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(54) Title: USES OF ANTI-CD40 ANTIBODIES IN COMBINATION THERAPY FOR B CELL-RELATED CANCERS

(57) Abstract: This invention relates to new uses of anti-CD40 antibodies in combination with bendamustine in the treatment of diseases or conditions associated with neoplastic B-cell growth. The invention is particularly useful for the treatment of patients who have previously been administered (i) the chimeric anti-CD20 monoclonal antibody rituximab, or (iii) combination therapy with rituximab and a chemotherapeutic regimen.



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USES OF ANTI-CD40 ANTIBODIES IN COMBINATION THERAPY FOR B CELL-RELATED CANCERS

FIELD OF THE INVENTION

This invention relates to new uses of anti-CD40 antibodies in the treatment of diseases or conditions associated with neoplastic B-cell growth. The invention is particularly useful for the treatment of patients having CD40⁺ B-cell lymphomas that are refractory to treatment with the chimeric anti-CD20 monoclonal antibody rituximab and
5 who have been exposed to at least one prior chemotherapeutic regimen.

BACKGROUND OF THE INVENTION

CD40 is a 50-55kDa cell-surface antigen present on the surface of both normal and neoplastic human B-cells. Malignant B-cells from tumors of B-cell lineage express
10 CD40 and appear to depend on CD40 signaling for survival and proliferation. Transformed cells from patients with low- and high-grade B-cell lymphomas, B-cell acute lymphoblastic leukemia, multiple myeloma, chronic lymphocytic leukemia, and Hodgkin's disease express CD40. CD40 expression is also detected in acute myeloblastic leukemia and 50% of AIDS-related lymphomas.

15 Anti-CD40 antibodies and uses thereof have been disclosed, *e.g.*, in co-owned international patent applications published as WO 2005/044294, WO 2005/044304, WO 2005/044305, WO 2005/044306, WO 2005/044307, WO 2005/044854, WO 2005/044855, WO 2006/073443, WO 2006/125117, WO 2006/125143, WO 2007/053661, and WO 2007/053767. Those applications specifically disclose a
20 human IgG₁ anti-CD40 monoclonal antibody, designated as CHIR-12.12 therein (but now known as HCD122), generated by immunization of transgenic mice bearing the human IgG₁ heavy chain locus and the human κ light chain locus (XenoMouse[®] technology; Abgenix, California). Those applications also disclose use of anti-CD40 antibodies, such as HCD122, for the treatment of diseases or conditions associated with neoplastic B-cell
25 growth.

Although any one therapeutic agent may provide a benefit to the patient, further methods are needed to reduce toxicity and to improve treatment outcomes. In addition,

diseases or conditions can often become refractory to treatment with single-agent therapy, either as a result of initial resistance or resistance that develops during therapy. Consequently, any discovery of a combination therapy that can improve treatment relative to single-agent therapy is of great interest.

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BRIEF SUMMARY OF THE INVENTION

Methods, compositions, uses, and kits useful in the treatment of diseases or conditions associated with neoplastic B-cell growth are provided. The methods, compositions, uses, and kits are directed to combination therapy with bendamustine and
10 an anti-CD40 antibody, wherein at least one therapeutically effective dose of each of these anti-cancer agents is administered to a patient having a disease or condition that is associated with neoplastic B-cell growth. The invention is particularly useful for the treatment of patients who have previously been administered (i) the chimeric anti-CD20 monoclonal antibody rituximab, or (ii) combination therapy with rituximab and a
15 chemotherapeutic regimen.

DETAILED DESCRIPTION OF THE INVENTION

The invention provides methods for treating human patients for diseases or conditions associated with neoplastic B-cell growth. Such neoplastic B cells include, but
20 are not limited to, neoplastic B cells derived from lymphomas including low-, intermediate-, and high-grade B cell lymphomas, immunoblastic lymphomas, non-Hodgkin's lymphomas, Hodgkin's disease, Epstein-Barr Virus (EBV) induced lymphomas, and AIDS-related lymphomas, as well as B cell acute lymphoblastic leukemias, myelomas, chronic lymphocytic leukemias, acute myeloblastic leukemias, and
25 the like. The methods involve combination therapy with (i) an anti-CD40 antibody and (ii) the alkylating agent bendamustine (marketed under the name Treanda®; Cephalon, Inc., Frazer, PA). Combination therapy with an anti-CD40 antibody, such as HCD122, and bendamustine provides a therapeutic benefit that is greater than that provided by the use of either of these anti-cancer agents alone. In some embodiments, the combined
30 effect of these two therapies can be greater than the sum of the individual effects of each

therapy, *i.e.*, the combination of an anti-CD40 antibody with bendamustine can provide a synergistic therapeutic effect.

In addition, in some embodiments, these two types of anti-cancer agents are used in combination to treat tumors that are refractory to treatment with an anti-CD20 antibody therapy, particularly the anti-CD20 antibody rituximab, where the refractory status of the tumor is a result of initial resistance to the anti-CD20 antibody therapy, or is a result of resistance that develops during one or more time courses of therapy with the anti-CD20 antibody therapy. In yet other embodiments, combination therapy with an anti-CD40 antibody and bendamustine has a synergistic therapeutic effect against tumors that are refractory or non-refractory (*i.e.*, responsive) to anti-CD20 antibody therapy. Administering these two known therapies in combination results in a greater response rate with a more durable response in this patient population than when either agent is administered alone. Without wishing to be bound by any theory or mechanism of action, it is believed that the complimentary, pro-apoptotic mechanisms of an anti-CD40 antibody (such as HCD122) and bendamustine will beneficially provide for a greater response rate in patients having a CD40⁺ B-cell malignancy that is refractory to anti-CD20 antibody therapy, particularly rituximab therapy.

The invention thus provides a method for treating a human patient for a disease or condition associated with neoplastic B-cell growth, said method comprising administering to said patient bendamustine in combination with an anti-CD40 antibody.

In some embodiments, the anti-CD40 antibody (herein "the antibody therapy") and the bendamustine (herein "the bendamustine therapy") are administered to the patient at the same time. In these embodiments, the antibody therapy may be administered to the patient at exactly the same time as the bendamustine therapy (*i.e.*, the two therapies are administered simultaneously). Alternatively, the antibody therapy may be administered to the patient at approximately the same time as the bendamustine therapy (*i.e.*, the two therapies are not administered at precisely the same time), *e.g.*, during the same visit to a physician or other healthcare professional.

In other embodiments, the antibody therapy and the bendamustine therapy are not administered to the patient at the same time, but are administered sequentially (consecutively), in either order. In these embodiments, the methods of the invention may

comprise administering a first cycle of bendamustine therapy to the patient before a first dose of the anti-CD40 antibody is administered to the patient. Alternatively, the methods may comprise administering a first cycle of the bendamustine therapy to the patient after a first dose of the anti-CD40 antibody is administered to the patient. In embodiments
5 where the antibody therapy and the bendamustine therapy are administered sequentially, the therapies may be administered in such a way that both therapies exert a therapeutic effect on the patient at the same time (*i.e.*, the periods in which each therapy is effective may overlap) although this is not essential.

The invention therefore provides a method for treating a human patient for a
10 disease or condition associated with neoplastic B-cell growth, said method comprising administering to the patient one or more doses of an anti-CD40 antibody before, during, or after administering one or more doses of bendamustine.

In some embodiments, a first cycle of the bendamustine therapy is administered to the patient before a first dose of the anti-CD40 antibody. In some of these embodiments,
15 a first cycle of the bendamustine therapy may be administered from about one (1) day to about two weeks (about 14 days), from about 1 day to about 13 days, from about 1 day to about 12 days, from about 1 day to about 11 days, from about 1 day to about 10 days, from about 1 day to about 9 days, from about 1 day to about 8 days, from about 1 day to about one week (about 7 days), from about 1 day to about 6 days, from about 1 day to
20 about 5 days, from about 1 day to about 4 days, from about 1 day to about 3 days, or from about 1 day to about 2 days, or about 1 day before the first dose of an anti-CD40 antibody is administered to the patient. In this manner, the bendamustine therapy may be administered about 14 days, about 13 days, about 12 days, about 11 days, about 10 days, about 9 days, about 8 days, about 7 days, about 6 days, about 5 days, about 4 days, about
25 3 days, about 2 days, or about 1 day before a first dose of the anti-CD40 antibody is administered to the patient. In other words, the antibody therapy may be administered from about one (1) day to about two weeks (about 14 days), from about 1 day to about 13 days, from about 1 day to about 11 days, from about 1 day to about 10 days, from about 1 day to about 9 days, from about 1 day to about 8 days, from about 1 day to about one
30 week (about 7 days), from about 1 day to about 6 days, from about 1 day to about 5 days, from about 1 day to about 4 days, from about 1 day to about 3 days, from about 1 day to

about 2 days, or about 1 day after the first cycle of bendamustine therapy. In this manner, the antibody therapy may be administered about 1 day, about 2 days, about 3 days, about 4 days, about 5 days, about 6 days, about 7 days, about 8 days, about 9 days, about 10 days, about 11 days, about 12 days, about 13 days, or about 14 days after the first cycle of bendamustine therapy.

In other embodiments, a first cycle of the bendamustine therapy is administered to the patient after a first dose of the anti-CD40 antibody. In some of these embodiments, a first cycle of bendamustine therapy may be administered about one (1) day to about two weeks (about 14 days), from about 1 day to about 13 days, from about 1 day to about 12 days, from about 1 day to about 11 days, from about 1 day to about 10 days, from about 1 day to about 9 days, from about 1 day to about 8 days, from about 1 day to about one week (about 7 days), from about 1 day to about 6 days, from about 1 day to about 5 days, from about 1 day to about 4 days, from about 1 day to about 3 days, or from about 1 day to about 2 days, or about 1 day after the first dose of an anti-CD40 antibody is administered to the patient. In this manner, a first cycle of bendamustine therapy may be administered about 14 days, about 13 days, about 12 days, about 11 days, about 10 days, about 9 days, about 8 days, about 7 days, about 6 days, about 5 days, about 4 days, about 3 days, about 2 days, or about 1 day after a first dose of the anti-CD40 antibody is administered to the patient. In other words, the antibody therapy may be administered from about one (1) day to about two weeks (about 14 days), from about 1 day to about 13 days, from about 1 day to about 11 days, from about 1 day to about 10 days, from about 1 day to about 9 days, from about 1 day to about 8 days, from about 1 day to about one week (about 7 days), from about 1 day to about 6 days, from about 1 day to about 5 days, from about 1 day to about 4 days, from about 1 day to about 3 days, from about 1 day to about 2 days, or about 1 day before the first cycle of bendamustine therapy. In this manner, the antibody therapy may be administered about 1 day, about 2 days, about 3 days, about 4 days, about 5 days, about 6 days, about 7 days, about 8 days, about 9 days, about 10 days, about 11 days, about 12 days, about 13 days, or about 14 days before the first cycle of bendamustine therapy.

In yet other embodiments, the antibody therapy and the bendamustine therapy are administered to a patient in need of treatment for a disease or condition associated with

neoplastic B-cell growth, where the administration occurs according to a dosing regimen that comprises multiple cycles of combination therapy with these two anti-cancer agents. In some of these embodiments, the combination therapy comprises administration of these two agents to the patient over multiple cycles (for example, two or more cycles),
5 each of which consists of about 21 days (3 weeks) to about 35 days (about 5 weeks), including, for example, about 28 days (4 weeks). During each cycle of combination therapy, at least one therapeutically effective dose of bendamustine and at least one therapeutically effective dose of the anti-CD40 antibody (such as HCD122) are administered to the patient by the standard acceptable administration routes for these two
10 anti-cancer agents. Suitable therapeutically effective doses of these two anti-cancer agents are described herein below. In particular embodiments, these two agents are administered intravenously (iv), preferably by infusion over the time periods described elsewhere herein.

Upon completion of multiple cycles of combination anti-CD40
15 antibody/bendamustine therapy, a patient may receive a maintenance cycle of anti-CD40 antibody therapy. By "maintenance cycle of anti-CD40 antibody therapy" is intended a time period following combination anti-CD40 antibody/bendamustine therapy during which a patient is administered the anti-CD40 antibody therapy in the absence of bendamustine therapy. During this maintenance cycle, the anti-CD40 antibody is
20 administered according to a desired dosing regimen. In one such embodiment, the anti-CD40 antibody is administered at the same therapeutically effective dose and frequency (for example, bi-weekly) that the antibody was administered during the final cycle of combination anti-CD40 antibody/bendamustine therapy. The length of this maintenance cycle is generally about two weeks to about three months, about two weeks to about two
25 months, about two weeks to about one month, although it can be longer where the health of the patient, history of disease progression, and responsiveness of the patient to follow-up anti-CD40 antibody therapy provide for continued administration of this anti-cancer agent.

The invention thus encompasses multiple cycles of combination therapy with an
30 anti-CD40 antibody and bendamustine, optionally followed by a maintenance cycle of anti-CD40 antibody therapy, where the patient undergoing treatment is administered one

or more therapeutically effective doses of the antibody and bendamustine according to a suitable dosing regimen. For purposes of the present invention "multiple cycles" includes, without limitation, two, three, four, five, six, seven, eight, nine, ten or more cycles of combination therapy. It is to be understood that the dosing regimen followed within any given cycle of combination therapy may be different, or the same as, the dosing regimen of a prior cycle, or a subsequent cycle, of combination therapy, so long as the patient is administered at least one therapeutically effective dose of bendamustine and at least one therapeutically effective dose of the anti-CD40 antibody during a given cycle of combination therapy with these two anti-cancer agents.

It is also to be understood that multiple cycles of combination therapy with these two agents may be given successively, i.e., without interruption or time period off of combination therapy. In this manner, upon completion of any given cycle of combination therapy, a subsequent cycle of combination therapy is initiated the next day. Thus, for example, where 3 cycles of combination therapy are to be administered to a patient, and each cycle consists of 28 days, cycle 1 would occur during days 1-28, cycle 2 would occur during days 29-56, and cycle 3 would occur during days 57-84, with no time period elapsing between the completion of a prior cycle and the initiation of a subsequent cycle of combination therapy. Alternatively, if warranted, a patient may be given a time period off between multiple cycles of combination therapy. The length of this interruption between cycles of combination anti-CD40 antibody/bendamustine therapy will depend upon the health of the patient, history of disease progression, and responsiveness of the patient to the initial and/or prior cycles of combination anti-CD40 antibody/bendamustine therapy. Generally the length of this interruption is about 1 week to about 4 weeks, for example, about 1 week, about 2 weeks, about 3 weeks, or about 4 weeks.

In some embodiments, combination therapy with these two anti-cancer agents comprises the administration of two therapeutically effective doses of bendamustine and at least one therapeutically effective dose of an anti-CD40 antibody to the patient in need thereof over each cycle of combination therapy. Where two doses of bendamustine are to be administered over any given cycle of combination therapy with an anti-CD40 antibody, preferably bendamustine is administered on days 1 and 2 of the cycle, for example, on days 1 and 2 of a 21-day, 28-day, or 35-day cycle of combination therapy.

Preferably a first therapeutically effective dose of anti-CD40 antibody is administered to the patient within 3 days of the start of a given cycle of combination therapy (for example, on day 1, 2, 3, or 4 of that cycle), and a second therapeutically effective dose of the antibody is optionally administered to the patient about 12-14 days following the administration of the first dose of anti-CD40 antibody (i.e., on day 13, 14, 15, or 16 of that cycle). Where the first dose of anti-CD40 antibody is administered on the same day as the dose of bendamustine (i.e., on day 1 or 2 of each cycle), the agents may be administered simultaneously or sequentially, in either order. In some embodiments, the anti-CD40 antibody is administered prior to administering the bendamustine.

10 In one such embodiment, the combination therapy of the invention comprises administration of these two anti-cancer agents to a patient in need of treatment for a disease or condition associated with neoplastic B-cell growth, where the administration occurs over multiple cycles, each of which consists of about 28 days (4 weeks). For each such cycle, the combination therapy comprises administering a therapeutically effective dose of bendamustine to the patient on each of days 1 and 2 of the cycle, and administering a therapeutically effective dose of the anti-CD40 antibody (such as HCD122) to the patient according to a bi-weekly schedule (i.e., once every two weeks). In this manner, the patient receives two therapeutically effective doses of bendamustine and two therapeutically effective doses of the anti-CD40 antibody over the 28-day cycle.

20 An exemplary dosing regimen for each of said 28-day cycles of combination therapy comprises the administration of a therapeutically effective dose of bendamustine on each of days 1 and 2 of said 28-day cycle, and administration of a first therapeutically effective dose of said anti-CD40 antibody (such as HCD122) within the first three days of the 28-day cycle, for example, on day 1, day 2, or day 3 of the 28-day cycle. In one such embodiment, the first therapeutically effective dose of the anti-CD40 antibody (such as HCD122) is administered on day 1 of the 28-day cycle of combination therapy, and a second therapeutically effective dose of the anti-CD40 antibody is administered on day 15 of the 28-day cycle of combination therapy; in other embodiments, the first therapeutically effective dose of the anti-CD40 antibody (such as HCD122) is administered on day 2 of the 28-day cycle of combination therapy, and a second therapeutically effective dose of the anti-CD40 antibody is administered on day 15 of the

28-day cycle of combination therapy; in yet other embodiments, the first therapeutically effective dose of the anti-CD40 antibody (such as HCD122) is administered on day 3 of the 28-day cycle of combination therapy, and a second therapeutically effective dose of the anti-CD40 antibody is administered on day 15 of the 28-day cycle of combination
5 therapy. It is recognized that if the patient undergoing this particular regimen of multiple cycles of combination anti-CD40 antibody/bendamustine therapy is exhibiting symptoms of non-tolerance of the treatment schedule, an alternative anti-CD40 antibody dosing schedule can be implemented, wherein the second therapeutically effective dose of the anti-CD40 antibody is withheld (i.e., the day 15 antibody dose is not administered).

10 In particular embodiments, a patient in need of treatment for a disease or condition associated with neoplastic B-cell growth (for example, for a B-cell lymphoma, such as follicular lymphoma) is administered 3-8 cycles of combination anti-CD40 antibody/bendamustine therapy, each of which consists of a 28-day cycle, wherein a therapeutically effective dose of bendamustine in the range of about 60 mg/m² to about
15 120 mg/m² (for example, 60, 65, 70, 75, 80, 85, 90, 95, 100, 105, 110, 115, 120 mg/m², or other such value falling between about 60 mg/m² and about 120 mg/m²) is administered intravenously (iv) on day 1 and day 2 of each 28-day cycle, and a therapeutically effective dose of an anti-CD40 antibody (such as HCD122) in the range of about 2.5 mg/kg to about 5.0 mg/kg (body weight)(for example, about 2.5, 3.0, 3.5, 4.0
20 4.5, 5.0 mg/kg, or other such value falling between about 2.5 mg/kg and about 5.0 mg/kg) is administered intravenously (iv) on day 1, 2, or 3 of each 28-day cycle, and again on day 15 of each 28-day cycle. In some of these embodiments, the patient is administered 6 cycles of this combination therapy according to this dosing regimen. In one such embodiment, the therapeutically effective dose of bendamustine is about 90 mg/m² and
25 the therapeutically effective dose of the anti-CD40 antibody (such as HCD122) is about 3.0 mg/kg, 3.5 mg/kg, 4.0 mg/kg, or 4.5 mg/kg, which are administered according to the foregoing dosing regimen for six (6) 28-day cycles. Optionally, these multiple cycles of combination therapy are followed by a maintenance cycle of anti-CD40 antibody therapy, where the anti-CD40 antibody (such as HCD122) is administered bi-weekly for up to
30 three months at the same dose that was administered in the final cycle of combination anti-CD40 antibody/bendamustine therapy. In some of these embodiments, the patient

undergoing multiple cycles of this combination anti-CD40 antibody/bendamustine therapy has a B-cell lymphoma that is refractory to rituximab and has received at least one prior chemotherapeutic regimen, as described elsewhere herein.

When the antibody and bendamustine therapies are administered at the same time, they may be administered as a single pharmaceutical formulation or as two separate pharmaceutical formulations. When the therapies are not administered at the same time, they are administered as two separate pharmaceutical formulations. In embodiments where a pharmaceutical formulation contains the antibody therapy and bendamustine, this pharmaceutical formulation may be obtained by a method comprising the steps of (i) obtaining a lyophilized anti-CD40 antibody composition, (ii) obtaining a composition comprising bendamustine in a sterile diluent, and (iii) reconstituting the lyophilized antibody composition using the composition comprising bendamustine. The invention therefore provides a pharmaceutical composition comprising (i) bendamustine, (ii) an anti-CD40 antibody, and (iii) a pharmaceutically acceptable carrier or excipient.

The invention also provides the use of (i) bendamustine and (ii) an anti-CD40 antibody, in the manufacture of a medicament for treating a human patient for a disease or condition associated with neoplastic B-cell growth. In other embodiments, the invention provides the use of (i) bendamustine and (ii) an anti-CD40 antibody, in the manufacture of two separate medicaments for treating a human patient for a disease or condition associated with neoplastic B-cell growth by combination therapy.

The invention also provides a kit for treating a human patient for a disease or condition associated with neoplastic B-cell growth, said kit comprising (i) bendamustine and (ii) an anti-CD40 antibody. The kit may further comprise one or more devices for administering the combination therapy to a human patient, such as one or more of (i) a sterile needle and syringe, (ii) a sterile container (*e.g.*, a glass bottle, plastic bottle or plastic bag) and drip chamber, (iii) a sterile tube with a regulating clamp, and (iv) a catheter.

The invention provides a method for treating a human patient for a disease or condition associated with neoplastic B-cell growth, said method comprising administering to said patient bendamustine, wherein the patient has been pre-treated with an anti-CD40 antibody. The invention also provides a method for treating a human

patient for a disease or condition associated with neoplastic B-cell growth, said method comprising administering to said patient an anti-CD40 antibody, wherein the patient has been pre-treated with bendamustine.

The invention further provides the use of an anti-CD40 antibody in the
5 manufacture of a medicament for treating a human patient for a disease or condition associated with neoplastic B-cell growth, wherein said human patient has been pre-treated with bendamustine. The invention also provides the use of bendamustine in the manufacture of a medicament for treating a human patient for a disease or condition associated with neoplastic B-cell growth, wherein said human patient has been pre-
10 treated with an anti-CD40 antibody.

By “pre-treated” or “pre-treatment” is intended the subject has received one or more doses of a first therapy prior to a second therapy. “Pre-treated” or “pre-treatment” includes patients that have been treated with a first therapy within 2 years, within 18 months, within 1 year, within 6 months, within 2 months, within 6 weeks, within 1
15 month, within 4 weeks, within 3 weeks, within 2 weeks, within 1 week, within 6 days, within 5 days, within 4 days, within 3 days, within 2 days, or within 1 day prior to initiation of treatment with a second therapy. In the combination methods of the invention, “pre-treated” or “pre-treatment” thus includes patients that have been treated with an anti-CD40 antibody within 2 years, within 18 months, within 1 year, within 6
20 months, within 2 months, within 6 weeks, within 1 month, within 4 weeks, within 3 weeks, within 2 weeks, within 1 week, within 6 days, within 5 days, within 4 days, within 3 days, within 2 days, or within 1 day prior to initiation of treatment with the bendamustine therapy. In the combination methods of the invention, “pre-treated” or “pre-treatment” also includes patients that have been treated with the bendamustine
25 therapy within 2 years, within 18 months, within 1 year, within 6 months, within 2 months, within 6 weeks, within 1 month, within 4 weeks, within 3 weeks, within 2 weeks, within 1 week, within 6 days, within 5 days, within 4 days, within 3 days, within 2 days, or within 1 day, prior to initiation of treatment with an anti-CD40 antibody.

Patients who have been pre-treated with an anti-CD40 antibody can be
30 distinguished from other patients, *e.g.*, by consulting patients’ medical records or carrying out suitable *in vitro* test(s). Patients who have been pre-treated with

bendamustine can be distinguished from other patients, *e.g.*, by consulting patients' medical records or carrying out suitable *in vitro* test(s).

The invention also provides the use of an anti-CD40 antibody in the manufacture of a medicament for treating a human patient for a disease or condition associated with neoplastic B-cell growth, wherein the medicament is administered prior to administration of bendamustine. In alternative embodiments, the invention provides the use of an anti-CD40 antibody in the manufacture of a medicament for treating a human patient for a disease or condition associated with neoplastic B-cell growth, wherein the medicament is administered subsequent to administration of bendamustine. The invention also provides the use of bendamustine in the manufacture of a medicament for treating a human patient for a disease or condition associated with neoplastic B-cell growth, wherein the medicament is administered prior to administration of an anti-CD40 antibody. In alternative embodiments, the invention provides the use of bendamustine in the manufacture of a medicament for treating a human patient for a disease or condition associated with neoplastic B-cell growth, wherein the medicament is administered subsequent to administration of an anti-CD40 antibody.

The invention also provides an anti-CD40 antibody and bendamustine for simultaneous, separate, or sequential use in treating a human patient for a disease or condition associated with neoplastic B-cell growth by combination therapy. The invention also provides the use of an anti-CD40 antibody in the manufacture of a medicament for simultaneous or sequential use in combination with bendamustine for treating a human patient for a disease or condition associated with neoplastic B-cell growth. The invention also provides the use of bendamustine in the manufacture of a medicament for simultaneous or sequential use in combination with an anti-CD40 antibody for treating a human patient for a disease or condition associated with neoplastic B-cell growth.

The methods of the invention may comprise administering a dose of an anti-CD40 antibody at any time during a first or subsequent cycle of the bendamustine therapy. Alternatively, the methods of the invention may comprise administering a dose of an anti-CD40 antibody between cycles of bendamustine therapy.

In some embodiments of the methods, uses, compositions, and kits disclosed herein, the combination therapy provides a synergistic improvement in therapeutic efficacy relative to the individual therapeutic agents when administered alone. The term “synergy” is used to describe a combined effect of two or more active agents that is
5 greater than the sum of the individual effects of each respective active agent. Thus, where the combined effect of two or more agents results in “synergistic inhibition” of an activity or process, for example, tumor growth, it is intended that the inhibition of the activity or process is greater than the sum of the inhibitory effects of each respective active agent. The term “synergistic therapeutic effect” therefore refers to a therapeutic
10 effect observed with a combination of two or more therapies wherein the therapeutic effect (as measured by any of a number of parameters, *e.g.*, tumour growth delay) is greater than the sum of the individual therapeutic effects observed with the respective individual therapies.

A summary of standard techniques and procedures which may be employed in
15 order to utilize the invention is given below. It will be understood that this invention is not limited to the particular methodology, protocols, cell lines, vectors and reagents described. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only and it is not intended that this terminology should limit the scope of the present invention. The extent of the invention is limited only
20 by the terms of the appended claims.

Standard abbreviations for nucleotides and amino acids are used in this specification. The practice of the present invention will employ, unless otherwise indicated, conventional techniques of molecular biology, microbiology, recombinant DNA technology and immunology, which are within the skill of those working in the art.
25 Such techniques are explained fully in the literature.

The invention involves the use of anti-CD40 antibodies for the treatment of human patients having diseases or conditions associated with neoplastic B-cell growth. By “CD40”, “CD40 antigen”, or “CD40 receptor” is intended the 50-55 kDa transmembrane glycoprotein of the tumor necrosis factor (TNF) receptor family (see, for
30 example, U.S. Patent Nos. 5,674,492 and 4,708,871; Stamenkovic *et al.* (1989) *EMBO* 8:1403; Clark (1990) *Tissue Antigens* 36:33; Barclay *et al.* (1997) *The Leucocyte Antigen*

Facts Book (2d ed.; Academic Press, San Diego)). Two isoforms of human CD40, encoded by alternatively spliced transcript variants of this gene, have been identified. The first isoform (also known as the "long isoform" or "isoform 1") is expressed as a 277-amino-acid precursor polypeptide (SEQ ID NO:9; first reported as GenBank Accession No. CAA43045, and identified as isoform 1 in GenBank Accession No. NP_001241), encoded by SEQ ID NO:8 (see GenBank Accession Nos. X60592 and NM_001250), which has a signal sequence represented by the first 19 residues. The second isoform (also known as the "short isoform" or "isoform 2") is expressed as a 203-amino-acid precursor polypeptide (SEQ ID NO:7; GenBank Accession No. NP_690593), encoded by SEQ ID NO:6 (GenBank Accession No. NM_152854), which also has a signal sequence represented by the first 19 residues. The precursor polypeptides of these two isoforms of human CD40 share in common their first 165 residues (*i.e.*, residues 1-165 of SEQ ID NO:7 and SEQ ID NO:9). The precursor polypeptide of the short isoform (shown in SEQ ID NO:7) is encoded by a transcript variant (SEQ ID NO:6) that lacks a coding segment, which leads to a translation frame shift; the resulting CD40 isoform contains a shorter and distinct C-terminus (residues 166-203 of SEQ ID NO:7) from that contained in the long isoform of CD40 (C-terminus shown in residues 166-277 of SEQ ID NO:9). For purposes of the present invention, the term "CD40," or "CD40 antigen," "CD40 cell surface antigen," or "CD40 receptor" encompasses both the short and long isoforms of CD40.

By "CD40-expressing cells" herein is intended any normal or malignant cells that express detectable levels of the CD40 antigen. Methods for detecting CD40 antigen expression in cells are well known in the art and include, but are not limited to, PCR techniques, immunohistochemistry, flow cytometry, Western blot, ELISA, and the like. These methods allow for the detection of CD40 mRNA, CD40 antigen and cell-surface CD40 antigen. Preferably, the CD40-expressing cells are cells that express detectable levels of cell-surface CD40 antigen.

By "CD40 ligand" or "CD40L" is intended the 32-33 kDa transmembrane protein that also exists in two smaller biologically active soluble forms, 18 kDa and 31 kDa, respectively (Graf *et al.* (1995) *Eur. J. Immunol.* 25:1749-1754; Mazzei *et al.* (1995) *J.*

Biol. Chem. 270:7025-7028; Pietravalle *et al.* (1996) *J. Biol. Chem.* 271:5965-5967).

Human CD40L is also known as CD154 or gp39.

By "human patient" is intended a human who is afflicted with, at risk of developing or relapsing with, any disease or condition associated with neoplastic B-cell growth.

By "disease or condition associated with neoplastic B-cell growth" is intended any disease or condition (including pre-malignant conditions) involving uncontrolled growth of cells of B-cell lineage. Such diseases and conditions include, but are not limited to, acute lymphoblastic leukemia (ALL), acute myelogenous leukemia (AML), chronic myelogenous leukemia (CML), chronic lymphocytic leukemia (CLL), prolymphocytic leukemia (PLL), small lymphocytic leukemia (SLL), diffuse small lymphocytic leukemia (DSLL), diffuse large B-cell lymphoma (DLBCL), hairy cell leukemia, non-Hodgkin's lymphomas, including follicular lymphoma, Hodgkin's disease, Epstein-Barr Virus (EBV) induced lymphomas, myelomas such as multiple myeloma, Waldenstrom's macroglobulinemia, heavy chain disease, mucosal associated lymphoid tissue lymphoma, monocytoid B cell lymphoma, splenic lymphoma, lymphomatoid granulomatosis, intravascular lymphomatosis, immunoblastic lymphomas, AIDS-related lymphomas, and the like.

The methods of the invention find use in the treatment of subjects having non-Hodgkin's lymphomas related to abnormal B cell proliferation or accumulation. For purposes of the present invention, such lymphomas will be referred to according to the *Working Formulation* classification scheme, that is those B cell lymphomas categorized as low grade, intermediate grade, and high grade (see "The Non-Hodgkin's Lymphoma Pathologic Classification Project," *Cancer* 49(1982):2112-2135). Thus, low-grade B cell lymphomas include small lymphocytic, follicular small-cleaved cell, and follicular mixed small-cleaved and large cell lymphomas; intermediate-grade lymphomas include follicular large cell, diffuse small cleaved cell, diffuse mixed small and large cell, and diffuse large cell lymphomas; and high-grade lymphomas include large cell immunoblastic, lymphoblastic, and small non-cleaved cell lymphomas of the Burkitt's and non-Burkitt's type. The methods of the invention can be used to treat low-, intermediate-, and high-grade B cell lymphomas.

The methods of the invention are useful in the therapeutic treatment of B cell lymphomas that are classified according to the Revised European and American Lymphoma Classification (REAL) system. Such B cell lymphomas include, but are not limited to, lymphomas classified as precursor B cell neoplasms, such as B lymphoblastic leukemia/lymphoma; peripheral B cell neoplasms, including B cell chronic lymphocytic leukemia/small lymphocytic lymphoma, lymphoplasmacytoid lymphoma/immunocytoma, mantle cell lymphoma (MCL), follicle center lymphoma (follicular lymphoma) (including diffuse small cell, diffuse mixed small and large cell, and diffuse large cell lymphomas), marginal zone B-cell lymphoma (including

5 leukemic, nodal, and splenic types, *e.g.*, extranodal marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue), plasmacytoma/ myeloma, diffuse large cell B cell lymphoma of the subtype primary mediastinal (thymic), Burkitt's lymphoma, and Burkitt's like high-grade B cell lymphoma; and unclassifiable low-grade or high-grade B cell lymphomas.

15 In the methods of the invention, combination therapy is used to provide a positive therapeutic response with respect to a disease or condition. By "positive therapeutic response" is intended an improvement in the disease or condition, and/or an improvement in the symptoms associated with the disease or condition, as a result of the therapeutic activity of the combination therapy. That is, an anti-proliferative effect, the prevention of

20 further tumor outgrowths, a reduction in tumor size, a reduction in the number of neoplastic cells, and/or a decrease in one or more symptoms associated with CD40-expressing cells can be observed. Thus, for example, a positive therapeutic response would refer to one or more of the following improvements in the disease: (1) a reduction in tumor size; (2) a reduction in the number of neoplastic cells; (3) an increase in

25 neoplastic cell death; (4) inhibition of neoplastic cell survival; (4) inhibition (*i.e.*, slowing to some extent, preferably halting) of tumor growth; (5) inhibition (*i.e.*, slowing to some extent, preferably halting) of neoplastic cell infiltration into peripheral organs; (6) inhibition (*i.e.*, slowing to some extent, preferably halting) of tumor metastasis; (7) the prevention of further tumor outgrowths; (8) an increased patient survival rate; and (9)

30 some relief from one or more symptoms associated with the disease or condition.

Positive therapeutic responses in any given disease or condition can be determined by standardized response criteria specific to that disease or condition. Tumor response can be assessed for changes in tumor morphology (*i.e.*, overall tumor burden, tumor size, and the like) using screening techniques such as magnetic resonance imaging (MRI) scan, x-radiographic imaging, computed tomographic (CT) scan, bone scan imaging, endoscopy, and tumor biopsy sampling including bone marrow aspiration (BMA) and counting of tumor cells in the circulation. In addition to these positive therapeutic responses, the subject undergoing therapy may experience the beneficial effect of an improvement in the symptoms associated with the disease. Thus for B cell tumors, the subject may experience a decrease in the so-called B symptoms, *i.e.*, night sweats, fever, weight loss, and/or urticaria. For pre-malignant conditions, therapy with an anti-CD40 therapeutic agent may block and/or prolong the time before development of a related malignant condition, for example, development of multiple myeloma in subjects suffering from monoclonal gammopathy of undetermined significance (MGUS).

An improvement in the disease may be characterized as a complete response. By "complete response" is intended an absence of clinically detectable disease with normalisation of any previously abnormal radiographic studies, bone marrow, and cerebrospinal fluid (CSF) or abnormal monoclonal protein in the case of myeloma. Such a response may persist for at least 4 to 8 weeks, or sometimes 6 to 8 weeks, following treatment according to the methods of the invention. Alternatively, an improvement in the disease may be categorized as being a partial response. By "partial response" is intended at least about a 50% decrease in all measurable tumor burden (*i.e.*, the number of malignant cells present in the subject, or the measured bulk of tumor masses or the quantity of abnormal monoclonal protein) in the absence of new lesions, which may persist for 4 to 8 weeks, or 6 to 8 weeks. Guidelines for determining efficacy of a cancer therapy, including the combination anti-CD40 antibody/bendamustine therapy described herein, include the Cheson response criteria outlined in Cheson *et al.* (1999) *J. Clin. Oncol.* 17:1244-1253, also revised in Cheson *et al.* (2007) *J. Clin. Oncol.* 25:579-586, and further clarified in Cheson (2007) *Hematol. Oncol. Clin. N. Am.* 21:841-854, the contents of each of which are herein incorporated by reference in their entirety.

The methods and products of the invention involve use of therapeutically or prophylactically effective amounts of an anti-CD40 antibody and bendamustine. By "an effective amount" or "therapeutically or prophylactically effective amount" is intended an amount of antibody therapy or bendamustine therapy that, when administered as a part of
5 a combination therapy, brings about a positive therapeutic response with respect to patient treatment. Suitable amounts are described in more detail elsewhere herein.

"Tumor" (or "tumour"), as used herein, refers to all neoplastic cell growth and proliferation, whether malignant or benign, and all pre-cancerous and cancerous cells and tissues. "Neoplastic", as used herein, refers to any form of dysregulated or unregulated
10 cell growth, whether malignant or benign, resulting in abnormal growth. Thus, "neoplastic cells" include malignant and benign cells having dysregulated or unregulated cell growth. The terms "cancer" and "cancerous" refer to or describe the physiological condition in mammals that is typically characterized by unregulated cell growth.

"Treatment" is herein defined as the application or administration of combination
15 therapy to a patient, or application or administration of combination therapy to an isolated tissue from a patient, where the patient has a disease, a symptom of a disease, or a predisposition toward a disease, where the purpose is to cure, heal, alleviate, relieve, alter, remedy, ameliorate, improve, or affect the disease, the symptoms of the disease, or the predisposition toward the disease.

20 The methods of the invention are particularly useful for treating patients who have previously been administered other oncotherapeutic treatments. This includes patients who have been administered another oncotherapeutic treatment at any time prior to initiation of the combination therapy according to the invention, *e.g.*, within 15 years, within 14 years, within 13 years, within 12 years, within 11 years, within 10 years, within
25 9 years, within 8 years, within 7 years, within 6 years, within 5 years, within 4 years, within 3 years, within 2 years, within 18 months, within 1 year, within 6 months, within 2 months, within 6 weeks, within 1 month, within 4 weeks, within 3 weeks, within 2 weeks, within 1 week, within 6 days, within 5 days, within 4 days, within 3 days, within 2 days, or within 1 day, prior to initiation of the combination therapy according to the
30 invention.

In particular, the methods of the invention are useful for treating a disease or condition associated with neoplastic B-cell growth, including the B cell lymphomas listed above, that are refractory to (i.e., resistant to, or have become resistant to) first-line oncotherapeutic treatments. The term “oncotherapeutic” or “oncotherapy” is intended to mean a treatment for cancer such as chemotherapy, surgery, radiation therapy, single anti-cancer antibody therapy, and combinations thereof.

Such oncotherapy can include chemotherapy regimens such as treatment with CVP (cyclophosphamide, vincristine and prednisone), R-CVP (rituximab plus CVP), CHOP (cyclophosphamide, doxorubicin, vincristine and prednisone), R-CHOP (rituximab plus CHOP), ICE (ifosfamide, carboplatin, and etoposide), Mitozantrone, Cytarabine, DVP (daunorubicin, prednisone, and vincristine), ATRA (all-trans-retinoic acid), Idarubicin, hoelzer chemotherapy regime, La La chemotherapy regime, ABVD (adriamycin, bleomycin, vinblastine, and dacarbazine), CEOP (cyclophosphamide, epirubicin, vincristine, and prednisone), CEOP-BE (cyclophosphamide, epirubicin, vincristine, prednisone, bleomycin, and etoposide), 2-CdA (2-chlorodeoxyadenosine (2-CdA), fludarabine-based chemotherapy such as FLAG & IDA (fludarabine, cytarabine, and idarubicin; with or without subsequent G-CSF treatment), VAD (vincristine, doxorubicin, and dexamethasone), M & P (melphalan and prednisone), C-Weekly (cyclophosphamide and prednisone), ABCM (adriamycin (doxorubicin), BCNU, cyclophosphamide, and melphalan), MOPP (nitrogen mustard, oncovin, procarbazine, and prednisone), and DHAP (dexamethasone, high-dose ara-C, and platinol). Alternatively, such oncotherapies can include radiation treatment, including myeloablative therapies, or radioimmunotherapy, for example, with yttrium-90-labeled ibritumomab tiuxetan or iodine-131-labeled tositumomab.

In particular, the combination therapy of the invention may be useful for treating a human patient who has previously been administered (i) anti-CD40 antibody therapy alone, (ii) bendamustine therapy alone, (iii) a chemotherapeutic regimen alone, (iv) anti-CD20 antibody therapy (such as the chimeric anti-CD20 antibody rituximab) alone, or (iv) an anti-CD20 antibody-containing regimen (such as a rituximab-containing regimen), particularly combination therapy with CHOP and an anti-CD20 antibody (such as rituximab, wherein the combination therapy is commonly termed R-CHOP) or

combination therapy with CVP and an anti-CD20 antibody (such as rituximab, wherein the combination therapy is commonly termed R-CVP).

The invention may be particularly useful for treating diseases or conditions that are refractory to therapy with other oncotherapeutic treatments. The invention may therefore be useful in treating diseases or conditions that are refractory to therapy with (i) a chemotherapeutic regimen alone (such as CHOP), (ii) an anti-CD40 antibody (such as HCD122) alone, (iii) an anti-CD20 antibody (such as rituximab) alone, or (iv) combination therapy with CHOP and an anti-CD20 antibody (for example, R-CHOP) or CVP and an anti-CD20 antibody (for example, R-CVP). By “refractory” is intended the particular disease or condition is resistant to, or non-responsive to, therapy with a particular oncotherapeutic agent. A disease or condition can be refractory to therapy with a particular therapeutic agent either from the onset of treatment with the particular therapeutic agent (*i.e.*, non-responsive to initial exposure to the therapeutic agent), or as a result of developing resistance to the therapeutic agent, either over the course of a first treatment period with the therapeutic agent or during a subsequent treatment period with the therapeutic agent. The invention therefore provides methods, compositions, uses and kits for treating a human patient for a disease or condition associated with neoplastic B-cell growth, wherein said disease or condition is refractory to an oncotherapeutic treatment other than the combination therapy of the invention.

The invention may also be particularly useful for treating patients who have relapsed after therapy with other oncotherapeutic treatments. The invention may therefore be useful in treating patients who have relapsed after therapy with (i) CHOP alone, (ii) an anti-CD40 antibody (such as HCD122) alone, (iii) bendamustine alone, (iv) an anti-CD20 antibody (such as rituximab) alone, or (v) combination therapy with CHOP and an anti-CD20 antibody (R-CHOP). By “relapsed” is meant that the patient achieved a partial or complete response to a prior oncotherapeutic treatment, but has subsequently had a recurrence of the disease or condition. The invention therefore provides methods, compositions, uses and kits for treating a human patient for a disease or condition associated with neoplastic B-cell growth, wherein said patient has relapsed after therapy with an oncotherapeutic treatment other than the combination therapy of the invention.

The combination therapy of the invention addresses problems associated with therapy using rituximab (the IDEC-C2B8 monoclonal antibody (Biogen Idec or Genentech) commercially available under the tradename Rituxan®). Rituximab is a chimeric anti-CD20 monoclonal antibody containing human IgG1 and kappa constant regions with murine variable regions isolated from a murine anti-CD20 monoclonal antibody (Reff *et al.* (1994) *Blood* 83:435-445). The methods of the invention enable the treatment of patients having a disease or condition associated with CD40-expressing B-cells, which might otherwise have been treated with rituximab or by combination therapy with rituximab and chemotherapeutic agents (*e.g.*, CHOP, CVP).

Accordingly, the invention also provides methods, compositions, uses, and kits for treating a human patient for a disease or condition associated with neoplastic B-cell growth, including the B cell lymphomas listed above, by combination therapy, wherein the patient has previously been administered the chimeric anti-CD20 antibody rituximab, or has previously been administered rituximab and has received at least one prior chemotherapeutic regimen. The invention may be useful in treating patients with a disease or condition associated with neoplastic B-cell growth that is refractory to therapy with (i) rituximab alone, or (ii) combination therapy with rituximab and a chemotherapeutic regimen (for example, CHOP and rituximab (R-CHOP) or CVP and rituximab (R-CVP)). The invention may also be useful in treating patients who have relapsed after therapy with (i) rituximab alone, or (ii) combination therapy with rituximab and a chemotherapeutic regimen (for example, CHOP and rituximab (R-CHOP) or CVP and rituximab (R-CVP)). In some embodiments, the methods, compositions, uses, and kits are directed to treatment of patients for a non-Hodgkin's lymphoma, including CD40⁺ follicular lymphoma, where this disease has progressed during or within 6 months of treatment with rituximab or a rituximab-containing regimen and who have received treatment with at least one prior chemotherapeutic regimen, for example, CHOP, CVP, or a fludarabine-based chemotherapy.

Patients who have been pre-treated with rituximab can be distinguished from other patients, *e.g.*, by consulting patients' medical records or carrying out suitable *in vitro* test(s). For example, the number of circulating CD19⁺ B-cells is depleted in patients treated with rituximab, and numbers of circulating CD19⁺ B-cells can be

monitored using suitable methods, *e.g.*, FACS (McLaughlin *et al.* (1998) *J. Clin. Oncol.* 16(8):2825-2833; Maloney *et al.* (1997) *Blood* 90(6):2188-2195).

The methods of the invention involve the use of anti-CD40 antibodies. Natural antibodies are usually heterotetrameric glycoproteins of about 150,000 daltons, composed of two identical light (L) chains and two identical heavy (H) chains. Each light chain is linked to a heavy chain by one covalent disulfide bond, while the number of disulfide linkages varies among the heavy chains of different isotypes. Each heavy and light chain also has regularly spaced intrachain disulfide bridges. Each heavy chain has at one end a variable domain (V_H) followed by a number of constant domains. Each light chain has a variable domain at one end (V_L) and a constant domain at its other end; the constant domain of the light chain is aligned with the first constant domain of the heavy chain, and the light chain variable domain is aligned with the variable domain of the heavy chain. Particular amino acid residues are believed to form an interface between the light and heavy-chain variable domains. The term "variable" refers to the fact that certain portions of the variable domains differ extensively in sequence among antibodies. The variable regions confer antigen-binding specificity. The constant domains are not involved directly in binding an antibody to an antigen, but exhibit various effector functions, such as Fc receptor (FcR) binding, participation of the antibody in antibody-dependent cellular toxicity, initiation of complement dependent cytotoxicity, and mast cell degranulation.

The "light chains" of antibodies from any vertebrate species can be assigned to one of two clearly distinct types, called kappa (κ) and lambda (λ), based on the amino acid sequences of their constant domains.

Depending on the amino acid sequence of the constant domain of their "heavy chains", antibodies can be assigned to different classes. There are five major classes of human antibodies: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into subclasses (isotypes), *e.g.*, IgG1, IgG2, IgG3, IgG4, IgA1, and IgA2. The heavy-chain constant domains that correspond to the different classes of antibodies are called alpha, delta, epsilon, gamma, and mu, respectively. The subunit structures and three-dimensional configurations of different classes of antibodies are well known. Different isotypes have different effector functions. For example, human IgG1 and IgG3 isotypes have ADCC (antibody dependent cell-mediated cytotoxicity) activity. IgG1

antibodies, in particular human IgG1 antibodies, are particularly useful in the methods of the invention.

“Human effector cells” are leukocytes that express one or more FcRs and perform effector functions. Preferably, the cells express at least FcγRIII and carry out antigen-dependent cell-mediated cytotoxicity (ADCC) effector function. Examples of human leukocytes that mediate ADCC include peripheral blood mononuclear cells (PBMC), natural killer (NK) cells, monocytes, macrophages, eosinophils, and neutrophils, with PBMCs and NK cells being preferred. Antibodies that have ADCC activity are typically of the IgG1 or IgG3 isotype. Note that in addition to isolating IgG1 and IgG3 antibodies, ADCC-mediating antibodies can be made by combining a variable region from a non-ADCC antibody with an IgG1 or IgG3 isotype constant region.

The terms “Fc receptor” or “FcR” are used to describe a receptor that binds to the Fc region of an antibody. The preferred FcR is a native-sequence human FcR. Moreover, a preferred FcR is one that binds an IgG antibody (a gamma receptor) and includes receptors of the FcγRI, FcγRII, and FcγRIII subclasses, including allelic variants and alternatively spliced forms of these receptors. FcγRII receptors include FcγRIIA (an “activating receptor”) and FcγRIIB (an “inhibiting receptor”), which have similar amino acid sequences that differ primarily in the cytoplasmic domains thereof. Activating receptor FcγRIIA contains an immunoreceptor tyrosine-based activation motif (ITAM) in its cytoplasmic domain. Inhibiting receptor FcγRIIB contains an immunoreceptor tyrosine-based inhibition motif (ITIM) in its cytoplasmic domain (see Dairon (1997) *Annu. Rev. Immunol.* 15:203-234). FcRs are reviewed in Ravetch and Kinet (1991) *Annu. Rev. Immunol.* 9:457-492 (1991); Capel *et al.* (1994) *Immunomethods* 4:25-34; and de Haas *et al.* (1995) *J. Lab. Clin. Med.* 126:330-341. Other FcRs, including those to be identified in the future, are encompassed by the term “FcR” herein. The term also includes the neonatal receptor, FcRn, which is responsible for the transfer of maternal IgGs to the fetus (Guyer *et al.* (1976) *J. Immunol.* 117:587 and Kim *et al.* (1994) *J. Immunol.* 24:249 (1994)).

The term “antibody” is used herein in the broadest sense and covers fully assembled antibodies, antibody fragments which retain the ability to specifically bind to the CD40 antigen (*e.g.*, Fab, F(ab')₂, Fv, and other fragments), single chain antibodies

(scFv), diabodies, bispecific antibodies, chimeric antibodies, humanized antibodies, fully human antibodies, and the like, and recombinant peptides comprising the foregoing. The term “antibody” covers both polyclonal and monoclonal antibodies.

As used herein “anti-CD40 antibody” encompasses any antibody that specifically
5 recognizes the CD40 antigen. In some embodiments, anti-CD40 antibodies for use in the methods of the present invention, in particular monoclonal anti-CD40 antibodies, exhibit a strong single-site binding affinity for the CD40 antigen. Such monoclonal antibodies exhibit an affinity for CD40 (K_D) of at least 10^{-5} M, preferably at least 10^{-6} M, at least 10^{-7} M, at least 10^{-8} M, at least 10^{-9} M, at least 10^{-10} M, at least 10^{-11} M or at least 10^{-12} M,
10 when measured using a standard assay such as Biacore™. Biacore analysis is known in the art and details are provided in the “BIAApplications handbook”.

By “specifically recognizes” or “specifically binds to” is intended that the anti-CD40 antibody binds to the CD40 antigen on the surface of human B-cells, but does not bind to a significant extent other antigens on the surface of human B-cells, such as the
15 CD20 antigen.

The anti-CD40 antibodies for use in the methods of the present invention can be produced using any suitable antibody production method known to those of skill in the art.

The anti-CD40 antibody used in the methods of the present invention may be a
20 monoclonal antibody. The term “monoclonal antibody” (and “mAb”) as used herein refers to an antibody obtained from a substantially homogeneous population of antibodies, *i.e.*, the individual antibodies comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts. The term is not limited regarding the species of the antibody and does not require production
25 of the antibody by any particular method. In contrast to polyclonal antibody preparations, which typically include different antibodies directed against different antigenic determinants (epitopes), each monoclonal antibody is directed against a single determinant (epitope) on the antigen.

The term “monoclonal” as originally used in relation to antibodies referred to
30 antibodies produced by a single clonal line of immune cells, as opposed to “polyclonal” antibodies that, while all recognizing the same target protein, were produced by different

B cells and would be directed to different epitopes on that protein. As used herein, the word “monoclonal” does not imply any particular cellular origin, but refers to any population of antibodies that all have the same amino acid sequence and recognize the same epitope in the same target protein. Thus a monoclonal antibody may be produced
5 using any suitable protein synthesis system, including immune cells, non-immune cells, acellular systems, *etc.* This usage is usual in the field *e.g.*, the product datasheets for the CDR-grafted humanized antibody Synagis™ expressed in a murine myeloma NS0 cell line, the humanized antibody Herceptin™ expressed in a CHO cell line, and the phage-displayed antibody Humira™ expressed in a CHO cell line all refer to the products as
10 monoclonal antibodies.

By “epitope” is intended the part of an antigenic molecule to which an antibody is produced and to which the antibody will bind. Epitopes can comprise linear amino acid residues (*i.e.*, residues within the epitope are arranged sequentially in a linear fashion), non-linear amino acid residues (referred to herein as “non-linear epitopes”; these epitopes
15 are not arranged sequentially), or both linear and non-linear amino acid residues.

Monoclonal antibodies may be made by the hybridoma method first described by Kohler *et al.* (1975) *Nature* 256:495, or may be made by recombinant DNA methods (see, *e.g.*, U.S. Patent No. 4,816,567). Monoclonal antibodies may also be isolated from antibody phage libraries generated using the techniques described in, for example,
20 McCafferty *et al.* (1990) *Nature* 348:552-554 (1990) and U.S. Patent No. 5,514,548. Clackson *et al.* (1991) *Nature* 352:624-628 and Marks *et al.* (1991) *J. Mol. Biol.* 222:581-597 describe the isolation of murine and human antibodies, respectively, using phage libraries. Subsequent publications describe the production of high affinity (nM range) human antibodies by chain shuffling (Marks *et al.* (1992) *Bio/Technology* 10:779-
25 783), as well as combinatorial infection and *in vivo* recombination as a strategy for constructing very large phage libraries (Waterhouse *et al.* (1993) *Nucleic. Acids Res.* 21:2265-2266). Thus, these techniques are viable alternatives to traditional monoclonal antibody hybridoma techniques for isolation of monoclonal antibodies.

Where anti-CD40 antibodies for use in the methods of the invention are to be
30 prepared using recombinant DNA methods, the DNA encoding the monoclonal antibodies is readily isolated and sequenced using conventional procedures. Once

isolated, the DNA may be placed into expression vectors, which are then transfected into host cells such as *E. coli* cells, simian COS cells, Chinese Hamster Ovary (CHO) cells, or myeloma cells that do not otherwise produce immunoglobulin protein, to obtain the synthesis of monoclonal antibodies in the recombinant host cells. Review articles on
5 recombinant expression in bacteria of DNA encoding the antibody include Skerra *et al.* (1993) *Curr. Opinion in Immunol.* 5:256 and Phickthun (1992) *Immunol. Revs.* 130:151. Alternatively, antibody can be produced in a cell line such as a CHO cell line, as disclosed in U.S. Patent Nos. 5,545,403; 5,545,405; and 5,998,144. Briefly the cell line is transfected with vectors capable of expressing a light chain and a heavy chain,
10 respectively. By transfecting the two proteins on separate vectors, chimeric antibodies can be produced. Another advantage is the mammalian glycosylation of the antibody in CHO cells. CHO cells are a preferred source of recombinant antibodies for use in the combination therapy of the invention.

A "host cell," as used herein, refers to a microorganism or a eukaryotic cell or cell
15 line cultured as a unicellular entity that can be, or has been, used as a recipient for a recombinant vector or other transfer polynucleotides, and include the progeny of the original cell that has been transfected. It is understood that the progeny of a single cell may not necessarily be completely identical in morphology or in genomic or total DNA complement as the original parent, due to natural, accidental, or deliberate mutation.

20 Monoclonal antibodies to CD40 are known in the art. See, for example, the sections dedicated to B-cell antigen in McMichael, ed. (1987; 1989) *Leukocyte Typing III and IV* (Oxford University Press, New York); U.S. Patent Nos. 5,674,492; 5,874,082; 5,677,165; 6,056,959; WO 00/63395; International Publication Nos. WO 02/28905 and WO 02/28904; Gordon *et al.* (1988) *J. Immunol.* 140:1425; Valle *et al.* (1989) *Eur. J.*
25 *Immunol.* 19:1463; Clark *et al.* (1986) *PNAS* 83:4494; Paulie *et al.* (1989) *J. Immunol.* 142:590; Gordon *et al.* (1987) *Eur. J. Immunol.* 17:1535; Jabara *et al.* (1990) *J. Exp. Med.* 172:1861; Zhang *et al.* (1991) *J. Immunol.* 146:1836; Gascan *et al.* (1991) *J. Immunol.* 147:8; Banchereau *et al.* (1991) *Clin. Immunol. Spectrum* 3:8; and Banchereau *et al.* (1991) *Science* 251:70.

30 As noted above, the term antibody as used herein encompasses chimeric antibodies. By "chimeric" antibodies is intended antibodies that are most preferably

derived using recombinant DNA techniques and which comprise both human (including immunologically “related” species, *e.g.*, chimpanzee) and non-human components. Thus, the constant region of the chimeric antibody is most preferably substantially identical to the constant region of a natural human antibody; the variable region of the chimeric antibody is most preferably derived from a non-human source and has the desired antigenic specificity to CD40. The non-human source can be any vertebrate source that can be used to generate antibodies to CD40 antigen. Such non-human sources include, but are not limited to, rodents (*e.g.*, rabbit, rat, mouse, etc.; see, for example, U.S. Patent No. 4,816,567) and non-human primates (*e.g.*, Old World Monkey, Ape, etc.; see, for example, U.S. Patent Nos. 5,750,105 and 5,756,096). The phrase “constant region” refers to the portion of the antibody molecule that confers effector functions. In previous work directed towards producing non-immunogenic antibodies for use in therapy of human disease, mouse constant regions were substituted by human constant regions. The constant regions of the subject humanized antibodies were derived from human antibodies. However, these antibodies can elicit an unwanted and potentially dangerous immune response in humans and there was a loss of affinity.

As noted above, the term antibody as used herein encompasses humanized antibodies. By “humanized” is intended forms of antibodies that contain minimal sequence derived from non-human antibody sequences. For the most part, humanized antibodies are human antibodies (recipient antibody) in which residues from a hypervariable region (also known as complementarity determining region or CDR) of the recipient are replaced by residues from a hypervariable region of a non-human species (donor antibody) such as mouse, rat, rabbit, or nonhuman primate having the desired specificity, affinity, and capacity. The phrase “complementarity determining region” refers to amino acid sequences which together define the binding affinity and specificity of the natural Fv region of a native antibody binding site. See, *e.g.*, Chothia *et al* (1987) *J. Mol. Biol.* 196:901-917; Kabat *et al* (1991) U. S. Dept. of Health and Human Services, NIH Publication No. 91-3242).

Humanization can be performed following the method of Winter and co-workers (Jones *et al.* (1986) *Nature* 321:522-525; Riechmann *et al.* (1988) *Nature* 332:323-327; Verhoeven *et al.* (1988) *Science* 239:1534-1536), by substituting rodent or mutant rodent

- CDRs or CDR sequences for the corresponding sequences of a human antibody. See also U.S. Patent Nos. 5,225,539; 5,585,089; 5,693,761; 5,693,762; 5,859,205. In some instances, residues within the framework regions of one or more variable regions of the human antibody are replaced by corresponding non-human residues (see, for example,
- 5 U.S. Patent Nos. 5,585,089; 5,693,761; 5,693,762; and 6,180,370). Furthermore, humanized antibodies may comprise residues that are not found in the recipient antibody or in the donor antibody. These modifications are made to further refine antibody performance (*e.g.*, to obtain desired affinity). In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which
- 10 all or substantially all of the hypervariable regions correspond to those of a non-human antibody and all or substantially all of the framework regions are those of a human antibody sequence. The humanized antibody optionally also will comprise at least a portion of an antibody constant region (Fc), typically that of a human antibody. For further details see Jones *et al.* (1986) *Nature* 331:522-525; Riechmann *et al.* (1988)
- 15 *Nature* 332:323-329; and Presta (1992) *Curr. Op. Struct. Biol.* 2:593-596. Accordingly, such "humanized" antibodies may include antibodies wherein substantially less than an intact human variable domain has been substituted by the corresponding sequence from a non-human species. In practice, humanized antibodies are typically human antibodies in which some CDR residues and possibly some framework residues are substituted by
- 20 residues from analogous sites in rodent antibodies. See, for example, U.S. Patent Nos. 5,225,539; 5,585,089; 5,693,761; 5,693,762; 5,859,205. See also U.S. Patent No. 6,180,370, and International Publication No. WO 01/27160, where humanized antibodies and techniques for producing humanized antibodies having improved affinity for a predetermined antigen are disclosed.
- 25 Humanized anti-CD40 antibodies can also be produced using the Human Engineering™ technology (Xoma Ltd., Berkeley, California), which has been described as a method for reducing immunogenicity while maintaining binding activity of antibody molecules (*e.g.*, see Studnicka *et al.* (1994) *Protein Engineering* 7:805-814 and U.S. Patent No. 5,766,886).
- 30 Humanized anti-CD40 monoclonal antibodies include antibodies such as SGN-40 (Tai *et al.* (2004) *Cancer Res.* 64:2846-52; U.S. Patent No. 6,838,261), which is the

humanized form of the murine anti-CD40 antibody SGN-14 (Francisco *et al.* (2000) *Cancer Res.* 60:3225-31), and the antibodies disclosed in U.S. Patent Application Publication No. 2004/0120948.

The present invention can also be practiced using xenogeneic or modified antibodies produced in a non-human mammalian host, more particularly a transgenic mouse, characterized by inactivated endogenous immunoglobulin (Ig) loci. In such transgenic animals, competent endogenous genes for the expression of light and heavy subunits of host immunoglobulins are rendered non-functional and substituted with the analogous human immunoglobulin loci. These transgenic animals produce human antibodies in the substantial absence of light or heavy host immunoglobulin subunits. See, for example, U.S. Patent Nos. 5,877,397 and 5,939,598.

Thus, in some embodiments, fully human antibodies to CD40, for example, are obtained by immunizing transgenic mice. One such mouse is obtained using XenoMouse® technology (Abgenix; Fremont, California), and is disclosed in U.S. Patent Nos. 6,075,181, 6,091,001, and 6,114,598. For example, to produce the HCD122 antibody, mice transgenic for the human IgG₁ heavy chain locus and the human κ light chain locus were immunized with Sf9 cells expressing human CD40. Mice can also be transgenic for other isotypes.

In some embodiments, the anti-CD40 antibody will have a light chain variable domain (V_L) that comprises the light chain CDR sequences of HCD122. Thus, in some embodiments, the anti-CD40 antibody will have a light chain variable domain that comprises an amino acid sequence as shown in SEQ ID NO:10 for CDR-L1, an amino acid sequence as shown in SEQ ID NO:11 for CDR-L2, and an amino acid sequence as shown in SEQ ID NO:12 for CDR-L3. In other embodiments, the anti-CD40 antibody will have a heavy chain variable domain (V_H) that comprises the heavy chain CDR sequences of HCD122. Thus, in some embodiments, the anti-CD40 antibody will have a heavy chain variable domain (V_H) that comprises an amino acid sequence as shown in SEQ ID NO:13 for CDR-H1, an amino acid sequence as shown in SEQ ID NO:14 for CDR-H2, and an amino acid sequence as shown in SEQ ID NO:15 for CDR-H3.

In further embodiments, the anti-CD40 antibody will have a light chain variable domain (V_L) that comprises the light chain CDR sequences of HCD122, and a heavy

chain variable domain (V_H) that comprises the heavy chain CDR sequences of HCD122. Thus, in further embodiments, the anti-CD40 antibody will have a light chain variable domain (V_L) that comprises an amino acid sequence as shown in SEQ ID NO:10 for CDR-L1, an amino acid sequence as shown in SEQ ID NO:11 for CDR-L2, and an amino acid sequence as shown in SEQ ID NO:12 for CDR-L3, and a heavy chain variable domain (V_H) that comprises an amino acid sequence as shown in SEQ ID NO:13 for CDR-H1, an amino acid sequence as shown in SEQ ID NO:14 for CDR-H2, and an amino acid sequence as shown in SEQ ID NO:15 for CDR-H3.

There are various schemes for defining the CDR residues in a given antibody variable domain (*e.g.*, see the web site designated as “bioinf.org.uk/abs” located on the World Wide Web (www)). The most commonly used is the Kabat numbering scheme (Kabat *et al.* (1991) *Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD). According to the Kabat numbering scheme, the CDRs in a light chain variable region are amino acids 24-34 (CDR-L1), 50-56 (CDR-L2) and 89-97 (CDR-L3), and the CDRs in a heavy chain variable region are amino acids 31-35 (CDR-H1), 50-65 (CDR-H2) and 95-102 (CDR-H3). Another well-known scheme is the Chothia numbering scheme (Chothia & Lesk (1987) *Mol. Biol.* 196:901- 917). By Chothia numbering, the CDRs in a light chain variable region are amino acids 26-32 (CDR-L1), 50-52 (CDR-L2) and 91-96 (CDR-L3), and the CDRs in a heavy chain variable region are amino acids 26-32 (CDR-H1), 53-55 (CDR-H2) and 96-101 (CDR-H3). Using one or more of the known schemes, the skilled person will readily be able to determine whether a given antibody meets the light chain and heavy chain CDR sequence requirements specified above.

“Antibody fragments” comprise a portion of an intact antibody, preferably the antigen-binding or variable region of the intact antibody. Examples of antibody fragments include Fab, F(ab')₂, and Fv fragments.

By “Fab” is intended a monovalent antigen-binding fragment of an antibody that contains the constant domain of the light chain and the first constant domain (C_H1) of the heavy chain. Papain digestion of antibodies produces two identical Fab fragments, and a residual “Fc” fragment, whose name reflects its ability to crystallize readily. By “F(ab')₂” is intended a bivalent antigen-binding fragment of an antibody that contains both light

chains and part of both heavy chains, and which retains the ability to cross-link antigen. Pepsin treatment yields an F(ab')₂ fragment. "Fv" is the minimum antibody fragment that contains a complete antigen recognition and binding site. This region consists of a dimer of one heavy- and one light-chain variable domain in tight, non-covalent association. It is in this configuration that the three CDRs of each variable domain interact to define an antigen-binding site on the surface of the V_H-V_L dimer. Collectively, the six CDRs confer antigen-binding specificity to the antibody. However, even a single variable domain (or half of an Fv comprising only three CDRs specific for an antigen) has the ability to recognize and bind antigen, although at a lower affinity than the entire binding site.

The invention may also use a single-chain Fv (scFv), which is a polypeptide comprising the V_H and V_L domains of an antibody, wherein these domains are present in a single polypeptide chain (see e.g., U.S. Patents 4,946,778, 5,260,203, 5,455,030, and 5,856,456). Generally, the scFv polypeptide comprises a polypeptide linker between the V_H and V_L domains that enables the scFv to form the desired structure for antigen binding. For a review of scFv see Pluckthun (1994) in *The Pharmacology of Monoclonal Antibodies*, Vol. 113, ed. Rosenberg and Moore (Springer-Verlag, New York), pp. 269-315.

Fragments of an anti-CD40 antibody are suitable for use in the methods of the invention so long as they retain the ability to bind to the CD40 antigen on the surface of human B-cells. Such fragments are referred to herein as "antigen-binding" fragments. Such fragments are preferably characterized by functional properties similar to the corresponding full-length antibody. Thus, for example, a fragment of a full-length anti-CD40 antibody will preferably be capable of specifically binding a human CD40 antigen expressed on the surface of a human cell, and is free of significant agonist activity as described elsewhere herein. Fragments of an anti-CD40 antibody for use in the methods of the invention may in some instances retain the ability to bind to the relevant FcR or FcRs.

Various techniques have been developed for the production of antibody fragments. Traditionally, these fragments were derived via proteolytic digestion of intact antibodies (see, e.g., Morimoto *et al.* (1992) *Journal of Biochemical and Biophysical*

Methods 24:107-117 (1992) and Brennan *et al.* (1985) *Science* 229:81). However, these fragments can now be produced directly by recombinant host cells. For example, the antibody fragments can be isolated from the antibody phage libraries discussed above. Alternatively, Fab'-SH fragments can be directly recovered from *E. coli* and chemically coupled to form F(ab')₂ fragments (Carter *et al.* (1992) *Bio/Technology* 10:163-167). According to another approach, F(ab')₂ fragments can be isolated directly from recombinant host cell culture. Other techniques for the production of antibody fragments will be apparent to the skilled practitioner.

The anti-CD40 antibodies used in the combination therapy of the invention are free of significant agonist activity when bound to CD40 antigen on the surface of human B-cells. In some embodiments, their binding to CD40 on the surface of human B-cells may result in inhibition of the proliferation and differentiation of the B-cells. The anti-CD40 antibodies suitable for use in the methods of the invention include those antibodies that can exhibit antagonist activity toward human B-cells expressing the cell-surface CD40 antigen. These anti-CD40 antibodies and antigen-binding fragments thereof are referred to herein as "antagonist anti-CD40 antibodies."

An "agonist" combines with a receptor on a cell and initiates a reaction or activity that is similar to or the same as that initiated by a natural ligand of the receptor. An agonist of CD40 induces any or all of, but not limited to, the following responses: B cell proliferation and/or differentiation; upregulation of intercellular adhesion via such molecules as ICAM-1, E-selectin, VCAM, and the like; secretion of pro-inflammatory cytokines such as IL-1, IL-6, IL-8, IL-12, TNF, and the like; signal transduction through the CD40 receptor by such pathways as TRAF (*e.g.*, TRAF2 and/or TRAF3), MAP kinases such as NIK (NF- κ B inducing kinase), I-kappa B kinases (IKK α/β), transcription factor NF- κ B, Ras and the MEK/ERK pathway, the PI3K/AKT pathway, the P38 MAPK pathway, and the like; transduction of an anti-apoptotic signal by such molecules as XIAP, mcl-1, bcl-x, and the like; B and/or T cell memory generation; B cell antibody production; B cell isotype switching, up-regulation of cell-surface expression of MHC Class II and CD80/86, and the like.

By "significant" agonist activity is intended an agonist activity of at least 30%, 35%, 40%, 45%, 50%, 60%, 70%, 75%, 80%, 85%, 90%, 95%, or 100% greater than the

agonist activity induced by a negative control as measured in an assay of a B cell response. Preferably, "significant" agonist activity is an agonist activity that is at least 2-fold greater or at least 3-fold greater than the agonist activity induced by a negative control as measured in an assay of a B cell response. Thus, for example, where the B cell response of interest is B cell proliferation, "significant" agonist activity would be induction of a level of B cell proliferation that is at least 2-fold greater or at least 3-fold greater than the level of B cell proliferation induced by a negative control. In one embodiment, an antibody that does not bind to CD40 serves as the negative control. A substance "free of significant agonist activity" would exhibit an agonist activity of not more than about 25% greater than the agonist activity induced by a negative control, preferably not more than about 20% greater, 15% greater, 10% greater, 5% greater, 1% greater, 0.5% greater, or even not more than about 0.1% greater than the agonist activity induced by a negative control as measured in an assay of a B cell response.

An "antagonist" of CD40 prevents or reduces induction of any of the responses induced by binding of the CD40 receptor to an agonist ligand, particularly CD40L. The antagonist may reduce induction of a response to CD40L binding by 5%, 10%, 15%, 20%, 25%, 30%, 35%, preferably 40%, 45%, 50%, 55%, 60%, more preferably 70%, 80%, 85%, and most preferably 90%, 95%, 99%, or 100%.

Preferred antibodies and fragments for use in the methods of the invention are anti-CD40 antibodies that are free of significant agonist activity when bound to CD40 antigen on human B cells, and which exhibit antagonist activity when bound to CD40 antigen on human B cells. In some embodiments, the anti-CD40 antibody is free of significant agonist activity in one B cell response. In other embodiments, the anti-CD40 antibody is free of significant agonist activity in assays of more than one B cell response (*e.g.*, proliferation and differentiation, or proliferation, differentiation, and antibody production).

Methods for measuring antagonist activity of an anti-CD40 therapeutic agent (*e.g.*, an anti-CD40 antibody) are known in the art and include, but are not limited to, standard competitive binding assays, assays for monitoring antibody secretion by B cells, B cell proliferation assays, Banchereau-Like-B cell proliferation assays, T cell helper assays for antibody production, co-stimulation of B cell proliferation assays, and assays

for up-regulation of B cell activation markers. Relevant assays are described in *e.g.*, United States Patent No. 6,087,329 and the international patent applications published as WO 00/75348, WO 2005/044294, WO 2005/044304, WO 2005/044305, WO 2005/044306, WO 2005/044307, WO 2005/044854, WO 2005/044855, WO 2006/073443, WO 2006/125117, WO 2006/125143, WO 2007/053661 and WO 2007/053767.

Any of the assays known in the art can be used to determine whether an anti-CD40 antibody acts as an antagonist of one or more B cell responses. In some embodiments, the anti-CD40 antibody acts as an antagonist of at least one B cell response selected from the group consisting of B cell proliferation, B cell differentiation, antibody
5 production, intercellular adhesion, B cell memory generation, isotype switching, up-regulation of cell-surface expression of MHC Class II and CD80/86, and secretion of pro-inflammatory cytokines such as IL-8, IL-12, and TNF. Of particular interest are antagonist anti-CD40 antibodies that are free of significant agonist activity with respect to B cell proliferation when bound to the human CD40 antigen on the surface of a human
10 B cell.

The anti-CD40 antibody may be an antagonist of B cell proliferation induced by soluble or cell-surface CD40L, as measured in a B cell proliferation assay. Suitable B cell proliferation assays are known in the art. Suitable B cell proliferation assays are also described below. In some embodiments, the antagonist anti-CD40 antibody stimulates B
15 cell proliferation at a level that is not more than about 25% greater than the B cell proliferation induced by a negative control (*i.e.*, at least 75% inhibition), preferably not more than about 20% greater, 15% greater, 10% greater, 5% greater, 1% greater, 0.5% greater, or even not more than about 0.1% greater than the B cell proliferation induced by a negative control.

20 In other embodiments, the anti-CD40 antibody is an antagonist of B cell proliferation induced by another anti-CD40 antibody (*e.g.*, the S2C6 anti-CD40 antibody; Kwekkeboom *et al.* (1993) *Immunology* 79:439-444), as measured in a B cell proliferation assay, and the level of B cell proliferation stimulated by the other anti-CD40 antibody in the presence of the antagonist anti-CD40 antibody is not more than about
25 25% of the B cell proliferation induced by the other anti-CD40 antibody in the absence of

the antagonist anti-CD40 antibody (*i.e.*, at least 75% inhibition), preferably not more than about 20%, 15%, 10%, 5%, 1%, 0.5%, or even not more than about 0.1% of the B cell proliferation induced by the other anti-CD40 antibody in the absence of the antagonist anti-CD40 antibody.

- 5 In yet other embodiments, the anti-CD40 antibody is an antagonist of B cell proliferation that is induced by the cell line EL4B5 (Kwekkeboom *et al.* (1993) *Immunology* 79:439-444) as measured in a B cell activation assay, and the level of B cell proliferation stimulated by the EL4B5 cell line in the presence of the antagonist anti-CD40 antibody is not more than about 25% of the B cell proliferation induced by this cell
- 10 line in the absence of the antagonist anti-CD40 antibody (*i.e.*, at least 75% inhibition), preferably not more than about 20%, 15%, 10%, 5%, 1%, 0.5%, or even not more than about 0.1% of the B cell proliferation induced by this cell line in the absence of the antagonist anti-CD40 antibody.

- In still other embodiments, the anti-CD40 antibody is an antagonist of human T-
- 15 cell-induced antibody production by human B cells as measured in the human T-cell helper assay for antibody production by B cells. In this manner, the level of IgG antibody production, IgM antibody production, or both IgG and IgM antibody production by B cells stimulated by T cells in the presence of the antagonist anti-CD40 antibody is not more than about 50% of the respective antibody production by B cells stimulated by T
- 20 cells in the absence of the antagonist anti-CD40 antibody (*i.e.*, at least 75% inhibition), preferably not more than about 25%, 20%, 15%, 10%, 5%, 1%, 0.5%, or even not more than about 0.1% of the respective antibody production by B cells stimulated by T cells in the absence of the antagonist anti-CD40 antibody.

- For example, the following assays can be used to assess the antagonist activity of
- 25 an anti-CD40 antibody. Human B cells for these assays can be obtained, for example, by isolation from tonsils obtained from individuals undergoing tonsillectomies, essentially as described in De Groot *et al.* (1990) *Lymphokine Research* (1990) 9:321. Briefly, the tissue is dispersed with scalpel blades, phagocytic and NK cells are depleted by treatment with 5 mM L-leucine methyl ester and T cells are removed by one cycle of rosetting with
- 30 sheep erythrocytes (SRBC) treated with 2-aminoethyl isothiuronium bromide. The purity of the resulting B lymphocyte preparations can be checked by indirect

immunofluorescent labelling with anti-(CD20) mAb B1 (Coulter Clone, Hialeah, FA) or anti-(CD3) mAb OKT3 (Ortho, Raritan, NJ) and a FITC-conjugated F(ab')₂ fragment of rabbit anti-(mouse Ig) (Zymed, San Francisco, CA), and FACS analysis.

5 *B-cell Proliferation Assay*

B cells (4×10^4 per well) are cultured in 200 μ l IMDM supplemented with 10% fetal calf serum in flat bottom 96-well microtiter plates. B cells are stimulated by addition of immobilized anti-(IgM) antibodies (Immunobeads; 5 μ g/ml; BioRad, Richmond, California). Where desired, 100 U/ml recombinant IL-2 is added. Varying concentrations of test monoclonal antibodies (mAbs) are added at the onset of the microcultures and proliferation is assessed at day 3 by measurement of the incorporation of (³H)-thymidine after 18 hour pulsing. An antagonist anti-CD40 antibody does not significantly costimulate human B-cell proliferation in the presence of immobilized anti-IgM or in the presence of immobilized anti-IgM and IL-2.

15

Banchereau-Like B-Cell Proliferation Assay

For testing the ability of anti-CD40 monoclonal antibodies to stimulate B-cell proliferation in a culture system analogous to that described by Banchereau *et al.* (1991) *Science* (1991) 251:70, mouse 3T6 transfectant cells expressing the HR allelic form of human Fc γ RII are used. B cells (2×10^4 per well) are cultured in flat-bottom microwells in the presence of 1×10^4 transfectant cells (irradiated with 5000 Rad) in 200 μ l IMDM supplemented with 10% fetal calf serum and 100 U/ml recombinant IL-4. Before addition of the B cells, the 3T6 cells are allowed to adhere to the culture plastic for at least 5 hours. Anti-CD40 mAbs are added at concentrations varying from 15 ng/ml to 2000 ng/ml and proliferation of B cells is assessed by measurement of thymidine incorporation at day 7, upon 18 hour pulsing with [³H] thymidine.

Inhibition of S2C6-Stimulated B-Cell Proliferation Using Antagonist Anti-CD40 mAbs

30 Antagonist anti-CD40 monoclonal antibodies (mAbs) may also be characterized by their ability to inhibit stimulation of B-cell proliferation by an anti-CD40 antibody

such as S2C6 (also known as SGN-14, which is reportedly an agonist of CD40 stimulation of proliferation of normal B cells; Francisco *et al.* (2000) *Cancer Res.* 60:3225-3231) using the B-cell Proliferation Assay described above. Human tonsillar B cells (4×10^4 per well) are cultured in 200 μ l in microwells in the presence of anti-IgM coupled to Sepharose beads (5 μ g/ml) and anti-CD40 mAb S2C6 (1.25 μ g/ml). Varying concentrations of an anti-CD40 mAb of interest are added and [^{3} H]-thymidine incorporation is assessed after 3 days. As a control anti-(glucocerebrosidase) mAb 8E4 can be added in similar concentrations. Barneveld *et al.* (1983) *Eur. J. Biochem.* 134:585. An antagonist anti-CD40 antibody can inhibit the costimulation of anti-IgM induced human B-cell proliferation by mAb S2C6, for example, by at least 75% or more (*i.e.*, S2C6-stimulated proliferation in the presence of an antagonist anti-CD40 antibody is no more than 25% of that observed in the absence of the antagonist anti-CD40 antibody). In contrast, no significant inhibition would be seen with equivalent amounts of non-relevant mAb 8E4, directed to β -glucocerebrosidase. Barneveld *et al.*, *supra*. Such a result would indicate that the anti-CD40 mAbs does not deliver stimulatory signals for the proliferation of human B cells, but, conversely, can inhibit stimulatory signals exerted by triggering CD40 with another mAb.

B-Cell Activation Assay with EL4B5 Cells

Zubler *et al.* (1985) *J. Immunol.* (1985) 134:3662 observed that a mutant subclone of the mouse thymoma EL-4 line, known as EL4B5, could strongly stimulate B cells of both murine and human origin to proliferate and differentiate into immunoglobulin-secreting plasma cells *in vitro*. This activation was found to be antigen-independent and not MHC restricted. For optimal stimulation of human B cells, the presence of supernatant from activated human T cells was needed but a B-cell response also occurred when EL4B5 cells were preactivated with phorbol-12-myristate 13-acetate (PMA) or IL-1. Zubler *et al.* (1987) *Immunological Reviews* 99:281; and Zhang *et al.* (1990) *J. Immunol.* 144:2955. B-cell activation in this culture system is efficient — limiting dilution experiments have shown that the majority of human B cells can be activated to proliferate and differentiate into antibody-secreting cells. Wen *et al.* (1987) *Eur. J. Immunol.* 17:887.

B cells (1000 per well) are cultured together with irradiated (5000 Rad) EL4B5 cells (5×10^4 per well) in flat bottom microtiter plates in 200 μ l IMDM supplemented with 10% heat-inactivated fetal calf serum, 5 ng/ml phorbol-12-myristate 13-acetate and 5% human T-cell supernatant. mAbs are added at varying concentrations at the onset of the cultures and thymidine incorporation is assessed at day 6 after 18 hour pulsing with [^{3} H]-thymidine. For the preparation of T-cell supernatant, purified T cells are cultured at a density of 10^6 /ml for 36 hours in the presence of 1 μ g/ml PHA and 10 ng/ml PMA. Wen *et al.* (1987) *Eur. J. Immunol.* (1987) 17:887. T-cell supernatant is obtained by centrifugation of the cells and stored at -20°C. The effectiveness of T-cell supernatants in enhancing proliferation of human B cells in EL4B5-B cell cultures is tested and the most effective supernatants are pooled for use in experiments. When assessing the effect of an anti-CD40 antibody on EL4B5-induced human B-cell proliferation, a monoclonal antibody such as MOPC-141 (IgG2b) can be added as a control.

15 *Human T Cell Helper Assay for Antibody Production by B Cells*

An antagonist anti-CD40 antibody may function as an antagonist of antibody production by B cells. An anti-CD40 antibody can be tested for this type of antagonist activity by assessing the antibody's ability to inhibit antibody production by B cells that have been stimulated in a contact-dependent manner with activated T cells in a T cell helper assay. In this manner, 96-well tissue culture plates are coated with a 1:500 dilution of ascites fluid of anti-CD3 mAb CLB-T3/3 (CLB, Amsterdam, The Netherlands). As indicated costimulatory mAbs are added: anti CD2 mAbs CLB-T11.1/1 and CLB-T11.2/1 (CLB, Amsterdam, The Netherlands), both ascites 1:1000 and anti-CD28 mAb CLB-28/1 (CLB, Amsterdam, The Netherlands). Subsequently, tonsillar T cells (irradiated, 3000 Rad; 10^5 per well), tonsillar B cells (10^4 per well), and rIL-2 (20 U/ml) are added. The final volume of each cell culture is 200 μ l. After 8 days, cells are spun down, and cell-free supernatant is harvested. The concentrations of human IgM and IgG in (diluted) samples is estimated by ELISA as described below.

In one embodiment, human tonsillar B cells (10^4 /well) are cultured together with irradiated purified T cells (3000 rad, 10^5 /well) in 96-well plates, coated with anti-CD3 mAb and with or without different mAbs to costimulate the T cells. After 8 days of

culture the supernatants are harvested for the determination of antibody production by the B cells. Antibody production by the B cells is assessed by the ELISA assay described below. The anti-CD40 antibody of interest is added in varying concentrations from the onset of the cultures. As a control, mAb MOPC-141 can be added.

- 5 An antagonist anti-CD40 antibody can inhibit IgG and IgM antibody production of B cells stimulated by human T cells by at least 50% or more (*i.e.*, T cell-induced antibody production by B cells in the presence of an antagonist anti-CD40 antibody is no more than 50% of that observed in the absence of the antagonist anti-CD40 antibody). In contrast, a control antibody such as MOPC-141 would have no significant effect on T
10 cell-induced antibody production by B cells.

ELISA Assay for Antibody Quantification

- The concentrations of human IgM and IgG are estimated by ELISA. 96-well ELISA plates are coated with 4 µg/ml mouse anti-human IgG mAb MH 16-01 (CLB,
15 Amsterdam, The Netherlands) or with 1.2 µg/ml mouse anti-human IgM mAb 4102 (Tago, Burlingame, CA) in 0.05 M carbonate buffer (pH = 9.6), by incubation for 16 h at 4°C. Plates are washed 3 times with PBS-0.05% Tween-20 (PBS-Tween) and saturated with BSA for 1 hour. After 2 washes the plates are incubated for 1 h at 37°C with different dilutions of the test samples. After 3 washes, bound Ig is detected by incubation
20 for 1 h at 37°C with 1 µg/ml peroxidase-labeled mouse anti-human IgG mAb MH 16-01 (CLB) or mouse anti-human IgM mAb MH 15-01 (CLB). Plates are washed 4 times and bound peroxidase activity is revealed by the addition of O-phenylenediamine as a substrate. Human standard serum (H00, CLB) is used to establish a standard curve for each assay.

Antagonist anti-CD40 antibodies are known in the art. See, for example, the human anti-CD40 antibody produced by the hybridoma designated F4-465 disclosed in U.S. Patent Application Publication Nos. 20020142358 and 20030059427. F4-465 was obtained from the HAC mouse (Kuroiwa *et al.* (2000) *Nature Biotech.* 10:1086 (2000)) and therefore expresses the human lambda light chain.

In addition to antagonist activity, the anti-CD40 antibody for use in the methods of the present invention will preferably have another mechanism of action against a target cell. The anti-CD40 antibody will preferably have ADCC activity.

Of particular interest to the present invention are anti-CD40 antibodies that share
5 the binding characteristics of HCD122 (produced by the hybridoma cell line deposited with the ATCC (American Type Culture Collection; 10801 University Blvd., Manassas, Virginia 20110-2209 (USA)) on September 17, 2003, as Patent Deposit No. PTA-5543). Such antibodies include, but are not limited to:

- a) the monoclonal antibody HCD122, produced by the hybridoma cell line
10 deposited with the ATCC as Patent Deposit No. PTA-5543;
- b) an antibody comprising an amino acid sequence selected from the group consisting of the sequence shown in SEQ ID NO:2, the sequence shown in SEQ ID NO:4, the sequence shown in SEQ ID NO:5, both the sequences shown in SEQ ID NO:2 and SEQ ID NO:4, and both the sequences shown in SEQ ID NO:2 and SEQ ID NO:5;
- c) an antibody comprising an amino acid sequence selected from the group consisting of the sequence shown in SEQ ID NO:17, the sequence shown in SEQ ID NO:19, the sequence shown in SEQ ID NO:20, both the sequences shown in SEQ ID NO:17 and SEQ ID NO:19, and both the sequences shown in SEQ ID NO:17 and SEQ ID NO:20;
- d) an antibody comprising an amino acid sequence selected from the group consisting of the sequence shown in SEQ ID NO:16, the sequence shown in SEQ ID NO:18, and both the sequences shown in SEQ ID NO:16 and SEQ ID NO:18;
- e) an antibody having an amino acid sequence encoded by a nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of the sequence shown in SEQ ID NO:1, the sequence shown in SEQ ID NO:3, and both the sequences shown in SEQ ID NO:1 and SEQ ID NO:3;
- f) an antibody having a light chain variable domain (V_L) that comprises the amino acid sequence as shown in SEQ ID NO:10 for CDR-L1, the amino acid sequence as shown in SEQ ID NO:11 for CDR-L2, and the amino acid sequence as shown in SEQ ID NO:12 for CDR-L3;

g) an antibody having a heavy chain variable domain (V_H) that comprises the amino acid sequence as shown in SEQ ID NO:13 for CDR-H1, the amino acid sequence as shown in SEQ ID NO:14 for CDR-H2, and the amino acid sequence as shown in SEQ ID NO:15 for CDR-H3;

h) an antibody having a light chain variable domain (V_L) that comprises the amino acid sequence as shown in SEQ ID NO:10 for CDR-L1, the amino acid sequence as shown in SEQ ID NO:11 for CDR-L2, and the amino acid sequence as shown in SEQ ID NO:12 for CDR-L3, and having a heavy chain variable domain (V_H) that comprises the amino acid sequence as shown in SEQ ID NO:13 for CDR-H1, the amino acid sequence as shown in SEQ ID NO:14 for CDR-H2, and the amino acid sequence as shown in SEQ ID NO:15 for CDR-H3;

i) an antibody comprising a light chain variable domain containing the complementarity determining region (CDR) residues of SEQ ID NO:2;

j) an antibody comprising a heavy chain variable domain containing the complementarity determining region (CDR) residues of SEQ ID NO:4;

k) an antibody comprising a light chain variable domain containing the complementarity determining region (CDR) residues of SEQ ID NO:2 and a heavy chain variable domain containing the complementarity determining region (CDR) residues of SEQ ID NO:4;

l) an antibody that binds domain 2 of human CD40 antigen;

m) an antibody that binds to a CD40 epitope capable of binding the monoclonal antibody HCD122;

n) an antibody that binds to an epitope comprising residues 82-87 of the human CD40 sequence shown in SEQ ID NO:7 or SEQ ID NO:9; and

o) an antibody that competes with the monoclonal antibody HCD122 in a competitive binding assay.

An anti-CD40 antibody obtained from a CHO cell containing one or more expression vectors encoding the antibody can be used in the methods of the invention.

The monoclonal antibody HCD122, produced by the hybridoma cell line deposited with the ATCC as Patent Deposit No. PTA-5543, is particularly preferred for
5 use in the methods, compositions, uses, and kits of the invention.

The monoclonal antibody HCD122 binds domain 2 of human CD40 antigen, whereas earlier anti-CD40 antibodies having antagonistic properties were found to bind to other domains of human CD40.

The HCD122 monoclonal antibody binds soluble CD40 in ELISA-type assays, 5 prevents the binding of CD40-ligand to cell-surface CD40, and displaces the pre-bound CD40-ligand, as determined by flow cytometric assays. When tested *in vitro* for effects on proliferation of B cells from normal human subjects, HCD122 acts as antagonist anti-CD40 antibody. Furthermore, HCD122 does not induce strong proliferation of human lymphocytes from normal subjects. The antibody is able to kill CD40-expressing target 10 cells by antibody dependent cellular cytotoxicity (ADCC). The binding affinity of HCD122 for human CD40 is 5×10^{-10} M, as determined by the Biacore™ assay.

The nucleotide and amino acid sequences of the HCD122 antibody are known (e.g., see WO 2005/044854). Further, the mouse hybridoma line 153.8E2.D10.D6.12.12 (CMCC#12056), which expresses the HCD122 antibody, has been deposited with the 15 American Type Culture Collection [ATCC; 10801 University Blvd., Manassas, Virginia 20110-2209 (USA)] on September 17, 2003, under Patent Deposit Number PTA-5543.

The complete sequence for the light chain of HCD122 is set forth in SEQ ID NO:2, which includes the leader sequence (residues 1-20 of SEQ ID NO:2), the variable region (residues 21-132 of SEQ ID NO:2), and the constant region (residues 133-239 of 20 SEQ ID NO:2). The complete sequence for the heavy chain of HCD122 is set forth in SEQ ID NO:4, which includes the leader sequence (residues 1-19 of SEQ ID NO:4), the variable region (residues 20-139 of SEQ ID NO:4), and the constant regions (residues 140-469 of SEQ ID NO:4). The complete sequence for a variant of HCD122 is set forth in SEQ ID NO:5, which includes the leader sequence (residues 1-19 of SEQ ID NO:5), 25 the variable region (residues 20-139 of SEQ ID NO:5), and the constant regions (residues 140-469 of SEQ ID NO:5). This variant differs from HCD122 in that it contains a substitution of a serine residue for the alanine residue at position 153 of SEQ ID NO:4, which is within the constant region. The nucleotide sequences encoding the light and heavy chains of HCD122 are set forth in SEQ ID NO:1 (coding sequence for the light 30 chain of HCD122) and SEQ ID NO:3 (coding sequence for the heavy chain of HCD122).

The amino acid sequence for the variable region of the HCD122 light chain without the leader sequence (*i.e.*, residues 21-132 of SEQ ID NO:2) is set forth in SEQ ID NO:16. The amino acid sequence for the variable and constant regions of the HCD122 light chain without the leader sequence (*i.e.*, residues 21-239 of SEQ ID NO:2) is set forth in SEQ ID NO:17. The amino acid sequence for the variable region of the HCD122 heavy chain without the leader sequence (*i.e.*, residues 20-139 of SEQ ID NO:4) is set forth in SEQ ID NO:18. The amino acid sequence for the variable and constant regions of the HCD122 heavy chain without the leader sequence (*i.e.*, residues 20-469 of SEQ ID NO:4) is set forth in SEQ ID NO:19. The amino acid sequence for the variable and constant regions of the HCD122 heavy chain variant (*i.e.*, residues 20-469 of SEQ ID NO:5) is set forth in SEQ ID NO:20.

Anti-CD40 antibodies for use in the methods, compositions, uses, and kits of the present invention include antibodies differing from the HCD122 monoclonal antibody but retaining the CDRs, and antibodies with one or more amino acid addition(s), deletion(s), or substitution(s). HCD122 is a fully human antibody, but can be further de-immunized if desired. De-immunized anti-CD40 antibodies can be produced using known methods, *e.g.*, as described in WO 98/52976 and WO 00/34317. In this manner, residues within the anti-CD40 antibodies may be modified so as to render the antibodies less immunogenic to humans while retaining their therapeutic activity.

Any known antibody having the binding specificity of interest can have sequence variations produced using methods described in, for example, EP 0983303, WO 00/34317, and WO 98/52976. For example, it has been shown that sequences within the CDR can cause an antibody to bind to MHC Class II and trigger an unwanted helper T-cell response in certain patients. A conservative substitution can allow the antibody to retain binding activity yet lose its ability to trigger an unwanted T-cell response. Any such conservative or non-conservative substitutions can be made using art-recognized methods, such as those noted elsewhere herein, and the resulting antibodies can also be used in the methods of the present invention. The variant antibodies can be routinely tested for the particular activity, for example, antagonist activity, affinity, and specificity using methods described herein.

For example, amino acid sequence variants of an antagonist anti-CD40 antibody, for example, the HCD122 monoclonal antibody, can be prepared by mutations in the cloned DNA sequence encoding the antibody of interest. Methods for mutagenesis and nucleotide sequence alterations are well known in the art. See, for example, Walker and

5 Gastra, eds. (1983) *Techniques in Molecular Biology* (MacMillan Publishing Company, New York); Kunkel (1985) *Proc. Natl. Acad. Sci. USA* 82:488-492; Kunkel *et al.* (1987) *Methods Enzymol.* 154:367-382; Sambrook *et al.* (1989) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor, New York); U.S. Patent No. 4,873,192; and the references cited therein. Guidance as to appropriate amino acid substitutions that do not

10 affect biological activity of the polypeptide of interest may be found in the model of Dayhoff *et al.* (1978) in *Atlas of Protein Sequence and Structure* (Natl. Biomed. Res. Found., Washington, D.C.). Conservative substitutions, such as exchanging one amino acid with another having similar properties, may be preferred. Examples of conservative substitutions include, but are not limited to, Gly $\leftrightarrow$ Ala, Val $\leftrightarrow$ Ile $\leftrightarrow$ Leu, Asp $\leftrightarrow$ Glu,

15 Lys $\leftrightarrow$ Arg, Asn $\leftrightarrow$ Gln, and Phe $\leftrightarrow$ Trp $\leftrightarrow$ Tyr.

In constructing variants of an antibody of interest, for example, an antagonist anti-CD40 antibody polypeptide of interest, modifications may be made such that variants continue to possess the desired activity, *i.e.*, similar binding affinity and, in the case of antagonist anti-CD40 antibodies, are capable of specifically binding to a human CD40

20 antigen expressed on the surface of a human cell, and being free of significant agonist activity but exhibiting antagonist activity when bound to a CD40 antigen on a human CD40-expressing cell. Obviously, any mutations made in the DNA encoding the variant polypeptide must not place the sequence out of reading frame and preferably will not create complementary regions that could produce secondary mRNA structure (*e.g.*, see

25 EP 0075444).

In addition, the constant region of an antibody, for example, an antagonist anti-CD40 antibody, can be mutated to alter effector function in a number of ways. For example, see U.S. Patent 6,737,056 B1 and U.S. Patent Application Publication No. 2004/0132101A1, which disclose Fc mutations that optimize antibody binding to Fc

30 receptors.

Preferably, variants of a reference antibody, for example, an antagonist anti-CD40 antibody, have amino acid sequences that have at least 70% or 75% sequence identity, preferably at least 80% or 85% sequence identity, more preferably at least 90%, 91%, 92%, 93%, 94% or 95% sequence identity to the amino acid sequence for the reference antibody, for example, an antagonist anti-CD40 antibody molecule, for example, the HCD122 monoclonal antibody described herein. More preferably, the molecules share at least 96%, 97%, 98% or 99% sequence identity. For purposes of the present invention, percent sequence identity is determined using the Smith-Waterman homology search algorithm using an affine gap search with a gap open penalty of 12 and a gap extension penalty of 2, BLOSUM matrix of 62. The Smith-Waterman homology search algorithm is taught in Smith and Waterman (1981) *Adv. Appl. Math.* 2:482-489. A variant may, for example, differ from the reference antibody, for example, an antagonist anti-CD40 antibody, by as few as 1 to 15 amino acid residues, as few as 1 to 10 amino acid residues, such as 6-10, as few as 5, as few as 4, 3, 2, or even 1 amino acid residue.

With respect to optimal alignment of two amino acid sequences, the contiguous segment of the variant amino acid sequence may have additional amino acid residues or deleted amino acid residues with respect to the reference amino acid sequence. The contiguous segment used for comparison to the reference amino acid sequence will include at least 20 contiguous amino acid residues, and may be 30, 40, 50, or more amino acid residues. Corrections for sequence identity associated with conservative residue substitutions or gaps can be made (see Smith-Waterman homology search algorithm).

The precise chemical structure of an antibody capable of specifically binding CD40 and retaining antagonist activity, particularly when bound to CD40 antigen on malignant B cells, depends on a number of factors. As ionizable amino and carboxyl groups are present in an antibody molecule, a particular polypeptide may be obtained as an acidic or basic salt, or in neutral form. All such preparations that retain their biological activity when placed in suitable environmental conditions are included in the definition of antagonist anti-CD40 antibodies as used herein. Further, the primary amino acid sequence of the polypeptide may be augmented by derivatization using sugar moieties (glycosylation) or by other supplementary molecules such as lipids, phosphate, acetyl groups and the like. It may also be augmented by conjugation with saccharides.

Certain aspects of such augmentation are accomplished through post-translational processing systems of the producing host; other such modifications may be introduced *in vitro*. In any event, such modifications are included in the definition of an anti-CD40 antibody used herein. It is expected that such modifications may quantitatively or
5 qualitatively affect the activity, either by enhancing or diminishing the activity of the polypeptide, in the various assays. Further, individual amino acid residues in the chain may be modified by oxidation, reduction, or other derivatization, and the polypeptide may be cleaved to obtain fragments that retain activity.

The art provides substantial guidance regarding the preparation and use of
10 antibody variants. In preparing the anti-CD40 antibody variants, one of skill in the art can readily determine which modifications to the native protein nucleotide or amino acid sequence will result in a variant that is suitable for use as a therapeutically active component of a pharmaceutical composition used in the methods of the present invention.

The anti-CD40 antibody for use in the methods of the invention preferably
15 possesses at least one of the following biological activities *in vitro* and/or *in vivo*: inhibition of antibody secretion by normal human peripheral B cells stimulated by T cells; inhibition of survival and/or proliferation of normal human peripheral B cells stimulated by CD40L-expressing cells or soluble CD40 ligand (sCD40L); inhibition of survival and/or proliferation of normal human peripheral B cells stimulated by Jurkat T
20 cells; inhibition of "survival" anti-apoptotic intracellular signals in any cell stimulated by sCD40L or solid-phase CD40L; and, inhibition of CD40 signal transduction in any cell upon ligation with sCD40L or solid-phase CD40L, deletion, anergy and/or tolerance induction of CD40-bearing target cells or cells bearing cognate ligands to CD40 including, but not limited to, T cells and B cells, induction of expansion or activation of
25 CD4⁺CD25⁺ regulatory T cells (see for example, donor alloantigen-specific tissue rejection via CD40-CD40L interference, van Maurik *et al.* (2002) *J. Immunol.* 169:5401-5404), cytotoxicity via any mechanism (including, but not limited to, antibody-dependent cell-mediated cytotoxicity (ADCC), complement-dependent cytotoxicity (CDC), down-regulation of proliferation, and/or apoptosis in target cells), modulation of target cell
30 cytokine secretion and/or cell surface molecule expression, and combinations thereof. Assays for such biological activities can be performed as described herein. See also the

assays described in Schultze *et al.* (1998) *Proc. Natl. Acad. Sci. USA* 92:8200-8204; Denton *et al.* (1998) *Pediatr. Transplant.* 2:6-15; Evans *et al.* (2000) *J. Immunol.* 164:688-697; Noelle (1998) *Agents Actions Suppl.* 49:17-22; Lederman *et al.* (1996) *Curr. Opin. Hematol.* 3:77-86; Coligan *et al.* (1991) *Current Protocols in Immunology* 13:12; Kwekkeboom *et al.* (1993) *Immunology* 79:439-444; and U.S. Patent Nos. 5,674,492 and 5,847,082.

It is possible to engineer an antibody to have increased ADCC activity. In particular, the carboxy-terminal half of the CH2 domain is important for ADCC mediated through the FcRIII receptor. Since the CH2 and hinge regions have an important role in effector functions, a series of multiple-domain antibodies that contain extra CH2 and/or hinge regions may be created and investigated for any changes in effector potency (see Greenwood *et al.* (1994) *Ther. Immunol.* 1(5):247-55). An alternative approach may be to engineer extra domains in parallel, for example, through creation of dimers by engineering a cysteine into the H-chain of a chimeric Ig (see Shopes (1992) *J. Immunol.* 148(9):2918-2922). Furthermore, changes to increase ADCC activity may be engineered by introducing mutations into the Fc region (see, for example, U.S. Patent No. 6,737,056 B1), expressing cells in fucosyl transferase deficient cell lines (see, for example, U.S. Patent Application Publication No. 2003/0115614), or effecting other changes to antibody glycosylation (see, for example, U.S. Patent No. 6,602,684).

A representative assay to detect antagonist anti-CD40 antibodies specific to the CD40-antigen epitopes identified herein is a "competitive binding assay". Competitive binding assays are serological assays in which unknowns are detected and quantitated by their ability to inhibit the binding of a labeled known ligand to its specific antibody. This is also referred to as a competitive inhibition assay. In a representative competitive binding assay, labeled CD40 polypeptide is precipitated by candidate antibodies in a sample, for example, in combination with monoclonal antibodies raised against one or more epitopes of anti-CD40 monoclonal antibodies. Anti-CD40 antibodies that specifically react with an epitope of interest can be identified by screening a series of antibodies prepared against a CD40 protein or fragment of the protein comprising the particular epitope of the CD40 protein of interest. For example, for human CD40, epitopes of interest include epitopes comprising linear and/or nonlinear amino acid

residues of the short isoform of human CD40 (see GenBank Accession No. NP_690593) set forth in SEQ ID NO:7, encoded by the sequence set forth SEQ ID NO:6; see also GenBank Accession No. NM_152854), or of the long isoform of human CD40 (see GenBank Accession Nos. CAA43045 and NP_001241, set forth in SEQ ID NO:9, 5 encoded by the sequence set forth in SEQ ID NO:8; see GenBank Accession Nos. X60592 and NM_001250). Alternatively, competitive binding assays with previously identified suitable antagonist anti-CD40 antibodies could be used to select monoclonal antibodies comparable to the previously identified antibodies.

Antibodies employed in such immunoassays may be labeled or unlabeled.

- 10 Unlabeled antibodies may be employed in agglutination; labeled antibodies may be employed in a wide variety of assays, employing a wide variety of labels. Detection of the formation of an antibody-antigen complex between an anti-CD40 antibody and an epitope of interest can be facilitated by attaching a detectable substance to the antibody. Suitable detection means include the use of labels such as radionuclides, enzymes, 15 coenzymes, fluorescers, chemiluminescers, chromogens, enzyme substrates or co-factors, enzyme inhibitors, prosthetic group complexes, free radicals, particles, dyes, and the like. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, β -galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials 20 include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material is luminol; examples of bioluminescent materials include luciferase, luciferin, and aequorin; and examples of suitable radioactive material include ^{125}I , ^{131}I , ^{35}S , or ^3H . Such labeled reagents may be used in a variety of well-known assays, such as 25 radioimmunoassays, enzyme immunoassays, *e.g.*, ELISA, fluorescent immunoassays, and the like. See for example, U.S. Patent Nos. 3,766,162; 3,791,932; 3,817,837; and 4,233,402.

- As noted above, the combination therapy of the invention addresses problems associated with known therapies for diseases or conditions associated with neoplastic B- 30 cell growth, including therapy using rituximab (commercially available under the tradename Rituxan®). Rituximab has been shown to be an effective treatment for low-,

intermediate-, and high-grade non-Hodgkin's lymphoma (NHL) and active in other B-cell malignancies (see for example, Maloney *et al.* (1994) *Blood* 84:2457-2466), McLaughlin *et al.* (1998) *J. Clin. Oncol.* 16:2825-2833, Maloney *et al.* (1997) *Blood* 90:2188-2195, Hainsworth *et al.* (2000) *Blood* 95:3052-3056, Colombat *et al.* (2001) *Blood* 97:101-106, 5 Coiffer *et al.* (1998) *Blood* 92:1927-1932), Foran *et al.* (2000) *J. Clin. Oncol.* 18:317-324, Anderson *et al.* (1997) *Biochem. Soc. Trans.* 25:705-708, or Vose *et al.* (1999) *Ann. Oncol.* 10:58a). Rituximab is licensed for treatment of relapsed B cell low-grade or follicular non-Hodgkin's lymphoma (NHL). In follicular lymphoma, which is the most common indolent non-Hodgkin lymphoma (NHL), rituximab combined with 10 chemotherapy has led to notable improvements in response rates, progression-free survival (PFS), and overall survival (OS) (Marcus *et al.* (2005) *Blood*. 105:1417-1423; Hiddemann *et al.* (2005) *Blood* 106:3725-3732; Herold *et al.* (20007) *J Clin Oncol.* 25:1986-1992; Foussard *et al.* (2006) *J. Clin. Oncol.* 18S(June 20 suppl):424. Abstract 7508). Treatment guidelines from the National Comprehensive Cancer Network now 15 recommend a rituximab-based regimen as initial therapy for patients with B-cell lymphoma (National Comprehensive Cancer Network (NCCN) NCCN Clinical Practice Guidelines in Oncology. Non-Hodgkin's Lymphomas. Version 3. [January 13, 2009]; available on the world-wide web at nccn.org/professionals/physician_gls/PDF/nhl.pdf). However, patients tend to become refractory to rituximab over time (see Witzig *et al.* 20 (2002) *J. Clin. Oncol.* 20:3262, Grillo- Lopez *et al.* (1998) *J. Clin. Oncol.* 16:2825, or Jazirehi *et al.* (2003) *Mol. Cancer Ther.* 2:1183-1193). For example, some patients lose CD20 expression on malignant B cells after anti-CD20 antibody therapy (Davis *et al.* (1999) *Clin. Cancer Res.* 5:611). Furthermore, 30% to 50% of patients with low-grade NHL exhibit no clinical response to this monoclonal antibody (Hainsworth *et al.* (2000) 25 *Blood* 95:3052-3056; Colombat *et al.* (2001) *Blood* 97:101-106). Although yttrium-90 ibritumomab tiuxetan and iodine-131 tositumomab have demonstrated activity in patients who are refractory to single-agent rituximab, their use has been limited by strict eligibility criteria and other factors. Moreover, patients with indolent B-cell lymphoma currently are more likely to be treated with rituximab-chemotherapy combinations than 30 with single-agent rituximab. Consequently, rituximab resistance often develops within

the context of generalized chemotherapy resistance, and innovative treatments are needed for this “rituximab-refractory” patient population.

For patients developing resistance to this monoclonal antibody, or having a B-cell lymphoma that is resistant to initial therapy with this antibody, alternative forms of therapeutic intervention are needed. Alternative therapies are also desirable for patients who relapse after therapy with rituximab. The discovery of combination therapies with superior therapeutic response, in particular improvement in overall survival, compared to rituximab and other chemotherapeutic regimens could drastically improve methods of therapy for diseases or condition associated with neoplastic B cell growth, such as B cell lymphomas, particularly B cell non-Hodgkin's lymphomas.

The invention uses combination therapy with bendamustine and anti-CD40 antibodies for treating diseases or conditions associated with neoplastic B-cell growth. Bendamustine (Treanda™; Cephalon, Inc., Frazer, Pa) is a novel alkylator whose mechanisms of action involve induction of apoptosis through activation of DNA-damage stress responses, inhibition of mitotic checkpoints, and induction of mitotic catastrophe (Leoni *et al.* (2008) *Clin Cancer Res.* 2008;14:309-317). The compound also contains a benzimidazole ring, which may confer purine analogue-like properties in addition to the alkylating properties. In vitro studies indicate that the DNA repair mechanisms that operate after exposure to the drug are different from those evoked by other agents, potentially explaining observed antitumor effects in cell lines that are resistant to other alkylating agents (Strumberg *et al.* (1996) *Anticancer Drugs.* 1996;7:415-421).

In accordance with the combination therapy of the present invention, bendamustine is administered at therapeutically effective doses ranging between about 60 mg/m² and about 120 mg/m², including, for example, 60 mg/m², 65 mg/m², 70 mg/m², 75 mg/m², 80 mg/m², 85 mg/m², 90 mg/m², 95 mg/m², 100 mg/m², 105 mg/m², 110 mg/m², 115 mg/m², and 120 mg/m², and other such doses falling within the range of about 60 mg/m² and 120 mg/m². In order to reduce hematologic toxicity, dose modifications, and treatment delays, bendamustine preferably is administered at a dose of about about 85 mg/m², about 90 mg/m², or about 95 mg/m² on Days 1 and 2 of any given cycle of combination therapy with the anti-CD40 antibody (such as HCD122). Preferably the bendamustine is administered intravenously (iv) by infusion over 60 minutes

according to the most up-to-date prescription guidelines or other applicable institutional or regional guidelines.

The anti-CD40 antibodies of this invention are administered at a concentration that is therapeutically effective to treat a disease or condition associated with neoplastic
5 CD40-expressing B cells. To accomplish this goal, the antibodies may be formulated using a variety of acceptable carrier and/or excipients known in the art. The anti-CD40 antibody may be administered by a parenteral route of administration. Typically, the antibodies are administered by injection, either intravenously or subcutaneously. Methods to accomplish this administration are known to those of ordinary skill in the art.

10 Intravenous administration occurs preferably by infusion over a period of about less than 1 hour to about 10 hours (more preferably less than 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 hours). Subsequent infusions may be administered over a period of about less than 1 to about 6 hours, including, for example, about 1 to about 4 hours, about 1 to about 3 hours, or about 1 to about 2 hours. In some embodiments, the anti-CD40 antibody is
15 administered by infusion over a period of about 2 to about 6 hours. Alternatively, a dose can be administered subcutaneously.

A pharmaceutical composition of the invention is formulated to be compatible with its intended route of administration. Solutions or suspensions used for parenteral application can include the following components: a sterile diluent such as water for
20 injection, saline solution; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose. pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. The
25 parenteral preparation can be enclosed in ampoules, disposable syringes, or multiple dose vials made of glass or plastic.

The anti-CD40 antibodies are typically provided by standard technique within a pharmaceutically acceptable buffer, for example, sterile saline, sterile buffered water, combinations of the foregoing, etc. Methods for preparing parenterally administrable
30 agents are described in *Remington: The Science and Practice of Pharmacy* (21st edition, Lippincott Williams & Wilkins, May 2005). See also, for example, WO 98/56418, which

describes stabilized antibody pharmaceutical formulations suitable for use in the methods of the present invention.

The amount of anti-CD40 antibody to be administered is readily determined by one of ordinary skill in the art in view of the guidance provided herein. Factors
5 influencing the mode of administration and the respective amount of anti-CD40 antibody include, but are not limited to, the severity of the disease, the history of the disease, and the age, height, weight, health, type of disease, and physical condition of the individual undergoing therapy or response to antibody infusion. Similarly, the amount of anti-CD40 antibody to be administered will be dependent upon the mode of
10 administration and whether the subject will undergo a single dose or multiple doses of this anti-cancer agent. Generally, a higher dosage of anti-CD40 antibody is preferred with increasing weight of the subject undergoing therapy.

For a single dose of anti-CD40 antibody the amount to be administered may be in the range from about 0.1 mg/kg to about 35 mg/kg, from about 0.5 mg/kg to about 30
15 mg/kg, from about 1 mg/kg to about 30 mg/kg, from about 3 mg/kg to about 30 mg/kg, from about 3 mg/kg to about 25 mg/kg, from about 3 mg/kg to about 20 mg/kg, or from about 5 mg/kg to about 15 mg/kg. Thus, for example, the dose can be 0.3 mg/kg, 0.5 mg/kg, 1 mg/kg, 1.5 mg/kg, 2 mg/kg, 2.5 mg/kg, 3 mg/kg, 3.5 mg/kg, 4 mg/kg, 4.5 mg/kg, 5 mg/kg, 5.5 mg/kg, 6 mg/kg, 6.5 mg/kg, 7 mg/kg, 7.5 mg/kg, 8 mg/kg, 8.5
20 mg/kg, 9 mg/kg, 9.5 mg/kg, 10 mg/kg, 15 mg/kg, 20 mg/kg, 25 mg/kg, 30 mg/kg, or 35 mg/kg, or other such doses falling within the range of about 0.3 mg/kg to about 35 mg/kg.

Treatment of a subject with a therapeutically effective amount of an antibody can include a single treatment or, preferably, can include a series of treatments. Thus, the
25 methods of the invention may comprise administration of multiple doses of anti-CD40 antibody. The method may comprise administration of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, or more therapeutically effective separate doses of a pharmaceutical composition comprising an anti-CD40 antibody. The frequency and duration of administration of multiple doses of the pharmaceutical compositions comprising anti-
30 CD40 antibody can be readily determined by one of skill in the art without undue experimentation when equipped with the disclosure provided herein. The same

therapeutically effective dose of an anti-CD40 antibody can be administered over the course of a treatment period. Alternatively, different therapeutically effective doses of an anti-CD40 antibody can be used over the course of a treatment period.

In an example, a subject is treated with anti-CD40 antibody in the range of
5 between about 0.1 to 20 mg/kg body weight, once per week for between about 1 to 10 weeks, preferably between about 2 to 8 weeks, more preferably between about 3 to 7 weeks, and even more preferably for about 4, 5, or 6 weeks. Treatment may occur at intervals of every 2 to 12 months to prevent relapse or upon indication of relapse. It will also be appreciated that the effective dosage of antibody used for treatment may increase
10 or decrease over the course of a particular treatment. Changes in dosage may result and become apparent from the results of diagnostic assays.

Thus, in one embodiment, the dosing regimen includes a first administration of a therapeutically effective dose of at least one anti-CD40 antibody on days 1, 8, 15, and 22 of a treatment period.

15 In another embodiment, the dosing regimen includes a dosing regimen having a first administration of a therapeutically effective dose of at least one anti-CD40 antibody daily, or on days 1, 3, 5, and 7 of a week in a treatment period; a dosing regimen including a first administration of a therapeutically effective dose of at least one anti-CD40 antibody on days 1 and 3-4 of a week in a treatment period; and a dosing regimen
20 including a first administration of a therapeutically effective dose of at least one anti-CD40 antibody on day 1 of a week in a treatment period. The treatment period may comprise at least 1 week, at least 2 weeks, at least 3 weeks, at least a month, at least 2 months, at least 3 months, at least 6 months, or at least a year. Treatment periods may be subsequent or separated from each other by at least a week, at least 2 weeks, at least a
25 month, at least 3 months, at least 6 months, or at least a year.

In other embodiments, the initial therapeutically effective dose of an anti-CD40 antibody as defined elsewhere herein can be in the lower dosing range (*i.e.*, about 0.3 mg/kg to about 20 mg/kg) with subsequent doses falling within the higher dosing range (*i.e.*, from about 20 mg/kg to about 50 mg/kg).

30 In alternative embodiments, the initial therapeutically effective dose of an anti-CD40 antibody as defined elsewhere herein can be in the upper dosing range (*i.e.*, about

20 mg/kg to about 50 mg/kg) with subsequent doses falling within the lower dosing range (*i.e.*, 0.3 mg/kg to about 20 mg/kg). Thus, in some embodiments of the invention, anti-CD40 antibody therapy may be initiated by administering a “loading dose” of the antibody to the subject in need therapy. By “loading dose” is intended an initial dose of the anti-CD40 antibody that is administered to the subject, where the dose of the antibody administered falls within the higher dosing range (*i.e.*, from about 20 mg/kg to about 50 mg/kg). The “loading dose” can be administered as a single administration, for example, a single infusion where the antibody is administered IV, or as multiple administrations, for example, multiple infusions where the antibody is administered IV, so long as the complete “loading dose” is administered within about a 24-hour period. Following administration of the “loading dose,” the subject is then administered one or more additional therapeutically effective doses of the anti-CD40 antibody. Subsequent therapeutically effective doses can be administered, for example, according to a weekly dosing schedule, or once every two weeks, once every three weeks, or once every four weeks. In such embodiments, the subsequent therapeutically effective doses generally fall within the lower dosing range (*i.e.*, 0.3 mg/kg to about 20 mg/kg).

Alternatively, in some embodiments, following the “loading dose”, the subsequent therapeutically effective doses of the anti-CD40 antibody are administered according to a “maintenance schedule”, wherein the therapeutically effective dose of the antibody is administered once a month, once every 6 weeks, once every two months, once every 10 weeks, once every three months, once every 14 weeks, once every four months, once every 18 weeks, once every five months, once every 22 weeks, once every six months, once every 7 months, once every 8 months, once every 9 months, once every 10 months, once every 11 months, or once every 12 months. In such embodiments, the therapeutically effective doses of the anti-CD40 antibody fall within the lower dosing range (*i.e.*, 0.3 mg/kg to about 20 mg/kg), particularly when the subsequent doses are administered at more frequent intervals, for example, once every two weeks to once every month, or within the higher dosing range (*i.e.*, from about 20 mg/kg to about 50 mg/kg), particularly when the subsequent doses are administered at less frequent intervals, for example, where subsequent doses are administered about one month to about 12 months apart.

The anti-CD40 antibodies present in the pharmaceutical compositions described herein for use in the methods of the invention may be native or obtained by recombinant techniques, and may be from any source, including mammalian sources such as, *e.g.*, mouse, rat, rabbit, primate, pig, and human. Preferably such polypeptides are derived
5 from a human source, and more preferably are recombinant, human proteins from hybridoma cell lines.

Any pharmaceutical composition comprising an anti-CD40 antibody having the binding properties described herein as the therapeutically active component can be used in the methods of the invention. Thus liquid, lyophilized, or spray-dried compositions
10 comprising one or more of the anti-CD40 antibodies may be prepared as an aqueous or nonaqueous solution or suspension for subsequent administration to a subject in accordance with the methods of the invention. Each of these compositions will comprise at least one anti-CD40 antibody as a therapeutically or prophylactically active component. By "therapeutically or prophylactically active component" is intended the
15 anti-CD40 antibody is specifically incorporated into the composition to bring about a desired therapeutic or prophylactic response with regard to treatment, prevention, or diagnosis of a disease or condition within a subject when the pharmaceutical composition is administered to that subject. Preferably the pharmaceutical compositions comprise appropriate stabilizing agents, bulking agents, or both to minimize problems associated
20 with loss of protein stability and biological activity during preparation and storage.

Formulants may be added to pharmaceutical compositions comprising an anti-CD40 antibody. These formulants may include, but are not limited to, oils, polymers, vitamins, carbohydrates, amine acids, salts, buffers, albumin, surfactants, or bulking agents. Preferably carbohydrates include sugar or sugar alcohols such as mono-, di-, or
25 polysaccharides, or water soluble glucans. The saccharides or glucans can include fructose, glucose, trehalose, mannose, sorbose, xylose, maltose, sucrose, dextran, pullulan, dextrin, α - and β -cyclodextrin, soluble starch, hydroxyethyl starch, and carboxymethylcellulose, or mixtures thereof. "Sugar alcohol" is defined as a C₄ to C₈ hydrocarbon having a hydroxyl group and includes galactitol, inositol, mannitol, xylitol,
30 sorbitol, glycerol, and arabitol. These sugars or sugar alcohols may be used individually or in combination. The sugar or sugar alcohol concentration is between 1.0% and 7%

w/v., more preferably between 2.0% and 6.0% w/v. Preferably amino acids include levorotary (L) forms of carnitine, arginine, and betaine; however, other amino acids may be added. Preferred polymers include polyvinylpyrrolidone (PVP) with an average molecular weight between 2,000 and 3,000, or polyethylene glycol (PEG) with an
5 average molecular weight between 3,000 and 5,000. Surfactants that can be added to the formulation are shown in EP Nos. 270,799 and 268,110.

The formulants to be incorporated into a pharmaceutical composition should provide for the stability of the anti-CD40 antibody. That is, the anti-CD40 antibody should retain its physical and/or chemical stability and have the desired biological
10 activity, *i.e.*, one or more of the antagonist activities defined herein above.

Methods for monitoring protein stability are well known in the art. See, for example, Jones (1993) *Adv. Drug Delivery Rev.* 10:29-90; Lee, ed. (1991) *Peptide and Protein Drug Delivery* (Marcel Dekker, Inc., New York, New York); and the stability assays disclosed herein below. Generally, protein stability is measured at a chosen
15 temperature for a specified period of time. In preferred embodiments, a stable antibody pharmaceutical formulation provides for stability of the anti-CD40 antibody when stored at room temperature (about 25°C) for at least 1 month, at least 3 months, or at least 6 months, and/or is stable at about 2-8°C for at least 6 months, at least 9 months, at least 12 months, at least 18 months, at least 24 months.

20 A protein such as an antibody, when formulated in a pharmaceutical composition, is considered to retain its physical stability at a given point in time if it shows no visual signs (*i.e.*, discoloration or loss of clarity) or measurable signs (for example, using size-exclusion chromatography (SEC) or UV light scattering) of precipitation, aggregation, and/or denaturation in that pharmaceutical composition. With respect to chemical
25 stability, a protein such as an antibody, when formulated in a pharmaceutical composition, is considered to retain its chemical stability at a given point in time if measurements of chemical stability are indicative that the protein (*i.e.*, antibody) retains the biological activity of interest in that pharmaceutical composition. Methods for monitoring changes in chemical stability are well known in the art and include, but are
30 not limited to, methods to detect chemically altered forms of the protein such as result from clipping, using, for example, SDS-PAGE, SEC, and/or matrix-assisted laser

desorption ionization/time of flight mass spectrometry; and degradation associated with changes in molecular charge (for example, associated with deamidation), using, for example, ion-exchange chromatography. See, for example, the methods disclosed herein below.

- 5 An anti-CD40 antibody, when formulated in a pharmaceutical composition, is considered to retain a desired biological activity at a given point in time if the desired biological activity at that time is within about 30%, preferably within about 20% of the desired biological activity exhibited at the time the pharmaceutical composition was prepared as determined in a suitable assay for the desired biological activity. Assays for
- 10 measuring the desired biological activity of the anti-CD40 antibodies can be performed as described in the Examples herein. See also the assays described in Schultze *et al.* (1998) *Proc. Natl. Acad. Sci. USA* 92:8200-8204; Denton *et al.* (1998) *Pediatr. Transplant.* 2:6-15; Evans *et al.* (2000) *J. Immunol.* 164:688-697; Noelle (1998) *Agents Actions Suppl.* 49:17-22; Lederman *et al.* (1996) *Curr. Opin. Hematol.* 3:77-86; Coligan *et al.* (1991)
- 15 *Current Protocols in Immunology* 13:12; Kwekkeboom *et al.* (1993) *Immunology* 79:439-444; and U.S. Patent Nos. 5,674,492 and 5,847,082.

- In some embodiments of the invention, the anti-CD40 antibody is formulated in a liquid pharmaceutical formulation. The anti-CD40 antibody can be prepared using any method known in the art, including those methods disclosed herein above. The anti-CD40
- 20 antibody may be recombinantly produced in a CHO cell line.

- Where the anti-CD40 antibody is to be stored prior to its formulation, it can be frozen, for example, at $\leq -20^{\circ}\text{C}$, and then thawed at room temperature for further formulation. The liquid pharmaceutical formulation comprises a therapeutically effective amount of the anti-CD40 antibody. The amount of antibody thereof present in the
- 25 formulation takes into consideration the route of administration and desired dose volume.

- In this manner, the liquid pharmaceutical composition comprises the anti-CD40 antibody at a concentration of about 0.1 mg/ml to about 50.0 mg/ml, about 0.5 mg/ml to about 40.0 mg/ml, about 1.0 mg/ml to about 30.0 mg/ml, about 5.0 mg/ml to about 25.0 mg/ml, about 5.0 mg/ml to about 20.0 mg/ml, or about 15.0 mg/ml to about 25.0 mg/ml.
- 30 In some embodiments, the liquid pharmaceutical composition comprises the anti-CD40 antibody at a concentration of about 0.1 mg/ml to about 5.0 mg/ml, about 5.0 mg/ml to

about 10.0 mg/ml, about 10.0 mg/ml to about 15.0 mg/ml, about 15.0 mg/ml to about 20.0 mg/ml, about 20.0 mg/ml to about 25.0 mg/ml, about 25.0 mg/ml to about 30.0 mg/ml, about 30.0 mg/ml to about 35.0 mg/ml, about 35.0 mg/ml to about 40.0 mg/ml, about 40.0 mg/ml to about 45.0 mg/ml, or about 45.0 mg/ml to about 50.0 mg/ml. In
5 other embodiments, the liquid pharmaceutical composition comprises the anti-CD40 antibody at a concentration of about 15.0 mg/ml, about 16.0 mg/ml, about 17.0 mg/ml, about 18.0 mg/ml, about 19.0 mg/ml, about 20.0 mg/ml, about 21.0 mg/ml, about 22.0 mg/ml, about 23.0 mg/ml, about 24.0 mg/ml, or about 25.0 mg/ml. The liquid pharmaceutical composition comprises the anti-CD40 antibody and a buffer that
10 maintains the pH of the formulation in the range of about pH 5.0 to about pH 7.0, including about pH 5.0, 5.1, 5.2, 5.3, 5.4, 5.5, 5.6, 5.7, 5.8, 5.9, 6.0, 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6.9, 7.0. In some embodiments, the buffer maintains the pH of the formulation in the range of about pH 5.0 to about pH 6.5, about pH 5.0 to about pH 6.0, about pH 5.0 to about pH 5.5, about pH 5.5 to about 7.0, about pH 5.5 to about pH 6.5, or
15 about pH 5.5 to about pH 6.0.

Any suitable buffer that maintains the pH of the liquid anti-CD40 antibody formulation in the range of about pH 5.0 to about pH 7.0 can be used in the formulation, so long as the physicochemical stability and desired biological activity of the antibody are retained as noted herein above. Suitable buffers include, but are not limited to,
20 conventional acids and salts thereof, where the counter ion can be, for example, sodium, potassium, ammonium, calcium, or magnesium. Examples of conventional acids and salts thereof that can be used to buffer the pharmaceutical liquid formulation include, but are not limited to, succinic acid or succinate, citric acid or citrate, acetic acid or acetate, tartaric acid or tartarate, phosphoric acid or phosphate, gluconic acid or gluconate,
25 glutamic acid or glutamate, aspartic acid or aspartate, maleic acid or maleate, and malic acid or malate buffers. The buffer concentration within the formulation can be from about 1 mM to about 50 mM, including about 1 mM, 2 mM, 5 mM, 8 mM, 10 mM, 15 mM, 20 mM, 25 mM, 30 mM, 35 mM, 40 mM, 45 mM, 50 mM, or other such values within the range of about 1 mM to about 50 mM. In some embodiments, the buffer
30 concentration within the formulation is from about 5 mM to about 15 mM, including

about 5 mM, 6 mM, 7 mM, 8 mM, 9 mM, 10 mM, 11 mM, 12 mM, 13 mM, 14 mM, 15 mM, or other such values within the range of about 5 mM to about 15 mM.

In some embodiments of the invention, the liquid pharmaceutical formulation comprises a therapeutically effective amount of the anti-CD40 antibody and succinate buffer or citrate buffer at a concentration that maintains the pH of the formulation in the range of about pH 5.0 to about pH 7.0, preferably about pH 5.0 to about pH 6.5. By “succinate buffer” or “citrate buffer” is intended a buffer comprising a salt of succinic acid or a salt of citric acid, respectively. In a preferred embodiment, the succinate or citrate counterion is the sodium cation, and thus the buffer is sodium succinate or sodium citrate, respectively. However, any cation is expected to be effective. Other possible succinate or citrate cations include, but are not limited to, potassium, ammonium, calcium, and magnesium. As noted above, the succinate or citrate buffer concentration within the formulation can be from about 1 mM to about 50 mM, including about 1 mM, 2 mM, 5 mM, 8 mM, 10 mM, 15 mM, 20 mM, 25 mM, 30 mM, 35 mM, 40 mM, 45 mM, 50 mM, or other such values within the range of about 1 mM to about 50 mM. In some embodiments, the buffer concentration within the formulation is from about 5 mM to about 15 mM, including about 5 mM, 6 mM, 7 mM, 8 mM, 9 mM, 10 mM, 11 mM, 12 mM, 13 mM, 14 mM, or about 15 mM. In other embodiments, the liquid pharmaceutical formulation comprises the anti-CD40 antibody at a concentration of about 0.1 mg/ml to about 50.0 mg/ml, or about 5.0 mg/ml to about 25.0 mg/ml, and succinate or citrate buffer, for example, sodium succinate or sodium citrate buffer, at a concentration of about 1 mM to about 20 mM, about 5 mM to about 15 mM, preferably about 10 mM.

Where it is desirable for the liquid pharmaceutical formulation to be near isotonic, the liquid pharmaceutical formulation comprising the anti-CD40 antibody and a buffer can further comprise an amount of an isotonicizing agent sufficient to render the formulation near isotonic. By “near isotonic” is intended the aqueous formulation has an osmolarity of about 240 mmol/kg to about 360 mmol/kg, preferably about 240 to about 340 mmol/kg, more preferably about 250 to about 330 mmol/kg, even more preferably about 260 to about 320 mmol/kg, still more preferably about 270 to about 310 mmol/kg. Methods of determining the isotonicity of a solution are known to those skilled in the art.

Those skilled in the art are familiar with a variety of pharmaceutically acceptable solutes useful in providing isotonicity in pharmaceutical compositions. The isotonicizing agent can be any reagent capable of adjusting the osmotic pressure of the liquid pharmaceutical formulation of the present invention to a value nearly equal to that of a
5 body fluid. It is desirable to use a physiologically acceptable isotonicizing agent. Thus, the liquid pharmaceutical formulation comprising a therapeutically effective amount of the anti-CD40 antibody and a buffer can further comprise components that can be used to provide isotonicity, for example, sodium chloride; amino acids such as alanine, valine, and glycine; sugars and sugar alcohols (polyols), including, but not limited to, glucose,
10 dextrose, fructose, sucrose, maltose, mannitol, trehalose, glycerol, sorbitol, and xylitol; acetic acid, other organic acids or their salts, and relatively minor amounts of citrates or phosphates. The ordinary skilled person would know of additional agents that are suitable for providing optimal tonicity of the liquid formulation.

In some preferred embodiments, the liquid pharmaceutical formulation
15 comprising an anti-CD40 antibody and a buffer further comprises sodium chloride as the isotonicizing agent. The concentration of sodium chloride in the formulation will depend upon the contribution of other components to tonicity. In some embodiments, the concentration of sodium chloride is about 50 mM to about 300 mM, about 50 mM to about 250 mM, about 50 mM to about 200 mM, about 50 mM to about 175 mM, about 50
20 mM to about 150 mM, about 75 mM to about 175 mM, about 75 mM to about 150 mM, about 100 mM to about 175 mM, about 100 mM to about 200 mM, about 100 mM to about 150 mM, about 125 mM to about 175 mM, about 125 mM to about 150 mM, about 130 mM to about 170 mM, about 130 mM to about 160 mM, about 135 mM to about 155 mM, about 140 mM to about 155 mM, or about 145 mM to about 155 mM. In one such
25 embodiment, the concentration of sodium chloride is about 150 mM. In other such embodiments, the concentration of sodium chloride is about 150 mM, the buffer is sodium succinate or sodium citrate buffer at a concentration of about 5 mM to about 15 mM, the liquid pharmaceutical formulation comprises a therapeutically effective amount of the anti-CD40 antibody and the formulation has a pH of about pH 5.0 to about pH 7.0,
30 about pH 5.0 to about pH 6.0, or about pH 5.5 to about pH 6.5. In other embodiments, the liquid pharmaceutical formulation comprises the anti-CD40 antibody at a concentration

of about 0.1 mg/ml to about 50.0 mg/ml or about 5.0 mg/ml to about 25.0 mg/ml, about 150 mM sodium chloride, and about 10 mM sodium succinate or sodium citrate, at a pH of about pH 5.5.

Protein degradation due to freeze thawing or mechanical shearing during
5 processing of a liquid pharmaceutical formulation of the present invention can be inhibited by incorporation of surfactants into the formulation in order to lower the surface tension at the solution-air interface. Thus, in some embodiments, the liquid pharmaceutical formulation comprises a therapeutically effective amount of the anti-CD40 antibody, a buffer, and further comprises a surfactant. In other embodiments, the
10 liquid pharmaceutical formulation comprises an anti-CD40 antibody, a buffer, an isotonicizing agent, and further comprises a surfactant.

Typical surfactants employed are nonionic surfactants, including polyoxyethylene sorbitol esters such as polysorbate 80 (Tween 80) and polysorbate 20 (Tween 20); polyoxypropylene-polyoxyethylene esters such as Pluronic F68; polyoxyethylene
15 alcohols such as Brij 35; simethicone; polyethylene glycol such as PEG400; lysophosphatidylcholine; and polyoxyethylene-p-t-octylphenol such as Triton X-100. Classic stabilization of pharmaceuticals by surfactants or emulsifiers is described, for example, in Levine *et al.* (1991) *J. Parenteral Sci. Technol.* 45(3):160-165. A preferred surfactant employed in the practice of the present invention is polysorbate 80. Where a
20 surfactant is included, it is typically added in an amount from about 0.001 % to about 1.0% (w/v), about 0.001% to about 0.5%, about 0.001% to about 0.4%, about 0.001% to about 0.3%, about 0.001% to about 0.2%, about 0.005% to about 0.5%, about 0.005% to about 0.2%, about 0.01% to about 0.5%, about 0.01% to about 0.2%, about 0.03% to about 0.5%, about 0.03% to about 0.3%, about 0.05% to about 0.5%, or about 0.05% to
25 about 0.2%.

Thus, in some embodiments, the liquid pharmaceutical formulation comprises a therapeutically effective amount of the anti-CD40 antibody, the buffer is sodium succinate or sodium citrate buffer at a concentration of about 1 mM to about 50 mM, about 5 mM to about 25 mM, or about 5 mM to about 15 mM; the formulation has a pH
30 of about pH 5.0 to about pH 7.0, about pH 5.0 to about pH 6.0, or about pH 5.5 to about pH 6.5; and the formulation further comprises a surfactant, for example, polysorbate 80,

in an amount from about 0.001% to about 1.0% or about 0.001% to about 0.5%. Such formulations can optionally comprise an isotonicizing agent, such as sodium chloride at a concentration of about 50 mM to about 300 mM, about 50 mM to about 200 mM, or about 50 mM to about 150 mM. In other embodiments, the liquid pharmaceutical

5 formulation comprises the anti-CD40 antibody at a concentration of about 0.1 mg/ml to about 50.0 mg/ml or about 5.0 mg/ml to about 25.0 mg/ml, including about 20.0 mg/ml; about 50 mM to about 200 mM sodium chloride, including about 150 mM sodium chloride; sodium succinate or sodium citrate at about 5 mM to about 20 mM, including about 10 mM sodium succinate or sodium citrate; sodium chloride at a concentration of

10 about 50 mM to about 200 mM, including about 150 mM; and optionally a surfactant, for example, polysorbate 80, in an amount from about 0.001% to about 1.0%, including about 0.001% to about 0.5%; where the liquid pharmaceutical formulation has a pH of about pH 5.0 to about pH 7.0, about pH 5.0 to about pH 6.0, about pH 5.0 to about pH 5.5, about pH 5.5 to about pH 6.5, or about pH 5.5 to about pH 6.0.

15 The liquid pharmaceutical formulation can be essentially free of any preservatives and other carriers, excipients, or stabilizers noted herein above. Alternatively, the formulation can include one or more preservatives, for example, antibacterial agents, pharmaceutically acceptable carriers, excipients, or stabilizers described herein above provided they do not adversely affect the physicochemical stability of the anti-CD40

20 antibody. Examples of acceptable carriers, excipients, and stabilizers include, but are not limited to, additional buffering agents, co-solvents, surfactants, antioxidants including ascorbic acid and methionine, chelating agents such as EDTA, metal complexes (for example, Zn-protein complexes), and biodegradable polymers such as polyesters. A thorough discussion of formulation and selection of pharmaceutically acceptable carriers,

25 stabilizers, and isomolytes can be found in *Remington: The Science and Practice of Pharmacy* (21st edition, Lippincott Williams & Wilkins, May 2005).

"Carriers" as used herein include pharmaceutically acceptable carriers, excipients, or stabilizers that are nontoxic to the cell or mammal being exposed thereto at the dosages and concentrations employed. Often the physiologically acceptable carrier is an

30 aqueous pH buffered solution. Examples of physiologically acceptable carriers include buffers such as phosphate, citrate, succinate, and other organic acids; antioxidants

including ascorbic acid; 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, arginine or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrans; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; salt-forming counterions such as sodium; and/or nonionic surfactants such as TWEEN, polyethylene glycol (PEG), and Pluronic.

After the liquid pharmaceutical formulation or other pharmaceutical composition described herein is prepared, it can be lyophilized to prevent degradation. Methods for lyophilizing liquid compositions are known to those of ordinary skill in the art. Just prior to use, the composition may be reconstituted with a sterile diluent (Ringer's solution, distilled water, or sterile saline, for example) that may include additional ingredients. Upon reconstitution, the composition is preferably administered to subjects using those methods that are known to those skilled in the art.

The anti-CD40 antibody-containing pharmaceutical composition may be a composition as described in co-owned International Patent Application No. PCT/US2007/066757 published as WO 2007/124299. In particular, a pharmaceutical composition for use in the combination therapy of the invention may comprise (i) an anti-CD40 antibody, a buffering agent to maintain the pH of the composition between around pH 5.0 and pH 7.0, and (iii) an amount of arginine-HCl sufficient to render the liquid composition near isotonic. In these compositions, the buffering agent may be a citrate/citric acid buffer. The composition may further comprise a non-ionic surfactant and/or L-methionine as further stabilizing agents. The composition may have an osmolarity of about 240 mmol/kg to about 360 mmol/kg. The concentration of the buffering agent may be from about 5 mM to about 100 mM, from about 5 mM to about 20 mM, or from about 5 mM to about 15 mM (*e.g.*, 10 mM). The composition may have a pH of from pH 5.0 to pH 6.0 (*e.g.*, around pH 5.5). The composition may comprise arginine-HCl at a concentration of about 50 mM to about 200 mM, or from about 100 mM to about 175 mM (*e.g.*, about 150 mM). The composition may further comprise the surfactant polysorbate, for example at a concentration of about 0.001% to about 1.0% (w/v), or at a concentration of about 0.025% to about 0.1% (w/v). The composition may

further comprise methionine at a concentration of about 0.5 mM to about 20.0 mM or at a concentration of about 1.0 mM to about 20.0 mM (*e.g.*, about 5.0 mM). The anti-CD40 antibody may be present in the composition at about 0.1 mg/ml to about 50.0 mg/ml, or at about 1.0 mg/ml to about 35.0 mg/ml, or at about 10.0 mg/ml to about 35.0 mg/ml.

- 5 Any pharmaceutical composition comprising bendamustine as the therapeutically active component can be used in the methods of the invention. These will contain bendamustine and a pharmaceutically acceptable carrier or excipient, *e.g.*, a pharmaceutically acceptable carrier or excipient as described elsewhere herein. Suitable pharmaceutical compositions are well known in the art. By “therapeutically active
- 10 component” is intended that the relevant therapeutic agent is specifically incorporated into the composition to bring about a desired therapeutic response with regard to treatment of a disease or condition within a subject when the pharmaceutical composition is administered to that subject. The bendamustine component is administered at concentrations that are “therapeutically effective” to treat a disease or condition
- 15 associated with neoplastic B-cell growth.

The bendamustine may be administered by any appropriate route of administration. Bendamustine is normally administered intravenously. Methods to accomplish this administration are known to those of ordinary skill in the art.

- Bendamustine is normally administered in cycles of treatment, each cycle
- 20 comprising administration of bendamustine at about 60 mg/m² to about 120 mg/m² on day 1 and day 2 of a treatment cycle, preferably at about 90 mg/m² on day 1 and day 2 of a treatment cycle. The cycle is generally repeated every three weeks (21 days) or every four weeks (28 days). A usual course of treatment consists of four to eight cycles in total, including, for example, four cycles, five cycles, six cycles, seven cycles, or eight cycles.

- 25 In the methods, uses, compositions and kits of the invention the bendamustine may be used at 60-120 mg/m², 60-110 mg/m², 60-100 mg/m², 60-90 mg/m², 70-120 mg/m², 70-110 mg/m², 70-100 mg/m², 70-90 mg/m², 80-120 mg/m², 80-110 mg/m², 80-100 mg/m², or 80-95 mg/m² (*e.g.*, at 90 mg/m²). The skilled person will readily be able to select an appropriate bendamustine regimen for use in the combination therapy of
- 30 the invention when equipped with the disclosure provided herein.

In the methods, uses, compositions and kits of the invention the bendamustine regimen will preferably be repeated every four weeks, but may be repeated every three weeks, every four weeks, every five weeks, every six weeks, every seven weeks, or every eight weeks, if desired. The combination therapy of the invention may enable lower doses of bendamustine to be used while retaining therapeutic efficacy, thereby allowing the bendamustine regimen to be repeated more frequently, such as every week or every two weeks. The bendamustine can be administered for any desired number of cycles, *e.g.*, 1-20 cycles, preferably 3-15 cycles, more preferably 5-10 cycles.

The term “comprising” encompasses “including” as well as “consisting”. For example, a composition “comprising” X may consist exclusively of X or may include something additional, *e.g.*, X + Y.

The word “substantially” does not exclude “completely” *e.g.*, a composition which is “substantially free” from Y may be completely free from Y. Where necessary, the word “substantially” may be omitted from the definition of the invention.

The term “about” in relation to a numerical value *x* means, for example, $x \pm 10\%$.

Various aspects and embodiments of the present invention will now be described in more detail by way of example only. It will be appreciated that modification of detail may be made without departing from the scope of the invention.

EXPERIMENTAL

The anti-CD40 antibody used in the examples below is the monoclonal antibody HCD122 (formerly known as CHIR-12.12). The production, sequencing and characterization of HCD122 has already been described. The pharmacology of HCD122 has been studied in a variety of *in vitro*, *ex vivo*, and *in vivo* systems to support its use as an investigational drug in B-cell malignancies (Luqman *et al.* (2008) *Blood* 112(3):117-120). HCD122 is a potent CD40 antagonist that blocks CD40L-induced proliferation and survival. HCD122 prevents binding of CD40L to cell surface CD40 and is able to compete with unbound, soluble CD40L. HCD122 does not internalize upon binding cell surface, human CD40. When HCD122 was evaluated for its ability to induce antibody-dependent cell-mediated cytotoxicity (ADCC), the potency and maximal amount of cell lysis induced by HCD122 was greater than that observed for the anti-CD20 antibody,

rituximab, in both primary NHL tumor cells and cell lines (Daudi, Namalwa). Thus, HCD122 is anticipated to have activity against lymphoid malignancies by blocking the CD40/CD40L activation pathway and via ADCC.

5 Example 1: Determination of MTD for Bendamustine in SCID Mice

This experiment was designed to establish the maximum tolerated dose (MTD) for bendamustine in SCID mice. Two regimens were tested: once daily for two days (which is the clinical dose regimen) and once daily for 5 days to provide a more sustained dose regimen. In this study, twenty CR female CB.17 SCID mice, ages 6 to 10 weeks, were administered bendamustine in saline intravenously at a dose of 10 mg/kg daily for 2 days (N=5), 20 mg/kg daily for 2 days (N=5), 30 mg/kg daily for 2 days (N=5), or 15 mg/kg daily for 5 days (N=5). Each animal was monitored for weight loss over the 14-day study period during the active dosing phase.

The MTD of > 15% body weight loss was not reached, but the once daily for 5 days regimen approached MTD. See Table 1 below.

Table 1. MTD Response Summary for Day 14 (Day 15, MTV on day 1 for Group 1)										
Group	N	Agent	mg/kg	Route	Dosing	BW Nadir	TR	NTR	NTRm	Mean Day of Death
1#	5	Bendmaustine	10	iv	qd x 2	-1.7% (15)	0	0	0	-
2	5	Bendamustine	20	iv	qd x 2	-7.6% (15)	0	0	0	-
3	5	Bendamustine	30	iv	qd x 2	-6.6% (9)	0	0	0	-
4	5	Bendamustine	15	iv	qd x 5	-11.5% (13)	0	0	0	-

- Control Group

BW Nadir – Lowest mean body weight for group (based on at least half of the remaining animals, expressed as % of initial mean body weight).

TR – Number of treatment related deaths.

NTR – Number of non-treatment-related deaths, due to accident/unknown etiology

5 NTRm – Number of non-treatment-related deaths due to metastasis.

Example 2: Dose-Ranging Efficacy Study of Bendamustine in the Human DLBCL Xenograft Models RL and SU-DHL-4 in SCID Mice

The Group 3 and Group 4 dosing regimens identified in Example 1 (i.e.,

10 bendamustine dosed iv at 30 mg/kg daily for 2 days and 15 mg/kg daily for 5 days) are tested for efficacy in the human xenograft models RL and SU-DHL-4 in SCID mice. The RL cell line (ATCC; CRL-2261) is a human B cell lymphoma cell line established from a 52 year old Caucasian male patient with NHL. The SU-DHL-4 cell line (DSMZ; ACC 495) is a human B cell lymphoma cell line established from the peritoneal effusion of a

15 38 year old man with B-NHL (diffuse large cell, cleaved cell type). These cell lines are both reported to be negative for the Epstein-Barr virus genome, in contrast to many of the common lymphoma cell lines used in the field. The use of cell lines that are positive for the Epstein-Barr virus may lead to problems when interpreting experimental data, due to influences on signalling by the oncogenic EBV in those cell lines. The RL and SU-DHL-

20 4 lymphoma cell lines are specifically chosen because they are EBV negative, which allows greater confidence that the results are indeed authentic.

Example 3: Combination Study of HCD122 Plus Bendamustine Human DLBCL Xenograft Models.

25 The human monoclonal antibody HCD122 and bendamustine have each shown anti-tumor efficacy in RL and SU-DHL-4 lymphoma models when used alone.

The anti-tumor activity of HCD122 is tested in RL DLBCL xenograft models in combination with bendamustine in CB17.SCID mice. HCD122 is administered at high (30 mg/kg) and low (10 mg/kg) dose levels in combination with the fully efficacious dose

30 of bendamustine (30 mg/kg, dosed once daily for two days), and compared to comparable dosing regimens for these single agents, to study HCD122 effects on tumor growth

delay. This study design is thought to best represent the clinical setting where bendamustine is given at the established efficacious dose level.

In this study, 10×10^6 RL cells are subcutaneously implanted with equal volume of MatrigelTM in the animals' midline thoracic vertebral region in a 200 μ l volume. The antibody administration is initiated when the mean tumor volume is 150-200 mm³ in size. HCD122, and the negative control human IgG1 antibody, are each administered by intraperitoneal injection (ip) on a once-a-week schedule, or bi-weekly (once every two weeks) and the length of treatment is 4 weeks, where the dose of HCD122 is 10 mg/kg or 30 mg/kg. HCD122 is administered alone (as a single agent) or in combination with bendamustine. The bendamustine is administered intravenously (iv), alone or in combination with HCD122 ip administration, at the efficacious dose of 30 mg/kg, once daily on day 1 and day 2 of the treatment period. For tumor volume measurements, length then width are measured with a digital caliper. The measurements are recorded twice a week once the tumors become visible. Tumor volumes and doubling times are calculated based on the formula, $\text{Volume} = L \times W^2/2$. The animals' weights are recorded and evaluated as a per group average.

All of the therapies significantly reduce tumor growth at day 25 when compared to treatment with huIgG1 control antibody. The observed tumor growth inhibition (TGI) with HCD122 plus bendamustine is improved over that observed with bendamustine alone or HCD122 alone. Tumor growth delay (time to reach tumor size of 500 mm³) is longer for the HCD122 plus bendamustine treatment group than for the groups receiving either agent alone, and reduction in tumor growth is greater in the treatment group receiving both HCD122 and bendamustine than in the single-agent groups.

In a second approach, HCD122 is combined with a sub-efficacious dose of bendamustine to reveal potential combination effects on tumor growth during the dosing period.

Example 4: Phase Ib Study of HCD122 Administered Intravenously in Combination with Bendamustine in Patients with CD40+ Follicular Lymphoma Who Are Refractory to Rituximab

Follicular lymphoma (FL) is the most common indolent non-Hodgkin's lymphoma (NHL) and the second most common form of NHL worldwide, comprising approximately 20% to 25% of all NHL cases in the United States and Europe (Salles (2007) "Clinical features, prognosis and treatment of follicular lymphoma," *Hematology Am. Soc. Hemtol. Educ. Program* 216-25. The incidence of FL is increasing, with over 24,000 new cases diagnosed worldwide each year (Friedberg (2008) *Semin. Hematol.* 45(Suppl. 2):S2-S6). The majority of patients diagnosed with FL are 50 years and older, present with widespread disease at diagnosis, and have a median survival of 8 to 15 years from initial diagnosis (Peterson *et al.* (2003) *J. Clin. Oncol.* 21(1):5-15; Swenson *et al.* (2005) *J. Clin. Oncol.* 23(22):5019-5026; Liu *et al.* (2006) *J. Clin. Oncol.* 24(10):1582-9).

Therapy options for symptomatic patients after initial diagnosis include chemotherapy, rituximab monotherapy, rituximab and chemotherapy, or radioimmunotherapy (National Comprehensive Cancer Network (NCCN), v1.2010, NCCN clinical practice guidelines in oncology, NCCN, Fort Washington, PA). A number of different chemotherapy agents and regimens are used for the initial treatment and include cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP); cyclophosphamide, vincristine, and prednisone (CVP); and fludarabine. Radioimmunotherapy with yttrium-90- labeled ibritumomab tiuxetan or iodine-131- labeled tositumomab has also been used in this setting. Upon clinical relapse or for nonresponsive disease, second-line treatment options include rituximab plus chemotherapy (e.g. R-CHOP, R-CVP), fludarabine-based chemotherapy, bendamustine, or high-dose chemotherapy with autologous or allogeneic stem-cell rescue.

The anti-CD20 monoclonal antibody, rituximab, has been a valuable addition to the treatment of B-cell NHL and has improved response rates in FL. However, 50% of patients with relapsed or refractory CD20-positive FL do not respond to initial therapy with rituximab, and approximately 60% of patients who were previously treated with rituximab no longer benefit from retreatment (Tay *et al.* (2010) *Blood Rev.* 24(2):69-82). The alkylating agent, bendamustine, was recently approved as a single agent for rituximab-refractory NHL, and is an effective treatment option for indolent B-cell NHLs that are refractory to rituximab (Friedberg *et al.* (2008) *J. Clin. Oncol.* 26:204-210;

Kahl *et al.* (2010) *Cancer* 116(1):106-114). In the first-line setting, preliminary studies with bendamustine plus rituximab demonstrate improved progression free survival (PFS) and complete response (CR) rates compared with R-CHOP (Rummel *et al.* (2009) *Blood* 114:168-169 (Abstract 405).

- 5 Although the introduction of these new agents and regimens for the treatment of NHL has resulted in improved CR rates and survival rates in some settings, the lack of any significant improvement in overall survival indicates a continued need for novel drugs and interventions (Tay *et al.* (2010) *Blood Rev.* 24(2):69-82). Also, few treatment options for FL patients who are refractory to rituximab-containing regimens are available.
- 10 Those that are available, such as radioimmunotherapies, have not been incorporated into broad clinical use. In particular, an unmet medical need exists for effective treatments for patients who are refractory to rituximab and have received at least one prior chemotherapeutic regimen.

- The purpose of this study is to assess the safety and preliminary efficacy of
- 15 combining HCD122, an antagonist anti-CD40 monoclonal antibody, with the alkylating agent, bendamustine, in patients who have confirmed CD40⁺ follicular lymphoma that is rituximab refractory, with the aim of meeting the unmet medical need of patients with rituximab-refractory FL. Without being bound by any theory or mechanism of action, it is hypothesized that by combining two agents with
- 20 complimentary, pro-apoptotic mechanisms of action, the addition of HCD122 to bendamustine therapy will result in a greater response rate with a more durable response in this population of patients than when either agent is used alone.

Study Design

- 25 This phase Ib, multicenter, open-label study will investigate the safety, pharmacokinetics, pharmacodynamics, and preliminary efficacy of HCD122 in combination with bendamustine in patients with documented CD40⁺ follicular lymphoma (FL) that is refractory to treatment with rituximab and who have received treatment with at least one prior chemotherapeutic regimen. The patient population is
- 30 adult patients (18 years of age or older) having this clinical presentation. For purposes

of this trial, "refractory" is defined as disease progression during rituximab treatment or disease progression within 6 months of the last rituximab dose.

In dose escalation (n=15-30), successive cohorts of newly enrolled patients receive increasing doses of HCD122 (2.0 mg/kg, 3.0 mg/kg, 4.0 mg/kg, up to 6.0 mg/kg) in combination with bendamustine (90 mg/m²) until the MTD of HCD122 is determined. Once the MTD is established, additional patients are enrolled into the dose expansion phase of the study to better characterize the safety, tolerability, and make a preliminary assessment of anti-tumor activity of the combination. A minimum of 20 total patients (escalation + expansion) are treated at the MTD.

DLTs that primarily contribute to the determination of the MTD are identified during cycle 1 of dose escalation. A DLT is defined as an adverse event (AE) or abnormal laboratory value assessed as clinically relevant and related to study treatment. Type, frequency, and severity of AEs are monitored during the dose escalation and dose expansion phases, as are changes in hematology and blood chemistry values, assessments of physical examinations, vital signs, and electrocardiograms. All patients remain on treatment until disease progression, unacceptable toxicity, or patient withdrawal.

Treatment Duration and Study Periods

The study is subdivided into four sequential periods, including screening (where patients having documented CD40⁺ follicular lymphoma are identified and enrolled), combination treatment, HCD122 maintenance treatment, and follow-up.

The combination treatment period begins on day 1 of cycle 1. During this period, HCD122 is administered in combination with bendamustine for 6 treatment cycles or until disease progression or study discontinuation. Continuation of the combination treatment period for up to 8 cycles may be considered. Treatment cycles are 28-days. During the 28-day cycles of the combination treatment period, both HCD122 and bendamustine are administered intravenously (iv). During cycle 1 of dose escalation, 90 mg/m² bendamustine is administered over 60 minutes on days 1 and 2; HCD122 is administered on days 3 and 15 at the assigned dose (estimated duration of HCD122 infusion is 2-6 hours). In all cycles of dose expansion and cycles 2-6 of dose escalation,

90 mg/m² bendamustine is administered iv over 60 minutes on days 1 and 2; HCD122 is administered on days 1 and 15 at the assigned dose (dose escalation phase) or at the MTD (dose expansion phase). On days that require infusions of both HCD122 and bendamustine, HCD122 is administered before bendamustine.

- 5 The HCD122 maintenance treatment period follows the combination treatment period for patients enrolled in the dose escalation and dose expansion phases of the trial. During this period, HCD122 is administered as a single agent bi-weekly (i.e., once every 14 days) on day 1 and day 15 of the maintenance treatment period, at the assigned dose (dose escalation phase) or MTD (dose expansion phase)
- 10 until disease progression or study discontinuation. A patient may be considered to begin the HCD122 maintenance treatment period prior to the completion of the sixth combination treatment cycle if the patient is unable to tolerate additional bendamustine infusions and remains eligible for the study.

- The end-of-treatment (EOT) visit occurs within seven days after the last
- 15 administration of either HCD122 or bendamustine. All participating patients must complete this visit even if they have had to discontinue prematurely. The follow-up period starts after the EOT visit and continues until the completion of all follow-up assessments. The visits and assessments that occur during the follow-up period may include assessment of AEs 28 days following the last dose of study treatment; and tumor
- 20 response assessments until disease progression or start of new anti-cancer therapy (every 3 months).

Assessment Types

- Throughout the study period, patients will be assessed for type, frequency, and
- 25 severity of AEs during dosing, and overall response rate (complete response, CR; partial response, PR, as defined by the Cheson response criteria (see, Cheson *et al.* (1999) *J. Clin. Oncol.* 17:1244-1253, also revised in Cheson *et al.* (2007) *J. Clin. Oncol.* 25:579-586, and further clarified in Cheson (2007) *Hematol. Oncol. Clin. N. Am.* 21:841-854)) to determine efficacy of combination therapy with HCD122 and bendamustine.

30

Many modifications and other embodiments of the inventions set forth herein will come to mind to one skilled in the art to which these inventions pertain having the benefit of the teachings presented in the foregoing descriptions and the associated drawings.

Therefore, it is to be understood that the inventions are not to be limited to the specific
5 embodiments disclosed and that modifications and other embodiments are intended to be included within the scope of appended claims. Although specific terms are employed herein, they are used in a generic and descriptive sense only and not for purposes of limitation.

All publications and patent applications cited herein are incorporated in full by
10 reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

SEQUENCE LISTING

<110> Baeck, Johan
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 Ewald, Brett
 Rediske, John
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 gct gtc cat cca gaa cca ccc act gca tgc aga gaa aaa cag tac cta 96
 Ala Val His Pro Glu Pro Pro Thr Ala Cys Arg Glu Lys Gln Tyr Leu
 20 25 30
 ata aac agt cag tgc tgt tct ttg tgc cag cca gga cag aaa ctg gtg 144
 Ile Asn Ser Gln Cys Cys Ser Leu Cys Gln Pro Gly Gln Lys Leu Val
 35 40 45
 agt gac tgc aca gag ttc act gaa acg gaa tgc ctt cct tgc ggt gaa 192
 Ser Asp Cys Thr Glu Phe Thr Glu Thr Glu Cys Leu Pro Cys Gly Glu
 50 55 60
 agc gaa ttc cta gac acc tgg aac aga gag aca cac tgc cac cag cac 240
 Ser Glu Phe Leu Asp Thr Trp Asn Arg Glu Thr His Cys His Gln His
 65 70 75 80
 aaa tac tgc gac ccc aac cta ggg ctt cgg gtc cag cag aag ggc acc 288
 Lys Tyr Cys Asp Pro Asn Leu Gly Leu Arg Val Gln Gln Lys Gly Thr
 85 90 95
 tca gaa aca gac acc atc tgc acc tgt gaa gaa ggc tgg cac tgt acg 336
 Ser Glu Thr Asp Thr Ile Cys Thr Cys Glu Glu Gly Trp His Cys Thr
 100 105 110
 agt gag gcc tgt gag agc tgt gtc ctg cac cgc tca tgc tgg ccc ggc 384
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 115 120 125

ttt ggg gtc aag cag att gct aca ggg gtt tct gat acc atc tgc gag 432
 Phe Gly Val Lys Gln Ile Ala Thr Gly Val Ser Asp Thr Ile Cys Glu
 130 135 140

ccc tgc cca gtc ggc ttc ttc tcc aat gtg tca tct gct ttc gaa aaa 480
 Pro Cys Pro Val Gly Phe Phe Ser Asn Val Ser Ser Ala Phe Glu Lys
 145 150 155 160

tgt cac cct tgg aca agg tcc cca gga tgc gct gag agc cct ggt ggt 528
 Cys His Pro Trp Thr Arg Ser Pro Gly Ser Ala Glu Ser Pro Gly Gly
 165 170 175

gat ccc cat cat ctt cgg gat cct gtt tgc cat cct ctt ggt gct ggt 576
 Asp Pro His His Leu Arg Asp Pro Val Cys His Pro Leu Gly Ala Gly
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<210> 7
 <211> 203
 <212> PRT
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 35 40 45
 Ser Asp Cys Thr Glu Phe Thr Glu Thr Glu Cys Leu Pro Cys Gly Glu
 50 55 60
 Ser Glu Phe Leu Asp Thr Trp Asn Arg Glu Thr His Cys His Gln His
 65 70 75 80
 Lys Tyr Cys Asp Pro Asn Leu Gly Leu Arg Val Gln Gln Lys Gly Thr
 85 90 95
 Ser Glu Thr Asp Thr Ile Cys Thr Cys Glu Glu Gly Trp His Cys Thr
 100 105 110
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 115 120 125
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 130 135 140
 Pro Cys Pro Val Gly Phe Phe Ser Asn Val Ser Ser Ala Phe Glu Lys
 145 150 155 160
 Cys His Pro Trp Thr Arg Ser Pro Gly Ser Ala Glu Ser Pro Gly Gly
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<223> Coding sequence for long isoform of human CD40

<400> 8

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atg gtt cgt ctg cct ctg cag tgc gtc ctc tgg ggc tgc ttg ctg acc 48
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1 5 10 15

gct gtc cat cca gaa cca ccc act gca tgc aga gaa aaa cag tac cta 96
Ala Val His Pro Glu Pro Pro Thr Ala Cys Arg Glu Lys Gln Tyr Leu
20 25 30

ata aac agt cag tgc tgt tct ttg tgc cag cca gga cag aaa ctg gtg 144
Ile Asn Ser Gln Cys Cys Ser Leu Cys Gln Pro Gly Gln Lys Leu Val
35 40 45

agt gac tgc aca gag ttc act gaa acg gaa tgc ctt cct tgc ggt gaa 192
Ser Asp Cys Thr Glu Phe Thr Glu Thr Glu Cys Leu Pro Cys Gly Glu
50 55 60

agc gaa ttc cta gac acc tgg aac aga gag aca cac tgc cac cag cac 240
Ser Glu Phe Leu Asp Thr Trp Asn Arg Glu Thr His Cys His Gln His
65 70 75 80

aaa tac tgc gac ccc aac cta ggg ctt cgg gtc cag cag aag ggc acc 288
Lys Tyr Cys Asp Pro Asn Leu Gly Leu Arg Val Gln Gln Lys Gly Thr
85 90 95

tca gaa aca gac acc atc tgc acc tgt gaa gaa ggc tgg cac tgt acg 336
Ser Glu Thr Asp Thr Ile Cys Thr Cys Glu Glu Gly Trp His Cys Thr
100 105 110

agt gag gcc tgt gag agc tgt gtc ctg cac cgc tca tgc tcg ccc ggc 384
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115 120 125

ttt ggg gtc aag cag att gct aca ggg gtt tct gat acc atc tgc gag 432
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130 135 140

ccc tgc cca gtc ggc ttc ttc tcc aat gtg tca tct gct ttc gaa aaa 480
Pro Cys Pro Val Gly Phe Phe Ser Asn Val Ser Ser Ala Phe Glu Lys
145 150 155 160

tgt cac cct tgg aca agc tgt gag acc aaa gac ctg gtt gtg caa cag 528
Cys His Pro Trp Thr Ser Cys Glu Thr Lys Asp Leu Val Val Gln Gln
165 170 175

gca ggc aca aac aag act gat gtt gtc tgt ggt ccc cag gat cgg ctg 576
Ala Gly Thr Asn Lys Thr Asp Val Val Cys Gly Pro Gln Asp Arg Leu
180 185 190

aga gcc ctg gtg gtg atc ccc atc atc ttc ggg atc ctg ttt gcc atc 624
Arg Ala Leu Val Val Ile Pro Ile Ile Phe Gly Ile Leu Phe Ala Ile
195 200 205

ctc ttg gtg ctg gtc ttt atc aaa aag gtg gcc aag aag cca acc aat 672
Leu Leu Val Leu Val Phe Ile Lys Lys Val Ala Lys Lys Pro Thr Asn
210 215 220

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35    40    45  
Ser Asp Cys Thr Glu Phe Thr Glu Thr Glu Cys Leu Pro Cys Gly Glu  
50    55    60  
Ser Glu Phe Leu Asp Thr Trp Asn Arg Glu Thr His Cys His Gln His  
65    70    75    80  
Lys Tyr Cys Asp Pro Asn Leu Gly Leu Arg Val Gln Gln Lys Gly Thr  
85    90    95  
Ser Glu Thr Asp Thr Ile Cys Thr Cys Glu Glu Gly Trp His Cys Thr  
100   105  110  
Ser Glu Ala Cys Glu Ser Cys Val Leu His Arg Ser Cys Ser Pro Gly  
115   120  125  
Phe Gly Val Lys Gln Ile Ala Thr Gly Val Ser Asp Thr Ile Cys Glu  
130   135  140  
Pro Cys Pro Val Gly Phe Ser Asn Val Ser Ser Ala Phe Glu Lys  
145   150  155  160  
Cys His Pro Trp Thr Ser Cys Glu Thr Lys Asp Leu Val Val Gln Gln  
165   170  175  
Ala Gly Thr Asn Lys Thr Asp Val Val Cys Gly Pro Gln Asp Arg Leu  
180   185  190  
Arg Ala Leu Val Val Ile Pro Ile Phe Gly Ile Leu Phe Ala Ile  
195   200  205  
Leu Leu Val Leu Val Phe Ile Lys Lys Val Ala Lys Lys Pro Thr Asn  
210   215  220  
Lys Ala Pro His Pro Lys Gln Glu Pro Gln Glu Ile Asn Phe Pro Asp  
225   230  235  240  
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82

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<223> HCD122 light chain CDR2

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<210> 12

<211> 9

<212> PRT

<213> Artificial Sequence

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<223> HCD122 light chain CDR3

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<211> 10

<212> PRT

<213> Artificial Sequence

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<223> HCD122 heavy chain CDR1

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<211> 17

<212> PRT

<213> Artificial Sequence

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<223> HCD122 heavy chain CDR2

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<210> 15

<211> 11

<212> PRT
 <213> Artificial Sequence

<220>
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<210> 16
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 <212> PRT
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 35 40 45
 Pro Gln Val Leu Ile Ser Leu Gly Ser Asn Arg Ala Ser Gly Val Pro
 50 55 60
 Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
 65 70 75 80
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 100 105 110

<210> 17
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 20 25 30
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 35 40 45
 Pro Gln Val Leu Ile Ser Leu Gly Ser Asn Arg Ala Ser Gly Val Pro
 50 55 60
 Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
 65 70 75 80
 Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Met Gln Ala
 85 90 95
 Arg Gln Thr Pro Phe Thr Phe Gly Pro Gly Thr Lys Val Asp Ile Arg
 100 105 110
 Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu
 115 120 125
 Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe
 130 135 140

Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln
 145 150 155 160
 Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser
 165 170 175
 Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu
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 195 200 205
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<210> 18
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 35 40 45
 Ala Val Ile Ser Tyr Glu Glu Ser Asn Arg Tyr His Ala Asp Ser Val
 50 55 60
 Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Ile Thr Leu Tyr
 65 70 75 80
 Leu Gln Met Asn Ser Leu Arg Thr Glu Asp Thr Ala Val Tyr Tyr Cys
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 35 40 45
 Ala Val Ile Ser Tyr Glu Glu Ser Asn Arg Tyr His Ala Asp Ser Val
 50 55 60
 Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Ile Thr Leu Tyr
 65 70 75 80
 Leu Gln Met Asn Ser Leu Arg Thr Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95
 Ala Arg Asp Gly Gly Ile Ala Ala Pro Gly Pro Asp Tyr Trp Gly Gln
 100 105 110
 Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val

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      115              120              125
Phe Pro Leu Ala Pro Ala Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala
130              135              140
Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser
145              150              155              160
Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val
      165              170              175
Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro
      180              185              190
Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys
      195              200              205
Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Pro Lys Ser Cys Asp
210              215              220
Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly
225              230              235              240
Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
      245              250              255
Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
      260              265              270
Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
      275              280              285
Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg
      290              295              300
Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys
305              310              315              320
Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu
      325              330              335
Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
      340              345              350
Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu
      355              360              365
Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
      370              375              380
Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val
385              390              395              400
Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp
      405              410              415
Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His
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Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
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Gly Lys
450

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<210> 20

<211> 450

<212> PRT

<213> Artificial Sequence

<220>

<223> Variable and constant domains of HCD122 heavy chain variant

<400> 20

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      20              25              30
Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
      35              40              45
Ala Val Ile Ser Tyr Glu Glu Ser Asn Arg Tyr His Ala Asp Ser Val
      50              55              60
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Ile Thr Leu Tyr

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65					70					75				80	
Leu	Gln	Met	Asn	Ser	Leu	Arg	Thr	Glu	Asp	Thr	Ala	Val	Tyr	Tyr	Cys
				85					90					95	
Ala	Arg	Asp	Gly	Gly	Ile	Ala	Ala	Pro	Gly	Pro	Asp	Tyr	Trp	Gly	Gln
			100					105					110		
Gly	Thr	Leu	Val	Thr	Val	Ser	Ser	Ala	Ser	Thr	Lys	Gly	Pro	Ser	Val
			115					120				125			
Phe	Pro	Leu	Ala	Pro	Ser	Ser	Lys	Ser	Thr	Ser	Gly	Gly	Thr	Ala	Ala
			130				135				140				
Leu	Gly	Cys	Leu	Val	Lys	Asp	Tyr	Phe	Pro	Glu	Pro	Val	Thr	Val	Ser
145					150					155					160
Trp	Asn	Ser	Gly	Ala	Leu	Thr	Ser	Gly	Val	His	Thr	Phe	Pro	Ala	Val
				165					170					175	
Leu	Gln	Ser	Ser	Gly	Leu	Tyr	Ser	Leu	Ser	Ser	Val	Val	Thr	Val	Pro
			180					185					190		
Ser	Ser	Ser	Leu	Gly	Thr	Gln	Thr	Tyr	Ile	Cys	Asn	Val	Asn	His	Lys
			195				200					205			
Pro	Ser	Asn	Thr	Lys	Val	Asp	Lys	Arg	Val	Glu	Pro	Lys	Ser	Cys	Asp
			210			215					220				
Lys	Thr	His	Thr	Cys	Pro	Pro	Cys	Pro	Ala	Pro	Glu	Leu	Leu	Gly	Gly
225					230					235					240
Pro	Ser	Val	Phe	Leu	Phe	Pro	Pro	Lys	Pro	Lys	Asp	Thr	Leu	Met	Ile
				245					250					255	
Ser	Arg	Thr	Pro	Glu	Val	Thr	Cys	Val	Val	Val	Asp	Val	Ser	His	Glu
			260					265					270		
Asp	Pro	Glu	Val	Lys	Phe	Asn	Trp	Tyr	Val	Asp	Gly	Val	Glu	Val	His
			275				280					285			
Asn	Ala	Lys	Thr	Lys	Pro	Arg	Glu	Glu	Gln	Tyr	Asn	Ser	Thr	Tyr	Arg
			290			295					300				
Val	Val	Ser	Val	Leu	Thr	Val	Leu	His	Gln	Asp	Trp	Leu	Asn	Gly	Lys
305				310						315					320
Glu	Tyr	Lys	Cys	Lys	Val	Ser	Asn	Lys	Ala	Leu	Pro	Ala	Pro	Ile	Glu
				325					330					335	
Lys	Thr	Ile	Ser	Lys	Ala	Lys	Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val	Tyr
			340					345					350		
Thr	Leu	Pro	Pro	Ser	Arg	Glu	Glu	Met	Thr	Lys	Asn	Gln	Val	Ser	Leu
			355				360					365			
Thr	Cys	Leu	Val	Lys	Gly	Phe	Tyr	Pro	Ser	Asp	Ile	Ala	Val	Glu	Trp
			370			375					380				
Glu	Ser	Asn	Gly	Gln	Pro	Glu	Asn	Asn	Tyr	Lys	Thr	Thr	Pro	Pro	Val
385				390						395					400
Leu	Asp	Ser	Asp	Gly	Ser	Phe	Phe	Leu	Tyr	Ser	Lys	Leu	Thr	Val	Asp
				405					410				415		
Lys	Ser	Arg	Trp	Gln	Gln	Gly	Asn	Val	Phe	Ser	Cys	Ser	Val	Met	His
			420				425						430		
Glu	Ala	Leu	His	Asn	His	Tyr	Thr	Gln	Lys	Ser	Leu	Ser	Leu	Ser	Pro
			435				440					445			
Gly	Lys														
			450												

THAT WHICH IS CLAIMED:

1. A method for treating a human patient for a disease or condition associated with neoplastic B-cell growth, said method comprising administering to said patient bendamustine in combination with an anti-CD40 antibody, wherein said anti-
5 CD40 antibody is free of significant agonist activity when bound to CD40 antigen on the surface of human B-cells, and wherein said patient has previously been administered (i) the chimeric anti-CD20 monoclonal antibody rituximab, or (ii) the chimeric anti-CD20 monoclonal antibody rituximab and at least one prior chemotherapeutic regimen.
- 10 2. The method according to claim 1, wherein said disease or condition is refractory to therapy with (i) the chimeric anti-CD20 monoclonal antibody rituximab, or (iii) combination therapy with rituximab and said chemotherapeutic regimen.
3. The method according to claim 1, wherein said patient has relapsed after
15 therapy with (i) the chimeric anti-CD20 monoclonal antibody rituximab, or (iii) combination therapy with rituximab and said chemotherapeutic regimen.
4. The method according to claim 3, wherein said patient has relapsed after therapy with (i) CHOP, (ii) the chimeric anti-CD20 monoclonal antibody rituximab, or
20 (iii) combination therapy with CHOP and rituximab.
5. The method according to claim 1, wherein the bendamustine and the anti-CD40 antibody are administered to the patient at the same time.
- 25 6. The method according to claim 1, wherein the bendamustine and the anti-CD40 antibody are administered to the patient sequentially.
7. The method according to claim 6, wherein a first cycle of bendamustine therapy is administered to the patient before a first dose of an anti-CD40 antibody is
30 administered to the patient.

8. The method according to claim 6, wherein a first cycle of bendamustine therapy is administered to the patient after a first dose of an anti-CD40 antibody is administered to the patient.

5 9. The method according to claim 1, wherein multiple cycles of combination therapy with bendamustine and an anti-CD40 antibody are administered to the patient.

10 10. The method according to claim 9, wherein said multiple cycles are selected from the group consisting of 21-day cycles, 28-day cycles, 35-day cycles, and any combination thereof.

11. The method according to claim 10, wherein each of said multiple cycles is a 28-day cycle.

15 12. The method according to claim 10 or 11, wherein two cycles, three cycles, four cycles, five cycles, six cycles, seven cycles, or eight cycles of combination therapy are administered to the patient.

20 13. The method according to any of claims 10-12, wherein a therapeutically effective dose of bendamustine is administered on day 1 and day 2 of each of said cycles.

14. The method according to any of claims 10-13, wherein a first therapeutically effective dose of said anti-CD40 antibody is administered on day 1, day 2, or day 3 of each of said cycles.

25 15. The method according to any of claims 10-14, wherein a second therapeutically effective dose of said anti-CD40 antibody is administered on day 15 of each of said cycles.

30 16. The method according to any of claims 10-15, wherein said therapeutically effective dose of bendamustine is about 85 mg/m² to about 95 mg/m².

17. The method according to any of claims 10-16, wherein said therapeutically effective dose of anti-CD40 antibody is about 3.0 mg/kg to about 4.5 mg/kg.

5

18. The method according to any of claims 10-17, wherein the patient is administered a maintenance cycle of anti-CD40 antibody therapy upon completion of administration of said multiple cycles of combination therapy.

10 19. Use of (i) bendamustine and (ii) an anti-CD40 antibody in the manufacture of a medicament for treating a human patient for a disease or condition associated with neoplastic B-cell growth, wherein said anti-CD40 antibody is free of significant agonist activity when bound to CD40 antigen on the surface of human B-cells, and wherein said patient has previously been administered (i) the chimeric anti-CD20 monoclonal antibody
15 rituximab, or (ii) the chimeric anti-CD20 monoclonal antibody rituximab and at least one prior chemotherapeutic regimen.

20. Use of (i) bendamustine and (i) an anti-CD40 antibody in the manufacture of at least two separate medicaments for treating a human patient for a disease or
20 condition associated with neoplastic B-cell growth by combination therapy, wherein said anti-CD40 antibody is free of significant agonist activity when bound to CD40 antigen on the surface of human B-cells, and wherein said patient has previously been administered (i) the chimeric anti-CD20 monoclonal antibody rituximab, or (ii) the chimeric anti-CD20 monoclonal antibody rituximab and at least one prior chemotherapeutic regimen.

25

21. A kit for treating a human patient for a disease or condition associated with neoplastic B-cell growth, said kit comprising:

- (i) bendamustine; and
- (ii) an anti-CD40 antibody, wherein said anti-CD40 antibody is free of
30 significant agonist activity when bound to CD40 antigen on the surface of human B-cells.

22. A method, use, or kit of any preceding claim, wherein said disease or condition is selected from the group consisting of acute lymphoblastic leukemia (ALL), acute myelogenous leukemia (AML), chronic myelogenous leukemia (CML), chronic lymphocytic leukemia (CLL), prolymphocytic leukemia (PLL), small lymphocytic leukemia (SLL), diffuse small lymphocytic leukemia (DSLL), diffuse large B-cell lymphoma (DLBCL), hairy cell leukemia, non-Hodgkin's lymphomas, Hodgkin's disease, Epstein-Barr Virus (EBV) induced lymphomas, myelomas such as multiple myeloma, Waldenstrom's macroglobulinemia, heavy chain disease, mucosal associated lymphoid tissue lymphoma, monocytoid B cell lymphoma, splenic lymphoma, lymphomatoid granulomatosis, intravascular lymphomatosis, immunoblastic lymphomas, and AIDS-related lymphomas.

23. A method, use, or kit of claim 22, wherein said disease or condition is a non-Hodgkin's lymphoma.

24. A method, use, or kit of claim 23, wherein said non-Hodgkin's lymphoma is follicular lymphoma.

25. A method, use, or kit of any preceding claim, wherein said anti-CD40 antibody is a monoclonal antibody that binds domain 2 of human CD40 antigen.

26. A method, use, or kit of any preceding claim, wherein said anti-CD40 antibody is a monoclonal antibody that binds to an epitope comprising residues 82-87 of the human CD40 sequence shown in SEQ ID NO:7 or SEQ ID NO:9.

27. A method, use, or kit of any preceding claim, wherein said anti-CD40 antibody is selected from the group consisting of:

- a) the monoclonal antibody HCD122, produced by the hybridoma cell line deposited with the ATCC as Patent Deposit No. PTA-5543;
- b) an antibody comprising an amino acid sequence selected from the group consisting of the sequence shown in SEQ ID NO:2, the sequence shown in SEQ ID

NO:4, the sequence shown in SEQ ID NO:5, both the sequences shown in SEQ ID NO:2 and SEQ ID NO:4, and both the sequences shown in SEQ ID NO:2 and SEQ ID NO:5;

c) an antibody comprising an amino acid sequence selected from the group consisting of the sequence shown in SEQ ID NO:17, the sequence shown in SEQ ID NO:19, the sequence shown in SEQ ID NO:20, both the sequences shown in SEQ ID NO:17 and SEQ ID NO:19, and both the sequences shown in SEQ ID NO:17 and SEQ ID NO:20;

d) an antibody comprising an amino acid sequence selected from the group consisting of the sequence shown in SEQ ID NO:16, the sequence shown in SEQ ID NO:18, and both the sequences shown in SEQ ID NO:16 and SEQ ID NO:18;

i) an antibody comprising a light chain variable domain containing the complementarity determining region (CDR) residues of SEQ ID NO:2;

e) an antibody comprising a heavy chain variable domain containing the complementarity determining region (CDR) residues of SEQ ID NO:4;

f) an antibody comprising a light chain variable domain containing the complementarity determining region (CDR) residues of SEQ ID NO:2 and a heavy chain variable domain containing the complementarity determining region (CDR) residues of SEQ ID NO:4;

g) an antibody having an amino acid sequence encoded by a nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of the sequence shown in SEQ ID NO:1, the sequence shown in SEQ ID NO:3, and both the sequences shown in SEQ ID NO:1 and SEQ ID NO:3;

h) an antibody having a light chain variable domain (V_L) that comprises the amino acid sequence as shown in SEQ ID NO:10 for CDR-L1, the amino acid sequence as shown in SEQ ID NO:11 for CDR-L2, and the amino acid sequence as shown in SEQ ID NO:12 for CDR-L3;

i) an antibody having a heavy chain variable domain (V_H) that comprises the amino acid sequence as shown in SEQ ID NO:13 for CDR-H1, the amino acid sequence as shown in SEQ ID NO:14 for CDR-H2, and the amino acid sequence as shown in SEQ ID NO:15 for CDR-H3; and

j) an antibody having a light chain variable domain (V_L) that comprises the amino acid sequence as shown in SEQ ID NO:10 for CDR-L1, the amino acid sequence as shown in SEQ ID NO:11 for CDR-L2, and the amino acid sequence as shown in SEQ ID NO:12 for CDR-L3, and having a heavy chain variable domain (V_H)
5 that comprises the amino acid sequence as shown in SEQ ID NO:13 for CDR-H1, the amino acid sequence as shown in SEQ ID NO:14 for CDR-H2, and the amino acid sequence as shown in SEQ ID NO:15 for CDR-H3.

28. A method, use, or kit according to any of claims 1-27, wherein said anti-
10 CD40 antibody is obtained from a CHO cell containing one or more expression vectors encoding the antibody.

29. A method, use, or kit of any preceding claim, wherein said anti-CD40
antibody is the monoclonal antibody HCD122 (CHIR-12.12) produced by the hybridoma
15 cell line deposited with the ATCC as Patent Deposit No. PTA-5543.

30. A method, composition, use or kit of any of claims 1-29, wherein said
anti-CD40 antibody is an antigen-binding antibody fragment selected from the group
consisting of a Fab fragment, a $F(ab')_2$ fragment, and a Fv fragment, wherein the
20 fragment is free of significant agonist activity when bound to CD40 antigen on the
surface of human B-cells.

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2011/062584

A. CLASSIFICATION OF SUBJECT MATTER

INV. A61K39/395 A61K31/4184 A61P35/02
ADD. C07K16/28

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A61K C07K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EP0-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2007/053767 A1 (NOVARTIS AG [CH]; XOMA TECHNOLOGY LTD [US]; AUKERMAN SHARON LEA [US];) 10 May 2007 (2007-05-10) claims 38-39 page 9, line 11 - line 17 page 76, line 5	1-30
X	US 2007/098718 A1 (LONG LI [US] ET AL) 3 May 2007 (2007-05-03) claims 10,26 claims 19, 25 paragraph [0144] page 24, left-hand column, line 30 - line 31 ----- -/--	1-30

☒ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

10 February 2012

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INTERNATIONAL SEARCH REPORT

International application No

PCT/US2011/062584

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	FRIEDBERG ET AL: "Bendamustine in Patients With Rituximab-Refractory Indolent and Transformed Non-Hodgkin's Lymphoma: Results From a Phase II Multicenter, Single-Agent Study", JOURNAL OF CLINICAL ONCOLOGY, AMERICAN SOCIETY OF CLINICAL ONCOLOGY, US, vol. 26, no. 2, 10 January 2008 (2008-01-10), pages 204-210, XP008140662, ISSN: 0732-183X, DOI: 10.1200/JCO.2007.12.5070 the whole document -----	1-30
A	ROBINSON K SUE ET AL: "Phase II multicenter study of bendamustine plus rituximab in patients with relapsed indolent B-cell and mantle cell non-Hodgkin's lymphoma.", JOURNAL OF CLINICAL ONCOLOGY : OFFICIAL JOURNAL OF THE AMERICAN SOCIETY OF CLINICAL ONCOLOGY 20 SEP 2008 LNKD-PUBMED:18626004, vol. 26, no. 27, 20 September 2008 (2008-09-20), pages 4473-4479, XP002668734, ISSN: 1527-7755 the whole document -----	1-30

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US2011/062584

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US 2007098718	A1	03-05-2007	NONE