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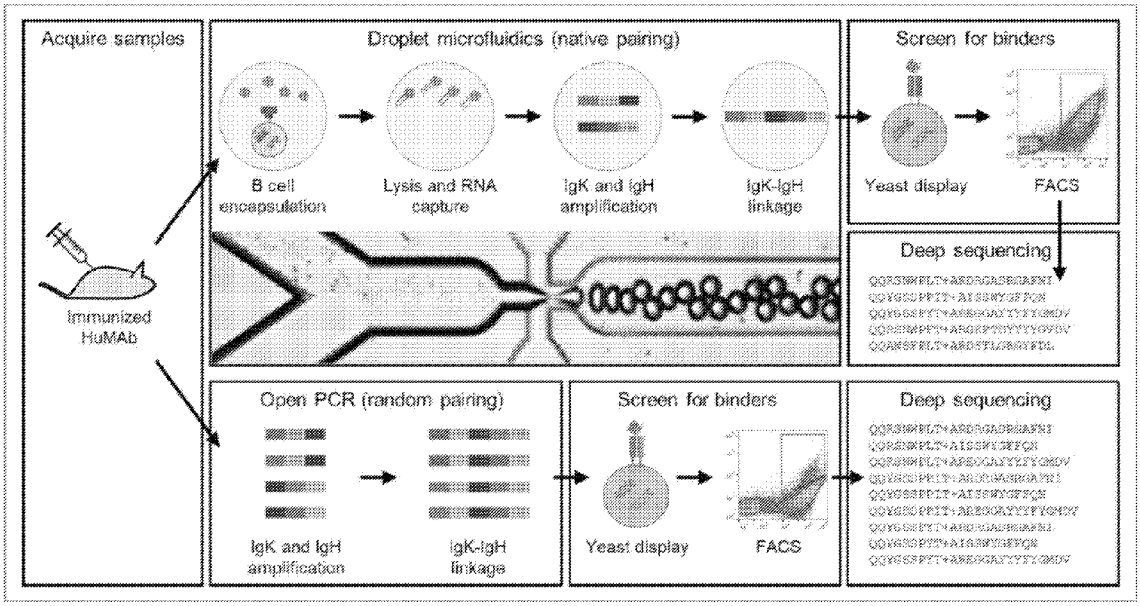


FIG. 1

(57) Abstract: Provided herein are antigen-binding proteins (ABPs) that selectively bind to B7-H4 and its isoforms and homologs, and compositions comprising the ABPs. Also provided are methods of using the ABPs, such as therapeutic and diagnostic methods.

[Continued on next page]

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ANTI-B7-H4 BINDING PROTEINS AND METHODS OF USE THEREOF

1. CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to and the benefit of U.S. Provisional Patent Application No. 62/785,654, filed on December 27, 2018, the entire contents of which are incorporated by reference herein.

2. SEQUENCE LISTING

[0002] The instant application contains a Sequence Listing with 8702 sequences which has been submitted via EFS-Web and is hereby incorporated by reference in its entirety. Said ASCII copy, created on December 20, 2019, is named GGN-009WO_SL.txt, and is 588,377 bytes in size.

3. FIELD

[0003] Provided herein are antigen-binding proteins (ABPs) with binding specificity for B7-H4 and compositions comprising such ABPs, including pharmaceutical compositions, diagnostic compositions, and kits. Also provided are methods of making B7-H4 ABPs, and methods of using B7-H4 ABPs, for example, for therapeutic purposes, diagnostic purposes, and research purposes.

4. BACKGROUND

[0004] B7-H4, also known as CD80 (cluster of differentiation 80) and V-set domain-containing T cell activation inhibitor (VTCN1), is a cell surface receptor that suppresses T cell inflammatory activity. B7-H4 is a member of the B7 family of molecules involved in immune regulation and is over-expressed in many solid tumor cells, cancer stem-like cells, supporting vasculature, and stroma. B7-H4 negatively regulates T-cell-mediated immune response by inhibiting T-cell activation, proliferation, cytokine production and development of cytotoxicity. When expressed on the cell surface of tumor macrophages, B7-H4 plays an important role, together with regulatory T-cells (Treg), in the suppression of tumor-associated antigen-specific T-cell immunity. B7-H4 is also involved in promoting epithelial cell transformation.

[0005] Tumor cells hijack the B7-H4 pathway by up-regulating B7-H4 and thus suppress the anti-tumor immune response. Recently, it has been proposed that B7-H4 inhibitors could antagonize binding of B7-H4 to its ligands, thereby activating the immune system to attack tumors. B7-H4 antibodies could also be used to induce antibody-dependent cell-mediated cytotoxicity (ADCC) cells specific to the tumor microenvironment, thus directly killing the tumor. B7-H4 antibodies could therefore be used to treat some types of cancer.

[0006] Though the biological function of B7-H4 is still being elucidated, the molecule is also a promising target for therapy of autoimmune disease. Because B7-H4 has both immune stimulatory and immune inhibitory properties, drugs that agonize or antagonize B7-H4 could be used to treat autoimmune diseases.

[0007] Thus, there is a need for developing B7-H4 ABPs that can be used for treatment, diagnosis, and research of various diseases, including cancer and autoimmune disease.

5. SUMMARY

[0008] Provided herein are novel ABPs with binding specificity for B7-H4 and methods of using such ABPs. The B7-H4 is a human B7-H4 (SEQ ID: 7001) or a fragment of the human B7-H4.

[0009] The ABP can comprise an antibody. In some embodiments, the antibody is a monoclonal antibody. In some embodiments, the antibody is a chimeric antibody. In some embodiments, the antibody is a humanized antibody. In some embodiments, the antibody is a human antibody. In some embodiments, the ABP comprises an antibody fragment. In some embodiments, the ABP comprises an alternative scaffold. In some embodiments, the ABPs comprises a single-chain variable fragment (scFv).

[0010] The ABPs provided herein can induce various biological effects associated with inhibition or activation of B7-H4. In some embodiments, an ABP provided herein prevents binding between B7-H4 and its ligands. In some embodiments, an ABP provided herein prevents inhibition of an effector T cell. In some embodiments, the ABP directly kills tumor cells by ADCC, for example mediated by binding of NK cell-expressed CD16 to the ABP Fc domain.

[0011] Also provided are kits comprising one or more of the pharmaceutical compositions comprising the ABPs, and instructions for use of the pharmaceutical composition.

[0012] Also provided are isolated polynucleotides encoding the ABPs provided herein, and portions thereof.

[0013] Also provided are vectors comprising such polynucleotides.

[0014] Also provided are recombinant host cells comprising such polynucleotides and recombinant host cells comprising such vectors.

[0015] Also provided are methods of producing the ABP using the polynucleotides, vectors, or host cells provided herein.

[0016] Also provided are pharmaceutical compositions comprising the ABPs and a pharmaceutically acceptable excipient.

[0017] Also provided are methods of treating or preventing a disease or condition in a subject in need thereof, comprising administering to the subject an effective amount of an ABP provided herein, or a pharmaceutical composition comprising such ABP. In some aspects, the disease or condition is a cancer or autoimmune disease. In some aspects, the disease or condition is a viral or bacterial infection. In some aspects the method further comprises administering one or more additional therapeutic agents. In some aspects, the additional therapeutic agent is an immune stimulatory agent.

[0018] More specifically, the present disclosure provides an isolated antigen binding protein (ABP) that specifically binds a human cluster of differentiation 80 (B7-H4), comprising: (a) a CDR3-L having a sequence selected from SEQ ID NOS: 3001-3012 and a CDR3-H having a sequence selected from SEQ ID NOS: 6001-6012; or (b) a CDR3-L having a sequence selected from SEQ ID NOS: 8336-8419; and a CDR3-H having a sequence selected from SEQ ID NOS: 8588-8671; or (c) a CDR3-L having a sequence of the CD3-L of any one of the clones in the library deposited under ATCC Accession No. PTA-125511 and a CDR3-L having a sequence of the CD3-L of any one of the clones in the library deposited under ATCC Accession No. PTA-125511. In some embodiments, the CDR3-L and the CDR3-H are a cognate pair. In some embodiments, the ABP comprises (a) a CDR1-L having a sequence selected from SEQ ID NOS: 1001-1012 and a CDR2-L having a sequence selected from SEQ ID NOS: 2001-2012; and a CDR1-H having a sequence selected from SEQ ID NOS: 4001-4012; and a CDR2-H having a sequence selected from SEQ ID NOS: 5001-5012; or (b) a CDR1-L having a sequence selected from SEQ ID NOS: 8168-8251; and a CDR2-L having a sequence selected from SEQ ID NOS: 8252-8335; and a CDR1-H having a sequence selected from SEQ ID NOS: 8420-8503; and a CDR2-H having a sequence selected from SEQ ID NOS: 8504-8587; or (c) a CDR1-L having a sequence selected from a CDR1-L of any one of the clones in the library deposited under ATCC Accession No. PTA-125511; and a CDR2-L having a sequence selected from a CDR2-L of any one of the clones in the library deposited under ATCC Accession No. PTA-125511; and a CDR1-H having a sequence selected from a CDR1-H of any one of the clones in the library deposited under ATCC Accession No. PTA-125511; and a CDR2-H having a sequence selected from a CDR2-H of any one of the clones in the library deposited under ATCC Accession No. PTA-125511.

[0019] In some embodiments, the ABP comprises a CDR1-L, a CDR2-L, a CDR3-L, a CDR1-H, a CDR2-H and a CDR3-H, wherein the CDR1-L consists of SEQ ID NO: 1001, the CDR2-L consists of SEQ ID NO: 2001, the CDR3-L consists of SEQ ID NO: 3001, the CDR1-H consists of SEQ ID NO: 4001, the CDR2-H consists of SEQ ID NO: 5001 and the CDR3-H consists of SEQ ID NO: 6001; or the CDR1-L consists of SEQ ID NO: 1002, CDR2-L consists of SEQ ID NO: 2002, the CDR3-L consists of SEQ ID NO: 3002, the CDR1-H consists of SEQ ID NO: 4002, the CDR2-H consists of SEQ ID NO: 5002 and the CDR3-H consists of SEQ ID NO: 6002; or the CDR1-L consists of SEQ ID NO: 1003, the CDR2-L consists of SEQ ID NO: 2003, the CDR3-L consists of SEQ ID NO: 3003, the CDR1-H consists of SEQ ID NO: 4003, the CDR2-H consists of SEQ ID NO: 5003 and the CDR3-H consists of SEQ ID NO: 6003; or the CDR1-L consists of SEQ ID NO: 1004, the CDR2-L consists of SEQ ID NO: 2004, the CDR3-L

consists of SEQ ID NO: 3004, the CDR1-H consists of SEQ ID NO: 4004, the CDR2-H consists of SEQ ID NO: 5004 and the CDR3-H consists of SEQ ID NO: 6004; or the CDR1-L consists of SEQ ID NO: 1005, the CDR2-L consists of SEQ ID NO: 2005, the CDR3-L consists of SEQ ID NO: 3005, the CDR1-H consists of SEQ ID NO: 4005, the CDR2-H consists of SEQ ID NO: 5005 and the CDR3-H consists of SEQ ID NO: 6005; or the CDR1-L consists of SEQ ID NO: 1006, the CDR2-L consists of SEQ ID NO: 2006, the CDR3-L consists of SEQ ID NO: 3006, the CDR1-H consists of SEQ ID NO: 4006, the CDR2-H consists of SEQ ID NO: 5006 and the CDR3-H consists of SEQ ID NO: 6006; or the CDR1-L consists of SEQ ID NO: 1007, the CDR2-L consists of SEQ ID NO: 2007, the CDR3-L consists of SEQ ID NO: 3007, the CDR1-H consists of SEQ ID NO: 4007, the CDR2-H consists of SEQ ID NO: 5007 and the CDR3-H consists of SEQ ID NO: 6007; or the CDR1-L consists of SEQ ID NO: 1008, the CDR2-L consists of SEQ ID NO: 2008, the CDR3-L consists of SEQ ID NO: 3008, the CDR1-H consists of SEQ ID NO: 4008, the CDR2-H consists of SEQ ID NO: 5008 and the CDR3-H consists of SEQ ID NO: 6008 or the CDR1-L consists of SEQ ID NO: 1009, the CDR2-L consists of SEQ ID NO: 2009, the CDR3-L consists of SEQ ID NO: 3009, the CDR1-H consists of SEQ ID NO: 4009, the CDR2-H consists of SEQ ID NO: 5009 and the CDR3-H consists of SEQ ID NO: 6009; or the CDR1-L consists of SEQ ID NO: 1010, the CDR2-L consists of SEQ ID NO: 2010, the CDR3-L consists of SEQ ID NO: 3010, the CDR1-H consists of SEQ ID NO: 4010, the CDR2-H consists of SEQ ID NO: 5010 and the CDR3-H consists of SEQ ID NO: 6010; or the CDR1-L consists of SEQ ID NO: 1011, the CDR2-L consists of SEQ ID NO: 2011, the CDR3-L consists of SEQ ID NO: 3011, the CDR1-H consists of SEQ ID NO: 4011, the CDR2-H consists of SEQ ID NO: 5011 and the CDR3-H consists of SEQ ID NO: 6011; or the CDR1-L consists of SEQ ID NO: 1012, the CDR2-L consists of SEQ ID NO: 2012, the CDR3-L consists of SEQ ID NO: 3012, the CDR1-H consists of SEQ ID NO: 4012, the CDR2-H consists of SEQ ID NO: 5012 and the CDR3-H consists of SEQ ID NO: 6012.

[0020] In some embodiments, the ABP comprises a variable light chain (V_L) comprising a sequence at least 97% identical to a sequence selected from SEQ ID NOS: 1-12 and a variable heavy chain (V_H) comprising a sequence at least 97% identical to a sequence selected from SEQ ID NOS: 101-112; or a variable light chain (V_L) comprising a sequence at least 97% identical to a sequence selected from SEQ ID NOS: 8000-8083; and a variable heavy chain (V_H) comprising a sequence at least 97% identical to a sequence selected from SEQ ID NOS: 8084-8167; or a variable light chain (V_L) comprising a sequence at least 97% identical to a V_L sequence of any one of the clones in the library deposited under ATCC Accession No. PTA-125511 and a variable heavy chain (V_H) comprising a sequence at least 97% identical to a V_H sequence of any

one of the clones in the library deposited under ATCC Accession No. PTA-125511. In some embodiments, the V_L and the V_H are a cognate pair.

[0021] In some embodiments, the ABP comprises a variable light chain (V_L) comprising a sequence selected from SEQ ID NOS: 1-12 and a variable heavy chain (V_H) comprising a a sequence selected from SEQ ID NOS: 101-112 or a variable light chain (V_L) comprising a sequence selected from SEQ ID NOS: 8000-8083; and a variable heavy chain (V_H) comprising a sequence selected from SEQ ID NOS: 8084-8167; or a variable light chain (V_L) comprising a V_L sequence of any one of the clones in the library deposited under ATCC Accession No. PTA-125511 and a variable heavy chain (V_H) comprising a V_H sequence of any one of the clones in the library deposited under ATCC Accession No. PTA-125511. In some embodiments, the V_L and the V_H are a cognate pair.

[0022] In some embodiments, the ABP comprises an scFv or a full length monoclonal antibody. In some embodiments, the ABP comprises an immunoglobulin constant region.

[0023] In some embodiments, the ABP binds human B7-H4 with a K_D of less than 500nM, as measured by surface plasmon resonance. In some embodiments, the ABP binds human B7-H4 with a K_D of less than 200nM, as measured by surface plasmon resonance. In some embodiments, the the ABP binds human B7-H4 with a K_D of less than 25nM, as measured by surface plasmon resonance. In some embodiments, the ABP binds to human B7-H4 on a cell surface with a KD of less than 25nM.

[0024] Another aspect of the present disclosure provides a pharmaceutical composition comprising any of the disclosed ABPs and an excipient.

[0025] Another aspect of the present disclosure provides a method of treating a disease comprising the step of: administering to a subject in need thereof an effective amount of the ABP disclosed herein or the pharmaceutical composition disclosed herein. In some embodiments, the disease is selected from the group consisting of cancer, AIDS, Alzheimer's disease and viral or bacterial infection. In some embodiments, the further comprises the step of administering one or more additional therapeutic agents to the subject. In some embodiments, the additional therapeutic agent is selected from CTLA-4 inhibitor, TIGIT inhibitor, a chemotherapy agent, an immune-stimulatory agent, radiation, a cytokine, a polynucleotide encoding a cytokine and a combination thereof.

[0026] The present disclosure further provides an isolated polynucleotide encoding the ABP provided herein. In another aspect, the present disclosure provides a vector comprising the isolated polynucleotide. In yet another aspect, the present disclosure provides a host cell comprising the isolated polynucleotide or the vector.

[0027] The present disclosure further provides a method of producing an isolated antigen binding protein (ABP) that specifically binds human B7-H4, comprising: expressing the ABP in the host cell, and isolating the ABP.

6. BRIEF DESCRIPTION OF THE DRAWINGS

[0028] **FIG. 1** summarized the method of generating scFv libraries from B cells isolated from fully human mice and selecting a B cell expressing an antibody having high-affinity to the antigen. FIG. 1 discloses 8675-8702, respectively, in order of appearance.

[0029] **FIG. 2** illustrates scFv amplification procedure. First, a mixture of primers directed against the IgK C region, the IgG C region, and all V regions is used to separately amplify IgK and IgH. Second, the V-H and C-K primers contain a region of complementarity that results in the formation of an overlap extension amplicon that is a fusion product between IgK and IgH. The region of complementarity comprises a DNA sequence that encodes a Gly-Ser rich scFv linker sequence. Third, semi-nested PCR is performed to add adapters for Illumina sequencing or yeast display.

7. DETAILED DESCRIPTION

7.1. Definitions

[0030] Unless otherwise defined herein, scientific and technical terms used in connection with the present disclosure shall have the meanings that are commonly understood by those of ordinary skill in the art. Further, unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular. Generally, nomenclatures used in connection with, and techniques of, cell and tissue culture, molecular biology, immunology, microbiology, genetics and protein and nucleic acid chemistry and hybridization described herein are those well-known and commonly used in the art. The methods and techniques of the present disclosure are generally performed according to conventional methods well known in the art and as described in various general and more specific references that are cited and discussed throughout the present specification unless otherwise indicated. *See, e.g.*, Sambrook *et al. Molecular Cloning: A Laboratory Manual, 2d ed.*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989) and Ausubel *et al., Current Protocols in Molecular Biology*, Greene Publishing Associates (1992), and Harlow and Lane *Antibodies: A Laboratory Manual* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1990), which are incorporated herein by reference. Enzymatic reactions and purification techniques are performed according to manufacturer's specifications, as commonly accomplished in the art or as described herein. The terminology used in connection with, and the laboratory procedures and techniques of, analytical chemistry, synthetic organic chemistry, and medicinal and pharmaceutical chemistry described herein are those well-known and commonly used in the art. Standard techniques can be used for

chemical syntheses, chemical analyses, pharmaceutical preparation, formulation, and delivery, and treatment of patients.

[0031] The following terms, unless otherwise indicated, shall be understood to have the following meanings:

[0032] The terms "B7-H4," "B7-H4 protein," and "B7-H4 antigen" are used interchangeably herein to refer to human B7-H4, or any variants (*e.g.*, splice variants and allelic variants), isoforms, and species homologs of human B7-H4 that are naturally expressed by cells, or that are expressed by cells transfected with a *vtcn1* gene. In some aspects, the B7-H4 protein is a B7-H4 protein naturally expressed by a primate (*e.g.*, a monkey or a human), a rodent (*e.g.*, a mouse or a rat), a dog, a camel, a cat, a cow, a goat, a horse, or a sheep. In some aspects, the B7-H4 protein is human B7-H4 (hB7-H4; SEQ ID NO: 7001).

[0033] The term "immunoglobulin" refers to a class of structurally related proteins generally comprising two pairs of polypeptide chains: one pair of light (L) chains and one pair of heavy (H) chains. In an "intact immunoglobulin," all four of these chains are interconnected by disulfide bonds. The structure of immunoglobulins has been well characterized. *See, e.g.,* Paul, *Fundamental Immunology* 7th ed., Ch. 5 (2013) Lippincott Williams & Wilkins, Philadelphia, PA. Briefly, each heavy chain typically comprises a heavy chain variable region (V_H) and a heavy chain constant region (C_H). The heavy chain constant region typically comprises three domains, abbreviated C_{H1}, C_{H2}, and C_{H3}. Each light chain typically comprises a light chain variable region (V_L) and a light chain constant region. The light chain constant region typically comprises one domain, abbreviated C_L.

[0034] The term "antigen-binding protein" (ABP) refers to a protein comprising one or more antigen-binding domains that specifically bind to an antigen or epitope. In some embodiments, the antigen-binding domain binds the antigen or epitope with specificity and affinity similar to that of naturally occurring antibodies. In some embodiments, the ABP comprises an antibody. In some embodiments, the ABP consists of an antibody. In some embodiments, the ABP consists essentially of an antibody. In some embodiments, the ABP comprises an alternative scaffold. In some embodiments, the ABP consists of an alternative scaffold. In some embodiments, the ABP consists essentially of an alternative scaffold. In some embodiments, the ABP comprises an antibody fragment. In some embodiments, the ABP consists of an antibody fragment. In some embodiments, the ABP consists essentially of an antibody fragment. A "B7-H4 ABP," "anti- B7-H4 ABP," or "B7-H4-specific ABP" is an ABP, as provided herein, which specifically binds to the antigen B7-H4. In some embodiments, the ABP binds the extracellular domain of B7-H4. In

certain embodiments, a B7-H4 ABP provided herein binds to an epitope of B7-H4 that is conserved between or among B7-H4 proteins from different species.

[0035] The term "antibody" is used herein in its broadest sense and includes certain types of immunoglobulin molecules comprising one or more antigen-binding domains that specifically bind to an antigen or epitope. An antibody specifically includes intact antibodies (*e.g.*, intact immunoglobulins), antibody fragments, and multi-specific antibodies. One example of an antigen-binding domain is an antigen-binding domain formed by a V_H -V_L dimer. An antibody is one type of ABP.

[0036] The term "alternative scaffold" refers to a molecule in which one or more regions may be diversified to produce one or more antigen-binding domains that specifically bind to an antigen or epitope. In some embodiments, the antigen-binding domain binds the antigen or epitope with specificity and affinity similar to that of naturally occurring antibodies. Exemplary alternative scaffolds include those derived from fibronectin (*e.g.*, Adnectins™), the β-sandwich (*e.g.*, iMab), lipocalin (*e.g.*, Anticalins®), EETI-II/AGRP, BPTI/LACI-D1/ITI-D2 (*e.g.*, Kunitz domains), thioredoxin peptide aptamers, protein A (*e.g.*, Affibody®), ankyrin repeats (*e.g.*, DARPins), gamma-B-crystallin/ubiquitin (*e.g.*, Affilins), $CTLD_3$ (*e.g.*, Tetranectins), Fynomers, and (LDLR-A module) (*e.g.*, Avimers). Additional information on alternative scaffolds is provided in Binz et al., *Nat. Biotechnol.*, 2005 23:1257-1268; Skerra, *Current Opin. in Biotech.*, 2007 18:295-304; and Silacci et al., *J. Biol. Chem.*, 2014, 289:14392-14398; each of which is incorporated by reference in its entirety. An alternative scaffold is one type of ABP.

[0037] The term "antigen-binding domain" means the portion of an ABP that is capable of specifically binding to an antigen or epitope.

[0038] The terms "full length antibody," "intact antibody," and "whole antibody" are used herein interchangeably to refer to an antibody having a structure substantially similar to a naturally occurring antibody structure and having heavy chains that comprise an Fc region.

[0039] The term "Fc region" means the C-terminal region of an immunoglobulin heavy chain that, in naturally occurring antibodies, interacts with Fc receptors and certain proteins of the complement system. The structures of the Fc regions of various immunoglobulins, and the glycosylation sites contained therein, are known in the art. *See* Schroeder and Cavacini, *J. Allergy Clin. Immunol.*, 2010, 125:S41-52, incorporated by reference in its entirety. The Fc region may be a naturally occurring Fc region, or an Fc region modified as described elsewhere in this disclosure.

[0040] The V_H and V_L regions may be further subdivided into regions of hypervariability ("hypervariable regions (HVRs)") also called "complementarity determining regions" (CDRs))

interspersed with regions that are more conserved. The more conserved regions are called framework regions (FRs). Each V_H and V_L generally comprises three CDRs and four FRs, arranged in the following order (from N-terminus to C-terminus): FR1 - CDR1 - FR2 - CDR2 - FR3 - CDR3 - FR4. The CDRs are involved in antigen binding, and influence antigen specificity and binding affinity of the antibody. *See* Kabat et al., *Sequences of Proteins of Immunological Interest* 5th ed. (1991) Public Health Service, National Institutes of Health, Bethesda, MD, incorporated by reference in its entirety.

[0041] The light chain from any vertebrate species can be assigned to one of two types, called kappa (κ) and lambda (λ), based on the sequence of its constant domain.

[0042] The heavy chain from any vertebrate species can be assigned to one of five different classes (or isotypes): IgA, IgD, IgE, IgG, and IgM. These classes are also designated α, δ, ε, γ, and μ, respectively. The IgG and IgA classes are further divided into subclasses on the basis of differences in sequence and function. Humans express the following subclasses: IgG1, IgG2, IgG3, IgG4, IgA1, and IgA2.

[0043] The amino acid sequence boundaries of a CDR can be determined by one of skill in the art using any of a number of known numbering schemes, including those described by Kabat et al., *supra* ("Kabat" numbering scheme); Al-Lazikani et al., 1997, *J. Mol. Biol.*, 273:927-948 ("Chothia" numbering scheme); MacCallum et al., 1996, *J. Mol. Biol.* 262:732-745 ("Contact" numbering scheme); Lefranc et al., *Dev. Comp. Immunol.*, 2003, 27:55-77 ("IMGT" numbering scheme); and Honegge and Plückthun, *J. Mol. Biol.*, 2001, 309:657-70 ("AHo" numbering scheme); each of which is incorporated by reference in its entirety.

[0044] Table 1 provides the positions of CDR1-L (CDR1 of V_L), CDR2-L (CDR2 of V_L), CDR3-L (CDR3 of V_L), CDR1-H (CDR1 of V_H), CDR2-H (CDR2 of V_H), and CDR3-H (CDR3 of V_H), as identified by the Kabat and Chothia schemes. For CDR1-H, residue numbering is provided using both the Kabat and Chothia numbering schemes.

[0045] CDRs may be assigned, for example, using antibody numbering software, such as Abnum, available at www.bioinf.org.uk/abs/abnum/, and described in Abhinandan and Martin, *Immunology*, 2008, 45:3832-3839, incorporated by reference in its entirety.

TABLE 1
Residues in CDRs according to Kabat and Chothia numbering schemes.

CDR	Kabat	Chothia
CDR1-L	24-34	24-34
CDR2-L	50-56	50-56
CDR3-L	89-97	89-97
CDR1-H (Kabat Numbering)	31-35B	26-32 or 34*
CDR1-H (Chothia Numbering)	31-35	26-32

CDR2-H	50-65	52-56
CDR3-H	95-102	95-102

* The C-terminus of CDR1-H, when numbered using the Kabat numbering convention, varies between 32 and 34, depending on the length of the CDR.

[0046] The "EU numbering scheme" is generally used when referring to a residue in an antibody heavy chain constant region (*e.g.*, as reported in Kabat et al., *supra*).

[0047] An "antibody fragment" comprises a portion of an intact antibody, such as the antigen-binding or variable region of an intact antibody. Antibody fragments include, for example, Fv fragments, Fab fragments, $F(ab')_2$ fragments, Fab' fragments, scFv (sFv) fragments, and scFv-Fc fragments.

[0048] "Fv" fragments comprise a non-covalently-linked dimer of one heavy chain variable domain and one light chain variable domain.

[0049] "Fab" fragments comprise, in addition to the heavy and light chain variable domains, the constant domain of the light chain and the first constant domain (C_{H1}) of the heavy chain. Fab fragments may be generated, for example, by recombinant methods or by papain digestion of a full-length antibody.

[0050] "$F(ab')_2$" fragments contain two Fab' fragments joined, near the hinge region, by disulfide bonds. $F(ab')_2$ fragments may be generated, for example, by recombinant methods or by pepsin digestion of an intact antibody. The F(ab') fragments can be dissociated, for example, by treatment with ß-mercaptoethanol.

[0051] "Single-chain Fv" or "sFv" or "scFv" antibody fragments comprise a V_H domain and a V_L domain in a single polypeptide chain. The V_H and V_L are generally linked by a peptide linker. *See* Plückthun A. (1994). In some embodiments, the linker is a $(GGGGS)_n$ (SEQ ID NO: 8672). In some embodiments, n = 1, 2, 3, 4, 5, or 6. *See* Antibodies from *Escherichia coli*. In Rosenberg M. & Moore G.P. (Eds.), *The Pharmacology of Monoclonal Antibodies* vol. 113 (pp. 269-315). Springer-Verlag, New York, incorporated by reference in its entirety.

[0052] "scFv-Fc" fragments comprise an scFv attached to an Fc domain. For example, an Fc domain may be attached to the C-terminal of the scFv. The Fc domain may follow the V_H or V_L, depending on the orientation of the variable domains in the scFv (i.e., V_H -V_L or V_L -V_H). Any suitable Fc domain known in the art or described herein may be used. In some cases, the Fc domain comprises an IgG4 Fc domain.

[0053] The term "single domain antibody" refers to a molecule in which one variable domain of an antibody specifically binds to an antigen without the presence of the other variable domain. Single domain antibodies, and fragments thereof, are described in Arabi Ghahroudi et al., *FEBS*

Letters, 1998, 414:521-526 and Muyldermans et al., *Trends in Biochem. Sci.*, 2001, 26:230-245, each of which is incorporated by reference in its entirety.

[0054] A "monospecific ABP" is an ABP that comprises a binding site that specifically binds to a single epitope. An example of a monospecific ABP is a naturally occurring IgG molecule which, while divalent, recognizes the same epitope at each antigen-binding domain. The binding specificity may be present in any suitable valency.

[0055] The term "monoclonal antibody" refers to an antibody from a population of substantially homogeneous antibodies. A population of substantially homogeneous antibodies comprises antibodies that are substantially similar and that bind the same epitope(s), except for variants that may normally arise during production of the monoclonal antibody. Such variants are generally present in only minor amounts. A monoclonal antibody is typically obtained by a process that includes the selection of a single antibody from a plurality of antibodies. For example, the selection process can be the selection of a unique clone from a plurality of clones, such as a pool of hybridoma clones, phage clones, yeast clones, bacterial clones, or other recombinant DNA clones. The selected antibody can be further altered, for example, to improve affinity for the target ("affinity maturation"), to humanize the antibody, to improve its production in cell culture, and/or to reduce its immunogenicity in a subject.

[0056] The term "chimeric antibody" refers to an antibody in which a portion of the heavy and/or light chain is derived from a particular source or species, while the remainder of the heavy and/or light chain is derived from a different source or species.

[0057] "Humanized" forms of non-human antibodies are chimeric antibodies that contain minimal sequence derived from the non-human antibody. A humanized antibody is generally a human antibody (recipient antibody) in which residues from one or more CDRs are replaced by residues from one or more CDRs of a non-human antibody (donor antibody). The donor antibody can be any suitable non-human antibody, such as a mouse, rat, rabbit, chicken, or non-human primate antibody having a desired specificity, affinity, or biological effect. In some instances, selected framework region residues of the recipient antibody are replaced by the corresponding framework region residues from the donor antibody. Humanized antibodies may also comprise residues that are not found in either the recipient antibody or the donor antibody. Such modifications may be made to further refine antibody function. For further details, *see* Jones et al., *Nature*, 1986, 321:522-525; Riechmann et al., *Nature*, 1988, 332:323-329; and Presta, *Curr. Op. Struct. Biol.*, 1992, 2:593-596, each of which is incorporated by reference in its entirety.

[0058] A "human antibody" is one which possesses an amino acid sequence corresponding to that of an antibody produced by a human or a human cell, or derived from a non-human source

that utilizes a human antibody repertoire or human antibody-encoding sequences (*e.g.*, obtained from human sources or designed *de novo*). Human antibodies specifically exclude humanized antibodies. In some embodiments, rodents are genetically engineered to replace their rodent antibody sequences with human antibody sequences.

[0059] An "isolated ABP" or "isolated nucleic acid" is an ABP or nucleic acid that has been separated and/or recovered from a component of its natural environment. Components of the natural environment may include enzymes, hormones, and other proteinaceous or nonproteinaceous materials. In some embodiments, an isolated ABP is purified to a degree sufficient to obtain at least 15 residues of N-terminal or internal amino acid sequence, for example by use of a spinning cup sequenator. In some embodiments, an isolated ABP is purified to homogeneity by gel electrophoresis (*e.g.*, SDS-PAGE) under reducing or nonreducing conditions, with detection by Coomassie blue or silver stain. An isolated ABP includes an ABP *in situ* within recombinant cells, since at least one component of the ABP's natural environment is not present. In some aspects, an isolated ABP or isolated nucleic acid is prepared by at least one purification step. In some embodiments, an isolated ABP or isolated nucleic acid is purified to at least 80%, 85%, 90%, 95%, or 99% by weight. In some embodiments, an isolated ABP or isolated nucleic acid is purified to at least 80%, 85%, 90%, 95%, or 99% by volume. In some embodiments, an isolated ABP or isolated nucleic acid is provided as a solution comprising at least 85%, 90%, 95%, 98%, 99% to 100% ABP or nucleic acid by weight. In some embodiments, an isolated ABP or isolated nucleic acid is provided as a solution comprising at least 85%, 90%, 95%, 98%, 99% to 100% ABP or nucleic acid by volume.

[0060] "Affinity" refers to the strength of the sum total of non-covalent interactions between a single binding site of a molecule (*e.g.*, an ABP) and its binding partner (*e.g.*, an antigen or epitope). Unless indicated otherwise, as used herein, "affinity" refers to intrinsic binding affinity, which reflects a 1:1 interaction between members of a binding pair (*e.g.*, ABP and antigen or epitope). The affinity of a molecule X for its partner Y can be represented by the dissociation equilibrium constant (K_D). The kinetic components that contribute to the dissociation equilibrium constant are described in more detail below. Affinity can be measured by common methods known in the art, including those described herein. Affinity can be determined, for example, using surface plasmon resonance (SPR) technology (*e.g.*, BIACORE®) or biolayer interferometry (*e.g.*, FORTEBIO®).

[0061] With regard to the binding of an ABP to a target molecule, the terms "bind," "specific binding," "specifically binds to," "specific for," "selectively binds," and "selective for" a particular antigen (*e.g.*, a polypeptide target) or an epitope on a particular antigen mean binding

that is measurably different from a non-specific or non-selective interaction (*e.g.*, with a non-target molecule). Specific binding can be measured, for example, by measuring binding to a target molecule and comparing it to binding to a non-target molecule. Specific binding can also be determined by competition with a control molecule that mimics the epitope recognized on the target molecule. In that case, specific binding is indicated if the binding of the ABP to the target molecule is competitively inhibited by the control molecule. In some aspects, the affinity of a B7-H4 ABP for a non-target molecule is less than about 50% of the affinity for B7-H4. In some aspects, the affinity of a B7-H4 ABP for a non-target molecule is less than about 40% of the affinity for B7-H4. In some aspects, the affinity of a B7-H4 ABP for a non-target molecule is less than about 30% of the affinity for B7-H4. In some aspects, the affinity of a B7-H4 ABP for a non-target molecule is less than about 20% of the affinity for B7-H4. In some aspects, the affinity of a B7-H4 ABP for a non-target molecule is less than about 10% of the affinity for B7-H4. In some aspects, the affinity of a B7-H4 ABP for a non-target molecule is less than about 1% of the affinity for B7-H4. In some aspects, the affinity of a B7-H4 ABP for a non-target molecule is less than about 0.1% of the affinity for B7-H4.

[0062] The term "k_d" (sec^{-1}), as used herein, refers to the dissociation rate constant of a particular ABP -antigen interaction. This value is also referred to as the k_{off} value.

[0063] The term "k_a" ($M^{-1} \times sec^{-1}$), as used herein, refers to the association rate constant of a particular ABP -antigen interaction. This value is also referred to as the k_{on} value.

[0064] The term "K_D" (M), as used herein, refers to the dissociation equilibrium constant of a particular ABP -antigen interaction. $K_D = k_d/k_a$.

[0065] The term "K_A" (M^{-1}), as used herein, refers to the association equilibrium constant of a particular ABP -antigen interaction. $K_A = k_a/k_d$.

[0066] An "affinity matured" ABP is one with one or more alterations (*e.g.*, in one or more CDRs or FRs) that result in an improvement in the affinity of the ABP for its antigen, compared to a parent ABP which does not possess the alteration(s). In one embodiment, an affinity matured ABP has nanomolar or picomolar affinity for the target antigen. Affinity matured ABPs may be produced using a variety of methods known in the art. For example, Marks et al. (*Bio/Technology*, 1992, 10:779-783, incorporated by reference in its entirety) describes affinity maturation by V_H and V_L domain shuffling. Random mutagenesis of CDR and/or framework residues is described by, for example, Barbas et al. (*Proc. Nat. Acad. Sci. U.S.A.*, 1994, 91:3809-3813); Schier et al., *Gene*, 1995, 169:147-155; Yelton et al., *J. Immunol.*, 1995, 155:1994-2004; Jackson et al., *J. Immunol.*, 1995, 154:3310-33199; and Hawkins et al, *J. Mol. Biol.*, 1992, 226:889-896; each of which is incorporated by reference in its entirety.

[0067] An "immunoconjugate" is an ABP conjugated to one or more heterologous molecule(s).

[0068] "Effector functions" refer to those biological activities mediated by the Fc region of an antibody, which activities may vary depending on the antibody isotype. Examples of antibody effector functions include C1q binding to activate complement dependent cytotoxicity (CDC), Fc receptor binding to activate antibody-dependent cellular cytotoxicity (ADCC), and antibody dependent cellular phagocytosis (ADCP).

[0069] When used herein in the context of two or more ABPs, the term "competes with" or "cross-competes with" indicates that the two or more ABPs compete for binding to an antigen (*e.g.*, B7-H4). In one exemplary assay, B7-H4 is coated on a surface and contacted with a first B7-H4 ABP, after which a second B7-H4 ABP is added. In another exemplary assay, a first B7-H4 ABP is coated on a surface and contacted with B7-H4, and then a second B7-H4 ABP is added. If the presence of the first B7-H4 ABP reduces binding of the second B7-H4 ABP, in either assay, then the ABPs compete. The term "competes with" also includes combinations of ABPs where one ABP reduces binding of another ABP, but where no competition is observed when the ABPs are added in the reverse order. However, in some embodiments, the first and second ABPs inhibit binding of each other, regardless of the order in which they are added. In some embodiments, one ABP reduces binding of another ABP to its antigen by at least 25%, at least 50%, at least 60%, at least 70%, at least 80%, at least 85%, at least 90%, or at least 95%. A skilled artisan can select the concentrations of the antibodies used in the competition assays based on the affinities of the ABPs for B7-H4 and the valency of the ABPs. The assays described in this definition are illustrative, and a skilled artisan can utilize any suitable assay to determine if antibodies compete with each other. Suitable assays are described, for example, in Cox et al., "Immunoassay Methods," in *Assay Guidance Manual [Internet]*, Updated December 24, 2014 (www.ncbi.nlm.nih.gov/books/NBK92434/; accessed September 29, 2015); Silman et al., *Cytometry*, 2001, 44:30-37; and Finco et al., *J. Pharm. Biomed. Anal.*, 2011, 54:351-358; each of which is incorporated by reference in its entirety.

[0070] The term "epitope" means a portion of an antigen the specifically binds to an ABP. Epitopes frequently consist of surface-accessible amino acid residues and/or sugar side chains and may have specific three dimensional structural characteristics, as well as specific charge characteristics. Conformational and non-conformational epitopes are distinguished in that the binding to the former but not the latter may be lost in the presence of denaturing solvents. An epitope may comprise amino acid residues that are directly involved in the binding, and other amino acid residues, which are not directly involved in the binding. The epitope to which an ABP binds can be determined using known techniques for epitope determination such as, for example,

testing for ABP binding to B7-H4 variants with different point-mutations, or to chimeric B7-H4 variants.

[0071] Percent "identity" between a polypeptide sequence and a reference sequence, is defined as the percentage of amino acid residues in the polypeptide sequence that are identical to the amino acid residues in the reference sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity. Alignment for purposes of determining percent amino acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN, MEGALIGN (DNASTAR), CLUSTALW, CLUSTAL OMEGA, or MUSCLE software. Those skilled in the art can determine appropriate parameters for aligning sequences, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared.

[0072] A "conservative substitution" or a "conservative amino acid substitution," refers to the substitution an amino acid with a chemically or functionally similar amino acid. Conservative substitution tables providing similar amino acids are well known in the art. By way of example, the groups of amino acids provided in TABLES 2-4 are, in some embodiments, considered conservative substitutions for one another.

TABLE 2: Selected groups of amino acids that are considered conservative substitutions for one another, in certain embodiments.

Acidic Residues	D and E
Basic Residues	K, R, and H
Hydrophilic Uncharged Residues	S, T, N, and Q
Aliphatic Uncharged Residues	G, A, V, L, and I
Non-polar Uncharged Residues	C, M, and P
Aromatic Residues	F, Y, and W

TABLE 3: Additional selected groups of amino acids that are considered conservative substitutions for one another, in certain embodiments.

Group 1	A, S, and T
Group 2	D and E
Group 3	N and Q
Group 4	R and K
Group 5	I, L, and M
Group 6	F, Y, and W

TABLE 4: Further selected groups of amino acids that are considered conservative substitutions for one another, in certain embodiments.

Group A	A and G
Group B	D and E
Group C	N and Q
Group D	R, K, and H
Group E	I, L, M, V
Group F	F, Y, and W
Group G	S and T
Group H	C and M

[0073] Additional conservative substitutions may be found, for example, in Creighton, *Proteins: Structures and Molecular Properties* 2nd ed. (1993) W. H. Freeman & Co., New York, NY. An ABP generated by making one or more conservative substitutions of amino acid residues in a parent ABP is referred to as a "conservatively modified variant."

[0074] The term "treating" (and variations thereof such as "treat" or "treatment") refers to clinical intervention in an attempt to alter the natural course of a disease or condition in a subject in need thereof. Treatment can be performed both for prophylaxis and during the course of clinical pathology. Desirable effects of treatment include preventing occurrence or recurrence of disease, alleviation of symptoms, diminish of any direct or indirect pathological consequences of the disease, preventing metastasis, decreasing the rate of disease progression, amelioration or palliation of the disease state, and remission or improved prognosis.

[0075] As used herein, the term "therapeutically effective amount" or "effective amount" refers to an amount of an ABP or pharmaceutical composition provided herein that, when administered to a subject, is effective to treat a disease or disorder.

[0076] As used herein, the term "subject" means a mammalian subject. Exemplary subjects include humans, monkeys, dogs, cats, mice, rats, cows, horses, camels, goats, rabbits, and sheep. In certain embodiments, the subject is a human. In some embodiments the subject has a disease or condition that can be treated with an ABP provided herein. In some aspects, the disease or condition is a cancer. In some aspects, the disease or condition is a viral infection.

[0077] The term "package insert" is used to refer to instructions customarily included in commercial packages of therapeutic or diagnostic products (*e.g.*, kits) that contain information about the indications, usage, dosage, administration, combination therapy, contraindications and/or warnings concerning the use of such therapeutic or diagnostic products.

[0078] The term "cytotoxic agent," as used herein, refers to a substance that inhibits or prevents a cellular function and/or causes cell death or destruction.

[0079] A "chemotherapeutic agent" refers to a chemical compound useful in the treatment of cancer. Chemotherapeutic agents include "anti-hormonal agents" or "endocrine therapeutics" which act to regulate, reduce, block, or inhibit the effects of hormones that can promote the growth of cancer.

[0080] The term "cytostatic agent" refers to a compound or composition which arrests growth of a cell either *in vitro* or *in vivo*. In some embodiments, a cytostatic agent is an agent that reduces the percentage of cells in S phase. In some embodiments, a cytostatic agent reduces the percentage of cells in S phase by at least about 20%, at least about 40%, at least about 60%, or at least about 80%.

[0081] The term "tumor" refers to all neoplastic cell growth and proliferation, whether malignant or benign, and all pre-cancerous and cancerous cells and tissues. The terms "cancer," "cancerous," "cell proliferative disorder," "proliferative disorder" and "tumor" are not mutually exclusive as referred to herein. The terms "cell proliferative disorder" and "proliferative disorder" refer to disorders that are associated with some degree of abnormal cell proliferation. In some embodiments, the cell proliferative disorder is a cancer.

[0082] The term "pharmaceutical composition" refers to a preparation which is in such form as to permit the biological activity of an active ingredient contained therein to be effective in treating a subject, and which contains no additional components which are unacceptably toxic to the subject.

[0083] The terms "modulate" and "modulation" refer to reducing or inhibiting or, alternatively, activating or increasing, a recited variable.

[0084] The terms "increase" and "activate" refer to an increase of 10%, 20%, 30%, 40%, 50%, 60%, 70%, 75%, 80%, 85%, 90%, 95%, 100%, 2-fold, 3-fold, 4-fold, 5-fold, 10-fold, 20-fold, 50-fold, 100-fold, or greater in a recited variable.

[0085] The terms "reduce" and "inhibit" refer to a decrease of 10%, 20%, 30%, 40%, 50%, 60%, 70%, 75%, 80%, 85%, 90%, 95%, 2-fold, 3-fold, 4-fold, 5-fold, 10-fold, 20-fold, 50-fold, 100-fold, or greater in a recited variable.

[0086] The term "agonize" refers to the activation of receptor signaling to induce a biological response associated with activation of the receptor. An "agonist" is an entity that binds to and agonizes a receptor.

[0087] The term "antagonize" refers to the inhibition of receptor signaling to inhibit a biological response associated with activation of the receptor. An "antagonist" is an entity that binds to and antagonizes a receptor.

[0088] The term "effector T cell" includes T helper (i.e., CD4+) cells and cytotoxic (i.e., CD8+) T cells. CD4+ effector T cells contribute to the development of several immunologic processes, including maturation of B cells into plasma cells and memory B cells, and activation of cytotoxic T cells and macrophages. CD8+ effector T cells destroy virus-infected cells and tumor cells. *See* Seder and Ahmed, *Nature Immunol.*, 2003, 4:835-842, incorporated by reference in its entirety, for additional information on effector T cells.

[0089] The term "regulatory T cell" includes cells that regulate immunological tolerance, for example, by suppressing effector T cells. In some aspects, the regulatory T cell has a CD4+CD25+Foxp3+ phenotype. In some aspects, the regulatory T cell has a CD8+CD25+

phenotype. *See* Nocentini et al., *Br. J. Pharmacol.*, 2012, 165:2089-2099, incorporated by reference in its entirety, for additional information on regulatory T cells.

[0090] The term "dendritic cell" refers to a professional antigen-presenting cell capable of activating a naïve T cell and stimulating growth and differentiation of a B cell.

[0091] A "variant" of a polypeptide (*e.g.*, an antibody) comprises an amino acid sequence wherein one or more amino acid residues are inserted into, deleted from and/or substituted into the amino acid sequence relative to the native polypeptide sequence, and retains essentially the same biological activity as the native polypeptide. The biological activity of the polypeptide can be measured using standard techniques in the art (for example, if the variant is an antibody, its activity may be tested by binding assays, as described herein). Variants of the present disclosure include fragments, analogs, recombinant polypeptides, synthetic polypeptides, and/or fusion proteins.

[0092] A "derivative" of a polypeptide is a polypeptide (*e.g.*, an antibody) that has been chemically modified, *e.g.*, via conjugation to another chemical moiety such as, for example, polyethylene glycol, albumin (*e.g.*, human serum albumin), phosphorylation, and glycosylation. Unless otherwise indicated, the term "antibody" includes, in addition to antibodies comprising two full-length heavy chains and two full-length light chains, derivatives, variants, fragments, and muteins thereof, examples of which are described below.

[0093] A nucleotide sequence is "operably linked" to a regulatory sequence if the regulatory sequence affects the expression (*e.g.*, the level, timing, or location of expression) of the nucleotide sequence. A "regulatory sequence" is a nucleic acid that affects the expression (*e.g.*, the level, timing, or location of expression) of a nucleic acid to which it is operably linked. The regulatory sequence can, for example, exert its effects directly on the regulated nucleic acid, or through the action of one or more other molecules (*e.g.*, polypeptides that bind to the regulatory sequence and/or the nucleic acid). Examples of regulatory sequences include promoters, enhancers and other expression control elements (*e.g.*, polyadenylation signals). Further examples of regulatory sequences are described in, for example, Goeddel, 1990, Gene Expression Technology: Methods in Enzymology 185, Academic Press, San Diego, CA and Baron et al., 1995, Nucleic Acids Res. 23:3605–06. [0078]

[0094] A "host cell" is a cell that can be used to express a nucleic acid, *e.g.*, a nucleic acid of the present disclosure. A host cell can be a prokaryote, for example, *E. coli*, or it can be a eukaryote, for example, a single-celled eukaryote (*e.g.*, a yeast or other fungus), a plant cell (*e.g.*, a tobacco or tomato plant cell), an animal cell (*e.g.*, a human cell, a monkey cell, a hamster cell, a rat cell, a mouse cell, or an insect cell) or a hybridoma. Examples of host cells include CS-9 cells, the

COS-7 line of monkey kidney cells (ATCC CRL 1651) (*see* Gluzman et al., 1981, Cell 23:175), L cells, C127 cells, 3T3 cells (ATCC CCL 163), Chinese hamster ovary (CHO) cells or their derivatives such as Veggie CHO and related cell lines which grow in serum-free media (*see* Rasmussen et al., 1998, Cytotechnology 28:31), HeLa cells, BHK (ATCC CRL 10) cell lines, the CV1/EBNA cell line derived from the African green monkey kidney cell line CV1 (ATCC CCL 70) (*see* McMahan et al., 1991, EMBO J. 10:2821), human embryonic kidney cells such as 293, 293 EBNA or MSR 293, human epidermal A431 cells, human Colo205 cells, other transformed primate cell lines, normal diploid cells, cell strains derived from *in vitro* culture of primary tissue, primary explants, HL-60, U937, HaK or Jurkat cells. Typically, a host cell is a cultured cell that can be transformed or transfected with a polypeptide-encoding nucleic acid, which can then be expressed in the host cell.

[0095] The phrase "recombinant host cell" can be used to denote a host cell that has been transformed or transfected with a nucleic acid to be expressed. A host cell also can be a cell that comprises the nucleic acid but does not express it at a desired level unless a regulatory sequence is introduced into the host cell such that it becomes operably linked with the nucleic acid. It is understood that the term host cell refers not only to the particular subject cell but to the progeny or potential progeny of such a cell. Because certain modifications may occur in succeeding generations due to, *e.g.*, mutation or environmental influence, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term as used herein.

7.2. Other interpretational conventions

[0096] Ranges recited herein are understood to be shorthand for all of the values within the range, inclusive of the recited endpoints. For example, a range of 1 to 50 is understood to include any number, combination of numbers, or sub-range from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, and 50.

[0097] Unless otherwise indicated, reference to a compound that has one or more stereocenters intends each stereoisomer, and all combinations of stereoisomers, thereof.

7.3. Nucleic acids

[0098] In one aspect, the present disclosure provides isolated nucleic acid molecules. The nucleic acids comprise, for example, polynucleotides that encode all or part of an antigen binding protein, for example, one or both chains of an antibody of the present disclosure, or a fragment, derivative, mutein, or variant thereof, polynucleotides sufficient for use as hybridization probes, PCR primers or sequencing primers for identifying, analyzing, mutating or amplifying a polynucleotide encoding a polypeptide, anti-sense nucleic acids for inhibiting expression of a polynucleotide, and complementary sequences of the foregoing. The nucleic acids can be any

length. They can be, for example, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 75, 100, 125, 150, 175, 200, 250, 300, 350, 400, 450, 500, 750, 1,000, 1,500, 3,000, 5,000 or more nucleotides in length, and/or can comprise one or more additional sequences, for example, regulatory sequences, and/or be part of a larger nucleic acid, for example, a vector. The nucleic acids can be single-stranded or double-stranded and can comprise RNA and/or DNA nucleotides, and artificial variants thereof (*e.g.*, peptide nucleic acids).

[0099] Nucleic acids encoding antibody polypeptides (*e.g.*, heavy or light chain, variable domain only, or full length) can be isolated from B-cells of mice that have been immunized with B7-H4. The nucleic acid can be isolated by conventional procedures such as polymerase chain reaction (PCR).

[00100] Nucleic acid sequences encoding the variable regions of the heavy and light chain variable regions are shown herein. The skilled artisan will appreciate that, due to the degeneracy of the genetic code, each of the polypeptide sequences disclosed herein is encoded by a large number of other nucleic acid sequences. The present disclosure provides each degenerate nucleotide sequence encoding each antigen binding protein of the present disclosure.

[00101] The present disclosure further provides nucleic acids that hybridize to other nucleic acids (*e.g.*, nucleic acids comprising a nucleotide sequence of any of *vtcn1* gene) under particular hybridization conditions. Methods for hybridizing nucleic acids are well-known in the art. *See*, *e.g.*, Curr. Prot. in Mol. Biol., John Wiley & Sons, N.Y. (1989), 6.3.1-6.3.6. As defined herein, a moderately stringent hybridization condition uses a prewashing solution containing 5x sodium chloride/sodium citrate (SSC), 0.5% SDS, 1.0 mM EDTA (pH 8.0), hybridization buffer of about 50% formamide, 6X SSC, and a hybridization temperature of 55° C (or other similar hybridization solutions, such as one containing about 50% formamide, with a hybridization temperature of 42° C), and washing conditions of 60° C, in 0.5X SSC, 0.1% SDS. A stringent hybridization condition hybridizes in 6X SSC at 45° C, followed by one or more washes in 0.1x SSC, 0.2% SDS at 68° C. Furthermore, one of skill in the art can manipulate the hybridization and/or washing conditions to increase or decrease the stringency of hybridization such that nucleic acids comprising nucleotide sequences that are at least 65, 70, 75, 80, 85, 90, 95, 98, or 99% identical to each other typically remain hybridized to each other. The basic parameters affecting the choice of hybridization conditions and guidance for devising suitable conditions are set forth by, for example, Sambrook, Fritsch, and Maniatis (1989, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., chapters 9 and 11; and Curr. Prot. in Mol. Biol. 1995, Ausubel et al., eds., John Wiley & Sons, Inc., sections

2.10 and 6.3-6.4), and can be readily determined by those having ordinary skill in the art based on, for example, the length and/or base composition of the DNA.

[00102] Changes can be introduced by mutation into a nucleic acid, thereby leading to changes in the amino acid sequence of a polypeptide (*e.g.*, an antigen binding protein) that it encodes. Mutations can be introduced using any technique known in the art. In one embodiment, one or more particular amino acid residues are changed using, for example, a site-directed mutagenesis protocol. In another embodiment, one or more randomly selected residues are changed using, for example, a random mutagenesis protocol. However it is made, a mutant polypeptide can be expressed and screened for a desired property (*e.g.*, binding to B7-H4).

[00103] Mutations can be introduced into a nucleic acid without significantly altering the biological activity of a polypeptide that it encodes. For example, one can make nucleotide substitutions leading to amino acid substitutions at non-essential amino acid residues. In one embodiment, a nucleotide sequence provided herein for B7-H4, or a desired fragment, variant, or derivative thereof, is mutated such that it encodes an amino acid sequence comprising one or more deletions or substitutions of amino acid residues that are shown herein for B7-H4 to be residues where two or more sequences differ. Alternatively, one or more mutations can be introduced into a nucleic acid that selectively change the biological activity (*e.g.*, binding of B7-H4) of a polypeptide that it encodes. For example, the mutation can quantitatively or qualitatively change the biological activity. Examples of quantitative changes include increasing, reducing or eliminating the activity. Examples of qualitative changes include changing the antigen specificity of an antigen binding protein.

[00104] In another aspect, the present disclosure provides nucleic acid molecules that are suitable for use as primers or hybridization probes for the detection of nucleic acid sequences of the present disclosure. A nucleic acid molecule of the present disclosure can comprise only a portion of a nucleic acid sequence encoding a full-length polypeptide of the present disclosure, for example, a fragment that can be used as a probe or primer or a fragment encoding an active portion (*e.g.*, a B7-H4 binding portion) of a polypeptide of the present disclosure.

[00105] Probes based on the sequence of a nucleic acid of the present disclosure can be used to detect the nucleic acid or similar nucleic acids, for example, transcripts encoding a polypeptide of the present disclosure. The probe can comprise a label group, *e.g.*, a radioisotope, a fluorescent compound, an enzyme, or an enzyme co-factor. Such probes can be used to identify a cell that expresses the polypeptide

7.4. Expression vectors

[00106] The present disclosure provides vectors comprising a nucleic acid encoding a polypeptide of the present disclosure or a portion thereof. Examples of vectors include, but are

not limited to, plasmids, viral vectors, non-episomal mammalian vectors and expression vectors, for example, recombinant expression vectors.

[00107] In another aspect of the present disclosure, expression vectors containing the nucleic acid molecules and polynucleotides of the present disclosure are also provided, and host cells transformed with such vectors, and methods of producing the polypeptides are also provided. The term "expression vector" refers to a plasmid, phage, virus or vector for expressing a polypeptide from a polynucleotide sequence. Vectors for the expression of the polypeptides contain at a minimum sequences required for vector propagation and for expression of the cloned insert. An expression vector comprises a transcriptional unit comprising an assembly of (1) a genetic element or elements having a regulatory role in gene expression, for example, promoters or enhancers, (2) a sequence that encodes polypeptides and proteins to be transcribed into mRNA and translated into protein, and (3) appropriate transcription initiation and termination sequences. These sequences may further include a selection marker. Vectors suitable for expression in host cells are readily available and the nucleic acid molecules are inserted into the vectors using standard recombinant DNA techniques. Such vectors can include promoters which function in specific tissues, and viral vectors for the expression of polypeptides in targeted human or animal cells.

[00108] The recombinant expression vectors of the present disclosure can comprise a nucleic acid of the present disclosure in a form suitable for expression of the nucleic acid in a host cell. The recombinant expression vectors include one or more regulatory sequences, selected on the basis of the host cells to be used for expression, which is operably linked to the nucleic acid sequence to be expressed. Regulatory sequences include those that direct constitutive expression of a nucleotide sequence in many types of host cells (*e.g.*, SV40 early gene enhancer, Rous sarcoma virus promoter and cytomegalovirus promoter), those that direct expression of the nucleotide sequence only in certain host cells (*e.g.*, tissue-specific regulatory sequences, *see* Voss et al., 1986, Trends Biochem. Sci. 11:287, Maniatis et al., 1987, Science 236:1237, incorporated by reference herein in their entireties), and those that direct inducible expression of a nucleotide sequence in response to particular treatment or condition (*e.g.*, the metallothionin promoter in mammalian cells and the tet-responsive and/or streptomycin responsive promoter in both prokaryotic and eukaryotic systems (*see* id.). It will be appreciated by those skilled in the art that the design of the expression vector can depend on such factors as the choice of the host cell to be transformed, the level of expression of protein desired, etc. The expression vectors of the present disclosure can be introduced into host cells to thereby produce

proteins or peptides, including fusion proteins or peptides, encoded by nucleic acids as described herein.

[00109] In some embodiments, the expression vector is an expression vector purified from one of the clones of the library of B7-H4 binding clones deposited under ATCC Accession No. PTA-125511. In some embodiments, the expression vector is generated by genetic modification of one of an expression vector in one of the clones purified from the library of B7-H4 binding clones deposited under ATCC Accession No. PTA-125511. In some embodiments, the expression vector is generated by using variable region sequences of heavy and light chains of one of the clones of the library of B7-H4 binding clones deposited under ATCC Accession No. PTA-125511.

[00110] The present disclosure further provides methods of making polypeptides. A variety of other expression/host systems may be utilized. Vector DNA can be introduced into prokaryotic or eukaryotic systems via conventional transformation or transfection techniques. These systems include but are not limited to microorganisms such as bacteria (for example, E. coli) transformed with recombinant bacteriophage, plasmid or cosmid DNA expression vectors; yeast transformed with yeast expression vectors; insect cell systems infected with virus expression vectors (*e.g.*, baculovirus); plant cell systems transfected with virus expression vectors (*e.g.*, cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or transformed with bacterial expression vectors (*e.g.*, Ti or pBR322 plasmid); or animal cell systems. Mammalian cells useful in recombinant protein production include but are not limited to VERO cells, HeLa cells, Chinese hamster ovary (CHO) cell lines, or their derivatives such as Veggie CHO and related cell lines which grow in serum-free media (*see* Rasmussen et al., 1998, Cytotechnology 28:31) or CHO strain DX-B11, which is deficient in DHFR (*see* Urlaub et al., 1980, Proc. Natl. Acad. Sci. USA 77:4216-20) COS cells such as the COS-7 line of monkey kidney cells (ATCC CRL 1651) (*see* Gluzman et al., 1981, Cell 23:175), W138, BHK, HepG2, 3T3 (ATCC CCL 163), RIN, MDCK, A549, PC12, K562, L cells, C127 cells, BHK (ATCC CRL 10) cell lines, the CV1/EBNA cell line derived from the African green monkey kidney cell line CV1 (ATCC CCL 70) (*see* McMahan et al., 1991, EMBO J. 10:2821), human embryonic kidney cells such as 293, 293 EBNA or MSR 293, human epidermal A431 cells, human Colo205 cells, other transformed primate cell lines, normal diploid cells, cell strains derived from *in vitro* culture of primary tissue, primary explants, HL-60, U937, HaK or Jurkat cells. Mammalian expression allows for the production of secreted or soluble polypeptides which may be recovered from the growth medium.

[00111] For stable transfection of mammalian cells, it is known that, depending upon the expression vector and transfection technique used, only a small fraction of cells may integrate the

foreign DNA into their genome. In order to identify and select these integrants, a gene that encodes a selectable marker (*e.g.*, for resistance to antibiotics) is generally introduced into the host cells along with the gene of interest. Once such cells are transformed with vectors that contain selectable markers as well as the desired expression cassette, the cells can be allowed to grow in an enriched media before they are switched to selective media, for example. The selectable marker is designed to allow growth and recovery of cells that successfully express the introduced sequences. Resistant clumps of stably transformed cells can be proliferated using tissue culture techniques appropriate to the cell line employed. An overview of expression of recombinant proteins is found in Methods of Enzymology, v. 185, Goeddell, D.V., ed., Academic Press (1990). Preferred selectable markers include those which confer resistance to drugs, such as G418, hygromycin and methotrexate. Cells stably transfected with the introduced nucleic acid can be identified by drug selection (*e.g.*, cells that have incorporated the selectable marker gene will survive, while the other cells die), among other methods.

[00112] The transformed cells can be cultured under conditions that promote expression of the polypeptide, and the polypeptide recovered by conventional protein purification procedures (as defined above). One such purification procedure includes the use of affinity chromatography, e.g., over a matrix having all or a portion (e.g., the extracellular domain) of B7-H4 bound thereto. Polypeptides contemplated for use herein include substantially homogeneous recombinant mammalian anti- B7-H4 antibody polypeptides substantially free of contaminating endogenous materials.

[00113] In some cases, such as in expression using prokaryotic systems, the expressed polypeptides of this disclosure may need to be "refolded" and oxidized into a proper tertiary structure and disulfide linkages generated in order to be biologically active. Refolding can be accomplished using a number of procedures well known in the art. Such methods include, for example, exposing the solubilized polypeptide to a pH usually above 7 in the presence of a chaotropic agent. The selection of chaotrope is similar to the choices used for inclusion body solubilization; however a chaotrope is typically used at a lower concentration. Exemplary chaotropic agents are guanidine and urea. In most cases, the refolding/oxidation solution will also contain a reducing agent plus its oxidized form in a specific ratio to generate a particular redox potential which allows for disulfide shuffling to occur for the formation of cysteine bridges. Some commonly used redox couples include cysteine/cystamine, glutathione/dithiobisGSH, cupric chloride, dithiothreitol DTT/dithiane DTT, and 2-mercaptoethanol (bME)/dithio-bME. In many instances, a co-solvent may be used to increase

the efficiency of the refolding. Commonly used cosolvents include glycerol, polyethylene glycol of various molecular weights, and arginine.

[00114] In addition, the polypeptides can be synthesized in solution or on a solid support in accordance with conventional techniques. Various automatic synthesizers are commercially available and can be used in accordance with known protocols. *See*, for example, Stewart and Young, Solid Phase Peptide Synthesis, 2d.Ed., Pierce Chemical Co. (1984); Tam et al., J Am Chem Soc, 105:6442, (1983); Merrifield, Science 232:341-347 (1986); Barany and Merrifield, The Peptides, Gross and Meienhofer, eds, Academic Press, New York, 1-284; Barany et al., Int J Pep Protein Res, 30:705-739 (1987).

[00115] The polypeptides and proteins of the present disclosure can be purified according to protein purification techniques well known to those of skill in the art. These techniques involve, at one level, the crude fractionation of the proteinaceous and non-proteinaceous fractions. Having separated the peptide polypeptides from other proteins, the peptide or polypeptide of interest can be further purified using chromatographic and electrophoretic techniques to achieve partial or complete purification (or purification to homogeneity). The term "purified polypeptide" as used herein, is intended to refer to a composition, isolatable from other components, wherein the polypeptide is purified to any degree relative to its naturally-obtainable state. A purified polypeptide therefore also refers to a polypeptide that is free from the environment in which it may naturally occur. Generally, "purified" will refer to a polypeptide composition that has been subjected to fractionation to remove various other components, and which composition substantially retains its expressed biological activity. Where the term "substantially purified" is used, this designation will refer to a peptide or polypeptide composition in which the polypeptide or peptide forms the major component of the composition, such as constituting about 50 %, about 60 %, about 70 %, about 80 %, about 85 %, or about 90 % or more of the proteins in the composition.

[00116] Various techniques suitable for use in purification will be well known to those of skill in the art. These include, for example, precipitation with ammonium sulphate, PEG, antibodies (immunoprecipitation) and the like or by heat denaturation, followed by centrifugation; chromatography such as affinity chromatography (Protein-A columns), ion exchange, gel filtration, reverse phase, hydroxylapatite, hydrophobic interaction chromatography, isoelectric focusing, gel electrophoresis, and combinations of these techniques. As is generally known in the art, it is believed that the order of conducting the various purification steps may be changed, or that certain steps may be omitted, and still result in a suitable method for the

preparation of a substantially purified polypeptide. Exemplary purification steps are provided in the Examples below.

[00117] Various methods for quantifying the degree of purification of polypeptide will be known to those of skill in the art in light of the present disclosure. These include, for example, determining the specific binding activity of an active fraction, or assessing the amount of peptide or polypeptide within a fraction by SDS/PAGE analysis. A preferred method for assessing the purity of a polypeptide fraction is to calculate the binding activity of the fraction, to compare it to the binding activity of the initial extract, and to thus calculate the degree of purification, herein assessed by a "-fold purification number." The actual units used to represent the amount of binding activity will, of course, be dependent upon the particular assay technique chosen to follow the purification and whether or not the polypeptide or peptide exhibits a detectable binding activity.

7.5. Antibody

[00118] B7-H4 antibodies can be purified from host cells that have been transfected by a gene encoding the antibodies by elution of filtered supernatant of host cell culture fluid using a Heparin HP column, using a salt gradient.

[00119] A Fab fragment is a monovalent fragment having the V_L, V_H, C_L and C_{H1} domains; a $F(ab')_2$ fragment is a bivalent fragment having two Fab fragments linked by a disulfide bridge at the hinge region; a Fd fragment has the V_H and C_{H1} domains; an Fv fragment has the V_L and V_H domains of a single arm of an antibody; and a dAb fragment has a V_H domain, a V_L domain, or an antigen-binding fragment of a V_H or V_L domain (US Pat. No. 6,846,634, 6,696,245, US App. Pub. No. 05/0202512, 04/0202995, 04/0038291, 04/0009507, 03/0039958, Ward et al., Nature 341:544-546, 1989).

[00120] Polynucleotide and polypeptide sequences of particular light and heavy chain variable domains are described below. Antibodies comprising a light chain and heavy chain are designated by combining the name of the light chain and the name of the heavy chain variable domains. For example, "L4H7," indicates an antibody comprising the light chain variable domain of L4 (comprising a sequence of SEQ ID NO:4) and the heavy chain variable domain of H7 (comprising a sequence of SEQ ID NO:107). Light chain variable sequences are provided in SEQ ID Nos: 1-12, and heavy chain variable sequences are provided in SEQ ID Nos:101-112.

[00121] In other embodiments, an antibody may comprise a specific heavy or light chain, while the complementary light or heavy chain variable domain remains unspecified. In particular, certain embodiments herein include antibodies that bind a specific antigen (such as B7-H4) by way of a specific light or heavy chain, such that the complementary heavy or light chain may be promiscuous, or even irrelevant, but may be determined by, for example, screening

combinatorial libraries. Portolano et al., J. Immunol. V. 150 (3), pp. 880-887 (1993); Clackson et al., Nature v. 352 pp. 624-628 (1991); Adler *et al.,* A natively paired antibody library yields drug leads with higher sensitivity and specificity than a randomly paired antibody library, MAbs (2018)); Adler *et al.,* Rare, high-affinity mouse anti-B7-H4 antibodies that function in checkpoint blockade, discovered using microfluidics and molecular genomics, MAbs (2017).

[00122] Naturally occurring immunoglobulin chains exhibit the same general structure of relatively conserved framework regions (FR) joined by three hypervariable regions, also called complementarity determining regions or CDRs. From N-terminus to C-terminus, both light and heavy chains comprise the domains FR1, CDR1, FR2, CDR2, FR3, CDR3 and FR4. The assignment of amino acids to each domain is in accordance with the definitions of Kabat et al. in Sequences of Proteins of Immunological Interest, 5th Ed., US Dept. of Health and Human Services, PHS, NIH, NIH Publication no. 91-3242, 1991.

[00123] The term "human antibody," also referred to as "fully human antibody," includes all antibodies that have one or more variable and constant regions derived from human immunoglobulin sequences. In one embodiment, all of the variable and constant domains are derived from human immunoglobulin sequences (a fully human antibody). These antibodies may be prepared in a variety of ways, examples of which are described below, including through the immunization with an antigen of interest of a mouse that is genetically modified to express antibodies derived from human heavy and/or light chain-encoding genes.

[00124] A humanized antibody has a sequence that differs from the sequence of an antibody derived from a non-human species by one or more amino acid substitutions, deletions, and/or additions, such that the humanized antibody is less likely to induce an immune response, and/or induces a less severe immune response, as compared to the non-human species antibody, when it is administered to a human subject. In one embodiment, certain amino acids in the framework and constant domains of the heavy and/or light chains of the non-human species antibody are mutated to produce the humanized antibody. In another embodiment, the constant domain(s) from a human antibody are fused to the variable domain(s) of a non-human species. In another embodiment, one or more amino acid residues in one or more CDR sequences of a non-human antibody are changed to reduce the likely immunogenicity of the non-human antibody when it is administered to a human subject, wherein the changed amino acid residues either are not critical for immunospecific binding of the antibody to its antigen, or the changes to the amino acid sequence that are made are conservative changes, such that the binding of the humanized antibody to the antigen is not significantly worse than the binding of the non-human

antibody to the antigen. Examples of how to make humanized antibodies may be found in U.S. Pat. Nos. 6,054,297, 5,886,152 and 5,877,293.

[00125] The term "chimeric antibody" refers to an antibody that contains one or more regions from one antibody and one or more regions from one or more other antibodies. In one embodiment, one or more of the CDRs are derived from a human anti-B7-H4 antibody. In another embodiment, all of the CDRs are derived from a human anti-B7-H4 antibody. In another embodiment, the CDRs from more than one human anti-B7-H4 antibodies are mixed and matched in a chimeric antibody. For instance, a chimeric antibody may comprise a CDR1 from the light chain of a first human anti-B7-H4 antibody, a CDR2 and a CDR3 from the light chain of a second human anti-B7-H4 antibody, and the CDRs from the heavy chain from a third anti-B7-H4 antibody. Further, the framework regions may be derived from one of the same anti-B7-H4 antibodies, from one or more different antibodies, such as a human antibody, or from a humanized antibody. In one example of a chimeric antibody, a portion of the heavy and/or light chain is identical with, homologous to, or derived from an antibody from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is/are identical with, homologous to, or derived from an antibody (-ies) from another species or belonging to another antibody class or subclass. Also included are fragments of such antibodies that exhibit the desired biological activity (i.e., the ability to specifically bind B7-H4).

[00126] Fragments or analogs of antibodies can be readily prepared by those of ordinary skill in the art following the teachings of this specification and using techniques well-known in the art. Preferred amino- and carboxy-termini of fragments or analogs occur near boundaries of functional domains. Structural and functional domains can be identified by comparison of the nucleotide and/or amino acid sequence data to public or proprietary sequence databases. Computerized comparison methods can be used to identify sequence motifs or predicted protein conformation domains that occur in other proteins of known structure and/or function. Methods to identify protein sequences that fold into a known three-dimensional structure are known. *See*, *e.g.*, Bowie et al., 1991, Science 253:164.

[00127] Antigen binding fragments derived from an antibody can be obtained, for example, by proteolytic hydrolysis of the antibody, for example, pepsin or papain digestion of whole antibodies according to conventional methods. By way of example, antibody fragments can be produced by enzymatic cleavage of antibodies with pepsin to provide a 5S fragment termed F(ab')2. This fragment can be further cleaved using a thiol reducing agent to produce 3.5S Fab' monovalent fragments. Optionally, the cleavage reaction can be performed using a blocking group for the sulfhydryl groups that result from cleavage of disulfide linkages. As an

alternative, an enzymatic cleavage using papain produces two monovalent Fab fragments and an Fc fragment directly. These methods are described, for example, by Goldenberg, U.S. Patent No. 4,331,647, Nisonoff et al., Arch. Biochem. Biophys. 89:230, 1960; Porter, Biochem. J. 73:119, 1959; Edelman et al., in Methods in Enzymology 1:422 (Academic Press 1967); and by Andrews, S.M. and Titus, J.A. in Current Protocols in Immunology (Coligan J.E., et al., eds), John Wiley & Sons, New York (2003), pages 2.8.1 2.8.10 and 2.10A.1 2.10A.5. Other methods for cleaving antibodies, such as separating heavy chains to form monovalent light heavy chain fragments (Fd), further cleaving of fragments, or other enzymatic, chemical, or genetic techniques may also be used, so long as the fragments bind to the antigen that is recognized by the intact antibody.

[00128] An antibody fragment may also be any synthetic or genetically engineered protein. For example, antibody fragments include isolated fragments consisting of the light chain variable region, "Fv" fragments consisting of the variable regions of the heavy and light chains, recombinant single chain polypeptide molecules in which light and heavy variable regions are connected by a peptide linker (scFv proteins).

[00129] Another form of an antibody fragment is a peptide comprising one or more complementarity determining regions (CDRs) of an antibody. CDRs (also termed "minimal recognition units", or "hypervariable region") can be incorporated into a molecule either covalently or noncovalently to make it an antigen binding protein. CDRs can be obtained by constructing polynucleotides that encode the CDR of interest. Such polynucleotides are prepared, for example, by using the polymerase chain reaction to synthesize the variable region using mRNA of antibody producing cells as a template (*see*, for example, Larrick et al., Methods: A Companion to Methods in Enzymology 2:106, 1991; Courtenay Luck, "Genetic Manipulation of Monoclonal Antibodies," in Monoclonal Antibodies: Production, Engineering and Clinical Application, Ritter et al. (eds.), page 166 (Cambridge University Press 1995); and Ward et al., "Genetic Manipulation and Expression of Antibodies," in Monoclonal Antibodies: Principles and Applications, Birch et al., (eds.), page 137 (Wiley Liss, Inc. 1995).

[00130] Thus, in one embodiment, the binding agent comprises at least one CDR as described herein. The binding agent may comprise at least two, three, four, five or six CDR's as described herein. The binding agent may further comprise at least one variable region domain of an antibody described herein. The variable region domain may be of any size or amino acid composition and will generally comprise at least one CDR sequence responsible for binding to human B7-H4, for example CDR1-H, CDR2-H, CDR3-H, CDR1-L, CDR2-L, and CDR3-L, specifically described herein and which is adjacent to or in frame with one or more framework

sequences. In general terms, the variable (V) region domain may be any suitable arrangement of immunoglobulin heavy (V_H) and/or light (V_L) chain variable domains. Thus, for example, the V region domain may be monomeric and be a V_H or V_L domain, which is capable of independently binding human B7-H4 with an affinity at least equal to 1 x 10^7M or less as described below. Alternatively, the V region domain may be dimeric and contain V_H V_H, V_H V_L, or V_L V_L, dimers. The V region dimer comprises at least one V_H and at least one V_L chain that may be non covalently associated (hereinafter referred to as F_V). If desired, the chains may be covalently coupled either directly, for example via a disulfide bond between the two variable domains, or through a linker, for example a peptide linker, to form a single chain Fv (scFV).

[00131] The variable region domain may be any naturally occurring variable domain or an engineered version thereof. By engineered version is meant a variable region domain that has been created using recombinant DNA engineering techniques. Such engineered versions include those created, for example, from a specific antibody variable region by insertions, deletions, or changes in or to the amino acid sequences of the specific antibody. Particular examples include engineered variable region domains containing at least one CDR and optionally one or more framework amino acids from a first antibody and the remainder of the variable region domain from a second antibody.

[00132] The variable region domain may be covalently attached at a C terminal amino acid to at least one other antibody domain or a fragment thereof. Thus, for example, a V_H domain that is present in the variable region domain may be linked to an immunoglobulin CH1 domain, or a fragment thereof. Similarly a V_L domain may be linked to a CK domain or a fragment thereof. In this way, for example, the antibody may be a Fab fragment wherein the antigen binding domain contains associated V_H and V_L domains covalently linked at their C termini to a CH1 and CK domain, respectively. The CH1 domain may be extended with further amino acids, for example to provide a hinge region or a portion of a hinge region domain as found in a Fab' fragment, or to provide further domains, such as antibody CH2 and CH3 domains.

[00133] As described herein, antibodies comprise at least one of these CDRs. For example, one or more CDR may be incorporated into known antibody framework regions (IgG1, IgG2, *etc.*), or conjugated to a suitable vehicle to enhance the half-life thereof. Suitable vehicles include, but are not limited to Fc, polyethylene glycol (PEG), albumin, transferrin, and the like. These and other suitable vehicles are known in the art. Such conjugated CDR peptides may be in monomeric, dimeric, tetrameric, or other form. In one embodiment, one or more water-soluble polymer is bonded at one or more specific position, for example at the amino terminus, of a binding agent.

[00134] In another example, individual V_L or V_H chains from an antibody (i.e. B7-H4 antibody) can be used to search for other V_H or V_L chains that could form antigen-binding fragments (or Fab), with the same specificity. Thus, random combinations of V_H and V_L chain Ig genes can be expressed as antigen-binding fragments in a bacteriophage library (such as fd or lambda phage). For instance, a combinatorial library may be generated by utilizing the parent V_L or V_H chain library combined with antigen-binding specific V_L or V_H chain libraries, respectively. The combinatorial libraries may then be screened by conventional techniques, for example by using radioactively labeled probe (such as radioactively labeled B7-H4). *See*, for example, Portolano et al., J. Immunol. V. 150 (3) pp. 880-887 (1993).

[00135] Diabodies are bivalent antibodies comprising two polypeptide chains, wherein each polypeptide chain comprises V_H and V_L domains joined by a linker that is too short to allow for pairing between two domains on the same chain, thus allowing each domain to pair with a complementary domain on another polypeptide chain (*see, e.g.*, Holliger *et al.*, 1993, *Proc. Natl. Acad. Sci.* USA 90:6444-48, and Poljak *et al.*, 1994, *Structure* 2:1121-23). If the two polypeptide chains of a diabody are identical, then a diabody resulting from their pairing will have two identical antigen binding sites. Polypeptide chains having different sequences can be used to make a diabody with two different antigen binding sites. Similarly, tribodies and tetrabodies are antibodies comprising three and four polypeptide chains, respectively, and forming three and four antigen binding sites, respectively, which can be the same or different.

[00136] Antibody polypeptides are also disclosed in U. S. Patent No. 6,703,199, including fibronectin polypeptide monobodies. Other antibody polypeptides are disclosed in U.S. Patent Publication 2005/0238646, which are single-chain polypeptides.

[00137] In certain embodiments, an antibody comprises one or more water soluble polymer attachments, including, but not limited to, polyethylene glycol, polyoxyethylene glycol, or polypropylene glycol. *See, e.g.*, U.S. Pat. Nos. 4,640,835, 4,496,689, 4,301,144, 4,670,417, 4,791,192 and 4,179,337. In certain embodiments, a derivative binding agent comprises one or more of monomethoxy-polyethylene glycol, dextran, cellulose, or other carbohydrate based polymers, poly-(N-vinyl pyrrolidone)-polyethylene glycol, propylene glycol homopolymers, a polypropylene oxide/ethylene oxide co-polymer, polyoxyethylated polyols (*e.g.*, glycerol) and polyvinyl alcohol, as well as mixtures of such polymers. In certain embodiments, one or more water-soluble polymer is randomly attached to one or more side chains. In certain embodiments, PEG can act to improve the therapeutic capacity for a binding agent, such as an antibody. Certain such methods are discussed, for example, in U.S. Pat. No. 6,133,426, which is hereby incorporated by reference for any purpose.

7.6. Antigen binding protein

[00138] In one aspect, the present disclosure provides antigen binding proteins (*e.g.*, antibodies, antibody fragments, antibody derivatives, antibody muteins, and antibody variants), that bind to B7-H4.

[00139] An antigen binding protein can have, for example, the structure of a naturally occurring immunoglobulin. An "immunoglobulin" is a tetrameric molecule. In a naturally occurring immunoglobulin, each tetramer is composed of two identical pairs of polypeptide chains, each pair having one "light" (about 25 kDa) and one "heavy" chain (about 50-70 kDa). The amino-terminal portion of each chain includes a variable region of about 100 to 110 or more amino acids primarily responsible for antigen recognition. The carboxy-terminal portion of each chain defines a constant region primarily responsible for effector function. Human light chains are classified as kappa and lambda light chains. Heavy chains are classified as mu, delta, gamma, alpha, or epsilon, and define the antibody's isotype as IgM, IgD, IgG, IgA, and IgE, respectively. Within light and heavy chains, the variable and constant regions are joined by a "J" region of about 12 or more amino acids, with the heavy chain also including a "D" region of about 10 more amino acids. *See* generally, Fundamental Immunology Ch. 7 (Paul, W., ed., 2nd ed. Raven Press, N.Y. (1989)) (incorporated by reference in its entirety for all purposes). The variable regions of each light/heavy chain pair form the antibody binding site such that an intact immunoglobulin has two binding sites.

[00140] Antigen binding proteins in accordance with the present disclosure include antigen binding proteins that inhibit a biological activity of B7-H4.

[00141] Different antigen binding proteins may bind to different domains of B7-H4 or act by different mechanisms of action. As indicated herein *inter alia*, the domain region are designated such as to be inclusive of the group, unless otherwise indicated. For example, amino acids 4-12 refers to nine amino acids: amino acids at positions 4, and 12, as well as the seven intervening amino acids in the sequence. Other examples include antigen binding proteins that inhibit binding of B7-H4 to its ligands. An antigen binding protein need not completely inhibit a B7-H4-induced activity to find use in the present disclosure; rather, antigen binding proteins that reduce a particular activity of B7-H4 are contemplated for use as well. (Discussions herein of particular mechanisms of action for B7-H4-binding antigen binding proteins in treating particular diseases are illustrative only, and the methods presented herein are not bound thereby.)

[00142] In another aspect, the present disclosure provides antigen binding proteins that comprise a light chain variable region selected from the group consisting of A1LC-A12LC or a heavy chain variable region selected from the group consisting of A1HC-A12HC, and fragments, derivatives, muteins, and variants thereof. Such an antigen binding protein can be denoted using

the nomenclature "LxHy," wherein "x" corresponds to the number of the light chain variable region and "y" corresponds to the number of the heavy chain variable region as they are labeled in the sequences below. That is to say, for example, that "A1HC" denotes the heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 101; "A1LC" denotes the light chain variable region comprising the amino acid sequence of SEQ ID NO:1, and so forth. More generally speaking, "L2H1" refers to an antigen binding protein with a light chain variable region comprising the amino acid sequence of L2 (SEQ ID NO:2) and a heavy chain variable region comprising the amino acid sequence of H1 (SEQ ID NO:101). For clarity, all ranges denoted by at least two members of a group include all members of the group between and including the end range members. Thus, the group range A1-A12, includes all members between A1 and A12, as well as members A1 and A12 themselves. The group range A4-A6 includes members A4, A5, and A6, etc.

[00143] In some embodiments, antigen binding proteins comprise variable (V(D)J) regions of both heavy and light chain sequences identical to one of the clones in the library of B7-H4 binding clones, deposited under ATCC Accession NO. PTA-125511. In some embodiments, antigen binding proteins comprise variable (V(D)J) regions of either heavy or light chain sequence identical to one of the clones in the library of B7-H4 binding clones, deposited under ATCC Accession NO. PTA-125511. In some embodiments, antigen binding proteins are expressed from the expression vector in one of the clones in the library of B7-H4 binding clones, deposited under ATCC Accession NO. PTA-125511.

[00144] Also shown below are the locations of the CDRs (underlined) that create part of the antigen-binding site, while the Framework Regions (FRs) are the intervening segments of these variable domain sequences. In both light chain variable regions and heavy chain variable regions there are three CDRs (CDR1-3) and four FRs (FR 1-4). The CDR regions of each light and heavy chain also are grouped by antibody type (A1, A2, A3, etc.). Antigen binding proteins of the present disclosure include, for example, antigen binding proteins having a combination of light chain and heavy chain variable domains selected from the group of combinations consisting of L1H1 (antibody A1), L2H2 (antibody A2), L3H3 (antibody A3), L4H4 (antibody A4), L5H5 (antibody A5), L6H6 (antibody A6), L7H7 (antibody A7), L8H8 (antibody A8), L9H9 (antibody A9), L10H10 (antibody A10), L11H11 (antibody A11), and L12H12 (antibody A12).

[00145] In some embodiments, antigen binding proteins comprise all six CDR sequences (three CDRs of light chain and three CDRs of heavy chain) identical to one of the clones in the library of B7-H4 binding clones, deposited under ATCC Accession NO. PTA-125511. In some embodiments, antigen binding proteins comprise three out of six CDR sequences (three CDRs of

light chain or three CDRs of heavy chain) identical to one of the clones in the library of B7-H4 binding clones, deposited under ATCC Accession NO. PTA-125511. In some embodiments, antigen binding proteins comprise one, two, three, four, or five out of six CDR sequences identical to one of the clones in the library of B7-H4 binding clones, deposited under ATCC Accession NO. PTA-125511.

[00146] In one embodiment, the present disclosure provides an antigen binding protein comprising a light chain variable domain comprising a sequence of amino acids that differs from the sequence of a light chain variable domain selected from the group consisting of L1 through L12 only at 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 residues, wherein each such sequence difference is independently either a deletion, insertion, or substitution of one amino acid residue. In another embodiment, the light-chain variable domain comprises a sequence of amino acids that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to the sequence of a light chain variable domain selected from the group consisting of L1-L12. In another embodiment, the light chain variable domain comprises a sequence of amino acids that is encoded by a nucleotide sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to a nucleotide sequence that encodes a light chain variable domain selected from the group consisting of L1-L12 (which includes L1, L2, L3, L4, L5, L6, L7, L8, L9, L10, L11, and L12). In another embodiment, the light chain variable domain comprises a sequence of amino acids that is encoded by a polynucleotide that hybridizes under moderately stringent conditions to the complement of a polynucleotide that encodes a light chain variable domain selected from the group consisting of L1-L12. In another embodiment, the light chain variable domain comprises a sequence of amino acids that is encoded by a polynucleotide that hybridizes under moderately stringent conditions to the complement of a polynucleotide that encodes a light chain variable domain selected from the group consisting of L1-L12. In another embodiment, the light chain variable domain comprises a sequence of amino acids that is encoded by a polynucleotide that hybridizes under moderately stringent conditions to a complement of a light chain polynucleotide of L1-L12.

[00147] In one embodiment, the present disclosure provides an antigen binding protein comprising a light chain variable domain comprising a sequence of amino acids that differs from the sequence of a light chain variable domain encoded by one of the clones of the library of B7-H4 binding clones, deposited under ATCC Accession NO. PTA-125511, only at 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 residues, wherein each such sequence difference is independently either a deletion, insertion, or substitution of one amino acid residue. In another embodiment, the light-chain variable domain comprises a sequence of amino acids that is at least 70%, 75%, 80%,

85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to the sequence of a light chain variable domain encoded by one of the clones of the library of B7-H4 binding clones, deposited under ATCC Accession NO. PTA-125511. In another embodiment, the light chain variable domain comprises a sequence of amino acids that is encoded by a nucleotide sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to a nucleotide sequence of one of the clones of the library of B7-H4 binding clones, deposited under ATCC Accession NO. PTA-125511.

[00148] In another embodiment, the present disclosure provides an antigen binding protein comprising a heavy chain variable domain comprising a sequence of amino acids that differs from the sequence of a heavy chain variable domain selected from the group consisting of H1-H12 only at 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 residue(s), wherein each such sequence difference is independently either a deletion, insertion, or substitution of one amino acid residue. In another embodiment, the heavy chain variable domain comprises a sequence of amino acids that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to the sequence of a heavy chain variable domain selected from the group consisting of H1-H12. In another embodiment, the heavy chain variable domain comprises a sequence of amino acids that is encoded by a nucleotide sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to a nucleotide sequence that encodes a heavy chain variable domain selected from the group consisting of H1-H12. In another embodiment, the heavy chain variable domain comprises a sequence of amino acids that is encoded by a polynucleotide that hybridizes under moderately stringent conditions to the complement of a polynucleotide that encodes a heavy chain variable domain selected from the group consisting of H1-H12. In another embodiment, the heavy chain variable domain comprises a sequence of amino acids that is encoded by a polynucleotide that hybridizes under moderately stringent conditions to the complement of a polynucleotide that encodes a heavy chain variable domain selected from the group consisting of H1-H12. In another embodiment, the heavy chain variable domain comprises a sequence of amino acids that is encoded by a polynucleotide that hybridizes under moderately stringent conditions to a complement of a heavy chain polynucleotide disclosed herein.

[00149] In one embodiment, the present disclosure provides an antigen binding protein comprising a heavy chain variable domain comprising a sequence of amino acids that differs from the sequence of a heavy chain variable domain encoded by one of the clones of the library of B7-H4 binding clones, deposited under ATCC Accession NO. PTA-125511, only at 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 residues, wherein each such sequence difference is

independently either a deletion, insertion, or substitution of one amino acid residue. In another embodiment, the heavy chain variable domain comprises a sequence of amino acids that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to the sequence of a heavy chain variable domain encoded by one of the clones of the library of B7-H4 binding clones, deposited under ATCC Accession NO. PTA-125511. In another embodiment, the heavy chain variable domain comprises a sequence of amino acids that is encoded by a nucleotide sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to a nucleotide sequence of one of the clones of the library of B7-H4 binding clones, deposited under ATCC Accession NO. PTA-125511.

[00150] Particular embodiments of antigen binding proteins of the present disclosure comprise one or more amino acid sequences that are identical to the amino acid sequences of one or more of the CDRs and/or FRs referenced herein. In one embodiment, the antigen binding protein comprises a light chain CDR1 sequence illustrated above. In another embodiment, the antigen binding protein comprises a light chain CDR2 sequence illustrated above. In another embodiment, the antigen binding protein comprises a light chain CDR3 sequence illustrated above. In another embodiment, the antigen binding protein comprises a heavy chain CDR1 sequence illustrated above. In another embodiment, the antigen binding protein comprises a heavy chain CDR2 sequence illustrated above. In another embodiment, the antigen binding protein comprises a heavy chain CDR3 sequence illustrated above.

[00151] In one embodiment, the present disclosure provides an antigen binding protein that comprises one or more CDR sequences that differ from a CDR sequence shown above by no more than 5, 4, 3, 2, or 1 amino acid residues.

[00152] In some embodiments, at least one of the antigen binding protein's CDR1 sequences is a CDR1 sequence from A1-A12, CDR1-L1 to 12, or CDR1-H1 to 12 as shown in TABLE 5. In some embodiments, at least one of the antigen binding protein's CDR2 sequences is a CDR2 sequence from A1-A12, CDR2-L1 to 12, or CDR2-H1 to 12 as shown in TABLE 5. In some embodiments, at least one of the antigen binding protein's CDR3 sequences is a CDR3 sequence from A1-A12, CDR3-L1 to 12, or CDR3-H1 to 12 as shown in TABLE 5.

[00153] In another embodiment, the antigen binding protein's light chain CDR3 sequence is a light chain CDR3 sequence from A1-A12 or CDR3-L1 to 12, as shown in TABLE 5 and the antigen binding protein's heavy chain CDR3 sequence is a heavy chain sequence from A1-A12 or CDR-H1 to 12, as shown in TABLE 5.

[00154] In another embodiment, the antigen binding protein comprises 1, 2, 3, 4, or 5 CDR sequence(s) that each independently differs by 6, 5, 4, 3, 2, 1, or 0 single amino acid additions,

substitutions, and/or deletions from a CDR sequence of A1-A23, and the antigen binding protein further comprises 1, 2, 3, 4, or 5 CDR sequence(s) that each independently differs by 6, 5, 4, 3, 2, 1, or 0 single amino acid additions, substitutions, and/or deletions from a CDR sequence. In some embodiments, the antigen binding protein comprises 1, 2, 3, 4, or 5 CDR sequence(s) that each has at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to a CDR sequence of A1-A12.

[00155] The nucleotide sequences of A1-A12, or the amino acid sequences of A1-A12, can be altered, for example, by random mutagenesis or by site-directed mutagenesis (*e.g.*, oligonucleotide-directed site-specific mutagenesis) to create an altered polynucleotide comprising one or more particular nucleotide substitutions, deletions, or insertions as compared to the non-mutated polynucleotide. Examples of techniques for making such alterations are described in Walder *et al.*, 1986, Gene 42:133; Bauer *et al.* 1985, Gene 37:73; Craik, BioTechniques, January 1985, 12-19; Smith *et al.*, 1981, *Genetic Engineering: Principles and Methods*, Plenum Press; and U.S. Patent Nos. 4,518,584 and 4,737,462. These and other methods can be used to make, for example, derivatives of anti-B7-H4 antibodies that have a desired property, for example, increased affinity, avidity, or specificity for B7-H4, increased activity or stability *in vivo* or *in vitro*, or reduced *in vivo* side-effects as compared to the underivatized antibody.

[00156] Other derivatives of anti-B7-H4 antibodies within the scope of this disclosure include covalent or aggregative conjugates of anti-B7-H4 antibodies, or fragments thereof, with other proteins or polypeptides, such as by expression of recombinant fusion proteins comprising heterologous polypeptides fused to the N-terminus or C-terminus of an anti-B7-H4 antibody polypeptide. For example, the conjugated peptide may be a heterologous signal (or leader) polypeptide, *e.g.*, the yeast alpha-factor leader, or a peptide such as an epitope tag. Antigen binding protein-containing fusion proteins can comprise peptides added to facilitate purification or identification of antigen binding protein (*e.g.*, poly-His). An antigen binding protein also can be linked to the FLAG peptide Asp-Tyr-Lys-Asp-Asp-Asp-Asp-Lys (DYKDDDDK) (SEQ ID NO: 7002) as described in Hopp *et al.*, *Bio/Technology* 6:1204, 1988, and U.S. Patent 5,011,912. The FLAG peptide is highly antigenic and provides an epitope reversibly bound by a specific monoclonal antibody (mAb), enabling rapid assay and facile purification of expressed recombinant protein. Reagents useful for preparing fusion proteins in which the FLAG peptide is fused to a given polypeptide are commercially available (Sigma, St. Louis, MO).

[00157] One suitable Fc polypeptide, described in PCT application WO 93/10151 (hereby incorporated by reference), is a single chain polypeptide extending from the N-terminal hinge

region to the native C-terminus of the Fc region of a human IgG1 antibody. Another useful Fc polypeptide is the Fc mutein described in U.S. Patent 5,457,035 and in Baum *et al.*, 1994, EMBO J. 13:3992-4001. The amino acid sequence of this mutein is identical to that of the native Fc sequence presented in WO 93/10151, except that amino acid 19 has been changed from Leu to Ala, amino acid 20 has been changed from Leu to Glu, and amino acid 22 has been changed from Gly to Ala. The mutein exhibits reduced affinity for Fc receptors.

[00158] In other embodiments, the variable portion of the heavy and/or light chains of an anti-B7-H4 antibody may be substituted for the variable portion of an antibody heavy and/or light chain.

[00159] Oligomers that contain one or more antigen binding proteins may be employed as B7-H4 antagonists or agonists. Oligomers may be in the form of covalently-linked or non-covalently-linked dimers, trimers, or higher oligomers. Oligomers comprising two or more antigen binding protein are contemplated for use, with one example being a homodimer. Other oligomers include heterodimers, homotrimers, heterotrimers, homotetramers, heterotetramers, *etc*.

[00160] One embodiment is directed to oligomers comprising multiple antigen binding proteins joined *via* covalent or non-covalent interactions between peptide moieties fused to the antigen binding proteins. Such peptides may be peptide linkers (spacers), or peptides that have the property of promoting oligomerization. Leucine zippers and certain polypeptides derived from antibodies are among the peptides that can promote oligomerization of antigen binding proteins attached thereto, as described in more detail below.

[00161] In particular embodiments, the oligomers comprise from two to four antigen binding proteins. The antigen binding proteins of the oligomer may be in any form, such as any of the forms described above, *e.g.*, variants or fragments. Preferably, the oligomers comprise antigen binding proteins that have B7-H4 binding activity.

[00162] In one embodiment, an oligomer is prepared using polypeptides derived from immunoglobulins. Preparation of fusion proteins comprising certain heterologous polypeptides fused to various portions of antibody-derived polypeptides (including the Fc domain) has been described, *e.g.*, by Ashkenazi *et al.*, 1991, PNAS USA 88:10535; Byrn *et al.*, 1990, Nature 344:677; and Hollenbaugh *et al.*, 1992 *Curr. Prot.s in Immunol.*, Suppl. 4, pages 10.19.1 - 10.19.11.

[00163] One embodiment of the present disclosure is directed to a dimer comprising two fusion proteins created by fusing a B7-H4 binding fragment of an anti-B7-H4 antibody to the Fc region of an antibody. The dimer can be made by, for example, inserting a gene fusion encoding

the fusion protein into an appropriate expression vector, expressing the gene fusion in host cells transformed with the recombinant expression vector, and allowing the expressed fusion protein to assemble much like antibody molecules, whereupon interchain disulfide bonds form between the Fc moieties to yield the dimer.

[00164] Alternatively, the oligomer is a fusion protein comprising multiple antigen binding proteins, with or without peptide linkers (spacer peptides). Among the suitable peptide linkers are those described in U.S. Patents 4,751,180 and 4,935,233.

[00165] Another method for preparing oligomeric antigen binding proteins involves use of a leucine zipper. Leucine zipper domains are peptides that promote oligomerization of the proteins in which they are found. Leucine zippers were originally identified in several DNA-binding proteins (Landschulz *et al.*, 1988, Science 240:1759), and have since been found in a variety of different proteins. Among the known leucine zippers are naturally occurring peptides and derivatives thereof that dimerize or trimerize. Examples of leucine zipper domains suitable for producing soluble oligomeric proteins are described in PCT application WO 94/10308, and the leucine zipper derived from lung surfactant protein D (SPD) described in Hoppe *et al.*, 1994, FEBS Letters 344:191, hereby incorporated by reference. The use of a modified leucine zipper that allows for stable trimerization of a heterologous protein fused thereto is described in Fanslow *et al.*, 1994, Semin. Immunol. 6:267-78. In one approach, recombinant fusion proteins comprising an anti-B7-H4 antibody fragment or derivative fused to a leucine zipper peptide are expressed in suitable host cells, and the soluble oligomeric anti-B7-H4 antibody fragments or derivatives that form are recovered from the culture supernatant.

[00166] In one aspect, the present disclosure provides antigen binding proteins that interfere with the binding of B7-H4 to its ligands. Such antigen binding proteins can be made against B7-H4, or a fragment, variant or derivative thereof, and screened in conventional assays for the ability to interfere with binding of B7-H4 to its ligands. Examples of suitable assays are assays that test the antigen binding proteins for the ability to inhibit binding of B7-H4 ligands to cells expressing B7-H4, or that test antigen binding proteins for the ability to reduce a biological or cellular response that results from the binding of B7-H4 ligands to cell surface B7-H4. For example, antibodies can be screened according to their ability to bind to immobilized antibody surfaces (B7-H4). Antigen binding proteins that block the binding of B7-H4 to a ligand can be employed in treating any B7-H4-related condition, including but not limited to cancer or autoimmune disease. In an embodiment, a human anti-B7-H4 monoclonal antibody generated by procedures involving immunization of transgenic mice is employed in treating such conditions.

[00167] Antigen-binding fragments of antigen binding proteins of the present disclosure can be produced by conventional techniques. Examples of such fragments include, but are not limited to, Fab and $F(ab')_2$ fragments. Antibody fragments and derivatives produced by genetic engineering techniques also are contemplated.

[00168] Additional embodiments include chimeric antibodies, *e.g.*, humanized versions of non-human (*e.g.*, murine) monoclonal antibodies. Such humanized antibodies may be prepared by known techniques, and offer the advantage of reduced immunogenicity when the antibodies are administered to humans. In one embodiment, a humanized monoclonal antibody comprises the variable domain of a murine antibody (or all or part of the antigen binding site thereof) and a constant domain derived from a human antibody. Alternatively, a humanized antibody fragment may comprise the antigen binding site of a murine monoclonal antibody and a variable domain fragment (lacking the antigen-binding site) derived from a human antibody. Procedures for the production of chimeric and further engineered monoclonal antibodies include those described in Riechmann *et al.*, 1988, Nature 332:323, Liu *et al.*, 1987, Proc. Nat. Acad. Sci. USA 84:3439, Larrick *et al.*, 1989, Bio/Technology 7:934, and Winter *et al.*, 1993, TIPS 14:139. In one embodiment, the chimeric antibody is a CDR grafted antibody. Techniques for humanizing antibodies are discussed in, *e.g.*, U.S. Pat. No.s 5,869,619, 5,225,539, 5,821,337, 5,859,205, 6,881,557, Padlan *et al.*, 1995, FASEB J. 9:133-39, and Tamura *et al.*, 2000, J. Immunol. 164:1432-41.

[00169] Procedures have been developed for generating human or partially human antibodies in non-human animals. For example, mice in which one or more endogenous immunoglobulin genes have been inactivated by various means have been prepared. Human immunoglobulin genes have been introduced into the mice to replace the inactivated mouse genes. Antibodies produced in the animal incorporate human immunoglobulin polypeptide chains encoded by the human genetic material introduced into the animal. In one embodiment, a non-human animal, such as a transgenic mouse, is immunized with a B7-H4 polypeptide, such that antibodies directed against the B7-H4 polypeptide are generated in the animal.

[00170] One example of a suitable immunogen is a soluble human B7-H4, such as a polypeptide comprising the extracellular domain of the protein having the following sequence: SEQ ID: 7001 or other immunogenic fragment of the protein. Examples of techniques for production and use of transgenic animals for the production of human or partially human antibodies are described in U.S. Patents 5,814,318, 5,569,825, and 5,545,806, Davis et al., 2003, Production of human antibodies from transgenic mice in Lo, ed. Antibody Engineering: Methods and Protocols, Humana Press, NJ:191-200, Kellermann et al., 2002, *Curr Opin Biotechnol.*

13:593-97, Russel et al., 2000, *Infect Immun.* 68:1820-26, Gallo et al., 2000, *Eur J Immun.* 30:534-40, Davis et al., 1999, *Cancer Metastasis Rev.* 18:421-25, Green, 1999, *J Immunol Methods.* 231:11-23, Jakobovits, 1998, *Advanced Drug Delivery Reviews* 31:33-42, Green et al., 1998, *J Exp Med.* 188:483-95, Jakobovits A, 1998, *Exp. Opin. Invest. Drugs.* 7:607-14, Tsuda et al., 1997, *Genomics.* 42:413-21, Mendez et al., 1997, *Nat Genet.* 15:146-56, Jakobovits, 1994, *Curr Biol.* 4:761-63, Arbones et al., 1994, *Immunity.* 1:247-60, Green et al., 1994, *Nat Genet.* 7:13-21, Jakobovits et al., 1993, Nature. 362:255-58, Jakobovits et al., 1993, *Proc Natl Acad Sci* U S A. 90:2551-55. Chen, J., M. Trounstine, F. W. Alt, F. Young, C. Kurahara, J. Loring, D. Huszar. *Inter'l Immunol.* 5 (1993): 647-656, Choi et al., 1993, *Nature Genetics* 4: 117-23, Fishwild et al., 1996, *Nature Biotech.* 14: 845-51, Harding et al., 1995, Annals of the New York Academy of Sciences, Lonberg et al., 1994, *Nature* 368: 856-59, Lonberg, 1994, Transgenic Approaches to Human Monoclonal Antibodies in Handbook of Experimental Pharmacology 113: 49-101, Lonberg et al., 1995, Internal Review of Immunology 13: 65-93, Neuberger, 1996, *Nature Biotechnology* 14: 826, Taylor et al., 1992, *Nucleic Acids Res.* 20: 6287-95, Taylor et al., 1994, *Inter'l Immunol.* 6: 579-91, Tomizuka et al., 1997, *Nature Genetics* 16: 133-43, Tomizuka et al., 2000, *Pro. Nat'lAcad. Sci.* USA 97: 722-27, Tuaillon et al., 1993, *Pro.Nat'lAcad.Sci.* USA 90: 3720-24, and Tuaillon et al., 1994, *J.Immunol.* 152: 2912-20.

[00171] Antigen binding proteins (*e.g.*, antibodies, antibody fragments, and antibody derivatives) of the present disclosure can comprise any constant region known in the art. The light chain constant region can be, for example, a kappa- or lambda-type light chain constant region, *e.g.*, a human kappa- or lambda-type light chain constant region. The heavy chain constant region can be, for example, an alpha-, delta-, epsilon-, gamma-, or mu-type heavy chain constant regions, *e.g.*, a human alpha-, delta-, epsilon-, gamma-, or mu-type heavy chain constant region. In one embodiment, the light or heavy chain constant region is a fragment, derivative, variant, or mutein of a naturally occurring constant region.

[00172] Techniques are known for deriving an antibody of a different subclass or isotype from an antibody of interest, *i.e.*, subclass switching. Thus, IgG antibodies may be derived from an IgM antibody, for example, and *vice versa*. Such techniques allow the preparation of new antibodies that possess the antigen-binding properties of a given antibody (the parent antibody), but also exhibit biological properties associated with an antibody isotype or subclass different from that of the parent antibody. Recombinant DNA techniques may be employed. Cloned DNA encoding particular antibody polypeptides may be employed in such procedures, *e.g.*, DNA encoding the constant domain of an antibody of the desired isotype. *See* also Lantto *et al.*, 2002, *Methods Mol. Biol.* 178:303-16.

[00173] In one embodiment, an antigen binding protein of the present disclosure comprises the IgG1 heavy chain domain of any of A1-A12 (H1-H12) or a fragment of the IgG1 heavy chain domain of any of A1-A12 (H1-H12). In another embodiment, an antigen binding protein of the present disclosure comprises the kappa light chain constant chain region of A1-A12 (L1-L12), or a fragment of the kappa light chain constant region of A1-A12 (L1-L12). In another embodiment, an antigen binding protein of the present disclosure comprises both the IgG1 heavy chain domain, or a fragment thereof, of A1-A12 (L1-L12) and the kappa light chain domain, or a fragment thereof, of A1-A12 (L1-L12).

[00174] Accordingly, the antigen binding proteins of the present disclosure include those comprising, for example, the variable domain combinations L1H1, L2H2, L3H3, L4H4, L5H5, L6H6, L7H7, L8H8, L9H9, L10H10, L11H11, and L12 H12, having a desired isotype (for example, IgA, IgG1, IgG2, IgG3, IgG4, IgM, IgE, and IgD) as well as Fab or $F(ab')_2$ fragments thereof. Moreover, if an IgG4 is desired, it may also be desired to introduce a point mutation (CPSCP (SEQ ID NO: 8673) -> CPPCP (SEQ ID NO: 8674)) in the hinge region as described in Bloom *et al.*, 1997, *Protein Science* 6:407, incorporated by reference herein) to alleviate a tendency to form intra-H chain disulfide bonds that can lead to heterogeneity in the IgG4 antibodies.

[00175] In one embodiment, the antigen binding protein has a K_{off} of $1x10^{-4}$ s^{-1} or lower. In another embodiment, the K_{off} is $5x10^{-5}$ s^{-1} or lower. In another embodiment, the K_{off} is substantially the same as an antibody having a combination of light chain and heavy chain variable domain sequences selected from the group of combinations consisting of L1H1, L2H2, L3H3, L4H4, L5H5, L6H6, … and L12H12. In another embodiment, the antigen binding protein binds to B7-H4 with substantially the same K_{off} as an antibody that comprises one or more CDRs from an antibody having a combination of light chain and heavy chain variable domain sequences selected from the group of combinations consisting of L1H1, L2H2, L3H3, L4H4, L5H5, L6H6, … and L12H12. In another embodiment, the antigen binding protein binds to B7-H4 with substantially the same K_{off} as an antibody that comprises one of the amino acid sequences illustrated above. In another embodiment, the antigen binding protein binds to B7-H4 with substantially the same K_{off} as an antibody that comprises one or more CDRs from an antibody that comprises one of the amino acid sequences illustrated above.

[00176] In one aspect, the present disclosure provides antigen-binding fragments of an anti-B7-H4 antibody of the present disclosure. Such fragments can consist entirely of antibody-derived sequences or can comprise additional sequences. Examples of antigen-binding fragments

include Fab, F(ab')2, single chain antibodies, diabodies, triabodies, tetrabodies, and domain antibodies. Other examples are provided in Lunde *et al.*, 2002, Biochem. Soc. Trans. 30:500-06.

[00177] Single chain antibodies (scFv) may be formed by linking heavy and light chain variable domain (Fv region) fragments via an amino acid bridge (short peptide linker, *e.g.*, a synthetic sequence of amino acid residues), resulting in a single polypeptide chain. Such single-chain Fvs (scFvs) have been prepared by fusing DNA encoding a peptide linker between DNAs encoding the two variable domain polypeptides (V_L and V_H). The resulting polypeptides can fold back on themselves to form antigen-binding monomers, or they can form multimers (*e.g.*, dimers, trimers, or tetramers), depending on the length of a flexible linker between the two variable domains (Kortt *et al.*, 1997, *Prot. Eng.* 10:423; Kortt *et al.*, 2001, *Biomol. Eng.* 18:95-108, Bird *et al.*, 1988, *Science* 242:423-26 and Huston *et al.*, 1988, *Proc. Natl. Acad. Sci.* USA 85:5879-83). By combining different V_L and V_H-comprising polypeptides, one can form multimeric scFvs that bind to different epitopes (Kriangkum *et al.*, 2001, *Biomol. Eng.* 18:31-40). Techniques developed for the production of single chain antibodies include those described in U.S. Patent No. 4,946,778; Bird, 1988, *Science* 242:423; Huston *et al.*, 1988, *Proc. Natl. Acad. Sci.* USA 85:5879; Ward *et al.*, 1989, *Nature* 334:544, de Graaf *et al.*, 2002, *Methods Mol Biol.* 178:379-87. ScFvs comprising the variable domain combinations L1H1, L2H2, L3H3, L4H4, L5H5, L6H6, …, and L12H12 are encompassed by the present disclosure.

7.7. Monoclonal antibody

[00178] In another aspect, the present disclosure provides monoclonal antibodies that bind to B7-H4. Monoclonal antibodies of the present disclosure may be generated using a variety of known techniques. In general, monoclonal antibodies that bind to specific antigens may be obtained by methods known to those skilled in the art (*see*, for example, Kohler *et al.*, *Nature* 256:495, 1975; Coligan *et al.* (eds.), *Current Protocols in Immunology*, 1:2.5.12.6.7 (John Wiley & Sons 1991); U.S. Patent Nos. RE 32,011, 4,902,614, 4,543,439, and 4,411,993; *Monoclonal Antibodies, Hybridomas: A New Dimension in Biological Analyses*, Plenum Press, Kennett, McKearn, and Bechtol (eds.) (1980); and *Antibodies: A Laboratory Manual*, Harlow and Lane (eds.), Cold Spring Harbor Laboratory Press (1988); Picksley *et al.*, "Production of monoclonal antibodies against proteins expressed in *E. coli*," in *DNA Cloning 2: Expression Systems, 2nd Edition*, Glover *et al.* (eds.), page 93 (Oxford University Press 1995)). Antibody fragments may be derived therefrom using any suitable standard technique such as proteolytic digestion, or optionally, by proteolytic digestion (for example, using papain or pepsin) followed by mild reduction of disulfide bonds and alkylation. Alternatively, such fragments may also be generated by recombinant genetic engineering techniques as described herein.

[00179] Monoclonal antibodies can be obtained by injecting an animal, for example, a rat, hamster, a rabbit, or preferably a mouse, including for example a transgenic or a knock-out, as known in the art, with an immunogen comprising human B7-H4 [sequence SEQ ID 7001] or a fragment thereof, according to methods known in the art and described herein. The presence of specific antibody production may be monitored after the initial injection and/or after a booster injection by obtaining a serum sample and detecting the presence of an antibody that binds to human B7-H4 or peptide using any one of several immunodetection methods known in the art and described herein. From animals producing the desired antibodies, lymphoid cells, most commonly cells from the spleen or lymph node, are removed to obtain B-lymphocytes. The B lymphocytes are then fused with a drug-sensitized myeloma cell fusion partner, preferably one that is syngeneic with the immunized animal and that optionally has other desirable properties (*e.g.*, inability to express endogenous Ig gene products, *e.g.*, P3X63 - Ag 8.653 (ATCC No. CRL 1580); NSO, SP20) to produce hybridomas, which are immortal eukaryotic cell lines.

[00180] The lymphoid (*e.g.*, spleen) cells and the myeloma cells may be combined for a few minutes with a membrane fusion-promoting agent, such as polyethylene glycol or a nonionic detergent, and then plated at low density on a selective medium that supports the growth of hybridoma cells but not unfused myeloma cells. A preferred selection media is HAT (hypoxanthine, aminopterin, thymidine). After a sufficient time, usually about one to two weeks, colonies of cells are observed. Single colonies are isolated, and antibodies produced by the cells may be tested for binding activity to human B7-H4, using any one of a variety of immunoassays known in the art and described herein. The hybridomas are cloned (*e.g.*, by limited dilution cloning or by soft agar plaque isolation) and positive clones that produce an antibody specific to B7-H4 are selected and cultured. The monoclonal antibodies from the hybridoma cultures may be isolated from the supernatants of hybridoma cultures.

[00181] An alternative method for production of a murine monoclonal antibody is to inject the hybridoma cells into the peritoneal cavity of a syngeneic mouse, for example, a mouse that has been treated (*e.g.*, pristane-primed) to promote formation of ascites fluid containing the monoclonal antibody. Monoclonal antibodies can be isolated and purified by a variety of well-established techniques. Such isolation techniques include affinity chromatography with Protein-A Sepharose, size-exclusion chromatography, and ion-exchange chromatography (*see*, for example, Coligan at pages 2.7.1-2.7.12 and pages 2.9.1-2.9.3; Baines *et al.*, "Purification of Immunoglobulin G (IgG)," in *Methods in Molecular Biology, Vol. 10*, pages 79-104 (The Humana Press, Inc. 1992)). Monoclonal antibodies may be purified by affinity chromatography using an appropriate ligand selected based on particular properties of the antibody (*e.g.*, heavy or

light chain isotype, binding specificity, etc.). Examples of a suitable ligand, immobilized on a solid support, include Protein A, Protein G, an anticonstant region (light chain or heavy chain) antibody, an anti-idiotype antibody, and a TGF-beta binding protein, or fragment or variant thereof.

[00182] Monoclonal antibodies may be produced using any technique known in the art, *e.g.*, by immortalizing spleen cells harvested from the transgenic animal after completion of the immunization schedule. The spleen cells can be immortalized using any technique known in the art, *e.g.*, by fusing them with myeloma cells to produce hybridomas. Hybridoma cell lines are identified that produce an antibody that binds a B7-H4 polypeptide. Such hybridoma cell lines, and anti-B7-H4 monoclonal antibodies produced by them, are encompassed by the present disclosure. Myeloma cells for use in hybridoma-producing fusion procedures preferably are non-antibody-producing, have high fusion efficiency, and enzyme deficiencies that render them incapable of growing in certain selective media which support the growth of only the desired fused cells (hybridomas). Examples of suitable cell lines for use in mouse fusions include Sp-20, P3-X63/Ag8, P3-X63-Ag8.653, NS1/1.Ag 4 1, Sp210-Ag14, FO, NSO/U, MPC-11, MPC11-X45-GTG 1.7 and S194/5XX0 Bul; examples of cell lines used in rat fusions include R210.RCY3, Y3-Ag 1.2.3, IR983F and 4B210. Other cell lines useful for cell fusions are U-266, GM1500-GRG2, LICR-LON-HMy2 and UC729-6. Hybridomas or mAbs may be further screened to identify mAbs with particular properties, such as the ability to block a B7-H4-induced activity.

[00183] An antibody of the present disclosure may also be a fully human monoclonal antibody. An isolated fully human antibody is provided that specifically binds to the B7-H4, wherein the antigen binding protein possesses at least one *in vivo* biological activity of a human anti-B7-H4 antibody.

7.8. Method of generating antibodies

[00184] Fully human monoclonal antibodies may be generated by any number of techniques with which those having ordinary skill in the art will be familiar. Such methods include, but are not limited to, Epstein Barr Virus (EBV) transformation of human peripheral blood cells (*e.g.*, containing B lymphocytes), *in vitro* immunization of human B-cells, fusion of spleen cells from immunized transgenic mice carrying inserted human immunoglobulin genes, isolation from human immunoglobulin V region phage libraries, or other procedures as known in the art and based on the disclosure herein. For example, fully human monoclonal antibodies may be obtained from transgenic mice that have been engineered to produce specific human antibodies in response to antigenic challenge. Methods for obtaining fully human antibodies from transgenic mice are described, for example, by Green *et al.*, *Nature Genet.* 7:13, 1994;

Lonberg *et al.*, *Nature* 368:856, 1994; Taylor *et al.*, *Int. Immun.* 6:579, 1994; U.S. Patent No. 5,877,397; Bruggemann *et al.*, 1997 *Curr. Opin. Biotechnol.* 8:455-58; Jakobovits *et al.*, 1995 *Ann. N. Y. Acad. Sci.* 764:525-35. In this technique, elements of the human heavy and light chain locus are introduced into strains of mice derived from embryonic stem cell lines that contain targeted disruptions of the endogenous heavy chain and light chain loci (*see also* Bruggemann *et al.*, *Curr. Opin. Biotechnol.* 8:455-58 (1997)). For example, human immunoglobulin transgenes may be mini-gene constructs, or transloci on yeast artificial chromosomes, which undergo B-cell-specific DNA rearrangement and hypermutation in the mouse lymphoid tissue. Fully human monoclonal antibodies may be obtained by immunizing the transgenic mice, which may then produce human antibodies specific for B7-H4. Lymphoid cells of the immunized transgenic mice can be used to produce human antibody-secreting hybridomas according to the methods described herein. Polyclonal sera containing fully human antibodies may also be obtained from the blood of the immunized animals.

[00185] Another method for generating human antibodies of the present disclosure includes immortalizing human peripheral blood cells by EBV transformation. *See, e.g.*, U.S. Patent No. 4,464,456. Such an immortalized B-cell line (or lymphoblastoid cell line) producing a monoclonal antibody that specifically binds to B7-H4 can be identified by immunodetection methods as provided herein, for example, an ELISA, and then isolated by standard cloning techniques. The stability of the lymphoblastoid cell line producing an anti-B7-H4 antibody may be improved by fusing the transformed cell line with a murine myeloma to produce a mouse-human hybrid cell line according to methods known in the art (*see, e.g.*, Glasky *et al.*, *Hybridoma* 8:377-89 (1989)). Still another method to generate human monoclonal antibodies is *in vitro* immunization, which includes priming human splenic B-cells with human B7-H4, followed by fusion of primed B-cells with a heterohybrid fusion partner. *See, e.g.*, Boerner *et al.*, 1991 *J. Immunol.* 147:86-95.

[00186] In certain embodiments, a B-cell that is producing an anti-human B7-H4 antibody is selected and the light chain and heavy chain variable regions are cloned from the B-cell according to molecular biology techniques known in the art (WO 92/02551; U.S. Patent 5,627,052; Babcook *et al.*, *Proc. Natl. Acad. Sci. USA* 93:7843-48 (1996)) and described herein. B-cells from an immunized animal may be isolated from the spleen, lymph node, or peripheral blood sample by selecting a cell that is producing an antibody that specifically binds to B7-H4. B-cells may also be isolated from humans, for example, from a peripheral blood sample.

[00187] Methods for detecting single B-cells that are producing an antibody with the desired specificity are well known in the art, for example, by plaque formation,

fluorescence-activated cell sorting, *in vitro* stimulation followed by detection of specific antibody, and the like. Methods for selection of specific antibody-producing B-cells include, for example, preparing a single cell suspension of B-cells in soft agar that contains human B7-H4. Binding of the specific antibody produced by the B-cell to the antigen results in the formation of a complex, which may be visible as an immunoprecipitate.

[00188] In some embodiments, specific antibody-producing B-cells are selected by using a method that allows identification natively paired antibodies. For example, a method described in Adler *et al.,* A natively paired antibody library yields drug leads with higher sensitivity and specificity than a randomly paired antibody library, MAbs (2018), which is incorporated by reference in its entirety herein, can be employed. The method combines microfluidic technology, molecular genomics, yeast single-chain variable fragment (scFv) display, fluorescence-activated cell sorting (FACS) and deep sequencing as summarized in FIG. 1 adopted from Adler *et al.* In short, B cells can be isolated from immunized animals and then pooled. The B cells are encapsulated into droplets with oligo-dT beads and a lysis solution, and mRNA-bound beads are purified from the droplets, and then injected into a second emulsion with an OE-RT-PCR amplification mix that generates DNA amplicons that encode scFv with native pairing of heavy and light chain Ig. Libraries of natively paired amplicons are then electroporated into yeast for scFv display. FACS is used to identify high affinity scFv. Finally, deep antibody sequencing can be used to identify all clones in the pre- and post-sort scFv libraries.

[00189] After the B-cells producing the desired antibody are selected, the specific antibody genes may be cloned by isolating and amplifying DNA or mRNA according to methods known in the art and described herein.

[00190] The methods for obtaining antibodies of the present disclosure can also adopt various phage display technologies known in the art. *See, e.g.*, Winter *et al.*, 1994 *Annu. Rev. Immunol.* 12:433-55; Burton *et al.*, 1994 *Adv. Immunol.* 57:191-280. Human or murine immunoglobulin variable region gene combinatorial libraries may be created in phage vectors that can be screened to select Ig fragments (Fab, Fv, sFv, or multimers thereof) that bind specifically to B7-H4 binding protein or variant or fragment thereof. *See, e.g.*, U.S. Patent No. 5,223,409; Huse *et al.*, 1989 *Science* 246:1275-81; Sastry *et al.*, *Proc. Natl. Acad. Sci. USA* 86:5728-32 (1989); Alting-Mees *et al.*, *Strategies in Molecular Biology* 3:1-9 (1990); Kang *et al.*, 1991 *Proc. Natl. Acad. Sci. USA* 88:4363-66; Hoogenboom *et al.*, 1992 *J. Molec. Biol.* 227:381-388; Schlebusch *et al.*, 1997 *Hybridoma* 16:47-52 and references cited therein. For example, a library containing a plurality of polynucleotide sequences encoding Ig variable region fragments may be inserted into the genome of a filamentous bacteriophage, such as M13 or a

variant thereof, in frame with the sequence encoding a phage coat protein. A fusion protein may be a fusion of the coat protein with the light chain variable region domain and/or with the heavy chain variable region domain. According to certain embodiments, immunoglobulin Fab fragments may also be displayed on a phage particle (*see, e.g.*, U.S. Patent No. 5,698,426).

[00191] Antibody fragments fused to another protein, such as a minor coat protein, can be also used to enrich phage with antigen. Then, using a random combinatorial library of rearranged heavy (V_H) and light (V_L) chains from mice immune to the antigen (*e.g.* B7-H4), diverse libraries of antibody fragments are displayed on the surface of the phage. These libraries can be screened for complementary variable domains, and the domains purified by, for example, affinity column. *See* Clackson et al., Nature, V. 352 pp. 624-628 (1991).

[00192] Heavy and light chain immunoglobulin cDNA expression libraries may also be prepared in lambda phage, for example, using λImmunoZap^{TM}(H) and λImmunoZap^{TM}(L) vectors (Stratagene, La Jolla, California). Briefly, mRNA is isolated from a B-cell population, and used to create heavy and light chain immunoglobulin cDNA expression libraries in the λImmunoZap(H) and λImmunoZap(L) vectors. These vectors may be screened individually or co-expressed to form Fab fragments or antibodies (*see* Huse *et al.*, *supra*; *see also* Sastry *et al.*, *supra*). Positive plaques may subsequently be converted to a non-lytic plasmid that allows high level expression of monoclonal antibody fragments from *E. coli*.

[00193] In one embodiment, in a hybridoma the variable regions of a gene expressing a monoclonal antibody of interest are amplified using nucleotide primers. These primers may be synthesized by one of ordinary skill in the art, or may be purchased from commercially available sources. (*See*, *e.g.*, Stratagene (La Jolla, California), which sells primers for mouse and human variable regions including, among others, primers for V_{Ha}, V_{Hb}, V_{Hc}, V_{Hd}, C_{H1}, V_L and C_L regions.) These primers may be used to amplify heavy or light chain variable regions, which may then be inserted into vectors such as ImmunoZAP^{TM}H or ImmunoZAP^{TM}L (Stratagene), respectively. These vectors may then be introduced into *E. coli*, yeast, or mammalian-based systems for expression. Large amounts of a single-chain protein containing a fusion of the V_H and V_L domains may be produced using these methods (*see* Bird *et al.*, *Science* 242:423-426, 1988).

[00194] Once cells producing antibodies according to the present disclosure have been obtained using any of the above-described immunization and other techniques, the specific antibody genes may be cloned by isolating and amplifying DNA or mRNA therefrom according to standard procedures as described herein. The antibodies produced therefrom may be

sequenced and the CDRs identified and the DNA coding for the CDRs may be manipulated as described previously to generate other antibodies according to the present disclosure.

[00195] B7-H4 binding agents of the present disclosure preferably modulate B7-H4 function in the cell-based assay described herein and/or the *in vivo* assay described herein and/or bind to one or more of the domains described herein and/or cross-block the binding of one of the antibodies described in this application and/or are cross-blocked from binding B7-H4 by one of the antibodies described in this application. Accordingly such binding agents can be identified using the assays described herein.

[00196] In certain embodiments, antibodies are generated by first identifying antibodies that bind to one or more of the domains provided herein and/or neutralize in the cell-based and/or *in vivo* assays described herein and/or cross-block the antibodies described in this application and/or are cross-blocked from binding B7-H4 by one of the antibodies described in this application. The CDR regions from these antibodies are then used to insert into appropriate biocompatible frameworks to generate B7-H4 binding agents**.** The non-CDR portion of the binding agent may be composed of amino acids, or may be a non-protein molecule. The assays described herein allow the characterization of binding agents. Preferably the binding agents of the present disclosure are antibodies as defined herein.

[00197] Other antibodies according to the present disclosure may be obtained by conventional immunization and cell fusion procedures as described herein and known in the art.

[00198] Molecular evolution of the complementarity determining regions (CDRs) in the center of the antibody binding site also has been used to isolate antibodies with increased affinity, for example, antibodies having increased affinity for c-erbB-2, as described by Schier *et al.*, 1996, *J. Mol. Biol.* 263:551. Accordingly, such techniques are useful in preparing antibodies to B7-H4. Antigen binding proteins directed against a B7-H4 can be used, for example, in assays to detect the presence of B7-H4 polypeptides, either *in vitro* or *in vivo*. The antigen binding proteins also may be employed in purifying B7-H4 proteins by immunoaffinity chromatography.

[00199] Although human, partially human, or humanized antibodies will be suitable for many applications, particularly those involving administration of the antibody to a human subject, other types of antigen binding proteins will be suitable for certain applications. Non-human antibodies can be derived from any antibody-producing animal, such as mouse, rat, rabbit, goat, donkey, or non-human primate (such as monkey (*e.g.*, cynomolgus or rhesus monkey) or ape (*e.g.*, chimpanzee)). An antibody from a particular species can be made by, for example, immunizing an animal of that species with the desired immunogen (*e.g.*, a B7-H4 polypeptide) or using an artificial system for generating antibodies of that species (*e.g.*, a bacterial or phage

display-based system for generating antibodies of a particular species), or by converting an antibody from one species into an antibody from another species by replacing, *e.g.*, the constant region of the antibody with a constant region from the other species, or by replacing one or more amino acid residues of the antibody so that it more closely resembles the sequence of an antibody from the other species. In one embodiment, the antibody is a chimeric antibody comprising amino acid sequences derived from antibodies from two or more different species.

[00200] Antigen binding proteins may be prepared, and screened for desired properties, by any of a number of conventional techniques. Certain of the techniques involve isolating a nucleic acid encoding a polypeptide chain (or portion thereof) of an antigen binding protein of interest (*e.g.*, an anti- B7-H4 antibody), and manipulating the nucleic acid through recombinant DNA technology. The nucleic acid may be fused to another nucleic acid of interest, or altered (*e.g.*, by mutagenesis or other conventional techniques) to add, delete, or substitute one or more amino acid residues, for example. Furthermore, the antigen binding proteins may be purified from cells that naturally express them (*e.g.*, an antibody can be purified from a hybridoma that produces it), or produced in recombinant expression systems, using any technique known in the art. *See*, for example, *Monoclonal Antibodies, Hybridomas: A New Dimension in Biological Analyses*, Kennet *et al.* (eds.), Plenum Press, New York (1980); and *Antibodies: A Laboratory Manual*, Harlow and Land (eds.), Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, (1988).

[00201] Any expression system known in the art can be used to make the recombinant polypeptides of the present disclosure. Expression systems are detailed comprehensively above. In general, host cells are transformed with a recombinant expression vector that comprises DNA encoding a desired polypeptide. Among the host cells that may be employed are prokaryotes, yeast or higher eukaryotic cells. Prokaryotes include gram negative or gram positive organisms, for example *E. coli* or *Bacilli*. Higher eukaryotic cells include insect cells and established cell lines of mammalian origin. Examples of suitable mammalian host cell lines include the COS-7 line of monkey kidney cells (ATCC CRL 1651) (Gluzman *et al.*, 1981, Cell 23:175), L cells, 293 cells, C127 cells, 3T3 cells (ATCC CCL 163), Chinese hamster ovary (CHO) cells, HeLa cells, BHK (ATCC CRL 10) cell lines, and the CVI/EBNA cell line derived from the African green monkey kidney cell line CVI (ATCC CCL 70) as described by McMahan *et al.*, 1991, EMBO J. 10: 2821. Appropriate cloning and expression vectors for use with bacterial, fungal, yeast, and mammalian cellular hosts are described by Pouwels *et al.* (*Cloning Vectors: A Laboratory Manual*, Elsevier, New York, 1985).

[00202] It will be appreciated that an antibody of the present disclosure may have at least one amino acid substitution, providing that the antibody retains binding specificity. Therefore,

modifications to the antibody structures are encompassed within the scope of the present disclosure. These may include amino acid substitutions, which may be conservative or non-conservative that do not destroy the B7-H4 binding capability of an antibody. Conservative amino acid substitutions may encompass non-naturally occurring amino acid residues, which are typically incorporated by chemical peptide synthesis rather than by synthesis in biological systems. These include peptidomimetics and other reversed or inverted forms of amino acid moieties. A conservative amino acid substitution may also involve a substitution of a native amino acid residue with a normative residue such that there is little or no effect on the polarity or charge of the amino acid residue at that position.

[00203] Non-conservative substitutions may involve the exchange of a member of one class of amino acids or amino acid mimetics for a member from another class with different physical properties (*e.g.* size, polarity, hydrophobicity, charge). Such substituted residues may be introduced into regions of the human antibody that are homologous with non-human antibodies, or into the non-homologous regions of the molecule.

[00204] Moreover, one skilled in the art may generate test variants containing a single amino acid substitution at each desired amino acid residue. The variants can then be screened using activity assays known to those skilled in the art. Such variants could be used to gather information about suitable variants. For example, if one discovered that a change to a particular amino acid residue resulted in destroyed, undesirably reduced, or unsuitable activity, variants with such a change may be avoided. In other words, based on information gathered from such routine experiments, one skilled in the art can readily determine the amino acids where further substitutions should be avoided either alone or in combination with other mutations.

[00205] A skilled artisan will be able to determine suitable variants of the polypeptide as set forth herein using well-known techniques. In certain embodiments, one skilled in the art may identify suitable areas of the molecule that may be changed without destroying activity by targeting regions not believed to be important for activity. In certain embodiments, one can identify residues and portions of the molecules that are conserved among similar polypeptides. In certain embodiments, even areas that may be important for biological activity or for structure may be subject to conservative amino acid substitutions without destroying the biological activity or without adversely affecting the polypeptide structure.

[00206] Additionally, one skilled in the art can review structure-function studies identifying residues in similar polypeptides that are important for activity or structure. In view of such a comparison, one can predict the importance of amino acid residues in a protein that correspond to amino acid residues which are important for activity or structure in similar

proteins. One skilled in the art may opt for chemically similar amino acid substitutions for such predicted important amino acid residues.

[00207] One skilled in the art can also analyze the three-dimensional structure and amino acid sequence in relation to that structure in similar polypeptides. In view of such information, one skilled in the art may predict the alignment of amino acid residues of an antibody with respect to its three dimensional structure. In certain embodiments, one skilled in the art may choose not to make radical changes to amino acid residues predicted to be on the surface of the protein, since such residues may be involved in important interactions with other molecules.

[00208] A number of scientific publications have been devoted to the prediction of secondary structure. *See* Moult *J., Curr. Op. in Biotech.*, 7(4):422-427 (1996), Chou *et al.*, *Biochem.*, 13(2):222-245 (1974); Chou *et al.*, *Biochem.,* 113(2):211-222 (1974); Chou *et al.*, *Adv. Enzymol. Relat. Areas Mol. Biol.*, 47:45-148 (1978); Chou *et al.*, *Ann. Rev. Biochem.*, 47:251-276 and Chou *et al.*, *Biophys. J.*, 26:367-384 (1979). Moreover, computer programs are currently available to assist with predicting secondary structure. One method of predicting secondary structure is based upon homology modeling. For example, two polypeptides or proteins which have a sequence identity of greater than 30%, or similarity greater than 40% often have similar structural topologies. The recent growth of the protein structural database (PDB) has provided enhanced predictability of secondary structure, including the potential number of folds within a polypeptide's or protein's structure. *See* Holm *et al.*, *Nucl. Acid. Res.*, 27(1):244-247 (1999). It has been suggested (Brenner *et al.*, *Curr. Op. Struct. Biol.*, 7(3):369-376 (1997)) that there are a limited number of folds in a given polypeptide or protein and that once a critical number of structures have been resolved, structural prediction will become dramatically more accurate.

[00209] Additional methods of predicting secondary structure include "threading" (Jones, D., *Curr. Opin. Struct. Biol.*, 7(3):377-87 (1997); Sippl *et al.*, *Structure,* 4(1):15-19 (1996)), "profile analysis" (Bowie *et al.*, *Science,* 253:164-170 (1991); Gribskov *et al.*, *Meth. Enzym.*, 183:146-159 (1990); Gribskov *et al.*, *Proc. Nat. Acad. Sci.*, 84(13):4355-4358 (1987)), and "evolutionary linkage" (*See* Holm, supra (1999), and Brenner, supra (1997)).

[00210] In certain embodiments, variants of antibodies include glycosylation variants wherein the number and/or type of glycosylation site has been altered compared to the amino acid sequences of a parent polypeptide. In certain embodiments, variants comprise a greater or a lesser number of N-linked glycosylation sites than the native protein. An N-linked glycosylation site is characterized by the sequence: Asn-X-Ser or Asn-X-Thr, wherein the amino acid residue designated as X can be any amino acid residue except proline. The substitution of amino acid residues to create this sequence provides a potential new site for the addition of an N-linked

carbohydrate chain. Alternatively, substitutions which eliminate this sequence will remove an existing N-linked carbohydrate chain. Also provided is a rearrangement of N-linked carbohydrate chains wherein one or more N-linked glycosylation sites (typically those that are naturally occurring) are eliminated and one or more new N-linked sites are created. Additional preferred antibody variants include cysteine variants wherein one or more cysteine residues are deleted from or substituted for another amino acid (*e.g.*, serine) as compared to the parent amino acid sequence. Cysteine variants can be useful when antibodies must be refolded into a biologically active conformation such as after the isolation of insoluble inclusion bodies. Cysteine variants generally have fewer cysteine residues than the native protein, and typically have an even number to minimize interactions resulting from unpaired cysteines.

[00211] Desired amino acid substitutions (whether conservative or non-conservative) can be determined by those skilled in the art at the time such substitutions are desired. In certain embodiments, amino acid substitutions can be used to identify important residues of antibodies to B7-H4, or to increase or decrease the affinity of the antibodies to B7-H4 described herein.

[00212] According to certain embodiments, preferred amino acid substitutions are those which: (1) reduce susceptibility to proteolysis, (2) reduce susceptibility to oxidation, (3) alter binding affinity for forming protein complexes, (4) alter binding affinities, and/or (4) confer or modify other physiochemical or functional properties on such polypeptides. According to certain embodiments, single or multiple amino acid substitutions (in certain embodiments, conservative amino acid substitutions) may be made in the naturally-occurring sequence (in certain embodiments, in the portion of the polypeptide outside the domain(s) forming intermolecular contacts). In certain embodiments, a conservative amino acid substitution typically may not substantially change the structural characteristics of the parent sequence (*e.g.*, a replacement amino acid should not tend to break a helix that occurs in the parent sequence, or disrupt other types of secondary structure that characterizes the parent sequence). Examples of art-recognized polypeptide secondary and tertiary structures are described in Proteins, Structures and Molecular Principles (Creighton, Ed., W. H. Freeman and Company, New York (1984)); Introduction to Protein Structure (C. Branden and J. Tooze, eds., Garland Publishing, New York, N.Y. (1991)); and Thornton *et al. Nature* 354:105 (1991), which are each incorporated herein by reference.

[00213] In certain embodiments, antibodies of the present disclosure may be chemically bonded with polymers, lipids, or other moieties.

[00214] The binding agents may comprise at least one of the CDRs described herein incorporated into a biocompatible framework structure. In one example, the biocompatible framework structure comprises a polypeptide or portion thereof that is sufficient to form a

conformationally stable structural support, or framework, or scaffold, which is able to display one or more sequences of amino acids that bind to an antigen (*e.g.*, CDRs, a variable region, etc.) in a localized surface region. Such structures can be a naturally occurring polypeptide or polypeptide "fold" (a structural motif), or can have one or more modifications, such as additions, deletions or substitutions of amino acids, relative to a naturally occurring polypeptide or fold. These scaffolds can be derived from a polypeptide of any species (or of more than one species), such as a human, other mammal, other vertebrate, invertebrate, plant, bacteria or virus.

[00215] Typically the biocompatible framework structures are based on protein scaffolds or skeletons other than immunoglobulin domains. For example, those based on fibronectin, ankyrin, lipocalin, neocarzinostain, cytochrome b, CP1 zinc finger, PST1, coiled coil, LACI-D1, Z domain and tendamistat domains may be used (*See e.g.*, Nygren and Uhlen, 1997, *Curr. Opin. in Struct. Biol.*, 7, 463-469).

[00216] Humanized antibodies can be produced using techniques known to those skilled in the art (Zhang, W., *et al.*, *Molecular Immunology. 42(12)*:1445-1451, 2005; Hwang W. *et al.*, *Methods. 36(1)*:35-42, 2005; Dall'Acqua WF, *et al.*, *Methods 36(1)*:43-60, 2005; and Clark, M., I*mmunology Today. 21(8)*:397-402, 2000).

[00217] Additionally, one skilled in the art will recognize that suitable binding agents include portions of these antibodies, such as one or more of CDR1-L1 to 12 with SEQ ID NOS 1001-1012; CDR2-L1 to 12 with SEQ ID NOS 2001-2012; CDR3-L1 to 12 with SEQ ID NOS 3001-3012; CDR1-H1to 12 with SEQ ID NOS 4001-4012; CDR2-H1to 12 with SEQ ID NOS 5001-5012; and CDR3-H1to 12 with SEQ ID NOS 6001-6012, as specifically disclosed herein. At least one of the regions of CDR regions may have at least one amino acid substitution from the sequences provided here, provided that the antibody retains the binding specificity of the non-substituted CDR. The non-CDR portion of the antibody may be a non-protein molecule, wherein the binding agent cross-blocks the binding of an antibody disclosed herein to B7-H4 and/or neutralizes B7-H4. The non-CDR portion of the antibody may be a non-protein molecule in which the antibody exhibits a similar binding pattern to human B7-H4 peptides in a competition binding assay as that exhibited by at least one of antibodies A1-A12, and/or neutralizes B7-H4. The non-CDR portion of the antibody may be composed of amino acids, wherein the antibody is a recombinant binding protein or a synthetic peptide, and the recombinant binding protein cross-blocks the binding of an antibody disclosed herein to B7-H4 and/or neutralizes B7-H4. The non-CDR portion of the antibody may be composed of amino acids, wherein the antibody is a recombinant antibody, and the recombinant antibody exhibits a similar binding pattern to human B7-H4 peptides in the human B7-H4 peptide epitope competition binding assay (described

hereinbelow) as that exhibited by at least one of the antibodies A1-A12, and/or neutralizes B7-H4.

[00218] Where an antibody comprises one or more of CDR1-H, CDR2-H, CDR3-H, CDR1-L, CDR2-L and CDR3-L as described above, it may be obtained by expression from a host cell containing DNA coding for these sequences. A DNA coding for each CDR sequence may be determined on the basis of the amino acid sequence of the CDR and synthesized together with any desired antibody variable region framework and constant region DNA sequences using oligonucleotide synthesis techniques, site-directed mutagenesis and polymerase chain reaction (PCR) techniques as appropriate. DNA coding for variable region frameworks and constant regions is widely available to those skilled in the art from genetic sequences databases such as GenBank®.

[00219] Once synthesized, the DNA encoding an antibody of the present disclosure or fragment thereof may be propagated and expressed according to any of a variety of well-known procedures for nucleic acid excision, ligation, transformation, and transfection using any number of known expression vectors. Thus, in certain embodiments expression of an antibody fragment may be preferred in a prokaryotic host, such as *Escherichia coli* (*see, e.g.*, Pluckthun *et al.*, 1989 *Methods Enzymol.* 178:497-515). In certain other embodiments, expression of the antibody or a fragment thereof may be preferred in a eukaryotic host cell, including yeast (*e.g.*, *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, and *Pichia pastoris*), animal cells (including mammalian cells) or plant cells. Examples of suitable animal cells include, but are not limited to, myeloma (such as a mouse NSO line), COS, CHO, or hybridoma cells. Examples of plant cells include tobacco, corn, soybean, and rice cells.

[00220] One or more replicable expression vectors containing DNA encoding an antibody variable and/or constant region may be prepared and used to transform an appropriate cell line, for example, a non-producing myeloma cell line, such as a mouse NSO line or a bacteria, such as *E. coli*, in which production of the antibody will occur. In order to obtain efficient transcription and translation, the DNA sequence in each vector should include appropriate regulatory sequences, particularly a promoter and leader sequence operatively linked to the variable domain sequence. Particular methods for producing antibodies in this way are generally well-known and routinely used. For example, basic molecular biology procedures are described by Maniatis *et al.* (*Molecular Cloning, A Laboratory Manual*, 2nd ed., Cold Spring Harbor Laboratory, New York, 1989; *see also* Maniatis *et al*, 3rd ed., Cold Spring Harbor Laboratory, New York, (2001)). DNA sequencing can be performed as described in Sanger *et al.* (PNAS 74:5463, (1977)) and the Amersham International plc sequencing handbook, and site directed mutagenesis can be carried

out according to methods known in the art (Kramer *et al., Nucleic Acids Res.* 12:9441, (1984); Kunkel *Proc. Natl. Acad. Sci. USA* 82:488-92 (1985); Kunkel *et al., Methods in Enzymol.* 154:367-82 (1987); the Anglian Biotechnology Ltd. handbook). Additionally, numerous publications describe techniques suitable for the preparation of antibodies by manipulation of DNA, creation of expression vectors, and transformation and culture of appropriate cells (Mountain A and Adair, J R in *Biotechnology and Genetic Engineering Reviews* (ed. Tombs, M P, 10, Chapter 1, 1992, Intercept, Andover, UK); "Current Protocols in Molecular Biology", 1999, F.M. Ausubel (ed.), Wiley Interscience, New York).

[00221] Where it is desired to improve the affinity of antibodies according to the present disclosure containing one or more of the above-mentioned CDRs can be obtained by a number of affinity maturation protocols including maintaining the CDRs (Yang *et al., J. Mol. Biol.*, 254, 392-403, 1995), chain shuffling (Marks *et al., Bio/Technology*, 10, 779-783, 1992), use of mutation strains of *E. coli.* (Low *et al., J. Mol. Biol.*, 250, 350-368, 1996), DNA shuffling (Patten *et al., Curr. Opin. Biotechnol.*, 8, 724-733, 1997), phage display (Thompson *et al., J. Mol. Biol.*, 256, 7-88, 1996) and sexual PCR (Crameri, *et al., Nature*, 391, 288-291, 1998). All of these methods of affinity maturation are discussed by Vaughan *et al.* (*Nature Biotech.*, 16, 535-539, 1998).

[00222] It will be understood by one skilled in the art that some proteins, such as antibodies, may undergo a variety of posttranslational modifications. The type and extent of these modifications often depends on the host cell line used to express the protein as well as the culture conditions. Such modifications may include variations in glycosylation, methionine oxidation, diketopiperizine formation, aspartate isomerization and asparagine deamidation. A frequent modification is the loss of a carboxy-terminal basic residue (such as lysine or arginine) due to the action of carboxypeptidases (as described in Harris, R.J. *Journal of Chromatography* 705:129-134, 1995).

7.9. Sequences

[00223] Antibodies A1-A12 comprise heavy and light chain V(J)D polynucleotides (also referred to herein as L1-L12 and H1-H12, respectively). Antibodies A1-A12 comprise the sequences listed in TABLES 5 and 7. For example, antibody A1 comprises light chain L1 (SEQ ID NO:1) and heavy chain H1 (SEQ ID NO:101). CDR sequences in the light chain (L1-L12) and heavy chain (H1-H12) are also provided with a specific SEQ ID NOs. For example, three CDR sequences (CDR1, CDR 2 and CDR3) for L1 are CDR1-L1 (SEQ ID NO:1001), CDR2-L1 (SEQ ID NO:2001) and CDR3-L1 (SEQ ID NO:3001), respectively and three CDR sequences (CDR1, CDR 2 and CDR3) for H1 are CDR1-H1 (SEQ ID NO:4001), CDR2-H1 (SEQ ID NO:5001) and CDR3-H1 (SEQ ID NO:6001).

TABLE 5		
Antibodies	**Light Chain**	**Heavy Chain**
A1	L1 (SEQ ID NO:1) L1 comprises CDR1-L1 (SEQ ID NO:1001), CDR2-L1 (SEQ ID NO:2001) and CDR3-L1 (SEQ ID NO:3001)	H1 (SEQ ID NO: 101) H1 comprises CDR1-H1 (SEQ ID NO: 4001), CDR2-H1 (SEQ ID NO: 5001) and CDR3-H1 (SEQ ID NO: 6001)
A2	L2 (SEQ ID NO:2) L2 comprises CDR1-L2 (SEQ ID NO:1002), CDR2-L2 (SEQ ID NO:2002) and CDR3-L2 (SEQ ID NO:3002)	H2 (SEQ ID NO: 102) He comprises CDR1-H2 (SEQ ID NO: 4002), CDR2-H2 (SEQ ID NO: 5002) and CDR3-H2 (SEQ ID NO: 6002)
A3	L3 (SEQ ID NO:3) L3 comprises CDR1-L3 (SEQ ID NO:1003), CDR2-L3 (SEQ ID NO:2003) and CDR3-L3 (SEQ ID NO:3003)	H3 (SEQ ID NO: 103) H3 comprises CDR1-H3 (SEQ ID NO:4003), CDR2-H3 (SEQ ID NO:5003) and CDR3-H3 (SEQ ID NO:6003)
A4	L4 (SEQ ID NO:4) L4 comprises CDR1-L4 (SEQ ID NO:1004), CDR2-L4 (SEQ ID NO:2004) and CDR3-L4 (SEQ ID NO:3004)	H4 (SEQ ID NO:104) H4 comprises CDR1-H4 (SEQ ID NO:4004), CDR2-H4 (SEQ ID NO:5004) and CDR3-H4 (SEQ ID NO:6004)
A5	L5 (SEQ ID NO:5) L5 comprises CDR1-L5 (SEQ ID NO:1005), CDR2-L5 (SEQ ID NO:2005) and CDR3-L5 (SEQ ID NO:3005)	H5 (SEQ ID NO:105) H4 comprises CDR1-H5 (SEQ ID NO:4005), CDR2-H5 (SEQ ID NO:5005) and CDR3-H5 (SEQ ID NO:6005)
A6	L6 (SEQ ID NO:6) L6 comprises CDR1-L6 (SEQ ID NO:1006), CDR2-L6 (SEQ ID NO:2006) and CDR3-L6 (SEQ ID NO:3006)	H6 (SEQ ID NO:106) H6 comprises CDR1-H6 (SEQ ID NO:4006), CDR2-H6 (SEQ ID NO:5006) and CDR3-H6 (SEQ ID NO:6006)
A7	L7 (SEQ ID NO:7) L7 comprises CDR1-L7 (SEQ ID NO:1007), CDR2-L7 (SEQ ID NO:2007) and CDR3-L7 (SEQ ID NO:3007)	H7 (SEQ ID NO:107) H7 comprises CDR1-H7 (SEQ ID NO:4007), CDR2-H7 (SEQ ID NO:5007) and CDR3-H7 (SEQ ID NO:6007)
A8	L8 (SEQ ID NO:8) L8 comprises CDR1-L8 (SEQ ID NO:1008), CDR2-L8 (SEQ ID NO:2008) and CDR3-L6 (SEQ ID NO:3008)	H8 (SEQ ID NO:108) H8 comprises CDR1-H8 (SEQ ID NO:4008), CDR2-H8 (SEQ ID NO:5008) and CDR3-H8 (SEQ ID NO:6008)
A9	L9 (SEQ ID NO:9) L9 comprises CDR1-L9 (SEQ ID NO:1009), CDR2-L9 (SEQ ID NO:2009) and CDR3-L9 (SEQ ID NO:3009)	H9 (SEQ ID NO:109) H9 comprises CDR1-H9 (SEQ ID NO:4009), CDR2-H9 (SEQ ID NO:5009) and CDR3-H9 (SEQ ID NO:6009)
A10	L10 (SEQ ID NO:10) L10 comprises CDR1-L10 (SEQ ID NO:1010), CDR2-L10 (SEQ ID NO:2010) and CDR3-L10 (SEQ ID NO:3010)	H10 (SEQ ID NO:110) H10 comprises CDR1-H10 (SEQ ID NO:4010), CDR2-H10 (SEQ ID NO:5010) and CDR3-H10 (SEQ ID NO:6010)
A11	L11 (SEQ ID NO:11) L11 comprises CDR1-L11 (SEQ ID NO:1011), CDR2-L11 (SEQ ID NO:2011) and CDR3-L11 (SEQ ID NO:3011)	H11 (SEQ ID NO:11) H11 comprises CDR1-H11 (SEQ ID NO:4011), CDR2-H11 (SEQ ID NO:5011) and CDR3-H11 (SEQ ID NO:6011)
A12	L12 (SEQ ID NO:12) L12 comprises CDR1-L12 (SEQ ID NO:1012), CDR2-L12 (SEQ ID NO:2012) and CDR3-L12 (SEQ ID NO:3012)	H12 (SEQ ID NO:122) H12 comprises CDR1-H12 (SEQ ID NO:4012), CDR2-H12 (SEQ ID NO:5012) and CDR3-H12 (SEQ ID NO:6012)

7.10. Pharmaceutical compositions

[00224] Pharmaceutical compositions containing the proteins and polypeptides of the present disclosure are also provided. Such compositions comprise a therapeutically or prophylactically effective amount of the polypeptide or protein in a mixture with pharmaceutically acceptable materials, and physiologically acceptable formulation materials.

[00225] The pharmaceutical composition may contain formulation materials for modifying, maintaining or preserving, for example, the pH, osmolarity, viscosity, clarity, color, isotonicity, odor, sterility, stability, rate of dissolution or release, adsorption or penetration of the composition.

[00226] Suitable formulation materials include, but are not limited to, amino acids (such as glycine, glutamine, asparagine, arginine or lysine); antimicrobials; antioxidants (such as ascorbic acid, sodium sulfite or sodium hydrogen-sulfite); buffers (such as borate, bicarbonate, Tris-HCl, citrates, phosphates, other organic acids); bulking agents (such as mannitol or glycine), chelating agents (such as ethylenediamine tetraacetic acid (EDTA)); complexing agents (such as caffeine, polyvinylpyrrolidone, beta-cyclodextrin or hydroxypropyl-beta-cyclodextrin); fillers; monosaccharides; disaccharides and other carbohydrates (such as glucose, mannose, or dextrins); proteins (such as serum albumin, gelatin or immunoglobulins); coloring; flavoring and diluting agents; emulsifying agents; hydrophilic polymers (such as polyvinylpyrrolidone); low molecular weight polypeptides; salt-forming counterions (such as sodium); preservatives (such as benzalkonium chloride, benzoic acid, salicylic acid, thimerosal, phenethyl alcohol, methylparaben, propylparaben, chlorhexidine, sorbic acid or hydrogen peroxide); solvents (such as glycerin, propylene glycol or polyethylene glycol); sugar alcohols (such as mannitol or sorbitol); suspending agents; surfactants or wetting agents (such as pluronics, PEG, sorbitan esters, polysorbates such as polysorbate 20, polysorbate 80, triton, tromethamine, lecithin, cholesterol, tyloxapal); stability enhancing agents (sucrose or sorbitol); tonicity enhancing agents (such as alkali metal halides (preferably sodium or potassium chloride, mannitol sorbitol); delivery vehicles; diluents; excipients and/or pharmaceutical adjuvants. Neutral buffered saline or saline mixed with conspecific serum albumin are examples of appropriate diluents. In accordance with appropriate industry standards, preservatives such as benzyl alcohol may also be added. The composition may be formulated as a lyophilizate using appropriate excipient solutions (*e.g.*, sucrose) as diluents. Suitable components are nontoxic to recipients at the dosages and concentrations employed. Further examples of components that may be employed in pharmaceutical formulations are presented in Remington's Pharmaceutical Sciences, 16th Ed. (1980) and 20th Ed. (2000), Mack Publishing Company, Easton, PA.

[00227] Optionally, the composition additionally comprises one or more physiologically active agents, for example, an anti-angiogenic substance, a chemotherapeutic substance (such as capecitabine, 5-fluorouracil, or doxorubicin), an analgesic substance, *etc.*, non-exclusive examples of which are provided herein. In various particular embodiments, the composition

comprises one, two, three, four, five, or six physiologically active agents in addition to a B7-H4-binding protein.

[00228] In another embodiment of the present disclosure, the compositions disclosed herein may be formulated in a neutral or salt form. Illustrative pharmaceutically-acceptable salts include the acid addition salts (formed with the free amino groups of the protein) and which are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids as acetic, oxalic, tartaric, mandelic, and the like. Salts formed with the free carboxyl groups can also be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, histidine, procaine and the like. Upon formulation, solutions will be administered in a manner compatible with the dosage formulation and in such amount as is therapeutically effective.

[00229] The carriers can further comprise any and all solvents, dispersion media, vehicles, coatings, diluents, antibacterial and antifungal agents, isotonic and absorption delaying agents, buffers, carrier solutions, suspensions, colloids, and the like. The use of such media and agents for pharmaceutical active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active ingredient, its use in the therapeutic compositions is contemplated. Supplementary active ingredients can also be incorporated into the compositions. The phrase "pharmaceutically-acceptable" refers to molecular entities and compositions that do not produce an allergic or similar untoward reaction when administered to a human.

[00230] The optimal pharmaceutical composition will be determined by one skilled in the art depending upon, for example, the intended route of administration, delivery format, and desired dosage. *See* for example, Remington's Pharmaceutical Sciences, *supra.* Such compositions may influence the physical state, stability, rate of *in vivo* release, and rate of *in vivo* clearance of the polypeptide. For example, suitable compositions may be water for injection, physiological saline solution for parenteral administration.

7.10.1. Content of pharmaceutically active ingredient

[00231] In typical embodiments, the active ingredient (*i.e.,* the proteins and polypeptides of the present disclosure) is present in the pharmaceutical composition at a concentration of at least 0.01mg/ml, at least 0.1mg/ml, at least 0.5mg/ml, or at least 1mg/ml. In certain embodiments, the active ingredient is present in the pharmaceutical composition at a concentration of at least 1 mg/ml, 2 mg/ml, 3 mg/ml, 4 mg/ml, 5 mg/ml, 10 mg/ml, 15 mg/ml, 20 mg/ml, or 25 mg/ml. In certain embodiments, the active ingredient is present in the

pharmaceutical composition at a concentration of at least 30 mg/ml, 35 mg/ml, 40 mg/ml, 45 mg/ml or 50 mg/ml.

[00232] In some embodiments, the pharmaceutical composition comprises one or more additional active ingredients in addition to the proteins or polypeptides of the present disclosure. The one or more additional active ingredients can be a drug targeting a different check point receptor, such as PD-1 inhibitor (*e.g.*, anti-PD-1 antibody) or TIGIT inhibitor (*e.g.*, anti-TIGIT antibody).

7.10.2. Formulation Generally

[00233] The pharmaceutical composition can be in any form appropriate for human or veterinary medicine, including a liquid, an oil, an emulsion, a gel, a colloid, an aerosol or a solid.

[00234] The pharmaceutical composition can be formulated for administration by any route of administration appropriate for human or veterinary medicine, including enteral and parenteral routes of administration.

[00235] In various embodiments, the pharmaceutical composition is formulated for administration by inhalation. In certain of these embodiments, the pharmaceutical composition is formulated for administration by a vaporizer. In certain of these embodiments, the pharmaceutical composition is formulated for administration by a nebulizer. In certain of these embodiments, the pharmaceutical composition is formulated for administration by an aerosolizer.

[00236] In various embodiments, the pharmaceutical composition is formulated for oral administration, for buccal administration, or for sublingual administration.

[00237] In some embodiments, the pharmaceutical composition is formulated for intravenous, intramuscular, or subcutaneous administration.

[00238] In some embodiments, the pharmaceutical composition is formulated for intrathecal or intracerebroventricular administration.

[00239] In some embodiments, the pharmaceutical composition is formulated for topical administration.

7.10.3. Pharmacological compositions adapted for injection

[00240] For intravenous, cutaneous or subcutaneous injection, or injection at the site of affliction, the active ingredient will be in the form of a parenterally acceptable aqueous solution which is pyrogen-free and has suitable pH, isotonicity and stability. Those of relevant skill in the art are well able to prepare suitable solutions using, for example, isotonic vehicles such as Sodium Chloride Injection, Ringer's Injection, Lactated Ringer's Injection. Preservatives, stabilisers, buffers, antioxidants and/or other additives can be included, as required.

[00241] In various embodiments, the unit dosage form is a vial, ampule, bottle, or pre-filled syringe. In some embodiments, the unit dosage form contains 0.01 mg, 0.1 mg, 0.5 mg, 1

mg, 2.5 mg, 5 mg, 10 mg, 12.5 mg, 25 mg, 50 mg, 75 mg, or 100 mg of the pharmaceutical composition. In some embodiments, the unit dosage form contains 125 mg, 150 mg, 175 mg, or 200 mg of the pharmaceutical composition. In some embodiments, the unit dosage form contains 250 mg of the pharmaceutical composition.

[00242] In typical embodiments, the pharmaceutical composition in the unit dosage form is in liquid form. In various embodiments, the unit dosage form contains between 0.1 mL and 50 ml of the pharmaceutical composition. In some embodiments, the unit dosage form contains 1 ml, 2.5 ml, 5 ml, 7.5 ml, 10 ml, 25 ml, or 50 ml of pharmaceutical composition.

[00243] In particular embodiments, the unit dosage form is a vial containing 1 ml of the pharmaceutical composition at a concentration of 0.01 mg/ml, 0.1 mg/ml, 0.5 mg/ml, or 1mg/ml. In some embodiments, the unit dosage form is a vial containing 2 ml of the pharmaceutical composition at a concentration of 0.01 mg/ml, 0.1 mg/ml, 0.5 mg/ml, or 1mg/ml.

[00244] In some embodiments, the pharmaceutical composition in the unit dosage form is in solid form, such as a lyophilate, suitable for solubilization.

[00245] Unit dosage form embodiments suitable for subcutaneous, intradermal, or intramuscular administration include preloaded syringes, auto-injectors, and autoinject pens, each containing a predetermined amount of the pharmaceutical composition described hereinabove.

[00246] In various embodiments, the unit dosage form is a preloaded syringe, comprising a syringe and a predetermined amount of the pharmaceutical composition. In certain preloaded syringe embodiments, the syringe is adapted for subcutaneous administration. In certain embodiments, the syringe is suitable for self-administration. In particular embodiments, the preloaded syringe is a single use syringe.

[00247] In various embodiments, the preloaded syringe contains about 0.1 mL to about 0.5 mL of the pharmaceutical composition. In certain embodiments, the syringe contains about 0.5 mL of the pharmaceutical composition. In specific embodiments, the syringe contains about 1.0 mL of the pharmaceutical composition. In particular embodiments, the syringe contains about 2.0 mL of the pharmaceutical composition.

[00248] In certain embodiments, the unit dosage form is an autoinject pen. The autoinject pen comprises an autoinject pen containing a pharmaceutical composition as described herein. In some embodiments, the autoinject pen delivers a predetermined volume of pharmaceutical composition. In other embodiments, the autoinject pen is configured to deliver a volume of pharmaceutical composition set by the user.

[00249] In various embodiments, the autoinject pen contains about 0.1 mL to about 5.0 mL of the pharmaceutical composition. In specific embodiments, the autoinject pen contains about

0.5 mL of the pharmaceutical composition. In particular embodiments, the autoinject pen contains about 1.0 mL of the pharmaceutical composition. In other embodiments, the autoinject pen contains about 5.0 mL of the pharmaceutical composition.

7.11. Unit dosage forms

[00250] The pharmaceutical compositions may conveniently be presented in unit dosage form.

[00251] The unit dosage form will typically be adapted to one or more specific routes of administration of the pharmaceutical composition.

[00252] In various embodiments, the unit dosage form is adapted for administration by inhalation. In certain of these embodiments, the unit dosage form is adapted for administration by a vaporizer. In certain of these embodiments, the unit dosage form is adapted for administration by a nebulizer. In certain of these embodiments, the unit dosage form is adapted for administration by an aerosolizer.

[00253] In various embodiments, the unit dosage form is adapted for oral administration, for buccal administration, or for sublingual administration.

[00254] In some embodiments, the unit dosage form is adapted for intravenous, intramuscular, or subcutaneous administration.

[00255] In some embodiments, the unit dosage form is adapted for intrathecal or intracerebroventricular administration.

[00256] In some embodiments, the pharmaceutical composition is formulated for topical administration.

[00257] The amount of active ingredient which can be combined with a carrier material to produce a single dosage form will generally be that amount of the compound which produces a therapeutic effect.

7.12. Methods of use

[00258] Therapeutic antibodies may be used that specifically bind to intact B7-H4.

[00259] *In vivo* and/or *in vitro* assays may optionally be employed to help identify optimal dosage ranges. The precise dose to be employed in the formulation will also depend on the route of administration, and the seriousness of the condition, and should be decided according to the judgment of the practitioner and each subject's circumstances. Effective doses may be extrapolated from dose-response curves derived from in vitro or animal model test systems.

[00260] An oligopeptide or polypeptide is within the scope of the present disclosure if it has an amino acid sequence that is at least 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to least one of the CDRs provided herein; and/or to a CDR of a B7-H4 binding agent

that cross-blocks the binding of at least one of antibodies A1-A12 to B7-H4, and/or is cross-blocked from binding to B7-H4 by at least one of antibodies A1-A12; and/or to a CDR of a B7-H4 binding agent wherein the binding agent can block the binding of B7-H4 to its ligands.

[00261] B7-H4 binding agent polypeptides and antibodies are within the scope of the present disclosure if they have amino acid sequences that are at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to a variable region of at least one of antibodies A1-A12, and cross-block the binding of at least one of antibodies A1-A12 to B7-H4, and/or are cross-blocked from binding to B7-H4 by at least one of antibodies A1-A2; and/or can block the inhibitory effect of B7-H4 on its ligands.

[00262] Antibodies according to the present disclosure may have a binding affinity for human B7-H4 of less than or equal to 5 x 10^{-7}M, less than or equal to 1 x 10^{-7}M, less than or equal to 0.5 x 10^{-7}M, less than or equal to 1 x 10^{-8}M, less than or equal to 1 x 10^{-9}M, less than or equal to 1 x 10^{-10}M, less than or equal to 1 x 10^{-11}M, or less than or equal to 1 x 10^{-12} M.

[00263] The affinity of an antibody or binding partner, as well as the extent to which an antibody inhibits binding, can be determined by one of ordinary skill in the art using conventional techniques, for example those described by Scatchard *et al.* (*Ann. N.Y. Acad. Sci.* 51:660-672 (1949)) or by surface plasmon resonance (SPR; BIAcore, Biosensor, Piscataway, NJ). For surface plasmon resonance, target molecules are immobilized on a solid phase and exposed to ligands in a mobile phase running along a flow cell. If ligand binding to the immobilized target occurs, the local refractive index changes, leading to a change in SPR angle, which can be monitored in real time by detecting changes in the intensity of the reflected light. The rates of change of the SPR signal can be analyzed to yield apparent rate constants for the association and dissociation phases of the binding reaction. The ratio of these values gives the apparent equilibrium constant (affinity) (*see, e.g.,* Wolff *et al.*, *Cancer Res.* 53:2560-65 (1993)).

[00264] An antibody according to the present disclosure may belong to any immunoglobin class, for example IgG, IgE, IgM, IgD, or IgA. It may be obtained from or derived from an animal, for example, fowl (*e.g.,* chicken) and mammals, which includes but is not limited to a mouse, rat, hamster, rabbit, or other rodent, cow, horse, sheep, goat, camel, human, or other primate. The antibody may be an internalizing antibody. Production of antibodies is disclosed generally in U.S. Patent Publication No. 2004/0146888 A1.

[00265] In the methods described above to generate antibodies according to the present disclosure, including the manipulation of the specific A1-A12 CDRs into new frameworks and/or constant regions, appropriate assays are available to select the desired antibodies (*i.e.* assays for

determining binding affinity to B7-H4; cross-blocking assays; Biacore-based competition binding assay;" *in vivo* assays).

7.12.1. Methods of treating a disease responsive to a B7-H4 inhibitor or activator

[00266] In another aspect, methods are presented for treating a subject having a disease responsive to a B7-H4 inhibitor or activator. The disease can be cancer, autoimmune disease, or viral or bacterial infection.

[00267] The terms "treatment," "treating," and the like are used herein to generally mean obtaining a desired pharmacologic and/or physiologic effect. The effect may be prophylactic, in terms of completely or partially preventing a disease, condition, or symptoms thereof, and/or may be therapeutic in terms of a partial or complete cure for a disease or condition and/or adverse effect, such as a symptom, attributable to the disease or condition. "Treatment" as used herein covers any treatment of a disease or condition of a mammal, particularly a human, and includes: (a) preventing the disease or condition from occurring in a subject which may be predisposed to the disease or condition but has not yet been diagnosed as having it; (b) inhibiting the disease or condition (*e.g.*, arresting its development); or (c) relieving the disease or condition (*e.g.*, causing regression of the disease or condition, providing improvement in one or more symptoms). Improvements in any conditions can be readily assessed according to standard methods and techniques known in the art. The population of subjects treated by the method of the disease includes subjects suffering from the undesirable condition or disease, as well as subjects at risk for development of the condition or disease.

[00268] By the term "therapeutically effective dose" or "effective amount" is meant a dose or amount that produces the desired effect for which it is administered. The exact dose or amount will depend on the purpose of the treatment, and will be ascertainable by one skilled in the art using known techniques (*see*, *e.g.*, Lloyd (1999) The Art, Science and Technology of Pharmaceutical Compounding).

[00269] The term "sufficient amount" means an amount sufficient to produce a desired effect.

[00270] The term "therapeutically effective amount" is an amount that is effective to ameliorate a symptom of a disease. A therapeutically effective amount can be a "prophylactically effective amount" as prophylaxis can be considered therapy.

[00271] The term "ameliorating" refers to any therapeutically beneficial result in the treatment of a disease state, *e.g.*, a neurodegenerative disease state, including prophylaxis, lessening in the severity or progression, remission, or cure thereof.

[00272] The actual amount administered, and rate and time-course of administration, will depend on the nature and severity of protein aggregation disease being treated. Prescription of treatment, *e.g.* decisions on dosage *etc.*, is within the responsibility of general practitioners and other medical doctors, and typically takes account of the disorder to be treated, the condition of the individual patient, the site of delivery, the method of administration and other factors known to practitioners. Examples of the techniques and protocols mentioned above can be found in Remington's Pharmaceutical Sciences, 16th edition, Osol, A. (ed), 1980.

[00273] In some embodiments, the pharmaceutical composition is administered by inhalation, orally, by buccal administration, by sublingual administration, by injection or by topical application.

[00274] In some embodiments, the pharmaceutical composition is administered in an amount sufficient to modulate survival of neurons or dopamine release. In some embodiments, the major cannabinoid is administered in an amount less than 1g, less than 500 mg, less than 100 mg, less than 10 mg per dose.

[00275] In some embodiments, the pharmaceutical composition is administered once a day, 2-4 times a day, 2-4 times a week, once a week, or once every two weeks.

[00276] A composition can be administered alone or in combination with other treatments, either simultaneously or sequentially dependent upon the condition to be treated. For example, the pharmaceutical composition can be administered in combination with one or more drugs targeting a different check point receptor, such as PD-1 inhibitor (*e.g.*, anti-PD-1 antibody) or TIGIT inhibitor (*e.g.,* anti-TIGIT antibody).

7.13. Examples

[00277] Below are examples of specific embodiments for carrying out the present disclosure. The examples are offered for illustrative purposes only, and are not intended to limit the scope of the present disclosure in any way. Efforts have been made to ensure accuracy with respect to numbers used (*e.g.*, amounts, temperatures, *etc.*), but some experimental error and deviation should, of course, be allowed for.

[00278] The practice of the present disclosure will employ, unless otherwise indicated, conventional methods of protein chemistry, biochemistry, recombinant DNA techniques and pharmacology, within the skill of the art. Such techniques are explained fully in the literature. *See*, *e.g.*, T.E. Creighton, *Proteins: Structures and Molecular Properties* (W.H. Freeman and Company, 1993); A.L. Lehninger, *Biochemistry* (Worth Publishers, Inc., current addition); Sambrook, et al., *Molecular Cloning: A Laboratory Manual* (2nd Edition, 1989); *Methods In Enzymology* (S. Colowick and N. Kaplan eds., Academic Press, Inc.); *Remington's Pharmaceutical Sciences*, 18th Edition (Easton, Pennsylvania: Mack Publishing Company,

1990); Carey and Sundberg *Advanced Organic Chemistry 3rd Ed.* (Plenum Press) Vols A and B(1992). Furthermore, methods of generating and selecting antibodies explained in Adler *et al.*, A natively paired antibody library yields drug leads with higher sensitivity and specificity than a randomly paired antibody library, MAbs (2018), and Adler *et al.*, Rare, high-affinity mouse anti-B7-H4 antibodies that function in checkpoint blockade, discovered using microfluidics and molecular genomics, MAbs (2017), which are incorporated by reference in its entirety herein, can be employed.

7.13.1. Example 1: Generation of antigen binding protein

[00279] ***Mouse Immunization and Sample Preparation:***

[00280] First, transgenic mice carrying inserted human immunoglobulin genes were immunized with soluble B7-H4 immunogen of SEQ ID NO: 7001 (*i.e.*, His-tagged B7-H4 protein (R&D Systems)) using TiterMax as an adjuvant. One µg of immunogen was injected into each hock and 3 µg of immunogen was administered intraperitoneally, every third day for 15 days. Titer was assessed by enzyme-linked immunosorbent assay (ELISA) on a 1:2 dilution series of each animal's serum, starting at a 1:200 dilution. A final intravenous boost of 2.5 µg/hock without adjuvant was given to each animal before harvest. Lymph nodes (popliteal, inguinal, axillary, and mesenteric) were surgically removed after sacrifice. Single cell suspensions for each animal were made by manual disruption followed by passage through a 70 µm filter. Next, the EasySep™ Mouse Pan-B Cell Isolation Kit (Stemcell Technologies) negative selection kit was used to isolate B cells from each sample. The lymph node B cell populations were quantified by counting on a C-Chip hemocytometer (Incyto) and assessed for viability using Trypan blue. The cells were then diluted to 5,000–6,000 cells/mL in phosphate-buffered saline (PBS) with 12% OptiPrep™ Density Gradient Medium (Sigma). This cell mixture was used for microfluidic encapsulation. Approximately one million B cells were run from each of the six animals through an emulsion droplet microfluidics platform.

[00281] Generating paired heavy and light chain libraries:

[00282] A DNA library encoding scFv from RNA of single cells, with native heavy-light Ig pairing intact, was generated using the emulsion droplet microfluidics platform or vortex emulsions. The method for generating the DNA library was divided into 1) poly(A) + mRNA capture, 2) multiplexed overlap extension reverse transcriptase polymerase chain reaction (OE-RT-PCR), and 3) nested PCR to remove artifacts and add adapters for deep sequencing or yeast display libraries. The scFv libraries were generated from approximately one million B cells from each animal that achieved a positive ELISA titer.

[00283] For poly(A) + mRNA capture, a custom designed co-flow emulsion droplet microfluidic chip fabricated from glass (Dolomite) was used. The microfluidic chip has two input

channels for fluorocarbon oil (Dolomite), one input channel for the cell suspension mix described above, and one input channel for oligo-dT beads (NEB) at 1.25 mg/ml in cell lysis buffer (20 mM Tris pH 7.5, 0.5 M NaCl, 1 mM ethylenediaminetetraacetic acid (EDTA), 0.5% Tween-20, and 20 mM dithiothreitol). The input channels were etched to 50 μm by 150 μm for most of the chip's length, narrow to 55 μm at the droplet junction, and were coated with hydrophobic Pico-Glide (Dolomite). Three Mitos P-Pump pressure pumps (Dolomite) were used to pump the liquids through the chip. Droplet size depends on pressure, but typically droplets of ~45 mm diameter are optimally stable. Emulsions were collected into chilled 2 ml microcentrifuge tubes and incubated at 40 °C for 15 minutes for mRNA capture. The beads were extracted from the droplets using Pico-Break (Dolomite). In some embodiments, similar single cell partitioning emulsions were made using a vortex.

[00284] For multiplex OE-RT-PCR, glass Telos droplet emulsion microfluidic chips were used (Dolomite). mRNA-bound beads were re-suspended into OE-RT-PCR mix and injected into the microfluidic chips with a mineral oil-based surfactant mix (available commercially from GigaGen) at pressures that generate 27 μm droplets. The OE-RT-PCR mix contains 2x one-step RT-PCR buffer, 2.0 mM $MgSO_4$, SuperScript III reverse transcriptase, and Platinum Taq (Thermo Fisher Scientific), plus a mixture of primers directed against the IgK C region, the IgG C region, and all V regions (FIG. 2). The overlap region was a DNA sequence that encodes a Gly-Ser rich scFv linker sequence. The DNA fragments were recovered from the droplets using a droplet breaking solution (available commercially from GigaGen) and then purified using QIAquick PCR Purification Kit (Qiagen). In some embodiments, similar OE-RT-PCR emulsions were made using a vortex.

[00285] For nested PCR (FIG. 2), the purified OE-RT-PCR product was first run on a 1.7% agarose gel for 80 minutes at 150 V. A band at 1200–1500 base pair (bp) corresponding to the linked product was excised and purified using NucleoSpin Gel and PCR Clean-up Kit (Macherey Nagel). PCR was then performed to add adapters for Illumina sequencing or yeast display; for sequencing, a randomer of seven nucleotides is added to increase base calling accuracy in subsequent next generation sequencing steps. Nested PCR was performed with 2x NEBNext High-Fidelity amplification mix (NEB) with either Illumina adapter containing primers or primers for cloning into the yeast expression vector. The nested PCR product was run on a 1.2% agarose gel for 50 minutes at 150V. A band at 800–1100 bp was excised and purified using NucleoSpin Gel and PCR Clean-up Kit (Macherey Nagel).

[00286] In some embodiments, scFv libraries were not natively paired, for example, randomly paired by amplifying scFv directly from RNA isolated from B cells.

7.13.2. Example 2: Isolation of B7-H4 binders by yeast display

[00287] *Library Screening:*

[00288] Human IgG1-Fc (Thermo Fisher Scientific) and B7-H4 (R&D Systems) proteins were biotinylated using the EZ-Link Micro Sulfo-NHS-LC-Biotinylation kit (Thermo Fisher Scientific). The biotinylation reagent was resuspended to 9 mM and added to the protein at a 50-fold molar excess. The reaction was incubated on ice for 2 hours and then the biotinylation reagent was removed using Zeba desalting columns (Thermo Fisher Scientific). The final protein concentration was calculated with a Bradford assay.

[00289] Next, the six DNA libraries were expressed as surface scFv in yeast. A yeast surface display vector (pYD) that contains a GAL1/10 promoter, an Aga2 cell wall tether, and a C-terminal c-Myc tag was built. The GAL1/10 promoter induces expression of the scFv protein in medium that contains galactose. The Aga2 cell wall tether was required to shuttle the scFv to the yeast cell surface and tether the scFv to the extracellular space. The c-Myc tag was used during the flow sort to stain for yeast cells that express in-frame scFv protein. *Saccharomyces cerevisiae* cells (ATCC) were electroporated (Bio-Rad Gene Pulser II; 0.54 kV, 25 uF, resistance set to infinity) with gel-purified nested PCR product and linearized pYD vector for homologous recombination *in vivo*. Transformed cells were expanded and induced with galactose to generate yeast scFv display libraries.

[00290] Two million yeast cells from the expanded scFv libraries were stained with anti-c-Myc (Thermo Fisher Scientific A21281) and an AF488-conjugated secondary antibody (Thermo Fisher Scientific A11039). To select scFv-expressing cells that bind to B7-H4, biotinylated B7-H4 antigen was added to the yeast culture (7 nM final) during primary antibody incubation and then stained with PE-streptavidin (Thermo Fisher Scientific). Yeast cells were flow sorted on a BD Influx (Stanford Shared FACS Facility) for double- positive cells (AF488C/PEC), and recovered clones were then plated on SD-CAA plates with kanamycin, streptomycin, and penicillin (Teknova) for expansion. The expanded first round FACS clones were then subjected to a second round of FACS with the same antigen at the same molarity (7 nM final). Plasmid minipreps (Zymo Research) were prepared from yeast recovered from the final FACS sort. Tailed-end PCR was used to add Illumina adapters to the plasmid libraries for deep sequencing.

[00291] In a typical FACS dot plot, the upper right quadrant contains yeast that stain for both antigen binding and scFv expression (identified by a C-terminal c-Myc tag). The lower left quadrant contains yeast that do not stain for either the antigen or scFv expression. The lower right quadrant contains yeast that express the scFv but do not bind the antigen. the frequency of binders in each repertoire was estimated by dividing the count of yeast that double stain for antigen and scFv expression by the count of yeast that express an scFv. Libraries generated from

immunized mice yielded low percentages of scFv binders (ranging from 0.08%–1.28%) when sorted at 7 nM final antigen concentration. There was no clear association between serum titer and the frequency of binders in a repertoire. Following expansion of these sorted cells, a second round of FACS at 7 nM final antigen concentration was used to increase the specificity of the screen. The frequency of binders in the second FACS was always substantially higher than the first FACS, ranging from 8.39%–84.4%. Generally, lower frequency of binders in the first sort yielded lower frequency of binders in the second sort. Presumably, this is due to lower gating specificity for samples that have fewer bona fide binders in the original repertoire.

[00292] ***Deep repertoire sequencing:***

[00293] B7-H4-binding clones were recovered as a library ("a library of B7-H4 binding clones"), and subjected to deep repertoire sequencing. Deep repertoire sequencing determines the sequences of all paired variable (V(D)J) regions of both heavy and light chain sequences. The library of B7-H4 binding clones were deposited under ATCC Accession No. PTA-125511 under the Budapest Treaty on November 20, 2018, under ATCC Account No. 197361 (American Type Culture Collection (ATCC) 10801 University Boulevard, Manassas, VA 20110 USA). Each clone in the library contains an scFv comprising a paired variable (V(D)J) regions of both heavy and light chain sequences originating from a single cell. Deep repertoire sequencing determines the sequences of all paired variable (V(D)J) regions of both heavy and light chain sequences. Some of the heavy and light chain sequences obtained from sequencing the yeast scFv library are provided in SEQ ID NOS: 1-12 and SEQ ID NOS: 101-112. Additional sequences obtained from sequencing the yeast scFv library are provided in SEQ ID NOS: 8000-8167. Specifically, their variable light chain (V_L) sequences include SEQ ID NOS: 8000-8083. Their heavy chain (V_H) sequences include SEQ ID NOS: 8084-8167.

[00294] Deep antibody sequencing libraries were quantified using a quantitative PCR Illumina Library Quantification Kit (KAPA) and diluted to 17.5 pM. Libraries were sequenced on a MiSeq (Illumina) using a 500 cycle MiSeq Reagent Kit v2, according to the manufacturer's instructions. To obtain high quality sequence reads with maintained heavy and light chain linkage, sequencing was performed in two separate runs. In the first run ("linked run"), the scFv libraries were directly sequenced to obtain forward read of 340 cycles for the light chain V-gene and CDR3, and reverse read of 162 cycles that cover the heavy chain CDR3 and part of the heavy chain V-gene. In the second run ("unlinked run"), the scFv library was first used as a template for PCR to separately amplify heavy and light chain V-genes. Then, forward reads of 340 cycles and reverse reads of 162 cycles for the heavy and light chain Ig were obtained

separately. This produces forward and reverse reads that overlap at the CDR3 and part of the V-gene, which increases confidence in nucleotide calls.

[00295] To remove base call errors, the expected number of errors (E) for a read were calculated from its Phred scores. By default, reads with E >1 were discarded, leaving reads for which the most probable number of base call errors is zero. As an additional quality filter, singleton nucleotide reads were discarded because sequences found two or more times have a high probability of being correct. Finally, high-quality, linked antibody sequences were generated by merging filtered sequences from the linked and unlinked runs. Briefly, a series of scripts that first merged forward and reverse reads from the unlinked run was written in Python. Any pairs of forward and reverse sequences that contained mismatches were discarded. Next, the nucleotide sequences from the linked run were used to query merged sequences in the unlinked run. The final output from the scripts is a series of full-length, high-quality variable (V(D)J) sequences, with native heavy and light chain Ig pairing.

[00296] To identify reading frame and FR/CDR junctions, a database of well-curated immunoglobulin sequences was first processed to generate position-specific sequence matrices (PSSMs) for each FR/CDR junction. These PSSMs were used to identify FR/CDR junctions for each of the merged nucleotide sequences generated using the processes described above. This identified the protein reading frame for each of the nucleotide sequences. CDR sequences that have a low identify score to the PSSMs are indicated by an exclamation point. Python scripts were then used to translate the sequences. Reads were required to have a valid predicted CDR3 sequence, so, for example, reads with a frame-shift between the V and J segments were discarded. Next, UBLAST was run using the scFv nucleotide sequences as queries and V and J gene sequences from the IMGT database as the reference sequences. The UBLAST alignment with the lowest E-value was used to assign V and J gene families and compute %ID to germline.

[00297] Each animal yielded 38–50 unique scFv sequences present at 0.1% frequency or greater after the second FACS selection, including a total of 12 unique scFv candidate binders (SEQ ID Nos: 1-12 for light chains; SEQ ID Nos: 101-112 for heavy chains). The light chain having a sequence of SEQ ID NO: [n] and the heavy chain having a sequence of SEQ ID NO: [100+n] are a cognate pair from a single cell, and forming a single scFv. For example, the light chain of SEQ ID NO:1 and the heavy chain of SEQ ID NO:101 are a cognate pair, the light chain of SEQ ID NO:12 and the heavy chain of SEQ ID NO:112 are a cognate pair, *etc.*

[00298] In this method, the two rounds of FACS resulted in enrichment of the B7-H4-binding scFvs. In addition, many scFv were not detected in the sequencing data from the initial population of B cells from the immunized mice and most of the scFv present in the pre-sort

mouse repertoires were eliminated following FACS. Therefore, this work suggests that most of the antibodies present in the repertoires of immunized mice are not strong binders to the immunogen and that this method can enrich for rare nM-affinity binders from the initial population of B cells from immunized mice.

7.13.3. Example 3: Biological characteristics of antigen binding protein

[00299] scFv sequences that were present at low frequency in pre-sort libraries and became high frequency in post-sort libraries were then synthesized as full-length mAbs in Chinese hamster ovary (CHO) cells. These mAbs comprise the 2–3 most abundant sequences in the second round of FACS for each animal.

[00300] ***B7-H4 Target Binding Profiles:***

[00301] The binding specificity and affinity of each full-length antibody towards human B7-H4 were determined using surface plasmon resonance (SPR).

[00302] For SPR, a moderate density (»1,000 Response Units) of an antihuman IgG-Fc reagent (Southern Biotech 2047-01) was amine-coupled to a Xantec CMD-50M chip (50nm carboxymethyldextran medium density of functional groups) activated with 133 mM EDC (Sigma) and 33.3 mM S-NHS (ThermoFisher) in 100 mM MES pH 5.5. Then, goat anti-Human IgG Fc (Southern Biotech 2047-01) was coupled for 10 minutes at 25 mg/m L in 10 mM Sodim Acetate pH 4.5 (Carterra Inc.). The surface was then deactivated with 1 M ethanolamine pH 8.5 (Carterra Inc.). Running buffer used for the lawn immobilization was HBS-EPC (10 mM HEPES, 150 mM NaCl, 3 mM EDTA, 0.05% Tween 20, pH 7.4; Teknova).

[00303] The sensor chip was then transferred to a continuous flow microspotter (CFM; Carterra Inc.) for array capturing. The mAb supernatants were diluted 50-fold (3–10 mg/mL final concentration) into HBS-EPC with 1 mg/mL BSA. The samples were each captured twice with 15-minute and 4-minute capture steps on the first and second prints, respectively, to create multiple densities, using a 65 mL/min flow rate. The running buffer in the CFM was also HBS-EPC.

[00304] Next, the sensor chip was loaded onto an SPR reader (MX- 96 system; Ibis Technologies) for the kinetic analysis. B7-H4 was injected at five increasing concentrations in a four-fold dilution series with concentrations of 1.95, 7.8, 31.25, 125, and 500 nM in running buffer (HBS-EPC with 1.0 mg/mL BSA). B7-H4 injections were 5 minutes with a 15-minute dissociation at 8 mL/second in a non-regenerative kinetic series. An injection of the goat anti-Human IgG Fc capture antibody at 75 mg/mL was injected at the end of the series to verify the capture level of each mAb. Binding data was double referenced by subtracting an interspot surface and a blank injection and analyzed for ka (on-rate), kd (off-rate), and KD (affinity) using the Kinetic Interaction Tool software (Carterra Inc.).

[00305] For cell surface binding studies, stable B7-H4 expressing Flp-In CHO (Thermo Fisher Scientific) cells were generated and mixed at a 50:50 ratio. One million cells were stained with 1 µg of the disclosed anti-B7-H4 recombinant antibodies in 200 µl of MACS Buffer (DPBS with 0.5% bovine serum albumin and 2 mM EDTA) for 30 minutes at 4°C. Cells were then co-stained with anti-human irrelevant target and anti-human IgG Fc-PE antibodies for 30 minutes at 4°C. An anti-human B7-H4-FITC was used as a control for these mixing experiments and cell viability was assessed with DAPI. Flow cytometry analysis was conducted on a BD Influx at the Stanford Shared FACS Facility and data was analyzed using FlowJo.

[00306] Antibodies that specifically bind to B7-H4 were identified. On rate, off rate, and affinity to B7-H4 (K_D) of each antibody is provided in TABLE 6.

TABLE 6

Ab#	Binds to B7-H4 (FACS)?	k_{on} to B7-H4 (M^{-1} s^{-1})
sc209*	No	1.20E+04
A1	Yes	3.00E+05
A2	Yes	3.90E+04
A3	Yes	3.10E+05
A4	No	2.50E+04
A5	No	3.10E+04
A6	No	1.00E+04
A7	No	1.10E+04
A8	No	2.10E+05
A9	No	7.70E+04
A10	No	1.90E+04
A11	No	7.10E+04
A12	No	6.30E+03

**(from Millennium patent US 8,323,645)*

[00307] ***In vitro cellular assay:***

[00308] In vitro cell killing assays are used to test antibodies for efficacy against tumor cells. Fresh tumor samples are first obtained from donors. Single cell suspensions are generated from the tumors using a commercial kit (Miltenyi), and then isolate B7-H4-positive cells are isolated using FACS. Next, using a commercial kit (BioVision), populations of target cells are labeled with carboxyfluorosuccinimide ester (CFSE) and 7-aminoactinomycin D (7-AAD). CFSE labels live cells and 7-AAD labels apoptotic and necrotic cells. A dilution series of anti-B7-H4 antibodies and NK cells are then mixed with the B7-H4-expressing tumor cells. Cell killing, or ADCC, is then quantified using FACS.

[00309] Similar assays are performed with tumor cell lines instead of primary tumor cells. Cell killing is also measured directly using label-free measurement devices such as the xCELLigence Sytem from Acea Biosciences.

[00310] ***Epitope binning:***

[00311] Epitope binning is performed using high-throughput Array SPR in a modified classical sandwich approach. A sensor chip is functionalized using the Carterra CFM and methods similar to the SPR affinity studies, except a CMD-200M chip type was used (200nm carboxymethyl dextran, Xantec) and mAbs are coupled at 50 mg/mL to create a surface with higher binding capacity (~3,000 reactive units immobilized). The mAb supernatants are diluted at 1:1 or 1:10 in running buffer, depending on the concentration of the mAb in the supernatant.

[00312] The sensor chip is placed in the MX-96 instrument, and the captured mAbs ("ligands") are crosslinked to the surface using the bivalent amine reactive linker bis(sulfosuccinimidyl) suberate (BS3, ThermoFisher), which is injected for 10 minutes at 0.87 mM in water. Excess activated BS3 is neutralized with 1 M ethanolamine pH 8.5. For each binning cycle, a 7-minute injection of 250 mg/mL human IgG (Jackson ImmunoResearch 009-000-003) is used to block reference surfaces and any remaining capacity of the target spots.

[00313] Next, 250 nM B7-H4 protein is injected onto the sensor chip, followed by injections of the diluted mAb supernatants ("analytes") or buffer blanks as negative controls. Thus, the analyte mAb only binds to the antigen if it is not competitive with the ligand mAb. At the end of each cycle, a one minute regeneration injection is performed using a solution of 4 parts Pierce IgG Elution Buffer (ThermoFisher #21004), one part 5 M NaCl (0.83 M final), and 1.25 parts 0.85% H3PO4 (0.17% final).

[00314] A network community plot algorithm in an SPR epitope data analysis software package (Carterra Inc.) is then used to determine epitope bins. Note that the clustering algorithm groups mAbs for which only analyte data are available separately from the mAbs for which both ligand and analyte data are available. This phenomenon is an artifact of the incomplete competitive matrix. mAbs with both ligand and analyte data have more mAb-mAb measurements, resulting in more mAb-mAb connections, which leads to a closer relationship in the community plot.

8. INCORPORATION BY REFERENCE

[00315] All publications, patents, patent applications and other documents cited in this application are hereby incorporated by reference in their entireties for all purposes to the same extent as if each individual publication, patent, patent application or other document were individually indicated to be incorporated by reference for all purposes.

9. EQUIVALENTS

[00316] While various specific embodiments have been illustrated and described, the above specification is not restrictive. It will be appreciated that various changes can be made without departing from the spirit and scope of the present disclosure(s). Many variations will become apparent to those skilled in the art upon review of this specification.

Table 7 provides sequences for the antibody light chains, heavy chains, CDRs, and B7-H4.

	Table 7	
SEQ ID NO	**Sequence**	**Chain (Antibody)**
1	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLIY GASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYGSSPWTFG QGTKVEIK	L1 (A1)
2	DIQMTQSPSSLSASVGDRVTITCRASQAIRNDLGWYQQKPGKAPKRLIY AASSLQSGVPSRFSGSGSGTEFTLTISSLQPEDFATYYCLQHNSYPRTSG QGTKVEIK	L2 (A2)
3	EIVLTQSPGTLSLSPGERATLSCRASQSVSSTYLAWYQQKPGQAPRLLIY GASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYGSSMYTFG QGTKLEIK	L3 (A3)
4	EIVMTQSPATLSLSPGERATLSCRASQSVSSTYLSWYQQKPGQAPRLLIY GASTRATGIPARFSGSGSGTDFTLTISSLQPEDFAVYYCQQDYNLPLTFG GGTKVEIK	L4 (A4)
5	EIVMTQSPATLSLSPGERATLSCRASQSGSSSYLSWYQQKPGQAPRLLIY GASTRATGIPARFSGSGSGTDFTLTISSLQPEDFAVYYCHQDYNLPYTLG QGTKLEIK	L5 (A5)
6	EIVMTQSPAPLSLSPGDRATLSCRVSQSGSSSYLSWYQQKPGQAPRLLIY GASTRATGIPARFSGSGSGTDFTLTISSLQPEDFAVYYCHQDYNLPYTFG QGTKLETK	L6 (A6)
7	EIVMTQSPATLSLSPGERATLSCRASQSVSSTYLSWYQQKPGQAPRLLIY GASTRATGIPARFSGSGSGTDFTLTISSLQPEDFAVYYCQQDYNLPLTFG GGTKVEIK	L7 (A7)
8	EIVMTQSPATLSLSPGERATLSCRASQIVSSTYLSWFQQKPGQAPRLLIY GASTRATGIPARFSGSGSGTDFTLTISSLKPEDFAVYYCQQDYNLPYTFG QGTKLEIK	L8 (A8)
9	DIQMTQSPSSLSASVGDRVTITCRASQGISNYLAWYQQKPGKVPKLLIY AASTLQSGVPSRFSGSGSGTDFTLTISSLQPEDVATYYCQKYNSAPYTFG QGTKLEIK	L9 (A9)
10	AIQMTQSPSSLSASVGDRVTITCRASQGIRNDLGWYQQKPGKAPNLLIY AASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCLQDYSSPLTFGG GTKVEIK	L10 (A10)
11	EIVMTQSPATLSLSPGERATLSCRASQSVSSTYLSWFQQNPGQAPRLLIY GASTRATGIPARFSGSGSGTDFTLTISSLQPEDFAVYYCQQDYKSPYTFG QGTKLEIK	L11 (A11)
12	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSFLAWYQQKPGQAPRLLIY GASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYGTSLTFGG GTKVEIK	L12 (A12)
101	EVQLVESGGGLVQPGGSLRLSCAASGFTFSNYAMSWVRQAPGKGLEW VSAISATGGSTKSADSVKGRFTISRDNSKNTLYLQMSSLRADDTAGYYC VKGPNWGLYYFDYWGQGTLVTVSSA	H1 (A1)
102	QVQLQQWGAGLLKPSETLSLTCAVYGGSFSGFYWNWIRQPPGKGLEWI GEINHSGTTNYNPSLKSRVTISVDTSKNQFSLKLSSVTAADTAVYYCAR GLYTWNLDSWGQGTLVTVSSA	H2 (A2)
103	QLQLQESGPGQVKPSETLSLTCTVSGGSISSSSYYWGWIRQPPGKGLEWI GSIYYSGRTYYNPSFKSRVTISVDTSKNQFSLKLSSVTAADTAVYYCAR HGHFDWLLHWYFDLWGRGTLVTVSSA	H3 (A3)

104	EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEW VSAISGSGGSTFYADSVKGRFTISRDNSKNTLSLQMKSLRAEDTAGYYC AKGFEGDYGFFDYWGQGTLVTVSSA	H4 (A4)
105	QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKGLEW VAVISYDGNNKYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYY CAREDYYGSGSYYKAYYGMDVWGQGTTVTVSSA	H5 (A5)
106	QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKGLEW VAVMSYDGSNKYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVY YCAREDDILTGYYYYGMDVWGQGTTVTVSSA	H6 (A6)
107	EVQLVESGGGLVQPGGSLRLSCAASGFTFSSHAMSWIRQAPGRGLEWV SAISGSGGSSFFADSVKGRFTISRDNSKNTLYLQMHSLRVEDTAVYYCA KGEYSYGYFDYWGQGTLVTVSSA	H7 (A7)
108	QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGLHWVRQAPGKGLEW VAVISYDGSHKYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVFY CARDDDILTGFYGMDVWGQGTTVTVSSA	H8 (A8)
109	EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEW VSVISGSGGSTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYC AKWRWFGEPHWFDPWGQGTPVTVSSA	H9 (A9)
110	QLQLQESGPGLVKPSETLSLTCTVSGGSISSSSYYWGWIRQPPGKGLEWI GSINYSGNTYYNPSLKSRVTISVDTSKNQFSLMMSSVTAADTAVYFCAR RRYFDWLLSNAFDIWGQGTMVTVSSA	H10 (A10)
111	QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGIHWVRQAPGKGLEWV AVISYDGSNKYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYC ARDDDILTGSYGMDVWGQGTTVTVSSA	H11 (A11)
112	EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEW VSAISSSGGSTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDRAVYYC AKWATLVRGVIINHWFFDLWGRGTLVTVSSA	H12 (A12)
1001	QSVSSSY	CDR1-L1 (A1)
1002	QAIRND	CDR1-L2 (A2)
1003	QSVSSTY	CDR1-L3 (A3)
1004	QSVSSTY	CDR1-L4 (A4)
1005	QSGSSSY	CDR1-L5 (A5)
1006	QSGSSSY	CDR1-L6 (A6)
1007	QSVSSTY	CDR1-L7 (A7)
1008	QIVSSTY	CDR1-L8 (A8)
1009	QGISNY	CDR1-L9 (A9)
1010	QGIRND	CDR1-L10 (A10)
1011	QSVSSTY	CDR1-L11 (A11)
1012	QSVSSSF	CDR1-L12 (A12)
2001	GAS	CDR2-L1 (A1)
2002	AAS	CDR2-L2 (A2)
2003	GAS	CDR2-L3 (A3)
2004	GAS	CDR2-L4 (A4)
2005	GAS	CDR2-L5 (A5)
2006	GAS	CDR2-L6 (A6)
2007	GAS	CDR2-L7 (A7)
2008	GAS	CDR2-L8 (A8)
2009	AAS	CDR2-L9 (A9)
2010	AAS	CDR2-L10 (A10)
2011	GAS	CDR2-L11 (A11)
2012	GAS	CDR2-L12 (A12)
3001	QQYGSSPWT	CDR3-L1 (A1)
3002	LQHNSYPRT	CDR3-L2 (A2)
3003	QQYGSSMYT	CDR3-L3 (A3)
3004	QQDYNLPLT	CDR3-L4 (A4)
3005	HQDYNLPYT	CDR3-L5 (A5)
3006	HQDYNLPYT	CDR3-L6 (A6)
3007	QQDYNLPLT	CDR3-L7 (A7)
3008	QQDYNLPYT	CDR3-L8 (A8)

3009	QKYNSAPYT	CDR3-L9 (A9)
3010	LQDYSSPLT	CDR3-L10 (A10)
3011	QQDYKSPYT	CDR3-L11 (A11)
3012	QQYGTSLT	CDR3-L12 (A12)
4001	GFTFSNYA	CDR1-H1 (A1)
4002	GGSFSGFY	CDR1-H2 (A2)
4003	GGSISSSSYY	CDR1-H3 (A3)
4004	GFTFSSYA	CDR1-H4 (A4)
4005	GFTFSSYG	CDR1-H5 (A5)
4006	GFTFSSYG	CDR1-H6 (A6)
4007	GFTFSSHA	CDR1-H7 (A7)
4008	GFTFSSYG	CDR1-H8 (A8)
4009	GFTFSSYA	CDR1-H9 (A9)
4010	GGSISSSSYY	CDR1-H10 (A10)
4011	GFTFSSYG	CDR1-H11 (A11)
4012	GFTFSSYA	CDR1-H12 (A12)
5001	ISATGGST	CDR2-H1 (A1)
5002	INHSGTT	CDR2-H2 (A2)
5003	IYYSGRT	CDR2-H3 (A3)
5004	ISGSGGST	CDR2-H4 (A4)
5005	ISYDGNNK	CDR2-H5 (A5)
5006	MSYDGSNK	CDR2-H6 (A6)
5007	ISGSGGSS	CDR2-H7 (A7)
5008	ISYDGSHK	CDR2-H8 (A8)
5009	ISGSGGST	CDR2-H9 (A9)
5010	INYSGNT	CDR2-H10 (A10)
5011	ISYDGSNK	CDR2-H11 (A11)
5012	ISSSGGST	CDR2-H12 (A12)
6001	VKGPNWGLYYFDY	CDR3-H1 (A1)
6002	ARGLYTWNLDS	CDR3-H2 (A2)
6003	ARHGHFDWLLHWYFDL	CDR3-H3 (A3)
6004	AKGFEGDYGFFDY	CDR3-H4 (A4)
6005	AREDYYGSGSYYKAYYGMDV	CDR3-H5 (A5)
6006	AREDDILTGYYYYGMDV	CDR3-H6 (A6)
6007	AKGEYSYGYFDY	CDR3-H7 (A7)
6008	ARDDDILTGFYGMDV	CDR3-H8 (A8)
6009	AKWRWFGEPHWFDP	CDR3-H9 (A9)
6010	ARRRYFDWLLSNAFDI	CDR3-H10 (A10)
6011	ARDDDILTGSYGMDV	CDR3-H11 (A11)
6012	AKWATLVRGVIINHWFFDL	CDR3-H12 (A12)
7001	MASLGQILFWSIISIIIILAGAIALIIGFGISGRHSITVTTVASAGNIGEDGILS CTFEPDIKLSDIVIQWLKEGVLGLVHEFKEGKDELSEQDEMFRGRTAVF ADQVIVGNASLRLKNVQLTDAGTYKCYIITSKGKGNANLEYKTGAFSM PEVNVDYNASSETLRCEAPRWFPQPTVVWASQVDQGANFSEVSNTSFE LNSENVTMKVVSVLYNVTINNTYSCMIENDIAKATGDIKVTESEIKRRS HLQLLNSKASLCVSSFFAISWALLPLSPYLMLK	Human B7-H4

Table 8 provides the sequence identifiers for the light chain, heavy chain, and CDRs of the indicated clones

Table 8								
	SEQ ID NO							
Antibody Clone Number	**Light Chain**	**Heavy Chain**	**CDR1 (Light)**	**CDR2 (Light)**	**CDR3 (Light)**	**CDR1 (Heavy)**	**CDR2 (Heavy)**	**CDR3 (Heavy)**
1	8000	8084	8168	8252	8336	8420	8504	8588
2	8001	8085	8169	8253	8337	8421	8505	8589

3	8002	8086	8170	8254	8338	8422	8506	8590
4	8003	8087	8171	8255	8339	8423	8507	8591
5	8004	8088	8172	8256	8340	8424	8508	8592
6	8005	8089	8173	8257	8341	8425	8509	8593
7	8006	8090	8174	8258	8342	8426	8510	8594
8	8007	8091	8175	8259	8343	8427	8511	8595
9	8008	8092	8176	8260	8344	8428	8512	8596
10	8009	8093	8177	8261	8345	8429	8513	8597
11	8010	8094	8178	8262	8346	8430	8514	8598
12	8011	8095	8179	8263	8347	8431	8515	8599
13	8012	8096	8180	8264	8348	8432	8516	8600
14	8013	8097	8181	8265	8349	8433	8517	8601
15	8014	8098	8182	8266	8350	8434	8518	8602
16	8015	8099	8183	8267	8351	8435	8519	8603
17	8016	8100	8184	8268	8352	8436	8520	8604
18	8017	8101	8185	8269	8353	8437	8521	8605
19	8018	8102	8186	8270	8354	8438	8522	8606
20	8019	8103	8187	8271	8355	8439	8523	8607
21	8020	8104	8188	8272	8356	8440	8524	8608
22	8021	8105	8189	8273	8357	8441	8525	8609
23	8022	8106	8190	8274	8358	8442	8526	8610
24	8023	8107	8191	8275	8359	8443	8527	8611
25	8024	8108	8192	8276	8360	8444	8528	8612
26	8025	8109	8193	8277	8361	8445	8529	8613
27	8026	8110	8194	8278	8362	8446	8530	8614
28	8027	8111	8195	8279	8363	8447	8531	8615
29	8028	8112	8196	8280	8364	8448	8532	8616
30	8029	8113	8197	8281	8365	8449	8533	8617
31	8030	8114	8198	8282	8366	8450	8534	8618
32	8031	8115	8199	8283	8367	8451	8535	8619
33	8032	8116	8200	8284	8368	8452	8536	8620
34	8033	8117	8201	8285	8369	8453	8537	8621
35	8034	8118	8202	8286	8370	8454	8538	8622
36	8035	8119	8203	8287	8371	8455	8539	8623
37	8036	8120	8204	8288	8372	8456	8540	8624
38	8037	8121	8205	8289	8373	8457	8541	8625
39	8038	8122	8206	8290	8374	8458	8542	8626
40	8039	8123	8207	8291	8375	8459	8543	8627
41	8040	8124	8208	8292	8376	8460	8544	8628
42	8041	8125	8209	8293	8377	8461	8545	8629
43	8042	8126	8210	8294	8378	8462	8546	8630
44	8043	8127	8211	8295	8379	8463	8547	8631
45	8044	8128	8212	8296	8380	8464	8548	8632

46	8045	8129	8213	8297	8381	8465	8549	8633
47	8046	8130	8214	8298	8382	8466	8550	8634
48	8047	8131	8215	8299	8383	8467	8551	8635
49	8048	8132	8216	8300	8384	8468	8552	8636
50	8049	8133	8217	8301	8385	8469	8553	8637
51	8050	8134	8218	8302	8386	8470	8554	8638
52	8051	8135	8219	8303	8387	8471	8555	8639
53	8052	8136	8220	8304	8388	8472	8556	8640
54	8053	8137	8221	8305	8389	8473	8557	8641
55	8054	8138	8222	8306	8390	8474	8558	8642
56	8055	8139	8223	8307	8391	8475	8559	8643
57	8056	8140	8224	8308	8392	8476	8560	8644
58	8057	8141	8225	8309	8393	8477	8561	8645
59	8058	8142	8226	8310	8394	8478	8562	8646
60	8059	8143	8227	8311	8395	8479	8563	8647
61	8060	8144	8228	8312	8396	8480	8564	8648
62	8061	8145	8229	8313	8397	8481	8565	8649
63	8062	8146	8230	8314	8398	8482	8566	8650
64	8063	8147	8231	8315	8399	8483	8567	8651
65	8064	8148	8232	8316	8400	8484	8568	8652
66	8065	8149	8233	8317	8401	8485	8569	8653
67	8066	8150	8234	8318	8402	8486	8570	8654
68	8067	8151	8235	8319	8403	8487	8571	8655
69	8068	8152	8236	8320	8404	8488	8572	8656
70	8069	8153	8237	8321	8405	8489	8573	8657
71	8070	8154	8238	8322	8406	8490	8574	8658
72	8071	8155	8239	8323	8407	8491	8575	8659
73	8072	8156	8240	8324	8408	8492	8576	8660
74	8073	8157	8241	8325	8409	8493	8577	8661
75	8074	8158	8242	8326	8410	8494	8578	8662
76	8075	8159	8243	8327	8411	8495	8579	8663
77	8076	8160	8244	8328	8412	8496	8580	8664
78	8077	8161	8245	8329	8413	8497	8581	8665
79	8078	8162	8246	8330	8414	8498	8582	8666
80	8079	8163	8247	8331	8415	8499	8583	8667
81	8080	8164	8248	8332	8416	8500	8584	8668
82	8081	8165	8249	8333	8417	8501	8585	8669
83	8082	8166	8250	8334	8418	8502	8586	8670
84	8083	8167	8251	8335	8419	8503	8587	8671

WHAT IS CLAIMED IS:

1. An isolated antigen binding protein (ABP) that specifically binds a human cluster of differentiation 80 (B7-H4), comprising:

(a) a CDR3-L having a sequence selected from SEQ ID NOS: 3001-3012 and a CDR3-H having a sequence selected from SEQ ID NOS: 6001-6012; or

(b) a CDR3-L having a sequence selected from SEQ ID NOS: 8336-8419; and a CDR3-H having a sequence selected from SEQ ID NOS: 8588-8671; or

(c) a CDR3-L having a sequence of the CD3-L of any one of the clones in the library deposited under ATCC Accession No. PTA-125511 and a CDR3-L having a sequence of the CD3-L of any one of the clones in the library deposited under ATCC Accession No. PTA-125511.

2. The ABP of claim 1, wherein the CDR3-L and the CDR3-H are a cognate pair.

3. The ABP of claim 1, comprising

(a) a CDR1-L having a sequence selected from SEQ ID NOS: 1001-1012 and a CDR2-L having a sequence selected from SEQ ID NOS: 2001-2012; and a CDR1-H having a sequence selected from SEQ ID NOS: 4001-4012; and a CDR2-H having a sequence selected from SEQ ID NOS: 5001-5012; or

(b) a CDR1-L having a sequence selected from SEQ ID NOS: 8168-8251; and a CDR2-L having a sequence selected from SEQ ID NOS: 8252-8335; and a CDR1-H having a sequence selected from SEQ ID NOS: 8420-8503; and a CDR2-H having a sequence selected from SEQ ID NOS: 8504-8587; or

(c) a CDR1-L having a sequence selected from a CDR1-L of any one of the clones in the library deposited under ATCC Accession No. PTA-125511; and a CDR2-L having a sequence selected from a CDR2-L of any one of the clones in the library deposited under ATCC Accession No. PTA-125511; and a CDR1-H having a sequence selected from a CDR1-H of any one of the clones in the library deposited under ATCC Accession No. PTA-125511; and a CDR2-H having a sequence selected from a CDR2-H of any one of the clones in the library deposited under ATCC Accession No. PTA-125511;

4. The ABP of claim 1, comprising a CDR1-L, a CDR2-L, a CDR3-L, a CDR1-H, a CDR2-H and a CDR3-H, wherein

the CDR1-L consists of SEQ ID NO: 1001, the CDR2-L consists of SEQ ID NO: 2001, the CDR3-L consists of SEQ ID NO: 3001, the CDR1-H consists of SEQ ID NO: 4001, the CDR2-H consists of SEQ ID NO: 5001 and the CDR3-H consists of SEQ ID NO: 6001; or

the CDR1-L consists of SEQ ID NO: 1002, CDR2-L consists of SEQ ID NO: 2002, the CDR3-L consists of SEQ ID NO: 3002, the CDR1-H consists of SEQ ID NO: 4002, the CDR2-H consists of SEQ ID NO: 5002 and the CDR3-H consists of SEQ ID NO: 6002; or

the CDR1-L consists of SEQ ID NO: 1003, the CDR2-L consists of SEQ ID NO: 2003, the CDR3-L consists of SEQ ID NO: 3003, the CDR1-H consists of SEQ ID NO: 4003, the CDR2-H consists of SEQ ID NO: 5003 and the CDR3-H consists of SEQ ID NO: 6003; or

the CDR1-L consists of SEQ ID NO: 1004, the CDR2-L consists of SEQ ID NO: 2004, the CDR3-L consists of SEQ ID NO: 3004, the CDR1-H consists of SEQ ID NO: 4004, the CDR2-H consists of SEQ ID NO: 5004 and the CDR3-H consists of SEQ ID NO: 6004; or

the CDR1-L consists of SEQ ID NO: 1005, the CDR2-L consists of SEQ ID NO: 2005, the CDR3-L consists of SEQ ID NO: 3005, the CDR1-H consists of SEQ ID NO: 4005, the CDR2-H consists of SEQ ID NO: 5005 and the CDR3-H consists of SEQ ID NO: 6005; or

the CDR1-L consists of SEQ ID NO: 1006, the CDR2-L consists of SEQ ID NO: 2006, the CDR3-L consists of SEQ ID NO: 3006, the CDR1-H consists of SEQ ID NO: 4006, the CDR2-H consists of SEQ ID NO: 5006 and the CDR3-H consists of SEQ ID NO: 6006; or

the CDR1-L consists of SEQ ID NO: 1007, the CDR2-L consists of SEQ ID NO: 2007, the CDR3-L consists of SEQ ID NO: 3007, the CDR1-H consists of SEQ ID NO: 4007, the CDR2-H consists of SEQ ID NO: 5007 and the CDR3-H consists of SEQ ID NO: 6007; or

the CDR1-L consists of SEQ ID NO: 1008, the CDR2-L consists of SEQ ID NO: 2008, the CDR3-L consists of SEQ ID NO: 3008, the CDR1-H consists of SEQ ID NO: 4008, the CDR2-H consists of SEQ ID NO: 5008 and the CDR3-H consists of SEQ ID NO: 6008 or

the CDR1-L consists of SEQ ID NO: 1009, the CDR2-L consists of SEQ ID NO: 2009, the CDR3-L consists of SEQ ID NO: 3009, the CDR1-H consists of SEQ ID NO: 4009, the CDR2-H consists of SEQ ID NO: 5009 and the CDR3-H consists of SEQ ID NO: 6009; or

the CDR1-L consists of SEQ ID NO: 1010, the CDR2-L consists of SEQ ID NO: 2010, the CDR3-L consists of SEQ ID NO: 3010, the CDR1-H consists of SEQ ID NO: 4010, the CDR2-H consists of SEQ ID NO: 5010 and the CDR3-H consists of SEQ ID NO: 6010; or

the CDR1-L consists of SEQ ID NO: 1011, the CDR2-L consists of SEQ ID NO: 2011, the CDR3-L consists of SEQ ID NO: 3011, the CDR1-H consists of SEQ ID NO: 4011, the CDR2-H consists of SEQ ID NO: 5011 and the CDR3-H consists of SEQ ID NO: 6011; or

the CDR1-L consists of SEQ ID NO: 1012, the CDR2-L consists of SEQ ID NO: 2012, the CDR3-L consists of SEQ ID NO: 3012, the CDR1-H consists of SEQ ID NO: 4012, the CDR2-H consists of SEQ ID NO: 5012 and the CDR3-H consists of SEQ ID NO: 6012.

5. The ABP of claim 1, comprising

a variable light chain (V_L) comprising a sequence at least 97% identical to a sequence selected from SEQ ID NOS: 1-12 and a variable heavy chain (V_H) comprising a sequence at least 97% identical to a sequence selected from SEQ ID NOS: 101-112; or

a variable light chain (V_L) comprising a sequence at least 97% identical to a sequence selected from SEQ ID NOS: 8000-8083; and a variable heavy chain (V_H) comprising a sequence at least 97% identical to a sequence selected from SEQ ID NOS: 8084-8167; or

a variable light chain (V_L) comprising a sequence at least 97% identical to a V_L sequence of any one of the clones in the library deposited under ATCC Accession No. PTA-125511 and a variable heavy chain (V_H) comprising a sequence at least 97% identical to a V_H sequence of any one of the clones in the library deposited under ATCC Accession No. PTA-125511.

6. The ABP of claim 5, wherein the V_L and the V_H are a cognate pair.

7. The ABP of claim 1, comprising

a variable light chain (V_L) comprising a sequence selected from SEQ ID NOS: 1-12 and a variable heavy chain (V_H) comprising a a sequence selected from SEQ ID NOS: 101-112 or

a variable light chain (V_L) comprising a sequence selected from SEQ ID NOS: 8000-8083; and a variable heavy chain (V_H) comprising a sequence selected from SEQ ID NOS: 8084-8167; or

a variable light chain (V_L) comprising a V_L sequence of any one of the clones in the library deposited under ATCC Accession No. PTA-125511 and a variable heavy chain (V_H) comprising a V_H sequence of any one of the clones in the library deposited under ATCC Accession No. PTA-125511.

8. The ABP of claim 7, wherein the V_L and the V_H are a cognate pair.

9. The ABP of any of claims 1-8, wherein the ABP comprises an scFv or a full length monoclonal antibody.

10. The ABP of any of claims 1-8, wherein the ABP comprises an immunoglobulin constant region.

11. The ABP of any of the above claims, wherein the ABP binds human B7-H4 with a K_D of less than 500nM, as measured by surface plasmon resonance.

12. The ABP of claim 11, wherein the ABP binds human B7-H4 with a K_D of less than 200nM, as measured by surface plasmon resonance.

13. The ABP of claim 12, wherein the ABP binds human B7-H4 with a K_D of less than 25nM, as measured by surface plasmon resonance.

14. The ABP of any of claims 1-13, wherein the ABP binds to human B7-H4 on a cell surface with a K_D of less than 25nM.

15. A pharmaceutical composition comprising the ABP of any of claims 1-14 and an excipient.

16. A method of treating a disease comprising the step of:

administering to a subject in need thereof an effective amount of the ABP of any of claims 1-14 or the pharmaceutical composition of claim 15.

17. The method of claim 16, wherein the disease is selected from the group consisting of cancer, AIDS, Alzheimer's disease and viral or bacterial infection.

18. The method of any of claims 16-17, further comprising the step of administering one or more additional therapeutic agents to the subject.

19. The method of claim 18, wherein the additional therapeutic agent is selected from CTLA-4 inhibitor, TIGIT inhibitor, a chemotherapy agent, an immune-stimulatory agent, radiation, a cytokine, a polynucleotide encoding a cytokine and a combination thereof.

20. An isolated polynucleotide encoding the ABP of any of claims 1-10.

21. A vector comprising the isolated polynucleotide of claim 20.

22. A host cell comprising the isolated polynucleotide of claim 20 or the vector of claim 21.

23. A method of producing an isolated antigen binding protein (ABP) that specifically binds human B7-H4, comprising:

expressing the ABP in the host cell of claim 22, and isolating the ABP.

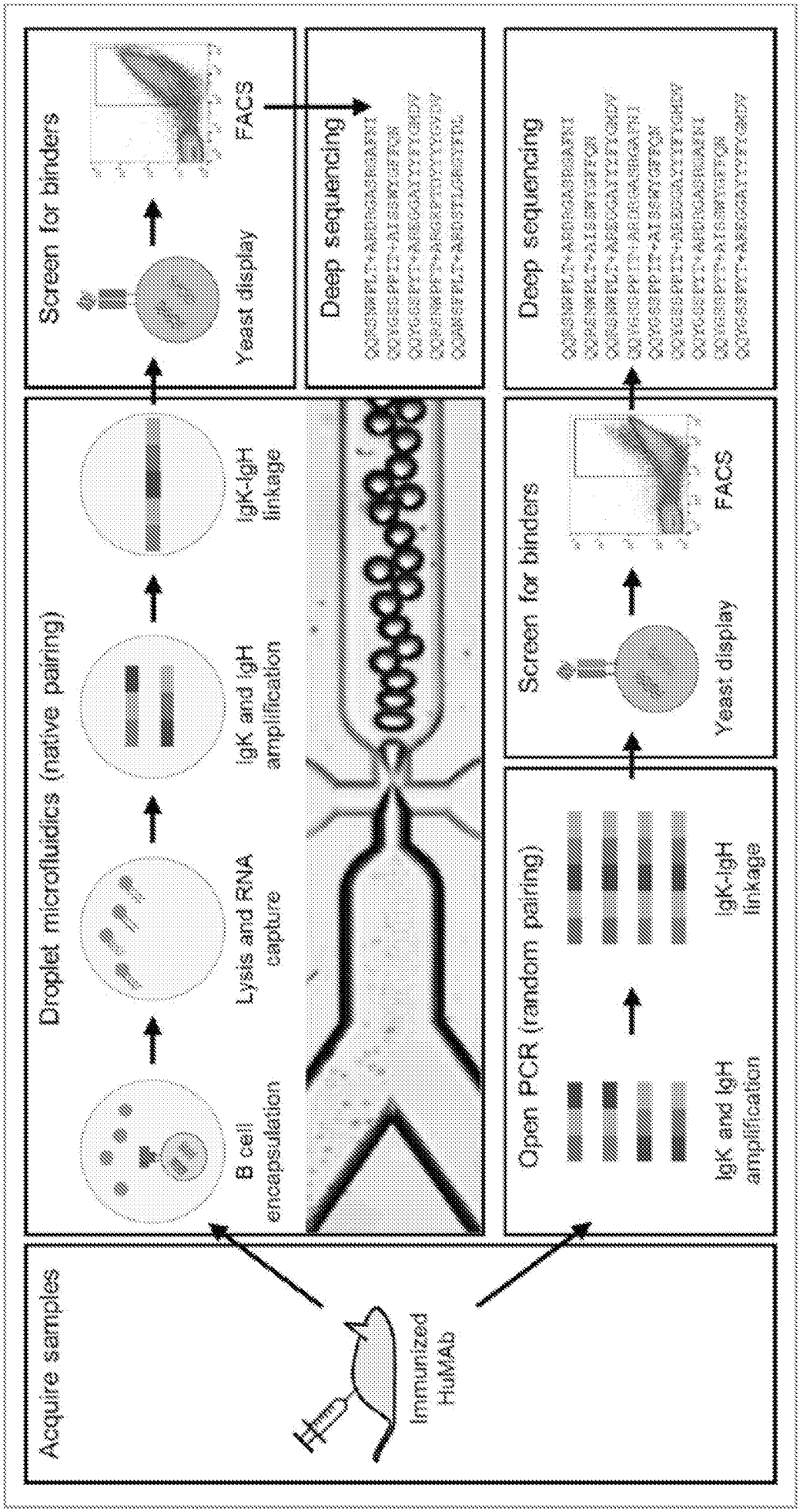

Acquire samples
Immunized HuMAb
Droplet microfluidics (native pairing)
B cell encapsulation
Lysis and RNA capture
IgK and IgH amplification
IgK-IgH linkage
Screen for binders
Yeast display
FACS
Deep sequencing
Open PCR (random pairing)
IgK and IgH amplification
IgK-IgH linkage
Screen for binders
Yeast display
FACS
Deep sequencing

FIG. 1

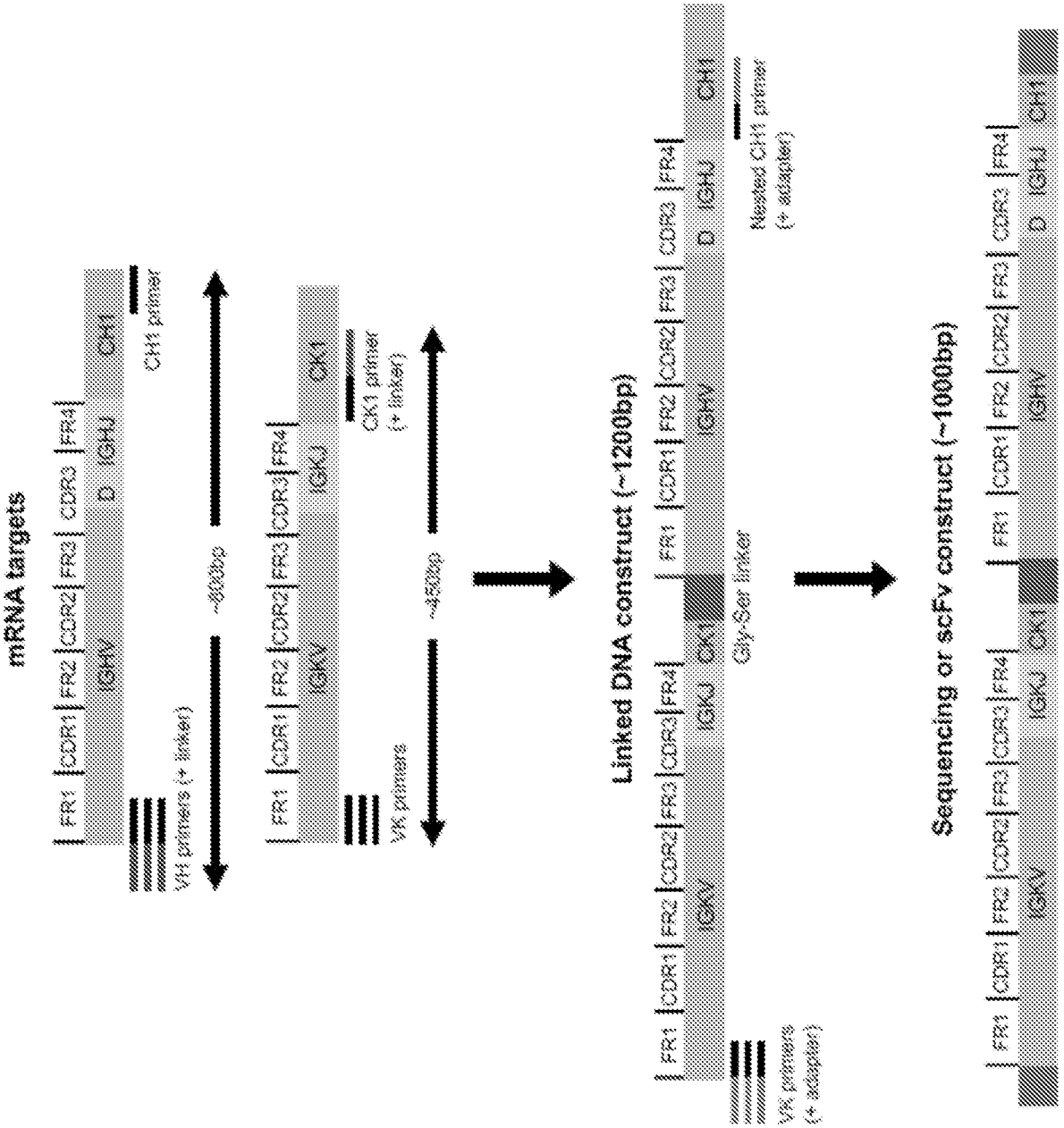

mRNA targets
FR1 CDR1 FR2 CDR2 FR3 CDR3 FR4
IGHV D IGHJ CH1
CH1 primer
VH primers (+ linker)
~600bp
FR1 CDR1 FR2 CDR2 FR3 CDR3 FR4
IGKV IGKJ CK1
CK1 primer (+ linker)
VK primers
~450bp
Linked DNA construct (~1200bp)
IGKV IGKJ CK1 Gly-Ser linker IGHV D IGHJ CH1
VK primers (+ adapter)
Nested CH1 primer (+ adapter)
Sequencing or scFv construct (~1000bp)
IGKV IGKJ CK1 Gly-Ser linker IGHV D IGHJ CH1

FIG. 2

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2019/068800

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A61K 39/395; C07K 16/28; C07K 16/46; C07K 19/00; C12N 15/13; C12P 21/08 (2020.01)

CPC - A61K 2039/505; C07K 16/28; C07K 16/2827; C07K 16/2896; C07K 2317/56; C07K 2317/565; C07K 2319/00 (2020.02)

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

USPC - 424/130.1; 424/133.1; 424/134.1; 435/328; 530/387.3; 530/388.1; 536/23.53 (keyword delimited)

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.
A	US 2015/0315275 A1 (AMPLIMMUNE, INC. et al) 05 November 2015 (05.11.2015) entire document	1-10
A	US 2013/0197202 A1 (BRISTOL-MYERS SQUIBB COMPANY) 01 August 2013 (01.08.2013) entire document	1-10
A	US 2017/0239366 A1 (GENENTECH, INC.) 24 August 2017 (24.08.2017) entire document	1-10
A	WO 2016/070001 A1 (JOUNCE THERAPEUTICS, INC.) 06 May 2016 (06.05.2016) entire document	1-10
A	US 2014/0356364 A1 (LANGERMANN et al) 04 December 2014 (04.12.2014) entire document	1-10
A	US 2011/0085970 A1 (TERRETT et al) 14 April 2011 (14.04.2011) entire document	1-10

☐ 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

"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
20 April 2020	05 JUN 2020
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, VA 22313-1450 Facsimile No. 571-273-8300	Authorized officer Blaine R. Copenheaver PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2019/068800

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. ☒ forming part of the international application as filed:

      ☒ in the form of an Annex C/ST.25 text file.

      ☐ on paper or in the form of an image file.

   b. ☐ furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

   c. ☐ furnished subsequent to the international filing date for the purposes of international search only:

      ☐ in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).

      ☐ on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

SEQ ID NOs: 1-12 and 101-108 were searched.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2019/068800

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.: 11-23
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 extra sheet(s).

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.:

1-10 to the extent that they read on an anti-human B7-H4 binding protein of SEQ ID NOs: 1, 101, 1001, 2001, 3001, 4001, 5001, and 6001.

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.

Continued from 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 examined, the appropriate additional examination fees need to be paid.

Group I+: claims 1-10 are drawn to isolated anti-human B7-H4 binding proteins.

The first invention of Group I+ is restricted to an anti-human B7-H4 binding protein, the anti-human B7-H4 binding protein comprising a heavy chain variable region, wherein in the heavy chain variable region is selected to be SEQ ID NO:101, the heavy chain variable region further comprising heavy chain complementarity determining regions CDR1, CDR2, and CDR3, wherein CDR1 is SEQ ID NO:4001, CDR2 is SEQ ID NO:5001, and CDR3 is SEQ ID NO:6001; and a light chain variable region, wherein the light chain variable region is selected to be SEQ ID NO:1, the light chain variable region further comprising light chain complementarity determining regions CDR1, CDR2, and CDR3, where CDR1 is SEQ ID NO:1001, CDR2 is SEQ ID NO:2001, and CDR3 is SEQ ID NO:3001. It is believed that claims 1-10 read on this first named invention and thus these claims will be searched without fee to the extent that they read on an anti-human B7-H4 binding protein of SEQ ID NOs: 1, 101, 1001, 2001, 3001, 4001, 5001, and 6001.

Applicant is invited to elect additional anti-human B7-H4 binding proteins, each with a specified SEQ ID NO for each heavy chain variable region and light chain variable region, to be searched in a specific combination by paying an additional fee for each set of election. An exemplary election would be an anti-human B7-H4 binding protein, the anti-human B7-H4 binding protein comprising a heavy chain variable region, wherein in the heavy chain variable region is selected to be SEQ ID NO:102, the heavy chain variable region further comprising heavy chain complementarity determining regions CDR1, CDR2, and CDR3, wherein CDR1 is SEQ ID NO:4002, CDR2 is SEQ ID NO:5002, and CDR3 is SEQ ID NO:6002; and a light chain variable region, wherein the light chain variable region is selected to be SEQ ID NO:2, the light chain variable region further comprising light chain complementarity determining regions CDR1, CDR2, and CDR3, where CDR1 is SEQ ID NO:1002, CDR2 is SEQ ID NO:2002, and CDR3 is SEQ ID NO:3002. Additional anti-human B7-H4 binding proteins will be searched upon the payment of additional fees. Applicants must specify the claims that read on any additional elected inventions. Applicants must further indicate, if applicable, the claims which read on 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/examined.

The inventions listed in Groups I+ 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:

The Groups I+ formulas do not share a significant structural element responsible for binding human B7-H4, requiring the selection of alternatives for the amino acid sequence of the heavy and light chain variable chain regions, where "...[the] variable light chain (VL) compris[es] a sequence at least 97% identical to a sequence selected from SEQ ID NOs: 1-12 and ...[the] variable heavy chain (VH) compris[es] a sequence at least 97% identical to a sequence selected from SEQ ID NOs: 101-112; or ...[the] variable light chain (VL) compris[es] a sequence at least 97% identical to a sequence selected from SEQ ID NOs: 8000-8083; and ...[the] variable heavy chain (VH) compris[es] a sequence at least 97% identical to a sequence selected from SEQ ID NOs: 8084-8167; or ...[the] variable light chain (VL) compris[es] a sequence at least 97% identical to a VL sequence of any one of the clones in the library deposited under ATCC Accession No. PTA-125511 and a variable heavy chain (VH) compris[es] a sequence at least 97% identical to a VH sequence of any one of the clones in the library deposited under ATCC Accession No. PTA-125511".

Additionally, even if Groups I+ were considered to share the technical features of an isolated antigen binding protein (ABP) that specifically binds a human cluster of differentiation 80 (B7-H4), comprising: a variable light chain (VL) comprising a CDR1-L, a CDR2-L, and a CDR3-L and a variable heavy chain (VH) comprising a CDR1-H, a CDR2-H, and a CDR3-H; these shared technical features do not represent a contribution over the prior art.

Specifically, WO 2016/070001 A1 to Jounce Therapeutics, Inc. discloses an isolated antigen binding protein (ABP) that specifically binds a human cluster of differentiation 80 (B7-H4) (a human B7-H4 antibody, Para. [0197]; 8E7 antibody clone which recognizes the human B7-H4, Para. [0255]), comprising: a variable light chain (VL) comprising a CDR1-L, a CDR2-L, and a CDR3-L and a variable heavy chain (VH) comprising a CDR1-H, a CDR2-H, and a CDR3-H (a B7-H4 antibody ....the antibody has six of the CDRs provided ....the antibody has the heavy chain and the light chain variable region provided, Para. [0161]; [t]he various CDRs within an antibody can be designated by their appropriate number and chain type, including... CDR-L1, CDR-L2, CDR-L3, CDR-H1, CDR-H2, and CDR-H3, Para. [075]; the B7-H4 antibody comprises... three heavy chain CDRs that are the heavy chain CDRs ...and three light chain CDRs that are the light chain, Claim 28).

The inventions listed in Groups I+ therefore lack unity under Rule 13 because they do not share a same or corresponding special technical features.