGGATTTCG
CCTGCGATTTTTTGGGCCCTGGTCGTCGTCGCAGGAGTTTTGTTTTGCTATGGACTGCTCG
TCACAGTTGCTTTGTGTGTTATCTGGACAAGGAAACGGTGGCAAAATGAGAAGTTTGGGG
TGGACATGCCAGATGACTATGAAGATGAAAATCTCTATGAGGGCCTGAACCTTGATGACTGTT
CTATGTATGAGGACATCTCCAGGGGACTCCAGGGCACCTACCAGGATGTGGGCAACCTCCA
CATTGGAGATGCCAGCTGGAAAAGCCATGA

anti-human PSMA(XENP14484) CAR 79a

(SEQ ID NO: 129)

EVQLVQSGAEVKKPGASVKVSCKTSGYTFTEYTIHWVRQAPGQSLEWMGNINPNNGGTT
YNQKFQGRVITITVDKSTSTAYMELSSLRSEDTAVYYCAAGWNFDYWGQGLTVTVSSGKP
GSGKPGSGKPGSGKPGSDIVMTQSPDSLAVSLGERATLSCRASQDVGTAVDWYQQKPDQS
PKLLIYWASTRHTGVPDRFTGSGSGTDFTLTISSLQAEDVAVYFCQQYNSYPLTFGAGTKV
EIKVVPVLQKVNSTTTKPVLRTPSPVHPTGTSQPQRPEDCRPRGSVKGTGLDFACDFWALVVV
AGVLFQYGLLVTVALCVIWRKRWQNEKFGVDMPPDDYEDENLYEGLNLDDCSMYEDISRGL
QGTQDVGNLHIGDAQLEKP

mouse IL 12a-mouse IgG2a Fc

(SEQ ID NO: 130)

ATGTGTCAGTCACGCTATCTTCTCTTCTTGCTACTCTGGCCTTGCTCAATCACTTGTC
CTTGCTCGTGTGATTCTGTGTCCGGCCAGCTAGGTGTCTCTCCAGTACCGGAATCT
CCTGAAAACACCGGATGACATGGTAAAGACAGCTAGGGAGAACTCAAGCACTACTC
CTGCACAGCTGAGGATATCGATCATGAGGACATCACCAAGGACCAGACATCCACTCTG
AAAACCTTGCCTGCCTTTGGAACCTCACAGAAGCAATCTTGTCTGGCAACGCGTGAAA
CGAGTTCTACTACAAGAGGGTCCTGTCTTCCCCCTCAAAAAGACAAGCCTTATGATGAC
CTTGTGTCTCGGTAGCATTATGAGGACCTAAAGATGTATCAAACCGAGTTTCAGGCTA
TCAATGCAGCGCTCCAGAATCATAACCATCAGCAGATCATTCTTGACAAAGGAATGCT
CGTGGCCATTGATGAACTAATGCAGAGCCTAAACCACAATGGCGAGACTCTTCGACAG
AAACCGCCTGTGGGCGAGGCCGATCCATATAGAGTCAAAATGAACTGTGTATTCTCC
TGCATGCATTTAGTACTCGTGTAGTGACTATTAACAGAGTGATGGGTACCTTTCTCA
GCTCCCAGAGGGGCCACAATCAAGCCCTGTCTCCATGCAAATGCCAGCACCTAACC
TCTTGGGTGGACCATCCGTCTTCATCTTCCCTCCAAAGATCAAGGATGTACTCATGATC
TCCCTGAGCCCCATAGTCACATGTGTGGTGGTGGATGTGAGCGAGGATGACCCAGATG
TCCAGATCAGCTGGTTTGTGAACAACGTGGAAGTACACACAGCTCAGACACAAACCCA
TAGAGAGGATTACAACAGTACTCTCCGGGTGGTCACTGCCCCTCCCCATCCAGCACCAG
GACTGGATGAGTGGCAAGGAGTTCAATGCAAGGTCAACAACAAAGACCTCCAGCG
CCCATCGAGAGAACCATCTCAAAACCCAAAGGGTCAGTAAGAGCTCCACAGGTATATG
TCTTGCCTCCACCAGAAGAAGAGATGACTAAGAAACAGGTCACTCTGACCTGCATGGT
CACAGACTTCATGCCTGAAGACATTTACGTGGAGTGGACCAACAACGGGAAAACAGA
GCTAAACTACAAGAACACTGAACCAGTCTGGACTCTGATGGTTCTTACTTCATGTACA
GCAAGCTGAGAGTGGAAAAGAAGAACTGGGTGGAAAGAAATAGCTACTCCTGTTTCAG
TGGTCCACGAGGTCTGCACAATCACACACGACTAAGAGCTTCTCCCGGACTCCGGG
TAAATAG

mouse IL 12a-mouse IgG2a Fc

(SEQ ID NO: 131)

MCQSRYLFLATLALLNHLSLARVIPVSGPARCLSQSRNLLKTDDMVKTAREKLKHYSCTAEDIDHEDITRDQSTLKTCLPLELHKNESCLATRETSSTTRGSCLPPQKTSLMMTLCLGSIYEDLKMYQTEFQAINAALQNHNHQQIILDKGMLVAIDELMQSLNHNGETLRQKPPVGEADPYRVKMKLCILLHAFSTRVVTINRVMGYLSSAPRGPTIKPCPPCKCPAPNLLGGPSVFIFPPKIKDVLMSLSPIVTCVVVDVSEDDPDVQISWVFNNEVHTAQTQTHREDYNSTLRVVSALPIHQHDWMSGKEFKCKVNNKDLPAPIERTISKPKGSVRAPQVYVLPPEEEMTKKQVTLTCMVTDFMPEDIYVEWTNNGKTELNYKNTEPVLDSGYSYFMYSKLRVEKKNWVERNSYSCSVVHEGLHNHHTTKSFSRTPGK*

mouse IL 12b-mouse IgG2a Fc

(SEQ ID NO: 132)

ATGTGCCACAGAACTCACAAATTTCTTGGTTCGCAATCGTCCTGCTGGTGTACCCCTGATGGCAATGTGGGAGTTGAAAAGGATGTATACGTCGTCGAGGTGCGACTGGACACCTGACGCTCCGGGTGAACTGTCAACCTCACTTGCCTGACTCCTGAAGAGGACGACATCAGCTGGACGAGCGACCAGCGACATGGAGTGATAGGGTCTGGCAAGACGCTTACTATCACGGTTAAGGAATTTCTCGACGCAGGGCAGTACACATGTCACAAGGGCGGCGAGACTCTGAGCCACTCCCATTGCTGCTGCACAAGAAGGAGAATGGTATCTGGTCTACCGAAATCCGTAAGAATTTTAAGAACAAGACTTTTCTGAAATGCGAGGCCCAAATTATTCCGGACGTTTCACTTGCAGTTGGCTCGTTCAAAGAAATATGGACTTGAAATTTAACATTAATCCAAGCTCTTCATCTCCTGACAGCAGGGCCGTAACCTGTGGAATGGCTTCATTGTCAGCTGAGAAAGTTACGCTTGACCAAAGGGATTATGAGAAATACAGCGTGAGTTGCCAGGAAGATGTGACATGTCCAACGGCAGAGGAAACGTTGCCAATTGAGCTCGCTTTGGAAGCTCGTCAACAAAACAAGTATGAAAACCTATAGTACTAGCTTCTTCATACGGGACATCATCAAACAGATCCACCTAAGAATTTGCAGATGAAGCCTCTGAAGAATTCACAAGTCGAGGTATCCGGAATACCCAGATTCATGGTCCACTCCTCATAGTTACTTTAGCCTGAAATTTCTTTGTACGCATACAGCGGAAGAAGGAGAAAATGAAGGAGACGGAAGAAGGCTGCAATCAGAAAGGCGCTTTTCTTGTGAAAAGACGAGCACTGAGGTTCAATGCAAAGGCGGGAATGTATGTGTTCAAGCCCAAGATAGGTATTATAATAGTCTCTGCTCTAAGTGGGCTTGCGTACCATGCAGAGTTAGAAAGTCCCAGAGGGCCCAACAATCAAGCCCTGTCTCCATGAGAAATGCCAGCACCTAACCTCTTGGGTGGACCATCCGTCTTCATCTTCCCTCCAAGATCAAGGATGTACTCATGATCTCCCTGAGCCCCATAGTCACATGTGTGGTGGTGGATGTGAGCGAGGATGACCCAGATGTCCAGATCAGCTGGTTTGTGAACAACGTGGAAGTACACACAGCTCAGACACAAACCCATAGAGAGGATTACAACAGTACTCTCCGGGTGGTCAGTGCCCTCCCATCCAGCACCAGGACTGGATGAGTGGCAAGGAGTTCAAATGCAAGGTCAACAACAAGACCTCCCAGCGCCCATCGAGAGAACCATCTCAAACCCAAAGGGTCAGTAAGAGCTCCACAGGTATATGTCTTGCCTCCACCAGAAGAAGAGATGACTAAGAAACAGGTACCTCTGACCTGCATGGTACAGACTTCATGCCTGAAGACATTTACGTGGAGTGGACCAACAACGGGAAAACAGAGCTAACTACAAGAACAAGTGAACCAGTCTGACTCTGATGGTTCTTACTTCATGTACAGCAAGCTGAGAGTGGAAAAGAAGAACTGGGTGGAAAGAAATAGCTACTCCTGTTCAGTGGTCCACGAGGGTCTGCACAATCACCACACGACTAAGAGCTTCTCCCGGACTCCGGGTAAATAG

mouse IL 12b-mouse IgG2a Fc

(SEQ ID NO: 133)

MCPQKLTIWFAIVLLVSPLMAMWELEKDVYVVEVDWTPDAPGETVNLTCDTPEEDDITWTSQDRHGVIGSGKLTITVKEFLDAGQYCHKGGETLSHSHLLLHKKENGIWSTEILKNFKNKTFLKCEAPNYSGRFTCSWLVRNMDLKFNIKSSSSSPDSRAVTCGMASLSAEKVTLDRDYEKYSVSCQEDVTCPTAEETLPIELALEARQONKYENYSTSFFIRDIIKPDPPKNLQMKPLKNSQVEVSWEPDSWSTPHSYFSLKFFVRIQRKKEKMKETEEGCNQKGAFLVEKTSTE VQCKGNGVCVQAQDRYYNSSCSKWACVPCRVRSPRGPTIKPCPPCKCPAPNLLGGPSVFIFPPKIKDVLMSLSPIVTCVVVDVSEDDPDVQISWVFNNEVHTAQTQTHREDYNSTLRVVSALPIHQHDWMSGKEFKCKVNNKDLPAPIERTISKPKGSVRAPQVYVLPPEEEMTKKQVTLTCMVTDFMPEDIYVEWTNNGKTELNYKNTEPVLDSGYSYFMYSKLRVEKKNWVERNSYSCSVVHEGLHNHHTTKSFSRTPGK

mouse IL 12a-mouse IgG2a Fc (Silent)

(SEQ ID NO: 134)

ATGTGTCAGTCACGCTATCTTCTCTTCCTTGCTACTCTGGCCTTGCTCAATCACTTGTCC
CTTGCTCGTGTGATTCTGTGTCCGGCCAGCTAGGTGTCTCTCCAGTCACGGAATCT
CCTGAAAACACGGATGACATGGTAAAGACAGCTAGGGAGAACTCAAGCACTACTC
CTGCACAGCTGAGGATATCGATCATGAGGACATCACCAGGGACCAGACATCCACTCTG
AAAACCTTGCCTTGTGAACTCCACAAGAACGAATCTTGTCTGGCAACGCGTGA
CGAGTTCTACTACAAGAGGGTCTGTCTTCCCCCTCAAAAGACAAGCCTTATGATGAC
CTTGTGTCTCGGTAGCATTATGAGGACCTAAAGATGTATCAAACCGAGTTTCAGGCTA
TCAATGCAGCGCTCCAGAATCATAACCATCAGCAGATCATTCTTGACAAAGGAATGCT
CGTGGCCATTGATGAACTAATGCAGAGCCTAAACCACAATGGCGAGACTCTTCGACAG
AAACCGCCTGTGGGCGAGGCCGATCCATATAGAGTCAAAATGAACTGTGTATTCTCC
TGCATGCATTTAGTACTCGTGTAGTGACTATTAACAGAGTGATGGGTACCTTTCTCA
GCTCCCAGAGGGCCCAATCAAGCCCTGTCTCCATGCAAATGCCAGCACCTAACG
CTGCCGGTGGACCATCCGTCTTCATCTTCCCTCCAAAGATCAAGGATGTACTCATGATC
TCCCTGAGCCCCATAGTCACATGTGTGGTGGTGGATGTGAGCGAGGATGACCCAGATG
TCCAGATCAGCTGGTTTGTGAACAACGTGGAAGTACACACAGCTCAGACACAAACCCA
TAGAGAGGATTACAACAGTACTCTCCGGGTGGTCAAGTGCCTCCCCATCCAGCACCAG
GACTGGATGAGTGGCAAGGAGTTCAAATGCAAGGTCAACAACAAAGACCTCGGAGCG
CCCATCGAGAGAACCATCTCAAACCCAAAGGGTCAGTAAGAGCTCCACAGGTATATG
TCTTGCCTCCACCAGAAGAAGAGATGACTAAGAAACAGGTCACTCTGACCTGCATGGT
CACAGACTTCATGCCTGAAGACATTTACGTGGAGTGGACCAACAACGGGAAAACAGA
GCTAAACTACAAGAACTGAACCAGTCTGGACTCTGATGGTTCTTACTTCATGTACA
GCAAGCTGAGAGTGGAAAAGAAGAACTGGGTGGAAAGAAATAGCTACTCCTGTTCAG
TGGTCCACGAGGGTCTGCACAATCACCACACGACTAAGAGCTTCTCCCGGACTCCGGG
TAAATGA

mouse IL 12a-mouse IgG2a Fc (Silent)

(SEQ ID NO: 135)

MCQSRYLFLATLALLNHLRLARVIPVSGPARCLSQSRNLLKTDDMVKTAREKLKHYSC
TAEDIDHEDITRDQSTLKTCLPLELHKNESCLATRETSSTTRGSCLPPQKTSMLMTLCGSI
YEDLKMYQTEFQAINAALQNHNHQQIILDKGMLVAIDELMQSLNHNGETLRQKPPVGEAD
PYRVKMKLCILLHAFSTRVVTINRVMGYLSSAPRGPTIKPCPPCKCPAPNAAGGPSVFIFPP
KIKDVLMSLSPIVTCVVVDVSEDDPDVQISWFVNNVEVHTAQQTTHREDYNSTLRVVSAL
PIQHQQWMSGKEFKCKVNNKDLGAPIERTISKPKGSVRAPQVYVLPPEEEMTKKQVTLT
CMVTDMPEDIYVEWTNNGKTELNYKNTEPVLDSDGSYFMYSKLRVEKKNWVERNSYSC
SVVHEGLHNHHTTKSFSRTPGK*

mouse IL 12b-mouse IgG2a Fc (Silent)

(SEQ ID NO: 136)

ATGTGCCCACAGAACTCACAAATTTCTTGGTTCGCAATCGTCCTGCTGGTGTCAACCCCT
GATGGCAATGTGGGAGTTGGAAAAGGATGTATACGTCGTCGAGGTGCACTGGACACCT
GACGCTCCGGGTGAAACTGTCAACCTCACTTGCGATACTCCTGAAGAGGACGACATCA
CGTGACGAGCGACCAGCGACATGGAGTGATAGGTCTGGCAAGACGCTTACTATCAC
GGTTAAGGAATTTCTCGACGCAGGGCAGTACACATGTCACAAGGGCGGCGAGACTCTG
AGCCACTCCCATTTGCTGCTGCACAAGAAGGAGAATGGTATCTGGTCTACCGAAATCC
TGAAGAATTTTAAGAACAAGACTTTTCTGAAATGCGAGGCCCAATTATTCCGGACG
TTTCACTTGCAGTTGGCTCGTTCAAAGAAATATGGACTTGAAATTTAACATTAATCCA
GCTCTTCATCTCCTGACAGCAGGGCCGTAACCTGTGGAATGGCTTCATTGTCAGCTGAG
AAAGTTACGCTTGACCAAAGGGATTATGAGAAATACAGCGTGAGTTGCCAGGAAGAT
GTGACATGTCCAACGGCAGAGGAAACGTTGCCAATTGAGCTCGCTTTGGAAGCTCGTC
AACAAAACAAGTATGAAAATAAGTACTAGCTTCTTCATACGGGACATCATCAAACC
AGATCCACCTAAGAATTTGCAGATGAAGCCTCTGAAGAATTCACAAGTCGAGGTATCC
TGGGAATACCCAGATTTCATGGTCCACTCCTCATAGTTACTTTAGCCTGAAATCTTTGT
ACGCATACAGCGGAAGAAGGAGAAAATGAAGGAGACGGAAGAAGGCTGCAATCAGA

AAGGCGCTTTTCTTGTTGAAAAGACGAGCACTGAGGTTCAATGCAAAGGCGGGAATGT
ATGTGTTCAAGCCCAAGATAGGTATTATAATAGCTCCTGCTCTAAGTGGGCTTGCGTAC
CATGCAGAGTTAGAAAGTCCCAGAGGGCCCCACAATCAAGCCCTGTCTCCATGCAAATG
CCCAGCACCTAACGCTGCCGGTGGACCATCCGTCTTCATCTTCCCTCCAAAGATCAAGG
ATGTACTCATGATCTCCCTGAGCCCCATAGTCACATGTGTGGTGGTGGATGTGAGCGA
GGATGACCCAGATGTCCAGATCAGCTGGTTTGTGAACAACGTGGAAGTACACACAGCT
CAGACACAAACCCATAGAGAGGATTACAACAGTACTCTCCGGGTGGTCAGTGCCCTCC
CCATCCAGCACCAAGGACTGGATGAGTGGCAAGGAGTTCAAATGCAAGGTCAACAACA
AAGACCTCGGAGCGCCCATCGAGAGAACCATCTCAAAACCCAAAGGGTCAGTAAGAG
CTCCACAGGTATATGTCTTGCCTCCACCAGAAGAAGAGATGACTAAGAAACAGGTCAC
TCTGACCTGCATGGTACAGACTTCATGCCTGAAGACATTTACGTGGAGTGGACCAAC
AACGGGAAAACAGAGCTAAACTACAAGAACTGAACCAGTCCTGGACTCTGATGGT
TCTTACTTCATGTACAGCAAGCTGAGAGTGGAAAAGAAGAACTGGGTGGAAAGAAAT
AGTACTCCTGTTCAAGTGGTCCACGAGGGTCTGCACAATCACCACACGACTAAGAGCT
TCTCCCGGACTCCGGGTAAATGA
mouse IL 12b-mouse IgG2a Fc (Silent)

(SEQ ID NO: 137)

MCPQKLTISWFAIVLLVSPLMAMWELEKDVYVVEVDWTPDAPGETVNLTCDTPEEDDIT
WTSDQRHGVIGSGKLTITVKEFLDAGQYTCHKGGETLSHSHLLLHKKENGIWSTEILKNF
KNKTFCLKCEAPNYSGRFTCSWLVRNMDLKFNIKSSSSSPDSRAVTCGMASLSAEKVTL
QRDYEKYSVSCQEDVTCPTAEETLPIELALEARQQNKYENYSTSFIRDIIKPDPPKNLQMK
PLKNSQVEVSWEYPDSWSTPHSYFSLKFFVRIQRKKEKMKETEEGCNQKGAFLVEKTSTE
VQCKGGNVVCVQAQDRYNNSSCSKWACVPCRVRSPRGPTIKPCPPCKCPAPNAAGGPSVFI
FPPKIKDVLMSLSPIVTCVVVDVSEDDPDVQISWVFNVEVHTAQTQTHREDYNSTLRVV
SALPIQHQQDWMSGKEFKCKVNNKDLGAPIERTISKPGSVRAPQVYVLPPEEEMTKKQV
TLTCMVTDFMPEDIYVEWTNNGKTELNYKNTEPVLDSGDSYFMYSKLRVEKKNWVERNS
YSCSVVHEGLHNHHTTKSFSRTPGK

CD3 zeta cytoplasmic domain-human

(SEQ ID NO: 138)

RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPGEMGGKQRRKNPQEG
YNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQUALPPR

CD3 zeta cytoplasmic domain-human

(SEQ ID NO: 139)

AGAGTGAAGTTCAGCAGGAGCGCAGACGCCCCCGCGTACCAGCAGGGCCAGAACCAG
CTCTATAACGAGCTCAATCTAGGACGAAGAGAGGAGTACGATGTTTTGGACAAGAGAC
GTGGCCGGGACCCTGAGATGGGGGGAAAGCCGCAGAGAAGGAAGAACCCTCAGGAA
GGCCTGTACAATGAACTGCAGAAAGATAAGATGGCGGAGGCCTACAGTGAGATTGGG
ATGAAAGGCGAGCGCCGGAGGGGCAAGGGGCACGATGGCCTTTACCAGGGTCTCAGT
ACAGCCACCAAGGACACCTACGACGCCCTTCACATGCAGGCCCTGCCCCCTCGCTAA

pWF-506

(SEQ ID NO: 140)

METDTLLLWVLLLWVPGSTGQSVLTQPPSVSAAPGQRTVISCSTGTRSNIGSDYVSWYQHLP
GTAPKLLVYGDNLPSGIPDRFSASKSGTSATLGITGLQTGDEADYYCGTWDYTLNGVVF
GGGKLTVLGSRGGGSGGGGSGGGGSLMAQVQLVESGGGLVQPGGSLRLSCAASGFT
FSSYAMSWVRQAPGKGLEWVSIVYSGGSSTYYADSVKGRFTISRDNKNLTLYLQMNSLRA
EDTAVYYCARTSYLNHGDYWGQGLTVTVSSPKSCDKTHTCPPCPAPELLGGPSVFLFPPKP
KDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVL
TVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLT
CLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFS

VMHEALHNHYTQKSLSLSPQLLEESCAEAQDGLDGLWTTITIFITLFLLSVCYSATVTFF
KVKWIFSSVVDLKQTIIPDYRNMIGQGA

pWF-506

(SEQ ID NO: 141)

ATGGAAACCGATACACTGCTGCTGTGGGTGCTGCTGCTGTGGGTGCCAGGATCTACCG
GTCAGTCTGTGTTGACGCAGCCGCCCTCAGTGTCTGCGGCCCCAGGACAGAGGGTCAC
CATCTCCTGCTCTGGAACCAAGGTCCAACATTGGGAGTGATTATGTTTCCTGGTACCAAC
ACCTCCCAGGAACAGCCCCCAAACCTCCTCGTTTATGGCGATAATCTGCGACCCCTCAGG
GATTCTGACCGATTCTCTGCCTCCAAGTCTGGCACGTCAGCCACCCTGGGCATCACCG
GACTCCAGACTGGGGACGAGGCCGATTATTACTGCGGCACATGGGATTACACCCTGAA
TGGTGTGGTGTTTCGGCGGAGGGACCAAGCTGACCGTCCTAGGTTCTAGAGGTGGTGGT
GGTAGCGGCGGCGGCGGCTCTGGTGGTGGTGGATCCCTCGAGATGGCCCAGGTGCAGC
TGGTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGGGGGTCCCTGAGACTCTCCTGTGC
AGCCTCTGGATTACCTTTAGCAGCTATGCCATGAGCTGGGTCCGCCAGGCTCCAGGG
AAGGGGCTGGAGTGGGTCTCAGTTATTTATAGCGGTGGTAGTAGCACATACTATGCAG
ACTCCGTGAAGGGCCGGTTCACCATCTCCAGAGATAATTCCAAGAACACGCTGTATCT
GCAAATGAACAGCCTGAGAGCCGAGGACACGGCCGTATATTACTGTGCGCGCACTTCT
TACCTGAACCATGGTGATTACTGGGGTCAAGGTACTCTGGTGACCGTGTCTAGCCCCA
AGAGCTGCGACAAGACCCACACCTGCCCCCCCTGCCAGCCCCAGAGCTGCTGGGCGG
ACCCTCCGTGTTCTGTTCCCCCCCCAAGCCCAAGGACACCCTGATGATCAGCAGGACC
CCCGAGGTGACCTGCGTGGTGGTGGACGTGAGCCACGAGGACCCAGAGGTGAAGTTC
AACTGGTACGTGGACGGCGTGGAGGTGCACAACGCCAAGACCAAGCCCAGAGAGGAG
CAGTACAACAGCACCTACAGGGTGGTGTCCGTGCTGACCGTGTCTGCACCAGGACTGGC
TGAACGGCAAGGAATACAAGTGCAAGGTCTCCAACAAGGCCCTGCCAGCCCCCATCG
AAAAGACCATCAGCAAGGCCAAGGGCCAGCCACGGGAGCCCCAGGTGTACACCCTGC
CCCCCTCCCGGGAGGAGATGACCAAGAACCAGGTGTCCCTGACCTGTCTGGTGAAGGG
CTTCTACCCCAGCGACATCGCCGTGGAGTGGGAGAGCAACGGCCAGCCCCGAGAACA
CTACAAGACCAACCCCCAGTGCTGGACAGCGACGGCAGCTTCTTCTGTACGCAAG
CTGACCGTGGACAAGTCCAGGTGGCAGCAGGGCAACGTGTTCAAGTGCAGCGTGATGC
ACGAGGCCCTGCACAACCACTACACCCAGAAGAGCCTGAGCCTGTCCCCCGAGCTGCA
ACTGGAGGAGAGCTGTGCGGAGGCGCAGGACGGGGAGCTGGACGGGCTGTGGACGAC
CATCACCATCTTCATCACACTCTTCTGTAAAGCGTGTGCTACAGTGCCACCGTCACT
TCTTCAAGGTGAAGTGGATCTTCTCTCGGTGGTGGACCTGAAGCAGACCATCATCCCC
GACTACAGGAACATGATCGGACAGGGGGCCTGA

pWF-507:

(SEQ ID NO: 142)

METDTLLLWVLLLWVPGSTGQSVLTQPPSVSAAPGQRVTISCSGTRSNIQSDYVSWYQHLP
GTAPKLLVYGDNLRPSPGIPDRFSASKSGTSATLGITGLQTGDEADYYCGTWDYTLNGVVF
GGGTKLTVLGQPKANPTVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADGSPVKA
GVETTKPSKQSNKYAASSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS

pWF-507:

(SEQ ID NO: 143)

ATGGAAACCGATACACTGCTGCTGTGGGTGCTGCTGCTGTGGGTGCCAGGATCTACCG
GTCAGTCTGTGTTGACGCAGCCGCCCTCAGTGTCTGCGGCCCCAGGACAGAGGGTCAC
CATCTCCTGCTCTGGAACCAAGGTCCAACATTGGGAGTGATTATGTTTCCTGGTACCAAC
ACCTCCCAGGAACAGCCCCCAAACCTCCTCGTTTATGGCGATAATCTGCGACCCCTCAGG
GATTCTGACCGATTCTCTGCCTCCAAGTCTGGCACGTCAGCCACCCTGGGCATCACCG
GACTCCAGACTGGGGACGAGGCCGATTATTACTGCGGCACATGGGATTACACCCTGAA
TGGTGTGGTGTTTCGGCGGAGGGACCAAGCTGACCGTCCTAGGTCAGCCCAAGGCCAAC
CCCACTGTCACCTCTGTTCCCGCCCTCCTCTGAGGAGCTCCAAGCCAACAAGGCCACACT
AGTGTGTCTGATCAGTGACTTCTACCCGGGAGCTGTGACAGTGGCCTGGAAGGCAGAT

GGCAGCCCCGTCAAGGCGGGAGTGGAGACCACCAAACCCTCCAAACAGAGCAACAAC
AAGTACGCGGCCAGCAGCTACCTGAGCCTGACGCCCAGCAGTGGAAGTCCACAGA
AGCTACAGCTGCCAGGTCACGCATGAAGGGAGCACCGTGGAGAAGACAGTGGCCCCCT
ACAGAATGTTTCATAG

pWF-508:

(SEQ ID NO: 144)

MVFTPQILGLMLFWISASRGQVQLVESGGGLVQPGGSLRLSCAASGFTFSYAMSWVRQA
PGKGLEWVSVIYSGGSSTYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCARTS
YLNHGDYWGQGT LTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTWSN
SGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKRVKPKSC
DKHTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDG
VEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKG
QPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDG
SFFLYSKLTVDKSRWQQGNVFC SVMHEALHNHYTQKSLSLPELQLEESCAEAQDGELD
GLWTTITIFITLFLSVCYSATVTFFKVKWIFSSVVDLKQTIIPDYRNMIGQGA

pWF-508:

(SEQ ID NO: 145)

ATGGTGTTTACACCGCAAATATTGGGGCTCATGCTTTTCTGGATCAGTGCAAGCAGGG
GACAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGGGGGTCCCTGA
GACTCTCCTGTGCAGCCTCTGGATTACCTTTAGCAGCTATGCCATGAGCTGGGTCCGC
CAGGCTCCAGGGAAGGGGCTGGAGTGGGTCTCAGTTATTTATAGCGGTGGTAGTAGCA
CATACTATGCAGACTCCGTGAAGGGCCGGTTCACCATCTCCAGAGATAATTCCAAGAA
CACGCTGTATCTGCAATGAACAGCCTGAGAGCCGAGGACACGGCCGTATATTACTGT
GCGCGCACTTCTTACCTGAACCATGGTGATTACTGGGGTCAAGGTACTCTGGTGACCGT
GTCTAGCGCCTCCACCAAGGGCCCATCGGTCTTCCCCCTGGCACCTCCTCCAAGAGCA
CCTCTGGGGGACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGT
GACGGTGTCGTGGAACCTCAGGCGCCCTGACCAGCGCGGTGCACACCTTCCCGGTCTC
TGACAGTCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGGCTCCAGCAGCTT
GGGCACCCAGACCTACATCTGCAACGTGAACCACAAGCCCAGCAACACCAAGGTGGA
CAAGAGAGTGGAGCCCAAGAGCTGCGACAAGACCCACACCTGCCCCCCTGCCAGC
CCCAGAGCTGCTGGGCGGACCTCCGTGTTCTGTTCCCCC AAGCCCAAGGACACC
CTGATGATCAGCAGGACCCCCGAGGTGACCTGCGTGGTGGTGACGTGAGCCACGAG
GACCCAGAGGTGAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCACAACGCCAAG
ACCAAGCCCAGAGAGGAGCAGTACAACAGCACCTACAGGGTGGTGTCCGTGCTGACC
GTGCTGCACCAGGACTGGCTGAACGGCAAGGAATACAAGTGCAAGGTCTCCAACAAG
GCCCTGCCAGCCCCCATCGAAAAGACCATCAGCAAGGCCAAGGGCCAGCCACGGGAG
CCCCAGGTGTACACCCTGCCCCCTCCCGGGAGGAGATGACCAAGAACCAGGTGTCCC
TGACCTGTCTGGTGAAGGGCTTCTACCCAGCGACATCGCCGTGGAGTGGGAGAGCAA
CGGCCAGCCGAGAACAACTACAAGACCACCCCCCAGTGCTGGACAGCGACGGCAG
CTTCTTCCTGTACAGCAAGCTGACCGTGGACAAGTCCAGGTGGCAGCAGGGCAACGTG
TTCAGCTGCAGCGTGATGCACGAGGCCCTGCACAACCACTACACCCAGAAGAGCCTGA
GCCTGTCCCCGAGCTGCAACTGGAGGAGAGCTGTGCGGAGGCGCAGGACGGGGAGC
TGGACGGGCTGTGGACGACCATCACCATCTTCATCACACTCTTCCTGTAAAGCGTGTGC
TACAGTGCCACCGTCACCTTCTTCAAGGTGAAGTGGATCTTCTCCTCGGTGGTGGACCT
GAAGCAGACCATCATCCCCGACTACAGGAACATGATCGGACAGGGGGCCTGA

pWF-509:

(SEQ ID NO: 146)

METDTLLLWVLLLWVPGSTGQSVLTQPPSVSAAPGQRV TISCSGTRSNIGSDYVSWYQHL P
GTAPKLLVYGDNL RPSGIPDRFSASKSGTSATLGITGLQTGDEADYYCGTWDYTLNGVVF
GGGTKLTVLGSRRGGGSGGGGSGGGGSLEMAQVQLVESGGGLVQPGGSLRLSCAASGFT

pWF-509:

[illegible]

pWF-510:

METDTLLLVWLLLVWPGSTGQSVLTQPPSVSAAPGQRTVISCSTGRSNIGSDYVSWYQHLPL
GTAPKLLVYGDNLRPSPGIPDRFSASKSGTSATLGITGLQTGDEADYYCGTWDYTLNGVVF
GGGTKLTVLGSRRGGGSGGGGSGGGGSLEMAQVQLVESGGGLVQPGGSLRLSCAASGFT
FSSYAMSWVRQAPGKGLEWVSVIYSGGSSTYYADSVKGRFTISRDNKNTLYLQMNSLRA
EDTAVYYCARTSYLNHGDYWGQGLTVTVSSAAAFVPVFLPAKPTTTPAPRPPTPAPTIAAQ
PLSLRPEACRPAAGGAVHTRGLDFACDFWVLVVGGLVACYSLLVTVAFIIFWVRKRWQ
NEKLGLDAGDEYEDENLYEGLNLDDCSMYEDISRGLQGTQYODVGSNIGDVOLEKP

pWF-510:

ATGGA AACCGATA CACTGCTGCTGTGGGTGCTGCTGCTGTGGGTGCCAGGATCTACCG
GTCAGTCTGTGTTGACGCAGCCGCCCTCAGTGTCTGCGGCCCCAGGACAGAGGGTCAC
CATCTCCTGCTCTGGAACCAGGTCCAACATTGGGAGTGATTATGTTTCTGGTACCAAC
ACCTCCCAGGAACAGCCCCCAAACCTCCTCGTTTATGGCGATAATCTGCGACCCTCAGG
GATTCTTGACCGATTCTCTGCCTCCAAGTCTGGCACGTCAGCCACCCTGGGCATCACCG
GACTCCAGACTGGGGACGAGGCCGATTATTACTGCGGCACATGGGATTACACCCTGAA
TGGTGTGGTGTTCGGCGGAGGGACCAAGCTGACCGTCCTAGGTTCTAGAGGTGGTGGT
GGTAGCGGCGGCGGCGGCTCTGGTGGTGGTGGATCCCTCGAGATGGCCCAGGTGCAGC
TGGTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGGGGGTCCCTGAGACTCTCCTGTGC
AGCCTCTGGATTACCTTTAGCAGCTATGCCATGAGCTGGGTCCGCCAGGCTCCAGGG
AAGGGGCTGGAGTGGGTCTCAGTTATTTATAGCGGTGGTAGTAGCACATACTATGCAG
ACTCCGTGAAGGGCCGGTTCACCATCTCCAGAGATAATTC AAGAACACGCTGTATCT
GCA AATGAACAGCCTGAGAGCCGAGGACACGGCCGTATATTACTGTGCGCGCACTTCT
TACCTGAACCATGGTGATTACTGGGGTCAAGGTACTCTGGTGACCGTGTCTAGCGCCG
CTGCATTCTGTGCCTGTGTTTCTCCAGCTAAGCCCACTACCACCCCCGCTCCAAGGCCG

CCCACGCCCCGCTCCTACTATTGCTAGTCAGCCTTTAAGTTTACGACCCGAAGCTTGCAG
 GCCCCGCCGCCGGCGGCGCTGTGCACACCAGGGGGCTTGATTTTGCCTGCGACTTTTGG
 GTATTGGTAGTGGTGGGCGGAGTTTTAGCCTGCTACAGCCTCCTGGTAACAGTGGCTTT
 TATCATCTTTTGGGTGAGGAAACGATGGCAGAACGAGAAGCTCGGGTTGGATGCCGGG
 GATGAATATGAAGATGAAAACCTTTATGAAGGCCTGAACCTGGACGACTGCTCCATGT
 ATGAGGACATCTCCCGGGGCCTCCAGGGCACCTACCAGGATGTGGGCAGCCTCAACAT
 AGGAGATGTCCAGCTGGAGAAGCCGTGA

INCORPORATION BY REFERENCE

[0168] All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference. However, the citation of a reference herein should not be construed as an acknowledgement that such reference is prior art to the present invention. To the extent that any of the definitions or terms provided in the references incorporated by reference differ from the terms and discussion provided herein, the present terms and definitions control.

EQUIVALENTS

[0169] The foregoing written specification is considered to be sufficient to enable one skilled in the art to practice the invention. The foregoing description and examples detail certain preferred embodiments of the invention and describe 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.

[0170] The following examples, including the experiments conducted and results achieved, are provided for illustrative purposes only and are not to be construed as limiting the present invention.

EXAMPLES

Example 1 – Chimeric Antigen Receptor for B Cells (CAR-B) Constructs to Bind PSMA.

[0171] **DNA Constructs.** Exemplary CAR-B constructs were designed to recognize Prostate Specific Membrane Antigen (“PSMA”). PSMA is an antigen that is expressed more highly on prostate cancer cells than on other non-cancerous cells. Various construct were made comprising an extracellular domain that comprised an scFv specific for PSMA, an extracellular hinge region from CD8, a CD28 transmembrane domain, and various intracellular signaling domains. A list of the constructs is provided in Table 6:

TABLE 6

Construct	Description
pWF-82	pTLPW-SFFV-XENP14484 scFv-hCD8H-hCD28M-hCD19E (SEQ ID NOS. 39 and 40)

pWF-83	pTLPW-SFFV-XENP14484 scFv-hCD8H-hCD28M-hCD40E (SEQ ID NOS. 41 and 42)
pWF-84	pTLPW-SFFV-XENP14484 scFv-hCD8H-hCD28M-h(CD40+CD79b)E (SEQ ID NOS. 43 and 44)
pWF-85	pTLPW-SFFV-XENP14484 scFv-hCD8H-hCD28M-h(CD40+CD137)E (SEQ ID NOS. 45 and 46)
pWF-86	pTLPW-SFFV-XENP14484 scFv-hCD8H-hCD28M-h(CD40+Fcγr2a)E (SEQ ID NOS. 47)
pWF-87	pTLPW-SFFV-XENP14484 scFv-hCD8H-hCD28M-h(hMyd88+CD40)E (SEQ ID NOS. 48 and 49)
pWF-88	pTLPW-SFFV-XENP14484 scFv-hCD8H-hCD28M-hCD79aE (SEQ ID NOS. 50 and 51)
pWF-89	pTLPW-SFFV-XENP14484 scFv-hCD8H-hCD28M-hCD79bE (SEQ ID NOS. 52 and 53)

[0172] Expression of anti-PSMA CAR-B on HEK-293 Cells. The constructs encoding pWF82 to pWF89 were used to prepare lentivirus in Lentix cells using the Takara lentivirus preparation kit. Expression of the various CAR-B constructs was measured using flow cytometry using antibodies specific for PSMA (biotin-PSMA, Sinobiological and is depicted in FIG. 5.

[0173] Expression of anti-PSMA CAR-B in Human B Cells. To measure expression and binding of anti-PSMA CAR-B's in B cells, two additional constructs were made:

TABLE 7

Construct	Description
pWF-391	pMMLV(LTR)-hEF1a promoter-anti hPSMA(XENP14484)-CBCR (SEQ ID NOS. 54 and 55)
pWF-394	pMMLV(LTR)-hEF1a promoter-anti sarcoglycan CBCR1 (SEQ ID NOS. 56 and 57)

[0174] A MMLV based vector was used for the preparation of the retrovirus. The retrovirus was used to infect mouse B cells isolated from the spleen. After transduction, B cells were further expanded on feeder cells expressing CD40L and soluble IL-4. The expression of anti-PSMA CAR-B was detected by using recombinant biotin-PSMA. PE-labeled streptavidin was used to detect PSMA binding in HEK-293 cells.

[0175] Results. The results of this experiment are depicted in FIG. 6 and demonstrate that it is possible to create mouse B cell that expresses a CAR-B that can bind with specificity to an antigen. For example, B cells expressing pWF396 or pWF397 bound to PSMA whereas the B cells expressing pWF394 did not bind PSMA pWF398 was designed to bind sarcoglycan not PSMA).

Example 2 – Chimeric Antigen Receptor on B cells (CAR-B) Constructs to Bind GPC3.

[0176] DNA Construct. Exemplary CAR constructs were designed to recognize glypican-3 (GPC-3). Glypican-3 is expressed on hepatocellular carcinoma cells among other tumor types, but

not on most non-cancer cells. GPC3 can be used to target an anti-GPC3 CAR to hepatocellular carcinoma, as well as other cancers in which GPC3 is expressed (e.g. ovarian clear cell carcinoma, pediatric cancers, lung cancers (i.e. lung adenocarcinoma and lung squamous cell carcinoma), urothelial carcinoma, thyroid cancer, gastric cancer, and others). Various construct were made comprising an extracellular domain that comprised an scFv specific for GPC-3, an extracellular hinge region from CD8, a CD28 transmembrane domain, and various intracellular signaling domains. An additional anti-PSMA CAR-B was constructed as a control for these experiments. A list of the constructs is provided in Table 8.

TABLE 8

Construct	Description
pWF-396	pMMLV(LTR)-hEF1a promoter-anti-GPC3 scFv-hCD8H-hCD28M-hCD79aE (SEQ ID NOS. 58 and 59)
pWF-397	pMMLV(LTR)-hEF1a promoter-anti-GPC3 scFv-hCD8H-hCD28M-hCD79bE (SEQ ID NOS. 60 and 61)
pWF-398	pMMLV(LTR)-hEF1a promoter-anti-hPSMA(XENP14484) scFv-hCD8H-hCD28M-hCD79aE (SEQ ID NOS. 62 and 63)

[0177] Expression of anti-GPC-3 on HEK-293 Cell. Lentiviral transductions were used to express GPC3 CAR-B proteins on the surface of HEK293 cells. Expression was determined by flow cytometry with an anti-idiotypic antibody specific for GPC-3 (Eureka Therapeutics).

[0178] Expression of anti-GPC-3 CAR-B in Human B Cells. pWF 396, 397 and 398 encoding CAR constructs were used to prepare MMLV retrovirus. This retrovirus was used to transduce mouse B cells isolated by negative selection (Stem Cell Technologies) and activated for 24 hours by co-culture with HeLa cells expressing CD40L and the addition of soluble IL-4. 48 hours post-transduction, expression was confirmed using flow cytometry. The expression of the CAR-B was detected using an anti-idiotypic antibody against human GPC3. The anti-idiotypic antibody was obtained from Eureka Therapeutics.

[0179] Results. Mouse B cells expressing anti-GPC-3 CAR-Bs, pWF-396 and 397, were expressed and specifically bound by anti-GPC3 idiotypic antibody.

Example 3 – Adenovirus Variant F35 Expressing GFP

[0180] Adenovirus variant F35 expressing GFP was demonstrated to efficiently infect human B cells. Human B cells were isolated from the peripheral blood. The B cells were infected with adenovirus encoding GFP at volumes of 0, 1, 3, 10 μ L. The titer of the adenovirus preparations were approximately 1×10^{12} particles/ml.

Example 4 – Delivering Payloads to Tumor Cells

[0181] A large screening study was conducted to examine the effect of payloads on NIH3T3 fibroblasts in a CT26 Model. Payloads included various immunomodulators, including cytokines and chemokines. First, BALB/c mice were injected with CT26 tumors into their left and right flank. *See* FIG. 8. Twelve and sixteen days later, mice were injected into the right flank tumor, with various combinations of 4-5 payloads. Tumor volume was measured for up to 35 days.

[0182] Generation of the BALB/C CT26 tumor model. A total of 139 mice were injected with CT26 tumors into their left and right flanks.

[0183] Selection of Payload. Twelve peptides were identified for their potential to (i) recruit and activate dendritic cells; (ii) initiate homing and guidance of dendritic cells and T cells into the tumor site; and (iii) activate effector T cells. The payloads screened are listed in Table 9.

TABLE 9

Payload	SEQ ID NO
FLT3L	70, 71
XCL1	72, 73
TIM4-Fc	74, 75
CXCL13	68, 69
mCCL21	92, 93
mCD80 – membrane bound	86, 87
mCD40L – membrane bound	88, 89
mIFNa A2	84, 85
mIL-12	80, 81
mIL-21	90, 91
mLIGHT mutant	78, 79
M4-1BBL-membrane bound	76, 77
mIL-15	124, 125

[0184] Each was either given a combination of 4-5 payloads, all 12 payloads, or 3T3 cells (without payload) or saline as a control. In total, there were twenty-seven groups (n = 5 mice/group). The experimental groups are identified in Table 10.

TABLE 10

Group #	Treatment
1	FLT3L, XCL1, CXCL13, TIM4-Fc, TLR
2	FLT3L, XCL1, CXCL13, CD80-MB
3	FLT3L, XCL1, CXCL13, CD40L-MB, TLR
4	FLT3L, XCL1, CXCL13, IL-12 and TM
5	FLT3L, XCL1, CXCL13, 4-1BBL-MB
6	FLT3L, XCL1, CXCL13, IFNa A2
7	FLT3L, XCL1, LIGHT, TIM4-Fc
8	FLT3L, XCL1, LIGHT, CD80-MB
9	FLT3L, XCL1, LIGHT, CD40L-MB, TLR

10	FLT3L, XCL1, LIGHT, IL-12 and TM
11	FLT3L, XCL1, LIGHT, 4-1BBL-MB
12	FLT3L, XCL1, LIGHT, IFNa A2
13	FLT3L, XCL1, IL-21, TIM4-Fc
14	FLT3L, XCL1, IL-21, CD80-MB
15	FLT3L, XCL1, IL-21, CD40L-MB
16	FLT3L, XCL1, IL-21, IL-12 and TM
17	FLT3L, XCL1, IL-21, 4-1BBL-MB
18	FLT3L, XCL1, IL-21, IFNa A2
19	FLT3L, XCL1, CCL21, TIM4-Fc
20	FLT3L, XCL1, CCL21, CD80-MB
21	FLT3L, XCL1, CCL21, CD40L-MB
22	FLT3L, XCL1, CCL21, IL-12 and TM
23	FLT3L, XCL1, CCL21, 4-1BBL-MB
24	FLT3L, XCL1, CCL21, IFNa A2
25	All Payloads
26	Saline
27	3t3 cells (no payload)

[0185] Dosing. Tumor volume was between 100 mm³ and 150 mm³ at the time of the first injection. For the groups receiving 4 payloads, each pay load was delivered at 2.5×10^5 cells per injection for a total of 10^6 cells. For the groups receiving 5 payloads, each pay load was delivered at [x] cells per injection for a total of 3×10^6 cells. The fifth payload was co-administered with Poly(I:C), which is a ds-RNA analog. Payloads were administered by intra-tumor injection. The volume of administration was 50 µL for all groups except the poly (I:C) group and the large 12-way group, where the volume was 150 µL.

[0186] Payload Administration Procedures. Cells were harvested with versene (not in the presences of trypsin). Once collected, the cells were counted, spun and resuspended in a volume that could be adjusted to 20×10^6 /ml after the cells are recounted. TLR agonist (Invivogen Cat# ODN:1826) by resuspending lyophilized powder in water provided. TLR agonist was resuspended at 10 mg/ml and heated to 70degC and then let to sit at RT for 1 hour prior to using. The dose of TLR agonist is 50 µg in 50 µl.

[0187] Results. The results are depicted in FIGS 9-11. Several combinations of payloads injected ipsilaterally demonstrated antitumor activity in the contralateral tumors manifested as delayed tumor growth in this model. Groups 3, 8 and 21 showed the most significant impairment of tumor growth over 30 days.

Example 5 – Modified B Cells that Express and Secrete Payloads

[0188] Experimental Design. A BALB/c mouse CT26 tumor model was used to evaluate the efficacy of modified B cells expressing various payload on tumor volume and survival. Mice were injected with tumor cells at a volume of 100 µL. On day 6 once tumors had reached a volume of

175mm³, mice were injected with modified B cells expressing various payloads as described below. Tumor volume and survival were measured for 17 days.

[0189] Isolation of Mouse PBMCs. Mouse PBMCs or splenocytes are isolated from blood or spleen, respectively. PBMCs are isolated using Lympholyte-M (CedarLane, Cat#CL5030). Splenocytes are isolated by manual cell separation through a 70 micron nylon cell strainer. B cells are then isolated from PBMCs or splenocytes via immunomagnetic negative selection using EasySep® Mouse B cell Isolation Kit (Stem Cell Technologies, Cat #19854).

[0190] Selection of Payloads. Nucleic acid sequences expressing payload peptides or proteins are transfected or transduced into isolated B cells. The following twelve peptides were identified for their potential to (i) recruit and activate dendritic cells; (ii) initiate homing and guidance of dendritic cells and T cells into the tumor site; and (iii) activate effector T cells. The payloads screened are listed in Table 9.

[0191] Each mouse was either given a combination of 4-5 payloads, or isolated B cells (without payload) or saline as a control. In total, there were twenty-seven groups (n = 5 mice/group). The experimental groups are identified in Table 11.

TABLE 11

Group #	Treatment
3	FLT3L, XCL1, CXCL13, CD40L-MB, TLR
8	FLT3L, XCL1, mLIGHT, CD80-MB
21	FLT3L, XCL1, CCL21, CD40L-MB
26	Saline
27	B cells (no payload)

[0192] Generation of Payload Expressing B Cells. For transfection, purified or cultured B cells are washed and suspended in Cytoporation Medium T (BTX, Cat # 47-0002) at 5×10^6 to 25×10^6 cells per ml and mixed with 7.5 µg to 50 µg RNA (RNA constructs are designed and prepped in house or purchased from TriLink using CleanCap® and fully substituted with Pseudo-U). 200 µL cell/RNA suspension electroporated using BTX Agilpulse® Electroporation System.

[0193] Dosing. Tumor volume was between 100 mm³ and 150 mm³ at the time of the first injection. For the groups receiving 4 payloads, each payload was delivered at 2.5×10^5 cells per injection for a total of 10^6 cells delivered. For the groups receiving 5 payloads, each payload was delivered at 2.5×10^5 cells per injection for a total of 1.25×10^6 cells delivered. Payloads were injected intra-tumor. The volume of administration was 50 µL for groups receiving 4 payloads, the volume of administration was 100 µL for groups receiving 5 payloads.

[0194] Payload Administration Procedures. Cells were harvested with versene (not in the presence of trypsin). Once collected, the cells were counted, spun and resuspended in a volume

that could be adjusted to $20 \times 10^6/\text{ml}$. TLR agonist (InvivoGen Cat# ODN:1826) by resuspending lyophilized powder in water provided. TLR agonist was resuspended at 10 mg/ml and heated to 70°C and then let to sit at RT for 1 hour prior to using. The dose of TLR agonist is 50 µg in 50 µl.

Example 6 – Anti-tumor activity of intratumorally injected B cells

[0195] Mouse splenocytes were obtained and isolated via manual cell separation utilizing a 70 micron nylon cell strainer. Autologous (BALB/c) or allogeneic (C57Bl/6) donor mice were used (data shown utilized allogeneic B cells). B cells were isolated from the splenocytes above using immunomagnetic negative selection via the EasySep® Mouse B Cell Isolation Kit (Stem Cell Technologies®, Cat #19854).

[0196] B cells were then injected either (i) fresh or (ii) first stimulated for 16-24 hours in growth media (RPMI, 10% FBS, 1% Pen/Strep, 5ng/ml recombinant mouse IL-4, 100uM beta-mercaptoethanol) with 5 µg/ml Lipopolysaccharide. 5×10^6 B cells were then intratumorally injected into the CT26 mouse model, and anti-tumor responses in the distal (abscopal) tumor were measured. Tumors were implanted at day 0, and at day 6 palpable tumor mass was observed. Treatment was initiated on day 6 intratumorally. The results are set forth in Figure 12.

Example 7 – Expression of chimeric antigen receptor (CAR) in B cells using RNA electroporation to make CAR B cells

[0197] Mouse PBMCs or splenocytes were isolated from blood or spleen as follows. Mouse PBMCs were isolated using Lympholyte-M (CedarLane, Cat #CL5030), and splenocytes were isolated by manual cell separation via passage through a 70 micron nylon cell strainer. B cells were then isolated from PBMCs or splenocytes, respectively, via immunomagnetic negative selection using the EasySep® Mouse B Cell Isolation Kit (Stem Cell Technologies, Cat #19854).

[0198] B cells were then stimulated for 16-24 hours in growth media (RPMI, 10% FBS, 1% Pen/Strep, 5ng/ml recombinant mouse IL-4, and 100uM beta-mercaptoethanol) with 5-15 µg/ml lipopolysaccharide. B cells were then transduced or transfected using known techniques (viral transfection or electroporation) to achieve either stable or transient expression of CAR-B. A strep II tag was incorporated for post-translational detection. Representative CAR-Bs depicted are as follows:

1. XENP PSMA CBCR (3X strep II tag)
2. HyHEL10 CBCR (3X strep II tag)
3. D1.3-M3 HEL CBCR (3X strep II tag)

[0199] For transfection, purified or cultured B cells were washed and suspended in Cytoporation Medium T (BTX, Cat #47-0002) at 5×10^6 to 25×10^6 cells per ml and mixed with 7.5ug to 50ug RNA (RNA constructs were designed and prepped either in-house or purchased from TriLink using CleanCap® and fully substituted with Pseudo-U). A 200ul cell/RNA suspension was obtained and electroporated using the BTX AgilePulse® Electroporation System. Cells were then washed in

PBS and prepped for IV injection into immune-incompetent mice with established HepG2 tumor cells that express respective antigen (e.g. GPC3, HEL, PSMA). Translation and expression of protein of interest was then measured using an anti-Strep II tag antibody. The results are set forth in Figure 13. In Figure 13, the X axis shows strength of expression signal as measured by flow cytometry, and the Y axis sets forth percent of cells expressing the desired protein of interest (PSMA, HEL).

[0200] This experiment demonstrates that the desired RNA sequence/s are successfully transfected or transduced (accordingly), the RNA is successfully translated, and the desired protein of interest is expressed on the cell surface.

Example 8 – Modified B cells expressing integrins and homing receptors

[0201] Nucleic acid constructs expressing an integrin, a homing receptor, or both are constructed using known techniques. Mouse and Human B cells are transfected or transduced (accordingly) with the nucleic acid constructs to express the integrin, the homing receptor, or both. These modified cells are administered intravenously into mice or a human host. Time-lapse imaging will measure accumulation of the modified B cells at the site/target of interest, such as a homing or target tissue, an inflammatory site in a specific location or tissue, or a tumor or tumor microenvironment, to establish that expression of an integrin and/or a homing receptor of defined homing specificity endows the B cells with the ability to home to and accumulate at the site/target of interest where delivery of therapeutic payloads is desirable. A screening study is conducted according to the techniques of Example 5 to examine delivery and effect of payloads at the site/target of interest.

Example 9 – Altering B cell trafficking

[0202] Isolated B cells are cultured with a specific concentration of all-trans-retinoic acid (ATRA) or derivatives thereof that induce expression of $\alpha 4\beta 7$ integrin and the homing receptor CCR9. Thereafter, the B cells are harvested and administered intravenously into mice. There are two experimental groups of the recipient mice. The first group of mice are pre-treated with DSS or TNBS to induce gut inflammation. The second group of mice are not treated with DSS or TNBS. Inflammation similar to that observed in human intestinal bowl diseases is induced by pretreatment with DSS or TNBS. Administered B cells treated with ATRA or derivative thereof will home to areas of inflammation consistent with their homing potential due to increased expression of $\alpha 4\beta 7$ integrin and the homing receptor CCR9.

Example 10 – Modified B cells expressing immune inhibitory molecules

[0203] Nucleic acid constructs expressing an immune inhibitory molecule selected from IL-10, TGF- β , PD-L1, PD-L2, LAG-3, and TIM-3, or any combinations thereof, are constructed using known techniques. Mouse and Human B cells are transfected or transduced (accordingly) with the nucleic acid constructs to express one or more of the immune inhibitory molecules listed above.

These modified cells are administered intravenously into mice or a human host or elsewhere near or at sites of inflammation. Time-lapse imaging will measure accumulation of the modified B cells at a site/target of interest, such as a homing or target tissue, an inflammatory site in a specific location or tissue, or a tumor or tumor microenvironment, to establish that inflammation at the site and autoimmune activity of the B cells localized to the site are decreased, thereby leading to a positive therapeutic response.

Example 11 – Activation of B cells with TLRs

[0204] B cells are treated with TLR agonists and/or modified to express a constitutively active TLR for use in potentiating B cells for immune responses and producing potent effector B cells to increase antigen-specific immune responses in a subject. Isolated mouse or human B cells are treated *in vitro* with a TLR agonist at the same time or in advance of the administration of the B cells. In some instances, the mouse or human B cells are treated with more than one TLR agonists.

[0205] A modified B cell, transfected or transduced with or without a CAR-B construct of the foregoing examples, is engineered to express one or more constitutively active TLRs. Each TLR is introduced into the modified B cell (transduced or transfected using known techniques) as a DNA construct under the control of a constitutively activated transcriptional pathway. A modified B cell, expressing one or more constitutively active TLRs (with or without a CAR-B construct), is also treated with one or more TLR agonists at the same time or in advance of the administration of the modified B cells to a subject or patient in need thereof. Time-lapse imaging and other known techniques will measure accumulation of the modified B cells in the desired location and confirm expression of the TLR(s) and any expressed CAR-B of a defined specificity.

[0206] This experiment will demonstrate that the desired DNA sequence/s encoding specific TLR(s) of interest are successfully transfected or transduced (accordingly) into B cells with or without a CAR-B construct and treated with or without TLR agonist(s), the RNA is successfully translated, the desired TLR(s) are expressed in the B cells for producing potent effector B cells potentiating B cells for immune responses.

Example 12 – Antigen presentation both in HLA Class I and Class II molecules using RNA electroporated B cells

[0207] **mRNA Constructs.** Exemplary mRNA constructs are designed by fusing a specific antigen, e.g., a tumor antigen or an infectious disease antigen, to the targeting signal of a the lysosomal protein LAMP1, to target the specific antigen to the lysosomes and present the antigen simultaneously and efficiently in both HLA class I and class II molecules. Tumor antigens and infectious disease antigens are well known in the art and can include any antigen of interest against which an immune response is desired. Various mRNA constructs are made encoding at least one specific antigen of interest fused to the targeting signal of LAMP1 that is capable of presenting the

specific antigen simultaneously and efficiently by both HLA class I and class II molecules when transfected into a suitable immune cell.

[0208] Experimental Design. Isolated mouse or human B cells are electroporated *in vitro* with an mRNA construct described above (i.e., encoding a specific antigen of interest fused to the targeting signal of LAMP1) using known mRNA electroporation techniques. In some instances, the mouse or human B cells are also transduced or transfected using known techniques with a CAR-B construct according to any of the foregoing examples. The mRNA electroporated B cells, transduced with or without a CAR-B construct of interest, are introduced intravenously into mice or a human host. Time-lapse imaging will measure accumulation of the modified B cells in the desired location and also confirm expression of CAR-B of a defined specificity. Translation and expression of the specific tumor antigens or infectious disease antigens of interest are measured using known techniques to establish that the antigens of interest are targeted to the lysosomes and presented simultaneously and efficiently by both HLA class I and class II molecules.

[0209] This experiment will demonstrate that the desired mRNA sequence/s encoding specific antigens of interest fused to a targeting signal are successfully transfected into B cells (which, if desired, are also transduced with a CAR-B construct), the mRNA is successfully translated, and the electroporated and modified B cells simultaneously and efficiently present the specific antigen of interest by both HLA class I and class II molecules for increasing antigen-specific immune responses in a subject.

Example 13 – B Cells Expressing a PSMA-specific CAR Reduce Tumor Growth in CT26-PSMA Tumors

[0210] Mouse Tumor Model. A BALB/c CT26-PSMA tumor model engineered to express human PSMA was used to evaluate the efficacy of PSMA-specific CAR engineered murine B cells on tumor volume and survival. Eight-week-old BALB/c mice were injected on one hind flank with 1.0×10^6 CT26-PSMA tumor cells in a volume of 50 μ l. On day 5 when the tumor volume reached approximately 60 mm³ the mice were distributed equally into 3 groups of 10 mice. Treatment of mice was started on day 6 using murine B cells engineered with mRNA encoding two different PSMA-specific CAR formats or un-engineered B cells administered intravenously at a dose of 1.5×10^6 cells in 100 μ l, or saline on day 6. Tumor volume was measured using calipers on day 5, 9, 11, and 13. There was a statistically significant tumor reduction of 57% in the PSMA-CAR group (format 79a) relative to saline on day 13. There was not a significant reduction of tumor volume on day 13 in the PSMA-CAR treatment group (format 79b) relative to saline (FIG. 14).

[0211] Engineering of Murine B Cells. Mouse splenocytes were isolated from BALB/c donor spleens by manual cell separation through a 70 micron nylon cell strainer. B cells were then isolated from splenocytes via immunomagnetic negative selection using EasySep Mouse B cell Isolation Kit (Stem Cell Technologies, Cat #19854). B cells were stimulated for 24 hours in growth media

(RPMI, 10% FBS, 25 mM HEPES, 1% Pen/Strep, 5ng/ml recombinant mouse IL-4, 100μM beta-mercaptoethanol) with anti-CD40 (250 ng/ml). Cells were then electroporated with 20 μg CAR mRNA construct per 3.6×10^6 B cells using BTX AgilePulse electroporation system set at 280V for 1ms. Cells were washed and resuspended in PBS at a concentration of 15×10^6 B cells /ml. 100 μl of cell suspension were used per dose.

PSMA construct CD79a: pmRNA_d7_13_anti hPSMA(XENP14484) scFv-mCD8H-mCD28M-mCD79aE #ab-1

PSMA construct CD79b: pmRNA_d7_13_anti hPSMA(XENP14484) scFv-mCD8H-mCD28M-mCD79bE #ac-1

Example 14 – Allogeneic B Cells Expressing a PSMA-specific CAR Reduce Tumor Growth in CT26-PSMA Tumors

[0212] Mouse Tumor Model. A BALB/c CT26-PSMA tumor model engineered to express human PSMA was used to evaluate the efficacy of PSMA-specific CAR engineered allogeneic murine B cells on tumor volume and survival. Eight-week-old BALB/c mice were injected on one hind flank with 1.0×10^6 CT26-PSMA tumor cells in a volume of 50 μl. On day 5 when the tumor volume reached approximately 70 mm³ the mice were distributed equally into 3 groups of 10 mice. Treatment of mice was started on day 6 using autologous murine B cells engineered with mRNA encoding a PSMA-specific CAR and an mRNA encoding CCR7 or allogeneic murine B cells engineered with mRNA encoding a PSMA-specific CAR administered intravenously at a dose of 1.5×10^6 cells in 100μl, or saline. Tumor volume was measured using calipers on day 5, 8, and 10. There was a statistically significant tumor reduction of 51% in the allogeneic and autologous engineered B cell groups relative to saline on day 10 (FIG. 15). ($p < 0.005$).

[0213] Engineering of Murine B Cells. Mouse splenocytes were isolated from autologous BALB/c and allogeneic C57Bl/6 donor spleens by manual cell separation through a 70 micron nylon cell strainer. B cells were then isolated from splenocytes via immunomagnetic negative selection using EasySep Mouse B cell Isolation Kit (Stem Cell Technologies, Cat #19854). B cells were stimulated for 24 hours in growth media (RPMI, 10% FBS, 25 mM HEPES, 1% Pen/Strep, 5ng/ml recombinant mouse IL-4, 100μM beta-mercaptoethanol) with anti-CD40 (250 ng/ml). Cells were then electroporated with 20 ug CAR mRNA construct per 3.6×10^6 B cells using BTX AgilePulse electroporation system set at 280V for 1ms. Cells were washed and resuspended in PBS at a concentration of 15×10^6 B cells /ml. 100 μl of cell suspension were used per dose.

Example 15 – The Antitumor Activity of PSMA-CAR-Engineered B Cells Depends on an Intact Host Immune System

[0214] Mouse Tumor Models. The effect of antitumor PSMA-CAR B cells was studied in WT and immunocompromised NSG mice.

[0215] WT Mice. A BALB/c CT26-PSMA tumor model engineered to express human PSMA was used to evaluate the efficacy of PSMA-specific CAR engineered murine B cells on tumor

volume and survival in WT mice. Eight-week-old BALB/c mice were injected on one hind flank with 1.0×10^6 CT26-PSMA tumor cells in a volume of 50 μ l. On day 5 when the tumor volume reached approximately 60 mm³ the mice were distributed equally into 4 groups of 10 mice. Treatment of mice was started on day 6 using murine B cells engineered with mRNA encoding two different PSMA-specific CAR formats or un-engineered B cells administered intravenously at a dose of 1.5×10^6 cells in 100 μ l, or saline on day 6. Tumor volume was measured using calipers on day 5, 9, 11, and 13. There was a statistically significant tumor reduction of 57% in the PSMA-CAR group (format 79a) relative to saline on day 13. There was not a significant reduction of tumor volume on day 13 in the PSMA-CAR treatment group (format 79b) or un-engineered B cells, relative to saline (FIG. 14).

[0216] NSG Mice. A BALB/c CT26-PSMA tumor model engineered to express human PSMA was used to evaluate the efficacy of PSMA-specific CAR engineered murine B cells on tumor volume and survival in immunocompromised mice. Eight-week-old NSG mice were injected on one hind flank with 1.0×10^6 CT26-PSMA tumor cells in a volume of 50 μ l. On day 5 when the tumor volume reached approximately 60 mm³ the mice were distributed equally into 2 groups of 10 mice. Treatment of mice was started on day 6 using murine B cells engineered with mRNA encoding a PSMA-specific CAR format administered intravenously at a dose of 1.5×10^6 cells in 100 μ l, or saline on day 6. Tumor volume was measured using calipers on day 5, 8, 10, and 13. There was no significant reduction in tumor volume in the PSMA-CAR group (format 79a) relative to saline on day 13 (FIG. 16B).

[0217] Engineering of Murine B Cells. Mouse splenocytes were isolated from autologous BALB/c and allogeneic C57Bl/6 donor spleens by manual cell separation through a 70 micron nylon cell strainer. B cells were then isolated from splenocytes via immunomagnetic negative selection using EasySep Mouse B cell Isolation Kit (Stem Cell Technologies, Cat #19854). B cells were stimulated for 24 hours in growth media (RPMI, 10% FBS, 25 mM HEPES, 1% Pen/Strep, 5ng/ml recombinant mouse IL-4, 100 μ M beta-mercaptoethanol) with anti-CD40 (250 ng/ml). Cells were then electroporated with 20 μ g CAR mRNA construct per 3.6×10^6 B cells using BTX AgilePulse electroporation system set at 280V for 1ms. Cells were washed and resuspended in PBS at a concentration of 15×10^6 B cells / ml. 100 μ l of cell suspension were used per dose.

PSMA construct CD79a: pmRNA_d7_13_anti hPSMA(XENP14484) scFv-mCD8H-mCD28M-mCD79aE #ab-1

PSMA construct CD79b: pmRNA_d7_13_anti hPSMA(XENP14484) scFv-mCD8H-mCD28M-mCD79bE #ac-1

Example 16 – B Cells Expressing a GPC3-specific CAR Reduce Tumor Growth in HEPA 1-6 GPC3 Tumors

[0218] Mouse Tumor Model. A C57Bl/6 HEPA 1-6 tumor model engineered to express human GPC3 (HEPA 1-6-GPC3) was used to evaluate the efficacy of murine B cells on tumor volume and survival. Eight-week-old C57Bl/6 mice were injected on one hind flank with 5.0×10^6 HEPA 1-6-GPC3 tumor cells at a volume of 200 μ l. On day 19 when the tumors volume reached approximately 250 mm³ the mice were distributed equally into 3 groups of 10 mice. Treatment of mice was started on day 20 using murine B cells engineered with mRNA encoding a GPC3-specific CAR or a PSMA-specific CAR administered intravenously at a dose of 1.5×10^6 cells in 100 μ l, or saline on day 20 and day 27. Tumor volume was measured using calipers on day 19, 23, 26, and 30. There was a statistically significant tumor reduction of 68% in the GPC3-CAR group relative to saline on day 30. There was not a significant reduction of tumor volume on day 30 in the PSMA-CAR treatment group relative to saline. (note: this study is still in progress on 1-19-2021) (FIG. 17).

[0219] Engineering of Murine B Cells. Mouse splenocytes were isolated from C57Bl/6 donor spleens by manual cell separation through a 70 micron nylon cell strainer. B cells were then isolated from splenocytes via immunomagnetic negative selection using EasySep Mouse B cell Isolation Kit (Stem Cell Technologies, Cat #19854). B cells were stimulated for 24 hours in growth media (RPMI, 10% FBS, 25 mM HEPES, 1% Pen/Strep, 5ng/ml recombinant mouse IL-4, 100 μ M beta-mercaptoethanol) with anti-CD40 (250 ng/ml). Cells were then electroporated with 20 μ g CAR mRNA construct per 3.6×10^6 B cells using BTX AgilePulse electroporation system set at 280V for 1ms. Cells were washed and resuspended in PBS at a concentration of 15×10^6 B cells /ml. 100 μ l of cell suspension were used per dose.

GPC3 mRNA construct: pmRNA_d7_13_anti-hGPC3 scFv-mCD8H-mCD28M-mCD79aE #15-1

PSMA construct: pmRNA_d7_13_anti hPSMA(XENP14484) scFv-mCD8H-mCD28M-mCD79aE #ab-1

Example 17 – Multimerized GPC3 can activate NF κ B expression of luciferase in cells expressing a GPC3 CAR in a dose-responsive manner

[0220] CAR-B Construct Design. Five CAR-B constructs were designed using three basic formats (i) CAR 2 (an scFv, a hinge domain, a transmembrane domain and a signaling domain (see FIG XA)); (ii) CAR 3 (a multimerized receptor complex with 2 of each of the following: an scFv, a hinge domain, an FC domain, a transmembrane domain and a cytoplasmic tail (see FIG XB)); (iii) CAR 4 (a multimerized receptor complex with 2 of each of the following: (a FAB domain, a hinge domain, an FC domain, a transmembrane domain and a cytoplasmic tail (see FIG XC). The five CAR-B constructs are as follows:

TABLE 12

pWF-506 (SEQ ID NO. 140/141)	pmRNA_d7_13_anti-hGPC3 scFv-hIgG1 Fc [TM + cyto] A-1 (CAR 3)
pWF-507	pmRNA_d7_13_anti-hGPC3 v1-hcLamda / pmRNA_d7_13_anti-hGPC3 vH-hlgHg1 [TM+ cyto]

(SEQ ID NO. 142/143) / pWF-508 (SEQ ID NO. 144/145)	(CAR 4)
pWF-509 (SEQ ID NO. 146/147)	pmRNA_d7_13_anti-hGPC3 scFv-hCD8H-hCD28M-hCD79bE (CAR 2)
pWF-510 (SEQ ID NO. 148/149)	pmRNA_d7_13_anti-hGPC3 scFv-hCD8H-hCD28M-hCD79aE (CAR 2)

[0221] *NFκB Reporter Assay: Antigen induced signaling.* Ramos NFκB-luciferase reporter cells were transduced with mRNA coding for one of the CAR-B constructs listed above. Ramos NFκB-luciferase reporter cells were transfected at 280 V and 1 msec with 10 μg of RNA in 200 μL of electroporation buffer followed by culturing overnight in growth medium. The cells were left at room temperature for 4 hours to quiesce the cells to reduce background. 30,000 of the transfected cells were transferred to each well in a multi-well plate in a volume of 30 μL per well. The transfected Ramos cells were then incubated with GPC3 protein multimerized with streptavidin, streptavidin control or GPC3-Fc protein for 3 hours in growth medium. 30 μL of Bioglo substrate (Promega) was added to each well and the plate was read within 5 minutes using a luminometer. As demonstrated in FIG. 18, multimerized GPC3 was capable of activating NFκB expression of luciferase in cells expressing three of the four GPC3 CAR-Bs except pWF-509 (GPC3-CD79b). All four constructs displayed good binding to GPC3 in FACS assays. Therefore, CD79b was an example where a CAR, which had good binding affinity, did not signal.

[0222] *NFκB Reporter Assay: Tonic Signaling.* Tonic signaling was also assessed, using the NFκB Reporter Assay. CAR constructs, which induced elevated tonic signaling in the absence of cognate antigen binding, were generated. FIG. 19 shows that the four CAR-B constructs were expressed in a human B cell reporter line and NFκB luciferase activity was measured in the absence of cognate target antigen. Each construct displayed significant tonic signaling activity. Engineered B cells with tonic signaling CAR Bs remained at a high number in vivo and led to high and durable expression of replacement factors or other payloads.

Example 18 – A CD80 payload enhances the antitumor activity of Anti-GPC3CAR-CD79a B cells

[0223] *Experimental Design.* A syngeneic C57Bl/6 mouse HEPA1-6GPC3 tumor is a model of human HCC engineered to express human GPC3. This model was used to evaluate the efficacy of murine B cells electroporated with anti-GPC3CAR-CD79a and a CD80 payload mRNAs. Mice were injected on one hind flank with 5.0×10^6 HEPA1-6GPC3 tumor cells at a volume of 200 μl in matrigel. On day 11, 14, and 17 the mice were administered a 200 μl IV dose of 1.5×10^6 B cells,

B cells engineered with anti-GPC3CAR-CD79a, B cells engineered with anti-GPC3CAR-CD79a and CD80, or saline as indicated in FIG. 20. The B cells were engineered with mRNA as described below. Tumor volume was monitored on multiple days as indicated in FIG. 20.

[0224] In FIG. 20, both the anti-GPC3CAR-CD79a, and anti-GPC3CAR-CD79a plus CD80 combo displayed a statistically significant effect relative to saline or un-engineered B cells on day 44 and at multiple earlier time points. Additionally, by day 44 there were no complete responses in the saline control or B cell control groups but the anti-GPC3CAR-CD79a, and anti-GPC3CAR-CD79a plus CD80 combo resulted in 4 and 7 complete responses, respectively, as indicated in FIGS. 21A-21C. These data demonstrate that inclusion of the CD80 payload as mRNA potentiated the antitumor activity of B cells co-electroporated with an antigen-specific GPC3 CAR.

[0225] *B Cell preparation.* Mouse splenocytes were isolated from C57Bl/6 donor spleens by mechanical cell separation through a 70 micron nylon cell strainer. B cells were then isolated from splenocytes via immunomagnetic negative selection using EasySep Mouse B cell Isolation Kit (Stem Cell Technologies, Cat #19854). B cells were stimulated for 24 hours in growth media (RPMI, 10% FBS, 1% Pen/Strep, 5ng/ml recombinant mouse IL-4, 100uM beta-mercaptoethanol) with 250 ng/ml CD40 antibody (anti-murine CD40 Ab). Cells were then electroporated with 20ug mRNA per 1.0×10^7 B cells using BTX AgilePulse electroporation system set at 400V for 1ms, 2ms interval for 5 pulses. When two mRNA's were cotransfected, 20ug of each mRNA was used. Immediately after electroporation, the cells were washed in PBS and prepared for IV administration at a dose of 1.0×10^7 per 200ul. The electroporated cells were administered to mice within 90 minutes after electroporation.

[0226] Twelve hours after electroporation, a small aliquot of the cells were stained for expression of anti-GPC3CAR-CD79a and CD80 expression. For detection of anti-GPC3CAR-CD79a expression, GPC3-Avitag and Streptavidin-BV421 were used. CD80 expression was measured with an anti-CD80-PE FACS antibody. The FACS plots in FIGS. 22A-22C show expression of the GPC3 CAR post-electroporation. CD80 was expressed at a basal level in un-engineered B cells, thus accounting for the ~10% positivity. This level remained in the CAR sample, but was increased dramatically in the CAR + CD80 sample. The latter suggested efficient expression of CD80.

What is Claimed

1. An isolated modified B cell, capable of expressing a chimeric receptor (CAR-B), wherein said chimeric receptor comprises
 - a) an extracellular domain, wherein the extracellular domain comprises an extracellular binding domain and a hinge domain;
 - b) a transmembrane domain; and
 - c) a cytoplasmic domain that comprises at least one signaling domain.
2. The isolated modified B cell of claim 1, wherein said extracellular binding domain(s), recognizes at least one antigen or protein expressed on the surface of a target cell.
3. The isolated modified B cell of claim 1, wherein the extracellular binding domain(s) recognizes at least one antigen that is a secreted protein.
4. The isolated modified B cell of claim 3, wherein the secreted protein accumulates in the extracellular matrix.
5. The isolated modified B cell of claim 2, wherein said target cell is selected from the group consisting of a tumor cell, a cardiac muscle cell, a skeletal muscle cell, a bone cell, a blood cell, a nerve cell, a fat cell, a skin cell, an endothelial cell, a hepatocyte, a pulmonary epithelial cell, and a fibroblast cell.
6. The isolated modified B cell of claim 1, wherein said B cell expresses more than one CAR-B receptor construct.
7. The isolated modified B cell of claim 1, wherein said CAR-B receptor comprises more than one extracellular binding domain.
8. The isolated modified B cell of claim 1, wherein said extracellular binding domain is a single chain variable fragment (scFv), or a full-length antibody or an antibody fragment, or the extracellular domain of a receptor or ligand.

9. The isolated modified B cell of claim 1, wherein said extracellular binding domain is capable of binding to an antigen or protein selected from the group consisting of: PSMA, GPC3, ASGR1, ASGR2, Sarcoglycan, Corin, FAP, MUC1, CEA153, JAM-1, LAF-1 and Her2.
10. The isolated modified B cell of claim 1, wherein said hinge domain is derived from the group consisting of an immunoglobulin (e.g., IgG, IgM), CD28 and CD8.
11. The isolated modified B cell of claim 1, wherein said cytoplasmic domain comprises at least one signaling domain native to B cells receptors.
12. The isolated modified B cell of claim 1, wherein said cytoplasmic domain comprises at least one signaling domain that is an immunoglobulin.
13. The isolated modified B cell of claim 1, wherein said cytoplasmic domain comprises a domain that is selected from the group consisting of: CD79a (Immunoglobulin α), CD40, CD19, CD137, Fc γ 2a, MyD88, CD21, Syk, FYN, LYN, PI3K, BTK, PLC γ 2, CD3 ζ and BLNK.
14. The isolated modified B cell of claim 13, wherein said cytoplasmic domain comprises domain that is CD79a.
15. The isolated modified B cell of claim 13, wherein said cytoplasmic domain further comprises a costimulatory domain.
16. An isolated modified B cell, wherein said B cell is capable of expressing and secreting a payload, wherein the payload is not naturally expressed in a B cell or is expressed at higher levels than is naturally expressed in a B cell.
17. The isolated modified B cell of claim 16, wherein the payload is an antibody or fragment thereof.
18. The isolated modified B cell of claim 17, wherein the antibody is a secreted antibody or fragment thereof
19. The isolated modified B cell of claim 17, wherein the antibody is membrane bound.
20. The modified B cell of claim 16, wherein said payload is selected from a group of cytokines, chemokines, T cell costimulatory molecules, and checkpoint molecules, the group consisting of: IL-1, IL-7, IL-8, IL-10, IL-12, IL-13, IL-17, IL-18, IL-21, interferon α , interferon β ,

interferon γ , TSLP, CCL21, FLT3L, XCL1, LIGHT(TNFSF14), OX40L, CD137L, CD40L, ICOSL, anti-CD3 antibody, CD47, TIM4-FC, CXCL13, CCL21, CD80, CD40L, IFN α A2, LIGHT, 4-1BBL, MDGF (C19orf10), FGF10, PDGF, agrin, TNF- α , GM-CSF, an anti-FAP antibody, an anti-TGF- β antibody; a TGF- β trap, decoy or other inhibitory molecule; an anti-BMP antibody; a BMP trap, decoy or other inhibitory molecule.

21. The modified B cell of claim 16, wherein said B cell is capable of expressing more than one payload.

22. The modified B cell of claim 16, wherein said B cell is capable of expressing more than 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 payloads.

23. A method of treating a patient comprising administering the modified B cell of claim 14.

24. The method of claim 23, wherein the modified B cell is administered intra-tumorally, intravenously, subcutaneously, intradermally, or within an inflammatory lesion

25. The method of claim 23, further comprising administering one or more checkpoint inhibitors, with or without an additional chemotherapeutic agent.

26. The method of claim 25, wherein the checkpoint inhibitor is capable of inhibiting a checkpoint molecule selected from the group consisting of: PD-1, PD-L1, CTLA-4, LAG3, TIM-3 and NKG2A.

27. The method of claim 23, further comprising administering a T cell costimulatory molecule.

28. The method of claim 27, wherein the T cell costimulatory molecule is selected from the group consisting of: CD80, CD86, ICOSL, 4-1BBL, OX40L, CD27, and LIGHT.

29. An isolated modified B cell, capable of expressing a chimeric receptor, wherein said chimeric receptor comprises

- a) an extracellular domain, wherein the extracellular domain comprises an extracellular binding domain and a hinge domain;
- b) a transmembrane domain; and
- c) a cytoplasmic domain that comprises at least one signaling domain

wherein said modified B cell is further capable of expressing a payload, wherein the payload is not naturally expressed in a B cell.

30. The isolated modified B cell of claim 29, wherein said extracellular binding domain, recognizes an antigen or protein expressed on the surface of a target cell.

31. The isolated modified B cell of claim 29, wherein said extracellular binding domain, recognizes an antigen or protein that is secreted.

32. The isolated modified B cell of claim 29, wherein said target cell is selected from the group consisting of a tumor cell, a cardiac muscle cell, a skeletal muscle cell, a bone cell, a blood cell, a nerve cell, a fat cell, a skin cell, an endothelial cell, a hepatocyte, a pulmonary epithelial cell and a fibroblast.

33. The isolated modified B cell of claim 29, wherein said B cell expresses more than one CAR-B receptor construct.

34. The isolated modified B cell of claim 29, wherein said CAR-B receptor comprises more than one extracellular binding domain.

35. The isolated modified B cell of claim 29, wherein said extracellular binding domain is a single chain variable fragment (scFv), a full-length antibody, an antibody fragment or an extracellular domain of a receptor or ligand.

36. The isolated modified B cell of claim 29, wherein said extracellular binding domain is capable of binding to an antigen or protein selected from the group consisting of: PSMA, GP3, ASGR1, ASGR2, Sarcoglycan, Corin, and Her2, FAP, MUC1, CEA153, JAM-1, and LFA-1.

37. The isolated modified B cell of claim 29, wherein said hinge domain is derived from the group consisting of an immunoglobulin (for example, IgG or IgM), CD28 and CD8.

38. The isolated modified B cell of claim 29, wherein said cytoplasmic domain comprises at least one signaling domain native to B cells.

39. The isolated modified B cell of claim 29, wherein said cytoplasmic domain comprises at least one signaling domain that is an immunoglobulin.

40. The isolated modified B cell of claim 29, wherein said cytoplasmic domain comprises a domain selected from the group consisting of: CD79a (Immunoglobulin α), , CD40, CD19, CD137, Fc γ 2a, MyD88, CD21, Syk, FYN, LYN, PI3K, BTK, PLC γ 2, CD ζ and BLNK.
41. The isolated modified B cell of claim 40, wherein the cytoplasmic domain comprises a domain that is CD79a (Immunoglobulin α).
42. The isolated modified B cell of claim 41, wherein said cytoplasmic domain further comprises a costimulatory domain.
43. The isolated modified B cell of claim 29, wherein the payload is a secreted or membrane bound antibody or fragment thereof.
44. The isolated modified B cell of claim 29, wherein said payload is selected from a group of cytokines, chemokines, T cell costimulatory molecules, and checkpoint molecules, the group consisting of: IL-1, IL-7, IL-8, IL-10, IL-12, IL-13, IL-17, IL-18, IL-21, interferon α , interferon β , interferon γ , TSLP, CCL21, FLT3L, XCL1, LIGHT(TNFSF14), OX40L, CD137L, CD40L, ICOSL, anti-CD3 antibody, CD47, TIM4-FC, CXCL13, CCL21, CD80, CD40L, IFN α A2, LIGHT , 4-1BBL, MDGF (C19orf10), FGF10, PDGF, agrin, TNF- α , GM-CSF, an anti-FAP antibody, an anti-TGF- β antibody; a TGF- β trap, decoy or other inhibitory molecule; an anti-BMP antibody; a BMP trap, decoy or other inhibitory molecule.
45. The isolated modified B cell of claim 29, wherein said B cell is capable of expressing more than one payload.
46. The isolated modified B cell of claim 29, wherein said B cell is capable of expressing more than 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 payloads.
47. The isolated modified B cell of claim 29, wherein the modified B cell further encodes at least one protein selected from the group consisting of: the cytoplasmic domains of CD79a (Immunoglobulin α), CD79b (Immunoglobulin β), CD40, CD19, CD137, Fc γ 2a, CD ζ and MyD88.
48. A method of treating a patient comprising administering the modified B cell of claim 29.
49. The method of claim 48, further comprising administering a checkpoint inhibitor.

50. The method of claim 48, wherein the checkpoint inhibitor is capable of inhibiting a checkpoint molecule selected from the group consisting of: PD-1, PD-L1, CTLA-4, LAG3, TIM-3 and NKG2A.

51. The method of claim 50, wherein the checkpoint inhibitor is a monoclonal antibody.

52. An isolated modified B cell, capable of expressing a chimeric receptor, wherein said chimeric receptor comprises an extracellular domain, wherein said extracellular domain comprises a hinge domain and an extracellular binding domain, wherein said extracellular binding domain is not naturally expressed on a B cell; and wherein said extracellular binding domain is capable of recognizing a target of interest.

53. The isolated modified B cell of claim 52, wherein said binding domain is a single chain variable fragment (scFv), a full length antibody, an antibody fragment, an extracellular domain of a ligand or receptor.

54. The isolated modified B cell of claim 53, wherein said binding domain is an scFv.

55. The isolated modified B cell of claim 52, wherein herein said B cell is capable of expressing more than one chimeric receptor.

56. The isolated modified B cell of claim 52, wherein said B cell is further capable of expressing a payload.

57. A method of treating a patient comprising administering the modified B cell of claim 52.

58. A nucleic acid capable of expressing a chimeric B cell receptor, wherein said chimeric receptor comprises:

- a) an extracellular domain, wherein said extracellular domain comprises an extracellular binding domain and a hinge domain;
- b) a transmembrane domain; and
- c) a cytoplasmic domain that comprises at least one signaling domain.

59. The nucleic acid of claim 58, wherein said extracellular binding domain, recognizes an antigen or protein expressed on the surface of a target cell.
60. The nucleic acid of claim 58, wherein said extracellular binding domain, recognizes an antigen or protein that is a secreted protein.
61. The nucleic acid of claim 58, wherein the secreted protein accumulates in the extracellular matrix.
62. The nucleic acid of claim 58, wherein said extracellular binding domain is a single chain variable fragment (scFv), a full length antibody, an antibody fragment or an extracellular domain of a receptor or ligand.
63. The nucleic acid of claim 58, wherein said target cell is selected from the group consisting of a tumor cell, a cardiac muscle cell, a skeletal muscle cell, a bone cell, a blood cell, a nerve cell, a fat cell, a skin cell, an endothelial cell, a hepatocyte, a pulmonary epithelial cell or a fibroblast.
64. The nucleic acid of claim 58, wherein said vector expresses more than one CAR-B receptor.
65. The nucleic acid of claim 58, wherein said CAR-B receptor expresses more than one extracellular binding domain.
66. The nucleic acid of claim 58, wherein said extracellular binding domain is capable of binding to an antigen or protein selected from the group consisting of: PSMA, GP3, ASGR1, AGSR2, Sarcoglycan, Corin, Her2, PAF1, MUC1, CEA153, JAM-1, and LFA-1.
67. The nucleic acid of claim 58, wherein said hinge domain is derived from the group consisting of an immunoglobulin domain (for example, IgG or IgM), CD28 and CD8.
68. The nucleic acid of claim 58, wherein said cytoplasmic domain comprises at least one signaling domain native to B cell receptors.
69. The nucleic acid of claim 58, wherein said cytoplasmic domain comprises a domain selected from the group consisting of: CD79a (Immunoglobulin α), CD79b (Immunoglobulin β), CD40, CD19, CD137, Fc γ 2a, MyD88, CD21, Syk, FYN, LYN, PI3K, BTK, PLC γ 2, CD3 ζ and BLNK.

70. The nucleic acid of claim 69, wherein said cytoplasmic domain comprises a domain that is CD79a (Immunoglobulin α).
71. The nucleic acid of claim 69, wherein said cytoplasmic domain further comprises a costimulatory domain.
72. A vector comprising a nucleic acid capable of expressing a chimeric B cell receptor, wherein said chimeric receptor comprises:
- a) an extracellular domain, wherein said extracellular domain comprises an extracellular binding domain and a hinge domain;
 - b) a transmembrane domain; and
 - c) a cytoplasmic domain that comprises at least one signaling domain.
73. The vector of claim 72, wherein said extracellular binding domain recognizes an antigen or protein expressed on the surface of a target cell.
74. The vector of claim 72, wherein said extracellular binding domain, recognizes an antigen or protein that is a secreted protein.
75. The vector of claim 72, wherein the secreted protein accumulates in the extracellular matrix.
76. The vector of claim 72, wherein said target cell is selected from the group consisting of a tumor cell, a cardiac muscle cell, a skeletal muscle cell, a bone cell, a blood cell, a nerve cell, a fat cell, a skin cell, an endothelial cell, a hepatocyte, a pulmonary epithelial cell or a fibroblast.
77. The vector of claim 72, wherein said vector expresses more than one CAR-B receptor.
78. The vector of claim 72, wherein said CAR-B expresses more than one extracellular binding domain.
79. The vector of claim 72, wherein said extracellular binding domain is a single chain variable fragment (scFv), a full-length antibody, an antibody fragment, an extracellular domain of a receptor or ligand.

80. The vector of claim 72, wherein said extracellular binding domain is capable of binding to an antigen or protein selected from the group consisting of: PSMA, GPC3, ASGR1, ASGR2, Sarcoglycan, Corin, Her2, PAF1, MUC1, CEA153, JAM-1, and LFA-1.
81. The vector of claim 72, wherein said hinge domain is derived from the group consisting of an immunoglobulin domain (for example, IgG or IgM), CD28 and CD8.
82. The vector of claim 72, wherein said cytoplasmic domain comprises at least one signaling domain native to B cells.
83. The vector of claim 72, wherein said cytoplasmic domain comprises a signaling domain selected from the group consisting of: CD79a (Immunoglobulin α), CD79b (Immunoglobulin β), CD40, CD19, CD137, Fc γ 2a, MyD88, CD21, Syk, FYN, LYN, PI3K, BTK, PLC γ 2, CD3 ζ and BLNK.
84. The vector of claim 72, wherein said cytoplasmic domain further comprises a costimulatory domain.
85. An isolated modified B cell, capable of expressing an integrin, a homing antibody, protein, a receptor, or combinations thereof, wherein said integrin, homing antibody, protein, or receptor is not naturally expressed in a B cell or is expressed at higher levels than is naturally expressed in a B cell; and wherein said integrin, homing antibody, protein, receptor, or combinations thereof is attracted to a site or target of interest.
86. The isolated modified B cell of claim 85, wherein the integrin, homing antibody, protein, and receptor is selected from CLA (PSGL-1 glycoform), CLA (PSGL-1 glycoform), CCR10, CCR3, CCR4, CCR5, CCR6, CCR9, CD43E, CD44, c-Met, CXCR3, CXCR4, LFA-1, LFA-1 (α L β 2), selectin ligands, VLA-4, VLA-4 (α 4 β 1), and α 4 β 7, or combinations thereof.
87. The isolated modified B cell of claim 86, wherein the site of interest is a homing or target tissue, an inflammatory site in a specific location or tissue, or a tumor or tumor microenvironment, where delivery of payloads is desirable.
88. The isolated modified B cell of claim 87, wherein the homing or target tissue is selected from skin, gut (intestine, colon, mesenteric lymph nodes (mLN), Peyer's Patch (PP), small

intestine), liver, lung, bone marrow, heart, peripheral lymph node (LN), CNS, thymus, and bone marrow.

89. The isolated modified B cell of claim 88, wherein the target of interest is selected from CXCL16, CCL17, CCL17(22), CCL20 (MIP-3 α), CCL21, CCL25, CCL27, CCL28, CCL4, CCL5, CD62E, CD62P, CXCL10, CXCL12, CXCL13, CXCL16, CXCL9/CXCL10, CXCR3, E/P-selectin, E-selectin, GPR15L, HGF, Hyaluronate, ICAM-1, ligands for CCR1, 2, 5, MAdCAM, MAdCAM-1, PNAd, VAP-1, VCAM, and VCAM-1, or combinations thereof.

90. A method of treating a patient comprising administering the isolated modified B cell of claim 82.

91. The method of claim 90, further comprising administering a compound or a derivative thereof, wherein the compound or derivative thereof is capable of modulating the expression of the integrin, homing antibody, protein, and receptor, or combinations thereof.

92. The method of claim 91, wherein the compound or a derivative thereof is capable of altering trafficking of B cells to a site or target of interest in the patient.

93. The method of claim 92, wherein the compound is all-trans-retinoic acid (ATRA) or a derivative thereof.

94. An isolated modified B cell, capable of expressing an immune inhibitory molecule, wherein said immune inhibitory molecule is not naturally expressed in a B cell or is expressed at higher levels than is naturally expressed in a B cell.

95. The isolated modified B cell of claim 94, wherein said immune inhibitory molecule is selected from IL-10, TGF- β , PD-L1, PD-L2, LAG-3, and TIM-3, or combinations thereof.

96. The isolated modified B cell of claim 95, wherein said immune inhibitory molecule is capable of decreasing inflammation and autoimmune activity of B cells at a site or target of interest in a patient.

97. A method of treating a patient comprising administering the isolated modified B cell of claim 94.

98. The method of claim 97, wherein said immune inhibitory molecule is selected from IL-10, TGF- β , PD-L1, PD-L2, LAG-3, and TIM-3, or combinations thereof.

99. The method of claim 98, wherein said immune inhibitory molecule is capable of decreasing inflammation and autoimmune activity of B cells at a site or target of interest in the patient.

100. The method of claim 99, further comprising administering a compound or a derivative thereof capable of increasing the expression of an integrin, a homing antibody, a protein, a receptor, or combinations thereof in the B cell.

101. The method of claim 100, wherein said compound or derivative thereof is capable of altering trafficking of B cells to a site or target of interest in the patient.

102. The method of claim 101, wherein said compound is all-trans-retinoic acid (ATRA) or a derivative thereof.

103. An isolated modified B cell, wherein the isolated modified B cell is treated with a compound or a derivative thereof, wherein said compound or derivative thereof is capable of increasing the expression of an integrin, a homing antibody, a protein, a receptor, or combinations thereof in B cells.

104. The isolated modified B cell of claim 103, wherein said compound or derivative thereof is capable of altering trafficking of B cells to a site or target of interest in the patient.

105. The isolated modified B cell of claim 104, wherein said compound is all-trans-retinoic acid (ATRA) or a derivative thereof

106. A method of treating a patient comprising administering the isolated modified B cell of claim 100, wherein said compound or derivative thereof is capable of (i) modulating the expression of an integrin, a homing antibody, a protein, a receptor, or combinations thereof in B cells, and (ii) altering trafficking of B cells to a site or target of interest in the patient.

107. The method of claim 106, wherein said compound is all-trans-retinoic acid (ATRA) or a derivative thereof.

108. An isolated modified B cell, capable of expressing at least one or more of a constitutively active Toll-like receptor (TLR), wherein said TLR is not naturally expressed in a B cell or is expressed at higher levels than is naturally expressed in a B cell.

109. The isolated modified B cell of claim 108, wherein said TLR is selected from TLR1, TLR2, TLR3, TLR4, TLR5, TLR6, TLR7, TLR8, TLR9, TLR10, TLR11, TLR12, and TLR13, or combinations thereof.

110. The isolated modified B cell of claim 109, wherein said TLR is capable of potentiating B cells for increasing immune responses in a patient.

111. The isolated modified B cell of claim 110, wherein said TLR is capable of producing potent effector B cells for increasing immune responses in a patient.

112. A method of treating a patient comprising administering the isolated modified B cell of claim 108, wherein said immune inhibitory molecule is capable of decreasing inflammation and autoimmune activity of B cells at a site or target of interest in a patient.

113. The method of claim 112, wherein said TLR is selected from TLR1, TLR2, TLR3, TLR4, TLR5, TLR6, TLR7, TLR8, TLR9, TLR10, TLR11, TLR12, and TLR13, or combinations thereof.

114. The method of claim 113, wherein said TLR is capable of (i) potentiating B cells, and (ii) producing potent effector B cells, for increasing immune responses in a patient.

115. The method of claim 114, further comprising administering at least one or more of a TLR agonist to the patient.

116. An isolated modified B cell, wherein the isolated modified B cell is treated with at least one or more of a TLR agonist.

117. The isolated modified B cell of claim 116, wherein said TLR agonist is capable of (i) potentiating B cells, and (ii) producing potent effector B cells, for increasing immune responses in a patient.

118. The isolated modified B cell of claim 117, wherein said TLR agonist binds to one or more TLRs selected from TLR1, TLR2, TLR3, TLR4, TLR5, TLR6, TLR7, TLR8, TLR9, TLR10, TLR11, TLR12, and TLR13, or combinations thereof.

119. The isolated modified B cell of claim 118, wherein said TLR agonist is selected from CpG-rich oligonucleotides, double-stranded RNA mimic, polyinosinic acid:polycytidylic acid (poly-I:C).
120. The isolated modified B cell of claim 119, wherein said TLR agonist comprises CpG oligonucleotides.
121. A method of treating a patient comprising administering the isolated modified B cell of claim 116.
122. The method of claim 121, wherein said TLR agonist is capable of (i) potentiating B cells, and (ii) producing potent effector B cells, for increasing immune responses in the patient.
123. The method of claim 122, wherein said TLR agonist binds to one or more TLRs selected from TLR1, TLR2, TLR3, TLR4, TLR5, TLR6, TLR7, TLR8, TLR9, TLR10, TLR11, TLR12, and TLR13, or combinations thereof.
124. The method of claim 123, wherein said TLR agonist is selected from CpG-rich oligonucleotides, double-stranded RNA mimic, polyinosinic acid:polycytidylic acid (poly-I:C).
125. The method of claim 124, wherein said TLR agonist comprises CpG oligonucleotides.
126. An isolated modified B cell, wherein said B cell is electroporated with an mRNA encoding at least one or more of an antigen fused to a targeting signal.
127. The isolated modified B cell of claim 126, wherein said antigen is (i) not naturally presented by a B cell, (ii) not presented by a B cell simultaneously in both HLA class I and class II molecules naturally, or (iii) not presented by a B cell with high efficiencies in both HLA class I and class II molecules naturally.
128. The isolated modified B cell of claim 127, wherein said targeting signal is targeting signal of a lysosomal protein.
129. The isolated modified B cell of claim 128, wherein said targeting signal is a targeting signal of lysosome-associated membrane protein-1 (LAMP1).

130. The isolated modified B cell of claim 129, wherein said antigen is capable of being targeted to the lysosomes and presented simultaneously and efficiently in both HLA class I and class II molecules.
131. The isolated modified B cell of claim 130, wherein said B cells is capable of increasing antigen-specific immune responses in a patient.
132. A method of treating a patient comprising administering the isolated modified B cell of claim 126.
133. The method of claim 132, wherein said antigen is (i) not naturally presented by a B cell, (ii) not presented by a B cell simultaneously by both HLA class I and class II molecules naturally, or (iii) not presented by a B cell with high efficiencies by both HLA class I and class II molecules naturally.
134. The method of claim 133, wherein said targeting signal is targeting signal of a lysosomal protein.
135. The method of claim 134, wherein said targeting signal is a targeting signal of lysosome-associated membrane protein-1 (LAMP1).
136. The method of claim 135, wherein said antigen is capable of being targeted to the lysosomes and presented simultaneously and efficiently in both HLA class I and class II molecules.
137. The method of claim 136, wherein said B cells is capable of increasing antigen-specific immune responses in the patient.
138. The method of any of the claims 132-137, wherein the patient is treated with more than one differently modified B cells.
139. The method of any one of the claims 132-137, wherein the patient is treated with more than two differently modified B cells.
140. An isolated modified B cell, capable of expressing a chimeric receptor (CAR-B), wherein said chimeric receptor comprises
- a) an extracellular domain, wherein the extracellular domain comprises an extracellular binding domain and a hinge domain;

- b) a transmembrane domain; and
- c) a cytoplasmic domain.

141. The isolated modified B cell of claim 140, wherein the extracellular domain comprises an immunoglobulin domain.

142. The isolated modified B cell of claim 140, wherein the chimeric receptor is capable of forming a homologous dimer.

143. The isolated modified B cell of claim 140, wherein the extracellular binding domain, recognizes an antigen or protein expressed on the surface of a target cell.

144. The isolated modified B cell of claim 143, wherein said extracellular binding domain, recognizes an antigen or protein that is secreted.

145. The isolated modified B cell of claim 143, wherein said target cell is selected from the group consisting of a tumor cell, a cardiac muscle cell, a skeletal muscle cell, a bone cell, a blood cell, a nerve cell, a fat cell, a skin cell, an endothelial cell, a hepatocyte, a pulmonary epithelial cell and a fibroblast.

146. The isolated modified B cell of claim 143, wherein said extracellular binding domain is a single chain variable fragment (scFv), a full-length antibody, an antibody fragment or an extracellular domain of a receptor or ligand.

147. The isolated modified B cell of claim 143, wherein said extracellular binding domain is capable of binding to an antigen or protein selected from the group consisting of: PSMA, GP3, ASGR1, ASGR2, Sarcoglycan, Corin, and Her2, FAP, MUC1, CEA153, JAM-1, and LFA-1.

148. The isolated modified B cell of claim 14, wherein said hinge domain is derived from the group consisting of an immunoglobulin (for example, IgG or IgM), CD28 and CD8.

149. An isolated modified B cell, capable of expressing a chimeric receptor (CAR-B), wherein said chimeric receptor comprises the amino acid sequence SEQ ID NO: 146.

150. An isolated modified B cell, capable of expressing a chimeric receptor (CAR-B), wherein said chimeric receptor comprises the amino acid sequence SEQ ID NO: 142, SEQ ID NO: 144 or both.