

CLAIMS

What is claimed is:

1. A heterodimeric protein comprising:
 - (a) a first domain comprising one or more butyrophilin family proteins, or a fragment thereof, wherein the fragments are capable of binding a gamma delta T cell receptor and are optionally comprising extracellular domains, or one or both of immunoglobulin V (IgV)- and IgC-like domains;
 - (b) a second domain comprising a targeting domain, the targeting domain being selected from an (i) antibody, antibody-like molecule, or antigen binding fragment thereof, and (ii) an extracellular domain; and
 - (c) a linker that adjoins the first and second domain, wherein the linker comprises (a) a first charge polarized core domain adjoined to one or more butyrophilin family protein, optionally at the carboxy terminus, and (b) a second charge polarized core domain adjoined to one or more butyrophilin family protein, optionally at the carboxy terminus, wherein the first and second charge polarized core domains comprise peptides having positively and/or negatively charged amino acid residues at the amino and/or carboxy terminus of the charge polarized core domain, wherein the linker forms a heterodimer through electrostatic interactions between positively charged amino acid residues and negatively charged amino acid residues on the first and second charge polarized core domains.
2. The heterodimeric protein of claim 1, wherein the first domain comprises two of the same butyrophilin family proteins, or the first domain comprises two different butyrophilin family proteins.
3. The heterodimeric protein of claim 1 or claim 2, wherein the butyrophilin family proteins, or a fragment thereof comprise a V-type domain, and/or a B30.2 domain, optionally wherein the first domain comprises one or more fragments of the butyrophilin family proteins, wherein the fragment is capable of binding a gamma delta T cell receptor and is optionally an extracellular domain.

4. The heterodimeric protein of ~~any one of claims 1-3~~ claim 2 or claim 3, wherein the butyrophilin family proteins are selected from BTN3A1, BTN2A1, BTN3A2, BTNL3, BTNL8, BTNL1, BTN1A1, BTNL2, BTN2A2, BTN2A3, BTN3A3, BTNL6, BTNL9, BTNL10, and SKINT;

~~optionally wherein the first domain comprises:~~

~~(a) any one of BTN3A1, BTN2A1, BTN3A2, BTNL3, BTNL8, BTNL1, BTN1A1, BTNL2, BTN2A2, BTN2A3, BTN3A3, BTNL6, BTNL9, BTNL10, and SKINT; and~~

~~(b) any one of BTN3A1, BTN2A1, BTN3A2, BTNL3, BTNL8, BTNL1, BTN1A1, BTNL2, BTN2A2, BTN2A3, BTN3A3, BTNL6, BTNL9, BTNL10, and SKINT;~~

optionally wherein the two butyrophilin family proteins of the first domain comprises:

(a) any one of human human BTN3A1, human BTN2A1, human BTN3A2, human BTNL3, human BTNL8, BTNL1, human BTN1A1, human BTNL2, human BTN2A2, human BTN2A3, human BTN3A3, human BTNL6, human BTNL9, and human BTNL10, and

(b) any one of human human BTN3A1, human BTN2A1, human BTN3A2, human BTNL3, human BTNL8, BTNL1, human BTN1A1, human BTNL2, human BTN2A2, human BTN2A3, human BTN3A3, human BTNL6, human BTNL9, and human BTNL10;

optionally wherein the two butyrophilin family proteins of the first domain comprises a polypeptide having an amino acid sequence of:

(a) any one of SEQ ID NOs: 49-52 and 99-111; and

(b) any one of SEQ ID NOs: 49-52 and 99-111; and/or

optionally wherein the two butyrophilin family proteins of the first domain comprises:

(a) BTNL3 and BTNL8;

(b) BTN3A1 and BTN3A2;

(c) BTN3A1 and BTN2A1; or

(d) BTNL1 and BTNL6; and/or

optionally wherein the two butyrophilin family proteins of the first domain comprises:

(a) human BTNL3 and human BTNL8;

(b) human BTN3A1 and human BTN3A2; or

(c) human BTN3A1 and human BTN2A1; and/or

~~optionally wherein the first domain comprises a polypeptide having~~

~~(a) an amino acid sequence having at least 90%, or 95%, or 97%, or 98%, or 99% identity with SEQ ID NO: 49, and an amino acid sequence having at least 90%, or 95%, or 97%, or 98%, or 99% identity with SEQ ID NO: 52; or~~

~~(b) an amino acid sequence having at least 90%, or 95%, or 97%, or 98%, or 99% identity with SEQ ID NO: 50, and an amino acid sequence having at least 90%, or 95%, or 97%, or 98%, or 99% identity with SEQ ID NO: 51; and/or~~

~~optionally wherein the first domain comprises a polypeptide having an amino acid sequence of~~

~~(a) SEQ ID NO: 49 and SEQ ID NO: 52; or~~

~~(b) SEQ ID NO: 50 and SEQ ID NO: 51.~~

5. The heterodimeric protein of any one of claims 1-4, wherein the targeting domain is an antibody, an antibody-like molecule, or antigen binding fragment thereof, optionally wherein the antibody-like molecule is selected from a single-chain antibody (scFv), a single-domain antibody, a recombinant heavy-chain-only antibody (VHH), a shark heavy-chain-only antibody (VNAR), a microprotein (cysteine knot protein, knottin), a DARPin; a Tetranectin; an Affibody; a Transbody; an Anticalin; an AdNectin; an Affilin; an Affimer, a Microbody; an aptamer; an alterase; a plastic antibody; a phylomer; a stradobody; a maxibody; an evibody; a fynomer, an armadillo repeat protein, a Kunitz domain, an avimer, an atrimer, a probody, an immunobody, a triomab, a troybody; a pepbody; a vaccibody, a UniBody; a DuoBody, a Fv, a Fab, a Fab', and a F(ab')₂; optionally wherein the antibody-like molecule is an scFv.

6. The heterodimeric protein of any one of claims 1-5, wherein:

~~the targeting domain is an extracellular domain, optionally wherein the targeting domain comprises a portion of the extracellular domain of LAG-3, PD-1, TIGIT, CD19, or PSMA; and/or~~

~~optionally~~ the targeting domain is capable of binding an antigen ~~on the surface of a cancer cell, optionally wherein the targeting domain specifically binds one of~~ selected from CD19, PSMA, GD2, PSCA, BCMA, CD123, B7-H3, CD20, CD30, CD33, CD38, CEA, CLEC12A, DLL3, EGFRvIII, EpCAM, CD307, FLT3, GPC3, gpA33, HER2, MUC16, P-cadherin, SSTR2, and mesothelin.

7. The heterodimeric protein of claim 6, wherein the targeting domain specifically binds CD19 or PSMA.

8. The heterodimeric protein of any one of claims 1-7, wherein the targeting domain is a polypeptide having an amino acid sequence ~~with at least 90%, or 95%, or 97%, or 98%, or 99% identity with a polypeptide selected~~ from SEQ ID NOs: 53-60, optionally wherein the targeting domain is a polypeptide having an amino acid sequence of selected from one or more of SEQ ID NOs: 53-60.

~~9. The heterodimeric protein of any one of claims 1-8, wherein the linker comprises (a) a first charge polarized core domain adjoined to a butyrophilin family protein, optionally at the carboxy terminus, and (b) a second charge polarized core domain adjoined to a butyrophilin family protein, optionally at the carboxy terminus.~~

910. The heterodimeric protein of claim 19, ~~wherein the linker forms a heterodimer through electrostatic interactions between positively charged amino acid residues and negatively charged amino acid residues on the first and second charge polarized core domains,~~
~~optionally~~ wherein the first and/or second charge polarized core domain comprises a polypeptide linker, optionally selected from a flexible amino acid sequence, IgG hinge region, or antibody sequence, and/or

optionally wherein the linker comprises the hinge-CH2-CH3 Fc domain derived from IgG1, optionally human IgG1; or IgG4, optionally human IgG4.

1011. The heterodimeric protein of claim 910, wherein:

~~the first and/or second charge polarized core domain further comprise peptides having positively and/or negatively charged amino acid residues at the amino and/or carboxy terminus of the charge polarized core domain; and/or~~

~~optionally~~ wherein the positively charged amino acid residues are present in a peptide comprising positively charged amino acid residues in the first and/or the second charge polarized core domains;

optionally wherein the positively charged amino acid residues include one or more of amino acids selected from His, Lys, and Arg; and/or

~~optionally~~ wherein the negatively charged amino acid residues are present in a peptide comprising negatively charged amino acid residues in the first and/or the second charge polarized core domains;

optionally wherein the negatively charged amino acid residues may include one or more amino acids selected from Asp and Glu.

1142. The heterodimeric protein of claim 1044, wherein: the peptide comprising positively charged amino acid residues comprises the sequence RKGGKR (SEQ ID NO: 11) or GSGSRKGGKRGS (SEQ ID NO: 12); and/or the peptide comprising negatively charged amino acid residues comprises the sequence DEGGED (SEQ ID NO: 13) or GSGSDEGGEDGS (SEQ ID NO: 14).

~~13. — The heterodimeric protein of any one of claims 1-12, wherein the first domain and/or the heterodimeric protein modulates or is capable of modulating a $\gamma\delta$ (gamma delta) T cell; optionally wherein the gamma delta T cell is selected from a cell expressing V γ 4, V γ 9 δ 2, or V γ 7 δ 4; and/or~~

~~optionally wherein the first domain comprises BTNL3 and BTNL8 and it modulates a V γ 4-expressing T cell;~~

~~optionally wherein the first domain comprises:~~

~~(a) BTN3A1 and BTN3A2 and it modulates a V γ 9 δ 2-expressing T cell;~~

~~(b) BTN3A1 and BTN2A1 and it modulates a V γ 9 δ 2-expressing T cell; or~~

~~(c) BTNL1 and BTNL6 and it modulates a V γ 7 δ 4-expressing T cell; and/or~~

~~optionally wherein the modulation of a gamma delta T cell is activation of a gamma delta T cell; and/or~~

~~optionally wherein the heterodimeric protein is capable of forming a synapse between a gamma delta T cell and a tumor cell and/or the heterodimeric protein is capable of contemporaneous activation and targeting of gamma delta T cells to tumor cells.~~

1214. The heterodimeric protein of any one of claims 1-~~1113~~, wherein:

the heterodimeric protein is a complex of two polypeptide chains; and/or

wherein the heterodimeric protein comprises an alpha chain and a beta chain wherein the alpha chain and the beta chain each independently comprise

(a) a first domain comprising a butyrophilin family protein, or fragment thereof;

(b) a second domain comprising a targeting domain, the targeting domain being selected from an (i) antibody, antibody-like molecule, or antigen binding fragment thereof, ~~and (ii) a extracellular domain; and~~

(c) a linker that adjoins the first and second domain;

~~optionally~~ wherein the alpha chain and the beta chain self-associate to form the heterodimer.

1315. The heterodimeric protein of any one of claims 1-~~1214~~, wherein the heterodimeric protein comprises one or more amino acid sequences having at least 90%, or at least 91%, or at least 92%, or at least 93%, or at least 94%, or at least 95%, or at least 96%, or at least 97%, or at least 98%, or at least 98%, or at least 99% sequence identity to:

~~(a) any extracellular domain (“ECD”) sequence of Table 1, and/or~~

~~(b) one or more of SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 33, SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 37, SEQ ID NO: 38, SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 43, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 46, SEQ ID NO: 66, SEQ ID NO: 67, SEQ ID NO: 68, SEQ ID NO: 69, SEQ ID NO: 70, SEQ ID NO: 71, SEQ ID NO: 72, SEQ ID NO: 73, SEQ ID NO: 74, SEQ ID NO: 75, SEQ ID NO: 76, SEQ ID NO: 77, SEQ ID NO: 78, SEQ ID NO: 79, SEQ ID NO: 80, SEQ ID NO: 81, SEQ ID NO: 82, SEQ ID NO: 83, SEQ ID NO: 84, SEQ ID~~

~~NO: 85, SEQ ID NO: 86, SEQ ID NO: 87, SEQ ID NO: 94, SEQ ID NO: 95, SEQ ID NO: 96, SEQ ID NO: 97~~, each optionally with a leader sequence omitted.

~~1416~~. A pharmaceutical composition, comprising the heterodimeric protein of any of claims 1-~~1315~~.

~~15~~. A nucleic acid encoding the heterodimeric protein of any of claims 1-13 or the first and/or second polypeptide chains of the heterodimeric protein of claim 12 or claim 13.

~~1617~~. An expression vector, comprising a nucleic acid encoding the heterodimeric protein of any of claims 1-13 or the first and/or second polypeptide chains of the heterodimeric protein of ~~any of claims 1-15~~ claim 12 or claim 13, optionally wherein the expression vector is a mammalian expression vector; and/or optionally wherein the expression vector comprises DNA or RNA.

~~1718~~. A host cell, comprising the expression vector of claim ~~1617~~.

~~1819~~. Use of the pharmaceutical composition of claim ~~1416~~ in the manufacture of a medicament for the treatment of cancer, for contemporaneous activation and targeting of gamma delta T cells to tumor cells; or modulating a patient's immune response; or for stimulating proliferation of gamma delta T cells, wherein

the pharmaceutical composition of claim ~~1416~~ is to be administered to a subject in need thereof; and/or

the pharmaceutical composition of claim ~~1416~~ is to be contacted with a cell derived from a subject in need thereof thereby causing an ex vivo proliferation of gamma delta T cells;

optionally wherein the subject's T cells are to be activated by the first domain; and/or

optionally wherein the subject has a tumor and the gamma delta T cells are to modulate cells of the tumor; and/or

~~for the treatment of cancer~~,

optionally wherein the cancer is a lymphoma or a leukemia;

optionally wherein the cancer is a Hodgkin's and non-Hodgkin's lymphoma, B-cell lymphoma (including low grade/follicular non-Hodgkin's lymphoma (NHL); small lymphocytic

(SL) NHL; intermediate grade/follicular NHL; intermediate grade diffuse NHL; high grade immunoblastic NHL; high grade lymphoblastic NHL; high grade small non-cleaved cell NHL; bulky disease NHL; mantle cell lymphoma; AIDS-related lymphoma; and Waldenstrom's Macroglobulinemia; chronic lymphocytic leukemia (CLL); acute lymphoblastic leukemia (ALL); Hairy cell leukemia; or chronic myeloblastic leukemia; and/or

optionally wherein the cancer is basal cell carcinoma, biliary tract cancer; bladder cancer; bone cancer; brain and central nervous system cancer; breast cancer; cancer of the peritoneum; cervical cancer; choriocarcinoma; colon and rectum cancer; connective tissue cancer; cancer of the digestive system; endometrial cancer; esophageal cancer; eye cancer; cancer of the head and neck; gastric cancer (including gastrointestinal cancer); glioblastoma; hepatic carcinoma; hepatoma; intra-epithelial neoplasm; kidney or renal cancer; larynx cancer; leukemia; liver cancer; lung cancer (e.g., small-cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung, and squamous carcinoma of the lung); melanoma; myeloma; neuroblastoma; oral cavity cancer (lip, tongue, mouth, and pharynx); ovarian cancer; pancreatic cancer; prostate cancer; retinoblastoma; rhabdomyosarcoma; rectal cancer; cancer of the respiratory system; salivary gland carcinoma; sarcoma; skin cancer; squamous cell cancer; stomach cancer; testicular cancer; thyroid cancer; uterine or endometrial cancer; cancer of the urinary system; vulval cancer; lymphoma including Hodgkin's and non-Hodgkin's lymphoma, as well as B-cell lymphoma (including low grade/follicular non-Hodgkin's lymphoma (NHL); small lymphocytic (SL) NHL; intermediate grade/follicular NHL; intermediate grade diffuse NHL; high grade immunoblastic NHL; high grade lymphoblastic NHL; high grade small non-cleaved cell NHL; bulky disease NHL; mantle cell lymphoma; AIDS-related lymphoma; and Waldenstrom's Macroglobulinemia; chronic lymphocytic leukemia (CLL); acute lymphoblastic leukemia (ALL); Hairy cell leukemia; chronic myeloblastic leukemia; as well as other carcinomas and sarcomas; and post-transplant lymphoproliferative disorder (PTLD), as well as abnormal vascular proliferation associated with phakomatoses, edema (e.g. that associated with brain tumors), and Meigs' syndrome, and/or

optionally wherein the cancer is prostate cancer; and/or

optionally wherein the cancer is an epithelial-derived carcinoma; and/or

optionally wherein the cancer is known to express the antigenic target of the second domain of the heterodimeric protein; and/or

optionally wherein the cancer has mutations which limit recognition by alpha beta T cells, optionally selected from mutations in MHC I, beta 2 microglobulin, and Transporter associated with antigen processing (TAP); and/or

optionally wherein the subject is to be further administered autologous or allogeneic gamma delta T cells that were expanded ex vivo;

optionally wherein the autologous or allogeneic gamma delta T cells express a Chimeric Antigen Receptor; and/or

optionally wherein the subject is further administered autologous or allogeneic T cells that express a Chimeric Antigen Receptor.

1920. Use of the pharmaceutical composition of claim 1416 in the manufacture of a medicament for treating an autoimmune disease or disorder, wherein the autoimmune disease or disorder is optionally selected from rheumatoid arthritis, systemic lupus erythematosus, diabetes mellitus, ankylosing spondylitis, Sjögren's syndrome, inflammatory bowel diseases (e.g., colitis ulcerosa, Crohn's disease), multiple sclerosis, sarcoidosis, psoriasis, Grave's disease, Hashimoto's thyroiditis, psoriasis, hypersensitivity reactions (e.g., allergies, hay fever, asthma, and acute edema cause type I hypersensitivity reactions), and vasculitis.