CD40 ANTIBODY FORMULATION AND METHODS

BACKGROUND OF THE INVENTION

CD40 is a member of the tumor necrosis factor receptor (TNFR) superfamily. It is expressed on antigen presenting cells (B cells, dendritic cells, monocytes), hematopoietic precursors, endothelial cells, smooth muscle cells, epithelial cells, as well as the majority of human tumors. (Grewal & Flavell, Ann. Rev. Immunol., 1996, 16: 111-35; Toes & Schoenberger, Seminars in Immunology, 1998, 10(6) : 443-8). Studies using CD40 agonist agents have reported that stimulation of the CD40 receptor elicits a cascade of effects associated with anti-tumor activity. For example, stimulation of the CD40 receptor on antigen presenting cells has been shown to enhance their maturation, antigen-presenting function, costimulatory potential and their release of immunoregulatory cytokines (Lee et al., PNAS USA, 1999, 96(4) : 1421-6 ; Cella et al., J. Exp. Med., 1996; 184(2): 747-52). CD40 agonists have also been reported to promote the apoptosis of CD40+ tumors and enhance their ability to be processed by dendritic cells (von Leoprechting et al., Cancer Res., 1999, 59 :1287-94; Sotomayo et al., Nature Medicine,1999, 5(7) : 780-87 ; Eliopoulos et al., Mol. Cell Biol., 2000, 29(15) : 5503-15 ; Ziebold et al., Arch. Immunol. Therapiae Experimentalis, 2000, 48(4) :225-33 ; Hoffmann et al., J. Immunol., 2001, 24(2) : 162-71). The significance of these immune stimulatory and direct anti-tumor effects has been illustrated in animal models in which CD40 agonist antibodies have been shown to prevent tumor growth and reverse tumor tolerance (Diehl et al., Nature Med.,1999, 5(7) : 774-9; Francisco et al., Cancer Res., 2000, 60(12) : 32225-31). CD40 antibodies are referred to in the following patent publications: U.S. 5,786,456; U.S. 5,674,492; WO 02/088186; US 2003059427; US 20020142358; WO 01/56603; U.S. 5,801,227; EP 806963; WO 88/06891; and WO 94/04570. However, highly effective methods of administration and formulations for CD40 antibodies have not been described. Also useful would be a stable formulation suitable for use in such treatment.

SUMMARY OF THE INVENTION

The present invention relates to a method of treating cancer in a patient in need of such treatment comprising administering to said patient a CD40 agonist antibody or a fragment thereof, wherein said antibody is administered according to an intermittent dosing regimen of at least two cycles, each cycle comprising (a) a dosing period during which a therapeutically effective amount of said CD40 agonist antibody is administered to said patient and thereafter (b) a resting period. In one embodiment, the administration produces a plasma concentration of the antibody of 0.01 μg/ml to 10 μg/ml for at least three hours and the resting period is for at least 1 week. In other embodiments, the dosing period is for at least one day, 1-5 days, or 1-3 days. In other embodiments, the resting period is from 1-8, 1-6 weeks, 2-5 weeks, or 3-4 weeks.

In certain embodiments, the therapeutically effective amount of the CD40 agonist antibody produces the plasma concentration of said antibody of about 0.03 μg/ml to 10 μg/ml, about 0.03 μg/ml to 1μg/ml, about 0.03 μg/ml to 0.3 μg/ml, or about 0.1 μg/ml to 0.3 μg/ml for 3 to 120 hours. In some embodiments, the specified plasma concentration is maintained for at least one day, 24 to 30 hours, 24 to 36 hours, 24 to 48 hours, 24 to 72 hours, 24 to 96 hours, or 24 to 120 hours. In some embodiments, the plasma concentration is maintained for 3 to 96 or 12 to 72 hours.

In certain embodiments, the therapeutically effective amount of the CD40 agonist antibody administered during the dosing period is about 0.03 to 3.0 mg/kg/day, 0.1 to 3.0 mg/kg/day, 0.1 to 1.0 mg/kg/day, or about 0.1 to 0.3 mg/kg/day. In one embodiment the dosage is administered for 1-5 days or 1-3 days, either consecutively or on alternate days.

The intermittent dosing regimen of CD40 agonist antibodies, as described above in connection with tumor treatment, is also useful in enhancing immune responses in patients and such use, therefore, is also provided by the present invention. In certain embodiments, the enhancement of a patient's immune response results in increased CD23 or MHC-II expression on B-cells in patient's whole blood, which, for example, may be measured at the end of a dosing period.

In some embodiments, the anti-CD40 antibody is administered to a patient who suffers from primary and/or combined immunodeficiencies, including CD40- dependent immunodeficiency with Hyper-IgM syndrome, Common Variable Immunodeficiency, Bruton's Agammaglobulinemia, IgG subclass deficiencies, and X-linked SCID (common gamma chain mutations). In some embodiments, the anti-CD40 antibody is administered to treat a patient who is immunosuppressed, for example due to chemotherapy, or has an immune-debilitating disease, including any acquired immune deficiency disease, such as HIV. In some embodiments, the anti-CD40 antibody is administered to enhance the immunity of an elderly patient. In some embodiments, the anti-CD40 antibody is administered to treat a patient who has a bacterial, viral, fungal or parasitic infection. In some embodiments, a human agonist anti-CD40 antibody may be administered prophylactically to a patient who, because of age, illness or general poor health is susceptible to infection to prevent or to reduce the number or severity of infections.

The present invention also provides a method of treating a tumor in a patient comprising administering a CD40 agonist antibody and a DNA replication inhibitor, preferably a platin-derivative, especially cisplatin. In certain embodiments, cisplatin is administered intravenously. In some embodiments, cisplatin is administered in an amount of from about 25 to 300 mg per m^2, about 50 to 150 mg per m^2, or about 75 to 100 mg per m^2 of the patient's body surface area. In one embodiment, the cisplatin is administered in one dose (e.g., a single intravenous infusion). In another embodiment, it is administered over 2-5 days. In

certain embodiments, the amount of the CD40 antibody being administered in combination with cisplatin is administered in a dosage of about 0.1 to 3.0 mg/kg, or about 0.1 to 1.0 mg/kg, or about 0.1 to 0.3 mg/kg.

In another aspect, administration of cisplatin is combined with the intermittent dosing regimen of the CD40 antibody, with cisplatin being administered during one or more of the dosing periods or rest periods.

In another aspect, the invention relates to a method of treating a tumor in a patient in need of such treatment by administering to the patient a CD40 agonist antibody or a fragment thereof in a dosage of less than 1 mg/kg/day, wherein the C_{max} serum concentration in the patient resulting from administration of the antibody is less than 50 μg/ml. In one embodiment, the dosage is between 0.1 to 0.3 mg/kg and the C_{max} serum concentration of the antibody in the patient is between 0.5 and 10 μg/ml.

In another aspect, the invention relates to a stable liquid pharmaceutical formulation suitable for parenteral administration comprising an anti-CD40 antibody at a pH of from 5.0-6.0 and a pharmaceutically acceptable carrier, the formulation being stable for a period of at least three months. The formulation preferably has a concentration of CD40 antibody of at least about 5 mg/ml. In one embodiment, the formulation comprises an anti-CD40 antibody, sodium acetate, sodium chloride, and polysorbate 80. Preferably, it comprises 20 mM sodium acetate, 140 mM sodium chloride, and 0.2 mg/mL polysorbate 80. The anti-CD40 antibody preferably has the amino acid sequence of an antibody selected from the group consisting of antibody 21.4.1 or 3.1.1.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows inhibition of growth of the CD40(-) Tumor K562 by a CD40 agonist antibody in the presence of immune cells. Animals received a single injection (IP) of 21.4.1 or KLH at the time of tumor challenge. Tumor size is reported for each individual animal on Day 21 in mm^2 (10 animals per group). The study is representative of at least 5 separate studies.

Figure 2 shows inhibition of growth of the human breast tumor cell line BT 474 by a CD40 agonist antibody. The values represent individual tumor measurements taken on Day 53 after injection using 6 animals per group. The study is representative of two separate experiments. The mean for each treatment group is indicated by the horizontal line.

Figure 3 shows inhibition of CD40(+) tumor growth by a CD40 agonist antibody, alone or in the presence of immune cells. Animals received a single injection of 21.4.1 at the time of tumor challenge. (a) Tumors were injected alone or (b) together with human peripheral blood T cells and DC. The data points represent the tumor size (mm^2) for each individual animal. The mean for each treatment group (N=10) is indicated by the horizontal line. The study is representative of at least 3 separate experiments.

Figure 4 shows effects of a CD40 agonist antibody in delaying mortality induced by a B cell Lymphoma (Daudi). The data points refer to the mean number of surviving animals, N=10 per group.

Figure 5 shows tumor regression caused by a combination therapy with a CD40 agonist antibody and cisplatin.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

As used herein, the term "agonist CD40 antibody" or "agonist anti-CD40 antibody" means an antibody that specifically binds to human CD40 molecule and increases one or more CD40 activities by at least about 20% when added to a cell, tissue or organism expressing CD40. In some embodiments, the antibody activates CD40 activity by at least 40%, 50%, 60%, 70%, 80%, or 85%. In some embodiments, the activation occurs in the presence of CD40L. In some embodiments, the activity of the activating antibody is measured using a whole blood surface molecule upregulation assay. In another embodiment, the activity of the activating antibody is measured using a dendritic cell assay to measure IL-12 release. In another embodiment the activity of the activating antibody is measured using an *in vivo* tumor model.

The term "antibody" as used herein refers to an intact antibody, or a binding fragment thereof that competes with the intact antibody for specific binding. Binding fragments are produced by recombinant DNA techniques, or by enzymatic or chemical cleavage of intact antibodies. Binding fragments include Fab, Fab', $F(ab')_2$, Fv, and single-chain antibodies. It is understood that reference to an intact (*e.g.*, whole, full-length, etc.) antibody herein includes an antibody having a terminal lysine deletion in the heavy chain, which commonly occurs during recombinant expression.

Preferably, the agonist CD40 antibody is a human antibody. As used herein, the term "human antibody" means an antibody in which the variable and constant domain sequences are derived from human sequences. Human CD40 antibodies are described in detail in U.S. provisional application no. 60/348,980, filed November 9, 2001, and PCT International Application No. PCT/US02/36107 (now published as WO 03/040170) filed November 8, 2002, the entire disclosure of which is hereby incorporated by reference. Human antibodies provide a substantial advantage in the treatment methods of the present invention, as they are expected to minimize the immunogenic and allergic responses that are associated with use of non-human antibodies in human patients.

Exemplary human anti-CD40 antibodies useful for the present invention include antibodies having the amino acid sequences of antibodies designated 3.1.1, 3.1.1H-A78T, 3.1.1H-A78T-V88A-V97A, 7.1.2, 10.8.3, 15.1.1, 21.4.1, 21.2.1, 22.1.1, 22.1.1H-C109A, 23.5.1, 23.25.1, 23.28.1, 23.28.1H-D16E, 23.29.1, 24.2.1, 3.1.1H-A78T-V88A-V97A/3.1.1L-

L4M-L83V and 23.28.1L-C92A, as well as an antibody comprising a CDR or variable region of any of the exemplary antibodies.

Antibodies that recognize the same or similar epitopes, or a portion thereof, as any of the exemplary antibodies may also be useful for the present invention. That is, as would be understood by one skilled in the art based upon the disclosure provided herein, an antibody that competes with an antibody of the invention (*e.g.*, 3.1.1, 3.1.1H-A78T, 3.1.1H-A78T-V88A-V97A, 7.1.2, 10.8.3, 15.1.1, 21.4.1, 21.2.1, 22.1.1, 22.1.1H-C109A, 23.5.1, 23.25.1, 23.28.1, 23.28.1H-D16E, 23.29.1, 24.2.1, 3.1.1H-A78T-V88A-V97A/3.1.1L-L4M-L83V and 23.28.1L-C92A, and the like) can be useful as disclosed elsewhere herein. An antibody of interest that competes with an antibody exemplified herein can be readily identified using methods well known in the art for the characterization of antibodies. More specifically, assays for assessing the binding characteristics of an antibody, as well as for comparing those binding characteristics to those of another antibody, are well known in the art. Such methods include, but are not limited to, ELISA-based assays, use of BIAcore binding studies, as well as those detailed in US Patent Application Publication No. 2003/0157730A1 to Walker et al.

By the term "compete", as used herein with regard to an antibody, is meant that a first antibody competes for binding with a second antibody where binding of the first antibody with its cognate epitope is detectably decreased in the presence of the second antibody compared to the binding of the first antibody in the absence of the second antibody. The alternative, where the binding of the second antibody to its epitope is also detectably decreased in the presence of the first antibody, can, but need not be the case. That is, a first antibody can inhibit the binding of a second antibody to its epitope without that second antibody inhibiting the binding of the first antibody to its respective epitope. However, where each antibody detectably inhibits the binding of the other antibody with its cognate epitope or ligand, whether to the same, greater, or lesser extent, the antibodies are said to "cross-compete" with each other for binding of their respective epitope(s). For instance, cross-competing antibodies can bind to the epitope, or potion of the epitope, to which the antibodies of the invention (*e.g.*, 3.1.1, 3.1.1H-A78T, 3.1.1H-A78T-V88A-V97A, 7.1.2, 10.8.3, 15.1.1, 21.4.1, 21.2.1, 22.1.1, 22.1.1H-C109A, 23.5.1, 23.25.1, 23.28.1, 23.28.1H-D16E, 23.29.1, 24.2.1, 3.1.1H-A78T-V88A-V97A/3.1.1L-L4M-L83V and 23.28.1L-C92A) bind. Both competing and cross-competing antibodies are encompassed by the present invention. Regardless of the mechanism by which such competition or cross-competition occurs (*e.g.*, steric hindrance, conformational change, or binding to a common epitope, or portion thereof, and the like), the skilled artisan would appreciate, based upon the teachings provided herein, that such competing and/or cross-competing antibodies are encompassed and can be useful for the methods disclosed herein.

In addition the exemplary antibodies may be further modified by substitution, addition or deletion of one or more amino acid residues without eliminating the antibody's ability to bind the antigen and exert its agonistic function. Indeed, an antibody designated "3.1.1H-A78T-V88A-V97A/3.1.1L-L4M-L83V", comprises three amino acid substitutions in the heavy chain variable region, *i.e.*, a substitution from alanine to threonine at amino acid residue number 78, a substitution from valine to alanine at amino acid residue number 88, and a substitution from valine to alanine at amino acid residue number 97 (SEQ ID NO:9), all with respect to the amino acid sequence of the heavy chain variable region of antibody 3.1.1 (SEQ ID NO:1). In addition, the 3.1.1H-A78T-V88A-V97A/3.1.1L-L4M-L83V antibody further comprises an amino acid substitution from leucine to methionine at amino acid residue number 4 and a substitution from leucine to valine at amino acid residue number 83 in the light chain variable region (SEQ ID NO:10) compared with the amino acid sequence of the variable region of the light chain of antibody 3.1.1 (SEQ ID NO:3). The amino acid sequences of the constant regions of the heavy chains (SEQ ID NO:2) and light chains (SEQ ID NO:4) of 3.1.1 and 3.1.1H-A78T-V88A-V97A/3.1.1L-L4M-L83V antibodies are the same. Antibody 3.1.1H-A78T-V88A-V97A/3.1.1L-L4M-L83V is also referred to as "3.1.1H3L2" to reflect that the antibody comprises three amino acid substitutions in the heavy chain and two amino acid substitutions in the light chain relative to antibody 3.1.1.

Thus, in some embodiments, the exemplary antibodies may be modified by substitution, addition, or deletion of one to ten, one to five, or one to three amino acid residues, e.g., in a CDR or framework region. These exemplary antibodies and methods of producing them are described in detail in U.S. provisional application no. 60/348,980, filed November 9, 2001, and PCT International Application No. PCT/US02/36107 (WO 03/040170), filed November 8, 2002. However, the invention is not limited to these, or any other, amino acid substitutions. Rather, the skilled artisan, armed with the teachings provided herein, would appreciate that a wide variety of amino acid substitutions are encompassed by the invention.

Hybridomas 3.1.1, 7.1.2, 10.8.3, 15.1.1 and 21.4.1 were deposited in accordance with the Budapest Treaty, in the American Type Culture Collection (ATCC), 10801 University Boulevard, Manassas, VA 20110-2209, on August 6, 2001. Hybridomas 21.2.1, 22.1.1, 23.5.1, 23.25.1, 23.28.1, 23.29.1 and 24.2.1 were deposited in the ATCC on July 16, 2002. The hybridomas have been assigned the following deposit numbers:

Hybridoma	Deposit No.
3.1.1 (LN 15848)	PTA-3600
7.1.2 (LN 15849)	PTA-3601
10.8.3 (LN 15850)	PTA-3602
15.1.1 (LN 15851)	PTA-3603
21.4.1 (LN 15853)	PTA-3605
21.2.1 (LN 15874)	PTA-4549
22.1.1 (LN 15875)	PTA-4550
23.5.1 (LN 15855)	PTA-4548
23.25.1 (LN 15876)	PTA-4551
23.28.1 (LN 15877)	PTA-4552
23.29.1 (LN 15878)	PTA-4553
24.2.1 (LN 15879)	PTA-4554

The sequences of these antibodies are known, and described in WO 03/040170. For convenience, the amino acid sequences of heavy and light chains of two of these antibodies are shown below:

Antibody 3.1.1:

3.1.1: Heavy Chain Protein Sequence	Variable (SEQ ID NO:1): QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQA PGKGLEWVAVISKDGGNKYHADSVKGRFTISRDNSKNALYL QMNSLRVEDTAVYYCVRRGHQLVLGYYYYNGLDVWGQGTT VTVSS Constant (SEQ ID NO:2): ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWN SGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSNFGTQTYTCN VDHKPSNTKVDKTVERKCCVECPPCPAPPVAGPSVFLFPPK PKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNA KTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVSNKGL PAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVK GFYPSDIAVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLT VDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
3.1.1: Light Chain Protein Sequence	Variable (SEQ ID NO:3): DIVLTQSPLSLPVTPGEPASISCRSSQSLLYSNGYNFLDWYLQ KPGQSPQLLIYLGSNRASGVPDRFSGSGSGTDFTLKISRLEA EDVGVYYCMQALQTPRTFGQGTKVEIK Constant (SEQ ID NO:4): RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWK VDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHK VYACEVTHQGLSSPVTKSFNRGEC

3.1.1H-A78T-V88A-V97A: Heavy Chain Protein Sequence	Variable (SEQ ID NO:9): QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQA PGKGLEWVAVISKDGGNKYHADSVKGRFTISRDNSKNTLYL QMNSLRAEDTAVYYCARRGHQLVLGYYYYNGLDVWGQGTT VTVSS Constant (SEQ ID NO:2): ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWN SGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSNFGTQTYTCN VDHKPSNTKVDKTVERKCCVECPPCPAPPVAGPSVFLFPPK PKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNA KTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVSNKGL PAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVK GFYPSDIAVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLT VDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
3.1.1L-L4M-L83V: Light Chain Protein Sequence	Variable (SEQ ID NO:10): DIVMTQSPLSLPVTPGEPASISCRSSQSLLYSNGYNFLDWYL QKPGQSPQLLIYLGSNRASGVPDRFSGSGSGTDFTLKISRVE AEDVGVYYCMQALQTPRTFGQGTKVEIK Constant (SEQ ID NO:4): RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWK VDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHK VYACEVTHQGLSSPVTKSFNRGEC

Antibody 21.4.1:

21.4.1: Heavy Chain Protein Sequence	Variable (SEQ ID NO:5): QVQLVQSGAEVKKPGASVKVSCKASGYTFTGYYMHWVRQA PGQGLEWMGWINPDSGGTNYAQKFQGRVTMTRDTSISTAY MELNRLRSDDTAVYYCARDQPLGYCTNGVCSYFDYWGQGT LVTVSS Constant (SEQ ID NO:6): ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWN SGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSNFGTQTYTCN VDHKPSNTKVDKTVERKCCVECPPCPAPPVAGPSVFLFPPK PKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNA KTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVSNKGL PAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVK GFYPSDIAVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLT VDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

21.4.1: Light Chain Protein Sequence	Variable (SEQ ID NO:7): DIQMTQSPSSVSASVGDRVTITCRASQGIYSWLAWYQQKPG KAPNLLIYTASTLQSGVPSRFSGSGSGTDFTLTISSLQPEDFA TYYCQQANIFPLTFGGGTKVEIK Constant (SEQ ID NO:8): RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWK VDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHK VYACEVTHQGLSSPVTKSFNRGEC

Thus, the amino acid sequence of 21.4.1 antibody comprises the amino acid sequences set forth in SEQ ID NOs:5-8, the amino acid sequence of 3.1.1. antibody comprises the amino acid sequences set forth in SEQ ID NOs:1-4, and the amino acid sequence of 3.1.1H-A78T-V88A-V97A/3.1.1L-L4M-L83V antibody comprises the sequences set forth in SEQ ID NO:9, SEQ ID NO:2, SEQ ID NO10 and SEQ ID NO4. The amino acids which differ between 3.1.1 and 3.1.1H-A78T-V88A-V97A/3.1.1L-L4M-L83V are underlined.

It would be understood, based upon the disclosure provided herein, that a 3.1.1 antibody of the invention encompasses any combination of the heavy and/or light variable regions set forth herein. That is, an antibody can comprise any combination of variable regions, including, but not limited to, 3.1.1H (SEQ ID NO:1)/3.1.1L (SEQ ID NO:3), 3.1.1H (SEQ ID NO:1)/3.1.1L-L4M-L83V (SEQ ID NO:10), 3.1.1H-A78T-V88A-V97A (SEQ ID NO:9)/3.1.1L (SEQ ID NO3), and, more preferably, 3.1.1H-A78T-V88A-V97A (SEQ ID NO:9)/3.1.1L-L4M-L83V (SEQ ID NO:10). However, the invention is in no way limited to these or any other particular combinations.

In certain embodiments, the tumor treatment inhibits cancer cell proliferation, inhibits or prevents an increase in tumor weight or volume, and/or causes a decrease in tumor weight or volume. In some embodiments, the tumor treatment prolongs patient survival. In certain embodiments, tumor growth is inhibited at least 50%, 55%, 60%, 65%, 70% or 75%, compared to those not treated. In some embodiments, the tumor is CD40 positive. In some embodiments, the tumor is CD40 negative. The tumor can be a solid tumor or a non-solid tumor such as lymphoma. In some embodiments, an anti-CD40 antibody is administered to a patient who has a tumor that is cancerous

Patients that can be treated with anti-CD40 antibodies or antibody portions include, but are not limited to, patients that have been diagnosed as having brain cancer, lung cancer, bone cancer, pancreatic cancer, skin cancer, cancer of the head and neck, cutaneous or intraocular melanoma, uterine cancer, ovarian cancer, rectal cancer, cancer of the anal region, stomach cancer, gastric cancer, colorectal cancer, colon cancer, gynecologic tumors (e.g., uterine sarcomas, carcinoma of the fallopian tubes, carcinoma of the endometrium, carcinoma of the cervix, carcinoma of the vagina or carcinoma of the vulva), cancer of the esophagus, cancer of the small intestine, cancer of the endocrine system (e.g., cancer of the

thyroid, parathyroid or adrenal glands), sarcomas of soft tissues, leukemia, myeloma, multiple myeloma, cancer of the urethra, cancer of the penis, prostate cancer, chronic or acute leukemia, solid tumors of childhood, Hodgkin's disease, lymphocytic lymphomas, non-Hodgkin lymphoma, cancer of the bladder, liver cancer, renal cancer, cancer of the kidney or ureter (e.g., renal cell carcinoma, carcinoma of the renal pelvis), or neoplasms of the central nervous system (e.g., primary CNS lymphoma, spinal axis tumors, brain stem gliomas or pituitary adenomas), glioma or fibrosarcoma.

As used herein, the term "patient" refers to a human or a non-human mammal that expresses a cross-reacting CD40 (e.g., a primate, cynomolgus or rhesus monkey). Preferably a patient being treated is human.

As used herein, the term "intermittent dosing regimen" means a dosing regimen that comprises administering a CD40 agonist antibody, followed by a rest period.

As used herein, the term "resting period" means a period of time during which the patient is not given a CD40 agonist antibody. For example, if the antibody has been given on a daily basis, there would be rest period if the daily administration is discontinued, e.g., for some number of days or weeks. If a dose is administered on a different schedule a rest period would occur where that dosing is discontinued for some time. Alternately, a rest period may occur where the concentration of the antibody is maintained at a sub-therapeutic level.

In one embodiment, the antibody is not given after the second rest period, i.e., when the method of the invention involves two cycles, the drug need not be administered following the second rest cycle.

Preferably, during the rest period, the plasma concentration of the antibody is maintained at sub-therapeutic level.

The dosing period and/or the dose of the antibody can be the same or different between cycles.

The total treatment time (i.e., the number of cycles for treatment) will vary from patient to patient based on sound medical judgment and factors particular to the patient being treated. In general, the treatment is administered until a satisfactory response is obtained. In certain embodiments of the invention, the treatment period comprises 2-20, 2-15, 2-10, 2-7, 2-5 cycles or 2-3 cycles.

The antibody may be administered by any means desired, including, e.g., intravenous, subcutaneous, intramuscular, parenteral, intratumor, and transdermal administration. In one embodiment the CD40 antibody is administered intravenously. In another, it is administered using a microneedle device; such devices are well known and include, e.g., the device described in WO 03/084598.

When administered in combination with a DNA replication inhibitor, e.g., cisplatin, the antibody may be administered before, during, or after administration of the inhibitor.

In one aspect, the invention relates to an aqueous solution for intravenous injection, with the pH of about 5.0 to 6.0, preferably pH of about 5.5. Such a solution may be formulated with sodium acetate (trihydrate), acetic acid (glacial), Polysorbate 80, sodium chloride and water. It is preferred that the antibody solution be stored at refrigerated temperatures between 2° C and 8° C, and not be frozen.

In accordance with the present invention, also provided are methods of treating a tumor in a patient in need of such treatment comprising administering to said patient a combination of a therapeutically effective amount of a CD40 agonist antibody and a therapeutically effective amount of a DNA replication inhibitor, e.g., a platin-derivative. In certain embodiments, a CD40 agonist antibody works in synergistic combination with the platin-derivative compound, especially cisplatin, such that anti-tumor effect of the combination is greater than what would be predicted from administration of each compound alone.

Platin-derivatives are well-known group of compounds that exhibit their anti-tumor activity by interfering with DNA replication. In certain embodiments, platin derivatives are selected from the group consisting of cisplatin (cis-diaminedichloroplatinum, See Merck Index), carboplatin and oxaliplatin.

The invention will be more fully understood by reference to the following examples. They should not, however, be construed as limiting the scope of the invention. All literature citations are incorporated by reference.

EXAMPLES

Example 1: Effects Of Antibody On Lymph Node Cells From Cancer Patients

Effects of a human anti-CD40 antibody (21.4.1) on lymph node cells obtained from cancer patients stimulated with autologous tumor cells was examined.

Lymph node cells and tumors were collected from patients with renal cell carcinoma, non-small cell lung cancer, transitional cell carcinoma of the bladder, colon cancer, prostate cancer, and head and neck cancer. The lymph node cells were placed into culture together with irradiated collagenase treated tumors (recovered from the same patient) in the presence or absence of 21.4.1 (1 μg/mL; 6.7 nM). Proliferation was assessed using ^{3}H-thymidine 96 hours later. The number of INFγ producing cells was assessed by ELISPOT, following restimulation.

The antibody enhanced the number of IFNγ+ positive T cells in cultures of lymph node cells stimulated with tumor antigen. Further, the proliferation of these lymph node cells in response to tumor antigen was enhanced 3-4 fold.

The antibody enhanced the proliferation and cytokine producing capacity of lymph node cells obtained from cancer patients when stimulated with tumor antigen.

Example 2 : Binding of Antibody to Fc Receptor

The binding of an anti-CD40 antibody (21.4.1) to Fc receptors on human and cynomolgus leukocytes was examined.

Flow cytometric studies indicated that FcR types FcγRII (CD32) and FcγRIII (CD16), as well as very low levels of FcγRI (CD64), were expressed on human leukocytes. The binding of 21.4.1 to Fc receptors (FcR) on human or cynomolgus peripheral blood leukocytes was determined by using ^{125}I-21.4.1 and a human IgG1 control mAb. Human leukocytes from normal donors or cynomolgus leukocytes were isolated from whole blood using plasma gel and washed thoroughly to allow dissociation of receptor-bound serum immunoglobulins. Centrifugation through a sucrose cushion was used to separate cell-bound and free antibodies. Studies were performed at 4°C in the presence of sodium azide to prevent receptor internalization.

21.4.1 was tested for specific binding to FcR by using excess unlabeled human IgG2 isotype matched antibody as a competitor. ^{125}I-21.4.1 specific binding to FcR on human leukocytes (n=5 donors) was –1.0±8.5%, and specific binding to FcR on cynomolgus leukocytes (n=4) was 15±13%. Addition of 500-fold excess unlabeled 21.4.1, which would block any specific binding of ^{125}I-21.4.1 to leukocyte CD40 receptors as well as FcR, demonstrated 49% and 67% specific binding of ^{125}I-21.4.1 to CD40 receptors on human and cynomolgus leukocytes, respectively (% specific binding to CD40 was calculated by subtracting % binding to FcR from total % specific binding). As a control, ^{125}I-IgG1 consistently demonstrated specific binding to human and cynomolgus leukocytes. The specific binding of the IgG1 control antibody to FcR on human and cynomolgus leukocytes accounted for 56% and 51% of the total bound radioactivity, respectively.

These studies indicate that the antibody shows minimal specific binding to Fc receptors on human and cynomolgus leukocytes.

Example 3 : Whole-Blood Cytokine Release Assay

An anti-CD40 antibody (21.4.1) was tested for its ability to induce the release of cytokines from unstimulated human whole blood using an in vitro whole blood assay which correlates with induction of antibody-mediated cytokine release in humans. 21.4.1 was tested at 1, 10 and 100 µg/mL, along with a murine anti-human CD3 IgG1 as a positive control that induces cytokine release through an Fc mediated pathway, and LPS as a second positive control that induces cytokines by stimulating macrophages. The donors used included individuals that responded to both the murine antibody and LPS (4 donors), as well as individuals who only responded to the LPS (3 donors). Heparinized whole blood was cultured with 21.4.1 for 5 hours and plasma was collected and analyzed for tumor necrosis factor alpha (TNF-α), interferon gamma (INF-γ) and interleukin-6 (IL-6) by ELISA (using

commercially available kits). Cultures were also incubated for 48 hours and analyzed for interleukin-1 beta (IL-1β).

Cytokines were not detected in the plasma of human blood cultured with 1 or 10 µg/mL 21.4.1. Only one donor treated with 100 µg/mL of the antibody showed low but measurable levels of two cytokines (34 pg/mL of TNF-α in and 90 pg/mL IL-6). This donor was re-tested subsequently and showed no detectable induction of TNF-α or IL-6. There was no elevation of INFγ or IL-1β in any of the samples.

These studies indicate that 21.4.1 does not induce inflammatory cytokines in human whole blood.

Example 4 : Pharmacodynamics and Pharmacokinetics of Antibody

A CD40 antibody (21.4.1) was administered intravenously at various doses (1 mg/kg n = 4, 3 mg/kg n = 4, 5 mg/kg n = 2 and 10 mg/kg n = 2) to cynomolgus monkeys. Heparinized blood was drawn from the monkeys at various time points pre- and postdose. The blood was aliquoted and stained. Data were acquired using a Becton Dickinson FACSCalibur and analyzed with CellQuest software. Results were calculated as fold increases in median fluorescence intensity as compared to pre-dose values.

MHC Class II expression, reflecting activation state and antigen presenting capacity of B-cells, increased by 2.5 to 3 fold by 24 hours after dosing for all doses tested, with no clear dose-response relationship observed. CD23 expression, another marker of B-cell activation, was evaluated in 2 animals at 3 mg/kg, and one animal at 10 mg/kg. CD23 expression increased ≥20-fold at 24 hours after dosing with no dose effect observed. Upregulation of both surface markers persisted (≥2-fold increase) while 21.4.1 levels remained above 1 µg/ml. CD71 (transferrin receptor) and CD86 costimulatory molecule levels also showed moderate upregulation, while CD80 expression did not change significantly.

21.4.1 upregulates surface markers in cynomolgus B-cells in vivo. MHC Class II and CD23 expression on CD20+ cells increase with treatment, and 1 mg/kg (corresponding to a C_{max} of ~ 20 µg/mL and an exposure of ≥0.1 µg/mL for 4 days) appears to produce a saturating pharmacodynamic response in cynomolgus B-cells. The duration of this response was longer at higher doses.

The pharmacokinetic properties of an anti-CD40 antibody (21.4.1) were examined in cynomolgus monkeys following intravenous (IV) administration of a single dose of 1, 3, 5 or 10 mg/kg. 21.4.1 was characterized by low systemic clearance (0.0133 to 0.0635 mL/min/kg) and small volume of distribution at steady state (0.0459 to 0.0757 L/kg), resulting in an apparent mean elimination half-life of 0.75 to 2.0 days (Table 1). The pharmacokinetics of 21.4.1 appeared to be dose-dependent over the dose range examined. Clearance values generally decreased with increasing dose from 1 to 10 mg/kg and the apparent mean

elimination half-life increased from 0.75 day at 1 mg/kg to 2.0 days at 10 mg/kg. The volume of distribution at steady state was similar at different doses (mean of 0.0575 L/kg).

The observed dose-dependent clearance may be in part due to the binding of 21.4.1 to CD40 receptors that are widely expressed in normal tissues and the subsequent internalization and elimination of the antibody-receptor complex. Development of primate anti-human antibody (PAHA) response may also contribute to the accelerated clearance in some monkeys. PAHA was evaluated only after individual serum concentrations of 21.4.1 reached the lower limit of quantitation (LLOQ, 0.03 μg/mL) since the presence of 21.4.1 in test serum interferes with the assay for PAHA. Anti-21.4.1 antibodies were detected in all monkeys in the 3, 5, and 10 mg/kg dose groups at 14 to 28 days following administration of the antibody.

Table 1

Mean (± SD) Pharmacokinetic Parameters of 21.4.1 in Cynomolgus Monkeys Following a Single IV Administration at 1, 3, 5, and 10 mg/kg

Dose (mg/kg)	N/ Gender	CL (mL/min/kg)	Vdss (L/kg)	$t_{1/2}$ (day)	$AUC_{(0-\infty)}$ (μg·h/mL)
1	2/sex	0.0635 ± 0.0245	0.0757 ± 0.0265	0.75 ± 0.21	298 ± 126
3	2/sex	0.0213 ± 0.0055	0.0459 ± 0.0055	1.4 ± 0.3	2460 ± 600
5	2F	0.0174	0.0488	1.4	4790
10	1/sex	0.0133	0.0529	2.0	12500

Example 5: Anti-Tumor Activity of Antibody

The tumor growth inhibitory activity of a CD40 antibody (21.4.1) was determined in SCID-beige mice injected SC with tumor cells alone (1×10^7) or with human DC (1×10^5) and T cells (5×10^5) from the same donor. The ratio of tumor cells to DC and T cells was 100:1:5. Unless otherwise indicated, the results are presented in terms of the tumor size in mm^2 at one fixed time point pre-determined (from kinetic experiments) to be the time when tumor growth in control animals reached a size of 300-400 mm^2 and it was no longer humane to continue the experiment. In all cases, only one injection of 21.4.1 was administered which had a $T_{1/2}$ of >30 days in SCID-beige mice.

Example 5(a): Effects of Antibody on CD40(-) Human Tumors

The effects of a CD40 antibody (21.4.1) on the growth of CD40(-) tumors (e.g., erythroleukemia and colon carcinoma) were examined. In particular, K562 tumors were chosen to assess the efficacy of 21.4.1 against a CD40(-) low immunogenic (class I and II negative) tumor.

SCID-beige mice were injected SC with the CD40(-) erythroleukemic tumor, K562 (ATCC CCL-243) alone or in the presence of human peripheral blood T cells and DCs.

Animals received a single IP injection of 21.4.1 either at the time of tumor injection or 5 days later using various dose levels.

A single IP injection of 21.4.1 resulted in the dose-dependent inhibition of K562 tumor growth when immune cells were present as illustrated on Day 21 after tumor challenge (Figure 1). The amount of 21.4.1 to cause a 50% inhibition of tumor growth was 0.005 mg/kg corresponding to a C_{max} serum concentration of 0.05 µg/mL. Similar results were observed with the CD40(-) colon carcinoma, Lovo (ATCC CCL-229). The results were identical when 21.4.1 was administered on Day 0 or Day +5 relative to tumor challenge. The growth of these CD40(-) tumors was not inhibited by 21.4.1 in the absence of immune cells.

21.4.1 prevents the growth of CD40(-) tumors when immune cells are present, suggesting enhancement of immune mediated anti-tumor activity. This was demonstrated against a colon carcinoma and an erythroleukemic tumor. This anti-tumor activity was also demonstrated using antibody 3.1.1 for the colon carcinoma and for 3.1.1H-A78T-V88A-V97A/3.1.1L-L4M-L83V antibody (IC50 < 0.01 mg/kg) in the erythroleukemic tumor. Thus, the data disclosed herein demonstrate that 3.1.1H-A78T-V88A-V97A/3.1.1L-L4M-L83V antibody has the *in vivo* activity of 3.1.1 antibody. These *in vivo* tumor results further support that given the similar *in vitro* data obtained where the two antibodies were compared, antibody 3.1.1 and antibody 3.1.1H-A78T-V88A-V97A/3.1.1L-L4M-L83V will perform in a similar manner *in vivo*. Thus, results obtained using 3.1.1 apply to 3.1.1H-A78T-V88A-V97A/3.1.1L-L4M-L83V in this and other assays.

Example 5(b): Effects of Antibody on Human Breast and Prostate Tumor Growth

The effects of an anti-D40 antibody (21.4.1) on preventing the growth of breast and prostate tumors was examined.

SCID-beige mice were challenged with the human breast tumor, BT 474 (ATCC HTB-20), SC, together with human peripheral blood T cells and DC. Animals received a single dose of 21.4.1 (IP) at the time of tumor injection.

As shown in Figure 2, a single injection of 21.4.1 prevented the growth of BT 474 cells in the presence of immune cells. The amount of 21.4.1 necessary to cause a 50% reduction in tumor growth was 0.005 mg/kg corresponding to a C_{max} serum concentration of 0.05 µg/mL. Similar results were observed against the human prostate cancer cell line, PC-3 (ATCC CRL-1435). This was also demonstrated using antibody 3.1.1 and can be expected for 3.1.1H-A78T-V88A-V97A/3.1.1L-L4M-L83V.

21.4.1 prevents the growth of human breast and prostate tumors.

Example 5(c): Anti-Tumor Effects of Antibody on CD40(+) Tumors

The effects of an anti-CD40 antibody (21.4.1) on anti-tumor activity against CD40(+) tumors and changes in efficacy in the presence and absence of immune cells was studied.

SCID-beige mice were injected subcutaneously with the CD40(+) Raji B cell lymphoma (ATCC CCL-86) (SC) followed by a single dose of 21.4.1 (IP) at the time of tumor injection. Some animals were also injected with human T cells and DC. Tumor growth was assessed on Day 21.

As shown in Figure 3, the amount of 21.4.1 to cause a 50% inhibition of tumor growth in the absence of immune cells was 0.02 mg/kg, corresponding to a C_{max} serum concentration of 0.2 µg/mL. When tumor cells were co-injected with immune cells, the amount of 21.4.1 necessary to cause a 50% inhibition of tumor growth was decreased 20-fold to 0.001 mg/kg (C_{max} serum concentration = 0.01 µg/mL).

These results illustrate that 21.4.1 has direct anti-tumor activity against CD40(+) tumors. This observation was also made for 3.1.1H-A78T-V88A-V97A/3.1.1L-L4M-L83V (IC50 < 0.01 mg/kg). This anti-tumor activity for antibody 21.4.1 was enhanced when immune cells were present and this was also demonstrated with antibody 3.11 and is expected for antibody 3.1.1H-A78T-V88A-V97A/3.1.1L-L4M-L83V.

Example 5(d): Anti-Tumor Effects of Antibody on B-Cell Lymphoma

The ability of an anti-CD40 antibody according to the invention (21.4.1) to delay mortality in a CD40(+) systemic tumor model using a B cell lymphoma was assessed.

SCID-beige mice were injected IV with the B cell lymphoma Daudi (ATCC CCL-213). 21.4.1 was administered as a single injection (IP) at the time of tumor injection. Mortality was monitored for 58 days.

As shown in Figure 4, a single injection of 21.4.1 prevented mortality induced by a systemically administered tumor cell line.

21.4.1 delays mortality in a CD40(+) systemic tumor model using a B cell lymphoma. This was also demonstrated using 3.1.1 and similar results are expected for 3.1.1H-A78T-V88A-V97A/3.1.1L-L4M-L83V.

Example 6 : Therapeutic Effects Of Antibody In Combination With Cisplatin

The therapeutic effects of an anti-CD40 antibody (21.4.1) in preventing the growth of human breast tumors alone and in the presence of cisplatin was examined.

SCID-beige mice were injected SC with the breast tumor, BT 474. The antibody (1 mg/kg , IP) and/or cisplatin (2.5 mg/kg, IP) were administered as a single injection once tumors reached a size of 200 mm^2. Tumor growth was measured on Day 84 after challenge.

As shown in Figure 4, a single injection of 21.4.1 or cisplatin prevented tumor growth. However, the combination of both treatments lead to complete tumor regression in 7/8 animals.

21.4.1 prevents tumor growth when administered alone once tumors are established and causes tumor regression when administered in combination with cisplatin. This was also

demonstrated using antibody 3.1.1 as is likely for 3.1.1H-A78T-V88A-V97A/3.1.1L-L4M-L83V as well.

Example 7: Multidose Pharmacokinetics of Antibody

In a multiple-dose study, 21.4.1 was administered intravenously to cynomolgus monkeys (2/sex/dose) at doses of 0.3, 1.0, and 10 mg/kg on Days 1, 3, 5, 7, and 9 for 5 total doses. Blood was collected on Days 1 and 9 before dosing and 0.5, 6, and 24 hours after dosing and before dosing and 0.5 hour after dosing on Day 5 to measure serum drug concentrations. Systemic exposure to 21.4.1, as assessed by mean C_{max} and mean $AUC_{(0-24)}$, increased with increasing dose from 0.3 to 10 mg/kg on both Day 1 and Day 9 (Table 2). Similar exposures (mean C_{max} and mean AUC) were observed on Days 1 and 9 in the 0.3 and 1 mg/kg dose groups. In the 10 mg/kg dose group, the mean C_{max} and mean $AUC_{(0-24)}$ values increased 2.6- and 2.8-fold, respectively, from Day 1 to Day 9. Gender-related differences in exposure were not observed.

Table 2

Mean (± SD) Pharmacokinetic Parameters of 21.4.1 in Cynomolgus Monkeys on Days 1 and 9 Following Every Other Day IV Administration

Dose[a] (mg/kg)	Day	C_{max} (µg/mL)	T_{max} (h)	$AUC_{(0-24)}$ (µg·h/mL)
0.3	1	4.67 ± 1.71	1.9 ± 2.8	47.7 ± 15.4
	9	7.4 ± 2.9	0.5 ± 0.0	55.6 ± 47.1
1.0	1	26.7 ± 5.1	0.5 ± 0.0	387 ± 59
	9	12.3 ± 9.1	1.9 ± 2.8	219 ± 151
10	1	226 ± 29	1.9 ± 2.8	4130 ± 600
	9	577 ± 163	3.3 ± 3.2	11400 ± 2100

[a] N = 2/sex/dose

Example 8: Antibody Formulation

CD40 antibody was concentrated to approximately 11.0 mg/mL ± 0.8 mg/mL using an ultrafiltration unit containing 30 kDa molecular weight cut-off cassettes. The concentrate was then diafiltered into 20 mM sodium acetate / 140 mM sodium chloride, pH 5.5 buffer. 2% polysorbate 80 solution was added to the concentrated diafiltered product to achieve a final concentration of 0.02% Polysorbate 80.

WHAT IS CLAIMED IS :

1. Use of a combination of a therapeutically effective amount of a CD40 agonist antibody and a therapeutically effective of a DNA replication inhibitor in the preparation of a medicament for treating tumors.

2. The use according to claim 1, wherein the DNA replication inhibitor is a platin-derivative.

3. The use according to claim 2, wherein the platin-derivative is selected from the group consisting of cisplatin, carboplatin, and oxaliplatin.

4. The use according to claim 2, wherein the platin-derivative is cisplatin.

5. The use according to any one of claims 1-4, wherein the CD40 agonist antibody is antibody 21.4.1.

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