

METHODS OF TREATING CANCER WITH A COMBINATION OF A NONFUCOSYLATED ANTI-CD70 ANTIBODY AND A CD47 ANTAGONIST

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Patent Application No. 63/216,233, filed June 29, 2021 and U.S. Provisional Patent Application No. 63/318,920, filed March 11, 2022, both of which are incorporated herein by reference in their entirety.

REFERENCE TO SEQUENCE LISTING

[0002] A sequence listing designated 0070-00812PC_ST25.txt, and has a size of 15 kilobytes, is incorporated by reference.

TECHNICAL FIELD

[0003] The present invention relates to methods of treating cancer, such as myeloid malignancies including myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML), with a nonfucosylated anti-CD70 antibody in combination with a CD47 antagonist.

BACKGROUND

[0004] CD70 is a member of the tumor necrosis factor (TNF) family of cell membrane-bound and secreted molecules that are expressed by a variety of normal and malignant cell types. The primary amino acid (AA) sequence of CD70 predicts a transmembrane type II protein with its carboxyl terminus exposed to the outside of cells and its amino terminus found in the cytosolic side of the plasma membrane (Bowman et al., 1994, *J. Immunol.* 152:1756-61; Goodwin et al., 1993, *Cell* 73:447-56). Human CD70 is composed of a 20 AA cytoplasmic domain, an 18 AA transmembrane domain, and a 155 AA extracytoplasmic domain with two potential N-linked glycosylation sites (Bowman et al., supra; Goodwin et al., supra). Specific immunoprecipitation of radioisotope-labeled CD70-expressing cells by anti-CD70 antibodies yields polypeptides of 29 and 50 kDa (Goodwin et al., supra; Hintzen et al., 1994, *J. Immunol.* 152:1762-73). Based on its homology to TNF-alpha and TNF-beta, especially in

structural strands C, D, H and I, a trimeric structure is predicted for CD70 (Petsch et al., 1995, *Mol. Immunol.* 32:761-72).

[0005] Original immunohistological studies revealed that CD70 is expressed on germinal center B cells and rare T cells in tonsils, skin, and gut (Hintzen et al., 1994, *Int. Immunol.* 6:477-80). Subsequently, CD70 was reported to be expressed on the cell surface of recently antigen-activated T and B lymphocytes, and its expression wanes after the removal of antigenic stimulation (Lens et al, 1996, *Eur. J. Immunol.* 26:2964-71; Lens et al., 1997, *Immunology* 90:38-45). Within the lymphoid system, activated natural killer cells (Orengo et al., 1997, *Clin. Exp. Immunol.* 107:608-13) and mouse mature peripheral dendritic cells (Akiba et al., 2000, *J. Exp. Med.* 191:375-80) also express CD70. In non-lymphoid lineages, CD70 has been detected on thymic medullary epithelial cells (Hintzen et al., 1994, *supra*; Hishima et al., 2000, *Am. J. Surg Pathol.* 24:742-46).

[0006] CD70 is not expressed on normal non-hematopoietic cells. CD70 expression is mostly restricted to recently antigen-activated T and B cells under physiological conditions, and its expression is down-regulated when antigenic stimulation ceases. Evidence from animal models suggests that CD70 may contribute to immunological disorders such as, e.g., rheumatoid arthritis (Brugnoni et al., 1997, *Immunol. Lett.* 55:99-104), psoriatic arthritis (Brugnoni et al., 1997, *Immunol. Lett.* 55:99-104), and lupus (Oelke et al., 2004, *Arthritis Rheum.* 50:1850-60). In addition to its potential role in inflammatory responses, CD70 is also expressed on a variety of transformed cells including lymphoma B cells, Hodgkin's and Reed-Sternberg cells, malignant cells of neural origin, and a number of carcinomas. Studies have shown that stem cells from acute myeloid leukemia (AML) and myelodysplastic disease (MDS) patients express both CD70 and its receptor, CD27. Interactions between this ligand-receptor pair may promote leukemia blast survival and proliferation.

[0007] Monoclonal antibodies produced in mammalian host cells can have a variety of post-translational modifications, including glycosylation. Monoclonal antibodies, such as IgG1s, have an N-linked glycosylation site at asparagine 297 (Asn297) of each heavy chain (two per intact antibody). The glycans attached to Asn297 on antibodies are typically complex biantennary structures with very low or no bisecting N-acetylglucosamine (bisecting GlcNAc) with low amounts of terminal sialic acid and variable amounts of galactose. The glycans also usually have

high levels of core fucosylation. Reduction of core fucosylation in antibodies has been shown to alter Fc effector functions, in particular Fcγ receptor binding and ADCC activity. This observation has led to interest in the engineering cell lines so they produce antibodies with reduced core fucosylation.

[0008] Methods for engineering cell lines to reduce core fucosylation include gene knock-outs, gene knock-ins and RNA interference (RNAi). In gene knock-outs, the gene encoding FUT8 (alpha 1,6-fucosyltransferase enzyme) is inactivated. FUT8 catalyzes the transfer of a fucosyl residue from GDP-fucose to position 6 of Asn-linked (N-linked) GlcNAc of an N-glycan. FUT8 is reported to be the only enzyme responsible for adding fucose to the N-linked biantennary carbohydrate at Asn297. Gene knock-ins add genes encoding enzymes such as GNTIII or a golgi alpha mannosidase II. An increase in the levels of such enzymes in cells diverts monoclonal antibodies from the fucosylation pathway (leading to decreased core fucosylation), and having increased amount of bisecting N-acetylglucosamines. RNAi typically also targets FUT8 gene expression, leading to decreased mRNA transcript levels or knock out gene expression entirely.

[0009] Alternatives to engineering cell lines include the use of small molecule inhibitors that act on enzymes in the glycosylation pathway. Inhibitors such as catanospermine act early in the glycosylation pathway, producing antibodies with immature glycans (e.g., high levels of mannose) and low fucosylation levels. Antibodies produced by such methods generally lack the complex N-linked glycan structure associated with mature antibodies. Small molecule fucose analogs can also be used to generate recombinant antibodies that have complex N-linked glycans, but have reduced core fucosylation.

[0010] Cluster of Differentiation 47 (CD47), also known as integrin associated protein (IAP), is a transmembrane receptor belonging to the immunoglobulin superfamily of proteins. CD47 is ubiquitously expressed on cells and serves as a marker for self-recognition, preventing phagocytosis by serving as a “don’t eat me” signal. CD47 mediates its effects through interactions with several other proteins, including thrombospondin (TSP) and signal regulatory protein-alpha (SIRPα). The interaction between SIRPα on phagocytic cells and CD47 on target cells helps ensure that target cells do not become engulfed. Certain cancers co-opt the CD47-

based immune evasion mechanism of a cell by increasing expression of CD47 on the cell surface of the cancer cell, thus avoiding clearance by the immune system.

[0011] There is a need for improved therapies for treating cancers associated with CD70 expression. Provided herein are methods of treating cancer, such as cancers that express CD70, with a combination of anti-CD70 antibodies, such as anti-CD70 antibodies with reduced core fucosylation that can exert a clinically useful cytotoxic, cytostatic, or immunomodulatory effect on CD70-expressing cells, particularly without exerting undesirable effects on non-CD70-expressing cells, and agents that antagonize CD47. In particular, provided herein are methods of treating myeloid malignancies, including Acute Myeloid leukemia (AML), Myeloproliferative disorders (MPDS), myelodysplastic syndrome (MDS) and myelodysplastic/myeloproliferative syndromes, which are all clonal stem-cell (HSC) or progenitor malignant disorders (TIU et al., *Leukemia*, vol. 21(8), p: 1648-57, 2007).

[0012] MDS encompasses multiple subtypes, including MDS with single-lineage dysplasia, MDS with ring sideroblasts, MDS with multilineage dysplasia, MDS with excess blasts, MDS with isolated del(5q), and MDS, unclassifiable (ARBER et al., *Blood*, vol. 127, p: 2391-405, 2016). MDS is characterized by ineffective hematopoiesis in one or more of the lineage of the bone marrow. Early MDS mostly demonstrates excessive apoptosis and hematopoietic cell dysplasia (CLAESSENS et al., *Blood*, vol. 99, p: 1594-601, 2002; CLASESENS et al., *Blood*, vol. 105, p: 4035-42, 2005). In about a third of MDS patients, this ineffective hematopoiesis precedes progression to secondary AML (sAML). Although some molecular events associated with specific MDS subtypes (ELBERT et al., *Nature*, vol. 451(7176), p: 335-9, 2008) or disease transformation (BRAUN et al., *Blood*, vol. 107(3), p: 1156-65, 2006) have been identified, the underlying molecular defects are still poorly understood. No biological markers, except morphological features, are currently available for early diagnosis and prognosis.

[0013] Acute myeloid leukemia (AML) is a malignant tumor of the myeloid lineage of white blood cells. This blood stasis formation is usually fatal blood and bone marrow disease within weeks to months if left untreated. There are 30,000 AMLs in the United States and 47,000 AML estimates in the European Union (2010 prevalence data confirmed by Mattson-Jack, 2010). AML is the most prevalent form of adult acute leukemia (about 90%) and contains about 33% of

new leukemia cases. The median age of patients diagnosed with AML was 67 years. In the United States, AML accounts for approximately 1.2% of cancer deaths.

[0014] AML causes non-specific symptoms such as weight loss, fatigue, fever and night sweats. AML is diagnosed by blood tests, bone marrow tests, and laboratory tests to determine AML subtypes and to determine treatment decisions.

[0015] All references cited herein, including patent applications, patent publications, and scientific literature, are herein incorporated by reference in their entirety, as if each individual reference were specifically and individually indicated to be incorporated by reference.

SUMMARY

[0016] Provided herein is a method of treating a cancer in a subject, the method comprising administering to the subject a nonfucosylated anti-CD70 antibody and a CD47 antagonist, wherein the method results in a depletion of cancer cells in the subject, wherein the method does not result in a depletion of CD70+ T regulatory cells (CD70+ Tregs) in the subject, wherein the anti-CD70 antibody comprises a heavy chain variable region, a light chain variable region and an Fc domain, wherein the heavy chain variable region comprises:

- (i) a CDR-H1 comprising the amino acid sequence of SEQ ID NO:8;
- (ii) a CDR-H2 comprising the amino acid sequence of SEQ ID NO:9; and
- (iii) a CDR-H3 comprising the amino acid sequence of SEQ ID NO:10; and

wherein the light chain variable region comprises:

- (i) a CDR-L1 comprising the amino acid sequence of SEQ ID NO:11;
- (ii) a CDR-L2 comprising the amino acid sequence of SEQ ID NO:12; and
- (iii) a CDR-L3 comprising the amino acid sequence of SEQ ID NO:13, wherein the

cancer is selected from the group consisting of myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML). In some embodiments, the anti-CD70 antibody comprises a heavy chain variable region comprising an amino acid sequence at least 85% identical to the amino acid sequence of SEQ ID NO:1 and a light chain variable region comprising an amino acid sequence at least 85% identical to the amino acid sequence of SEQ ID NO:2. In some embodiments, the anti-CD70 antibody comprises a heavy chain variable region comprising the amino acid

sequence of SEQ ID NO:1 and a light chain variable region comprising the amino acid sequence of SEQ ID NO:2. In some embodiments, the Fc domain of the anti-CD70 antibody is an antibody effector domain mediating one or more of antibody-dependent cellular cytotoxicity (ADCC), antibody-dependent cellular phagocytosis (ADCP), and complement-dependent cellular cytotoxicity (CDC). In some embodiments, the Fc domain of the anti-CD70 antibody is an antibody effector domain mediating ADCC. In some embodiments, the Fc domain of the anti-CD70 antibody is a human Fc domain. In some embodiments, the anti-CD70 antibody is a nonfucosylated form of vorsetuzumab. In some embodiments, the anti-CD70 antibody is conjugated to a therapeutic agent. In some embodiments, the therapeutic agent is a chemotherapeutic agent or an immunomodulatory agent. In some embodiments, the therapeutic agent is a chemotherapeutic agent. In some embodiments, the chemotherapeutic agent is monomethyl auristatin E (MMAE) or monomethyl auristatin F (MMAF). In some embodiments, the therapeutic agent is an immunomodulatory agent. In some embodiments, the method comprises administering a population of anti-CD70 antibodies, wherein each antibody in the population of anti-CD70 antibodies comprises a heavy chain variable region, a light chain variable region, and an Fc domain, wherein the heavy chain variable region comprises:

- (i) a CDR-H1 comprising the amino acid sequence of SEQ ID NO:8;
- (ii) a CDR-H2 comprising the amino acid sequence of SEQ ID NO:9; and
- (iii) a CDR-H3 comprising the amino acid sequence of SEQ ID NO:10; and

wherein the light chain variable region comprises:

- (i) a CDR-L1 comprising the amino acid sequence of SEQ ID NO:11;
- (ii) a CDR-L2 comprising the amino acid sequence of SEQ ID NO:12; and
- (iii) a CDR-L3 comprising the amino acid sequence of SEQ ID NO:13, wherein at least

50% of the anti-CD70 antibodies in the population of the anti-CD70 antibodies lack core fucosylation. In some embodiments, at least 70% of the anti-CD70 antibodies in the population of the anti-CD70 antibodies lack core fucosylation. In some embodiments, at least 90% of the anti-CD70 antibodies in the population of the anti-CD70 antibodies lack core fucosylation. In some embodiments, the anti-CD70 antibody is administered at a dose of about 1-30 mg/kg of the subject's body weight. In some embodiments, the anti-CD70 antibody is administered at a dose of about 10-20 mg/kg of the subject's body weight. In some embodiments, the anti-CD70 antibody is administered at a dose of about 10 mg/kg of the subject's body weight. In some

embodiments, the anti-CD70 antibody is administered at a dose of about 15 mg/kg of the subject's body weight. In some embodiments, the anti-CD70 antibody is administered at a dose of about 20 mg/kg of the subject's body weight. In some embodiments, the anti-CD70 antibody is administered once about every 1-4 weeks. In some embodiments, the anti-CD70 antibody is administered once about every 2 weeks. In some embodiments, the CD47 antagonist inhibits the interaction between CD47 and SIRP α . In some embodiments, the CD47 antagonist increases phagocytosis of tumor cells. In some embodiments, the CD47 antagonist is selected from the group consisting of an antibody, or antigen-binding fragment thereof, that binds to CD47, and antibody or antigen-binding fragment thereof, that binds to SIRP α , and a fusion protein comprising SIRP α , or a fragment thereof, and an antibody, or fragment thereof. In some embodiments, the fusion protein comprising SIRP α , or a fragment thereof, and an antibody, or fragment thereof, comprises SIRP α , or the immunoglobulin V-like domain thereof, covalently linked to the Fc region of an antibody. In some embodiments, the CD47 antagonist is an IgG1 or IgG4 antibody. In some embodiments, the CD47 antagonist is selected from the group consisting of magrolimab, CC-90002, ALX148, RRx-001, TTI-622, TTI-621, and KWAR23. In some embodiments, the CD47 antagonist is magrolimab. In some embodiments, the CD47 antagonist is administered at a dose of 1-50 mg/kg of the subject's body weight. In some embodiments, the CD47 antagonist is administered at a dose of 1-30 mg/kg of the subject's body weight. In some embodiments, the CD47 antagonist is administered at a dose of 1 mg/kg of the subject's body weight. In some embodiments, the CD47 antagonist is administered at a dose of 15 mg/kg of the subject's body weight. In some embodiments, the CD47 antagonist is administered at a dose of 30 mg/kg of the subject's body weight. In some embodiments, the CD47 antagonist is administered at a sub-optimal dose. In some embodiments, the CD47 antagonist is administered once about every 1-4 weeks. In some embodiments, the CD47 antagonist is administered once about every week. In some embodiments, the CD47 antagonist is administered once about every 2 weeks. In some embodiments, the CD47 antagonist is initially administered on days 1, 4, 8, 11, 15, and 22 of a first four-week cycle. In some embodiments, the CD47 antagonist is administered on days 1, 8, 15, and 22 of a second four-week cycle. In some embodiments, the CD47 antagonist is administered on days 1 and 15 of a third four-week cycle. In some embodiments, the cancer is MDS. In some embodiments, the MDS is relapsed or refractory MDS. In some embodiments, the subject experienced treatment failure after prior hypomethylating agent

(HMA) therapy for the MDS. In some embodiments, the cancer is AML. In some embodiments, the AML is relapsed or refractory AML. In some embodiments, the subject received 2 prior treatment regimens to treat the AML. In some embodiments, the subject received 3 prior treatment regimens to treat the AML. In some embodiments, at least about 0.1%, at least about 1%, at least about 2%, at least about 3%, at least about 4%, at least about 5%, at least about 6%, at least about 7%, at least about 8%, at least about 9%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 60%, at least about 70%, or at least about 80% of the cancer cells express CD70. In some embodiments, at least about 0.1%, at least about 1%, at least about 2%, at least about 3%, at least about 4%, at least about 5%, at least about 6%, at least about 7%, at least about 8%, at least about 9%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 60%, at least about 70%, or at least about 80% of the cancer cells express CD47. In some embodiments, administering the nonfucosylated anti-CD70 antibody and CD47 antagonist to the subject results in a depletion of cancer cells by at least about 5%, at least about 6%, at least about 7%, at least about 8%, at least about 9%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 95%, or about 100% compared to the amount of cancer cells before administering the nonfucosylated anti-CD70 antibody and CD47 antagonist to the subject. In some embodiments, administering the nonfucosylated anti-CD70 antibody and CD47 antagonist to the subject results in a depletion of CD70+ Tregs of no more than about 20%, about 10%, about 9%, about 8%, about 7%, about 6%, about 5%, about 4%, about 3%, about 2%, about 1%, or about 0.1% compared to the amount of CD70+ Tregs before administering the afucosylated anti-CD70 antibody and CD47 antagonist to the subject. In some embodiments, one or more therapeutic effects in the subject is improved after administration of the nonfucosylated anti-CD70 antibody and CD47 antagonist relative to a baseline. In some embodiments, the one or more therapeutic effects is selected from the group consisting of: objective response rate, duration of response, time to response, progression free survival and overall survival. In some embodiments, the objective response rate is at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least

about 45%, at least about 50%, at least about 60%, at least about 70%, or at least about 80%. In some embodiments, the subject exhibits progression-free survival of at least about 1 month, at least about 2 months, at least about 3 months, at least about 4 months, at least about 5 months, at least about 6 months, at least about 7 months, at least about 8 months, at least about 9 months, at least about 10 months, at least about 11 months, at least about 12 months, at least about eighteen months, at least about two years, at least about three years, at least about four years, or at least about five years after administration of the nonfucosylated anti-CD70 antibody and CD47 antagonist. In some embodiments, the subject exhibits overall survival of at least about 1 month, at least about 2 months, at least about 3 months, at least about 4 months, at least about 5 months, at least about 6 months, at least about 7 months, at least about 8 months, at least about 9 months, at least about 10 months, at least about 11 months, at least about 12 months, at least about eighteen months, at least about two years, at least about three years, at least about four years, or at least about five years after administration of the nonfucosylated anti-CD70 antibody and CD47 antagonist. In some embodiments, the duration of response to the anti-CD70 antibody and CD47 antagonist is at least about 1 month, at least about 2 months, at least about 3 months, at least about 4 months, at least about 5 months, at least about 6 months, at least about 7 months, at least about 8 months, at least about 9 months, at least about 10 months, at least about 11 months, at least about 12 months, at least about eighteen months, at least about two years, at least about three years, at least about four years, or at least about five years after administration of the nonfucosylated anti-CD70 antibody and CD47 antagonist. In some embodiments, the route of administration for the anti-CD70 antibody is intravenous. In some embodiments, the route of administration for the CD47 antagonist is intravenous. In some embodiments, the subject is a human. In some embodiments, the method further comprises the administration of azacitidine. In some embodiments, the azacitidine is administered at a dose of 75 mg/m² of the subject's body surface area. In some embodiments, the azacitidine is administered on days 1 to 7 of a 4-week cycle. In some embodiments, the azacitidine is administered on days 1 to 5 and 8 to 9 of a 4-week cycle. In some embodiments, the method further comprises the administration of venetoclax. In some embodiments, the method further comprises the administration of fluoroquinolone.

[0017] Also provided herein is a pharmaceutical composition for the treatment of cancer, the composition comprising a nonfucosylated anti-CD70 antibody, wherein the anti-CD70 antibody

comprises a heavy chain variable region, a light chain variable region, and an Fc domain, wherein the heavy chain variable region comprises:

- (i) a CDR-H1 comprising the amino acid sequence of SEQ ID NO:8;
- (ii) a CDR-H2 comprising the amino acid sequence of SEQ ID NO:9; and
- (iii) a CDR-H3 comprising the amino acid sequence of SEQ ID NO:10; and

wherein the light chain variable region comprises:

- (i) a CDR-L1 comprising the amino acid sequence of SEQ ID NO:11;
- (ii) a CDR-L2 comprising the amino acid sequence of SEQ ID NO:12; and
- (iii) a CDR-L3 comprising the amino acid sequence of SEQ ID NO:13, and at least one

pharmaceutically compatible ingredient, wherein the pharmaceutical composition is for use in combination with a CD47 antagonist, wherein the composition is for use in the method of any of the embodiments herein.

[0018] Also provided herein is a kit comprising a nonfucosylated anti-CD70 antibody and a CD47 antagonist, wherein the anti-CD70 antibody comprises a heavy chain variable region, a light chain variable region, and an Fc domain, wherein the heavy chain variable region comprises:

- (i) a CDR-H1 comprising the amino acid sequence of SEQ ID NO:8;
- (ii) a CDR-H2 comprising the amino acid sequence of SEQ ID NO:9; and
- (iii) a CDR-H3 comprising the amino acid sequence of SEQ ID NO:10; and

wherein the light chain variable region comprises:

- (i) a CDR-L1 comprising the amino acid sequence of SEQ ID NO:11;
- (ii) a CDR-L2 comprising the amino acid sequence of SEQ ID NO:12; and
- (iii) a CDR-L3 comprising the amino acid sequence of SEQ ID NO:13, and instructions

for using the anti-CD70 antibodies in the method of any of the embodiments herein.

[0019] It is to be understood that one, some, or all of the properties of the various embodiments described herein may be combined to form other embodiments of the present invention. These and other aspects of the invention will become apparent to one of skill in the art. These and other embodiments of the invention are further described by the detailed description that follows.

BRIEF DESCRIPTION OF THE DRAWINGS

[0020] FIG. 1 is a graph evaluating the effect of SEA-CD70 alone, h5F9-G4 (Magrolimab) alone, or SEA-CD70 in combination with h5F9-G4 on tumor growth in the MV4-11 AML xenograft model. Mean tumor volume ($\pm$ SEM) is reported for each treatment arm. For each treatment group, data are plotted until the first animal in each group was sacrificed for reaching a tumor size $>1000 \text{ mm}^3$.

[0021] FIG. 2 is a graph evaluating the effect of SEA-CD70 in combination with h5F9-G4 (Magrolimab) and azacitidine (Vidaza®) on tumor growth in the MV411 acute myeloid leukemia xenograft mouse model. Mean tumor volume ($\pm$ SEM) is reported for each treatment arm. For each single treatment group, data are plotted until the first animal in each group was sacrificed for reaching a tumor size $>750 \text{ mm}^3$.

DETAILED DESCRIPTION

I. Definitions

[0022] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art pertinent to the methods and compositions described. When trade names are used herein, applicants intend to independently include the trade name product formulation, the generic drug, and the active pharmaceutical ingredient(s) of the trade name product. As used herein, the following terms and phrases have the meanings ascribed to them unless specified otherwise.

[0023] The term "and/or" where used herein is to be taken as specific disclosure of each of the two specified features or components with or without the other. Thus, the term "and/or" as used in a phrase such as "A and/or B" herein is intended to include "A and B," "A or B," "A" (alone), and "B" (alone). Likewise, the term "and/or" as used in a phrase such as "A, B, and/or C" is intended to encompass each of the following aspects: A, B, and C; A, B, or C; A or C; A or B; B or C; A and C; A and B; B and C; A (alone); B (alone); and C (alone).

[0024] It is understood that aspects and embodiments of the invention described herein include "comprising," "consisting," and "consisting essentially of" aspects and embodiments.

[0025] Units, prefixes, and symbols are denoted in their Système International de Unites (SI) accepted form. Numeric ranges are inclusive of the numbers defining the range. The headings provided herein are not limitations of the various aspects of the disclosure, which can be had by reference to the specification as a whole. Accordingly, the terms defined immediately below are more fully defined by reference to the specification in its entirety.

[0026] The terms “CD70 binding agent” and “anti-CD70 binding agent” as used herein means an anti-CD70 antibody, a derivative or a fragment of an anti-CD70 antibody, or other agent that binds to CD70 and comprises at least one CDR or variable region of a CD70 binding antibody, or a derivative thereof.

[0027] The term “CD47” as used herein refers to the cell surface antigen CD47, which is a transmembrane protein ubiquitously expressed on a variety of normal cells and tumor cells. CD47 is a ligand for the immunoglobulin superfamily receptor SIRP α . CD47 is also referred to as “Antigenic surface determinant protein OA3”, “Integrin-associated protein (IAP)” and “Protein MER6”. The term CD47 as used herein is intended to encompass all polymorphic variants of the CD47 protein.

[0028] The term “SIRP α ” as used herein refers to “Signal-regulatory protein alpha”, which is also known as SHP substrate 1 (SHPS-1), Brain Ig-like molecule with tyrosine-based activation motifs (Bit), CD172 antigen-like family member A, Inhibitory receptor SHPS-1, Macrophage fusion receptor, MyD-1 antigen, SIRP α 1, SIRP α 2, SIRP α 3, p84, and CD172a. SIRP α is a member of the immunoglobulin superfamily and is a transmembrane protein expressed on phagocytic cells, including macrophages and dendritic cells. It is a receptor for CD47. The term SIRP α as used herein is intended to encompass all polymorphic variants of the SIRP α protein.

[0029] The term “SIRP α antibody molecule fusion protein” is intended to mean a fusion protein comprising the SIRP α protein or a fragment thereof and an antibody molecule. The antibody molecule may be a full-length antibody molecule as defined elsewhere herein, for example a full-length IgG antibody. Alternatively, the antibody molecule may be an antigen binding fragment of an antibody as defined elsewhere herein. The SIRP α protein or fragment thereof may be fused to the antibody molecule at any suitable location on the antibody molecule. For example, the SIRP α protein or fragment thereof may be fused to the N-terminus or C-terminus of the heavy chain or light chain of the antibody molecule. In certain embodiments, the

SIRP α antibody molecule fusion protein will not include the full-length SIRP α protein but will include a fragment thereof, particularly a fragment capable of binding to CD47. For example, the SIRP α antibody molecule fusion protein may include one or more copies of the SIRP α immunoglobulin V-like domain.

[0030] The term “specifically binds” means that the binding agent will react, in a highly selective manner, with its corresponding antigen and not with the multitude of other antigens (*e.g.*, non-CD70 molecules or non-CD47 molecules).

[0031] As used herein, the term “functional” in the context of a CD70 binding agent or CD47 binding agent indicates that the binding agent is capable of binding to CD70 or CD47.

[0032] The terms “inhibit” or “inhibition of” as used herein means to reduce by a measurable amount, or to prevent entirely.

[0033] The term “deplete” in the context of the effect of a CD70-binding agent on CD70-expressing cells refers to a reduction in the number of or elimination of the CD70-expressing cells. Similarly, the term “deplete” in the context of the effect of a CD47-binding agent on CD47-expressing cells refers to a reduction in the number of or elimination of the CD47-expressing cells.

[0034] “Intact antibodies” and “intact immunoglobulins” are defined herein as heterotetrameric glycoproteins, typically of about 150,000 daltons, composed of two identical light (L) chain and two identical heavy (H) chains. Each light chain is covalently linked to a heavy chain by a disulfide bond to form a heterodimer. The heterotetramer is formed by covalent disulfide linkage between the two identical heavy chains of such heterodimers. Although the light and heavy chains are linked together by a disulfide bond, the number of disulfide linkages between the two heavy chains varies by immunoglobulin (Ig) isotype. Each heavy and light chain also has regularly spaced intrachain disulfide bridges. Each heavy chain has at the amino-terminus a variable domain (V_H), followed by three or four constant domains (C_{H1} , C_{H2} , C_{H3} , and/or C_{H4}), as well as a hinge (J) region between C_{H1} and C_{H2} . Each light chain has two domains, an amino-terminal variable domain (V_L) and a carboxy-terminal constant domain (C_L). The V_L domain associates non-covalently with the V_H domain, whereas the C_L domain is commonly covalently linked to the C_{H1} domain via a disulfide bond. Particular amino

acid residues are believed to form an interface between the light and heavy chain variable domains (Chothia *et al.*, 1985, *J. Mol. Biol.* 186:651-663).

[0035] The term “hypervariable” refers to certain sequences within the variable domains that differ extensively in sequence among antibodies and contain residues that are directly involved in the binding and specificity of each particular antibody for its specific antigenic determinant. Hypervariability, both in the light chain and the heavy chain variable domains, is concentrated in three segments known as complementarity determining regions (CDRs) or hypervariable loops (HVLs). CDRs are defined by sequence comparison in Kabat *et al.*, 1991, In: Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, M.D., whereas HVLs are structurally defined according to the three-dimensional structure of the variable domain, as described by Chothia and Lesk, 1987, *J. Mol. Biol.* 196:901-917. Where these two methods result in slightly different identifications of a CDR, the structural definition is preferred. As defined by Kabat (*see* Kabat *et al.*, "Sequences of proteins of immunological interest, 5th ed., Pub. No. 91-3242, U.S. Dept. Health & Human Services, NIH, Bethesda, M.D., 1991), CDR-L1 is positioned at about residues 24-34, CDR-L2, at about residues 50-56, and CDR-L3, at about residues 89-97 in the light chain variable domain and at about 31-35 in CDR-H1, at about 50-65 in CDR-H2, and at about 95-102 in CDR-H3 in the heavy chain variable domain.

[0036] The three CDRs within each of the heavy and light chains are separated by framework regions (FRs), which contain sequences that tend to be less variable. From the amino terminus to the carboxy terminus of the heavy and light chain variable domains, the FRs and CDRs are arranged in the order: FR1, CDR1, FR2, CDR2, FR3, CDR3, and FR4. The largely β -sheet configuration of the FRs brings the CDRs within each of the chains to close proximity to each other as well as to the CDRs from the other chain. The resulting conformation contributes to the antigen binding site (*see* Kabat *et al.*, 1991, NIH Publ. No. 91-3242, Vol. I, pages 647-669), although not all CDR residues are necessarily directly involved in antigen binding.

[0037] FR residues and Ig constant domains typically are not directly involved in antigen binding, but can contribute to antigen binding or mediate antibody effector function. Some FR residues can have a significant effect on antigen binding in at least three ways: by noncovalently binding directly to an epitope, by interacting with one or more CDR residues, and by affecting

the interface between the heavy and light chains. The constant domains mediate various Ig effector functions, such as participation of the antibody in antibody dependent cellular cytotoxicity (ADCC), complement dependent cytotoxicity (CDC) and/or antibody dependent cellular phagocytosis (ADCP).

[0038] The light chains of vertebrate immunoglobulins are assigned to one of two clearly distinct classes, kappa (κ) and lambda (λ), based on the amino acid sequence of the constant domain. By comparison, the heavy chains of mammalian immunoglobulins are assigned to one of five major classes, according to the sequence of the constant domains: IgA, IgD, IgE, IgG, and IgM. IgG and IgA are 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 immunoglobulins are called α , δ , ϵ , γ , and μ , respectively. The subunit structures and three-dimensional configurations of the classes of native immunoglobulins are well known.

[0039] The terms “antibody”, “anti-CD70 antibody”, “humanized anti-CD70 antibody”, “variant humanized anti-CD70 antibody”, “anti-CD47 antibody”, “humanized anti-CD47 antibody”, and “variant humanized anti-CD47 antibody” are used herein in the broadest sense and specifically encompass full-length and native antibodies, monoclonal antibodies (including full-length monoclonal antibodies), polyclonal antibodies, multispecific antibodies (*e.g.*, bispecific antibodies), and antibody or antigen-binding fragments thereof, such as variable domains and other portions of antibodies that exhibit a desired biological activity, *e.g.*, CD70 binding or CD47 binding.

[0040] The term “monoclonal antibody” (mAb) refers to an antibody obtained from a population of substantially homogeneous antibodies; that is, the individual antibodies comprising the population are identical except for naturally occurring mutations that may be present in minor amounts. Monoclonal antibodies are highly specific, being directed against a single antigenic determinant, also referred to as an epitope. The modifier “monoclonal” is indicative of a substantially homogeneous population of antibodies directed to the identical epitope and is not to be construed as requiring production of the antibody by any particular method. Monoclonal antibodies can be made by any technique or methodology known in the art; for example, the hybridoma method first described by Köhler *et al.*, 1975, *Nature* 256:495, or recombinant DNA methods known in the art (*see, e.g.*, U.S. Patent No. 4,816,567). In another example, monoclonal

antibodies can also be isolated from phage antibody libraries, using techniques described in Clackson *et al.*, 1991, *Nature* 352: 624-628, and Marks *et al.*, 1991, *J. Mol. Biol.* 222:581-597.

[0041] In contrast, the antibodies in a preparation of polyclonal antibodies are typically a heterogeneous population of immunoglobulin isotypes and/or classes and also exhibit a variety of epitope specificity.

[0042] The term “chimeric” antibody, as used herein, is a type of monoclonal antibody in which a portion of or the complete amino acid sequence in one or more regions or domains of the heavy and/or light chain is identical with, homologous to, or a variant of the corresponding sequence in a monoclonal antibody from another species or belonging to another immunoglobulin class or isotype, or from a consensus sequence. Chimeric antibodies include fragments of such antibodies, provided that the antibody fragment exhibits the desired biological activity of its parent antibody, for example binding to the same epitope (*see, e.g.*, U.S. Patent No. 4,816,567; and Morrison *et al.*, 1984, *Proc. Natl. Acad. Sci. USA* 81:6851-6855). Methods for producing chimeric antibodies are known in the art. (*See, e.g.*, Morrison, 1985, *Science* 229:1202; Oi *et al.*, 1986, *BioTechniques* 4:214; Gillies *et al.*, 1989, *J. Immunol. Methods* 125:191-202; U.S. Patent Nos. 5,807,715; 4,816,567; and 4,816,397.)

[0043] The terms “antibody fragment”, “anti-CD70 antibody fragment”, “humanized anti-CD70 antibody fragment”, “variant humanized anti-CD70 antibody fragment”, “anti-CD47 antibody fragment”, “humanized anti-CD47 antibody fragment”, and “variant humanized anti-CD47 antibody fragment” refer to a portion of a full-length anti-CD70 antibody or anti-CD47 antibody in which a variable region or a functional capability is retained, for example, specific CD70 or CD47 epitope binding. Examples of antibody fragments include, but are not limited to, a Fab, Fab', F(ab')₂, Fd, Fv, scFv and scFv-Fc fragment, diabody, triabody, tetrabody, linear antibody, single-chain antibody, and other multispecific antibodies formed from antibody fragments. (*See* Holliger and Hudson, 2005, *Nat. Biotechnol.* 23:1126-1136.)

[0044] A “single-chain Fv” or “scFv” antibody fragment is a single chain Fv variant comprising the V_H and V_L domains of an antibody, in which the domains are present in a single polypeptide chain and which is capable of recognizing and binding antigen. The scFv polypeptide optionally contains a polypeptide linker positioned between the V_H and V_L domains that enables the scFv to form a desired three-dimensional structure for antigen binding (*see, e.g.*,

Pluckthun, 1994, In *The Pharmacology of Monoclonal Antibodies*, Vol. 113, Rosenberg and Moore eds., Springer-Verlag, New York, pp. 269-315).

[0045] The term “diabody” refers to small antibody fragment having two antigen-binding sites. Each fragment contains a heavy chain variable domain (V_H) concatenated to a light chain variable domain (V_L) to form a $V_H - V_L$ or $V_L - V_H$ polypeptide. By using a linker that is too short to allow pairing between the two domains on the same chain, the linked $V_H - V_L$ domains are forced to pair with complementary domains of another chain, creating two antigen-binding sites. Diabodies are described more fully, for example, in EP 404 097; WO 93/11161; and Hollinger *et al.*, 1993, *Proc. Natl. Acad. Sci. USA* 90:6444-6448.

[0046] The term “linear antibody” refers to antibodies that comprises a pair of tandem Fd segments ($V_H - C_H1 - V_H - C_H1$) that form a pair of antigen binding regions. Linear antibodies can be bispecific or monospecific, as described in Zapata *et al.*, 1995, *Protein Eng.* 8(10):1057-1062.

[0047] A “humanized antibody” refers to an immunoglobulin amino acid sequence variant or fragment thereof which is capable of binding to a predetermined antigen and which comprises a variable region polypeptide chain having framework regions having substantially the amino acid sequence of a human immunoglobulin and a CDR(s) having substantially the amino acid sequence of a non-human immunoglobulin.

[0048] Generally, a humanized antibody has one or more amino acid residues introduced into it from a source which is non-human. These non-human amino acid residues are referred to herein as “import” residues, which are typically taken from an “import” antibody domain, particularly a variable domain. An import residue, sequence, or antibody has a desired affinity and/or specificity, or other desirable antibody biological activity as discussed herein.

[0049] In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin and all or substantially all of the framework regions are those of a human immunoglobulin sequence, such as from, for example, a consensus or germline sequence. The humanized antibody optionally also will comprise at least a portion of an immunoglobulin Fc domain, typically that of a human immunoglobulin. For example, the antibody may contain both the light chain as well as at least the variable domain of a heavy

chain. The antibody also may include the C_{H1}, hinge (J), C_{H2}, C_{H3}, and/or C_{H4} regions of the heavy chain, as appropriate.

[0050] The humanized antibody can be selected from any class of immunoglobulins, including IgM, IgG, IgD, IgA and IgE, and any isotype, including IgG₁, IgG₂, IgG₃ and IgG₄. The constant region or domain can include, for example, a complement fixing constant domain where it is desired that the humanized antibody exhibit cytotoxic activity (*e.g.*, IgG₁). Where such cytotoxic activity is not desirable, the constant domain may be of another class (*e.g.*, IgG₂). The humanized antibody may comprise sequences from more than one class or isotype, and selecting particular constant domains to optimize desired effector functions is within the ordinary skill in the art.

[0051] The FR and CDR regions of the humanized antibody need not correspond precisely to the parental sequences, *e.g.*, the import CDR or the consensus FR may be altered by substitution, insertion or deletion of at least one residue so that the CDR or FR residue at that site does not correspond to either the consensus or the import antibody. Such mutations typically will not be extensive. Usually, at least 75% of the humanized antibody residues will correspond to those of the parental FR and CDR sequences, more often at least 90%, and most often greater than 95%.

[0052] The term “antibody effector function(s)” as used herein refers to a function contributed by an Fc domain(s) of an Ig. Such functions can be, for example, antibody-dependent cellular cytotoxicity, antibody-dependent cellular phagocytosis or complement-dependent cytotoxicity. Such function can be effected by, for example, binding of an Fc effector domain(s) to an Fc receptor on an immune cell with phagocytic or lytic activity or by binding of an Fc effector domain(s) to components of the complement system. Typically, the effect(s) mediated by the Fc-binding cells or complement components result in inhibition and/or depletion of the CD70 targeted cell. Without intending to be bound by any particular theory, Fc regions of antibodies can recruit Fc receptor (FcR)-expressing cells and juxtapose them with antibody-coated target cells. Cells expressing surface FcR for IgGs including FcγRIII (CD16), FcγRII (CD32) and FcγRIII (CD64) can act as effector cells for the destruction of IgG-coated cells. Such effector cells include monocytes, macrophages, natural killer (NK) cells, neutrophils and eosinophils. Engagement of FcγR by IgG activates antibody-dependent cellular cytotoxicity (ADCC) or antibody-dependent cellular phagocytosis (ADCP). ADCC is mediated by CD16+

effector cells through the secretion of membrane pore-forming proteins and proteases, while phagocytosis is mediated by CD32⁺ and CD64⁺ effector cells (*see Fundamental Immunology*, 4th ed., Paul ed., Lippincott-Raven, N.Y., 1997, Chapters 3, 17 and 30; Uchida *et al.*, 2004, *J. Exp. Med.* 199:1659-69; Akewanlop *et al.*, 2001, *Cancer Res.* 61:4061-65; Watanabe *et al.*, 1999, *Breast Cancer Res. Treat.* 53:199-207). In addition to ADCC and ADCP, Fc regions of cell-bound antibodies can also activate the complement classical pathway to elicit complement-dependent cytotoxicity (CDC). C1q of the complement system binds to the Fc regions of antibodies when they are complexed with antigens. Binding of C1q to cell-bound antibodies can initiate a cascade of events involving the proteolytic activation of C4 and C2 to generate the C3 convertase. Cleavage of C3 to C3b by C3 convertase enables the activation of terminal complement components including C5b, C6, C7, C8 and C9. Collectively, these proteins form membrane-attack complex pores on the antibody-coated cells. These pores disrupt the cell membrane integrity, killing the target cell (*see Immunobiology*, 6th ed., Janeway *et al.*, Garland Science, N. Y., 2005, Chapter 2).

[0053] The term “antibody-dependent cellular cytotoxicity”, or ADCC, is a mechanism for inducing cell death that depends upon the interaction of antibody-coated target cells with immune cells possessing lytic activity (also referred to as effector cells). Such effector cells include natural killer cells, monocytes/macrophages and neutrophils. The effector cells attach to an Fc effector domain(s) of Ig bound to target cells via their antigen-combining sites. Death of the antibody-coated target cell occurs as a result of effector cell activity.

[0054] The term “antibody-dependent cellular phagocytosis”, or ADCP, refers to the process by which antibody-coated cells are internalized, either in whole or in part, by phagocytic immune cells (*e.g.*, macrophages, neutrophils and dendritic cells) that bind to an Fc effector domain(s) of Ig.

[0055] The term “complement-dependent cytotoxicity”, or CDC, refers to a mechanism for inducing cell death in which an Fc effector domain(s) of a target-bound antibody activates a series of enzymatic reactions culminating in the formation of holes in the target cell membrane. Typically, antigen-antibody complexes such as those on antibody-coated target cells bind and activate complement component C1q which in turn activates the complement cascade leading to target cell death. Activation of complement may also result in deposition of complement

components on the target cell surface that facilitate ADCC by binding complement receptors (*e.g.*, CR3) on leukocytes.

[0056] “Immune cell” as used herein refers to a cell of hematopoietic lineage involved in regulating an immune response. In typical embodiments, an immune cell is a T lymphocyte, a B lymphocyte, an NK cell, a monocyte/macrophage, or a dendritic cell.

[0057] “Effector cell” as used herein refers to a cell that expresses a surface receptor for the Fc domain of an immunoglobulin (FcR). For example, cells that express surface FcR for IgGs including FcγRIII (CD16), FcγRII (CD32) and FcγRIII (CD64) can act as effector cells. Such effector cells include monocytes, macrophages, natural killer (NK) cells, neutrophils and eosinophils.

[0058] A “therapeutic agent” is an agent that exerts a cytotoxic, cytostatic, and/or immunomodulatory effect on cancer cells, activated immune cells or other target cell population. Examples of therapeutic agents include cytotoxic agents, chemotherapeutic agents, cytostatic agents, and immunomodulatory agents.

[0059] A “cytotoxic effect” refers to the depletion, elimination and/or the killing of a target cell. A “cytotoxic agent” refers to an agent that has a cytotoxic effect on a cell. The term is intended to include radioactive isotopes (such as I¹³¹, I¹²⁵, Y⁹⁰, and Re¹⁸⁶), chemotherapeutic agents, and toxins such as enzymatically active toxins of bacterial, fungal, plant, or animal origin, and fragments thereof. Such cytotoxic agents can be coupled to an antibody, *e.g.*, a humanized anti-CD70 antibody or anti-CD47 antibody, and used, for example, to treat a patient indicated for therapy with the antibody. In one embodiment, “cytotoxic agent” includes monoclonal antibodies, *e.g.*, antibodies used in combination with the humanized antibodies described herein.

[0060] A “chemotherapeutic agent” is a chemical compound useful in the treatment of cancer. Examples of chemotherapeutic agents include alkylating agents such as thiotepa and cyclophosphamide (CYTOXAN™); alkyl sulfonates such as busulfan, improsulfan, and piposulfan; aziridines such as benzodopa, carboquone, meturedopa, and uredopa; ethylenimines and methylamelamines including altretamine, triethylenemelamine, triethylenephosphoramide, triethylenethiophosphoramide, and trimethylolomelamine; acetogenins (especially bullatacin and bullatacinone); camptothecin (including the synthetic analogue topotecan); bryostatin;

callystatin; CC-1065 (including its adozelesin, carzelesin, and bizelesin synthetic analogues) and derivatives thereof; cryptophycines (particularly cryptophycin 1 and cryptophycin 8); dolastatin, auristatins (including analogues monomethyl-auristatin E and monomethyl-auristatin F (*see, e.g.*, U.S. Published Application No. 2005-0238649, published October 27, 2005, incorporated herein in its entirety); duocarmycin (including the synthetic analogues, KW-2189 and CBI-TMI); eleutherobin; pancratistatin; sarcodictyin; spongistatin; nitrogen mustards such as chlorambucil, chlornaphazine, cholophosphamide, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, melphalan, novembichin, phenesterine, prednimustine; trofosfamide, uracil mustard; nitrosureas such as carmustine, chlorozotocin, fotemustine, lomustine, nimustine, ranimustine; antibiotics such as the enediyne antibiotics (*e.g.*, calicheamicin, especially calicheamicin gammaII and calicheamicin phiII, *see for example, Agnew, Chem. Intl. Ed. Engl.*, 33:183-186; dynemicin, including dynemicin A; bisphosphonates, such as clodronate; esperamicin; as well as neocarzinostatin chromophore and related chromoprotein enediyne antibiotic chromomorphores), aclacinomysins, actinomycin, authramycin, azaserine, bleomycins, cactinomycin, carabycin, carminomycin, carzinophilin, chromomycins, dactinomycin, daunorubicin, detorubicin, 6-diazo-5-oxo-L-norleucine, doxorubicin (Adriamycin™) (including morpholino-doxorubicin, cyanomorpholino-doxorubicin, 2-pyrrolino-doxorubicin, and deoxydoxorubicin), epirubicin, esorubicin, idarubicin, marcellomycin, mitomycins such as mitomycin C, mycophenolic acid, nogalamycin, olivomycins, peplomycin, potfiromycin, puromycine, quelamycin, rodorubicin, streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin, zorubicin; anti-metabolites such as methotrexate and 5-fluorouracil (5-FU); folic acid analogues such as denopterin, methotrexate, pteropterin, trimetrexate; purine analogs such as fludarabine, 6-mercaptopurine, thiamiprine, thioguanine; pyrimidine analogs such as ancitabine, azacitidine, 6-azauridine, carmofur, cytarabine, dideoxyuridine, doxifluridine, enocitabine, floxuridine; androgens such as calusterone, dromostanolone propionate, epitioestanol, mepitioestane, testolactone; anti-adrenals such as aminoglutethimide, mitotane, trilostane; folic acid replenisher such as frolinic acid; aceglatone; aldophosphamide glycoside; aminolevulinic acid; eniluracil; amsacrine; bestrabucil; bisantrene; edatraxate; defofamine; democolcine; diaziquone; elfornithine; elliptinium acetate; an epothilone; etoglucid; gallium nitrate; hydroxyurea; lentinan; lonidamine; maytansinoids such as maytansine and ansamitocins; mitoguazone, mitoxantrone; mopidamol; nitracrine; pentostatin;

phenamet; pirarubicin; losoxantrone; podophyllinic acid; 2-ethylhydrazide; procarbazine; PSK[®]; razoxane; rhizoxin; sizofiran; spirogermanium; tenuazonic acid; triaziquone; 2,2',2''-trichlorotriethylamine; trichothecenes (especially T-2 toxin, verracurin A, roridin A and anguidine); urethan; vindesine; dacarbazine; mannomustine; mitabronitol; mitolactol; pipobroman; gacytosine; arabinoside ("Ara-C"); cyclophosphamide; thiotepa; taxoids, *e.g.*, paclitaxel (TAXOL[®], Bristol-Myers Squibb Oncology, Princeton, NJ) and doxetaxel (TAXOTERE[®], Rhône-Poulenc Rorer, Antony, France); chlorambucil; gemcitabine (Gemzar[™]); 6-thioguanine; mercaptopurine; methotrexate; platinum analogs such as cisplatin and carboplatin; vinblastine; platinum; etoposide (VP-16); ifosfamide; mitoxantrone; vincristine; vinorelbine (Navelbine[™]); novantrone; teniposide; edatrexate; daunomycin; aminopterin; xeloda; ibandronate; CPT-11; topoisomerase inhibitor RFS 2000; difluoromethylornithine (DMFO); retinoids such as retinoic acid; capecitabine; and pharmaceutically acceptable salts, acids, or derivatives of any of the above. Also included in this definition are anti-hormonal agents that act to regulate or inhibit hormone action on tumors such as anti-estrogens and selective estrogen receptor modulators (SERMs), including, for example, tamoxifen (including Nolvadex[™]), raloxifene, droloxifene, 4-hydroxytamoxifen, trioxifene, keoxifene, LY117018, onapristone, and toremifene (Fareston[™]); aromatase inhibitors that inhibit the enzyme aromatase, which regulates estrogen production in the adrenal glands, such as, for example, 4(5)-imidazoles, aminoglutethimide, megestrol acetate (Megace[™]), exemestane, formestane, fadrozole, vorozole (Rivisor[™]), letrozole (Femara[™]), and anastrozole (Arimidex[™]); and anti-androgens such as flutamide, nilutamide, bicalutamide, leuprolide, and goserelin; and pharmaceutically acceptable salts, acids, or derivatives of any of the above.

[0061] The term "prodrug" as used herein refers to a precursor or derivative form of a pharmaceutically active substance that is less cytotoxic to tumor cells compared to the parent drug and is capable of being enzymatically activated or converted into the more active parent form. *See*, for example, Wilman, 1986, "Prodrugs in Cancer Chemotherapy", In *Biochemical Society Transactions*, 14, pp. 375-382, 615th Meeting Belfast; and Stella *et al.*, 1985, "Prodrugs: A Chemical Approach to Targeted Drug Delivery, In: *Directed Drug Delivery*, Borchardt *et al.*, (ed.), pp. 247-267, Humana Press. Useful prodrugs include, but are not limited to, phosphate-containing prodrugs, thiophosphate-containing prodrugs, sulfate-containing prodrugs, peptide-containing prodrugs, D-amino acid-modified prodrugs, glycosylated prodrugs, β -lactam-

containing prodrugs, optionally substituted phenoxyacetamide-containing prodrugs, and optionally substituted phenylacetamide-containing prodrugs, 5-fluorocytosine and other 5-fluorouridine prodrugs that can be converted into the more active cytotoxic free drug. Examples of cytotoxic drugs that can be derivatized into a prodrug form include, but are not limited to, those chemotherapeutic agents described above.

[0062] A “cytostatic effect” refers to the inhibition of cell proliferation. A “cytostatic agent” refers to an agent that has a cytostatic effect on a cell, thereby inhibiting the growth and/or expansion of a specific subset of cells.

[0063] The term “immunomodulatory effect” as used herein refers to a stimulation (immunostimulatory) or inhibition (immunosuppressive) of the development or maintenance of an immunologic response. Inhibition can be effected by, for example, by elimination of immune cells (*e.g.*, T or B lymphocytes); induction or generation of immune cells that can modulate (*e.g.*, down-regulate) the functional capacity of other cells; induction of an unresponsive state in immune cells (*e.g.*, anergy); or increasing, decreasing or changing the activity or function of immune cells, including, for example, altering the pattern of proteins expressed by these cells (*e.g.*, altered production and/or secretion of certain classes of molecules such as cytokines, chemokines, growth factors, transcription factors, kinases, costimulatory molecules or other cell surface receptors, and the like). An “immunomodulatory agent” refers to an agent that has an immunomodulatory effect on a cell. In some embodiments, an immunomodulatory agent has a cytotoxic or cytostatic effect on an immune cell that promotes an immune response.

[0064] The term “label” refers to a detectable compound or composition that is conjugated directly or indirectly to the antibody. The label may itself be detectable (*e.g.*, radioisotope labels or fluorescent labels) or, in the case of an enzymatic label, may catalyze chemical alteration of a substrate compound or composition that is detectable. Labeled anti-CD70 antibody can be prepared and used in various applications including *in vitro* and *in vivo* diagnostics.

[0065] An “isolated” nucleic acid molecule is a nucleic acid molecule that is identified and separated from at least one contaminant nucleic acid molecule with which it is ordinarily associated in the natural source of the nucleic acid. An isolated nucleic acid molecule is other than in the form or setting in which it is found in nature. Isolated nucleic acid molecules therefore are distinguished from the nucleic acid molecule as it exists in natural cells. However,

an isolated nucleic acid molecule includes a nucleic acid molecule contained in cells that ordinarily express the antibody where, for example, the nucleic acid molecule is in a chromosomal location different from that of natural cells.

[0066] The term “control sequences” refers to polynucleotide sequences necessary for expression of an operably linked coding sequence in a particular host organism. The control sequences suitable for use in prokaryotic cells include, for example, promoter, operator, and ribosome binding site sequences. Eukaryotic control sequences include, but are not limited to, promoters, polyadenylation signals, and enhancers. These control sequences can be utilized for expression and production of anti-CD70 binding agent in prokaryotic and eukaryotic host cells.

[0067] A nucleic acid sequence is “operably linked” when it is placed into a functional relationship with another nucleic acid sequence. For example, a nucleic acid presequence or secretory leader is operably linked to a nucleic acid encoding a polypeptide if it is expressed as a preprotein that participates in the secretion of the polypeptide; a promoter or enhancer is operably linked to a coding sequence if it affects the transcription of the sequence; or a ribosome binding site is operably linked to a coding sequence if it is positioned so as to facilitate translation. Generally, “operably linked” means that the DNA sequences being linked are contiguous, and, in the case of a secretory leader, contiguous and in reading frame. However, enhancers are optionally contiguous. Linking can be accomplished by ligation at convenient restriction sites. If such sites do not exist, synthetic oligonucleotide adaptors or linkers can be used to link the DNA sequences.

[0068] The term “polypeptide” refers to a polymer of amino acids and its equivalent and does not refer to a specific length of a product; thus, “peptides” and “proteins” are included within the definition of a polypeptide. Also included within the definition of polypeptides are “antibodies” as defined herein. A “polypeptide region” refers to a segment of a polypeptide, which segment may contain, for example, one or more domains or motifs (*e.g.*, a polypeptide region of an antibody can contain, for example, one or more complementarity determining regions (CDRs)). The term “fragment” refers to a portion of a polypeptide typically having at least 20 contiguous or at least 50 contiguous amino acids of the polypeptide. A “derivative” is a polypeptide or fragment thereof having one or more non-conservative or conservative amino acid substitutions relative to a second polypeptide; or a polypeptide or fragment thereof that is

modified by covalent attachment of a second molecule such as, *e.g.*, by attachment of a heterologous polypeptide, or by glycosylation, acetylation, phosphorylation, and the like. Further included within the definition of “derivative” are, for example, polypeptides containing one or more analogs of an amino acid (*e.g.*, unnatural amino acids and the like), polypeptides with unsubstituted linkages, as well as other modifications known in the art, both naturally and non-naturally occurring.

[0069] An “isolated” polypeptide is one which has been identified and separated and/or recovered from a component of its natural environment. Contaminant components of its natural environment are materials which would interfere with diagnostic or therapeutic uses for the polypeptide, and may include enzymes, hormones, and other proteinaceous or nonproteinaceous solutes. An isolated polypeptide includes an isolated antibody, or a fragment or derivative thereof. “Antibody” includes the antibody *in situ* within recombinant cells since at least one component of the antibody's natural environment will not be present.

[0070] In certain embodiments, the antibody will be purified (1) to greater than 95% by weight of antibody as determined by the Lowry method, and in other aspects to more than 99% by weight, (2) to a degree sufficient to obtain at least 15 residues of N-terminal or internal amino acid sequence by use of a spinning cup sequenator, or (3) to homogeneity by SDS-PAGE under reducing or nonreducing conditions using Coomassie blue or, preferably, silver stain.

[0071] The term “heterologous,” in the context of a polypeptide, means from a different source (*e.g.*, a cell, tissue, organism, or species) as compared with another polypeptide, so that the two polypeptides are different. Typically, a heterologous polypeptide is from a different species.

[0072] In the context of immunoglobulin polypeptides or fragments thereof, “conservative substitution” means one or more amino acid substitutions that do not substantially reduce specific binding (*e.g.*, as measured by the K_D) of the immunoglobulin polypeptide or fragment thereof to an antigen (*i.e.*, substitutions that increase binding affinity, that do not significantly alter binding affinity, or that reduce binding affinity by no more than about 40%, typically no more than about 30%, more typically no more than about 20%, even more typically no more than about 10%, or most typically no more than about 5%, as determined by standard binding assays such as, *e.g.*, ELISA).

[0073] The terms “identical” or “percent identity,” in the context of two or more nucleic acids or polypeptide sequences, refer to two or more sequences or subsequences that are the same or have a specified percentage of nucleotides or amino acid residues that are the same, when compared and aligned for maximum correspondence. To determine the percent identity, the sequences are aligned for optimal comparison purposes (*e.g.*, gaps can be introduced in the sequence of a first amino acid or nucleic acid sequence for optimal alignment with a second amino or nucleic acid sequence). The amino acid residues or nucleotides at corresponding amino acid positions or nucleotide positions are then compared. When a position in the first sequence is occupied by the same amino acid residue or nucleotide as the corresponding position in the second sequence, then the molecules are identical at that position. The percent identity between the two sequences is a function of the number of identical positions shared by the sequences (*i.e.*, % identity = # of identical positions/total # of positions (*e.g.*, overlapping positions) x 100). In some embodiments, the two sequences are the same length.

[0074] The term “substantially identical,” in the context of two nucleic acids or polypeptides, refers to two or more sequences or subsequences that have at least 50%, at least 55%, at least 60%, or at least 65% identity; typically at least 70% or at least 75% identity; more typically at least 80% or at least 85% identity; and even more typically at least 90%, at least 95%, or at least 98% identity (*e.g.*, as determined using one of the methods set forth *infra*).

[0075] The terms “similarity” or “percent similarity” in the context of two or more polypeptide sequences refer to two or more sequences or subsequences that have a specified percentage of amino acid residues that are the same or conservatively substituted when compared and aligned for maximum correspondence, as measured using one of the methods set forth *infra*. By way of example, a first amino acid sequence can be considered similar to a second amino acid sequence when the first amino acid sequence is at least 50%, 60%, 70%, 75%, 80%, 90%, or 95% identical, or conservatively substituted, to the second amino acid sequence when compared to an equal number of amino acids as the number contained in the first sequence, or when compared to an alignment of polypeptides that has been aligned by, *e.g.*, one of the methods set forth *infra*.

[0076] The terms “substantial similarity” or “substantially similar,” in the context of polypeptide sequences, indicate that a polypeptide region has a sequence with at least 70%,

typically at least 80%, more typically at least 85%, or at least 90% or at least 95% sequence similarity to a reference sequence. For example, a polypeptide is substantially similar to a second polypeptide, for example, where the two peptides differ by one or more conservative substitution(s).

[0077] In the context of anti-CD70 antibodies, or derivatives thereof, a protein that has one or more polypeptide regions substantially identical or substantially similar to one or more antigen-binding regions (*e.g.*, a heavy or light chain variable region, or a heavy or light chain CDR) of an anti-CD70 antibody retains specific binding to an epitope of CD70 recognized by the anti-CD70 antibody, as determined using any of various standard immunoassays known in the art or as referred to herein.

[0078] In the context of anti-CD47 antibodies, or derivatives thereof, a protein that has one or more polypeptide regions substantially identical or substantially similar to one or more antigen-binding regions (*e.g.*, a heavy or light chain variable region, or a heavy or light chain CDR) of an anti-CD47 antibody retains specific binding to an epitope of CD47 recognized by the anti-CD47 antibody, as determined using any of various standard immunoassays known in the art or as referred to herein.

[0079] In the context of anti-SIRP α antibodies, or derivatives thereof, a protein that has one or more polypeptide regions substantially identical or substantially similar to one or more antigen-binding regions (*e.g.*, a heavy or light chain variable region, or a heavy or light chain CDR) of an anti-SIRP α antibody retains specific binding to an epitope of SIRP α recognized by the anti-SIRP α antibody, as determined using any of various standard immunoassays known in the art or as referred to herein.

[0080] The determination of percent identity or percent similarity between two sequences can be accomplished using a mathematical algorithm. A preferred, non-limiting example of a mathematical algorithm utilized for the comparison of two sequences is the algorithm of Karlin and Altschul, 1990, *Proc. Natl. Acad. Sci. USA* 87:2264-2268, modified as in Karlin and Altschul, 1993, *Proc. Natl. Acad. Sci. USA* 90:5873-5877. Such an algorithm is incorporated into the NBLAST and XBLAST programs of Altschul *et al.*, 1990, *J. Mol. Biol.* 215:403-410. BLAST nucleotide searches can be performed with the NBLAST program, score = 100, wordlength = 12, to obtain nucleotide sequences homologous to a nucleic acid encoding a

protein of interest. BLAST protein searches can be performed with the XBLAST program, score = 50, wordlength = 3, to obtain amino acid sequences homologous to protein of interest. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul *et al.*, 1997, *Nucleic Acids Res.* 25:3389-3402. Alternatively, PSI-Blast can be used to perform an iterated search which detects distant relationships between molecules (*Id.*). When utilizing BLAST, Gapped BLAST, and PSI-Blast programs, the default parameters of the respective programs (*e.g.*, XBLAST and NBLAST) can be used. Another non-limiting example of a mathematical algorithm utilized for the comparison of sequences is the algorithm of Myers and Miller, CABIOS (1989). Such an algorithm is incorporated into the ALIGN program (version 2.0) which is part of the GCG sequence alignment software package. When utilizing the ALIGN program for comparing amino acid sequences, a PAM120 weight residue table, a gap length penalty of 12, and a gap penalty of 4 can be used. Additional algorithms for sequence analysis are known in the art and include ADVANCE and ADAM as described in Torellis and Robotti, 1994, *Comput. Appl. Biosci.* 10:3-5; and FASTA described in Pearson and Lipman, 1988, *Proc. Natl. Acad. Sci. USA* 85:2444-8. Within FASTA, ktup is a control option that sets the sensitivity and speed of the search. If ktup=2, similar regions in the two sequences being compared are found by looking at pairs of aligned residues; if ktup=1, single aligned amino acids are examined. ktup can be set to 2 or 1 for protein sequences, or from 1 to 6 for DNA sequences. The default if ktup is not specified is 2 for proteins and 6 for DNA. Alternatively, protein sequence alignment may be carried out using the CLUSTAL W algorithm, as described by Higgins *et al.*, 1996, *Methods Enzymol.* 266:383-402.

[0081] As used herein, the expressions “cell”, “cell line”, and “cell culture” are used interchangeably and all such designations include the progeny thereof. Thus, “transformants” and “transformed cells” include the primary subject cell and cultures derived therefrom without regard for the number of transfers. It is also understood that all progeny may not be precisely identical in DNA content, due to deliberate or naturally occurring mutations. Mutant progeny that have the same function or biological activity as screened for in the originally transformed cell are included. Where distinct designations are intended, it will be clear from the context.

[0082] The term “subject” for purposes of treatment refers to any animal, particularly an animal classified as a mammal, including humans, domesticated and farm animals, and zoo,

sports, or pet animals, such as dogs, horses, cats, cows, and the like. Preferably, the subject is human.

[0083] A “disorder”, as used herein, and the terms “CD70-associated disorder” and “CD70-associated disease” refer to any condition that would benefit from treatment with an anti-CD70 binding agent, as described herein. A “CD70-associated disorder” and “CD70-associated disease” typically express CD70, or a fragment thereof, on the cell surface. This includes chronic and acute disorders or diseases including those pathological conditions that predispose the mammal to the disorder in question. Non-limiting examples of disorders to be treated herein include cancer, myeloid malignancies, hematological malignancies, benign and malignant tumors, leukemias and lymphoid malignancies, carcinomas, and inflammatory, angiogenic and immunologic disorders. Specific examples of disorders are disclosed *infra*. Similarly, the terms “CD47-associated disorder” and “CD47-associated disease” refer to any condition that would benefit from treatment with an anti-CD47 binding agent or other CD47 antagonist, as described herein. A “CD47-associated disorder” and “CD47-associated disease” typically express CD47, or a fragment thereof, on the cell surface. This includes chronic and acute disorders or diseases including those pathological conditions that predispose the mammal to the disorder in question. Non-limiting examples of disorders to be treated herein include cancer, myeloid malignancies, hematological malignancies, benign and malignant tumors, leukemias and lymphoid malignancies, carcinomas, and inflammatory, angiogenic and immunologic disorders. Specific examples of disorders are disclosed *infra*.

[0084] The terms “treatment” and “therapy”, and the like, as used herein, are meant to include therapeutic as well as prophylactic, or suppressive measures for a disease or disorder leading to any clinically desirable or beneficial effect, including but not limited to alleviation or relief of one or more symptoms, regression, slowing or cessation of progression of the disease or disorder. Thus, for example, the term treatment includes the administration of an agent prior to or following the onset of a symptom of a disease or disorder, thereby preventing or removing all signs of the disease or disorder. As another example, the term includes the administration of an agent after clinical manifestation of the disease to combat the symptoms of the disease. Further, administration of an agent after onset and after clinical symptoms have developed where administration affects clinical parameters of the disease or disorder, such as the degree of tissue

injury or the amount or extent of metastasis, whether or not the treatment leads to amelioration of the disease, comprises “treatment” or “therapy” as used herein.

[0085] As used herein, the terms “prevention” or “prevent” refer to administration of an anti-CD70 binding agent and/or CD47 antagonist to a subject before the onset of a clinical or diagnostic symptom of a CD70-expressing and/or CD47-expressing cancer or immunological disorder (*e.g.*, administration to an individual with a predisposition or at a high risk of acquiring the CD70-expressing and/or CD47-expressing cancer or immunological disorder) to (a) block the occurrence or onset of the CD70-expressing and/or CD47-expressing cancer or immunological disorder, or one or more of clinical or diagnostic symptoms thereof, (b) inhibit the severity of onset of the CD70-expressing and/or CD47-expressing cancer or immunological disorder, or (c) to lessen the likelihood of the onset of the CD70-expressing and/or CD47-expressing cancer or immunological disorder.

[0086] The term “intravenous infusion” refers to introduction of an agent, *e.g.*, a therapeutic agent, into the vein of an animal or human patient over a period of time greater than approximately 15 minutes, generally between approximately 30 to 90 minutes.

[0087] The term “intravenous bolus” or “intravenous push” refers to drug administration into a vein of an animal or human such that the body receives the drug in approximately 15 minutes or less, generally 5 minutes or less.

[0088] The term “subcutaneous administration” refers to introduction of an agent, *e.g.*, a therapeutic agent, under the skin of an animal or human patient, typically within a pocket between the skin and underlying tissue, by relatively slow, sustained delivery from a drug receptacle. Pinching or drawing the skin up and away from underlying tissue may create the pocket.

[0089] The term “package insert” is used to refer to instructions customarily included in commercial packages of therapeutic products, that contain information about the indications, usage, administration, contraindications and/or warnings concerning the use of such therapeutic products.

[0090] A “liposome” is a small vesicle composed of various types of lipids, phospholipids and/or surfactant which is useful for delivery of a drug (such as an antibody) to a mammal. The

components of the liposome are commonly arranged in a bilayer formation, similar to the lipid arrangement of biological membranes.

[0091] The term “subcutaneous infusion” refers to introduction of a drug under the skin of an animal or human patient, preferably within a pocket between the skin and underlying tissue, by relatively slow, sustained delivery from a drug receptacle for a period of time including, but not limited to, 30 minutes or less, or 90 minutes or less. Optionally, the infusion may be made by subcutaneous implantation of a drug delivery pump implanted under the skin of the animal or human patient, wherein the pump delivers a predetermined amount of drug for a predetermined period of time, such as 30 minutes, 90 minutes, or a time period spanning the length of the treatment regimen.

[0092] The term “subcutaneous bolus” refers to drug administration beneath the skin of an animal or human patient, where bolus drug delivery is less than approximately 15 minutes; in another aspect, less than 5 minutes, and in still another aspect, less than 60 seconds. In yet even another aspect, administration is within a pocket between the skin and underlying tissue, where the pocket may be created by pinching or drawing the skin up and away from underlying tissue.

[0093] The term “effective amount” refers to the amount of an anti-CD70 binding agent (*e.g.*, an antibody or derivative or other binding agent) or CD47 antagonist that is sufficient to inhibit the occurrence or ameliorate one or more clinical or diagnostic symptoms of a CD70-expressing and/or CD47-expressing cancer or immunological disorder in a subject. An effective amount of an agent is administered according to the methods described herein in an “effective regimen.” The term “effective regimen” refers to a combination of amount of the agent and dosage frequency adequate to accomplish treatment or prevention of a CD70-expressing and/or CD47-expressing cancer or immunological disorder.

[0094] The term “therapeutically effective amount” is used to refer to an amount of a therapeutic agent having beneficial patient outcome, for example, a growth arrest effect or deletion of the cell. In one aspect, the therapeutically effective amount has apoptotic activity, or is capable of inducing cell death. In another aspect, the therapeutically effective amount refers to a target serum concentration that has been shown to be effective in, for example, slowing disease progression. Efficacy can be measured in conventional ways, depending on the condition to be treated. For example, in neoplastic diseases or disorders characterized by cells expressing CD70

and/or CD47, efficacy can be measured by assessing the time to disease progression (TTP) or determining the response rates (RR).

[0095] As used herein, "complete response" or "CR" refers to disappearance of all target lesions; "partial response" or "PR" refers to at least a 30% decrease in the sum of the longest diameters (SLD) of target lesions, taking as reference the baseline SLD; and "stable disease" or "SD" refers to neither sufficient shrinkage of target lesions to qualify for PR, nor sufficient increase to qualify for PD, taking as reference the smallest SLD since the treatment started.

[0096] As used herein, "progression free survival" or "PFS" refers to the length of time during and after treatment during which the disease being treated (*e.g.*, cancer) does not get worse. Progression-free survival may include the amount of time patients have experienced a complete response or a partial response, as well as the amount of time patients have experienced stable disease.

[0097] As used herein, "overall response rate" or "ORR" refers to the sum of complete response (CR) rate and partial response (PR) rate.

[0098] As used herein, "overall survival" or "OS" refers to the percentage of individuals in a group who are likely to be alive after a particular duration of time.

[0099] An "adverse event" (AE) as used herein is any unfavorable and generally unintended or undesirable sign (including an abnormal laboratory finding), symptom, or disease associated with the use of a medical treatment. A medical treatment can have one or more associated AEs and each AE can have the same or different level of severity. Reference to methods capable of "altering adverse events" means a treatment regime that decreases the incidence and/or severity of one or more AEs associated with the use of a different treatment regime.

[0100] A "serious adverse event" or "SAE" as used herein is an adverse event that meets one of the following criteria:

- Is fatal or life-threatening (as used in the definition of a serious adverse event, "life-threatening" refers to an event in which the patient was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it was more severe.
- Results in persistent or significant disability/incapacity
- Constitutes a congenital anomaly/birth defect

- Is medically significant, *i.e.*, defined as an event that jeopardizes the patient or may require medical or surgical intervention to prevent one of the outcomes listed above. Medical and scientific judgment must be exercised in deciding whether an AE is “medically significant”
- Requires inpatient hospitalization or prolongation of existing hospitalization, excluding the following: 1) routine treatment or monitoring of the underlying disease, not associated with any deterioration in condition; 2) elective or pre-planned treatment for a pre-existing condition that is unrelated to the indication under study and has not worsened since signing the informed consent; and 3) social reasons and respite care in the absence of any deterioration in the patient’s general condition.

[0101] The use of the alternative (*e.g.*, “or”) should be understood to mean either one, both, or any combination thereof of the alternatives. As used herein, the indefinite articles “a” or “an” should be understood to refer to “one or more” of any recited or enumerated component.

[0102] The terms “about” or “comprising essentially of” refer to a value or composition that is within an acceptable error range for the particular value or composition as determined by one of ordinary skill in the art, which will depend in part on how the value or composition is measured or determined, *i.e.*, the limitations of the measurement system. For example, “about” or “comprising essentially of” can mean within 1 or more than 1 standard deviation per the practice in the art. Alternatively, “about” or “comprising essentially of” can mean a range of up to 20%. Furthermore, particularly with respect to biological systems or processes, the terms can mean up to an order of magnitude or up to 5-fold of a value. When particular values or compositions are provided in the application and claims, unless otherwise stated, the meaning of “about” or “comprising essentially of” should be assumed to be within an acceptable error range for that particular value or composition.

[0103] The term “pharmaceutically acceptable” as used herein means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans. The term “pharmaceutically compatible ingredient” refers to a pharmaceutically acceptable diluent, adjuvant, excipient, or vehicle with which an anti-CD70-binding agent or CD47 antagonist is administered.

[0104] The phrase “pharmaceutically acceptable salt,” as used herein, refers to pharmaceutically acceptable organic or inorganic salts of an anti-CD70 binding agent or therapeutic agent or CD47 antagonist or therapeutic agent. The anti-CD70 binding agent or therapeutic agent or CD47 antagonist or therapeutic agent contains at least one amino group, and accordingly acid addition salts can be formed with this amino group or other suitable groups. Exemplary salts include, but are not limited, to sulfate, citrate, acetate, oxalate, chloride, bromide, iodide, nitrate, bisulfate, phosphate, acid phosphate, isonicotinate, lactate, salicylate, acid citrate, tartrate, oleate, tannate, pantothenate, bitartrate, ascorbate, succinate, maleate, gentisinate, fumarate, gluconate, glucuronate, saccharate, formate, benzoate, glutamate, methanesulfonate, ethanesulfonate, benzenesulfonate, p toluenesulfonate, and pamoate (i.e., 1,1’ methylene bis -(2 hydroxy 3 naphthoate)) salts. A pharmaceutically acceptable salt may involve the inclusion of another molecule such as an acetate ion, a succinate ion or other counterion. The counterion may be any organic or inorganic moiety that stabilizes the charge on the parent compound. Furthermore, a pharmaceutically acceptable salt may have more than one charged atom in its structure. Instances where multiple charged atoms are part of the pharmaceutically acceptable salt can have multiple counter ions. Hence, a pharmaceutically acceptable salt can have one or more charged atoms and/or one or more counterion.

[0105] “Pharmaceutically acceptable solvate” or “solvate” refer to an association of one or more solvent molecules and an anti-CD70 binding agent and/or therapeutic agent or CD47 antagonist and/or therapeutic agent. Examples of solvents that form pharmaceutically acceptable solvates include, but are not limited to, water, isopropanol, ethanol, methanol, DMSO, ethyl acetate, acetic acid, and ethanolamine.

[0106] The abbreviation “AFP” refers to dimethylvaline-valine-dolaisoleuine-dolaproine-phenylalanine-p-phenylenediamine.

[0107] The abbreviation “MMAE” refers to monomethyl auristatin E.

[0108] The abbreviation “AEB” refers to an ester produced by reacting auristatin E with paraacetyl benzoic acid.

[0109] The abbreviation “AEVB” refers to an ester produced by reacting auristatin E with benzoylvaleric acid.

[0110] The abbreviation "MMAF" refers to dovaline-valine-dolaisoleunine-dolaproine-phenylalanine.

[0111] The abbreviations "fk" and "phe-lys" refer to the linker phenylalanine-lysine.

[0112] The terms "Treg" or "regulatory T cell" refer to CD4⁺ T cells that suppresses CD4⁺CD25⁺ and CD8⁺ T cell proliferation and/or effector function, or that otherwise down-modulate an immune response. Notably, Treg may down-regulate immune responses mediated by Natural Killer cells, Natural Killer T cells as well as other immune cells.

[0113] The terms "regulatory T cell function" or "a function of Treg" are used interchangeably to refer to any biological function of a Treg that results in a reduction in CD4⁺CD25⁺ or CD8⁺ T cell proliferation or a reduction in an effector T cell-mediated immune response. Treg function can be measured via techniques established in the art. Non-limiting examples of useful in vitro assays for measuring Treg function include Transwell suppression assays as well as in vitro assays in which the target conventional T cells (Tconv) and Tregs purified from human peripheral blood or umbilical cord blood (or murine spleens or lymph nodes) are optionally activated by anti-CD3⁺ anti-CD28 coated beads (or antigen-presenting cells (APCs) such as, e.g., irradiated splenocytes or purified dendritic cells (DCs) or irradiated PBMCs) followed by in vitro detection of conventional T cell proliferation (e.g., by measuring incorporation of radioactive nucleotides (such as, e.g., [³H]-thymidine) or fluorescent nucleotides, or by Cayman Chemical MTT Cell Proliferation Assay Kit, or by monitoring the dilution of a green fluorochrome ester CFSE or Seminaphtharhodafluor (SNARF-1) dye by flow cytometry). Other common assays measure T cell cytokine responses. Useful in vivo assays of Treg function include assays in animal models of diseases in which Tregs play an important role, including, e.g., (1) homeostasis model (using naïve homeostatically expanding CD4⁺ T cells as target cells that are primarily suppressed by Tregs), (2) inflammatory bowel disease (IBD) recovery model (using Th1 T cells (Th17) as target cells that are primarily suppressed by Tregs), (3) experimental autoimmune encephalomyelitis (EAE) model (using Th1 7 and Th1 T cells as target cells that are primarily suppressed by Tregs), (4) B16 melanoma model (suppression of antitumor immunity) (using CD8⁺ T cells as target cells that are primarily suppressed by Tregs), (5) suppression of colon inflammation in adoptive transfer colitis where naïve CD4⁺CD45RB^M Tconv cells are transferred into RagV mice, and (6) Foxp3 rescue model (using lymphocytes as

target cells that are primarily suppressed by Tregs). According to one protocol, all of the models require mice for donor T cell populations as well as Ragl^{-/-} or Foxp3 mice for recipients. For more details on various useful assays see, e.g., Collison and Vignali, In Vitro Treg Suppression Assays, Chapter 2 in Regulatory T Cells: Methods and Protocols, Methods in Molecular Biology, Kassiotis and Liston eds., Springer, 2011, 707:21-37; Workman et al, In Vivo Treg Suppression Assays, Chapter 9 in Regulatory T Cells: Methods and Protocols, Methods in Molecular Biology, Kassiotis and Liston eds., Springer, 2011, 119-156; Takahashi et al, Int. Immunol, 1998, 10: 1969-1980; Thornton et al, J. Exp. Med., 1998, 188:287-296; Collison et al, J. Immunol, 2009, 182:6121-6128; Thornton and Shevach, J. Exp. Med., 1998, 188:287-296; Asseman et al, J. Exp. Med., 1999, 190:995-1004; Dieckmann et al, J. Exp. Med., 2001, 193: 1303-1310; Belkaid, Nature Reviews, 2007, 7:875-888; Tang and Bluestone, Nature Immunology, 2008, 9:239-244; Bettini and Vignali, Curr. Opin. Immunol, 2009, 21 :612-618; Dannull et al, J Clin Invest, 2005, 115(12):3623-33; Tsaknaridis, et al, J Neurosci Res., 2003, 74:296-308.

[0114] As described herein, any concentration range, percentage range, ratio range, or integer range is to be understood to include the value of any integer within the recited range and, when appropriate, fractions thereof (such as one tenth and one hundredth of an integer), unless otherwise indicated.

[0115] Various aspects of the disclosure are described in further detail in the following subsections.

II. Anti-CD70 Antibodies

[0116] The invention provides anti-CD70 antibodies, such as humanized antibodies derived from the mouse antibody 1F6. 1F6 is a murine immunoglobulin G1 (IgG1) monoclonal antibody against CD70. 1F6 and humanized 1F6 variants are described in U.S. Pat. No. 8,067,546 and International Patent Publication WO 2006/113909. In some embodiments, the anti-CD70 antibody is nonfucosylated.

[0117] The binding affinity of humanized forms of the mouse 1F6 antibody (*i.e.*, dissociation constant, K_D) is preferably within a factor of five or a factor of two of that of the mouse antibody 1F6 for human CD70. Humanized 1F6 antibodies specifically bind to human CD70 in native form and/or recombinantly expressed from Chinese hamster ovary (CHO) cells as does the mouse antibody from which they were derived. Preferred humanized 1F6 antibodies have an

affinity the same as or greater than (i.e., greater than beyond margin of error in measurement) that of 1F6 for human CD70 (e.g., 1.1-5 fold, 1.1 to 3 fold, 1.5 to 3- fold, 1.7 to 2.3-fold or 1.7-2.1-fold the affinity or about twice the affinity of 1F6). Preferred humanized 1F6 antibodies bind to the same epitope and/or compete with 1F6 for binding to human CD70.

[0118] In some embodiments, antibodies of the invention inhibit cancer (e.g., growth of cells, metastasis and/or lethality to the organisms) as shown on cancerous cells propagating in culture, in an animal model or clinical trial. Animal models can be formed by implanting CD70-expressing human tumor cell lines into appropriate immunodeficient rodent strains, e.g., athymic nude mice or SCID mice. These tumor cell lines can be established in immunodeficient rodent hosts either as solid tumor by subcutaneous injections or as disseminated tumors by intravenous injections.

[0119] Once established within a host, these tumor models can be applied to evaluate the therapeutic efficacies of the anti-CD70 antibodies, or conjugated forms thereof, as described in the Examples.

[0120] Generally, anti-CD70 antibodies of the disclosure bind CD70, *e.g.*, human CD70, and exert cytostatic and cytotoxic effects on malignant cells, such as cancer cells. Anti-CD70 antibodies of the disclosure are preferably monoclonal, and may be multispecific, human, humanized or chimeric antibodies, single chain antibodies, Fab fragments, F(ab') fragments, fragments produced by a Fab expression library, and CD70 binding fragments of any of the above. In some embodiments, the anti-CD70 antibodies of the disclosure specifically bind CD70. The immunoglobulin molecules of the disclosure can be of any type (*e.g.*, IgG, IgE, IgM, IgD, IgA and IgY), class (*e.g.*, IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2) or subclass of immunoglobulin molecule.

[0121] In certain embodiments of the disclosure, the anti-CD70 antibodies are antigen-binding fragments (*e.g.*, human antigen-binding fragments) as described herein and include, but are not limited to, Fab, Fab' and F(ab')₂, Fd, single-chain Fvs (scFv), single-chain antibodies, disulfide-linked Fvs (sdFv) and fragments comprising either a V_L or V_H domain. Antigen-binding fragments, including single-chain antibodies, may comprise the variable region(s) alone or in combination with the entirety or a portion of the following: hinge region, CH1, CH2, CH3 and CL domains. Also included in the present disclosure are antigen-binding fragments

comprising any combination of variable region(s) with a hinge region, CH1, CH2, CH3 and CL domains. In some embodiments, the anti-CD70 antibodies or antigen-binding fragments thereof are human, murine (*e.g.*, mouse and rat), donkey, sheep, rabbit, goat, guinea pig, camelid, horse, or chicken.

[0122] The anti-CD70 antibodies of the present disclosure may be monospecific, bispecific, trispecific or of greater multi specificity. Multispecific antibodies may be specific for different epitopes of CD70 or may be specific for both CD70 as well as for a heterologous protein. *See, e.g.*, PCT publications WO 93/17715; WO 92/08802; WO 91/00360; WO 92/05793; Tutt, *et al.*, 1991, J. Immunol. 147:60 69; U.S. Pat. Nos. 4,474,893; 4,714,681; 4,925,648; 5,573,920; 5,601,819; Kostelny *et al.*, 1992, J. Immunol. 148:1547 1553.

[0123] The anti-CD70 antibodies of the present disclosure may be humanized antibodies. In some embodiments, the anti-CD70 antibodies of the present disclosure are humanized antibodies of the mouse antibody 1F6. Humanized versions of 1F6 are described in U.S. Pat. No. 8,067,546. A humanized antibody is a genetically engineered antibody in which the CDRs from a non-human "donor" antibody are grafted into human "acceptor" antibody sequences (*see, e.g.*, Queen, US 5,530,101 and 5,585,089; Winter, US 5,225,539; Carter, US 6,407,213; Adair, US 5,859,205; and Foote, US 6,881,557). The acceptor antibody sequences can be, for example, a mature human antibody sequence, a composite of such sequences, a consensus sequence of human antibody sequences, or a germline region sequence. A preferred acceptor sequence for the heavy chain is the germline V_H exon V_HL-2 (also referred to in the literature as HV1-2) (Shin *et al.*, 1991, EMBO J. 10:3641-3645) and for the hinge region (J_H), exon J_H-6 (Mattila *et al.*, 1995, Eur. J. Immunol. 25:2578-2582). For the light chain, a preferred acceptor sequence is exon VK2-30 (also referred to in the literature as KV2-30) and for the hinge region exon JK-4 (Hieter *et al.*, 1982, J. Biol. Chem. 257:1516-1522). Thus, a humanized antibody is an antibody having some or all CDRs entirely or substantially from a donor antibody and variable region framework sequences and constant regions, if present, entirely or substantially from human antibody sequences. Similarly, a humanized heavy chain has at least one, two and usually all three CDRs entirely or substantially from a donor antibody heavy chain, and a heavy chain variable region framework sequence and heavy chain constant region, if present, substantially from human heavy chain variable region framework and constant region sequences. Similarly a humanized light chain has at least one, two and usually all three CDRs entirely or substantially from a donor

antibody light chain, and a light chain variable region framework sequence and light chain constant region, if present, substantially from human light chain variable region framework and constant region sequences. Other than nanobodies and dAbs, a humanized antibody comprises a humanized heavy chain and a humanized light chain. A CDR in a humanized antibody is substantially from a corresponding CDR in a non-human antibody when at least 60%, 85%, 90%, 95% or 100% of corresponding residues (as defined by Kabat) are identical between the respective CDRs. The variable region framework sequences of an antibody chain or the constant region of an antibody chain are substantially from a human variable region framework sequence or human constant region respectively when at least 85%, 90%, 95% or 100% of corresponding residues defined by Kabat are identical.

[0124] Although humanized antibodies often incorporate all six CDRs (preferably as defined by Kabat) from a mouse antibody, they can also be made with less than all CDRs (e.g., at least 3, 4, or 5) CDRs from a mouse antibody (e.g., Pascalis et al., *J. Immunol.* 169:3076, 2002; Vajdos et al., *Journal of Molecular Biology*, 320: 415-428, 2002; Iwahashi et al., *Mol. Immunol.* 36:1079-1091, 1999; Tamura et al, *Journal of Immunology*, 164:1432-1441, 2000).

[0125] Certain amino acids from the human variable region framework residues can be selected for substitution based on their possible influence on CDR conformation and/or binding to antigen. Investigation of such possible influences is by modeling, examination of the characteristics of the amino acids at particular locations, or empirical observation of the effects of substitution or mutagenesis of particular amino acids.

[0126] For example, when an amino acid differs between a murine variable region framework residue and a selected human variable region framework residue, the human framework amino acid can be substituted by the equivalent framework amino acid from the mouse antibody when it is reasonably expected that the amino acid:

- (1) noncovalently binds antigen directly,
- (2) is adjacent to a CDR region,
- (3) otherwise interacts with a CDR region (e.g. is within about 6 Å of a CDR region); or
- (4) mediates interaction between the heavy and light chains.

[0127] Anti-CD70 antibodies of the present disclosure may be described or specified in terms of the particular CDRs they comprise. The precise amino acid sequence boundaries of a given CDR or FR can be readily determined using any of a number of well-known schemes, including those described by Kabat *et al.* (1991), “Sequences of Proteins of Immunological Interest,” 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD (“Kabat” numbering scheme); Al-Lazikani *et al.*, (1997) JMB 273,927-948 (“Chothia” numbering scheme); MacCallum *et al.*, J. Mol. Biol. 262:732-745 (1996), “Antibody-antigen interactions: Contact analysis and binding site topography,” J. Mol. Biol. 262, 732-745.” (“Contact” numbering scheme); Lefranc MP *et al.*, “IMGT unique numbering for immunoglobulin and T cell receptor variable domains and Ig superfamily V-like domains,” Dev Comp Immunol, 2003 Jan;27(1):55-77 (“IMGT” numbering scheme); Honegger A and Plückthun A, “Yet another numbering scheme for immunoglobulin variable domains: an automatic modeling and analysis tool,” J Mol Biol, 2001 Jun 8;309(3):657-70, (“Aho” numbering scheme); and Martin *et al.*, “Modeling antibody hypervariable loops: a combined algorithm,” PNAS, 1989, 86(23):9268-9272, (“AbM” numbering scheme). The boundaries of a given CDR may vary depending on the scheme used for identification. In some embodiments, a “CDR” or “complementarity determining region,” or individual specified CDRs (*e.g.*, CDR-H1, CDR-H2, CDR-H3), of a given antibody or region thereof (*e.g.*, variable region thereof) should be understood to encompass a (or the specific) CDR as defined by any of the aforementioned schemes. For example, where it is stated that a particular CDR (*e.g.*, a CDR-H3) contains the amino acid sequence of a corresponding CDR in a given V_H or V_L region amino acid sequence, it is understood that such a CDR has a sequence of the corresponding CDR (*e.g.*, CDR-H3) within the variable region, as defined by any of the aforementioned schemes. The scheme for identification of a particular CDR or CDRs may be specified, such as the CDR as defined by the Kabat, Chothia, AbM or IMGT method.

[0128] CDR sequences of the anti-CD70 antibodies and of the anti-CD70 antibody-drug conjugates described herein are according to the Kabat numbering scheme as described in Kabat *et al.* (1991), “Sequences of Proteins of Immunological Interest,” 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD, unless specified otherwise.

[0129] In some embodiments of the anti-CD70 antibodies described herein, the heavy chain variable region CDR sequences comprise the following:

- a) CDR-H1: NYGMN (SEQ ID NO:8);
- b) CDR-H2: WINTYTGEPTYADAFKG (SEQ ID NO:9); and
- c) CDR-H3: DYGDYGMDY (SEQ ID NO:10).

[0130] In some embodiments of the anti-CD70 antibodies described herein, the light chain variable region CDR sequences comprise the following:

- a) CDR-L1: RASKSVSTSGYSFMH (SEQ ID NO:11);
- b) CDR-L2: LASNLES (SEQ ID NO:12); and
- c) CDR-L3: QHSREVPWT (SEQ ID NO:13).

[0131] In one aspect, provided herein is an anti-CD70 antibody comprising a heavy chain variable region and a light chain variable region, wherein the heavy chain variable region comprises (i) CDR-H1 comprising the amino acid sequence of SEQ ID NO:8, (ii) CDR-H2 comprising the amino acid sequence of SEQ ID NO:9, and (iii) CDR-H3 comprising the amino acid sequence of SEQ ID NO:10; and wherein the light chain variable region comprises (i) CDR-L1 comprising the amino acid sequence of SEQ ID NO:11, (ii) CDR-L2 comprising the amino acid sequence of SEQ ID NO:12, and (iii) CDR-L3 comprising the amino acid sequence of SEQ ID NO:13, wherein the CDRs of the anti-CD70 antibody are defined by the Kabat numbering scheme.

[0132] In one aspect, provided herein is an anti-CD70 antibody comprising a heavy chain variable region comprising the three CDRs of SEQ ID NO:1 and a light chain variable region comprising the three CDRs of SEQ ID NO:2, wherein the CDRs of the anti-CD70 antibody are defined by the Kabat numbering scheme. In some embodiments, the anti-CD70 antibody further comprises an Fc domain. In some embodiments, the anti-CD70 antibody is nonfucosylated.

[0133] An anti-CD70 antibody described herein may comprise any suitable framework variable domain sequence, provided that the antibody retains the ability to bind CD70 (*e.g.*, human CD70). As used herein, heavy chain framework regions are designated "HC-FR1-FR4," and light chain framework regions are designated "LC-FR1-FR4."

[0134] In some embodiments of the anti-CD70 antibodies described herein, the heavy chain variable domain comprises the amino acid sequence of
QVQLVQSGAEVKKPGASVKVSCKASGYTFTNYGMNWVRQAPGQGLKWMGWINTYTG

EPTYADAFKGRVTMTRDTSISTAYMELSRLRSDDTAVYYCARDYGDYGM DYWGQGT
VTVSS (SEQ ID NO:1) and the light chain variable domain comprises the amino acid sequence
of
DIVMTQSPDSLAVSLGERATINCRASKSVSTSGYSFMHWYQQKPGQPPKLLIYLASNLES
GVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQHSREVPWTFGQGTKVEIK (SEQ ID
NO:2).

[0135] In some embodiments of the anti-CD70 antibodies described herein, the heavy chain
variable domain comprises the amino acid sequence of
QVQLVQSGAEVKKPGASVKVSKASGYTFTNYGMNWRQAPGQGLKWMGWINTYTG
EPTYADAFKGRVTMTRDTSISTAYMELSRLRSDDTAVYYCARDYGDYGM DYWGQGT
VTVSS (SEQ ID NO:1) and the light chain variable domain comprises the amino acid sequence
of
DIVMTQSPDSLAVSLGERATINCRASKSVSTSGYSFMHWYQQKPGQPPKLLIYLASNLES
GVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQHSREVPWTFGQGTKVEIKR (SEQ ID
NO:7).

[0136] In one aspect, provided herein is an anti-CD70 antibody comprising a heavy chain
variable domain comprising the amino acid sequence of SEQ ID NO:1 or comprising a light
chain variable domain comprising the amino acid sequence of SEQ ID NO:2. In some
embodiments, the N-terminal glutamine of the heavy chain variable domain is cyclized to form
pyroglutamic acid. In one aspect, provided herein is an anti-CD70 antibody comprising a heavy
chain variable domain comprising the amino acid sequence of SEQ ID NO:1 and comprising a
light chain variable domain comprising the amino acid sequence of SEQ ID NO:2. In some
embodiments, the N-terminal glutamine of the heavy chain variable domain is cyclized to form
pyroglutamic acid.

[0137] In one aspect, provided herein is an anti-CD70 antibody comprising a heavy chain
variable domain comprising the amino acid sequence of SEQ ID NO:1 or comprising a light
chain variable domain comprising the amino acid sequence of SEQ ID NO:7. In some
embodiments, the N-terminal glutamine of the heavy chain variable domain is cyclized to form
pyroglutamic acid. In one aspect, provided herein is an anti-CD70 antibody comprising a heavy
chain variable domain comprising the amino acid sequence of SEQ ID NO:1 and comprising a

light chain variable domain comprising the amino acid sequence of SEQ ID NO:7. In some embodiments, the N-terminal glutamine of the heavy chain variable domain is cyclized to form pyroglutamic acid.

[0138] In some embodiments, provided herein is an anti-CD70 antibody comprising a heavy chain variable domain comprising an amino acid sequence having at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the amino acid sequence of SEQ ID NO:1. In some embodiments, the N-terminal glutamine of the heavy chain variable domain is cyclized to form pyroglutamic acid. In certain embodiments, a heavy chain variable domain comprising an amino acid sequence having at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the amino acid sequence of SEQ ID NO:1 contains substitutions (*e.g.*, conservative substitutions), insertions, or deletions relative to the reference sequence and retains the ability to bind to a CD70 (*e.g.*, human CD70). In certain embodiments, a total of 1 to 10 amino acids have been substituted, inserted and/or deleted in SEQ ID NO:1. In certain embodiments, substitutions, insertions, or deletions (*e.g.*, 1, 2, 3, 4, or 5 amino acids) occur in regions outside the CDRs (*i.e.*, in the FRs). In some embodiments, the anti-CD70 antibody comprises a heavy chain variable domain sequence of SEQ ID NO:1 including post-translational modifications of that sequence. In some embodiments, the N-terminal glutamine of the heavy chain variable domain is cyclized to form pyroglutamic acid.

[0139] In some embodiments, provided herein is an anti-CD70 antibody comprising a light chain variable domain comprising an amino acid sequence having at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the amino acid sequence of SEQ ID NO:2. In certain embodiments, a light chain variable domain comprising an amino acid sequence having at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the amino acid sequence of SEQ ID NO:2 contains substitutions (*e.g.*, conservative substitutions), insertions, or deletions relative to the reference sequence and retains the ability to bind to a CD70 (*e.g.*, human CD70). In certain embodiments, a total of 1 to 10 amino acids have been substituted, inserted and/or deleted in SEQ ID NO:2. In certain embodiments, substitutions, insertions, or deletions (*e.g.*, 1, 2, 3, 4, or 5 amino acids) occur in regions outside the CDRs (*i.e.*, in the FRs). In some embodiments,

the anti-CD70 antibody comprises a light chain variable domain sequence of SEQ ID NO:2 including post-translational modifications of that sequence.

[0140] In some embodiments, provided herein is an anti-CD70 antibody comprising a light chain variable domain comprising an amino acid sequence having at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the amino acid sequence of SEQ ID NO:7. In certain embodiments, a light chain variable domain comprising an amino acid sequence having at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the amino acid sequence of SEQ ID NO:5 contains substitutions (*e.g.*, conservative substitutions), insertions, or deletions relative to the reference sequence and retains the ability to bind to a CD70 (*e.g.*, human CD70). In certain embodiments, a total of 1 to 10 amino acids have been substituted, inserted and/or deleted in SEQ ID NO:7. In certain embodiments, substitutions, insertions, or deletions (*e.g.*, 1, 2, 3, 4, or 5 amino acids) occur in regions outside the CDRs (*i.e.*, in the FRs). In some embodiments, the anti-CD70 antibody comprises a light chain variable domain sequence of SEQ ID NO:7 including post-translational modifications of that sequence.

[0141] In some embodiments, provided herein is an anti-CD70 antibody comprising a heavy chain comprising an amino acid sequence having at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the amino acid sequence QVQLVQSGAE VKKPGASVKV SCKASGYTFT NYGMNWVRQA PGQGLKWMGW INTYTGEPTY ADAFKGRVTM TRDTSISTAY MELSRLRSDD TAVYYCARDY GDYGM DYWGQ GTTVTVSSAS TKGPSVFPLA PSSKSTSGGT AALGCLVKDY FPEPVTVSWN SGALTSGVHT FPAVLQSSGL YSLSSVVTVP SSSLGTQTYI CNVNHKPSNT KVDKKVEPKS CDKTHTCPPC PAPELLGGPS VFLFPPKPKD TLMISRTPEV TCVVVDVSHE DPEVKFNWYV DGVEVHNAKT KPREEQYNST YRVVSVLTVL HQDWLNGKEY KCKVSNKALP APIEKTISKA KGQPREPQVY TLPPSRDEL TKNQVSLTCLV KGFYPSDIAV EWESNGQPEN NYKTTTPVLD SDGSFFLYSK LTVDKSRWQQ GNVFSCSVMH EALHNHYTQK SLSLSPGK (SEQ ID NO:3). In certain embodiments, a heavy chain comprising an amino acid sequence having at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the amino acid sequence of SEQ ID NO:3 contains substitutions (*e.g.*, conservative substitutions), insertions, or deletions relative to the reference

sequence and retains the ability to bind to a CD70 (*e.g.*, human CD70). In certain embodiments, a total of 1 to 10 amino acids have been substituted, inserted and/or deleted in SEQ ID NO:3. In certain embodiments, substitutions, insertions, or deletions (*e.g.*, 1, 2, 3, 4, or 5 amino acids) occur in regions outside the CDRs (*i.e.*, in the FRs). In some embodiments, the anti-CD70 antibody comprises a heavy chain sequence of SEQ ID NO:3 including post-translational modifications of that sequence.

[0142] In some embodiments, provided herein is an anti-CD70 antibody comprising a light chain comprising an amino acid sequence having at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the amino acid sequence of DIVMTQSPDS LAVSLGERAT INCRASKSVS TSGYSFMHWY QQKPGQPPKL LIYLASNLES GVPDRFSGSG SGTDFLTIS SLQAEDVAVY YCQHSREVPW TFGQGKTKVEI KRTVAAPSVF IFPPSDEQLK SGTASVVCLL NNFYPREAKV QWKVDNALQS GNSQESVTEQ DSKDSTYLS STLTLKADY EKHKVYACEV THQGLSSPVT KSFNRGEC (SEQ ID NO:4). In certain embodiments, a light chain comprising an amino acid sequence having at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the amino acid sequence of SEQ ID NO:4 contains substitutions (*e.g.*, conservative substitutions), insertions, or deletions relative to the reference sequence and retains the ability to bind to a CD70 (*e.g.*, human CD70). In certain embodiments, a total of 1 to 10 amino acids have been substituted, inserted and/or deleted in SEQ ID NO:4. In certain embodiments, substitutions, insertions, or deletions (*e.g.*, 1, 2, 3, 4, or 5 amino acids) occur in regions outside the CDRs (*i.e.*, in the FRs). In some embodiments, the anti-CD70 antibody comprises a light chain sequence of SEQ ID NO:4 including post-translational modifications of that sequence.

[0143] In some embodiments, the anti-CD70 antibody comprises a heavy chain variable domain as in any of the embodiments provided above, and a light chain variable domain as in any of the embodiments provided above. In one embodiment, the antibody comprises the heavy chain variable domain sequence of SEQ ID NO:1 and the light chain variable domain sequence of SEQ ID NO:2, including post-translational modifications of those sequences. In some embodiments, the N-terminal glutamine of the heavy chain variable domain is cyclized to form pyroglutamic acid.

[0144] In some embodiments, the anti-CD70 antibody comprises: i) an amino acid sequence having at least 85% sequence identity to a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:1, and ii) an amino acid sequence having at least 85% sequence identity to a light chain variable region comprising the amino acid sequence of SEQ ID NO:2. In some embodiments, the N-terminal glutamine of the heavy chain variable domain is cyclized to form pyroglutamic acid.

[0145] In some embodiments, the anti-CD70 antibody is a monoclonal antibody.

[0146] In some embodiments, the anti-CD70 antibody comprises a heavy chain variable region comprising the three CDRs or a light chain variable region comprising the three CDRs of an anti-CD70 antibody described in U.S. Pat. No. 8,067,546, U.S. Pat. No. 8,562,987, U.S. Pat. No. 9,428,585, U.S. Pat. No. 9,701,752, US 2009/0148942, US 2012/0045436, US 2014/0178936, US 2017/0022282 or International Patent Publication WO 2006/113909. In some embodiments, the anti-CD70 antibody comprises a heavy chain variable region comprising the three CDRs and a light chain variable region comprising the three CDRs of an anti-CD70 antibody described in U.S. Pat. No. 8,067,546, U.S. Pat. No. 8,562,987, U.S. Pat. No. 9,428,585, U.S. Pat. No. 9,701,752, US 2009/0148942, US 2012/0045436, US 2014/0178936, US 2017/0022282 or International Patent Publication WO 2006/113909. In some embodiments, the CDRs are defined by the Kabat numbering scheme.

[0147] In some embodiments, the anti-CD70 antibody comprises a heavy chain variable region or a light chain variable region of an anti-CD70 antibody described in U.S. Pat. No. 8,067,546, U.S. Pat. No. 8,562,987, U.S. Pat. No. 9,428,585, U.S. Pat. No. 9,701,752, US 2009/0148942, US 2012/0045436, US 2014/0178936, US 2017/0022282 or International Patent Publication WO 2006/113909. In some embodiments, the anti-CD70 antibody comprises a heavy chain variable region and a light chain variable region of an anti-CD70 antibody described in U.S. Pat. No. 8,067,546, U.S. Pat. No. 8,562,987, U.S. Pat. No. 9,428,585, U.S. Pat. No. 9,701,752, US 2009/0148942, US 2012/0045436, US 2014/0178936, US 2017/0022282 or International Patent Publication WO 2006/113909.

[0148] In some embodiments, the anti-CD70 antibody is an anti-CD70 antibody, such as a humanized 1F6 variant, as described in U.S. Pat. No. 8,067,546, U.S. Pat. No. 8,562,987, U.S. Pat. No. 9,428,585, U.S. Pat. No. 9,701,752, US 2009/0148942, US 2012/0045436, US

2014/0178936, US 2017/0022282 or International Patent Publication WO 2006/113909. In some embodiments, the anti-CD70 antibody is an anti-CD70 antibody, such as a nonfucosylated form of a humanized 1F6 variant, as described in U.S. Pat. No. 8,067,546, U.S. Pat. No. 8,562,987, U.S. Pat. No. 9,428,585, U.S. Pat. No. 9,701,752, US 2009/0148942, US 2012/0045436, US 2014/0178936, US 2017/0022282 or International Patent Publication WO 2006/113909.

[0149] In some embodiments, the anti-CD70 antibody comprises a heavy chain variable region comprising the three CDRs or a light chain variable region comprising the three CDRs of the anti-CD70 antibody vorsetuzumab. In some embodiments, the anti-CD70 antibody comprises a heavy chain variable region comprising the three CDRs and a light chain variable region comprising the three CDRs of the anti-CD70 antibody vorsetuzumab. In some embodiments, the CDRs are defined by the Kabat numbering scheme.

[0150] In some embodiments, the anti-CD70 antibody comprises a heavy chain variable region or a light chain variable region of the anti-CD70 antibody vorsetuzumab. In some embodiments, the anti-CD70 antibody comprises a heavy chain variable region and a light chain variable region of the anti-CD70 antibody vorsetuzumab.

[0151] In some embodiments, the anti-CD70 antibody is a nonfucosylated form of vorsetuzumab.

[0152] Anti-CD70 antibodies of the present invention may also be described or specified in terms of their binding affinity to CD70 (*e.g.*, human CD70). Preferred binding affinities include those with a dissociation constant or K_D less than 5×10^{-2} M, 10^{-2} M, 5×10^{-3} M, 10^{-3} M, 5×10^{-4} M, 10^{-4} M, 5×10^{-5} M, 10^{-5} M, 5×10^{-6} M, 10^{-6} M, 5×10^{-7} M, 10^{-7} M, 5×10^{-8} M, 10^{-8} M, 5×10^{-9} M, 10^{-9} M, 5×10^{-10} M, 10^{-10} M, 5×10^{-11} M, 10^{-11} M, 5×10^{-12} M, 10^{-12} M, 5×10^{-13} M, 10^{-13} M, 5×10^{-14} M, 10^{-14} M, 5×10^{-15} M, or 10^{-15} M.

[0153] There are five classes of immunoglobulins: IgA, IgD, IgE, IgG and IgM, having heavy chains designated α , δ , ϵ , γ and μ , respectively. The γ and α classes are further divided into subclasses *e.g.*, humans express the following subclasses: IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2. IgG1 antibodies can exist in multiple polymorphic variants termed allotypes (reviewed in Jefferis and Lefranc 2009. *mAbs* Vol 1 Issue 4 1-7) any of which are suitable for use in some of the embodiments herein. Common allotypic variants in human populations are those designated by the letters a, f, n, z or combinations thereof. In any of the embodiments

herein, the antibody may comprise a heavy chain Fc region comprising a human IgG Fc region.

In further embodiments, the human IgG Fc region comprises a human IgG1.

[0154] In some embodiments, the anti-CD70 antibody comprises a heavy chain variable domain as in any of the embodiments provided above, and a light chain variable domain as in any of the embodiments provided above. In one embodiment, the antibody comprises a heavy chain constant region comprising the amino acid sequence of AS TKGPSVFPLA PSSKSTSGGT AALGCLVKDY FPEPVTVSWN SGALTSGVHT FPAVLQSSGL YSLSSVVTVP SSSLGTQTYI CNVNHKPSNT KVDKKVEPKS CDKTHTCPPC PAPELLGGPS VFLFPPKPKD TLMISRTPEV TCVVVDVSHE DPEVKFNWYV DGVEVHNAKT KPREEQYNST YRVVSVLTVL HQDWLNGKEY KCKVSNKALP APIEKTISKA KGQPREPQVY TLPPSRDELT KNQVSLTCLV KGFYPSDIAV EWESNGQPEN NYKTTTPVLD SDGSFFLYSK LTVDKSRWQQ GNVFSCSVMH EALHNHYTQK SLSLSPGK (SEQ ID NO:5) and a light chain constant region comprising the amino acid sequence of TVAAPSVF IFPPSDEQLK SGTASVVCLL NNFYPREAKV QWKVDNALQS GNSQESVTEQ DSKDSTYSLT STLTLKADY EKHKVYACEV THQGLSSPVT KSFNRGEC (SEQ ID NO:6), including post-translational modifications of those sequences.

[0155] The antibodies also include derivatives that are modified, *i.e.*, by the covalent attachment of any type of molecule to the antibody such that covalent attachment does not prevent the antibody from binding to CD70 or from exerting a cytostatic or cytotoxic effect on cells. For example, but not by way of limitation, the antibody derivatives include antibodies that have been modified, *e.g.*, by glycosylation, acetylation, PEGylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to a cellular ligand or other protein, etc. Any of numerous chemical modifications may be carried out by known techniques, including, but not limited to specific chemical cleavage, acetylation, formylation, metabolic synthesis of tunicamycin, etc. Additionally, the derivative may contain one or more non-classical amino acids.

[0156] The CD70-binding agent can optionally include an antibody effector domain that mediates or stimulates an ADCC, ADCP and/or CDC response against a CD70-expressing target cell. The effector domain(s) can be, for example, an Fc domain or domains of an Ig molecule. Such a CD70-binding agent can exert a cytotoxic or cytostatic effect on CD70-expressing cancer

cells, or exert a cytotoxic, cytostatic, or immunomodulatory effect on activated lymphocytes or dendritic cells, for example, in the treatment of a CD70-expressing cancer or an immunological disorder, respectively. Typically, the CD70-binding agent recruits and/or activates cytotoxic white blood cells (*e.g.*, natural killer (NK) cells, phagocytotic cells (*e.g.*, macrophages), and/or serum complement components).

[0157] The anti-CD70 antibody can be a humanized antibody, a single chain antibody, an scFv, a diabody, an Fab, a minibody, an scFv-Fc, an Fv, or the like. In some embodiments, a CD70 antigen-binding region can be joined to an effector domain or domains such as, for example, the hinge-C_H2-C_H3 domains of an immunoglobulin, or a portion or fragment of an effector domain(s) having effector function. Antigen-binding antibody fragments, including single-chain antibodies, can comprise, for example, the variable region(s) in combination with the entirety or a portion of an effector domain (*e.g.*, a C_H2 and/or C_H3 domain alone or in combination with a C_H1, hinge and/or C_L domain). Also, antigen-binding fragments can comprise any combination of effector domains. In some embodiments, the anti-CD70 antibody can be a single chain antibody comprising a CD70-binding variable region joined to hinge-C_H2-C_H3 domains.

[0158] The effector domains of the anti-CD70 antibody can be from any suitable human immunoglobulin isotype. For example, the ability of human immunoglobulin to mediate CDC and ADCC/ADCP is generally in the order of IgM≈IgG1≈IgG3>IgG2>IgG4 and IgG1≈IgG3>IgG2/IgM/IgG4, respectively. A CD70-binding polypeptide can be expressed as a recombinant fusion protein comprising of the appropriate constant domains to yield the desired effector function(s). Upon binding to target cells, the anti-CD70 antibodies or derivatives can trigger *in vitro* and *in vivo* target cell destruction through an antibody effector function, such as ADCC, CDC, and ADCP.

[0159] The CD70-binding agent optionally can be conjugated to a therapeutic agent, such as a cytotoxic, cytostatic or immunomodulatory agent. Useful classes of cytotoxic or immunomodulatory agents include, for example, antitubulin agents, auristatins, DNA minor groove binders, DNA replication inhibitors, alkylating agents (*e.g.*, platinum complexes such as *cis*-platin, mono(platinum), bis(platinum) and tri-nuclear platinum complexes and carboplatin), anthracyclines, antibiotics, antifolates, antimetabolites, chemotherapy sensitizers, duocarmycins,

etoposides, fluorinated pyrimidines, ionophores, lexitropsins, nitrosoureas, platinols, pre-forming compounds, purine antimetabolites, puromycins, radiation sensitizers, steroids, taxanes, topoisomerase inhibitors, vinca alkaloids, and the like. In some typical embodiments, the therapeutic agent is a cytotoxic agent. Suitable cytotoxic agents include, for example, dolastatins (*e.g.*, auristatin E, AFP, MMAF, MMAE), DNA minor groove binders (*e.g.*, enediynes and lexitropsins), duocarmycins, taxanes (*e.g.*, paclitaxel and docetaxel), puromycins, vinca alkaloids, CC-1065, SN-38, topotecan, morpholino-doxorubicin, rhizoxin, cyanomorpholino-doxorubicin, echinomycin, combretastatin, netropsin, epothilone A and B, estramustine, cryptophysins, cemadotin, maytansinoids, discodermolide, eleutherobin, and mitoxantrone. In specific embodiments, the cytotoxic or cytostatic agent is auristatin E (also known in the art as dolastatin-10) or a derivative thereof. Typically, the auristatin E derivative is, *e.g.*, an ester formed between auristatin E and a keto acid. For example, auristatin E can be reacted with paraacetyl benzoic acid or benzoylvaleric acid to produce AEB and AEVB, respectively. Other typical auristatin derivatives include AFP, MMAF, and MMAE. The synthesis and structure of auristatin E and its derivatives are described in U.S. Patent Application Publication Nos. 20030083263 and 20050009751), International Patent Application No. PCT/US03/24209, International Patent Application No. PCT/US02/13435, and U.S. Patent Nos. 6,323,315; 6,239,104; 6,034,065; 5,780,588; 5,665,860; 5,663,149; 5,635,483; 5,599,902; 5,554,725; 5,530,097; 5,521,284; 5,504,191; 5,410,024; 5,138,036; 5,076,973; 4,986,988; 4,978,744; 4,879,278; 4,816,444; and 4,486,414. In specific embodiments, the cytotoxic agent is a DNA minor groove binding agent. (*See, e.g.*, U.S. Patent No. 6,130,237.) For example, in some embodiments, the minor groove binding agent is a CBI compound. In other embodiments, the minor groove binding agent is an enediyne (*e.g.*, calicheamicin). Examples of anti-tubulin agents include, but are not limited to, taxanes (*e.g.*, Taxol[®] (paclitaxel), Taxotere[®] (docetaxel)), T67 (Tularik), vinca alkaloids (*e.g.*, vincristine, vinblastine, vindesine, and vinorelbine), and dolastatins (*e.g.*, auristatin E, AFP, MMAF, MMAE, AEB, AEVB). Other antitubulin agents include, for example, baccatin derivatives, taxane analogs (*e.g.*, epothilone A and B), nocodazole, colchicine and colcimid, estramustine, cryptophysins, cemadotin, maytansinoids, combretastatins, discodermolide, and eleutherobin. In some embodiments, the cytotoxic agent is a maytansinoid, another group of anti-tubulin agents. For example, in specific embodiments, the

maytansinoid is maytansine or DM-1 (ImmunoGen, Inc.; *see also* Chari *et al.*, 1992, *Cancer Res.* 52:127-131).

[0160] In some embodiments, an anti-CD70 antibody can be chimeric, comprising a human or non-human Fc region or portion thereof. For example, the antibody can include an Fc domain or portion of non-human origin, *e.g.*, rodent (*e.g.*, mouse or rat), donkey, sheep, rabbit, goat, guinea pig, camelid, horse, chicken or monkey (*e.g.*, macaque, rhesus or the like).

[0161] An anti-CD70 binding agent, such as an antibody, can be monospecific, bispecific, trispecific, or of greater multispecificity. Multispecific antibodies may be specific for different epitopes of CD70 and/or may be specific for both CD70 as well as for a heterologous protein. (*See, e.g.*, PCT Publications WO 93/17715, WO 92/08802, WO 91/00360, and WO 92/05793; Tutt *et al.*, 1991, *J. Immunol.* 147:60-69; U.S. Patent Nos. 4,474,893; 4,714,681; 4,925,648; 5,573,920; and 5,601,819; Kostelny *et al.*, 1992, *J. Immunol.* 148:1547-1553.) Multispecific antibodies, including bispecific and trispecific antibodies, useful for practicing the methods described herein are antibodies that immunospecifically bind to both CD70 (including but not limited to antibodies that have the CDRs of the monoclonal antibody 1F6) and a second cell surface receptor or receptor complex that mediates ADCC, ADCP, and/or CDC, such as CD16/Fc γ RIII, CD64/Fc γ RI, killer inhibitory or activating receptors, or the complement control protein CD59. In some embodiments, the binding of the portion of the multispecific antibody to the second cell surface molecule or receptor complex may enhance the effector functions of the anti-CD70 antibody or other CD70 binding agent.

[0162] The antibodies can be generated by methods known in the art. For example, monoclonal antibodies can be prepared using a wide variety of techniques including, *e.g.*, the use of hybridoma, recombinant, and phage display technologies, or a combination thereof. Hybridoma techniques are generally discussed in, for example, Harlow *et al.*, *Antibodies: A Laboratory Manual* (Cold Spring Harbor Laboratory Press, 2nd ed., 1988); and Hammerling *et al.*, In *Monoclonal Antibodies and T-Cell Hybridomas*, pp. 563-681 (Elsevier, N.Y., 1981). Examples of phage display methods that can be used to make the anti-CD70 antibodies include, *e.g.*, those disclosed in Hoogenboom and Winter, 1991, *J. Mol. Biol.* 227:381; Marks *et al.*, 1991, *J. Mol. Biol.* 222:581; Quan and Carter, 2002, *The rise of monoclonal antibodies as therapeutics* in *Anti-IgE and Allergic Disease*, Jardieu and Fick Jr., eds., Marcel Dekker, New

York, NY, Chapter 20, pp. 427–469; Brinkman *et al.*, 1995, *J. Immunol. Methods* 182:41-50; Ames *et al.*, 1995, *J. Immunol. Methods* 184:177-186; Kettleborough *et al.*, 1994, *Eur. J. Immunol.* 24:952-958; Persic *et al.*, 1997, *Gene* 187:9-18; Burton *et al.*, 1994, *Advances in Immunology* 57:191-280; PCT Application No. PCT/GB91/01134; PCT Publications WO 90/02809, WO 91/10737, WO 92/01047, WO 92/18619, WO 93/11236, WO 95/15982, WO 95/20401, and U.S. Patent Nos. 5,698,426; 5,223,409; 5,403,484; 5,580,717; 5,427,908; 5,750,753; 5,821,047; 5,571,698; 5,427,908; 5,516,637; 5,780,225; 5,658,727; 5,733,743 and 5,969,108 (the disclosures of which are incorporated by reference herein).

[0163] Examples of techniques that can be used to produce single-chain antibodies include those described in U.S. Patents 4,946,778 and 5,258,498; Huston *et al.*, 1991, *Methods in Enzymology* 203:46-88; Shu *et al.*, 1993, *Proc. Natl. Acad. Sci. USA* 90:7995-7999; and Skerra *et al.*, 1988, *Science* 240:1038-1040.

[0164] Methods for making bispecific antibodies are known in the art. Traditional production of full-length bispecific antibodies is based on the coexpression of two immunoglobulin heavy chain-light chain pairs, where the two chains have different specificities (*see, e.g.*, Milstein *et al.*, 1983, *Nature* 305:537-39). Because of the random assortment of immunoglobulin heavy and light chains, these hybridomas (quadromas) produce a potential mixture of 10 different antibody molecules, of which some have the correct bispecific structure. Similar procedures are disclosed in International Publication No. WO 93/08829, and in Traunecker *et al.*, 1991, *EMBO J.* 10:3655-59.

[0165] According to a different approach, antibody variable domains with the desired binding specificities (antibody-antigen combining sites) are fused to immunoglobulin constant domain sequences. The fusion typically is with an immunoglobulin heavy chain constant domain, comprising at least part of the hinge, C_H2, and C_H3 regions. In some embodiments, the fusion includes a first heavy-chain constant region (C_H1) containing the site necessary for light chain binding, present in at least one of the fusions. Nucleic acids with sequences encoding the immunoglobulin heavy chain fusions and, if desired, the immunoglobulin light chain, are inserted into separate expression vectors, and are co-transfected into a suitable host organism. This provides for great flexibility in adjusting the mutual proportions of the three polypeptide fragments in embodiments when unequal ratios of the three polypeptide chains used in the

construction provide the optimum yields. It is, however, possible to insert the coding sequences for two or all three polypeptide chains in one expression vector when the expression of at least two polypeptide chains in equal ratios results in high yields or when the ratios are of no particular significance.

[0166] In an embodiment of this approach, the bispecific antibodies have a hybrid immunoglobulin heavy chain with a first binding specificity in one arm, and a hybrid immunoglobulin heavy chain-light chain pair (providing a second binding specificity) in the other arm. This asymmetric structure facilitates the separation of the desired bispecific compound from unwanted immunoglobulin chain combinations, as the presence of an immunoglobulin light chain in only one half of the bispecific molecule provides for a facile way of separation (*see, e.g.*, International Publication No. WO 94/04690, which is incorporated herein by reference in its entirety).

[0167] For further discussion of bispecific antibodies see, for example, Suresh *et al.*, 1986, *Methods in Enzymology* 121:210; Rodrigues *et al.*, 1993, *J. Immunology* 151:6954-61; Carter *et al.*, 1992, *Bio/Technology* 10:163-67; Carter *et al.*, 1995, *J. Hematotherapy* 4:463-70; Merchant *et al.*, 1998, *Nature Biotechnology* 16:677-81. Using such techniques, bispecific antibodies can be prepared for use in the treatment or prevention of disease as defined herein.

[0168] Bifunctional antibodies are also described in European Patent Publication No. EPA 0 105 360. As disclosed in this reference, hybrid or bifunctional antibodies can be derived either biologically, *i.e.*, by cell fusion techniques, or chemically, especially with cross-linking agents or disulfide-bridge forming reagents, and may comprise whole antibodies or fragments thereof. Methods for obtaining such hybrid antibodies are disclosed for example in International Publication WO 83/03679 and European Patent Publication No. EPA 0 217 577, both of which are incorporated herein by reference.

[0169] In some embodiments, framework residues in the human framework regions will be substituted with the corresponding residue from the CDR donor antibody to alter, preferably improve, antigen binding. These framework substitutions are identified by methods well known in the art, *e.g.*, by modeling of the interactions of the CDR and framework residues to identify framework residues important for antigen binding and sequence comparison to identify unusual framework residues at particular positions. (*See, e.g.*, U.S. Patent No. 5,585,089; Riechmann *et*

al., 1988, *Nature* 332:323.) Antibodies can be humanized using a variety of techniques known in the art including, for example, CDR-grafting (*see, e.g.*, EP 0 239 400; PCT Publication WO 91/09967; U.S. Patent Nos. 5,225,539; 5,530,101; and 5,585,089), veneering or resurfacing (*see, e.g.*, EP 0 592 106; EP 0 519 596; Padlan, 1991, *Molecular Immunology* 28(4/5):489-498; Studnicka *et al.*, 1994, *Protein Engineering* 7(6):805-814; Roguska *et al.*, 1994, *Proc. Natl. Acad. Sci. USA* 91:969-973), and chain shuffling (*see, e.g.*, U.S. Patent No. 5,565,332) (all of these references are incorporated by reference herein).

[0170] Humanized monoclonal antibodies can be produced by recombinant DNA techniques known in the art, for example using methods described in International Publication No. WO 87/02671; European Patent Publication No. 0 184 187; European Patent Publication No. 0 171 496; European Patent Publication No. 0 173 494; International Publication No. WO 86/01533; U.S. Patent No. 4,816,567; European Patent Publication No. 0 012 023; Berter *et al.*, 1988, *Science* 240:1041-43; Liu *et al.*, 1987, *Proc. Natl. Acad. Sci. USA* 84:3439-43; Liu *et al.*, 1987, *J. Immunol.* 139:3521-26; Sun *et al.*, 1987, *Proc. Natl. Acad. Sci. USA* 84:214-18; Nishimura *et al.*, 1987, *Cancer. Res.* 47:999-1005; Wood *et al.*, 1985, *Nature* 314:446-449; Shaw *et al.*, 1988, *J. Natl. Cancer Inst.* 80:1553-59; Morrison, 1985, *Science* 229:1202-07; Oi *et al.*, 1986, *BioTechniques* 4:214; U.S. Patent No. 5,225,539; Jones *et al.*, 1986, *Nature* 321:552-25; Verhoeyan *et al.*, 1988, *Science* 239:1534; and Beidler *et al.*, 1988, *J. Immunol.* 141:4053-60; each of which is incorporated herein by reference in its entirety.

[0171] As set forth *supra*, a CD70 binding agent can be a derivative of an anti-CD70 antibody. Generally, an anti-CD70 antibody derivative comprises an anti-CD70 antibody (including *e.g.*, an antigen-binding fragment or conservatively substituted polypeptides) and at least one polypeptide region or other moiety heterologous to the anti-CD70 antibody. For example, an anti-CD70 antibody can be modified, *e.g.*, by the covalent attachment of any type of molecule. Typical modifications include, *e.g.*, glycosylation, acetylation, pegylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to a cellular ligand (*e.g.*, an albumin-binding molecule) or other protein, and the like. Any of numerous chemical modifications may be carried out by known techniques, including, but not limited to specific chemical cleavage, acetylation, formylation, metabolic synthesis of tunicamycin, *etc.*

[0172] In some embodiments, the covalent attachment does not interfere with effector function, *e.g.*, prevent the antibody derivative from specifically binding to CD70 via the antigen-binding region or region derived therefrom, or the effector domains(s) from specifically binding Fc receptor.

[0173] In some embodiments, the antibody derivative is a multimer, such as, for example, a dimer, comprising one or more monomers, where each monomer includes (i) an antigen-binding region of an anti-CD70 antibody, or a polypeptide region derived therefrom (such as, *e.g.*, by conservative substitution of one or more amino acids), and (ii) a multimerizing (*e.g.*, dimerizing) polypeptide region, such that the antibody derivative forms multimers (*e.g.*, homodimers) that specifically bind to CD70. In typical embodiments, an antigen-binding region of an anti-CD70 antibody, or a polypeptide region derived therefrom, is recombinantly or chemically fused with a heterologous protein, wherein the heterologous protein comprises a dimerization or multimerization domain. Prior to administration of the antibody derivative to a subject for the purpose of treating or preventing immunological disorders or CD70-expressing cancers, the derivative is subjected to conditions that allow formation of a homodimer or heterodimer. A heterodimer, as used herein, may comprise identical dimerization domains but different CD70 antigen-binding regions, identical CD70 antigen-binding regions but different dimerization domains, or different CD70 antigen-binding regions and dimerization domains.

[0174] Typical dimerization domains are those that originate from transcription factors. In one embodiment, the dimerization domain is that of a basic region leucine zipper (“bZIP”) (see Vinson *et al.*, 1989, *Science* 246:911-916). Useful leucine zipper domains include, for example, those of the yeast transcription factor GCN4, the mammalian transcription factor CCAAT/enhancer-binding protein C/EBP, and the nuclear transform in oncogene products, Fos and Jun. (See, *e.g.*, Landschultz *et al.*, 1988, *Science* 240:1759-64; Baxevanis and Vinson, 1993, *Curr. Op. Gen. Devel.* 3:278-285; O’Shea *et al.*, 1989, *Science* 243:538-542.) In another embodiment, the dimerization domain is that of a basic-region helix-loop-helix (“bHLH”) protein. (See, *e.g.*, Murre *et al.*, 1989, *Cell* 56:777-783. See also Davis *et al.*, 1990, *Cell* 60:733-746; Voronova and Baltimore, 1990, *Proc. Natl. Acad. Sci. USA* 87:4722-26.) Particularly useful hHLH proteins are myc, max, and mac.

[0175] In yet other embodiments, the dimerization domain is an immunoglobulin constant region such as, for example, a heavy chain constant region or a domain thereof (*e.g.*, a C_H1 domain, a C_H2 domain, and/or a C_H3 domain). (*See, e.g.*, U.S. Patent Nos. 5,155,027; 5,336,603; 5,359,046; and 5,349,053; EP 0 367 166; and WO 96/04388.)

[0176] Heterodimers are known to form between Fos and Jun (Bohmann *et al.*, 1987, *Science* 238:1386-1392), among members of the ATF/CREB family (Hai *et al.*, 1989, *Genes Dev.* 3:2083-2090), among members of the C/EBP family (Cao *et al.*, 1991, *Genes Dev.* 5:1538-52; Williams *et al.*, 1991, *Genes Dev.* 5:1553-67; Roman *et al.*, 1990, *Genes Dev.* 4:1404-15), and between members of the ATF/CREB and Fos/Jun families (Hai and Curran, 1991, *Proc. Natl. Acad. Sci. USA* 88:3720-24). Therefore, when a CD70-binding protein is administered to a subject as a heterodimer comprising different dimerization domains, any combination of the foregoing may be used.

[0177] In other embodiments, an anti-CD70 antibody derivative is an anti-CD70 antibody conjugated to a second antibody (an “antibody heteroconjugate”) (*see, e.g.*, U.S. Patent No. 4,676,980). Heteroconjugates useful for practicing the present methods comprise an antibody that binds to CD70 (*e.g.*, an antibody that has the CDRs and/or heavy chains of the monoclonal antibody 1F6) and an antibody that binds to a surface receptor or receptor complex that mediates ADCC, phagocytosis, and/or CDC, such as CD16/FcγRIII, CD64/FcγRI, killer cell activating or inhibitory receptors, or the complement control protein CD59. In a typical embodiment, the binding of the portion of the multispecific antibody to the second cell surface molecule or receptor complex enhances the effector functions of an anti-CD70 antibody. In other embodiments, the antibody can be a therapeutic agent. Suitable antibody therapeutic agents are described herein.

[0178] In some embodiments, any of the anti-CD70 antibodies described herein is nonfucosylated.

[0179] In some embodiments, provided herein is a population of anti-CD70 antibodies comprising a plurality of anti-CD70 antibodies as described herein, wherein the anti-CD70 antibodies in the population of anti-CD70 antibodies have reduced core fucosylation. In some embodiments, at least 20% of antibodies in the population of anti-CD70 antibodies lack core fucosylation. In some embodiments, at least 30% of antibodies in the population of anti-CD70

antibodies lack core fucosylation. In some embodiments, at least 40% of antibodies in the population of anti-CD70 antibodies lack core fucosylation. In some embodiments, at least 50% of antibodies in the population of anti-CD70 antibodies lack core fucosylation. In some embodiments, at least 60% of antibodies in the population of anti-CD70 antibodies lack core fucosylation. In some embodiments, at least 70% of antibodies in the population of anti-CD70 antibodies lack core fucosylation. In some embodiments, at least 80% of antibodies in the population of anti-CD70 antibodies lack core fucosylation. In some embodiments, at least 90% of antibodies in the population of anti-CD70 antibodies lack core fucosylation. In some embodiments, at least 95% of antibodies in the population of anti-CD70 antibodies lack core fucosylation. In some embodiments, at least 98% of antibodies in the population of anti-CD70 antibodies lack core fucosylation. In some embodiments, at least 99% of antibodies in the population of anti-CD70 antibodies lack core fucosylation. In some embodiments, at least 99.5% of antibodies in the population of anti-CD70 antibodies lack core fucosylation. In some embodiments, substantially none (i.e., less than 0.5%) of the antibodies in the population of anti-CD70 antibodies have core fucosylation. In some embodiments, all of the antibodies in the population of anti-CD70 antibodies lack core fucosylation.

[0180] As described in U.S. Patent No. 10,196,445, modification of antibody glycosylation can be accomplished by, for example, expressing the antibody in a host cell with altered glycosylation machinery. Cells with altered glycosylation machinery have been described and can be used as host cells in which to express recombinant antibodies of this disclosure to thereby produce an antibody with altered glycosylation. For example, the cell lines Ms704, Ms705, and Ms709 lack the fucosyltransferase gene, FUT8 (α -(1,6) fucosyltransferase (see U.S. Pat. App. Publication No. 20040110704; Yamane-Ohnuki et al. (2004) *Biotechnol. Bioeng.* 87: 614), such that antibodies expressed in these cell lines lack fucose on their carbohydrates. As another example, EP 1176195 also describes a cell line with a functionally disrupted FUT8 gene as well as cell lines that have little or no activity for adding fucose to the N-acetylglucosamine that binds to the Fc region of the antibody, for example, the rat myeloma cell line YB2/0 (ATCC CRL 1662). PCT Publication WO 03/035835 describes a variant CHO cell line, Lec13, with reduced ability to attach fucose to Asn(297)-linked carbohydrates, also resulting in hypofucosylation of antibodies expressed in that host cell. See also Shields et al. (2002) *J. Biol. Chem.* 277:26733. Antibodies with a modified glycosylation profile can also be produced in chicken eggs, as

described in PCT Publication No. WO 2006/089231. Alternatively, antibodies with a modified glycosylation profile can be produced in plant cells, such as Lemna. See e.g. U.S. Publication No. 2012/0276086. PCT Publication No. WO 99/54342 describes cell lines engineered to express glycoprotein-modifying glycosyl transferases (e.g., beta(1,4)-N-acetylglucosaminyltransferase III (GnTIII)) such that antibodies expressed in the engineered cell lines exhibit increased bisecting GlcNAc structures which results in increased ADCC activity of the antibodies. See also Umaña et al. (1999) *Nat. Biotech.* 17:176. Alternatively, the fucose residues of the antibody may be cleaved off using a fucosidase enzyme. For example, the enzyme alpha-L-fucosidase removes fucosyl residues from antibodies. Tarentino et al. (1975) *Biochem.* 14:5516. Antibodies with reduced core fucosylation can be prepared by producing the antibodies in cell lines that have been engineered to reduce core fucosylation using gene knock-outs, gene knock-ins, or RNAi. Small molecule inhibitors that act on enzymes in the glycosylation pathway can also be used to generate antibodies with reduced core fucosylation. Such methods are described in U.S. Patent No. 8,163,551. In some embodiments, anti-CD70 antibodies as described herein with reduced core fucosylation are generated by culturing a host cell expressing the antibodies in a culture medium comprising an effective amount of a fucose analog that reduces the incorporation of fucose into complex N-glycoside-linked sugar chains of antibodies or antibody derivatives produced by host cell. See U.S. Patent No. 8,163,551. Methods of producing nonfucosylated antibodies are also described in Pereira et al. (2018) *MAbs* 10(5):693-711.

[0181] In some embodiments, the anti-CD70 antibody or derivative thereof competitively inhibits binding of mAb 1F6 to CD70, as determined by any method known in the art for determining competitive binding (such as e.g., the immunoassays described herein). In typical embodiments, the antibody competitively inhibits binding of 1F6 to CD70 by at least 50%, at least 60%, at least 70%, or at least 75%. In other embodiments, the antibody competitively inhibits binding of 1F6 to CD70 by at least 80%, at least 85%, at least 90%, or at least 95%.

[0182] Antibodies can be assayed for specific binding to CD70 by any of various known methods. Immunoassays which can be used include, for example, competitive and non-competitive assay systems using techniques such as Western blots, radioimmunoassays, ELISA (enzyme linked immunosorbent assay), “sandwich” immunoassays, immunoprecipitation assays, precipitin reactions, gel diffusion precipitin reactions, immunodiffusion assays, agglutination

assays, complement-fixation assays, immunoradiometric assays, fluorescent immunoassays, and protein A immunoassays. Such assays are routine and well-known in the art. (*See, e.g.*, Ausubel *et al.*, eds., *Short Protocols in Molecular Biology* (John Wiley and Sons, Inc., New York, 4th ed. 1999); Harlow and Lane, *Using Antibodies: A Laboratory Manual* (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1999).)

[0183] Further, the binding affinity of an antibody to CD70 and the off-rate of an antibody CD70 interaction can be determined by competitive binding assays. One example of a competitive binding assay is a radioimmunoassay comprising the incubation of labeled CD70 (*e.g.*, ^3H or ^{125}I) with the antibody of interest in the presence of increasing amounts of unlabeled CD70, and the detection of the antibody bound to the labeled CD70. The affinity of the antibody for CD70 and the binding off-rates can then be determined from the data by Scatchard plot analysis. Competition with a second antibody (such as *e.g.*, mAb 1F6) can also be determined using radioimmunoassays. In this case, CD70 is incubated with the antibody of interest conjugated to a labeled compound (*e.g.*, ^3H or ^{125}I) in the presence of increasing amounts of an unlabeled second antibody. Alternatively, the binding affinity of an antibody to CD70 and the on- and off-rates of an antibody-CD70 interaction can be determined by surface plasmon resonance. In some embodiments, the anti-CD70 antibodies or derivatives thereof can be targeted to and accumulate on the membrane of a CD70-expressing cell.

[0184] Anti-CD70 antibodies and derivatives thereof can be produced by methods known in the art for the synthesis of proteins, typically, *e.g.*, by recombinant expression techniques. Recombinant expression of an antibody or derivative thereof that binds to CD70 typically includes construction of an expression vector containing a nucleic acid that encodes the antibody or derivative thereof. A vector for the production of the protein molecule may be produced by recombinant DNA technology using techniques known in the art. Standard techniques such as, for example, those described in Sambrook and Russell, *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 3rd ed., 2001); Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 2nd ed., 1989); *Short Protocols in Molecular Biology* (Ausubel *et al.*, John Wiley and Sons, New York, 4th ed., 1999); and Glick and Pasternak, *Molecular Biotechnology: Principles and Applications of Recombinant DNA* (ASM Press, Washington,

D.C., 2nd ed., 1998) can be used for recombinant nucleic acid methods, nucleic acid synthesis, cell culture, transgene incorporation, and recombinant protein expression.

[0185] For example, for recombinant expression of an anti-CD70 antibody, an expression vector may encode a heavy or light chain thereof, or a heavy or light chain variable domain, operably linked to a promoter. An expression vector may include, for example, the nucleotide sequence encoding the constant region of the antibody molecule (*see, e.g.*, PCT Publication WO 86/05807; PCT Publication WO 89/01036; and U.S. Patent No. 5,122,464), and the variable domain of the antibody may be cloned into such a vector for expression of the entire heavy or light chain. The expression vector is transferred to a host cell by conventional techniques, and the transfected cells are then cultured by conventional techniques to produce the anti-CD70 antibody. In typical embodiments for the expression of double-chained antibodies, vectors encoding both the heavy and light chains can be co-expressed in the host cell for expression of the entire immunoglobulin molecule.

[0186] A variety of prokaryotic and eukaryotic host-expression vector systems can be utilized to express an anti-CD70 antibody or derivative thereof. Typically, eukaryotic cells, particularly for whole recombinant anti-CD70 antibody molecules, are used for the expression of the recombinant protein. For example, mammalian cells such as Chinese hamster ovary cells (CHO), in conjunction with a vector such as the major intermediate early gene promoter element from human cytomegalovirus, is an effective expression system for the production of anti-CD70 antibodies and derivatives thereof (*see, e.g.*, Foecking *et al.*, 1986, *Gene* 45:101; Cockett *et al.*, 1990, *Bio/Technology* 8:2).

[0187] Other host-expression systems include, for example, plasmid-based expression systems in bacterial cells (*see, e.g.*, Ruther *et al.*, 1983, *EMBO* 1,2:1791; Inouye and Inouye, 1985, *Nucleic Acids Res.* 13:3101-3109; Van Heeke and Schuster, 1989, *J. Biol. Chem.* 24:5503-5509); insect systems such as, *e.g.*, the use of *Autographa californica* nuclear polyhedrosis virus (AcNPV) expression vector in *Spodoptera frugiperda* cells; and viral-based expression systems in mammalian cells, such as, *e.g.*, adenoviral-based systems (*see, e.g.*, Logan and Shenk, 1984, *Proc. Natl. Acad. Sci. USA* 81:355-359; Bittner *et al.*, 1987, *Methods in Enzymol.* 153:51-544).

[0188] In addition, a host cell strain can be chosen that modulates the expression of the inserted sequences, or modifies and processes the gene product in the specific fashion desired.

Appropriate cell lines or host systems can be chosen to ensure the correct modification and processing (*e.g.*, glycosylation, phosphorylation, and cleavage) of the protein expressed. To this end, eukaryotic host cells which possess the cellular machinery for proper processing of the primary transcript and gene product can be used. Such mammalian host cells include, for example, CHO, VERO, BHK, HeLa, COS, MDCK, 293, 3T3, and W138.

[0189] A stable expression system is typically used for long-term, high-yield production of recombinant anti-CD70 antibody or derivative thereof or other CD70 binding agent. For example, cell lines that stably express the anti-CD70 antibody or derivative thereof can be engineered by transformation of host cells with DNA controlled by appropriate expression control elements (*e.g.*, promoter and enhancer sequences, transcription terminators, polyadenylation sites) and a selectable marker, followed by growth of the transformed cells in a selective media. The selectable marker confers resistance to the selection and allows cells to stably integrate the DNA into their chromosomes and grow to form foci which in turn can be cloned and expanded into cell lines. A number of selection systems can be used, including, for example, the herpes simplex virus thymidine kinase, hypoxanthineguanine phosphoribosyltransferase, and adenine phosphoribosyltransferase genes, which can be employed in *tk*⁻, *hgprt*⁻ or *aprt*⁻ cells, respectively. Also, antimetabolite resistance can be used as the basis of selection for the following genes: *dhfr*, which confers resistance to methotrexate; *gpt*, which confers resistance to mycophenolic acid; *neo*, which confers resistance to the aminoglycoside G-418; and *hygro*, which confers resistance to hygromycin. Methods commonly known in the art of recombinant DNA technology can be routinely applied to select the desired recombinant clone, and such methods are described, for example, in *Current Protocols in Molecular Biology* (Ausubel *et al.* eds., John Wiley and Sons, N.Y., 1993); Kriegler, *Gene Transfer and Expression, A Laboratory Manual* (Stockton Press, N.Y., 1990); *Current Protocols in Human Genetics* (Dracopoli *et al.* eds., John Wiley and Sons, N.Y., 1994, Chapters 12 and 13); and Colberre-Garapin *et al.*, 1981, *J. Mol. Biol.* 150:1.

[0190] The expression levels of an antibody or derivative can be increased by vector amplification. (*See generally, e.g.*, Bebbington and Hentschel, *The Use of Vectors Based on Gene Amplification for the Expression of Cloned Genes in Mammalian Cells in DNA Cloning*, Vol. 3 (Academic Press, New York, 1987).) When a marker in the vector system expressing an anti-CD70 antibody or derivative thereof is amplifiable, an increase in the level of inhibitor

present in host cell culture media will select host cells that have increased copy number of a marker gene conferring resistance to the inhibitor. The copy number of an associated antibody gene will also be increased, thereby increasing expression of the antibody or derivative thereof (*see* Crouse *et al.*, 1983, *Mol. Cell. Biol.* 3:257).

[0191] Where the anti-CD70 antibody comprises both a heavy and a light chain or derivatives thereof, the host cell may be co-transfected with two expression vectors, the first vector encoding the heavy chain protein and the second vector encoding the light chain protein. The two vectors may contain identical selectable markers which enable equal expression of heavy and light chain proteins. Alternatively, a single vector may be used which encodes, and is capable of expressing, both heavy and light chain proteins. In such situations, the light chain is typically placed before the heavy chain to avoid an excess of toxic free heavy chain (*see* Proudfoot, 1986, *Nature* 322:52; Kohler, 1980, *Proc. Natl. Acad. Sci. USA* 77:2197). The coding sequences for the heavy and light chains may comprise cDNA or genomic DNA.

[0192] Once an anti-CD70 antibody or derivative thereof has been produced (*e.g.*, by an animal, chemical synthesis, or recombinant expression), it can be purified by any suitable method for purification of proteins, including, for example, by chromatography (*e.g.*, ion exchange or affinity chromatography (such as, for example, Protein A chromatography for purification of antibodies having an intact Fc region)), centrifugation, differential solubility, or by any other standard technique for the purification of proteins. An anti-CD70 antibody or derivative thereof can, for example, be fused to a marker sequence, such as a peptide, to facilitate purification by affinity chromatography. Suitable marker amino acid sequences include, *e.g.*, a hexa-histidine peptide, such as the tag provided in a pQE vector (QIAGEN, Inc., Chatsworth, CA, 91311), and the “HA” tag, which corresponds to an epitope derived from the influenza hemagglutinin protein (Wilson *et al.*, 1984, *Cell* 37:767), and the “flag” tag.

[0193] Once an anti-CD70 antibody or derivative thereof is produced, its ability to exert a cytostatic or cytotoxic effect on CD70-expressing cancer cells or an immunomodulatory effect on a CD70-expressing immune cell is determined by the methods described *infra* or as known in the art.

[0194] To minimize activity of the anti-CD70 antibody outside the activated immune cells or CD70-expressing cancer cells, an antibody that specifically binds to cell membrane-bound

CD70, but not to soluble CD70, can be used, so that the anti-CD70 antibody is concentrated at the cell surface of the activated immune cell or CD70-expressing cancer cell.

[0195] Typically, the anti-CD70 antibody or derivative is substantially purified (*e.g.*, substantially free from substances that limit its effect or produce undesired side-effects). In some embodiments, the anti-CD70 antibody or derivative is at least about 40% pure, at least about 50% pure, or at least about 60% pure. In some embodiments, the anti-CD70 antibody or derivative is at least about 60-65%, 65-70%, 70-75%, 75-80%, 80-85%, 85-90%, 90-95%, or 95-98% pure. In some embodiments, the anti-CD70 antibody or derivative is approximately 99% pure.

III. CD47 Antagonists

[0196] The invention provides CD47 antagonists. In some embodiments, the CD47 antagonist inhibits the interaction between CD47 and SIRP α . In some embodiments, the CD47 antagonist increases phagocytosis of tumor cells. In some embodiments, the CD47 antagonist is selected from the group consisting of a small molecule inhibitor of CD47, a small molecule inhibitor of SIRP α , an antibody, or antigen-binding fragment thereof, that binds to CD47, an antibody or antigen-binding fragment thereof, that binds to SIRP α , and a fusion protein comprising SIRP α , or a fragment thereof, and an antibody, or fragment thereof. In some embodiments, the CD47 antagonist is a small molecule inhibitor of CD47. In some embodiments, the CD47 antagonist is a small molecule inhibitor of SIRP α . In some embodiments, the CD47 antagonist is an antibody, or antigen-binding fragment thereof, that binds to CD47. In some embodiments, the CD47 antagonist is an antibody or antigen-binding fragment thereof, that binds to SIRP α . In some embodiments, the CD47 antagonist is a fusion protein comprising SIRP α , or a fragment thereof. In some embodiments, the fusion protein comprising SIRP α , or a fragment thereof, and an antibody, or fragment thereof, comprises SIRP α , or the immunoglobulin V-like domain thereof, covalently linked to the Fc region of an antibody.

[0197] In some embodiments, the CD47 antagonist is an antibody, or antigen-binding fragment thereof. Antibodies of the disclosure are preferably monoclonal, and may be multispecific, human, humanized or chimeric antibodies, single chain antibodies, Fab fragments, F(ab') fragments, fragments produced by a Fab expression library, and CD47 binding fragments

of any of the above. In some embodiments, the CD47 antagonist antibodies of the disclosure specifically bind CD47. In some embodiments, the CD47 antagonist antibodies of the disclosure specifically bind SIRP α . The immunoglobulin molecules of the disclosure can be of any type (*e.g.*, IgG, IgE, IgM, IgD, IgA and IgY), class (*e.g.*, IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2) or subclass of immunoglobulin molecule. In some embodiments, the antibody is an IgG1 or IgG4 antibody. In some embodiments, the antibody is an IgG1 antibody. In some embodiments, the antibody is an IgG4 antibody. In certain embodiments of the disclosure, the antibodies are antigen-binding fragments (*e.g.*, human antigen-binding fragments) as described herein and include, but are not limited to, Fab, Fab' and F(ab')₂, Fd, single-chain Fvs (scFv), single-chain antibodies, disulfide-linked Fvs (sdFv) and fragments comprising either a V_L or V_H domain. Antigen-binding fragments, including single-chain antibodies, may comprise the variable region(s) alone or in combination with the entirety or a portion of the following: hinge region, CH1, CH2, CH3 and CL domains. Also included in the present disclosure are antigen-binding fragments comprising any combination of variable region(s) with a hinge region, CH1, CH2, CH3 and CL domains. In some embodiments, the CD47 antagonist antibodies or antigen-binding fragments thereof are human, murine (*e.g.*, mouse and rat), donkey, sheep, rabbit, goat, guinea pig, camelid, horse, or chicken.

[0198] The CD47 antagonist antibodies of the present disclosure may be monospecific, bispecific, trispecific or of greater multi specificity. Multispecific antibodies may be specific for different epitopes of the same molecule or may be specific for heterologous proteins. *See, e.g.*, PCT publications WO 93/17715; WO 92/08802; WO 91/00360; WO 92/05793; Tutt, *et al.*, 1991, J. Immunol. 147:60 69; U.S. Pat. Nos. 4,474,893; 4,714,681; 4,925,648; 5,573,920; 5,601,819; Kostelny *et al.*, 1992, J. Immunol. 148:1547 1553.

[0199] In some embodiments, the CD47 antagonist antibody is a human antibody. In some embodiments, the CD47 antagonist antibody is a humanized antibody. In some embodiments, the CD47 antagonist antibody is a chimeric antibody.

[0200] CD47 antagonist antibodies of the present disclosure may be described or specified in terms of the particular CDRs they comprise. The precise amino acid sequence boundaries of a given CDR or FR can be readily determined using any of a number of well-known schemes, including those described by Kabat *et al.* (1991), "Sequences of Proteins of Immunological

Interest,” 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD (“Kabat” numbering scheme); Al-Lazikani *et al.*, (1997) JMB 273,927-948 (“Chothia” numbering scheme); MacCallum *et al.*, J. Mol. Biol. 262:732-745 (1996), “Antibody-antigen interactions: Contact analysis and binding site topography,” J. Mol. Biol. 262, 732-745.” (“Contact” numbering scheme); Lefranc MP *et al.*, “IMGT unique numbering for immunoglobulin and T cell receptor variable domains and Ig superfamily V-like domains,” Dev Comp Immunol, 2003 Jan;27(1):55-77 (“IMGT” numbering scheme); Honegger A and Plückthun A, “Yet another numbering scheme for immunoglobulin variable domains: an automatic modeling and analysis tool,” J Mol Biol, 2001 Jun 8;309(3):657-70, (“Aho” numbering scheme); and Martin *et al.*, “Modeling antibody hypervariable loops: a combined algorithm,” PNAS, 1989, 86(23):9268-9272, (“AbM” numbering scheme). The boundaries of a given CDR may vary depending on the scheme used for identification. In some embodiments, a “CDR” or “complementarity determining region,” or individual specified CDRs (*e.g.*, CDR-H1, CDR-H2, CDR-H3), of a given antibody or region thereof (*e.g.*, variable region thereof) should be understood to encompass a (or the specific) CDR as defined by any of the aforementioned schemes. For example, where it is stated that a particular CDR (*e.g.*, a CDR-H3) contains the amino acid sequence of a corresponding CDR in a given V_H or V_L region amino acid sequence, it is understood that such a CDR has a sequence of the corresponding CDR (*e.g.*, CDR-H3) within the variable region, as defined by any of the aforementioned schemes. The scheme for identification of a particular CDR or CDRs may be specified, such as the CDR as defined by the Kabat, Chothia, AbM or IMGT method.

[0201] CDR sequences of the CD47 antagonist antibodies are according to the Kabat numbering scheme as described in Kabat *et al.* (1991), “Sequences of Proteins of Immunological Interest,” 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD, unless specified otherwise.

[0202] CD47 antagonist antibodies of the present invention may also be described or specified in terms of their binding affinity (*e.g.*, human CD47 or human SIRPα). Preferred binding affinities include those with a dissociation constant or K_D less than 5 × 10⁻² M, 10⁻² M, 5 × 10⁻³ M, 10⁻³ M, 5 × 10⁻⁴ M, 10⁻⁴ M, 5 × 10⁻⁵ M, 10⁻⁵ M, 5 × 10⁻⁶ M, 10⁻⁶ M, 5 × 10⁻⁷ M, 10⁻⁷ M, 5 × 10⁻⁸ M, 10⁻⁸ M, 5 × 10⁻⁹ M, 10⁻⁹ M, 5 × 10⁻¹⁰ M, 10⁻¹⁰ M, 5 × 10⁻¹¹ M, 10⁻¹¹ M, 5 × 10⁻¹² M, 10⁻¹² M, 5 × 10⁻¹³ M, 10⁻¹³ M, 5 × 10⁻¹⁴ M, 10⁻¹⁴ M, 5 × 10⁻¹⁵ M, or 10⁻¹⁵ M.

[0203] There are five classes of immunoglobulins: IgA, IgD, IgE, IgG and IgM, having heavy chains designated α , δ , ϵ , γ and μ , respectively. The γ and α classes are further divided into subclasses *e.g.*, humans express the following subclasses: IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2. IgG1 antibodies can exist in multiple polymorphic variants termed allotypes (reviewed in Jefferis and Lefranc 2009. *mAbs* Vol 1 Issue 4 1-7) any of which are suitable for use in some of the embodiments herein. Common allotypic variants in human populations are those designated by the letters a, f, n, z or combinations thereof. In any of the embodiments herein, the antibody may comprise a heavy chain Fc region comprising a human IgG Fc region. In further embodiments, the human IgG Fc region comprises a human IgG1. In further embodiments, the human IgG Fc region comprises a human IgG4.

[0204] The antibodies also include derivatives that are modified, *i.e.*, by the covalent attachment of any type of molecule to the antibody such that covalent attachment does not prevent the antibody from binding, *e.g.*, to CD47 or SIRP α , or from exerting a cytostatic or cytotoxic effect on cells. For example, but not by way of limitation, the antibody derivatives include antibodies that have been modified, *e.g.*, by glycosylation, acetylation, PEGylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to a cellular ligand or other protein, etc. Any of numerous chemical modifications may be carried out by known techniques, including, but not limited to specific chemical cleavage, acetylation, formylation, metabolic synthesis of tunicamycin, etc. Additionally, the derivative may contain one or more non-classical amino acids.

[0205] The CD47 antagonist antibody can optionally include an antibody effector domain that mediates or stimulates an ADCC, ADCP and/or CDC response against a CD47-expressing target cell. The effector domain(s) can be, for example, an Fc domain or domains of an Ig molecule. Such a CD47 antagonist antibody can exert a cytotoxic or cytostatic effect on CD47-expressing cancer cells, or exert a cytotoxic, cytostatic, or immunomodulatory effect on activated lymphocytes or dendritic cells, for example, in the treatment of a CD47-expressing cancer or an immunological disorder, respectively. Typically, the CD47 antagonist antibody recruits and/or activates cytotoxic white blood cells (*e.g.*, natural killer (NK) cells, phagocytotic cells (*e.g.*, macrophages), and/or serum complement components).

[0206] The CD47 antagonist antibodies described herein can be assayed for specific binding to a target, e.g., CD47 or SIRP α , and for binding affinity using techniques as described herein for anti-CD70 antibodies.

[0207] The CD47 antagonist antibodies described herein can be produced using techniques as described herein for anti-CD70 antibodies.

[0208] In some embodiments the CD47 antagonist is selected from the group consisting of magrolimab (Forty Seven, Inc.; Gilead Sciences, Inc.), CC-90002 (Celgene Corporation), ALX148 (ALX Oncology), Vx-1004 (Corvus Pharmaceutical), NI-1701 (Novimmune S.A.), NI-1801 (Novimmune S.A.), RCT-1938 (Radiation Control Technologies, Inc.), KWAR23 (See WO2015138600), FSI-189 (Forty Seven Inc.; Gilead Sciences, Inc. (also known as GS-0189)), ES-004 (Elpiscience), BI765063 (OSE Immunotherapeutics (also known as OSE-172)), ADU-1805 (Aduro Biotech), CC-95251 (Celgene), AL-008 (Alector), RRx-001 (EpicentRx), CTX-5861 (Compass Therapeutics), TTI-621 (Trillium Therapeutics), and TTI-622 (Trillium Therapeutics). In some embodiments, the CD47 antagonist is disclosed in WO200140307, WO2002092784, WO2007133811, WO2009046541, WO2010083253, WO2011076781, WO2013056352, WO2015138600, WO2016179399, WO2016205042, WO2017178653, WO2018026600, WO2018057669, WO2018107058, WO2018190719, WO2018210793, WO2019023347, WO2019042470, WO2019175218, WO2019183266, WO2020013170 or WO2020068752.

[0209] In some embodiments, the CD47 antagonist is magrolimab, which is also known as Hu5F9-G4 and h5F9-G4. See US Patent No. 9,017,675. In some embodiments, the CD47 antagonist comprises the three heavy chain CDRs and the three light chain CDRs of magrolimab. In some embodiments, the CD47 antagonist comprises the heavy chain variable region and the light chain variable region of magrolimab. In some embodiments, the CD47 antagonist is a biosimilar of magrolimab.

[0210] In some embodiments, the CD47 antagonist is an antibody disclosed in US Patent No. 9,017,675. In some embodiments, the CD47 antagonist comprises the three heavy chain CDRs and the three light chain CDRs of an antibody disclosed in US Patent No. 9,017,675. In some embodiments, the CD47 antagonist comprises the heavy chain variable region and the light chain

variable region of an antibody disclosed in US Patent No. 9,017,675. In some embodiments, the CD47 antagonist is a biosimilar of an antibody disclosed in US Patent No. 9,017,675.

[0211] In some embodiments, the CD47 antagonist is an antibody disclosed in US2019/0185561. In some embodiments, the CD47 antagonist comprises the three heavy chain CDRs and the three light chain CDRs of an antibody disclosed in US2019/0185561. In some embodiments, the CD47 antagonist comprises the heavy chain variable region and the light chain variable region of an antibody disclosed in US2019/0185561. In some embodiments, the CD47 antagonist is a biosimilar of an antibody disclosed in US2019/0185561.

[0212] Typically, the CD47 antagonist or derivative is substantially purified (*e.g.*, substantially free from substances that limit its effect or produce undesired side-effects). In some embodiments, the CD47 antagonist or derivative is at least about 40% pure, at least about 50% pure, or at least about 60% pure. In some embodiments, the CD47 antagonist or derivative is at least about 60-65%, 65-70%, 70-75%, 75-80%, 80-85%, 85-90%, 90-95%, or 95-98% pure. In some embodiments, the CD47 antagonist or derivative is approximately 99% pure.

IV. Methods of Treatment

[0213] The invention provides methods of treating cancers, such as myeloid malignancies, in a subject comprising administering to the subject a therapeutically effective amount of an anti-CD70 antibody, such as a nonfucosylated anti-CD70 antibody, as described herein and a CD47 antagonist as described herein. In some embodiments, the cancer expresses CD70. In some embodiments, the cancer expresses CD47. In some embodiments, the cancer expresses CD70 and CD47. Myeloid malignancies include Acute Myeloid leukemia (AML), Myeloproliferative disorders (MPDS), myelodysplastic syndrome (MDS) and myelodysplastic/myeloproliferative syndromes that are all clonal stem-cell (HSC) or progenitor malignant disorders. In some embodiments, the cancer is MDS. In some embodiments, the cancer is AML. MDS encompasses multiple subtypes, including MDS with single-lineage dysplasia, MDS with ring sideroblasts, MDS with multilineage dysplasia, MDS with excess blasts, MDS with isolated del(5q), and MDS, unclassifiable. MDS is characterized by ineffective hematopoiesis in one or more of the lineage of the bone marrow. Early MDS mostly demonstrate excessive apoptosis and hematopoietic cell dysplasia. In about a third of MDS patients, this ineffective hematopoiesis precedes progression to secondary AML (sAML). AML is a malignant tumor of

the myeloid lineage of white blood cells. In some embodiments, the method comprises administering a nonfucosylated anti-CD70 antibody and a CD47 antagonist to the subject, wherein the anti-CD70 antibody comprises a heavy chain variable region comprising the three CDRs of SEQ ID NO:1, a light chain variable region comprising the three CDRs of SEQ ID NO:2, wherein the CDRs of the anti-CD70 antibody are defined by the Kabat numbering scheme, and an Fc domain. In some embodiments, the amount of anti-CD70 antibody administered to the subject is a therapeutically effective amount. In some embodiments, the amount of anti-CD70 antibody administered to the subject is a sub-therapeutic or sub-optimal amount. In some embodiments, the amount of CD47 antagonist administered to the subject is a therapeutically effective amount. In some embodiments, the amount of CD47 antagonist administered to the subject is a sub-therapeutic or sub-optimal amount. In some embodiments, the method comprises administering a CD47 antagonist and a population of anti-CD70 antibodies to the subject, wherein at least 30% of the anti-CD70 antibodies in the population of the anti-CD70 antibodies lack core fucosylation. In some embodiments, the method comprises administering a CD47 antagonist and a population of anti-CD70 antibodies to the subject, wherein at least 40% of the anti-CD70 antibodies in the population of the anti-CD70 antibodies lack core fucosylation. In some embodiments, the method comprises administering a CD47 antagonist and a population of anti-CD70 antibodies to the subject, wherein at least 50% of the anti-CD70 antibodies in the population of the anti-CD70 antibodies lack core fucosylation. In some embodiments, the method comprises administering a CD47 antagonist and a population of anti-CD70 antibodies to the subject, wherein at least 60% of the anti-CD70 antibodies in the population of the anti-CD70 antibodies lack core fucosylation. In some embodiments, the method comprises administering a CD47 antagonist and a population of anti-CD70 antibodies to the subject, wherein at least 70% of the anti-CD70 antibodies in the population of the anti-CD70 antibodies lack core fucosylation. In some embodiments, the method comprises administering a CD47 antagonist and a population of anti-CD70 antibodies to the subject, wherein at least 80% of the anti-CD70 antibodies in the population of the anti-CD70 antibodies lack core fucosylation. In some embodiments, the method comprises administering a CD47 antagonist and a population of anti-CD70 antibodies to the subject, wherein at least 90% of the anti-CD70 antibodies in the population of the anti-CD70 antibodies lack core fucosylation. In some embodiments, the method comprises administering a CD47 antagonist and a population of anti-CD70 antibodies to

the subject, wherein at least 95% of the anti-CD70 antibodies in the population of the anti-CD70 antibodies lack core fucosylation. In some embodiments, the method comprises administering a CD47 antagonist and a population of anti-CD70 antibodies to the subject, wherein at least 98% of the anti-CD70 antibodies in the population of the anti-CD70 antibodies lack core fucosylation. In some embodiments, the method comprises administering a CD47 antagonist and a population of anti-CD70 antibodies to the subject, wherein at least 99% of the anti-CD70 antibodies in the population of the anti-CD70 antibodies lack core fucosylation. In some embodiments, the method comprises administering a CD47 antagonist and a population of anti-CD70 antibodies to the subject, wherein at least 99.5% of the anti-CD70 antibodies in the population of the anti-CD70 antibodies lack core fucosylation. In some embodiments, the CD47 antagonist and anti-CD70 antibody are administered in combination with a hypomethylating agent (HMA). In some embodiments, the HMA is azacitidine. In some embodiments, the CD47 antagonist and anti-CD70 antibody are administered in combination with a BH3-mimetic. In some embodiments, the CD47 antagonist and anti-CD70 antibody are administered in combination with venetoclax (VENCLEXTA®). In some embodiments, the CD47 antagonist and anti-CD70 antibody are administered in combination with an HMA and a BH3-mimetic. In some embodiments, the CD47 antagonist and anti-CD70 antibody are administered in combination with an HMA and venetoclax. In some embodiments, the CD47 antagonist and anti-CD70 antibody are administered in combination with azacitidine and a BH3-mimetic. In some embodiments, the CD47 antagonist and anti-CD70 antibody are administered in combination with azacitidine and a venetoclax.

[0214] In some embodiments, provided herein is a method of treating MDS in a subject comprising administering an anti-CD70 antibody described herein and a CD47 antagonist described herein. In some embodiments, the cancer cells of the MDS express CD70. In some embodiments, the cancer cells of the MDS express CD47. In some embodiments, the cancer cells of the MDS express CD70 and CD47. In some embodiments, the anti-CD70 antibody is nonfucosylated. In some embodiments, the MDS is relapsed or refractory MDS. In some embodiments, the MDS is relapsed MDS. In some embodiments, the MDS is refractory MDS. In some embodiments, the amount of anti-CD70 antibody administered to the subject is a therapeutically effective amount. In some embodiments, the amount of anti-CD70 antibody administered to the subject is a sub-therapeutic or sub-optimal amount. In some embodiments,

the amount of CD47 antagonist administered to the subject is a therapeutically effective amount. In some embodiments, the amount of CD47 antagonist administered to the subject is a sub-therapeutic or suboptimal amount. In some embodiments, the subject experienced treatment failure after prior hypomethylating agent (HMA) therapy for the MDS. A HMA (also known as a demethylating agent) is a drug that inhibits DNA methylation. In some embodiments, the HMA is a DNA methyltransferase inhibitor. In some embodiments, the HMA is azacitidine. In some embodiments, the HMA is decitabine.

[0215] In some embodiments, provided herein is a method of treating AML in a subject comprising administering an anti-CD70 antibody described herein and a CD47 antagonist described herein. In some embodiments, the cancer cells of the AML express CD70. In some embodiments, the cancer cells of the AML express CD47. In some embodiments, the cancer cells of the AML express CD70 and CD47. In some embodiments, the anti-CD70 antibody is nonfucosylated. In some embodiments, the AML is relapsed or refractory AML. In some embodiments, the AML is relapsed AML. In some embodiments, the AML is refractory AML. In some embodiments, the amount of anti-CD70 antibody administered to the subject is a therapeutically effective amount. In some embodiments, the amount of anti-CD70 antibody administered to the subject is a sub-therapeutic or sub-optimal amount. In some embodiments, the amount of CD47 antagonist administered to the subject is a therapeutically effective amount. In some embodiments, the amount of CD47 antagonist administered to the subject is a sub-therapeutic or sub-optimal amount. In some embodiments, the subject received 1 prior treatment regimen to treat the AML. In some embodiments, the subject received 2 prior treatment regimens to treat the AML. In some embodiments, the subject received 3 prior treatment regimens to treat the AML.

[0216] In some embodiments, at least about 0.1%, at least about 1%, at least about 2%, at least about 3%, at least about 4%, at least about 5%, at least about 6%, at least about 7%, at least about 8%, at least about 9%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 60%, at least about 70%, or at least about 80% of the cancer cells from the subject express CD70. In some embodiments, at least 0.1%, at least 1%, at least 2%, at least 3%, at least 4%, at least 5%, at least 6%, at least 7%, at least 8%, at least 9%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at

least 50%, at least 60%, at least 70%, or at least 80% of the cancer cells from the subject express CD70. In some embodiments, the percentage of cells that express CD70 is determined using immunohistochemistry (IHC). In some embodiments, the percentage of cells that express CD70 is determined using flow cytometry. In some embodiments, the percentage of cells that express CD70 is determined using an enzyme-linked immunosorbent assay (ELISA).

[0217] In some embodiments, at least about 0.1%, at least about 1%, at least about 2%, at least about 3%, at least about 4%, at least about 5%, at least about 6%, at least about 7%, at least about 8%, at least about 9%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 60%, at least about 70%, or at least about 80% of the cancer cells from the subject express CD47. In some embodiments, at least 0.1%, at least 1%, at least 2%, at least 3%, at least 4%, at least 5%, at least 6%, at least 7%, at least 8%, at least 9%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 60%, at least 70%, or at least 80% of the cancer cells from the subject express CD47. In some embodiments, the percentage of cells that express CD47 is determined using immunohistochemistry (IHC). In some embodiments, the percentage of cells that express CD70 is determined using flow cytometry. In some embodiments, the percentage of cells that express CD47 is determined using an enzyme-linked immunosorbent assay (ELISA).

[0218] In one aspect, a method of treating cancer with an anti-CD70 antibody as described herein and a CD47 antagonist as described herein results in an improvement in one or more therapeutic effects in the subject after administration of the antibody relative to a baseline. In some embodiments, the one or more therapeutic effects is the objective response rate, the duration of response, the time to response, progression free survival, overall survival, or any combination thereof. In one embodiment, the one or more therapeutic effects is stable disease. In one embodiment, the one or more therapeutic effects is partial response. In one embodiment, the one or more therapeutic effects is complete response. In one embodiment, the one or more therapeutic effects is the objective response rate. In one embodiment, the one or more therapeutic effects is the duration of response. In one embodiment, the one or more therapeutic effects is the time to response. In one embodiment, the one or more therapeutic effects is progression free survival. In one embodiment, the one or more therapeutic effects is overall survival. In one embodiment, the one or more therapeutic effects is cancer regression.

[0219] In one embodiment of the methods or uses or product for uses provided herein, response to treatment with an anti-CD70 antibody as described herein and a CD47 antagonist as described herein may include the following criteria (Cheson criteria):

Term	Definition (all criteria must be met unless otherwise specified)a
Morphologic complete remission (CR)	Absolute neutrophil count (ANC) $\geq 1000/\mu\text{L}$ and platelets $\geq 100,000/\mu\text{L}$ without transfusions and/or exogenous growth factor support (i.e., no transfusion or exogenous growth factor within 7 days of assessment). Bone marrow with $<5\%$ blasts No evidence of extramedullary disease
Morphologic complete remission with incomplete blood count recovery (CRi)	CRi(p) (morphologic CR with incomplete platelet recovery) Bone marrow with $<5\%$ blasts Platelets $<100,000/\mu\text{L}$ or $\geq 100,000/\mu\text{L}$ if subject transfused in last 7 days ANC $\geq 1000/\mu\text{L}$ without exogenous growth factor support No evidence of extramedullary disease CRi(n) (morphologic CR with incomplete neutrophil recovery) Bone marrow with $<5\%$ blasts ANC $<1000/\mu\text{L}$ or ANC $\geq 1000/\mu\text{L}$ with use of exogenous growth factors in last 7 days Platelets $\geq 100,000/\mu\text{L}$ without transfusions in last 7 days No evidence of extramedullary disease
Morphologic complete remission with partial hematologic recovery (CRh)	Bone marrow with $<5\%$ blasts ANC $>500/\mu\text{L}$ and platelets $\geq 50,000/\mu\text{L}$ without transfusions and/or exogenous growth factor support in last 7 days without qualifying as full CR No evidence of extramedullary disease
Morphologic leukemia free state (mLFS)	Bone marrow with $<5\%$ blasts No evidence of extramedullary disease Criteria for blood count recovery not met for CR, CRi, or CRh
Partial remission (PR)	ANC $\geq 1000/\mu\text{L}$ and platelets $\geq 100,000/\mu\text{L}$ without transfusions and/or exogenous growth factor support (i.e., no transfusion or exogenous growth factor within 7 days of assessment). Bone marrow with 5% to 25% blasts and at least a 50% decrease in bone marrow blast percent from baseline No evidence of extramedullary disease
Antileukemic Effect	$>25\%$ reduction of bone marrow blasts relative to baseline and criteria for PR not met
Stable Disease (SD)	Absence of CR, CRi, CRh, mLFS, PR, or antileukemic effect. Criteria for progressive disease (PD) not met

Progressive Disease (PD)	>25% absolute rise in bone marrow blast percent from baseline or appearance of new extramedullary disease after 4 or more cycles of treatment. In subjects with baseline bone marrow blasts >75%, a 25% proportional (instead of absolute) increase in bone marrow blasts is considered PD.
Relapse from CR/CRi/CRh	Reappearance of blasts in the blood (unless consistent with regenerating bone marrow), or bone marrow (>5%), or in any extramedullary site after achieving CR, CRi or CRh

a Modified from the Revised Recommendations of the International Working Group for Diagnosis, Standardization of Response Criteria, Treatment Outcomes, and Reporting Standards for Therapeutic Trials in Acute Myeloid Leukemia (Cheson BD, Bennett JM, Kopecky KJ, Buchner T, Willman CL, Estey EH, Schiffer CA, Doehner H, Tallman MS, Lister TA, Lo-Coco F, Willemze R, Biondi A, Hiddemann W, Larson RA, Lowenberg B, Sanz MA, Head DR, Ohno R, Bloomfield CD (2003). Revised recommendations of the International Working Group for Diagnosis, Standardization of Response Criteria, Treatment Outcomes, and Reporting Standards for Therapeutic Trials in Acute Myeloid Leukemia. J Clin Oncol 21(24): 4642-9).

[0220] In one embodiment of the methods or uses or product for uses provided herein, response to treatment with an anti-CD70 antibody as described herein and a CD47 antagonist described herein may include the following criteria (Cheson criteria):

<u>Category</u>	<u>Response criteria (responses must last at least 4 weeks)</u>
Complete remission	Bone marrow $\leq 5\%$ myeloblasts with normal maturation of all cell lines* Persistent dysplasia will be noted*† Peripheral blood‡: Hgb ≥ 11 g/dL Platelets $\geq 100 \times 10^9/L$ Neutrophils $\geq 1.0 \times 10^9/L$ † Blasts 0%
Partial remission	All CR criteria if abnormal before treatment except: Bone marrow blasts decreased by $\geq 50\%$ over pretreatment but still $> 5\%$ Cellularity and morphology not relevant
Marrow CR†	Bone marrow: $\leq 5\%$ myeloblasts and decrease by $\geq 50\%$ over pretreatment† Peripheral blood: if HI responses, they will be noted in addition to Marrow CR†
Stable disease	Failure to achieve at least PR, but no evidence of progression for > 8 weeks
Failure	Death during treatment or disease progression characterized by worsening of cytopenias, increase in percentage of bone marrow blasts, or progression to a more advanced MDS FAB subtype than pretreatment
Relapse after CR or PR	At least 1 of the following: Return to pretreatment bone marrow blast percentage Decrement of $\geq 50\%$ from maximum remission/response levels in granulocytes or platelets Reduction in Hgb concentration by ≥ 1.5 g/dL or transfusion dependence
Cytogenetic response	Complete

Disease progression	Disappearance of the chromosomal abnormality without appearance of new ones Partial At least 50% reduction of the chromosomal abnormality For subjects with: Less than 5% blasts: $\geq 50\%$ increase in blasts to $>5\%$ blasts 5%–10% blasts: $\geq 50\%$ increase in blasts to $>10\%$ blasts 10%–20% blasts $\geq 50\%$ increase in blasts to $>20\%$ blasts 20%–30% blasts $\geq 50\%$ increase in blasts to $>30\%$ blasts Any of the following: At least 50% decrement from maximum remission/response in granulocytes or platelets Reduction in Hgb by ≥ 2 g/dL Transfusion dependence
Survival	Endpoints: Overall: death from any cause Event free: failure or death from any cause PFS: disease progression or death from MDS DFS: time to relapse Cause-specific death: death related to MDS

Deletions to IWG response criteria are not shown.

To convert hemoglobin from grams per deciliter to grams per liter, multiply grams per deciliter by 10. MDS indicates myelodysplastic syndromes; Hgb, hemoglobin; CR, complete remission; HI, hematologic improvement; PR, partial remission; FAB, French-American-British; PFS, progression-free survival; DFS, disease-free survival.

*Dysplastic changes should consider the normal range of dysplastic changes (modification). (Ramos F, Fernandez-Ferrero S, Suarez D, et al. Myelodysplastic syndrome: a search for minimal diagnostic criteria. Leuk Res. 1999;23:283-290)

†Modification to IWG response criteria.

‡In some circumstances, protocol therapy may require the initiation of further treatment (e.g., consolidation, maintenance) before the 4-week period. Such subjects can be included in the response category into which they fit at the time the therapy is started. Transient cytopenias during repeated chemotherapy courses should not be considered as interrupting durability of response, as long as they recover to the improved counts of the previous course. (Cheson BD, Greenberg PL, Bennett JM, Lowenberg B, Wijermans PW, Nimer SD, Pinto A, Beran M, de Witte TM, Stone RM, Mittelman M, Sanz GF, Gore SD, Schiffer CA, Kantarjian H (2006). Clinical application and proposal for modification of the International Working Group (IWG) response criteria in myelodysplasia. Blood 108(2): 419-25).

Hematologic Improvement ^a	Response criteria (responses must last at least 8 weeks) ^b
Erythroid response (pretreatment, <11 g/dL)	Hgb increase by ≥ 1.5 g/dL Relevant reduction of units of RBC transfusions by an absolute number of at least 4 RBC transfusion per 8 week compared with the pretreatment transfusion number in the previous 8 weeks. Only RBC transfusions given for a Hgb of ≤ 9.0 g/dL pretreatment will count in the RBC transfusion response evaluation
Platelet response (pretreatment, $<100 \times 10^9/L$)	Absolute increase of $\geq 30 \times 10^9/L$ for subjects starting with $>20 \times 10^9/L$ platelets Increase from $< 20 \times 10^9/L$ to $> 20 \times 10^9/L$ and by at least 100%^b
Neutrophil response (pretreatment, $<1.0 \times 10^9/L$)	At least 100% increase and an absolute increase $> 0.5 \times 10^9/L$^b
Progression or relapse after HI ^c	At least 1 of the following: At least 50% decrement from maximum response levels in granulocytes or platelets

	Reduction in Hgb by ≥ 1.5 g/dL Transfusion dependence
--	---

RBC= red blood cell

a Pretreatment counts average of at least 2 measurements (not influenced by transfusions) ≥ 1 week apart (modification).

b Modification to IWG response criteria.

c In the absence of another explanation, such as acute infection, repeated courses of chemotherapy (modification), gastrointestinal bleeding, hemolysis, and so forth. It is recommended that 2 kinds of erythroid and platelet responses be reported overall as well as by the individual response pattern (Cheson 2006)

[0221] In one embodiment of the methods or uses or product for uses provided herein, the effectiveness of treatment with an anti-CD70 antibody as described herein and a CD47 antagonist described herein is assessed by measuring the objective response rate. In some embodiments, the objective response rate is the proportion of patients with tumor size reduction of a predefined amount and for a minimum period of time. In some embodiments the objective response rate is based upon Cheson criteria. In one embodiment, the objective response rate is at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 60%, at least about 70%, or at least about 80%. In one embodiment, the objective response rate is at least about 20%-80%. In one embodiment, the objective response rate is at least about 30%-80%. In one embodiment, the objective response rate is at least about 40%-80%. In one embodiment, the objective response rate is at least about 50%-80%. In one embodiment, the objective response rate is at least about 60%-80%. In one embodiment, the objective response rate is at least about 70%-80%. In one embodiment, the objective response rate is at least about 80%. In one embodiment, the objective response rate is at least about 85%. In one embodiment, the objective response rate is at least about 90%. In one embodiment, the objective response rate is at least about 95%. In one embodiment, the objective response rate is at least about 98%. In one embodiment, the objective response rate is at least about 99%. In one embodiment, the objective response rate is at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 60%, at least 70%, or at least 80%. In one embodiment, the objective response rate is at least 20%-80%. In one embodiment, the objective response rate is at least 30%-80%. In one embodiment, the objective response rate is at least 40%-80%. In one embodiment, the objective response rate is at least 50%-80%. In one embodiment, the objective response rate is at least 60%-80%. In one embodiment, the objective response rate is at least 70%-80%. In one embodiment, the objective response rate is at least 80%. In one embodiment, the objective response rate is at least 85%. In one embodiment, the objective response rate is at least 90%. In

one embodiment, the objective response rate is at least 95%. In one embodiment, the objective response rate is at least 98%. In one embodiment, the objective response rate is at least 99%. In one embodiment, the objective response rate is 100%.

[0222] In one embodiment of the methods or uses or product for uses described herein, response to treatment with an anti-CD70 antibody as described herein and a CD47 antagonist described herein is assessed by measuring the time of progression free survival after administration of the anti-CD70 antibody described herein and the CD47 antagonist described herein. In some embodiments, the subject exhibits progression-free survival of at least about 1 month, at least about 2 months, at least about 3 months, at least about 4 months, at least about 5 months, at least about 6 months, at least about 7 months, at least about 8 months, at least about 9 months, at least about 10 months, at least about 11 months, at least about 12 months, at least about eighteen months, at least about two years, at least about three years, at least about four years, or at least about five years after administration of the anti-CD70 antibody described herein and the CD47 antagonist described herein. In some embodiments, the subject exhibits progression-free survival of at least about 6 months after administration of the anti-CD70 antibody described herein and the CD47 antagonist described herein. In some embodiments, the subject exhibits progression-free survival of at least about one year after administration of the anti-CD70 antibody described herein and the CD47 antagonist described herein. In some embodiments, the subject exhibits progression-free survival of at least about two years after administration of the anti-CD70 antibody described herein and the CD47 antagonist described herein. In some embodiments, the subject exhibits progression-free survival of at least about three years after administration of the anti-CD70 antibody described herein and the CD47 antagonist described herein. In some embodiments, the subject exhibits progression-free survival of at least about four years after administration of the anti-CD70 antibody described herein and the CD47 antagonist described herein. In some embodiments, the subject exhibits progression-free survival of at least about five years after administration of the anti-CD70 antibody described herein and the CD47 antagonist described herein. In some embodiments, the subject exhibits progression-free survival of at least 1 month, at least 2 months, at least 3 months, at least 4 months, at least 5 months, at least 6 months, at least 7 months, at least 8 months, at least 9 months, at least 10 months, at least 11 months, at least 12 months, at least eighteen months, at least two years, at least three years, at least four years, or at least five years after

administration of the anti-CD70 antibody described herein and the CD47 antagonist described herein. In some embodiments, the subject exhibits progression-free survival of at least 6 months after administration of the anti-CD70 antibody described herein and the CD47 antagonist described herein. In some embodiments, the subject exhibits progression-free survival of at least one year after administration of the anti-CD70 antibody described herein and the CD47 antagonist described herein. In some embodiments, the subject exhibits progression-free survival of at least two years after administration of the anti-CD70 antibody described herein and the CD47 antagonist described herein. In some embodiments, the subject exhibits progression-free survival of at least three years after administration of the anti-CD70 antibody described herein and the CD47 antagonist described herein. In some embodiments, the subject exhibits progression-free survival of at least four years after administration of the anti-CD70 antibody described herein and the CD47 antagonist described herein. In some embodiments, the subject exhibits progression-free survival of at least five years after administration of the anti-CD70 antibody described herein and the CD47 antagonist described herein.

[0223] In one embodiment of the methods or uses or product for uses described herein, response to treatment with an anti-CD70 antibody described herein and a CD47 antagonist described herein is assessed by measuring the time of overall survival after administration of the anti-CD70 antibody described herein and the CD47 antagonist described herein. In some embodiments, the subject exhibits overall survival of at least about 1 month, at least about 2 months, at least about 3 months, at least about 4 months, at least about 5 months, at least about 6 months, at least about 7 months, at least about 8 months, at least about 9 months, at least about 10 months, at least about 11 months, at least about 12 months, at least about eighteen months, at least about two years, at least about three years, at least about four years, or at least about five years after administration of the anti-CD70 antibody described herein and the CD47 antagonist described herein. In some embodiments, the subject exhibits overall survival of at least about 6 months after administration of the anti-CD70 antibody described herein and the CD47 antagonist described herein. In some embodiments, the subject exhibits overall survival of at least about one year after administration of the anti-CD70 antibody described herein and the CD47 antagonist described herein. In some embodiments, the subject exhibits overall survival of at least about two years after administration of the anti-CD70 antibody described herein and the CD47 antagonist described herein. In some embodiments, the subject exhibits overall survival of

at least about three years after administration of the anti-CD70 antibody described herein and the CD47 antagonist described herein. In some embodiments, the subject exhibits overall survival of at least about four years after administration of the anti-CD70 antibody described herein and the CD47 antagonist described herein. In some embodiments, the subject exhibits overall survival of at least about five years after administration of the anti-CD70 antibody described herein and the CD47 antagonist described herein. In some embodiments, the subject exhibits overall survival of at least 1 month, at least 2 months, at least 3 months, at least 4 months, at least 5 months, at least 6 months, at least 7 months, at least 8 months, at least 9 months, at least 10 months, at least 11 months, at least about 12 months, at least eighteen months, at least two years, at least three years, at least four years, or at least five years after administration of the anti-CD70 antibody described herein and the CD47 antagonist described herein. In some embodiments, the subject exhibits overall survival of at least 6 months after administration of the anti-CD70 antibody described herein and the CD47 antagonist described herein. In some embodiments, the subject exhibits overall survival of at least one year after administration of the anti-CD70 antibody described herein and the CD47 antagonist described herein. In some embodiments, the subject exhibits overall survival of at least two years after administration of the anti-CD70 antibody described herein and the CD47 antagonist described herein. In some embodiments, the subject exhibits overall survival of at least three years after administration of the anti-CD70 antibody described herein and the CD47 antagonist described herein. In some embodiments, the subject exhibits overall survival of at least four years after administration of the anti-CD70 antibody described herein and the CD47 antagonist described herein. In some embodiments, the subject exhibits overall survival of at least five years after administration of the anti-CD70 antibody described herein and the CD47 antagonist described herein.

[0224] In one embodiment of the methods or uses or product for uses described herein, response to treatment with an anti-CD70 antibody described herein and a CD47 antagonist described herein is assessed by measuring the duration of response to the anti-CD70 antibody described herein and the CD47 antagonist described herein after administration of the anti-CD70 antibody described herein and the CD47 antagonist described herein. In some embodiments, the duration of response to the anti-CD70 antibody described herein and the CD47 antagonist described herein is at least about 1 month, at least about 2 months, at least about 3 months, at least about 4 months, at least about 5 months, at least about 6 months, at least about 7 months, at

least about 8 months, at least about 9 months, at least about 10 months, at least about 11 months, at least about 12 months, at least about eighteen months, at least about two years, at least about three years, at least about four years, or at least about five years after administration of the anti-CD70 antibody described herein and the CD47 antagonist described herein. In some embodiments, the duration of response to the anti-CD70 antibody described herein and the CD47 antagonist described herein is at least about 6 months after administration of the anti-CD70 antibody described herein and the CD47 antagonist described herein. In some embodiments, the duration of response to the anti-CD70 antibody described herein and the CD47 antagonist described herein is at least about one year after administration of the anti-CD70 antibody described herein and the CD47 antagonist described herein. In some embodiments, the duration of response to the anti-CD70 antibody described herein and the CD47 antagonist described herein is at least about two years after administration of the anti-CD70 antibody described herein and the CD47 antagonist described herein. In some embodiments, the duration of response to the anti-CD70 antibody described herein and the CD47 antagonist described herein is at least about three years after administration of the anti-CD70 antibody described herein and the CD47 antagonist described herein. In some embodiments, the duration of response to the anti-CD70 antibody described herein and the CD47 antagonist described herein is at least about four years after administration of the anti-CD70 antibody described herein and the CD47 antagonist described herein. In some embodiments, the duration of response to the anti-CD70 antibody described herein and the CD47 antagonist described herein is at least about five years after administration of the anti-CD70 antibody described herein and the CD47 antagonist described herein. In some embodiments, the duration of response to the anti-CD70 antibody described herein and the CD47 antagonist described herein is at least 1 month, at least 2 months, at least 3 months, at least 4 months, at least 5 months, at least 6 months, at least 7 months, at least 8 months, at least 9 months, at least 10 months, at least 11 months, at least 12 months, at least eighteen months, at least two years, at least three years, at least four years, or at least five years after administration of the anti-CD70 antibody described herein and the CD47 antagonist described herein. In some embodiments, the duration of response to the anti-CD70 antibody described herein and the CD47 antagonist described herein is at least 6 months after administration of the anti-CD70 antibody described herein and the CD47 antagonist described herein. In some embodiments, the duration of response to the anti-CD70 antibody described

herein and the CD47 antagonist described herein is at least one year after administration of the anti-CD70 antibody described herein and the CD47 antagonist described herein. In some embodiments, the duration of response to the anti-CD70 antibody described herein and the CD47 antagonist described herein is at least two years after administration of the anti-CD70 antibody described herein and the CD47 antagonist described herein. In some embodiments, the duration of response to the anti-CD70 antibody described herein and the CD47 antagonist described herein is at least three years after administration of the anti-CD70 antibody described herein and the CD47 antagonist described herein. In some embodiments, the duration of response to the anti-CD70 antibody described herein and the CD47 antagonist described herein is at least four years after administration of the anti-CD70 antibody described herein and the CD47 antagonist described herein. In some embodiments, the duration of response to the anti-CD70 antibody described herein and the CD47 antagonist described herein is at least five years after administration of the anti-CD70 antibody described herein and the CD47 antagonist described herein.

[0225] In some embodiments of the methods or uses or product for uses described herein, administering an anti-CD70 antibody described herein, such as a nonfucosylated anti-CD70 antibody, and a CD47 antagonist described herein to a subject results in a depletion of cancer cells in the subject. In some embodiments, administering an anti-CD70 antibody described herein, such as a nonfucosylated anti-CD70 antibody, and a CD47 antagonist described herein results in a depletion of cancer cells by at least about 5%, at least about 6%, at least about 7%, at least about 8%, at least about 9%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 95%, or about 100% compared to the amount of cancer cells before administering the anti-CD70 antibody and the CD47 antagonist to the subject. In some embodiments, the cancer cells are depleted by at least about 5% compared to the amount of cancer cells before administering the anti-CD70 antibody and the CD47 antagonist to the subject. In some embodiments, the cancer cells are depleted by at least about 10% compared to the amount of cancer cells before administering the anti-CD70 antibody and the CD47 antagonist to the subject. In some embodiments, the cancer cells are depleted by at least about 20% compared to the amount of cancer cells before administering the anti-CD70 antibody and the CD47

antagonist to the subject. In some embodiments, the cancer cells are depleted by at least about 30% compared to the amount of cancer cells before administering the anti-CD70 antibody and the CD47 antagonist to the subject. In some embodiments, the cancer cells are depleted by at least about 40% compared to the amount of cancer cells before administering the anti-CD70 antibody and the CD47 antagonist to the subject. In some embodiments, the cancer cells are depleted by at least about 50% compared to the amount of cancer cells before administering the anti-CD70 antibody and the CD47 antagonist to the subject. In some embodiments, the cancer cells are depleted by at least about 60% compared to the amount of cancer cells before administering the anti-CD70 antibody and the CD47 antagonist to the subject. In some embodiments, the cancer cells are depleted by at least about 70% compared to the amount of cancer cells before administering the anti-CD70 antibody and the CD47 antagonist to the subject. In some embodiments, the cancer cells are depleted by at least about 80% compared to the amount of cancer cells before administering the anti-CD70 antibody and the CD47 antagonist to the subject. In some embodiments, the cancer cells are depleted by at least about 90% compared to the amount of cancer cells before administering the anti-CD70 antibody and the CD47 antagonist to the subject. In some embodiments, the cancer cells are depleted by at least about 95% compared to the amount of cancer cells before administering the anti-CD70 antibody and the CD47 antagonist to the subject. In some embodiments, the cancer cells are depleted by at least about 99% compared to the amount of cancer cells before administering the anti-CD70 antibody and the CD47 antagonist to the subject. In some embodiments, the cancer cells are depleted by about 100% compared to the amount of cancer cells before administering the anti-CD70 antibody and the CD47 antagonist to the subject. In some embodiments, administering an anti-CD70 antibody described herein, such as a nonfucosylated anti-CD70 antibody, and a CD47 antagonist described herein results in a depletion of cancer cells by at least 5%, at least 6%, at least 7%, at least 8%, at least 9%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 60%, at least 70%, at least about 80%, at least about 90%, at least 95%, or 100% compared to the amount of cancer cells before administering the anti-CD70 antibody and the CD47 antagonist to the subject. In some embodiments, the cancer cells are depleted by at least 5% compared to the amount of cancer cells before administering the anti-CD70 antibody and the CD47 antagonist to the subject. In some embodiments, the cancer cells are depleted by at least 10% compared to the amount of

cancer cells before administering the anti-CD70 antibody and the CD47 antagonist to the subject. In some embodiments, the cancer cells are depleted by at least 20% compared to the amount of cancer cells before administering the anti-CD70 antibody and the CD47 antagonist to the subject. In some embodiments, the cancer cells are depleted by at least 30% compared to the amount of cancer cells before administering the anti-CD70 antibody and the CD47 antagonist to the subject. In some embodiments, the cancer cells are depleted by at least 40% compared to the amount of cancer cells before administering the anti-CD70 antibody and the CD47 antagonist to the subject. In some embodiments, the cancer cells are depleted by at least 50% compared to the amount of cancer cells before administering the anti-CD70 antibody and the CD47 antagonist to the subject. In some embodiments, the cancer cells are depleted by at least 60% compared to the amount of cancer cells before administering the anti-CD70 antibody and the CD47 antagonist to the subject. In some embodiments, the cancer cells are depleted by at least 70% compared to the amount of cancer cells before administering the anti-CD70 antibody and the CD47 antagonist to the subject. In some embodiments, the cancer cells are depleted by at least 80% compared to the amount of cancer cells before administering the anti-CD70 antibody and the CD47 antagonist to the subject. In some embodiments, the cancer cells are depleted by at least 90% compared to the amount of cancer cells before administering the anti-CD70 antibody and the CD47 antagonist to the subject. In some embodiments, the cancer cells are depleted by at least 95% compared to the amount of cancer cells before administering the anti-CD70 antibody and the CD47 antagonist to the subject. In some embodiments, the cancer cells are depleted by at least 99% compared to the amount of cancer cells before administering the anti-CD70 antibody and the CD47 antagonist to the subject. In some embodiments, the cancer cells are depleted by 100% compared to the amount of cancer cells before administering the anti-CD70 antibody and the CD47 antagonist to the subject.

[0226] In some embodiments of the methods or uses or product for uses described herein, administering an anti-CD70 antibody described herein, such as a nonfucosylated anti-CD70 antibody, and a CD47 antagonist described herein to a subject does not result in a depletion of CD70+ T regulatory cells (CD70+ Tregs) in the subject. In some embodiments, administering an anti-CD70 antibody described herein, such as a nonfucosylated anti-CD70 antibody, and a CD47 antagonist described herein results in a depletion of CD70+ Tregs of no more than about 50%, about 40%, about 30%, about 20%, about 10%, about 9%, about 8%, about 7%, about 6%, about 5%, about 4%, about 3%, about 2%, about 1%, or about 0.1% compared to the amount of CD70+

Tregs before administering the anti-CD70 antibody and the CD47 antagonist to the subject. In some embodiments, the CD70+ Tregs are depleted by no more than about 50% compared to the amount of CD70+ Tregs before administering the anti-CD70 antibody and the CD47 antagonist to the subject. In some embodiments, the CD70+ Tregs are depleted by no more than about 40% compared to the amount of CD70+ Tregs before administering the anti-CD70 antibody and the CD47 antagonist to the subject. In some embodiments, the CD70+ Tregs are depleted by no more than about 30% compared to the amount of CD70+ Tregs before administering the anti-CD70 antibody and the CD47 antagonist to the subject. In some embodiments, the CD70+ Tregs are depleted by no more than about 20% compared to the amount of CD70+ Tregs before administering the anti-CD70 antibody and the CD47 antagonist to the subject. In some embodiments, the CD70+ Tregs are depleted by no more than about 10% compared to the amount of CD70+ Tregs before administering the anti-CD70 antibody and the CD47 antagonist to the subject. In some embodiments, the CD70+ Tregs are depleted by no more than about 5% compared to the amount of CD70+ Tregs before administering the anti-CD70 antibody and the CD47 antagonist to the subject. In some embodiments, the CD70+ Tregs are depleted by no more than about 1% compared to the amount of CD70+ Tregs before administering the anti-CD70 antibody and the CD47 antagonist to the subject. In some embodiments, the CD70+ Tregs are depleted by no more than about 0.1% compared to the amount of CD70+ Tregs before administering the anti-CD70 antibody and the CD47 antagonist to the subject. In some embodiments, administering an anti-CD70 antibody described herein, such as a nonfucosylated anti-CD70 antibody, and a CD47 antagonist described herein results in a depletion of CD70+ Tregs of no more than 50%, 40%, 30%, 20%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, or 0.1% compared to the amount of CD70+ Tregs before administering the anti-CD70 antibody and the CD47 antagonist to the subject. In some embodiments, the CD70+ Tregs are depleted by no more than 50% compared to the amount of CD70+ Tregs before administering the anti-CD70 antibody and the CD47 antagonist to the subject. In some embodiments, the CD70+ Tregs are depleted by no more than 40% compared to the amount of CD70+ Tregs before administering the anti-CD70 antibody and the CD47 antagonist to the subject. In some embodiments, the CD70+ Tregs are depleted by no more than 30% compared to the amount of CD70+ Tregs before administering the anti-CD70 antibody and the CD47 antagonist to the subject. In some embodiments, the CD70+ Tregs are depleted by no more than 20% compared to the amount of

CD70+ Tregs before administering the anti-CD70 antibody and the CD47 antagonist to the subject. In some embodiments, the CD70+ Tregs are depleted by no more than 10% compared to the amount of CD70+ Tregs before administering the anti-CD70 antibody and the CD47 antagonist to the subject. In some embodiments, the CD70+ Tregs are depleted by no more than 5% compared to the amount of CD70+ Tregs before administering the anti-CD70 antibody and the CD47 antagonist to the subject. In some embodiments, the CD70+ Tregs are depleted by no more than 1% compared to the amount of CD70+ Tregs before administering the anti-CD70 antibody and the CD47 antagonist to the subject. In some embodiments, the CD70+ Tregs are depleted by no more than 0.1% compared to the amount of CD70+ Tregs before administering the anti-CD70 antibody and the CD47 antagonist to the subject.

[0227] In some embodiments, a fucosylated anti-CD70 antibody depletes CD70+ Tregs in a subject to a greater extent than the nonfucosylated form of an anti-CD70 antibody comprising the same heavy and light chain amino acid sequences. In some embodiments, the fucosylated anti-CD70 antibody depletes CD70+ Tregs in a subject to a greater extent than the nonfucosylated form of an anti-CD70 antibody comprising the same heavy and light chain amino acid sequences when the subject is homozygous for the high affinity FcγRIIIa receptor (V/V 158). In some embodiments, the fucosylated anti-CD70 antibody depletes CD70+ Tregs in a subject to the same extent as the nonfucosylated form of an anti-CD70 antibody comprising the same heavy and light chain amino acid sequences when the subject is homozygous for the low affinity FcγRIIIa receptor (F/F 158). In some embodiments, neither the fucosylated anti-CD70 antibody nor the nonfucosylated form of an anti-CD70 antibody comprising the same heavy and light chain amino acid sequences deplete CD8 T cells when the subject is homozygous for the high affinity FcγRIIIa receptor (V/V 158). In some embodiments, neither the fucosylated anti-CD70 antibody nor the nonfucosylated form of an anti-CD70 antibody comprising the same heavy and light chain amino acid sequences deplete CD8 T cells when the subject is homozygous for the low affinity FcγRIIIa receptor (F/F 158).

V. Assays for Cytotoxic, Cytostatic, and Immunomodulatory Activities

[0228] Methods of determining whether an antibody mediates effector function against a target cell are known. Illustrative examples of such methods are described *infra*.

[0229] For determining whether an anti-CD70 antibody and/or CD47 antagonist mediates antibody-dependent cellular cytotoxicity against activated immune cells, CD70-expressing cancer cells, and/or CD47-expressing cancer cells, an assay that measures target cell death in the presence of antibody and effector immune cells may be used. An assay used to measure this type of cytotoxicity can be based on determination of ^{51}Cr release from metabolically-labeled target cells after incubation in the presence of effector cells and target-specific antibody (*see, e.g.*, Perussia and Loza, 2000, *Methods in Molecular Biology* 121:179-92; and “ ^{51}Cr Release Assay of Antibody-Dependent Cell-Mediated Cytotoxicity (ADCC)” in *Current Protocols in Immunology*, Coligan *et al.* eds., Wiley and Sons, 1993). For example, activated immune cells (*e.g.*, activated lymphocytes) or CD70-expressing cancer cells labeled with $\text{Na}_2^{51}\text{CrO}_4$ and plated at a density of 5,000 cells per well of a 96-well plate can be treated with varying concentrations of anti-CD70 antibody for 30 minutes then mixed with normal human peripheral blood mononuclear cells (PBMC) for 4 hours. The membrane disruption that accompanies target cell death releases ^{51}Cr into the culture supernatant which may be collected and assessed for radioactivity as a measure of cytotoxic activity. Other assays to measure ADCC may involve nonradioactive labels or be based on induced release of specific enzymes. For example, a non-radioactive assay based on time-resolved fluorometry is commercially available (Delphia, Perkin Elmer). This assay is based on loading target cells with an acetoxymethyl ester of fluorescence enhancing ligand (BATDA) that penetrates the cell membrane then hydrolyses to form a membrane impermeable hydrophilic ligand (TDA). When mixed with target specific antibody and PBMC effector cells, TDA is released from lysed cells and is available to form a highly fluorescent chelate when mixed with Europium. The signal, measured with a time-resolved fluorometer, correlates with the amount of cell lysis. Similar assays can be conducted with CD47 antagonists.

[0230] To determine whether an anti-CD70 antibody or CD47 antagonist mediates antibody-dependent cellular phagocytosis against activated immune cells, CD70-expressing cancer cells, and/or CD47-expressing cancer cells, an assay that measures target cell internalization by effector immune cells (*e.g.*, fresh cultured macrophages or established macrophage-like cell line) may be used (*see, e.g.*, Munn and Cheung, 1990, *J. Exp. Med.* 172:231-37; Keler *et al.*, 2000, *J. Immunol.* 164:5746-52; Akewanlop *et al.*, 2001, *Cancer Res.* 61:4061-65). For example, target cells may be labeled with a lipophilic membrane dye such as PKH67 (Sigma), coated with target-specific antibody, and mixed with effector immune cells for 4-24 hours. The effector cells may

then be identified by counterstaining with a fluorochrome-labeled antibody specific for a phagocytic cell surface marker (*e.g.*, CD14) and the cells analyzed by two-color flow cytometry or fluorescence microscopy. Dual-positive cells represent effector cells that have internalized target cells. For these assays, effector cells may be monocytes derived from PBMC that have been differentiated into macrophages by culture for 5-10 days with M-CSF or GM-CSF (*see, e.g.*, Munn and Cheung, *supra*). Human macrophage-like cell lines U937 (Larrick *et al.*, 1980, *J. Immunology* 125:6-12) or THP-1 (Tsuchiya *et al.*, 1980, *Int. J. Cancer* 26:171-76) which are available from ATCC may be used as an alternative phagocytic cell source.

[0231] Methods of determining whether an antibody mediates complement-dependent cytotoxicity upon binding to target cells are also known. The same methods can be applied to determine whether an anti-CD70 antibody mediates CDC on activated immune cells or CD70-expressing cancer cells. The same methods can also be applied to determine whether a CD47 antagonist mediates CDC on activated immune cells or CD47-expressing cancer cells. Illustrative examples of such methods are described *infra*.

[0232] The source of active complement can either be normal human serum or purified from laboratory animal including rabbits. In a standard assay, an anti-CD70 antibody is incubated with CD70-expressing activated immune cells (*e.g.*, activated lymphocytes) or CD70-expressing cancer cells in the presence of complement. The ability of such an anti-CD70 antibody to mediate cell lysis can be determined by several readouts. In one example, a $\text{Na}^{51}\text{CrO}_4$ release assay is used. In this assay, target cells are labeled with $\text{Na}^{51}\text{CrO}_4$. Unincorporated $\text{Na}^{51}\text{CrO}_4$ is washed off and cells are plated at a suitable density, typically between 5,000 to 50,000 cells/well, in a 96-well plate. Incubation with the anti-CD70 antibody in the presence of normal serum or purified complement typically last for 2-6 hours at 37°C in a 5% CO_2 atmosphere. Released radioactivity, indicating cell lysis, is determined in an aliquot of the culture supernatant by gamma ray counting. Maximum cell lysis is determined by releasing incorporated $\text{Na}^{51}\text{CrO}_4$ by detergent (0.5-1% NP-40 or Triton X-100) treatment. Spontaneous background cell lysis is determined in wells where only complement is present without any anti-CD70 antibodies. Percentage cell lysis is calculated as (anti-CD70 antibody-induced lysis – spontaneous lysis)/maximum cell lysis). The second readout is a reduction of metabolic dyes, *e.g.*, Alamar Blue, by viable cells. In this assay, target cells are incubated with anti-CD70 antibodies with complement and incubated as described above. At the end of incubation, 1/10 volume of Alamar

Blue (Biosource International, Camarillo, CA) is added. Incubation is continued for up to 16 hours at 37°C in a 5% CO₂ atmosphere. Reduction of Alamar Blue as an indication of metabolically active viable cells is determined by fluorometric analysis with excitation at 530 nm and emission at 590 nm. The third readout is cellular membrane permeability to propidium iodide (PI). Formation of pores in the plasma membrane as a result of complement activation facilitates entry of PI into cells where it will diffuse into the nuclei and bind DNA. Upon binding to DNA, PI fluorescence in the 600 nm significantly increases. Treatment of target cells with anti-CD70 antibodies and complement is carried out as described above. At end of incubation, PI is added to a final concentration of 5 µg/ml. The cell suspension is then examined by flow cytometry using a 488 nm argon laser for excitation. Lysed cells are detected by fluorescence emission at 600 nm. Similar assays can be conducted with CD47 antagonists.

VI. Pharmaceutical Compositions Comprising Anti-CD70 Antibodies and Administration Thereof

[0233] A composition comprising an anti-CD70 antibody can be administered to a subject having or at risk of having a cancer, such as a CD70-expressing cancer. The invention further provides for the use of an anti-CD70 antibody in the manufacture of a medicament for prevention or treatment of cancer, such as a CD70-expressing cancer. The term “subject” as used herein means any mammalian patient to which a CD70-binding agent can be administered, including, *e.g.*, humans and non-human mammals, such as primates, rodents, and dogs. Subjects specifically intended for treatment using the methods described herein include humans. The antibodies can be administered either alone or in combination with other compositions in the prevention or treatment of the cancer, such as a CD70-expressing cancer.

[0234] A composition comprising a CD47 antagonist can be administered to a subject having or at risk of having cancer, such as a CD47-expressing cancer. The invention further provides for the use of a CD47 antagonist in the manufacture of a medicament for prevention or treatment of cancer, such as a CD47-expressing cancer. The term “subject” as used herein means any mammalian patient to which a CD47 antagonist can be administered, including, *e.g.*, humans and non-human mammals, such as primates, rodents, and dogs. Subjects specifically intended for treatment using the methods described herein include humans. The antagonists can be

administered either alone or in combination with other compositions in the prevention or treatment of the cancer, such as a CD47-expressing cancer.

[0235] Various delivery systems are known and can be used to administer the anti-CD70 antibody or CD47 antagonist. Methods of introduction include but are not limited to intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural, and oral routes. The anti-CD70 antibody or CD47 antagonist can be administered, for example by infusion or bolus injection (*e.g.*, intravenous or subcutaneous), by absorption through epithelial or mucocutaneous linings (*e.g.*, oral mucosa, rectal and intestinal mucosa, and the like) and can be administered together with other biologically active agents such as chemotherapeutic agents. Administration can be systemic or local. In one embodiment, the anti-CD70 antibody described herein is administered parenterally. In one embodiment, the CD47 antagonist described herein is administered parenterally. Parenteral administration refers to modes of administration other than enteral and topical administration, usually by injection, and include epidermal, intravenous, intramuscular, intraarterial, intrathecal, intracapsular, intraorbital, intracardiac, intradermal, intraperitoneal, intratendinous, transtracheal, subcutaneous, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal, intracranial, intrathoracic, epidural and intrasternal injection and infusion. In some embodiments, the route of administration of an anti-CD70 antibody described herein is intravenous injection or infusion. In some embodiments, the route of administration of an anti-CD70 antibody described herein is intravenous infusion. In some embodiments, the route of administration of a CD47 antagonist described herein is intravenous injection or infusion. In some embodiments, the route of administration of CD47 antagonist described herein is intravenous infusion.

[0236] In specific embodiments, the anti-CD70 antibody and/or CD47 antagonist composition is administered by injection, by means of a catheter, by means of a suppository, or by means of an implant, the implant being of a porous, non-porous, or gelatinous material, including a membrane, such as a sialastic membrane, or a fiber. Typically, when administering the composition, materials to which the anti-CD70 antibody and/or CD47 antagonist does not absorb are used.

[0237] An anti-CD70 antibody or CD47 antagonist can be administered as pharmaceutical compositions comprising a therapeutically effective amount of the antibody and one or more

pharmaceutically compatible ingredients. For example, the pharmaceutical composition typically includes one or more pharmaceutical carriers (*e.g.*, sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like). Water is a more typical carrier when the pharmaceutical composition is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. Suitable pharmaceutical excipients include, for example, starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol, and the like. The composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents. These compositions can take the form of solutions, suspensions, emulsion, tablets, pills, capsules, powders, sustained-release formulations and the like. The composition can be formulated as a suppository, with traditional binders and carriers such as triglycerides. Oral formulations can include standard carriers such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, *etc.* Examples of suitable pharmaceutical carriers are described in “Remington’s Pharmaceutical Sciences” by E.W. Martin. Such compositions will contain a therapeutically effective amount of the protein, typically in purified form, together with a suitable amount of carrier so as to provide the form for proper administration to the patient. The formulations correspond to the mode of administration.

[0238] In typical embodiments, the pharmaceutical composition is formulated in accordance with routine procedures as a pharmaceutical composition adapted for intravenous administration to human beings. Typically, compositions for intravenous administration are solutions in sterile isotonic aqueous buffer. Where necessary, the pharmaceutical can also include a solubilizing agent and a local anesthetic such as lignocaine to ease pain at the site of the injection. Generally, the ingredients are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent. Where the pharmaceutical is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the pharmaceutical is administered by injection, an

ampoule of sterile water for injection or saline can be provided so that the ingredients can be mixed prior to administration.

[0239] Further, the pharmaceutical composition can be provided as a pharmaceutical kit comprising (a) a container containing an anti-CD70 antibody or CD47 antagonist in lyophilized form and (b) a second container containing a pharmaceutically acceptable diluent (*e.g.*, sterile water) for injection. The pharmaceutically acceptable diluent can be used for reconstitution or dilution of the lyophilized anti-CD70 antibody or CD47 antagonist. Optionally associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration.

[0240] The amount of the anti-CD70 antibody and/or CD47 antagonist that is effective in the treatment or prevention of the cancer can be determined by standard clinical techniques. In addition, *in vitro* assays may optionally be employed to help identify optimal dosage ranges. The precise dose to be employed in the formulation will also depend on the route of administration, and the stage of the cancer, and should be decided according to the judgment of the practitioner and each patient's circumstances. Effective doses may be extrapolated from dose-response curves derived from *in vitro* or animal model test systems.

[0241] For example, toxicity and therapeutic efficacy of the anti-CD70 antibody and/or CD47 antagonist can be determined in cell cultures or experimental animals by standard pharmaceutical procedures for determining the LD₅₀ (the dose lethal to 50% of the population) and the ED₅₀ (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD₅₀/ED₅₀. An anti-CD70 antibody and/or CD47 antagonist that exhibits a large therapeutic index is preferred. Where an anti-CD70 antibody exhibits toxic side effects, a delivery system that targets the anti-CD70 antibody to the site of affected tissue can be used to minimize potential damage to non-CD70-expressing cells and, thereby, reduce side effects. Where a CD47 antagonist exhibits toxic side effects, a delivery system that targets the CD47 antagonist to the site of affected tissue can be used to minimize potential damage to non-CD47-expressing cells and, thereby, reduce side effects.

[0242] The data obtained from the cell culture assays and animal studies can be used in formulating a range of dosage for use in humans. The dosage of the anti-CD70 antibody or CD47 antagonist typically lies within a range of circulating concentrations that include the ED₅₀ with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. For an anti-CD70 antibody or CD47 antagonist used in the method, the therapeutically effective dose can be estimated initially from cell culture assays. A dose can be formulated in animal models to achieve a circulating plasma concentration range that includes the IC₅₀ (*i.e.*, the concentration of the test compound that achieves a half-maximal inhibition of symptoms) as determined in cell culture. Such information can be used to more accurately determine useful doses in humans. Levels in plasma can be measured, for example, by high performance liquid chromatography.

[0243] Generally, the dosage of an anti-CD70 antibody administered to a patient with cancer is about 0.1 mg/kg to 100 mg/kg of the subject's body weight. More typically, the dosage of the anti-CD70 antibody administered to a subject is 0.1 mg/kg to 50 mg/kg of the subject's body weight, even more typically 1 mg/kg to 30 mg/kg, 1 mg/kg to 20 mg/kg, 1 mg/kg to 15 mg/kg, 1 mg/kg to 12 mg/kg, 1 mg/kg to 10 mg/kg, or 1 mg/kg to 7.5 mg/kg of the subject's body weight. In some embodiments, the dose of the anti-CD70 antibody is about 1.5 mg/kg. In some embodiments, the dose of the anti-CD70 antibody is about 5 mg/kg. In some embodiments, the dose of the anti-CD70 antibody is about 10 mg/kg to about 20 mg/kg. In some embodiments, the dose of the anti-CD70 antibody is about 10 mg/kg. In some embodiments, the dose of the anti-CD70 antibody is about 15 mg/kg. In some embodiments, the dose of the anti-CD70 antibody is about 20 mg/kg. Generally, human antibodies have a longer half-life within the human body than antibodies from other species due to the immune response to the foreign proteins. Thus, lower dosages of anti-CD70 antibody comprising humanized or chimeric antibodies and less frequent administration is often possible.

[0244] A dose of an anti-CD70 antibody can be administered, for example, daily, once per week (weekly), twice per week, thrice per week, four times per week, five times per week, biweekly, monthly or otherwise as needed. In some embodiments, the anti-CD70 antibody is administered once about every 2 weeks. In some embodiments, the anti-CD70 antibody is administered once every 2 weeks.

[0245] In some embodiments, the dosage of an anti-CD70 antibody corresponds to a sub-optimal dosage (*i.e.*, below the EC₅₀ for the anti-CD70 antibody). For example, the dosage of an anti-CD70 antibody can comprise a dosage selected from the lowest 25%, lowest 15%, lowest 10% or lowest 5% of the therapeutic window. As used herein, the term “therapeutic window” refers to the range of dosage of a drug or of its concentration in a bodily system that provides safe and effective therapy.

[0246] In some embodiments, the dosage of an anti-CD70 antibody is from about 0.05 mg/kg to about 1 mg/kg, or about 0.1 mg/kg to about 0.9 mg/kg, or about 0.15 to about 0.75 mg/kg of the subject's body weight. Such a dosage can be administered from 1 to about 15 times per week. Each dose can be the same or different. For example, a dosage of about 0.15 mg/kg of an anti-CD70 antibody can be administered from 1 to 10 times per four day, five day, six day or seven day period.

[0247] Generally, the dosage of a CD47 antagonist administered to a patient with cancer is about 0.1 mg/kg to 100 mg/kg of the subject's body weight. More typically, the dosage of the CD47 antagonist administered to a subject is 0.1 mg/kg to 50 mg/kg of the subject's body weight, even more typically 1 mg/kg to 30 mg/kg, 1 mg/kg to 20 mg/kg, 1 mg/kg to 15 mg/kg, 1 mg/kg to 12 mg/kg, 1 mg/kg to 10 mg/kg, or 1 mg/kg to 7.5 mg/kg of the subject's body weight. In some embodiments, the dose of the CD47 antagonist is about 1 mg/kg to about 50 mg/kg. In some embodiments, the dose of the CD47 antagonist is about 1 mg/kg to about 30 mg/kg. In some embodiments, the dose of the CD47 antagonist is about 1 mg/kg. In some embodiments, the dose of CD47 antagonist is about 1.5 mg/kg. In some embodiments, the dose of the CD47 antagonist is about 5 mg/kg. In some embodiments, the dose of the CD47 antagonist is about 10 mg/kg. In some embodiments, the dose of the CD47 antagonist is about 15 mg/kg. In some embodiments, the dose of the CD47 antagonist is about 20 mg/kg. In some embodiments, the dose of the CD47 antagonist is about 30 mg/kg. Generally, human antibodies have a longer half-life within the human body than antibodies from other species due to the immune response to the foreign proteins. Thus, lower dosages of anti-CD47 antibody comprising humanized or chimeric antibodies and less frequent administration is often possible.

[0248] A dose of CD47 antagonist can be administered, for example, daily, once per week (weekly), twice per week, thrice per week, four times per week, five times per week, biweekly, monthly or otherwise as needed.

[0249] In some embodiments, the CD47 antagonist is administered on days 1, 4, 8, 11, 15, and 22 of a first four-week treatment cycle. In some embodiments, the CD47 antagonist is administered at a dose of 1 mg/kg of the subject's body weight on days 1 and 4 of the first four-week treatment cycle. In some embodiments, the CD47 antagonist is administered at a dose of 15 mg/kg on day 8 of the first four-week treatment cycle. In some embodiments, the CD47 antagonist is administered at a dose of 30 mg/kg on days 11, 15 and 22 of the first four-week treatment cycle. In some embodiments, the CD47 antagonist is administered on days 1, 8, 15, and 22 of a second four-week treatment cycle. In some embodiments, the CD47 antagonist is administered at a dose of 30 mg/kg of the subject's body weight on days 1, 8, 15, and 22 of the second four-week treatment cycle. In some embodiments, the CD47 antagonist is administered on days 1 and 15 of a third four-week treatment cycle. In some embodiments, the CD47 antagonist is administered at a dose of 30 mg/kg of the subject's body weight on days 1 and 15 of the third four-week treatment cycle. In some embodiments, the CD47 antagonist is administered at a dose of 30 mg/kg of the subject's body weight on days 1 and 15 for each four-week treatment cycle after the third four-week treatment cycle. In some embodiments, the CD47 is magrolimab.

[0250] In some embodiments, the dosage of CD47 antagonist corresponds to a sub-optimal dosage (*i.e.*, below the EC_{50} for the CD47 antagonist). For example, the dosage of CD47 antagonist can comprise a dosage selected from the lowest 25%, lowest 15%, lowest 10% or lowest 5% of the therapeutic window. As used herein, the term "therapeutic window" refers to the range of dosage of a drug or of its concentration in a bodily system that provides safe and effective therapy.

[0251] In some embodiments, the dosage of CD47 antagonist is from about 0.05 mg/kg to about 1 mg/kg, or about 0.1 mg/kg to about 0.9 mg/kg, or about 0.15 to about 0.75 mg/kg of the subject's body weight. Such a dosage can be administered from 1 to about 15 times per week. Each dose can be the same or different. For example, a dosage of about 0.15 mg/kg of CD47

antagonist can be administered from 1 to 10 times per four day, five day, six day or seven day period.

[0252] In some embodiments, the pharmaceutical compositions comprising the anti-CD70 antibody and/or CD47 antagonist can further comprise a therapeutic agent (*e.g.*, a non-conjugated cytotoxic or immunomodulatory agent such as, for example, any of those described herein). The anti-CD70 antibody and/or CD47 antagonist also can be co-administered in combination with one or more therapeutic agents for the treatment or prevention of cancers, such as CD70-expressing and/or CD47-expressing cancers. For example, combination therapy can include a therapeutic agent (*e.g.*, a cytostatic, cytotoxic, or immunomodulatory agent, such as an unconjugated cytostatic, cytotoxic, or immunomodulatory agent such as those conventionally used for the treatment of cancers). Combination therapy can also include, *e.g.*, administration of an agent that targets a receptor or receptor complex other than CD70 and/or CD47 on the surface of activated lymphocytes, dendritic cells or CD70-expressing and/or CD47-expressing cancer cells. An example of such an agent includes an antibody that binds to a molecule other than CD70 or CD47 at the surface of an activated lymphocyte, dendritic cell, or CD70-expressing and/or CD47-expressing cancer cell. Another example includes a ligand that targets such a receptor or receptor complex. Typically, such an antibody or ligand binds to a cell surface receptor on activated lymphocytes, dendritic cell, or CD70-expressing and/or CD47-expressing cancer cell and enhances the cytotoxic or cytostatic effect of the anti-CD70 antibody and/or CD47 antagonist by delivering a cytostatic or cytotoxic signal to the activated lymphocyte, dendritic cell or CD70-expressing and/or CD47-expressing cancer cell. Such combinatorial administration can have an additive or synergistic effect on disease parameters (*e.g.*, severity of a symptom, the number of symptoms, or frequency of relapse). Another example includes a hypomethylating agent (HMA). In some embodiments, the HMA is azacitidine (VIDAZA®). Another example includes a BH3-mimetic. In some embodiments, the BH3-mimetic is venetoclax (VENCLEXTA®). In some embodiments, the pharmaceutical composition comprises an anti-CD70 antibody, a CD47 antagonist, an HMA, and a BH3-mimetic. In some embodiments, the pharmaceutical composition comprises an anti-CD70 antibody, a CD47 antagonist, an HMA, and venetoclax. In some embodiments, the pharmaceutical composition comprises an anti-CD70 antibody, a CD47 antagonist, azacitidine, and a BH3-mimetic. In some embodiments, the pharmaceutical composition comprises an anti-CD70 antibody, a CD47

antagonist, azacitidine, and a venetoclax. Combination therapy can also include a hypomethylating agent (HMA). In some embodiments, the HMA is azacitidine (VIDAZA®). Combination therapy can also include a BH3-mimetic. In some embodiments, the BH3-mimetic is venetoclax (VENCLEXTA®). In some embodiments, the combination therapy comprises an anti-CD70 antibody, a CD47 antagonist, an HMA, and a BH3-mimetic. In some embodiments, the combination therapy comprises an anti-CD70 antibody, a CD47 antagonist, an HMA, and venetoclax. In some embodiments, the combination therapy comprises an anti-CD70 antibody, a CD47 antagonist, azacitidine, and a BH3-mimetic. In some embodiments, the combination therapy comprises an anti-CD70 antibody, a CD47 antagonist, azacitidine, and a venetoclax. In some embodiments, azacitidine is administered at a dose of 75 mg/m² of the subject's body surface area. In some embodiments, azacitidine is administered on days 1 to 7 of each four-week treatment cycle. In some embodiments, azacitidine is administered on days 1 to 5 and 8 to 9 of each four-week treatment cycle.

[0253] In some embodiments, an anti-CD70 antibody is administered concurrently with a CD47 antagonist. In some embodiments, a CD47 antagonist is administered prior or subsequent to an anti-CD70 antibody, by at least an hour and up to several months, for example at least an hour, five hours, 12 hours, a day, a week, a month, or three months, prior or subsequent to administration of the anti-CD70 antibody. In some embodiments, the subject is monitored following administration of the anti-CD70 antibody the CD47 antagonist.

VII. Articles of Manufacture and Kits

[0254] In another aspect, an article of manufacture or kit is provided which comprises an anti-CD70 antibody described herein and/or a CD47 antagonist described herein. The article of manufacture or kit may further comprise instructions for use of the anti-CD70 antibody described herein and/or CD47 antagonist described herein in the methods of the invention. Thus, in certain embodiments, the article of manufacture or kit comprises instructions for the use of an anti-CD70 antibody described herein and/or CD47 antagonist described herein in methods for treating cancer (*e.g.*, myeloid malignancies) in a subject comprising administering to the subject an anti-CD70 antibody described herein and a CD47 antagonist described herein. In some embodiments, the cancer is MDS. In some embodiments, the cancer is AML. In some

embodiments the cancer is a relapsed or refractory cancer. In some embodiments, the subject is a human.

[0255] The article of manufacture or kit may further comprise a container. Suitable containers include, for example, bottles, vials (*e.g.*, dual chamber vials), syringes (such as single or dual chamber syringes) and test tubes. In some embodiments, the container is a vial. The container may be formed from a variety of materials such as glass or plastic. The container holds the formulation.

[0256] The article of manufacture or kit may further comprise a label or a package insert, which is on or associated with the container, may indicate directions for reconstitution and/or use of the formulation. The label or package insert may further indicate that the formulation is useful or intended for subcutaneous, intravenous (*e.g.*, intravenous infusion), or other modes of administration for treating cancer in a subject. The container holding the formulation may be a single-use vial or a multi-use vial, which allows for repeat administrations of the reconstituted formulation. The article of manufacture or kit may further comprise a second container comprising a suitable diluent. The article of manufacture or kit may further include other materials desirable from a commercial, therapeutic, and user standpoint, including other buffers, diluents, filters, needles, syringes, and package inserts with instructions for use.

[0257] The article of manufacture or kit herein optionally further comprises a container comprising a further medicament, wherein the anti-CD70 antibody and/or CD47 antagonist is a first and/or second medicament, and which article or kit further comprises instructions on the label or package insert for treating the subject with the further medicament, in an effective amount. In some embodiments, the label or package insert indicates that the anti-CD70 antibody and/or CD47 antagonist are to be administered sequentially or simultaneously with the further medicament.

[0258] In some embodiments, the anti-CD70 antibody and/or CD47 antagonist described herein is present in the container as a lyophilized powder. In some embodiments, the lyophilized powder is in a hermetically sealed container, such as a vial, an ampoule or sachette, indicating the quantity of the active agent. Where the pharmaceutical is administered by injection, an ampoule of sterile water for injection or saline can be, for example, provided, optionally as part of the kit, so that the ingredients can be mixed prior to administration. Such kits can further

include, if desired, one or more of various conventional pharmaceutical components, such as, for example, containers with one or more pharmaceutically acceptable carriers, additional containers, etc., as will be readily apparent to those skilled in the art. Printed instructions, either as inserts or as labels, indicating quantities of the components to be administered, guidelines for administration, and/or guidelines for mixing the components can also be included in the kit.

[0259] The invention will be more fully understood by reference to the following examples. They should not, however, be construed as limiting the scope of the invention. It is understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and scope of the appended claims.

EXAMPLES

Example 1. Effect of SEA-CD70 (h1F6 SEA) in combination with anti-CD47 antibody clone h5F9 on tumor growth in the MV4-11 AML xenograft mouse model.

[0260] CD47 is a cell surface protein that functions as a regulator of phagocytosis mediated by cells of the innate immune system, such as macrophages and dendritic cells. CD47 serves as the ligand for a receptor on these innate immune cells, SIRP-alpha, which in turn delivers an inhibitory signal for phagocytosis. Human acute myeloid leukemia (AML) cells express CD47, therefore, blocking monoclonal antibodies directed against CD47 could enable phagocytosis and elimination of cancer cells.

[0261] In this study, tumor growth in response to administration of the afucosylated anti-CD70 antibody h1F6 SEA (SEA-CD70) alone, the anti-CD47 monoclonal antibody h5F9-G4 (h5F9 hIgG4k, Magrolimab) alone, or SEA-CD70 in combination with h5F9 hIgG4k was assessed in a CD70-expressing AML xenograft mouse model, MV4-11. Tumor growth was reported as a volume and calculated as an average across animals within each treatment group (FIG. 1). SCID mice were implanted with 5×10^6 MV4-11 cells subcutaneously in the flank on day 0. When mean tumor size of 50 mm^3 (measured by using the formula: $\text{Volume (mm}^3\text{)} = 0.5 * \text{Length} * \text{Width}^2$, where the length is the longer dimension) was reached, mice were randomized into treatment groups of 9 mice per group. Treatments were given intraperitoneally.

Stock concentrations of antibody and chemotherapy were diluted to the appropriate concentration and injected into animals at 10 µl/g of body weight. Tumor length and width, and animal weight were measured two times weekly throughout the study and tumor volume was calculated using the formula above. Animals were followed until tumor volume measured ~1000 mm³, at which time the animals were euthanized. Animals were treated with h5F9-G4 at a dose of 0.3 and 1 mg/kg or h1F6 SEA at 10 mg/kg every 4 days for a total of 5 cycles (Q4dx5). Animals receiving the combination of treatments received each treatment at the same dose and schedule as the single treatments. Analysis of tumor volume changes over time shows that combination of h1F6 SEA and h5F9-G4 elicits a greater antitumor activity than each single agent. Notably, combination of h1F6 SEA with sub-efficacious doses of h5F9-G4 which are >10 fold lower than what is usually used in preclinical models, shows a synergistic effect, and induces sustainable complete remission of the tumors within the experimental timeline.

Example 2. Effect of SEA-CD70 (h1F6 SEA) in combination with anti-CD47 clone hu5F9-G4, and hypomethylating agent azacitidine (Vidaza®), on tumor growth in the MV4-11 acute myeloid leukemia mouse model.

[0262] In this study, tumor growth in response to administration of the afucosylated anti-CD70 antibody h1F6 SEA (SEA-CD70) in combination with single agents anti-CD47 monoclonal antibody hu5F9-G4 (hu5F9 hIgG4k, hu5F9 hIgG4kappa, Magrolimab), or single hypomethylating agent azacitidine (Vidaza®), or with a combination of both (triplet combination), was assessed in a CD70 expressing cell xenograft mouse model MV4-11 line. Tumor growth was reported as a volume (mm³) and calculated as an average across animals within each treatment group (FIG. 2). SCID mice were implanted with 5x10⁶ MV4-11 cells subcutaneously in the flank on day 0. When mean tumor size of 50 mm³ (measured by using the formula: Volume (mm³) = 0.5*Length*Width², where the length is the longer dimension) was reached, mice were randomized into treatment groups of 5 mice per group. Treatments were given intraperitoneally. Stock concentrations of antibody and chemotherapy were diluted to the appropriate concentration and injected into animals at 10 µl/g of body weight. Tumor length and width, and animal weight were measured two times weekly throughout the study and tumor volume was calculated using the formula above. Animals were followed until tumor volume

measured $\sim 750 \text{ mm}^3$, at which time the animals were euthanized. To allow for the proper assessment of the drug combinatorial effects, animals were treated with sub-efficacious doses of hu5F9-G4 (0.1 mg/kg every 4 days for a total of 3 cycles (Q4dx3)), or azacitidine (Vidaza®) (2 mg/kg every day for 5 consecutive days (Q1dx5) for three cycles (3 weeks total)). h1F6-SEA was dosed at 10 mg/kg every 4 days for 5 cycles (Q5x5). Animal receiving combination of treatments received each treatment at the same dose and schedule as the single treatments indicated above. Analysis of tumor volume changes over time shows that addition of h1F6-SEA, to the combination of hu5F9-G4 and azacitidine (Vidaza®) is well tolerated and elicits a greater antitumor activity than each possible double combination (h1F6-SEA + hu5F9-G4, h1F6-SEA + azacitidine (Vidaza®), or hu5F9-G4 + azacitidine (Vidaza®)).

CLAIMS

What is claimed is:

1. A method of treating a cancer in a subject, the method comprising administering to the subject a nonfucosylated anti-CD70 antibody and a CD47 antagonist, wherein the method results in a depletion of cancer cells in the subject, wherein the method does not result in a depletion of CD70+ T regulatory cells (CD70+ Tregs) in the subject, wherein the anti-CD70 antibody comprises a heavy chain variable region, a light chain variable region and an Fc domain, wherein the heavy chain variable region comprises:

- (i) a CDR-H1 comprising the amino acid sequence of SEQ ID NO:8;
- (ii) a CDR-H2 comprising the amino acid sequence of SEQ ID NO:9; and
- (iii) a CDR-H3 comprising the amino acid sequence of SEQ ID NO:10; and

wherein the light chain variable region comprises:

- (i) a CDR-L1 comprising the amino acid sequence of SEQ ID NO:11;
- (ii) a CDR-L2 comprising the amino acid sequence of SEQ ID NO:12; and
- (iii) a CDR-L3 comprising the amino acid sequence of SEQ ID NO:13,

wherein the cancer is selected from the group consisting of myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML).

2. The method of claim 1, wherein the anti-CD70 antibody comprises a heavy chain variable region comprising an amino acid sequence at least 85% identical to the amino acid sequence of SEQ ID NO:1 and a light chain variable region comprising an amino acid sequence at least 85% identical to the amino acid sequence of SEQ ID NO:2.

3. The method of claim 1, wherein the anti-CD70 antibody comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:1 and a light chain variable region comprising the amino acid sequence of SEQ ID NO:2.

4. The method of any one of claims 1-3, wherein the Fc domain of the anti-CD70 antibody is an antibody effector domain mediating one or more of antibody-dependent cellular cytotoxicity

(ADCC), antibody-dependent cellular phagocytosis (ADCP), and complement-dependent cellular cytotoxicity (CDC).

5. The method of any one of claims 1-3, wherein the Fc domain of the anti-CD70 antibody is an antibody effector domain mediating ADCC.

6. The method of any one of claims 1-5, wherein the Fc domain of the anti-CD70 antibody is a human Fc domain.

7. The method of any one of claims 1-6, wherein the anti-CD70 antibody is a nonfucosylated form of vorsetuzumab.

8. The method of any one of claims 1-7, wherein the anti-CD70 antibody is conjugated to a therapeutic agent.

9. The method of claim 8, wherein the therapeutic agent is a chemotherapeutic agent or an immunomodulatory agent.

10. The method of claim 8, wherein the therapeutic agent is a chemotherapeutic agent.

11. The method of claim 10, wherein the chemotherapeutic agent is monomethyl auristatin E (MMAE) or monomethyl auristatin F (MMAF).

12. The method of claim 8, wherein the therapeutic agent is an immunomodulatory agent.

13. The method of any one of claims 1-12, wherein the method comprises administering a population of anti-CD70 antibodies, wherein each antibody in the population of anti-CD70 antibodies comprises a heavy chain variable region, a light chain variable region, and an Fc domain, wherein the heavy chain variable region comprises:

- (i) a CDR-H1 comprising the amino acid sequence of SEQ ID NO:8;

(ii) a CDR-H2 comprising the amino acid sequence of SEQ ID NO:9; and
(iii) a CDR-H3 comprising the amino acid sequence of SEQ ID NO:10; and
wherein the light chain variable region comprises:
(i) a CDR-L1 comprising the amino acid sequence of SEQ ID NO:11;
(ii) a CDR-L2 comprising the amino acid sequence of SEQ ID NO:12; and
(iii) a CDR-L3 comprising the amino acid sequence of SEQ ID NO:13, wherein at least 50% of the anti-CD70 antibodies in the population of the anti-CD70 antibodies lack core fucosylation.

14. The method of claim 13, wherein at least 70% of the anti-CD70 antibodies in the population of the anti-CD70 antibodies lack core fucosylation.

15. The method of claim 13, wherein at least 90% of the anti-CD70 antibodies in the population of the anti-CD70 antibodies lack core fucosylation.

16. The method of any one of claims 1-15, wherein the anti-CD70 antibody is administered at a dose of about 1-30 mg/kg of the subject's body weight.

17. The method of claim 16, wherein the anti-CD70 antibody is administered at a dose of about 10-20 mg/kg of the subject's body weight.

18. The method of claim 16, wherein the anti-CD70 antibody is administered at a dose of about 10 mg/kg of the subject's body weight.

19. The method of claim 16, wherein the anti-CD70 antibody is administered at a dose of about 15 mg/kg of the subject's body weight.

20. The method of claim 16, wherein the anti-CD70 antibody is administered at a dose of about 20 mg/kg of the subject's body weight.

21. The method of any one of claims 1-20, wherein the anti-CD70 antibody is administered once about every 1-4 weeks.
22. The method of claim 21, wherein the anti-CD70 antibody is administered once about every 2 weeks.
23. The method of any one of claims 1-22, wherein the CD47 antagonist inhibits the interaction between CD47 and SIRP α .
24. The method of any one of claims 1-23, wherein the CD47 antagonist increases phagocytosis of tumor cells.
25. The method of any one of claims 1-24, wherein the CD47 antagonist is selected from the group consisting of an antibody, or antigen-binding fragment thereof, that binds to CD47, and antibody or antigen-binding fragment thereof, that binds to SIRP α , and a fusion protein comprising SIRP α , or a fragment thereof, and an antibody, or fragment thereof.
26. The method of claim 25, wherein the fusion protein comprising SIRP α , or a fragment thereof, and an antibody, or fragment thereof, comprises SIRP α , or the immunoglobulin V-like domain thereof, covalently linked to the Fc region of an antibody.
27. The method of claim 25, wherein the CD47 antagonist is an IgG1 or IgG4 antibody.
28. The method of claim 25, wherein the CD47 antagonist is selected from the group consisting of magrolimab, CC-90002, ALX148, RRx-001, TTI-622, TTI-621, and KWAR23.
29. The method of claim 28, wherein the CD47 antagonist is magrolimab.
30. The method of any one of claims 1-29, wherein the CD47 antagonist is administered at a dose of 1-50 mg/kg of the subject's body weight.

31. The method of claim 30, wherein the CD47 antagonist is administered at a dose of 1-30 mg/kg of the subject's body weight.
32. The method of claim 31, wherein the CD47 antagonist is administered at a dose of 1 mg/kg of the subject's body weight.
33. The method of claim 31, wherein the CD47 antagonist is administered at a dose of 15 mg/kg of the subject's body weight.
34. The method of claim 31, wherein the CD47 antagonist is administered at a dose of 30 mg/kg of the subject's body weight.
35. The method of any one of claims 1-29, wherein the CD47 antagonist is administered at a sub-optimal dose.
36. The method of any one of claims 1-35, wherein the CD47 antagonist is administered once about every 1-4 weeks.
37. The method of claim 36, wherein the CD47 antagonist is administered once about every week.
38. The method of claim 36, wherein the CD47 antagonist is administered once about every 2 weeks.
39. The method of any one of claims 1-35, wherein the CD47 antagonist is initially administered on days 1, 4, 8, 11, 15, and 22 of a first four-week cycle.
40. The method of claim 39, wherein the CD47 antagonist is administered on days 1, 8, 15, and 22 of a second four-week cycle.

41. The method of claim 40, wherein the CD47 antagonist is administered on days 1 and 15 of a third four-week cycle.
42. The method of any one of claims 1-41, wherein the cancer is MDS.
43. The method of claim 42, wherein the MDS is relapsed or refractory MDS.
44. The method of claim 43, wherein the subject experienced treatment failure after prior hypomethylating agent (HMA) therapy for the MDS.
45. The method of any one of claims 1-41, wherein the cancer is AML.
46. The method of claim 45, wherein the AML is relapsed or refractory AML.
47. The method of claim 46, wherein the subject received 2 prior treatment regimens to treat the AML.
48. The method of claim 46, wherein the subject received 3 prior treatment regimens to treat the AML.
49. The method of any one of claims 1-48, wherein at least about 0.1%, at least about 1%, at least about 2%, at least about 3%, at least about 4%, at least about 5%, at least about 6%, at least about 7%, at least about 8%, at least about 9%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 60%, at least about 70%, or at least about 80% of the cancer cells express CD70.
50. The method of any one of claims 1-49, wherein at least about 0.1%, at least about 1%, at least about 2%, at least about 3%, at least about 4%, at least about 5%, at least about 6%, at least about 7%, at least about 8%, at least about 9%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at

least about 45%, at least about 50%, at least about 60%, at least about 70%, or at least about 80% of the cancer cells express CD47.

51. The method of any one of claims 1-50, wherein administering the nonfucosylated anti-CD70 antibody and CD47 antagonist to the subject results in a depletion of cancer cells by at least about 5%, at least about 6%, at least about 7%, at least about 8%, at least about 9%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 95%, or about 100% compared to the amount of cancer cells before administering the nonfucosylated anti-CD70 antibody and CD47 antagonist to the subject.

52. The method of any one of claims 1-51, wherein administering the nonfucosylated anti-CD70 antibody and CD47 antagonist to the subject results in a depletion of CD70+ Tregs of no more than about 20%, about 10%, about 9%, about 8%, about 7%, about 6%, about 5%, about 4%, about 3%, about 2%, about 1%, or about 0.1% compared to the amount of CD70+ Tregs before administering the afucosylated anti-CD70 antibody and CD47 antagonist to the subject.

53. The method of any one of claims 1-52, wherein one or more therapeutic effects in the subject is improved after administration of the nonfucosylated anti-CD70 antibody and CD47 antagonist relative to a baseline.

54. The method of claim 53, wherein the one or more therapeutic effects is selected from the group consisting of: objective response rate, duration of response, time to response, progression free survival and overall survival.

55. The method of any one of claims 1-54, wherein the objective response rate is at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 60%, at least about 70%, or at least about 80%.

56. The method of any one of claims 1-55, wherein the subject exhibits progression-free survival of at least about 1 month, at least about 2 months, at least about 3 months, at least about 4 months, at least about 5 months, at least about 6 months, at least about 7 months, at least about 8 months, at least about 9 months, at least about 10 months, at least about 11 months, at least about 12 months, at least about eighteen months, at least about two years, at least about three years, at least about four years, or at least about five years after administration of the nonfucosylated anti-CD70 antibody and CD47 antagonist.

57. The method of any one of claims 1-56, wherein the subject exhibits overall survival of at least about 1 month, at least about 2 months, at least about 3 months, at least about 4 months, at least about 5 months, at least about 6 months, at least about 7 months, at least about 8 months, at least about 9 months, at least about 10 months, at least about 11 months, at least about 12 months, at least about eighteen months, at least about two years, at least about three years, at least about four years, or at least about five years after administration of the nonfucosylated anti-CD70 antibody and CD47 antagonist.

58. The method of any one of claims 1-57, wherein the duration of response to the anti-CD70 antibody and CD47 antagonist is at least about 1 month, at least about 2 months, at least about 3 months, at least about 4 months, at least about 5 months, at least about 6 months, at least about 7 months, at least about 8 months, at least about 9 months, at least about 10 months, at least about 11 months, at least about 12 months, at least about eighteen months, at least about two years, at least about three years, at least about four years, or at least about five years after administration of the nonfucosylated anti-CD70 antibody and CD47 antagonist.

59. The method of any one of claims 1-58, wherein the route of administration for the anti-CD70 antibody is intravenous.

60. The method of any one of claims 1-59, wherein the route of administration for the CD47 antagonist is intravenous.

61. The method of any one of claims 1-60, wherein the subject is a human.

62. The method of any one of claims 1-61, further comprising the administration of azacitidine.
63. The method of claim 62, wherein the azacitidine is administered at a dose of 75 mg/m² of the subject's body surface area.
64. The method of claim 62 or 63, wherein the azacitidine is administered on days 1 to 7 of a 4-week cycle.
65. The method of claim 62 or 63, wherein the azacitidine is administered on days 1 to 5 and 8 to 9 of a 4-week cycle.
66. The method of any one of claims 1-65, further comprising the administration of venetoclax.
67. The method of any one of claims 1-66, further comprising the administration of fluoroquinolone.
68. A pharmaceutical composition for the treatment of cancer, the composition comprising a nonfucosylated anti-CD70 antibody, wherein the anti-CD70 antibody comprises a heavy chain variable region, a light chain variable region, and an Fc domain, wherein the heavy chain variable region comprises:
- (i) a CDR-H1 comprising the amino acid sequence of SEQ ID NO:8;
 - (ii) a CDR-H2 comprising the amino acid sequence of SEQ ID NO:9; and
 - (iii) a CDR-H3 comprising the amino acid sequence of SEQ ID NO:10; and
- wherein the light chain variable region comprises:
- (i) a CDR-L1 comprising the amino acid sequence of SEQ ID NO:11;
 - (ii) a CDR-L2 comprising the amino acid sequence of SEQ ID NO:12; and
 - (iii) a CDR-L3 comprising the amino acid sequence of SEQ ID NO:13, and at least one pharmaceutically compatible ingredient, wherein the pharmaceutical composition is for use in combination with a CD47 antagonist, wherein the composition is for use in the method of any one of claims 1-67.

69. A kit comprising a nonfucosylated anti-CD70 antibody and a CD47 antagonist, wherein the anti-CD70 antibody comprises a heavy chain variable region, a light chain variable region, and an Fc domain, wherein the heavy chain variable region comprises:

- (i) a CDR-H1 comprising the amino acid sequence of SEQ ID NO:8;
- (ii) a CDR-H2 comprising the amino acid sequence of SEQ ID NO:9; and
- (iii) a CDR-H3 comprising the amino acid sequence of SEQ ID NO:10; and

wherein the light chain variable region comprises:

- (i) a CDR-L1 comprising the amino acid sequence of SEQ ID NO:11;
- (ii) a CDR-L2 comprising the amino acid sequence of SEQ ID NO:12; and
- (iii) a CDR-L3 comprising the amino acid sequence of SEQ ID NO:13, and instructions

for using the anti-CD70 antibodies in the method of any one of claims 1-67.