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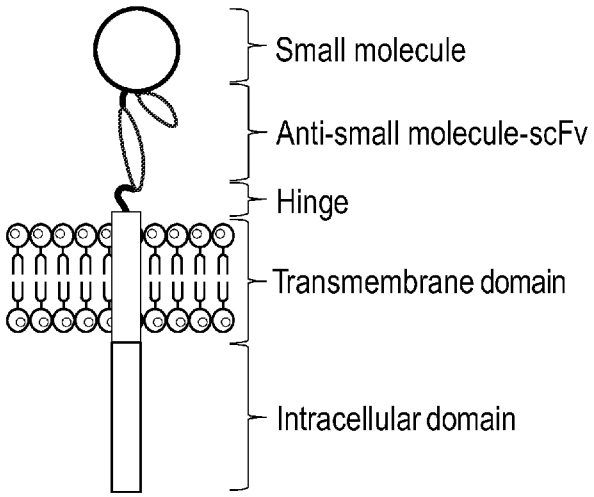
(54) Title: ENGINEERED CYTOKINE RECEPTORS FOR TUNABLE ADOPTIVE CELL THERAPY

FIG. 1A



(57) Abstract: Disclosed are receptor cytokine switches, immune cells containing them, and uses thereof for controllable adoptive cell therapy.

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ENGINEERED CYTOKINE RECEPTORS FOR TUNABLE ADOPTIVE CELL THERAPY

RELATED APPLICATIONS

[0001] This application claims the benefit of priority under 35 U.S.C. § 119(e) to U.S. Provisional Application No: 63/252,850, filed October 6, 2021, which is incorporated herein by reference in its entirety.

GOVERNMENT LICENSE RIGHTS

[0002] This disclosure was made with government support under grant number 1DP1 DK105602-01 awarded by the National Institutes of Health. The government has certain rights in the disclosure.

SEQUENCE LISTING

[0003] The instant application contains a Sequence Listing which has been submitted electronically in XML format and is hereby incorporated by reference in its entirety. Said XML copy, created on August 27, 2022, is named 52095-631001WO_ST.xml and is 69 KB bytes in size.

BACKGROUND OF THE DISCLOSURE

[0004] Immunotherapy using adoptive cell transfer (ACT) aims to stimulate or suppress immunity. One of the most promising approaches of ACT is the administration of antigen-specific T or chimeric antigen receptor-T (CAR T) cells. CAR T cells targeting the B cell antigen CD19 have had remarkable clinical responses in patients with B cell malignancies (Davila and Brentjens, Clin. Adv. Hematol. Oncol. *14(10)*:802–808 (2016); Halim and Maher, Ther. Adv. Vaccines Immunother. *8*:1-17 (2020)). Despite successes in hematological cancers, the effect of CAR-T cells against solid tumors has been limited partly because of the limited persistence, survival, expansion, and the efficacy of CAR-T cells after infusion (Kosti, *et al.*, Front. Immunol. *9*:1104-9 (2018); Jafarzadeh, *et al.*, Front. Immunol. *11*:702-17 (2020).

[0005] Generation of memory T cells is essential for prolonged persistence of CAR T cell therapy. Their formation requires three signals, namely antigen, co-stimulation and pro-inflammatory cytokines. Cytokines have crucial roles in the development, proliferation, survival, and differentiation of various immune cells (Foster, Int. J. Exp. Pathol. *82(3)*:171–

192 (2001)). Cytokine-targeted immunotherapies can modulate immune responses by promoting or inhibiting specific immune-cell functions. Interleukin-2 (IL-2) stimulates T cell development and survival. High-dose IL-2 therapy was approved by the United States Food and Drug Administration (FDA) for treatment of metastatic melanoma and kidney cancer (Jiang, *et al.*, Oncoimmunology *5(6)*:e1163462-10 (2016)). However, systemic administration of cytokines has been largely unsuccessful due to uncontrolled cytokine release syndrome and intolerable toxicity. Therefore, controllable activation of cytokine signaling in specific cells is necessary to prevent systemic toxicity and to improve engineered immune cell-driven efficacy.

SUMMARY OF THE DISCLOSURE

[0006] A first aspect of the present disclosure is directed to a cytokine receptor switch comprising a signal peptide, a single chain antibody fragment (scFv) that specifically binds a synthetic, substantially nonimmunogenic small molecule (hereinafter "synthetic small molecule"), a hinge domain, a transmembrane domain, and an intracellular domain of a cytokine receptor.

[0007] In some embodiments, the signal peptide is native to or derived from a cytokine receptor in which case the signal peptide and the intracellular domain may be native to or derived from different cytokine receptors. In some embodiments, the signal peptide is native to or derived from interleukin-2 receptor alpha chain (IL-2RA), IL-2RB, IL-2RG, IL-4RA, IL-7RA, IL-9R, IL-15RA, or IL-21R. In other embodiments, the signal peptide is native to or derived from cluster of differentiation 8 (CD8).

[0008] In some embodiments, the synthetic small molecule that specifically binds the scFv is a fluorescein (*e.g.*, fluorescein isothiocyanate (FITC)), 4-[(6-methylpyrazin-2-yl) oxy] benzoate (MPOB), anthraquinone-2-carboxylate (AQ), tetraxetan (DOTA), or polyhistidine-tag (His-tag).

[0009] In some embodiments, the hinge domain is native to or derived from CD8.

[0010] In some embodiments, the transmembrane domain and intracellular domain are each independently native to or derived from IL-2RA, IL-2RB, IL-2RG, IL-4RA, IL-7RA, IL-9R, IL-15RA, or IL-21R.

[0011] Another aspect of the present disclosure is directed to nucleic acids encoding the cytokine receptor switches described herein.

[0012] A further aspect is directed to a composition comprising an immune cell that comprises an exogenous nucleic acid encoding the cytokine receptor switch. In some embodiments, the immune cells are T cells such as $CD8^+$ and $CD4^+$ T cells. In other embodiments, the immune cells are NK cells.

[0013] In some embodiments, the immune cell comprises at least two nucleic acid which encode that encode different cytokine receptor switches that comprise different signal peptides from different cytokine receptors, different transmembrane domains, and/or different intracellular domains of the cytokine receptors.

[0014] In some embodiments, the immune cells contain an exogenous nucleic acid that encodes a chimeric antigen receptor (CAR) directed against a cell surface antigen. In some embodiments, the cell surface antigen is CD19, B-cell maturation antigen (BCMA), human epidermal growth factor receptor 2 (HER2), epidermal growth factor receptor (EGFR), mucin 1 (MUC1), or TNF receptor superfamily member 13B (TNFRSF13B).

[0015] In some embodiments, the immune cells comprise a nucleic acid that encodes a binary activated chimeric antigen receptor (BAT-CAR).

[0016] In some aspects, the present disclosure is directed to a method for stimulating an immune cell that comprise an exogenous nucleic acid that encodes the cytokine receptor switch, comprising contacting the immune cells with a sufficient concentration of the synthetic, small molecule, wherein the contacting promotes proliferation of the immune cells. It may also promote a change in phenotype (*e.g.*, memory, cytotoxic, and regulatory phenotypes) of the immune cells.

[0017] In some embodiments, the contacting is conducted *ex vivo*. Immune cells that contain an exogenous nucleic acid encoding the cytokine receptor switch are placed in suitable medium and contacted with an effective concentration of the synthetic small molecule, *e.g.*, from 0.1 to 1000 μg/mL based on total volume of the medium. In some embodiments, the concentration of the synthetic small molecule ranges from 0.1 to 100 μg/mL based on total volume of the medium. The duration of the contact (also referred to herein as "treatment" or "treating") may be in the order of hours, days and weeks (*e.g.*, 1, 2, 3, 4 or more weeks). In some embodiments, the contacting may be conducted in a high-affinity plate, dish, or flask wherein the synthetic small molecule is conjugated to the carrier, which may be polymeric in nature, *e.g.*, bovine serum albumin (BSA) affixed to a surface thereof.

[0018] In some other embodiments, the immune cells are contacted with the synthetic small molecule *in vivo*. In some embodiments, the immune cells are contacted with the synthetic small molecule systemically. In other embodiments, the immune cells are CAR-containing immune cells that due to the binding specificity of the CAR, are directed to specific cell surface antigens, *e.g.*, tumor surface antigens, thus allowing for local stimulation of the immune cells. Further aspects of the present disclosure are directed to methods of treating cancer. In some embodiments, a therapeutically effective concentration of a composition containing the *ex-vivo*-stimulated immune cells are administered to a subject. In other embodiments, the method comprises: administering to a subject in need thereof a therapeutically effective concentration of immune cells; administering to the subject an effective concentration of a synthetic small molecule for a suitable time period, thereby stimulating the immune cells; and decreasing the stimulation of the immune cells by administering to the subject a composition containing synthetic monomeric or polymeric small molecules. To optimize stimulation, the synthetic small molecules are advantageously administered as multimers, *e.g.*, wherein monomers of the small molecule are conjugated to a carrier such as BSA.

[0019] In some embodiments, the cancer is a hematological cancer such as leukemia, lymphoma or multiple myeloma. In some embodiments, the cancer is characterized by the presence of a solid tumor such as breast cancer, ovarian cancer, lung cancer, or brain cancer, *e.g.*, glioblastoma multiforme.

[0020] Methods of stimulating immune cells comprising nucleic acids encoding cytokine receptor switches are known in the art, as described for example, in U.S. Patent 5,747,292 and International Publication Nos. WO/2018/111834 and WO 2019/193197, each of which is incorporated herein by reference in its entirety. Methods are making and stimulating immune cells comprising nucleic acids encoding cytokine receptor switches are also described in Nelson *et al.*, Nature, *369(6478)*:333-336 (1994); Gerhartz *et al.*, J. Biol. Chem., *271*:12991 – 12998 (1996); Sockolosky *et al.*, Science, 2018, *359(6379)*: 1037-1042 (1996); Chang *et al.*, Nat. Chem. Biol. *14(3)*:317-324 (2018), Leung *et al.*, JCI Insight *4(11)*:e124430-18 (2019), and Yang *et al.*, PNAS *118*:e2106612118-12 (2021).

[0021] The inventive cytokine receptor switches differ from known chimeric cytokine receptors. For example, chimeric receptors taught in U.S. Patent 5,747,292 are activated by endogenous proteins which cross-react with natural receptors. The chimeric receptors taught in

WO 2019/193197 are activated by bacterial proteins (*e.g.*, GFP and mCherry) that are not substantially nonimmunogenic (*i.e.*, the bacterial proteins GFP and mCherry are immunogenic).

[0022] The present disclosure provides compositions and methods to direct controllable cytokine receptor signaling to a desired set of immune cells independent of their natural ligands. In contrast with the prior art, the present disclosure requires use of a synthetic small molecule that serves as an artificial ligand and a substitute for the native cytokine, and which is also substantially nonimmunogenic. Therefore, the cytokine receptor switch is under the control of an exogenous moiety, namely the synthetic small molecule, allowing for precise control of activation or stimulation of the cytokine receptor and the ensuing signaling, both *ex vivo* and *in vivo*. Basically, the synthetic small molecule acts as an on/off switch.

[0023] The present disclosure may offer several additional advantages, especially in the context of CAR-T cells. Combinations of different cytokine receptor switches and CARs may pre-condition T cells with memory, effector, or regulatory phenotypes, which may greatly improve persistency and cytotoxicity of the CAR T cells. In addition to controlling their activation or stimulation, the CAR-T cells that contain a cytokine receptor switch may exhibit greater resistance to the immunosuppressive environments characteristic of solid tumor microenvironments. As shown in working examples below, T cell subsets expressing memory markers were increased using compositions of the present disclosure. Use of synthetic small molecules provide additional advantages, particularly with respect to *ex vivo* expansion of CAR-T cells that contain the cytokine receptor switch, namely in terms of cost relative to use of antibodies and recombinant cytokines.

BRIEF DESCRIPTION OF THE DRAWINGS

[0024] FIGs. 1A-1D are images showing structures and primary sequences for inventive cytokine receptor switches. FIG. 1A is an image showing the components of a cytokine receptor switch. A synthetic small molecule serves as an artificial ligand instead of a natural cytokine of the cytokine receptor. FIG. 1B is an image showing systemic and local activations of cytokine receptor switches with small molecules conjugated to nonimmunogenic scaffold or antibodies. Cytokine receptor switches may be combined with a chimeric antigen receptor (CAR). Either the same single chain antibody against a synthetic small molecule (scFv) against the same small molecule (homotargeted) or different scFvs against different small molecules (heterotargeted) were fused in the cytokine receptor switches or CAR. FIG. 1C is an image

showing a representative primary sequence for the inventive cytokine receptor switches. FIG. 1D is an image showing primary sequences for inventive cytokine receptor switches.

[0025] FIG. 2 is an image showing cytokine receptor switches expressed on primary human T cells that were stimulated by fluorescein isothiocyanate (FITC) conjugated to bovine serum albumin (BSA) coated on a culture plate.

[0026] FIGs. 3A-3D are sets of graphs showing that inventive cytokine receptor switches increased effector and central memory markers on T cells with CD3/CD28-costimulation. FIG. 3A is a set of graphs showing that inventive IL2RA- and IL15RA-cytokine receptor switches increased effector memory markers on $CD4^+$ T cells with CD3/CD28-costimulation, and IL2RA-, IL2RB-, IL2RG-, IL7RA-, and IL15RA-cytokine receptor switches increased effector memory markers on $CD8^+$ T cells with CD3/CD28-costimulation. FIG. 3B a set of graphs showing that inventive IL2RA- and IL7RA-cytokine receptor switches increased central memory markers on $CD8^+$ T cells with CD3/CD28-costimulation. FIG. 3C is a set of graphs that show that inventive cytokine receptor switches had little effect on effector memory markers $CD4^+$ and $CD8^+$ T cells without CD3/CD28-costimulation. FIG. 3D is a set of graphs showing that inventive IL7RA-cytokine receptor switch increased central memory markers on $CD4^+$ T cells without CD3/CD28-costimulation.

[0027] FIG. 4 is an image showing the use of inventive cytokine receptor switches with CARs or binary activated T cells comprising nucleic acids encoding chimeric antigen receptors (BAT-CARs).

[0028] FIGs. 5A-5C are a set of graphs showing that BSA-FITC bound on high-affinity plate efficiently stimulated fluorescein-specific BAT-CAR-T cells. FIG. 5A is graph showing the expression of interleukin-2 in BAT-CAR-$CD8^+$ T cells stimulated by BSA-FITC bound on normal tissue culture plate (plate-bound (normal)), BSA-FITC bound on high-affinity plate (plate-bound high affinity), and BSA-FITC solution added to directly cells (free). FIG. 5B is graph showing the expression of interferon gamma $(IFNg)^+$ in BAT-CAR-$CD8^+$ T cells stimulated by BSA-FITC bound on normal tissue culture plate, BSA-FITC bound on high-affinity plate, and BSA-FITC solution added to directly cells. FIG. 5C is graph showing the expression of CD69 on BAT-CAR-$CD8^+$ T cells stimulated by BSA-FITC bound on normal tissue culture plate, BSA-FITC bound on high-affinity plate, and BSA-FITC solution added directly to the cells.

[0029] FIGs. 6A-6B are a set of graphs showing that inventive cytokine receptor switches activated NK cells. FIG. 6A is a line graph showing that the combination of inventive IL15RA-, IL2RB- and IL2RG-cytokine receptor switches promoted cell proliferation of human natural killer (NK) cells, NK92. FIG. 6B is a bar graph showing that the combination of inventive IL7RA- and IL2RG-cytokine receptor switches increased the expression of activation marker CD69 on NK92 cells.

DETAILED DESCRIPTION OF THE DISCLOSURE

[0030] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as is commonly understood by one of skill in art to which the subject matter herein belongs. As used in the specification and the appended claims, unless specified to the contrary, the following terms have the meaning indicated in order to facilitate the understanding of the present disclosure.

[0031] As used in the description and the appended claims, the singular forms "a", "an", and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a composition" includes mixtures of two or more such compositions, reference to "an inhibitor" includes mixtures of two or more such inhibitors, and the like.

[0032] Unless stated otherwise, the term "about" means within 10% (*e.g.*, within 5%, 2% or 1%) of the particular value modified by the term "about."

[0033] The transitional term "comprising," which is synonymous with "including," "containing," or "characterized by," is inclusive or open-ended and does not exclude additional, unrecited elements or method steps. By contrast, the transitional phrase "consisting of" excludes any element, step, or ingredient not specified in the claim. The transitional phrase "consisting essentially of" limits the scope of a claim to the specified materials or steps "and those that do not materially affect the basic and novel characteristic(s)" of the claimed disclosure.

[0034] As used herein, "immune cell" refers to a cell of hematopoietic origin functionally involved in the initiation and/or execution of innate and/or adaptative immune response. Representative examples of immune cells include T cells and natural killer (NK) cells. The term "immune cell" as used herein also refers to cells derived from stem cells. The stem cells can be adult stem cells, non-human embryonic stem cells, more particularly non-human stem

cells, cord blood stem cells, progenitor cells, bone marrow stem cells, induced pluripotent stem cells, totipotent stem cells or hematopoietic stem cells.

[0035] In some embodiments, the immune cells are $CD8^+$ T cells. In some embodiments, the immune cells are $CD4^+$ T cells. In some embodiments, the immune cells are a combination of $CD8^+$ T cells and $CD4^+$ T cells. In some embodiments, the immune cells are NK cells.

[0036] The term "memory phenotype", as used herein, refers to a readiness of the immune cell that comprises an exogenous nucleic acid encoding an inventive cytokine receptor switch to respond to an antigen more quickly than a naïve immune cell (that does not contain the exogenous nucleic acid).

[0037] The term "cytotoxic phenotype" as used herein refers to immune cells that are toxic, *i.e.*, immune cells that induce the death of other cells such as tumor cells, infected cells or cells that are otherwise damaged or dysfunctional. For example, cytotoxic T cells mediate the lysis of target cells (*e.g.*, cancer cells) bearing cognate antigens. Cytotoxic T cells are generally antigen-specific and major histocompatibility complex (MHC)-restricted in that they recognize antigenic peptides only in association with the MHC molecules on the surface of target cells. NK cells target and kill aberrant cells including stressed, virus- or microbe-infected cells or malignant cells.

[0038] The term "antibody" is used herein in the broadest sense and includes polyclonal and monoclonal antibodies, including intact antibodies and functional (antigen-binding) antibody fragments, including fragment antigen binding (Fab) fragments, $F(ab')_2$ fragments, Fab' fragments, Fv fragments, recombinant IgG (rIgG) fragments, variable heavy chain (VH) regions capable of specifically binding the antigen, single chain antibody fragments, including single chain variable fragments (scFv), and single domain antibodies (*e.g.*, sdAb, sdFv, nanobody) fragments. The scFv includes the variable regions of the VH and light chains (VL) of an antibody, and typically includes up to about 50, *e.g.*, about 10 amino acid residues. The linker can either connect the N-terminus of the VH with the C-terminus of the VL, or vice versa. ScFvs can be prepared according to methods known in the art (*see*, *e.g.*, Bird *et al.*, Science *242*:423-426 (1988) and Huston *et al.*, Proc. Natl. Acad. Sci. USA *85*:5879-5883 (1988)). In some embodiments, the linker sequence includes amino acids glycine and serine, and in some cases, sets of glycine and serine repeats such as (Gly4Ser)n, where n is an integer equal to or greater than 1. The length and amino acid composition of the linker may be varied *e.g.*, to achieve optimal folding and interaction between the VH and VL to create a functional

epitope. *See*, *e.g.*, Hollinger *et al.*, Proc. Natl. Acad. Sci. U.S.A. *90*:6444-6448 (1993). The term "antibody" encompasses genetically engineered and/or otherwise modified forms of immunoglobulins, such as intrabodies, peptibodies, chimeric antibodies, fully human antibodies, humanized antibodies, and heteroconjugated antibodies, multispecific, *e.g.*, bispecific, antibodies, nanobodies, diabodies, triabodies, and tetrabodies, tandem di-scFv, tandem tri-scFv. Unless otherwise stated, the term "antibody" should be understood to encompass functional antibody fragments thereof. The term also encompasses intact or full-length antibodies, including antibodies of any class or sub-class, including IgG and sub-classes thereof, IgM, IgE, IgA, and IgD.

[0039] As used herein, "synthetic substantially nonimmunogenic small molecule" refers to an organic molecule or compound that is monofuctional and that ranges in size from about 50 to about 10,000 daltons, usually from about 50 to about 5,000 daltons and more usually from about 100 to about 1000 daltons that can bind an scFv on the inventive cytokine receptor switches without provoking a significant immune response in the subject.

[0040] Examples of substantially nonimmunogenic small molecules as used herein include fluorescein and fluorescein derivatives (*e.g.*, fluorescein isothiocyanate (FITC)), 4-[(6-methylpyrazin-2-yl) oxy] benzoate (MPOB), anthraquinone-2-carboxylate (AQ), tetraxetan (DOTA), or polyhistidine-tag (His-tag). A "significant immune" response is any immune response that would limit or restrict the *in vivo* utility of the synthetic small molecule as used in accordance with the teachings of the present disclosure. A detectable immune response is not necessarily a "significant immune response." That is, "substantially nonimmunogenic" embraces immune responses that are detectable but not significant. In some embodiments, the substantially nonimmunogenic small molecule is conjugated to a carrier.

[0041] With respect to synthetic small molecules described herein, and to the extent the following terms are used herein to further describe them, the following definitions apply.

[0042] The term "specific binding" as it relates to interaction between the synthetic small molecule and the single chain antibody fragment (scFv) refers to an inter-molecular interaction that is substantially specific in that binding of the synthetic small molecule with other endogenous entities (proteinaceous and non-proteinaceous alike), although detectable, may be functionally insignificant.

[0043] As used herein in the context of elements of the cytokine receptor switch, CARs and BAT-CARs, the term "derived from" (also referred to as "derived from, *e.g.*, native to")

embraces an element having a at least a portion of a sequence identical to the sequence of that element in a native gene, *e.g.*, a signal peptide natively associated with an IL-2RA receptor (*e.g.*, a signal peptide "native to" an IL-2RA receptor), and elements that have non-naturally occurring sequences that differ from a native sequence in terms of at least one modification such as an amino acid substitution, addition (*e.g.*, at either or both termini) or deletion (*e.g.*, "derived from"), provided that the modification does not compromise the function that the element performs as part of the cytokine receptor switch. In some embodiments, an element of a cytokine receptor switch, CAR, and BAT-CAR derived from a protein includes a sequence a portion of a native gene that has one or more alterations from the native gene or may be identical to the native gene for a range of amino acids or nucleic acids but has alterations, substitions, or deleteions outside of the range of identical sequence.

[0044] A used herein the term "sufficient concentration" refers to the concentration of a synthetic small molecule needed to activate a cytokine switch in the context in which the synthetic small molecule is being used (*e.g.*, *in vitro,* or *in vivo*).

Cytokine receptor switches

[0045] A key aspect of the present disclosure is directed to cytokine receptor switch comprising a signal peptide, *e.g.*, which is native to or derived from cytokine receptor, a single chain antibody fragment (scFv) that specifically binds a synthetic small molecule, a hinge domain, a transmembrane domain, and an intracellular domain of a cytokine receptor (FIG. 1A, FIG. 1C, and FIG. 1D). DNA and amino acid sequences of representative components of cytokine receptor switches described herein are set forth in Table 1.

Table 1

Domain	DNA Sequence	Amino Acid Sequence
IL2RA signal peptide	ATGGACAGCTACCTGCTGATGTGG GGCCTGCTGACCTTCATCATGGTG CCTGGCTGTCAGGCC (SEQ ID NO: 1)	MDSYLLMWGLLTFIMV PGCQA (SEQ ID NO: 2)
IL2RB signal peptide	ATGGCTGCTCCAGCTCTGTCTTGGA GACTGCCCCTGCTGATTCTGCTGCT GCCTCTGGCTACATCTTGGGCCTCT GCC (SEQ ID NO: 3)	MAAPALSWRLPLLILLL PLATSWASA (SEQ ID NO: 4)
IL2RG signal peptide	ATGCTGAAGCCCAGCCTGCCTTTT ACCAGCCTGCTGTTCCTGCAGCTG CCTCTGCT TGGCGTGGGA (SEQ ID NO: 5)	MLKPSLPFTSLLFLQLPL LGVG (SEQ ID NO: 6)

Domain	DNA Sequence	Amino Acid Sequence
IL4RA signal peptide	ATGGGGTGGCTTTGCTCTGGGCTC CTGTTCCCTGTGCTGCCTGGTCCTG CTGCAGGTGGCAAGCTCTGGGAAC (SEQ ID NO: 7)	MGWLCSGLLFPVSCLV LLQVASSGN (SEQ ID NO: 8)
IL7RA signal peptide	ATGACAATCCTGGGCACCACCTTC GGCATGGTGTTCAGTCTGCTGCAG GTCGTG TCTGGC (SEQ ID NO: 9)	MTILGTTFGMVFSLLQV VSG (SEQ ID NO: 10)
IL9R signal peptide	ATGGGACTGGGCAGATGCATCTGG GAAGGCTGGACCTTGGAGAGTGAG GCCCTGAGGCGAGACATGGGCACT GGCTCCTGGCCTGCATCTGCATCT GCACCTGTGTCTGCTTGGGAGTC (SEQ ID NO: 11)	MGLGRCIWEGWTLESE ALRRDMGTWLLACICIC TCVCLGV (SEQ ID NO: 12)
IL15RA signal peptide	ATGGCTCCTCGGAGAGCCAGAGGC TGTAGAACACTTGGACTGCCCGCT CTGCTGCTGCTCCTGCTTCTTAGAC CTCCTGCCACAAGAGGC (SEQ ID NO: 13)	MAPRRARGCRTLGLPA LLLLLLLRPPATRG (SEQ ID NO: 14)
IL21R signal peptide	ATGCCAAGAGGATGGGCCGCTCCT CTTCTCCTGTTGCTGCTTCAAGGCG GCTGG GGC (SEQ ID NO: 15)	MPRGWAAPLLLLLLQG GWG (SEQ ID NO: 16)
IL2RA transmembrane domain	GTGGCCGTGGCCGGATGTGTGTTT CTGCTGATCTCTGTGCTGCTGCTGA GCGGC CTG (SEQ ID NO: 17)	VAVAGCVFLLISVLLLS GL (SEQ ID NO: 18)
IL2RB transmembrane domain	ATCCCCTGGCTGGGACATCTGCTT GTTGGACTGTCTGGCGCCTTCGGCT TCATCATCCTGGTGTATCTGCTGAT C (SEQ ID NO: 19)	IPWLGHLLVGLSGAFGF IILVYLLI (SEQ ID NO: 20)
IL2RG transmembrane domain	GTGGTCATCTCTGTGGGCTCTATGG GCCTGATCATCTCCCTGCTGTGTGT GTACT TTTGGCTG (SEQ ID NO: 21)	VVISVGSMGLIISLLCVY FWL (SEQ ID NO: 22)
IL4RA transmembrane domain	CTCCTGCTGGGCGTCAGCGTTTCCT GCATTGTCATCCTGGCCGTCTGCCT GTTGTGCTATGTCAGCATCACC (SEQ ID NO: 23)	LLLGVSVSCIVILAVCLL CYVSIT (SEQ ID NO: 24)
IL7RA transmembrane domain	CCCATCCTGCTGACAATCAGCATCC TGAGCTTTTTCAGCGTGGCCCTGCT GGTCATCCTGGCCTGTGTGCTGTGG (SEQ ID NO: 25)	PILLTISILSFFSVALLVIL ACVLW (SEQ ID NO: 26)
IL9R transmembrane domain	GGCAACACCCTTGTTGCTGTGTCC ATCTTTCTCCTGCTGACTGGCCCGA CCTACCTCCTGTTC (SEQ ID NO: 27)	GNTLVAVSIFLLLTGPT YLLF (SEQ ID NO: 28)
IL15RA transmembrane domain	GTGGCCATCAGCACAAGCACCGTT CTGCTGTGTGGCCTGTCAGCCGTTA GCCTGC TGGCTTGCTACCTG (SEQ ID NO: 29)	VAISTSTVLLCGLSAVS LLACYL (SEQ ID NO: 30)

Domain	DNA Sequence	Amino Acid Sequence
IL21R transmembrane domain	GGCTGGAATCCTCATCTGTTGTTGT TGCTCCTCCTGGTCATCGTGTTCAT CCCCGC CTTTTGG (SEQ ID NO: 31)	GWNPHLLLLLLLVIVFIP AFW (SEQ ID NO: 32)
IL2RA intracellular domain	ACTTGGCAAAGACGGCAGAGAAA GAGCCGGCGGACCATC (SEQ ID NO: 33)	TWQRRQRKSRRTI (SEQ ID NO: 34)
IL2RB intracellular domain	AACTGCCGGAACACAGGCCCTTGG CTGAAGAAAGTGCTGAAGTGCAAC ACCCCTGATCCGAGCAAGTTCTTTA GCCAGCTGAGCAGCGAGCATGGCG GCGACGTTCAGAAATGGCTGTCTA GCCCATTTCCTAGCAGCAGCTTCAG CCCAGGTGGACTGGCCCCTGAGAT TAGCCCTCTGGAAGTGCTGGAACG GGACAAAGTGACCCAGCTGCTCCT CCAGCAGGATAAGGTGCCAGAACC TGCCAGCCTGTCCAGCAATCACAG CCTGACCAGCTGCTTTACCAACCA GGGCTACTTCTTCTTCCATCTGCCT GACGCTCTGGAAATCGAGGCCTGC CAGGTGTACTTCACCTACGATCCCT ACAGCGAAGAGGACCCCGATGAAG GTGTTGCTGGCGCCCCTACAGGAT CTTCTCCACAGCCTCTGCAACCTCT GAGCGGCGAGGATGATGCCTACTG CACCTTTCCAAGCAGGGACGACCT GCTCCTGTTCAGCCCATCTCTGCTC GGAGGACCATCTCCTCCATCTACA GCTCCAGGCGGATCTGGCGCTGGC GAGGAAAGAATGCCACCTAGCCTG CAAGAGCGGGTGCCCAGAGATTGG GATCCTCAACCTCTCGGCCCTCCAA CACCTGGCGTGCCAGATCTCGTGG ACTTTCAGCCTCCTCCAGAGCTGGT GCTGAGAGAAGCTGGCGAAGAAGT GCCAGACGCTGGCCCTAGAGAGGG CGTTAGCTTTCCTTGGAGCAGACCT CCTGGACAGGGCGAGTTTAGGGCC CTGAATGCAAGACTGCCTCTGAAC ACCGACGCCTACCTGTCTCTGCAA GAACTGCAGGGACAAGACCCCACA CACCTGGTG (SEQ ID NO: 35)	NCRNTGPWLKKVLKCN TPDPSKFFSQLSSEHGG DVQKWLSSPFPSSSFSP GGLAPEISPLEVLERDK VTQLLLQQDKVPEPASL SSNHSLTSCFTNQGYFF FHLPDALEIEACQVYFT YDPYSEEDPDEGVAGA PTGSSPQPLQPLSGEDD AYCTFPSRDDLLLFSPSL LGGPSPPSTAPGGSGAG EERMPPSLQERVPRDW DPQPLGPPTPGVPDLVD FQPPPELVLREAGEEVP DAGPREGVSFPWSRPPG QGEFRALNARLPLNTD AYLSLQELQGQDPTHL V (SEQ ID NO: 36)
IL2RG intracellular domain	GAACGGACCATGCCTCGGATCCCC ACACTGAAGAACCTGGAAGATCTG GTCACCGAGTACCACGGCAACTTC AGTGCTTGGAGCGGCGTGTCAAAA	ERTMPRIPTLKNLEDLV TEYHGNFSAWSGVSKG LAESLQPDYSERLCLVS EIPPKGGALGEGPGASP

Domain	DNA Sequence	Amino Acid Sequence
	GGACTGGCCGAAAGCCTGCAGCCT GACTACTCCGAGAGACTGTGCCTG GTGTCTGAGATCCCTCCTAAAGGC GGCGCTCTCGGAGAAGGACCTGGT GCCTCTCCATGCAATCAGCACAGC CCTTATTGGGCCCCTCCTTGCTACA CCCTGAAACCTGAGACA (SEQ ID NO: 37)	CNQHSPYWAPPCYTLK PET (SEQ ID NO: 38)
IL4RA intracellular domain	AAGATTAAGAAAGAATGGTGGGAT CAGATTCCCAACCCAGCCCGCAGC CGCCTCGTGGCTATAATAATCCAG GATGCTCAGGGGTCACAGTGGGAG AAGCGGTCCCGAGGCCAGGAACCA GCCAAGTGCCCACACTGGAAGAAT TGTCTTACCAAGCTCTTGCCCTGTT TTCTGGAGCACAACATGAAAAGGG ATGAAGATCCTCACAAGGCTGCCA AAGAGATGCCTTTCCAGGGCTCTG GAAAATCAGCATGGTGCCCAGTGG AGATCAGCAAGACAGTCCTCTGGC CAGAGAGCATCAGCGTGGTGCGAT GTGTGGAGTTGTTTGAGGCCCCGG TGGAGTGTGAGGAGGAGGAGGAG GTAGAGGAAGAAAAAGGGAGCTT CTGTGCATCGCCTGAGAGCAGCAG GGATGACTTCCAGGAGGGAAGGG AGGGCATTGTGGCCCGGCTAACAG AGAGCCTGTTCCTGGACCTGCTCG GAGAGGAGAATGGGGGCTTTTGCC AGCAGGACATGGGGGAGTCATGCC TTCTTCCACCTTCGGGAAGTACGA GTGCTCACATGCCCTGGGATGAGT TCCCAAGTGCAGGGCCCAAGGAGG CACCTCCCTGGGGCAAGGAGCAGC CTCTCCACCTGGAGCCAAGTCCTC CTGCCAGCCCGACCCAGAGTCCAG ACAACCTGACTTGCACAGAGACGC CCCTCGTCATCGCAGGCAACCCTG CTTACCGCAGCTTCAGCAACTCCCT GAGCCAGTCACCGTGTCCCAGAGA GCTGGGTCCAGACCCACTGCTGGC CAGACACCTGGAGGAAGTAGAACC CGAGATGCCCTGTGTCCCCCAGCT CTCTGAGCCAACCACTGTGCCCCA ACCTGAGCCAGAAACCTGGGAGCA GATCCTCCGCCGAAATGTCCTCCA GCATGGGGCAGCTGCAGCCCCCGT	KIKKEWWDQIPNPARSR LVAIIIQDAQGSQWEKR SRGQEPAKCPHWKNCL TKLLPCFLEHNMKRDE DPHKAAKEMPFQGSGK SAWCPVEISKTVLWPESI SVVRCVELFEAPVECEE EEEVEEEKGSFCASPESS RDDFQEGREGIVARLTE SLFLDLLGEENGGFCQQ DMGESCLLPPSGSTSAH MPWDEFPSAGPKEAPP WGKEQPLHLEPSPPASP TQSPDNLTCTETPLVIAG NPAYRSFSNSLSQSPCPR ELGPDPLLARHLEEVEP EMPCVPQLSEPTTVPQP EPETWEQILRRNVLQHG AAAAPVSAPTSGYQEFV HAVEQGGTQASAVVGL GPPGEAGYKAFSSLLAS SAVSPEKCGFGASSGEE GYKPFQDLIPGCPGDPA PVPVPLFTFGLDREPPRS PQSSHLPSSSPEHLGLEP GEKVEDMPKPPLPQEQ ATDPLVDSLGSGIVYSA LTCHLCGHLKQCHGQE DGGQTPVMASPCCGCC CGDRSSPPTTPLRAPDPS PGGVPLEASLCPASLAPS GISEKSKSSSSFHPAPGN AQSSSQTPKIVNFVSVGP TYMRVS (SEQ ID NO: 40)

Domain	DNA Sequence	Amino Acid Sequence
	CTCGGCCCCCACCAGTGGCTATCA GGAGTTTGTACATGCGGTGGAGCA GGGTGGCACCCAGGCCAGTGCGGT GGTGGGCTTGGGTCCCCCAGGAGA GGCTGGTTACAAGGCCTTCTCAAG CCTGCTTGCCAGCAGTGCTGTGTCC CCAGAGAAATGTGGGTTTGGGGCT AGCAGTGGGGAAGAGGGGTATAA GCCTTTCCAAGACCTCATTCCTGGC TGCCCTGGGGACCCTGCCCCAGTC CCTGTCCCCTTGTTCACCTTTGGAC TGGACAGGGAGCCACCTCGCAGTC CGCAGAGCTCACATCTCCCAAGCA GCTCCCCAGAGCACCTGGGTCTGG AGCCGGGGGAAAAGGTAGAGGAC ATGCCAAAGCCCCCACTTCCCCAG GAGCAGGCCACAGACCCCCTTGTG GACAGCCTGGGCAGTGGCATTGTC TACTCAGCCCTTACCTGCCACCTGT GCGGCCACCTGAAACAGTGTCATG GCCAGGAGGATGGTGGCCAGACCC CTGTCATGGCCAGTCCTTGCTGTGG CTGCTGCTGTGGAGACAGGTCCTC GCCCCCTACAACCCCCCTGAGGGC CCCAGACCCCTCTCCAGGTGGGGT TCCACTGGAGGCCAGTCTGTGTCC GGCCTCCCTGGCACCCTCGGGCAT CTCAGAGAAGAGTAAATCCTCATC ATCCTTCCATCCTGCCCCTGGCAAT GCTCAGAGCTCAAGCCAGACCCCC AAAATCGTGAACTTTGTCTCCGTG GGACCCACATACATGAGGGTCTCT (SEQ ID NO: 39)	
IL7RA intracellular domain	AAGAAGCGGATCAAGCCCATCGTG TGGCCCAGCCTGCCTGACCACAAG AAAACCCTGGAACACCTGTGCAAG AAGCCCCGGAAGAACCTGAACGTG TCCTTCAATCCCGAGAGCTTCCTGG ACTGCCAGATCCACAGAGTGGACG ACATCCAGGCCAGGGACGAAGTGG AAGGCTTTCTGCAGGACACATTCC CTCAGCAGCTGGAAGAGAGCGAGA AGCAGAGACTCGGAGGCGACGTGC AGAGCCCTAATTGCCCTTCTGAGG ACGTCGTGATCACCCCTGAGAGCT TCGGCAGAGATAGCAGCCTGACAT GTCTGGCCGGCAATGTGTCCGCCT	KKRIKPIVWPSLPDHKK TLEHLCKKPRKNLNVSF NPESFLDCQIHRVDDIQ ARDEVEGFLQDTFPQQL EESEKQRLGGDVQSPNC PSEDVVITPESFGRDSSL TCLAGNVSACDAPILSSS RSLDCRESGKNGPHVY QDLLLSLGTTNSTLPPPF SLQSGILTLNPVAQGQPI LTSLGSNQEEAYVTMSS FYQNQ (SEQ ID NO: 42)

Domain	DNA Sequence	Amino Acid Sequence
	GTGATGCCCCTATCCTGAGCAGCA GCAGAAGCCTGGATTGCAGAGAGA GCGGCAAGAACGGCCCTCACGTGT ACCAGGATCTGCTCCTGAGCCTGG GAACCACCAATAGCACACTGCCTC CACCATTCAGCCTGCAGAGCGGCA TCCTGACACTGAACCCTGTTGCTCA GGGCCAGCCAATCCTGACCAGCCT GGGCAGCAATCAAGAAGAGGCCTA CGTCACCATGAGCAGCTTCTACCA GAACCAG (SEQ ID NO: 41)	
IL9R intracellular domain	AAGCTGTCACCTAGAGTGAAGCGC ATATTCTATCAGAATGTGCCCAGC CCTGCGATGTTCTTCCAGCCATTGT ATAGCGTACACAATGGCAATTTCC AGACCTGGATGGGCGCCCACGGAG CTGGTGTTTTGTTGTCTCAAGATTG TGCAGGGACGCCCCAAGGCGCGTT GGAGCCTTGTGTCCAGGAAGCAAC CGCTCTTCTCACATGTGGCCCTGCT AGGCCATGGAAAAGCGTGGCTTTG GAGGAGGAGCAAGAGGGCCCAGG CACGAGACTTCCAGGAAACCTCTC CAGCGAGGACGTTCTGCCCGCTGG ATGCACAGAATGGCGAGTGCAGAC GCTGGCTTATTTGCCTCAGGAGGA CTGGGCCCCAACAAGCCTTACCAG ACCCGCGCCACCAGATTCAGAGGG AAGCCGATCCAGTTCTAGCTCCTC ATCAAGCAACAATAACAATTATTG CGCTCTGGGATGTTATGGTGGATG GCACTTGAGCGCGTTGCCAGGCAA CACGCAATCATCTGGTCCCATACC CGCGCTTGCATGCGGACTGAGTTG CGACCATCAGGGTCTCGAAACTCA GCAAGGTGTTGCGTGGGTCCTGGC GGGTCATTGCCAAAGACCTGGCCT GCACGAGGATCTTCAGGGAATGCT TTTGCCAAGTGTGCTCTCCAAGGCT CGATCTTGGACGTTC (SEQ ID NO: 43)	KLSPRVKRIFYQNVPSP AMFFQPLYSVHNGNFQ TWMGAHGAGVLLSQD CAGTPQGALEPCVQEA TALLTCGPARPWKSVA LEEEQEGPGTRLPGNLS SEDVLPAGCTEWRVQT LAYLPQEDWAPTSLTRP APPDSEGSRSSSSSSSSN NNNYCALGCYGGWHL SALPGNTQSSGPIPALAC GLSCDHQGLETQQGVA WVLAGHCQRPGLHEDL QGMLLPSVLSKARSWT F (SEQ ID NO: 44)
IL15RA intracellular domain	AAGTCCAGACAGACACCTCCTCTG GCCAGCGTGGAAATGGAAGCCATG GAAGCTCTGCCAGTGACCTGGGGC ACCTCCAGCAGAGATGAGGATCTG GAAAACTGCAGCCACCACCTGTGA TGA (SEQ ID NO: 45)	KSRQTPPLASVEMEAM EALPVTWGTSSRDEDLE NCSHHL (SEQ ID NO: 46)

Domain	DNA Sequence	Amino Acid Sequence
IL21R intracellular domain	AGCCTGAAAACACACCCTCTGTGGC GGCTGTGGAAGAAAATCTGGGCCG TGCCATCTCCTGAGCGGTTCTTCAT GCCTCTGTACAAGGGCTGCAGCGG CGACTTCAAGAAATGGGTCGGAGC CCCTTTTACCGGCAGCTCTCTGGAA CTTGGACCTTGGAGCCCTGAAGTGC CCAGCACACTGGAAGTGTACAGCT GTCACCCTCCTAGAAGCCCCGCCAA GAGACTGCAGCTCACAGAGCTGCA AGAGCCTGCCGAGCTGGTGGAATC TGATGGCGTGCCCAAGCCTAGCTTC TGGCCCACCGCTCAAAATTCTGGCG GCAGCGCCTACAGCGAGGAAAGAG ATAGACCTTACGGCCTGGTGTCCAT CGACACCGTGACAGTGCTGGATGC CGAGGGACCTTGTACCTGGCCTTGT AGCTGCGAGGACGATGGCTACCCT GCTCTGGATCTGGACGCAGGACTG GAACCTTCTCCAGGCCTCGAAGATC CTCTGCTGGACGCCGGAACAACAG TGCTGTCTTGTGGCTGTGTGTCCGC CGGATCTCCTGGACTTGGAGGACCT CTGGGAAGCCTGCTGGACAGACTG AAACCTCCTCTGGCCGATGGCGAA GATTGGGCTGGTGGACTTCCTTGGG GCGGAAGATCTCCAGGCGGAGTGT CTGAATCTGAGGCCGGTTCTCCACT GGCCGGCCTGGATATGGATACCTTC GATAGCGGCTTCGTGGGCAGCGATT GCAGCAGCCCTGTGGAATGCGACTT CACATCTCCTGGCGACGAGGGCCC ACCTAGAAGCTATCTCAGACAGTG GTCGTGATCCCTCCACCTCTGTCTA GTCCTGGACCACAGGCCAGC (SEQ ID NO: 47)	SLKTHPLWRLWKKIWA VPSPERFFMPLYKGCSG DFKKWVGAPFTGSSLEL GPWSPEVPSTLEVYSCH PPRSPAKRLQLTELQEP AELVESDGVPKPSFWPT AQNSGGSAYSEERDRP YGLVSIDTVTVLDAEGP CTWPCSCEDDGYPALD LDAGLEPSPGLEDPLLD AGTTVLSCGCVSAGSPG LGGPLGSLLDRLKPPLA DGEDWAGGLPWGGRSP GGVSESEAGSPLAGLD MDTFDSGFVGSDCSSPV ECDFTSPGDEGPPRSYL RQWVVIPPPLSSPGPQA S (SEQ ID NO: 48)
CD8 hinge	ACAACAACCCCTGCTCCTCGGCCTC CTACACCAGCTCCTACAATTGCCA GCCAGCCACTGTCTCTGAGGCCCG AAGCTTGTAGACCTGCTGCAGGCG GAGCCGTGCATACAAGAGGACTGG ACTTCGCCTGTGAC (SEQ ID NO: 49)	TTTPAPRPPTPAPTIASQ PLSLRPEACRPAAGGAV HTRGLDFACD (SEQ ID NO: 50)
anti-fluorescein scFv 4M5.4	GACGTGGTCATGACACAGACCCCT CTGTCTCTGCCTGTGTCTCTGGGAG ATCAGGCCAGCATCAGCTGCAGAT CTAGCCAGAGCCTGGTGCACAGCA	DVVMTQTPLSLPVSLGD QASISCRSSQSLVHSNG NTYLRWYLQKPGQSPK VLIYKVSNRVSGVPDRF

Domain	DNA Sequence	Amino Acid Sequence
	ACGGCAACACCTACCTGCGGTGGT ATCTGCAGAAGCCCGGCCAGTCTC CTAAGGTGCTGATCTACAAGGTGT CCAACAGAGTGTCCGGCGTGCCCG ATAGATTTTCTGGCAGCGGCTCTG GCACCGACTTCACCCTGAAGATCA ATAGAGTGGAAGCCGAGGACCTGG GCGTGTACTTCTGTAGCCAGTCTAC CCACGTGCCATGGACCTTTGGCGG CGGAACAAAGCTGGAAATCAAGA GCAGCGCCGACGACGCCAAGAAG GACGCCGCTAAGAAGGATGACGCC AAAAAAGACGATGCCAAAAAGGA TGGCGGCGTGAAGCTGGACGAAAC AGGCGGAGGACTTGTTCAGCCTGG CGGAGCCATGAAGCTGAGCTGTGT GACCAGCGGCTTCACCTTCGGCCA CTACTGGATGAACTGGGTCCGACA GAGCCCTGAGAAAGGCCTGGAATG GGTCGCCCAGTTCAGAAACAAGCC CTACAACTACGAAACCTACTACAG CGACAGCGTGAAGGGCAGATTCAC CATCAGCCGGGACGACAGCAAGTC CAGCGTGTACCTGCAGATGAACAA CCTGCGCGTGGAAGATACCGGCAT CTACTACTGTACCGGCGCCAGCTA CGGCATGGAATATCTCGGCCAGGG CACCAGCGTGACCGTGTCT (SEQ ID NO: 51)	SGSGSGTDFTLKINRVE AEDLGVYFCSQSTHVP WTFGGGTKLEIKSSADD AKKDAAKKDDAKKDD AKKDGGVKLDETGGGL VQPGGAMKLSCVTSGF TFGHYWMNWVRQSPE KGLEWVAQFRNKPYNY ETYYSDSVKGRFTISRD DSKSSVYLQMNNLRVE DTGIYYCTGASYGMEY LGQGTSVTVS (SEQ ID NO: 52)
anti-MPOB scFv	CAAGTGCGGCTGCAAGAGTCTGGA CCTAGCCTGGTCAAGCCCAGCCAG ACACTGAGCCTGACCTGTACCGTG TCCGGCTTCAGCCTGACCAACTAC TATGTCGGCTGGGTCCGACAGGCC CCTGGAAAAGCTCTTGAGTGGGTC GGAGTGATGGATAGCGGCGGAGG CACCTACTACAACCCCGCTCTGAA AAGCCGGCTGAGCATCACCAGAGA CACCAGCAAGTCTCAGGTGTCCCT GAGCCTGTCTAGCGTGACCACAGA GGATACCGCCGTGTACTACTGCGC CAGCTTCAGCTTCGGCAGAGACTG GAATTATTGGGGCCCTGGCCTGCT GCTGACAATCCCTTCTGAGGGCAA GTCTAGCGGCAGCGGCTCTGAGAG CAAGGTGGACAGCTATGAGCTGAC CCAGCCTAGCAGCGTGTCCAGATC	QVRLQESGPSLVKPSQT LSLTCTVSGFSLTNYYV GWVRQAPGKALEWVG VMDSGGGTYYNPALKS RLSITRDTSKSQVSLSLS SVTTEDTAVYYCASFSF GRDWNYWGPGLLLTIP SEGKSSGSGSESKVDSY ELTQPSSVSRSLGQSVSI TCSGSSSNVGYGNYVG WFQQVPGSAPKLLIYD ATSRASGVPDRFSGSRS SNTATLTISSLQAEDEA DYYCAHWDSSANIAIFG SGSRLTVLG (SEQ ID NO: 54)

Domain	DNA Sequence	Amino Acid Sequence
	TCTGGGCCAGTCCGTGTCCATCAC CTGTAGCGGAAGCAGCAGCAATGT CGGCTACGGCAATTACGTCGGCTG GTTCCAGCAGGTCCCAGGCTCTGC TCCTAAGCTGCTGATCTACGACGC CACCTCTAGAGCCAGCGGCGTGCC AGATAGATTCAGCGGCAGCAGAAG CAGCAACACCGCCACACTGACAAT CAGCAGTCTGCAGGCCGAGGACGA GGCCGATTACTATTGTGCCCACTG GGACAGCAGCGCCAATATCGCCAT CTTTGGCTCCGGCAGCAGGCTGAC AGTTCTGGGA (SEQ ID NO: 53)	
anti-Anthraquione scFv	CAAGTTCGGCTGCAAGGCTCTGGA CCTAGCCTGGTCAAGCCTAGCCAG ACACTGAGCCTGACCTGTACCGTG TCCGGCTTCAGCCTGACAAGCAAC GCTGTGGACTGGGTCCGACAGGCT CCTGGAAAAGTGCCTGAGTGGCTG GGCTTCATCAGAGGCGGCGGAAGC ACCTTCTACAACAGCGCCCTGAAG TCCAGACTGAGCATCACCAGAGAC ACCAGCAAGAGCCAGGTGTCCCTG AGCCTGTCTAGCGTGACCACAGAG GACACCGCCGTGTACTACTGTGCC AGAGCCAGCTGTAGCGGCGACATC TACACAGACACCTGTGGCATCGAC TACTGGGGCCCTGGACTGCTGGTT ACCGTTAGCTCTGAGGGCAAGTCT AGCGGCAGCGGAAGCGAGAGCAA AGTGGACCAGTCTGCCCTGACACA GCCTAGCAGCGTGTCCAGATCTCT GGGCCAGTCCGTGTCCATCACCTG TTCTGGCAGCAGCTCTAACGTCGG CGCTGGCAACTACGTGAACTGGTT CAGACTGATCCCTGGCAGCGCCCC TAAGAGCCTGATCTACGCTGCCAC AACAAGAGCCTCTGGCGTGCCCGA TAGATTCAGCGGCTCTAGAAGCGG CAACACCGCCACACTGACAATCAG CAGTCTGCAGGCCGAGGACGAGGC CGACTACTACTGTAGCAGCTACGA CATCACAGCCGTGAACCTGTTCGG CTCCGGCACCAGACTGACAGTTCT TGGA (SEQ ID NO: 55)	QVRLQGSGPSLVKPSQT LSLTCTVSGFSLTSNAV DWVRQAPGKVPEWLG FIRGGGSTFYNSALKSR LSITRDTSKSQVSLSLSS VTTEDTAVYYCARASC SGDIYTDTCGIDYWGPG LLVTVSSEGKSSGSGSE SKVDQSALTQPSSVSRS LGQSVSITCSGSSSNVG AGNYVNWFRLIPGSAP KSLIYAATTRASGVPDR FSGSRSGNTATLTISSLQ AEDEADYYCSSYDITAV NLFGSGTRLTVLG (SEQ ID NO: 56)
anti-DOTA scFv	GCCTCTCACGTGAAGCTGCAAGAG TCTGGACCTGGCCTGGTGCAGCCT	ASHVKLQESGPGLVQPS QSLSLTCTVSGFSLTDY

Domain	DNA Sequence	Amino Acid Sequence
	AGCCAAAGCCTGTCTCTGACCTGT ACCGTGTCCGGCTTCAGCCTGACA GATTACGGCGTGCACTGGGTCCGA CAGAGCCCTGGAAAAGGACTGGA ATGGCTGGGAGTGATTTGGAGCGG CGGAGGCACAGCCTATAACACAGC CCTGATCAGCAGACTGAACATCTA CCGGGACAACAGCAAGAACCAGG TGTTCCTGGAAATGAACTCCCTGC AGGCCGAGGACACCGCCATGTACT ACTGTGCCAGAAGAGGCAGCTACC CCTACAACTACTTCGACGCCTGGG GCTGTGGCACCACCGTGACAGTTT CTAGCGGAGGCGGAGGATCTGGTG GCGGAGGTAGTGGTGGCGGTGGAT CTCAGGCCGTGGTCATCCAAGAAA GCGCCCTGACAACACCTCCTGGCG AGACAGTGACACTGACCTGTGGAT CTTCTACAGGCGCCGTGACCGCCA GCAACTACGCCAATTGGGTGCAAG AGAAGCCCGACCACTGCTTCACAG GCCTGATCGGCGGCCACAACAATA GACCTCCAGGCGTGCCAGCCAGAT TCAGCGGATCTCTGATCGGAGACA AGGCCGCTCTGACAATCGCCGGCA CACAGACAGAGGACGAGGCCATCT ACTTTTGCGCCCTGTGGTACAGCG ACCACTGGGTTATCGGCGGAGGAA CCAGACTGACAGTGCTCGGCGGAT CTGAGCAGAAGCTGATCTCCGAAG AGGACCTG (SEQ ID NO: 57)	GVHWVRQSPGKGLEW LGVIWSGGGTAYNTALI SRLNIYRDNSKNQVFLE MNSLQAEDTAMYYCA RRGSYPYNYFDAWGCG TTVTVSSGGGGSGGGG SGGGGSQAVVIQESALT TPPGETVTLTCGSSTGA VTASNYANWVQEKPD HCFTGLIGGHNNRPPGV PARFSGSLIGDKAALTIA GTQTEDEAIYFCALWYS DHWVIGGGTRLTVLGG SE QKLISEEDL (SEQ ID NO: 58)

[0046] The signal peptide directs the nascent protein into the endoplasmic reticulum. In some embodiments, the signal peptide contained in the cytokine receptor switch is native to or derived from an interleukin-2 receptor alpha chain (IL-2RA), IL-2RB, IL-2RG, IL-4RA, IL-7RA, IL-9R, IL-15RA, or IL-21R. In some embodiments, the signal peptide is native to CD8, *i.e.*, a CD8 signal peptide. In some embodiments, the signal peptide and the intracellular domain are native to the same cytokine receptor. In some embodiments, the signal peptide and the intracellular domain are native to different cytokine receptors.

[0047] In some embodiments, the signal peptide is native to IL-2RA and has nucleic acid sequence SEQ ID NO: 1 and amino acid sequence SEQ ID NO: 2.

[0048] In some embodiments, the signal peptide is native to IL-2RB and has nucleic acid sequence SEQ ID NO: 3 and amino acid sequence SEQ ID NO: 4.

[0049] In some embodiments, the signal peptide is native to IL-2RG and has nucleic acid sequence SEQ ID NO: 5 and amino acid sequence SEQ ID NO: 6.

[0050] In some embodiments, the signal peptide is native to IL-4RA and has nucleic acid sequence SEQ ID NO: 7 and amino acid sequence SEQ ID NO: 8.

[0051] In some embodiments, the signal peptide is native to IL-7RA and has nucleic acid sequence SEQ ID NO: 9 and amino acid sequence SEQ ID NO: 10.

[0052] In some embodiments, the signal peptide is native to IL-9R and has nucleic acid sequence SEQ ID NO: 11 and amino acid sequence SEQ ID NO: 12.

[0053] In some embodiments, the signal peptide is native to IL-15RA and has nucleic acid sequence SEQ ID NO: 13 and amino acid sequence SEQ ID NO: 14.

[0054] In some embodiments, the signal peptide is native to IL-21R and has nucleic acid sequence SEQ ID NO: 15 and amino acid sequence SEQ ID NO: 16.

[0055] The scFv binds a synthetic small molecule. The term "synthetic small molecule" as used herein refers to an organic molecule or compound that is monofuctional and that ranges in size from about 50 to about 10,000 daltons, usually from about 50 to about 5,000 daltons and more usually from about 100 to about 1000 daltons.

[0056] Representative examples of synthetic small molecules include fluorescein and fluorescein derivatives (*e.g.*, FITC, 5-carboxyfluorescein, 6-carboxyfluorescein, 5/6-carboxyfluorescein, NHS-fluorescein (5(6)-Carboxyfluorescein N-hydroxysuccinimide ester), 5-(iodoacetamido)fluorescein, 5-([4,6-dichlorotriazin-2-yl]amino)fluorescein hydrochloride, 5-(bromomethyl)fluorescein, and fluorescein 5-carbamoylmethylthiopropanoic acid), 4-[(6-methylpyrazin-2-yl) oxy] benzoate (MPOB), anthraquinone-2-carboxylate (AQ), and tetraxetan (DOTA). In some embodiments, the synthetic small molecule is polymeric, wherein the polymer is a monopolymer, a heteropolymer, or a branched polymer. A representative example of a polymeric synthetic small molecule is a polyhistidine-tag (His-tag), *e.g.*, having from about 6 to about 9 histidine (His) residues. An exemplary 6 His-tag has a molecular weight of about 800 daltons.

[0057] In some embodiments, the scFv binds a synthetic small molecule which is fluorescein and fluorescein derivatives, 4-[(6-methylpyrazin-2-yl) oxy] benzoate (MPOB), anthraquinone-2-carboxylate (AQ), tetraxetan (DOTA), a polyhistidine-tag (His-tag).

[0058] The synethetic small molecule is substantially nonimmunogenic. In some embodiments, the synthetic small molecule is nonimmunogenic such that if injected by itsself into an animal, it would not cause that animal to produce antibodies or T cells reactive thereto. In some embodiments, the synthetic small molecule generates IgM antibodies in an animal but does not cause antibody class switching. In some embodiments, the synthetic small molecule generates a low level of antibodies in an animal such that the synthetic small molecule may still bind one or more cytokine receptor switches without being neutralized. In some embodiments, the synthetic small molecule does not generate a significant immune response. A "significant immune" response is any immune response that would limit or restrict the *in vivo* utility of the synthetic small molecule as used in accordance with the teachings of the present disclosure.

[0059] A representative example of an scFv that binds fluorescein has nucleic acid sequence SEQ ID NO: 51 and amino acid sequence SEQ ID NO: 52.

[0060] A representative example of an scFv that binds MPOB has nucleic acid sequence SEQ ID NO: 53 and amino acid sequence SEQ ID NO: 54.

[0061] A representative example of an scFv that binds AQ has nucleic acid sequence SEQ ID NO: 55 and amino acid sequence SEQ ID NO: 56.

[0062] A representative example of an scFv that binds DOTA has nucleic acid sequence SEQ ID NO: 57 and amino acid sequence SEQ ID NO: 58.

[0063] The transmembrane (TM) domain allows the cytokine receptor switch to be stably anchored into the cell membrane of the immune cell. The transmembrane domain may be derived from the same protein or from a different protein from which the other domains of the cytokine receptor switch are derived. The transmembrane domain may be derived from a natural or from a recombinant source. Where the source is natural, the domain may be derived from any membrane-bound or transmembrane protein.

[0064] In some embodiments, the transmembrane domain is derived from IL-2RA, IL-2RB, IL-2RG, IL-4RA, IL-7RA, IL-9RA, IL-15RA, or IL-21R.

[0065] In some embodiments, the transmembrane domain peptide is derived from IL-2RA and has nucleic acid sequence SEQ ID NO: 17 and amino acid sequence SEQ ID NO: 18.

[0066] In some embodiments, the transmembrane domain peptide is derived from IL-2RB and has nucleic acid sequence SEQ ID NO: 19 and amino acid sequence SEQ ID NO: 20.

[0067] In some embodiments, the transmembrane domain peptide is derived from IL-2RG and has nucleic acid sequence SEQ ID NO: 21 and amino acid sequence SEQ ID NO: 22.

[0068] In some embodiments, the transmembrane domain peptide is derived from IL-4RA and has nucleic acid sequence SEQ ID NO: 23 and amino acid sequence SEQ ID NO: 24.

[0069] In some embodiments, the transmembrane domain peptide is derived from IL-7RA and has nucleic acid sequence SEQ ID NO: 25 and amino acid sequence SEQ ID NO: 26.

[0070] In some embodiments, the transmembrane domain peptide is derived from IL-9R and has nucleic acid sequence SEQ ID NO: 27 and amino acid sequence SEQ ID NO: 28.

[0071] In some embodiments, the transmembrane domain peptide is derived from IL-15RA and has nucleic acid sequence SEQ ID NO: 29 and amino acid sequence SEQ ID NO: 30.

[0072] In some embodiments, the transmembrane domain peptide is derived from IL-21R and has nucleic acid sequence SEQ ID NO: 31 and amino acid sequence SEQ ID NO: 32.

[0073] The cytokine receptor switch can be designed to include a transmembrane domain that is indirectly attached to the scFv. In such embodiments, the transmembrane domain is attached to the scFv via a hinge domain. As used herein, the term "hinge domain" refers to a domain that links the extracellular binding domain to the transmembrane domain, and may confer flexibility to the extracellular binding domain. In some embodiments, the hinge domain positions the extracellular domain close to the plasma membrane of the immune cell to minimize the potential for recognition by antibodies or binding fragments thereof. The hinge domain may be natural (such as a hinge from a human protein) or synthetic. Sources of hinge domains include human Ig (immunoglobulin) hinges (*e.g.*, an IgG4 hinge, an IgD hinge), and a CD8 (*e.g.*, CD8α hinge).

[0074] In some embodiments, the hinge domain is derived from cluster of differentiation 8 (CD8).

[0075] In some embodiments, the hinge domain peptide has nucleic acid sequence SEQ ID NO: 49 and amino acid sequence SEQ ID NO: 50.

[0076] As used herein, the term "intracellular domain" refers to a signaling moiety that provides to immune cells, such as T-cells, a signal which mediates a cellular response such as, for example, activation, proliferation, differentiation, and/or cytokine secretion. In some embodiments, the intracellular domain is native to or derived from IL-2RA, IL-2RB, IL-2RG, IL-4RA, IL-7RA, IL-9RA, IL-15RA, or IL-21R.

[0077] In some embodiments, the intracellular domain is derived from IL-2RA and has nucleic acid sequence SEQ ID NO: 33 and amino acid sequence SEQ ID NO: 34.

[0078] In some embodiments, the intracellular domain is derived from IL-2RB and has nucleic acid sequence SEQ ID NO: 35 and amino acid sequence SEQ ID NO: 36.

[0079] In some embodiments, the transmembrane domain peptide is derived from IL-2RG and has nucleic acid sequence SEQ ID NO: 37 and amino acid sequence SEQ ID NO: 38.

[0080] In some embodiments, the transmembrane domain peptide is derived from IL-4RA and has nucleic acid sequence SEQ ID NO: 39 and amino acid sequence SEQ ID NO: 40.

[0081] In some embodiments, the transmembrane domain peptide is derived from IL-7RA and has nucleic acid sequence SEQ ID NO: 41 and amino acid sequence SEQ ID NO: 42.

[0082] In some embodiments, the transmembrane domain peptide is derived from IL-9R and has nucleic acid sequence SEQ ID NO: 43 and amino acid sequence SEQ ID NO: 44.

[0083] In some embodiments, the transmembrane domain peptide is derived from IL-15RA and has nucleic acid sequence SEQ ID NO: 45 and amino acid sequence SEQ ID NO: 46.

[0084] In some embodiments, the transmembrane domain peptide is derived from IL-21R and has nucleic acid sequence SEQ ID NO: 47 and amino acid sequence SEQ ID NO: 48.

[0085] Representative cytokine receptor switches include combinations of the sequences of the signal peptides, scFvs, transmembrane domains, hinge domains and intracellular domains disclosed above.

[0086] In some embodiments, the cytokine receptor switch comprises an IL-2RB signal peptide, an anti-small molecule scFv, a CD8 hinge, an IL-2RB transmembrane domain, and an IL-2RB intracellular domain. In some embodiments, the cytokine receptor switch comprises an IL-2RG signal peptide, an anti-small molecule scFv, a CD8 hinge, an IL-2RG transmembrane domain, an IL-2RG intracellular domain. In some embodiments, the cytokine receptor switch comprises an IL-7RA signal peptide, an anti-small molecule scFv, a CD8 hinge, an IL-7RA transmembrane domain, and an IL-7RA intracellular domain. In some embodiments, the cytokine receptor switch comprises an IL-15RA signal peptide, an anti-small molecule scFv, a CD8 hinge, an IL-15RA transmembrane domain, and an IL-15RA intracellular domain.

[0087] In some embodiments, the cytokine receptor switch of the present disclosure is an anti-fluorescein-IL2-RA cytokine receptor switch and has an amino acid sequence (SEQ ID NO: 59):

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  1 mdsyllmwgl ltfimvpgcq advvmtqtpl slpvslgdqa siscrssqsl vhsngntylr
 61 wylqkpgqsp kvliykvsnr vsgvpdrfsg sgsgtdftlk inrveaedlg vyfcsqsthv
121 pwtfgggtkl eikssaddak kdaakkddak kddakkdggv kldetggglv qpggamklsc
181 vtsgftfghy wmnwvrqspe kglewvaqfr nkpynyetyy sdsvkgrfti srddskssvy
241 lqmnnlrved tgiyyctgas ygmeylgqgt svtvstttpa prpptpapti asqplsirpe
301 acrpaaggav htrgldfacd vavagcvfll isvlilsglt wqrrqrksrr ti
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Immune cells comprising nucleic acids encoding inventive cytokine receptor switches

[0088] In some aspects, the present disclosure is directed to a composition comprising an immune cell comprising at least one nucleic acid encoding a cytokine receptor switch. Immune cells useful in the present disclosure are mammalian, preferably primate immune cells such as immune cells from monkeys, and humans. In some embodiments, the immune cells are T cells. In some embodiments, the immune cells are NK cells. In some embodiments, the immune cells are allogeneic (from the same species but different donor) as the recipient subject; in some embodiments the immune cells are autologous (the donor and the recipient are the same); in some embodiments the immune cells are syngeneic (the donor and the recipients are different but are identical twins). Natural killer (NK) cells are an important effector cell type for adoptive cancer immunotherapy. Similar to T cells, in some embodiments, the NK cells useful in the present disclosure are allogeneic, autologous, or syngeneic. For example, in some embodiments, the T cells are $CD8^+$ or $CD4^+$ T cells. In some embodiments, NK cells are $CD56^{dim}$ $CD16^+$ NK cells. In some embodiments, NK cells are $CD56^{bright}$ $CD16^-$ NK cells. In some embodiments, the NK cells are primary NK cells, memory-like NK cells, or induced memory like NK cells. The compositions may include combinations of two or more types of immune cells that comprise the same or different cytokine receptor switch encoded by a nucleic acid.

[0089] In some embodiments, the composition comprises an immune cell that comprises a nucleic acid that encodes the anti-fluorescein-IL2-RA cytokine receptor switch having the amino acid sequence SEQ ID NO: 59.

[0090] In some embodiments, the immune cells comprise at leaset two nucleic acids that encode at least two cytokine receptor switches wherein at least one of the respective signal peptides, transmembrane domains, and intracellular domains are different.

[0091] For example, in some embodiments, the at least two cytokine receptor switches comprise different scFvs.

[0092] In some embodiments, the immune cells comprise at least two nucleic acids that encode at least two cytokine receptor switches, the nucleic acids encoding a first cytokine switch comprising an IL-2RG signal peptide, an anti-small molecule scFv, a CD8 hinge, an IL-2RG transmembrane domain, an IL-2RG intracellular domain, and a second cytokine switch

comprising an IL-7RA signal peptide, an anti-small molecule scFv, a CD8 hinge, an IL-7RA transmembrane domain, and an IL-7RA intracellular domain.

[0093] In some embodiments, the immune cells comprise at least three nucleic acids that encodes at least three cytokine receptor switches wherein at least one of the respective signal peptides, transmembrane domains, and intracellular domains are different.

[0094] In some embodiments, the immune cells comprise at least three nucleic acids that encode at least three cytokine receptor switches comprising a first cytokine switch comprising an IL-2RB signal peptide, an anti-small molecule scFv, a CD8 hinge, an IL-2RB transmembrane domain, and an IL-2RB intracellular domain, a second cytokine switch comprising an IL-2RG signal peptide, an anti-small molecule scFv, a CD8 hinge, an IL-2RG transmembrane domain, and an IL-2RG intracellular domain, and a third cytokine switch comprising an IL-15RA signal peptide, an anti-small molecule scFv, a CD8 hinge, an IL-15RA transmembrane domain, and an IL-15RA intracellular domain.

[0095] In some embodiments, the immune cells are derived from induced pluripotent stem cells (iPSC), cord blood, or peripheral blood mononuclear cells (PBMCs). Precursor harvesting, generation, and maintenance of iPSC are known in the art. *See*, for example, U.S. Patent Nos: 9,260,696, 10,214,722, 10,428,309, 10,844,356, and 11,193,108. Similar to iPSC, methods for harvesting, generating, and maintenaning stem cells from cord blood are known in the art. *See,* U.S. Patent Nos: 6,338,942, 7,311,905, 8,889,411 and 9,260,696. Similarly, methods for harvesting, generating, and maintenaning immune cells from PBMCs are known in the art. *See,* U.S. Patent Nos. 9,476,028, 11,162,072, 11,229,689 and U.S. Patent Publication No. 2017/0051252. Methods for differentiating and isolating T and NK cells from progenitor, pluripotent, or stem cells into a desired cell subset are known in the art.

Chimeric Antigen Receptor (CAR)-Immune Cells

[0096] In some embodiments, the immune cells are chimeric antigen receptor (CAR)-immune cells (*e.g.*, CAR-T cells) and also contain a CAR directed against a cell surface antigen (FIG. 1B, FIG. 4).

[0097] The term "Chimeric antigen receptor" (CAR) as used herein refers to a synthetically designed receptor comprising an extracellular binding domain that includes an antibody or binding fragment thereof, nanobody or other protein sequence that binds to a cell antigen associated with a disease or disorder (a "cell associated antigen") and is linked via a spacer

domain to an intracellular domain of a T cell. The spacer domain includes a transmembrane domain, and in some embodiments, a hinge domain.

Extracellular binding domain

[0098] As used herein, an extracellular binding domain is a moiety that specifically binds a target antigen, namely a cell surface antigen such as a tumor associated antigen. Extracellular binding domains may include a protein, polypeptide, oligopeptide, or peptide. The extracellular binding domain may be naturally occurring, synthetic, semi-synthetic, or recombinantly produced.

[0099] In some embodiments, the extracellular binding domain binds a cell associated antigen on a tumor cell (a "tumor associated antigen" (TAA)).

[00100] In some embodiments, the extracellular binding domain of the CAR is a single-chain variable fragment (scFv) of an antibody (as defined above).

[00101] Other types of antibody fragments having specificity for cell associated antigens that may be useful as components of the CAR include Fv, Fab, and 'Fab')$_2$ fragments. *See, e.g.*, U.S. Patent 4,946,788.

[00102] Representative examples of such extracellular binding domains are set forth in Table 2:

Table 2

TAA	**Targeting Moiety**	**Source**
ACVR1	mAb C-5	Santa Cruz Biotechnology
ALK	Antibody or fragment thereof	Mino-Kenudson *et al.*, Clin. Cancer Res. *16(5)*:1561-1571 (2010)
B7H3 (also known as CD276)	MGA271	Macrogenics
BCMA	Antibody or fragment thereof, Belantamab (anti-BCMA antibody conjugated with MMAF mitotic agent), anti-BCMA antibody J6M0 (belantamab biosimilar).	Friedman *et al.*, Hum. Gene Ther. *29*(*5*):585-601 (2018), Raje *et al.*, N Engl. J. Med., *380*(*18*):1726-1737 (2019), WO2010104949A2 and WO2003014294A2, Abeomics
BST2 (also known as CD317)	Anti-CD317 antibody, Monoclonal[3H4]	Antibodies-Online

BST2 (also known as CD317)	Anti-CD317 antibody, Monoclonal[696739]	R&D Systems
CAIX	antibody clone 303123	R&D Systems
CEA	Antibody or fragment thereof	Chmielewski *et al.*, Gastoenterology *143(4)*:1095-1107 (2012)
CLDN6	antibody IMAB027	Ganymed Pharmaceuticals; clinicaltrial.gov/show/NCT02054351
CS1	Antibody or fragment thereof, Elotuzumab	WO2004100898A2
CYP1B1	Antibody or fragment thereof	Maecker *et al.*, Blood *102(9)*:3287-3294 (2003)
EGFR	cetuximab, panitumumab, zalutumumab, nimotuzumab, matuzumab	Commercial sources
EPCAM	MT110, EpCAM-CD3 bispecific Ab	clinicaltrials.gov/ct2/show/NCT00635596)
EPCAM	Edrecolomab; 3622W94; ING-1; and adecatumumab (MT201)	Commercial sources
EphA2	Antibody or fragment thereof	Yu *et al.*, Mol. Ther. *22(1)*:102-111 (2014)
Ephrin B2	Antibody or fragment thereof	Abengozar *et al.*, Blood *119(19)*:4565-4576 (2012)
ERBB2 (HER2/neu)	trastuzumab, or pertuzumab	Commercial sources
FAP	Antibody or fragment thereof	Ostermann *et al.*, Clinical Cancer Research *14*:4584-4592 (2008)
FAP	sibrotuzumab	Hofheinz *et al.*, Oncology Research and Treatment *26*:44-48 (2003); and Tran *et al.*, J. Exp. Med. *210(6)*:1125-1135 (2013)
FCAR	CD89/FCAR Antibody (Catalog#10414-H08H)	Sino Biological Inc.

Folate receptor alpha	Antibody IMGN853	Commercial sources
Folate receptor alpha	Antibody or fragment thereof	U.S. Patent Application Publication 20120009181; U.S. Patent 4,851,332 and U.S. Patent 5,952,484
Fos-related antigen 1	Antibody 12F9	Novus Biologicals
Fucosyl GM1	Antibody or fragment thereof	U.S. Patent Application Publication 20100297138; WO2007067992
GD2	Antibody or fragment thereof	Mujoo *et al.*, Cancer Res. *47(4)*:1098-1104 (1987); Cheung *et al.*, Cancer Res. *45(6)*:2642-2649 (1985), Cheung *et al.*, J. Clin. Oncol. *5(9)*:1430-1440 (1987), Cheung *et al.*, J. Clin. Oncol. *16(9)*:3053-3060 (1998), Handgretinger *et al.*, Cancer Immunol. Immunother. *35(3)*:199-204 (1992); U.S. Publication No.: 20100150910; WO2011160119
GD2	mAb 14.18, 14G2a, ch14.18, hul4.18, 3F8, hu3F8, 3G6, 8B6, 60C3, 10B8, ME36.1, and 8H9; or fragments thereof	WO2012033885, WO2013040371, WO2013192294, WO2013061273, WO2013123061, WO2013074916, and WO201385552
GD3	Antibody or fragment thereof	U.S. Patent 7,253,263; U.S. Patent 8,207,308; U.S. Patent Application Publication 20120276046; EP1013761; WO2005035577; and U.S. Patent 6,437,098
GD3	Antibody or fragment thereof	U.S. Patent 7,253,263; U.S. Patent 8,207,308; U.S. Patent Application Publication 20120276046; EP1013761 A3; U.S. Patent

		Application Publication 20120276046; WO2005035577; U.S. Patent 6,437,098
GloboH	Antibody or fragment thereof	Kudryashov *et al.*, Glycoconj J. *15(3)*:243-9 (1998), Lou *et al.*, Proc. Natl. Acad. Sci. USA. *111(7)*:2482-2487 (2014)
GloboH	Antibody MBr1	Bremer *et al.*, J. Biol. Chem. *259*:14773-14777 (1984)
GM3	antibody CA 2523449 (mAb 14F7)	Commercial sources
Gp100	antibody HMB45, NKIbetaB	Commercial sources
Gp100	Antibody or fragment thereof	WO2013165940; U.S. Patent Application Publication 20130295007
GPC3	Antibody hGC33	Nakano *et al.*, Anticancer Drugs *21(10)*:907-916 (2010)
GPC3	Antibody MDX-1414, HN3, YP7	Feng *et al.*, FEBS Lett. *588(2)*:377-382 (2014)
GPRC5D	antibody FAB6300A	R&D Systems
GPRC5D	Antibody LS-A4180	Lifespan Biosciences
HER2	Antibody or fragment thereof, Trastuzumab, Pertuzumab	U.S. Patent 8,591,897 B2, WO2001000245
HMWMAA	mAb9.2.27	Kmiecik *et al.*, Oncoimmunology *3(1)*:e27185-3 (2014)
HMWMAA	Antibody or fragment thereof	U.S. Patent 6,528,481; WO2010033866; U.S. Patent Application Publication 20140004124
Human telomerase reverse transcriptase	Antibody cat no: LS-B95-100	Lifespan Biosciences

IGF-1	Antibody or fragment thereof	U.S. Patent 8,344,112 B2; EP2322550 A1; WO2006138315; WO2006138315
IL-11Rα	Antibody or fragment thereof	Abcam® (cat# ab55262); Novus Biologicals (cat# EPR5446)
IL-11Rα	Peptide	Huang *et al.*, Cancer Res. *72(1)*:271-281 (2012)
IL-13Rα2	Antibody or fragment thereof	WO2008146911, WO2004087758, several commercial catalog antibodies, and WO2004087758
Intestinal carboxyl esterase	Antibody 4F12: cat no: LS-B6190-50	Lifespan Biosciences
KIT (also known as CD117)	Antibody or fragment thereof	U.S. Patent No. 7,915,391, U.S. Patent Application Publication 20120288506, and several commercial catalog antibodies
LewisY	Hu3S193 Ab (scFvs)	Kelly *et al.*, Cancer Biother. Radiopharm. *23(4)*:411-423 (2008)
LewisY	NC10 scFv	Dolezal *et al.*, Protein Engineering *16(1)*:47-56 (2003)
LMP2	Antibody or fragment thereof	U.S. Patent 7,410,640; U.S. Patent Application Publication 20050129701
MAD-CT-2	Antibody or fragment thereof	Rodman *et al.*, J. Exp. Med. *1(167)*:1228-46 (1988); U.S. Patent 7,635,753
MAGE-A1	Antibody or fragment thereof	Willemsen *et al.*, J. Immunol. *174(12)*:7853-7858 (2005)
MelanA/MART1	Antibody or fragment thereof	EP2514766 A2; U.S. Patent 7,749,719
Mesothelin	Antibody or fragment thereof	Morello *et al.*, Cancer Discov. *6*:133–46 (2016), Liu *et al.*, Proc.

		Natl. Acad. Sci. USA *119*:e2202439119-9 (2022)
MUC1	Antibody SAR566658	Commercial sources
MUC1c	Antibody or fragment thereof	Kufe, Oncogene *32*:1073–1081 (2013)
MUC1* (truncated, membrane-bound)	Antibody or fragment thereof	U.S. Patent Application Publication 20200239594A1
Mut hsp70-2	Monoclonal: cat no: LS-C133261-100	Lifespan Biosciences
NCAM also known as CD56	antibody clone 2-2B: MAB5324, Lorvotuzumab	EMD Millipore
NY-BR-1	Antibody or fragment thereof	Jager *et al.*, Appl. Immunohistochem. Mol. Morphol. *15(1)*:77-83 (2007)
o-acetyl-GD2	antibody 8B6	Commercial sources
PDGFR-β	ab32570	Abcam®
PDGFRα	mAb APA5[a] mAb 16A1, Olaratumab	Invitrogen™; Biolegend, U.S. Patent 8,128,929 B2
PLAC1	Antibody or fragment thereof	Ghods *et al.*, Biotechnol. Appl. Biochem. *61(3)*:363-369 (2013)
Polysialic acid	Antibody or fragment thereof	Nagae *et al.*, J. Biol. Chem. *288(47)*:33784-33796 (2013)
PRSS21	Antibody or fragment therof	U.S. Patent 8,080,650
PSCA	scFv 7F5	Morgenroth *et al.*, Prostate *67(10)*:1121-1131 (2007)
PSCA	scFv C5-II	Nejatollahi *et al.*, J. of Oncology article ID 839831-8 (2013)
PSCA	Antibody or fragment thereof	U.S. Patent Application Publication 20090311181
PSMA	Antibody or fragment thereof	Parker *et al.*, Protein Expr. Purif. *89(2)*:136-145 (2013)

PSMA	J591 ScFv	U.S. Patent Application Publication 20110268656
PSMA	scFvD2B	Frigerio *et al.*, European J. Cancer *49(9)*:2223-2232 (2013)
PSMA	mAbs 3/A12, 3/E7 and 3/F11 and single chain antibody fragments scFv A5 and D7	WO2006125481
RAGE-1	Antibody MAB5328	EMD Millipore
ROR1	Antibody or fragment thereof	Hudecek *et al.*, Clin. Cancer Res. *19(12)*:3153-3164 (2013); WO2011159847; and U.S. Patent Application Publication 20130101607
Sarcoma translocation breakpoints	Antibody or fragment thereof	Luo *et al.*, EMBO Mol. Med. *4(6)*:453-461 (2012)
sLe	antibody G193 (for lewis Y)	Scott *et al.*, Cancer Res. *60*:3254-3261 (2000); Neeson *et al.*, J. Immunol. *190(1 Supplement)*: 177.10 (2013)
Sperm protein 17	Antibody or fragment thereof	Song *et al.*, Target Oncol. *9(3)*:263-272 (2013); Song *et al.*, Med. Oncol. *29(4)*:2923-2931 (2012)
SSEA-4	Antibody MC813	Cell Signaling; other commercially available antibodies
TAG72	Antibody or fragment thereof	Hombach *et al.*, Gastroenterology *113(4)*:1163-1170 (1997)
TAG72	Ab691	Abcam®
TEM1/CD248	Antibody or fragment thereof	Marty *et al.*, Cancer Lett. *235(2)*:298-308 (2006); Zhao *et al.*, J. Immunol. Methods *363(2)*:221-232 (2011)

Tie2	Antibody AB33	Cell Signaling Technology
Tn antigen	Antibody or fragment thereof	U.S. Patent 8,440,798, Brooks *et al.*, PNAS *107(22)*:10056-10061 (2010), and Stone *et al.*, OncoImmunology *1(6)*:863-873 (2012)
TNFRSF13B (TACI)	Antibody or fragment thereof	WO2020247618A1
TRP-2	Antibody or fragment thereof	Wang *et al.*, J. Exp. Med. *184(6)*:2207-2216 (1996)
TSHR	Antibody or fragment thereof	U.S. Patent 8,603,466; U.S. Patent 8,501,415; U.S. Patent 8,309,693
Tyrosinase	Antibody or fragment thereof	U.S. Patent 5,843,674; U.S. Patent 5,843,674
VEGFR2	Antibody or fragment therof	Chinnasamy *et al.*, J. Clin. Invest. *120(11)*:3953-3968 (2010)
WT-1	Antibody 176ra33	Dao *et al.*, Sci. Transl. Med. *5(176)*:176ra33-22 (2013)
WT-1	Antibody or fragment thereof	WO2012135854

[00103] In some embodiments that entail treatment of brain cancer, for example, the targeting ligand binds a brain tumor associated antigen. For example, tumor associated antigens present on GBM cells include ACVR1, EGFRvIII, IL13Rα2 and HER2. For example, the multiplexing approach may be used to treat brain cancer that simultaneously targets EGFRvIII, IL13Rα2 and HER2. Other proteins that have been implicated in brain cancers and which may be targeted by the bifunctional compounds of the present disclosure include EphA2, CSPG4, GD2, PDGFRα and GRP78. Antibodies and/or functional fragments thereof that bind brain tumor associated antigens are known in the art. *See, e.g.*, Table 1, above, which *inter alia*, describes antibodies and/or fragments thereof that bind ACVR1, PDGFRα, GD2 and EphA2. Targeting moieties that bind PDGFRα may include scFvs based on Olaratumab (and binding variants thereof).

[00104] In some embodiments, the targeting ligand binds to HER2 on $HER2^+$ malignancies such as breast, lung, colorectal, brain, ovarian, and pancreatic cancer. Representative targeting

ligands that bind HER and which may be useful in the present disclosure include Trastuzumab and Pertuzumab which bind the extracellular domains IV and II, respectively, of HER, and their HER-binding fragments (*e.g.*, scFvs).

[00105] An antibody fragment that binds EGFRvIII, is described in O'Rourke, *et al.*, Sci. Transl. Med. *9(399)*:eaaa0984-30 (2017). Other antibodies or fragments thereof that bind EGFRvIII are commercially available siltuximab and mAb DH8.3 (Novus Biologicals). Further representative examples of amino acid or gene sequences that encode scFvs targeting EGFRvIII that might be useful in the present disclosure are found in U.S. Patent Application Publication 2015/0259423.

[00106] An antibody fragment that binds IL13Rα2 is described in Brown, *et al.,* N. Engl. J. Med. *375(26)*:2561-2569 (2016). Other antibodies or fragments thereof that bind IL13Rα2 are commercially available from Abnova and Millipore.

[00107] An antibody fragment that binds HER2 is described in Ahmed, *et al.,* JAMA Oncol. *3(8)*:1049-1101 (2017). Other antibodies or fragments thereof that bind HER2 are commercially available, including trastuzumab and FRP5. Further representative examples of amino acid or gene sequences that encode scFvs targeting HER2 that might be useful in the present disclosure are found in U.S. Patent Application Publication 2011/0313137.

[00108] Another example of an antibody fragment that binds EphA2 is described in Chow, *et al.,* Mol. Ther. *21(3)*:629-637 (2013). Yet other antibodies or fragments thereof that bind EphA2 are commercially available from Thermo Fisher (mAb4H5 and mAb 1C11A12) and RND Systems. Further representative examples of amino acid or gene sequences that encode for scFvs targeting EphA2 that might be useful in the present disclosure are described in U.S. Patent Application Publication 2010/436783.

[00109] An antibody fragment that binds CSPG4 is described in Pellegatta, *et al.,* Sci. Transl. Med., *10*:eaao2731-33 (2018). Another antibody that binds CSPG4 is described in Fenton *et al.*, Oncol. Res. *22(2)*:117-21 (2015). Other antibodies or fragments thereof that bind CSPG4 are commercially available bevacizumab and Creative Biolabs mAb 225.28. Yet other antibodies or fragments thereof that bind CSPG4 are commercially available from Aviva Systems Biology. Further representative examples of amino acid or gene sequences that encode scFvs targeting CSPG4 that might be useful in the present disclosure are described in U.S. Patent 9,801,928 and U.S. Patent Application Publication 2019/0008940.

[00110] Another example of an antibody fragment that binds GD2 is described in Mount *et al.*, Nat. Med. *24*:572-579 (2018). Other antibodies or fragments thereof that bind GD2 include Dinutuximab, mAb 3F8, mAb 14g2a, and mAb 14.18. Further representative examples of amino acid or gene sequences that encode scFvs targeting GD2 that might be useful in the present disclosure are described in U.S. Patent 4,675,287.

[00111] Another example of an antibody fragment that binds PDGFRα is described in Brennan *et al.*, PLoS One, *4(11)*:e7752-10 (2009). Other antibodies or fragments thereof that bind PDGFRα are commercially available from Abcam, LifeSpan Bio, Santa Cruz (sc-338) and Thermo Fisher (mAb APA5). Further representative examples of amino acid or gene sequences that encode scFvs targeting PDGFRα that might be useful in the present disclosure are described in U.S. Patent Application Publication 2012/0027767.

[00112] An antibody fragment that binds GRP78 is described in Kang *et al.*, Sci. Rep. *6*:34922-7 (2016). Other antibodies or fragments thereof that bind GRP78 are commercially available from Thermo Fisher (PA1-014A) and Abcam (N-20). Further representative examples of amino acid or gene sequences that encode scFvs targeting GRP78 that might be useful in the present disclosure are described in U.S. Patent 10,259,884.

[00113] Other proteins that have been implicated in brain cancers and which may be targeted by the bifunctional compounds of the present disclosure include neural cell adhesion molecule (NCAM), cluster of differentiation 276 (CD276), and neuroectodermal stem cell marker (Nestin).

[00114] An antibody that binds NCAM is described in Modak *et al.*, Cancer Res. *61*:4048-4054 (2001). Other antibodies or fragments thereof that bind NCAM are mAb UJ13A and mAb ERIC-1. Further representative examples of amino acid or gene sequences that encode scFvs targeting NCAM that might be useful in the present disclosure are described in U.S. Patent 7,402,560.

[00115] Antibodies or fragments thereof that bind Nestin are commercially available from Abcam (ab6142) and Novus Biologicals (NB100-1604). Antibodies or fragments thereof that bind βIII-Tubulin are available from Abcam (2G10) and RND Systems (mAB 1195).

[00116] Another example of an antibody fragment that binds CS-1 is described in Chu *et al.*, Blood, *122*:14 (2013). Other antibodies or fragments thereof that bind CS-1 are commercially available and include REA150 (Miltenyi) or 162.1 (Biolegend). Further representative examples of amino acid or gene sequences that encode scFvs targeting CS-1 that might be

useful in the present disclosure are described in International Publication Number WO 2004/100898 A2.

[00117] Another example of an antibody fragment that binds BCMA is described in Raje *et al.*, N. Engl. J. Med. *380*(*18*):1726-1737 (2019). Other antibodies or fragments thereof that bind BCMA are commercially available and include REA315 (Miltenyi), J6M0 (Abeomics) (the anti-BCMA antibody included in the Belantamab conjugation compsosion), and 19F2 (Biolegend). Further representative examples of amino acid or gene sequences that encode scFvs targeting BCMA that might be useful in the present disclosure are described in International Publication Numbers WO 2010/104949 A2 and WO 2003/014294 A2.

[00118] In some embodiments, the cell surface antigen is CD19, B-cell maturation antigen (BCMA), human epidermal growth factor receptor 2 (HER2), epidermal growth factor receptor (EGFR), CD38, CS1 (SLAM family member 7 (SLAMF7)), G Protein-Coupled Receptor Class C Group 5 Member D (GPRC5D), or TNFRSF13B (TACI).

[00119] The transmembrane (TM) domain allows the CAR to be stably anchored into the cell membrane of the immune cell. The transmembrane domain may be derived from the same protein or from a different protein from which the other domains of the CAR are derived. The transmembrane domain may be derived from a natural or from a recombinant source. Where the source is natural, the domain may be derived from any membrane-bound or transmembrane protein. Representative examples of transmembrane domains that may be useful in the present disclosure include the transmembrane regions of CD28, CD27, CD3 epsilon, CD45, CD4, CD5, CD8, CD9, CD16, CD22, CD33, CD37, CD64, CD80, CD86, CD134, CD137, CD154.

[00120] The CAR can be designed to include a transmembrane domain that is indirectly attached to the extracellular binding domain. In such embodiments, the transmembrane domain is attached to the extracellular region of the CAR via a hinge domain. As used herein, the term "hinge domain" refers to a domain that links the extracellular binding domain to the transmembrane domain and may confer flexibility to the extracellular binding domain. In some embodiments, the hinge domain positions the extracellular domain close to the plasma membrane of the immune cell to minimize the potential for recognition by antibodies or binding fragments thereof. The hinge domain may be natural (such as a hinge from a human protein) or synthetic. Sources of hinge domains include human Ig (immunoglobulin) hinges (*e.g.*, an IgG4 hinge, an IgD hinge), and a CD8 (*e.g.*, CD8α hinge).

[00121] In some embodiments, the hinge domain is derived from cluster of differentiation 8 (CD8), for example SEQ ID NO: 50.

[00122] The intracellular signaling domain aids in immune cell activation upon binding of the CAR (*e.g.*, 2nd generation, 3rd generation, engineered T cell receptor (TCR)) to the cell associated antigen on the target cell. Such domains are known in the art and are commonly referred to as second, third and fourth generation CARs and engineered T cell receptors (TCRs). An intracellular signaling domain is generally responsible for activation of at least one of the normal effector functions of the immune cell in which the CAR has been introduced. Examples of intracellular signaling domains include the cytoplasmic sequences of the TCR and co-receptors that act in concert to initiate signal transduction following antigen receptor engagement. As is known in the art, signals generated through the TCR alone are insufficient for full activation of T cells; therefore, a secondary or costimulatory signal is also required. Thus, T cell activation is mediated by two distinct classes of cytoplasmic signaling sequences, namely those that initiate antigen-dependent primary activation through the TCR (*i.e.*, the primary intracellular signaling domains) and those that act in an antigen-independent manner to provide a secondary or costimulatory signal (*i.e.*, the secondary cytoplasmic or costimulatory domain). The primary signaling domain regulates primary activation of the TCR complex either in a stimulatory way, or in an inhibitory way. Primary intracellular signaling domains that act in a stimulatory manner may contain signaling motifs known as immunoreceptor tyrosine-based activation motifs (ITAMs). Representative examples of ITAM-containing primary intracellular signaling domains that may be suitable for use in the present disclosure include those of CD3ζ, common FcRγ (FCER1G), Fc-γ RIIa, FcR-β (Fc-ε R1b), CD3γ, CD3δ, and CD3ε. In some embodiments, the CARs include an intracellular signaling domain that contains the primary signaling domain of CD3ζ.

[00123] The intracellular signaling domain of the CAR may also include at least one other intracellular signaling or co-stimulatory domain. A co-stimulatory molecule is a cell surface molecule other than an antigen receptor or its ligands that is required for an efficient response of lymphocytes to an antigen. Representative examples of co-stimulatory domains that may be useful in the CARs of the present disclosure include CD27, CD28, 4-1BB (CD137), OX40, CD30, CD40, PD-1, ICOS, HVEM (LIGHTR), lymphocyte function-associated antigen-1 (LFA-1), CD2, CD7, LIGHT, NKG2C, and B7-H3. CD27 co-stimulation, for example, has been demonstrated to enhance expansion, effector function, and survival of human CAR T cells

in vitro and augments human T cell persistence and antitumor activity *in vivo* (Song, *et al.*, Blood *119(3)*:696-706 (2012)).

[00124] The intracellular signaling domain may be designed to include one or more, *e.g.*, 1, 2, 3, 4, 5, or more costimulatory signaling domains, which may be linked to each other in a specified or random order, optionally via a linker molecule. Polypeptide linkers that are about 1-10 amino acids in length may join consecutive intracellular signaling sequences. Examples of such linkers include doublets such as Gly-Ser, and single amino acids, *e.g.*, Ala and Gly. Combinations that may constitute the T-cell activation domain may be based on the cytoplasmic regions of CD28, CD137 (4-1BB), OX40 and HVEM, which serve to enhance T cell survival and proliferation; and CD3 CD3ζ and FcRε. which induce T cell activation. For example, CD3ζ, which contains 3 ITAMs, is the most commonly used intracellular domain component of CARs, transmits an activation signal to the T cell after antigen is bound. However, to provide additional co-stimulatory signaling, CD28 and OX40 domains can be used with CD3ζ which enable the CAR – immune cells to transmit the proliferative/survival signals.

[00125] CARs that may be used in the present disclosure and methods of making them are known in the art and are described, for example, in U.S. Patent Application Publication 2018/0169109, each of which is incorporated herein by reference in its entirety. Additional CARs that may be useful have been approved by the FDA, and include tisagenlecleucel (Kymriah™), axicabtagene ciloleucel (Yescarta™), idecabtagene vicleucel (Abecma®), and lisocabtagene maraleucel (Breyanzi®), brexucabtagene autoleucel (Tecartus), and ciltacabtagene autoleucel (Carvykti).

[00126] Exemplary cytokine receptor switches and CAR constructs are shown below in Table 3.

Table 3. Cytokine receptor switch and CAR constructs.

	CAR						Cytokine Receptor Switch				
scFv	FL	MPOB	AQ	DOTA	His	Others*	FL	MPOB	AQ	DOTA	His
1	CAR										
2		CAR									
3			CAR								
4				CAR							
5					CAR						
6						CAR					
7							IL2RA				
8							IL2RB				
9							IL2RG				

10							IL4RA				
11							IL7RA				
12							IL9R				
13							IL15RA				
14							IL21R				
15	CAR						IL2RA				
16	CAR						IL2RB				
17	CAR						IL2RG				
18	CAR						IL4RA				
19	CAR						IL7RA				
20	CAR						IL9R				
21	CAR						IL15RA				
22	CAR						IL21R				
23		CAR					IL2RA				
24		CAR					IL2RB				
25		CAR					IL2RG				
26		CAR					IL4RA				
27		CAR					IL7RA				
28		CAR					IL9R				
29		CAR					IL15RA				
30		CAR					IL21R				
31						CAR	IL2RA				
32						CAR	IL2RB				
33						CAR	IL2RG				
34						CAR	IL4RA				
35						CAR	IL7RA				
36						CAR	IL9R				
37						CAR	IL15RA				
38						CAR	IL21R				
39							IL2RA IL2RB				
40							IL2RA IL2RB IL2RG				
41							IL2RB IL2RG				
42							IL4R IL2RG				
43							IL7R IL2RG				
44							IL9R IL2RG				
45							IL15RA IL2RB IL2RG				
46							IL21R IL2RG				

47							IL2RB IL2RG	IL2RA			
48							IL2RG	IL4RA			
49							IL2RG	IL7RA			
50							IL2RG	IL9R			
51							IL2RB IL2RG	IL15RA			
52							IL2RG	IL21R			
53							IL2RG	IL2RA		IL2RB	
54							IL2RG	IL4RA			
55							IL2RG	IL7RA			
56							IL2RG	IL9R			
57							IL2RG	IL15RA		IL2RB	
58							IL2RG	IL21R			
59						CAR	IL2RG	IL2RA		IL2RB	
60						CAR	IL2RG	IL4RA			
61						CAR	IL2RG	IL7RA			
62						CAR	IL2RG	IL9R			
63						CAR	IL2RG	IL15RA		IL2RB	
64						CAR	IL2RG	IL21R			

*CARs including CD19-, BCMA-, HER2-, and EGFR-CAR.

[00127] Table 3 illustrates exemplary combinations of CARs and cytokine receptor switches with same or different scFvs, that may be present in the immune cell.

BAT-CARs

[00128] In some embodiments, the immune cells are binary activated T cells comprising nucleic acids encoding chimeric antigen receptors (BAT-CARs). BAT-CARs are substantially identical to CARs in terms of design, and the respective spacer domains (*e.g.*, transmembrane, and intracellular domains). However, in contrast to CAR T cells that are engineered so directly bind a cell associated antigen such as a tumor associated antigen, BAT-CAR cells bind a synthetic antigen (which may be a masked pro-antigen or unmasked) that is not present on normal or cancer cells. The synthetic antigen is delivered and attached to a cell associated antigen in the form of a conjugate with an antibody or fragment thereof that binds the cell associated antigen. The BAT-CAR cells can be administered to a subject with a single conjugate or multiple conjugates that contain antibodies or fragments that bind different cell associated antigens. By uncoupling tumor cell targeting from tumor cell killing, a CAR T cell with a single specificity (to the synthetic antigen or unmasked pro-antigen) can simultaneously target a plurality of tumor associated antigens. Therefore, the design of BAT-CAR immune

cells differs from CAR-immune cells mainly if not exclusively with respect to the extracellular binding domains.

[00129] The extracellular domain of a BAT-CAR is typically present at the amino terminal end and displayed on the surface of the immune cell. Except for its specificity, the extracellular domain of a BAT-CAR is typically an antibody or an antigen-binding fragment thereof such as an scFv.

[00130] Representative examples of synthetic antigens include fluorescein and fluorescein derivatives such as FITC. Representative examples of extracellular binding domains that bind fluorescein and FITC are described above (in connection with the cytokine receptor switches, per se). Representative examples of other binding moieties that may be useful as extracellular binding domains in a BAT-CAR are known in the art, *e.g.,* 4M5.3 ScFv, disclosed in Midelfort *et al.* J. Mol. Biol. *343*:685-701 (2004) and 2D12.5, 2D12.5ds, or C8.2.5, disclosed in Orcutt *et al.* Nucl. Med. Biol. *38(2)*:223-233 (2011). Representative examples of masked pro-antigens are known in the art, *e.g.,* International Publication Nos WO2017/143094, WO2018/200713, WO2019/236522, and WO2020/006312, each of which is incorporated herein by reference .

[00131] A representative example of a masked pro-antigen and BAT-CAR is a BAT-CAR with specificity for fluorescein and a masked fluorescein pro-antigen. A stimulus, *e.g.*, UV light unmasks the masked fluorescein molecule and thereby activates cells expressing the fluorescein-specific BAT-CAR. *See, e.g.,* Kobayashi *et al.*, ChemMedChem *17*:e202100722-5 (2022), incorporated by reference. In some embodiments, the synthetic antigen is masked by the addition of one or more 5-carboxymethoxy-2-nitrobenzyl (CMNB) caging groups.

[00132] BAT-CARs that may be used in the present disclosure and methods of making them are known in the art and described, for example, in International Publication Nos WO2017/143094, WO2018/200713, WO2019/236522, and WO2020/006312, each of which is incorporated herein by reference in its entirety.

[00133] A representative example of a polynucleotide that encodes the BAT-CAR an anti-FL CAR-CD28-4-1BB-CD3ζ has the sequence designated as SEQ ID NO: 60:

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   1 atggctctgc ctgtgacagc tctgctgctg cctctggctc tgctttctgca tgccgccaga
  61 cctgacgtgg tcatgacaca gacacctctg agcctgcctg tgtctctggg agatcaggcc
 121 agcatcagct gcagatctag ccagagcctg gtgcacagca acggcaacac ctacctgcgg
 181 tggtatctgc agaagccccgg ccagtctcct aaggtgctga tctacaaggt gtccaacaga
 241 gtgtccggcg tgcccgatag attttctggc agcggctctg gcaccgactt caccctgaag
 301 atcaatagag tggaagccga ggacctgggc gtgtacttct gtagccagtc tacccacgtg
 361 ccatggacct ttggcggcgg aacaaagctg gaaatcaaga gcagcgccga cgacgccaag
 421 aaggacgccg ctaagaagga tgacgccaaa aaagacgatg ccaaaaagga tggcggcgtg
 481 aagctggacg aaacaggcgg aggacttgtt cagcctggcg gagccatgaa gctgagctgt
 541 gtgaccagcg gcttcacctt cggccactac tggatgaact gggtccgaca gagccctgag
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 601 aaaggcctgg aatgggtcgc ccagttcaga aacaagccct acaactacga aacctactac
 661 agcgacagcg tgaagggcag attcaccatc agccgggacg acagcaagtc cagcgtgtac
 721 ctgcagatga acaacctgcg cgtggaagat accggcatct actactgtac cggcgccagc
 781 tacggcatgg aatatctcgg ccagggcacc agcgtgaccg tgtctacaac aacccctgct
 841 cctcggcctc ctacaccagc tcctacaatt gccagccagc cactgtctct gaggccccgaa
 901 gcttgtagac ctgctgcagg cggagccgtg catacaagag gactggattt cgcctgcgac
 961 ttctgggtgc tcgtggttgt tggcggagtg ctggcttgtt actcctgct ggttaccgtg
1021 gccttcatca tcttttgggt ccgaagcaag cggagccggc tgctgcacag cgactacatg
1081 aacatgaccc ctagacggcc cggacctacc agaaagcact accagcctta cgctcctcct
1141 agagacttcg ccgcctacag atccaagcgg ggcagaaaga agctgctgta catcttcaag
1201 cagcccttca tgcggcccgt gcagaccaca caagaggaag atggctgctc ctgcagattc
1261 cccgaggaag aagaaggcgg ctgcgagctg agagtgaagt tcagcagatc cgccgacgct
1321 cctgcctatc agcagggaca gaaccagctg tacaacgagc tgaacctggg gagaagagaa
1381 gagtacgacg tgctggacaa gcggagaggc agagatcctg agatgggcgg aaagccccag
1441 cggagaaaga atcctcaaga gggcctgtat aatgagctgc agaaagacaa gatggccgag
1501 gcctacagcg agatcggaat gaagggcgag cgcagaagag gcaagggaca cgatggactg
1561 taccagggcc tgagcaccgc caccaaggat acctatgatg ccctgcacat gcaggccctg
1621 ccacctagat gatga
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Introduction of Polynucleotides encoding Cytokine Receptor Switches into Immune Cells

[00134] Immune cells such as T cells may be engineered to comprising nucleic acids encoding express cytokine receptor switches in accordance with known techniques. Generally, a polynucleotide vector is constructed that encodes the cytokine receptor switch and the vector is introduced (*e.g.*, transfected, or transduced) into a population of immune cells. The cells are then grown under conditions promoting expression of the polynucleotide encoding the cytokine receptor switch. Successful transfection (or transduction which refers to viral-mediated gene integration) and display of cytokine receptor switches may be conducted via standard techniques. In some embodiments, immune cells may be engineered to produce cytokine receptor switches by first constructing a retroviral vector encoding a selected cytokine receptor switch. Retroviral transduction may be performed using known techniques (*e.g.*, Johnson, *et al.*, Blood *114*:535-546 (2009)). The surface expression of cytokine receptor switch on transfected immune cells may be determined, for example, by flow cytometry.

[00135] Expression vectors that encode the cytokine receptor switches can be introduced as one or more DNA molecules or constructs, where there may be at least one marker that will allow for selection of host cells that contain the construct(s).

[00136] A DNA construct is an artificially constructed segment of nucleic acid for the introduction (*i.e.*, transfection or transduction) into a target cell or tissue. The term "nucleic acid" as used herein refers to a polymer of nucleotides, each of which are organic molecules consisting of a nucleoside (a nucleobase and a five-carbon sugar) and a phosphate. The term nucleotide, unless specifically sated or obvious from context, includes nucleosides that have a

ribose sugar (*i.e.*, a ribonucleotide that forms ribonucleic acid, RNA) or a 2'-deoxyribose sugar (*i.e.*, a deoxyribonucleotide that forms deoxyribonucleic acid, DNA). Nucleotides serve as the monomeric units of nucleic acid polymers or polynucleotides. The four nucleobases in DNA are guanine (G), adenine (A), cytosine (C) and thymine (T). The four nucleobases in RNA are guanine (G), adenine (A), cytosine (C) and uracil (U). Nucleic acids are linear chains of nucleotides (*e.g.*, at least 3 nucleotides) chemically bonded by a series of ester linkages between the phosphoryl group of one nucleotide and the hydroxyl group of the sugar (*i.e.*, ribose or 2'-deoxyribose) in the adjacent nucleotide.

[00137] The nucleic acids encode at least a cytokine receptor switch protein. The terms "protein" and "polypeptide" as used herein refer to a string of amino acids connected by amide linkages, typically at least ten (10) amino acids or longer in length. Proteins are ordinarily derived from organisms but are not limited thereto, and for example, they may be composed of an artificially designed sequence. They may also be any of naturally derived proteins, synthetic proteins, or recombinant proteins.

[00138] The constructs can be prepared in conventional ways, where the individual components of the cytokine receptor switches may be ligated in the desired order, cloned in an appropriate cloning host, analyzed by restriction or sequencing, or other convenient means. Particularly, using PCR, individual fragments including all or portions of a functional unit may be isolated, where one or more mutations may be introduced using "primer repair", ligation, in vitro mutagenesis, etc., as appropriate. The construct(s) once completed and demonstrated to have the appropriate sequences may then be packaged into a suitable vector which is then introduced into the immune cell (*i.e.*, T cell) by any convenient means. Vectors containing useful elements such as bacterial or yeast origins of replication, selectable and/or amplifiable markers (*e.g.*, hypoxanthine-guanine phosphoribosyltransferase (hprt), neomycin resistance, thymidine kinase, hygromycin resistance, etc.), promoter/enhancer elements for expression in prokaryotes or eukaryotes, one or more suitable sites for the insertion of the nucleic acid sequences, such as a multiple cloning site (MCS), and etc. that may be used to prepare stocks of construct DNAs and for carrying out transfections are well known in the art, and many are commercially available.

[00139] The constructs may be integrated and packaged into non-replicating, defective viral genomes like Adenovirus, Adeno-associated virus (AAV), or Herpes simplex virus (HSV) or others, including retroviral vectors or lentiviral vectors, for infection or transduction into cells.

The constructs may include viral sequences for transfection, if desired. Alternatively, the construct may be introduced by fusion, electroporation, biolistics, transfection, lipofection, or the like. The host immune cells may be grown and expanded in culture before introduction of the construct(s), followed by the appropriate treatment for introduction of the construct(s) and integration of the construct(s). The cells are then expanded and screened by virtue of a marker present in the construct.

[00140] In some instances, the construct may be engineered to have a target site for homologous recombination, where it is desired that the construct be integrated at a particular genomic locus. For example, an endogenous gene can be knocked out and replaced (at the same locus or elsewhere) with the gene(s) encoded for by the construct using materials and methods as are known in the art for homologous recombination. For homologous recombination, one may use either OMEGA or O-vectors. *See*, *e.g.* , Thomas and Capecchi, Cell *51*:503-512 (1987); Mansour *et al.*, Nature *336*:348-352 (1988); and Joyner *et al.*, Nature *338*:153-156 (1989).

[00141] In some embodiments, the vector is a a lentiviral vector or a recombinant lentivirus vector. In some embodiments, the expression vector is a non-integrative and non-replicative recombinant lentivirus vector. Exemplary lentiviral vectors include, for example, LentiVector and LentiStable from Oxford BioMedica, LV-Max from Gibco, and the like. The construction of lentiviral vectors has been described, for example, in U.S. Patents 5,665,577, 5,981,276, 6,013,516, 7,090,837, 8,119,119 and 10,954,530.

Formulations and Methods of use

Formulations containing Cytokine Receptor Switch-containing Immune Cells

[00142] The inventive immune cells may be formulated in pharmaceutically acceptable vehicles or carriers, the selection and amounts of which may be determined depending upon the mode of administration. The therapeutically effective amount of the formulation may depend upon the concentration of the cells in the overall volume of the formulation. The number of inventive immune cells administered to a subject may vary between wide limits, depending various factors including, for example, the location, type, and severity of the cancer, and the age and condition of the individual to be treated, and is within the level of skill of a treating physician. In general, formulations contain from about 1×10^4 to about 1×10^{10} inventive immune cells. In some embodiments, the formulation contains from about 1×10^5 to about 1×10^9 inventive immune cells, from about 5×10^5 to about 5×10^8 inventive immune cells, or from about 1×10^6 to about 1×10^7 inventive immune cells. *See*, for example,

International Publication No WO/2020/006312, which is incorporated herein by reference in its entirety.

[00143] The formulation of inventive immune cells may be administered to a subject in need thereof in accordance with acceptable medical practice. An exemplary mode of administration is intravenous injection. Other modes of administration may include intratumoral, intradermal, subcutaneous (*s.c.*, *s.q.*, sub-Q, Hypo), intramuscular (*i.m.*), intraperitoneal (*i.p.*), intra-arterial, intramedullary, intracardiac, intra-articular (joint), intrasynovial (joint fluid area), intracranial (including convection-enhanced delivery), intraspinal, and intrathecal (spinal fluids). Any known device useful for parenteral injection or infusion of the formulations can be used to affect such modes of administration. Representative vehicles and carriers include buffers such as neutral buffered saline, phosphate buffered saline and the like. The compositions may further include one or more pharmaceutically acceptable excipients. Examples of such excipients include 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.

Formulations containing the Synthetic Small Molecule

[00144] To stimulate the inventive immune cells *in vivo*, A therapeutically effective amount of the synthetic small molecule may be administered to a subject in need thereof in accordance with acceptable medical practice. The synthetic small molecule may be formulated in pharmaceutically acceptable vehicles or carriers, the selection and amounts of which may be determined depending upon the mode of administration. An exemplary mode of administration is intravenous injection. Other modes include intratumoral, intradermal, subcutaneous (*s.c.*, *s.q.*, sub-Q, Hypo), *i.m.*, *i.p.*, intra-arterial, intramedullary, intracardiac, intra-articular, intrasynovial, intracranial, intraspinal, and intrathecal. Any known device useful for parenteral injection or infusion of the formulations can be used to effect administration of the synthetic small molecule. A representative example of a pharmaceutically acceptable vehicle or carrier is serum albumin such as human serum albumin, dextrans, and antibodies.

[00145] In general, the amount of the synthetic small molecule ranges from 0.1 to 1000 μg/mL based on total volume of the composition. In some embodiments, the amount of the synthetic small molecule ranges is about 0.1 to 100 μg/mL based on total volume of the composition. The total amount of the small molecule may differ depending on the vehicle or

carrier. For example, for *in vivo* injection, the effective dose may be higher than *ex vivo* because not all the injected small molecules might be delivered to the tumor.

Methods for stimulating immune cells expressing inventive cytokine receptor switches

[00146] Broadly, the inventive methods entail treating or contacting the immune cells with a sufficient concentration of the synthetic small molecule.

[00147] In some embodiments, the method is conducted *ex vivo*. Immune cells that contain an exogenous nucleic acid encoding the cytokine receptor switch are placed in a suitable container suitable medium and contacted with an effective amount of the synthetic small molecule, *e.g.*, from 0.1 to 1000 μg/mL based on total volume of the medium. In some embodiments, the amount of the synthetic small molecule ranges is about 0.1 to 100 μg/mL based on total volume of the medium. Representative examples of suitable media that may be used in the practice of the methods include RPMI-1640 (Gibco™) and X-VIVO™ 15 (BioWhittaker™).

[00148] The duration of the contact (also referred to herein as "treatment" or "treating" or "stimulating") may be in the order of hours, days and even weeks (*e.g.*, 1, 2, 3, 4 or more weeks). In some embodiments, the contacting may be conducted in a high-affinity plate, dish, or flask wherein the small molecule is conjugated to the carrier affixed to a surface of the container. Representative examples of carriers include bovine or human serum albumin, dextran, and antibodies (*e.g.*, anti-HER2 antibodies, anti-EGFR antibodies, anti-BCMA antibodies, and anti-CD19 antibodies). In some embodiments, the antibody is Pertuzumab, Cetuximab, Belentamab, J6M0, or Daratuzumab.

[00149] In some embodiments, stimulation promotes proliferation or a change in phenotype of the immune cells. For example, the stimulation may promote an increase in the population of the stimulated immunes cells by at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 100%, or greater.

[00150] In some embodiments, the immune cell stimulation promotes a change in phenotype of the immune cells. Exemplary phenotypes include memory, cytotoxic, and regulatory phenotypes.

[00151] In some embodiments, the immune cells comprising a nucleic acid encoding a cytokine receptor switch are stimulated on fluorescein isothiocyanate (FITC)-conjugated-bovine serum albumin (BSA)-coated plate with increasing doses of FITC (0, 0.1, 1, 10, 100, and 1000 μg/mL) in the presence of CD3/CD28 co-stimulation for up to 2 weeks. Central memory

(CD45RA-, C-C chemokine receptor type 7 (CCR7)$^+$) and effector memory (CD45RA$^-$ CCR7$^-$) markers on CD4$^+$ and CD8$^+$ T cells may be assessed by flow cytometry.

[00152] In some embodiments, immune cells comprising a nucleic acid encoding a cytokine receptor switch are stimulated on FITC-conjugated-BSA-coated plate with increasing doses of FITC (0, 0.1, 1, 10, 100, and 1000 μg/mL) in the presence or absence of CD3/CD28 co-stimulation for up to 2 weeks. Effector memory (CD45RA$^-$ CCR7$^-$), central memory (CD45RA-, CCR7$^+$), and activation (CD69) markers on CD4$^+$ and CD8$^+$ T cells may be assessed by flow cytometry.

[00153] In some embodiments, the immune cells comprising a nucleic acid encoding a cytokine receptor switch are stimulated on carboxyfluorescein-conjugated-BSA-coated plate with increasing doses of carboxyfluorescein (0, 0.1, 1, 10, 100, and 1000 μg/mL) in the presence of CD3/CD28 co-stimulation for up to 2 weeks.

[00154] In some embodiments, immune cells comprising a nucleic acid encoding a CAR with or without one or a combination of different cytokine receptor switches are stimulated on small molecule-conjugated-antibody-coated plates with increasing doses of small molecule conjugates (0, 0.1, 1, 10, and 100 μg/mL) in the presence or absence of CD3/CD28 co-stimulation. Expression of IL-2, IFN-gamma and CD69 in CD4$^+$ and CD8$^+$ T cells may be assessed by flow cytometry.

[00155] The stimulated immune cells may be isolated from the medium and then formulated for delivery to a subject. In some embodiments, the immune cells are stimulated *in vivo*.

[00156] These embodiments entail administering to a subject in need thereof a therapeutically effective amount of the composition described herein; and administering to the subject a therapeutically effective amount of the synthetic small molecule. The synthetic small molecule and the immune cells may be administered via the same or different formulations and substantially simultaneously or sequentially. The method may further include administering the subject a formulation of synthetic monomeric or polymeric small molecules that serves to decrease the stimulation.

[00157] Administration of the *ex vivo* activated immune cells and the activation of the immune cells *in vivo* are typically performed in the context of treating a disease or disorder, namely cancer.

[00158] The term "subject" (or "patient") as used herein includes all members of the animal kingdom prone to or suffering from the indicated disease or disorder. In some embodiments,

the subject is a mammal, *e.g.*, a human or a non-human mammal. The methods are also applicable to companion animals such as dogs and cats as well as livestock such as cows, horses, sheep, goats, pigs, and other domesticated and wild animals. A subject "in need of" treatment according to the present disclosure may be "suffering from or suspected of suffering from" a specific disease or disorder may have been positively diagnosed or otherwise presents with a sufficient number of risk factors or a sufficient number or combination of signs or symptoms such that a medical professional could diagnose or suspect that the subject is suffering from the disease or disorder. Thus, subjects suffering from, and suspected of suffering from, a specific disease or disorder are not necessarily two distinct groups.

[00159] Broadly, the methods may be effective in the treatment of carcinomas (solid tumors including both primary and metastatic tumors), sarcomas, melanomas, and hematological cancers (cancers affecting blood including lymphocytes, bone marrow and/or lymph nodes) such as leukemia, lymphoma, and multiple myeloma. Adult tumors/cancers and pediatric tumors/cancers are included. The cancers may be vascularized, or not yet substantially vascularized, or non-vascularized tumors.

[00160] Representative examples of cancers include adrenocortical carcinoma, AIDS-related cancers (*e.g.*, Kaposi's and AIDS-related lymphoma), appendix cancer, childhood cancers (*e.g.*, childhood cerebellar astrocytoma, childhood cerebral astrocytoma), basal cell carcinoma, skin cancer (non-melanoma), biliary cancer, extrahepatic bile duct cancer, intrahepatic bile duct cancer, bladder cancer, urinary bladder cancer, brain cancer (*e.g.*, gliomas and glioblastomas such as brain stem glioma, gestational trophoblastic tumor glioma, cerebellar astrocytoma, cerebral astrocytoma/malignant glioma, ependymoma, medulloblastoma, supratentorial primitive neuroectodeimal tumors, visual pathway and hypothalamic glioma), breast cancer, bronchial adenomas/carcinoids, carcinoid tumor, nervous system cancer (*e.g.*, central nervous system cancer, central nervous system lymphoma), cervical cancer, chronic myeloproliferative disorders, colorectal cancer (*e.g.*, colon cancer, rectal cancer), lymphoid neoplasm, mycosis fungoids, Sezary Syndrome, endometrial cancer, esophageal cancer, extracranial germ cell tumor, extragonadal germ cell tumor, extrahepatic bile duct cancer, eye cancer, intraocular melanoma, retinoblastoma, gallbladder cancer, gastrointestinal cancer (*e.g.*, stomach cancer, small intestine cancer, gastrointestinal carcinoid tumor, gastrointestinal stromal tumor (GIST)), cholangiocarcinoma, germ cell tumor, ovarian germ cell tumor, head and neck cancer, neuroendocrine tumors, Hodgkin's lymphoma, Ann Arbor stage III and stage

IV childhood Non-Hodgkin's lymphoma, ROS1-positive refractory Non-Hodgkin's lymphoma, leukemia, lymphoma, multiple myeloma, hypopharyngeal cancer, intraocular melanoma, ocular cancer, islet cell tumors (endocrine pancreas), renal cancer (*e.g.*, Wilm's Tumor, renal cell carcinoma), liver cancer, lung cancer (*e.g.*, non-small cell lung cancer and small cell lung cancer), ALK-positive anaplastic large cell lymphoma, ALK-positive advanced malignant solid neoplasm, Waldenstrom's macroglobulinema, melanoma, intraocular (eye) melanoma, merkel cell carcinoma, mesothelioma, metastatic squamous neck cancer with occult primary, multiple endocrine neoplasia (MEN), myelodysplastic syndromes, myelodysplastic/myeloproliferative diseases, nasopharyngeal cancer, neuroblastoma, oral cancer (*e.g.*, mouth cancer, lip cancer, oral cavity cancer, tongue cancer, oropharyngeal cancer, throat cancer, laryngeal cancer), ovarian cancer (*e.g.*, ovarian epithelial cancer, ovarian germ cell tumor, ovarian low malignant potential tumor), pancreatic cancer, islet cell pancreatic cancer, paranasal sinus and nasal cavity cancer, parathyroid cancer, penile cancer, pharyngeal cancer, pheochromocytoma, pineoblastoma, metastatic anaplastic thyroid cancer, undifferentiated thyroid cancer, papillary thyroid cancer, pituitary tumor, plasma cell neoplasm/multiple myeloma, pleuropulmonary blastoma, prostate cancer, retinoblastoma, rhabdomyosarcoma, salivary gland cancer, uterine cancer (*e.g.*, endometrial uterine cancer, uterine sarcoma, uterine corpus cancer), squamous cell carcinoma, testicular cancer, thymoma, thymic carcinoma, thyroid cancer, juvenile xanthogranuloma, transitional cell cancer of the renal pelvis and ureter and other urinary organs, urethral cancer, gestational trophoblastic tumor, vaginal cancer, vulvar cancer, hepatoblastoma, rhabdoid tumor, and Wilms tumor.

[00161] Sarcomas that may be treatable with the methods of the present disclosure include both soft tissue and bone cancers alike, representative examples of which include osteosarcoma or osteogenic sarcoma (bone) (*e.g.*, Ewing's sarcoma), chondrosarcoma (cartilage), leiomyosarcoma (smooth muscle), rhabdomyosarcoma (skeletal muscle), mesothelial sarcoma or mesothelioma (membranous lining of body cavities), fibrosarcoma (fibrous tissue), angiosarcoma or hemangioendothelioma (blood vessels), liposarcoma (adipose tissue), glioma or astrocytoma (neurogenic connective tissue found in the brain), myxosarcoma (primitive embryonic connective tissue), mesenchymous or mixed mesodermal tumor (mixed connective tissue types), and histiocytic sarcoma (immune cancer).

[00162] In some embodiments, methods of the present disclosure entail treatment of subjects having cell proliferative diseases or disorders of the hematological system, liver, brain, lung, colon, pancreas, prostate, ovary, breast, skin, and endometrium.

[00163] As used herein, "cell proliferative diseases or disorders of the hematological system" include lymphoma, leukemia, myeloid neoplasms, mast cell neoplasms, myelodysplasia, benign monoclonal gammopathy, lymphomatoid papulosis, polycythemia vera, agnogenic myeloid metaplasia, and essential thrombocythemia. Representative examples of hematologic cancers may thus include multiple myeloma, lymphoma (including T-cell lymphoma, Hodgkin's lymphoma, non-Hodgkin's lymphoma (diffuse large B-cell lymphoma (DLBCL), follicular lymphoma (FL), mantle cell lymphoma (MCL) and ALK+ anaplastic large cell lymphoma (*e.g.,* B-cell non-Hodgkin's lymphoma selected from diffuse large B-cell lymphoma (*e.g.,* germinal center B-cell-like diffuse large B-cell lymphoma or activated B-cell-like diffuse large B-cell lymphoma), Burkitt's lymphoma/leukemia, mantle cell lymphoma, mediastinal (thymic) large B-cell lymphoma, follicular lymphoma, marginal zone lymphoma, lymphoplasmacytic lymphoma/Waldenstrom macroglobulinemia, metastatic pancreatic adenocarcinoma, refractory B-cell non-Hodgkin's lymphoma, and relapsed B-cell non-Hodgkin's lymphoma, childhood lymphomas, and lymphomas of lymphocytic and cutaneous origin, *e.g.,* small lymphocytic lymphoma, leukemia, including childhood leukemia, hairy-cell leukemia, acute lymphocytic leukemia, acute myelocytic leukemia, acute myeloid leukemia (*e.g.,* acute monocytic leukemia), chronic lymphocytic leukemia, small lymphocytic leukemia, chronic myelocytic leukemia, chronic myelogenous leukemia, and mast cell leukemia, myeloid neoplasms and mast cell neoplasms.

[00164] Multiple myeloma is a cell proliferative disease or disorder of the hematological system. Multiple myeloma is a plasma cell neoplasm. Healthy plasma cells produce antibodies that recognize and attack pathogens. In multiple myeloma, cancerous plasma cells accumulate in the bone marrow and crowd out healthy blood cells. Rather than produce antibodies, the cancerous myeloma plasma cells produce abnormal proteins that can cause complications. The overgrowth of plasma cells also results in crowding out of normal blood-forming cells, leading to low blood counts as well as thrombocytopenia and leukopenia. The myeloma plasma cells make abnormal antibodies known as monoclonal immunoglobulin, monoclonal protein (M-protein), M-spike, or paraprotein.

[00165] Other plasma cell cancers that do not meet the multiple myeloma criteria include monoclonal gammopathy of uncertain significance (MGUS), smoldering multiple myeloma (SMM), solitary plasmacytoma, and light chain amyloidosis. Minimal residual disease (MRD) refers to the small number of malignant cells below the limit of detection available with conventional morphologic assessment. MRD in multiple myeloma refers to myeloma cells that are present in the bone marrow after measuring a clinical response (CR) and the subject is in remission. These residual myeloma cells are clinically relevant, as they may lead to disease progression and relapse.

[00166] As used herein, "cell proliferative diseases or disorders of the liver" include all forms of cell proliferative disorders affecting the liver. Cell proliferative disorders of the liver may include liver cancer (*e.g.*, hepatocellular carcinoma, intrahepatic cholangiocarcinoma and hepatoblastoma), a precancer or precancerous condition of the liver, benign growths or lesions of the liver, and malignant growths or lesions of the liver, and metastatic lesions in tissue and organs in the body other than the liver. Cell proliferative disorders of the liver may include hyperplasia, metaplasia, and dysplasia of the liver.

[00167] As used herein, "cell proliferative diseases or disorders of the brain" include all forms of cell proliferative disorders affecting the brain. Cell proliferative disorders of the brain may include brain cancer (*e.g.*, gliomas, glioblastomas, meningiomas, pituitary adenomas, vestibular schwannomas, and primitive neuroectodermal tumors (medulloblastomas)), a precancer or precancerous condition of the brain, benign growths or lesions of the brain, and malignant growths or lesions of the brain, and metastatic lesions in tissue and organs in the body other than the brain. Cell proliferative disorders of the brain are also called central nervous system (CNS) tumors, which include astrocytic tumors, oligodendroglial tumors, mixed gliomas, ependymal tumors, medulloblastomas, pineal parenchymal tumors, meningeal tumors, germ cell tumors, craniopharyngioma (grade I). Cell proliferative disorders of the brain may include hyperplasia, metaplasia, and dysplasia of the brain. A cancer that has spread to the brain is referred to as a metastatic brain tumor. Approximately half of metastic brain tumors originate from lung tumors. Other tumors that have a propensity to spread to the brain include, for example, melanoma, breast cancer, colon cancer, kidney cancer, and nasopharyngeal cancer.

[00168] Glioblastoma (GBM), also referred to as a grade IV astrocytoma, is a fast-growing and aggressive cell proliferative disorder of the brain. GBM is an astrocytic tumor that begins

in astrocytes (a glial cell type) and may also be called gliomas. GBM invades the nearby brain tissue, but generally does not spread to distant organs. GBMs can arise in the brain *de novo* or evolve from a lower-grade astrocytoma.

[00169] As used herein, "cell proliferative diseases or disorders of the lung" include all forms of cell proliferative disorders affecting lung cells. Cell proliferative disorders of the lung include lung cancer, precancer and precancerous conditions of the lung, benign growths or lesions of the lung, hyperplasia, metaplasia, and dysplasia of the lung, and metastatic lesions in the tissue and organs in the body other than the lung. Lung cancer includes all forms of cancer of the lung, *e.g.,* malignant lung neoplasms, carcinoma *in situ,* typical carcinoid tumors, and atypical carcinoid tumors. Lung cancer includes small cell lung cancer ("SLCL"), non-small cell lung cancer ("NSCLC"), adenocarcinoma, small cell carcinoma, large cell carcinoma, squamous cell carcinoma, and mesothelioma. Lung cancer can include "scar carcinoma", bronchioveolar carcinoma, giant cell carcinoma, spindle cell carcinoma, and large cell neuroendocrine carcinoma. Lung cancer also includes lung neoplasms having histologic and ultrastructural heterogeneity (*e.g.,* mixed cell types). In some embodiments, a compound of the present disclosure may be used to treat non-metastatic or metastatic lung cancer (*e.g.*, NSCLC, ALK-positive NSCLC, NSCLC harboring ROS1 rearrangement, lung adenocarcinoma, and squamous cell lung carcinoma).

[00170] As used herein, "cell proliferative diseases or disorders of the colon" include all forms of cell proliferative disorders affecting colon cells, including colon cancer, a precancer or precancerous conditions of the colon, adenomatous polyps of the colon and metachronous lesions of the colon. Colon cancer includes sporadic and hereditary colon cancer, malignant colon neoplasms, carcinoma *in situ*, typical carcinoid tumors, and atypical carcinoid tumors, adenocarcinoma, squamous cell carcinoma, and squamous cell carcinoma. Colon cancer can be associated with a hereditary syndrome such as hereditary nonpolyposis colorectal cancer, familiar adenomatous polyposis, MYH associated polyposis, Gardner's syndrome, Peutz-Jeghers syndrome, Turcot's syndrome and juvenile polyposis. Cell proliferative disorders of the colon may also be characterized by hyperplasia, metaplasia, or dysplasia of the colon.

[00171] As used herein, "cell proliferative diseases or disorders of the pancreas" include all forms of cell proliferative disorders affecting pancreatic cells. Cell proliferative disorders of the pancreas may include pancreatic cancer, a precancer or precancerous condition of the pancreas, hyperplasia of the pancreas, dysplasia of the pancreas, benign growths or lesions of

the pancreas, and malignant growths or lesions of the pancreas, and metastatic lesions in tissue and organs in the body other than the pancreas. Pancreatic cancer includes all forms of cancer of the pancreas, including ductal adenocarcinoma, adenosquamous carcinoma, pleomorphic giant cell carcinoma, mucinous adenocarcinoma, osteoclast-like giant cell carcinoma, mucinous cystadenocarcinoma, acinar carcinoma, unclassified large cell carcinoma, small cell carcinoma, pancreatoblastoma, papillary neoplasm, mucinous cystadenoma, papillary cystic neoplasm, and serous cystadenoma, and pancreatic neoplasms having histologic and ultrastructural heterogeneity (*e.g.,* mixed cell).

[00172] As used herein, "cell proliferative diseases or disorders of the prostate" include all forms of cell proliferative disorders affecting the prostate. Cell proliferative disorders of the prostate may include prostate cancer, a precancer or precancerous condition of the prostate, benign growths or lesions of the prostate, and malignant growths or lesions of the prostate, and metastatic lesions in tissue and organs in the body other than the prostate. Cell proliferative disorders of the prostate may include hyperplasia, metaplasia, and dysplasia of the prostate.

[00173] As used herein, "cell proliferative diseases or disorders of the ovary" include all forms of cell proliferative disorders affecting cells of the ovary. Cell proliferative disorders of the ovary involve growth of cells that forms in one or both ovaries, the fallopian tubes, or the tissue that covers organs in the abdomen (*e.g.*, cystadenocarcinoma, ovarian embryonal carcinoma, ovarian adenocarcinoma) Cell proliferative disorders of the ovary may include a precancer or precancerous condition of the ovary, benign growths or lesions of the ovary, ovarian cancer, and metastatic lesions in tissue and organs in the body other than the ovary. Cell proliferative disorders of the ovary may include hyperplasia, metaplasia, and dysplasia of the ovary. Cell proliferative disorders of the ovary include ovarian epithelial cancer (epithelial ovarian carcinomas), germ cell tumors, and stromal cell tumors. Epithelial ovarian carcinomas are the most common type of ovarian cancer. About 85% to 90% of these cancers involve the cells that cover the outer surface of the ovary. They commonly spread first to the lining and organs of the pelvis and abdomen and then to other parts of the body. Nearly 70% of women with this type of ovarian cancer are diagnosed in the advanced stages. Ovarian epithelial cancer, fallopian tube cancer, and primary peritoneal cancer are epithelial ovarian carcinomas.

[00174] As used herein, "cell proliferative diseases or disorders of the breast" include all forms of cell proliferative disorders affecting breast cells. Cell proliferative disorders of the breast may include breast cancer, a precancer or precancerous condition of the breast, benign

growths or lesions of the breast, and metastatic lesions in tissue and organs in the body other than the breast. Cell proliferative disorders of the breast are a group of disorders in which cells in the breast grow out of control. Cell proliferative disorders of the breast may include hyperplasia, metaplasia, and dysplasia of the breast. Cell proliferative disorders of the breast can begin in different parts of the breast. A breast is made up of three main parts: lobules, ducts, and connective tissue. The lobules are the glands that produce milk. The ducts are tubes that carry milk to the nipple. The connective tissue (which consists of fibrous and fatty tissue) surrounds and connects the breast tissue. Most breast cancers begin in the ducts or lobules. Invasive ductal carcinoma and invasive lobular carcinoma are the two most common types of breast cancer. In invasive ductal carcinoma, cancer cells originate in the ducts and then spread, or metastasize, outside the ducts into other parts of the breast tissue. In invasive lobular carcinoma, cancer cells originate in the lobules and then spread from the lobules to the breast tissues that are close by. As used herein, "cell proliferative diseases or disorders of the skin" include all forms of cell proliferative disorders affecting skin cells. Cell proliferative disorders of the skin may include a precancer or precancerous condition of the skin, benign growths or lesions of the skin, melanoma, malignant melanoma or other malignant growths or lesions of the skin, and metastatic lesions in tissue and organs in the body other than the skin. Cell proliferative disorders of the skin may include hyperplasia, metaplasia, and dysplasia of the skin.

[00175] As used herein, "cell proliferative diseases or disorders of the endometrium" include all forms of cell proliferative disorders affecting cells of the endometrium. Cell proliferative disorders of the endometrium may include a precancer or precancerous condition of the endometrium, benign growths or lesions of the endometrium, endometrial cancer, and metastatic lesions in tissue and organs in the body other than the endometrium. Cell proliferative disorders of the endometrium may include hyperplasia, metaplasia, and dysplasia of the endometrium.

[00176] In some embodiments, the cancer is breast cancer, ovarian cancer, multiple myeloma, lung cancer, or glioblastoma multiforme. The therapeutic methods of the present disclosure may be "first-line", *i.e.,* an initial treatment in patients who not yet undergone any anti-cancer treatment, either alone or in combination with other treatments. The therapeutic methods of the present disclosure may be advantageously used as a "second-line" therapy in that they are administered to patients who have undergone at least one prior anti-cancer

treatment regimen, *e.g.*, chemotherapy, radioimmunotherapy, toxin therapy, prodrug-activating enzyme therapy, antibody therapy, surgical therapy, immunotherapy, radiation therapy, targeted therapy or any combination thereof either alone or in combination with other treatments. In some cases, the prior therapy may have been unsuccessful or partially successful but where the patient became intolerant to the particular treatment, and particularly in cases where the front-line therapy is no longer effective on account of antigen loss/escape.

Combination Therapy

[00177] In certain embodiments, the inventive methods of treating cancer may be part of a combination therapy wherein the subject is also treated with another agent that exerts an indirect or direct effect. In the case of administration of BAT-CAR immune cells, , the other agent is a synthetic antigen that is conjugated to a binding moiety that binds a cell surface antigen such as a tumor associated antigen. The BAT-CAR immune cells bind the cell surface antigen indirectly via the extracellular binding domain of the BAT-CAR which binds the synthetic antigen. Representative examples of synthetic antigens and conjugates thereof are described, for example, in International Publication Nos WO2017/143094, WO2018/200713, and WO2020/006312, each which is incorporated herein by reference in its entirety. In some embodiments, the synthetic antigen is masked or caged (a "pro-antigen") which renders it unable to bind the extracellular binding domain of the BAT-CAR. These embodiments require administration of a further agent that unmasks or uncages the antigen so that it can interact with the extracellular binding domain of the BAT-CAR. This feature adds yet another level of control to the inventive methods. Representative examples of synthetic pro-antigens and conjugates thereof are described, for example, in International Publication Nos WO2017/143094, WO2018/200713, and WO2020/006312, which is incorporated herein by reference in its entirety. In some embodiments, the synthetic antigen is masked by the addition of one or more CMNB caging groups and the unmasking agent is UV light.

[00178] In some embodiments, the methods entail administration of another anti-cancer agent. An "anti-cancer" agent is capable of negatively affecting cancer in a subject, for example, by killing cancer cells, inducing apoptosis in cancer cells, reducing the growth rate of cancer cells, reducing the incidence or number of metastases, reducing tumor size, inhibiting tumor growth, reducing the blood supply to a tumor or cancer cells, promoting an immune response against cancer cells or a tumor, preventing or inhibiting the progression of cancer, or increasing the lifespan of a subject with cancer. More generally, these other compositions

would be provided in a combined amount effective to kill or inhibit proliferation of the cell. This process may involve contacting the cancer cells with the expression construct and the agent(s) or multiple factor(s) at the same time. This may be achieved by contacting the cell with a single composition or pharmacological formulation that includes both agents, or by contacting the cell with two distinct compositions or formulations, at the same time, wherein one composition includes the expression construct and the other includes the second agent(s).

[00179] Tumor cell resistance to chemotherapy and radiotherapy agents represents a major problem in clinical oncology. One goal of current cancer research is to find ways to improve the efficacy of chemo- and radiotherapy by combining it with other therapies. In the context of the present disclosure, it is contemplated that cell therapy could be used similarly in conjunction with chemotherapeutic, radiotherapeutic, or immunotherapeutic intervention, as well as pro-apoptotic or cell cycle regulating agents.

[00180] Alternatively, the present inventive therapy may precede or follow the other agent treatment by intervals ranging from minutes to weeks. In embodiments where the other agent and present disclosure are applied separately to the individual, one would generally ensure that a significant period of time did not expire between the times of each delivery, such that the agent and inventive therapy would still be able to exert an advantageously combined effect on the cell. In such instances, it is contemplated that one may contact the cell with both modalities within about 12-24 h of each other and, more preferably, within about 6-12 h of each other. In some situations, it may be desirable to extend the time period for treatment significantly, however, where several days (2, 3, 4, 5, 6 or 7) to several weeks (1, 2, 3, 4, 5, 6, 7 or 8) lapse between the respective administrations.

[00181] It is expected that the treatment cycles would be repeated as necessary. It also is contemplated that various standard therapies, as well as surgical intervention, may be applied in combination with the inventive cell therapy.

[00182] Multiple myeloma therapeutics that are suitable for the combination with the inventive therapies described herein include belantamab mafodotin-blmf (Blenrep®), bortezomib (Velcade®), carfilzomib (Kyprolis®), carmustine (BiCNU®), ciltacabtagene autoleucel (Carvykti®), cyclophosphamide, daratumumab (Darzalex®), daratumumab and hyaluronidase-fihj (Darzalex Faspro®), doxorubicin hydrochloride liposome (Doxil®), elotuzumab (Empliciti®), idecabtagene vicleucel (Abecma®), isatuximab-irfc (Sarclisa®), ixazomib citrate (Ninlaro®), lenalidomide (Revlimid), melphalan and melphalan

hydrochloride (Alkeran® Tablets, Alkeran® for injection, Evomela®), pamidronate disodium (Aredia®), plerixafor (Mozobil®), pomalidomide (Pomalyst®), Selinexor (Xpovio®), thalidomide (Thalomid®), zoledronic acid (Zometa®), and the PAD combination of bortezomib (PS-341), doxorubicin hydrochloride (Adriamycin®), and dexamethasone.

[00183] Breast cancer prevention and therapeutics that are suitable for the combination with the inventive therapies described herein may also include raloxifene and tamoxifen citrate (Soltamox®), abemaciclib (Verzenio®), paclitaxel (Abraxane®), ado-trastuzumab emtansine (Kadcyla®), everolimus (Afinitor®, Zortress®, Afinitor Disperz®), alpelisib (Piqray®), anastrozole (Arimidex®), pamidronate disodium (Aredia®), exemestane (Aromasin®), cyclophosphamide, doxorubicin hydrochloride, epirubicin hydrochloride (Ellence®), fam-trastuzumab deruxtecan-nxki (Enhertu®), fluorouracil (5-FU; Adrucil®), toremifene (Fareston®), letrozole (Femara®), gemcitabine (Gemzar®, Infugem®), eribulin mesylate (Halaven®), trastuzumab and hyaluronidase-oysk (Herceptin Hylecta®), trastuzumab (Herceptin®), palbociclib (Ibrance®), ixabepilone (Ixempra®), pembrolizumab (Keytruda®), ribociclib (Kisqali®), olaparib (Lynparza®), margetuximab-cmkb (Margenza®), neratinib maleate (Nerlynx®), pertuzumab (Perjeta®), pertuzumab trastuzumab and hyaluronidase-zzxf (Phesgo®), talazoparib tosylate (Talzenna®), docetaxel (Taxotere®), atezolizumab (Tecentriq®), thiotepa (Tepadina®), methotrexate sodium (Trexall®), sacituzumab govitecan-hziy (Trodelvy®), tucatinib (Tukysa®), lapatinib ditosylate (Tykerb®), vinblastine sulfate, capecitabine (Xeloda®), and goserelin acetate (Zoladex®).

[00184] Ovarian cancer therapeutics that are suitable for the combination with the inventive therapies described herein include melphalan (Alkeran®), bevacizumab (Alymsys®, Avastin®, Mvasi®, Zirabev®), cisplatin, cyclophosphamide, doxorubicin hydrochloride, doxorubicin hydrochloride liposomes (Doxil®), gemcitabine hydrochloride (Gemzar®, Infugem®), topotecan hydrochloride (Hycamtin®), olaparib (Lynparza®), carboplatin (Paraplatin®), rucaparib camsylate (Rubraca®), thiotepa (Tepadina®), and niraparib tosylate monohydrate (Zejula®). Drugs approved for treating epithelial ovarian carcinomas may be applied to ovarian germ cell cancers.

[00185] Brain cancer therapeutics that are suitable for the combination with the inventive therapies described herein include Belzutifan (Welireg), Bevacizumab (Alymsys, Avastin, Mvasi, Zirabev), Carmustine (BiCNU), Carmustine Implant (Gliadel Wafer), Everolimus (Afinitor, Afinitor Disperz), Lomustine, Naxitamab-gqgk (Danyelza), and Temozolomide

(Temodar)

Immunotherapy

[00186] Checkpoint inhibitors (*e.g.*, pembrolizumab (Keytruda®), nivolumab (Opdivo®), cemiplimab (Libtayo®), atezolizumab (Tecentriq®), avelumab (Bavencio®), durvalumab (Imfinzi®) or other immune modulating anti-bodies or reagents may also be used as part of a combined therapy, in conjunction with the present cell therapy.

Chemotherapy

[00187] Cancer therapies also include a variety of combination therapies with both chemical and radiation-based treatments. Combination chemotherapies include, for example, Abraxane®, altretamine, docetaxel, Herceptin®, methotrexate, Novantrone®, Zoladex®, cisplatin (CDDP), carboplatin, procarbazine, mechlorethamine, cyclophosphamide, camptothecin, ifosfamide, melphalan, chlorambucil, busulfan, nitrosurea, dactinomycin, daunorubicin, doxorubicin, bleomycin, plicomycin, mitomycin, etoposide (VP16), tamoxifen, raloxifene, estrogen receptor binding agents, Taxol®, gemcitabien, Navelbine®, farnesyl-protein tansferase inhibitors, transplatinum, 5-fluorouracil, vincristine, vinblastine and methotrexate, or any analog or derivative variant of the foregoing and also combinations thereof.

[00188] In specific embodiments, chemotherapy for the individual is employed in conjunction with the disclosure, for example before, during and/or after administration of the disclosure

Radiotherapy

[00189] Other factors that cause DNA damage and have been used extensively include what are commonly known as gamma-rays, X-rays, and/or the directed delivery of radioisotopes to tumor cells. Other forms of DNA damaging factors are also contemplated such as microwaves and UV-irradiation. It is most likely that all of these factors effect a broad range of damage on DNA, on the precursors of DNA, on the replication and repair of DNA, and on the assembly and maintenance of chromosomes. Dosage ranges for X-rays range from daily doses of 50 to 200 roentgens for prolonged periods of time (3 to 4 weeks), to single doses of 2000 to 6000 roentgens. Dosage ranges for radioisotopes vary widely, and depend on the half-life of the isotope, the strength and type of radiation emitted, and the uptake by the neoplastic cells.

Genes

[00190] In yet another embodiment, the secondary treatment is a gene therapy in which a therapeutic polynucleotide (*e.g.*, a therapeutic RNA such as an mRNA or a replicon) is administered before, after, or at the same time as the present disclosure clinical embodiments. A variety of expression products are encompassed within the disclosure, including inducers of cellular proliferation, inhibitors of cellular proliferation, or regulators of programmed cell death.

Surgery

[00191] Approximately 60% of persons with cancer will undergo surgery of some type, which includes preventative, diagnostic or staging, curative and palliative surgery. Curative surgery is a cancer treatment that may be used in conjunction with other therapies, such as the treatment of the present disclosure, chemotherapy, radiotherapy, hormonal therapy, gene therapy, immunotherapy and/or alternative therapies.

[00192] Curative surgery includes resection in which all or part of cancerous tissue is physically removed, excised, and/or destroyed. Tumor resection refers to physical removal of at least part of a tumor. In addition to tumor resection, treatment by surgery includes laser surgery, cryosurgery, electrosurgery, and miscopically controlled surgery (Mohs surgery). It is further contemplated that the present disclosure may be used in conjunction with removal of superficial cancers, precancers, or incidental amounts of normal tissue.

[00193] Upon excision of part or all the cancerous cells, tissue, or tumor, a cavity may be formed in the body. Treatment may be accomplished by perfusion, direct injection, or local application of the area with an additional anti-cancer therapy. Such treatment may be repeated, for example, every 1, 2, 3, 4, 5, 6, or 7 days, or every 1, 2, 3, 4, and 5 weeks or every 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 months. These treatments may be of varying dosages as well.

Other Agents

[00194] Further agents may be used in the inventive methods. These additional agents include immunomodulatory agents, agents that affect the upregulation of cell surface receptors and GAP junctions, cytostatic and differentiation agents, inhibitors of cell adhesion, or agents that increase the sensitivity of the hyperproliferative cells to apoptotic inducers. Immunomodulatory agents include tumor necrosis factor; F42K and other cytokine analogs; or MIP-1, MIP-1beta, MCP-1, RANTES, and other chemokines. Upregulation of cell surface receptors or their ligands such as Fas/Fas ligand, DR4 or DR5/TRAIL may potentiate the apoptotic inducing abilities of the present compositions and methods by establishing an

autocrine or paracrine effect on hyperproliferative cells. Increasing intercellular signaling by elevating the number of GAP junctions may increase the anti-hyperproliferative effects on the neighboring hyperproliferative cell population. Therefore, in other embodiments, cytostatic or differentiation agents may be used in combination with the present disclosure to further enhance the anti-hyperproliferative efficacy. Inhibitors of cell adhesion may also enhance the efficacy of the present disclosure. Examples of cell adhesion inhibitors are focal adhesion kinase (FAKs) inhibitors and Lovastatin. Yet other agents that increase the sensitivity of a hyperproliferative cell to apoptosis, such as the antibody c225, may be used.

[00195] These and other aspects of the present disclosure will be further appreciated upon consideration of the following examples, which are intended to illustrate certain particular embodiments of the disclosure but are not intended to limit its scope, as defined by the claims.

EXAMPLES

Example 1: Effect of inventive cytokine receptor switches on $CD4^+$ and $CD8^+$ T cells with and without CD3/CD28-costimulation.

[00196] T cells comprising nucleic acids encoding cytokine receptor switches were stimulated on FITC-conjugated-BSA-coated plate (FIG. 2) with increasing dose of FITC (0, 0.1, 1, 10, 100, and 1000 μg/mL) in the presence or absence of CD3/CD28 co-stimulation for up to 2 weeks. The effector memory ($CD45RA^-$ $CCR7^-$), central memory ($CD45RA^-$, $CCR7^+$), and activation (CD69) markers on $CD4^+$ and $CD8^+$ T cells were assessed by flow cytometry.

[00197] FIG. 3A shows that IL2RA- IL2RB-, IL2RG-, IL7RA- and IL15RA-cytokine receptor switches increased effector memory markers on CD8+ T cells with CD3/CD28-costimulation. IL2RA- and IL15RA-cytokine receptor switches also increased effector memory markers on $CD4^+$ T cells with CD3/CD28-costimulation.

[00198] FIG. 3B shows that IL2RA- and IL7RA-cytokine receptor switches increased central memory markers on $CD8^+$ T cells with CD3/CD28-costimulation.

[00199] FIG. 3C shows that cytokine receptor switches had little effect on effector memory markers on T cells without CD3/CD28-costimulation.

[00200] FIG. 3D shows that IL7RA-cytokie receptor switch increased central memory makers on $CD4^+$ T cells even without CD3/CD28-costimulation.

Example 2: BSA-FITC bound on high-affinity plate efficiently stimulated chimeric antigen receptor (CAR)-T cells.

[00201] T cells comprising a nucleic acid encoding anti-fluorescein CAR were stimulated on FITC-conjugated-antibody-coated normal (Normal) or high affinity (High affinity) plates or antibody-FITC solution (Free) with increasing doses of FITC (0, 0.1, 1, 10, and 100 μg/mL) in the presence or absence of CD3/CD28 co-stimulation. Expression of IL-2, IFN-gamma and CD69 in $CD8^+$ T cells were assessed by flow cytometry.

[00202] FIG. 5A shows that expression of IL-2 was increased in CAR T cells in a FITC-dose-dependent manner. Antibody-FITC bound to high-affinity plate stimulated CAR-T cells more effectively than antibody-FITC bound to normal plate or in solution.

[00203] FIG. 5B shows that expression of IFN-gamma was increased in CAR T cells in a FITC-dose-dependent manner. Antibody-FITC bound to high-affinity plate stimulated CAR-T cells more effectively than antibody-FITC bound to normal plate or in solution.

[00204] FIG. 5C shows that expression of CD69 was increased in CAR T cells in a FITC-dose-dependent manner. Antibody-FITC bound to high-affinity plate stimulated CAR-T cells more effectively than antibody-FITC bound to normal plate or in solution.

Example 3: Effect of inventive cytokine receptor switches on NK cells

[00205] Human natural killer (NK) cell line, NK92, expressing different combinations of cytokine receptor switches (IL2RB- and IL2RG-cytokine receptor switches, or IL15RA-, IL2RB- and IL2RG-cytokine receptor switches) were stimulated on FITC-conjugated-BSA-coated plate with increasing dose of FITC (0, 0,1, 1, 10, 100 μM) for up to 7 days. The fold-increase of viable cells was assessed by cell proliferation assay. The activation marker CD69 on NK92 cells was assessed by flow cytometry. FIG. 6A shows that the combination of inventive IL15RA-, IL2RB- and IL2RG-cytokine receptor switches promoted cell proliferation of NK92 cells. These NK92 cells comprise three nucleic acids encoding three cytokine receptor switches, including a first cytokine switch comprising an IL-2RB signal peptide, an anti-small molecule scFv, a CD8 hinge, an IL-2RB transmembrane domain, and an IL-2RB intracellular domain, a second cytokine switch comprising an IL-2RG signal peptide, an anti-small molecule scFv, a CD8 hinge, an IL-2RG transmembrane domain, and an IL-2RG intracellular domain, and a third cytokine switch comprising an IL-15RA signal peptide, an anti-small molecule scFv, a CD8 hinge, an IL-15RA transmembrane domain, and an IL-15RA intracellular domain.

[00206] NK 92 cells expressing different combinations of cytokine receptor switches (IL7RA- and IL2RG-cytokine receptor switches or IL2RB- and IL2RG-cytokine receptor switches) were stimulated on FITC-conjugated-BSA-coated plate with increasing dose of FITC (0, 0,1, 1, 10, 100, 1000 μM) for up to 7 days. The activation marker CD69 on NK92 cells was assessed by flow cytometry. FIG. 6B shows that the combination of inventive IL7RA- and IL2RG-cytokine receptor switches increased the expression of activation marker CD69 on NK92 cells. These NK92 cells comprise two nucleic acids

encoding two cytokine receptor switches, including a first cytokine switch comprising an IL-2RG signal peptide, an anti-small molecule scFv, a CD8 hinge, an IL-2RG transmembrane domain, an IL-2RG intracellular domain, and a second cytokine switch comprising an IL-7RA signal peptide, an anti-small molecule scFv, a CD8 hinge, an IL-7RA transmembrane domain, and an IL-7RA intracellular domain.

[00207] All patent publications and non-patent publications are indicative of the level of skill of those skilled in the art to which this disclosure pertains. All these publications (including any specific portions thereof that are referenced) are herein incorporated by reference to the same extent as if each individual publication were specifically and individually indicated as being incorporated by reference.

[00208] Although the disclosure herein has been described with reference to particular embodiments, it is to be understood that these embodiments are merely illustrative of the principles and applications of the present disclosure. It is therefore to be understood that numerous modifications may be made to the illustrative embodiments and that other arrangements may be devised without departing from the spirit and scope of the present disclosure as defined by the appended claims.

What is claimed is:

1. A cytokine receptor switch comprising a signal peptide, a single chain antibody fragment (scFv) that specifically binds a synthetic, substantially nonimmunogenic small molecule ("synthetic small molecule"), a hinge domain, a transmembrane domain, and an intracellular domain native to or derived from a first cytokine receptor.

2. The cytokine receptor switch of claim 1, wherein the signal peptide is native to a second cytokine receptor, wherein the first and second cytokine receptors are the same or different.

3. The cytokine receptor switch of claim 2, wherein the signal peptide is native to IL-2RA, IL-2RB, IL-2RG, IL-4RA, IL-7RA, IL-9R, IL-15RA, or IL-21R.

4. The cytokine receptor switch of claim 3, wherein the signal peptide is native to IL-2RA and has nucleic acid sequence SEQ ID NO: 1 and amino acid sequence SEQ ID NO: 2.

5. The cytokine receptor switch of claim 3, wherein the signal peptide is native to IL-2RB and has nucleic acid sequence SEQ ID NO: 3 and amino acid sequence SEQ ID NO: 4.

6. The cytokine receptor switch of claim 3, wherein the signal peptide is native to IL-2RG and has nucleic acid sequence SEQ ID NO: 5 and amino acid sequence SEQ ID NO 6.

7. The cytokine receptor switch of claim 3, wherein the signal peptide is native to IL-4RA and has nucleic acid sequence SEQ ID NO: 7 and amino acid sequence SEQ ID NO 8.

8. The cytokine receptor switch of claim 3, wherein the signal peptide is native to IL-7RA and has nucleic acid sequence SEQ ID NO: 9 and amino acid sequence SEQ ID NO 10.

9. The cytokine receptor switch of claim 3, wherein the signal peptide is native to IL-9R and has nucleic acid sequence SEQ ID NO: 11 and amino acid sequence SEQ ID NO 12.

10. The cytokine receptor switch of claim 3, wherein the signal peptide is native to IL-15RA and has nucleic acid sequence SEQ ID NO: 13 and amino acid sequence SEQ ID NO 14.

11. The cytokine receptor switch of claim 3, wherein the signal peptide is native to IL-21R and has nucleic acid sequence SEQ ID NO: 15 and amino acid sequence SEQ ID NO 16.

12. The cytokine receptor switch of claim 1, wherein the scFv binds fluorescein or a fluorescein derivative, 4-[(6-methylpyrazin-2-yl) oxy] benzoate (MPOB), anthraquinone-2-carboxylate (AQ), or tetraxetan (DOTA).

13. The cytokine receptor switch of claim 12, wherein the scFv binds fluorescein and fluorescein derivatives and has nucleic acid sequence SEQ ID NO: 51 and amino acid sequence SEQ ID NO 52.

14. The cytokine receptor switch of claim 12, wherein the scFv binds MPOB and has nucleic acid sequence SEQ ID NO: 53 and amino acid sequence SEQ ID NO 54.

15. The cytokine receptor switch of claim 12, wherein the scFv binds AQ and has nucleic acid sequence SEQ ID NO: 55 and amino acid sequence SEQ ID NO 56.

16. The cytokine receptor switch of claim 12, wherein the scFv binds DOTA and has nucleic acid sequence SEQ ID NO: 57 and amino acid sequence SEQ ID NO 58.

17. The cytokine receptor switch of claim 1, wherein the hinge domain is derived from cluster of differentiation 8 (CD8).

18. The cytokine receptor switch of claim 17, wherein the hinge domain peptide has nucleic acid sequence SEQ ID NO: 49 and amino acid sequence SEQ ID NO 50.

19. The cytokine receptor switch of claim 1, wherein the transmembrane domain is derived from IL-2RA, IL-2RB, IL-2RG, IL-4RA, IL-7RA, IL-9RA, IL-15RA, or IL-21R.

20. The cytokine receptor switch of claim 19, wherein the transmembrane domain peptide is derived from IL-2RA and has nucleic acid sequence SEQ ID NO: 17 and amino acid sequence SEQ ID NO 18.

21. The cytokine receptor switch of claim 19, wherein the transmembrane domain peptide is derived from IL-2RB and has nucleic acid sequence SEQ ID NO: 19 and amino acid sequence SEQ ID NO 20.

22. The cytokine receptor switch of claim 19, wherein the transmembrane domain peptide is derived from IL-2RG and has nucleic acid sequence SEQ ID NO: 21 and amino acid sequence SEQ ID NO 22.

23. The cytokine receptor switch of claim 19, wherein the transmembrane domain peptide is derived from IL-4RA and has nucleic acid sequence SEQ ID NO: 23 and amino acid sequence SEQ ID NO 24.

24. The cytokine receptor switch of claim 19, wherein the transmembrane domain peptide is derived from IL-7RA and has nucleic acid sequence SEQ ID NO: 25 and amino acid sequence SEQ ID NO 26.

25. The cytokine receptor switch of claim 19, wherein the transmembrane domain peptide is derived from IL-9R and has nucleic acid sequence SEQ ID NO: 27 and amino acid sequence SEQ ID NO 28.

26. The cytokine receptor switch of claim 19, wherein the transmembrane domain peptide is derived from IL-15RA and has nucleic acid sequence SEQ ID NO: 29 and amino acid sequence SEQ ID NO 30.

27. The cytokine receptor switch of claim 19, wherein the transmembrane domain peptide is derived from IL-21R and has nucleic acid sequence SEQ ID NO: 31 and amino acid sequence SEQ ID NO 32.

28. The cytokine receptor switch of claim 1, wherein the intracellular domain is derived from IL-2RA, IL-2RB, IL-2RG, IL-4RA, IL-7RA, IL-9RA, IL-15RA, or IL-21R.

29. The cytokine receptor switch of claim 28, wherein the intracellular domain is derived from IL-2RA and has nucleic acid sequence SEQ ID NO: 33 and amino acid sequence SEQ ID NO 34.

30. The cytokine receptor switch of claim 28, wherein the intracellular domain is derived from IL-2RB and has nucleic acid sequence SEQ ID NO: 35 and amino acid sequence SEQ ID NO 36.

31. The cytokine receptor switch of claim 28, wherein the intracellular domain is derived from IL-2RG and has nucleic acid sequence SEQ ID NO: 37 and amino acid sequence SEQ ID NO 38.

32. The cytokine receptor switch of claim 28, wherein the intracellular domain is derived from IL-4RA and has nucleic acid sequence SEQ ID NO: 39 and amino acid sequence SEQ ID NO 40.

33. The cytokine receptor switch of claim 28, wherein the intracellular domain is derived from IL-7RA and has nucleic acid sequence SEQ ID NO: 41 and amino acid sequence SEQ ID NO 42.

34. The cytokine receptor switch of claim 28, wherein the intracellular domain is derived from IL-9R and has nucleic acid sequence SEQ ID NO: 43 and amino acid sequence SEQ ID NO 44.

35. The cytokine receptor switch of claim 28, wherein the intracellular domain is derived from IL-15RA and has nucleic acid sequence SEQ ID NO: 45 and amino acid sequence SEQ ID NO 46.

36. The cytokine receptor switch of claim 28, wherein the intracellular domain is derived from IL-21R and has nucleic acid sequence SEQ ID NO: 47 and amino acid sequence SEQ ID NO 48.

37. The cytokine receptor switch of claim 1, which is an anti-fluorescein-IL2-RA cytokine receptor switch and has amino acid sequence SEQ ID NO: 59.

38. A nucleic acid encoding the cytokine receptor switch of claim 1.

39. A composition comprising an immune cell containing the nucleic acid of claim 38.

40. The composition of claim 39, wherein the immune cell further comprises at least two of the nucleic acids, wherein the two nucleic acids encode different cytokine receptor switches that comprise different signal peptides, different transmembrane domains, and/or different intracellular domains of the cytokine receptors.

41. The composition of claim 40, wherein the at least two cytokine receptor switches comprise different scFvs.

42. The composition of claim 40, wherein the cytokine receptor switches comprise the same scFv.

43. The composition of claim 40, wherein the at least 2 cytokine receptor switches comprise a first cytokine receptor switch comprising an IL-2RG signal peptide, an anti-small molecule scFv, a CD8 hinge, an IL-2RG transmembrane domain, an IL-2RG intracellular domain, and a second cytokine receptor switch comprising an IL-7RA signal peptide, an anti-small molecule scFv, a CD8 hinge, an IL-7RA transmembrane domain, and an IL-7RA intracellular domain.

44. The composition of claim 40, wherein the immune cell further comprises at least a third nucleic acid that encodes a third cytokine receptor switch that differs from the first and second cytokine receptor switches.

45. The composition of claim 44, wherein the at least 3 cytokine receptor switches comprise a first cytokine receptor switch comprising an IL-2RB signal peptide, an anti-small molecule scFv, a CD8 hinge, an IL-2RB transmembrane domain, and an IL-2RB intracellular domain, a second cytokine receptor switch comprising an IL-2RG signal peptide, an anti-small molecule scFv, a CD8 hinge, an IL-2RG transmembrane domain, and an IL-2RG intracellular domain, and a third cytokine receptor switch comprising an IL-15RA signal peptide, an anti-small molecule scFv, a CD8 hinge, an IL-15RA transmembrane domain, and an IL-15RA intracellular domain.

46. The composition of any claim 39, wherein the immune cells are T cells or NK cells.

47. The composition of claim 46, wherein the T cells are $CD8^+$ or $CD4^+$.

48. The composition of claim 39, wherein the immune cells further comprise a nucleic acid encoding a chimeric antigen receptor (CAR) directed against a cell surface antigen.

49. The composition of claim 48, wherein the cell surface antigen is CD19, B-cell maturation antigen (BCMA), human epidermal growth factor receptor 2 (HER2), or epidermal growth factor receptor (EGFR), mucin 1 (MUC1), or TNF receptor superfamily member 13B (TNFRSF13B).

50. The composition of claim 46, wherein immune cells are binary activated T cells that encodes a chimeric antigen receptor (BAT-CAR).

51. The composition of claim 46, wherein immune cells are binary activated NK cells comprising a nucleic acid encoding a chimeric antigen receptor.

52. A method for stimulating the immune cells in the composition of claim 39, comprising contacting the immune cells with a sufficient concentration of the synthetic small molecule to promote proliferation.

53. The method of claim 52, wherein the contacting also promotes a change in phenotype of the immune cells.

54. The method of claim 53, wherein the treating promotes a change in phenotype selected from memory, cytotoxic, and regulatory phenotypes.

55. The method of claim 52, wherein the synthetic small molecule is fluorescein or a fluorescein derivative, MPOB, AQ, or DOTA.

56. The method of claim 52, wherein the synthetic small molecule is conjugated to a carrier.

57. The method of claim 56, wherein the synthetic small molecule conjugated to the carrier is bound to a high-affinity plate, dish, or flask.

58. The method of claim 56, wherein the carrier is bovine or human serum albumin, dextran, or an antibody.

59. The method of claim 52, wherein the synthetic small molecule is a polymer.

60. The method of claim 59, wherein the polymer is a monopolymer, heteropolymer, or a branched polymer.

61. The method of claim 58, wherein the antibody is an anti-HER2 antibody, anti-EGFR antibody, anti-BCMA, or anti-CD19 antibody.

62. The method of claim 61, wherein the antibody is Pertuzumab, Cetuximab, Belantamab, J6M0, or Daratuzumab.

63. The method of claim 52 or 53, wherein the concentration of the synthetic small molecule ranges from 0.1 to 1000 μg/mL, based on total volume of the composition.

64. The method of claim 63, wherein the concentration of the synthetic small molecule ranges from 0.1 to 100 μg/mL, based on total volume of the composition.

65. The method of claim 52, wherein the immune cells are treated with the sufficient concentration of the synthetic small molecule for up to two or more weeks.

66. The method of claim 52, wherein the immune cells are treated with the sufficient concentration of the synthetic small molecule for one week.

67. The method of claim 52, wherein the immune cells are contacted with the synthetic small molecule *ex vivo*.

68. The method of claim 52, wherein the immune cells are contacted with the synthetic small molecule *in vivo*.

69. The method of claim 68, wherein the immune cells are contacted with the synthetic small molecule systematically.

70. The method of claim 68, wherein the immune cells are contacted with the synthetic small molecule locally.

71. A method for treating a disease or disorder comprising:

administering to a subject in need thereof a therapeutically effective amount of the composition of claim 39; and

administering to the subject a sufficient concentration of at least one synthetic small molecule conjugated to a carrier thereby stimulating the immune cells in the composition.

72. The method of claim 71, wherein the synthetic small molecule is fluorescein or a fluorescein derivative, MPOB, AQ, or DOTA.

73. The method of claim 71, where the carrier is human serum albumin or an antibody.

74. The method of claim 73, wherein the antibody is an anti-HER2 antibody, anti-EGFR antibody, anti-BCMA antibody, or anti-CD19 antibody.

75. The method of claim 74, wherein the antibody is Pertuzumab, Cetuximab, Belentamab, J6M0, or Daratuzumab.

76. The method of claim 71, wherein the disease or disorder is cancer.

77. The method of claim 76, wherein the cancer is breast cancer, ovarian cancer, multiple myeloma, lung cancer, or glioblastoma multiforme.

FIG. 1A

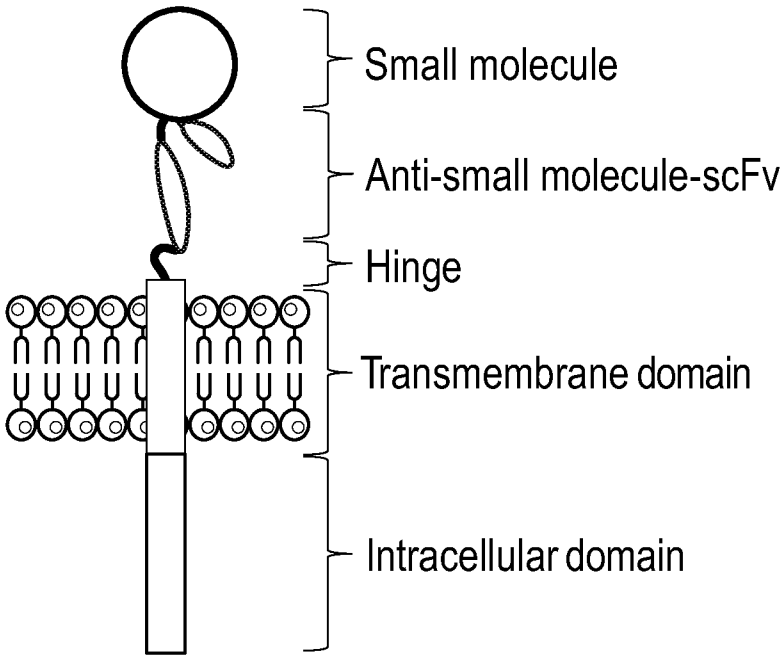

Small molecule
Anti-small molecule-scFv
Hinge
Transmembrane domain
Intracellular domain

FIG. 1B

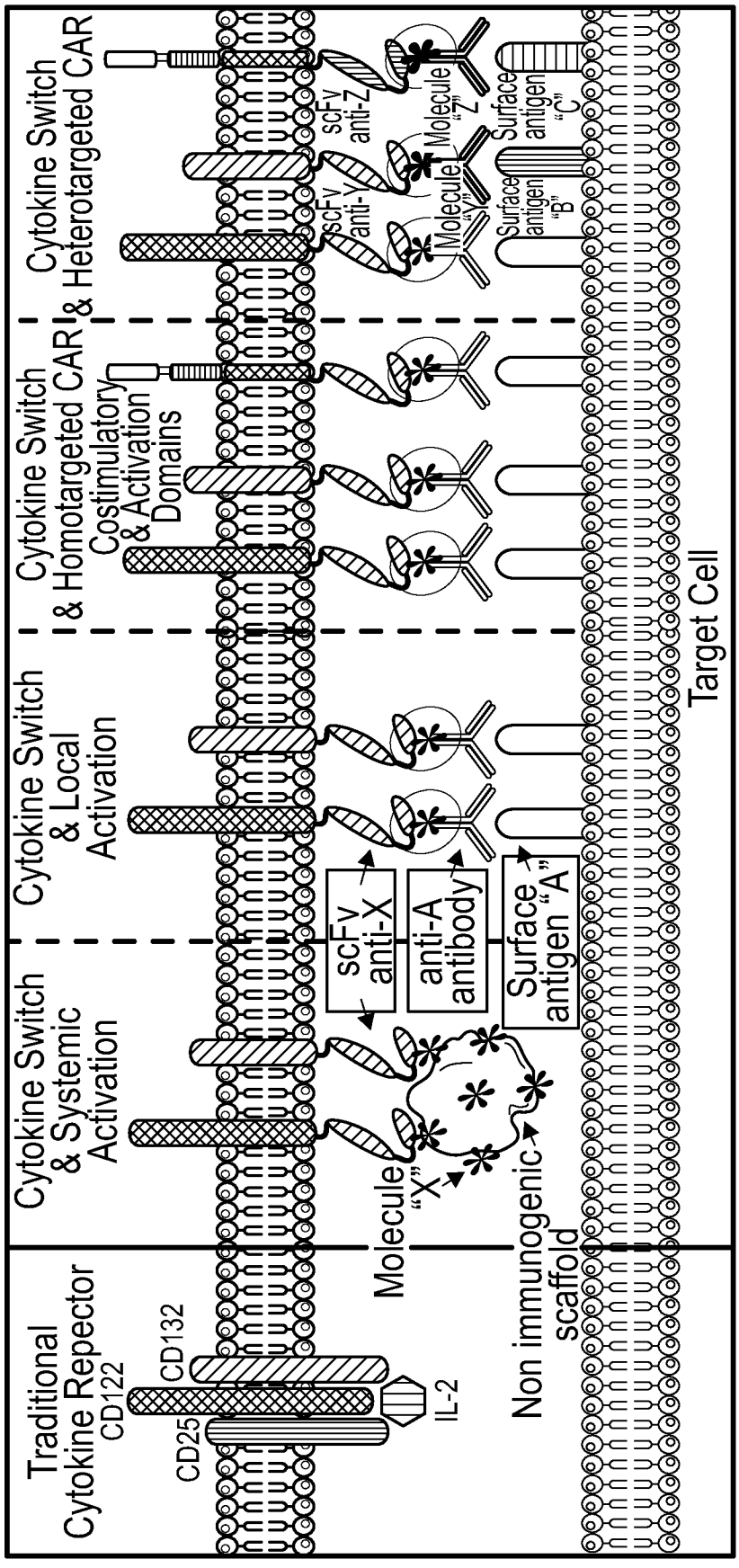

Traditional Cytokine Repector
CD122
CD132
CD25
IL-2
Cytokine Switch & Systemic Activation
Cytokine Switch & Local Activation
Cytokine Switch & Homotargeted CAR Costimulatory & Activation Domains
Cytokine Switch & Heterotargeted CAR
Molecule "X"
scFv anti-X
anti-A antibody
Surface antigen "A"
Non immunogenic scaffold
scFv anti-Y
Molecule "Y"
scFv anti-Z
Molecule "Z"
Surface antigen "B"
Surface antigen "C"
Target Cell

FIG. 1C

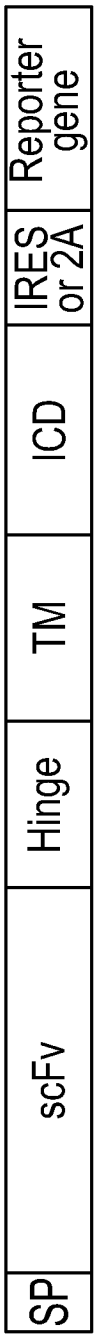

Cytokine Switch
SP
scFv
Hinge
TM
ICD
IRES or 2A
Reporter gene

FIG. 1D

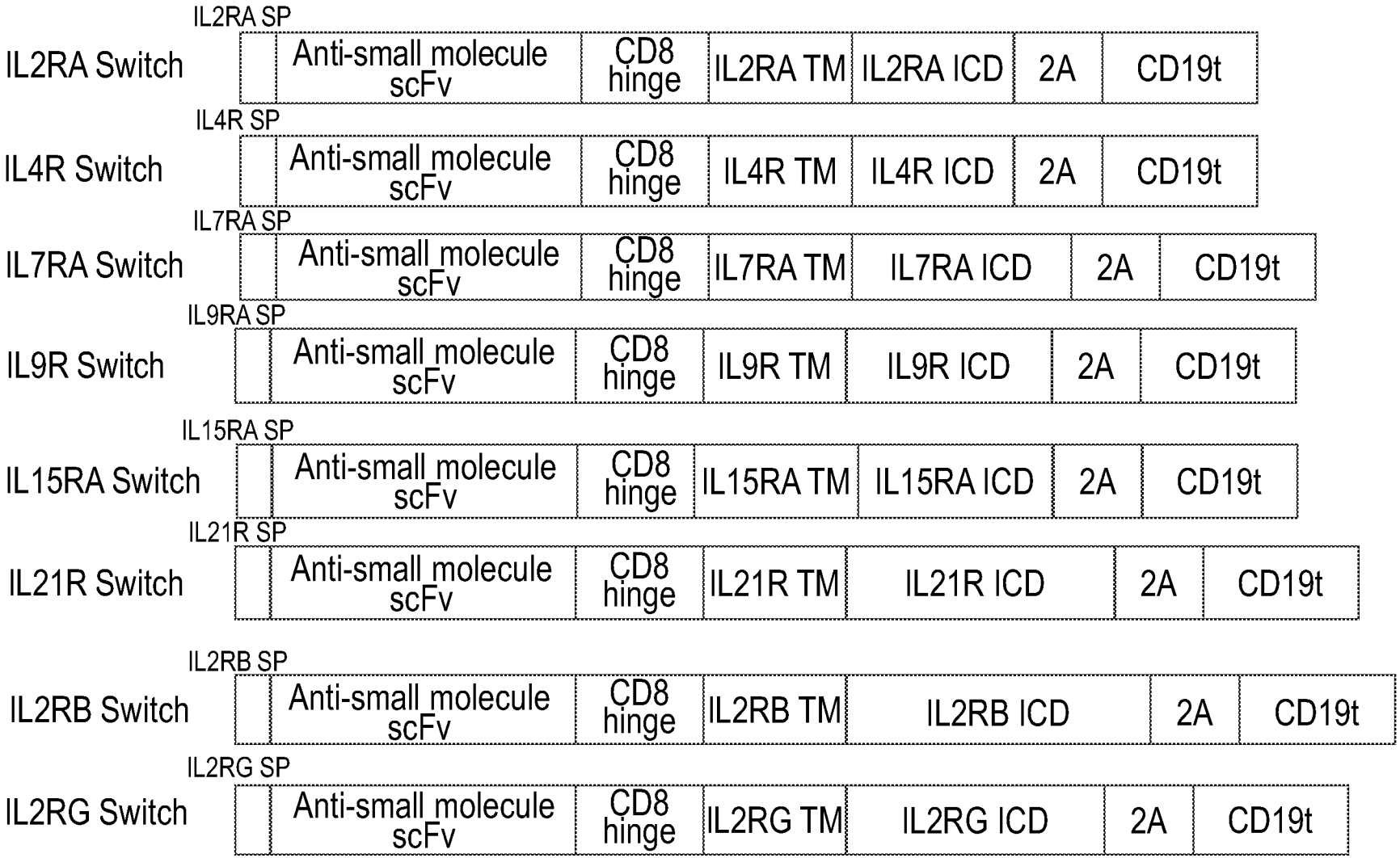

IL2RA SP
IL2RA Switch
Anti-small molecule scFv
CD8 hinge
IL2RA TM
IL2RA ICD
2A
CD19t
IL4R SP
IL4R Switch
Anti-small molecule scFv
CD8 hinge
IL4R TM
IL4R ICD
2A
CD19t
IL7RA SP
IL7RA Switch
Anti-small molecule scFv
CD8 hinge
IL7RA TM
IL7RA ICD
2A
CD19t
IL9RA SP
IL9R Switch
Anti-small molecule scFv
CD8 hinge
IL9R TM
IL9R ICD
2A
CD19t
IL15RA SP
IL15RA Switch
Anti-small molecule scFv
CD8 hinge
IL15RA TM
IL15RA ICD
2A
CD19t
IL21R SP
IL21R Switch
Anti-small molecule scFv
CD8 hinge
IL21R TM
IL21R ICD
2A
CD19t
IL2RB SP
IL2RB Switch
Anti-small molecule scFv
CD8 hinge
IL2RB TM
IL2RB ICD
2A
CD19t
IL2RG SP
IL2RG Switch
Anti-small molecule scFv
CD8 hinge
IL2RG TM
IL2RG ICD
2A
CD19t

FIG. 2

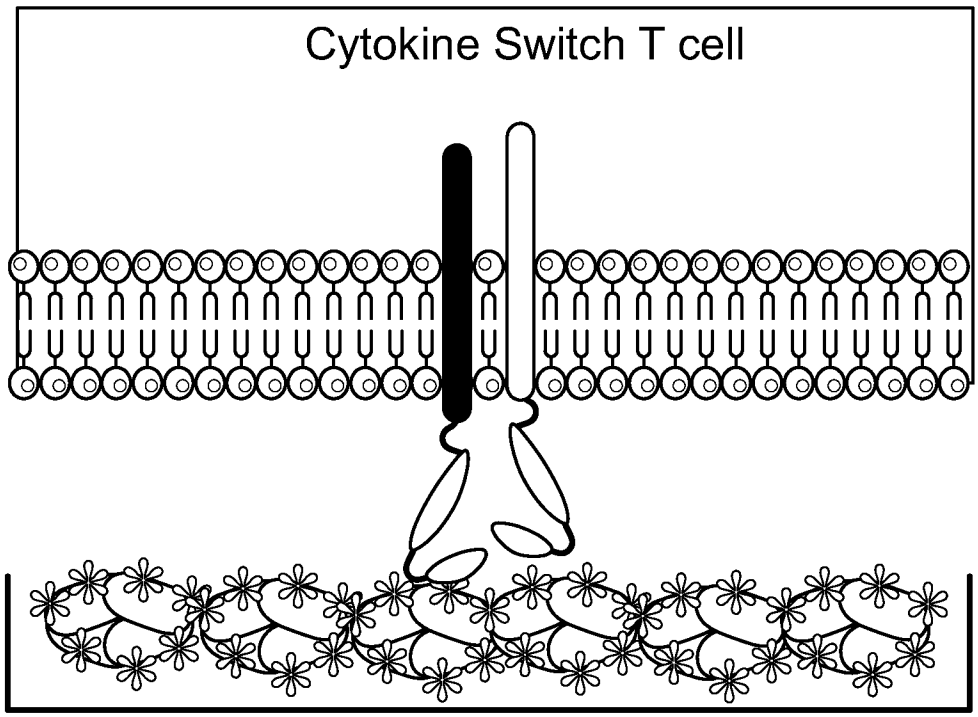


FIG. 3A

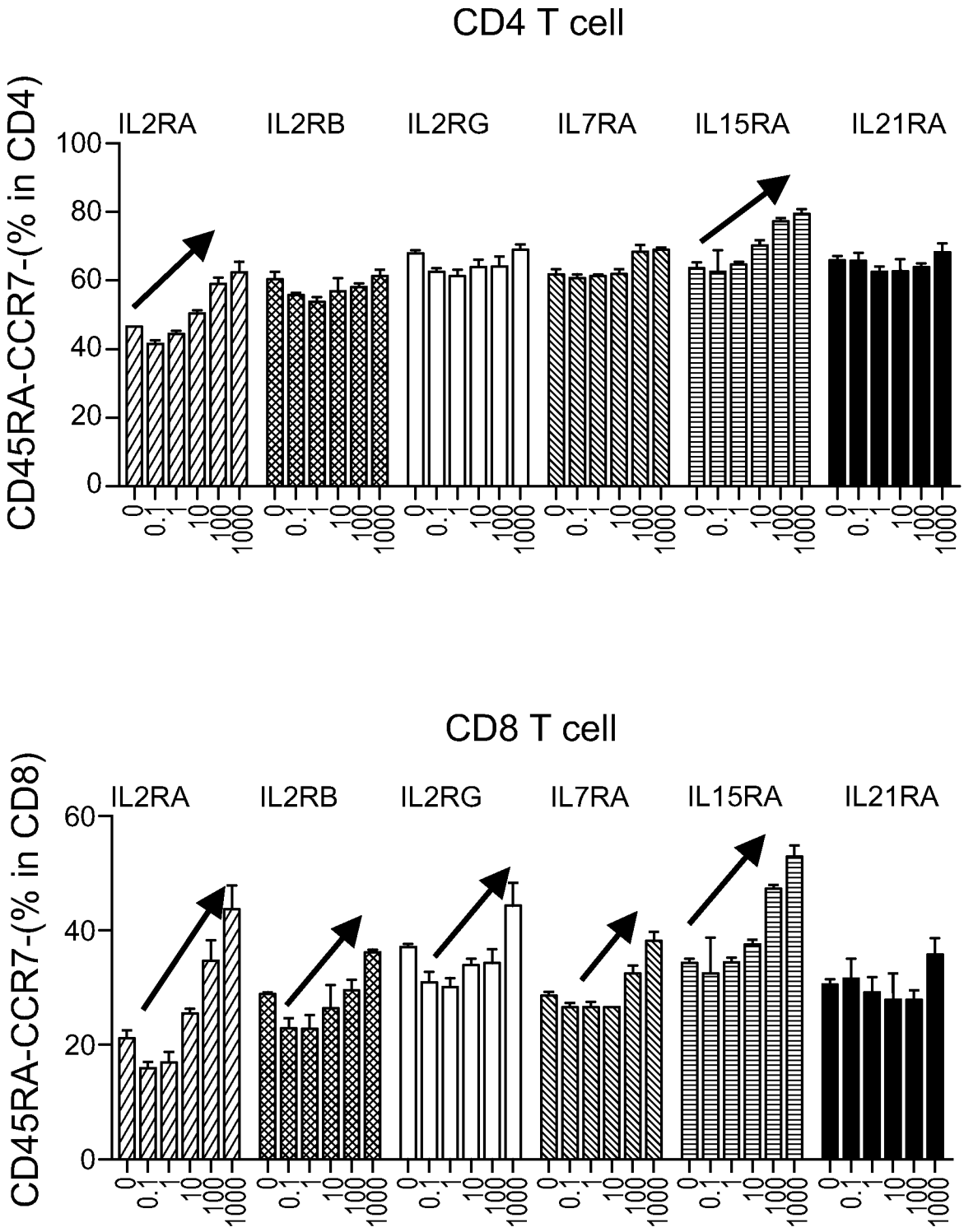
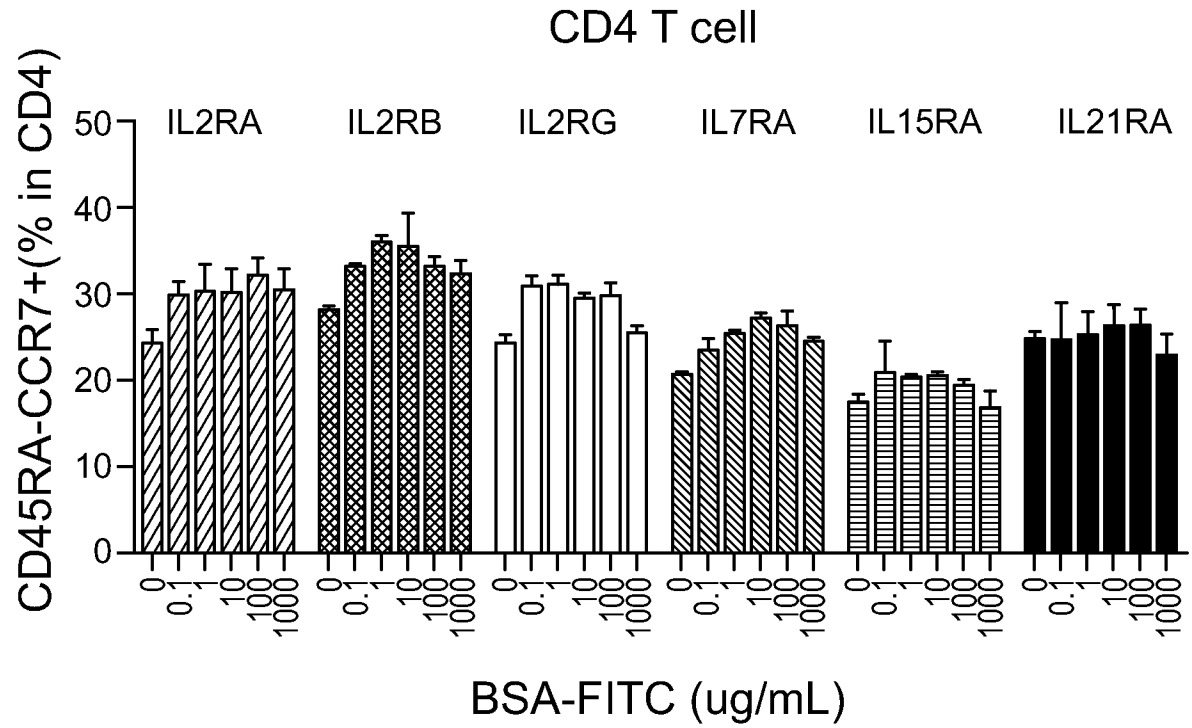

CD4 T cell
CD45RA-CCR7-(% in CD4)
IL2RA
IL2RB
IL2RG
IL7RA
IL15RA
IL21RA
100
80
60
40
20
0
0
0.1
1
10
100
1000
CD8 T cell
CD45RA-CCR7-(% in CD8)
60
40
20
0

FIG. 3B


CD4 T cell
CD45RA-CCR7+(% in CD4)
IL2RA
IL2RB
IL2RG
IL7RA
IL15RA
IL21RA
0
10
20
30
40
50
0
0.1
1
10
100
1000
BSA-FITC (ug/mL)

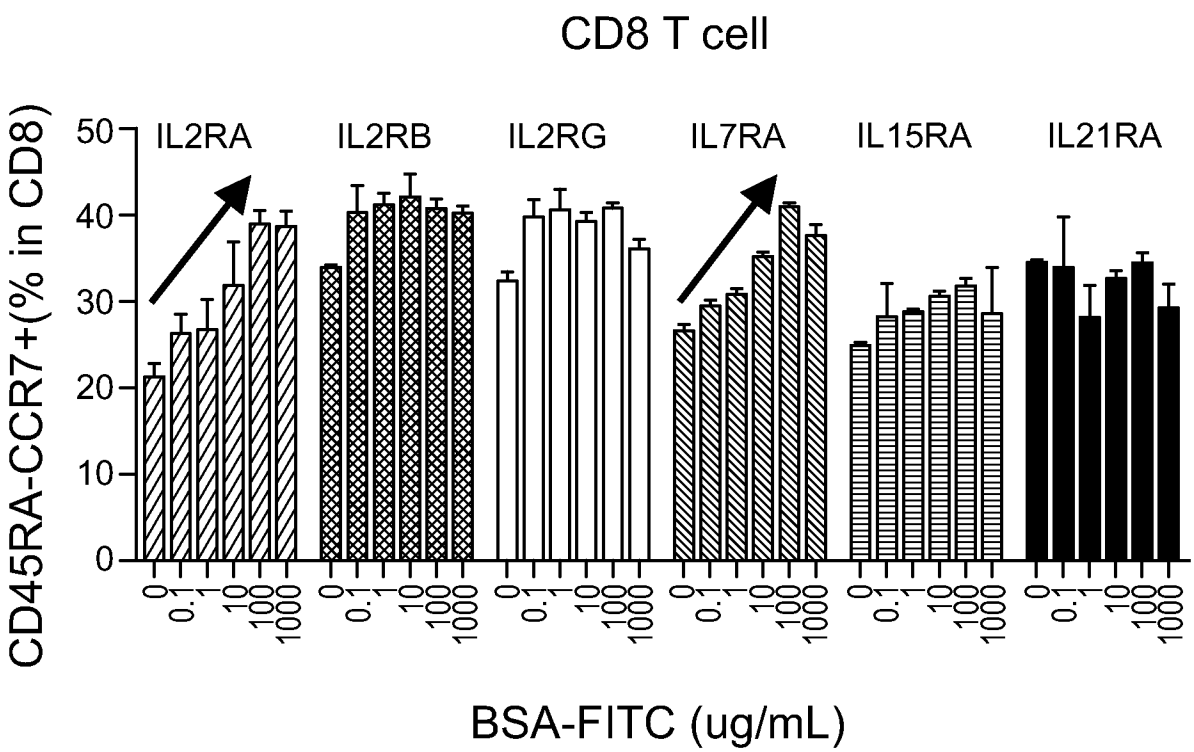

CD8 T cell
CD45RA-CCR7+(% in CD8)
IL2RA
IL2RB
IL2RG
IL7RA
IL15RA
IL21RA
0
10
20
30
40
50
0
0.1
1
10
100
1000
BSA-FITC (ug/mL)

FIG. 3C

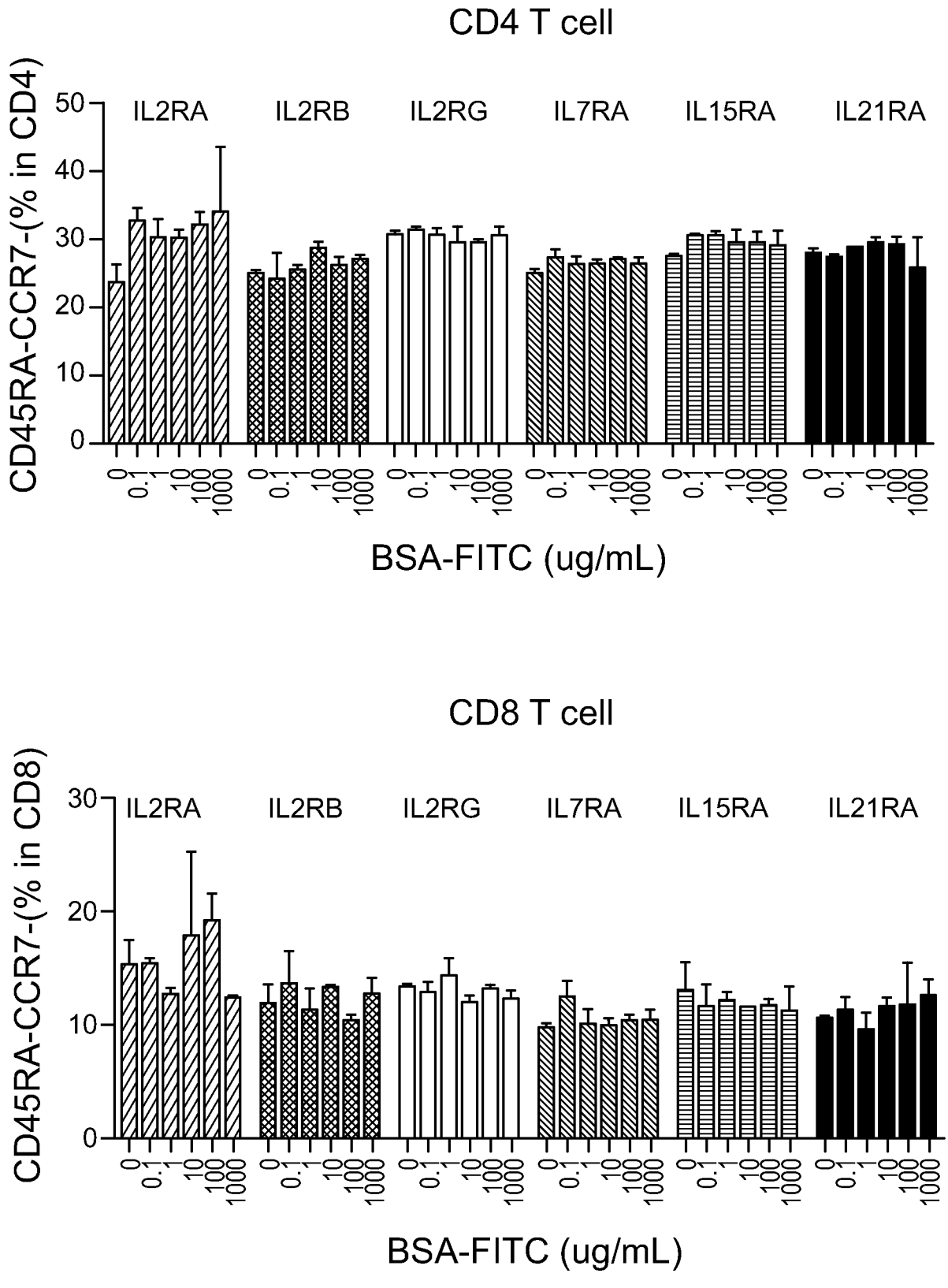
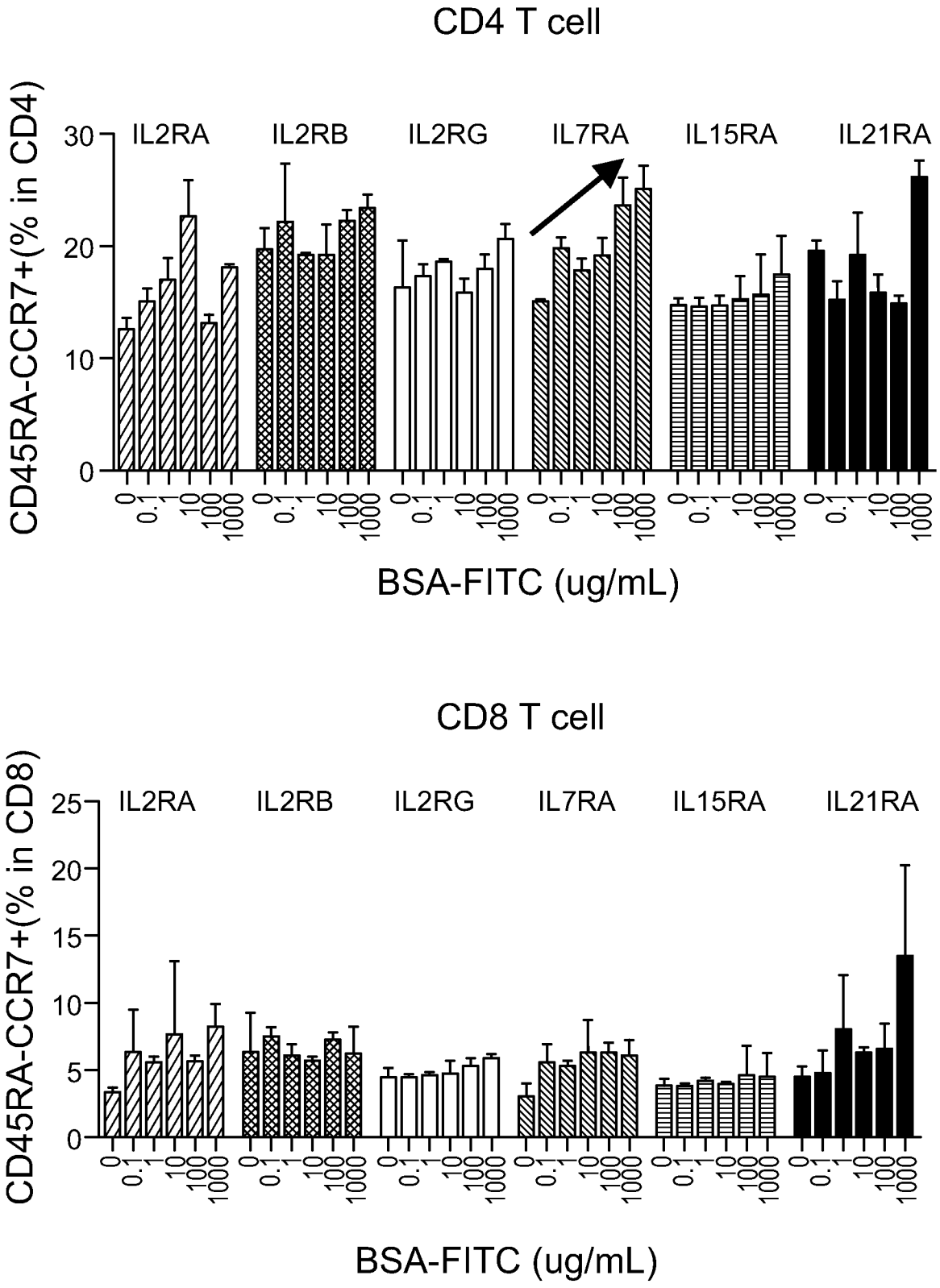

CD4 T cell
CD45RA-CCR7-(% in CD4)
IL2RA
IL2RB
IL2RG
IL7RA
IL15RA
IL21RA
BSA-FITC (ug/mL)
CD8 T cell
CD45RA-CCR7-(% in CD8)
BSA-FITC (ug/mL)

FIG. 3D


CD4 T cell
CD45RA-CCR7+(% in CD4)
IL2RA
IL2RB
IL2RG
IL7RA
IL15RA
IL21RA
BSA-FITC (ug/mL)
CD8 T cell
CD45RA-CCR7+(% in CD8)
BSA-FITC (ug/mL)

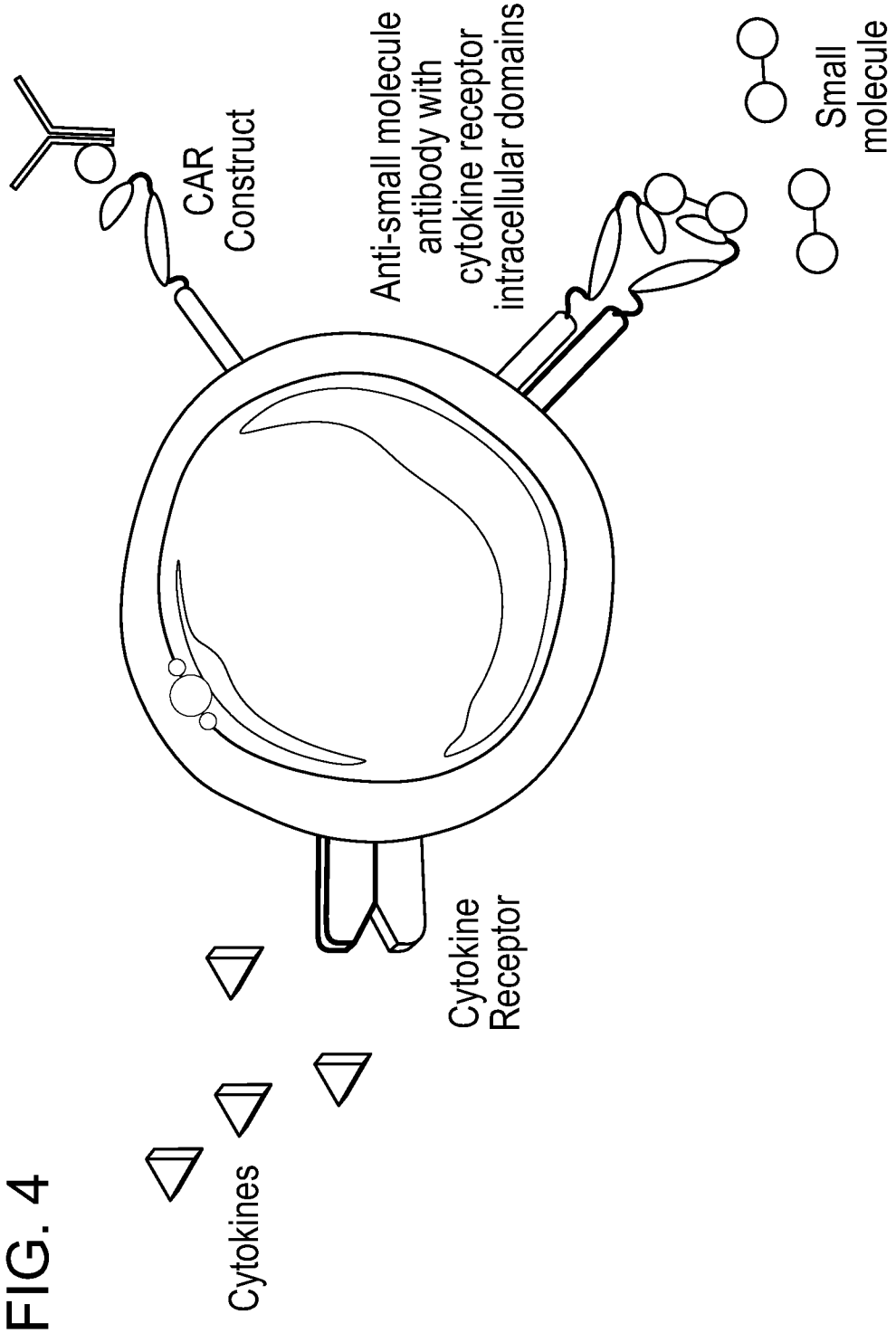

CAR
Construct
Anti-small molecule
antibody with
cytokine receptor
intracellular domains
Small
molecule
Cytokine
Receptor
Cytokines

FIG. 4

FIG. 5A

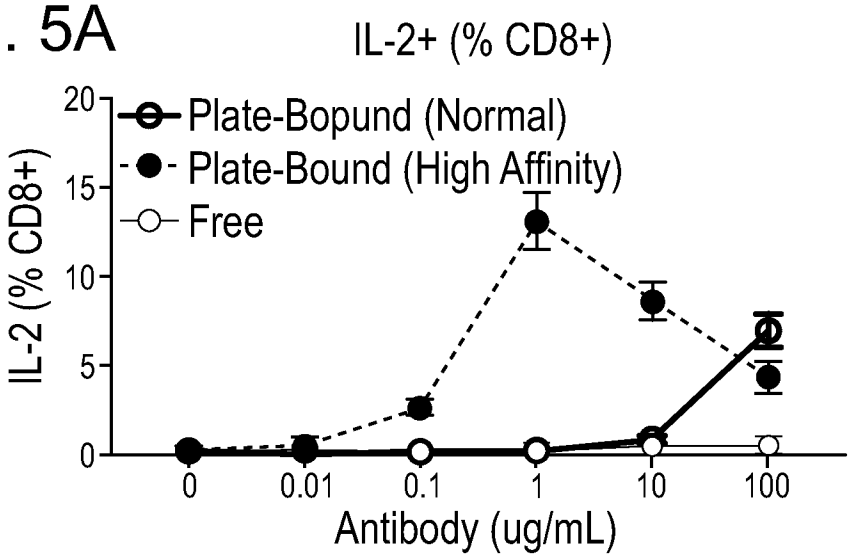


FIG. 5B

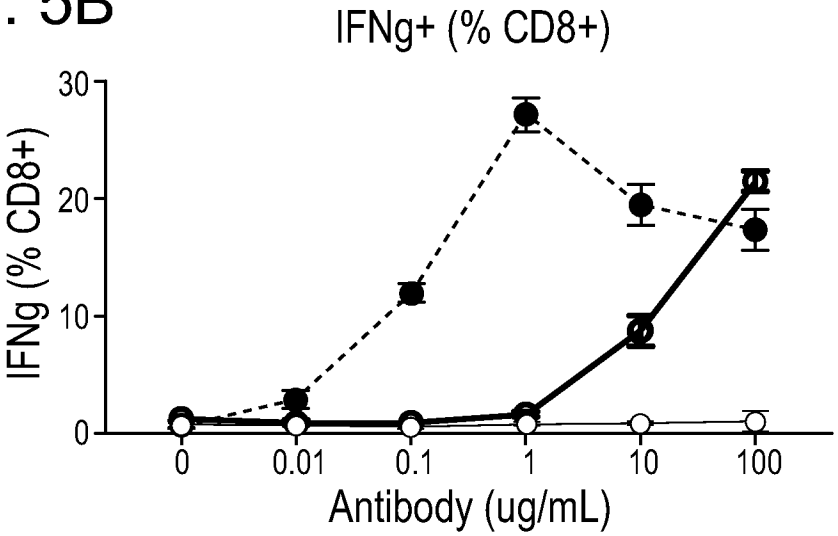


FIG. 5C

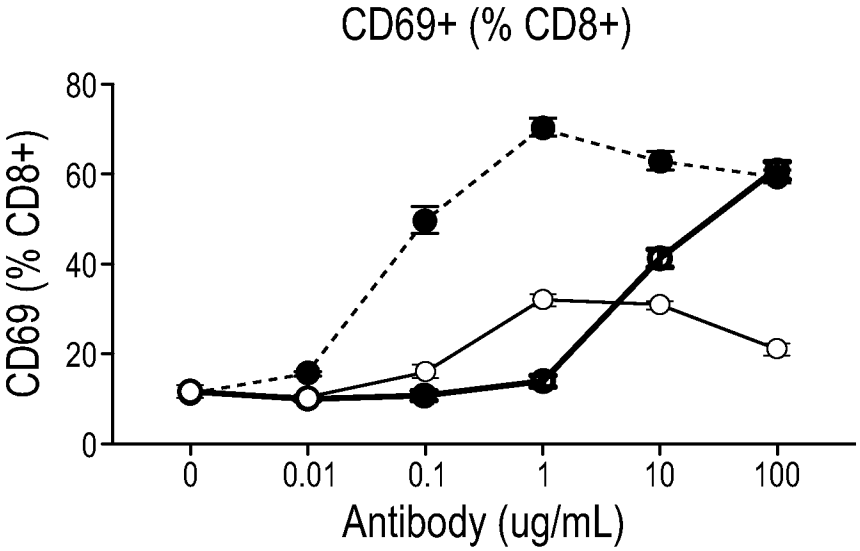


FIG. 6A

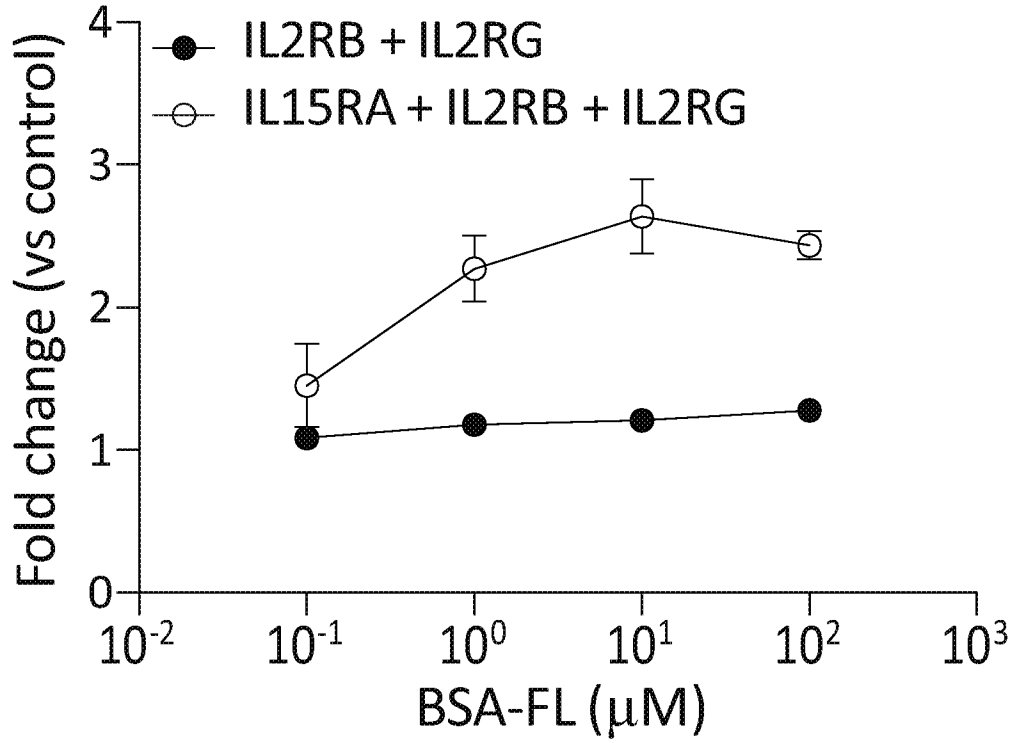
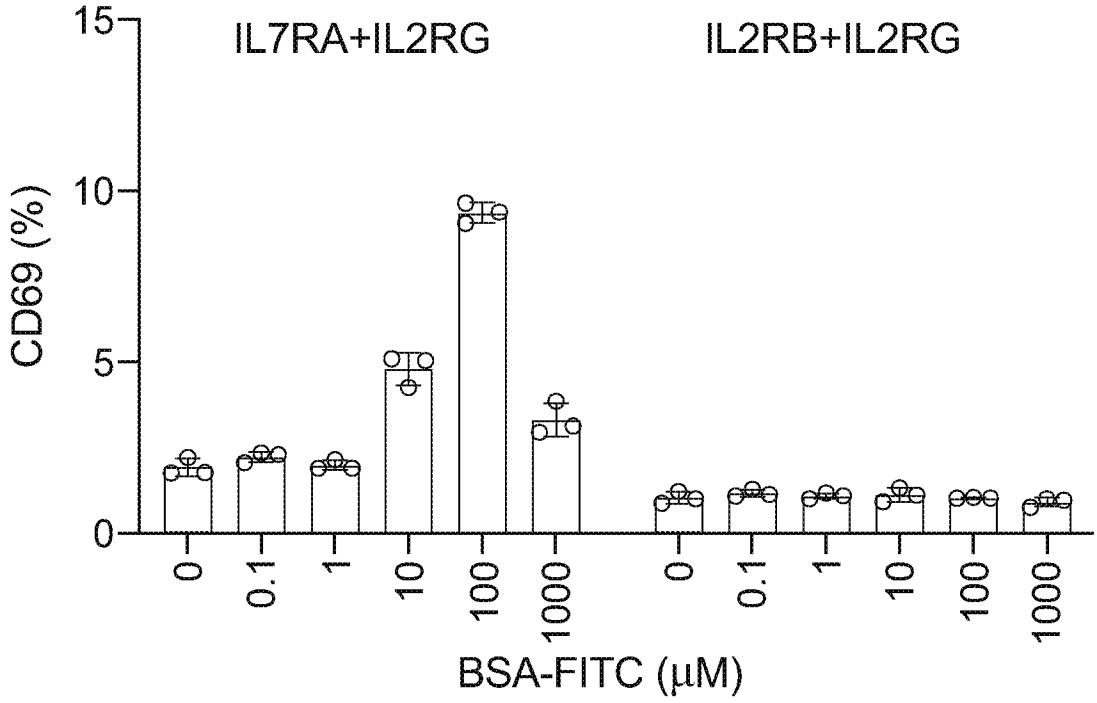

IL2RB + IL2RG
IL15RA + IL2RB + IL2RG
Fold change (vs control)
0
1
2
3
4
10^{-2}
10^{-1}
10^{0}
10^{1}
10^{2}
10^{3}
BSA-FL (μM)

FIG. 6B


IL7RA+IL2RG
IL2RB+IL2RG
CD69 (%)
0
5
10
15
0
0.1
1
10
100
1000
0
0.1
1
10
100
1000
BSA-FITC (μM)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 22/77610

A. CLASSIFICATION OF SUBJECT MATTER
IPC - INV. C07K 14/705 (2023.01)
ADD. C07K 14/715 (2023.01)
CPC - INV. C07K 2317/622, C07K 2319/75
ADD. C12N 5/0636, A61K 39/0011, C07K 16/3007, C07K 14/7051
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
See Search History document

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

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y --- A	US 2020/0102370 A1 (MASSACHUSETTS INSTITUTE OF TECHNOLOGY) 02 April 2020 (02.04.2020); para [0216], [0230], [0247], [0321], [0346], [0540], [0549], [0553], [0593]	1-2, 12, 17, 19, 28 --- 3 --- 4, 13, 18, 29
Y	US 2016/0362476 A1 (AXIOMX, INC.) 15 December 2016 (15.12.2016) para [0003], [0072], [0501]	3
A	WO 2016/170176 A1 (CUREVAC AG) 27 October 2016 (27.10.2016) abstract; pg 24, ln 9-10	4
A	US 9,061,059 B2 (MODERNA THERAPEUTICS, INC. et al.) 23 June 2015 (23.06.2015) abstract	29
A	WO 2016/174461 A1 (UCL BUSINESS PLC) 03 November 2016 (03.11.2016) abstract	29
A	WO 2019/236522 A1 (DANA-FARBER CANCER INSTITUTE) 12 December 2019 (12.12.2019) abstract; para [0126]	13, 18

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

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"D" document cited by the applicant in the international application
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
18 January 2023	FEB 17 2023
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer Kari Rodriquez Telephone No. PCT Helpdesk: 571-272-4300

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 22/77610

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. [X] forming part of the international application as fi led.

   b. [ ] furnished subsequent to the international fi ling date for the purposes of international search (Rule 13ter.1(a)),

      [ ] accompanied by a statement to the effect that the sequence listing does not go beyond the disclosure in the international application as filed.

2. [ ] With regard to any nucleotide and/or amino acid sequence disclosed in the international application, this report has been established to the extent that a meaningful search could be carried out without a WIPO Standard ST.26 compliant sequence listing.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 22/77610

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

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

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

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

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

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
---See Supplemental Box ---

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-4, 12-13, 17-19 and 28-29 limited to SEQ ID NOs: 1-2, 33-34, 51-52

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 22/77610

Box No. III Observations where unity of invention is lacking

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I+: Claims 1-37 directed to a cytokine receptor switch (protein, encoded by nucleic acid) composition, the switch comprising a signal peptide, a single chain antibody fragment (scFv) that binds a nonimmunogenic synthetic small molecule, a CD8-derived hinge domain peptide having a nucleic acid sequence SEQ ID NO: 49 and amino acid sequence SEQ ID NO: 50, a transmembrane domain, and a cytokine receptor-derived intracellular domain. The first named cytokine receptor switch composition will be searched to the extent that the signal peptide is native to IL-2RA, and has nucleic acid sequence SEQ ID NO: 1 and amino acid sequence SEQ ID NO: 2, the scFv binds fluorescein and has nucleic acid sequence SEQ ID NO: 51 and amino acid sequence SEQ ID NO: 52, a transmembrane domain derived from IL-2RA, and an intracellular domain derived from IL-2RA that has nucleic acid sequence SEQ ID NO: 33 and amino acid sequence SEQ ID NO 34. This first named invention has been selected based on the guidance set forth in section 10.54 of the PCT International Search and Preliminary Examination Guidelines. It is believed that claims 1-4, 12-13, 17-19 and 28-29 limited to said cytokine receptor switch encompass this first named invention, and thus these claims will be searched without fee to the extent that cytokine receptor switch encompasses said sequence elements. Additional cytokine receptor switch molecules will be searched upon the payment of additional fees. Applicants must specify the claims that encompass any additionally elected cytokine receptor switch molecules. Applicants must further indicate, if applicable, the claims which encompass the first named invention, if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched. An exemplary election would be a cytokine receptor switch comprising a signal peptide is native to IL-2RB, and has nucleic acid sequence SEQ ID NO: 3 and amino acid sequence SEQ ID NO: 4, the scFv binds MPOB and has nucleic acid sequence SEQ ID NO: 53 and amino acid sequence SEQ ID NO: 54, the transmembrane domain is derived from IL-2RB and has nucleic acid sequence SEQ ID NO: 19 and amino acid sequence SEQ ID NO 20, and an intracellular domain derived from IL-2RB that has nucleic acid sequence SEQ ID NO: 35 and amino acid sequence SEQ ID NO 36.(claims 1-3, 5, 12, 14, 19-20, 28 and 30).

Group II: Claims 38-51, drawn to a composition comprising a nucleic acid encoding one or more cytokine receptor switches and an immune cell comprising the nucleic acid[s], wherein the one or more nucleic acid[s] each comprise a signal peptide, a single chain antibody fragment (scFv) that binds a non-immunogenic synthetic small molecule, a hinge domain, a transmembrane domain, and a cytokine receptor-derived intracellular domain.

Group III: Claims 52-70, drawn to method for stimulating immune cells comprising contacting immune cells comprising one or more cytokine receptor switches with a synthetic small molecule sufficient to promote proliferation, wherein the one or more cytokine receptor switches each comprise a signal peptide, a single chain antibody fragment (scFv) that binds said synthetic small molecule, a hinge domain, a transmembrane domain, and a cytokine receptor-derived intracellular domain.

Group IV: Claims 71-77, drawn to a method for treating a disease or disorder comprising administering to a subject immune cells comprising one or more cytokine receptor switches, and further administering to the subject an immune cell-stimulating synthetic small molecule conjugated to a carrier, wherein the one or more cytokine receptor switches each comprise a signal peptide, a single chain antibody fragment (scFv) that binds said synthetic small molecule, a hinge domain, a transmembrane domain, and a cytokine receptor-derived intracellular domain.

The inventions listed as Groups I+, II, III and IV do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Special Technical Features

No technical features are shared between the nucleic and amino acid sequences of the cytokine receptor switch molecules of Group I+ and, accordingly, these groups lack unity a priori.

Additionally, even if the inventions listed as Group I+ were considered to share technical features, these shared technical features are previously disclosed by the prior art, as further discussed below.

Group I+ requires an isolated cytokine receptor switch composition having specified nucleic acid and amino acid sequences for a signal peptide, scFv, hinge domain, transmembrane domain and intracellular domain, not required by Groups II, III, IV.

Group II requires a composition comprising a nucleic acid encoding one or more cytokine receptor switches and an immune cell comprising the nucleic acid[s], not required by Groups I+, III, IV.

Group III requires a method of for stimulating immune cells comprising contacting immune cells comprising one or more cytokine receptor switches with a synthetic small molecule sufficient to promote proliferation, not required by Groups I+, II, IV.

Group IV requires a method for treating a disease or disorder comprising administering to a subject immune cell comprising one or more cytokine receptor switches, and further administering to the subject an immune cell-stimulating synthetic small molecule conjugated to a carrier, not required by Groups I+, II, III.

---See next Supplemental Box ---

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 22/77610

Box No. III Observations where unity of invention is lacking

Common Technical Features

The feature shared by Groups I+, II, III, and IV is one or more cytokine receptor switch molecules (encoded by nucleic acid molecules) each comprising a signal peptide, a single chain antibody fragment (scFv) that binds a nonimmunogenic synthetic small molecule, a hinge domain, a transmembrane domain, and a cytokine receptor-derived intracellular domain.

However, this shared technical feature does not represent a contribution over prior art, because the shared technical feature is taught by the article entitled "Engineered Cytokine Signaling to Improve CAR T Cell Effector Function", by Bell et al. (hereinafter 'Bell') (Frontiers in Immunology, 4 June 2021, Vol 12, Article No 684642, pp 1-16). Bell teaches said cytokine receptor switch molecules (pg 6, col 2, para 3 "Chimeric cytokine receptors or switch receptors, which convert one cytokine signal into another, are actively being explored", see Fig. 3C), and further teaches deriving said cytokine receptor switch molecules from chimeric antigen receptors- CARS (pg 1, col 1, para 1 "CARs consist of four components: i) an extracellular antigen recognition domain, most commonly a single chain variable fragment (scFv), ii) structural components, such as hinge and transmembrane domains, iii) a costimulatory domain that provides signals to sustain CAR T cell effector functions, and iv) a CD3z activation domain", See Fig. 2 and legend, pg 6, col 2, para 3 "Chimeric cytokine receptors or switch receptors, which convert one cytokine signal into another, are actively being explored to hijack immunosuppressive cytokines produced by tumor or tumor-associated cells to provide proliferative signals to CAR T cells, i. e., Switch receptors comprise CAR molecules that switch one cytokine signal into another.).

The common technical feature shared by Groups II-III, is immune cells comprising one or more cytokine receptor switches.

However, this shared technical feature does not represent a contribution over prior art, because the shared technical feature is taught by Bell (pg 6, col 2, para 3 "Chimeric cytokine receptors or switch receptors, which convert one cytokine signal into another, are actively being explored to hijack immunosuppressive cytokines produced by tumor or tumor-associated cells to provide proliferative signals to CAR T cells (Figure 3C). An IL-4/IL-7 switch receptor, which binds IL-4 but activates IL-7 signaling pathways, allowed PSCA-CAR T cells to maintain their cytolytic and proliferative capabilities in vitro and improved in vivo antitumor activity").

Another feature shared by Groups I+, II, III, and IV is a synthetic small molecule that binds a cytokine receptor switch molecule, e.g. binds to the extracellular scFv (e.g. a chimeric Switch receptor having the regions found in a CAR).

However, this shared technical feature does not represent a contribution over prior art, because the shared technical feature is taught by US 2019/0256597 A1 to Dana Farber Cancer Institute Inc (hereinafter 'DFCI') in view of Bell. DFCI teaches synthetic small molecules that have tumor-specific activation of CARs (para [0039] "the current invention is composed of three essential parts (1) a tumor-targeting binding molecule. (2) a masked small molecule, and (3) a CAR T cell specific for the small molecule.", para [0040] "A series of binary events determines whether CAR T cells will be activated by the synthetic small molecule. These Binary Activated T cells using Chimeric Antigen Receptors (BAT-CARs) should be completely inert in the absence of the small molecule and activated only at sites where the small molecule is unmasked", see Fig. 1, para [0056] "Preferably the small molecule is non-immunogenic", para [0010] "a chimeric antigen receptor (CAR) comprising an intracellular signaling domain, a transmembrane domain and an extracellular domain ... a scFV") thus, DFCI teaches a non-immunogenic synthetic small molecule able to activate a CAR). DFCI does not expressly teach that the CAR may be a cytokine receptor switch molecule, however, since Bell teaches Chimeric cytokine receptors or switch receptors, which convert one cytokine signal into another (pg 6, col 2, para 3 "Chimeric cytokine receptors or switch receptors, which convert one cytokine signal into another, are actively being explored to hijack immunosuppressive cytokines produced by tumor or tumor-associated cells to provide proliferative signals to CAR T cells"), it would have been obvious to an artisan of ordinary skill in the art to experiment with activating the switch chimeric receptors of Bell with non-immunogenic synthetic small molecules of DFCI, such as to create signaling effective to control the unwanted suppressive activity of immune cells (e.g. T-cells) against a specific tumor.

As the technical features were known in the art at the time of the invention, they cannot be considered special technical features that would otherwise unify the groups.

Groups I+, II, III, and IV therefore lack unity of invention under PCT Rule 13 because they do not share a same or corresponding special technical feature.