

epithelium, and B cells in tissues damaged by chronic autoimmunity including kidney, intestine, and joints (Borcherding, Am J Pathol. 2010 Apr;176(4):1816-27, ; Sawada-Hase, Am J Gastroenterol. 2000 Jun;95(6):1516-23). Soluble CD40L is elevated in patients suffering from SLE, IBD, and Sjogren's syndrome consistent with inflammatory burden in these patients.

5 Some of the earliest evidence the CD40/CD40L pathway in chronic intestinal inflammation came from preclinical models where anti-CD40L mAbs protected rodents from experimental colitis (de Jong, Gastroenterology. 2000 Sep;119(3):715-23; Liu, J Immunol. 2000 Jun 1;164(11):6005-14; Stuber, J Exp Med 1996 Feb 1, 183(2):693-8). Reduction in disease activity scores were associated with reduced pro-inflammatory cytokine production in the gut and protection from chronic body
10 weight loss. Similar results were observed in animals that were genetically deficient for CD40 or CD40L (de Jong, Gastroenterology. 2000 Sep;119(3):715-23). Treatment of mice with anti-CD40L mAbs after disease onset is still effective in reducing disease activity suggesting that this pathway is critical for maintenance of chronic inflammatory disease. In addition, CD40 agonist antibodies are sufficient to drive intestinal inflammation in mice that lack lymphocytes (Uhlig, Immunity. 2006
15 Aug;25(2):309-18). More recent data using CD40 siRNA also point to an important role for CD40 signaling in colitis (Arranz, J Control Release. 2013 Feb 10;165(3):163-72). In Crohn's disease, lamina propria monocytes and epithelium express high levels of CD40 and CD40+ monocytes are enriched in peripheral blood. Furthermore, polymorphisms in the *CD40* locus have been linked to increased susceptibility to IBD. In Crohn's patients treated with anti-TNF antibodies, transcriptional
20 profiling indicates that CD40 mRNA levels decrease in patients with an adequate drug treatment response. However, in patients with a poor response to TNF inhibitors, CD40 mRNA levels are unchanged suggesting that CD40-dependent, TNF-independent pathways may promote inflammation in these patients. Studies suggest that inhibition of CD40 mediated signaling is important in the pathogenesis of IBD as well as other autoimmune diseases. Accordingly, there
25 remains a need for antagonist anti-CD40 antibodies, and antigen-binding portions thereof, that can be used for therapeutic purposes for treating chronic inflammatory diseases and disorders, such Crohn's disease.

SUMMARY

30 This disclosure pertains to antagonist anti-CD40 antibodies, or antigen-binding portions thereof. Antibodies of the disclosures include, but are not limited to, antagonist humanized antibodies, and antigen-binding portions thereof, that are capable of binding human CD40 and are substantially free of agonist activity.

Disclosed is an isolated antibody, or antigen binding portion thereof, wherein the antibody, or antigen binding fragment thereof, binds an epitope of human CD40 defined by the topographic regions Cys62-Phe67, Gln79-Cys83, Arg90-Thr99, and Thr24-Cys37 of SEQ ID NO:1. In one embodiment, the antibody, or antigen binding portion thereof, is an antagonist antibody. In one
5 embodiment, the antibody, or antigen binding portion thereof, is an antagonist antibody which is substantially free of agonist activity.

In another embodiment, the antibody, or antigen binding portion thereof, comprises a heavy chain variable region comprising a CDR3 having the amino acid sequence of SEQ ID NO: 8 and a light chain variable region comprising a CDR3 having the amino acid sequence of SEQ ID NO: 12.
10 In a further embodiment, the antibody, or antigen binding portion thereof, comprises a heavy chain variable region comprising a CDR2 having the amino acid sequence of SEQ ID NO: 111 and a light chain variable region comprising a CDR2 having the amino acid sequence of SEQ ID NO: 11. In another further embodiment, the antibody, or antigen binding portion thereof, comprises a heavy chain variable region comprising a CDR2 having the amino acid sequence of SEQ ID NO: 42 and a
15 light chain variable region comprising a CDR2 having the amino acid sequence of SEQ ID NO: 11. In another embodiment, the antibody, or antigen binding portion thereof, comprises a heavy chain variable region comprising a CDR1 having the amino acid sequence of SEQ ID NO: 6 and a light chain variable region comprising a CDR1 having the amino acid sequence of SEQ ID NO: 21.

In one embodiment, the antibody, or antigen binding portion thereof, is an IgG isotype. In a
20 further related embodiment, the antibody, or antigen binding portion thereof, is an IgG1 or an IgG4 isotype.

In one embodiment, the antibody, or antigen binding portion thereof, has an IC50 of at least 50 nM in a Jurkat cell reporter assay.

In another aspect, there is provided an antagonist anti-CD40 antibody comprising a heavy
25 chain CDR1 comprising an amino acid sequence as set forth in SEQ ID NO:6, a heavy chain CDR2 comprising an amino acid sequence as set forth in SEQ ID NO:42, a heavy chain CDR3 comprising an amino acid sequence as set forth in SEQ ID NO:8, a light chain CDR1 comprising an amino acid sequence as set forth in SEQ ID NO:21, a light chain CDR2 comprising an amino acid sequence as set forth in SEQ ID NO:11, and a light chain CDR3 comprising an amino acid sequence as set forth in
30 SEQ ID NO:12. Disclosed is an antagonist anti-CD40 antibody, or antigen-binding portion thereof, comprising a light chain variable region comprising a CDR3 having an amino acid sequence as set forth in SEQ ID NO: 12 and/or a heavy chain variable region comprising a CDR3 having an amino acid sequence as set forth in SEQ ID NO: 8. In one embodiment, the light chain variable region of the antagonistic anti-CD40 antibody, or antigen-binding portion thereof, comprises a CDR3 having the
35 amino acid sequence as set forth in SEQ ID NO: 12 and wherein the heavy chain variable region comprises a CDR3 having the amino acid sequence as set forth in SEQ ID NO: 8. In another embodiment, the heavy chain variable region of the antagonist anti-CD40 antibody, or antigen-binding portion thereof, further comprises a

the antagonist anti-CD40 antibody, or antigen-binding portion thereof, is a human IgG1 constant domain. In a further related embodiment, the human IgG1 constant domain of the antagonist anti-CD40 antibody, or antigen-binding portion thereof, comprises an amino acid sequence of SEQ ID NO:2 or SEQ ID NO:3.

5 In another embodiment, the antagonist anti-CD40 antibody, or antigen-binding portion thereof, further comprises a light chain immunoglobulin constant domain comprising a human Ig kappa constant domain or a human Ig lambda constant domain. In a related embodiment, the human Ig kappa constant domain of the antagonist anti-CD40 antibody, or antigen-binding portion thereof, comprises an amino acid sequence of SEQ ID NO:4 or wherein the human Ig lambda constant domain
10 comprises an amino acid sequence SEQ ID NO:81.

The present disclosure also features, in certain embodiments, an antagonist anti-CD40 antibody, or antigen-binding portion thereof, that competes with the antibody, or antigen binding portion thereof, as set forth in any of the aspects and embodiments described herein.

Disclosed is an antagonist anti-CD40 antibody, or antigen-binding portion thereof, that
15 comprises a heavy chain CDR1 comprising an amino acid sequence as set forth in SEQ ID NO:6, a heavy chain CDR2 comprising an amino acid sequence as set forth in SEQ ID NO:42, a heavy chain CDR3 comprising an amino acid sequence as set forth in SEQ ID NO:8, a light chain CDR1 comprising an amino acid sequence as set forth in SEQ ID NO:21, a light chain CDR2 comprising an amino acid sequence as set forth in SEQ ID NO:11, and a light chain CDR3 comprising an amino acid
20 sequence as set forth in SEQ ID NO:12.

Disclosed is an antagonist anti-CD40 antibody, or antigen-binding portion thereof, comprising a heavy chain variable domain comprising an amino acid sequence set forth in SEQ ID NO: 28 and a light chain variable domain comprising an amino acid sequence set forth in SEQ ID NO: 20. Disclosed is an antagonist anti-CD40 antibody, or antigen-binding portion thereof,
25 comprising a heavy chain variable domain comprising an amino acid sequence having at least 90%, 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO: 28, and/or a light chain variable domain comprising an amino acid sequence having at least 90%, 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO: 20.

Disclosed is an antagonist anti-CD40 antibody, or antigen-binding portion thereof,
30 comprising a heavy chain comprising an amino acid sequence set forth in SEQ ID NO: 41, or a sequence having at least 90%, 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO: 41, and/or a light chain comprising an amino acid sequence set forth in SEQ ID NO: 40, or a sequence having at least 90%, 95%, 96%, 97%, 98%, or 99% identity to

SEQ ID NO: 40. In one embodiment, the heavy chain of the antagonist anti-CD40 antibody, or antigen binding portion thereof, comprises an amino acid sequence set forth in SEQ ID NO: 41, and the light chain of the antagonistic anti-CD40 antibody, or antigen binding portion thereof, comprises an amino acid sequence set forth in SEQ ID NO: 40.

5 In one aspect, there is provided an anti-CD40 antibody comprising a heavy chain comprising an amino acid sequence as set forth in SEQ ID NO: 41, and a light chain comprising an amino acid sequence as set forth in SEQ ID NO: 40.

In one embodiment, the antibodies, or antigen binding portions thereof, of the disclosure are recombinant.

10 In another aspect, there is provided a pharmaceutical composition comprising the anti-CD40 antibody as set forth in any of the aspects and embodiments described herein, and a pharmaceutically acceptable carrier. The present disclosure also features, in certain embodiments, a pharmaceutical composition comprising the anti-CD40 antibody, or antigen binding portion thereof, as set forth in any of the aspects and embodiments described herein, and a pharmaceutically acceptable carrier.

15 The present disclosure also features, in other certain embodiments, a pharmaceutical composition comprising the anti-CD40 antibody, or antigen binding portion thereof, as set forth in any of the aspects and embodiments described herein, and a polysorbate. In a further related embodiment, the polysorbate is polysorbate 80.

In another embodiment, the pharmaceutical composition comprises a histidine buffer.

20 In another further embodiment, the pharmaceutical composition comprises a polyol. In a related embodiment, the polyol is selected from mannitol, sorbitol, trehalose, or sucrose.

In another embodiment, the pharmaceutical composition has a pH of about 4 to about 8. In a related embodiment, the pharmaceutical composition has a pH of about 5 to about 7.

In another embodiment, the pharmaceutical composition is lyophilized.

25 In another aspect, there is provided an isolated nucleic acid encoding an anti-CD40 antibody amino acid sequence of any one of the aspects and embodiments described herein. The present disclosure also features, in other embodiments, an isolated nucleic acid encoding an antagonist anti-CD40 antibody amino acid sequence of any one of the aspects and embodiments described herein. In another aspect, there is provided a vector comprising the isolated nucleic acid. In a related
30 embodiment, the vector is selected from pcDNA, pTT, pTT3, pEFBOS, pBV, pJV, and pBJ vectors.

In another aspect, there is provided a host cell comprising the vector. In a related embodiment, the host cell is a prokaryotic cell or a eukaryotic cell. In a further embodiment, the eukaryotic cell is a protist cell, an animal cell, a plant cell, a fungal cell, a yeast cell, a mammalian cell, an avian cell, or an insect cell. In another further embodiment, the mammalian cell is a CHO cell
35 or a COS cell.

In another aspect, there is provided a method of producing an anti-CD40 antibody, the method comprising the steps of culturing a host cell of any one of the aspects and embodiments described herein in culture medium under conditions sufficient to produce the anti-CD40 antibody. The present disclosure also features, in certain embodiments, a method of producing an antagonist anti-CD40 antibody, or antigen binding portion thereof, the method comprising the steps of culturing a host cell of any one of the aspects and embodiments described herein in culture medium under conditions sufficient to produce the antagonist anti-CD40 antibody, or antigen binding portion thereof. In further embodiments, an antagonist anti-CD40 antibody, or antigen binding portion thereof, is produced by the method.

The present disclosure also features, in other embodiments, a method for reducing human CD40 activity, the method comprising the step of contacting human CD40 with the antibody, or antigen-binding portion thereof, of any one of the aspects and embodiments described herein, such that human CD40 activity is reduced. In a further embodiment, the method is an *in vitro* method.

The present disclosure also features, in other certain embodiments, a method for treating a human subject having a disorder in which CD40 is detrimental comprising administering an effective amount of the anti-CD40 antibody, or antigen binding portion thereof, of any one of the aspects and embodiments described herein, to the subject.

The present disclosure also features, in other embodiments, a method for reducing human CD40 activity in a human subject having a disorder in which CD40 activity is detrimental, the method comprising the step of administering to the human subject the antibody, or antigen binding portion thereof, of any one of aspects and embodiments described herein, such that human CD40 activity in the human subject is reduced.

In another aspect, there is provided an anti-CD40 antibody of any one of the aspects and embodiments described herein for use as a medicament.

In a further embodiment, the antibody, or antigen binding portion thereof, is administered before, concurrently, or after the administration of a second agent to the subject. In a further related embodiment, the second agent is selected from an antibody, or fragment thereof, capable of binding human IL-12; PGE2; LPA; NGF; CGRP; SubP; RAGE; histamine; a histamine receptor blocker; bradykinin; IL-1 alpha; IL-1beta; VEGF; PLGF; methotrexate; a corticosteroid, a glucocorticoid receptor modulator; cyclosporin, rapamycin, FK506, a non-steroidal anti-inflammatory agent, an inhaled steroid; beta-agonist; short-acting or long-acting beta-agonist; antagonist of leukotrienes or leukotriene receptors; ADVAIR; IgE inhibitor; anti-IgE antibodies; XOLAIR; phosphodiesterase inhibitor; PDE4 inhibitor; xanthine; anticholinergic drug; mast cell-stabilizing agent; Cromolyn; IL-4 inhibitor; IL-5 inhibitor; eotaxin/CCR3 inhibitors antagonists of histamine or its receptors including H1, H2, H3, and H4; antagonists of prostaglandin D or its receptors DP1 and CRTH2; TNF antagonist; a soluble fragment of a TNF receptor; ENBREL; TNF enzyme antagonist; TNF converting enzyme (TACE) inhibitor; muscarinic receptor antagonist; TGF-beta antagonist; interferon gamma; perfenidone; chemotherapeutic agent,

methotrexate; leflunomide; sirolimus (rapamycin) or an analog thereof, CCI-779; COX2 or cPLA2 inhibitor; NSAID; immunomodulator; p38 inhibitor; TPL-2, MK-2 and NFkB inhibitor; budenoside; epidermal growth factor; corticosteroid; cyclosporine; sulfasalazine; aminosalicylate; 6-mercaptopurine; azathioprine; metronidazole; lipoxygenase inhibitor; mesalamine; olsalazine; balsalazide; antioxidant; thromboxane inhibitor; IL-1 receptor antagonist; anti-IL-1 β antibody; anti-IL-6 antibody; growth factor; elastase inhibitor; pyridinyl-imidazole compound; antibody or agonist of LT, IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-14, IL-15, IL-16, IL-17, IL-18, IL-19, IL-20, IL-21, IL-22, IL-23, IL-24, IL-25, IL-26, IL-27, IL-28, IL-29, IL-30, IL-31, IL-32, IL-33, EMAP-II, GM-CSF, FGF, or PDGF; antibody of CD2, CD3, CD4, CD8, CD25, CD28, CD30, CD40, CD45, CD69, CD90 or their ligand; FK506; rapamycin; mycophenolate mofetil; ibuprofen; prednisolone; phosphodiesterase inhibitor; adenosine agonist; antithrombotic agent; complement inhibitor; adrenergic agent; IRAK, NIK, IKK, p38, or MAP kinase inhibitor; IL-1 β converting enzyme inhibitor; TNF- α quadrature converting enzyme inhibitor; T-cell signaling inhibitor; metalloproteinase inhibitor; 6-mercaptopurine; angiotensin converting enzyme inhibitor; soluble cytokine receptor; soluble p55 TNF receptor; soluble p75 TNF receptor; sIL-1RI; sIL-1RII; sIL-6R; anti-inflammatory cytokine; IL-4; IL-10; IL-11; or TGF- β .

In a further embodiment, the disorder is selected from a respiratory disorder; asthma; allergic and nonallergic asthma; asthma due to infection; asthma due to infection with respiratory syncytial virus (RSV); chronic obstructive pulmonary disease (COPD); a condition involving airway inflammation; eosinophilia; fibrosis and excess mucus production; cystic fibrosis; pulmonary fibrosis; an atopic disorder; atopic dermatitis; urticaria; eczema; allergic rhinitis; allergic enterogastritis; an inflammatory and/or autoimmune condition of the skin; an inflammatory and/or autoimmune condition of gastrointestinal organs; inflammatory bowel diseases (IBD); ulcerative colitis; Crohn's disease; an inflammatory and/or autoimmune condition of the liver; liver cirrhosis; liver fibrosis; liver fibrosis caused by hepatitis B and/or C virus; scleroderma; tumors or cancers; hepatocellular carcinoma; glioblastoma; lymphoma; Hodgkin's lymphoma; a viral infection; a bacterial infection; a parasitic infection; HTLV-1 infection; suppression of expression of protective type 1 immune responses, and suppression of expression of a protective type 1 immune response during vaccination.

In another further embodiment, the disorder is selected from an autoimmune or inflammatory disease, such as systemic lupus erythematosus (SLE), discoid lupus, lupus nephritis, sarcoidosis, inflammatory arthritis, including, but not limited to, juvenile arthritis, rheumatoid arthritis, psoriatic arthritis, Reiter's syndrome, ankylosing spondylitis, and gouty

arthritis, rejection of an organ or tissue transplant, hyperacute, acute, or chronic rejection and/or graft versus host disease, multiple sclerosis, hyper IgE syndrome, polyarteritis nodosa, primary biliary cirrhosis, inflammatory bowel disease, Crohn's disease, celiac's disease (gluten-sensitive enteropathy), autoimmune hepatitis, pernicious anemia, autoimmune hemolytic anemia, psoriasis, scleroderma, myasthenia gravis, autoimmune thrombocytopenic purpura, autoimmune thyroiditis, Grave's disease, Hasimoto's thyroiditis, immune complex disease, chronic fatigue immune dysfunction syndrome (CFIDS), polymyositis and dermatomyositis, cryoglobulinemia, thrombolysis, cardiomyopathy, pemphigus vulgaris, pulmonary interstitial fibrosis, sarcoidosis, Type I and Type II diabetes mellitus, type 1, 2, 3, and 4 delayed-type hypersensitivity, allergy or allergic disorders, unwanted/unintended immune responses to therapeutic proteins, asthma, Churg-Strauss syndrome (allergic granulomatosis), atopic dermatitis, allergic and irritant contact dermatitis, urticaria, IgE-mediated allergy, atherosclerosis, vasculitis, idiopathic inflammatory myopathies, hemolytic disease, Alzheimer's disease, chronic inflammatory demyelinating polyneuropathy, Sjogren's, and psoriasis.

In one embodiment, an anti-CD40 antibody, or antigen-binding portion thereof, of the disclosure, *e.g.* Ab102, is used to treat an inflammatory bowel disease (IBD).

In one embodiment, an anti-CD40 antibody, or antigen-binding portion thereof, of the disclosure, *e.g.* Ab102, is used to treat ulcerative colitis.

In one embodiment, an anti-CD40 antibody, or antigen-binding portion thereof, of the disclosure, *e.g.* Ab102, is used to treat Crohn's disease.

In one embodiment, an anti-CD40 antibody, or antigen-binding portion thereof, of the disclosure, *e.g.* Ab102, is used to treat systemic lupus erythematosus (SLE).

In one embodiment, an anti-CD40 antibody, or antigen-binding portion thereof, of the disclosure, *e.g.* Ab102, is used to treat sarcoidosis.

In one embodiment, an anti-CD40 antibody, or antigen-binding portion thereof, of the disclosure, *e.g.* Ab102, is used to treat juvenile arthritis.

In one embodiment, an anti-CD40 antibody, or antigen-binding portion thereof, of the disclosure, *e.g.* Ab102, is used to treat rheumatoid arthritis.

In one embodiment, an anti-CD40 antibody, or antigen-binding portion thereof, of the disclosure, *e.g.* Ab102, is used to treat psoriatic arthritis.

In one embodiment, an anti-CD40 antibody, or antigen-binding portion thereof, of the disclosure, *e.g.* Ab102, is used to treat ankylosing spondylitis.

In one embodiment, an anti-CD40 antibody, or antigen-binding portion thereof, of the disclosure, *e.g.* Ab102, is used to treat hidradenitis suppurativa.

In one embodiment, an anti-CD40 antibody, or antigen-binding portion thereof, of the disclosure, *e.g.* Ab102, is used to treat uveitis.

In one embodiment, an anti-CD40 antibody, or antigen-binding portion thereof, of the disclosure, *e.g.* Ab102, is used to treat Sjogren's.

5 In one embodiment, an anti-CD40 antibody, or antigen-binding portion thereof, of the disclosure, *e.g.* Ab102, is used to treat psoriasis.

In another further embodiment, the antibody, or antigen binding fragment thereof, is administered by at least one mode selected from parenteral, subcutaneous, intramuscular, intravenous, intra-articular, intrabronchial, intraabdominal, intracapsular, intracartilaginous, intracavitary, 10 intracelial, intracerebellar, intracerebroventricular, intracolic, intracervical, intragastric, intrahepatic, intramyocardial, intraosteal, intrapelvic, intrapericardiac, intraperitoneal, intrapleural, intraprostatic, intrapulmonary, intrarectal, intrarenal, intraretinal, intraspinal, intrasynovial, intrathoracic, intrauterine, intravesical, bolus, vaginal, rectal, buccal, sublingual, intranasal, and transdermal.

The present disclosure also features, in other certain embodiments, a method of determining 15 the presence of CD40 or fragment thereof in a test sample by an immunoassay, wherein the immunoassay comprises contacting the test sample with at least one antibody, or antigen-binding portion thereof, of any of the aspects and embodiments described herein, and at least one detectable label. In a further embodiment, the method further comprises the steps of: (i) contacting the test sample with the at least one antibody, or antigen-binding portion thereof, wherein the antibody, or 20 antigen-binding portion thereof, binds to an epitope on the CD40 or fragment thereof so as to form a first complex; (ii) contacting the complex with the at least one detectable label, wherein the detectable label binds to an epitope on the first complex, or on the CD40 or fragment thereof, that is not bound by the antibody, or antigen-binding portion thereof, to form a second complex; and (iii) detecting the presence of the CD40 or fragment thereof in the test sample based on the signal generated by the 25 detectable label in the second complex, wherein the presence of the CD40 or fragment thereof is directly correlated with the signal generated by the detectable label. In a further related embodiment, the method further comprises the steps of: (i) contacting the test sample with the at least one antibody, or antigen-binding portion thereof, wherein the antibody, or antigen-binding portion thereof, binds to an epitope on the CD40 or fragment thereof so as to form a first complex; (ii) contacting the complex 30 with the at least one detectable label, wherein the detectable label competes with the CD40 or fragment thereof for binding to the antibody, or antigen-binding portion thereof, so as to form a second complex; and (iii) detecting the presence of the CD40 or fragment thereof in the test sample based on the signal

generated by the detectable label in the second complex, wherein the presence of the CD40 or fragment thereof is indirectly correlated with the signal generated by the detectable label.

In one embodiment, the disclosure provides a DVD-Ig which comprises the binding regions, e.g. CDRs, described herein. In one embodiment, the DVD-Ig of the disclosure comprises four polypeptide chains, wherein two polypeptide chains comprise VD1-(X1)_n-VD2-C-(X2)_n, wherein VD1 is a first heavy chain variable domain, VD2 is a second heavy chain variable domain, C is a heavy chain constant domain, X1 is a linker with the proviso that it is not CH1, and X2 is an Fc region; and two polypeptide chains comprise VD1-(X1)_n-VD2-C-(X2)_n, wherein VD1 is a first light chain variable domain, VD2 is a second light chain variable domain, C is a light chain constant domain, X1 is a linker with the proviso that it is not CH1, and X2 does not comprise an Fc region; and n is 0 or 1; wherein said four polypeptide chains of said binding protein form four functional antigen binding sites. In one embodiment, the first (and/or second) heavy chain of the DVD comprises a CDR set as set forth in SEQ ID NOs: 6, 42, and 8. In one embodiment, the first (and/or second) light chain variable region comprises a CDR set as set forth in SEQ ID NOs: 21, 11, and 12. In one embodiment, the DVD-Ig of the disclosure is monospecific and binds huCD40. In another embodiment, the DVD-Ig of the disclosure is multispecific and binds CD40 and a second molecular target.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1A graphically depicts the antagonistic activity of chimeric antibody (Antibody 1 (Ab1)) versus an agonist control and known antagonist antibodies (4D11 (Astellas) and Bib (Boehringer)). **Figure 1B** graphically depicts the activity of the same chimeric antibody Ab1 in an agonist assay using the same agonist and antagonist controls as Figure 1A.

Figure 2 graphically depicts agonist activity (Figure 2A) and antagonist activity (Figure 2B) of humanized antibody Ab101 in comparison to antibody 4D11, antibody B1b, an IgG antibody (control), and an agonist control antibody (2141).

Figure 3 graphically depicts results from *in vivo* studies of humanized antibody Ab101. Figure 3A graphically depicts IgG production in *huscid* mice who have received human PBMCs in combination with an Ig control, a CTLA4-Ig fusion, or the Ab101 antibody. Figure 3B graphically depicts B cell survival in the same mouse model administered the same agents as Figure 3A.

CLAIMS

1. An anti-CD40 antibody comprising a heavy chain comprising an amino acid sequence as set forth in SEQ ID NO: 41, and a light chain comprising an amino acid sequence as set forth in SEQ ID NO: 40.
2. An antagonist anti-CD40 antibody comprising a heavy chain CDR1 comprising an amino acid sequence as set forth in SEQ ID NO:6, a heavy chain CDR2 comprising an amino acid sequence as set forth in SEQ ID NO:42, a heavy chain CDR3 comprising an amino acid sequence as set forth in SEQ ID NO:8, a light chain CDR1 comprising an amino acid sequence as set forth in SEQ ID NO:21, a light chain CDR2 comprising an amino acid sequence as set forth in SEQ ID NO:11, and a light chain CDR3 comprising an amino acid sequence as set forth in SEQ ID NO:12.
3. The anti-CD40 antibody of claim 2, wherein the antibody is an IgG isotype.
4. The anti-CD40 antibody of claim 3, wherein the antibody is an IgG1 isotype.
5. The anti-CD40 antibody of any one of claims 2-4, wherein the antibody is humanized.
6. The anti-CD40 antibody of any one of claims 2-5, wherein the antibody comprises a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 28.
7. The anti-CD40 antibody of any one of claims 2-6, wherein the antibody comprises a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 20.
8. The anti-CD40 antibody of any one of claims 2-7, wherein the antibody is substantially free of agonist activity.

9. The anti-CD40 antibody of any one of claims 2-8, wherein the antibody comprises a heavy chain immunoglobulin constant domain of a human IgG1 constant domain.
10. The anti-CD40 antibody of claim 9, wherein the human IgG1 constant domain comprises an amino acid sequence of SEQ ID NO:2 or SEQ ID NO:3.
11. The anti-CD40 antibody of any one of claims 2-10, wherein the antibody further comprises a light chain immunoglobulin constant domain comprising a human Ig kappa constant domain or a human Ig lambda constant domain.
12. The anti-CD40 antibody of claim 11, wherein the human Ig kappa constant domain comprises an amino acid sequence of SEQ ID NO:4 or wherein the human Ig lambda constant domain comprises an amino acid sequence SEQ ID NO:81.
13. A pharmaceutical composition comprising the anti-CD40 antibody of any one of claims 1-12, and a pharmaceutically acceptable carrier.
14. An isolated nucleic acid encoding an anti-CD40 antibody amino acid sequence of any one of claims 1-12.
15. A vector comprising an isolated nucleic acid of claim 14.
16. A host cell comprising a vector of claim 15.
17. The host cell of claim 16, wherein the host cell is a prokaryotic cell or a eukaryotic cell.
18. The host cell of claim 17, wherein the host cell is a CHO cell or a COS cell.

19. A method of producing an anti-CD40 antibody, the method comprising the steps of culturing a host cell of any one of claims 16-18 in culture medium under conditions sufficient to produce the anti-CD40 antibody.
20. An anti-CD40 antibody of any one of claims 1-12 for use as a medicament.