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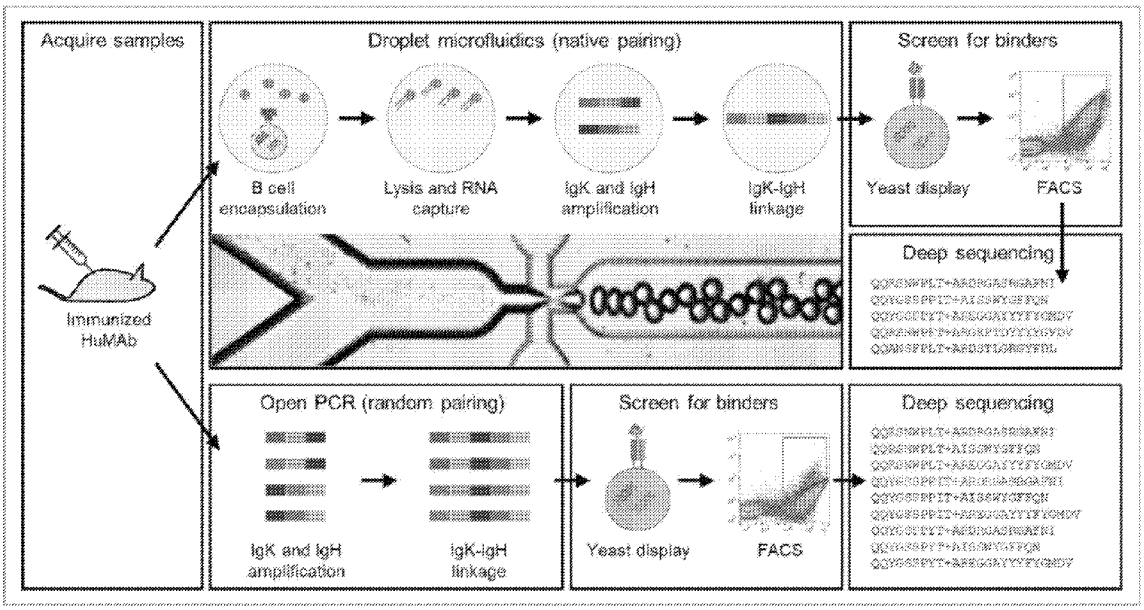

FIG. 1

(57) Abstract: Provided herein are antigen-binding proteins (ABPs) that selectively bind to CTLA-4 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-CTLA-4 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. 62/785,659, 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 11998 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-010WO_SL.txt, and is 1,927,908 bytes in size.

3. FIELD

[0003] Provided herein are antigen-binding proteins (ABPs) with binding specificity for CTLA-4 and compositions comprising such ABPs, including pharmaceutical compositions, diagnostic compositions, and kits. Also provided are methods of making CTLA-4 ABPs, and methods of using CTLA-4 ABPs, for example, for therapeutic purposes, diagnostic purposes, and research purposes.

4. BACKGROUND

[0004] CTLA-4, also known as cytotoxic T-lymphocyte associated protein 4 and CD152 (cluster of differentiation 152), is a cell surface receptor that suppresses T cell inflammatory activity. CTLA-4 is constitutively expressed by regulatory T cells (Tregs) and upregulated in stimulated T cells. CD80 and CD86, also expressed in antigen presenting cells (APCs) such as dendritic cells (DCs), are the primary ligands of CTLA-4. The interaction between CTLA-4 and its ligands is vitally important for downregulating the immune responses and promoting self-tolerance by suppressing T cell inflammatory activity. This activity prevents autoimmune diseases, as well as prevents the immune system from killing cancer cells.

[0005] CTLA-4 is a member of the immunoglobulin superfamily that is expressed by activated T cells and transmits an inhibitory signal to T cells. CTLA-4 binds CD80 and CD86 with greater affinity and avidity than CD28 thus enabling it to outcompete CD28 for its ligands. CTLA-4 transmits an inhibitory signal to T cells, whereas CD28 transmits a stimulatory signal. CTLA-4 is also found in regulatory T cells (Tregs) and contributes to their inhibitory function. T cell activation through the T cell receptor and CD28 leads to increased expression of CTLA-4. The mechanism by which CTLA-4 acts in T cells remains somewhat controversial. Biochemical evidence suggested that CTLA-4 recruits a phosphatase to the T cell receptor (TCR), thus attenuating the signal. This work remains unconfirmed in the literature since its first publication. More recent work has suggested that CTLA-4 may function in vivo by capturing and removing B7-1 and B7-2 from the membranes of antigen-presenting cells, thus making these unavailable for triggering of CD28.

[0006] Variants in CTLA-4 have been associated with insulin-dependent diabetes mellitus, Graves' disease, Hashimoto's thyroiditis, celiac disease, systemic lupus erythematosus, thyroid-associated orbitopathy, primary biliary cirrhosis and other autoimmune diseases. The comparatively high binding affinity of CTLA-4 for CD80 and CD86 has made it a potential therapy for autoimmune diseases. Soluble fusion proteins of CTLA-4 and antibodies (CTLA-4-Ig) have been used in clinical trials for rheumatoid arthritis.

[0007] Tumor cells suppress anti-tumor immune response through various mechanisms, including up-regulation of Tregs. Recently, CTLA-4 inhibitors have been shown to antagonize binding of CTLA-4 to its ligands, thereby activating the immune system to attack tumors. CTLA-4 antibodies have also been used to induce antibody-dependent cell-mediated cytotoxicity (ADCC) of Tregs specific to the tumor microenvironment, thus reducing immune tolerance to the tumor. CTLA-4 antibodies have been therefore used with varying success to treat some types of cancer.

[0008] Thus, there is a need for developing CTLA-4 ABPs that can be used for treatment, diagnosis, and research of various diseases, including cancer and autoimmune disease.

5. SUMMARY

[0009] Provided herein are novel ABPs with binding specificity for CTLA-4 and methods of using such ABPs. The CTLA-4 is a human CTLA-4 (SEQ ID: 7001) or a fragment of the human CTLA-4.

[0010] 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).

[0011] The ABPs provided herein can induce various biological effects associated with inhibition or activation of CTLA-4. In some embodiments, an ABP provided herein prevents binding between CTLA-4 and its ligands. In some embodiments, an ABP provided herein prevents inhibition of an effector T cell by a Treg. In some embodiments, the ABP directly kills or induces killing of Tregs or other CTLA-4-expressing cells in the tumor microenvironment by ADCC and/or ADCP, for example mediated by binding of NK cell-expressed CD16 to the ABP Fc domain. In some embodiments, the ABP inhibits the suppression of an effector T cell by a regulatory T cell by directly killing Tregs. In some embodiments, the tissue is a tumor. In some embodiments, the ABP activates CTLA-4, leading to Treg expansion and activation.

[0012] Also provided are kits comprising one or more of the pharmaceutical compositions comprising the ABPs, and instructions for use of the pharmaceutical composition.

[0013] Also provided are isolated polynucleotides encoding the ABPs provided herein, and portions thereof.

[0014] Also provided are vectors comprising such polynucleotides.

[0015] Also provided are recombinant host cells comprising such polynucleotides and recombinant host cells comprising such vectors.

[0016] Also provided are methods of producing the ABP using the polynucleotides, vectors, or host cells provided herein.

[0017] Also provided are pharmaceutical compositions comprising the ABPs and a pharmaceutically acceptable excipient.

[0018] 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.

[0019] More specifically, the present disclosure provides an isolated antigen binding protein (ABP) that specifically binds a human cytotoxic T-lymphocyte associated protein 4 (CTLA-4), comprising: (a) a CDR3-L having a sequence selected from SEQ ID NOS: 3001-3028 and a CDR3-H having a sequence selected from SEQ ID NOS: 6001-6028; or (b) a CDR3-L having a sequence selected from SEQ ID NOS: 9984-10479 and a CDR3-H having a sequence selected from SEQ ID NOS: 11472-11967; 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-125512 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-125512. In some embodiments, the CDR3-L and the CDR3-H are a cognate pair.

[0020] In some embodiments, the ABP comprises (a) a CDR1-L having a sequence selected from SEQ ID NOS: 1001-1028 and a CDR2-L having a sequence selected from SEQ ID NOS: 2001-2028; and a CDR1-H having a sequence selected from SEQ ID NOS: 4001-4028; and a CDR2-H having a sequence selected from SEQ ID NOS: 5001-5028; or (b) a CDR1-L having a sequence selected from SEQ ID NOS: 8992-9487; and a CDR2-L having a sequence selected from SEQ ID NOS: 9488-9983; and a CDR1-H having a sequence selected from SEQ ID NOS: 10480-10975;

and a CDR2-H having a sequence selected from SEQ ID NOS: 10976-11471; 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-125512; 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-125512; 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-125512; 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-125512;

[0021] 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; or the CDR1-L consists of SEQ ID NO: 1013, the CDR2-L consists of SEQ ID NO: 2013, the CDR3-L consists of SEQ ID NO: 3013, the CDR1-H consists of SEQ ID NO: 4013, the CDR2-H consists of SEQ ID NO: 5013 and the CDR3-H consists of SEQ ID NO: 6013; or the CDR1-L consists of SEQ ID NO: 1014, the CDR2-L consists of SEQ ID NO: 2014, the CDR3-L consists of SEQ ID NO: 3014, the CDR1-H consists of SEQ ID NO: 4014, the CDR2-H consists of SEQ ID NO: 5014 and the CDR3-H consists of SEQ ID NO: 6014; or the CDR1-L consists of SEQ ID NO: 1015, the CDR2-L consists of SEQ ID NO: 2015, the CDR3-L consists of SEQ ID NO: 3015, the CDR1-H consists of SEQ ID NO: 4015, the CDR2-H consists of SEQ ID NO: 5015 and the CDR3-H consists of SEQ ID NO: 6015; or the CDR1-L consists of SEQ ID NO: 1016, the CDR2-L consists of SEQ ID NO: 2016, the CDR3-L consists of SEQ ID NO: 3016, the CDR1-H consists of SEQ ID NO: 4016, the CDR2-H consists of SEQ ID NO: 5016 and the CDR3-H consists of SEQ ID NO: 6016; or the CDR1-L consists of SEQ ID NO: 1017, the CDR2-L consists of SEQ ID NO: 2017, the CDR3-L consists of SEQ ID NO: 3017, the CDR1-H consists of SEQ ID NO: 4017, the CDR2-H consists of SEQ ID NO: 5017 and the CDR3-H consists of SEQ ID NO: 6017; or the CDR1-L consists of SEQ ID NO: 1018, the CDR2-L consists of SEQ ID NO: 2018, the CDR3-L consists of SEQ ID NO: 3018, the CDR1-H consists of SEQ ID NO: 4018, the CDR2-H consists of SEQ ID NO: 5018 and the CDR3-H consists of SEQ ID NO: 6018; or the CDR1-L consists of SEQ ID NO: 1019, the CDR2-L consists of SEQ ID NO: 2019, the CDR3-L consists of SEQ ID NO: 3019, the CDR1-H consists of SEQ ID NO: 4019, the CDR2-H consists of SEQ ID NO: 5019 and the CDR3-H consists of SEQ ID NO: 6019; or the CDR1-L consists of SEQ ID NO: 1020, the CDR2-L consists of SEQ ID NO: 2020, the CDR3-L consists of SEQ ID NO: 3020, the CDR1-H consists of SEQ ID NO: 4020, the CDR2-H consists of SEQ ID NO: 5020 and the CDR3-H consists of SEQ ID NO: 6020; or the CDR1-L consists of SEQ ID NO: 1021, the CDR2-L consists of SEQ ID NO: 2021, the CDR3-L consists of SEQ ID NO: 3021, the CDR1-H

consists of SEQ ID NO: 4021, the CDR2-H consists of SEQ ID NO: 5021 and the CDR3-H consists of SEQ ID NO: 6021; or the CDR1-L consists of SEQ ID NO: 1022, the CDR2-L consists of SEQ ID NO: 2022, the CDR3-L consists of SEQ ID NO: 3022, the CDR1-H consists of SEQ ID NO: 4022, the CDR2-H consists of SEQ ID NO: 5022 and the CDR3-H consists of SEQ ID NO: 6022; or the CDR1-L consists of SEQ ID NO: 1023, the CDR2-L consists of SEQ ID NO: 2023, the CDR3-L consists of SEQ ID NO: 3023, the CDR1-H consists of SEQ ID NO: 4023, the CDR2-H consists of SEQ ID NO: 5023 and the CDR3-H consists of SEQ ID NO: 6023; or the CDR1-L consists of SEQ ID NO: 1024, the CDR2-L consists of SEQ ID NO: 2024, the CDR3-L consists of SEQ ID NO: 3024, the CDR1-H consists of SEQ ID NO: 4024, the CDR2-H consists of SEQ ID NO: 5024 and the CDR3-H consists of SEQ ID NO: 6024; or the CDR1-L consists of SEQ ID NO: 1025, the CDR2-L consists of SEQ ID NO: 2025, the CDR3-L consists of SEQ ID NO: 3025, the CDR1-H consists of SEQ ID NO: 4025, the CDR2-H consists of SEQ ID NO: 5025 and the CDR3-H consists of SEQ ID NO: 6025; or the CDR1-L consists of SEQ ID NO: 1026, the CDR2-L consists of SEQ ID NO: 2026, the CDR3-L consists of SEQ ID NO: 3026, the CDR1-H consists of SEQ ID NO: 4026, the CDR2-H consists of SEQ ID NO: 5026 and the CDR3-H consists of SEQ ID NO: 6026; or the CDR1-L consists of SEQ ID NO: 1027, the CDR2-L consists of SEQ ID NO: 2027, the CDR3-L consists of SEQ ID NO: 3027, the CDR1-H consists of SEQ ID NO: 4027, the CDR2-H consists of SEQ ID NO: 5027 and the CDR3-H consists of SEQ ID NO: 6027; or the CDR1-L consists of SEQ ID NO: 1028, the CDR2-L consists of SEQ ID NO: 2028, the CDR3-L consists of SEQ ID NO: 3028, the CDR1-H consists of SEQ ID NO: 4028, the CDR2-H consists of SEQ ID NO: 5028 and the CDR3-H consists of SEQ ID NO: 6028.

[0022] 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: 1-28 and a variable heavy chain (V_H) comprising a sequence at least 97% identical to a sequence selected from SEQ ID NOS: 101-128; or a variable light chain (V_L) comprising a sequence at least 97% identical to a sequence selected from SEQ ID NOS: 8000-8495 and a variable heavy chain (V_H) comprising a sequence at least 97% identical to a sequence selected from SEQ ID NOS: 8496-8991; 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-125512 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-125512. In some embodiments, the V_L and the V_H are a cognate pair.

[0023] In some embodiments, the ABP comprises a variable light chain (V_L) comprising a sequence selected from SEQ ID NOS: 1-28 and a variable heavy chain (V_H) comprising a a sequence selected from SEQ ID NOS: 101-128 or a variable light chain (V_L) comprising a sequence selected from SEQ ID NOS: 8000-8495 and a variable heavy chain (V_H) comprising a sequence selected from SEQ ID NOS: 8496-8991; 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-125512 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-125512. In some embodiments, the V_L and the V_H are a cognate pair.

[0024] In some embodiments, the ABP comprises an scFv or a full length monoclonal antibody. In some embodiments, the ABP comprises an immunoglobulin constant region.

[0025] In some embodiments, the ABP binds human CTLA-4 with a K_D of less than 500nM, as measured by surface plasmon resonance. In some embodiments, the ABP binds human CTLA-4 with a K_D of less than 200nM, as measured by surface plasmon resonance. In some embodiments, the ABP binds human CTLA-4 with a K_D of less than 25nM, as measured by surface plasmon resonance. In some embodiments, the ABP binds to human CTLA-4 on a cell surface with a K_D of less than 25nM.

[0026] 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 method 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.

6. BRIEF DESCRIPTION OF THE DRAWINGS

[0027] **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 SEQ ID NOS 11971-11998, respectively, in order of appearance.

[0028] **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.

[0029] FIG. 3 includes a schematic for the monoclonal antibodies sorted in their epitope bins.

[0030] FIG. 4 includes plots from the histopathological staining of hCTLA-4 KI mice bearing MC38 tumors. The plots show scoring of H&E, immunoglobulin (Ig), and C3 stains from the right kidney. ipi is Ipilimumab, and CTLA4.A14.2a is antibody A14 cloned onto a mouse IgG2a backbone.

[0031] FIG. 5 includes a plot showing the alkaline phosphatase levels in treated hCTLA4 KI mice bearing MC38 tumors. IPI is Ipilimumab, and CTLA4.A14.2a is antibody A14 cloned onto a mouse IgG2a backbone. U/L is units per liter.

[0032] FIG. 6 includes plots for the percentages of intratumoral regulatory T cells (Treg) cells and intratumoral natural killer (NK) cells after the indicated treatments.

[0033] FIG. 7 includes a plot showing the changes in body weight of the hCTLA4 mice receiving the indicated treatments. Ipi is Ipilimumab, and CTLA4.A14.2a is antibody A14 cloned onto a mouse IgG2a backbone. Error bars represent +/- standard error of the mean.

[0034] FIG. 8 includes plots showing the influence of the control, Ipi and the anti-CTLA4 treatments on percentage of the indicated cell populations. Ipi is Ipilimumab, and CTLA4.A14.2a is antibody A14 cloned onto a mouse IgG2a backbone.

[0035] FIG. 9 includes plots showing the influence of the control, Ipi and the anti-CTLA4 treatments on percentage of the indicated cell populations. Ipi is Ipilimumab, and CTLA4.A14.2a is antibody A14 cloned onto a mouse IgG2a backbone.

[0036] FIG. 10 includes plots showing the influence of the control, Ipi and the anti-CTLA4 treatments on percentage of dendritic cells (DCs) and activated dendritic cells (CD86+). Ipi is Ipilimumab, and CTLA4.A14.2a is antibody A14 cloned onto a mouse IgG2a backbone.

[0037] FIG. 11 includes a plot showing the mean tumor volume after treatment with 0.3 mg/kg of the indicated anti-CTLA4s.

7. DETAILED DESCRIPTION

7.1. Definitions

[0038] 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.

[0039] The following terms, unless otherwise indicated, shall be understood to have the following meanings:

[0040] The terms "CTLA-4," "CTLA-4 protein," and "CTLA-4 antigen" are used interchangeably herein to refer to human CTLA-4, or any variants (*e.g.*, splice variants and allelic variants), isoforms, and species homologs of human CTLA-4 that are naturally expressed by cells, or that are expressed by cells transfected with a *ctla4* gene. In some aspects, the CTLA-4 protein is a CTLA-4 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 CTLA-4 protein is human CTLA-4 (hCTLA-4; SEQ ID NO: 7001).

[0041] 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.

[0042] 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 "CTLA-4 ABP," "anti-CTLA-4 ABP," or "CTLA-4-specific ABP" is an ABP, as provided herein, which specifically binds to the antigen CTLA-4. In some embodiments, the ABP binds the extracellular domain of CTLA-4. In certain embodiments, a CTLA-4 ABP provided herein binds to an epitope of CTLA-4 that is conserved between or among CTLA-4 proteins from different species.

[0043] 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.

[0044] 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.

[0045] The term "antigen-binding domain" means the portion of an ABP that is capable of specifically binding to an antigen or epitope.

[0046] 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.

[0047] 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.

[0048] 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.

[0049] 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.

[0050] 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.

[0051] 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.

[0052] 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.

[0053] 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.

[0054] 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*).

[0055] 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.

[0056] "Fv" fragments comprise a non-covalently-linked dimer of one heavy chain variable domain and one light chain variable domain.

[0057] "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.

[0058] "$F(ab')_2$" 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.

[0059] "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: 11968).

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.

[0060] "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.

[0061] 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.

[0062] 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.

[0063] 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.

[0064] 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.

[0065] "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.

[0066] 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 antibodies.

[0067] 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.

[0068] "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®).

[0069] 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 CTLA-4 ABP for a non-target molecule is less than about 50% of the affinity for CTLA-4. In some aspects, the affinity of a CTLA-4 ABP for a non-target molecule is less than about 40% of the affinity for CTLA-4. In some aspects, the affinity of a CTLA-4 ABP for a non-target molecule is less than about 30% of the affinity for CTLA-4. In some aspects, the affinity of a CTLA-4 ABP for a non-target molecule is less than about 20% of the affinity for CTLA-4. In some aspects, the affinity of a CTLA-4 ABP for a non-target molecule is less than about 10% of the affinity for CTLA-4. In some aspects, the affinity of a CTLA-4 ABP for a non-target molecule is less than about 1% of the affinity for CTLA-4. In some aspects, the affinity of a CTLA-4 ABP for a non-target molecule is less than about 0.1% of the affinity for CTLA-4.

[0070] 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.

[0071] 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.

[0072] The term "K_D" (M), as used herein, refers to the dissociation equilibrium constant of a particular ABP -antigen interaction. $K_D = k_d/k_a$.

[0073] The term "K_A" (M^{-1}), as used herein, refers to the association equilibrium constant of a particular ABP -antigen interaction. $K_A = k_a/k_d$.

[0074] 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.

[0075] An "immunoconjugate" is an ABP conjugated to one or more heterologous molecule(s).

[0076] "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).

[0077] 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.*, CTLA-4). In one exemplary assay, CTLA-4 is coated on a surface and contacted with a first CTLA-4 ABP, after which a second CTLA-4 ABP is added. In another exemplary assay, a first CTLA-4 ABP is coated on a surface and contacted with CTLA-4, and then a second CTLA-4 ABP is added. If the presence of the first CTLA-4 ABP reduces binding of the second CTLA-4 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 CTLA-4 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.

[0078] 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 CTLA-4 variants with different point-mutations, or to chimeric CTLA-4 variants.

[0079] 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.

[0080] 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

[0081] 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."

[0082] 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.

[0083] 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.

[0084] 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.

[0085] 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.

[0086] 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.

[0087] 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.

[0088] 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%.

[0089] 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.

[0090] 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.

[0091] The terms "modulate" and "modulation" refer to reducing or inhibiting or, alternatively, activating or increasing, a recited variable.

[0092] 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.

[0093] 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.

[0094] 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.

[0095] 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.

[0096] 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.

[0097] 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.

[0098] 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.

[0099] 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.

[00100] 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.

[00101] 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]

[00102] 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.

[00103] 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

[00104] 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.

[00105] 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

[00106] 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).

[00107] 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 CTLA-4. The nucleic acid can be isolated by conventional procedures such as polymerase chain reaction (PCR).

[00108] 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.

[00109] The present disclosure further provides nucleic acids that hybridize to other nucleic acids (*e.g.*, nucleic acids comprising a nucleotide sequence of any of CTLA-4 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.

[00110] 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 CTLA-4).

[00111] 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 CTLA-4, 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 CTLA-4 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 CTLA-4) 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.

[00112] 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 CTLA-4 binding portion) of a polypeptide of the present disclosure.

[00113] 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

[00114] 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.

[00115] 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.

[00116] 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.

[00117] In some embodiments, the expression vector is an expression vector purified from one of the clones of the library of CTLA-4 binding clones deposited under ATCC Accession No. PTA-125512. 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 CTLA-4 binding clones deposited under ATCC Accession No. PTA-125512. 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 CTLA-4 binding clones deposited under ATCC Accession No. PTA-125512.

[00118] 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.

[00119] 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.

[00120] 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 CTLA-4 bound thereto. Polypeptides contemplated for use herein include substantially homogeneous recombinant mammalian anti- CTLA-4 antibody polypeptides substantially free of contaminating endogenous materials.

[00121] 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.

[00122] 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).

[00123] 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.

[00124] 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.

[00125] 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

[00126] CTLA-4 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.

[00127] 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).

[00128] 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: 1-28, and heavy chain variable sequences are provided in SEQ ID Nos:101-128.

[00129] 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 CTLA-4) 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-CTLA-4 antibodies that function in checkpoint blockade, discovered using microfluidics and molecular genomics, MAbs (2017).

[00130] 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.

[00131] 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.

[00132] 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.

[00133] 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-CTLA-4 antibody. In another embodiment, all of the CDRs are derived from a human anti-CTLA-4 antibody. In another embodiment, the CDRs from more than one human anti-CTLA-4 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-CTLA-4 antibody, a CDR2 and a CDR3 from the light chain of a second human anti-CTLA-4 antibody, and the CDRs from the heavy chain from a third anti-CTLA-4 antibody. Further, the framework regions may be derived from one of the same anti-CTLA-4 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 CTLA-4).

[00134] 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.

[00135] 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.

[00136] 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).

[00137] 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).

[00138] 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 CTLA-4, 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 CTLA-4 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).

[00139] 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.

[00140] 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.

[00141] 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.

[00142] In another example, individual V_L or V_H chains from an antibody (i.e. CTLA-4 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 CTLA-4). *See*, for example, Portolano et al., J. Immunol. V. 150 (3) pp. 880-887 (1993).

[00143] 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.

[00144] 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.

[00145] 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

[00146] 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 CTLA-4.

[00147] 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.

[00148] Antigen binding proteins in accordance with the present disclosure include antigen binding proteins that inhibit a biological activity of CTLA-4.

[00149] Different antigen binding proteins may bind to different domains of CTLA-4 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 CTLA-4 to its ligands. An antigen binding protein need not completely inhibit a CTLA-4-induced activity to find use in the present disclosure; rather, antigen binding proteins that reduce a particular activity of CTLA-4 are contemplated for use as well. (Discussions herein of particular mechanisms of action for CTLA-4-binding antigen binding proteins in treating particular diseases are illustrative only, and the methods presented herein are not bound thereby.)

[00150] In another aspect, the present disclosure provides antigen binding proteins that comprise a light chain variable region selected from the group consisting of A1LC-A28LC or a heavy chain variable region selected from the group consisting of A1HC-A28HC, 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-A28, includes all members between A1 and A28, as well as members A1 and A28 themselves. The group range A4-A6 includes members A4, A5, and A6, etc.

[00151] 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 CTLA-4 binding clones, deposited under ATCC Accession NO. PTA-125512. 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 CTLA-4 binding clones, deposited under ATCC Accession NO. PTA-125512. In some embodiments, antigen binding proteins are expressed from the expression vector in one of the clones in the library of CTLA-4 binding clones, deposited under ATCC Accession NO. PTA-125512.

[00152] 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), L12H12 (antibody A12), L13H13 (antibody A13), ... and L28H28 (antibody A28).

[00153] 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 CTLA-4 binding clones, deposited under ATCC Accession NO. PTA-125512. 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 CTLA-4 binding clones, deposited under ATCC Accession NO. PTA-125512. 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 CTLA-4 binding clones, deposited under ATCC Accession NO. PTA-125512.

[00154] 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 L28 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-L28. 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-L28 (which includes L1, L2, L3, L4, L5, L6, L7, L8, L9, L10, L11, L12, L13, ... and L28). 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-L28. 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-L28. 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-L28.

[00155] 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 CTLA-4 binding clones, deposited under ATCC Accession NO. PTA-125512, 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 CTLA-4 binding clones, deposited under ATCC Accession NO. PTA-125512. 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 CTLA-4 binding clones, deposited under ATCC Accession NO. PTA-125512.

[00156] 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-H28 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-H28. 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-H28. 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-H28. 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-H28. 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.

[00157] 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 CTLA-4 binding clones, deposited under ATCC Accession NO. PTA-125512, 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 CTLA-4 binding clones, deposited under ATCC Accession NO. PTA-125512. 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 CTLA-4 binding clones, deposited under ATCC Accession NO. PTA-125512.

[00158] 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.

[00159] 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.

[00160] In some embodiments, at least one of the antigen binding protein's CDR1 sequences is a CDR1 sequence from A1-A28, CDR1-L1 to 28, or CDR1-H1 to 28 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-A28, CDR2-L1 to 28, or CDR2-H1 to 28 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-A28, CDR3-L1 to 28, or CDR3-H1 to 28 as shown in TABLE 5.

[00161] In another embodiment, the antigen binding protein's light chain CDR3 sequence is a light chain CDR3 sequence from A1-A28 or CDR3-L1 to 28, as shown in TABLE 5, and the antigen binding protein's heavy chain CDR3 sequence is a heavy chain sequence from A1-A28 or CDR-H1 to 28, as shown in TABLE 5.

[00162] 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-A28.

[00163] The nucleotide sequences of A1-A28, or the amino acid sequences of A1-A28, 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-CTLA-4 antibodies that have a desired property, for example, increased affinity, avidity, or specificity for CTLA-4, increased activity or stability *in vivo* or *in vitro*, or reduced *in vivo* side-effects as compared to the underivatized antibody.

[00164] Other derivatives of anti-CTLA-4 antibodies within the scope of this disclosure include covalent or aggregative conjugates of anti-CTLA-4 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-CTLA-4 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).

[00165] 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.

[00166] In other embodiments, the variable portion of the heavy and/or light chains of an anti-CTLA-4 antibody may be substituted for the variable portion of an antibody heavy and/or light chain.

[00167] Oligomers that contain one or more antigen binding proteins may be employed as CTLA-4 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*.

[00168] 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.

[00169] 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 CTLA-4 binding activity.

[00170] 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. Prots in Immunol.*, Suppl. 4, pages 10.19.1 - 10.19.11.

[00171] One embodiment of the present disclosure is directed to a dimer comprising two fusion proteins created by fusing a CTLA-4 binding fragment of an anti-CTLA-4 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.

[00172] 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.

[00173] 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-CTLA-4 antibody fragment or derivative fused to a leucine zipper peptide are expressed in suitable host cells, and the soluble oligomeric anti-CTLA-4 antibody fragments or derivatives that form are recovered from the culture supernatant.

[00174] In one aspect, the present disclosure provides antigen binding proteins that interfere with the binding of CTLA-4 to its ligands. Such antigen binding proteins can be made against CTLA-4, or a fragment, variant or derivative thereof, and screened in conventional assays for the ability to interfere with binding of CTLA-4 to its ligands. Examples of suitable assays are assays that test the antigen binding proteins for the ability to inhibit binding of CTLA-4 ligands to cells expressing CTLA-4, or that test antigen binding proteins for the ability to reduce a biological or cellular response that results from the binding of CTLA-4 ligands to cell surface CTLA-4. For example, antibodies can be screened according to their ability to bind to immobilized antibody surfaces (CTLA-4). Antigen binding proteins that block the binding of CTLA-4 to a ligand can be employed in treating any CTLA-4-related condition, including but not limited to cancer. In an embodiment, a human anti-CTLA-4 monoclonal antibody generated by procedures involving immunization of transgenic mice is employed in treating such conditions.

[00175] 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.

[00176] 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.

[00177] 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 CTLA-4 polypeptide, such that antibodies directed against the CTLA-4 polypeptide are generated in the animal.

[00178] One example of a suitable immunogen is a soluble human CTLA-4, 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.

[00179] 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.

[00180] 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.

[00181] In one embodiment, an antigen binding protein of the present disclosure comprises the IgG1 heavy chain domain of any of A1-A28 (H1-H28) or a fragment of the IgG1 heavy chain domain of any of A1-A28 (H1-H28). In another embodiment, an antigen binding protein of the present disclosure comprises the kappa light chain constant chain region of A1-A28 (L1-L28), or a fragment of the kappa light chain constant region of A1-A28 (L1-L28). In another embodiment, an antigen binding protein of the present disclosure comprises both the IgG1 heavy chain domain, or a fragment thereof, of A1-A28 (L1-L28) and the kappa light chain domain, or a fragment thereof, of A1-A28 (L1-L28).

[00182] 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, L12H12, L13H13, … and L28H28, 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: 11969) -> CPPCP (SEQ ID NO: 11970)) 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.

[00183] 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 L28H28. In another embodiment, the antigen binding protein

binds to CTLA-4 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 L23H28. In another embodiment, the antigen binding protein binds to CTLA-4 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 CTLA-4 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.

[00184] In one aspect, the present disclosure provides antigen-binding fragments of an anti-CTLA-4 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.

[00185] 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 L28H28 are encompassed by the present disclosure.

7.7. Monoclonal antibody

[00186] In another aspect, the present disclosure provides monoclonal antibodies that bind to CTLA-4. 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.

[00187] 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 CTLA-4 [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 CTLA-4 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.

[00188] 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 CTLA-4, 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 CTLA-4 are selected and cultured. The monoclonal antibodies from the hybridoma cultures may be isolated from the supernatants of hybridoma cultures.

[00189] 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.

[00190] 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 CTLA-4 polypeptide. Such hybridoma cell lines, and anti-CTLA-4 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 CTLA-4-induced activity.

[00191] 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 CTLA-4,

wherein the antigen binding protein possesses at least one *in vivo* biological activity of a human anti-CTLA-4 antibody.

7.8. Method of generating antibodies

[00192] 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 CTLA-4. 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.

[00193] 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 CTLA-4 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-CTLA-4 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 CTLA-4, followed by fusion of primed B-cells with a heterohybrid fusion partner. *See, e.g.*, Boerner *et al.*, 1991 *J. Immunol.* 147:86-95.

[00194] In certain embodiments, a B-cell that is producing an anti-human CTLA-4 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 CTLA-4. B-cells may also be isolated from humans, for example, from a peripheral blood sample.

[00195] 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 CTLA-4. 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.

[00196] 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.

[00197] 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.

[00198] 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 CTLA-4 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).

[00199] 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.* CTLA-4), 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).

[00200] Heavy and light chain immunoglobulin cDNA expression libraries may also be prepared in lambda phage, for example, using λImmunoZap™(H) and λImmunoZap™(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*.

[00201] 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).

[00202] Once cells producing antibodies according to the 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 disclosure.

[00203] CTLA-4 binding agents of the present disclosure preferably modulate CTLA-4 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 CTLA-4 by one of the antibodies described in this application. Accordingly such binding agents can be identified using the assays described herein.

[00204] 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 CTLA-4 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 CTLA-4 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.

[00205] Other antibodies according to the disclosure may be obtained by conventional immunization and cell fusion procedures as described herein and known in the art.

[00206] 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

CTLA-4. Antigen binding proteins directed against a CTLA-4 can be used, for example, in assays to detect the presence of CTLA-4 polypeptides, either *in vitro* or *in vivo*. The antigen binding proteins also may be employed in purifying CTLA-4 proteins by immunoaffinity chromatography.

[00207] 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 of the present disclosure can be, for example, 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 CTLA-4 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.

[00208] 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- CTLA-4 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).

[00209] 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).

[00210] 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 CTLA-4 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.

[00211] 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.

[00212] 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.

[00213] 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.

[00214] 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.

[00215] 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.

[00216] 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.

[00217] 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)).

[00218] 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.

[00219] 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 CTLA-4, or to increase or decrease the affinity of the antibodies to CTLA-4 described herein.

[00220] 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.

[00221] In certain embodiments, antibodies of the present disclosure may be chemically bonded with polymers, lipids, or other moieties.

[00222] 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.

[00223] 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).

[00224] 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).

[00225] 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 28 with SEQ ID NOS 1001-1028; CDR2-L1 to 28 with SEQ ID NOS 2001-2028; CDR3-L1 to 28 with SEQ ID NOS 3001-3028; CDR1-H1to 28 with SEQ ID NOS 4001-4028; CDR2-H1to 28 with SEQ ID NOS

5001-5028; and CDR3-H1to 28 with SEQ ID NOS 6001-6028, 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 CTLA-4 and/or neutralizes CTLA-4. The non-CDR portion of the antibody may be a non-protein molecule in which the antibody exhibits a similar binding pattern to human CTLA-4 peptides in a competition binding assay as that exhibited by at least one of antibodies A1-A28, and/or neutralizes CTLA-4. 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 CTLA-4 and/or neutralizes CTLA-4. 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 CTLA-4 peptides in the human CTLA-4 peptide epitope competition binding assay (described hereinbelow) as that exhibited by at least one of the antibodies A1-A28, and/or neutralizes CTLA-4.

[00226] 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®.

[00227] 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.

[00228] 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).

[00229] Where it is desired to improve the affinity of antibodies according to the 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).

[00230] 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

[00231] Antibodies A1-A28 comprise heavy and light chain V(J)D polynucleotides (also referred to herein as L1-L28 and H1-H28, respectively). Antibodies A1-A28 comprise the sequences listed in TABLE 5. 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-L28) and heavy chain (H1-H28) 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:111) 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:112) H12 comprises CDR1-H12 (SEQ ID NO:4012), CDR2-H12 (SEQ ID NO:5012) and CDR3-H12 (SEQ ID NO:6012)
A13	L13 (SEQ ID NO:13) L13 comprises CDR1-L13 (SEQ ID NO:1013), CDR2-L13 (SEQ ID NO:2013) and CDR3-L13 (SEQ ID NO:3013)	H13 (SEQ ID NO:113) H13 comprises CDR1-H13 (SEQ ID NO:4013), CDR2-H13 (SEQ ID NO:5013) and CDR3-H13 (SEQ ID NO:6013)
A14	L14 (SEQ ID NO:14) L14 comprises CDR1-L14 (SEQ ID NO:1014), CDR2-L14 (SEQ ID NO:2014) and CDR3-L14 (SEQ ID NO:3014)	H14 (SEQ ID NO:114) H14 comprises CDR1-H14 (SEQ ID NO:4014), CDR2-H14 (SEQ ID NO:5014) and CDR3-H14 (SEQ ID NO:6014)
A15	L15 (SEQ ID NO:15) L15 comprises CDR1-L15 (SEQ ID NO:1015), CDR2-L15 (SEQ ID NO:2015) and CDR3-L15 (SEQ ID NO:3015)	H15 (SEQ ID NO:115) H15 comprises CDR1-H15 (SEQ ID NO:4015), CDR2-H15 (SEQ ID NO:5015) and CDR3-H15 (SEQ ID NO:6015)
A16	L16 (SEQ ID NO:16) L16 comprises CDR1-L16 (SEQ ID NO:1016), CDR2-L16 (SEQ ID NO:2016) and CDR3-L16 (SEQ ID NO:3016)	H16 (SEQ ID NO:116) H16 comprises CDR1-H16 (SEQ ID NO:4016), CDR2-H16 (SEQ ID NO:5016) and CDR3-H16 (SEQ ID NO:6016)
A17	L17 (SEQ ID NO:17) L17 comprises CDR1-L17 (SEQ ID NO:1017), CDR2-L17 (SEQ ID NO:2017) and CDR3-L17 (SEQ ID NO:3017)	H17 (SEQ ID NO:117) H17 comprises CDR1-H17 (SEQ ID NO:4017), CDR2-H17 (SEQ ID NO:5017) and CDR3-H17 (SEQ ID NO:6017)
A18	L18 (SEQ ID NO:18) L18 comprises CDR1-L18 (SEQ ID NO:1018), CDR2-L18 (SEQ ID NO:2018) and CDR3-L18 (SEQ ID NO:3018)	H18 (SEQ ID NO:118) H18 comprises CDR1-H18 (SEQ ID NO:4018), CDR2-H18 (SEQ ID NO:5018) and CDR3-H18 (SEQ ID NO:6018)
A19	L19 (SEQ ID NO:19) L19 comprises CDR1-L19 (SEQ ID NO:1019), CDR2-L19 (SEQ ID NO:2019) and CDR3-L19 (SEQ ID NO:3019)	H19 (SEQ ID NO:119) H19 comprises CDR1-H19 (SEQ ID NO:4019), CDR2-H19 (SEQ ID NO:5019) and CDR3-H19 (SEQ ID NO:6019)
A20	L20 (SEQ ID NO:20) L20 comprises CDR1-L20 (SEQ ID NO:1020), CDR2-L20 (SEQ ID NO:2020) and CDR3-L20 (SEQ ID NO:3020)	H20 (SEQ ID NO:120) H20 comprises CDR1-H20 (SEQ ID NO:4020), CDR2-H20 (SEQ ID NO:5020) and CDR3-H20 (SEQ ID NO:6020)
A21	L21 (SEQ ID NO:21)	H21 (SEQ ID NO:121)

	L21 comprises CDR1-L21 (SEQ ID NO:1021), CDR2-L21 (SEQ ID NO:2021) and CDR3-L21 (SEQ ID NO:3021)	H21 comprises CDR1-H21 (SEQ ID NO:4021), CDR2-H21 (SEQ ID NO:5021) and CDR3-H21 (SEQ ID NO:6021)
A22	L22 (SEQ ID NO:22) L22 comprises CDR1-L22 (SEQ ID NO:1022), CDR2-L22 (SEQ ID NO:2022) and CDR3-L22 (SEQ ID NO:3022)	H22 (SEQ ID NO:122) H22 comprises CDR1-H22 (SEQ ID NO:4022), CDR2-H22 (SEQ ID NO:5022) and CDR3-H22 (SEQ ID NO:6022)
A23	L23 (SEQ ID NO:23) L23 comprises CDR1-L23 (SEQ ID NO:1023), CDR2-L23 (SEQ ID NO:2023) and CDR3-L23 (SEQ ID NO:3023)	H23 (SEQ ID NO:123) H23 comprises CDR1-H23 (SEQ ID NO:4023), CDR2-H23 (SEQ ID NO:5023) and CDR3-H23 (SEQ ID NO:6023)
A24	L24 (SEQ ID NO:24) L24 comprises CDR1-L24 (SEQ ID NO:1024), CDR2-L24 (SEQ ID NO:2024) and CDR3-L24 (SEQ ID NO:3024)	H24 (SEQ ID NO:124) H24 comprises CDR1-H24 (SEQ ID NO:4024), CDR2-H24 (SEQ ID NO:5024) and CDR3-H24 (SEQ ID NO:6024)
A25	L25 (SEQ ID NO:25) L25 comprises CDR1-L25 (SEQ ID NO:1025), CDR2-L25 (SEQ ID NO:2025) and CDR3-L25 (SEQ ID NO:3025)	H25 (SEQ ID NO:125) H25 comprises CDR1-H25 (SEQ ID NO:4025), CDR2-H25 (SEQ ID NO:5025) and CDR3-H25 (SEQ ID NO:6025)
A26	L26 (SEQ ID NO:26) L26 comprises CDR1-L26 (SEQ ID NO:1026), CDR2-L26 (SEQ ID NO:2026) and CDR3-L26 (SEQ ID NO:3026)	H26 (SEQ ID NO:126) H26 comprises CDR1-H26 (SEQ ID NO:4026), CDR2-H26 (SEQ ID NO:5026) and CDR3-H26 (SEQ ID NO:6026)
A27	L27 (SEQ ID NO:27) L27 comprises CDR1-L27 (SEQ ID NO:1027), CDR2-L27 (SEQ ID NO:2027) and CDR3-L27 (SEQ ID NO:3027)	H27 (SEQ ID NO:127) H27 comprises CDR1-H27 (SEQ ID NO:4027), CDR2-H27 (SEQ ID NO:5027) and CDR3-H27 (SEQ ID NO:6027)
A28	L28 (SEQ ID NO:28) L28 comprises CDR1-L28 (SEQ ID NO:1028), CDR2-L28 (SEQ ID NO:2028) and CDR3-L28 (SEQ ID NO:3028)	H28 (SEQ ID NO:128) H28 comprises CDR1-H28 (SEQ ID NO:4028), CDR2-H28 (SEQ ID NO:5028) and CDR3-H28 (SEQ ID NO:6028)

7.10. Pharmaceutical compositions

[00232] 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.

[00233] 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.

[00234] 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.

[00235] 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 CTLA-4-binding protein.

[00236] 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.

[00237] 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.

[00238] 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

[00239] 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.

[00240] 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

[00241] 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.

[00242] 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.

[00243] 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.

[00244] In various embodiments, the pharmaceutical composition is formulated for oral administration, for buccal administration, or for sublingual administration.

[00245] In some embodiments, the pharmaceutical composition is formulated for intravenous, intramuscular, or subcutaneous administration.

[00246] In some embodiments, the pharmaceutical composition is formulated for intrathecal or intracerebroventricular administration.

[00247] In some embodiments, the pharmaceutical composition is formulated for topical administration.

7.10.3. Pharmacological compositions adapted for injection

[00248] 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.

[00249] 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.

[00250] 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.

[00251] 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.

[00252] In some embodiments, the pharmaceutical composition in the unit dosage form is in solid form, such as a lyophilate, suitable for solubilization.

[00253] 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.

[00254] 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.

[00255] 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.

[00256] 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.

[00257] 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

[00258] The pharmaceutical compositions may conveniently be presented in unit dosage form.

[00259] The unit dosage form will typically be adapted to one or more specific routes of administration of the pharmaceutical composition.

[00260] 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.

[00261] In various embodiments, the unit dosage form is adapted for oral administration, for buccal administration, or for sublingual administration.

[00262] In some embodiments, the unit dosage form is adapted for intravenous, intramuscular, or subcutaneous administration.

[00263] In some embodiments, the unit dosage form is adapted for intrathecal or intracerebroventricular administration.

[00264] In some embodiments, the pharmaceutical composition is formulated for topical administration.

[00265] 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

[00266] Therapeutic antibodies may be used that specifically bind to intact CTLA-4.

[00267] *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.

[00268] 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 CTLA-4 binding agent that cross-blocks the binding of at least one of antibodies A1-A28 to CTLA-4, and/or is cross-blocked from binding to CTLA-4 by at least one of antibodies A1-A28; and/or to a CDR of a CTLA-4 binding agent wherein the binding agent can block the binding of CTLA-4 to its ligands.

[00269] CTLA-4 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-A28, and cross-block the binding of at least one of antibodies A1-A28 to CTLA-4, and/or are cross-blocked from binding to CTLA-4 by at least one of antibodies A1-A28; and/or can block the inhibitory effect of CTLA-4 on its ligands.

[00270] Antibodies according to the disclosure may have a binding affinity for human CTLA-4 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.

[00271] 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)).

[00272] 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.

[00273] In the methods described above to generate antibodies according to the disclosure, including the manipulation of the specific A1-A28 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 CTLA-4; cross-blocking assays; Biacore-based competition binding assay;" *in vivo* assays).

7.12.1. Methods of treating a disease responsive to a CTLA-4 inhibitor or activator

[00274] In another aspect, methods are presented for treating a subject having a disease responsive to a CTLA-4 inhibitor or activator. The disease can be cancer, autoimmune disease, or viral or bacterial infection.

[00275] 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.

[00276] 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).

[00277] The term "sufficient amount" means an amount sufficient to produce a desired effect.

[00278] 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.

[00279] 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.

[00280] 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.

[00281] In some embodiments, the pharmaceutical composition is administered by inhalation, orally, by buccal administration, by sublingual administration, by injection or by topical application.

[00282] 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.

[00283] 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.

[00284] 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).

8. EXAMPLES

[00285] 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.

[00286] 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-CTLA-4 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.

8.1. Example 1: Generation of antigen binding protein

[00287] ***Mouse Immunization and Sample Preparation:***

[00288] First, transgenic mice carrying inserted human immunoglobulin genes were immunized with soluble CTLA-4 immunogen of SEQ ID NO: 7001 (*i.e.,* His-tagged CTLA-4 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.

[00289] ***Generating paired heavy and light chain libraries:***

[00290] 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.

[00291] 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.

[00292] 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.

[00293] 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).

[00294] In some embodiments, scFv libraries were not natively paired, for example, randomly paired by amplifying scFv directly from RNA isolated from B cells.

8.2. Example 2: Isolation of CTLA-4 binders by yeast display

[00295] ***Library Screening:***

[00296] Human IgG1-Fc (Thermo Fisher Scientific) and CTLA-4 (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.

[00297] 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.

[00298] 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 CTLA-4, biotinylated CTLA-4 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.

[00299] 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.

[00300] ***Deep repertoire sequencing:***

[00301] CTLA-4-binding clones were recovered as a library ("a library of CTLA-4 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 CTLA-4 binding clones were deposited under ATCC Accession No. PTA-125512 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: 1-28 and SEQ ID NOS: 101-128. Additional sequences obtained from sequencing the yeast scFv library are provided in SEQ ID NOS 8000-8991. Specifically, their variable light chain (V_L) sequences include SEQ ID NOS: 8000-8495. Their heavy chain (V_H) sequences include SEQ ID NOS: 8496-8991.

[00302] 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.

[00303] 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 by merging filtered sequences were generated from the linked and unlinked runs. Briefly, a series of scripts that first merged forward and reverse reads from the unlinked run were 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.

[00304] To identify reading frame and FR/CDR junctions, a database of well-curated immunoglobulin sequences were 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.

[00305] Each animal yielded 38–50 unique scFv sequences present at 0.1% frequency or greater after the second FACS selection, including a total of 28 unique scFv candidate binders (SEQ ID Nos: 1-28 for light chains; SEQ ID Nos: 101-128 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:28 and the heavy chain of SEQ ID NO:128 are a cognate pair, *etc.*

[00306] In this method, the two rounds of FACS resulted in enrichment of the CTLA-4-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.

8.3. Example 3: Biological characteristics of antigen binding protein

[00307] 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.

[00308] ***CTLA-4 Target Binding Profiles***

[00309] The binding specificity and affinity of each full-length antibody towards CTLA-4 were determined using biolayer interferometry (BLI) and/or surface plasmon resonance (SPR). Anti-cyno CTLA-4 and anti-mouse CTLA-4 affinities were tested using ForteBio (BLI). Anti-human CTLA-4 affinities were measured using Carterra (SPR).

[00310] For BLI, antibodies were loaded onto an Anti-Human IgG Fc (AHC) biosensor using the Octet Red96 system (ForteBio). Loaded biosensors were dipped into antigen dilutions beginning at 300 nM, with 6 serial dilutions at 1:3. Kinetic analysis was performed using a 1:1 binding model and global fitting.

[00311] For SPR, we amine-coupled a moderate density (»1,000 Response Units) of an antihuman IgG-Fc reagent (Southern Biotech 2047-01) 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).

[00312] 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.

[00313] Next, the sensor chip was loaded onto an SPR reader (MX- 96 system; Ibis Technologies) for the kinetic analysis. CTLA-4 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). CTLA-4 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.).

[00314] For cell surface binding studies, stable CTLA-4 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-CTLA-4 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 APC and anti-human IgG Fc-PE [M1310G05] (BioLegend 41070) antibodies for 30 minutes at 4°C. An anti-human CTLA-4-FITC antibody 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.

[00315] We identified antibodies that specifically bind to CTLA-4. Affinity to CTLA-4 (K_D) of each antibody is provided in TABLE 6. The Promega assay % inhibition was calculated relative to the strongest inhibitor, which is antibody A5. The affinity of each antibody against human CTLA-4, the on rate, off rate, and KD are shown in TABLE 7.

TABLE 6

Ab#	Binds by FACS?	Promega assay antagonist (EC50, ug/mL)	Promega assay % inhibition	Affinity to Human CTLA-4(nM)	Affinity to Cyno CTLA4 (nM)	Affinity to Mouse CTLA-4 (nM)
Ipilimumab	Yes	0.51	50.2%	4.9	1.3	no binding
A1	Yes	0.13	43.2%	0.77	0.96	51
A2	Yes	0.12	75.4%	5.1	3.1	no binding
A3	Yes	0.18	49.8%	1.5	1.2	no binding
A4	Yes	0.15	81%	3	1.2	no binding
A5	Yes	0.18	100%	4.6	2.2	39.4
A6	Yes	0.18	61.7%	5.7	1.6	87.8
A7	Yes	0.17	11.5%	5.7	0.75	no binding
A8	Yes	0.15	54.7%	4.6	1	no binding
A9	Yes	0.19	60.8%	6.8	0.92	no binding
A10	Yes	0.26	24.5%	22	3.1	no binding
A11	Yes	0.42	26.6%	7.5	1.6	no binding
A12	Yes	no blocking	0%	5.3	4.5	not tested

A13	Yes	0.09	13.3%	3.2	9.6	not tested
A14	Yes	0.09	6%	9.8	23.6	not tested
A15	Yes	no blocking	0%	10	2.6	not tested
A16	Yes	no blocking	0%	23	2.8	not tested
A17	Yes	no blocking	0%	51	6.3	not tested
A18	Yes	0.89	8.6%	48	no binding	not tested
A19	No	not tested	not tested	3.3	not tested	not tested
A20	No	not tested	not tested	20	not tested	not tested
A21	No	not tested	not tested	31	not tested	not tested
A22	No	not tested	not tested	32	not tested	not tested
A23	No	no blocking	0%	35	not tested	not tested
A24	No	not tested	not tested	55	not tested	not tested
A25	No	not tested	not tested	58	not tested	not tested
A26	No	not tested	not tested	74	not tested	not tested
A27	No	not tested	not tested	120	not tested	not tested
A28	No	no blocking	0%	46	not tested	not tested

TABLE 7

	kon (M-1 s-1)	**koff (s-1)**	**KD (M)**
Ipilimumab	6.50E+04	3.20E-04	4.90E-09
A1	2.10E+05	1.60E-04	7.70E-10
A2	1.00E+05	5.20E-04	5.10E-09
A3	1.90E+05	2.80E-04	1.50E-09
A4	7.60E+04	2.30E-04	3.00E-09
A5	9.00E+04	4.20E-04	4.60E-09
A6	6.10E+04	3.50E-04	5.70E-09
A7	6.40E+04	3.60E-04	5.70E-09
A8	8.50E+04	3.90E-04	4.60E-09
A9	6.30E+04	4.30E-04	6.80E-09
A10	2.30E+04	5.10E-04	2.20E-08
A11	4.70E+04	3.50E-04	7.50E-09
A12	5.10E+04	2.70E-04	5.30E-09
A13	1.30E+05	4.20E-04	3.20E-09
A14	5.40E+04	5.20E-04	9.80E-09
A15	7.00E+04	7.30E-04	1.00E-08
A16	3.10E+04	7.20E-04	2.30E-08
A17	6.60E+04	3.40E-03	5.10E-08
A18	4.20E+03	2.00E-04	4.80E-08
A19	1.20E+05	3.80E-04	3.30E-09
A20	3.20E+04	6.40E-04	2.00E-08
A21	1.80E+04	5.50E-04	3.10E-08
A22	1.10E+04	3.60E-04	3.20E-08
A23	3.40E+04	1.20E-03	3.50E-08
A24	2.50E+04	1.40E-03	5.50E-08
A25	3.00E+04	1.80E-03	5.80E-08
A26	3.50E+03	2.60E-04	7.40E-08
A27	3.10E+04	3.60E-03	1.20E-07
A28	5.20E+04	2.40E-03	4.60E-08

[00316] ***CTLA-4 ligand blocking assay:***

[00317] For analysis of the antibodies' ability to block the CTLA-4/ligand interaction, the CTLA-4 Blockade Bioassay (Promega) was used according to the manufacturer's instructions. On the day prior to the assay, aAPC/Raji cells that express CTLA-4 ligands CD80 and CD86 were thawed into 90% Ham's F-12/10% fetal bovine serum (FBS) and plated into the inner 60 wells of two 96-well plates. The cells were incubated overnight at 37 °C, 5% CO_2. On the day of assay, antibodies were diluted in 99% RPMI/1% FBS. The antibody dilutions were added to the wells containing the CTLA-4 ligand expressing aAPC/Raji cells, followed by addition of CTLA-4 effector cells (thawed into 99% RPMI/1% FBS). The cell/antibody mixtures were incubated at 37 °C, 5% CO_2 for 6 hours, after which Bio-Glo Reagent was added and luminescence was read using a Spectramax i3x plate reader (Molecular Devices). Fold-induction was plotted by calculating the ratio of [signal with antibody]/[signal with no antibody], and the plots were used to calculate the EC50 using SoftMax Pro (Molecular Devices). In-house produced ipilimumab was used as a positive control, and an antibody binding to an irrelevant antigen was used as a negative control.

[00318] Binding of CTLA-4 to its ligand leads to inhibition of T cell signaling. Antibodies that bind CTLA-4 and antagonize CTLA-4/ligand interactions can therefore remove this inhibition, allowing T cells to be activated. CTLA-4/ligand checkpoint blockade was tested through an *in vitro* cellular Nuclear Factor of Activated T cells (NFAT) luciferase reporter assay. In this assay, antibodies whose anti-CTLA-4 epitopes fall inside the ligand binding domain antagonize CTLA-4/ligand interactions, resulting in an increase of the NFAT-luciferase reporter. The full-length mAb candidates that can bind CTLA-4 expressed in CHO cells were assayed. To generate an EC50 value for each mAb, measurements were made across several concentrations. It was found that some full-length mAbs are functional in checkpoint blockade in a dose dependent manner as summarized in TABLE 6.

[00319] The ability of the CTLA4 antibodies (indicated in TABLE 8) to prevent the binding of CD80 or CD86 to plate-bound CTLA4 was evaluated using ELISA. The EC50 and the percent inhibition of each interaction is shown in the TABLE 8. Plates were coated with rhCTLA4-Fc and then blocked with 1x PBST with 5% w/v nonfat dry milk. After blocking a dilution series of the indicated antibody was added to the plate. Then, to determine how much CD80 or CD86 was still able to bind plate bound CTLA4, after the plates were washed, rhCD80-His or rhCD86-His, respectively, was added to the plate. Unbound CD80-His/CD86-His was washed away and mouse anti-His-HRP was added. TMB was used to determine how much CD80-His/CD86-His bound to the plate bound CTLA4 in the presence of each antibody.

[00320] In some embodiments of the present disclosure, the anti-CTLA-4 antibodies function pharmacologically by antibody-dependent cell-mediated cytotoxicity (ADCC). In some embodiments of the present disclosure, immune-related toxicities related to anti-CTLA-4 antibody therapy are abrogated with an antibody that functions in ADCC but which does not function in checkpoint blockade.

TABLE 8

Antibody	CD80 EC50 (ug/mL)	CD80 % inhibition	CD86 EC50 (ug/ml)	CD86 % inhibition
Ipilimumab	0.08211	96.5%	0.1136	90.6%
CTLA4.A7	0.9955	92.1%	1.98	78.8%
CTLA4.A2	0.1006	95.4%	0.1452	91.5%
CTLA4.A12	0.2427	89.2%	0.3704	77.4%
CTLA4.A14	0.2262	83.7%	0.2442	54.5%
CTLA4.A5	0.07049	96.3%	0.1218	90.6%

[00321] ***Epitope binning:***

[00322] Epitope binning was performed using high-throughput Array SPR in a modified classical sandwich approach. A sensor chip was 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 were coupled at 50 mg/mL to create a surface with higher binding capacity (~3,000 reactive units immobilized). The mAb supernatants were diluted at 1:1 or 1:10 in running buffer, depending on the concentration of the mAb in the supernatant.

[00323] The sensor chip was placed in the MX-96 instrument, and the captured mAbs ("ligands") were crosslinked to the surface using the bivalent amine reactive linker bis(sulfosuccinimidyl) suberate (BS3, ThermoFisher), which was injected for 10 minutes at 0.87 mM in water. Excess activated BS3 was 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) was used to block reference surfaces and any remaining capacity of the target spots.

[00324] Next, 250 nM CTLA-4 protein was 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 bound to the antigen if it was not competitive with the ligand mAb. At the end of each cycle, a one minute regeneration injection was 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).

[00325] A network community plot algorithm was then used in an SPR epitope data analysis software package (Carterra Inc.) 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 had more mAb-mAb

measurements, resulting in more mAb-mAb connections, which led to a closer relationship in the community plot.

[00326] The epitope binning showed that all the mAbs were in distinct bins from ipilimumab (FIG. 3).

8.4. Example 4: Influence of CTLA-4 ABPs on tumor growth

[00327] Transgenic mice expressing human CTLA-4 (hCTLA-4 KI mice) were implanted subcutaneously with MC38 tumor cells on the right flank. The hCTLA-4 KI mice were treated with one mg/kg of the indicated CTLA-4 antibody on Days 8, 11, and 14 post-implantation. Specifically, the mice were treated with a control antibody (n=8), ipilumumab (n=8), CTLA4.A2 antibody (n=8), CTLA4.A14 antibody (n=9), CTLA4.A14.2a antibody (n=8), CTLA4.A7 antibody (n=9), CTLA4.A7 antibody (n=9), and CTLA4.A12 antibody (n=8). CTLA-4.A14.2a antibody is the A14 antibody cloned onto a mouse IgG2a background, which enhances antibody-dependent cellular cytotoxicity (ADCC) activity. Tumor volume was measured and tumor growth inhibition was calculated using the formula below:

*Mean % Inhibition = (mean(C)-mean(T))/mean(C) * 100%*

T - current group value

C - control group value

[00328] Tumors were implanted subcutaneously in the right flank region with MC38 tumor cells ($1x10^6$) in 0.1 ml of PBS for tumor development. The cells in exponential growth phase were harvested and quantitated by cell counter before tumor implantation. Tumor volumes were measured twice per week in two dimensions using a caliper, and the volume will be expressed in mm^3 using the formula: "V = (L x W x W)/2, where V is tumor volume, L is tumor length (the longest tumor dimension) and W is tumor width (the longest tumor dimension perpendicular to L). Dosing as well as tumor and body weight measurements were conducted in a Laminar Flow Cabinet. The body weights and tumor volumes were measured by using StudyDirector™ software (version 3.1.399.19). Animals were dosed i.p. (intraperitoneally) with the indicated protein in a sterile saline solution including 0.1 mg/ml of the indicated protein. Each mouse received 10 microliters of the indicated solution per a gram of body weight, which leads to a dosing of 1 mg/kg. Animals were dosed days 0, 3, and 6 post randomization.

[00329] TABLE 9 shows the percentage of mice in which the tumor had a complete response (CR) to the treatment. At least 2 consecutive tumor measurements of 0 mm^3 following treatment initiation qualifies as a CR.

TABLE 9

Antibody	% of tumors with CR
Control	12.5% (1 of 8)
Ipilimumab	75% (6 of 8)

CTLA4.A2	100% (8 of 8)
CTLA4.A14	66.67% (6 of 9)
CTLA4.A14.2a	75% (6 of 8)
CTLA4.A7	55.6% (5 of 9)
CTLA4.A12	50% (4 of 8)

[00330] TABLE 10 shows the percentage of mice with tumors that had a CR but then later relapsed by day 56. The group treated with CTLA4.A14.2a that had previously shown a CR had 0% relapse by day 56, indicating that ADCC can prolong the anti-tumor immunity.

TABLE 10

Antibody	**% of relapse by Day 56 in tumors that had previously shown CR**
Ipilimumab	16.67% (1 of 6)
CTLA4.A2	25% (2 of 8)
CTLA4.A14	33.3% (2 of 6)
CTLA4.A14.2a	0% (0 of 6)

[00331] TABLE 11 shows the mean inhibition of tumor volume over time when the hCTLA-4 KI mice implanted with MC38 tumor cells were treated with one mg/kg of the control or one mg/kg of the indicated CTLA-4 antibodies.

TABLE 11: Mean Inhibition

Study day	**8**	**11**	**14**	**17**	**21**	**24**	**27**	**29**	**31**	**35**	**38**
Control	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Ipilimumab	-3.81%	0.44%	31.41%	69.65%	84.04%	90.73%	96.00%	96.92%	96.61%	87.45%	88.27%
CTLA4.A7	-7.49%	10.91%	34 54%	60.11%	70.73%	81.04%	78.25%	78.75%	70.60%	50.50%	43.41%
CTLA4.A2	-6.79%	-6.10%	26.70%	61.14%	85.22%	97.98%	99.58%	100.00%	100.00%	99.55%	99.36%
CTLA4.A12	-5.89%	12.35%	34.21%	49.59%	70.56%	86.82%	90.22%	91.82%	91.13%	68.63%	70.44%
CTLA4.A14	-7.37%	-6.91%	26.70%	58.54%	80.64%	93.71%	95.73%	97.23%	97.26%	92.22%	90.17%
CTLA4.A14.2a	-4.74%	-20.04%	9.74%	40.80%	65.98%	87.85%	93.48%	95.01%	93.66%	81.40%	82.75%

Example 5: Influence of CTLA4 ABPs on Systemic Anti-Tumor Immunity

[00332] The hCTLA4 KI mice bearing MC38 tumors were treated with the indicated anti-CTLA4 on days 8, 11, and 14 post tumor cell implantation, as explained *supra*. The mice in which tumors displayed a CR were re-challenged with implantation of MC38 cells on the opposite flank. TABLE 12 shows the individual mouse tumor volumes (mm^3) of the original or re-challenge tumors on the final day of the study (73 days after the original tumor cell implantation and 30 days after the re-challenge implantation). There was no growth of re-challenge tumors in mice in which the original tumor remained a CR. The 3 instances of growth seen in the re-challenge tumors were in mice in which the original tumor had started to re-grow (*see* TABLE 12). The results also indicated that CTLA4.A2 may induce protective systemic anti-tumor immunity even when the primary tumor (original tumor) relapses (*see* TABLE 13).

TABLE 12

	Ipilimumab (original tumor)	**Ipilimumab (re-challenge tumor)**	**CTLA4.A2 (original tumor)**	**CTLA4.A2 (re-challenge tumor)**	**CTLA4.A14 (original tumor)**	**CTLA4.A14 (re-challenge tumor)**
Tumor Volume (mm^3)	0	0	0	0	105.7	85.3
	1822.3	149.6	0	0	0	0
	0	0	0	0	913.3	98.1
	0	0	0	0	0	0
	0	0	1415	0	0	0
	0	0	0	0	0	0
			678.9	0		
			0	0		

TABLE 13

Antibody	**% of secondary tumors that grew if primary tumor relapsed**
Ipilimumab	100% (1 of 1)
CTLA4.A2	0% (0 of 2)
CTLA4.A14	100% (2 of 2)

8.5. Example 6: Influence of Increased Dosage of CLTA-4 ABPs

[00333] ***MC38 tumors treated with anti-CTLA-4***

[00334] Between 2 and 8 transgenic mice expressing human CTLA-4 (hCTLA-4 KI mice) were implanted with MC38 tumor cells on the right flank. Randomization started when the mean tumor size reached $98.5mm^2$. The hCTLA-4 KI mice were treated with 5 mg/kg of the indicated anti-CTLA4 bi-weekly for 5 doses starting on day 0 post-randomization. The administered antibodies are shown in TABLE 14. CTLA4.A14.2a is antibody A14 cloned onto a mouse IgG2a backbone, which enhances ADCC activity. The 297 suffix denotes that the hIgG1 Fc was

mutated at the N297 amino acid to eliminate glycosylation and thus Fc effector function including ADCC.

[00335] *A) Tumor growth inhibition*

[00336] Over the course of the study, tumor growth inhibition was determined using the formula below:

*Mean % Inhibition = (mean(C)-mean(T))/mean(C) * 100%*

T - current group value

C - control group value

[00337] The results showed that antibodies lacking Fc activity had reduced efficacy overall. These antibodies were still able to induce tumor regression in some animals, indicating that anti-CTLA4 works by both Fc-dependent and Fc-independent mechanisms of action, and indicating that anti-CTLA4s lacking Fc activity, including ADCC and ADCP, can induce anti-tumor responses (TABLEs 14 and 15).

TABLE 14

	Mean Inhibition						
Study Day	**0**	**3**	**6**	**10**	**13**	**17**	**20**
1x PBS (negative control)	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Ipilimumab	0.31%	-21.76%	12.56%	75.43%	91.55%	95.54%	98.71%
CTLA4.A5	0.74%	-28.09%	-6.22%	64.17%	87.32%	98.32%	100.00%
CTLA4.A14	-0.56%	-13.30%	5.33%	68.17%	86.21%	92.79%	97.52%
CTLA4.A12	0.40%	-32.59%	-14.13%	57.48%	83.00%	94.99%	98.04%
CTLA4.A8	0.26%	-18.48%	-14.18%	52.53%	78.37%	96.15%	97.96%
CTLA4.A7	-0.61%	-7.67%	10.04%	76.74%	94.36%	98.88%	100.00%
CTLA4.A2	0.27%	-23.97%	-9.95%	60.95%	89.33%	98.35%	100.00%
CTLA4.A13	-1.15%	-7.61%	6.23%	49.18%	64.82%	93.82%	95.23%
CTLA4.A5.2a	0.88%	11.78%	27.51%	66.64%	89.02%	96.85%	100.00%
CTLA4.A14.2a	1.04%	-7.89%	21.12%	45.60%	84.83%	97.30%	100.00%
Ipilimumab.297	0.68%	8.27%	21.37%	22.75%	16.02%	25.70%	26.39%
CTLA4.A5.297	-0.33%	17.56%	18.58%	5.70%	-15.40%	4.65%	13.61%

TABLE 15

% of each group in which tumor had started to regress by day 13 post treatment initiation	
1x PBS (negative control)	0%
Ipilimumab	100%
CTLA4.A5	100%
CTLA4.A14	100%
CTLA4.A12	88%
CTLA4.A8	100%
CTLA4.A7	100%
CTLA4.A2	100%

CTLA4.A13	86%
CTLA4.A5.2a	100%
CTLA4.A14.2a	100%
Ipilimumab.297	25%
CTLA4.A5.297	14%

[00338] *B) Histopathological Analysis:*

[00339] The hCTLA-4 mice were euthanized and their right kidneys were harvested for histopathological analysis. Tissue was formalin-fixed and paraffin-embedded, and cut in 5µm sections that were placed on glass slides for standard hematoxylin and eosin (H&E) staining as well as anti-IgG and anti-C3 immunohistochemistry (IHC) staining. Stained slides were prepared as digital images. A board-certified veterinary pathologist with experience in laboratory animals and toxicologic pathology evaluated the H&E images for any findings and evaluated the anti-IgG and C3 slides for location, intensity, and percent of positive staining. Findings in H&E images were scored on a scale from 0 to 5 (0=within normal limits, 1= minimal findings or the least change discernible, 2= mild findings, 3= moderate, 4=marked, and 5=severe or to the greatest extent possible). Findings in IHC images were scored on a scale of 1 to 4 for intensity (0=negative, 1=minimal or slightly positive and 4=very dark), and as a percent of the positive cells in the glomeruli (after reviewing at least 5 glomeruli).

[00340] The H&E, immunoglobulin, or C3 stain images were scored by a blinded pathologist and the results are shown in FIG. 4. The main H&E finding was that leukocytes in the renal interstitium are not usually involved in glomeruli. Ig and C3 deposition in the glomeruli scoring are also shown in FIG. 4.

[00341] *C) Alkaline Phosphatase:*

[00342] The hCTLA-4 mice were also analyzed for changes in alkaline phosphatase level. The level of alkaline phosphatase in the serum was determined using comprehensive diagnostic rotors on ABAXIS VetScan VS2.

[00343] The study found that ipilimumab (IPI) elevates alkaline phosphatase levels, which may be an indication of immune-mediated hepatitis. The CTLA4 antibodies (*e.g.*, CTLA4.A14.2A) showed a decrease in elevation of alkaline phosphatase levels (FIG. 5). This decreased elevation in alkaline phosphatase induced by the presently disclosed CTLA4 antibodies may indicate they are less likely to induce immune-mediated hepatitis than treatments such as ipilimumab.

8.6. Example 7: Influence of CTLA-4 ABPs on a second tumor model

[00344] ***RM1 tumors treated with anti-CTLA-4***

[00345] Transgenic mice expressing human CTLA-4 (hCTLA-4 KI mice) were implanted with RM1 tumor cells on the right flank. (Human IgG1 isotype negative control n=7, atezolimumab n=8, n=11 for all other groups). The hCTLA4 KI mice were treated with the antibodies indicated in TABLE 16. The CTLA4 antibodies were dosed at 5 mg/kg on days 0, 3, and 6 post randomization and atezolizumab was dosed at 5 mg/kg biweekly for 3 weeks starting at day 0 post randomization. Human IgG1 isotype negative control was dosed at 5mg/kg on Days 0, 3, and 6 post randomization. The mean inhibition of tumor growth was determined at Days 0, 4, 7, 11, 14, and 18 using the following formula:

$$Mean\ \%\ Inhibition = (mean(C)-mean(T))/mean(C) * 100\%$$

$$T - current\ group\ value$$

$$C - control\ group\ value$$

[00346] TABLE 16 shows that mean inhibition values for the control, the CTLA4 antibodies, and the atezolizumab treatments over the course of the study.

TABLE 16

	Mean Inhibition					
Study day	**0**	**4**	**7**	**11**	**14**	**18**
Human IgG1 isotype (negative control)	n/a	n/a	n/a	n/a	n/a	n/a
Ipilimumab	2.14%	11.33%	30.07%	41.96%	52.16%	57.56%
CTLA4.A2	0.09%	-12.28%	25.30%	43.83%	50.20%	59.41%
CTLA4.A14	-0.17%	-7.72%	22.10%	36.93%	48.84%	55.18%
Atezolizumab	1.83%	17.66%	0.41%	-3.02%	-7.89%	-2.89%
Atezolizumab + Ipilimumab	-0.47%	-3.31%	18.00%	29.81%	30.39%	35.21%
Atezolizumab + CTLA4.A2	1.50%	-6.72%	23.00%	41.64%	40.93%	44.30%
Atezolizumab + CTLA4.A14	0.87%	2.55%	31.58%	45.65%	47.43%	55.18%

8.7. Example 8: Combination treatment (pembrolizumab and anti-CTLA4s)

[00347] Transgenic mice expressing human CTLA-4 and PD-1 (hCTLA4-hPD1 KI mice, n=8 per treatment group) were implanted subcutaneously with 1x 10^6 MC38 tumor cells in the right flank. The hCTLA4-hPD1 KI mice were treated with a control (1x phosphate buffered saline, or PBS); 2 mg/kg pembrolizumab (pembro) or 2 mg/kg pembro + 5 mg/kg anti-CTLA4, administered i.p. with a dose volume of 10ml/kg per animal as indicated in TABLE 17 twice weekly for three weeks starting on day 1 post-randomization. The mean (%) delta inhibition of tumor growth induced by each treatment in comparison to control treatment was calculated using the formula below and the results are shown in TABLE 17.

$$Mean\ \%\ \Delta Inhibition = ((mean(C)-mean(C_0))-(mean(T)-mean(T_0)))/(mean(C)-mean(C_0))*100\%$$

T - current group value

T_0 - current group initial value

C - control group value

C_0 - control group initial value

TABLE 17

	Mean Delta Inhibition						
Study day	**4**	**7**	**10**	**14**	**17**	**21**	**24**
Control	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Pembrolizumab	44.33%	44.20%	30.78%	20.92%	10.08%	8.62%	-11.94%
Pembrolizumab + CTLA4.A5	29.57%	79.32%	85.83%	87.28%	86.27%	78.55%	97.36%
Pembrolizumab + CTLA4.A1	-82.05%	-5.30%	41.81%	50.97%	55.73%	52.23%	66.74%

[00348] The study showed that mice treated with pembro alone did not exhibit tumor growth inhibition at day 24, however the addition of the indicated CTLA4 antibodies increased the tumor growth inhibition over the course of the study.

[00349] At the end of the experiment, select tumors were harvested and flow cytometry was conducted to investigate intratumoral immune cell populations. The data indicate that anti-CTLA4s decrease intratumoral Treg populations while increasing intratumoral NK cell populations (FIG. 6).

8.8. Example 9: Immune Related Adverse Events

[00350] Transgenic mice expressing human CTLA-4 (hCTLA-4 KI mice) were implanted with MC38 tumor cells on the right flank. The hCTLA-4 KI mice were treated with one mg/kg of the indicated CTLA-4 antibody on days 8, 11, and 14 post-implantation. The mice were weighed on days 8, 11, 14, and 17 post-implantation. Animal counts were: n=8 for ipilimumab, *n*=9 for A7, *n*=8 for A2, *n*=9 for A14, and *n*=8 for A14.2. The percent changes in body weight of the mice receiving the indicated anti-CTLA4 treatments are shown in FIG. 7.

[00351] The mice treated with CTLA4.A7, CTLA4.A14, and CTLA4.A14.2a did not appear to exhibit weight loss after the final dose of anti-CTLA4 (FIG. 7). This finding was unexpected because immune-related Adverse Events (irAE) have been reported to be greater when anti-CTLA4s with enhanced ADCC (*e.g.*, CTLA.A14.2a) are administered. This data suggest that anti-CTLA4s with reduced blocking activity may limit induction of irAEs even when ADCC is enhanced.

8.9. Example 10: Peripheral Flow Cytometry

[00352] Transgenic mice expressing human CTLA-4 (hCTLA-4 KI mice) were implanted with MC38 tumor cells in the right flank. The hCTLA-4 KI mice were treated with one mg/kg of the indicated CTLA-4 antibody on days 8, 11, and 14 post-implantation. Peripheral flow

cytometry was performed on day 27. 100μL of blood was used for staining. The findings from the peripheral blood flow cytometry are shown in FIGs. 8-10.

[00353] The results indicated that CTLA4.A2 and CTLA4.A14 decrease the elevation of peripheral T cells (CD3+). Enhancing ADCC with CTLA4.A14.2a increased newly activated T cells (CD69+). CTLA4.A2 and CTLA4.A14 resulted in fewer non-conventional regulatory cells (CD4+PD1+, CD4+ICOS+). (*See* FIG. 8).

[00354] The results also indicated that CTLA4.A2 and CTLA4.A14 better enhance CD8+ T cells. CTLA4.A2 better enhanced newly activated T cells (CD8+CD69+) and led to decreased T cell exhaustion (CD8+PD1+) relative to Ipilimumab. ICOS has been described as a pharmacodynamic marker for anti-CTLA4. Enhancing ADCC with CTLA4.A14.2a appeared to further elevate CD8+ICOS+ cells (FIG. 9). The results also indicated that CTLA4.A2 and CTLA4.A14.2a lead to decreased peripheral immune activation relative to Ipilimumab, as judged by the frequency of dendritic cells (DCs) and activated DCs (CD86+). (*see* FIG. 10).

8.10. Example 11: Treatment with Low Dose CTLA-4 Study

[00355] Transgenic mice expressing human CTLA-4 (hCTLA-4 KI mice) were implanted subcutaneously in the right flank region with MC38 tumor cells (1E6) in 0.1 ml of PBS for tumor development. The cells in exponential growth phase were harvested and quantitated by cell counter before tumor implantation. The hCTLA-4 KI mice were randomized when the mean tumor volume was 96.15 mm^3 and treated with 0.3 mg/kg of the indicated anti-CTLA4, ipilimumab, or human IgG1 isotype control (Isotype) on days 0, 3 and 6 post-randomization. Tumor volume and mean % of inhibition was determined as described in Example 4. CTLA4.A2 and CTLA4.A14 resulted in significantly higher tumor inhibition over the 18 days of the study. The results of the study are shown in TABLE 18 and FIG. 11.

TABLE 18

Group	Dates/Study Days					
	12/6/2019	12/10/2019	12/13/2019	12/17/2019	12/20/2019	12/24/2019
	0	4	7	11	14	18
Isotype						
Ipilimumab	-0.17%	9.33%	18.68%	22.37%	27.23%	11.84%
CTLA4.A2	0.22%	13.00%	23.03%	49.09%	60.03%	59.54%
CTLA4.A14	0.01%	23.39%	35.06%	53.05%	57.50%	45.41%

9. INCORPORATION BY REFERENCE

[00356] 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.

10. EQUIVALENTS

[00357] 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 19 provides sequences for antibody light chains, heavy chains, CDRs, and human CTLA4.

TABLE 19

SEQ ID NO	Sequence	Chain (Antibody)
1	EIVLTQSPGTLSLSPGEGATLSCRASQSFSSNYLAWYQQKPGQAPRLLIYGASSRA TGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYGTSPFTFGPGTKVDIK	L1 (A1)
2	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLIYGASSRA TGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYGRSPFTFGPGTKVDIK	L2 (A2)
3	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLIYGASSRA TGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYGSSPFTFGPGTKVDIK	L3 (A3)
4	EIVLTQSPGTLSLSPGDRATLSCRASQSGSSSYLAWYQQKPGQAPRLLIYGASSRA TGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYGTSPFTSGPGTKVDIK	L4 (A4)
5	EIVLTQSPGTLSLSPGDRATLSCRASQSGSSSYLAWYQQKPGQAPRLLIYGASSRA TGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYGTSPFTSGPGTKVDIK	L5 (A5)
6	DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQS GVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYSTPFTFGPGTKVDIK	L6 (A6)
7	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLIYGASSRA TGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYGSSPFTFGPGTKVDIK	L7 (A7)
8	EIVLTQSPGTLSLSPGERATLSCRASQSVSYLAWYQQKPGQAPRLLIYGASSRATG IPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYGSSPWTFGQGTKVEIK	L8 (A8)
9	DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQS GVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYSTPFTFGPGTKVDIK	L9 (A9)
10	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLIYGASSRA TGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYGSSPFTFGPGTKVDIK	L10 (A10)
11	DIQLTQSPSFLSASVGDRVTITCRASQGISSYLAWYQQKPGKAPKLLIYAASTLQS GVPSRFSGSGSGTEFTLTISSLQPEDFATYYCQQLNSYPPTFGQGTKVEIK	L11 (A11)
12	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLIYGASSRA TGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYGTSPWTFGQGTKVEIK	L12 (A12)
13	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLIYGASSRA TGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYGSSPFTFGPGTKVDIK	L13 (A13)
14	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLIYGASSRA TGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYGSSPWTSGQGTKVEIK	L14 (A14)
15	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLIYGASSRA TGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYGSSPFTFGPGTKVDIK	L15 (A15)
16	DIQMTQSPSSLSASVGDRVTITCRASQGISNYLAWYQQKPGKVPKLLIYAASTLQS GVPSRFSGSGSGTDFTLTISSLQPEDVATYYCQNYNSAPWTFGQGTKVEIK	L16 (A16)
17	DIQMTQSPSSLSASVGDRVTITCRASQAIRNDLGWYQQKPGKAPKRLIYAASSLQS GVPPRFSGSGSGTEFTLTISSLQPEDFATYYCLQHNSYPLTFGGGTKVEIK	L17 (A17)
18	EIVLTQSPGTLSLSPGERATLSCRASQSVSYLAWYQQKPGQAPRLLIYGASSRATG IPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYGSSPWTFGQGTKVEIK	L18 (A18)
19	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLIYGASSRA TGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYGSSSMYTFGQGTKLEIK	L19 (A19)
20	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLIYGASSRA TGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYGRSPFTFGPGTKVDIK	L20 (A20)
21	DIVMTQSPLSLPVTPGEPASISCRSSQSLLHSNGYNYLDWYLQKPGQSPQLLIYLG SNRASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCMQTLQTPLTFGGGTKVEI K	L21 (A21)

22	DIQMTQSPSSLSASVGDRVTITCRASQAIRNDLGWYQQKPGKAPKRLIYAASSLQS GVPPRFSGSGSGTEFTLTISSLQPEDFATYYCLQHNSYPLTFGGGTKVEIK	L22 (A22)
23	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLIYGASSRA TGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYGPSPWTFGQGTKVEIK	L23 (A23)
24	DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQS GVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYRTPFTFGPGTKVDIK	L24 (A24)
25	EIVMTQSPATLSLSPGERATLSCRASQSVSSSYLSWYQQKPGQAPRLLIYGASTRA TGIPARFSGSGSGTDFTLTISNLQPEDFAVYYCQQGYNLPFTAGPGTKVDIK	L25 (A25)
26	DVVMTQSPLSLPVTLGQPASISCRSSQSLVYSDGNTYLNWFQQRPGQSPRRLIYK VSNRDSGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCMQGTHWPITSGQGTRLE IK	L26 (A26)
27	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLIYGASSRA TGIPDRFSGSGSGTDFTLTISRLEPEDFAVYHCQQYGRSPWTLGQGTKVEIK	L27 (A27)
28	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLIYGASSRA TGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYGSSPWTSGQGTKVEIK	L28 (A28)
101	QVQLVESGGGVVQPGRSLRLSCAASGFTFSSFGMHWVRQAPGKGLEWVAVIWY DGRNKYYVDSVKGRFTISRDNSNNTLYLQMNSLRAEDTAVYYCARGEFFGEFFD YWGQGTLVTVSSA	H1 (A1)
102	QVQLVESGGGVVQPGRSLRLSCAASGFTFSNYGMNWVRQAPGKGLEWVAVIWY DGRNKHYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARGGDWGPYF DYWGQGTLVTVSSA	H2 (A2)
103	QVQLVESGGGVVQPGRSLRLSCIASGFTFSSYGMHWVRQAPGKGLEWVAVNWY DGSNKHYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARGGVWGPYF DYWGQGTLVTVSSA	H3 (A3)
104	QVQLVESGGGVVQPGRSLRISCAASGFTFSSYGIHWVRQAPGKGLQWVAVIWYD GRNKYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCSRSGSFGAFDIW GQGTMVTVSSA	H4 (A4)
105	QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKGLEWVAVIWY DGNNKYYADSVKGRFTISRDNSKNTLYLQMYSLRAEDTAVYYCARGGILAAGIF DYWGQGTLVTVSSA	H5 (A5)
106	QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKGLEWVAVIWY DGSYKYYADSVKGRFTISRDDSKNTLYLQMSSLRAEDTAVYYCARAPHYAILTG YYEDYWGQGTLVTVSSA	H6 (A6)
107	QVQLVESGGGVVQPGRSLRLSCAASGFTLSSFGMHWVRQAPGKGLEWVAVIWY DGSNKYYADSVKGRFIISRDNSKTALYLQMNSLRAEDTAVYYCARAHYFGAFDI WGQGTMVTVSSA	H7 (A7)
108	QVQLVESGGGVVQPGRSLRLSCAASGFTFSRYGMHWVRQAPGKGLEWVAVIWY DGRNKYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTALYSCARAGELGPFDY WGQGTLVTVSSA	H8 (A8)
109	QVQLVESGGGVVQPGRSLRLSCAASGFTFSSHGMHWVRQAPGKGLEWVAVIWY DGSNKHYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARGDILTGYYG YWGQGTLVTVSSA	H9 (A9)
110	QVQLVESGGGVVQPGRSLRLSCVASGFTLSSYGMHWVRQAPGKGLEWVAVIWY DGSNKHYADSVKGRFTISRDNSKNTLSLQMNSLRAEDTAVYYCARGGQLGPFDY WGQGTLVTVSSA	H10 (A10)
111	QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKGLEWVAVIWY DVGNKYYIDSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARDYYGSGSPR HFDYWGQGTLVTVSSA	H11 (A11)
112	QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKGLEWVAVIWY DGSNKYYADSVKGRFTFSRDNSKNTLYLQMNSLRAEDTAVYYCARGGLMGAFD YWGQGTLVTVSSA	H12 (A12)
113	QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKGLEWVAVIWY DGRNKDYADSVKGRITISRDNSKNTLYLQMNSLRAEDTAVYYCARGGLLGPYFD YWGQGTLVTVSSA	H13 (A13)
114	QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGTGLEWVAVIWY EGRNKYYADPVKGRFTISRDNSKNTLYLQMNSLRDDDTAVYYCARAGDLGAFDI WGQGTMVTVSSA	H14 (A14)
115	QVQLVESGGGVVQPGRSLRLSCAASGFTFRSYGMHWVRQAPGKGLEWVAVIWY DGSNKHYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARNGLIGAFDI WGQGTMVTVSSA	H15 (A15)

116	QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGIHWVRQAPGKGLEWVAVIWYD GSNKYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARGSLLGPFDYW GQGTLVTVSSA	H16 (A16)
117	EVQLVESGGGLVQPGGSLRLSCAASGFTFSNYAMSWVRQAPGKGLEWVSAISGG GLSTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKDLLWLGFDY WGQGTLVTVSSA	H17 (A17)
118	QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKGLEWVAVIWY DGSNKFYADSVKGRFTISSDNSKNTLYLQMNSLRAEDTAVYYCARGGHLGSFDY WGQGTLVTVSSA	H18 (A18)
119	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYYMHWVRQAPGQGLEWMGIINP SVGSTSYAQKFQGRVTMTRDTSTSTVYMELSSLRSEDTAVYYCAREVRVRGVIIP FFDYWDQGTLVTVSSA	H19 (A19)
120	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYGISWVRQAPGQGLEWMGWISA YNGNTNYAQKFQGRVTMTTDTSTSTAYMELRSLRSDDTAVYYCAKVSGYFDYW GQGTLVTVSSA	H20 (A20)
121	QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKGLEWVAVIWY DGNNKYYADSVKGRFTISRDNSKNTLYLHMNSLRADDTAVYYCARMLRGAPYY YGMDVWGQGTTVTVSSA	H21 (A21)
122	EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSGISGS GGSTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKLGIAWYFDV WGRGTLVTVSSA	H22 (A22)
123	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYGISWVRQAPGQGLEWMGWISA YNGNTYYAQKFQGRVTMTTDTSTSTAYVELRSLRSDDTAVYYCARVTGRDAFDI WGQGTMVTVSSA	H23 (A23)
124	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYGISWVRQAPGQGLECMGWISA YNGNTNYAQKFQGRVTMITDTSTSTAYMELRSLRSDDTAVYYCARVGPINLDY WGQGTLVTVSSA	H24 (A24)
125	QVQLVQSGAEVKKPGASVKVSCKASGYTFTNYGISWVRQAPGQGLEWMGWISV YNGNTNYAQKFQGRVTMTTDTSTSTAYMELRSLISDDTAVYYCARLGKGLFDY WGQGTLVTVSSA	H25 (A25)
126	QVQLVQSGAEVKKPGASVKVSCKASDYTFTYYGISWVRQAPGQGLEWMGWISA YNGNTNYAQKLQGRVTMTTDTSTNTAYLELRSLRSDDTAVYYCARDYYDSSGY FDYWGQGTLVTVSSA	H26 (A26)
127	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYGISWVRQAPGQGLEWMGWISA YNGNTNYAQKLQGRVTMTTDTSTSTAYMELRSLRSDDTAVYYCGRWVRGVEY WGQGTLVTVSSA	H27 (A27)
128	QVQLVESGGGVVQPGRSLGLSCAASGFTFSTYGMHWVRQAPGKGLEWVAVTLY DGSNKYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARASLTGSFDY WGQGTLVTVSSA	H28 (A28)
1001	QSFSSNY	CDR1-L1 (A1)
1002	QSVSSSY	CDR1-L2 (A2)
1003	QSVSSSY	CDR1-L3 (A3)
1004	QSGSSSY	CDR1-L4 (A4)
1005	QSGSSSY	CDR1-L5 (A5)
1006	QSISSY	CDR1-L6 (A6)
1007	QSVSSSY	CDR1-L7 (A7)
1008	QSVSY	CDR1-L8 (A8)
1009	QSISSY	CDR1-L9 (A9)
1010	QSVSSSY	CDR1-L10 (A10)
1011	QGISSY	CDR1-L11 (A11)
1012	QSVSSSY	CDR1-L12 (A12)
1013	QSVSSSY	CDR1-L13 (A13)
1014	QSVSSSY	CDR1-L14 (A14)
1015	QSVSSSY	CDR1-L15 (A15)
1016	QGISNY	CDR1-L16 (A16)
1017	QAIRND	CDR1-L17 (A17)
1018	QSVSY	CDR1-L18 (A18)
1019	QSVSSSY	CDR1-L19 (A19)
1020	QSVSSSY	CDR1-L20 (A20)

1021	QSLLHSNGYNY	CDR1-L21 (A21)
1022	QAIRND	CDR1-L22 (A22)
1023	QSVSSSY	CDR1-L23 (A23)
1024	QSISSY	CDR1-L24 (A24)
1025	QSVSSSY	CDR1-L25 (A25)
1026	QSLVYSDGNTY	CDR1-L26 (A26)
1027	QSVSSSY	CDR1-L27 (A27)
1028	QSVSSSY	CDR1-L28 (A28)
2001	GAS	CDR2-L1 (A1)
2002	GAS	CDR2-L2 (A2)
2003	GAS	CDR2-L3 (A3)
2004	GAS	CDR2-L4 (A4)
2005	GAS	CDR2-L5 (A5)
2006	AAS	CDR2-L6 (A6)
2007	GAS	CDR2-L7 (A7)
2008	GAS	CDR2-L8 (A8)
2009	AAS	CDR2-L9 (A9)
2010	GAS	CDR2-L10 (A10)
2011	AAS	CDR2-L11 (A11)
2012	GAS	CDR2-L12 (A12)
2013	GAS	CDR2-L13 (A13)
2014	GAS	CDR2-L14 (A14)
2015	GAS	CDR2-L15 (A15)
2016	AAS	CDR2-L16 (A16)
2017	AAS	CDR2-L17 (A17)
2018	GAS	CDR2-L18 (A18)
2019	GAS	CDR2-L19 (A19)
2020	GAS	CDR2-L20 (A20)
2021	LGS	CDR2-L21 (A21)
2022	AAS	CDR2-L22 (A22)
2023	GAS	CDR2-L23 (A23)
2024	AAS	CDR2-L24 (A24)
2025	GAS	CDR2-L25 (A25)
2026	KVS	CDR2-L26 (A26)
2027	GAS	CDR2-L27 (A27)
2028	GAS	CDR2-L28 (A28)
3001	QQYGTSPFT	CDR3-L1 (A1)
3002	QQYGRSPFT	CDR3-L2 (A2)
3003	QQYGSSPFT	CDR3-L3 (A3)
3004	QQYGTSPFT	CDR3-L4 (A4)
3005	QQYGTSPFT	CDR3-L5 (A5)
3006	QQSYSTPFT	CDR3-L6 (A6)
3007	QQYGSSPFT	CDR3-L7 (A7)
3008	QQYGSSPWT	CDR3-L8 (A8)
3009	QQSYSTPFT	CDR3-L9 (A9)
3010	QQYGSSPFT	CDR3-L10 (A10)
3011	QQLNSYPPT	CDR3-L11 (A11)
3012	QQYGTSPWT	CDR3-L12 (A12)
3013	QQYGSSPFT	CDR3-L13 (A13)
3014	QQYGSSPWT	CDR3-L14 (A14)
3015	QQYGSSPFT	CDR3-L15 (A15)
3016	QNYNSAPWT	CDR3-L16 (A16)
3017	LQHNSYPLT	CDR3-L17 (A17)
3018	QQYGSSPWT	CDR3-L18 (A18)
3019	QQYGSSSMYT	CDR3-L19 (A19)
3020	QQYGRSPFT	CDR3-L20 (A20)
3021	MQTLQTPLT	CDR3-L21 (A21)
3022	LQHNSYPLT	CDR3-L22 (A22)

3023	QQYGPSPWT	CDR3-L23 (A23)
3024	QQSYRTPFT	CDR3-L24 (A24)
3025	QQGYNLPFT	CDR3-L25 (A25)
3026	MQGTHWPIT	CDR3-L26 (A26)
3027	QQYGRSPWT	CDR3-L27 (A27)
3028	QQYGSSPWT	CDR3-L28 (A28)
4001	GFTFSSFG	CDR1-H1 (A1)
4002	GFTFSNYG	CDR1-H2 (A2)
4003	GFTFSSYG	CDR1-H3 (A3)
4004	GFTFSSYG	CDR1-H4 (A4)
4005	GFTFSSYG	CDR1-H5 (A5)
4006	GFTFSSYG	CDR1-H6 (A6)
4007	GFTLSSFG	CDR1-H7 (A7)
4008	GFTFSRYG	CDR1-H8 (A8)
4009	GFTFSSHG	CDR1-H9 (A9)
4010	GFTLSSYG	CDR1-H10 (A10)
4011	GFTFSSYG	CDR1-H11 (A11)
4012	GFTFSSYG	CDR1-H12 (A12)
4013	GFTFSSYG	CDR1-H13 (A13)
4014	GFTFSSYG	CDR1-H14 (A14)
4015	GFTFRSYG	CDR1-H15 (A15)
4016	GFTFSSYG	CDR1-H16 (A16)
4017	GFTFSNYA	CDR1-H17 (A17)
4018	GFTFSSYG	CDR1-H18 (A18)
4019	GYTFTSYY	CDR1-H19 (A19)
4020	GYTFTSYG	CDR1-H20 (A20)
4021	GFTFSSYG	CDR1-H21 (A21)
4022	GFTFSSYA	CDR1-H22 (A22)
4023	GYTFTSYG	CDR1-H23 (A23)
4024	GYTFTSYG	CDR1-H24 (A24)
4025	GYTFTNYG	CDR1-H25 (A25)
4026	DYTFTYYG	CDR1-H26 (A26)
4027	GYTFTSYG	CDR1-H27 (A27)
4028	GFTFSTYG	CDR1-H28 (A28)
5001	IWYDGRNK	CDR2-H1 (A1)
5002	IWYDGRNK	CDR2-H2 (A2)
5003	NWYDGSNK	CDR2-H3 (A3)
5004	IWYDGRNK	CDR2-H4 (A4)
5005	IWYDGNNK	CDR2-H5 (A5)
5006	IWYDGSYK	CDR2-H6 (A6)
5007	IWYDGSNK	CDR2-H7 (A7)
5008	IWYDGRNK	CDR2-H8 (A8)
5009	IWYDGSNK	CDR2-H9 (A9)
5010	IWYDGSNK	CDR2-H10 (A10)
5011	IWYDVGNK	CDR2-H11 (A11)
5012	IWYDGSNK	CDR2-H12 (A12)
5013	IWYDGRNK	CDR2-H13 (A13)
5014	IWYEGRNK	CDR2-H14 (A14)
5015	IWYDGSNK	CDR2-H15 (A15)
5016	IWYDGSNK	CDR2-H16 (A16)
5017	ISGGGLST	CDR2-H17 (A17)
5018	IWYDGSNK	CDR2-H18 (A18)
5019	INPSVGST	CDR2-H19 (A19)
5020	ISAYNGNT	CDR2-H20 (A20)
5021	IWYDGNNK	CDR2-H21 (A21)
5022	ISGSGGST	CDR2-H22 (A22)
5023	ISAYNGNT	CDR2-H23 (A23)
5024	ISAYNGNT	CDR2-H24 (A24)

5025	ISVYNGNT	CDR2-H25 (A25)
5026	ISAYNGNT	CDR2-H26 (A26)
5027	ISAYNGNT	CDR2-H27 (A27)
5028	TLYDGSNK	CDR2-H28 (A28)
6001	ARGEFFGEFFDY	CDR3-H1 (A1)
6002	ARGGDWGPYFDY	CDR3-H2 (A2)
6003	ARGGVWGPYFDY	CDR3-H3 (A3)
6004	SRSGSFGAFDI	CDR3-H4 (A4)
6005	ARGGILAAGIFDY	CDR3-H5 (A5)
6006	ARAPHYAILTGYYEDY	CDR3-H6 (A6)
6007	ARAHYFGAFDI	CDR3-H7 (A7)
6008	ARAGELGPFDY	CDR3-H8 (A8)
6009	ARGDILTGYYGY	CDR3-H9 (A9)
6010	ARGGQLGPFDY	CDR3-H10 (A10)
6011	ARDYYGSGSPRHFDY	CDR3-H11 (A11)
6012	ARGGLMGAFDY	CDR3-H12 (A12)
6013	ARGGLLGPYFDY	CDR3-H13 (A13)
6014	ARAGDLGAFDI	CDR3-H14 (A14)
6015	ARNGLIGAFDI	CDR3-H15 (A15)
6016	ARGSLLGPFDY	CDR3-H16 (A16)
6017	AKDLLWLGFDY	CDR3-H17 (A17)
6018	ARGGHLGSFDY	CDR3-H18 (A18)
6019	AREVRVRGVIIPFFDY	CDR3-H19 (A19)
6020	AKVSGYFDY	CDR3-H20 (A20)
6021	ARMLRGAPYYYGMDV	CDR3-H21 (A21)
6022	AKLGIAWYFDV	CDR3-H22 (A22)
6023	ARVTGRDAFDI	CDR3-H23 (A23)
6024	ARVGPINLDY	CDR3-H24 (A24)
6025	ARLGKGLFDY	CDR3-H25 (A25)
6026	ARDYYDSSGYFDY	CDR3-H26 (A26)
6027	GRWVRGVEY	CDR3-H27 (A27)
6028	ARASLTGSFDY	CDR3-H28 (A28)
7001	MACLGFQRHKAQLNLAARTWPCTLLFFLLFIPVFCKAMHVAQPAVVLASSRGIAS FVCEYASPGKATEVRVTVLRQADSQVTEVCAATYMMGNELTFLDDSICTGTSSG NQVNLTIQGLRAMDTGLYICKVELMYPPPYYLGIGNGTQIYVIDPEPCPDSDFLLW ILAAVSSGLFFYSFLLTAVSLSKMLKKRSPLTTGVYVKMPPTEPECEKQFQPYFIPI N	Human CTLA4

Table 20 provides the sequence identifiers for the light chain, heavy chain, and CDRs of the indicated clones

TABLE 20								
	SEQ ID NO							
Antibody Clone Number	**Light Chain**	**Heavy Chain**	**CDR1 (Light)**	**CDR2 (Light)**	**CDR3 (Light)**	**CDR1 (Heavy)**	**CDR2 (Heavy)**	**CDR3 (Heavy)**
1	8000	8496	8992	9488	9984	10480	10976	11472
2	8001	8497	8993	9489	9985	10481	10977	11473
3	8002	8498	8994	9490	9986	10482	10978	11474
4	8003	8499	8995	9491	9987	10483	10979	11475
5	8004	8500	8996	9492	9988	10484	10980	11476
6	8005	8501	8997	9493	9989	10485	10981	11477
7	8006	8502	8998	9494	9990	10486	10982	11478
8	8007	8503	8999	9495	9991	10487	10983	11479
9	8008	8504	9000	9496	9992	10488	10984	11480
10	8009	8505	9001	9497	9993	10489	10985	11481
11	8010	8506	9002	9498	9994	10490	10986	11482
12	8011	8507	9003	9499	9995	10491	10987	11483
13	8012	8508	9004	9500	9996	10492	10988	11484
14	8013	8509	9005	9501	9997	10493	10989	11485

15	8014	8510	9006	9502	9998	10494	10990	11486
16	8015	8511	9007	9503	9999	10495	10991	11487
17	8016	8512	9008	9504	10000	10496	10992	11488
18	8017	8513	9009	9505	10001	10497	10993	11489
19	8018	8514	9010	9506	10002	10498	10994	11490
20	8019	8515	9011	9507	10003	10499	10995	11491
21	8020	8516	9012	9508	10004	10500	10996	11492
22	8021	8517	9013	9509	10005	10501	10997	11493
23	8022	8518	9014	9510	10006	10502	10998	11494
24	8023	8519	9015	9511	10007	10503	10999	11495
25	8024	8520	9016	9512	10008	10504	11000	11496
26	8025	8521	9017	9513	10009	10505	11001	11497
27	8026	8522	9018	9514	10010	10506	11002	11498
28	8027	8523	9019	9515	10011	10507	11003	11499
29	8028	8524	9020	9516	10012	10508	11004	11500
30	8029	8525	9021	9517	10013	10509	11005	11501
31	8030	8526	9022	9518	10014	10510	11006	11502
32	8031	8527	9023	9519	10015	10511	11007	11503
33	8032	8528	9024	9520	10016	10512	11008	11504
34	8033	8529	9025	9521	10017	10513	11009	11505
35	8034	8530	9026	9522	10018	10514	11010	11506
36	8035	8531	9027	9523	10019	10515	11011	11507
37	8036	8532	9028	9524	10020	10516	11012	11508
38	8037	8533	9029	9525	10021	10517	11013	11509
39	8038	8534	9030	9526	10022	10518	11014	11510
40	8039	8535	9031	9527	10023	10519	11015	11511
41	8040	8536	9032	9528	10024	10520	11016	11512
42	8041	8537	9033	9529	10025	10521	11017	11513
43	8042	8538	9034	9530	10026	10522	11018	11514
44	8043	8539	9035	9531	10027	10523	11019	11515
45	8044	8540	9036	9532	10028	10524	11020	11516
46	8045	8541	9037	9533	10029	10525	11021	11517
47	8046	8542	9038	9534	10030	10526	11022	11518
48	8047	8543	9039	9535	10031	10527	11023	11519
49	8048	8544	9040	9536	10032	10528	11024	11520
50	8049	8545	9041	9537	10033	10529	11025	11521
51	8050	8546	9042	9538	10034	10530	11026	11522
52	8051	8547	9043	9539	10035	10531	11027	11523
53	8052	8548	9044	9540	10036	10532	11028	11524
54	8053	8549	9045	9541	10037	10533	11029	11525
55	8054	8550	9046	9542	10038	10534	11030	11526
56	8055	8551	9047	9543	10039	10535	11031	11527
57	8056	8552	9048	9544	10040	10536	11032	11528
58	8057	8553	9049	9545	10041	10537	11033	11529
59	8058	8554	9050	9546	10042	10538	11034	11530
60	8059	8555	9051	9547	10043	10539	11035	11531
61	8060	8556	9052	9548	10044	10540	11036	11532
62	8061	8557	9053	9549	10045	10541	11037	11533
63	8062	8558	9054	9550	10046	10542	11038	11534
64	8063	8559	9055	9551	10047	10543	11039	11535
65	8064	8560	9056	9552	10048	10544	11040	11536
66	8065	8561	9057	9553	10049	10545	11041	11537
67	8066	8562	9058	9554	10050	10546	11042	11538
68	8067	8563	9059	9555	10051	10547	11043	11539
69	8068	8564	9060	9556	10052	10548	11044	11540
70	8069	8565	9061	9557	10053	10549	11045	11541
71	8070	8566	9062	9558	10054	10550	11046	11542
72	8071	8567	9063	9559	10055	10551	11047	11543
73	8072	8568	9064	9560	10056	10552	11048	11544

74	8073	8569	9065	9561	10057	10553	11049	11545
75	8074	8570	9066	9562	10058	10554	11050	11546
76	8075	8571	9067	9563	10059	10555	11051	11547
77	8076	8572	9068	9564	10060	10556	11052	11548
78	8077	8573	9069	9565	10061	10557	11053	11549
79	8078	8574	9070	9566	10062	10558	11054	11550
80	8079	8575	9071	9567	10063	10559	11055	11551
81	8080	8576	9072	9568	10064	10560	11056	11552
82	8081	8577	9073	9569	10065	10561	11057	11553
83	8082	8578	9074	9570	10066	10562	11058	11554
84	8083	8579	9075	9571	10067	10563	11059	11555
85	8084	8580	9076	9572	10068	10564	11060	11556
86	8085	8581	9077	9573	10069	10565	11061	11557
87	8086	8582	9078	9574	10070	10566	11062	11558
88	8087	8583	9079	9575	10071	10567	11063	11559
89	8088	8584	9080	9576	10072	10568	11064	11560
90	8089	8585	9081	9577	10073	10569	11065	11561
91	8090	8586	9082	9578	10074	10570	11066	11562
92	8091	8587	9083	9579	10075	10571	11067	11563
93	8092	8588	9084	9580	10076	10572	11068	11564
94	8093	8589	9085	9581	10077	10573	11069	11565
95	8094	8590	9086	9582	10078	10574	11070	11566
96	8095	8591	9087	9583	10079	10575	11071	11567
97	8096	8592	9088	9584	10080	10576	11072	11568
98	8097	8593	9089	9585	10081	10577	11073	11569
99	8098	8594	9090	9586	10082	10578	11074	11570
100	8099	8595	9091	9587	10083	10579	11075	11571
101	8100	8596	9092	9588	10084	10580	11076	11572
102	8101	8597	9093	9589	10085	10581	11077	11573
103	8102	8598	9094	9590	10086	10582	11078	11574
104	8103	8599	9095	9591	10087	10583	11079	11575
105	8104	8600	9096	9592	10088	10584	11080	11576
106	8105	8601	9097	9593	10089	10585	11081	11577
107	8106	8602	9098	9594	10090	10586	11082	11578
108	8107	8603	9099	9595	10091	10587	11083	11579
109	8108	8604	9100	9596	10092	10588	11084	11580
110	8109	8605	9101	9597	10093	10589	11085	11581
111	8110	8606	9102	9598	10094	10590	11086	11582
112	8111	8607	9103	9599	10095	10591	11087	11583
113	8112	8608	9104	9600	10096	10592	11088	11584
114	8113	8609	9105	9601	10097	10593	11089	11585
115	8114	8610	9106	9602	10098	10594	11090	11586
116	8115	8611	9107	9603	10099	10595	11091	11587
117	8116	8612	9108	9604	10100	10596	11092	11588
118	8117	8613	9109	9605	10101	10597	11093	11589
119	8118	8614	9110	9606	10102	10598	11094	11590
120	8119	8615	9111	9607	10103	10599	11095	11591
121	8120	8616	9112	9608	10104	10600	11096	11592
122	8121	8617	9113	9609	10105	10601	11097	11593
123	8122	8618	9114	9610	10106	10602	11098	11594
124	8123	8619	9115	9611	10107	10603	11099	11595
125	8124	8620	9116	9612	10108	10604	11100	11596
126	8125	8621	9117	9613	10109	10605	11101	11597
127	8126	8622	9118	9614	10110	10606	11102	11598
128	8127	8623	9119	9615	10111	10607	11103	11599
129	8128	8624	9120	9616	10112	10608	11104	11600
130	8129	8625	9121	9617	10113	10609	11105	11601
131	8130	8626	9122	9618	10114	10610	11106	11602
132	8131	8627	9123	9619	10115	10611	11107	11603

133	8132	8628	9124	9620	10116	10612	11108	11604
134	8133	8629	9125	9621	10117	10613	11109	11605
135	8134	8630	9126	9622	10118	10614	11110	11606
136	8135	8631	9127	9623	10119	10615	11111	11607
137	8136	8632	9128	9624	10120	10616	11112	11608
138	8137	8633	9129	9625	10121	10617	11113	11609
139	8138	8634	9130	9626	10122	10618	11114	11610
140	8139	8635	9131	9627	10123	10619	11115	11611
141	8140	8636	9132	9628	10124	10620	11116	11612
142	8141	8637	9133	9629	10125	10621	11117	11613
143	8142	8638	9134	9630	10126	10622	11118	11614
144	8143	8639	9135	9631	10127	10623	11119	11615
145	8144	8640	9136	9632	10128	10624	11120	11616
146	8145	8641	9137	9633	10129	10625	11121	11617
147	8146	8642	9138	9634	10130	10626	11122	11618
148	8147	8643	9139	9635	10131	10627	11123	11619
149	8148	8644	9140	9636	10132	10628	11124	11620
150	8149	8645	9141	9637	10133	10629	11125	11621
151	8150	8646	9142	9638	10134	10630	11126	11622
152	8151	8647	9143	9639	10135	10631	11127	11623
153	8152	8648	9144	9640	10136	10632	11128	11624
154	8153	8649	9145	9641	10137	10633	11129	11625
155	8154	8650	9146	9642	10138	10634	11130	11626
156	8155	8651	9147	9643	10139	10635	11131	11627
157	8156	8652	9148	9644	10140	10636	11132	11628
158	8157	8653	9149	9645	10141	10637	11133	11629
159	8158	8654	9150	9646	10142	10638	11134	11630
160	8159	8655	9151	9647	10143	10639	11135	11631
161	8160	8656	9152	9648	10144	10640	11136	11632
162	8161	8657	9153	9649	10145	10641	11137	11633
163	8162	8658	9154	9650	10146	10642	11138	11634
164	8163	8659	9155	9651	10147	10643	11139	11635
165	8164	8660	9156	9652	10148	10644	11140	11636
166	8165	8661	9157	9653	10149	10645	11141	11637
167	8166	8662	9158	9654	10150	10646	11142	11638
168	8167	8663	9159	9655	10151	10647	11143	11639
169	8168	8664	9160	9656	10152	10648	11144	11640
170	8169	8665	9161	9657	10153	10649	11145	11641
171	8170	8666	9162	9658	10154	10650	11146	11642
172	8171	8667	9163	9659	10155	10651	11147	11643
173	8172	8668	9164	9660	10156	10652	11148	11644
174	8173	8669	9165	9661	10157	10653	11149	11645
175	8174	8670	9166	9662	10158	10654	11150	11646
176	8175	8671	9167	9663	10159	10655	11151	11647
177	8176	8672	9168	9664	10160	10656	11152	11648
178	8177	8673	9169	9665	10161	10657	11153	11649
179	8178	8674	9170	9666	10162	10658	11154	11650
180	8179	8675	9171	9667	10163	10659	11155	11651
181	8180	8676	9172	9668	10164	10660	11156	11652
182	8181	8677	9173	9669	10165	10661	11157	11653
183	8182	8678	9174	9670	10166	10662	11158	11654
184	8183	8679	9175	9671	10167	10663	11159	11655
185	8184	8680	9176	9672	10168	10664	11160	11656
186	8185	8681	9177	9673	10169	10665	11161	11657
187	8186	8682	9178	9674	10170	10666	11162	11658
188	8187	8683	9179	9675	10171	10667	11163	11659
189	8188	8684	9180	9676	10172	10668	11164	11660
190	8189	8685	9181	9677	10173	10669	11165	11661
191	8190	8686	9182	9678	10174	10670	11166	11662

192	8191	8687	9183	9679	10175	10671	11167	11663
193	8192	8688	9184	9680	10176	10672	11168	11664
194	8193	8689	9185	9681	10177	10673	11169	11665
195	8194	8690	9186	9682	10178	10674	11170	11666
196	8195	8691	9187	9683	10179	10675	11171	11667
197	8196	8692	9188	9684	10180	10676	11172	11668
198	8197	8693	9189	9685	10181	10677	11173	11669
199	8198	8694	9190	9686	10182	10678	11174	11670
200	8199	8695	9191	9687	10183	10679	11175	11671
201	8200	8696	9192	9688	10184	10680	11176	11672
202	8201	8697	9193	9689	10185	10681	11177	11673
203	8202	8698	9194	9690	10186	10682	11178	11674
204	8203	8699	9195	9691	10187	10683	11179	11675
205	8204	8700	9196	9692	10188	10684	11180	11676
206	8205	8701	9197	9693	10189	10685	11181	11677
207	8206	8702	9198	9694	10190	10686	11182	11678
208	8207	8703	9199	9695	10191	10687	11183	11679
209	8208	8704	9200	9696	10192	10688	11184	11680
210	8209	8705	9201	9697	10193	10689	11185	11681
211	8210	8706	9202	9698	10194	10690	11186	11682
212	8211	8707	9203	9699	10195	10691	11187	11683
213	8212	8708	9204	9700	10196	10692	11188	11684
214	8213	8709	9205	9701	10197	10693	11189	11685
215	8214	8710	9206	9702	10198	10694	11190	11686
216	8215	8711	9207	9703	10199	10695	11191	11687
217	8216	8712	9208	9704	10200	10696	11192	11688
218	8217	8713	9209	9705	10201	10697	11193	11689
219	8218	8714	9210	9706	10202	10698	11194	11690
220	8219	8715	9211	9707	10203	10699	11195	11691
221	8220	8716	9212	9708	10204	10700	11196	11692
222	8221	8717	9213	9709	10205	10701	11197	11693
223	8222	8718	9214	9710	10206	10702	11198	11694
224	8223	8719	9215	9711	10207	10703	11199	11695
225	8224	8720	9216	9712	10208	10704	11200	11696
226	8225	8721	9217	9713	10209	10705	11201	11697
227	8226	8722	9218	9714	10210	10706	11202	11698
228	8227	8723	9219	9715	10211	10707	11203	11699
229	8228	8724	9220	9716	10212	10708	11204	11700
230	8229	8725	9221	9717	10213	10709	11205	11701
231	8230	8726	9222	9718	10214	10710	11206	11702
232	8231	8727	9223	9719	10215	10711	11207	11703
233	8232	8728	9224	9720	10216	10712	11208	11704
234	8233	8729	9225	9721	10217	10713	11209	11705
235	8234	8730	9226	9722	10218	10714	11210	11706
236	8235	8731	9227	9723	10219	10715	11211	11707
237	8236	8732	9228	9724	10220	10716	11212	11708
238	8237	8733	9229	9725	10221	10717	11213	11709
239	8238	8734	9230	9726	10222	10718	11214	11710
240	8239	8735	9231	9727	10223	10719	11215	11711
241	8240	8736	9232	9728	10224	10720	11216	11712
242	8241	8737	9233	9729	10225	10721	11217	11713
243	8242	8738	9234	9730	10226	10722	11218	11714
244	8243	8739	9235	9731	10227	10723	11219	11715
245	8244	8740	9236	9732	10228	10724	11220	11716
246	8245	8741	9237	9733	10229	10725	11221	11717
247	8246	8742	9238	9734	10230	10726	11222	11718
248	8247	8743	9239	9735	10231	10727	11223	11719
249	8248	8744	9240	9736	10232	10728	11224	11720
250	8249	8745	9241	9737	10233	10729	11225	11721

251	8250	8746	9242	9738	10234	10730	11226	11722
252	8251	8747	9243	9739	10235	10731	11227	11723
253	8252	8748	9244	9740	10236	10732	11228	11724
254	8253	8749	9245	9741	10237	10733	11229	11725
255	8254	8750	9246	9742	10238	10734	11230	11726
256	8255	8751	9247	9743	10239	10735	11231	11727
257	8256	8752	9248	9744	10240	10736	11232	11728
258	8257	8753	9249	9745	10241	10737	11233	11729
259	8258	8754	9250	9746	10242	10738	11234	11730
260	8259	8755	9251	9747	10243	10739	11235	11731
261	8260	8756	9252	9748	10244	10740	11236	11732
262	8261	8757	9253	9749	10245	10741	11237	11733
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264	8263	8759	9255	9751	10247	10743	11239	11735
265	8264	8760	9256	9752	10248	10744	11240	11736
266	8265	8761	9257	9753	10249	10745	11241	11737
267	8266	8762	9258	9754	10250	10746	11242	11738
268	8267	8763	9259	9755	10251	10747	11243	11739
269	8268	8764	9260	9756	10252	10748	11244	11740
270	8269	8765	9261	9757	10253	10749	11245	11741
271	8270	8766	9262	9758	10254	10750	11246	11742
272	8271	8767	9263	9759	10255	10751	11247	11743
273	8272	8768	9264	9760	10256	10752	11248	11744
274	8273	8769	9265	9761	10257	10753	11249	11745
275	8274	8770	9266	9762	10258	10754	11250	11746
276	8275	8771	9267	9763	10259	10755	11251	11747
277	8276	8772	9268	9764	10260	10756	11252	11748
278	8277	8773	9269	9765	10261	10757	11253	11749
279	8278	8774	9270	9766	10262	10758	11254	11750
280	8279	8775	9271	9767	10263	10759	11255	11751
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286	8285	8781	9277	9773	10269	10765	11261	11757
287	8286	8782	9278	9774	10270	10766	11262	11758
288	8287	8783	9279	9775	10271	10767	11263	11759
289	8288	8784	9280	9776	10272	10768	11264	11760
290	8289	8785	9281	9777	10273	10769	11265	11761
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292	8291	8787	9283	9779	10275	10771	11267	11763
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302	8301	8797	9293	9789	10285	10781	11277	11773
303	8302	8798	9294	9790	10286	10782	11278	11774
304	8303	8799	9295	9791	10287	10783	11279	11775
305	8304	8800	9296	9792	10288	10784	11280	11776
306	8305	8801	9297	9793	10289	10785	11281	11777
307	8306	8802	9298	9794	10290	10786	11282	11778
308	8307	8803	9299	9795	10291	10787	11283	11779
309	8308	8804	9300	9796	10292	10788	11284	11780

310	8309	8805	9301	9797	10293	10789	11285	11781
311	8310	8806	9302	9798	10294	10790	11286	11782
312	8311	8807	9303	9799	10295	10791	11287	11783
313	8312	8808	9304	9800	10296	10792	11288	11784
314	8313	8809	9305	9801	10297	10793	11289	11785
315	8314	8810	9306	9802	10298	10794	11290	11786
316	8315	8811	9307	9803	10299	10795	11291	11787
317	8316	8812	9308	9804	10300	10796	11292	11788
318	8317	8813	9309	9805	10301	10797	11293	11789
319	8318	8814	9310	9806	10302	10798	11294	11790
320	8319	8815	9311	9807	10303	10799	11295	11791
321	8320	8816	9312	9808	10304	10800	11296	11792
322	8321	8817	9313	9809	10305	10801	11297	11793
323	8322	8818	9314	9810	10306	10802	11298	11794
324	8323	8819	9315	9811	10307	10803	11299	11795
325	8324	8820	9316	9812	10308	10804	11300	11796
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328	8327	8823	9319	9815	10311	10807	11303	11799
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331	8330	8826	9322	9818	10314	10810	11306	11802
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333	8332	8828	9324	9820	10316	10812	11308	11804
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338	8337	8833	9329	9825	10321	10817	11313	11809
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348	8347	8843	9339	9835	10331	10827	11323	11819
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356	8355	8851	9347	9843	10339	10835	11331	11827
357	8356	8852	9348	9844	10340	10836	11332	11828
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360	8359	8855	9351	9847	10343	10839	11335	11831
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362	8361	8857	9353	9849	10345	10841	11337	11833
363	8362	8858	9354	9850	10346	10842	11338	11834
364	8363	8859	9355	9851	10347	10843	11339	11835
365	8364	8860	9356	9852	10348	10844	11340	11836
366	8365	8861	9357	9853	10349	10845	11341	11837
367	8366	8862	9358	9854	10350	10846	11342	11838
368	8367	8863	9359	9855	10351	10847	11343	11839

369	8368	8864	9360	9856	10352	10848	11344	11840
370	8369	8865	9361	9857	10353	10849	11345	11841
371	8370	8866	9362	9858	10354	10850	11346	11842
372	8371	8867	9363	9859	10355	10851	11347	11843
373	8372	8868	9364	9860	10356	10852	11348	11844
374	8373	8869	9365	9861	10357	10853	11349	11845
375	8374	8870	9366	9862	10358	10854	11350	11846
376	8375	8871	9367	9863	10359	10855	11351	11847
377	8376	8872	9368	9864	10360	10856	11352	11848
378	8377	8873	9369	9865	10361	10857	11353	11849
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384	8383	8879	9375	9871	10367	10863	11359	11855
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386	8385	8881	9377	9873	10369	10865	11361	11857
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388	8387	8883	9379	9875	10371	10867	11363	11859
389	8388	8884	9380	9876	10372	10868	11364	11860
390	8389	8885	9381	9877	10373	10869	11365	11861
391	8390	8886	9382	9878	10374	10870	11366	11862
392	8391	8887	9383	9879	10375	10871	11367	11863
393	8392	8888	9384	9880	10376	10872	11368	11864
394	8393	8889	9385	9881	10377	10873	11369	11865
395	8394	8890	9386	9882	10378	10874	11370	11866
396	8395	8891	9387	9883	10379	10875	11371	11867
397	8396	8892	9388	9884	10380	10876	11372	11868
398	8397	8893	9389	9885	10381	10877	11373	11869
399	8398	8894	9390	9886	10382	10878	11374	11870
400	8399	8895	9391	9887	10383	10879	11375	11871
401	8400	8896	9392	9888	10384	10880	11376	11872
402	8401	8897	9393	9889	10385	10881	11377	11873
403	8402	8898	9394	9890	10386	10882	11378	11874
404	8403	8899	9395	9891	10387	10883	11379	11875
405	8404	8900	9396	9892	10388	10884	11380	11876
406	8405	8901	9397	9893	10389	10885	11381	11877
407	8406	8902	9398	9894	10390	10886	11382	11878
408	8407	8903	9399	9895	10391	10887	11383	11879
409	8408	8904	9400	9896	10392	10888	11384	11880
410	8409	8905	9401	9897	10393	10889	11385	11881
411	8410	8906	9402	9898	10394	10890	11386	11882
412	8411	8907	9403	9899	10395	10891	11387	11883
413	8412	8908	9404	9900	10396	10892	11388	11884
414	8413	8909	9405	9901	10397	10893	11389	11885
415	8414	8910	9406	9902	10398	10894	11390	11886
416	8415	8911	9407	9903	10399	10895	11391	11887
417	8416	8912	9408	9904	10400	10896	11392	11888
418	8417	8913	9409	9905	10401	10897	11393	11889
419	8418	8914	9410	9906	10402	10898	11394	11890
420	8419	8915	9411	9907	10403	10899	11395	11891
421	8420	8916	9412	9908	10404	10900	11396	11892
422	8421	8917	9413	9909	10405	10901	11397	11893
423	8422	8918	9414	9910	10406	10902	11398	11894
424	8423	8919	9415	9911	10407	10903	11399	11895
425	8424	8920	9416	9912	10408	10904	11400	11896
426	8425	8921	9417	9913	10409	10905	11401	11897
427	8426	8922	9418	9914	10410	10906	11402	11898

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431	8430	8926	9422	9918	10414	10910	11406	11902
432	8431	8927	9423	9919	10415	10911	11407	11903
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435	8434	8930	9426	9922	10418	10914	11410	11906
436	8435	8931	9427	9923	10419	10915	11411	11907
437	8436	8932	9428	9924	10420	10916	11412	11908
438	8437	8933	9429	9925	10421	10917	11413	11909
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444	8443	8939	9435	9931	10427	10923	11419	11915
445	8444	8940	9436	9932	10428	10924	11420	11916
446	8445	8941	9437	9933	10429	10925	11421	11917
447	8446	8942	9438	9934	10430	10926	11422	11918
448	8447	8943	9439	9935	10431	10927	11423	11919
449	8448	8944	9440	9936	10432	10928	11424	11920
450	8449	8945	9441	9937	10433	10929	11425	11921
451	8450	8946	9442	9938	10434	10930	11426	11922
452	8451	8947	9443	9939	10435	10931	11427	11923
453	8452	8948	9444	9940	10436	10932	11428	11924
454	8453	8949	9445	9941	10437	10933	11429	11925
455	8454	8950	9446	9942	10438	10934	11430	11926
456	8455	8951	9447	9943	10439	10935	11431	11927
457	8456	8952	9448	9944	10440	10936	11432	11928
458	8457	8953	9449	9945	10441	10937	11433	11929
459	8458	8954	9450	9946	10442	10938	11434	11930
460	8459	8955	9451	9947	10443	10939	11435	11931
461	8460	8956	9452	9948	10444	10940	11436	11932
462	8461	8957	9453	9949	10445	10941	11437	11933
463	8462	8958	9454	9950	10446	10942	11438	11934
464	8463	8959	9455	9951	10447	10943	11439	11935
465	8464	8960	9456	9952	10448	10944	11440	11936
466	8465	8961	9457	9953	10449	10945	11441	11937
467	8466	8962	9458	9954	10450	10946	11442	11938
468	8467	8963	9459	9955	10451	10947	11443	11939
469	8468	8964	9460	9956	10452	10948	11444	11940
470	8469	8965	9461	9957	10453	10949	11445	11941
471	8470	8966	9462	9958	10454	10950	11446	11942
472	8471	8967	9463	9959	10455	10951	11447	11943
473	8472	8968	9464	9960	10456	10952	11448	11944
474	8473	8969	9465	9961	10457	10953	11449	11945
475	8474	8970	9466	9962	10458	10954	11450	11946
476	8475	8971	9467	9963	10459	10955	11451	11947
477	8476	8972	9468	9964	10460	10956	11452	11948
478	8477	8973	9469	9965	10461	10957	11453	11949
479	8478	8974	9470	9966	10462	10958	11454	11950
480	8479	8975	9471	9967	10463	10959	11455	11951
481	8480	8976	9472	9968	10464	10960	11456	11952
482	8481	8977	9473	9969	10465	10961	11457	11953
483	8482	8978	9474	9970	10466	10962	11458	11954
484	8483	8979	9475	9971	10467	10963	11459	11955
485	8484	8980	9476	9972	10468	10964	11460	11956
486	8485	8981	9477	9973	10469	10965	11461	11957

487	8486	8982	9478	9974	10470	10966	11462	11958
488	8487	8983	9479	9975	10471	10967	11463	11959
489	8488	8984	9480	9976	10472	10968	11464	11960
490	8489	8985	9481	9977	10473	10969	11465	11961
491	8490	8986	9482	9978	10474	10970	11466	11962
492	8491	8987	9483	9979	10475	10971	11467	11963
493	8492	8988	9484	9980	10476	10972	11468	11964
494	8493	8989	9485	9981	10477	10973	11469	11965
495	8494	8990	9486	9982	10478	10974	11470	11966
496	8495	8991	9487	9983	10479	10975	11471	11967

WHAT IS CLAIMED IS:

1. An isolated antigen binding protein (ABP) that specifically binds a human cytotoxic T-lymphocyte associated protein 4 (CTLA-4), comprising:

(a) a CDR3-L having a sequence selected from SEQ ID NOS: 3001-3028 and a CDR3-H having a sequence selected from SEQ ID NOS: 6001-6028; or

(b) a CDR3-L having a sequence selected from SEQ ID NOS: 9984-10479 and a CDR3-H having a sequence selected from SEQ ID NOS: 11472-11967; 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-125512 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-125512.

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: 1001-1028 and a CDR2-L having a sequence selected from SEQ ID NOS: 2001-2028; and a CDR1-H having a sequence selected from SEQ ID NOS: 4001-4028; and a CDR2-H having a sequence selected from SEQ ID NOS: 5001-5028; or

(b) a CDR1-L having a sequence selected from SEQ ID NOS: 8992-9487; and a CDR2-L having a sequence selected from SEQ ID NOS: 9488-9983; and a CDR1-H having a sequence selected from SEQ ID NOS: 10480-10975; and a CDR2-H having a sequence selected from SEQ ID NOS: 10976-11471; 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-125512; 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-125512; 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-125512; 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-125512;

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; or

the CDR1-L consists of SEQ ID NO: 1013, the CDR2-L consists of SEQ ID NO: 2013, the CDR3-L consists of SEQ ID NO: 3013, the CDR1-H consists of SEQ ID NO: 4013, the

CDR2-H consists of SEQ ID NO: 5013 and the CDR3-H consists of SEQ ID NO: 6013; or

the CDR1-L consists of SEQ ID NO: 1014, the CDR2-L consists of SEQ ID NO: 2014, the CDR3-L consists of SEQ ID NO: 3014, the CDR1-H consists of SEQ ID NO: 4014, the CDR2-H consists of SEQ ID NO: 5014 and the CDR3-H consists of SEQ ID NO: 6014; or

the CDR1-L consists of SEQ ID NO: 1015, the CDR2-L consists of SEQ ID NO: 2015, the CDR3-L consists of SEQ ID NO: 3015, the CDR1-H consists of SEQ ID NO: 4015, the CDR2-H consists of SEQ ID NO: 5015 and the CDR3-H consists of SEQ ID NO: 6015; or

the CDR1-L consists of SEQ ID NO: 1016, the CDR2-L consists of SEQ ID NO: 2016, the CDR3-L consists of SEQ ID NO: 3016, the CDR1-H consists of SEQ ID NO: 4016, the CDR2-H consists of SEQ ID NO: 5016 and the CDR3-H consists of SEQ ID NO: 6016; or

the CDR1-L consists of SEQ ID NO: 1017, the CDR2-L consists of SEQ ID NO: 2017, the CDR3-L consists of SEQ ID NO: 3017, the CDR1-H consists of SEQ ID NO: 4017, the CDR2-H consists of SEQ ID NO: 5017 and the CDR3-H consists of SEQ ID NO: 6017; or

the CDR1-L consists of SEQ ID NO: 1018, the CDR2-L consists of SEQ ID NO: 2018, the CDR3-L consists of SEQ ID NO: 3018, the CDR1-H consists of SEQ ID NO: 4018, the CDR2-H consists of SEQ ID NO: 5018 and the CDR3-H consists of SEQ ID NO: 6018; or

the CDR1-L consists of SEQ ID NO: 1019, the CDR2-L consists of SEQ ID NO: 2019, the CDR3-L consists of SEQ ID NO: 3019, the CDR1-H consists of SEQ ID NO: 4019, the CDR2-H consists of SEQ ID NO: 5019 and the CDR3-H consists of SEQ ID NO: 6019; or

the CDR1-L consists of SEQ ID NO: 1020, the CDR2-L consists of SEQ ID NO: 2020, the CDR3-L consists of SEQ ID NO: 3020, the CDR1-H consists of SEQ ID NO: 4020, the CDR2-H consists of SEQ ID NO: 5020 and the CDR3-H consists of SEQ ID NO: 6020; or

the CDR1-L consists of SEQ ID NO: 1021, the CDR2-L consists of SEQ ID NO: 2021, the CDR3-L consists of SEQ ID NO: 3021, the CDR1-H consists of SEQ ID NO: 4021, the CDR2-H consists of SEQ ID NO: 5021 and the CDR3-H consists of SEQ ID NO: 6021; or

the CDR1-L consists of SEQ ID NO: 1022, the CDR2-L consists of SEQ ID NO: 2022, the CDR3-L consists of SEQ ID NO: 3022, the CDR1-H consists of SEQ ID NO: 4022, the CDR2-H consists of SEQ ID NO: 5022 and the CDR3-H consists of SEQ ID NO: 6022; or

the CDR1-L consists of SEQ ID NO: 1023, the CDR2-L consists of SEQ ID NO: 2023, the CDR3-L consists of SEQ ID NO: 3023, the CDR1-H consists of SEQ ID NO: 4023, the CDR2-H consists of SEQ ID NO: 5023 and the CDR3-H consists of SEQ ID NO: 6023; or

the CDR1-L consists of SEQ ID NO: 1024, the CDR2-L consists of SEQ ID NO: 2024, the CDR3-L consists of SEQ ID NO: 3024, the CDR1-H consists of SEQ ID NO: 4024, the CDR2-H consists of SEQ ID NO: 5024 and the CDR3-H consists of SEQ ID NO: 6024; or

the CDR1-L consists of SEQ ID NO: 1025, the CDR2-L consists of SEQ ID NO: 2025, the CDR3-L consists of SEQ ID NO: 3025, the CDR1-H consists of SEQ ID NO: 4025, the CDR2-H consists of SEQ ID NO: 5025 and the CDR3-H consists of SEQ ID NO: 6025; or

the CDR1-L consists of SEQ ID NO: 1026, the CDR2-L consists of SEQ ID NO: 2026, the CDR3-L consists of SEQ ID NO: 3026, the CDR1-H consists of SEQ ID NO: 4026, the CDR2-H consists of SEQ ID NO: 5026 and the CDR3-H consists of SEQ ID NO: 6026; or

the CDR1-L consists of SEQ ID NO: 1027, the CDR2-L consists of SEQ ID NO: 2027, the CDR3-L consists of SEQ ID NO: 3027, the CDR1-H consists of SEQ ID NO: 4027, the CDR2-H consists of SEQ ID NO: 5027 and the CDR3-H consists of SEQ ID NO: 6027; or

the CDR1-L consists of SEQ ID NO: 1028, the CDR2-L consists of SEQ ID NO: 2028, the CDR3-L consists of SEQ ID NO: 3028, the CDR1-H consists of SEQ ID NO: 4028, the CDR2-H consists of SEQ ID NO: 5028 and the CDR3-H consists of SEQ ID NO: 6028.

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: 1-28 and a variable heavy chain (V_H) comprising a sequence at least 97% identical to a sequence selected from SEQ ID NOS: 101-128; or

a variable light chain (V_L) comprising a sequence at least 97% identical to a sequence selected from SEQ ID NOS: 8000-8495 and a variable heavy chain (V_H) comprising a sequence at least 97% identical to a sequence selected from SEQ ID NOS: 8496-8991; 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-125512 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-125512.

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: 1-28 and a variable heavy chain (V_H) comprising a a sequence selected from SEQ ID NOS: 101-128 or

a variable light chain (V_L) comprising a sequence selected from SEQ ID NOS: 8000-8495 and a variable heavy chain (V_H) comprising a sequence selected from SEQ ID NOS: 8496-8991; 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-125512 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-125512.

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 CTLA-4 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 CTLA-4 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 CTLA-4 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 CTLA-4 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 CTLA-4, comprising:

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

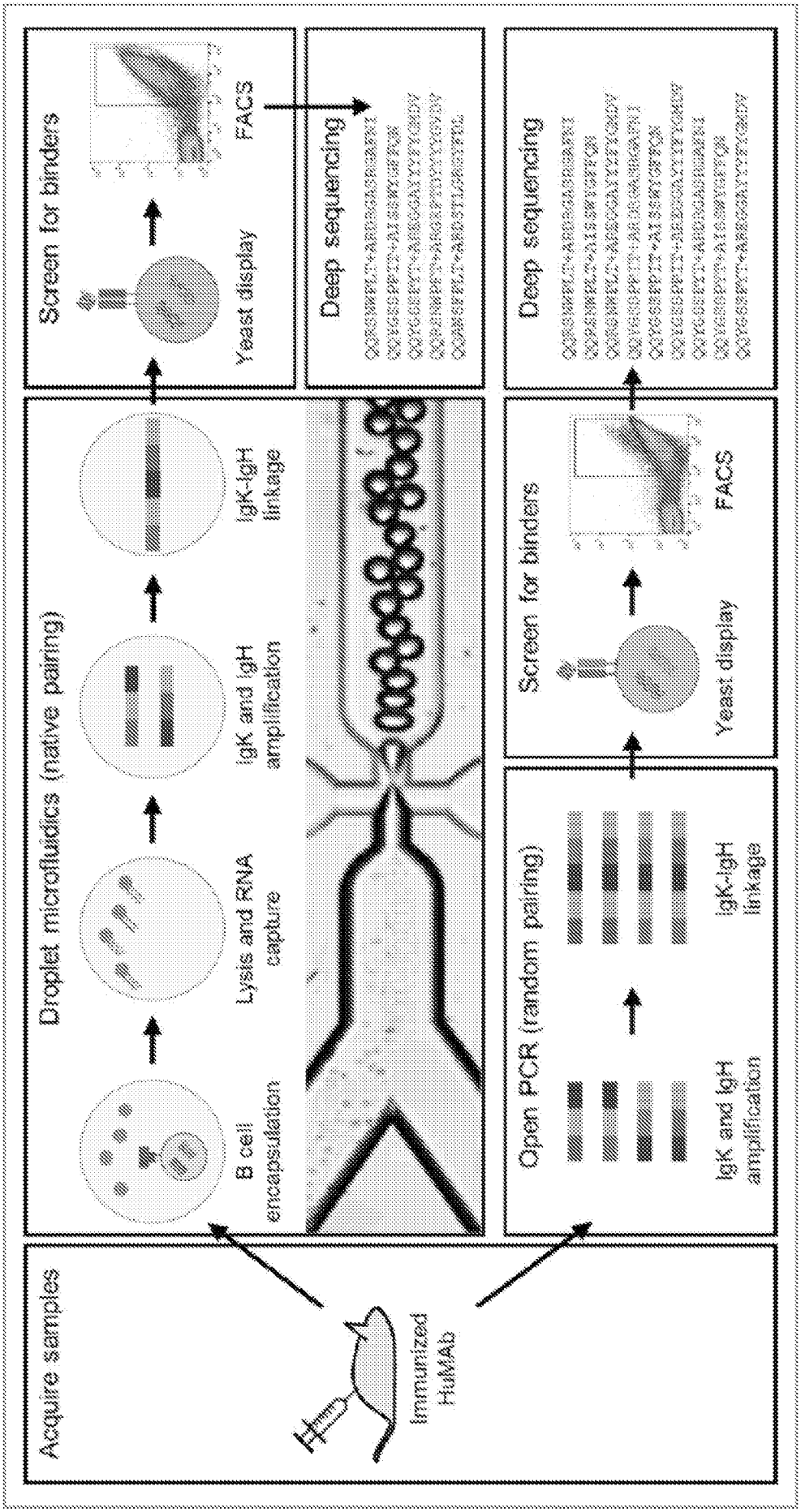
Acquire samples
Immunized HuMAb
Droplet microfluidics (native pairing)
B cell encapsulation
Lysis and RNA capture
IgK and IgH amplification
IgK-IgH linkage
Screen for binders
Yeast display
FACS
Deep sequencing
Open PCR (random pairing)
IgK and IgH amplification
IgK-IgH linkage
Screen for binders
Yeast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

FIG. 1

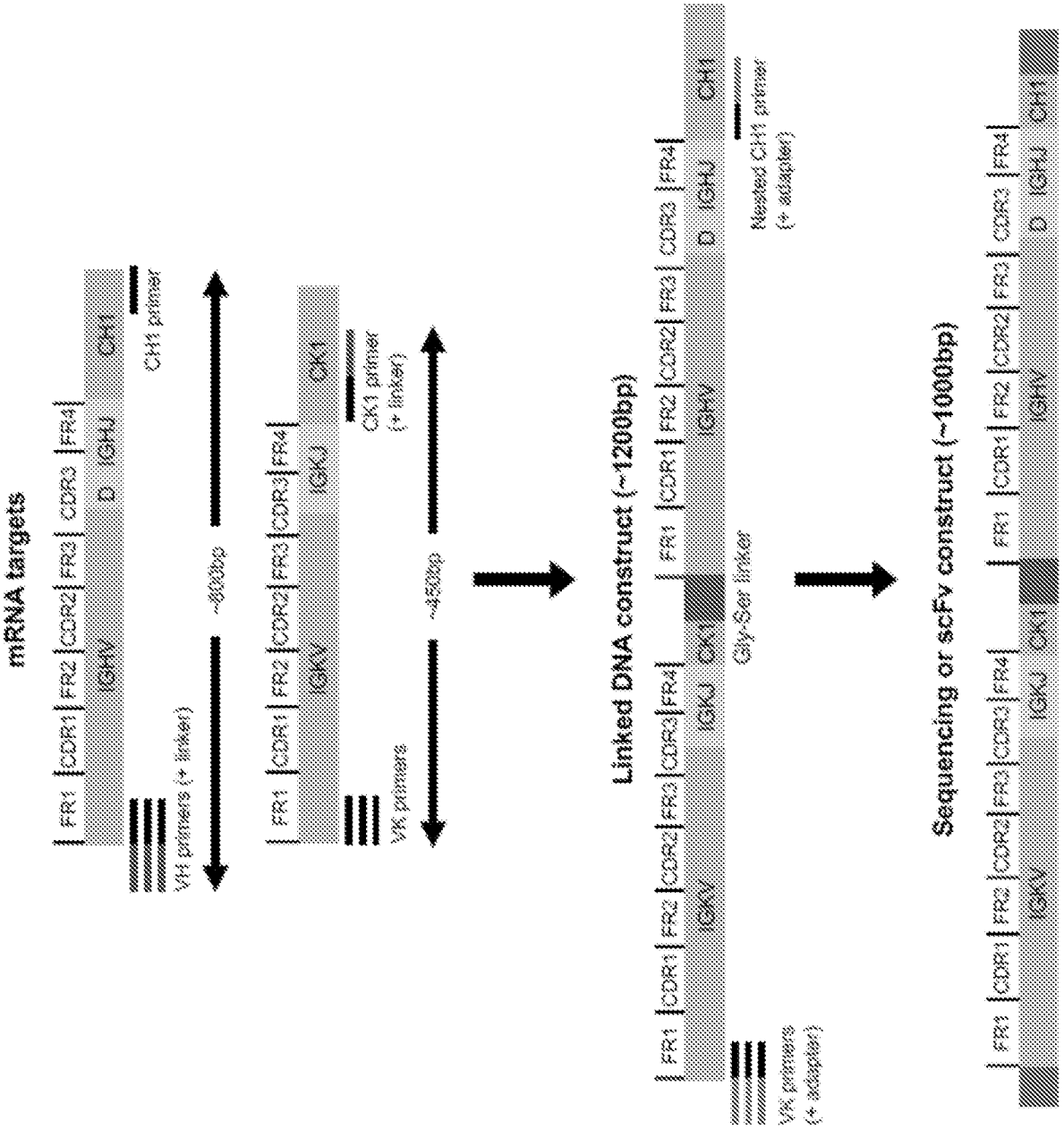
mRNA targets
FR1
CDR1
FR2
CDR2
FR3
CDR3
FR4
IGHV
D
IGHJ
CH1
CH1 primer
VH primers (+ linker)
~800bp
IGKV
IGKJ
CK1
CK1 primer (+ linker)
VK primers
~450bp
Linked DNA construct (~1200bp)
Gly-Ser linker
Nested CH1 primer (+ adapter)
VK primers (+ adapter)
Sequencing or scFv construct (~1000bp)

FIG. 2

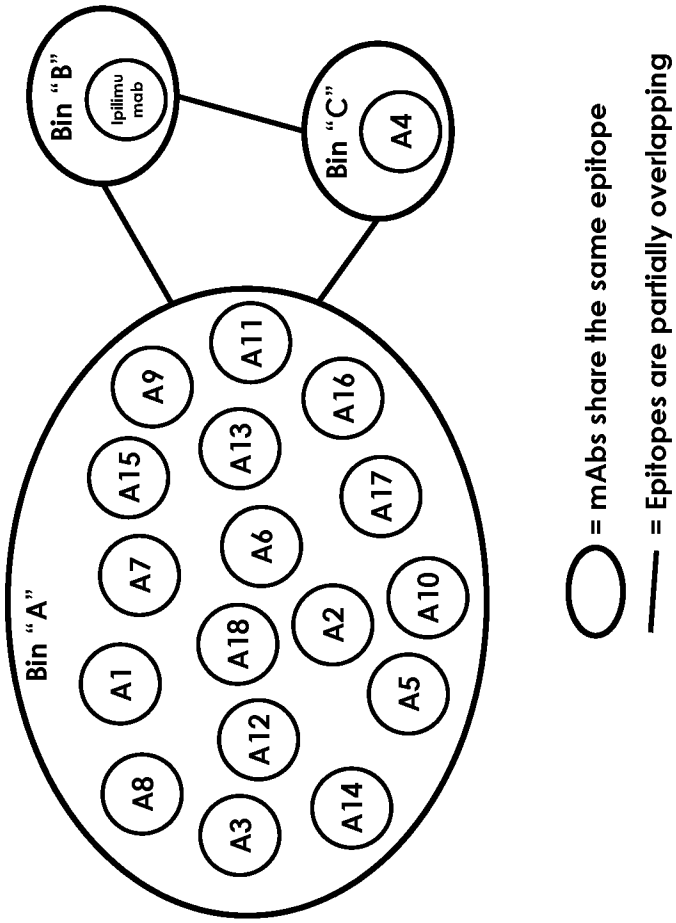
Bin "B"
Ipilimumab
Bin "C"
A4
Bin "A"
A1
A2
A3
A5
A6
A7
A8
A9
A10
A11
A12
A13
A14
A15
A16
A17
A18
= mAbs share the same epitope
= Epitopes are partially overlapping

FIG. 3

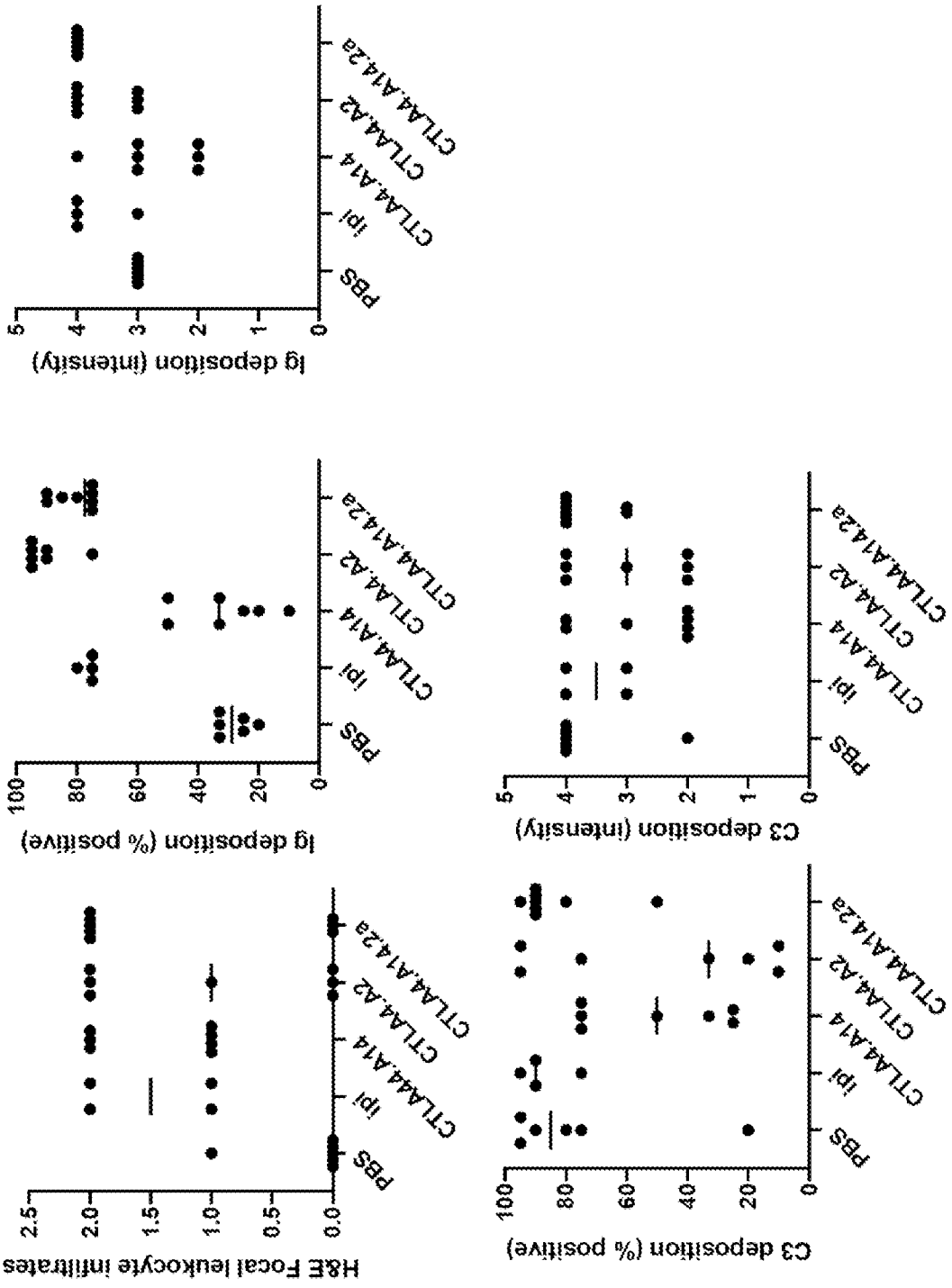
Ig deposition (intensity)
Ig deposition (% positive)
C3 deposition (intensity)
C3 deposition (% positive)
H&E Focal leukocyte infiltrates
PBS
Ipi
CTLA4.A14
CTLA4.A2
CTLA4.A14.2a

FIG. 4

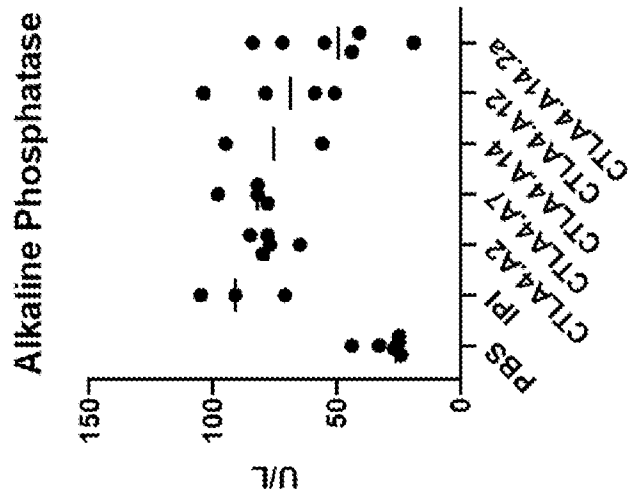
Alkaline Phosphatase
U/L
150
100
50
0
PBS
IPI
CTLA4.2
CTLA4.7
CTLA4.14
CTLA4.12
CTLA4.14.2a

FIG. 5

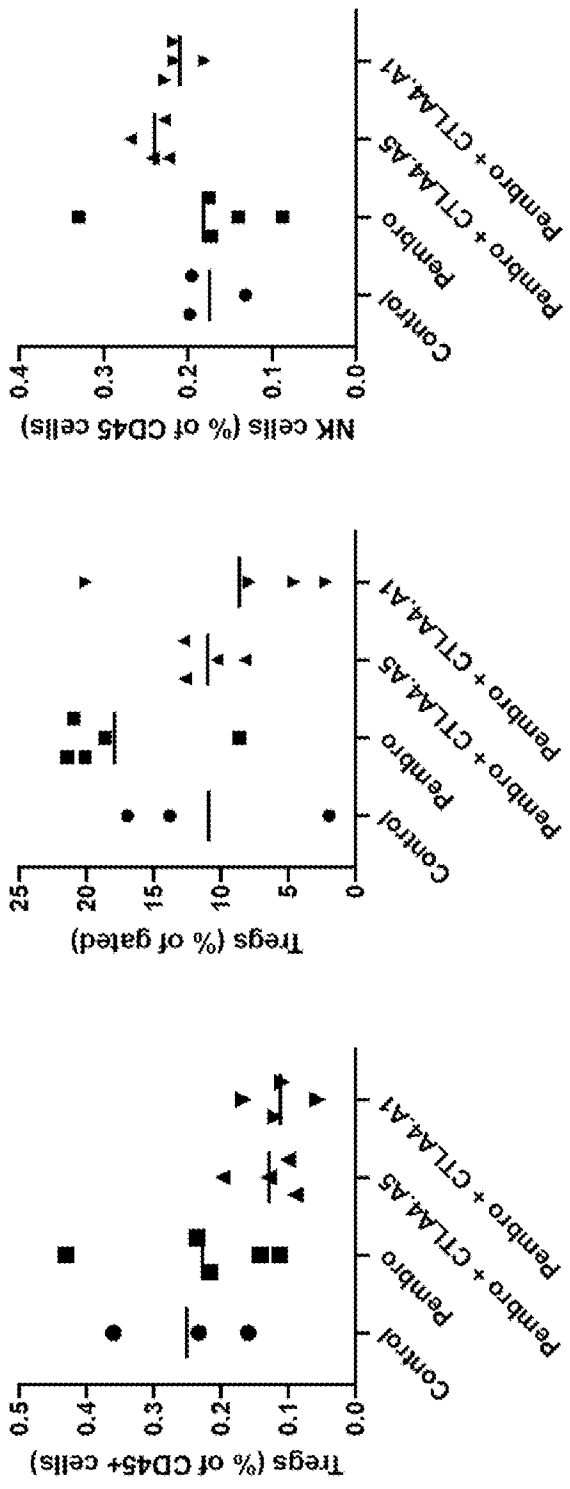
Tregs (% of CD45+ cells)
0.0
0.1
0.2
0.3
0.4
0.5
Tregs (% of gated)
0
5
10
15
20
25
NK cells (% of CD45 cells)
0.0
0.1
0.2
0.3
0.4
Control
Pembro
Pembro + CTLA4.A5
Pembro + CTLA4.A1

FIG. 6

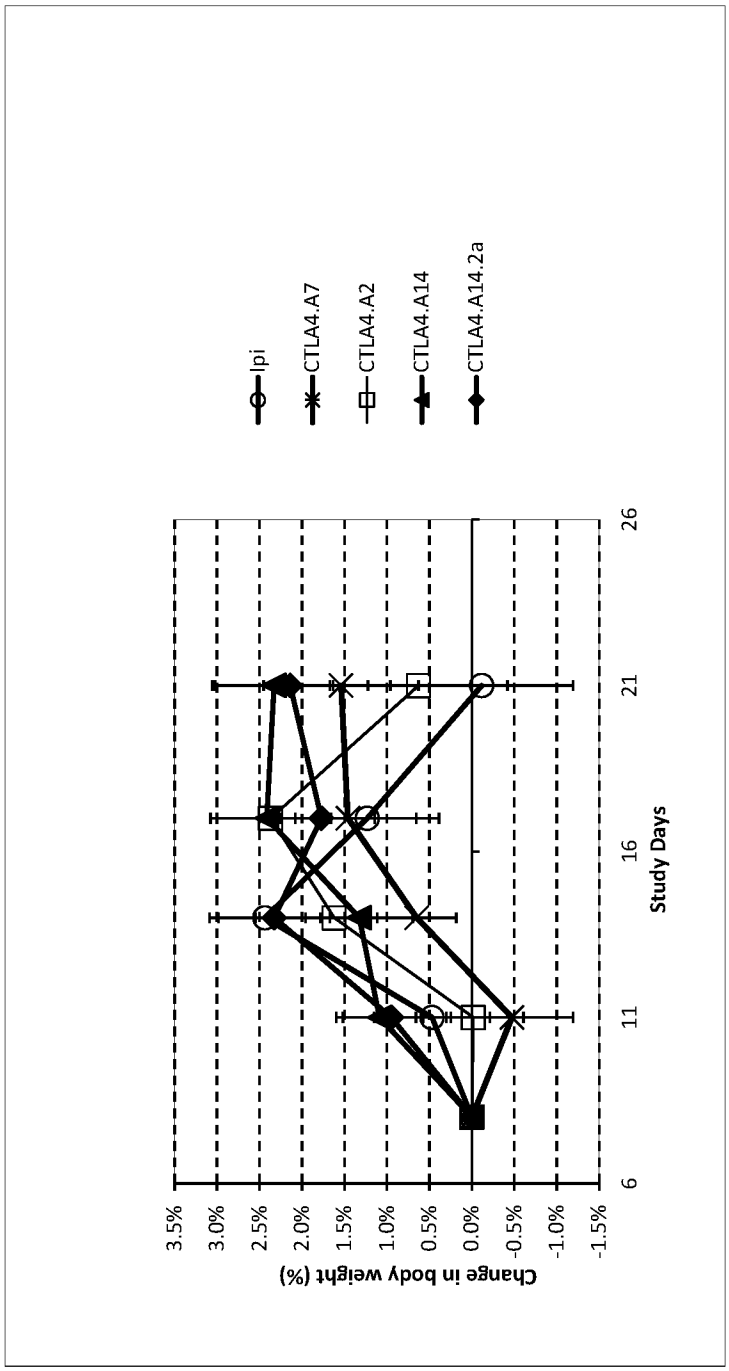
Ipi
CTLA4.A7
CTLA4.A2
CTLA4.A14
CTLA4.A14.2a
Change in body weight (%)
3.5%
3.0%
2.5%
2.0%
1.5%
1.0%
0.5%
0.0%
-0.5%
-1.0%
-1.5%
6
11
16
21
26
Study Days

FIG. 7

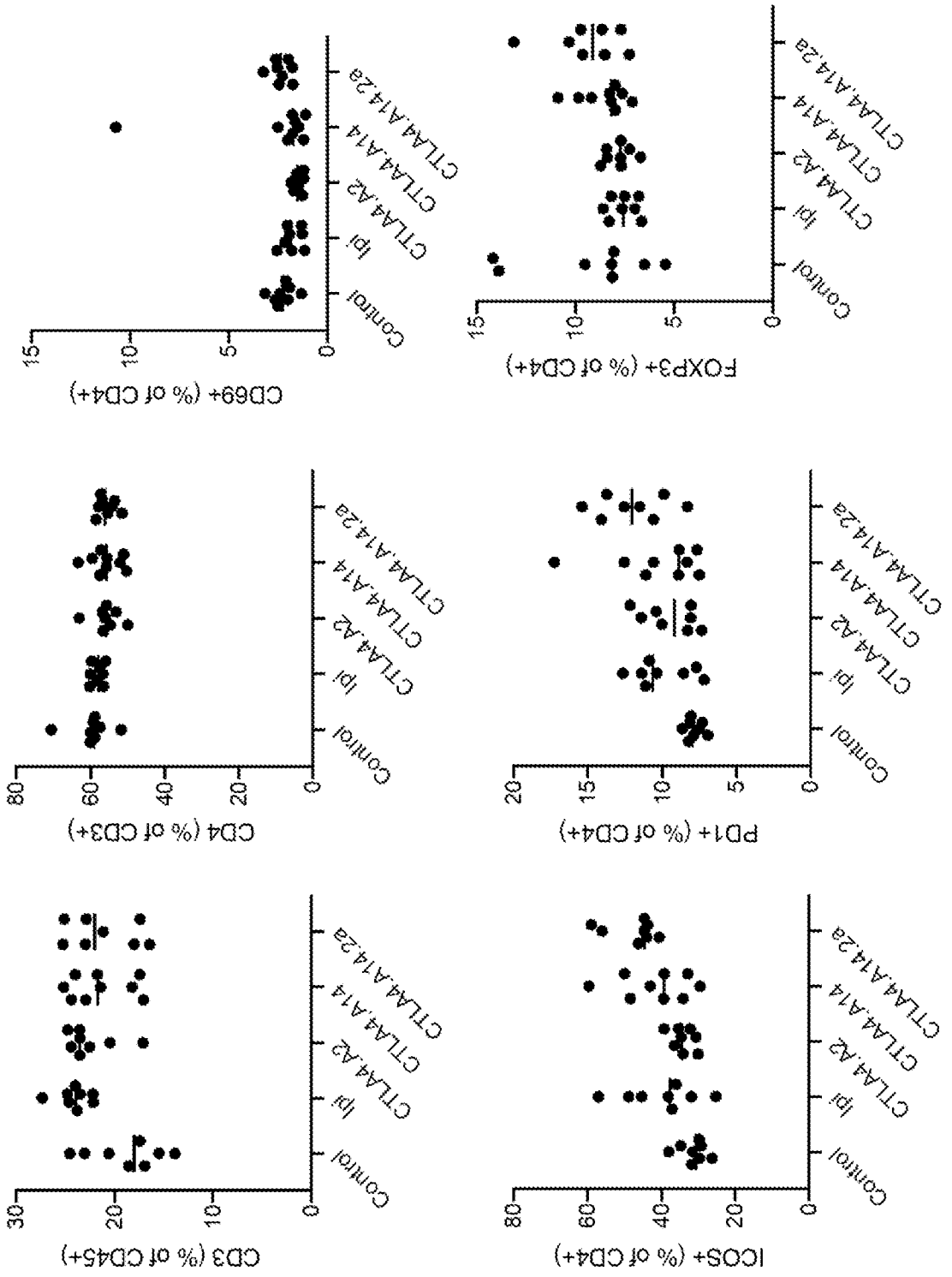
CD69+ (% of CD4+)
FOXP3+ (% of CD4+)
CD4 (% of CD3+)
PD1+ (% of CD4+)
CD3 (% of CD45+)
ICOS+ (% of CD4+)
Control
Ipi
CTLA4.A2
CTLA4.A14
CTLA4.A14.2a

FIG. 8

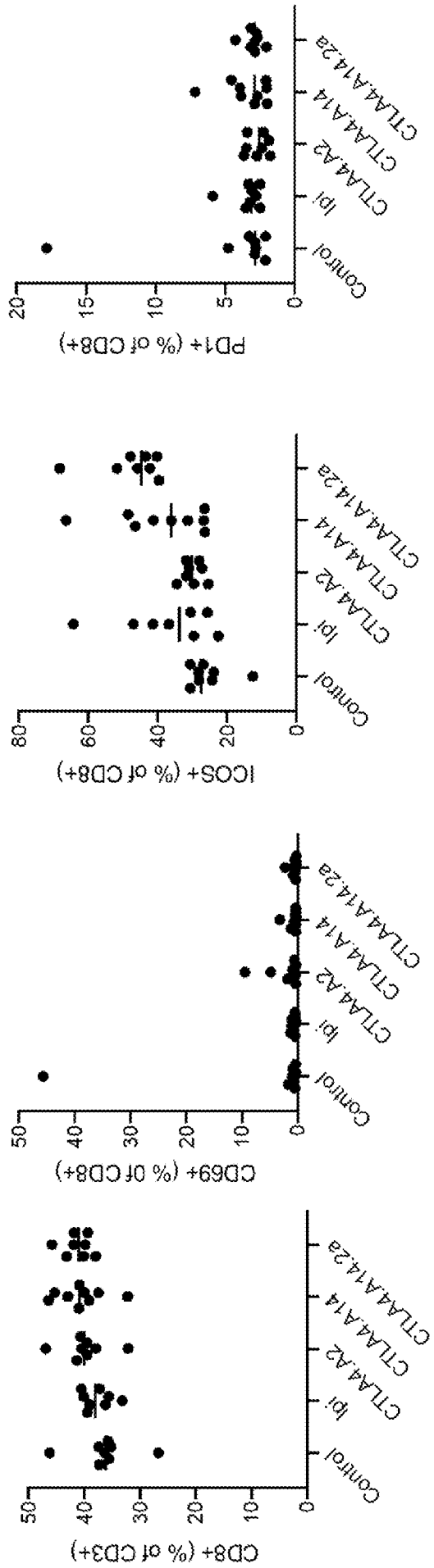
CD8+ (% of CD3+)
CD69+ (% of CD8+)
ICOS+ (% of CD8+)
PD1+ (% of CD8+)
Control
Ipi
CTLA4.A2
CTLA4.A14
CTLA4.A14.2a

FIG. 9

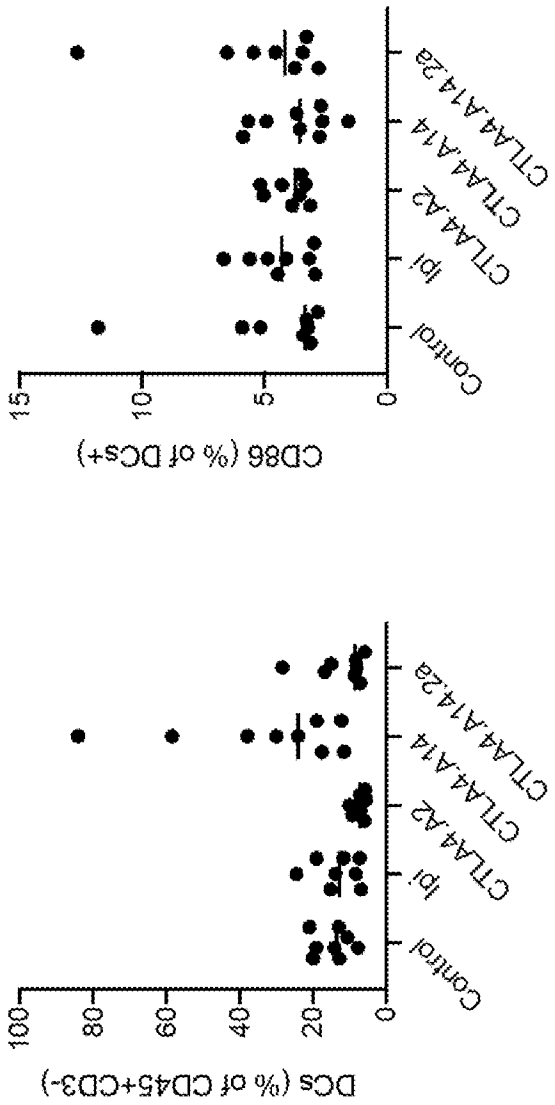
CD86 (% of DCs+)
15
10
5
0
Control
Ipi
CTLA4.A2
CTLA4.A14
CTLA4.A14.2a
DCs (% of CD45+CD3-)
100
80
60
40
20
0
Control
Ipi
CTLA4.A2
CTLA4.A14
CTLA4.A14.2a

FIG. 10

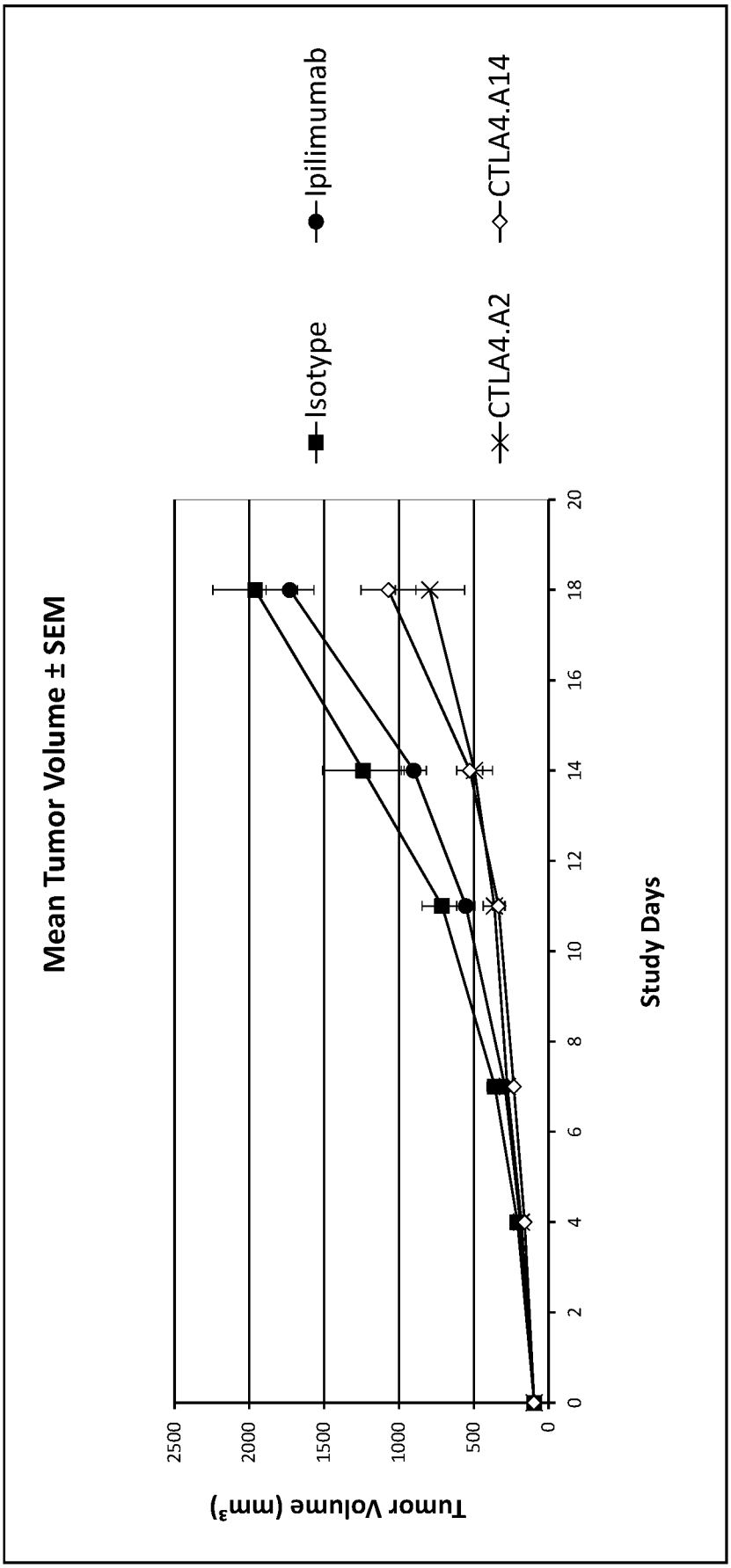
Mean Tumor Volume ± SEM
Isotype
Ipilimumab
CTLA4.A2
CTLA4.A14
Tumor Volume (mm³)
2500
2000
1500
1000
500
0
0
2
4
6
8
10
12
14
16
18
20
Study Days

FIG. 11

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2019/068820

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - C07K 14/725; C07K 14/74; C07K 16/00; C12N 15/10; C12Q 1/6806 (2020.01)

CPC - C12N 15/1075; C12Q 2525/161; C12Q 2535/122; C12Q 2563/159; C12Q 2565/629 (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; 530/387.1 (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	WO 2017/194265 A1 (AGENCY FOR SCIENCE, TECHNOLOGY AND RESEARCH et al) 16 November 2017 (16.11.2017) entire document	1-10
A	US 9,714,290 B2 (JONES et al) 25 July 2017 (25.07.2017) entire document	1-10
A	US 2013/0136749 A1 (MEDAREX, INC.) 30 May 2013 (30.05.2013) entire document	1-10
A	WO 2006/029219 A2 (OHIO STATE UNIVERSITY RESEARCH FOUNDATION et al) 16 March 2006 (16.03.2006) entire document	1-10
A	WO 2017/106372 A1 (ONCOIMMUNE, INC. et al) 22 June 2017 (22.06.2017) 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	20 MAY 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/US2019/068820

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: 1-10 and 101-110 were searched.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2019/068820

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 CTLA-4 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.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2019/068820

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 CTLA-4 binding proteins.

The first invention of Group I+ is restricted to an anti-human CTLA-4 binding protein, the anti-human CTLA-4 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 CTLA-4 binding protein of SEQ ID NOs: 1, 101, 1001, 2001, 3001, 4001, 5001, and 6001.

Applicant is invited to elect additional anti-human CTLA-4 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 CTLA-4 binding protein, the anti-human CTLA-4 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 CTLA-4 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 CTLA-4, 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: 1-28 and ...[the] variable heavy chain (VH) compris[es] a sequence at least 97% identical to a sequence selected from SEQ ID NOs: 101-128; or ...[the] variable light chain (VL) compris[es] a sequence at least 97% identical to a sequence selected from SEQ ID NOs: 8000-8495 and ...[the] variable heavy chain (VH) compris[es] a sequence at least 97% identical to a sequence selected from SEQ ID NOs: 8496-8991; 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-125512 and ...[the] 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-125512".

Additionally, even if Groups I+ were considered to share the technical features of an isolated antigen binding protein (ABP) that specifically binds a human cytotoxic T-lymphocyte associated protein 4 (CTLA-4), 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, US 2013/0136749 A1 to Medarex, Inc. discloses an isolated antigen binding protein (ABP) that specifically binds a human cytotoxic T-lymphocyte associated protein 4 (CTLA-4) ([t]he present invention provides a human sequence antibody that specifically binds to human CTLA-4, Para. [0017]), 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 ([t]he invention provides a human sequence antibody... comprising variable heavy and light chain sequences, Para. [0030]; antibodies of the invention comprise heavy chain CDR1, CDR2, and CDR3 sequences ...and light chain CDR1, CDR2, and CDR3 sequences, Para. [0034]).

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