HIGH AFFINITY ANTI-CD3 ANTIBODIES, AND METHODS FOR THEIR GENERATION AND USE

Related Applications

[0001] This application claims priority to U.S. Provisional Application No. 62/858,949, filed on June 7, 2019, the contents of which are incorporated by reference in its entirety.

Sequence Listing

[0002] The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created June 3, 2020, is named 1160430o001813.txt, and is 92,322 bytes in size.

Field of the Invention

[0003] The invention relates, *inter alia*, to anti-Cluster of Differentiation 3 (CD3) antibodies, including multispecific antibodies, and functional fragments thereof, and methods and reagents for their identification, isolation, preparation, and use.

Background of the Invention

[0004] Cell proliferative disorders, such as cancer, are characterized by the uncontrolled growth of cell subpopulations. They are the leading cause of death in the developed world and the second leading cause of death in developing countries, with a total number of new cancer cases per year expected to rise to 23.6 million by 2030. The National Cancer Institute estimates that almost 2 million new cases of cancer will be diagnosed in the U.S. and greater than 600,000 Americans will die of cancer in 2018. Cancer care thus represents a significant and ever-increasing societal burden.

[0005] The idea of using the cytotoxic capacity of T cells to kill tumor cells through use of CD3 targeting bispecific antibodies dates back to the mid-1980s. (Staerz et al. Nature 1985 314: 628-32). Many bispecific antibodies developed to date contain a first binding site specific to CD3 for T-cell recruitment and activation, and a second binding site for a targeted disease-associated antigen, such as an antigen produced by a tumor cell. CD3 bispecific antibodies trigger the CD3 surface receptor on T cells by binding to their second target protein expressed on tumors such that available T cells can bind to target-expressing cells via bridging by the CD3 bispecific antibody, irrespective of the peptide/MHC specificity of their

T-cell receptor. (*See*, e.g., Bassan, 2012, Blood 120:5094-95). Bridging of T cells and tumor cells using CD3 bispecific antibodies can induce dramatic regression of advanced-stage malignancies and, in some cases, lead to complete remission. Currently, more than 25 different CD3 bispecific antibodies are in clinical development for treatment of hematologic malignancies or solid cancers by targeting CD19, CD20, CD33, and CD123, or EpCAM, HER2, PSMA, and CEA, respectively. (*See* e.g., Liu et al. Front Immunol 2017 8:38).

[0006] While bispecific antibodies have shown considerable benefits over monospecific antibodies for the treatment and the detection of cancer, broad commercial application of bispecific antibodies has been hampered by the lack of efficient/low-cost production methods, the lack of stability of bispecific polypeptides and the lack of long half-lives in humans. A large variety of methods have been developed over the last few decades to produce bispecific monoclonal antibodies. However, many candidate bispecific antibodies with exquisite selectivity and high potency toward the target of interest often have problems in downstream development and clinical efficacy activities, including polyspecific binding (or "polyspecificity"); off-target binding; nonspecific binding; poor expression levels or profiles in eukaryotic host cells, such as mammalian host cells and yeast cells; poor chemical and physical properties, such as poor stability during storage (e.g., poor/low "shelf-life" stability), poor (low) solubility, poor (high) viscosity, propensity to aggregate, and the like; and poor clinical and biophysical profiles, such as poor pharmacokinetic profiles, poor pharmacodynamic profiles, fast or poor *in vivo* clearance rates, short circulation half-life, some of which result in termination of their development.

[0007] Certain techniques and assays exist to assess many of the aforementioned developability characteristics for discovered antibodies in the context of downstream development activities ("post-discovery antibodies"), such as CIC, SIC, BVP-ELISA, TMA, and other assays; however, such assays are typically not amenable to high-throughput formats in early antibody discovery platforms. Furthermore, assessment of these attributes typically requires milligram to gram quantities of protein, thus often imposing a *de facto* limitation on the number of leads that can be pragmatically considered for development, and consequently reducing the likelihood of program success. Consequently, significant resources are often expended attempting to fix poorly behaving lead candidates with few backups available in later stages of development.

[0008] A variety of CD3 antibodies are known in the art, including monoclonal and bispecific antibody formats. *See*, e.g., U.S. Pat. Nos. 7,262,276; 7,635,472; 7,862,813; 9,587,021; and 10,174,124. However, many of these CD3 antibodies possess developability issues, such as those outlined above, and/or possess insufficiently high binding affinity to CD3 for a particular multispecific format or chimeric antigen receptor (CAR) format or delivery approach or the like. Accordingly, there is an unmet need in the field of bispecific antibodies for anti-CD3 antibodies that display desirable developability profiles and possess high affinity for CD3 for use in cancer treatment.

Summary of the Invention

[0009] The present disclosure relates to anti-CD3 antibodies and methods of using them, which antibodies have particularly high affinity for CD3, e.g., a monovalent K_D of of about 250 picomolar or less.

[0010] The present disclosure provides an anti-CD3 antibody and/or antigen-binding fragment comprising a complementarity determining region (CDR) in a light chain variable region, CDRL3, wherein the CDRL3 comprises an amino acid sequence of X_1QSYFRRT (SEQ ID NO: 1), wherein X_1 is K, A, T, or V. In some embodiments, X_1 is A, T or V. In certain embodiments, CDRL3 comprises AQSYFRRT (SEQ ID NO: 2); TQSYFRRT (SEQ ID NO: 3); VQSYFRRT (SEQ ID NO: 4); or KQSYFRRT (SEQ ID NO: 5).

[0011] In some embodiments, an anti-CD3 antibody and/or antigen-binding fragment further comprises a complementarity determining region (CDR) in a heavy chain variable region,CDRH3, wherein the CDRH3 comprises an amino acid sequence of X_3RDAYGX_4YFYDV (SEQ ID NO: 6), wherein X_3 is A or V and X_4 is R or Q. In certain embodiments, X_3 is A.

[0012] In other embodiments, an anti-CD3 antibody and/or antigen-binding fragment further comprise a complementarity determining region (CDR) in a light chain variable region, CDRL1, wherein the CDRL1 comprises an amino acid sequence of KSSQSLLNARTX$_5$KNYLA (SEQ ID NO: 7), wherein X_5 is G, M, N, or R. In certain embodiments, X_5 is G or R. In some embodiments, CDRL1 may comprise KSSQSLLNARTGKNYLA (SEQ ID NO: 8) or KSSQSLLNARTRKNYLA (SEQ ID NO: 9).

[0013] In some embodiments, an anti-CD3 antibody and/or antigen-binding fragment further comprises a complementarity determining region (CDR) in a light chain variable region, CDRL2, wherein the CDRL2 comprises an amino acid sequence of WASTRES (SEQ ID NO: 10) or WASTRSS (SEQ ID NO: 11).

[0014] In some embodiments, an anti-CD3 antibody and/or antigen-binding fragment further comprises a complementarity determining region (CDR) in a heavy chain variable region, CDRH1, wherein the CDRH1 comprises an amino acid sequence of FNX_6KDYYX_7H (SEQ ID NO: 12), wherein X_6 is I, N or V and X_7 is M or I. In certain embodiments, X_6 is I and/or X_7 is M. In certain embodiments, CDRH1 comprises FNIKDYYMH (SEQ ID NO: 13).

[0015] In some embodiments, an anti-CD3 antibody and/or antigen-binding fragment further comprises a complementarity determining region (CDR) in a heavy chain variable region, CDRH2, wherein CDRH2 comprises an amino acid sequence of $WIDLX_8NANTVYDX_9KX_{10}QG$ (SEQ ID NO: 14), wherein X_8 is E or N, X_9 is A, H, or T, and X_{10} is F or L. In certain embodiments, X_8 is E and/or X_9 is A and/or X_{10} is F. In certain embodiments, CDRH2 comprises WIDLENANTVYDAKFQG (SEQ ID NO: 15) or WIDLENANTIYDAKFQG (SEQ ID NO: 16).

[0016] In some embodiments, the disclosure provides an anti-CD3 antibody and/or antigen-binding fragment thereof comprising one or more of a CDRL1, a CDRL2, and a CDRL3. Such an antibody, in some embodiments, further comprises a CDRH1, a CDRH2, and a CDRH3.

[0017] In some embodiments, anti-CD3 antibodies and/or antigen-binding fragments thereof as described herein comprise a binding affinity (K_D) of about 500 pM or less. In some embodiments, anti-CD3 antibodies and/or antigen-binding fragments thereof comprise a binding affinity (K_D) of about 250 pM or less. In some embodiments, anti-CD3 antibodies and/or antigen-binding fragments thereof comprise a binding affinity (K_D) of about 100 pM or less.

[0018] Moreover, the present disclosure generally relates to an anti-cluster of differentiation three ("CD3") antibody or antigen-binding antibody fragment, wherein said anti-CD3 antibody or antigen-binding antibody fragment comprises: a. a variable heavy (V_H) chain polypeptide comprising: i. a VH CDR1 (CDRH1) of FNIKDYYMH (SEQ ID NO: 13);

ii. a V_H CDR2 (CDRH2) of WIDLENANTVYDAKFQG (SEQ ID NO: 15); iii. a V_H CDR3 (CDRH3) of ARDAYGRYFYDV (SEQ ID NO: 103); and/or b. a variable light (V_L) chain polypeptide comprising: i. a V_L CDR1 (CDRL1) of KSSQSLLNARTGKNYLA (SEQ ID NO: 8); ii. a V_L CDR2 (CDRL2) of WASTRX$_1$S (SEQ ID NO: 133); and iii. a V_L CDR3 (CDRL3) of X$_2$QSYFRRT (SEQ ID NO: 134); X$_1$ and X$_2$ are each independently any amino acid. In some embodiments, X$_1$ is E or S; and/or X$_2$ is K or V. In some embodiments, said antibody or antigen-binding antibody fragment comprises a CDRL2 of SEQ ID NO: 10 or SEQ ID NO: 11; and/or a CDRL3 of SEQ ID NO: 5 or SEQ ID NO: 4. In some embodiments, said anti-CD3 antibody or antigen-binding antibody fragment is selected from the group consisting of: a. one which comprises a V_H chain CDR1 (CDRH1) of FNIKDYYMH (SEQ ID NO: 13), a V_H chain CDR2 (CDRH2) of WIDLENANTVYDAKFQG (SEQ ID NO: 15), and a V_H chain CDR3 (CDRH3) of ARDAYGRYFYDV (SEQ ID NO: 103); and/or a V_L chain CDR1 (CDRL1) of KSSQSLLNARTGKNYLA (SEQ ID NO: 8), a V_L chain CDR2 (CDRL2) of WASTRES (SEQ ID NO: 10), and a V_L chain CDR3 (CDRL3) of KQSYFRRT (SEQ ID NO: 5); and b. one which comprises a V_H chain CDR1 (CDRH1) of FNIKDYYMH (SEQ ID NO: 13), a V_H chain CDR2 (CDRH2) of WIDLENANTVYDAKFQG (SEQ ID NO: 15), and a V_H chain CDR3 (CDRH3) of ARDAYGRYFYDV (SEQ ID NO: 103); and/or a V_L chain CDR1 (CDRL1) of KSSQSLLNARTGKNYLA (SEQ ID NO: 8), a V_L chain CDR2 (CDRL2) of WASTRSS (SEQ ID NO: 11), and a V_L chain CDR3 (CDRL3) of VQSYFRRT (SEQ ID NO: 4).

[0019] In some embodiments, said anti-CD3 antibody or antigen-binding antibody fragment comprises a V_L chain polypeptide comprising an amino acid sequence that possesses at least 90% sequence identity to SEQ ID NO: 18 or SEQ ID NO: 34. In some embodiments, said anti-CD3 antibody or antigen-binding antibody fragment comprises a V_L chain polypeptide comprising an amino acid sequence that possesses at least 95% sequence identity to SEQ ID NO: 18 or SEQ ID NO: 34. In some embodiments, said anti-CD3 antibody or antigen-binding antibody fragment comprises a V_L chain polypeptide comprising the amino acid sequence of SEQ ID NO: 18 or SEQ ID NO: 34. In some embodiments, said anti-CD3 antibody or antigen-binding antibody fragment comprises a V_H chain polypeptide comprising an amino acid sequence that possesses at least 90% sequence identity to SEQ ID NO: 17. In some embodiments, said anti-CD3 antibody or antigen-binding antibody fragment comprises a V_H chain polypeptide comprising an amino acid sequence that possesses at least

95% sequence identity to SEQ ID NO: 17. In some embodiments, said anti-CD3 antibody or antigen-binding antibody fragment comprises a V_H chain comprising the amino acid sequence of SEQ ID NO: 17. In some embodiments, said anti-CD3 antibody or antigen-binding antibody fragment comprises a V_L chain polypeptide comprising an amino acid sequence that possesses at least 90% sequence identity to SEQ ID NO: 18 or SEQ ID NO: 34; and a V_H chain polypeptide comprising an amino acid sequence that possesses at least 90% sequence identity to SEQ ID NO: 17. In some embodiments, said anti-CD3 antibody or antigen-binding antibody fragment comprises a V_L chain polypeptide comprising an amino acid sequence that possesses at least 95% sequence identity to SEQ ID NO: 18 or SEQ ID NO: 34; and a V_H chain polypeptide comprising an amino acid sequence that possesses at least 95% sequence identity to SEQ ID NO: 17. In some embodiments, said anti-CD3 antibody or antigen-binding antibody fragment comprises a V_L chain polypeptide comprising the amino acid sequence of SEQ ID NO: 18 or SEQ ID NO: 34; and a V_H chain comprising the amino acid sequence of SEQ ID NO: 17. In some embodiments, said anti-CD3 antibody or antigen-binding antibody fragment comprises a V_L chain polypeptide comprising the amino acid sequence of SEQ ID NO: 18; and a V_H chain comprising the amino acid sequence of SEQ ID NO: 17. In some embodiments, said anti-CD3 antibody or antigen-binding antibody fragment comprises a V_L chain polypeptide comprising the amino acid sequence of SEQ ID NO: 34; and a V_H chain comprising the amino acid sequence of SEQ ID NO: 17.

[0020] In some embodiments, said anti-CD3 antibody or antigen-binding antibody fragment binds to CD3 with a dissociation constant (K_D) of about 500 pM or less, about 450 pM or less, about 400 pM or less, about 350 pM or less, about 300 pM or less, about 250 pM or less, about 200 pM or less, about 150 pM or less, or about 100 pM or less, optionally wherein said CD3 is human and/or cynomolgus, further optionally wherein said binding affinity is measured by surface plasmon resonance. In some embodiments, wherein said K_D is a monovalent K_D and/or wherein said K_D is measured using an scFv fragment of said anti-CD3 antibody or antibody fragment.

[0021] In some embodiments, said antibody or antigen-binding antibody fragment elicits T cell activation or T cell killing while displaying a decreased propensity to elicit cytokine production to levels capable of inducing cytokine release syndrome. In some embodiments, the antibody or antigen-binding antibody fragment comprises a multispecific antibody. In some embodiments, the antibody or antigen-binding antibody fragment

comprises a bispecific antibody. In some embodiments, the antibody or antigen-binding antibody fragment comprises an scFv. In some embodiments, the antibody or antigen-binding antibody fragment comprises at least a second antigen binding domain that specifically binds to an oncology target; an immune-oncology target; a neurodegenerative disease targets; an autoimmune disorder target; an infectious disease target; a metabolic disease target; a cognitive disorder target; a blood-brain barrier target; or a blood disease target. In some embodiments, the antibody or antigen-binding antibody fragment comprises at least a second antigen binding domain that specifically binds to an antigen selected from the group consisting of: 17-IA, 4-1BB, 4Dc, 6- keto-PGFla, 8-iso-PGF2a, 8-oxo-dG, Al Adenosine Receptor, A33, ACE, ACE-2, Activin, Activin A, Activin AB, Activin B, Activin C, Activin RIA, Activin RIA ALK-2, Activin RIB ALK-4, Activin RIIA, Activin RUB, ADAM, ADAM10, ADAM12, ADAM 15, ADAM 17/T ACE, ADAM8, ADAM9, ADAMTS, ADAMTS4, ADAMTS5, Addressins, aFGF, ALCAM, ALK, ALK-1, ALK-7, alpha-l-antitrypsin, alpha-V/beta-1 antagonist, ANG, Ang, APAF-1, APE, APJ, APP, APRIL, AR, ARC, ART, Artemin, anti-Id, ASPARTIC, Atrial natriuretic factor, av/b3 integrin, Axl, b2M, B7-1, B7-2, B7-H, B-lymphocyte Stimulator (BlyS), BACE, BACE-1, Bad, BAFF, BAFF-R, Bag-1, BAK, Bax, BCA-1, BCAM, Bel, BCMA, BDNF, b-ECGF, bFGF, BID, Bik, BFM, BLC, BL-CAM, BLK, BMP, BMP-2 BMP-2a, BMP-3 Osteogenin, BMP-4 BMP-2b, BMP-5, BMP-6 Vgr-1, BMP-7 (OP-1), BMP-8 (BMP-8a, OP-2), BMPR, BMPR-IA (ALK-3), BMPR-IB (ALK-6), BRK-2, RPK-1, BMPR-II (BRK-3), BMPs, b- NGF, BOK, Bombesin, Bone-derived neurotrophic factor, BPDE, BPDE-DNA, BTC, complement factor 3 (C3), C3a, C4, C5, C5a, CIO, CA125, CAD-8, Calcitonin, cAMP, carcinoembryonic antigen (CEA), carcinoma-associated antigen, Cathepsin A, Cathepsin B, Cathepsin C/DPPI, Cathepsin D, Cathepsin E, Cathepsin H, Cathepsin L, Cathepsin O, Cathepsin S, Cathepsin V, Cathepsin X/Z/P, CBL, CCI, CCK2, CCL, CCL1, CCL11, CCL12, CCL13, CCL 14, CCL15, CCL16, CCL1 7, CCL18, CCL19, CCL2, CCL20, CCL21, CCL22, CCL23, CCL24, CCL25, CCL26, CCL27, CCL28, CCL3, CCL4, CCL5, CCL6, CCL7, CCL8, CCL9/10, CCR, CCR1, CCR10, CCR10, CCR2, CCR3, CCR4, CCR5, CCR6, CCR7, CCR8, CCR9, CD1, CD2, CD4, CD5, CD6, CD7, CD8, CD10, CDlla, CDllb, CDllc, CD13, CD14, CD15, CD16, CD18, CD19, CD20, CD21, CD22, CD23, CD25, CD27L, CD28, CD29, CD30, CD30L, CD32, CD33 (p67 proteins), CD34, CD38, CD40, CD40L, CD44, CD45, CD46, CD49a, CD52, CD54, CD55, CD56, CD61, CD64, CD66e, CD74, CD80 (B7-1), CD89, CD95, CD123, CD137, CD138, CD140a, CD146, CD147, CD148, CD152, CD164,

CEACAM5, CFTR, cGMP, CINC, Clostridium botulinum toxin, Clostridium perfringens toxin, CKb8-l, CLC, CMV, CMV UL, CNTF, CNTN-1, COX, C-Ret, CRG-2, CT-1, CTACK, CTGF, CTLA-4, CX3CL1, CX3CR1, CXCL, CXCL1, CXCL2, CXCL3, CXCL4, CXCL5, CXCL6, CXCL7, CXCL8, CXCL9, CXCL10, CXCL11, CXCL12, CXCL13, CXCL14, CXCL15, CXCL16, CXCR, CXCR1, CXCR2, CXCR3, CXCR4, CXCR5, CXCR6, cytokeratin tumor-associated antigen, DAN, DCC, DcR3, DC-SIGN, Decay accelerating factor, des(l-3)-IGF-I (brain IGF-1), Dhh, digoxin, DNAM-1, Dnase, Dpp, DPPIV/CD26, Dtk, ECAD, EDA, EDA-A1, EDA-A2, EDAR, EGF, EGFR (ErbB-1), EMA, EMMPRIN, EN A, endothelin receptor, Enkephalinase, eNOS, Eot, eotaxinl, EpCAM, Ephrin B2/ EphB4, EPO, ERCC, E-selectin, ET-1, Factor Ila, Factor VII, Factor VIIIc, Factor IX, fibroblast activation protein (FAP), Fas, FcRl, FEN-1, Ferritin, FGF, FGF-19, FGF-2, FGF3, FGF-8, FGFR, FGFR-3, Fibrin, FL, FLIP, Flt-3, Flt-4, Follicle stimulating hormone, Fractalkine, FZD1, FZD2, FZD3, FZD4, FZD5, FZD6, FZD7, FZD8, FZD9, FZD10, G250, Gas 6, GCP-2, GCSF, GD2, GD3, GDF, GDF-1, GDF-3 (Vgr-2), GDF-5 (BMP-14, CDMP-1), GDF-6 (BMP-13, CDMP-2), GDF-7 (BMP-12, CDMP-3), GDF-8 (Myostatin), GDF-9, GDF- 15 (MIC-1), GDNF, GFAP, GFRa-1, GFR-alphal, GFR-alpha2, GFR-alpha3, GITR, Glucagon, Glut 4, glycoprotein Ilb/IIIa (GP Ilb/IIIa), GM-CSF, gpl30, gp72, GRO, Growth hormone releasing factor, Hapten (NP-cap or NIP-cap), HB-EGF, HCC, HCMV gB envelope glycoprotein, HCMV) gH envelope glycoprotein, HCMV UL, Hemopoietic growth factor (HGF), Hep B gpl20, heparanase, Her2, Her2/neu (ErbB-2), Her3 (ErbB-3), Her4 (ErbB-4), herpes simplex virus (HSV) gB glycoprotein, HSV gD glycoprotein, HGFA, High molecular weight melanoma-associated antigen (HMW-MAA), HIV gpl20, HIV IIIB gp 120 V3 loop, HLA, HLA-DR, HM1.24, HMFG PEM, HRG, Hrk, human cardiac myosin, human cytomegalovirus (HCMV), human growth hormone (HGH), HVEM, 1-309, IAP, ICAM, ICAM-1, ICAM-3, ICE, ICOS, IFNg, Ig, IgA receptor, IgE, IGF, IGF binding proteins, IGF-1R, IGFBP, IGF-I, IGF-II, IL, IL-1, IL-1R, IL-2, IL-2R, IL-4, IL-4R, IL-5, IL-5R, IL-6, IL-6R, IL-8, IL- 9, IL-10, IL-12, IL-13, IL-15, IL-18, IL-18R, IL-23, interferon (INF)-alpha, INF-beta, INF- gamma, Inhibin, iNOS, Insulin A-chain, Insulin B-chain, Insulin-like growth factor 1, integrin alpha2, integrin alpha3, integrin alpha4, integrin alpha4/betal, integrin, alpha4/beta7, integrin alpha5 (alphaV), integrin alpha5/betal, integrin alpha5/beta3, integrin alpha6, integrin betal, integrin beta2, interferon gamma, IP- 10, 1-TAC, JE, Kallikrein 2, Kallikrein 5, Kallikrein 6, , Kallikrein 11, Kallikrein 12, Kallikrein 14, Kallikrein 15, Kallikrein LI, Kallikrein L2, Kallikrein L3, Kallikrein L4, KC, KDR, Keratinocyte Growth

Factor (KGF), laminin 5, LAMP, LAP, LAP (TGF- 1), Latent TGF-1, Latent TGF-1 bpl, LBP, LDGF, LECT2, Lefty, Lewis-Y antigen, Lewis-Y related antigen, LFA-1, LFA-3, Lfo, LIF, LIGHT, lipoproteins, LIX, LKN, Lptn, L-Selectin, LT-a, LT-b, LTB4, LTBP-1, Lung surfactant, Luteinizing hormone, Lymphotoxin Beta Receptor, Mac-1, MAdCAM, MAG, MAP2, MARC, MCAM, MCAM, MCK-2, MCP, M-CSF, MDC, Mer, a metalloprotease, MGDF receptor, MGMT, MHC (HLA-DR), MIF, MIG, MIP, MIP-1-alpha, MK, MMAC1, MMP, MMP-1, MMP-10, MMP-11, MMP-12, MMP-13, MMP-14, MMP-15, MMP-2, MMP-24, MMP- 3, MMP-7, MMP-8, MMP-9, MPIF, Mpo, MSK, MSP, mucin (Mucl), MUC18, Muellerian- inhibiting substance, Mug, MuSK, NAIP, NAP, NCAD, N-Cadherin, NCA 90, NCAM, NCAM, Neprilysin, Neurotrophin-3,-4, or -6, Neurturin, Neuronal growth factor (NGF), NGFR, NGF-beta, nNOS, NO, NOS, Npn, NRG-3, NT, NTN, OB, OGG1, OPG, OPN, OSM, OX40L, OX40R, pl50, p95, PADPr, Parathyroid hormone, PARC, PARP, PBR, PBSF, PCAD, P-Cadherin, PCNA, PDGF, PDGF, PDK-1, PECAM, PEM, PF4, PGE, PGF, PGI2, PGJ2, PIN, PLA2, placental alkaline phosphatase (PLAP), PIGF, PLP, PP14, Proinsulin, Prorelaxin, Protein C, PS, PSA, PSCA, prostate specific membrane antigen (PSMA), PTEN, PTHrp, Ptk, PTN, R51, RANK, RANKL, RANTES, Relaxin A-chain, Relaxin B-chain, renin, respiratory syncytial virus (RSV) F, RSV Fgp, Ret, Rheumatoid factors, RLIP76, RPA2, RSK, S100, SCF/KL, SDF-1, SERINE, Serum albumin, sFRP-3, Shh, SIGIRR, SK-1, SLAM, SLPI, SMAC, SMDF, SMOH, SOD, SPARC, Stat, STEAP, STEAP-II, TACE, TACI, TAG-72 (tumor- associated glycoprotein-72), TARC, TCA-3, T-cell receptors (e.g., T-cell receptor alpha/beta), TdT, TECK, TEM1, TEM5, TEM7, TEM8, TERT, testicular PLAP-like alkaline phosphatase, TfR, TGF, TGF-alpha, TGF-beta, TGF-beta Pan Specific, TGF-beta RI (ALK-5), TGF-beta RII, TGF-beta Rllb, TGF-beta RIII, TGF-betal, TGF-beta2, TGF-beta3, TGF-beta4, TGF-beta5, Thrombin, Thymus Ck-1, Thyroid stimulating hormone, Tie, TIMP, TIQ, Tissue Factor, TMEFF2, Tmpo, TMPRSS2, TNF, TNF-alpha, TNF-alpha beta, TNF-beta2, TNFc, TNF-RI, TNF-RII, TNFRSF10A (TRAIL Rl Apo-2, DR4), TNFRSFIOB (TRAIL R2 DR5, KILLER, TRICK-2A, TRICK-B), TNFRSF10C (TRAIL R3 DcRl, LIT, TRID), TNFRSF10D (TRAIL R4 DcR2, TRUNDD), TNFRSF11A (RANK ODF R, TRANCE R), TNFRSFllB (OPG OCIF, TR1), TNFRSF12 (TWEAK R FN14), TNFRSF13B (TACI), TNFRSF13C (BAFF R), TNFRSF14 (HVEM ATAR, HveA, LIGHT R, TR2), TNFRSF16 (NGFR p75NTR), TNFRSF17 (BCMA), TNFRSF 18 (GITR AITR), TNFRSF19 (TROY TAJ, TRADE), TNFRSF19L (RELT), TNFRSFIA (TNF RI CD120a, p55-60), TNFRSFIB (TNF RII CD120b, p75-80), TNFRSF26

(TNFRH3), TNFRSF3 (LTbR TNF RIII, TNFC R), TNFRSF4 (OX40 ACT35, TXGP1 R), TNFRSF 5 (CD40 p50), TNFRSF6 (Fas Apo-1, APT1, CD95), TNFRSF6B (DcR3 M68, TR6), TNFRSF7 (CD27), TNFRSF8 (CD30), TNFRSF9 (4-1BB CD137, ILA), TNFRSF21 (DR6), TNFRSF22 (DcTRAIL R2 TNFRH2), TNFRST23 (DcTRAIL Rl TNFRH1), TNFRSF25 (DR3 Apo-3, LARD, TR-3, TRAMP, WSL-1), TNFSF10 (TRAIL Apo-2 Ligand, TL2), TNFSF11 (TRANCE/RANK Ligand ODF, OPG Ligand), TNFSF12 (TWEAK Apo-3 Ligand, DR3 Ligand), TNFSF13 (APRIL TALL2), TNFSF13B (BAFF BLYS, TALL1, THANK, TNFSF20), TNFSF14 (LIGHT HVEM Ligand, LTg), TNFSF15 (TLIA/VEGI), TNFSF18 (GITR Ligand AITR Ligand, TL6), TNFSFIA (TNF-a Conectin, DIF, TNFSF2), TNFSF1B (TNF-b LTa, TNFSF1), TNFSF3 (LTb TNFC, p33), TNFSF4 (OX40 Ligand gp34, TXGP1), TNFSF5 (CD40 Ligand CD154, gp39, HIGM1, IMD3, TRAP), TNFSF6 (Fas Ligand Apo-1 Ligand, APT1 Ligand), TNFSF7 (CD27 Ligand CD70), TNFSF8 (CD30 Ligand CD153), TNFSF9 (4-1BB Ligand CD137 Ligand), TP-1, t-PA, Tpo, TRAIL, TRAIL R, TRAIL-R1, TRAIL-R2, TRANCE, transferring receptor, TRF, Trk, TROP-2, TSG, TSLP, tumor-associated antigen CA 125, tumor-associated antigen expressing Lewis Y related carbohydrate, TWEAK, TXB2, Ung, uPAR, uPAR-1, Urokinase, VCAM, VCAM-1, VECAD, VE-Cadherin, VE-cadherin-2, VEFGR-1 (flt-1), VEGF, VEGFR, VEGFR-3 (flt-4), VEGI, VFM, Viral antigens, VLA, VLA-1, VLA-4, VNR integrin, von Willebrands factor, WIF- 1, WNT1, WNT2, WNT2B/13, WNT3, WNT3A, WNT4, WNT5A, WNT5B, WNT6, WNT7A, WNT7B, WNT8A, WNT8B, WNT9A, WNT9A, WNT9B, WNT10A, WNT10B, WNT11, WNT16, XCL1, XCL2, XCR1, XCR1, XEDAR, XIAP, XPD, CTLA4 (cytotoxic T lymphocyte antigen-4), PD1 (programmed cell death protein 1), PD-L1 (programmed cell death ligand 1), LAG-3 (lymphocyte activation gene-3), TIM-3 (T cell immunoglobulin and mucin protein-3), hormone receptors and growth factors.

[0022] In some embodiments, the antibody or antigen-binding antibody fragment comprises at least a second antigen binding domain that specifically binds to an antigen selected from the group consisting of: BCMA, CTLA4 (cytotoxic T lymphocyte antigen-4), PD1 (programmed cell death protein 1), PD-L1 (programmed cell death ligand 1), LAG-3 (lymphocyte activation gene-3), TIM-3, CD20, CD2, CD19, Her2, EGFR, EpCAM, FcyRIIIa (CD16), FcyRIIa (CD32a), FcyRIIb (CD32b), FcyRI (CD64), Toll-like receptors (TLRs), TLR4, TLR9, cytokines, IL-2, IL-5, IL-13, IL-6, IL-17, IL-12, IL-23, TNFa, TGFb, cytokine receptors, IL-2R, chemokines, chemokine receptors, growth factors, VEGF, and HGF. In some embodiments, the antibody or antigen-binding antibody fragment is comprised in a

chimeric antigen receptor (CAR), which optionally comprises at least one transmembrane domain, and at least one intracellular domain from a T-cell receptor, optionally a CD3ζ subunit, and at least one co-stimulatory domain. In some embodiments, the antibody or antigen-binding antibody fragment comprises an scFv2-Fc2 and/or scFv-IgG. In some embodiments, the antibody or antigen-binding antibody fragment comprises an IgG constant domain. In some embodiments, k. the antibody or antigen-binding antibody fragment comprises at least a second antigen binding domain that specifically binds to an antigen, wherein said antibody comprises a multispecific format selected from the group consisting of: Fab-Fc-scFv, "bottle-opener, Mab-scFv, Mab-Fv, Dual scFv, central Fv, central scFv, one-arm central scFv, Fab-Fab, Fab-Fv, mAb-Fv, mAb-Fab, DART, BiTE, common light chain-IgG, TandAb, Cross-Mab, SEED, BEAT, TrioMab, and DuetMab. Moreover, the present disclosure generally relates to an isolated or recombinant nucleic acid sequence encoding an antibody or antigen-binding antibody fragment as described herein. Furthermore, the present disclosure generally relates to an expression vector comprising an isolated or recombinant nucleic acid sequence as described herein. Furthermore, the present disclosure generally relates to a host cell transfected, transformed, or transduced with a nucleic acid sequence, optionally a mammalian or yeast cell, or a vector containing said nucleic acid sequence as described herein.

[0023] Moreover, the present disclosure generally relates to a pharmaceutical composition comprising an antibody or antigen-binding antibody fragment as described herein; and a pharmaceutically acceptable carrier and/or excipient.

[0024] Furthermore, the present disclosure generally relates to a method of treating a disorder in a mammal in need of such treatment, wherein the disorder comprises a proliferative disorder, an oncological disorder, an immuno-oncological disorder, a neurological disorder, a neurodegenerative disorder, or an autoimmune disorder, comprising administering an effective amount of one or more antibodies or antibody fragments as described herein, or a host cell which expresses one of said antibodies or antibody fragments as described herein, optionally an immune cell, further optionally a T or NK cell. In some embodiments, the method further comprises administering to the mammal an additional therapeutic agent, optionally wherein the mammal is a human.

[0025] Moreover, the present disclosure generally relates to an anti-cluster of differentiation three (“CD3”) antibody or antibody fragment, wherein said anti-CD3 antibody

or antigen-binding antibody fragment comprises one or more CDRs of any one or more of Ab1-Ab50. Furthermore, the present disclosure generally relates to an anti-cluster of differentiation three ("CD3") antibody or antibody fragment, wherein said anti-CD3 antibody or antigen-binding antibody fragment comprises one or more V_H and/or V_L chains comprising an amino acid sequence selected from those in Table 4.

Detailed Description of the Invention

[0026] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. As used herein, the term "about," when used in reference to a particular recited numerical value, means that the value may vary from the recited value by no more than 1 %. For example, as used herein, the expression "about 100" includes 99 and 101 and all values in between (e.g., 99.1, 99.2, 99.3, 99.4, etc.).

[0027] It is understood that aspects and embodiments of the invention described herein include "comprising," "consisting," and "consisting essentially of" aspects and embodiments.

[0028] Provided herein are anti-CD3 antibodies and/or antigen-binding fragments thereof having high affinity (K_D) for CD3. In some embodiments, the K_D of the anti-CD3 is about 500 pM or less. In some embodiments, the K_D is about 250 pM or less. In some embodiments, the K_D is about 100 pM or less. In some embodiments, said anti-CD3 antibody or antigen-binding antibody fragment binds to CD3 with a dissociation constant (K_D) of about 500 pM or more, about 500 pM or less, about 470 pM or less, about 450 pM or less, about 400 pM or less, about 350 pM or less, about 300 pM or less, about 250 pM or less, about 200 pM or less, about 150 pM or less, or about 100 pM or less, optionally wherein said CD3 is human and/or cynomolgus, further optionally wherein said binding affinity is measured by surface plasmon resonance. In some embodiments, said K_D is a monovalent K_D and/or wherein said K_D is measured using an scFv fragment of said anti-CD3 antibody or antibody fragment. In some embodiments, the anti-CD3 antibodies and/or antigen binding fragments thereof comprise certain CDR sequence motifs. In some embodiments, a CDR sequence motif comprises CDRL3 comprising SEQ ID NO: 1. In some embodiments, the anti-CD3 antibodies have favorable developability profiles.

Exemplary high affinity anti-CD3 antibodies and antigen-binding fragments thereof

[0029] "Cluster of Differentiation 3" or "CD3", generally refers to any native CD3 from any vertebrate source, including mammals such as primates (e.g., humans) and rodents (e.g., mice and rats), unless otherwise indicated, including, for example, CD3ε, CD3γ, CD3α, and CD3β chains. The term encompasses "full-length," unprocessed CD3 (e.g., unprocessed or unmodified CD3ε or CD3γ), as well as any form of CD3 that results from processing in the cell. The term also encompasses naturally occurring variants of CD3, including, for example, splice variants or allelic variants. CD3 includes, for example, human CD3ε protein (NCBI RefSeq No. NP_000724), which is 207 amino acids in length, and human CD3γ protein (NCBI RefSeq No. NP_000064), which is 182 amino acids in length. The term also refers to either the human or cynomolgus CD3epsilon protein, the amino acid sequence for which is SEQ ID NOs: 131 and 132, respectively (Table 7). "CD3εN27" and "CD3εN13" refer to the N-terminal 27 amino acids and the N-terminal 13 amino acids, respectively, of CD3, and optionally containing chemical modifications or conjugations made thereto.

[0030] An "anti-CD3 antibody" refers to an antibody or an antigen-binding fragment thereof capable of binding to CD3, e.g., CD3ε and/or CD3γ, e.g., human CD3ε and/or CD3γ, with sufficient affinity and/or specificity such that the antibody is useful as a diagnostic and/or therapeutic agent in targeting CD3. The anti-CD3 antibodies and antigen-binding fragments thereof described herein have particularly high affinity for CD3.

[0031] The term "high affinity" refers to those antibodies having a binding affinity to CD3, expressed as K_D. In some embodiments, the K_D is about 5×10^{-10} M or less. In some embodiments, the K_D is about 2.5×10^{-10} M or less. In some embodiments, the K_D is about 1×10^{-11} M or less. In some embodiments, said anti-CD3 antibody or antigen-binding antibody fragment binds to CD3 with a dissociation constant (K_D) of about 500 pM or more, about 500 pM or less, about 470 pM or less, about 450 pM or less, about 400 pM or less, about 350 pM or less, about 300 pM or less, about 250 pM or less, about 200 pM or less, about 150 pM or less, or about 100 pM or less, optionally wherein said CD3 is human and/or cynomolgus, further optionally wherein said binding affinity is measured by surface plasmon resonance.In some embodiments, the K_D is measured by surface plasmon resonance, e.g., BIACORE, biolayer interferometry measurements using, e.g., a FORTEBIO Octet HTX instrument (Pall Life Sciences), or solution-affinity ELISA. In some embodiments, the K_D is measured using an scFv fragment of the anti-CD3 antibody. In some embodiments, the monovalent K_D is measured.

[0032] In some embodiments, an anti-CD3 antibody binds to CD3 with a dissociation constant (K_D) of less than about 500 pM (5 x 10^{-10} M). In some embodiments, an anti-CD3 antibody binds to CD3 with a dissociation constant (K_D) of less than about 250 pM. In some embodiments, an anti-CD3 antibody binds to CD3 with a dissociation constant (K_D) of about 100 pM. In certain embodiments, the anti-CD3 antibody binds to an epitope of CD3 that is conserved among CD3 from different species, e.g., human and cyno cross-reactive.

[0033] The K_D can be measured using assays well-known to a skilled artisan, including, but not limited to: biolayer interferometry (BLI), surface plasmon resonance (SPR), solution equilibrium based kinetic exclusion assays, KinExA direct association, assays using FORTEBIO instruments and reagents, such as Octet RED 384 and HTX BLI-based instruments, enzyme-linked immunosorbent assays (ELISA), or radioimmunoassay (RIA). The K_D measurement may be performed with an intact anti-CD3 antibody or an antigen-binding fragment thereof, e.g., scFv, using a BIACORE® surface plasmon resonance assay.

[0034] While high affinity CD3 binders have been associated with triggering or contributing to cytokine release syndrome (CRS) in patients, the binding affinity of an antibody for CD3 is not the sole determinant of CRS. For example, a high affinity anti-CD3 antibody (~ low single digit nM K_D) used in a bispecific format, successfully demonstrated potent T-cell cytotoxicity for target tumor cells with limited cytokine (IFNγ, IL-6, TNFα) release (*See*, e.g., *Mol Cancer Ther. 2016 Sep; 15(9); 2155-65* and APTEVO AACR Poster regarding preclinical studies of APOV436, presented April 16, 2018). Without being bound by theory, a high affinity anti-CD3 antibody may be particularly beneficial for providing specific targeting and minimal CRS risk in multispecific antibodies with certain formats, e.g., scFv2-Fc2 or scFv-IgG or other like polymeric formats) or chimeric antigen receptors with various components assembled in different orders.

[0035] The term "cytokine release syndrome" (or "CRS") refers to a pro-inflammatory, positive feedback loop between cytokines and immune cells leading to excessive or uncontrolled release of pro-inflammatory cytokines by cells within the immune system (*see*, e.g., Lee et al., Blood, Vol. 124, pages 188-195 (2014) and Tisoncik et al., Microbiol Mol Biol Rev, Vol. 76, pages 16-32 (2012). Upon stimulation and activation, T cells release a series of cytokines to a level and degree that generates untoward biological/physiological effects of varying degree and severity, including acute inflammation characterized by, e.g., rubor (redness), swelling or edema, calor (heat), dolor (pain), and

"functio laesa" (loss of function). When localized in skin or other tissue, biological/physiological effects comprise increased blood flow, enabling vascular leukocytes and plasma proteins to reach extravascular sites of injury, increasing local temperatures and generation of pain, tissue edema and extravascular pressure and a reduction in tissue perfusion. Other biological/physiological effects comprise organ and system dysfunction, such as cardiac dysfunction, adult respiratory distress syndrome, neurologic toxicity, renal and/or hepatic failure, and disseminated intravascular coagulation. Elevated levels of IFNγ, IL-6, TNFα, TGFbeta, IL-2, granulocyte macrophage–colony-stimulating factor (GM-CSF), IL-10, IL-8, IL-5, and/or fractalkine are implicated as predictive and/or causative of CRS or the propensity to elicit CRS upon T-cell stimulation.

[0036] In certain embodiments, the anti-CD3 antibodies and/or antigen-binding fragments thereof described herein are detuned and/or modified to reduce the likelihood or severity of CRS induced by the antibody. Non-limiting exemplary modifications may include silent Fc regions (e.g., removing the Fc completely or modifying the Fc region to reduce or eliminate effector function), and/or masking (e.g., a polypeptide mask that is positioned such that it reduces or inhibits the ability of the antibody or antigen-binding fragment thereof to specifically bind CD3).

[0037] The term "antibody" is used herein in the broadest sense and encompasses various antibody structures, including but not limited to monoclonal antibodies, polyclonal antibodies, multispecific antibodies (e.g., bispecific antibodies), and/or antibody fragments (preferably those fragments that exhibit the desired antigen-binding activity).

[0038] A "monoclonal antibody" or "mAb" refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical and/or bind the same epitope, except for possible variant antibodies (e.g., containing naturally occurring mutations or arising during production of a monoclonal antibody preparation), such variants generally being present in minor amounts. In contrast to polyclonal antibody preparations, which typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody of a monoclonal antibody preparation is directed against a single determinant on an antigen.

[0039] With regard to multispecific antibodies, such antibodies comprise at least two different antigen binding domains which recognize and specifically bind to at least two different antigens. With regard to bispecific antibodies, such antibodies comprise two

different antigen binding domains which recognize and specifically bind to at least two different antigens. A "different antigen" may refer to different and/or distinct proteins, polypeptides, or molecules; as well as different and/or distinct epitopes, which epitopes may be contained within one protein, polypeptide, or other molecule.

[0040] The term "epitope" refers to an antigenic determinant that interacts with a specific antigen binding site in the variable region of an antibody molecule known as a paratope. A single antigen may have more than one epitope. Thus, different antibodies may bind to different areas on an antigen and may have different biological effects. The term "epitope" also refers to a site on an antigen to which B and/or T cells respond. It also refers to a region of an antigen that is bound by an antibody. Epitopes may be defined as structural or functional. Functional epitopes are generally a subset of the structural epitopes and have those residues that directly contribute to the affinity of the interaction. Epitopes may also be conformational, that is, composed of non-linear amino acids. In certain embodiments, epitopes may include determinants that are chemically active surface groupings of molecules such as amino acids, sugar side chains, phosphoryl groups, or sulfonyl groups, and, in certain embodiments, may have specific three-dimensional structural characteristics, and/or specific charge characteristics.

[0041] In some instances, an antibody comprises four polypeptide chains: two heavy (H) chains and two light (L) chains interconnected by disulfide bonds. There are five major classes of antibodies: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into subclasses (isotypes), e.g., IgG_1, IgG_2, IgG_3, IgG_4, IgA_1, and IgA_2. The heavy chain constant domains that correspond to the different classes of immunoglobulins are called α, δ, ε, γ, and μ, respectively.

[0042] In other instances, an antibody may instead comprise multimers thereof (e.g., IgM) or antigen-binding fragments thereof. Each heavy chain is comprised of a heavy chain variable region ("VH") and a heavy chain constant region ("CH"), which is comprised of domains C_H1, C_H2 and C_H3. Each light chain is comprised of a light chain variable region ("VL") and a light chain constant region ("CL"). The V_H and V_L regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDRs), interspersed with regions that are more conserved, termed framework regions (FRs). Each V_H and V_L is composed of three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. In

certain embodiments of the invention, the FRs of the antibody (or antigen-binding fragment thereof) may be identical to the human germline sequences or may be naturally or artificially modified. An amino acid consensus sequence may be defined based on a side-by-side analysis of two or more CDRs. Accordingly, the CDRs in a heavy chain are designated "CDRH1", "CDRH2", and "CDRH3", respectively, and the CDRs in a light chain are designated "CDRL1", "CDRL2", and "CDRL3".

[0043] Unless specifically indicated otherwise, the term "antibody" as used herein encompasses molecules comprising two immunoglobulin heavy chains and two immunoglobulin light chains (i.e., "full antibody molecules" or "intact antibodies") as well as antigen-binding fragments thereof.

[0044] An "antigen-binding fragment" refers to a portion of an intact antibody that binds the antigen to which the intact antibody binds (in this case, CD3). The terms "full-length antibody," "intact antibody," and "whole antibody" or the like are used herein interchangeably and refer to an antibody having a structure substantially similar to a native antibody.

[0045] An antigen-binding fragment of an antibody includes any naturally occurring, enzymatically obtainable, synthetic, or genetically engineered polypeptide or glycoprotein that specifically binds an antigen to form a complex. Exemplary antibody fragments include, but are not limited to, Fv, Fab, Fab', Fab'-SH, $F(ab')_2$; diabodies; linear antibodies; single-chain antibody molecules (e.g. scFv or VH or VL domains only); and multispecific antibodies formed from antibody fragments. In some embodiments, the antigen-binding fragments of the anti-CD3 antibodies described herein are scFvs.

[0046] As with full antibody molecules, antigen-binding fragments may be mono-specific or multispecific (e.g., bispecific). A multi-specific antigen-binding fragment of an antibody may comprise at least two different variable domains, wherein each variable domain is capable of specifically binding to a separate antigen or to a different epitope on the same antigen. A variety of multi-specific antibody formats may be used in the context of an antigen-binding fragment of anti-CD3 antibody described herein. Non-limiting examples of multispecific and bispecific formats include, e.g., Fab-Fc-scFv ("bottle-opener") (XENCOR), Mab-scFv (XENCOR), Mab-Fv (XENCOR), Dual scFv (XENCOR),central Fv (XENCOR), central scFv(XENCOR), one-arm central scFv (XENCOR), Fab-Fab (XENCOR), Fab-Fv (XENCOR), mAb-Fv (XENCOR), mAb-Fab (XENCOR), DART (MACROGENICS), BiTE

(AMGEN/MICROMET), KiTE, common light chain-IgG (Genentech), TandAb (SFFIMED) Cross-Mab (ROCHE), SEED (EMD SERONO), BEAT (GLENMARK), TrioMab (TRION PHARMA/FRESENIUS BIOTECH), DuetMab (MEDIMMUNE), and others, as disclosed, e.g., in (WO 95/09917; WO 2008/119566; WO 2008/119567; WO2011/121110; WO 2010/037835; WO 2007/042261; WO 2007/110205; WO 2011/121110; WO 2012/055961; WO 2012/16067; WO 2016/086189; WO 2016/182751; WO 2015/006749; WO 2014/049003; WO 2013/177101; WO 2015/128509; US 7,951,917; US 2009/0252729; US 2014/0348839; US 7,183,076; Mazor et al., *Mabs*, Vol. 7, pages 377-389 (2015); Muda et al., *Protein Engineering, Design, & Selection*, Vol. 24, pages 447-454 (2011); and Del Bano et al., *Antibodies*, Vol. 5, pages 1-23 (2016). In some embodiments, the anti-CD3 scFv fragments described herein comprise one or more variable domains of a multispecific (e.g., bispecific) antibody.

[0047] In certain embodiments, anti-CD3 antibodies and/or antigen-binding fragments thereof as described herein are contained in a multispecific antibody, in particular, a bispecific antibody that has binding specificity for a second antigen. Such a second antigen may be a different target altogether than the first target, or a different epitope present on the same target. In some embodiments, the binding specificities are to two different epitopes of CD3 (e.g., CD3ε or CD3γ). In other embodiments, one of the binding specificities is for CD3 (e.g., CD3ε or CD3γ) and the other is for a different biological molecule (e.g., a cell surface antigen, e.g., a tumor antigen).

[0048] Non-limiting examples of a second antigen, toward which a bispecific antibody comprising anti-CD3 antibodies and/or antigen-binding fragments thereof as described herein, comprises targets selected from the group consisting of: 17-IA, 4-1BB, 4Dc, 6- keto-PGFla, 8-iso-PGF2a, 8-oxo-dG, Al Adenosine Receptor, A33, ACE, ACE-2, Activin, Activin A, Activin AB, Activin B, Activin C, Activin RIA, Activin RIA ALK-2, Activin RIB ALK-4, Activin RIIA, Activin RUB, ADAM, ADAM10, ADAM12, ADAM15, ADAM17/TACE, ADAM8, ADAM9, ADAMTS, ADAMTS4, ADAMTS5, Addressins, aFGF, ALCAM, ALK, ALK-1, ALK-7, alpha-l-antitrypsin, alpha-V/beta-1 antagonist, ANG, Ang, APAF-1, APE, APJ, APP, APRIL, AR, ARC, ART, Artemin, anti-Id, ASPARTIC, Atrial natriuretic factor, av/b3 integrin, Axl, b2M, B7-1, B7-2, B7-H, B-lymphocyte Stimulator (BlyS), BACE, BACE-1, Bad, BAFF, BAFF-R, Bag-1, BAK, Bax, BCA-1, BCAM, Bel, BCMA, BDNF, b-ECGF, bFGF, BID, Bik, BIM, BLC, BL-CAM, BLK, BMP,

BMP-2 BMP-2a, BMP-3 Osteogenin, BMP-4 BMP-2b, BMP-5, BMP-6 Vgr-1, BMP-7 (OP-1), BMP-8 (BMP-8a, OP-2), BMPR, BMPR-IA (ALK-3), BMPR-IB (ALK-6), BRK-2, RPK-1, BMPR-II (BRK-3), BMPs, b- NGF, BOK, Bombesin, Bone-derived neurotrophic factor, BPDE, BPDE-DNA, BTC, complement factor 3 (C3), C3a, C4, C5, C5a, CIO, CA125, CAD-8, Calcitonin, cAMP, carcinoembryonic antigen (CEA), carcinoma-associated antigen, Cathepsin A, Cathepsin B, Cathepsin C/DPPI, Cathepsin D, Cathepsin E, Cathepsin H, Cathepsin L, Cathepsin O, Cathepsin S, Cathepsin V, Cathepsin X/Z/P, CBL, CCI, CCK2, CCL, CCLl, CCLll, CCL12, CCL13, CCL 14, CCL15, CCL16, CCLl 7, CCL18, CCL19, CCL2, CCL20, CCL21, CCL22, CCL23, CCL24, CCL25, CCL26, CCL27, CCL28, CCL3, CCL4, CCL5, CCL6, CCL7, CCL8, CCL9/10, CCR, CCR1, CCR10, CCR10, CCR2, CCR3, CCR4, CCR5, CCR6, CCR7, CCR8, CCR9, CD1, CD2, CD4, CD5, CD6, CD7, CD8, CD10, CDlla, CDllb, CDllc, CD13, CD14, CD15, CD16, CD18, CD19, CD20, CD21, CD22, CD23, CD25, CD27L, CD28, CD29, CD30, CD30L, CD32, CD33 (p67 proteins), CD34, CD38, CD40, CD40L, CD44, CD45, CD46, CD49a, CD52, CD54, CD55, CD56, CD61, CD64, CD66e, CD74, CD80 (B7-1), CD89, CD95, CD123, CD137, CD138, CD140a, CD146, CD147, CD148, CD152, CD164, CEACAM5, CFTR, cGMP, CINC, Clostridium botulinum toxin, Clostridium perfringens toxin, CKb8-l, CLC, CMV, CMV UL, CNTF, CNTN-1, COX, C-Ret, CRG-2, CT-1, CTACK, CTGF, CTLA-4, CX3CL1, CX3CR1, CXCL, CXCLl, CXCL2, CXCL3, CXCL4, CXCL5, CXCL6, CXCL7, CXCL8, CXCL9, CXCL10, CXCL11, CXCL12, CXCL13, CXCL14, CXCL15, CXCL16, CXCR, CXCR1, CXCR2, CXCR3, CXCR4, CXCR5, CXCR6, cytokeratin tumor-associated antigen, DAN, DCC, DcR3, DC-SIGN, Decay accelerating factor, des(l-3)-IGF-I (brain IGF-1), Dhh, digoxin, DNAM-1, Dnase, Dpp, DPPIV/CD26, Dtk, ECAD, EDA, EDA-A1, EDA-A2, EDAR, EGF, EGFR (ErbB-1), EMA, EMMPRIN, EN A, endothelin receptor, Enkephalinase, eNOS, Eot, eotaxinl, EpCAM, Ephrin B2/ EphB4, EPO, ERCC, E-selectin, ET-1, Factor Ila, Factor VII, Factor VIIIc, Factor IX, fibroblast activation protein (FAP), Fas, FcRl, FEN-1, Ferritin, FGF, FGF-19, FGF-2, FGF3, FGF-8, FGFR, FGFR-3, Fibrin, FL, FLIP, Flt-3, Flt-4, Follicle stimulating hormone, Fractalkine, FZD1, FZD2, FZD3, FZD4, FZD5, FZD6, FZD7, FZD8, FZD9, FZD10, G250, Gas 6, GCP-2, GCSF, GD2, GD3, GDF, GDF-1, GDF-3 (Vgr-2), GDF-5 (BMP-14, CDMP- 1), GDF-6 (BMP-13, CDMP-2), GDF-7 (BMP-12, CDMP-3), GDF-8 (Myostatin), GDF-9, GDF- 15 (MIC-1), GDNF, GDNF, GFAP, GFRa-1, GFR-alphal, GFR-alpha2, GFR-alpha3, GITR, Glucagon, Glut 4, glycoprotein Ilb/IIIa (GP Ilb/IIIa), GM-CSF, gpl30, gp72, GRO, Growth hormone releasing factor, Hapten (NP-cap or NIP-cap),

HB-EGF, HCC, HCMV gB envelope glycoprotein, HCMV) gH envelope glycoprotein, HCMV UL, Hemopoietic growth factor (HGF), Hep B gpl20, heparanase, Her2, Her2/neu (ErbB-2), Her3 (ErbB-3), Her4 (ErbB-4), herpes simplex virus (HSV) gB glycoprotein, HSV gD glycoprotein, HGFA, High molecular weight melanoma-associated antigen (HMW-MAA), HIV gpl20, HIV IIIB gp 120 V3 loop, HLA, HLA-DR, HM1.24, HMFG PEM, HRG, Hrk, human cardiac myosin, human cytomegalovirus (HCMV), human growth hormone (HGH), HVEM, 1-309, IAP, ICAM, ICAM-1, ICAM-3, ICE, ICOS, IFNg, Ig, IgA receptor, IgE, IGF, IGF binding proteins, IGF-1R, IGFBP, IGF-I, IGF-II, IL, IL-1, IL-1R, IL-2, IL-2R, IL-4, IL-4R, IL-5, IL-5R, IL-6, IL-6R, IL-8, IL- 9, IL-10, IL-12, IL-13, IL-15, IL-18, IL-18R, IL-23, interferon (INF)-alpha, INF-beta, INF- gamma, Inhibin, iNOS, Insulin A-chain, Insulin B-chain, Insulin-like growth factor 1, integrin alpha2, integrin alpha3, integrin alpha4, integrin alpha4/betal, integrin, alpha4/beta7, integrin alpha5 (alphaV), integrin alpha5/betal, integrin alpha5/beta3, integrin alpha6, integrin betal, integrin beta2, interferon gamma, IP-10, 1-TAC, JE, Kallikrein 2, Kallikrein 5, Kallikrein 6, , Kallikrein 11, Kallikrein 12, Kallikrein 14, Kallikrein 15, Kallikrein LI, Kallikrein L2, Kallikrein L3, Kallikrein L4, KC, KDR, Keratinocyte Growth Factor (KGF), laminin 5, LAMP, LAP, LAP (TGF- 1), Latent TGF-1, Latent TGF-1 bpl, LBP, LDGF, LECT2, Lefty, Lewis-Y antigen, Lewis-Y related antigen, LFA-1, LFA-3, Lfo, LIF, LIGHT, lipoproteins, LIX, LKN, Lptn, L-Selectin, LT-a, LT-b, LTB4, LTBP-1, Lung surfactant, Luteinizing hormone, Lymphotoxin Beta Receptor, Mac-1, MAdCAM, MAG, MAP2, MARC, MCAM, MCAM, MCK-2, MCP, M-CSF, MDC, Mer, METALLOPROTEASES, MGDF receptor, MGMT, MHC (HLA-DR), MIF, MIG, MIP, MIP-1-alpha, MK, MMAC1, MMP, MMP-1, MMP-10, MMP-11, MMP-12, MMP-13, MMP-14, MMP-15, MMP-2, MMP-24, MMP- 3, MMP-7, MMP-8, MMP-9, MPIF, Mpo, MSK, MSP, mucin (Mucl), MUC18, Muellerian- inhibitin substance, Mug, MuSK, NAIP, NAP, NCAD, N-Cadherin, NCA 90, NCAM, NCAM, Neprilysin, Neurotrophin-3,-4, or -6, Neurturin, Neuronal growth factor (NGF), NGFR, NGF-beta, nNOS, NO, NOS, Npn, NRG-3, NT, NTN, OB, OGG1, OPG, OPN, OSM, OX40L, OX40R, pl50, p95, PADPr, Parathyroid hormone, PARC, PARP, PBR, PBSF, PCAD, P-Cadherin, PCNA, PDGF, PDGF, PDK-1, PECAM, PEM, PF4, PGE, PGF, PGI2, PGJ2, PIN, PLA2, placental alkaline phosphatase (PLAP), P1GF, PLP, PP14, Proinsulin, Prorelaxin, Protein C, PS, PSA, PSCA, prostate specific membrane antigen (PSMA), PTEN, PTHrp, Ptk, PTN, R51, RANK, RANKL, RANTES, RANTES, Relaxin A-chain, Relaxin B-chain, renin, respiratory syncytial virus (RSV) F, RSV Fgp, Ret, Rheumatoid factors, RLIP76, RPA2, RSK, S100, SCF/KL, SDF-1,

SERINE, Serum albumin, sFRP-3, Shh, SIGIRR, SK-1, SLAM, SLPI, SMAC, SMDF, SMOH, SOD, SPARC, Stat, STEAP, STEAP-II, TACE, TACI, TAG-72 (tumor- associated glycoprotein-72), TARC, TCA-3, T-cell receptors (e.g., T-cell receptor alpha/beta), TdT, TECK, TEM1, TEM5, TEM7, TEM8, TERT, testicular PLAP-like alkaline phosphatase, TfR, TGF, TGF-alpha, TGF-beta, TGF-beta Pan Specific, TGF-beta RI (ALK-5), TGF-beta RII, TGF-beta Rllb, TGF-beta RIII, TGF-betal, TGF-beta2, TGF-beta3, TGF-beta4, TGF-beta5, Thrombin, Thymus Ck-1, Thyroid stimulating hormone, Tie, TIMP, TIQ, Tissue Factor, TMEFF2, Tmpo, TMPRSS2, TNF, TNF-alpha, TNF-alpha beta, TNF-beta2, TNFc, TNF-RI, TNF-RII, TNFRSF10A (TRAIL Rl Apo-2, DR4), TNFRSFIOB (TRAIL R2 DR5, KILLER, TRICK-2A, TRICK-B), TNFRSF10C (TRAIL R3 DcRl, LIT, TRID), TNFRSF10D (TRAIL R4 DcR2, TRUNDD), TNFRSF11A (RANK ODF R, TRANCE R), TNFRSFllB (OPG OCIF, TR1), TNFRSF12 (TWEAK R FN14), TNFRSF13B (TACI), TNFRSF13C (BAFF R), TNFRSF14 (HVEM ATAR, HveA, LIGHT R, TR2), TNFRSF16 (NGFR p75NTR), TNFRSF17 (BCMA), TNFRSF18 (GITR AITR), TNFRSF19 (TROY TAJ, TRADE), TNFRSF19L (RELT), TNFRSFIA (TNF RI CD120a, p55-60), TNFRSFIB (TNF RII CD120b, p75-80), TNFRSF26 (TNFRH3), TNFRSF3 (LTbR TNF RIII, TNFC R), TNFRSF4 (OX40 ACT35, TXGP1 R), TNFRSF5 (CD40 p50), TNFRSF6 (Fas Apo-1, APT1, CD95), TNFRSF6B (DcR3 M68, TR6), TNFRSF7 (CD27), TNFRSF8 (CD30), TNFRSF9 (4-lBB CD137, ILA), TNFRSF21 (DR6), TNFRSF22 (DcTRAIL R2 TNFRH2), TNFRST23 (DcTRAIL Rl TNFRH1), TNFRSF25 (DR3 Apo-3, LARD, TR-3, TRAMP, WSL-1), TNFSF10 (TRAIL Apo-2 Ligand, TL2), TNFSF11 (TRANCE/RANK Ligand ODF, OPG Ligand), TNFSF12 (TWEAK Apo-3 Ligand, DR3 Ligand), TNFSF13 (APRIL TALL2), TNFSF13B (BAFF BLYS, TALL1, THANK, TNFSF20), TNFSF14 (LIGHT HVEM Ligand, LTg), TNFSF15 (TL1A/VEGI), TNFSF18 (GITR Ligand AITR Ligand, TL6), TNFSFIA (TNF-a Conectin, DIF, TNFSF2), TNFSF1B (TNF-b LTa, TNFSF1), TNFSF3 (LTb TNFC, p33), TNFSF4 (OX40 Ligand gp34, TXGP1), TNFSF5 (CD40 Ligand CD154, gp39, HIGM1, IMD3, TRAP), TNFSF6 (Fas Ligand Apo-1 Ligand, APT1 Ligand), TNFSF7 (CD27 Ligand CD70), TNFSF8 (CD30 Ligand CD153), TNFSF9 (4-lBB Ligand CD137 Ligand), TP-1, t-PA, Tpo, TRAIL, TRAIL R, TRAIL-R1, TRAIL-R2, TRANCE, transferring receptor, TRF, Trk, TROP-2, TSG, TSLP, tumor-associated antigen CA 125, tumor-associated antigen expressing Lewis Y related carbohydrate, TWEAK, TXB2, Ung, uPAR, uPAR-1, Urokinase, VCAM, VCAM-1, VECAD, VE-Cadherin, VE-cadherin-2, VEFGR-1 (flt-1), VEGF, VEGFR, VEGFR-3 (flt-4), VEGI, VIM, Viral antigens, VLA, VLA-1, VLA-4,

VNR integrin, von Willebrands factor, WIF- 1, WNT1, WNT2, WNT2B/13, WNT3, WNT3A, WNT4, WNT5A, WNT5B, WNT6, WNT7A, WNT7B, WNT8A, WNT8B, WNT9A, WNT9A, WNT9B, WNT10A, WNT10B, WNT11, WNT16, XCL1, XCL2, XCR1, XCR1, XEDAR, XIAP, XPD, CTLA4 (cytotoxic T lymphocyte antigen-4), PD1 (programmed cell death protein 1), PD-L1 (programmed cell death ligand 1), LAG-3 (lymphocyte activation gene-3), TIM-3 (T cell immunoglobulin and mucin protein-3), receptors for hormones, and growth factors.

[0049] Multispecifics comprising anti-CD3 antibodies and antigen-binding fragments disclosed herein may be prepared according to a variety of techniques including, but not limited to, recombinant co-expression of two immunoglobulin heavy chain-light chain pairs having different specificities (*see* Milstein and Cuello, Nature 305: 537 (1983)), WO 93/08829, and Traunecker et al., EMBO J. 10: 3655 (1991)) "knob-in-hole" engineering (*see*, e.g., U.S. Pat. No. 5,731,168); immunoglobulin crossover (also known as Fab domain exchange or CrossMab format) technology (*see* e.g., WO2009/080253; Schaefer et al., Proc. Natl. Acad. Sci. USA, 108:11187-11192 (2011)); engineering electrostatic steering effects for antibody Fc-heterodimeric molecules (WO 2009/089004A1); cross-linking two or more antibodies or fragments (*see*, e.g., U.S. Pat. No. 4,676,980, and Brennan et al., Science, 229: 81 (1985)); leucine zippers (*see*, e.g., Kostelny et al., J. Immunol, 148(5):1547-1553 (1992)); "diabody" technology (*see*, e.g., Hollinger et al., Proc. Natl. Acad. Sci. USA, 90:6444-6448 (1993)); single-chain Fv (sFv) dimers (*see*, e.g. Gruber et al., J. ImmunoL, 152:5368 (1994)); and trispecific antibodies as described, e.g., in Tutt et al. J. ImmunoL 147: 60 (1991).

[0050] The present disclosure also contemplates modification of anti-CD3 antibodies disclosed herein, such modifications comprising one or more amino acid substitutions, insertions and/or deletions in the FR and/or CDR regions of the heavy and light chain variable domains. Once obtained, such derivative antibodies and/or antigen-binding fragments can be tested for one or more desired properties, such as improved binding specificity, increased binding affinity, improved developability, etc.

[0051] In some embodiments, anti-CD3 antibodies and/or antigen-binding fragments thereof comprise a heavy chain (HC) sequence, light chain (LC) sequence, CDRH3 sequence, CDRH2 sequence, CHRH1 sequence, CDRL3 sequence, CDRL2 sequence, CDRL1 sequence, and/or framework sequence. In some embodiments, anti-CD3 antibodies and/or antigen-binding fragments thereof have amino acid sequence identity to corresponding

sequences of anti-CD3 antibodies disclosed in Table 4 (Ab1-Ab50) by at least about 100%, at least about 99%, at least about 98%, at least about 97%, at least about 96%, at least about 95%, at least about 94%, at least about 93%, at least about 92%, at least about 91%, at least about 90%, at least about 89%, at least about 88%, at least about 87%, at least about 86%, at about 85%, at least about 84%, at least about 83%, at least about 82%, at least about 80%; and/or all percentages of identity in between. In some embodiments, percent identity is measured by any well-known algorithm of sequence identity, such as FASTA, BLAST or GAP.

[0052] In some embodiments, residue positions that are not identical differ by conservative amino acid substitutions. A "conservative amino acid substitution" is one in which an amino acid residue is substituted by another amino acid residue having a side chain (R group) with similar chemical properties (e.g., charge or hydrophobicity). In general, a conservative amino acid substitution will not substantially change the functional properties of a protein. In cases where two or more amino acid sequences differ from each other by conservative substitutions, the percent or degree of similarity may be adjusted upwards to correct for the conservative nature of the substitution. Means for making this adjustment are well known to those of skill in the art. (*See*, e.g., Pearson (1994) Methods Mol. Biol. 24: 307-331). Examples of groups of amino acids that have side chains with similar chemical properties include 1) aliphatic side chains: glycine, alanine, valine, leucine and isoleucine; 2) aliphatic- hydroxyl side chains: serine and threonine; 3) amide-containing side chains: asparagine and glutamine; 4) aromatic side chains: phenylalanine, tyrosine, and tryptophan; 5) basic side chains: lysine, arginine, and histidine; 6) acidic side chains: aspartate and glutamate, and 7) sulfur-containing side chains: cysteine and methionine. In some embodiments, conservative amino acids substitution groups are: valine-leucine-isoleucine, phenylalanine-tyrosine, lysine-arginine, alanine-valine, glutamate-aspartate, and asparagine-glutamine. Alternatively, in some embodiments, a conservative replacement comprises any change having a positive value in the PAM250 log-likelihood matrix disclosed in Gonnet et al. (1992) Science 256: 1443 45. In some embodiments, a "moderately conservative" replacement comprises any change having a nonnegative value in a PAM250 log-likelihood matrix.

[0053] Substitution of one or more CDR residues or omission of one or more CDRs is also possible. Antibodies have been described in which one or two CDRs can be dispensed to

alter binding in the scientific literature. Padlan et al. (1995 FASEB J. 9:133-139) analyzed contact regions between antibodies and their antigens based on published crystal structures, and concluded that only about one fifth to one third of CDR residues actually contact their associated antigen. Padlan also found many antibodies in which one or two CDRs had zero amino acids in contact with an antigen (*see* also, Vajdos et al. 2002 *J Mol Biol* 320:415-428). CDR residues not contacting an antigen can be identified based on previous studies (for example residues H60-H65 in CDRH2 are often not required), from regions of Kabat CDRs lying outside Chothia CDRs, by molecular modeling and/or empirically. If a CDR or residue(s) thereof is omitted, it is usually substituted with an amino acid occupying the corresponding position in another human antibody sequence or a consensus of such sequences. Positions for substitution within CDRs and amino acids to substitute can also be selected empirically.

[0054] In certain embodiments, substitutions, insertions, or deletions may occur within one or more CDRs of anti-CD3 antibodies described herein so long as such alterations do not substantially reduce the ability of the antibody to bind its antigen. For example, conservative alterations (e.g., conservative substitutions as provided herein) that do not substantially reduce binding affinity may be made in CDRs. Such alterations may, for example, be outside of antigen contacting residues in the CDRs. In certain embodiments of the variant VH and VL sequences provided above, each CDR either is unaltered, or contains no more than one, two or three amino acid substitutions.

[0055] A useful method for identification of residues or regions of an antibody that may be targeted for mutagenesis is called "alanine scanning mutagenesis" as described by Cunningham and Wells (1989) Science, 244:1081-1085. In this method, a residue or group of target residues (e.g., charged residues such as arg, asp, his, lys, and glu) are identified and replaced by a neutral or negatively charged amino acid (e.g., alanine or polyalanine) to determine whether the interaction of the antibody with antigen is affected. Further substitutions may be introduced at the amino acid locations demonstrating functional sensitivity to the initial substitutions. Alternatively, or additionally, a crystal structure of an antigen-antibody complex to identify contact points between the antibody and antigen. Such contact residues and neighboring residues may be targeted or eliminated as candidates for substitution. Variants may be screened to determine whether they contain the desired properties.

[0056] Amino acid sequence insertions include amino- and/or carboxyl-terminal fusions ranging in length from one residue to polypeptides containing a hundred or more residues, as well as intrasequence insertions of single or multiple amino acid residues. Examples of terminal insertions include an antibody with an N-terminal methionyl residue. Other insertional variants of an antibody molecule include fusion to the N- or C-terminus of the antibody to an enzyme (e.g. for ADEPT) or a polypeptide which increases serum half-life of the antibody.

[0057] As described throughout, anti-CD3 antibodies and/or antigen-binding fragments thereof as provided herein possess favorable developability and are, thus, relatively developable.

[0058] The term "developable" refers to the extent to which one or more polypeptides in a plurality of polypeptides possess desirable characteristics, such as, e.g., desirable expression, for example, in mammalian cells; solubility; viscosity; aggregation; chemical and/or physical stability; desirable shelf-life; melting temperature; pharmacokinetic profiles; circulation half-life; and clearance characteristics. Such characteristics may serve as indicia, independently, as combinations of sub-sets of such indicia, or in totality, for the likelihood that such one or more polypeptides may be successfully developed as a therapeutic candidate, and ultimately an approved drug. Accordingly, as understood in the art, generally, polypeptides with desirable developability characteristics possess, e.g., relatively high solubility, relatively low viscosity, relatively low propensity for aggregation, relatively high chemical stability, relatively high physical stability, relatively long shelf life, relatively high melting temperature, relatively long circulation half-life, relatively long clearance time, and the like. Polypeptides with undesirable developability characteristics possess, e.g., relatively low solubility, relatively high viscosity, relatively high propensity for aggregation, relatively poor chemical stability, relatively poor physical stability, relatively short shelf life, relatively low melting temperature, relatively short circulation half-life, relatively short clearance time, and the like.

[0059] Methods and assays that may be employed to ascertain the degree to which polypeptides, such as anti-CD3 antibodies and/or antigen-binding fragments thereof as described herein, possess desirable developability characteristics are available in the art, and include, for example; PSR assays (WO 2014/179363 and Xu et al., *Protein Eng Des Sol*, Vol. 26, pages 663-670 (2013)); SMP and SCP assays and the like; cross interaction

chromatography (CIC); self-interaction chromatography (SIC); dynamic light scattering; size exclusion chromatography (SEC), dynamic light scattering (DLS) spectroscopy; photon correlation spectroscopy; quasi-elastic light scattering, circular dichroism (CD), viscosity measurements; whole cell binding; tissue micro array methodologies; BVP ELISA assays; AC-SINS assays (Liu et al; *MAbs*, Vol. 6, pages 483-492 (2014); differential scanning calorimetry; and the like (*see*, e.g., He et al., *J. Pharm. Sci.*, Vol. 100(4), pp. 1330-1340 (2011); Wagner et al., *Pharm. Develop. & Technol* (posted online 2012; hyper-text transfer protocol: informahealthcare.com/doi/abs/10.3109/10837450.2011.649851); Hotzel et al., *mAbs*, Vol. 4(6), pages 753-7601 (2012); Weiqiang et al., *J. Pharm. Sci.*, Vol. 101(5), pp. 1701-1720 (2012); Banks et al., *J. Pharm. Sci.*, Vol. 101(8), pp. 2720-2732 (2012); Lie et al., *J. Pharm. Sci.*, Vol. 94(9), pp. 1928-1948 (2005); and Payne et al., *Biopolymers*, Vol. 85(5), pp. 527-533 (2006)).

[0060] In some embodiments, antibodies that are identified as possessing decreased developability are so detected by virtue of their interaction with a polyspecificity reagent ("PSR") and, as such, are referred to as "polyspecific" polypeptides. Such polyspecific antibodies may be referred to as relatively "undevelopable" or relatively "non-developable".

[0061] A "developability profile" refers to an index that may be assigned to antibodies upon assessing their developability. A developability profile is a measure or metric by which developability of anti-CD3 antibodies may be assessed, compared, and/or ranked. Such developability profiles serve as a measure of the degree of interaction of CD3 binders and antibodies comprising them. The degree of interaction may be assessed by any number of means available in the art that provides an output value that correlates with a strength or affinity of a polypeptide for a moiety to which it is bound. Exemplary means include flow cytometry means, such as FACS; ELISA; quantitative immunoaffinity assays or immunoprecipitation assays; mammalian two-hybrid or yeast two-hybrid assays, and the like. In the context of FACS, as demonstrated in the Examples, a degree of interaction between polypeptides in the plurality and the PSR may be ascertained by generating a mean fluorescence intensity (MFI) for each polypeptide-PSR interaction that is detected, and then ordering the MFI in either ascending or descending order, thereby ranking the polypeptides in the plurality according to the relative degree of interaction between each detected polypeptide and the PSR. Such a ranking provides for a ranking of polypeptides of the plurality such that

those polypeptides possessing enhanced developability are readily ascertained, as are those polypeptides possessing decreased developability.

[0062] A developability profile may also take the form of a normalized score, for example, by normalizing developability of anti-CD3 antibodies described herein to the developability of a standard (or control) antibody, e.g., anti-HEL antibody.

[0063] In certain embodiments, inventive CD3 binding domains and antibodies comprising them may be further modified to contain additional nonproteinaceous moieties that are known in the art and are readily available. Moieties suitable for derivatization of an antibody include but are not limited to water soluble polymers. Non-limiting examples of water soluble polymers include, but are not limited to, polyethylene glycol (PEG), copolymers of ethylene glycol/propylene glycol, carboxymethylcellulose, dextran, polyvinyl alcohol, polyvinyl pyrrolidone, poly-1,3-dioxolane, poly-1,3,6-trioxane, ethylene/maleic anhydride copolymer, polyaminoacids (either homopolymers or random copolymers), and dextran or poly(n-vinyl pyrrolidone)polyethylene glycol, polypropylene glycol homopolymers, polypropylene oxide/ethylene oxide co-polymers, polyoxyethylated polyols (e.g., glycerol), polyvinyl alcohol, and mixtures thereof. Polyethylene glycol propionaldehyde may have advantages in manufacturing due to its stability in water. The polymer may be of any molecular weight and may be branched or unbranched. The number of polymers attached to the antibody may vary, and if more than one polymer is attached, they can be the same or different molecules. In general, the number and/or type of polymers used for derivatization can be determined based on considerations including, but not limited to, the particular properties or functions of the antibody to be improved, whether the antibody derivative will be used in a therapy under defined conditions, etc.

[0064] In certain embodiments, the inventive CD3 binding domains and antibodies comprising them are provided which display an enhanced developability profile. The developability profile for anti-CD3 antibodies is obtained by performing one or more of a PSR assay; an SCP assay; AC-SINS; an ELISA; a DSF assay; a Tm assay; a HIC assay; a CIC assay; or combinations thereof.

[0065] In other embodiments, anti-CD3 antibodies and/or antigen-binding fragments thereof as described herein display a poly-specificity reagent (PSR) score of between about 0.0 and about 0.45; between about 0.0 and about 0.4; between about 0.0 and about 0.35; between about 0.0 and about 0.3; between about 0.0 and about 0.25; between about 0.0 and

about 0.2; between about 0.0 and about 0.15; or between about 0.0 and about 0.1. A score of 0.0-0.1 is "clean PSR". A score of .1 to .33 is "low PSR". A score of 0.33 to 0.66 is "medium PSR". A score of 0.66-1.00 is "high PSR". A high PSR score is indicative of decreased (or poor) developability. Generally, the lower the PSR score the more favorable the developability of the antibody.

[0066] In still other embodiments, anti-CD3 antibodies and/or antigen-binding fragments therof as described herein display a HIC score of less than about 10.5 minutes (a clean to low HIC score); between about 10.5 minutes and 11.5 minutes (a medium HIC score); or greater than about 11.5 minutes (a high HIC score). Generally, the lower the HIC score the more favorable the developability of the antibody.

[0067] In yet other embodiments, anti-CD3 antibodies and/or antigen-binding fragments thereof as described herein display an SEC score of less than about 95%, which indicates that the antibody is a monomer, i.e., not aggregating.

[0068] In still other embodiments, anti-CD3 antibodies and/or antigen-binding fragments thereof as described herein display a Tm of less than about 65°C.

[0069] In some embodiments, anti-CD3 antibodies and/or antigen-binding fragments thereof as described herein may be further modified to minimize effector function, e.g., a silent Fc.

[0070] "Effector function" refers to biological activities attributable to the Fc region of an antibody, which varies by antibody isotype. Exemplary effector functions include: C1q binding and complement dependent cytotoxicity (CDC); Fc receptor binding; antibody-dependent cell-mediated cytotoxicity (ADCC); phagocytosis; down regulation of cell surface receptors (e.g., B cell receptor); and B cell activation.

[0071] "Fc region" is a C-terminal region of an immunoglobulin heavy chain that contains at least a portion of the constant region, including native sequence Fc regions and variant Fc regions. A human IgG heavy chain Fc region can extend from Cys226, or from Pro230, to the carboxyl-terminus of the heavy chain. However, the C-terminal lysine (Lys447) of the Fc region may or may not be present. Unless otherwise specified herein, numbering of amino acid residues in the Fc region or constant region is according to the EU numbering system, also called the EU index, as described in Kabat et al., Sequences of

Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md., 1991.

[0072] In certain embodiments, one or more amino acid modifications may be introduced into the Fc region of an anti-CD3 antibody of the disclosure, thereby generating an Fc region variant (*see* e.g., US 2012/0251531). An Fc region variant may comprise a human Fc region sequence (e.g., a human IgG1, IgG2, IgG3 or IgG4 Fc region) comprising an amino acid modification (e.g., a substitution) at one or more amino acid positions.

[0073] In certain embodiments, the disclosure contemplates an anti-CD3 antibody variant that possesses some but not all effector functions, which make it a desirable candidate for applications in which the half-life of an antibody *in vivo* is important, yet certain effector functions (such as complement and ADCC) are unnecessary or deleterious. *In vitro* and/or *in vivo* cytotoxicity assays can be conducted to confirm reduction/depletion of CDC and/or ADCC activities. For example, Fc receptor (FcR) binding assays can be conducted to ensure that an antibody lacks FcγR binding (hence likely lacking ADCC activity) but retains FcRn binding ability. The primary cells for mediating ADCC (e.g. NK cells) express FcγRIII only, whereas monocytes express FcγRI, FcγRII and FcγRIII. FcR expression on hematopoietic cells is summarized in Table 3 on page 464 of Ravetch and Kinet, Annu. Rev. Immunol. 9:457-492 (1991). Non-limiting examples of *in vitro* assays to assess ADCC activity of a molecule of interest is described in U.S. Pat. No. 5,500,362 (*see*, e.g. Hellstrom, I. et al. Proc. Nat'l Acad. Sci. USA 83:7059-7063 (1986)) and Hellstrom, I et al., Proc. Nat'l Acad. Sci. USA 82:1499-1502 (1985); U.S. Pat. No. 5,821,337 (*see* Bruggemann, M. et al., J. Exp. Med. 166:1351-1361 (1987)). Alternatively, non-radioactive assay methods may be employed (*see*, for example, ACTI™ non-radioactive cytotoxicity assay for flow cytometry (Cell Technology, Inc. Mountain View, Calif.); and CytoTox 96® non-radioactive cytotoxicity assay (Promega, Madison, Wis.)). Useful effector cells for such assays include peripheral blood mononuclear cells (PBMC) and Natural Killer (NK) cells. Alternatively, or additionally, ADCC activity of the molecule of interest may be assessed *in vivo*, e.g., in an animal model such as that disclosed in Clynes et al. Proc. Nat'l Acad. Sci. USA 95:652-656 (1998). C1q binding assays may also be carried out to confirm that an antibody is unable to bind C1q and hence lacks CDC activity. *See*, e.g., C1q and C3c binding ELISA in WO 2006/029879 and WO 2005/100402. To assess complement activation, a CDC assay may be performed (*see*, for example, Gazzano-Santoro et al. J. ImmunoL Methods 202:163 (1996);

Cragg, M. S. et al. Blood. 101:1045-1052 (2003); and Cragg, M. S. and M. J. Glennie Blood. 103:2738-2743 (2004)). FcRn binding and *in vivo* clearance/half-life determinations can also be performed using methods known in the art (*see*, e.g., Petkova, S. B. et al. Int'l. ImmunoL 18(12):1759-1769 (2006)).

[0074] In some embodiments, antibodies with reduced effector function include those with substitution of one or more of Fc region residues 238, 265, 269, 270, 297, 327 and 329 (U.S. Pat. Nos. 6,737,056 and 8,219,149). In some embodiments, Fc mutants include Fc mutants with substitutions at two or more of amino acid positions 265, 269, 270, 297 and 327, including the so-called "DANA" Fc mutant with substitution of residues 265 and 297 to alanine (U.S. Pat. Nos. 7,332,581 and 8,219,149).

[0075] In other embodiments, anti-CD3 antibodies and/or antigen-binding fragments thereof as described herein are further modified to include a masking agent, e.g., a polypeptide mask, attached via a cleavable linker.

[0076] In some embodiments, anti-CD3 antibodies and/or antigen-binding fragments thereof as described herein are conjugated to a therapeutic moiety thereby forming an immunoconjugate. An "immunoconjugate" is an antibody conjugated to one or more heterologous molecule(s) such as, e.g., an antibiotic, a second anti-CD3 antibody, a vaccine, or a toxoid, or any other therapeutic moiety.

[0077] In certain embodiments, anti-CD3 antibodies and/or antigen-binding fragments thereof as described herein are altered to increase or decrease the extent to which the antibody is glycosylated. Addition or deletion of glycosylation sites to an anti-CD3 antibody of the disclosure may be conveniently accomplished by altering the amino acid sequence such that one or more glycosylation sites is created or removed.

[0078] Moreover, the present disclosure generally relates to an anti-CD3 antibody or antibody fragment, wherein said anti-CD3 antibody or antigen-binding antibody fragment comprises: a. a variable heavy (VH) chain polypeptide comprising: i. a V_H CDR1 (CDRH1) of FNIKDYYMH (SEQ ID NO: 13); ii. a V_H CDR2 (CDRH2) of WIDLENANTVYDAKFQG (SEQ ID NO: 15); iii. a V_H CDR3 (CDRH3) of ARDAYGRYFYDV (SEQ ID NO: 103); and/or b. a variable light (V_L) chain polypeptide comprising: i. a V_L CDR1 (CDRL1) of KSSQSLLNARTGKNYLA (SEQ ID NO: 8); a V_L CDR2 (CDRL2) of WASTRX_1S (SEQ ID NO: **133**); and a V_L CDR3 (CDRL3) of

X_2QSYFRRT (SEQ ID NO: **134**); wherein X_1 and X_2 are each independently any amino acid. In some embodiments, X_1 is E or S. In some embodiments, X_2 is K or V. In some embodiments, said antibody or antigen-binding antibody fragment comprises a CDRL2 of SEQ ID NO: 10 or SEQ ID NO: 11. In some embodiments, said antibody or antigen-binding antibody fragment comprises a CDRL3 of SEQ ID NO: 5 or SEQ ID NO: 4.

[0079] In some embodiments, said antibody or antigen-binding antibody fragment comprises a V_H chain CDR1 (CDRH1) of FNIKDYYMH (SEQ ID NO: 13), a V_H chain CDR2 (CDRH2) of WIDLENANTVYDAKFQG (SEQ ID NO: 15), and a V_H chain CDR3 (CDRH3) of ARDAYGRYFYDV (SEQ ID NO: 103); and/or a V_L chain CDR1 (CDRL1) of KSSQSLLNARTGKNYLA (SEQ ID NO: 8), a V_L chain CDR2 (CDRL2) of WASTRES (SEQ ID NO: 10), and a V_L chain CDR3 (CDRL3) of KQSYFRRT (SEQ ID NO: 5). In some embodiments, said antibody or antigen-binding antibody fragment comprises a V_H chain CDR1 (CDRH1) of FNIKDYYMH (SEQ ID NO: 13), a V_H chain CDR2 (CDRH2) of WIDLENANTVYDAKFQG (SEQ ID NO: 15), and a V_H chain CDR3 (CDRH3) of ARDAYGRYFYDV (SEQ ID NO: 103); and/or a V_L chain CDR1 (CDRL1) of KSSQSLLNARTGKNYLA (SEQ ID NO: 8), a V_L chain CDR2 (CDRL2) of WASTRSS (SEQ ID NO: 11), and a V_L chain CDR3 (CDRL3) of VQSYFRRT (SEQ ID NO: 4). In some embodiments, the anti-CD3 antibody may comprise any one or more CDRs of the antibody referred to as Ab1 and/or any one or more CDRs of the antibody referred to as Ab13. In some embodiments the anti-CD3 antibody may bind to the same or a substantially similar epitope or epitopes of CD3 as Ab1 and/or Ab13.

[0080] In some embodiments, said anti-CD3 antibody or antigen-binding antibody fragment comprises a V_L chain polypeptide comprising an amino acid sequence that possesses at least 90% sequence identity to SEQ ID NO: 18 or SEQ ID NO: 34. In some embodiments, said anti-CD3 antibody or antigen-binding antibody fragment comprises a V_L chain polypeptide comprising an amino acid sequence that possesses at least 95% sequence identity to SEQ ID NO: 18 or SEQ ID NO: 34. In some embodiments, said anti-CD3 antibody or antigen-binding antibody fragment comprises a V_L chain polypeptide comprising the amino acid sequence of SEQ ID NO: 18 or SEQ ID NO: 34. In some embodiments, said anti-CD3 antibody or antigen-binding antibody fragment comprises a V_H chain polypeptide comprising an amino acid sequence that possesses at least 90% sequence identity to SEQ ID NO: 17. In some embodiments, said anti-CD3 antibody or antigen-binding antibody fragment

comprises a V_H chain polypeptide comprising an amino acid sequence that possesses at least 95% sequence identity to SEQ ID NO: 17. In some embodiments, said anti-CD3 antibody or antigen-binding antibody fragment comprises a V_H chain comprising the amino acid sequence of SEQ ID NO: 17.

[0081] In some embodiments, said anti-CD3 antibody or antigen-binding antibody fragment comprises a V_L chain polypeptide comprising an amino acid sequence that possesses at least 90% sequence identity to SEQ ID NO: 18 or SEQ ID NO: 34; and a V_H chain polypeptide comprising an amino acid sequence that possesses at least 90% sequence identity to SEQ ID NO: 17. In some embodiments, said anti-CD3 antibody or antigen-binding antibody fragment comprises a V_L chain polypeptide comprising an amino acid sequence that possesses at least 95% sequence identity to SEQ ID NO: 18 or SEQ ID NO: 34; and a V_H chain polypeptide comprising an amino acid sequence that possesses at least 95% sequence identity to SEQ ID NO: 17. In some embodiments, said anti-CD3 antibody or antigen-binding antibody fragment comprises a V_L chain polypeptide comprising the amino acid sequence of SEQ ID NO: 18 or SEQ ID NO: 34; and a V_H chain comprising the amino acid sequence of SEQ ID NO: 17. In some embodiments, said anti-CD3 antibody or antigen-binding antibody fragment comprises a V_L chain polypeptide comprising the amino acid sequence of SEQ ID NO: 18; and a V_H chain comprising the amino acid sequence of SEQ ID NO: 17. In some embodiments, said anti-CD3 antibody or antigen-binding antibody fragment comprises a V_L chain polypeptide comprising the amino acid sequence of SEQ ID NO: 34; and a V_H chain comprising the amino acid sequence of SEQ ID NO: 17.

[0082] In some embodiments, said anti-CD3 antibody or antigen-binding antibody fragment binds to CD3 with a dissociation constant (K_D) of about 500 pM or less, about 470 pM or less, about 450 pM or less, about 400 pM or less, about 350 pM or less, about 300 pM or less, about 250 pM or less, about 200 pM or less, about 150 pM or less, or about 100 pM or less, optionally wherein said CD3 is human and/or cynomolgus, further optionally wherein said binding affinity is measured by surface plasmon resonance. In some embodiments, said K_D is a monovalent K_D and/or wherein said K_D is measured using an scFv fragment of said anti-CD3 antibody or antibody fragment.

[0083] Moreover, the present disclosure generally relates to an anti-cluster of differentiation three ("CD3") antibody or antibody fragment, wherein said anti-CD3 antibody or antigen-binding antibody fragment comprises one or more CDRs of any one or more of

Ab1-Ab50 and/or binds to the same epitope(s) as any one or more of Ab1-Ab50. Furthermore, the present disclosure generally relates to an anti-cluster of differentiation three ("CD3") antibody or antibody fragment, wherein said anti-CD3 antibody or antigen-binding antibody fragment comprises one or more V_H and/or V_L chains comprising an amino acid sequence selected from those in Table 4.

Production of the anti-CD3 antibodies and antigen-binding fragments thereof

[0084] Anti-CD3 antibodies and/or antigen-binding fragments thereof may be produced using recombinant methods. For example, isolated nucleic acids encoding an anti-CD3 antibody as described herein is provided. Such nucleic acids may encode an amino acid sequence comprising the VL, and/or an amino acid sequence comprising the VH of the antibody (e.g., the light and/or heavy chains of the antibody). In a further embodiment, one or more vectors (e.g., expression vectors) comprising such nucleic acids are provided. In a further embodiment, a host cell comprising such nucleic acids is provided. In one such embodiment, a host cell comprises (e.g., has been transformed with): (1) a vector comprising a nucleic acid sequence that encodes an amino acid sequence comprising the VL of the antibody and an amino acid sequence comprising the VH of the antibody, or (2) a first vector comprising a nucleic acid that encodes an amino acid sequence comprising the VL of the antibody and a second vector comprising a nucleic acid that encodes an amino acid sequence comprising the VH of the antibody. In one embodiment, the host cell is eukaryotic, e.g. a Chinese Hamster Ovary (CHO) cell or lymphoid cell (e.g., Y0, NS0, Sp20 cell). In one embodiment, a method of making an anti-CD3 antibody is provided, wherein the method comprises culturing a host cell comprising a nucleic acid encoding the antibody, as provided above, under conditions suitable for expression of the antibody, and optionally recovering the antibody from the host cell (or host cell culture medium).

[0085] The term "host cell" refers to cells into which an exogenous nucleic acid sequence has been introduced, including the progeny of such cells. Host cells include transformants and transformed cells, which include the primary transformed cell and progeny derived therefrom without regard to the number of passages.

[0086] For recombinant production of an anti-CD3 antibody, nucleic acids encoding an antibody, e.g., as described above, is isolated and inserted into one or more vectors for further cloning and/or expression in a host cell. Such nucleic acids may be readily isolated

and sequenced using conventional procedures (e.g., by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of the antibody).

[0087] Suitable host cells for cloning and/or expression of antibody-encoding vectors include prokaryotic or eukaryotic cells. For example, antibodies may be produced in bacteria, in particular when glycosylation and Fc effector function are not needed. For expression of antibody fragments and polypeptides in bacteria, *see*, e.g., U.S. Pat. Nos. 5,648,237, 5,789,199, and 5,840,523. (*See* also Charlton, Methods in Molecular Biology, Vol. 248 (B.K.C. Lo, ed., Humana Press, Totowa, N.J., 2003), pp. 245-254, describing expression of antibody fragments in E. coli.) After expression, the antibody may be isolated from the bacterial cell paste in a soluble fraction and can be further purified. In addition to prokaryotes, eukaryotic microbes such as filamentous fungi or yeast are suitable cloning or expression hosts for antibody-encoding vectors, including fungi and yeast strains whose glycosylation pathways have been "humanized," resulting in the production of an antibody with a partially or fully human glycosylation pattern. *See*, e.g., Gerngross, Nat. Biotech. 22:1409-1414 (2004), and Li et al., Nat. Biotech. 24:210-215 (2006); WO 2009/036379; WO 2010/105256; and WO 2012/009568. Plant cell cultures can also be utilized as hosts. *See*, e.g., U.S. Pat. Nos. 5,959,177, 6,040,498, 6,420,548, 7,125,978, and 6,417,429 (describing PLANTIBODIES™ technology for producing antibodies in transgenic plants). Vertebrate cells may also be used as hosts. For example, mammalian cell lines that are adapted to grow in suspension may be useful. Other examples of useful mammalian host cell lines are monkey kidney CV1 line transformed by SV40 (COS-7); human embryonic kidney line (293 or 293 cells as described, e.g., in Graham et al., J. Gen Virol. 36:59 (1977)); baby hamster kidney cells (BHK); mouse sertoli cells (TM4 cells as described, e.g., in Mather, Biol. Reprod. 23:243-251 (1980)); monkey kidney cells (CV1); African green monkey kidney cells (VERO-76); human cervical carcinoma cells (HELA); canine kidney cells (MDCK; buffalo rat liver cells (BRL 3A); human lung cells (W138); human liver cells (Hep G2); mouse mammary tumor (MMT 060562); TRI cells, as described, e.g., in Mather et al., Annals N.Y. Acad. Sci. 383:44-68 (1982); MRC 5 cells; and FS4 cells. Other useful mammalian host cell lines include Chinese hamster ovary (CHO) cells, including DHFR−CHO cells (Urlaub et al., Proc. Natl. Acad. Sci. USA 77:4216 (1980)); and myeloma cell lines such as Y0, NS0 and Sp2/0. For a review of certain mammalian host cell lines suitable for antibody production, *see*, e.g., Yazaki and Wu, Methods in Molecular Biology, Vol. 248 (B.K.C. Lo, ed., Humana Press, Totowa, N.J.), pp. 255-268 (2003).

[0088] Anti-CD3 antibodies and/or antigen-binding fragments thereof may be identified, screened for, selected for or characterized for their physical/chemical properties and/or biological activities by various assays known in the art, e.g., ELISA, Western blot, etc. or competition assays may be used to identify an antibody that competes with an anti-CD3 antibody of the invention for binding to CD3. In an exemplary competition assay, immobilized CD3 is incubated in a solution comprising a first labeled antibody that binds to CD3 and a second unlabeled antibody that is being tested for its ability to compete with the first antibody for binding to CD3. The second antibody may be present in a hybridoma supernatant. As a control, immobilized CD3 is incubated in a solution comprising the first labeled antibody but not the second unlabeled antibody. After incubation under conditions permissive for binding of the first antibody to CD3, excess unbound antibody is removed, and the amount of label associated with immobilized CD3 is measured. If the amount of label associated with immobilized CD3 is substantially reduced in the test sample relative to the control sample, then that indicates that the second antibody is competing with the first antibody for binding to CD3. *See*, e.g., Harlow and Lane (1988) Antibodies: A Laboratory Manual. Ch.14 (Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.).

[0089] Anti-CD3 antibodies and/or antigen-binding fragments thereof possessing biological activity may be identified using standard approaches. Biological activity may include, e.g., binding to CD3 on the surface of a T cell, either *in vivo*, *in vitro*, or *ex vivo*. In the case of a multispecific anti-CD3 antibody (such as a bispecific antibody with one arm that binds to CD3 and another arm that binds to a different target, e.g., a cell surface antigen, e.g., a tumor antigen), biological activity may also include effector cell activation (such as CD8+ and/or CD4+ T cell activation), effector cell population expansion (i.e., an increase in T cell count), target cell population reduction (i.e., a decrease in the population of cells expressing the second biological molecule on their cell surfaces), and/or target cell killing.

Diagnostic and therapeutic uses for the anti-CD3 antibodies and antigen-binding fragments thereof

[0090] Anti-CD3 antibodies and/or antigen-binding fragments described herein may be used for diagnosis and/or detection. "Detection" as used herein encompasses quantitative or qualitative detection. In one embodiment, a method of detecting the presence of CD3 in a biological sample is provided. Such method comprises (i) contacting the biological sample with an anti-CD3 antibody as described herein under conditions permissive for binding of the

anti-CD3 antibody to CD3, and (ii) detecting whether a complex is formed between the anti-CD3 antibody and CD3. Such method may be an *in vitro* or *in vivo* method. In certain embodiments, the biological sample comprises a cell or tissue(s).

[0091] In certain embodiments, labeled anti-CD3 antibodies are provided. The anti-CD3 antibodies and/or antigen-binding fragments thereof as described herein include a label or moiety that is detected directly (such as fluorescent, chromophoric, electron-dense, chemiluminescent, and radioactive labels or indirectly (such as enzymes or ligands). Non-limiting exemplary labels include, radioisotopes such as 32P, 14C, 125I, 3H, and 131I; fluorophores such as rare earth chelates or fluorescein and its derivatives, rhodamine and its derivatives, dansyl, umbelliferone, luceriferases, e.g., firefly luciferase and bacterial luciferase (U.S. Pat. No. 4,737,456), luciferin, 2,3-dihydrophthalazinediones, horseradish peroxidase (HRP), alkaline phosphatase, β-galactosidase, glucoamylase, lysozyme, saccharide oxidases, e.g., glucose oxidase, galactose oxidase, and glucose-6-phosphate dehydrogenase; heterocyclic oxidases such as uricase and xanthine oxidase, coupled with an enzyme that employs hydrogen peroxide to oxidize a dye precursor such as HRP, lactoperoxidase, or microperoxidase; biotin/avidin; spin labels; bacteriophage labels; stable free radicals; and the like.

[0092] CD3 antibodies and/or antigen-binding fragments thereof as described herein, as well as pharmaceutical compositions of such antibodies, may be used in therapeutic methods. In one embodiment, anti-CD3 antibodies and/or antigen-binding fragments thereof as described herein, or pharmaceutical compositions comprising such antibodies, may be used for treating or delaying progression of a cell proliferative disorder or an autoimmune disorder.

[0093] A "disorder" refers to any condition or disease that would benefit from treatment including, but not limited to, chronic and acute disorders or diseases including those pathological conditions which predispose a mammal to the disorder in question.

[0094] The terms "cell proliferative disorder" and "proliferative disorder" refer to disorders that are associated with some degree of abnormal cell proliferation. Cell proliferative disorders include cancer, e.g., a tumor.

[0095] "Tumor" as used herein refers to all neoplastic cell growth and proliferation, whether malignant or benign, and all pre-cancerous and cancerous cells and tissues.

[0096] "Cancer" refers to a physiological condition in mammals characterized by unregulated cell growth. Examples of cancer include, but are not limited to, carcinoma, lymphoma, blastoma, sarcoma, and leukemia or lymphoid malignancies; with more particular examples including squamous cell cancer (e.g., epithelial squamous cell cancer), lung cancer including small-cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung and squamous carcinoma of the lung, cancer of the peritoneum, hepatocellular cancer, gastric or stomach cancer including gastrointestinal cancer and gastrointestinal stromal cancer, pancreatic cancer, glioblastoma, cervical cancer, ovarian cancer, liver cancer, bladder cancer, cancer of the urinary tract, hepatoma, breast cancer, colon cancer, rectal cancer, colorectal cancer, endometrial or uterine carcinoma, salivary gland carcinoma, kidney or renal cancer, prostate cancer, vulval cancer, thyroid cancer, hepatic carcinoma, anal carcinoma, penile carcinoma, melanoma, superficial spreading melanoma, lentigo maligna melanoma, acral lentiginous melanomas, nodular melanomas, multiple myeloma and B-cell lymphoma (including low grade/follicular non-Hodgkin's lymphoma (NHL); small lymphocytic (SL) NHL; intermediate grade/follicular NHL; intermediate grade diffuse NHL; high grade immunoblastic NHL; high grade lymphoblastic NHL; high grade small non-cleaved cell NHL; bulky disease NHL; mantle cell lymphoma; AIDS-related lymphoma; and Waldenstrom's Macroglobulinemia); chronic lymphocytic leukemia (CLL); acute lymphoblastic leukemia (ALL); hairy cell leukemia; chronic myeloblastic leukemia; and post-transplant lymphoproliferative disorder (PTLD), as well as abnormal vascular proliferation associated with phakomatoses, edema (such as that associated with brain tumors), Meigs' syndrome, brain, as well as head and neck cancer, and associated metastases. In certain embodiments, cancers that are amenable to treatment by antibodies of the disclosure include breast cancer, colorectal cancer, rectal cancer, non-small cell lung cancer, glioblastoma, non-Hodgkins lymphoma (NHL), renal cell cancer, prostate cancer, liver cancer, pancreatic cancer, soft-tissue sarcoma, kaposi's sarcoma, carcinoid carcinoma, head and neck cancer, ovarian cancer, mesothelioma, and multiple myeloma. In some embodiments, the cancer is selected from: small cell lung cancer, gliblastoma, neuroblastomas, melanoma, breast carcinoma, gastric cancer, colorectal cancer (CRC), and hepatocellular carcinoma. Yet, in some embodiments, the cancer is selected from: non-small cell lung cancer, colorectal cancer, glioblastoma and breast carcinoma, including metastatic forms of those cancers. In other embodiments, the cancer is selected from a class of mature B-Cell cancers excluding Hodgkin's Lymphoma but including germinal-center B-cell-like

(GCB) DLBCL, activated B-cell-like (ABC) DLBCL, follicular lymphoma (FL), mantle cell lymphoma (MCL), acute myeloid leukemia (AML), chronic lymphoid leukemia (CLL), marginal zone lymphoma (MZL), small lymphocytic leukemia (SLL), lymphoplasmacytic lymphoma (LL), Waldenstrom macroglobulinemia (WM), central nervous system lymphoma (CNSL), Burkitt's lymphoma (BL), B-cell prolymphocytic leukemia, Splenic marginal zone lymphoma, Hairy cell leukemia, Splenic lymphoma/leukemia, unclassifiable, Splenic diffuse red pulp small B-cell lymphoma, Hairy cell leukemia variant, Waldenström macroglobulinemia, Heavy chain diseases, a Heavy chain disease, γ Heavy chain disease, μ Heavy chain disease, Plasma cell myeloma, Solitary plasmacytoma of bone, Extraosseous plasmacytoma, Extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma), Nodal marginal zone lymphoma, Pediatric nodal marginal zone lymphoma, Pediatric follicular lymphoma, Primary cutaneous follicle centre lymphoma, T-cell/histiocyte rich large B-cell lymphoma, Primary DLBCL of the CNS, Primary cutaneous DLBCL, leg type, EBV-positive DLBCL of the elderly, DLBCL associated with chronic inflammation, Lymphomatoid granulomatosis, Primary mediastinal (thymic) large B-cell lymphoma, Intravascular large B-cell lymphoma, ALK-positive large B-cell lymphoma, Plasmablastic lymphoma, Large B-cell lymphoma arising in HHV8-associated multicentric Castleman disease, Primary effusion lymphoma: B-cell lymphoma, unclassifiable, with features intermediate between diffuse large B-cell lymphoma and Burkitt lymphoma, and B-cell lymphoma, unclassifiable, with features intermediate between diffuse large B-cell lymphoma and classical Hodgkin lymphoma.

[0097] As used herein, “treatment” or “treat” or “treating” refer to clinical intervention in an attempt to alter the natural course of an individual being treated and can be performed either for prophylaxis or during the course of clinical pathology. Desirable effects of treatment include, but are not limited to, preventing occurrence or recurrence of disease, alleviation of symptoms, diminishment 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.

[0098] As used herein, the terms “prevent,” “preventing,” and “prevention” refer to the prevention or inhibition of the development or onset of a disorder or disease.

[0099] As used herein, the terms “ameliorate” and “alleviate” refer to a reduction or diminishment in the severity a condition or any symptoms thereof.

[0100] In some embodiments, antibodies of the invention are used to delay development of a disorder or disease or to delay the progression of a disorder or disease. As used herein, "delaying progression" of a disorder or disease means to defer, hinder, slow, retard, stabilize, and/or postpone development of the disease or disorder (e.g., a cell proliferative disorder, e.g., cancer). The delay can be of varying lengths of time, depending on the history of the disease and/or individual being treated.

[0101] An effective amount of such antibody or composition may be administered to an individual suffering from cancer or arthritis, rheumatoid arthritis, colitis, inflammatory bowel disease, autoimmune type I diabetes, etc. An "effective amount" of an anti-CD3 antibody disclosed herein or a composition (e.g., pharmaceutical composition) comprising such antibody, is at least the minimum amount required to achieve the desired therapeutic or prophylactic result, e.g., a measurable improvement or prevention of a particular disorder, e.g., a cell proliferative disorder, e.g., cancer, preferably with minimal or no toxic or detrimental effects. An effective amount may vary according to *inter alia* disease state, age, sex, and weight of the patient, and the ability of the antibody (or antigen-binding fragment thereof) to elicit a desired response in the individual and, in some instances, by co-administering one or more additional therapeutic agents.

[0102] In some embodiments, anti-CD3 antibodies and/or antigen-binding fragments thereof as described herein may be used to enhance immune function in an individual having a cell proliferative disorder or an autoimmune disorder. Following administration, such antibody or composition may enhance immune function in an individual having a cell proliferative disorder or an autoimmune disorder by activating effector cells (e.g., T cells, e.g., CD8+ and/or CD4+ T cells including Tregs), expanding (increasing) the effector cell population, reducing the population of target cells (e.g., a cell expressing a second biological molecule recognized by an anti-CD3 antibody of the invention, such as a bispecific antibody), and/or killing a target cell (e.g., target tumor cell).

[0103] Anti-CD3 antibodies and/or antigen-binding fragments thereof as disclosed herein may be used to treat disorders including, but not limited to, a proliferative disorder, an oncological disorder, an immune-oncological disorder, a neurological disorder, a cognitive disorder, a neurodegenerative disorder, an autoimmune disorder. In one embodiment, an effective amount of such anti-CD3 antibody may be administered, alone or in combination

with at least one additional agent, to an individual having such disorder. Such "individual" may be a mammal and, in particular, a human.

[0104] Non-limiting exemplary additional therapeutic agents include a chemotherapy agent, an antibody-drug conjugate (ADC), and/or a biological modifier. Chemotherapy agents may be selected from cyclophosphamide, doxorubicin, vincristine, and prednisolone (CHOP). ADC may be selected from an anti-CD79b antibody drug conjugate (such as anti-CD79b-MC-vc-PAB-MMAE or the anti-CD79b antibody drug conjugate described in any one of U.S. Pat. No. 8,088,378 and/or US 2014/0030280, or polatuzumab vedotin), an anti-CD19 antibody drug conjugate, an anti-CD22 antibody drug conjugate, an anti-CD45 antibody drug conjugate, and an anti-CD32 drug conjugate. A biological modifier may be selected from a BCL-2 inhibitor (such as GDC-0199/ABT-199), lenalidomide (Revlimid®), a PI3K-delta inhibitor (such as idelalisib (Zydelig®)), a PD-1 axis binding antagonist, an agonist, e.g., agonist antibody, directed against an activating co-stimulatory molecule, e.g., CD40, CD226, CD28, OX40 (e.g., AgonOX), GITR, CD137 (also known as TNFRSF9, 4-1 BB, or ILA), CD27 (e.g., CDX-1127), HVEM, or CD127, an antagonist, e.g., antagonist antibody, directed against an inhibitory co-stimulatory molecule, e.g., CTLA-4 (also known as CD152), PD-1, TIM-3, BTLA, VISTA, LAG-3, B7-H3, B7-H4, IDO (e.g., 1-methyl-D-tryptophan (also known as 1-D-MT)), TIGIT, MICA/B, GITR (e.g., TRX518) or arginase, ipilimumab (also known as MDX-010, MDX-101, or Yervoy®), tremelimumab (also known as ticilimumab or CP-675,206, urelumab (also known as BMS-663513), MGA271, an antagonist directed against a TGF beta, e.g., metelimumab (also known as CAT-192), fresolimumab (also known as GC1008), LY2157299k, and an adoptive transfer of a T cell (e.g., a cytotoxic T cell or CTL) expressing a chimeric antigen receptor (CAR), e.g., adoptive transfer of a T cell comprising a dominant-negative TGF beta receptor, e.g, a dominant-negative TGF beta type II receptor.

[0105] Anti-CD3 antibodies and/or antigen-binding fragments thereof as disclosed herein may be used to enhancing immune function in an individual, e.g., a human, having a disorder in an individual having such disorder. In one embodiment, a method of enhancing immune function comprises administering to an individual an effective amount of an anti-CD3 antibody to activate effector cells (e.g., T cells, e.g., CD8+ and/or CD4+ T cells), expand (increase) an effector cell population, reduce a target cell population, and/or kill a target cell (e.g., target tumor cell).

[0106] In a further aspect, pharmaceutical formulations comprising anti-CD3 antibodies and/or antigen-binding fragments as described herein are also provided, e.g., for use in any of the above therapeutic methods. A "pharmaceutical formulation" refers to a preparation in such form as to permit the biological activity of an active ingredient contained therein, such as the anti-CD3 antibodies described herein, to be effective, and which preferably contains no additional components which are unacceptably toxic to a subject to which the formulation would be administered.

[0107] In one embodiment, a pharmaceutical formulation comprises any of the anti-CD3 antibodies disclosed herein and a pharmaceutically acceptable carrier. A "pharmaceutically acceptable carrier" refers to an ingredient in a pharmaceutical formulation, other than an active ingredient, which is nontoxic to a subject. A pharmaceutically acceptable carrier includes, but is not limited to, a buffer, excipient, stabilizer, or preservative. In another embodiment, a pharmaceutical formulation comprises any of the anti-CD3 antibodies provided herein and at least one additional therapeutic agent.

[0108] Antibodies of the disclosure can be used either alone or in combination with other agents in a therapy, e.g., an anti-CD3 antibody and/or antigen-binding fragment thereof may be co-administered with at least one additional therapeutic agent. In certain embodiments, an additional therapeutic agent is a chemotherapeutic agent, growth inhibitory agent, cytotoxic agent, agent used in radiation therapy, anti-angiogenesis agent, apoptotic agent, anti-tubulin agent, or other agent, such as a epidermal growth factor receptor (EGFR) antagonist (e.g., a tyrosine kinase inhibitor), HER1/EGFR inhibitor (e.g., erlotinib (Tarceva™)), platelet derived growth factor inhibitor (e.g., Gleevec™ (Imatinib Mesylate)), a COX-2 inhibitor (e.g., celecoxib), interferon, cytokine, antibody other than the anti-CD3 antibody of the invention, such as an antibody that bind to one or more of the following targets ErbB2, ErbB3, ErbB4, PDGFR-beta, BIyS, APRIL, BCMA VEGF, or VEGF receptor(s), TRAIL/Apo2, PD-1, PD-L1, PD-L2, or another bioactive or organic chemical agent.

[0109] In some embodiments, the disclosure provides a method wherein the additional therapeutic agent is a glucocorticoid. In one embodiment, the glucocorticoid is dexamethasone.

[0110] Such combination therapies noted above encompass combined administration (where two or more therapeutic agents are included in the same or separate formulations), and

separate administration, in which case, administration of an antibody of the disclosure can occur prior to, simultaneously, and/or following, administration of additional therapeutic agent or agents. In one embodiment, administration of an anti-CD3 antibody and administration of an additional therapeutic agent occur within about one month, or within about one, two or three weeks, or within about one, two, three, four, five, or six days, of each other. Anti-CD3 antibodies of the disclosure (e.g., bispecific anti-CD3 antibodies of the invention that bind to CD3 and a second biological molecule, e.g., a cell surface antigen, e.g., a tumor antigen, such as a TDB antibody of the invention or variant thereof) can also be used in combination with radiation therapy.

[0111] An antibody of the disclosure (and/or any additional therapeutic agent) can be administered by any suitable means, including parenteral, intrapulmonary, and intranasal, and, if desired for local treatment, intralesional administration. Parenteral infusions include intramuscular, intravenous, intraarterial, intraperitoneal, or subcutaneous administration. In some embodiments, the antibody is administered by subcutaneous administration. In some embodiments, an anti-CD3 antibody administered by subcutaneous injection exhibits a less toxic response in a patient than the same anti-CD3 antibody administered by intravenous injection. Dosing can be by any suitable route, for example, by injections, such as intravenous or subcutaneous injections, depending in part on whether the administration is brief or chronic. Various dosing schedules including but not limited to single or multiple administrations over various time-points, bolus administration, and pulse infusion are contemplated herein.

[0112] Antibodies of the disclosure would be formulated, dosed, and administered in a fashion consistent with good medical practice. Factors for consideration in this context include the particular disorder being treated, the particular mammal being treated, the clinical condition of the individual patient, the cause of the disorder, the site of delivery of the agent, the method of administration, the scheduling of administration, and other factors known to medical practitioners. The antibody need not, but may optionally be, formulated with one or more agents currently used to prevent or treat the disorder in question. The effective amount of such other agents depends on the amount of antibody present in the formulation, the type of disorder or treatment, and other factors discussed above. These are generally used in the same dosages and with administration routes as described herein, or about from 1 to 99% of

the dosages described herein, or in any dosage and by any route that is empirically/clinically determined to be appropriate.

[0113] For the prevention or treatment of disease, the appropriate dosage of an antibody of the disclosure (when used alone or in combination with one or more other additional therapeutic agents) will depend on the type of disease to be treated, the type of antibody, the severity and course of the disease, whether the antibody is administered for preventive or therapeutic purposes, previous therapy, the patient's clinical history and response to the antibody, and the discretion of the attending physician. The antibody is suitably administered to the patient at one time or over a series of treatments.

[0114] As a general proposition, a therapeutically effective amount of the anti-CD3 antibody administered to human will be in the range of about 0.01 to about 100 mg/kg of patient body weight whether by one or more administrations. In some embodiments, an antibody used is administered in about 0.01 to about 45 mg/kg, about 0.01 to about 40 mg/kg, about 0.01 to about 35 mg/kg, about 0.01 to about 30 mg/kg, about 0.01 to about 25 mg/kg, about 0.01 to about 20 mg/kg, about 0.01 to about 15 mg/kg, about 0.01 to about 10 mg/kg, about 0.01 to about 5 mg/kg, or about 0.01 to about 1 mg/kg daily, for example. In one embodiment, an anti-CD3 antibody described herein is administered to a human at a dose of about 100 mg, about 200 mg, about 300 mg, about 400 mg, about 500 mg, about 600 mg, about 700 mg, about 800 mg, about 900 mg, about 1000 mg, about 1100 mg, about 1200 mg, about 1300 mg or about 1400 mg on day 1 of 21-day cycles. The dose may be administered as a single dose or as multiple doses (e.g., 2 or 3 doses), such as infusions. For repeated administrations over several days or longer, depending on the condition, the treatment would generally be sustained until a desired suppression of disease symptoms occurs. One exemplary dosage of the antibody would be in the range from about 0.05 mg/kg to about 10 mg/kg. Thus, one or more doses of about 0.5 mg/kg, 2.0 mg/kg, 4.0 mg/kg, or 10 mg/kg (or any combination thereof) may be administered to the patient. Such doses may be administered intermittently, for example, every week or every three weeks (e.g., such that the patient receives from about two to about twenty, or, for example, about six doses of the anti-CD3 antibody). An initial higher loading dose, followed by one or more lower doses, may be administered. The progress of this therapy is easily monitored by conventional techniques and assays.

[0115] In some embodiments, methods of the disclosure may further comprise an additional therapy. The additional therapy may be radiation therapy, surgery, chemotherapy, gene therapy, DNA therapy, viral therapy, RNA therapy, immunotherapy, bone marrow transplantation, nanotherapy, monoclonal antibody therapy, or a combination of the foregoing. The additional therapy may be in the form of adjuvant or neoadjuvant therapy. In some embodiments, the additional therapy is the administration of small molecule enzymatic inhibitor or anti-metastatic agent. In some embodiments, the additional therapy is the administration of side-effect limiting agents (e.g., agents intended to lessen the occurrence and/or severity of side effects of treatment, such as anti-nausea agents, etc.). In some embodiments, the additional therapy is radiation therapy. In some embodiments, the additional therapy is surgery. In some embodiments, the additional therapy is a combination of radiation therapy and surgery. In some embodiments, the additional therapy is gamma irradiation. In some embodiments, the additional therapy may be a separate administration of one or more of the therapeutic agents described above.

[0116] In another aspect of the disclosure, an article of manufacture containing materials useful for the treatment, prevention and/or diagnosis of the disorders described above is provided. The article of manufacture comprises a container and a label or package insert on or associated with the container. Suitable containers include, for example, bottles, vials, syringes, IV solution bags, etc. The containers may be formed from a variety of materials such as glass or plastic. The container holds a composition which is by itself or combined with another composition effective for treating, preventing and/or diagnosing the condition and may have a sterile access port (for example the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). At least one active agent in the composition is an antibody of the invention. The label or package insert indicates that the composition is used for treating the condition of choice. Moreover, the article of manufacture may comprise (a) a first container with a composition contained therein, wherein the composition comprises an antibody of the invention; and (b) a second container with a composition contained therein, wherein the composition comprises a further cytotoxic or otherwise therapeutic agent. The article of manufacture in this embodiment of the invention may further comprise a package insert indicating that the compositions can be used to treat a particular condition. Alternatively, or additionally, the article of manufacture may further comprise a second (or third) container comprising a pharmaceutically acceptable buffer, such as bacteriostatic water for injection

(BWFI), phosphate-buffered saline, Ringer's solution and dextrose solution. It may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, and syringes.

[0117] Accordingly, manufacture and/or preparation of a pharmaceutical composition comprising anti-CD3 antibodies and/or antigen-binding fragments as disclosed herein is also contemplated. The composition may be used alone or in combination with other active agents to treat a cell proliferative disorder (e.g., cancer) or an autoimmune disorder (e.g., arthritis, rheumatoid arthritis, colitis, inflammatory bowel disease, autoimmune type I diabetes, etc.).

[0118] In some embodiments, pharmaceutical compositions comprising anti-CD3 antibodies and/or antigen-binding fragments thereof as described herein are prepared, e.g., by mixing such an antibody having the desired degree of purity with one or more optional pharmaceutically acceptable carriers (Remington's Pharmaceutical Sciences 16th edition, Osol, A. Ed. (1980)), in the form of lyophilized formulations or aqueous solutions, optionally prepared for modified (e.g., sustained) release. Exemplary lyophilized antibody formulations are described in U.S. Pat. No. 6,267,958. Aqueous antibody formulations include those described in U.S. Pat. No. 6,171,586 and WO2006/044908, the latter formulations including a histidine-acetate buffer.

[0119] Pharmaceutically acceptable carriers are generally nontoxic to recipients at the dosages and concentrations employed, and include, but are not limited to: buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride; benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (e.g. Zn-protein complexes); and/or non-ionic surfactants such as polyethylene glycol (PEG). Exemplary pharmaceutically acceptable carriers herein further include insterstitial drug dispersion agents such as soluble neutral-active hyaluronidase glycoproteins

(sHASEGP), for example, human soluble PH-20 hyaluronidase glycoproteins, such as rHuPH20 (HYLENEX®, Baxter International, Inc.). Certain exemplary sHASEGPs and methods of use, including rHuPH20, are described in US Patent Publication Nos. 2005/0260186 and 2006/0104968.

[0120] Such formulations may contain more than one active ingredient as necessary for the particular indication being treated, preferably those with complementary activities that do not adversely affect each other and present in amounts that are effective for the purpose intended. For example, it may be desirable to further provide an additional therapeutic agent (e.g., a chemotherapeutic agent, a cytotoxic agent, a growth inhibitory agent, and/or an anti-hormonal agent).

[0121] Active ingredients may be entrapped in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or gelatin-microcapsules and poly-(methylmethacylate) microcapsules, respectively, in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles and nanocapsules) or in macroemulsions. Such techniques are disclosed in Remington's Pharmaceutical Sciences 16th edition, Osol, A. Ed. (1980).

EXAMPLES

Table 1. Binding data for exemplary high affinity anti-CD3 antibodies in a bispecific format.

Antibody	Biacore K_D Biotinylated Human CD3 εδ Fc (M) Monovalent	Cell Binding Human CD3+ Jurkat Fold Over Background (FOB)	Cell Binding Cyno CD3+ Jurkat Fold Over Background (FOB)
ADI-42594	4.7E-10	14	16
ADI-46357	2.5E-10	13	20
ADI-46359	2.2E-10	15	28
ADI-46366	2.3E-10	16	32
ADI-46378	1.2E-10	17	30
ADI-45959	1.2E-10	18	30
ADI-45975	2.1E-10	10	17

Table 2. Developability profile of anti-CD3 antibodies in a bispecific format.

	scFv-Fc or IgG K_D Human CD3εδ-Fc (M)	scFv-Fc or IgG K_D Cyno CD3εδ-Fc (M)	Cell Binding hCD3 (FOB)	Cell Binding cCD3 (FOB)	PSR Score (0-1)	HIC Retention Time (min)	SEC-HPLC % monomer ProA
ADI-42594	2.0E-10	2.0E-09	36	122	0.21	8.8	98.7
ADI-42596	1.6E-10	2.4E-09	29	46	0.40	8.8	99.8
ADI-45959	1.3E-10	1.7E-09	19	112	0.19	9.5	99.5
ADI-45960	1.4E-10	1.8E-09	63	58	0.20	8.9	93.9
ADI-45961	2.3E-10	3.0E-09	18	30	0.16	8.8	98.7
ADI-45962	1.4E-10	1.4E-09	18	46	0.15	8.9	99.2
ADI-45963	6.9E-11	2.1E-09	14	69	0.26	8.9	93.8
ADI-45964	9.7E-11	2.0E-09	56	63	0.28	8.8	93.6
ADI-45965	6.5E-11	1.4E-09	33	124	0.24	9.6	99.4
ADI-45966	6.1E-11	1.2E-09	14	27	0.26	9.5	99.3
ADI-45967	7.6E-11	1.4E-09	40	59	0.18	9.5	99.4
ADI-45968	6.8E-11	1.4E-09	16	18	0.24	9.5	99.3
ADI-45969	6.0E-11	1.5E-09	28	63	0.24	8.9	96.5
ADI-45970	1.5E-10	1.6E-09	53	68	0.20	8.9	99.2
ADI-45971	1.1E-10	1.9E-09	21	20	0.30	8.8	99.4
ADI-45972	8.1E-11	1.4E-09	32	45	0.17	9.5	99.2
ADI-45973	1.6E-10	1.8E-09	55	91	0.15	8.9	97.7
ADI-45974	1.9E-10	2.1E-09	47	67	0.28	8.9	99.4
ADI-45975	6.7E-11	1.4E-09	86	142	0.32	8.8	99.6
ADI-45976	1.2E-10	1.8E-09	45	109	0.19	11.4	99.4
ADI-45977	9.1E-11	1.1E-09	59	159	0.23	8.9	99.5

	scFv-Fc or IgG K_D Human CD3εδ-Fc (M)	scFv-Fc or IgG K_D Cyno CD3εδ-Fc (M)	Cell Binding hCD3 (FOB)	Cell Binding cCD3 (FOB)	PSR Score (0-1)	HIC Retention Time (min)	SEC-HPLC % monomer ProA
ADI-45978	7.4E-11	1.2E-09	25	37	0.26	9.5	99.7
ADI-45979	1.9E-10	1.7E-09	54	72	0.17	8.9	99.6
ADI-45980	8.0E-11	1.2E-09	8	85	0.22	9.5	99.7
ADI-45981	8.9E-11	1.6E-09	33	78	0.25	8.9	99.7
ADI-45982	1.2E-10	1.4E-09	24	31	0.21	9.0	99.2
ADI-45983	2.3E-10	2.0E-09	24	101	0.16	10.9	99.2
ADI-45984	9.5E-11	1.3E-09	88	92	0.29	9.5	100.0
ADI-45985	8.0E-11	1.2E-09	21	23	0.21	10.5	99.5
ADI-42596	1.1E-09	N.A.	11	81	0.36	8.9	99.7
ADI-46356	9.8E-10	N.A.	39	59	0.20	10.9	98.5
ADI-46357	9.7E-10	N.A.	24	80	0.21	8.9	94.4
ADI-46358	9.9E-10	N.A.	35	82	0.21	9.5	98.8
ADI-46359	8.9E-10	N.A.	43	102	0.26	9.0	98.4
ADI-46360	9.7E-10	N.A.	40	106	0.20	9.0	99.5
ADI-46361	9.3E-10	N.A.	23	84	0.21	9.5	98.9
ADI-46362	8.0E-10	N.A.	7	95	0.21	8.9	99.1
ADI-46363	9.3E-10	N.A.	49	129	0.20	9.5	98.6
ADI-46364	9.7E-10	N.A.	33	63	0.18	9.5	99.4
ADI-46365	1.0E-09	N.A.	36	84	0.16	10.1	99.4
ADI-46366	9.3E-10	N.A.	43	108	0.20	9.0	98.5
ADI-46367	9.5E-10	N.A.	8	62	0.21	8.9	94.8
ADI-46369	9.2E-10	N.A.	33	71	0.17	9.5	98.6
ADI-46370	1.0E-09	N.A.	40	46	0.20	8.9	98.8

	scFv-Fc or IgG K_D Human CD3εδ-Fc (M)	scFv-Fc or IgG K_D Cyno CD3εδ-Fc (M)	Cell Binding hCD3 (FOB)	Cell Binding cCD3 (FOB)	PSR Score (0-1)	HIC Retention Time (min)	SEC-HPLC % monomer ProA
ADI-46371	8.6E-10	N.A.	47	121	0.22	8.9	98.9
ADI-46372	1.1E-09	N.A.	14	80	0.16	10.3	99.2
ADI-46374	8.4E-10	N.A.	37	29	0.22	9.5	94.4
ADI-46375	8.6E-10	N.A.	48	40	0.25	9.5	99.7
ADI-46376	1.0E-09	N.A.	4	96	0.20	9.5	99.1
ADI-46378	9.2E-10	N.A.	22	91	0.31	9.5	98.8
ADI-46379	1.0E-09	N.A.	43	105	0.21	9.5	99.5
ADI-46381	7.2E-10	N.A.	71	174	0.18	0.0	99.6
ADI-26140	N.B.	N.B.	5	6	N.A.		N.A.

Table 3. Thermostability measurements for exemplary anti-CD3 antibodies.

ADI Name	scFv-Fc Tm DSF (°C)
ADI-42594	66.0
ADI-42596	66.0
ADI-46356	62.0
ADI-46357	66.5
ADI-46358	62.0
ADI-46359	62.5
ADI-46360	52.5
ADI-46361	58.5
ADI-46362	59.0
ADI-46363	58.5
ADI-46364	58.0
ADI-46365	58.5
ADI-46366	63.5
ADI-46367	66.5
ADI-46369	62.5
ADI-46370	62.0
ADI-46371	59.0
ADI-46372	59.0
ADI-46374	64.0
ADI-46375	58.5
ADI-46376	57.0
ADI-46378	58.5
ADI-46379	58.5
ADI-46381	56.5
ADI-26140	N.A.

MATERIALS AND METHODS

[0122] In addition to the description provided above, the following Materials and Methods were employed in the Examples.

[0123] *FACS affinity pressured selection methods.* Briefly, yeast cells (at least ~2 x 10^7 cells/labeling condition) were incubated with a volume of biotinylated antigen sufficient to represent a stoichiometric excess with respect to the average IgG presentation number. Antigen labeling conditions are 100 to 1 nM under equilibrium conditions, typically carried out for 20 min to several hours at room temperature in FACS wash buffer (phosphate-

buffered saline (PBS)/0.1% bovine serum albumin (BSA)). After washing three times with wash buffer, yeast were then stained with secondary reagents anti-human light chain FITC conjugate (LC-FITC) diluted 1:100 and either streptavidin-633 (SA-633) diluted 1:500 or extravidin-phycoerythrin (EA-PE) diluted 1:50 for 15 min at 4°C. After washing twice with ice-cold wash buffer, the cell pellets were resuspended in wash buffer in a typical volume of at least 1 mL per 1 x 10^7 yeast and transferred to strainer-capped sort tubes. Sorting is performed using a FACS ARIA sorter (BD Biosciences) and sort gates were determined to select for binders. After the final round of sorting, yeast were plated and individual colonies picked for characterization.

[0124] *FACS thermal pressured selection methods.* The parent antibody was diversified by error-prone PCR to derive an optimization library in yeast. This yeast library first proceeds through a positive antigen selection round to select for expressing binders, using CD3εN27. This enriched population then proceeded through a series of thermally pressured conditions ranging from ~50°C up to 65°C for 10 min with room temperature as a control. Optimally pressured conditions were gated on LC presentation (anti-human lambda PE) and antigen binding (SA-APC), reflecting the residual folded IgG competent to bind the selection reagents. Sorted cells are pelleted and the plasmids extracted using a commercial yeast plasmid purification kit (Zymo Research) in which the yeast cell walls are disrupted with Zymolase and the DNA is subsequently purified by a DNA mini-column. Plasmid DNA is then transformed into *E.coli* for amplification, followed by mini-prep isolation of the plasmid DNA with an *E. coli* plasmid purification kit (Qiagen). Plasmid DNA is then prepared for transformation into the appropriate yeast strain for subsequent cycles selection or sequencing and IgG production.

[0125] *Error-prone PCR optimization.* Error-prone PCR-based mutagenesis of the heavy chain (VH) and/or light chain (VL) using standard molecular biology techniques introduced stochastic diversity. Briefly, mutagenic nucleotide analogues dPTP and 8-oxo-dGTP were incorporated into the VH and VL amplification process at 1 uM concentration to increase the base mis-incorporation frequency up to approx. 0.01 bp. The mutated PCR product was recombined *in situ* by homologous recombination with a linearized vector containing the HC or LC constant region sequences. This typically results in a library of 1 x 10^{7-8} diversity. Affinity and expression co-pressures were applied by incubating the antigen antibody yeast complex at decreasing concentrations of antigen (equilibrium pressure) or

with parental Fab competition (equilibrium and kinetic pressures) for varying amounts of time to select for the highest affinity antibodies on FACS over successive rounds of selection.

[0126] *Oligonucleotide-based CDR H3 mutagenesis.* Discovered or previously optimized antibodies can progress through additional optimization by diversification of the CDR H3 sequence. To do so, the light chain variable region of the starting antibody is PCR amplified, and then, using yeast homologous recombination, is inserted into a yeast strain containing the light chain empty vector. This constitutes the parental light chain yeast strain. The heavy chain of the starting antibody is used as PCR input in combination with germline specific primers that generate a PCR product that contains from framework 1 through framework three of the heavy chain. This amplification is perform using the mutagenic nucleotide 8-oxo-dGTP to provide additional low levels of mutagenesis in the amplified heavy chain region. In order to create designed diversity in the CDR H3 region of the starting antibody, a library of CDR H3 oligos is generated/ordered (i.e. from IDT). The oligo pool is amplified with primers containing 5' tails that allow for germline specific recombination with the amplified FW1-FW3 region and the empty. A universal 3' primer is used for FW4. Alternatively, mutagenic PCR can be performed that incorporates 8-oxo-dGTP into a PCR reaction using germline specific 5' primers, the universal 3' primer and VH DNA. Once the LC strain, HC FW1-FW3 and diversified CDR H3-FW4 inputs have been generated, a three piece transformation is performed by introducing the two HC components along with a HC empty vector into the LC strain. Subsequently, the cells are grown out under selective pressure to ensure HC and LC components are present.

[0127] *Antibody yeast production and purification.* Yeast clones were grown to saturation and then induced for 48 h at 30°C with shaking. After induction, yeast cells were pelleted and the supernatants were harvested for purification. IgGs were purified using a Protein A column and eluted with acetic acid, pH 2.0. Fab fragments were generated by papain digestion and purified over KappaSelect or CaptureSelect IgG-CH1 (GE Healthcare LifeSciences).

[0128] *Antibody HEK production and purification.* Mammalian expression of IgG was done by sub-cloning antibodies into a new expression vector followed by transient transfection and expression in HEK293ADI1, a monoclonal cell line derived from HEK293 (DSMZ) selected for clump-free growth, growth rate, and transfectability. Briefly, expression vectors containing the antibody of interest were transfected by complexing with a transfection

reagent followed by exposure to HEK cells for one hour followed by dilution of culture media to a final density of 4 million cells per mL. The cells were then cultured for 7 days with fresh feed media every 48 hours. After 7 days, the supernatant was collected following centrifugation and purification was performed using protein A. If necessary, a CHT column purification was added to reach > 95 % monomer.

[0129] *ForteBio K_D measurements (Biolayer interferometry; BLI).* ForteBio affinity measurements were performed generally as previously described (Estep, P., *et al.*, High throughput solution-based measurement of antibody-antigen affinity and epitope binning. MAbs, 2013. 5(2): p. 270-8.). Briefly, ForteBio affinity measurements were performed by loading IgGs online onto AHQ sensors. Sensors were equilibrated off-line in assay buffer for 30 min and then monitored on-line for 60 seconds for baseline establishment. Sensors with loaded IgGs were exposed to 100 nM antigen for 5 min, afterwards they were transferred to assay buffer for 5 min for off-rate measurement. Kinetics was analyzed using the 1:1 binding model.

[0130] *BiaCore K_D measurements (Surface plasmon resonance; SPR).* Biosensor analysis was conducted at 25 °C in HBS-EP buffer system (10 mM HEPES pH 7.3, 150 mM NaCl, 3 mM EDTA, 0.05% Surfactant P20) using a Biacore 8K optical biosensor docked with a CM5 sensor chip (GE Healthcare, Marlboro, MA). The sample hotel was maintained at 10°C. Goat anti-human IgG capture antibody (Jackson ImmunoResearch Laboratories, Inc., West Grove, PA; 109-005-098) was immobilized (11700 +/- 400 RU) to both flow cells of the sensor chip using standard amine coupling chemistry. This surface type provided a format for reproducibly capturing fresh analysis antigen after each regeneration step. Flow cell 2 was used to analyze captured antigen (35.7 +/-0.8 RU) while flow cell 1 was used as a reference flow cell. Fab concentrations ranging from 100 to 0.412 nM (3-fold dilutions) were prepared in running buffer. Each of the Fab sample concentrations were run as a single replicate. Two blank (buffer) injections also were run and used to assess and subtract system artifacts. The association and dissociation phases for all Fab concentrations were monitored for 180 s each, at a flow rate of 30 µL/min. The surface was regenerated with 10 mM glycine, pH 1.5 for 30 s, at a flow rate of 30 µL/min. The data was aligned, double referenced, and fit using Biacore 8K Evaluation Software, version 1.0.

[0131] *Octet Red384 Epitope Binning/ligand blocking.* Epitope binning/ligand blocking was performed using a standard sandwich format cross-blocking assay. Control anti-

target IgG was loaded onto AHQ sensors and unoccupied Fc-binding sites on the sensor were blocked with an irrelevant human IgG1 antibody. The sensors were then exposed to 100 nM target antigen followed by a second anti-target antibody or ligand. Data was processed using ForteBio's Data Analysis Software 7.0. Additional binding by the second antibody or ligand after antigen association indicates an unoccupied epitope (non-competitor), while no binding indicates epitope blocking (competitor or ligand blocking).

[0132] *Size Exclusion Chromatography.* A TSKgel SuperSW mAb HTP column (22855) was used for fast SEC analysis of yeast and mammalian produced mAbs at 0.4 mL/min with a cycle time of 6 min/run. 200 mM Sodium Phosphate and 250 mM Sodium Chloride was used as the mobile phase.

[0133] *Dynamic Scanning Fluorimetry (DSF).* 10 µL of 20x Sypro Orange is added to 20 µL of 0.2-1mg/mL mAb or Fab solution. A RT-PCR instrument (BioRad CFX96 RT PCR) is used to ramp the sample plate temperature from 40 to 95°C at 0.5°C increments, with 2 min equilibrate at each temperature. The negative of first derivative for the raw data is used to extract Tm.

[0134] *PSR Preparation.* Polyspecific reactivity reagent (PSR) was prepared as described in, e.g., WO 2014/179363 and Xu et al., *mAbs*, 2013. In brief, 2.5 liters CHO-S cells were used as starting material. The cells were pelleted at 2,400 x g for 5 min in 500 mL centrifuge bottles filled to 400 mL. Cell pellets were combined and then resuspended in 25 ml Buffer B and pelleted at 2,400 x g for 3 min. The buffer was decanted and the wash repeated one time. Cell pellets were resuspend in 3x the pellet volume of Buffer B containing 1 x protease inhibitors (Roche, Complete, EDTA-free) using a polytron homogenizer with the cells maintained on ice. The homogenate was then centrifuged at 2,400 x g for 5 min and the supernatant retained and pelleted one additional time (2,400 x g/5min) to ensure the removal of unbroken cells, cell debris and nuclei; the resultant supernatant is the total protein preparation. The supernatant was then transferred into two Nalgene Oak Ridge 45 mL centrifuge tubes and pelleted at 40,000 x g for 40 min at 4°C. The supernatants containing the Separated Cytosolic Proteins (SCPs) were then transferred into clean Oak Ridge tubes, and centrifuged at 40,000 x g one more time. In parallel, the pellets containing the membrane fraction (EMF) were retained and centrifuged at 40,000 for 20 min to remove residual supernatant. The EMF pellets were then rinsed with Buffer B. 8 mL Buffer B was then added to the membrane pellets to dislodge the pellets and transfer into a Dounce Homogenizer.

After the pellets were homogenized, they were transferred to a 50 mL conical tube and represented the final EMF preparation.

[0135] One billion mammalian cells (e.g. CHO, HEK293, Sf9) at ~10^6 – 10^7 cells/mL were transferred from tissue culture environment into 4x 250 mL conical tubes and pelleted at 550 x g for 3 min. All subsequent steps were performed at 4°C or on ice with ice-cold buffers. Cells were washed with 100 mL of PBSF (1x PBS + 1 mg/mL BSA) and combined into one conical tube. After removing the supernatant, the cell pellet was then re-suspended in 30 mL Buffer B (50 mM HEPES, 0.15 M NaCl, 2 mM $CaCl_2$, 5 mM KCl, 5 mM $MgCl_2$, 10 % Glycerol, pH 7.2) and pelleted at 550 x g for 3 min. Buffer B supernatant was decanted and cells re-suspended in 3x pellet volume of Buffer B plus 2.5x protease inhibitor (Roche, cOmplete, EDTA-free). Protease inhibitors in Buffer B were included from here on forward. Cells were homogenized four times for 30 sec pulses (Polyton homogenizer, PT1200E) and the membrane fraction was pelleted at 40,000 x g for 1 hour at 4°C. The pellet is rinsed with 1 mL Buffer B; the supernatant is retained and represents the s. The pellet is transferred into a Dounce homogenizer with 3 mL of Buffer B and re-suspended by moving the pestle slowly up and down for 30-35 strokes. The enriched membrane fraction (EMF) is moved into a new collection tube, rinsing the pestle to collect all potential protein. Determine the protein concentration of the purified EMF using the Dc-protein assay kit (BioRad). To solubilize the EMF, transfer into Solubilization Buffer (50 mM HEPES, 0.15 M NaCl, 2 mM $CaCl_2$, 5 mM KCl, 5 mM $MgCl_2$, 1 % n-Dodecyl-b-D-Maltopyranoside (DDM), 1x protease inhibitor, pH 7.2) to a final concentration of 1 mg/mL. Rotate the mixture overnight at 4°C rotating followed by centrifugation in a 50 mL Oak Ridge tube (Fisher Scientific, 050529-ID) at 40,000 x g for 1 hour. Collect the supernatant which represents the soluble membrane proteins (SMPs) and quantify the protein yield as described above.

[0136] For biotinylation, prepare the NHS-LC-Biotin stock solution according to manufacturer's protocol (Pierce, Thermo Fisher). In brief, 20 µl of biotin reagent is added for every 1 mg of EMF sample and incubated at 4°C for 3 hours with gentle agitation. Adjust the volume to 25 mL with Buffer B and transfer to an Oak Ridge centrifuge tube. Pellet the biotinylated EMF (b-EMF) at 40,000 x g for 1 hour, and rinse two times with 3 mL of Buffer C (Buffer B minus the glycerol) without disturbing the pellet. Remove the residual solution. Re-suspended the pellet with a Dounce homogenizer in 3 mL of Buffer C as described

previously. The re-suspended pellet now represents biotinylated EMF (b-EMF). Solubilized as described above to prepare b-SMPs.

[0137] *PSR Binding Analyses.* Assays were performed generally as described in, e.g., Xu et al. To characterize the PSR profile of monoclonal antibodies presented on yeast, two million IgG-presenting yeast were transferred into a 96-well assay plate and pellet at 3000 x g for 3 min to remove supernatant. Re-suspend the pellet in 50 µl of freshly prepared 1:10 dilution of stock b-PSRs and incubate on ice for 20 minutes. Wash the cells twice with 200 µl of cold PBSF and pellet re-suspended in 50 µl of secondary labeling mix (Extravidin-R-PE, anti-human LC-FITC, and propidium iodide). Incubate the mix on ice for 20 minutes followed by two washes with 200 µl ice-cold PBSF. Re-suspend the cells in 100 µl of ice-cold PBSF and run the plate on a FACSCanto (BD Biosciences) using HTS sample injector. Flow cytometry data was analyzed for mean fluorescence intensity in the R-PE channel and normalized to proper controls in order to assess non-specific binding. Numerous methods for presentation or display of antibodies or antibody fragments on the surface of yeast have been described previously, all of which are consistent with this protocol (Blaise et al., 2004, Boder and Wittrup, 1997, Kuroda and Ueda, 2011, Orcutt and Wittrup, 2010, Rakestraw et al., 2011, Sazinsky et al., 2008, Tasumi et al., 2009, Vasquez et al., 2009).

[0138] *ForteBio Kinetics.* FortBio Octet HTX instruments were used in 12 channel mode (8 sensors per channel, 96 sensors per experiment) with either AHC, SA, or AHQ sensors. Instrumentation was driven by manufacturer supplied software (versions 8.2 and 9.0). Sample names and concentrations were input into the plate data page, and sensor associated proteins were identified in the "information" column on the sensor data page. Kinetic experiments are collected with either a 90 or 180 s baseline, 180 s association phase, and 180 s dissociation phase. Binning experiments were collected in 5 steps: 90 s of baseline1, 90 s of a sensor binding check with the secondary binder, 90 s of baseline2, 180 s of association, and 180 s of dissociation in the well containing the secondary mAb. All files were saved into a shared network drive with a naming convention that identifies the format of the experiment.

[0139] *HIC.* IgG1 samples were buffer exchanged into 1 M ammonium sulfate and 0.1 M sodium phosphate at pH 6.5 using a Zeba 40 kDa 0.5 mL spin column (Thermo Pierce, cat # 87766). A salt gradient was established on a Dionex ProPac HIC-10 column from 1.8 M ammonium sulfate, 0.1 M sodium phosphate at pH 6.5 to the same condition without

ammonium sulfate. The gradient ran for 17 min at a flow rate of 0.75 ml/min. An acetonitrile wash step was added at the end of the run to remove any remaining protein and the column was re-equilibrated over 7 column volumes before the next injection cycle. Peak retention times were monitored at A280 absorbance and concentrations of ammonium sulfate at elution were calculated based on gradient and flow rate.

[0140] *LCMS.* mAb samples were reduced by DTT, followed by middle down LCMS analysis on a Bruker maXis4G mass spectrometer coupled with an Agilent 1100 HPLC (Agilent). A POROS R2 10 µm (2.1 x 30 mm) reversed phase column was used to remove salt in the samples. A fast LC flow at 2 mL/min allows the separation between sample and salt and elution of samples and regeneration of column to finish within a 2.1 min cycle. A T-junction is used to deliver only 0.15 mL/min sample flow into the mass spectrometer for sample analysis. The Bruker maXis 4G mass spectrometer was run in positive ion mode with detection in the range of 750 to 2500 m/z. The remaining source parameters were set as follows; the capillary was set at 5500V, the Nebulizer at 4.0 Bar, dry gas at 4.0 l/min, and dry temp set at 200°C.

[0141] MS spectra were analyzed using Bruker Data Analysis version 4.1 and the deconvolution was accomplished using maximum entropy deconvolution with a mass range of 20 to 30 kDa.

[0142] An informal sequence listing is provided in Table 4. The informal sequence listing provides the heavy chain variable region ("HC") amino acid sequence, with each of the heavy chain variable region CDRs underlined, and the light chain variable region ("LC"), with each of the light chain variable region CDRs underlined.

Table 4. Informal sequence listing.

Antibody No.	SEQ ID NO:	Sequence	Clone # (ADI)	Descriptors
Ab1	17	QVQLVQSGAEVKKPGASVKVSCKASGFNIKDYYMH WVRQAPGQRLEWIGWIDLENANTVYDAKFQGRVTIT RDTSASTAYMELSSLRSEDTAVYYCARDAYGRYFYD VWGQGTLVTVSS	ADI-42594	HC amino acid sequence
Ab1	18	DIVMTQSPDSLAVSLGERATINCKSSQSLLNARTGKN YLAWYQQKPGQPPKLLIYWASTRESGVPDRFSGSGS GTDFTLTISSLQAEDVAVYYCKQSYFRRTFGGGTKVE IK	ADI-42594	LC amino acid sequence
Ab2	17	QVQLVQSGAEVKKPGASVKVSCKASGFNIKDYYMH WVRQAPGQRLEWIGWIDLENANTVYDAKFQGRVTIT RDTSASTAYMELSSLRSEDTAVYYCARDAYGRYFYD VWGQGTLVTVSS	ADI-42596	HC amino acid sequence
Ab2	19	DIVMTQSPDSLAVSLGERATINCKSSQSLLNARTRKN YLAWYQQKPGQPPKLLIYWASTRESGVPDRFSGSGS GTDFTLTISSLQAEDVAVYYCKQSYFRRTFGGGTKVE IK	ADI-42596	LC amino acid sequence
Ab3	20	QVQLVQSGAEVKKPGASVKVSCKASGFNIKDYYMH WVRQAPGQRLEWIGWIDLENANTVYDAKFQGRVTI ARDTSASTVYMELSSLRSEDTAVYYCARDAYGRYFY DVWGQGTLVTVSS	ADI-45967	HC amino acid sequence
Ab3	21	DIVMTQSPDSLAVSLGERAIINCKSSQSLLNARTGKN YLAWYQQKPGQPPKLLIYWASTRESGVPDRFSGSGS GTDFTLTISSLQAEDVAVYYCTQSYFRRTFGGGTKVE IK	ADI-45967	LC amino acid sequence
Ab4	22	QVQLVQSGAEVKKPGASVKVSCKASGFNIKDYYMH WVRQAPGQRLEWIGWIDLENANTVYDAKFQGRVTIT	ADI-45968	HC amino acid sequence

Antibody No.	SEQ ID NO:	Sequence	Clone # (ADI)	Descriptors
		RDASASTAYMELSSLRSEDTAVYYCARDAYGRYFY DVWGQGTLVTVSS		
Ab4	23	DIVMTQSPDSLAVSLGERATINCKSSQSLLNARTGKN YLAWYQQKPGQPPKLLIYWASTRESGVPDRFSGSGS GTDFTLTISSLQAEDVAVYYCVQSYFRRTFGGGTKVE IK	ADI-45968	LC amino acid sequence
Ab5	24	QVQLVQSGAEVKKPGASVKVSCKASGFNIKDYYMH WVRQAPGQRLEWIGWIDLENANTVYDAKFQGRVTIT RDTSASTAYMELGSLRSEDTAVYYCARDAYGRYFY DVWGQGTLVTVSS	ADI-45969	HC amino acid sequence
Ab5	25	DIVMTQSPDSLAVSLGERATINCKSSQSLLNARTGKN YLAWYQQKPGQPPKLLIYWASTRESGVPDRFSGSGS GTDFTLTISSLQAEDVAVYYCAQSYFRRTFGGGTKVE IK	ADI-45969	LC amino acid sequence
Ab6	26	QVQLVQSGAEVKKPGASVKVSCKASGFNVKDYYMH WVRQAPGQRLEWIGWIDLENANTVYDAKFQGRVTIT RDTSASTAYMELSSLRSEDTAVYYCARDAYGRYFYD VWGQGTLVTVSS	ADI-45970	HC amino acid sequence
Ab6	18	DIVMTQSPDSLAVSLGERATINCKSSQSLLNARTGKN YLAWYQQKPGQPPKLLIYWASTRESGVPDRFSGSGS GTDFTLTISSLQAEDVAVYYCKQSYFRRTFGGGTKVE IK	ADI-45970	LC amino acid sequence
Ab7	27	QVQLVQSGAEVKKPGATVKVSCKASGFNIKDYYIHW VRQAPGQRLEWIGWIDLENANTVYDAKFQGRVTITR DTSASTAYMELSSLRSEDTAVYYCARDAYGRYFYDV WGQGTLVTVSS	ADI-45971	HC amino acid sequence
Ab7	28	DIVMTQSPDSLAVSLGERATINCKSSQSLLNARTRKN YLAWYQQKPGQPPKLLIYWASTRESGVPDRFSGSGS	ADI-45971	LC amino acid sequence

Antibody No.	SEQ ID NO:	Sequence	Clone # (ADI)	Descriptors
		GTDFTLTISSLQAEDVAVYYCVQSYFRRTFGGGTKVE IK		
Ab8	29	QVQLVQSGAEVKRPGASVKVSCKASGFNIKDYYMH WVRQAPGQRLEWIGWIDLENANTVYDAKFQGRVTIT RDTSASTAYMELSSLRSEDTAVYYCARDAYGRYFYD VWGQGTLVTVSS	ADI-45972	HC amino acid sequence
Ab8	23	DIVMTQSPDSLAVSLGERATINCKSSQSLLNARTGKN YLAWYQQKPGQPPKLLIYWASTRESGVPDRFSGSGS GTDFTLTISSLQAEDVAVYYCVQSYFRRTFGGGTKVE IK	ADI-45972	LC amino acid sequence
Ab9	30	QVQLVQSEAEVKKPGASVKVSCKASGFNIKDYYMH WVRQAPGQRLEWIGWIDLENANTVYDAKFQGRVTIT RDTSASTAYMELSSLRSEDTAVYYCARDAYGRYFYD VWGQGTLVTVSS	ADI-45973	HC amino acid sequence
Ab9	18	DIVMTQSPDSLAVSLGERATINCKSSQSLLNARTGKN YLAWYQQKPGQPPKLLIYWASTRESGVPDRFSGSGS GTDFTLTISSLQAEDVAVYYCKQSYFRRTFGGGTKVE IK	ADI-45973	LC amino acid sequence
Ab10	31	QVQLVQSGAEVKKPGASVKVSCKASGFNIKDYYMH WVRQAPGQRLEWIGWIDLENANTVYDAKFQGRVTIT RDTSASTAYMELSNLRSEDTAVYYCARDAYGRYFY DVWGQGTLVTVSS	ADI-45974	HC amino acid sequence
Ab10	18	DIVMTQSPDSLAVSLGERATINCKSSQSLLNARTGKN YLAWYQQKPGQPPKLLIYWASTRESGVPDRFSGSGS GTDFTLTISSLQAEDVAVYYCKQSYFRRTFGGGTKVE IK	ADI-45974	LC amino acid sequence
Ab11	32	QVQLVQSGAEVKKPGASVKVSCKASGFNIKDYYMH WVRQAPGQRLEWIGWIDLENANTVYDAKFQGRVTIT	ADI-45975	HC amino acid sequence

Antibody No.	SEQ ID NO:	Sequence	Clone # (ADI)	Descriptors
		RDTSASTAYMELSSLRSEDTAVYYCARDAYGQYFYD VWGQGTLVTVSS		
Ab11	19	DIVMTQSPDSLAVSLGERATINCKSSQSLLNARTRKN YLAWYQQKPGQPPKLLIYWASTRESGVPDRFSGSGS GTDFTLTISSLQAEDVAVYYCKQSYFRRTFGGGTKVE IK	ADI-45975	LC amino acid sequence
Ab12	33	QVQLVQSGAEVKKPGASVKVSCKASGFNIKDYYMH WVRQAPGQRLEWIGWIDLENANTVYDAKFQGRVTIT RDTSASTAYMELSSLRSEDTAVYYCVRDAYGRYFYD VWGQGTLVTVSS	ADI-45976	HC amino acid sequence
Ab12	23	DIVMTQSPDSLAVSLGERATINCKSSQSLLNARTGKN YLAWYQQKPGQPPKLLIYWASTRESGVPDRFSGSGS GTDFTLTISSLQAEDVAVYYCVQSYFRRTFGGGTKVE IK	ADI-45976	LC amino acid sequence
Ab13	17	QVQLVQSGAEVKKPGASVKVSCKASGFNIKDYYMH WVRQAPGQRLEWIGWIDLENANTVYDAKFQGRVTIT RDTSASTAYMELSSLRSEDTAVYYCARDAYGRYFYD VWGQGTLVTVSS	ADI-45959	HC amino acid sequence
Ab13	34	DIVMSQSPDSLAVSLGERATINCKSSQSLLNARTGKN YLAWYQQKPGQPPKLLIYWASTRSSGVPDRFSGSGS GTDFTLTISSLQAEDVAVYYCVQSYFRRTFGGGTKVE IK	ADI-45959	LC amino acid sequence
Ab14	35	QVQLVQSGAEVKKPGASVKVSCKASGFNIKDYYMH WVRQAPGQRLEWIGWIDLENANTVYDAKLQGRVTI TRDTSASTAYMELSSLGSEDTAVYYCARDAYGRYFY DVWGQGTLVTVSS	ADI-45977	HC amino acid sequence
Ab14	36	DIVMTQSPDSLAVSLGERATINCKSSQSLLNARTGKN YLAWYQQKPGQPPKLLIYWASTRESGVPDRFSGSGS	ADI-45977	LC amino acid sequence

Antibody No.	SEQ ID NO:	Sequence	Clone # (ADI)	Descriptors
		GTDFTLTISSLQAEDVAVYYCTQSYFRRTFGGGTKVE IK		
Ab15	37	QVQLVQSGAEVKKPGASVKVSCKASGFNIKDYYMH WVRQAPGQRLEWLGWIDLENANTVYDAKFQGRVTI TRDTSASTAYMELSSLRSEDTAVYYCARDAYGRYFY DVWGQGTLVTVSS	ADI-45978	HC amino acid sequence
Ab15	38	DIVMTQSPDSLAVSLGERATINCKSSQSLLNARTGKN YLAWYQQKPGQPPKLLIYWASARESGVPDRFSGSGS GTDFTLTISSLQAEDVAVYYCTQSYFRRTFGGGTKVE IK	ADI-45978	LC amino acid sequence
Ab16	17	QVQLVQSGAEVKKPGASVKVSCKASGFNIKDYYMH WVRQAPGQRLEWIGWIDLENANTVYDAKFQGRVTIT RDTSASTAYMELSSLRSEDTAVYYCARDAYGRYFYD VWGQGTLVTVSS	ADI-45979	HC amino acid sequence
Ab16	39	DIVMTQSPDSLAVSLGERATINCKSSQELLNARTGKN YLAWYQQKPGQPPKLLIYWASTRESGVPDRFSGSGS GTDFTLTISSLQAEDVAVYYCKQSYFRRTFGGGTKVE IK	ADI-45979	LC amino acid sequence
Ab17	40	QVQLVQSGAEVKKPGASVKVSCKASGFNIKDYYMH WVRQAPGQRLEWIGWIDLENANTVYDAKFQGRVTIT RDTSASTAYMELNSLRSEDTAVYYCARDAYGRYFY DVWGQGTLVTVSS	ADI-45980	HC amino acid sequence
Ab17	41	DIVMTQSPDSLAVSLGERATINCKSSQSLLNARTGKN YLAWYQQKPGQPPKLLIYWAFTRESGVPDRFSGSGS GTDFTLTISSLQAEDVAVYYCTQSYFRRTFGGGTKVE IK	ADI-45980	LC amino acid sequence
Ab18	17	QVQLVQSGAEVKKPGASVKVSCKASGFNIKDYYMH WVRQAPGQRLEWIGWIDLENANTVYDAKFQGRVTIT	ADI-45981	HC amino acid sequence

Antibody No.	SEQ ID NO:	Sequence	Clone # (ADI)	Descriptors
		RDTSASTAYMELSSLRSEDTAVYYCARDAYGRYFYD VWGQGTLVTVSS		
Ab18	42	DIVMTQSPDSLAVSLGERATINCKSSQSLLNARTGKN YLAWYQQKPGQPPKLLIYWASMRESGVPDRFSGSRS GTDFTLTISSLQAEDVAVYYCSQSYFRRTFGGGTKVE IK	ADI-45981	LC amino acid sequence
Ab19	17	QVQLVQSGAEVKKPGASVKVSCKASGFNIKDYYMH WVRQAPGQRLEWIGWIDLENANTVYDAKFQGRVTIT RDTSASTAYMELSSLRSEDTAVYYCARDAYGRYFYD VWGQGTLVTVSS	ADI-45982	HC amino acid sequence
Ab19	43	DIVMTQSPDSLAVSLGERATINCKSSQSLLNARTGKN YLAWYQQKPGQPPKLLIYWASTRENGVPDRFSGSGS GTDFTLTISSLQAEDVAVYYCTQSYFRRTFGGGTKVE IK	ADI-45982	LC amino acid sequence
Ab20	44	QVQLVQSGAEVKKPGASVKVSCKASGFNIKDYYMH WVRQAPGQRLEWIGWIDLENANTVYDAKFQGRVTIT RDTSAGTAYMELSSLRSEDTAVYYCARDAYGRYFY DVWGQGTLVTVSS	ADI-45983	HC amino acid sequence
Ab20	45	DIVMTQSPDSLAVSLGERATINCKSSQSLLNARTMKN YLAWYQQKPGQPPKLLIYAASTRESGVPDRFSGSGS GTDFTLTISSLQAEDVAVYYCAQSYFRRTFGGGTKVE IK	ADI-45983	LC amino acid sequence
Ab21	17	QVQLVQSGAEVKKPGASVKVSCKASGFNIKDYYMH WVRQAPGQRLEWIGWIDLENANTVYDAKFQGRVTIT RDTSASTAYMELSSLRSEDTAVYYCARDAYGRYFYD VWGQGTLVTVSS	ADI-45984	HC amino acid sequence
Ab21	28	DIVMTQSPDSLAVSLGERATINCKSSQSLLNARTRKN YLAWYQQKPGQPPKLLIYWASTRESGVPDRFSGSGS	ADI-45984	LC amino acid sequence

Antibody No.	SEQ ID NO:	Sequence	Clone # (ADI)	Descriptors
		GTDFTLTISSLQAEDVAVYYCVQSYFRRTFGGGTKVE IK		
Ab22	17	QVQLVQSGAEVKKPGASVKVSCKASGFNIKDYYMH WVRQAPGQRLEWIGWIDLENANTVYDAKFQGRVTIT RDTSASTAYMELSSLRSEDTAVYYCARDAYGRYFYD VWGQGTLVTVSS	ADI-45985	HC amino acid sequence
Ab22	46	DIVMTQSPDSLAVSLGERATINCKTSQSLLNARTNKN YLAWYQQKPGQPPKLLIYWASTRESGVPDRFSGSGS GTDFTLTISSLQAEDVAVYYCVQSYFRRTFGGGTKVE IK	ADI-45985	LC amino acid sequence
Ab23	17	QVQLVQSGAEVKKPGASVKVSCKASGFNIKDYYMH WVRQAPGQRLEWIGWIDLENANTVYDAKFQGRVTIT RDTSASTAYMELSSLRSEDTAVYYCARDAYGRYFYD VWGQGTLVTVSS	ADI-45960	HC amino acid sequence
Ab23	47	DIVMTQSPDSLAVSLGERATINCKSSQSLLNARTGKN YLAWYQQKPGQPPKLLIYWASTRESGVPDRFSGSGS GTDFTLTISSLQAEDAAVYYCAQSYFRRTFGGGTKVE IK	ADI-45960	LC amino acid sequence
Ab24	17	QVQLVQSGAEVKKPGASVKVSCKASGFNIKDYYMH WVRQAPGQRLEWIGWIDLENANTVYDAKFQGRVTIT RDTSASTAYMELSSLRSEDTAVYYCARDAYGRYFYD VWGQGTLVTVSS	ADI-45961	HC amino acid sequence
Ab24	48	DIVMTQSPDSLAVSLGERATINCKSSQSLLNARTRRN YLAWYQQKPGQPPKLLIYWGSTRESGVPDRFSGSGS GTDFTLTISSLQAEDVAVYYCKQSYFRRTFGGGTKVE IK	ADI-45961	LC amino acid sequence
Ab25	49	QVQLVQSGAEVEEPGASVKVSCKASGFNIKDYYMH WVRQAPGQRLEWIGWIDLENANTVYDAKFQGRVTIT	ADI-45962	HC amino acid sequence

Antibody No.	SEQ ID NO:	Sequence	Clone # (ADI)	Descriptors
		RDTSASTAYMELSSLRSEDTAVYYCARDAYGRYFYD VWGQGTLVTVSS		
Ab25	23	DIVMTQSPDSLAVSLGERATINCKSSQSLLNARTGKN YLAWYQQKPGQPPKLLIYWASTRESGVPDRFSGSGS GTDFTLTISSLQAEDVAVYYCVQSYFRRTFGGGTKVE IK	ADI-45962	LC amino acid sequence
Ab26	50	QVQLVQSGAEVKKPGAPVKVSCKASGFNIKDYYMH WVRQAPGQRLEWIGWIDLENANTVYDAKFQGRVTIT RDTSASTAYMELSSLRSEDTAVYYCARDAYGRYFYD VWGQGTLVTVSS	ADI-45963	HC amino acid sequence
Ab26	18	DIVMTQSPDSLAVSLGERATINCKSSQSLLNARTGKN YLAWYQQKPGQPPKLLIYWASTRESGVPDRFSGSGS GTDFTLTISSLQAEDVAVYYCKQSYFRRTFGGGTKVE IK	ADI-45963	LC amino acid sequence
Ab27	51	QVQLVQSGAEVKKPGASVKVSCKASGFNIKDYYIHW VRQAPGQRLEWIGWIDLENANTVYDAKFQGRVTITR DTSASTAYMELSSLRSEDTAVYYCARDAYGRYFYDV WGQGTLVTVSS	ADI-45964	HC amino acid sequence
Ab27	25	DIVMTQSPDSLAVSLGERATINCKSSQSLLNARTGKN YLAWYQQKPGQPPKLLIYWASTRESGVPDRFSGSGS GTDFTLTISSLQAEDVAVYYCAQSYFRRTFGGGTKVE IK	ADI-45964	LC amino acid sequence
Ab28	52	QVQLVQSGAEVKKPGASVKVSCKASGFNIKDYYMH WVRQAPGQRLEWIGWIDLENANAVYDAKFQGRVTI TRDTSASTAYMELSSLRSEDTAVYYCARDAYGRYFY DVWGQGTLVTVSS	ADI-45965	HC amino acid sequence
Ab28	53	DIVMTQSPDSLAVSLGGRATINCKSSQSLLNARTGKN YLAWYQQKPGQPPKLLIYWASTRESGVPDRFSGSGS	ADI-45965	LC amino acid sequence

Antibody No.	SEQ ID NO:	Sequence	Clone # (ADI)	Descriptors
		GTDFTLTISSLQAEDVAVYYCVQSYFRRTFGGGTKVE IK		
Ab29	54	QVQLVQSGAEVKKPGASVKVSCKASGFNIKDYYMH WVRQAPGQRLEWIGWIDLENANTVYDAKFQGRVAI TRDTSASTAYMELSSLRSEDTAVYYCARDAYGRYFY DVWGQGTLVTVSS	ADI-45966	HC amino acid sequence
Ab29	36	DIVMTQSPDSLAVSLGERATINCKSSQSLLNARTGKN YLAWYQQKPGQPPKLLIYWASTRESGVPDRFSGSGS GTDFTLTISSLQAEDVAVYYCTQSYFRRTFGGGTKVE IK	ADI-45966	LC amino acid sequence
Ab30	17	QVQLVQSGAEVKKPGASVKVSCKASGFNIKDYYMH WVRQAPGQRLEWIGWIDLENANTVYDAKFQGRVTIT RDTSASTAYMELSSLRSEDTAVYYCARDAYGRYFYD VWGQGTLVTVSS	ADI-46357	HC amino acid sequence
Ab30	25	DIVMTQSPDSLAVSLGERATINCKSSQSLLNARTGKN YLAWYQQKPGQPPKLLIYWASTRESGVPDRFSGSGS GTDFTLTISSLQAEDVAVYYCAQSYFRRTFGGGTKVE IK	ADI-46357	LC amino acid sequence
Ab31	31	QVQLVQSGAEVKKPGASVKVSCKASGFNIKDYYMH WVRQAPGQRLEWIGWIDLENANTVYDAKFQGRVTIT RDTSASTAYMELSNLRSEDTAVYYCARDAYGRYFY DVWGQGTLVTVSS	ADI-46358	HC amino acid sequence
Ab31	36	DIVMTQSPDSLAVSLGERATINCKSSQSLLNARTGKN YLAWYQQKPGQPPKLLIYWASTRESGVPDRFSGSGS GTDFTLTISSLQAEDVAVYYCTQSYFRRTFGGGTKVE IK	ADI-46358	LC amino acid sequence
Ab32	55	QVQLVQSGAEVKKPGASVKVSCKASGFNIKDYYMH WVRQAPGQRLEWIGWIDLENANTVYDAKFQGRVTIT	ADI-46367	HC amino acid sequence

Antibody No.	SEQ ID NO:	Sequence	Clone # (ADI)	Descriptors
		RDTSASIAYMELSSLRSEDTAVYYCARDAYGRYFYD VWGQGTLVTVSS		
Ab32	25	DIVMTQSPDSLAVSLGERATINCKSSQSLLNARTGKN YLAWYQQKPGQPPKLLIYWASTRESGVPDRFSGSGS GTDFTLTISSLQAEDVAVYYCAQSYFRRTFGGGTKVE IK	ADI-46367	LC amino acid sequence
Ab33	56	QAQLVQSGAEVKKPGASVKVSCKASGFNIKDYYMH WVRQAPGQRLEWIGWIDLENANTVYDTKFQGRVTIT RDTSASTAYMELSSLRSEDTAVYYCARDAYGRYFYD VWGQGTLVTVSS	ADI-46369	HC amino acid sequence
Ab33	23	DIVMTQSPDSLAVSLGERATINCKSSQSLLNARTGKN YLAWYQQKPGQPPKLLIYWASTRESGVPDRFSGSGS GTDFTLTISSLQAEDVAVYYCVQSYFRRTFGGGTKVE IK	ADI-46369	LC amino acid sequence
Ab34	57	QVQLVQSGAEVKKPGASVKVSCKASGFNIKDYYMH WVRQAPGQRLEWIGWIDLENANTVYDHKFQGRVTIT RDTSASTAYMELSSLRSEDTAVYYCARDAYGRYFYD VWGQGTLVTVSS	ADI-46370	HC amino acid sequence
Ab34	23	DIVMTQSPDSLAVSLGERATINCKSSQSLLNARTGKN YLAWYQQKPGQPPKLLIYWASTRESGVPDRFSGSGS GTDFTLTISSLQAEDVAVYYCVQSYFRRTFGGGTKVE IK	ADI-46370	LC amino acid sequence
Ab35	58	QAQLVQSGAEVKKPGASVKVSCKASGFNIKDYYMH WVRQAPGQRPEWIGWIDLENANTVYDAKFQGRVTIT RDTSASTAYMELSSLRSEDTAAYYCARDAYGRYFYD VWGQGTLVTVSS	ADI-46371	HC amino acid sequence
Ab35	23	DIVMTQSPDSLAVSLGERATINCKSSQSLLNARTGKN YLAWYQQKPGQPPKLLIYWASTRESGVPDRFSGSGS	ADI-46371	LC amino acid sequence

Antibody No.	SEQ ID NO:	Sequence	Clone # (ADI)	Descriptors
		GTDFTLTISSLQAEDVAVYYCVQSYFRRTFGGGTKVE IK		
Ab36	59	QVQLVQSGAEVKKPGASVKVSCKASGFNIKDYYMH WVRQAPGQRLEWIGWIDLENANTVYDAKFQGRVTIT RDTSAGTAHMELSSLRSEDTAVYYCARDAYGRYFY DVWGQGTPVTVSS	ADI-46372	HC amino acid sequence
Ab36	23	DIVMTQSPDSLAVSLGERATINCKSSQSLLNARTGKN YLAWYQQKPGQPPKLLIYWASTRESGVPDRFSGSGS GTDFTLTISSLQAEDVAVYYCVQSYFRRTFGGGTKVE IK	ADI-46372	LC amino acid sequence
Ab37	60	QVQLVQSGAEVKKPGASVKVSCKASGFNIKDYYMH WVRQAPGQRLEWIGWIDLNNANTVYDAKFQGRVTI TRDTSASTAYMELSSLRSEDTAVYYCARDAYGRYFY DVWGQGTLVTVSS	ADI-46374	HC amino acid sequence
Ab37	23	DIVMTQSPDSLAVSLGERATINCKSSQSLLNARTGKN YLAWYQQKPGQPPKLLIYWASTRESGVPDRFSGSGS GTDFTLTISSLQAEDVAVYYCVQSYFRRTFGGGTKVE IK	ADI-46374	LC amino acid sequence
Ab38	17	QVQLVQSGAEVKKPGASVKVSCKASGFNIKDYYMH WVRQAPGQRLEWIGWIDLENANTVYDAKFQGRVTIT RDTSASTAYMELSSLRSEDTAVYYCARDAYGRYFYD VWGQGTLVTVSS	ADI-46375	HC amino acid sequence
Ab38	61	DIVMTQSPDSLAVSLGERATINCKSSQSLLNARTRKN YLAWYQQKPGQPPKLLIYWASTRESGVPDRFSGSGS GTDFTLTISSLQAEDVAVYYCTQSYFRRTFGGGTKVE IK	ADI-46375	LC amino acid sequence
Ab39	62	QVQLVQSGAEVKKPGASVKVSCKASGFNIKDYYMH WVRQAPGQRLEWIGWIDLENANTVYDAKLQGRVTI	ADI-46376	HC amino acid sequence

Antibody No.	SEQ ID NO:	Sequence	Clone # (ADI)	Descriptors
		TRDTSASTAYMELSSLRSEDTAVYYCARDAYGRYFY DVWGQGTPVTVSS		
Ab39	36	DIVMTQSPDSLAVSLGERATINCKSSQSLLNARTGKN YLAWYQQKPGQPPKLLIYWASTRESGVPDRFSGSGS GTDFTLTISSLQAEDVAVYYCTQSYFRRTFGGGTKVE IK	ADI-46376	LC amino acid sequence
Ab40	63	QVQLVQSGAEVKKPGASVKVSCKASGFNIKDYYMH WVRQAPGQRLEWIGWIDLENANTVYDAKFQGRVTIT RDTSASAAYMELSSLRSEDTAVYYCARDAYGRYFY DVWGQGTLVTVSS	ADI-46378	HC amino acid sequence
Ab40	64	DIVMTQSPDSLAVSLGERATINCKSSQSLLNARTRKN YLAWYQQKPGRPPKLLIYWASTRESGVPDRFSGSGS GTDFTLTISSLQAEDVAVYYCVQSYFRRTFGGGTKVE IK	ADI-46378	LC amino acid sequence
Ab41	63	QVQLVQSGAEVKKPGASVKVSCKASGFNIKDYYMH WVRQAPGQRLEWIGWIDLENANTVYDAKFQGRVTIT RDTSASAAYMELSSLRSEDTAVYYCARDAYGRYFY DVWGQGTLVTVSS	ADI-46379	HC amino acid sequence
Ab41	38	DIVMTQSPDSLAVSLGERATINCKSSQSLLNARTGKN YLAWYQQKPGQPPKLLIYWASARESGVPDRFSGSGS GTDFTLTISSLQAEDVAVYYCTQSYFRRTFGGGTKVE IK	ADI-46379	LC amino acid sequence
Ab42	32	QVQLVQSGAEVKKPGASVKVSCKASGFNIKDYYMH WVRQAPGQRLEWIGWIDLENANTVYDAKFQGRVTIT RDTSASTAYMELSSLRSEDTAVYYCARDAYGQYFYD VWGQGTLVTVSS	ADI-46359	HC amino acid sequence
Ab42	36	DIVMTQSPDSLAVSLGERATINCKSSQSLLNARTGKN YLAWYQQKPGQPPKLLIYWASTRESGVPDRFSGSGS	ADI-46359	LC amino acid sequence

Antibody No.	SEQ ID NO:	Sequence	Clone # (ADI)	Descriptors
		GTDFTLTISSLQAEDVAVYYCTQSYFRRTFGGGTKVE IK		
Ab43	65	QVQLVQSGAEVKKPGASVKVSCKASGFNIKDYYMH WVRQAPGQRLEWIGWIDLENANTVYDTKFQGRVTIT RDTSASTAYMELSSLRSEDTAVYYCARDAYGRYFYD VWGQGTLVTVSS	ADI-46381	HC amino acid sequence
Ab43	66	DIVMTQSPDSLAVSLGERATINCKTSQGLLNARTNKN YLAWYQQKPGQPPKLLIYWASTRESGVPDRFSGSGS GTDFTLTISSLQAEDVAVYYCVQSYFRRTFGGGTKVE IK	ADI-46381	LC amino acid sequence
Ab44	67	QVQLVQSGAEVKKPGASVKVSCKASGFNIKDYYMH WVRQAPGQRPEWIGWIDLENANTVYDAKFQGRVTIT RDTSASTAYMELSSLRSEDAAAYYCARDAYGRYFY DVWGQGTLVTVSS	ADI-46360	HC amino acid sequence
Ab44	68	DIVMTQSPDSLAVSLGERATINCKSSQSLLNARTGKN YLAWYQQKPGQPPELLIYWASTRESGVPDRFSGSGS GTDFTLTISSLQAEDVAVYYCTQSYFRRTFGGGTKVE IK	ADI-46360	LC amino acid sequence
Ab45	69	QVQLVQSGAEVKKPGASVKVSCKASGFNNKDYYMH WVRQAPGQRLEWIGWIDLENANTVYDAKFQGRVTIT RDTSASTAYMELSSLRSEDTAVYYCARDAYGRYFYD VWGQGTLVTVSS	ADI-46361	HC amino acid sequence
Ab45	36	DIVMTQSPDSLAVSLGERATINCKSSQSLLNARTGKN YLAWYQQKPGQPPKLLIYWASTRESGVPDRFSGSGS GTDFTLTISSLQAEDVAVYYCTQSYFRRTFGGGTKVE IK	ADI-46361	LC amino acid sequence
Ab46	70	QVQLVQSGAEVKKPGASVKVSCKASGFNIKDYYMH WVRQAPGQRPEWIGWIDLENANTVYDAKFQGRVTIT	ADI-46362	HC amino acid sequence

Antibody No.	SEQ ID NO:	Sequence	Clone # (ADI)	Descriptors
		RDTSASTAYMELSSLRSEDTAAYYCARDAYGRYFYD VWGQGTLVTVSS		
Ab46	23	DIVMTQSPDSLAVSLGERATINCKSSQSLLNARTGKN YLAWYQQKPGQPPKLLIYWASTRESGVPDRFSGSGS GTDFTLTISSLQAEDVAVYYCVQSYFRRTFGGGTKVE IK	ADI-46362	LC amino acid sequence
Ab47	17	QVQLVQSGAEVKKPGASVKVSCKASGFNIKDYYMH WVRQAPGQRLEWIGWIDLENANTVYDAKFQGRVTIT RDTSASTAYMELSSLRSEDTAVYYCARDAYGRYFYD VWGQGTLVTVSS	ADI-46363	HC amino acid sequence
Ab47	36	DIVMTQSPDSLAVSLGERATINCKSSQSLLNARTGKN YLAWYQQKPGQPPKLLIYWASTRESGVPDRFSGSGS GTDFTLTISSLQAEDVAVYYCTQSYFRRTFGGGTKVE IK	ADI-46363	LC amino acid sequence
Ab48	35	QVQLVQSGAEVKKPGASVKVSCKASGFNIKDYYMH WVRQAPGQRLEWIGWIDLENANTVYDAKLQGRVTI TRDTSASTAYMELSSLGSEDTAVYYCARDAYGRYFY DVWGQGTLVTVSS	ADI-46364	HC amino acid sequence
Ab48	23	DIVMTQSPDSLAVSLGERATINCKSSQSLLNARTGKN YLAWYQQKPGQPPKLLIYWASTRESGVPDRFSGSGS GTDFTLTISSLQAEDVAVYYCVQSYFRRTFGGGTKVE IK	ADI-46364	LC amino acid sequence
Ab49	59	QVQLVQSGAEVKKPGASVKVSCKASGFNIKDYYMH WVRQAPGQRLEWIGWIDLENANTVYDAKFQGRVTIT RDTSAGTAHMELSSLRSEDTAVYYCARDAYGRYFY DVWGQGTPVTVSS	ADI-46365	HC amino acid sequence
Ab49	36	DIVMTQSPDSLAVSLGERATINCKSSQSLLNARTGKN YLAWYQQKPGQPPKLLIYWASTRESGVPDRFSGSGS	ADI-46365	LC amino acid sequence

Antibody No.	SEQ ID NO:	Sequence	Clone # (ADI)	Descriptors
		GTDFTLTISSLQAEDVAVYYCTQSYFRRTFGGGTKVE IK		
Ab50	32	QVQLVQSGAEVKKPGASVKVSCKASGFNIKDYYMH WVRQAPGQRLEWIGWIDLENANTVYDAKFQGRVTIT RDTSASTAYMELSSLRSEDTAVYYCARDAYGQYFYD VWGQGTLVTVSS	ADI-46366	HC amino acid sequence
Ab50	23	DIVMTQSPDSLAVSLGERATINCKSSQSLLNARTGKN YLAWYQQKPGQPPKLLIYWASTRESGVPDRFSGSG GTDFTLTISSLQAEDVAVYYCVQSYFRRTFGGGTKVE IK	ADI-46366	LC amino acid sequence

Table 5. Antibody VH sequence information.

	VH FR1	SEQ ID NO:	VH CDR1	SEQ ID NO:	VH FR2	SEQ ID NO:	VH CDR2	SEQ ID NO:	VH FR3	SEQ ID NO:	VH CDR3	SEQ ID NO:	VH FR4	SEQ ID NO:
ADI-42594	QVQLVQS GAEVKKP GASVKVS CKASG	71	FNIKDY YMH	13	WVRQAP GQRLEW IG	81	WIDLEN ANTVY DAKFQ G	15	RVTITR DTSAST AYMELS SLRSED TAVYYC	89	ARDAYG RYFYDV	103	WGQG TLVTV SS	106
ADI-42596	QVQLVQS GAEVKKP GASVKVS CKASG	71	FNIKDY YMH	13	WVRQAP GQRLEW IG	81	WIDLEN ANTVY DAKFQ G	15	RVTITR DTSAST AYMELS SLRSED TAVYYC	89	ARDAYG RYFYDV	103	WGQG TLVTV SS	106
ADI-46356	QVQLVQS GAEVKKP GASVKVS CKASG	71	FNIKDY YMH	13	WVRQAP GQRLEW IG	81	WIDLEN ANTVY DAKFQ G	15	RVTITR DTSAST AYMELS SLRSED TAVYYC	89	ARDAYG RYFYDV	103	WGQG TLVTV SS	106
ADI-46357	QVQLVQS GAEVKKP GASVKVS CKASG	71	FNIKDY YMH	13	WVRQAP GQRLEW IG	81	WIDLEN ANTVY DAKFQ G	15	RVTITR DTSAST AYMELS SLRSED TAVYYC	89	ARDAYG RYFYDV	103	WGQG TLVTV SS	106
ADI-46358	QVQLVQS GAEVKKP GASVKVS CKASG	71	FNIKDY YMH	13	WVRQAP GQRLEW IG	81	WIDLEN ANTVY DAKFQ G	15	RVTITR DTSAST AYMELS NLRSED TAVYYC	90	ARDAYG RYFYDV	103	WGQG TLVTV SS	106

	VH FR1	SEQ ID NO:	VH CDR1	SEQ ID NO:	VH FR2	SEQ ID NO:	VH CDR2	SEQ ID NO:	VH FR3	SEQ ID NO:	VH CDR3	SEQ ID NO:	VH FR4	SEQ ID NO:
ADI-46359	QVQLVQSGAEVKKPGASVKVSCKASG	71	FNIKDYYMH	13	WVRQAPGQRLEWIG	81	WIDLENANTVYDAKFQG	15	RVTITRDTSASTAYMELSSLRSEDTAVYYC	89	ARDAYGQYFYDV	104	WGQGTLVTVSS	106
ADI-46360	QVQLVQSGAEVKKPGASVKVSCKASG	71	FNIKDYYMH	13	WVRQAPGQRPEWIG	82	WIDLENANTVYDAKFQG	15	RVTITRDTSASTAYMELSSLRSEDAAYYYC	91	ARDAYGRYFYDV	103	WGQGTLVTVSS	106
ADI-46361	QVQLVQSGAEVKKPGASVKVSCKASG	71	FNNKDYYMH	78	WVRQAPGQRLEWIG	81	WIDLENANTVYDAKFQG	15	RVTITRDTSASTAYMELSSLRSEDTAVYYC	89	ARDAYGRYFYDV	103	WGQGTLVTVSS	106
ADI-46362	QVQLVQSGAEVKKPGASVKVSCKASG	71	FNIKDYYMH	13	WVRQAPGQRPEWIG	82	WIDLENANTVYDAKFQG	15	RVTITRDTSASTAYMELSSLRSETAAYYC	92	ARDAYGRYFYDV	103	WGQGTLVTVSS	106
ADI-46363	QVQLVQSGAEVKKPGASVKVSCKASG	71	FNIKDYYMH	13	WVRQAPGQRLEWIG	81	WIDLENANTVYDAKFQG	15	RVTITRDTSASTAYMELSSLRSEDTAVYYC	89	ARDAYGRYFYDV	103	WGQGTLVTVSS	106

	VH FR1	SEQ ID NO:	VH CDR1	SEQ ID NO:	VH FR2	SEQ ID NO:	VH CDR2	SEQ ID NO:	VH FR3	SEQ ID NO:	VH CDR3	SEQ ID NO:	VH FR4	SEQ ID NO:
ADI-46364	QVQLVQSGAEVKKPGASVKVSCKASG	71	FNIKDYYMH	13	WVRQAPGQRLEWIG	81	WIDLENANTVYDAKLQG	84	RVTITRDTSASTAYMELSSLGSEDTAVYYC	93	ARDAYGRYFYDV	103	WGQGTLVTVSS	106
ADI-46365	QVQLVQSGAEVKKPGASVKVSCKASG	71	FNIKDYYMH	13	WVRQAPGQRLEWIG	81	WIDLENANTVYDAKFQG	15	RVTITRDTSAGTAHMELSSLRSEDTAVYYC	94	ARDAYGRYFYDV	103	WGQGTPVTVSS	107
ADI-46366	QVQLVQSGAEVKKPGASVKVSCKASG	71	FNIKDYYMH	13	WVRQAPGQRLEWIG	81	WIDLENANTVYDAKFQG	15	RVTITRDTSASTAYMELSSLRSEDTAVYYC	89	ARDAYGQYFYDV	104	WGQGTLVTVSS	106
ADI-46367	QVQLVQSGAEVKKPGASVKVSCKASG	71	FNIKDYYMH	13	WVRQAPGQRLEWIG	81	WIDLENANTVYDAKFQG	15	RVTITRDTSASIAYMELSSLRSEDTAVYYC	95	ARDAYGRYFYDV	103	WGQGTLVTVSS	106
ADI-46369	QAQLVQSGAEVKKPGASVKVSCKASG	72	FNIKDYYMH	13	WVRQAPGQRLEWIG	81	WIDLENANTVYDTKFQG	85	RVTITRDTSASTAYMELSSLRSEDTAVYYC	89	ARDAYGRYFYDV	103	WGQGTLVTVSS	106

	VH FR1	SEQ ID NO:	VH CDR1	SEQ ID NO:	VH FR2	SEQ ID NO:	VH CDR2	SEQ ID NO:	VH FR3	SEQ ID NO:	VH CDR3	SEQ ID NO:	VH FR4	SEQ ID NO:
ADI-46370	QVQLVQSGAEVKKPGASVKVSCKASG	71	FNIKDYYMH	13	WVRQAPGQRLEWIG	81	WIDLENANTVYDHKFQG	86	RVTITRDTSASTAYMELSSLRSEDTAVYYC	89	ARDAYGRYFYDV	103	WGQGTLVTVSS	106
ADI-46371	QAQLVQSGAEVKKPGASVKVSCKASG	72	FNIKDYYMH	13	WVRQAPGQRPEWIG	82	WIDLENANTVYDAKFQG	15	RVTITRDTSASTAYMELSSLRSEDTAAYYC	92	ARDAYGRYFYDV	103	WGQGTLVTVSS	106
ADI-46372	QVQLVQSGAEVKKPGASVKVSCKASG	71	FNIKDYYMH	13	WVRQAPGQRLEWIG	81	WIDLENANTVYDAKFQG	15	RVTITRDTSAGTAHMELSSLRSEDTAVYYC	94	ARDAYGRYFYDV	103	WGQGTPVTVSS	107
ADI-46374	QVQLVQSGAEVKKPGASVKVSCKASG	71	FNIKDYYMH	13	WVRQAPGQRLEWIG	81	WIDLNNANTVYDAKFQG	87	RVTITRDTSASTAYMELSSLRSEDTAVYYC	89	ARDAYGRYFYDV	103	WGQGTLVTVSS	106
ADI-46375	QVQLVQSGAEVKKPGASVKVSCKASG	71	FNIKDYYMH	13	WVRQAPGQRLEWIG	81	WIDLENANTVYDAKFQG	15	RVTITRDTSASTAYMELSSLRSEDTAVYYC	89	ARDAYGRYFYDV	103	WGQGTLVTVSS	106

	VH FR1	SEQ ID NO:	VH CDR1	SEQ ID NO:	VH FR2	SEQ ID NO:	VH CDR2	SEQ ID NO:	VH FR3	SEQ ID NO:	VH CDR3	SEQ ID NO:	VH FR4	SEQ ID NO:
ADI-46376	QVQLVQS GAEVKKP GASVKVS CKASG	71	FNIKDY YMH	13	WVRQAP GQRLEW IG	81	WIDLEN ANTVY DAKLQ G	84	RVTITR DTSAST AYMELS SLGSED TAVYYC	93	ARDAYG RYFYDV	103	WGQG TPVTV SS	107
ADI-46378	QVQLVQS GAEVKKP GASVKVS CKASG	71	FNIKDY YMH	13	WVRQAP GQRLEW IG	81	WIDLEN ANTVY DAKFQ G	15	RVTITR DTSASA AYMELS SLRSED TAVYYC	96	ARDAYG RYFYDV	103	WGQG TLVTV SS	106
ADI-46379	QVQLVQS GAEVKKP GASVKVS CKASG	71	FNIKDY YMH	13	WVRQAP GQRLEW IG	81	WIDLEN ANTVY DAKFQ G	15	RVTITR DTSASA AYMELS SLRSED TAVYYC	96	ARDAYG RYFYDV	103	WGQG TLVTV SS	106
ADI-46381	QVQLVQS GAEVKKP GASVKVS CKASG	71	FNIKDY YMH	13	WVRQAP GQRLEW IG	81	WIDLEN ANTVY DTKFQ G	85	RVTITR DTSAST AYMELS SLRSED TAVYYC	89	ARDAYG RYFYDV	103	WGQG TLVTV SS	106
ADI-42596	QVQLVQS GAEVKKP GASVKVS CKASG	71	FNIKDY YMH	13	WVRQAP GQRLEW IG	81	WIDLEN ANTVY DAKFQ G	15	RVTITR DTSAST AYMELS SLRSED TAVYYC	89	ARDAYG RYFYDV	103	WGQG TLVTV SS	106

	VH FR1	SEQ ID NO:	VH CDR1	SEQ ID NO:	VH FR2	SEQ ID NO:	VH CDR2	SEQ ID NO:	VH FR3	SEQ ID NO:	VH CDR3	SEQ ID NO:	VH FR4	SEQ ID NO:
ADI-45959	QVQLVQS GAEVKKP GASVKVS CKASG	71	FNIKDY YMH	13	WVRQAP GQRLEW IG	81	WIDLEN ANTVY DAKFQ G	15	RVTITR DTSAST AYMELS SLRSED TAVYYC	89	ARDAYG RYFYDV	103	WGQG TLVTV SS	106
ADI-45960	QVQLVQS GAEVKKP GASVKVS CKASG	71	FNIKDY YMH	13	WVRQAP GQRLEW IG	81	WIDLEN ANTVY DAKFQ G	15	RVTITR DTSAST AYMELS SLRSED TAVYYC	89	ARDAYG RYFYDV	103	WGQG TLVTV SS	106
ADI-45961	QVQLVQS GAEVKKP GASVKVS CKASG	71	FNIKDY YMH	13	WVRQAP GQRLEW IG	81	WIDLEN ANTVY DAKFQ G	15	RVTITR DTSAST AYMELS SLRSED TAVYYC	89	ARDAYG RYFYDV	103	WGQG TLVTV SS	106
ADI-45962	QVQLVQS GAEVEEP GASVKVS CKASG	73	FNIKDY YMH	13	WVRQAP GQRLEW IG	81	WIDLEN ANTVY DAKFQ G	15	RVTITR DTSAST AYMELS SLRSED TAVYYC	89	ARDAYG RYFYDV	103	WGQG TLVTV SS	106
ADI-45963	QVQLVQS GAEVKKP GAPVKVS CKASG	74	FNIKDY YMH	13	WVRQAP GQRLEW IG	81	WIDLEN ANTVY DAKFQ G	15	RVTITR DTSAST AYMELS SLRSED TAVYYC	89	ARDAYG RYFYDV	103	WGQG TLVTV SS	106

	VH FR1	SEQ ID NO:	VH CDR1	SEQ ID NO:	VH FR2	SEQ ID NO:	VH CDR2	SEQ ID NO:	VH FR3	SEQ ID NO:	VH CDR3	SEQ ID NO:	VH FR4	SEQ ID NO:
ADI-45964	QVQLVQS GAEVKKP GASVKVS CKASG	71	FNIKDY YIH	79	WVRQAP GQRLEW IG	81	WIDLEN ANTVY DAKFQ G	15	RVTITR DTSAST AYMELS SLRSED TAVYYC	89	ARDAYG RYFYDV	103	WGQG TLVTV SS	106
ADI-45965	QVQLVQS GAEVKKP GASVKVS CKASG	71	FNIKDY YMH	13	WVRQAP GQRLEW IG	81	WIDLEN ANAVY DAKFQ G	88	RVTITR DTSAST AYMELS SLRSED TAVYYC	89	ARDAYG RYFYDV	103	WGQG TLVTV SS	106
ADI-45966	QVQLVQS GAEVKKP GASVKVS CKASG	71	FNIKDY YMH	13	WVRQAP GQRLEW IG	81	WIDLEN ANTVY DAKFQ G	15	RVAITR DTSAST AYMELS SLRSED TAVYYC	97	ARDAYG RYFYDV	103	WGQG TLVTV SS	106
ADI-45967	QVQLVQS GAEVKKP GASVKVS CKASG	71	FNIKDY YMH	13	WVRQAP GQRLEW IG	81	WIDLEN ANTVY DAKFQ G	15	RVTIAR DTSAST VYMELS SLRSED TAVYYC	98	ARDAYG RYFYDV	103	WGQG TLVTV SS	106
ADI-45968	QVQLVQS GAEVKKP GASVKVS CKASG	71	FNIKDY YMH	13	WVRQAP GQRLEW IG	81	WIDLEN ANTVY DAKFQ G	15	RVTITR DASAST AYMELS SLRSED TAVYYC	99	ARDAYG RYFYDV	103	WGQG TLVTV SS	106

	VH FR1	SEQ ID NO:	VH CDR1	SEQ ID NO:	VH FR2	SEQ ID NO:	VH CDR2	SEQ ID NO:	VH FR3	SEQ ID NO:	VH CDR3	SEQ ID NO:	VH FR4	SEQ ID NO:
ADI-45969	QVQLVQS GAEVKKP GASVKVS CKASG	71	FNIKDY YMH	13	WVRQAP GQRLEW IG	81	WIDLEN ANTVY DAKFQ G	15	RVTITR DTSAST AYMELG SLRSED TAVYYC	100	ARDAYG RYFYDV	103	WGQG TLVTV SS	106
ADI-45970	QVQLVQS GAEVKKP GASVKVS CKASG	71	FNVKDY YMH	80	WVRQAP GQRLEW IG	81	WIDLEN ANTVY DAKFQ G	15	RVTITR DTSAST AYMELS SLRSED TAVYYC	89	ARDAYG RYFYDV	103	WGQG TLVTV SS	106
ADI-45971	QVQLVQS GAEVKKP GATVKVS CKASG	75	FNIKDY YIH	79	WVRQAP GQRLEW IG	81	WIDLEN ANTVY DAKFQ G	15	RVTITR DTSAST AYMELS SLRSED TAVYYC	89	ARDAYG RYFYDV	103	WGQG TLVTV SS	106
ADI-45972	QVQLVQS GAEVKRP GASVKVS CKASG	76	FNIKDY YMH	13	WVRQAP GQRLEW IG	81	WIDLEN ANTVY DAKFQ G	15	RVTITR DTSAST AYMELS SLRSED TAVYYC	89	ARDAYG RYFYDV	103	WGQG TLVTV SS	106
ADI-45973	QVQLVQS EAEVKKP GASVKVS CKASG	77	FNIKDY YMH	13	WVRQAP GQRLEW IG	81	WIDLEN ANTVY DAKFQ G	15	RVTITR DTSAST AYMELS SLRSED TAVYYC	89	ARDAYG RYFYDV	103	WGQG TLVTV SS	106

	VH FR1	SEQ ID NO:	VH CDR1	SEQ ID NO:	VH FR2	SEQ ID NO:	VH CDR2	SEQ ID NO:	VH FR3	SEQ ID NO:	VH CDR3	SEQ ID NO:	VH FR4	SEQ ID NO:
ADI-45974	QVQLVQS GAEVKKP GASVKVS CKASG	71	FNIKDY YMH	13	WVRQAP GQRLEW IG	81	WIDLEN ANTVY DAKFQ G	15	RVTITR DTSAST AYMELS NLRSED TAVYYC	90	ARDAYG RYFYDV	103	WGQG TLVTV SS	106
ADI-45975	QVQLVQS GAEVKKP GASVKVS CKASG	71	FNIKDY YMH	13	WVRQAP GQRLEW IG	81	WIDLEN ANTVY DAKFQ G	15	RVTITR DTSAST AYMELS SLRSED TAVYYC	89	ARDAYG QYFYDV	104	WGQG TLVTV SS	106
ADI-45976	QVQLVQS GAEVKKP GASVKVS CKASG	71	FNIKDY YMH	13	WVRQAP GQRLEW IG	81	WIDLEN ANTVY DAKFQ G	15	RVTITR DTSAST AYMELS SLRSED TAVYYC	89	VRDAYG RYFYDV	105	WGQG TLVTV SS	106
ADI-45977	QVQLVQS GAEVKKP GASVKVS CKASG	71	FNIKDY YMH	13	WVRQAP GQRLEW IG	81	WIDLEN ANTVY DAKLQ G	84	RVTITR DTSAST AYMELS SLGSED TAVYYC	93	ARDAYG RYFYDV	103	WGQG TLVTV SS	106
ADI-45978	QVQLVQS GAEVKKP GASVKVS CKASG	71	FNIKDY YMH	13	WVRQAP GQRLEW LG	83	WIDLEN ANTVY DAKFQ G	15	RVTITR DTSAST AYMELS SLRSED TAVYYC	89	ARDAYG RYFYDV	103	WGQG TLVTV SS	106

	VH FR1	SEQ ID NO:	VH CDR1	SEQ ID NO:	VH FR2	SEQ ID NO:	VH CDR2	SEQ ID NO:	VH FR3	SEQ ID NO:	VH CDR3	SEQ ID NO:	VH FR4	SEQ ID NO:
ADI-45979	QVQLVQS GAEVKKP GASVKVS CKASG	71	FNIKDY YMH	13	WVRQAP GQRLEW IG	81	WIDLEN ANTVY DAKFQ G	15	RVTITR DTSAST AYMELS SLRSED TAVYYC	89	ARDAYG RYFYDV	103	WGQG TLVTV SS	106
ADI-45980	QVQLVQS GAEVKKP GASVKVS CKASG	71	FNIKDY YMH	13	WVRQAP GQRLEW IG	81	WIDLEN ANTVY DAKFQ G	15	RVTITR DTSAST AYMELN SLRSED TAVYYC	101	ARDAYG RYFYDV	103	WGQG TLVTV SS	106
ADI-45981	QVQLVQS GAEVKKP GASVKVS CKASG	71	FNIKDY YMH	13	WVRQAP GQRLEW IG	81	WIDLEN ANTVY DAKFQ G	15	RVTITR DTSAST AYMELS SLRSED TAVYYC	89	ARDAYG RYFYDV	103	WGQG TLVTV SS	106
ADI-45982	QVQLVQS GAEVKKP GASVKVS CKASG	71	FNIKDY YMH	13	WVRQAP GQRLEW IG	81	WIDLEN ANTVY DAKFQ G	15	RVTITR DTSAST AYMELS SLRSED TAVYYC	89	ARDAYG RYFYDV	103	WGQG TLVTV SS	106
ADI-45983	QVQLVQS GAEVKKP GASVKVS CKASG	71	FNIKDY YMH	13	WVRQAP GQRLEW IG	81	WIDLEN ANTVY DAKFQ G	15	RVTITR DTSAGT AYMELS SLRSED TAVYYC	102	ARDAYG RYFYDV	103	WGQG TLVTV SS	106

	VH FR1	SEQ ID NO:	VH CDR1	SEQ ID NO:	VH FR2	SEQ ID NO:	VH CDR2	SEQ ID NO:	VH FR3	SEQ ID NO:	VH CDR3	SEQ ID NO:	VH FR4	SEQ ID NO:
ADI-45984	QVQLVQSGAEVKKPGASVKVSCKASG	71	FNIKDYYMH	13	WVRQAPGQRLEWIG	81	WIDLENANTVYDAKFQG	15	RVTITRDTSASTAYMELSSLRSEDTAVYYC	89	ARDAYGRYFYDV	103	WGQGTLVTVSS	106
ADI-45985	QVQLVQSGAEVKKPGASVKVSCKASG	71	FNIKDYYMH	13	WVRQAPGQRLEWIG	81	WIDLENANTVYDAKFQG	15	RVTITRDTSASTAYMELSSLRSEDTAVYYC	89	ARDAYGRYFYDV	103	WGQGTLVTVSS	106

Table 6. Antibody VL sequence information.

	VL FR1	SEQ ID NO:	VL CDR1	SEQ ID NO:	VL FR2	SEQ ID NO:	VL CDR2	SEQ ID NO:	VL FR3	SEQ ID NO:	VL CDR3	SEQ ID NO:	VL FR4	SEQ ID NO:
ADI-42594	DIVMTQ SPDSLA VSLGER ATINC	108	KSSQSLL NARTGK NYLA	8	WYQQKP GQPPKL LIY	117	WASTRE S	10	GVPDRF SGSGSGT DFTLTIS SLQAED VAVYYC	126	KQSYFR RT	5	FGGGT KVEIK	130
ADI-42596	DIVMTQ SPDSLA VSLGER ATINC	108	KSSQSLL NARTRK NYLA	9	WYQQKP GQPPKL LIY	117	WASTRE S	10	GVPDRF SGSGSGT DFTLTIS SLQAED VAVYYC	126	KQSYFR RT	5	FGGGT KVEIK	130
ADI-46356	DIVMTQ SPDSLA VSLGER ATINC	108	KSSQSLL NARTGK NYLA	8	WYQQKP GQPPKL LIY	117	WASTRE S	10	GVPDRF SGSGSGT DFTLTIS SLQAED VAVYYC	126	VQSYFR RT	4	FGGGT KVEIK	130
ADI-46357	DIVMTQ SPDSLA VSLGER ATINC	108	KSSQSLL NARTGK NYLA	8	WYQQKP GQPPKL LIY	117	WASTRE S	10	GVPDRF SGSGSGT DFTLTIS SLQAED VAVYYC	126	AQSYFR RT	2	FGGGT KVEIK	130
ADI-46358	DIVMTQ SPDSLA VSLGER ATINC	108	KSSQSLL NARTGK NYLA	8	WYQQKP GQPPKL LIY	117	WASTRE S	10	GVPDRF SGSGSGT DFTLTIS SLQAED VAVYYC	126	TQSYFR RT	3	FGGGT KVEIK	130

	VL FR1	SEQ ID NO:	VL CDR1	SEQ ID NO:	VL FR2	SEQ ID NO:	VL CDR2	SEQ ID NO:	VL FR3	SEQ ID NO:	VL CDR3	SEQ ID NO:	VL FR4	SEQ ID NO:
ADI-46359	DIVMTQ SPDSLA VSLGER ATINC	108	KSSQSLL NARTGK NYLA	8	WYQQKP GQPPKL LIY	117	WASTRE S	10	GVPDRF SGSGSGT DFTLTIS SLQAED VAVYYC	126	TQSYFR RT	3	FGGGT KVEIK	130
ADI-46360	DIVMTQ SPDSLA VSLGER ATINC	108	KSSQSLL NARTGK NYLA	8	WYQQKP GQPPELL IY	118	WASTRE S	10	GVPDRF SGSGSGT DFTLTIS SLQAED VAVYYC	126	TQSYFR RT	3	FGGGT KVEIK	130
ADI-46361	DIVMTQ SPDSLA VSLGER ATINC	108	KSSQSLL NARTGK NYLA	8	WYQQKP GQPPKL LIY	117	WASTRE S	10	GVPDRF SGSGSGT DFTLTIS SLQAED VAVYYC	126	TQSYFR RT	3	FGGGT KVEIK	130
ADI-46362	DIVMTQ SPDSLA VSLGER ATINC	108	KSSQSLL NARTGK NYLA	8	WYQQKP GQPPKL LIY	117	WASTRE S	10	GVPDRF SGSGSGT DFTLTIS SLQAED VAVYYC	126	VQSYFR RT	4	FGGGT KVEIK	130
ADI-46363	DIVMTQ SPDSLA VSLGER ATINC	108	KSSQSLL NARTGK NYLA	8	WYQQKP GQPPKL LIY	117	WASTRE S	10	GVPDRF SGSGSGT DFTLTIS SLQAED VAVYYC	126	TQSYFR RT	3	FGGGT KVEIK	130

	VL FR1	SEQ ID NO:	VL CDR1	SEQ ID NO:	VL FR2	SEQ ID NO:	VL CDR2	SEQ ID NO:	VL FR3	SEQ ID NO:	VL CDR3	SEQ ID NO:	VL FR4	SEQ ID NO:
ADI-46364	DIVMTQ SPDSLA VSLGER ATINC	108	KSSQSLL NARTGK NYLA	8	WYQQKP GQPPKL LIY	117	WASTRE S	10	GVPDRF SGSGSGT DFTLTIS SLQAED VAVYYC	126	VQSYFR RT	4	FGGGT KVEIK	130
ADI-46365	DIVMTQ SPDSLA VSLGER ATINC	108	KSSQSLL NARTGK NYLA	8	WYQQKP GQPPKL LIY	117	WASTRE S	10	GVPDRF SGSGSGT DFTLTIS SLQAED VAVYYC	126	TQSYFR RT	3	FGGGT KVEIK	130
ADI-46366	DIVMTQ SPDSLA VSLGER ATINC	108	KSSQSLL NARTGK NYLA	8	WYQQKP GQPPKL LIY	117	WASTRE S	10	GVPDRF SGSGSGT DFTLTIS SLQAED VAVYYC	126	VQSYFR RT	4	FGGGT KVEIK	130
ADI-46367	DIVMTQ SPDSLA VSLGER ATINC	108	KSSQSLL NARTGK NYLA	8	WYQQKP GQPPKL LIY	117	WASTRE S	10	GVPDRF SGSGSGT DFTLTIS SLQAED VAVYYC	126	AQSYFR RT	2	FGGGT KVEIK	130
ADI-46369	DIVMTQ SPDSLA VSLGER ATINC	108	KSSQSLL NARTGK NYLA	8	WYQQKP GQPPKL LIY	117	WASTRE S	10	GVPDRF SGSGSGT DFTLTIS SLQAED VAVYYC	126	VQSYFR RT	4	FGGGT KVEIK	130

	VL FR1	SEQ ID NO:	VL CDR1	SEQ ID NO:	VL FR2	SEQ ID NO:	VL CDR2	SEQ ID NO:	VL FR3	SEQ ID NO:	VL CDR3	SEQ ID NO:	VL FR4	SEQ ID NO:
ADI-46370	DIVMTQ SPDSLA VSLGER ATINC	108	KSSQSLL NARTGK NYLA	8	WYQQKP GQPPKL LIY	117	WASTRE S	10	GVPDRF SGSGSGT DFTLTIS SLQAED VAVYYC	126	VQSYFR RT	4	FGGGT KVEIK	130
ADI-46371	DIVMTQ SPDSLA VSLGER ATINC	108	KSSQSLL NARTGK NYLA	8	WYQQKP GQPPKL LIY	117	WASTRE S	10	GVPDRF SGSGSGT DFTLTIS SLQAED VAVYYC	126	VQSYFR RT	4	FGGGT KVEIK	130
ADI-46372	DIVMTQ SPDSLA VSLGER ATINC	108	KSSQSLL NARTGK NYLA	8	WYQQKP GQPPKL LIY	117	WASTRE S	10	GVPDRF SGSGSGT DFTLTIS SLQAED VAVYYC	126	VQSYFR RT	4	FGGGT KVEIK	130
ADI-46374	DIVMTQ SPDSLA VSLGER ATINC	108	KSSQSLL NARTGK NYLA	8	WYQQKP GQPPKL LIY	117	WASTRE S	10	GVPDRF SGSGSGT DFTLTIS SLQAED VAVYYC	126	VQSYFR RT	4	FGGGT KVEIK	130
ADI-46375	DIVMTQ SPDSLA VSLGER ATINC	108	KSSQSLL NARTRK NYLA	9	WYQQKP GQPPKL LIY	117	WASTRE S	10	GVPDRF SGSGSGT DFTLTIS SLQAED VAVYYC	126	TQSYFR RT	3	FGGGT KVEIK	130

	VL FR1	SEQ ID NO:	VL CDR1	SEQ ID NO:	VL FR2	SEQ ID NO:	VL CDR2	SEQ ID NO:	VL FR3	SEQ ID NO:	VL CDR3	SEQ ID NO:	VL FR4	SEQ ID NO:
ADI-46376	DIVMTQ SPDSLA VSLGER ATINC	108	KSSQSLL NARTGK NYLA	8	WYQQKP GQPPKL LIY	117	WASTRE S	10	GVPDRF SGSGSGT DFTLTIS SLQAED VAVYYC	126	TQSYFR RT	3	FGGGT KVEIK	130
ADI-46378	DIVMTQ SPDSLA VSLGER ATINC	108	KSSQSLL NARTRK NYLA	9	WYQQKP GRPPKL LIY	119	WASTRE S	10	GVPDRF SGSGSGT DFTLTIS SLQAED VAVYYC	126	VQSYFR RT	4	FGGGT KVEIK	130
ADI-46379	DIVMTQ SPDSLA VSLGER ATINC	108	KSSQSLL NARTGK NYLA	8	WYQQKP GQPPKL LIY	117	WASARE S	120	GVPDRF SGSGSGT DFTLTIS SLQAED VAVYYC	126	TQSYFR RT	3	FGGGT KVEIK	130
ADI-46381	DIVMTQ SPDSLA VSLGER ATINC	108	KTSQGL LNARTN KNYLA	112	WYQQKP GQPPKL LIY	117	WASTRE S	10	GVPDRF SGSGSGT DFTLTIS SLQAED VAVYYC	126	VQSYFR RT	4	FGGGT KVEIK	130
ADI-42596	DIVMTQ SPDSLA VSLGER ATINC	108	KSSQSLL NARTRK NYLA	9	WYQQKP GQPPKL LIY	117	WASTRE S	10	GVPDRF SGSGSGT DFTLTIS SLQAED VAVYYC	126	KQSYFR RT	5	FGGGT KVEIK	130

	VL FR1	SEQ ID NO:	VL CDR1	SEQ ID NO:	VL FR2	SEQ ID NO:	VL CDR2	SEQ ID NO:	VL FR3	SEQ ID NO:	VL CDR3	SEQ ID NO:	VL FR4	SEQ ID NO:
ADI-45959	DIVMSQ SPDSLA VSLGER ATINC	109	KSSQSLL NARTGK NYLA	8	WYQQKP GQPPKL LIY	117	WASTRS S	11	GVPDRF SGSGSGT DFTLTIS SLQAED VAVYYC	126	VQSYFR RT	4	FGGGT KVEIK	130
ADI-45960	DIVMTQ SPDSLA VSLGER ATINC	108	KSSQSLL NARTGK NYLA	8	WYQQKP GQPPKL LIY	117	WASTRE S	10	GVPDRF SGSGSGT DFTLTIS SLQAED AAVYYC	127	AQSYFR RT	2	FGGGT KVEIK	130
ADI-45961	DIVMTQ SPDSLA VSLGER ATINC	108	KSSQSLL NARTRR NYLA	113	WYQQKP GQPPKL LIY	117	WGSTRE S	121	GVPDRF SGSGSGT DFTLTIS SLQAED VAVYYC	126	KQSYFR RT	5	FGGGT KVEIK	130
ADI-45962	DIVMTQ SPDSLA VSLGER ATINC	108	KSSQSLL NARTGK NYLA	8	WYQQKP GQPPKL LIY	117	WASTRE S	10	GVPDRF SGSGSGT DFTLTIS SLQAED VAVYYC	126	VQSYFR RT	4	FGGGT KVEIK	130
ADI-45963	DIVMTQ SPDSLA VSLGER ATINC	108	KSSQSLL NARTGK NYLA	8	WYQQKP GQPPKL LIY	117	WASTRE S	10	GVPDRF SGSGSGT DFTLTIS SLQAED VAVYYC	126	KQSYFR RT	5	FGGGT KVEIK	130

	VL FR1	SEQ ID NO:	VL CDR1	SEQ ID NO:	VL FR2	SEQ ID NO:	VL CDR2	SEQ ID NO:	VL FR3	SEQ ID NO:	VL CDR3	SEQ ID NO:	VL FR4	SEQ ID NO:
ADI-45964	DIVMTQ SPDSLA VSLGER ATINC	108	KSSQSLL NARTGK NYLA	8	WYQQKP GQPPKL LIY	117	WASTRE S	10	GVPDRF SGSGSGT DFTLTIS SLQAED VAVYYC	126	AQSYFR RT	2	FGGGT KVEIK	130
ADI-45965	DIVMTQ SPDSLA VSLGGR ATINC	110	KSSQSLL NARTGK NYLA	8	WYQQKP GQPPKL LIY	117	WASTRE S	10	GVPDRF SGSGSGT DFTLTIS SLQAED VAVYYC	126	VQSYFR RT	4	FGGGT KVEIK	130
ADI-45966	DIVMTQ SPDSLA VSLGER ATINC	108	KSSQSLL NARTGK NYLA	8	WYQQKP GQPPKL LIY	117	WASTRE S	10	GVPDRF SGSGSGT DFTLTIS SLQAED VAVYYC	126	TQSYFR RT	3	FGGGT KVEIK	130
ADI-45967	DIVMTQ SPDSLA VSLGER AIINC	111	KSSQSLL NARTGK NYLA	8	WYQQKP GQPPKL LIY	117	WASTRE S	10	GVPDRF SGSGSGT DFTLTIS SLQAED VAVYYC	126	TQSYFR RT	3	FGGGT KVEIK	130
ADI-45968	DIVMTQ SPDSLA VSLGER ATINC	108	KSSQSLL NARTGK NYLA	8	WYQQKP GQPPKL LIY	117	WASTRE S	10	GVPDRF SGSGSGT DFTLTIS SLQAED VAVYYC	126	VQSYFR RT	4	FGGGT KVEIK	130

	VL FR1	SEQ ID NO:	VL CDR1	SEQ ID NO:	VL FR2	SEQ ID NO:	VL CDR2	SEQ ID NO:	VL FR3	SEQ ID NO:	VL CDR3	SEQ ID NO:	VL FR4	SEQ ID NO:
ADI-45969	DIVMTQ SPDSLA VSLGER ATINC	108	KSSQSLL NARTGK NYLA	8	WYQQKP GQPPKL LIY	117	WASTRE S	10	GVPDRF SGSGSGT DFTLTIS SLQAED VAVYYC	126	AQSYFR RT	2	FGGGT KVEIK	130
ADI-45970	DIVMTQ SPDSLA VSLGER ATINC	108	KSSQSLL NARTGK NYLA	8	WYQQKP GQPPKL LIY	117	WASTRE S	10	GVPDRF SGSGSGT DFTLTIS SLQAED VAVYYC	126	KQSYFR RT	5	FGGGT KVEIK	130
ADI-45971	DIVMTQ SPDSLA VSLGER ATINC	108	KSSQSLL NARTRK NYLA	9	WYQQKP GQPPKL LIY	117	WASTRE S	10	GVPDRF SGSGSGT DFTLTIS SLQAED VAVYYC	126	VQSYFR RT	4	FGGGT KVEIK	130
ADI-45972	DIVMTQ SPDSLA VSLGER ATINC	108	KSSQSLL NARTGK NYLA	8	WYQQKP GQPPKL LIY	117	WASTRE S	10	GVPDRF SGSGSGT DFTLTIS SLQAED VAVYYC	126	VQSYFR RT	4	FGGGT KVEIK	130
ADI-45973	DIVMTQ SPDSLA VSLGER ATINC	108	KSSQSLL NARTGK NYLA	8	WYQQKP GQPPKL LIY	117	WASTRE S	10	GVPDRF SGSGSGT DFTLTIS SLQAED VAVYYC	126	KQSYFR RT	5	FGGGT KVEIK	130

	VL FR1	SEQ ID NO:	VL CDR1	SEQ ID NO:	VL FR2	SEQ ID NO:	VL CDR2	SEQ ID NO:	VL FR3	SEQ ID NO:	VL CDR3	SEQ ID NO:	VL FR4	SEQ ID NO:
ADI-45974	DIVMTQ SPDSLA VSLGER ATINC	108	KSSQSLL NARTGK NYLA	8	WYQQKP GQPPKL LIY	117	WASTRE S	10	GVPDRF SGSGSGT DFTLTIS SLQAED VAVYYC	126	KQSYFR RT	5	FGGGT KVEIK	130
ADI-45975	DIVMTQ SPDSLA VSLGER ATINC	108	KSSQSLL NARTRK NYLA	9	WYQQKP GQPPKL LIY	117	WASTRE S	10	GVPDRF SGSGSGT DFTLTIS SLQAED VAVYYC	126	KQSYFR RT	5	FGGGT KVEIK	130
ADI-45976	DIVMTQ SPDSLA VSLGER ATINC	108	KSSQSLL NARTGK NYLA	8	WYQQKP GQPPKL LIY	117	WASTRE S	10	GVPDRF SGSGSGT DFTLTIS SLQAED VAVYYC	126	VQSYFR RT	4	FGGGT KVEIK	130
ADI-45977	DIVMTQ SPDSLA VSLGER ATINC	108	KSSQSLL NARTGK NYLA	8	WYQQKP GQPPKL LIY	117	WASTRE S	10	GVPDRF SGSGSGT DFTLTIS SLQAED VAVYYC	126	TQSYFR RT	3	FGGGT KVEIK	130
ADI-45978	DIVMTQ SPDSLA VSLGER ATINC	108	KSSQSLL NARTGK NYLA	8	WYQQKP GQPPKL LIY	117	WASARE S	120	GVPDRF SGSGSGT DFTLTIS SLQAED VAVYYC	126	TQSYFR RT	3	FGGGT KVEIK	130

	VL FR1	SEQ ID NO:	VL CDR1	SEQ ID NO:	VL FR2	SEQ ID NO:	VL CDR2	SEQ ID NO:	VL FR3	SEQ ID NO:	VL CDR3	SEQ ID NO:	VL FR4	SEQ ID NO:
ADI-45979	DIVMTQ SPDSLA VSLGER ATINC	108	KSSQELL NARTGK NYLA	114	WYQQKP GQPPKL LIY	117	WASTRE S	10	GVPDRF SGSGSGT DFTLTIS SLQAED VAVYYC	126	KQSYFR RT	5	FGGGT KVEIK	130
ADI-45980	DIVMTQ SPDSLA VSLGER ATINC	108	KSSQSLL NARTGK NYLA	8	WYQQKP GQPPKL LIY	117	WAFTRE S	122	GVPDRF SGSGSGT DFTLTIS SLQAED VAVYYC	126	TQSYFR RT	3	FGGGT KVEIK	130
ADI-45981	DIVMTQ SPDSLA VSLGER ATINC	108	KSSQSLL NARTGK NYLA	8	WYQQKP GQPPKL LIY	117	WASMRE S	123	GVPDRF SGSRSGT DFTLTIS SLQAED VAVYYC	128	SQSYFR RT	129	FGGGT KVEIK	130
ADI-45982	DIVMTQ SPDSLA VSLGER ATINC	108	KSSQSLL NARTGK NYLA	8	WYQQKP GQPPKL LIY	117	WASTRE N	124	GVPDRF SGSGSGT DFTLTIS SLQAED VAVYYC	126	TQSYFR RT	3	FGGGT KVEIK	130
ADI-45983	DIVMTQ SPDSLA VSLGER ATINC	108	KSSQSLL NARTMK NYLA	115	WYQQKP GQPPKL LIY	117	AASTRE S	125	GVPDRF SGSGSGT DFTLTIS SLQAED VAVYYC	126	AQSYFR RT	2	FGGGT KVEIK	130

	VL FR1	SEQ ID NO:	VL CDR1	SEQ ID NO:	VL FR2	SEQ ID NO:	VL CDR2	SEQ ID NO:	VL FR3	SEQ ID NO:	VL CDR3	SEQ ID NO:	VL FR4	SEQ ID NO:
ADI-45984	DIVMTQ SPDSLA VSLGER ATINC	108	KSSQSLL NARTRK NYLA	9	WYQQKP GQPPKL LIY	117	WASTRE S	10	GVPDRF SGSGSGT DFTLTIS SLQAED VAVYYC	126	VQSYFR RT	4	FGGGT KVEIK	130
ADI-45985	DIVMTQ SPDSLA VSLGER ATINC	108	KTSQSLL NARTNK NYLA	116	WYQQKP GQPPKL LIY	117	WASTRE S	10	GVPDRF SGSGSGT DFTLTIS SLQAED VAVYYC	126	VQSYFR RT	4	FGGGT KVEIK	130

Table 7. Human and Cynomolgus CD3ε Sequences

Name	Sequence:	SEQ ID NO:
Hu CD3ε Fc	QDGNEEMGGITQTPYKVSISGTTVILTCPQYPGSE ILWQHNDKNIGGDEDDKNIGSDEDHLSLKEFSEL EQSGYYVCYPRGSKPEDANFYLYLRARVCENCM EMDGGSDKTHTCPPCPAPELLGGPSVFLFPPKPK DTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDG VEVHNAKTKPREEQYASTYRVVSVLTVLHQDW LNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQ VYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEW ESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKS RWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK	131
Cy CD3ε Fc	QDGNEEMGSITQTPYQVSISGTTVILTCSQHLGSE AQWQHNGKNKEDSGDRLFLPEFSEMEQSGYYV CYPRGSNPEDASHHLYLKARVCENCMEMDGGS DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISR TPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNA KTKPREEQYASTYRVVSVLTVLHQDWLNGKEY KCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPS RDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPE NNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGN VFSCSVMHEALHNHYTQKSLSLSPGK	132

CLAIMS

1. An anti-cluster of differentiation three (CD3) antibody or antigen-binding antibody fragment, which comprises:

(A) a heavy chain variable region (VH) comprising a VH CDR1 (CDRH1), a VH CDR2 (CDRH2), and a VH CDR3 (CDRH3); and

(B) a light chain variable region (VL) comprising a VL CDR1 (CDRL1), a VL CDR2 (CDRL2), and a VL CDR3 (CDRL3),

wherein:

(i) (a) the amino acid sequences of the CDRH1, the CDRH2, and the CDRH3 comprise or consist of the heavy chain CDR 1, 2, and 3 sequences, respectively, contained in SEQ ID NO: 17, and

(b) the amino acid sequences of the CDRL1, the CDRL2, and the CDRL3 comprise or consist of the light chain CDR 1, 2, and 3 sequences, respectively, contained in SEQ ID NO: 34; or

(ii) the amino acid sequences of the CDRH1, the CDRH2, the CDRH3, the CDRL1, the CDRL2, and the CDRL3 comprise or consist of SEQ ID NO: 13, 15, 103, 8, 11, and 4, respectively.

2. The anti-CD3 antibody or antigen-binding antibody fragment of claim 1, wherein:

(A) the amino acid sequence of the VH has at least 90% sequence identity to SEQ ID NO: 17; and/or

(B) the amino acid sequence of the VL has at least 90% sequence identity to SEQ ID NO: 34.

3. The anti-CD3 antibody or antigen-binding antibody fragment of claim 1 or 2, wherein:

(A) the amino acid sequence of the VH has at least 95% sequence identity to SEQ ID NO: 17; and/or

(B) the amino acid sequence of the VL has at least 95% sequence identity to SEQ ID NO: 34.

4. The anti-CD3 antibody or antigen-binding antibody fragment of any one of claims 1-3, wherein the amino acid sequences of the VH and the VL comprise or consist of SEQ ID NOS: 17 and 34, respectively.

5. The anti-CD3 antibody or antigen-binding antibody fragment of any one of claims 1-4, which comprises an scFv, a Fv, a Fab, a Fab', a Fab'-SH, a F(ab')2, a diabody, a scFv2-Fc2, or a scFv-IgG.

6. The anti-CD3 antibody or antigen-binding antibody fragment of any one of claims 1-5, which comprises:

(A) a heavy chain polypeptide comprising the VH and a heavy chain constant region (CH); and

(B) a light chain polypeptide comprising the VL and a light chain constant region (CL),

optinally comprising an immunoglobulin molecule comprised of two said heavy chains and two said light chains interconnected by disulfide bonds or a multimer of said immunoglobulin molecule, optionally comprising an IgG, IgA, IgD, IgE, or IgM antibody, further optionally an IgG1, IgG2, IgG3, IgG4, IgA1, or IgA2 antibody.

7. The anti-CD3 antibody or antigen-binding antibody fragment of any one of claims 1-6, which:

(i) is comprised in a chimeric antigen receptor (CAR), which optionally comprises at least one transmembrane domain, at least one intracellular domain from a T-cell receptor or from a CD3ζ subunit, and at least one co-stimulatory domain; and/or

(ii) comprises an IgG constant domain.

8. The anti-CD3 antibody or antigen-binding antibody fragment of any one of claims 1-7, which:

(i) binds to CD3 with a dissociation constant (K_D) of about 500 pM or more, about 500 pM or less, about 450 pM or less, about 400 pM or less, about 350 pM or less, about 300 pM or less, about 250 pM or less, about 200 pM or less, about 150 pM or less, or about 100 pM or less, optionally wherein said CD3 is human and/or cynomolgus, further optionally wherein said K_D is measured by surface plasmon resonance; and/or

(ii) elicits T cell activation or T cell killing while displaying a decreased propensity to elicit cytokine production to levels capable of inducing cytokine release syndrome.

9. The anti-CD3 antibody or antigen-binding antibody fragment of any one of claims 1-8, which is or comprises a multispecific antibody, optionally a bispecific antibody.

10. The anti-CD3 antibody or antigen-binding antibody fragment of claim 9, which:

(i) comprises at least a second antigen binding domain that specifically binds to: an oncology target; an immune-oncology target; a neurodegenerative disease targets; an autoimmune disorder target; an infectious disease target; a metabolic disease target; a cognitive disorder target; a blood-brain barrier target; or a blood disease target;

(ii) comprises at least a second antigen binding domain that specifically binds to an antigen selected from the group consisting of: 17-IA, 4-1BB, 4Dc, 6- keto-PGFla, 8-iso-PGF2a, 8-oxo-dG, Al Adenosine Receptor, A33, ACE, ACE-2, Activin, Activin A, Activin AB, Activin B, Activin C, Activin RIA, Activin RIA ALK-2, Activin RIB ALK-4, Activin RIIA, Activin RUB, ADAM, ADAM10, ADAM12, ADAM 15, ADAM 17/T ACE, ADAM8, ADAM9, ADAMTS, ADAMTS4, ADAMTS5, Addressins, aFGF, ALCAM, ALK, ALK-1, ALK-7, alpha-l-antitrypsin, alpha-V/beta-1 antagonist, ANG, Ang, APAF-1, APE, APJ, APP, APRIL, AR, ARC, ART, Artemin, anti-Id, ASPARTIC, Atrial natriuretic factor, av/b3 integrin, Axl, b2M, B7-1, B7-2, B7-H, B-lymphocyte Stimulator (BlyS), BACE, BACE-1, Bad, BAFF, BAFF-R, Bag-1, BAK, Bax, BCA-1, BCAM, Bel, BCMA, BDNF, b-ECGF, bFGF, BID, Bik, BFM, BLC, BL-CAM, BLK, BMP, BMP-2 BMP-2a, BMP-3 Osteogenin, BMP-4 BMP-2b, BMP-5, BMP-6 Vgr-1, BMP-7 (OP-1), BMP-8 (BMP-8a, OP-2), BMPR, BMPR-IA (ALK-3), BMPR-IB (ALK-6), BRK-2, RPK-1, BMPR-II (BRK-3), BMPs, b- NGF, BOK, Bombesin, Bone-derived neurotrophic factor, BPDE, BPDE-DNA, BTC, complement factor 3 (C3), C3a, C4, C5, C5a, CIO, CA125, CAD-8, Calcitonin, cAMP, carcinoembryonic antigen (CEA), carcinoma-associated antigen, Cathepsin A, Cathepsin B, Cathepsin C/DPPI, Cathepsin D, Cathepsin E, Cathepsin H, Cathepsin L, Cathepsin O, Cathepsin S, Cathepsin V, Cathepsin X/Z/P, CBL, CCI, CCK2, CCL, CCL1, CCL11, CCL12, CCL13, CCL 14, CCL15, CCL16, CCL1 7, CCL18, CCL19, CCL2, CCL20, CCL21, CCL22, CCL23, CCL24, CCL25, CCL26, CCL27, CCL28, CCL3, CCL4, CCL5, CCL6, CCL7, CCL8, CCL9/10, CCR, CCR1, CCR10, CCR10, CCR2, CCR3, CCR4, CCR5, CCR6, CCR7, CCR8, CCR9, CD1, CD2, CD4, CD5, CD6, CD7, CD8, CD10, CDlla, CDllb, CDllc, CD13, CD14, CD15, CD16, CD18, CD19, CD20, CD21, CD22, CD23, CD25, CD27L, CD28, CD29, CD30, CD30L, CD32, CD33 (p67 proteins), CD34, CD38, CD40, CD40L, CD44, CD45, CD46, CD49a, CD52, CD54, CD55, CD56, CD61, CD64, CD66e, CD74, CD80 (B7-1), CD89, CD95, CD123, CD137, CD138, CD140a, CD146, CD147, CD148, CD152, CD164, CEACAM5, CFTR, cGMP, CINC, Clostridium botulinum toxin, Clostridium perfringens toxin, CKb8-l, CLC, CMV, CMV UL, CNTF, CNTN-1, COX, C-Ret, CRG-2, CT-1, CTACK, CTGF, CTLA-4, CX3CL1, CX3CR1, CXCL, CXCL1, CXCL2, CXCL3, CXCL4, CXCL5, CXCL6, CXCL7, CXCL8, CXCL9, CXCL10, CXCL11, CXCL12, CXCL13, CXCL14, CXCL15, CXCL16, CXCR, CXCR1, CXCR2, CXCR3, CXCR4, CXCR5, CXCR6, cytokeratin tumor-associated antigen, DAN, DCC, DcR3, DC-SIGN,

Decay accelerating factor, des(l-3)-IGF-I (brain IGF-1), Dhh, digoxin, DNAM-1, Dnase, Dpp, DPPIV/CD26, Dtk, ECAD, EDA, EDA-A1, EDA-A2, EDAR, EGF, EGFR (ErbB-1), EMA, EMMPRIN, EN A, endothelin receptor, Enkephalinase, eNOS, Eot, eotaxinl, EpCAM, Ephrin B2/ EphB4, EPO, ERCC, E-selectin, ET-1, Factor Ila, Factor VII, Factor VIIIc, Factor IX, fibroblast activation protein (FAP), Fas, FcRl, FEN-1, Ferritin, FGF, FGF-19, FGF-2, FGF3, FGF-8, FGFR, FGFR-3, Fibrin, FL, FLIP, Flt-3, Flt-4, Follicle stimulating hormone, Fractalkine, FZD1, FZD2, FZD3, FZD4, FZD5, FZD6, FZD7, FZD8, FZD9, FZD10, G250, Gas 6, GCP-2, GCSF, GD2, GD3, GDF, GDF-1, GDF-3 (Vgr-2), GDF-5 (BMP-14, CDMP- 1), GDF-6 (BMP-13, CDMP-2), GDF-7 (BMP-12, CDMP-3), GDF-8 (Myostatin), GDF-9, GDF- 15 (MIC-1), GDNF, GFAP, GFRa-1, GFR-alphal, GFR-alpha2, GFR-alpha3, GITR, Glucagon, Glut 4, glycoprotein Ilb/IIIa (GP Ilb/IIIa), GM-CSF, gpl30, gp72, GRO, Growth hormone releasing factor, Hapten (NP-cap or NIP-cap), HB-EGF, HCC, HCMV gB envelope glycoprotein, HCMV) gH envelope glycoprotein, HCMV UL, Hemopoietic growth factor (HGF), Hep B gpl20, heparanase, Her2, Her2/neu (ErbB-2), Her3 (ErbB-3), Her4 (ErbB-4), herpes simplex virus (HSV) gB glycoprotein, HSV gD glycoprotein, HGFA, High molecular weight melanoma-associated antigen (HMW-MAA), HIV gpl20, HIV IIIB gp 120 V3 loop, HLA, HLA-DR, HM1.24, HMFG PEM, HRG, Hrk, human cardiac myosin, human cytomegalovirus (HCMV), human growth hormone (HGH), HVEM, 1-309, IAP, ICAM, ICAM-1, ICAM-3, ICE, ICOS, IFNg, Ig, IgA receptor, IgE, IGF, IGF binding proteins, IGF-1R, IGFBP, IGF-I, IGF-II, IL, IL-1, IL-1R, IL-2, IL-2R, IL-4, IL-4R, IL-5, IL-5R, IL-6, IL-6R, IL-8, IL- 9, IL-10, IL-12, IL-13, IL-15, IL-18, IL-18R, IL-23, interferon (INF)-alpha, INF-beta, INF-gamma, Inhibin, iNOS, Insulin A-chain, Insulin B-chain, Insulin-like growth factor 1, integrin alpha2, integrin alpha3, integrin alpha4, integrin alpha4/betal, integrin, alpha4/beta7, integrin alpha5 (alphaV), integrin alpha5/betal, integrin alpha5/beta3, integrin alpha6, integrin betal, integrin beta2, interferon gamma, IP- 10, 1-TAC, JE, Kallikrein 2, Kallikrein 5, Kallikrein 6, , Kallikrein 11, Kallikrein 12, Kallikrein 14, Kallikrein 15, Kallikrein LI, Kallikrein L2, Kallikrein L3, Kallikrein L4, KC, KDR, Keratinocyte Growth Factor (KGF), laminin 5, LAMP, LAP, LAP (TGF- 1), Latent TGF-1, Latent TGF-1 bpl, LBP, LDGF, LECT2, Lefty, Lewis-Y antigen, Lewis-Y related antigen, LFA-1, LFA-3, Lfo, LIF, LIGHT, lipoproteins, LIX, LKN, Lptn, L-Selectin, LT-a, LT-b, LTB4, LTBP-1, Lung surfactant, Luteinizing hormone, Lymphotoxin Beta Receptor, Mac-1, MAdCAM, MAG, MAP2, MARC, MCAM, MCAM, MCK-2, MCP, M-CSF, MDC, Mer, a metalloprotease, MGDF receptor, MGMT, MHC (HLA-DR), MIF, MIG, MIP, MIP-1-alpha, MK, MMAC1, MMP, MMP-1, MMP-10, MMP-11, MMP-12, MMP-13, MMP-14, MMP-15, MMP-2, MMP-24, MMP- 3, MMP-7, MMP-8, MMP-9, MPIF, Mpo, MSK, MSP, mucin (Mucl), MUC18, Muellerian- inhibiting substance, Mug, MuSK, NAIP, NAP, NCAD, N-Cadherin, NCA 90, NCAM, NCAM, Neprilysin, Neurotrophin-3,-4, or -6, Neurturin, Neuronal growth factor (NGF), NGFR, NGF-beta, nNOS, NO, NOS, Npn, NRG-3, NT, NTN, OB, OGG1, OPG, OPN, OSM, OX40L, OX40R, pl50, p95, PADPr, Parathyroid hormone, PARC, PARP, PBR, PBSF, PCAD, P-Cadherin, PCNA, PDGF, PDGF, PDK-1, PECAM, PEM, PF4, PGE, PGF, PGI2, PGJ2, PIN, PLA2, placental alkaline phosphatase (PLAP), PIGF, PLP, PP14, Proinsulin,

Prorelaxin, Protein C, PS, PSA, PSCA, prostate specific membrane antigen (PSMA), PTEN, PTHrp, Ptk, PTN, R51, RANK, RANKL, RANTES, Relaxin A-chain, Relaxin B-chain, renin, respiratory syncytial virus (RSV) F, RSV Fgp, Ret, Rheumatoid factors, RLIP76, RPA2, RSK, S100, SCF/KL, SDF-1, SERINE, Serum albumin, sFRP-3, Shh, SIGIRR, SK-1, SLAM, SLPI, SMAC, SMDF, SMOH, SOD, SPARC, Stat, STEAP, STEAP-II, TACE, TACI, TAG-72 (tumor- associated glycoprotein-72), TARC, TCA-3, T-cell receptors (e.g., T-cell receptor alpha/beta), TdT, TECK, TEM1, TEM5, TEM7, TEM8, TERT, testicular PLAP-like alkaline phosphatase, TfR, TGF, TGF-alpha, TGF-beta, TGF-beta Pan Specific, TGF-beta RI (ALK-5), TGF-beta RII, TGF-beta Rllb, TGF-beta RIII, TGF-betal, TGF-beta2, TGF-beta3, TGF-beta4, TGF-beta5, Thrombin, Thymus Ck-1, Thyroid stimulating hormone, Tie, TIMP, TIQ, Tissue Factor, TMEFF2, Tmpo, TMPRSS2, TNF, TNF-alpha, TNF-alpha beta, TNF-beta2, TNFc, TNF-RI, TNF-RII, TNFRSF10A (TRAIL Rl Apo-2, DR4), TNFRSFIOB (TRAIL R2 DR5, KILLER, TRICK-2A, TRICK-B), TNFRSF10C (TRAIL R3 DcRl, LIT, TRID), TNFRSF10D (TRAIL R4 DcR2, TRUNDD), TNFRSF11A (RANK ODF R, TRANCE R), TNFRSFllB (OPG OCIF, TR1), TNFRSF12 (TWEAK R FN14), TNFRSF13B (TACI), TNFRSF13C (BAFF R), TNFRSF14 (HVEM ATAR, HveA, LIGHT R, TR2), TNFRSF16 (NGFR p75NTR), TNFRSF17 (BCMA), TNFRSF 18 (GITR AITR), TNFRSF19 (TROY TAJ, TRADE), TNFRSF19L (RELT), TNFRSFIA (TNF RI CD120a, p55-60), TNFRSFIB (TNF RII CD120b, p75-80), TNFRSF26 (TNFRH3), TNFRSF3 (LTbR TNF RIII, TNFC R), TNFRSF4 (OX40 ACT35, TXGP1 R), TNFRSF 5 (CD40 p50), TNFRSF6 (Fas Apo-1, APT1, CD95), TNFRSF6B (DcR3 M68, TR6), TNFRSF7 (CD27), TNFRSF8 (CD30), TNFRSF9 (4-1BB CD137, ILA), TNFRSF21 (DR6), TNFRSF22 (DcTRAIL R2 TNFRH2), TNFRST23 (DcTRAIL Rl TNFRH1), TNFRSF25 (DR3 Apo-3, LARD, TR-3, TRAMP, WSL-1), TNFSF10 (TRAIL Apo-2 Ligand, TL2), TNFSF11 (TRANCE/RANK Ligand ODF, OPG Ligand), TNFSF12 (TWEAK Apo-3 Ligand, DR3 Ligand), TNFSF13 (APRIL TALL2), TNFSF13B (BAFF BLYS, TALL1, THANK, TNFSF20), TNFSF14 (LIGHT HVEM Ligand, LTg), TNFSF15 (TLIA/VEGI), TNFSF18 (GITR Ligand AITR Ligand, TL6), TNFSFIA (TNF-a Conectin, DIF, TNFSF2), TNFSF1B (TNF-b LTa, TNFSF1), TNFSF3 (LTb TNFC, p33), TNFSF4 (OX40 Ligand gp34, TXGP1), TNFSF5 (CD40 Ligand CD154, gp39, HIGM1, IMD3, TRAP), TNFSF6 (Fas Ligand Apo-1 Ligand, APT1 Ligand), TNFSF7 (CD27 Ligand CD70), TNFSF8 (CD30 Ligand CD153), TNFSF9 (4-1BB Ligand CD137 Ligand), TP-1, t-PA, Tpo, TRAIL, TRAIL R, TRAIL-R1, TRAIL-R2, TRANCE, transferring receptor, TRF, Trk, TROP-2, TSG, TSLP, tumor-associated antigen CA 125, tumor-associated antigen expressing Lewis Y related carbohydrate, TWEAK, TXB2, Ung, uPAR, uPAR-1, Urokinase, VCAM, VCAM-1, VECAD, VE-Cadherin, VE-cadherin-2, VEFGR-1 (flt-1), VEGF, VEGFR, VEGFR-3 (flt-4), VEGI, VFM, Viral antigens, VLA, VLA-1, VLA-4, VNR integrin, von Willebrands factor, WIF- 1, WNT1, WNT2, WNT2B/13, WNT3, WNT3A, WNT4, WNT5A, WNT5B, WNT6, WNT7A, WNT7B, WNT8A, WNT8B, WNT9A, WNT9A, WNT9B, WNT10A, WNT10B, WNT11, WNT16, XCL1, XCL2, XCR1, XCR1, XEDAR, XIAP, XPD, CTLA4 (cytotoxic T lymphocyte antigen-4), PD1 (programmed cell death protein 1), PD-

L1 (programmed cell death ligand 1), LAG-3 (lymphocyte activation gene-3), TIM-3 (T cell immunoglobulin and mucin protein-3), hormone receptors and growth factors;

(iii) comprises at least a second antigen binding domain that specifically binds to an antigen selected from the group consisting of: BCMA, CTLA4 (cytotoxic T lymphocyte antigen-4), PD1 (programmed cell death protein 1), PD-L1 (programmed cell death ligand 1), LAG-3 (lymphocyte activation gene-3), TIM-3, CD20, CD2, CD19, Her2, EGFR, EpCAM, FcγRIIIa (CD16), FcγRIIa (CD32a), FcγRIIb (CD32b), FcγRI (CD64), Toll-like receptors (TLRs), TLR4, TLR9, cytokines, IL-2, IL-5, IL-13, IL-6, IL-17, IL-12, IL-23, TNFa, TGFb, cytokine receptors, IL-2R, chemokines, chemokine receptors, growth factors, VEGF, and HGF; and/or

(iv) comprises a multispecific format selected from the group consisting of: Fab-Fc-scFv, "bottle-opener, Mab-scFv, Mab-Fv, Dual scFv, central Fv, central scFv, one-arm central scFv, Fab-Fab, Fab-Fv, mAb-Fv, mAb-Fab, DART, BiTE, common light chain-IgG, TandAb, Cross-Mab, SEED, BEAT, TrioMab, and DuetMab.

11. An isolated or recombinant nucleic acid encoding an anti-CD3 antibody or antigen-binding antibody fragment of any one of claims 1-10.

12. An expression vector comprising an isolated or recombinant nucleic acid of claim 11.

13. A host cell transfected, transformed, or transduced with a nucleic acid of claim 11 or a vector containing said nucleic acid, optionally wherein the host cell is or comprises a eukaryotic cell or a prokaryotic cell, optionally a mammalian cell, an yeast cell, or a bacterial cell, further optionally a Chinese Hamster Ovary (CHO) cell, a human embryonic kidney (HEK) cell, or a lymphoid cell.

14. A pharmaceutical composition comprising: an anti-CD3 antibody or antigen-binding antibody fragment of any one of claims 1-10; and a pharmaceutically acceptable carrier and/or excipient.

15. An anti-CD3 antibody or antigen-binding antibody fragment of any one of claims 1-10 for use in the treatment of a disorder in a mammal in need of such treatment, wherein the disorder comprises a proliferative disorder, an oncological disorder, an immuno-oncological disorder, a neurological disorder, a neurodegenerative disorder, or an autoimmune disorder, optionally wherein: (i) the anti-CD3 antibody or antigen-binding antibody fragment is to be used in combination with an additional therapeutic agent; and/or (ii) the mammal is a human.

16. A host cell of claim 13 for use in the treatment of a disorder in a mammal in need of such treatment, wherein the disorder comprises a proliferative disorder, an oncological disorder, an immuno-oncological disorder, a neurological disorder, a neurodegenerative disorder, or an autoimmune disorder, optionally wherein the host cell is or comprises an immune cell, optionally a T or NK cell, and optionally wherein: (i) the anti-CD3 antibody or antigen-binding antibody fragment is to be used in combination with an additional therapeutic agent; and/or (ii) the mammal is a human.

17. Use of an anti-CD3 antibody or antigen-binding antibody fragment of any one of claims 1-10 in the manufacture of a medicament for the treatment of cancer or an autoimmune disease, optionally wherein: (i) the treatment further comprises use of an additional therapeutic agent; and/or (ii) the mammal is a human.

18. Use of a host cell of claim 13 in the manufacture of a medicament for the treatment of cancer or an autoimmune disease, optionally wherein the host cells is or comprises an immune cell, optionally a T or NK cell, and optionally wherein: (i) the treatment further comprises use of an additional therapeutic agent; and/or (ii) the mammal is a human.

19. A method of making a host cell of claim 13, the method comprising introducing an isolated or recombinant nucleic acid of claim 11 into a cell.

20. A method of making an anti-CD3 antibody or antigen-binding antibody fragment of any one of claims 1-10, the method comprising culturing a host cell comprising a nucleic nucleic acid encoding the anti-CD3 antibody or antigen-binding antibody fragment under a condition suitable for expression of the anti-CD3 antibody or antigen-binding antibody fragment.